CHROMELEON®

Chromatography Management System
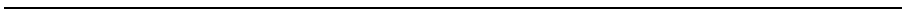

Tutorial

and

User Manual

Version: 6.80
June 2006

© Dionex 2006

CHROMELEON Software
Individual License Agreement

This legal document is an agreement between you, the end user, and Dionex Corporation. The CHROMELEON Chromatography Management System is provided to you only on the condition that you agree to become bound by the terms of this agreement, which includes the software license and the software disclaimer warranty. If you do not agree to the terms of this agreement, promptly return the disk package(s) and the accompanying items (including printed materials and binders or other containers) to Dionex for a full refund.

INDIVIDUAL CHROMELEON LICENSE

1. **GRANT OF LICENSE**   In exchange for the payment of the LICENSE FEE, Dionex grants the LICENSEE a non-exclusive right to use and display the CHROMELEON software (the "SOFTWARE") provided with this LICENSE in accordance with the terms and restrictions below. The LICENSEE is the end-user organization and its subsidiaries (excluding sub-contractors) billed by Dionex for this LICENSE. If the organization billed is not the end-user, the LICENSEE is the first end-user organization.

2. **OWNERSHIP OF THE SOFTWARE**   The LICENSEE owns the magnetic or other physical media on which the SOFTWARE is originally or subsequently recorded, but Dionex retains title and ownership of all SOFTWARE recorded on such media, regardless of the form in which it is recorded. This license is not a sale of the SOFTWARE or any copy.

3. **COPY RESTRICTIONS**   The SOFTWARE and all accompanying written materials are copyrighted, and may contain proprietary and confidential information of Dionex.

The LICENSEE may make one (1) copy of the original SOFTWARE diskettes provided with this LICENSE for backup purposes only. Any such copy automatically becomes the property of Dionex, and the LICENSEE must include the copyright notice and any confidentiality or proprietary legend with any such copy. The LICENSEE may copy the SOFTWARE from the original or backup diskettes to the computers used in accordance with the use restrictions below.

All other copying of the SOFTWARE and the accompanying materials is expressly forbidden. The LICENSEE may be held legally responsible for any copyright infringement that is caused or encouraged by the failure of the LICENSEE to abide by the terms of this license.

4. **USE RESTRICTIONS**   Under this LICENSE, the LICENSEE may use the SOFTWARE on one (1) single-user computer, or one (1) terminal of a multi-user computer. The LICENSEE must purchase a CHROMELEON Data Processing License for each additional computer on which the SOFTWARE provided under this LICENSE is used.

The SOFTWARE may be stored on the computer's local mass storage device, on a network file server, or both, as long as the above use restrictions are satisfied.

The LICENSEE may not modify, adapt, translate, reverse engineer, decompile nor disassemble the SOFTWARE, nor create derivative works based on it. The LICENSEE may not merge the SOFTWARE with another program. The LICENSEE may not modify, adapt, translate, or create derivative works based on the written materials without prior consent from Dionex.

5. **TRANSFER RESTRICTIONS**   The SOFTWARE is licensed only to the LICENSEE, and may not be transferred to anyone else without the prior written consent of Dionex. Any authorized transfer of the SOFTWARE shall be bound by the terms and conditions of this LICENSE.

6. **TERMINATION**   This LICENSE is effective until terminated. It will terminate automatically without notice from Dionex if the LICENSEE fails to comply with any of its provisions. Upon termination, the LICENSEE shall destroy the written materials and all copies of the SOFTWARE. The LICENSEE may terminate this LICENSE by destroying all copies of the software and accompanying written materials.

7. **UPDATE POLICY**   Dionex may (but is not obliged to) release updated versions of the SOFTWARE. One copy of any such versions will be provided to the LICENSEE at no additional charge for each registered SYSTEM that has a valid CHROMELEON Subscription Agreement ("SUBSCRIPTION") in effect at the time of the release. Any such software update that is provided under a SUBSCRIPTION may be used to collect data only on the one SYSTEM to which the SUBSCRIPTION applies. All terms of this LICENSE shall apply to any updates provided to the LICENSEE.

8. **MISCELLANEOUS**   This LICENSE, including the disclaimer of warranty and limited warranty, is governed by the laws of the State of California, excluding its choice of law rules.

DISCLAIMER OF WARRANTY AND LIMITED WARRANTY

The SOFTWARE and the accompanying materials (including instructions for use) are provided "as is" without warranty of any kind. Further, Dionex does not warranty, guarantee, or make any representations regarding the use, or the results of the use, of the SOFTWARE or written materials in terms of correctness, accuracy, reliability, currentness, or otherwise. The entire risk as to the results and performance of the SOFTWARE is assumed by the LICENSEE.

Dionex warrants to the original LICENSEE that the disks on which the software is recorded are free from defects in material and workmanship under normal use and service for a period of 90 days from the date of delivery of this individual LICENSE as evidenced by a copy of the receipt.

The entire liability of Dionex and the exclusive remedy to the LICENSEE shall be, at the discretion of Dionex, either (a) return of the purchase price or (b) replacement of any defective disks that are returned to Dionex with a copy of the receipt. If failure of the disks has resulted from accident, abuse, or misapplication, Dionex shall have no responsibility to replace the disks or refund the purchase price. Any replacement disk shall be warranted for the remainder of the original warranty period or thirty (30) days, whichever is longer.

THE ABOVE ARE THE ONLY WARRANTIES OF ANY KIND AND DIONEX HEREBY DISCLAIMS ALL OTHER WARRANTIES, EITHER EXPRESSED OR IMPLIED, INCLUDING (BUT NOT LIMITED TO) THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, THAT ARE MADE BY DIONEX ON THIS PRODUCT. No oral or written information or advice given by Dionex, its dealers, distributors, agents, or employees shall create a warranty or in any way increase the scope of this warranty, and the LICENSEE may not rely on such information or advice.

NEITHER DIONEX NOR ANYONE ELSE WHO HAS BEEN INVOLVED IN THE CREATION, PRODUCTION, OR DELIVERY OF THIS SOFTWARE SHALL BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL, OR INCIDENTAL DAMAGES (INCLUDING DAMAGES FOR LOSS OF BUSINESS PROFITS, BUSINESS INTERRUPTION, LOSS OF BUSINESS INFORMATION, AND THE LIKE) ARISING OUT OF THE USE OF OR INABILITY TO USE THIS SOFTWARE, EVEN IF DIONEX HAS BEEN

ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. IN THE EVENT THAT ANY LIABILITY IS IMPOSED ON DIONEX, THE LIABILITY OF DIONEX TO THE LICENSEE OR ANY THIRD PARTY SHALL NOT EXCEED THE PURCHASE PRICE OF THE SOFTWARE.

Some states do not allow exclusion or limitation of the duration of the implied warranties or exclusion or limitation of liability for incidental or consequential damages, so that the above limitations may not apply to you. This warranty gives you specific legal rights, and you may have other rights, which vary from state to state.

Trademarks and Product Names

Below please find a list containing various trademarks and product names. All trademarks (™) and registered trademarks (®) belong to Dionex Corporation unless indicated otherwise. All other trademarks or registered trademarks are the property of their respective owners.

A

AAA-*Direct*™ Amino Acid Analysis System

AAA-*Direct*™ Certified

AAA-*Certified*™

AAES™ Anion Atlas® Electrolytic Suppressor

Acclaim® columns

Acrodisk® (Gelman Sciences, Inc.)

Acurate™ Flow Splitter

Advanced Computer Interface (ACI)

Advanced Gradient Pump (AGP)

AD20 or AD25 UV/Vis Absorbance Detector

AES® Atlas Electrolytic Suppressor

Alliance® (Waters Corp.)

AminoPac® Columns

AminoTrap™ Columns

AMMS®, AMMS®-ICE, or AMMS-ICE II Anion MicroMembrane Suppressor

AnchorChips™ (Bruker BioSciences)

Anion Atlas® Electrolytic Suppressor

Anion Self-Regenerating Suppressor®

Anotop™ (Whatman)

AppliCard™

AQA™ Mass Spectrometer (Thermo Electron Corp.)

ARC™ Automated Run Completion

Aroclor™ (Monsanto Corp.)

AS Autosampler

AS-HV Autosampler

AS40 or AS50 Automated Sampler

AS3500 Autosampler

ASE® 100, 200, or 300 Accelerated Solvent Extractor

ASE® Solvent-Controller

ASI-100™, ASI-100T™, ASI-100P™, or ASI-100PT™ Autosampler

ASPEC™ (Gilson, Inc.)

ASRN™ Anion Self-Regenerating Neutralizer

ASRS®, ASRS® II, or ASRS® ULTRA Anion Self-Regenerating Suppressor

ATC Anion Trap Column

Atlas® Suppressor

Auto OnGuard™ (but OnGuard®)

AutoASE® software

autoflex™ (Bruker BioSciences)

Autoion®

AutoNeutralization™ system or technology

AutoQ™ equipment qualification

AutoRegen® System

AutoSelect™

AutoSuppression® device

AXIMA-QIT™ (Shimadzu Biotech)

AXP/AXP-MS Metering Pump

B

BAKER INSTRA-ANALYZED® Acids (J. T. Baker)

Biodialyser™ (AmiKa, Inc.)

BioLC® System

BioPlus™ Columns

BioSelect™ (The Separations Group)

BorateTrap™ Column

C

CA Carbamate Analyzer

CAES® Cation Atlas® Electrolytic Suppressor

CarboPac™ MA1, PA1, PA10, PA-100 columns

Cation Atlas™ Electrolytic Suppressor

Cation MicroMembrane Suppressor®

Cation Self-Regenerating Suppressor®

CD Builder™ (AppletWare, Inc.)

CD20, CD25, CD25A Conductivity Detector

Chemraz® (Greene, Tweed & Co.)

ChromatoCritters™

CH-2 Column Heater

CHX650 Column Temperature Controller

CHROMELEON® Chromatography Management Software

CHROMELEON® Purification Suite™

CMD™ Carbohydrate Membrane Desalter

CMMS® Cation MicroMembrane Suppressor

CMMS® II Cation MicroMembrane Suppressor®

Continuously Regenerating Anion Trap Column (CR-ATC)

Continuously Regenerating Cation Trap Column (CR-CTC)

Cryptand columns

CSRN™ Cation Self-Regenerating Neutralizer

CSRS®, CSRS® II, CSRS® ULTRA Cation Self-Regenerating Suppressor

D

DataDetective™ (AppletWare Inc.)

dBASE® (Borland International, Inc.)

Dequest® (Monsanto Corporation)

Dionex®

DNAPac® column (for NucleoPac)

DNAPhor™ SB1.5 kB Sieving Buffer Kit (for NucleoPhor)

Dowex® (Dow Chemical Company)

DX-80 Ion Analyzer

DX-120 Ion Chromatograph

DX-320 Ion Chromatograph

DX-500 or DX-600 Ion Chromatography System or HPLC System

DX-800 Process Analyzer

DX-LAN™ Instrument Interface

E

ED40, ED50, or ED50A Electrochemical Detector

EG40 or EG50 Eluent Generator

Electrochrom

EluGen®

EO1 Eluent/Solvent Organizer

esquire2000™ (Bruker BioSciences)

esquire3000 (Bruker BioSciences)

esquireHCT (Bruker BioSciences)

F

FAMOS™ Fully Automated Micro Autosampler

FastLoc™ (Thermo Electron Corp.)

Finnigan AQA™, Finnigan MSQ™

Flarefit®

FluoBoost Micro Fluorescence Flow Cell

Foxy® (Isco, Inc.)

FPLC® (Pharmacia LKB)

Freon® (E.I. du Pont de Nemours & Co.)

Fusica columns

G

GM-2, GM-3, or GM-4 Eluent Gradient Mixers

GP40 or GP50 Gradient Pump

GS50 Gradient Pump

H

HPICE® (mostly replaced by IonPac® ICE)

Hydromatrix™ (Varian Associates, Inc.)

I

ICAT® (University of Washington, Seattle, USA)

ICE (ion-exclusion columns, e.g., ICE-AS6)

ICS-90, ICS-1000, ICS-1500, ICS-2000, or ICS-2500 Ion Chromatography System

ICS-3000 Conductivity Detector

ICS-3000 Detector/Chromatography Module

ICS-3000 Dual Pump

ICS-3000 Electrochemical Detector

ICS-3000 Eluent Generator

ICS-3000 Single Pump

InkJet® (Hewlett-Packard)

IonPac® columns

IonPhor™ Electrolyte Buffers

IonSep® reagents

IP20, IP25, or IS25 Isocratic Pump

Irganox® (Ciba)

J

Just Add Water

K

Kalrez® (E.I. du Pont de Nemours & Co.)

KEL-F® (3M Corporation)

L

LANtastic® (Artisoft, Inc.)

LaserJet® (Hewlett-Packard Corporation)

LC5 Injection Module

LC10 Chromatography Organizer

LC20 Chromatography Enclosure

LC25 or LC30 Chromatography Oven

M

Mascot® (Matrix Science Ltd.)

MassLynx™ (Micromass)

µ-Guard™ columns

µ-Dumper

µ-Fluidics

MetPac™ Reagents

MFC-1 (Metal-Free Column)

MICRO® (International Products Corp.)

Microcolumn

Micro Precolumn

MicroBead™ resin

Microguard columns

MicroInjection valve

MicroMembrane™ Suppressor

Millennium® (Waters Corp.)

Milli-Q® (Millipore)

MMS™ MicroMembrane™ Suppressor

Mono Q® (Pharmacia LKB)

MonoDisk™

Monolithic capillary column

MonoStandard®

M Path™ (Thermo Electron Corp.)

MPIC® (Mobile Phase IC)

MRA™ Active Flow Splitter (Rheodyne)

MS-DOS® (Microsoft Corporation)

MSQ™ Mass Spectrometer (Thermo Electron Corp.)

N

Nano column

Nano LC

Nano precolumn

Nanospray

N-EVAP® (Organomation Associates, Inc.)

NovaPak® (Waters Corp.)

NucleoPac (now DNAPac®)

NucleoPhor (now DNAPhor™)

O

OligoStandards™

OmniFLEX™ (Bruker BioSciences)

OmniPac® Columns

OnGuard®, OnGuard® II Sample Prep Station (but Auto OnGuard™)

Optima™ (Fisher Scientific)

ORBO™ (Supelco, Inc.)

P

P680 HPLC Pump

PaintJet® (Hewlett-Packard)

PC10 Postcolumn Pneumatic Controller

PC10 Reagent Delivery Module

PD40 Diode Array Detector

PDA-100 Photodiode Array Detector

PeakNet® Chromatography Workstation

Pentium® (Intel)

PepMap™

Pico-Buffer®

PicoView™ (New Objective, Inc.)

PolyVial™

PowerPoint® (Microsoft)

Probot™ Microfraction Collector

Process 450 (data system for the 8200 series)

ProPac® Columns

ProteinChips® (Ciphergen)

Purification Suite™ (better: Chromeleon® Purification Suite™)

PWA™ Purification Workflow Automation

Q

QSTAR® (Applied Biosystems)

QTRAP® (Applied Biosystems)

R

Reacti-Block™ (Pierce Chemical Company)

Reacti-Therm™ (Pierce Chemical Company)

Reagent-Free™

RF 2000 Fluorescence Detector

RFIC™

S

SC-CSRS®

SELDI ProteinChips® (Ciphergen)

Self-Contained Cation Self-Regenerating Suppressor

SelectaPore™ Columns (The Separations Group)

Self-Regenerating Suppressor®

SEQUEST® (University of Washington, Seattle, USA)

Series 600 SFC and SFC/GC Systems

SFE-723 Supercritical Fluid Extractor

SFM™ Sample and Fraction Manager

SmartFlow™

SP10 AutoNeutralization™ System

SpeedVac™ (Savant Corp.)

SRC SRS Controller)

SRN™ Self-Regenerating Neutralizer

SRS® Self-Regenerating Suppressor

Summit® HPLC System

SUPELCOSIL™, Supelguard™ (Supelco, Inc.)

Superba columns

Superose® (Pharmacia)

Supor® (Pall Corporation)

SupraPur® (EM Industries, Inc.)

Switchos™ Microcolumn Switching Device

T

TAC-1 (Trace Anion Concentrator)

TCC-1 (Trace Cation Concentrator)

TCC-100 (Thermostatted Column Compartment)

Teflon®, Tefzel® (E.I. du Pont de Nemours & Co.)

ThermoFlare™

Thermos Column Oven

Triton® X-100 (Rohm & Haas)

TurboIonTrap® (Applied Biosystems)

TurboVap® (Zymark Corporation)

Tween® 20 (Atlas Chemical Co.)

U

UCI-100 or UCI-50 Universal Chromatography Interface

UI20 Universal Interface

ultraflex (Bruker BioSciences)

UltiChrom™ software

UltiMate™ 3000 LC System

UltiMate™ Dual Nano and Capillary LC System

UltiMate™ Nano and Capillary LC System

UltiMate™ Plus

UltiMate™ x2

Ultrex® (J.T. Baker)

UV-Booster

UVD 170S/170U HPLC UV/Vis Detectors

UVD 340S/340U HPLC Photodiode Array Detectors

U-Z View™ Capillary Flow Cell

V

Vespel® (E.I. du Pont de Nemours & Co.)

VHP™ (The Separations Group, Inc.)

Virtual Column™ separation simulation tool

Vortex-Genie® (Scientific Industries, Inc)

Voyager™ (Applied Biosystems)

Vydac® columns (The Separations Group, Inc.)

W

Windows® 2000/XP (Microsoft Corporation)

Windows NT® (Microsoft Corporation)

Wonderware InTouch® (Wonderware Corporation)

Z

Xcalibur® (Thermo Electron Corp.)

Zitex® (Norton Chemplast)

Zorbax® (E.I. du Pont de Nemours & Co.)

Abbreviations

Below please find a list of the most frequently used abbreviations in Chromeleon, the online Help, and the manual:
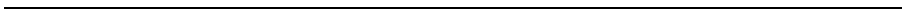
APS   Automated Purification System

➤*ARC*   Automated Run Completion

➤*AIA*   Analytical Instrument Association

BCD   ➤*Binary-Coded Decimal Code*

BOOTP Bootstrap Protocol (Internet protocol)

CAN   Controller Area Network

CD   Compact Disk

CDS   Chromatography Data System

CE   Capillary Electrophoresis

CFR   Code of Federal Regulations (often synonymous with 21 CFR Part 11)

CM   Chromeleon$^{®}$ Chromatography Management System

CMB   ➤*Backup* container/file (file extension)

CMS   Chromatography Management System

CS   Cluster Server

DAC   Digital Analog Converter

DHCP   Dynamic Host Communication Protocol

DNS   Domain Name Server

DX   Dionex

EMF   Early Maintenance Feedback

GC   Gas Chromatography

➤*GLP*   Good Laboratory Practice

GMP   Good Manufacturing Practice

GPIB   General Purpose Interface Bus

HPLC   High Performance Liquid Chromatography

IC	Ion Chromatography
IPC	Chromeleon Instruments Server or Instruments PC
➢*IQ*	Installation Qualification
➢*ISTD*	Internal Standard
LAN	Local Area Network
LIB	UV Spectra Library (file extension)
LIC	Chromeleon ➢*License Server*
➢*LIMS*	Laboratory Information Management System
LOD	➢*Limit of Detection*
MAC	Media Access Control address (unique address of each network interface card)
MRA	Mass Rate Attenuation (Flow Splitter)
MS	➢*Mass Spectrometer*
MSV	Motorized switching valve
NFP	➢*Network Failure Protection*
➢*ODBC*	Microsoft Open Database Connectivity
➢*OQ*	Operational Qualification
PAN	➢*Control Panel* for chromatographic system control (file extension)
➢*PDA*	Photodiode Array Detector
➢*PGM*	Control program for chromatographic system control (file extension)
PN	PeakNet® Chromatography Management System
➢*PPA*	Peak Purity Analysis
➢*PPI*	Peak Purity Index
➢*PQ*	Performance Qualification
➢*PWA*	Purification Workflow Automation
➢*QNT*	Quantification method for signal interpretation and evaluation (file extension)
QRF	➢*Query* file (file extension)

RDF ➤*Report Definition File*; includes the layout definition for the report and export (file extension)

➤*RPC* Remote Procedure Call

RSD Relative Standard Deviation

➤*SDK* Software Development Kit for Chromeleon

SEQ ➤*Sequence*; that is, the sequence of samples plus the corresponding files (file extension)

SFM 🌐 **Sample and Fraction Manager** (see the related topic in the **Administrator Help** section)

➤*SIM* Selected Ion Monitoring (MS channel for specific masses)

➤*SOR* Signed Off Results (File) = signed sequence

➤*SQL* Structured Query Language (computer language)

SSM Safety and Solvent Monitor

SST ➤*System Suitability Test*

TCP/IP Transmission Control Protocol/Internet Protocol (class of Internet protocols)

➤*TIC* Total Ion Current (MS channel for the entire mass range or a part thereof)

➤*TTL* Transistor-Transistor Logic

UDP User Datagram Protocol (IP communication protocol)

UDC ➤*User-Defined Column*

USB Universal Serial Bus

VCD ➤*Virtual Channel Driver* (driver for virtual devices)

WSP Workspace; that is, the definition of a screen layout (file extension)

For a list of the ➤*Institutes and Institutions for Industry Standards*, refer to the Glossary section.

Tutorial

Table of Contents

IX. Special Functions T-87

Addendum: Keyboard Shortcuts + Glossary T-105

Index... T-i

Chromeleon - Tutorial

Welcome to Chromeleon, the innovative chromatography management system.

Use this tutorial to become familiar with Chromeleon.

Learn how to

- Start the program

- Perform an analysis

- Reprocess your data

For an overview of the topics, refer to the ➔ *Table of Contents*.
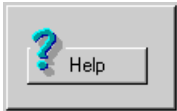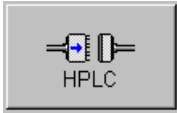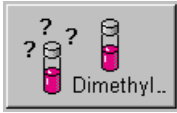
➡️ *Tip:*

The arrow (➔) references topics in the Tutorial.
There are no references between the Tutorial and the User and/or Administrator Manual.

Table of Contents

I.

➔*Starting Chromeleon*: If the computer and the Windows (2000 or XP) operating system are running, you can start Chromeleon by clicking the Windows **Start** button on the task bar.

II.

➔*Managing Data* Chromeleon allows you to manage data in folders and directories similar to Microsoft Windows. The tool that helps you to handle your chromatography data is the Browser.

III.

Online Help: The Tutorial can only deal with selected aspects of Chromeleon. For more information, refer to ➔*Online Help* and/or to the User Manual.

IV.

Controlling your HPLC system: Make sure that your chromatography or CE instruments are correctly connected to the PC via a serial port. If the instruments are connected correctly, you can operate the pump, autosampler, detector, etc. from a control panel on your PC. (For more information, refer to the ➔*Direct Control* section.) In addition, you can create a control program (PGM File) to operate the instruments automatically. (For more information, refer to ➔*Programmed Control (PGM File)*.

V.

Analyzing Samples: Can you control your instruments from the PC? If you can, you can start the chromatographic analysis. To use all functions provided by Chromeleon, we recommend that you create a ➔*Sample List (Sequence)* first:

Use the ➔*Sequence Wizard* to include the samples to process in the sample list. The wizard guides you in defining the number of samples, the order of sample processing, the injection volumes, the sample type (analysis or standard), and the location where Chromeleon finds information about how to perform the analysis.

V.
(Cont'd)

To make this information available to Chromeleon, create the corresponding files (PGM File, QNT File, see below) and enter a program and a method name.

When you have created the sample list, you can start the ➔*Analysis*. The resulting data is automatically saved.

VI.
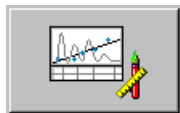
Quantifying: The ➔*Quantification Method (QNT Method)* allows you to minimize the reprocessing effort for single chromatograms. The QNT Method defines the detection parameters, the peak identification, and the calibration of the substances in several samples.

VII.
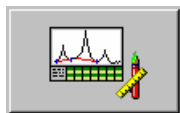
Data Analysis: You can display the result of a chromatographic analysis on the screen. ➔*Data Analysis* allows you to manually change integration limits directly in the single chromatogram or to re-evaluate samples based on new parameters ("offline.")

VIII.

Printing Results: You can ➔*print* your analysis data immediately after data acquisition or at any time later. For perfect adjustment to your personal requirements, create your own report templates in the ➔*Printer Layout*, define your own result variables, or embed your company logo.

IX.

Managing Data (Special Functions): In addition to the basic functions, the Chromeleon provides various special functions. For example, you can search samples according to defined criteria (query), backup and restore data, electronically sign sequences, and search spectra. For more information, refer to ➔*Special Functions*.

Sequence - Flow Chart
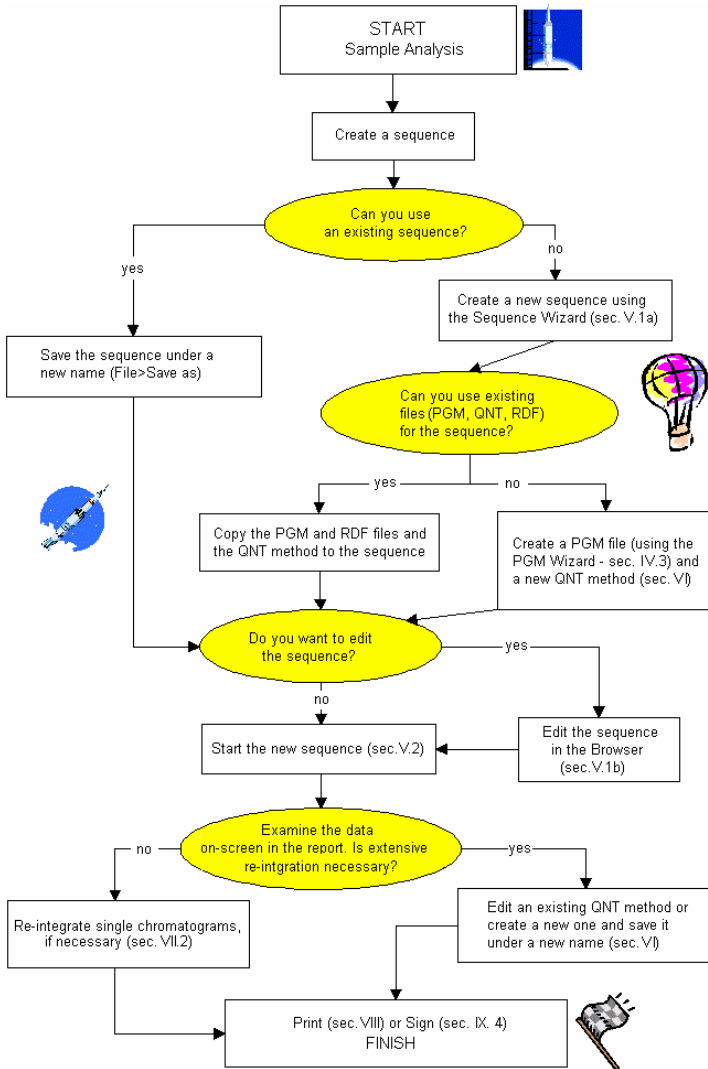
I. Starting Chromeleon

Objectives of this chapter:

- To make you familiar with the basics of a client/server software

- To start the client and server programs

Chromeleon is a client/server program, operable under Windows 2000 and Windows XP. The two independent program parts, the client and the server, allow you to operate the program from different PCs on a network:
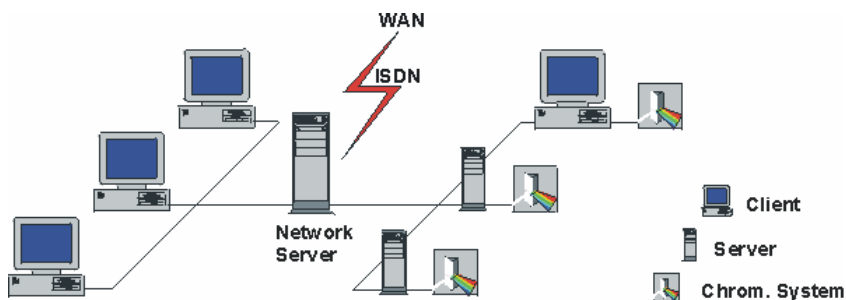
Server: The server PC is directly connected to the different modules of the chromatography systems. The server communicates with the individual modules of a ➔*Timebase* and controls them actively. You usually perform only two actions in the server program: you start and stop the server. In the client program, you specify which control actions the server shall perform.
Client: The client program allows you to work offline, i.e., to perform certain tasks independent from the server, for example, creating sequences, processing data, searching single spectra in a spectra library, etc. The client can be installed locally. In this case, the client and server programs can be installed on the same PC that is connected to the chromatography system. This is typical for a single-site installation (one timebase only).

In addition, remote client PCs can be connected to the chromatography server on the company network. You can then perform client functions on a PC that is not connected directly to the chromatography system but to the server PC on the network.

A characteristic of client/server systems is that certain processes are performed on the client, while others run on the server. The cooperation between the client and the server is crucial to the full functionality of the program.

Since the client and the ➔ *Server* are independent programs, you have to start them separately.

Starting the Client Program

• Click Start > Programs > **Chromeleon** to start the Chromeleon client.
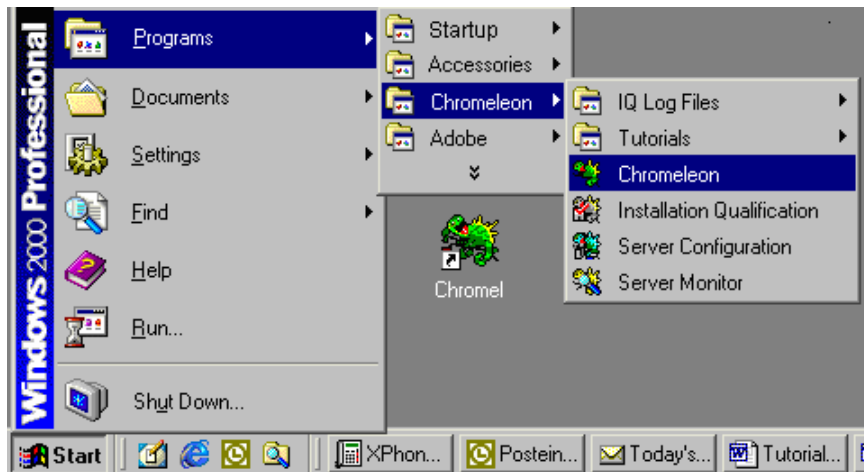
• If User Management was created and enabled during installation, a logon dialog box appears. In this case, enter your user ID and personal password.

Starting the Server Program

If you do not only want to view data but control an entire chromatography system and perform your own analyses, start the Server Monitor program. The server controls all connected instruments and allows direct instrument operation and control via the PC.

• Select **Server Monitor** to start the **Server Monitor** program. The Chromeleon icon appears on the Windows task bar next to the Windows system clock.

☞ *Tip:*
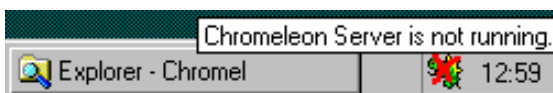
During installation, Chromeleon usually creates a link in the Autostart group and the corresponding icon is displayed. You can also configure the program in such a way that it starts whenever the computer is started.

- Move the mouse cursor to the icon. You will see the quick info message: **CHROMELEON Server is not running**.

- Open the context menu (right-click) and select **Start Server** (or double-click the icon and click **Start**). The icon can have different colors. Gray coloring indicates that the server is running idle.

- Both, the client program and the server program are active now. The client control panel allows direct access to all instruments that are connected to the server.

📝 *Note:*

The instruments of a system are combined in a common ➜ Timebase. Instruments and timebases are installed and configured in the Server Configuration program. Since your Dionex Service Representative usually performs this task during the initial installation of Chromeleon, the Tutorial does not provide more information about this.

II. Managing Files (Overview)

The Browser is Chromeleon's main tool for file management. In the Browser, you can open, manage, and/or save files and chromatographic data. The Browser window is opened automatically whenever Chromeleon is started.

Opening the Browser

- Click the Browser icon ▐▊ to open the Browser window or to display it on top.

Browser Structure and Functions

Browser functions and structure are similar to the Windows Explorer.

⚠ *Caution:*

Although the Browser is similar to the Windows Explorer, you should not confuse the Browser with the Windows Explorer. Do not use the Windows Explorer for operations within the Chromeleon datasources! Usually, the system administrator will prevent these operations by protecting the datasource directory.

The left pane shows the directory structure in which the directories and folders are arranged. The right pane provides detailed information about the currently selected item.
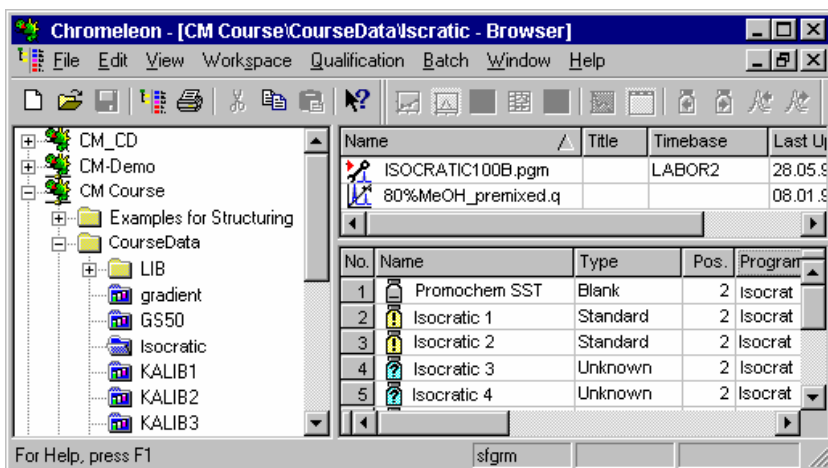
Top-level directories in the directory structure of the Browser are referred to as ➔*Datasources*, for example, ComputerName_local 🗔🐢. Datasources are used to save sequences and the related data, programs, and results.

- Click the **+** or **-** sign beside a datasource name to display or hide the items (yellow folders) underneath.

The ➔*Sequences* (blue folders: 🗃) created by the user are the low-level items in the directory structure. During the initial installation of Chromeleon, a default directory is created automatically. The directory name corresponds to the name of the timebase. The timebase contains a sequence that stores manually acquired data. Thus, it is called **manual**.

- Select a **directory** name to display the subdirectories on the right pane.
- Select a **sequence** to view the files and samples contained in the sequence.

When you select a sequence name, the files contained in the sequence appear in the upper right Browser pane. The different icons indicate the file types:

Symbol	**File Extension**	**File Type**
⚡	*.pgm	Control file or PGM File
📈	*.qnt	Quantification method
🖼	*.rdf	Report template or report definition
🖥	*.pan	Control panel

The sample list showing the individual samples of the sequence is displayed on the lower right Browser pane. The different icons indicate the sample types:

| **Symbol**	**Name**	**Sample Type**
🍶	Unknown	Unknown sample
🍶	Blank Run	Blank
🍶	Validation	Validation sample
🍶	Standard	Standard sample
🍶	Matrix	Matrix blank sample
🍶	Spiked	Spiked sample
🍶	Unspiked	Unspiked sample

II. Managing Files (Details)

Opening Files

Double-click to open a file in the Browser. Chromeleon loads the appropriate chromatographic environment according to the file type:

- Double-click a control file (PGM File) to open the PGM Editor.
- Double-click a quantification method (QNT File) to open the QNT editor.
- Double-click a PAN file to open a control panel.
- Double-click a report template (report definition file = RDF) to open the Printer Layout.

Opening Samples

When opening samples, keep the following in mind (independent of the file type):

- If raw data exist for several channels of a processed sample (Status = *finished, multiple, or interrupted*), double-clicking the sample opens one channel only (for example, a UV channel at a wavelength of 254 nm).

- Right-click a sample (here: **sample1**) and select **Open** from the context menu. A submenu is opened on which Chromeleon lists all channels that exist for this sample:
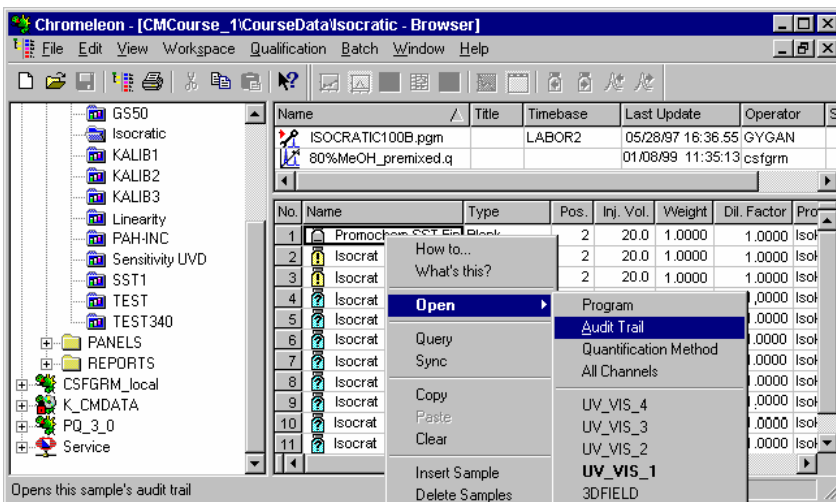
If you record four different channels of a sample with a diode array detector when you process the sample (as was in this example), a separate chromatogram will be available for each channel (here: **3DFIELD** and **UV_VIS-1** to **UV_VIS-4**).

• Select the channel or the chromatogram to display.

The highlighted channel (here: **UV_VIS-1**) is used as the "preferred channel." The preferred channel automatically opens when you double-click a sample. Select **Preferences** on the **File** menu, and then specify which channel Chromeleon shall use as the preferred channel.

Moving and Copying Items

Different ways are available to move or copy a subdirectory, sequence, or file:

• Left mouse button: Use this button to move or copy the element according to the settings made for the Left mouse button Drag/Drop behavior on the Browser tab page (select Preferences on the File menu).

• Right mouse button: Use this button to move or copy the element as desired.

• Keyboard shortcut: Use keyboard shortcuts to perform the desired action, for example, <Crtl> <C>, to copy the element or <Crtl> <V> to paste the element.

III. Online Help

There may be issues and questions that are not discussed in this Tutorial. In this case, refer to the User Manual and the online Help for more information. You can access online Help anywhere in the program:

- ➔*Context-sensitive Help* answers your questions that may occur at the current cursor position.

- ➔*Systematic Help* allows you to

    - Select an interesting topic on the Contents tab page

    - Search the index for any required information

    - Search for specific words and phrases in help topics by searching the entire text of the help system.

1. Context-sensitive Online Help

Access to the online Help depends on your current cursor position. Usually, a window opens from which you can access systematic online Help. In other cases, a popup window opens providing a short context-sensitive description and links for access to related topics.

Help via the Context Menu

Right-click to open the context menu:
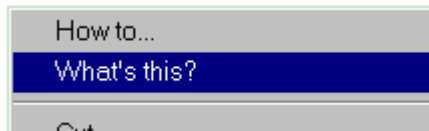
Select **How to ...** to display detailed information about actions that can be performed at the current cursor position. Select **What's this?** for a description of the selected item, command, etc. In some cases, **What's this?** topics provide information that is more general. Usually, links are available for specific information.

Help via the Help Button and Icon

First, click the [icon] icon on the toolbar to display a question mark appended to the cursor, and then click the item of interest for specific information.

In some cases, a **Help** button is available instead of the context menu or the question mark. Click Help to display information about the current screen.

In some windows in which you can enter parameters and variables, such as in the QNT Editor, click **Explain Param.** or **Explain Variable** for information about your selection.

Help via the F1 Key

In addition, you can always press the F1 key. This help information corresponds to the context-sensitive help information that is available via the question mark cursor or the **Help** button.

2. Systematic Online Help

Select **Index** on the **Help** menu for an overview of the topics that are available in the online Help. The help window provides the following tabs:

- **Contents**
- **Index**
- **Find**

Contents Tab

The Contents tab page contains the table of contents of the online Help. Double-click a book symbol to display the topic titles. Double-click an open book symbol to close the book again.

Topic titles are marked by a page symbol with a blue question mark. Double-click to open the topic.
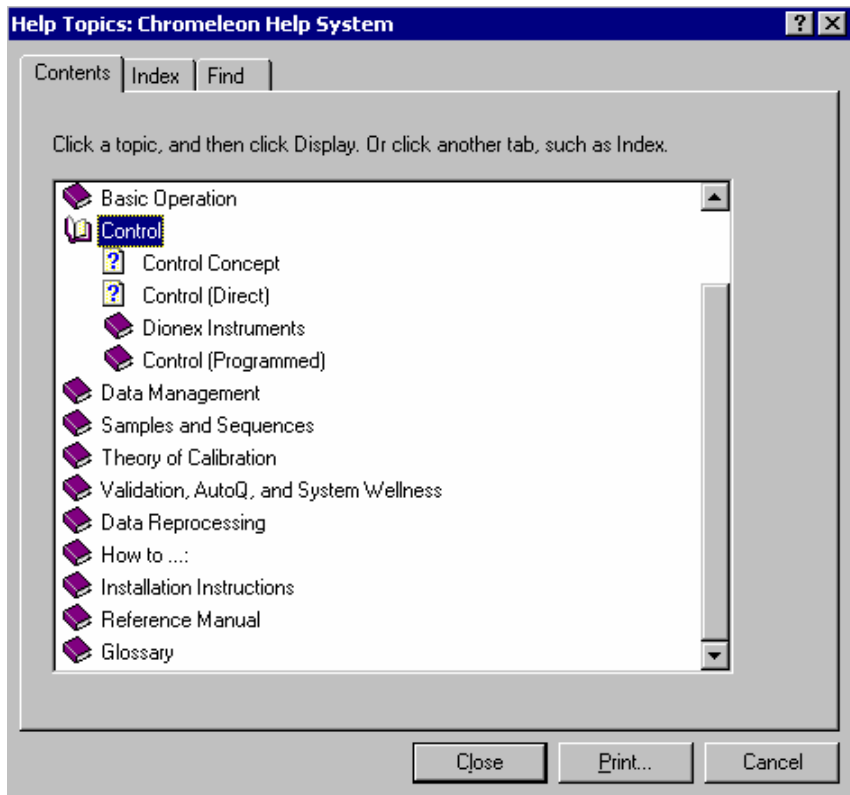
Index Tab

The Index tab page enables you to find a specific term by searching the index entries. Type the first few letters of the word you are looking for in the first field until the desired term appears in the second field. Click the index entry you want, and then click **Display** to open the desired topic. If several topics are found, the **Topics Found** window opens. Select an entry and then click **Display**.

Find Tab

The Find tab page enables you to search for specific words and phrases in help topics by searching the entire text of the help system. (The query is not limited to the index entries.) First, Chromeleon generates a list that contains every word from the online Help file. You can then search the resulting list for a specific term. The procedure is similar to searching for a specific term on the Index tab page.

The query finds all topics that contain the entered word(s). That is why the Topics Found list is far more extensive than the one found when searching the index. To narrow the search, you can select matching words in the second field:
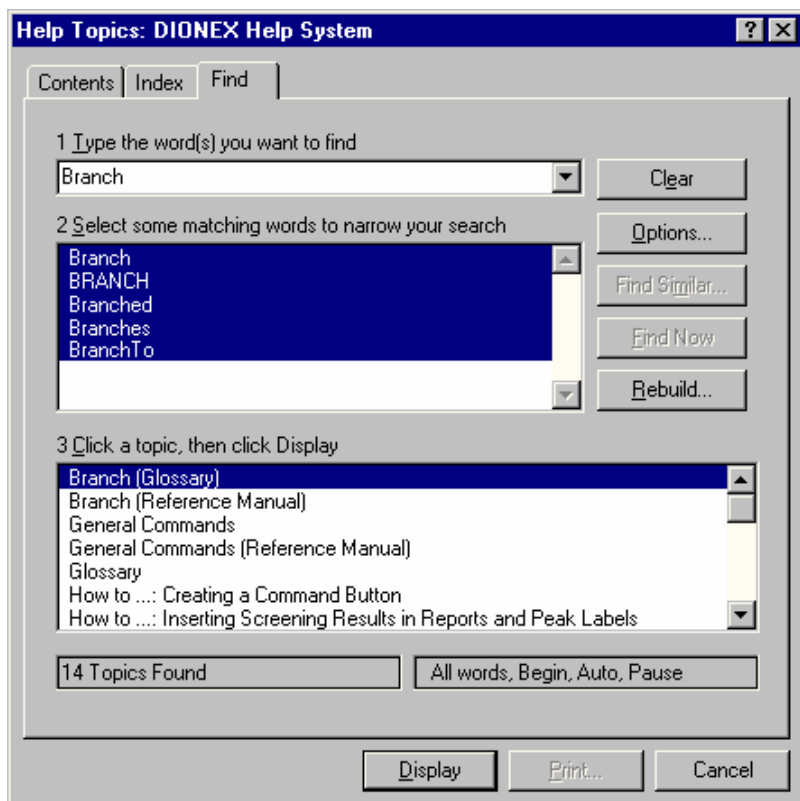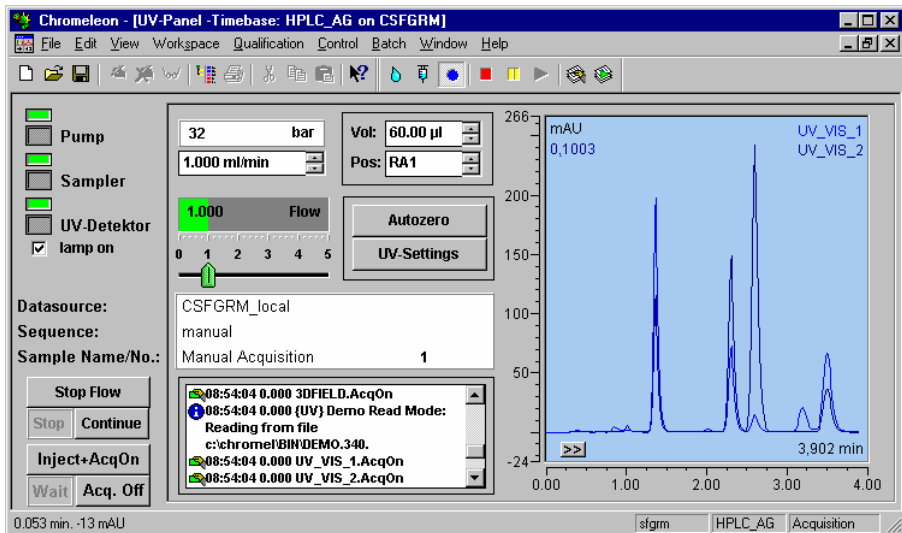
IV. Control

1. Direct Control

Objectives of this chapter:

- To describe the purpose and functions of a control panel

- To connect the devices to Chromeleon, using a control panel

- To display the current chromatogram

1a) The Control Panel

A control panel (often just called panel) allows you to control an entire chromatography system from your PC. The system may consist of one or several analytical instruments that are installed under the same timebase. The control panel comprises the different elements required to control your system and to display the current chromatogram.


The above image shows a control panel for controlling the basic functions of a pump, an autosampler, and a two-channel UV detector. On the right, you see the detector signal.

Chromeleon includes more than a hundred default control panels that cover most applications. Although it is possible to change the existing panels, you rarely have to do so. For more information, see ➔*Modifying a Control Panel* in the **Special Functions** section.
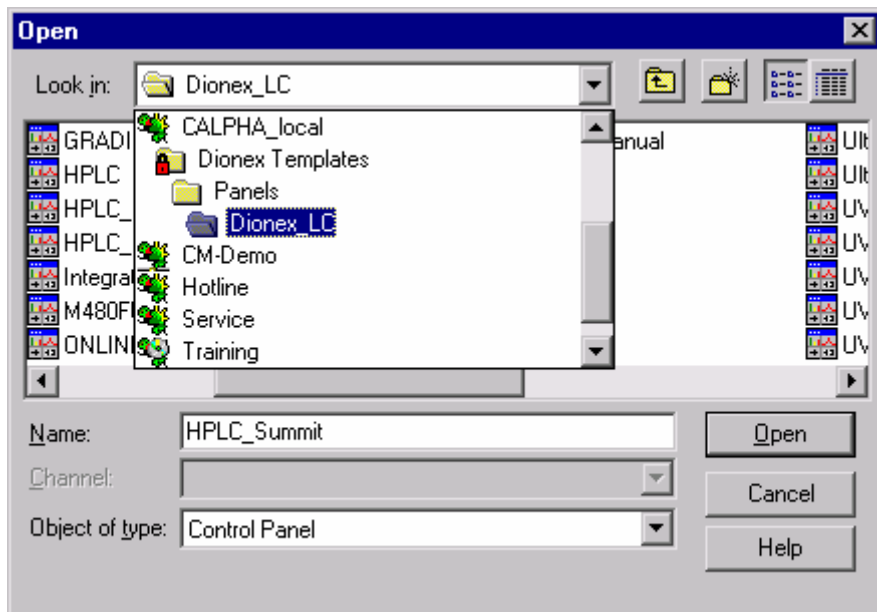
Opening a Default Control Panel

From the Browser

- Open the **Dionex Templates > Panels** directory and select the control panel that corresponds to your system.

- Double-click to open the panel.
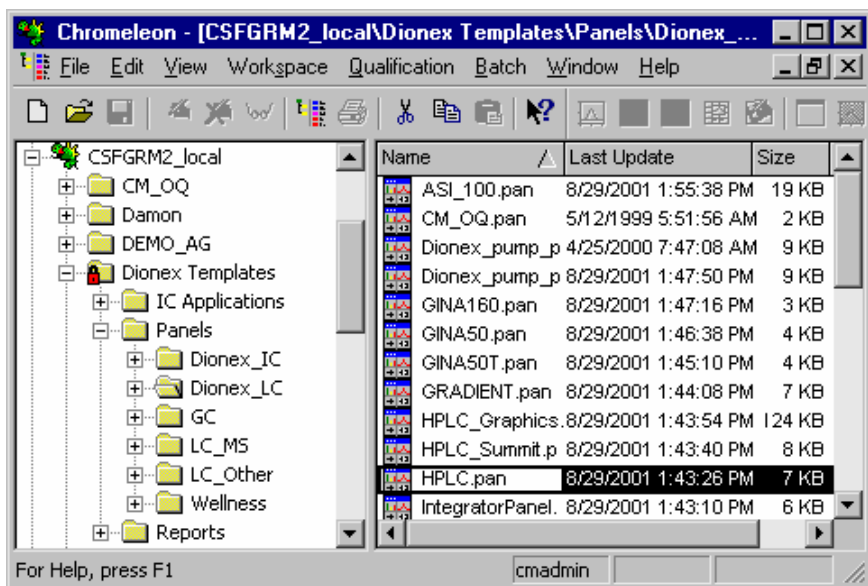
From the File or context Menu

- Select **Open** on the **File** or context menu.

- In the **Open** dialog box, select **Control Panel** in the **Object of type** field.

- In the **Look in** field, select the local ➔*Datasource* of the system. This datasource is automatically saved on your computer during the Chromeleon installation. The datasource is named <NAME_LOCAL>, wherein <NAME> is the computer name. If the **Look In** field contains further datasources, you can also search here for an appropriate control panel.

- Open the **Dionex Templates > Panels** directory and select one of the default control panels (*.**pan**).

- Double-click to open the panel.

As soon as a control panel has been opened, Chromeleon tries to connect to the ➔*Timebase* that is linked to this control panel.

If the connection cannot be established, the control elements are grayed and an error message is displayed. In this case, connect the control panel to the timebase as described in the next topic (see below).

1b) Connecting a Control Panel to a Timebase

When a control panel is open, you can connect to any Chromeleon timebase at any time. However, verify that the server on which the timebase is installed has been started as described in ➔ *Starting Chromeleon*.

- Select **Connect to Timebase** on the **Control** menu to create and/or to change the timebase assignment.

- In the dialog box that opens, determine the ➔ *Server* and the ➔ *Timebase* to which you want to connect the control panel.
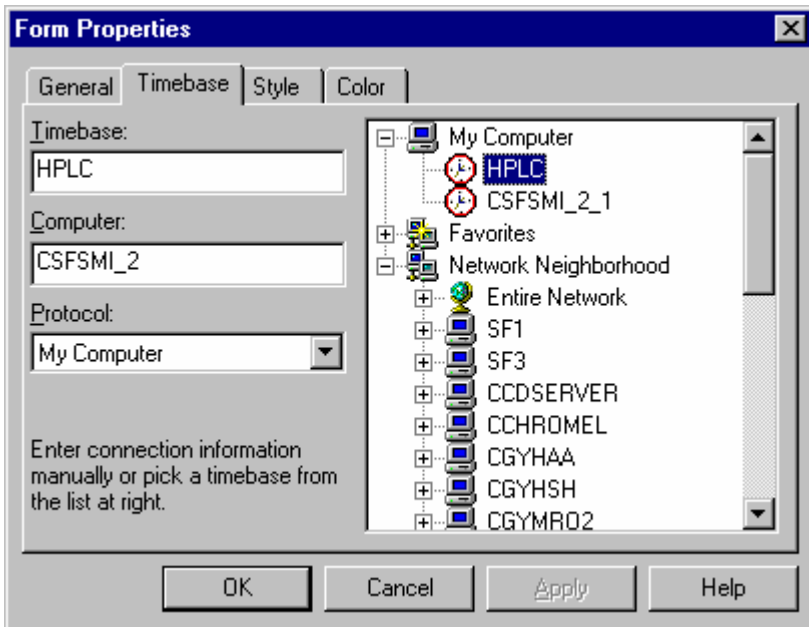
- If the server is started locally on your computer, click the **+** character to open the **My Computer** submenu. If the server is running, the names of all timebases that are installed on the respective server will be displayed. If the server is not running, a corresponding message will appear. In this case, start the server first and then select a timebase, for example, HPLC. The **Computer** field will automatically show your PC, that is, the name that was specified during the Windows installation. The **Protocol** field shows the entry **My Computer**.

- If your PC is connected to other computers via a network or modem, you can also access a server that was not started on your local computer, but on another PC. To do so, the select the network protocol under **Protocol**. (Access to other computers and timebases is usually via the Internet TCP/IP protocol.) Open the **Network Neighborhood** to browse for the required timebase.

- Chromeleon remembers the servers accessed on other computers. These servers are listed under **Favorites**.

As soon as you complete the dialog, Chromeleon tries to access the selected timebase. If communication fails, this may be due to several reasons.

Possible Problems when Connecting to a Timebase

Cannot connect to timebase "..."

$\Rightarrow$ The error message indicates that the corresponding server is not running, that the selected timebase does not exist on this server, or that a wrong communication protocol has been selected.

- Start the server as described in **Starting Chromeleon** or select the required timebase or protocol.

👉 *Tip:*

Chromeleon can communicate via various network protocols such as IPX, TCP/IP, or NetBEUI. Communication between two stations is possible only if the same (!) network protocol is installed and selected. Generally, it is sufficient to install the corresponding Microsoft ("IPX/SPX-compatible protocol"; "NetBEUI"; "TCP/IP") or Novell ("Novell IPX ODI Protocol") network protocols via **Settings > Control Panel > Network > Configuration**. *The protocol that is actually used depends on the current network installation. Please contact your network administrator.*

This timebase contains no device/object named "..."

$\Rightarrow$ The control panel tries to access an instrument (or a function of this instrument) that is not part of the current installation environment. The control panel and installation environment do not match. Load the appropriate control panel or change the existing configuration of the timebase in the Server Configuration program. For more information, refer to the online Help and/or the User Manual.

Device is not remote.

⇒ The control panel tries to access an instrument that is currently not ready for operation. Check whether all instruments listed in the configuration are actually connected to the server PC **and** that they are turned on. It may be necessary to restart the instrument to ensure proper functionality.

The connection is established correctly (no error message), but the controls are inactive.

⇒ The **Monitor Only** mode is enabled. It is not possible to actively operate the control panel. The **Monitor Only** mode is always enabled when the selected timebase is already controlled by a different workstation. Disable the **Monitor Only** mode on the **Control** menu. You can now actively control the timebase. (This is indicated by the controls on the control panel.) Simultaneously, the previously controlling workstation loses the control rights and is in **Monitor Only** mode.

🔷☞ *Tip:*

*Have a look at the **Audit Trail** entries. They often provide useful information that helps you to correct the error.*

1c) Using the Control Panel

As soon as a control panel is correctly connected to a timebase, you can operate the individual instruments of this timebase using the controls provided on the panel.

Depending on the control panel that is currently defined as default, various active and passive controls and display elements (**Lamp**, ... **Signal Plot**) are available for controlling instruments and displaying system functions. You can change the appearance of the controls (size, shape, and color).
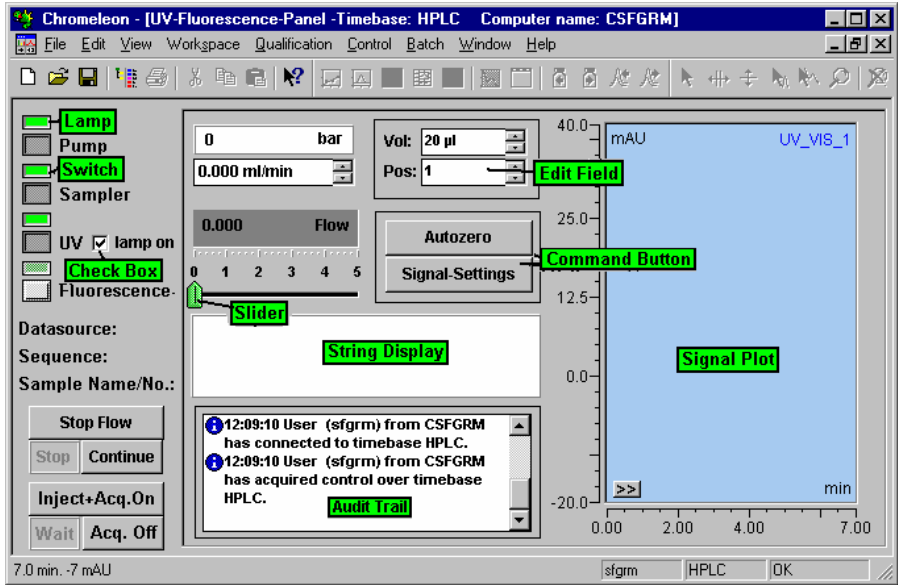
For example, the above control panel allows you to control an HPLC system that consists of a pump, an autosampler, a UV detector, and a fluorescence detector. In this example, all modules except the fluorescence detector are connected. Different buttons are available for issuing control commands such as Inject+Acq.On (for injection and data acquisition), autozero, etc. In addition, the current protocol data (Audit Trail) are displayed. After data acquisition has been started, the current chromatogram is displayed in the signal plot.

The control panel is usually configured during installation. We recommend storing the control panels in the directory of the corresponding timebase. (You cannot store them in the Dionex Templates directory because this directory is read-only).

Online-Toolbar: The basic commands such as the pump flow control, the inject command, or the start of the data acquisition are available on a separate toolbar. The toolbar is only active when a control panel is open.

The functions of the single buttons are as follows (from left to right):

- Flow...: Define the flow and gradient composition and start the flow

- Inject...

- Acquisition on/off: Start and stop data acquisition

- Stop Flow: Stop the flow, interrupt data acquisition, and hold the sample

- Hold: Freeze the solvent composition and hold data acquisition and the sample

- Continue: Continue the flow, gradient, and sample

- Edit Batch: Edit the order in which the samples are processed

- Start/Stop Batch

However, how does the system know that moving a slider should change the pump flow, and not the oven temperature? In addition to changing the size, color, and shape of each control, you can also change its functionality. These assignments are preset for the controls provided on the standard control panels. Although you can adapt them to your requirements, this is seldom required. For more information, see ➔*Modifying a Control Panel* in the **Special Functions** section.

Manual Sample Processing/Starting the Analysis

If the panel is connected to the corresponding timebase, you can use the **Online** toolbar to start sample analysis:

- Open the control panel and connect to the desired timebase.

- Select **Flow...** on the **Control** menu (or click the 🔵 icon on the **Online** toolbar). Enter the flow rate and the solvent composition the pump shall deliver. The pump immediately adjusts to the selected settings.

- Select **Inject...** on the **Control** menu (or click the 💉 icon on the **Online** toolbar). Specify the position from which to draw the solvent and the quantity (in µl) you want to draw and inject. This command, too, is executed immediately. Injection is performed directly after the command is issued.

- Select **Acquisition On** on the **Control** menu (or click the 🔵 icon on the **Online** toolbar). Define the signals to record and click OK to start data acquisition. Chromeleon records the data supplied by the detector.

You can also execute these commands directly from the control panel if your panel supports the corresponding option.

- To finish data acquisition and to complete the analysis, select **Acquisition Off** on the **Control** menu or click the **Acquisition on/off** icon again on the **Online** toolbar.

If your control panel is connected to a timebase on the local server, the recorded data is usually saved to the **Manual** sequence in the **<Timebase Name>** directory of the local datasource. This sequence is automatically generated during installation; it contains one sample only.

 Note:

With each new manual sample processing, the existing data is overwritten by the data of the newly analyzed sample (default setting). However, using the Save to sequence command, you may also save the data to any other sequence after data acquisition. Use this type of sample processing only for test runs etc.!

You will usually want to analyze several samples automatically one after another. To do so, you have to define both the order in which the different samples shall be processed and the commands that are required at the respective time. In Chromeleon, the processing information is contained in a ➔ *Sample List (Sequence)*. The information about the command order is part of a contro program (PGM File).

2. Programmed Control (PGM File)

Objectives of this chapter:

- To describe the definition and purpose of the program

- To introduce the Program Wizard that guides you through program creation

- To describe the functions for program reprocessing

The different commands instruct the single instruments of a chromatography system to perform specific tasks. Enter the commands via the controls on the control panel or by clicking the corresponding buttons (for more information, see the **Control Panel** section).

For automatic and synchronized operation, the single commands must be listed in a file. When starting the file, the listed commands are executed consecutively, observing the relative time differences. This type of file is called control file or PGM File.

Program Properties

For a very simple program, the PGM Editor could look like this, for example:
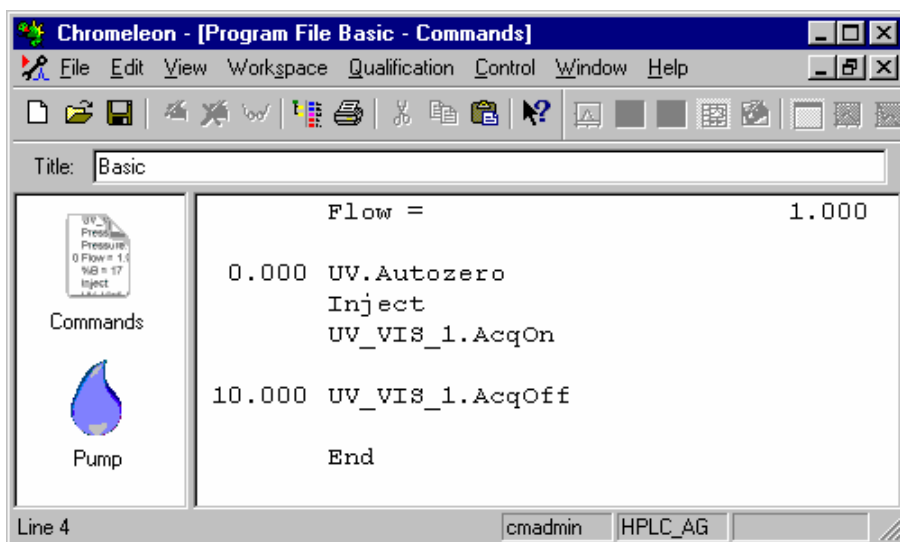
In the **Command** view, the various commands of the PGM File are listed on the right below the **Title** line. The commands are assigned to the corresponding retention time. For information about how to create a program, refer to the following sections.

3. The Program Wizard

Creating a Program

We recommend that you use the Chromeleon Program Wizard to create a basic program structure and thus, avoid syntax errors when you enter the commands.

- Verify that the server is running. If the server is not running, start the server as described in ➔ *Starting Chromeleon*.

- Select **New** on the **File** menu.

- Select **Program File** from the list and click **OK** to start the Program Wizard.
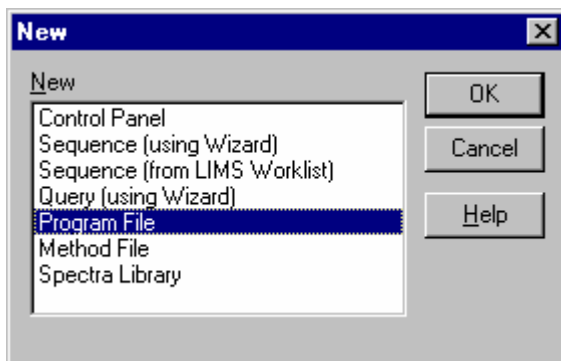
Depending on the installed instruments, the Program Wizard provides various steps. Below please find an example describing the different steps required to create a program for a typical HPLC timebase that contains the following devices:

- P680 Pump

- ASI-100 Autosampler (without cooling option) or AS50 Autosampler

- TCC-100 Thermostatted Column Compartment

- UVD340U Photodiode Array Detector

For more information about the wizard pages, press the F1 key or click the **Help** button. Click **Back** or **Next** to toggle between the pages.

Step 1: Timebase and Server

Determine the server and the timebase to be used:

- If the server was started locally, click the **+** character to display the items under **My Computer**, and then select a timebase. Select **My Computer** in the **Protocol** field. If you want to access a server that was not started on your computer but on a remote PC, click the **+** character to display the items under **Network Neighborhood**, and then select the appropriate timebase. In this case, you also have to select a network protocol in the **Protocol** field. For more information, press the F1 key.
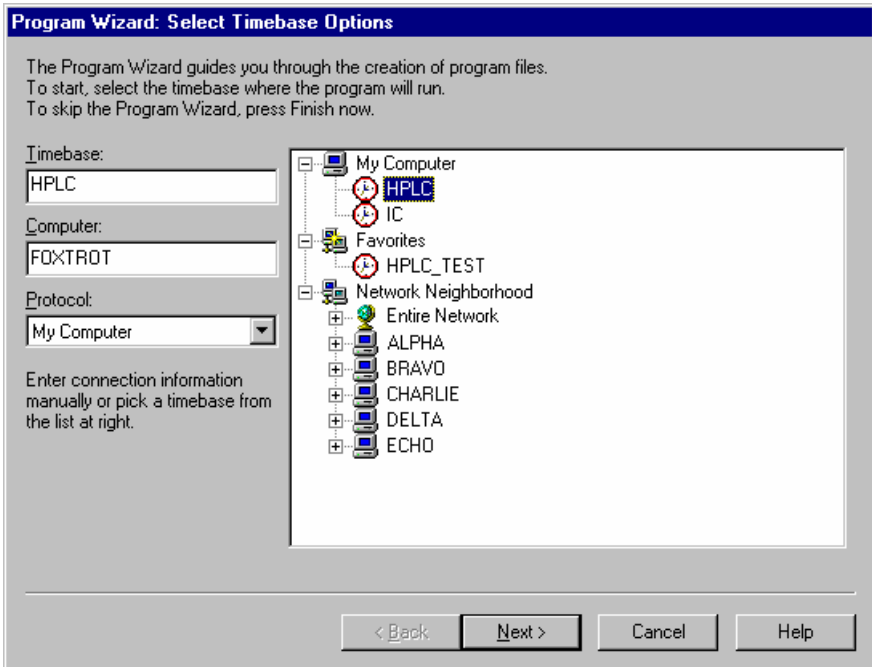
Clicking **Next** takes you to the next wizard page. Click **Finish** to generate a program with the settings selected on the different wizard pages.

Step 2: ColumnOven Options (here: Dionex TCC-100)

Set the nominal temperature and the upper and lower temperature limits:

Step 3: Pump Options (here: Dionex P680)

Define how to operate the pump:

- From the Type drop-down list, select **Isocratic** to deliver a constant solvent composition. If more than one solvent is used, the **Start** field determines the solvent composition in percent (for example: B = 45%, C = 0%, D = 5%). %A is calculated by the system from the portions of B, C, and D. Enter the flow rate in the **Start** field under **Total Flow**.

```
Program Wizard: Pump Options

  Type:    Isocratic          ▼
  ┌─Solvents────────────────────────────────────────────────────────┐
  │          Name                      Type        Start      End    │
  │ %A:   %A                        Automatic ▼                       │
  │ %B:   %B                        Automatic ▼    0.0    %   0.0   % │
  │ %C:   %C                        Automatic ▼    0.0    %   0.0   % │
  │ %D:   %D                        Automatic ▼    0.0    %   0.0   % │
  └──────────────────────────────────────────────────────────────────┘
  ┌─Pressure Limits──────────────────┐  ┌─Total Flow───────────────┐
  │ Lower Limit:  0      [0...400 Bar]│  │ Start:   1.000   ml/min  │
  │ Upper Limit:  200    [0...400 Bar]│  │ End:     0.000   ml/min  │
  └──────────────────────────────────┘  └──────────────────────────┘


           < Back      Next >       Finish      Cancel       Help
```

- If you select **Ramp** instead of **Isocratic**, use the **Start** and **End** input fields to set the start and end time of the gradient ramp. You can also select a **Multi-Step Gradient**. In this case, clicking **Next** opens an additional wizard page.

Step 3a: Flow Gradient Options (here: Dionex P680)

If you selected Multi-Step Gradient from the Type drop-down list, use this wizard page to specify the desired gradient by entering the:

- Retention time

- Flow

- Solvent composition

☞ *Tip:*

Before you enter a multi-step gradient, determine the flow rate and the start composition for the solvent on the previous wizard page. This facilitates the entry.

To append lines to the table, place the cursor in the last line of the table and press the arrow down key on the keyboard. The window will show the graphical representation of your gradient. A blue line indicates the flow, while the area represents the gradient composition. The following example shows a gradient at a flow rate of 1 ml/min, with a constant 5%D during the entire gradient. The portion of solvent B changes according to the entries made in the %B column.
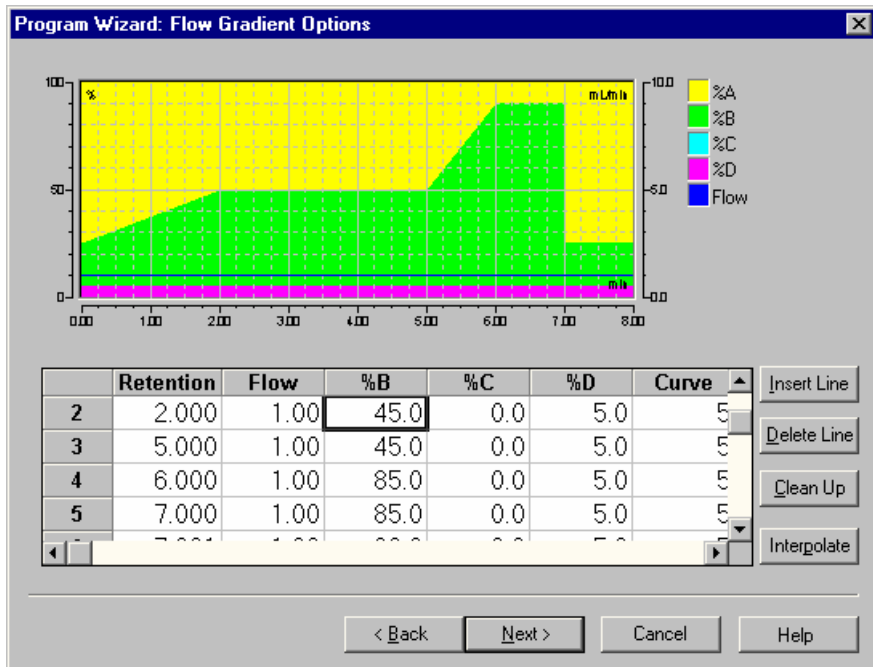
🖻 *Note*:

If you have a Dionex GP40, GP50, or GS50 pump installed, you can program curved or linear gradients. The default is Curve 5, that is, a linear gradient. Curves 1 to 4 result in a convex gradient. Use curves 6 to 9 for a concave gradient.

Step 4: Sampler Options (here: Dionex ASI-100)

Determine the autosampler options:

- Determine the syringe and sample options:

    - **Dispense Speed:** Enter the speed with which the injection shall be performed.

    - **Draw Speed:** Enter the speed with which the syringe is filled.

    - **Sample Height:** Enter the height at which the sample is drawn, measured from the vial bottom to the needle tip.

    - **Syringe Delay:** Enter the time that the needle will remain in the vial after loading.

- Determine the speed for the needle movements:

    - **Up/Down Speed:** Enter the speed for moving the needle up and down.

    - **Radial Speed:** Enter the speed for moving the needle radially.

- Select the **Synchronize injection with pump** check box to synchronize injection with the pump cycle.

Or else: Steps 4 + 4a: Sampler Options (here: Dionex AS50)

If you have an AS50 autosampler installed, use this wizard page to define the autosampler options that do not change during the program:

- If you have the respective option installed, define the **Column Temperature** and the **Tray Temperature**. Select the **Wait for stable temperature** check box to wait before the injection until the temperature is stable.

- In the **Cycle Time** field, enter the time between two injections.

- In the **Syringe Speed** field, define the speed for drawing the sample.

- In the **Sample Needle Height** field, define how deep the needle will dip into the vial, that is, the position from which the sample is drawn.

- In the **Cut Volume** field, enter the sample volume that is finally dispensed into the waste.

- In the **Flush Volume** field, specify the volume for flushing the injection port.

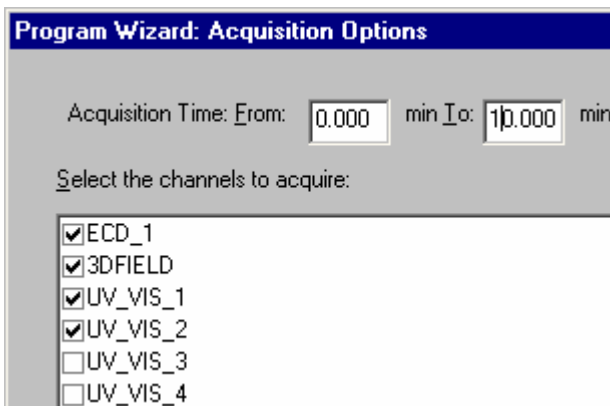Also, define the different AS50 sample preparation steps:

🗗 *Note***:**

In the example, the different functions have been entered one after the other. A "real" application would look different, of course.

- First, select a function in the **Function** column. Seven different functions are available.

- Determine the desired parameters. (For example, for the **Dispense** function these are the **Source**, the **Volume**, and the **Destination** parameters.)

- Click **Insert** to enter the sample preparation step. To delete an existing sample preparation step, select the respective step and click **Delete**.

Step 5: Acquisition Options (here: esp. Dionex UVD 340U)

Determine the signals to be recorded during sample processing.

- Select the signals. Under **Acquisition Time**, specify how long the individual sample shall be analyzed.

For a photodiode array detector, for example, an entire 3D field and the **UV_VIS_1** and **UV_VIS_2** channels are recorded for 10 minutes **(From 0.000 min to 10.000 min**). **0.000** means that data acquisition starts immediately after injection (t = 0.000 min).

Step 6: Signal Parameters (here: esp. Dionex UVD 340U)

Define the signal parameters for each signal selected in step 4.

- Depending on the signal type, you must set various parameters (for example, **Excitation Wavelength** for the fluorescence detector). In the case of a UV detector, specify the wavelength, the bandwidth, and the step used for recording (for example: Wavelength = 225nm, Bandwidth = 1nm, Step = Auto, Average = On (selected)).

- In case of ECD channels, specify the **Data Collection Rate** (= number of collected data points per second) and the **SRS Current** (= suppressor current).

- In addition, enter the temperature compensation factor and the cell temperature as desired.

Step 7: Completing and Saving the Program

- Click **Finish** to complete the Program Wizard. In addition to your input, the complete program displayed in the PGM Editor also includes commands that are automatically added by Chromeleon, such as the **Inject** and **End** commands. These commands are required to execute the file. When these commands are missing, the program can be executed in exceptions only. The complete program could look as follows:
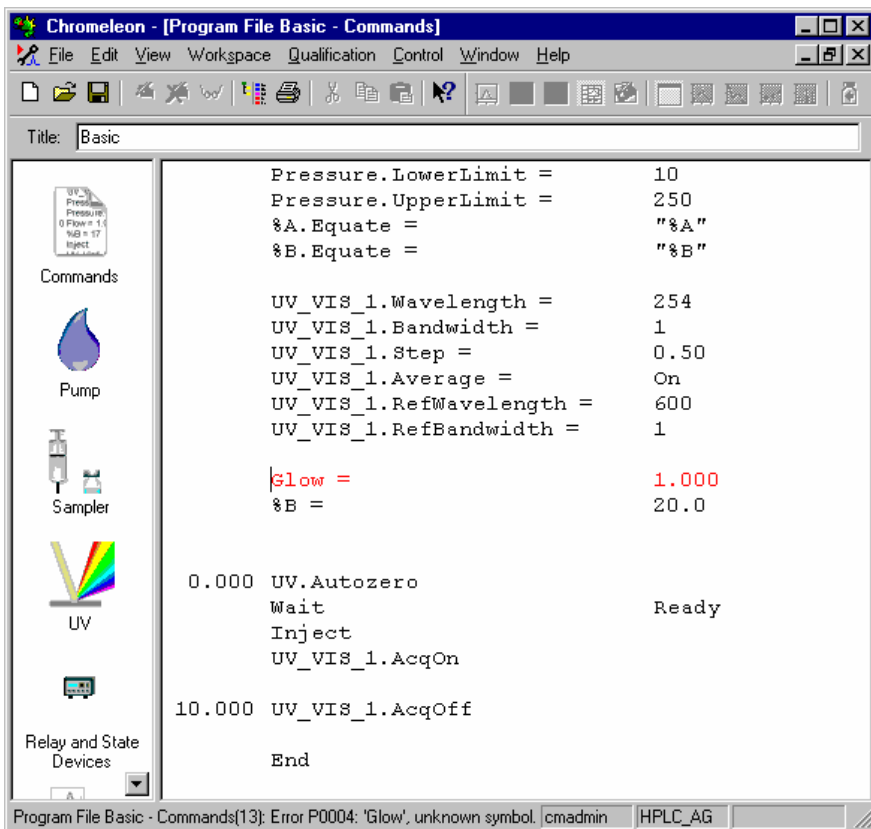
- You can edit a program later, as necessary. If you enter invalid commands, Chromeleon recognizes them and marks the respective line in red color (**Glow** instead of **Flow** in the above example). For more information about the error, refer to the status bar.

- Select **Save as** on the **File** menu to save the PGM File under a descriptive name.

4. Editing the PGM File

You can edit each line directly via the keyboard. Make sure that the control file is connected to a timebase. If it is not, Chromeleon cannot perform the Ready Check and input via the F8 key (see below) is not supported.

- To open the PGM File, select **Open** on the **File** menu or double-click the corresponding file name directly in the Browser.

- Verify that your server is running. If the server is not running, start the server as described in ➜ *Starting Chromeleon*.

- Select **Connect to Timebase** on the **Control** menu.

 Note:

If the timebase to which the program is connected is not available, select **Connect to Timebase** *on the* **Control** *menu to connect the program to a different timebase.*

You can now edit the program. Instead of overwriting the command syntax directly in the PGM File, we recommend to proceed as follows:

- Move the mouse cursor in the line to edit and press <F8 key>. Chromeleon opens the **Commands** dialog box for the currently selected command. You can now edit the input by selecting defined values, without having to worry about the correct command syntax.

- Save the edited PGM File using the **Save as** command.

V. The Analysis

1. The Sample List (Sequence)

Objectives of this chapter:

To describe the definition and purpose of a sequence

To introduce the Sequence Wizard that guides you through sequence creation

To describe the functions for sequence reprocessing

To automate the analysis by using a sample batch

To allow a chromatographic system to process several samples one after another without interruption, you have to define the order and the program to be used for sample processing. Chromeleon stores this information in a sequence.

Blue folders mark sequences in the left Browser pane (see "Isocratic" in the following picture). When you click a sequence, the right pane displays all files and samples contained in the sequence. The upper right Browser pane is reserved for files that are required for sample processing. The lower section lists all samples of the selected sequence. This section is referred to as **Sample List**.

Sample List Contents

The sample list forms the basis for sample processing. It lists the samples intended for processing (one line corresponds to one sample) and indicates how processing is or was performed (contents of individual columns).

No.	Number of the sample in the sequence.
Name	Sample name. The symbol indicates the sample type.
Type	Sample type

        Unknown: unknown sample (symbol in front of the sample name: 🔵)

        Standard: calibration sample (🟡)

        Validate: sample for validating the calibration (🟢)

        Blank: blank value (usually without injection) (⚪)

        Matrix: blank value of the sample matrix (🟢)

        Spiked: spiked sample (🟣)

        Unspiked: unspiked sample (🟣)

Position	Sample position in the autosampler.
Inj. Vol.	Injection volume in µl
Program	➔ *Control File (PGM File)*
Method	➔ *Quantification Method File (QNT Method)*
Status	Sample status

        Single: The sample has not yet been processed.

        Multiple: The sample can be analyzed several times (the old sample will be overwritten).

        Finished: The sample has already been processed.

        Running: The sample is currently being processed (green background).

        Interrupted: The sample was interrupted during processing.

Inj. Date/Time	Injection date and time
Weight	Sample weight
Dil. Factor	Dilution factor
ISTD Amount	Amount (quantity or concentration) of the used internal standard

Samples are always processed in the order of their appearance in the sample list.

1.a) Creating a New Sequence (Sequence Wizard)

The easiest way to create a new sequence is to use the Sequence Wizard. As an alternative, you may as well save an existing sequence under a new name and then modify the sequence as desired.

Start the Sequence Wizard to create the basic structure of a sample list.

Starting the Sequence Wizard

- Select **New** on the **File** menu.

- From the list, select **Sequence (using Wizard)**.

- Click **OK** to start the Sequence Wizard.
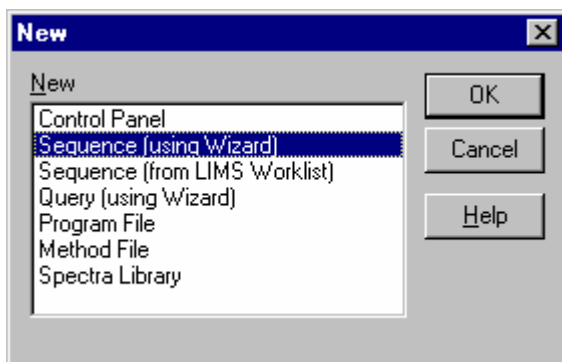
A sequence is created in **five steps**: Each step is performed on a separate page. For each page, detailed information is available via the F1 key or the **Help** button. Click **Back** or **Next** to toggle between the pages.

Step 1

Specify the timebase on which the sequence to be created shall run. Select a timebase in the right window section. The corresponding entries for **Computer** and **Protocol** are automatically made.
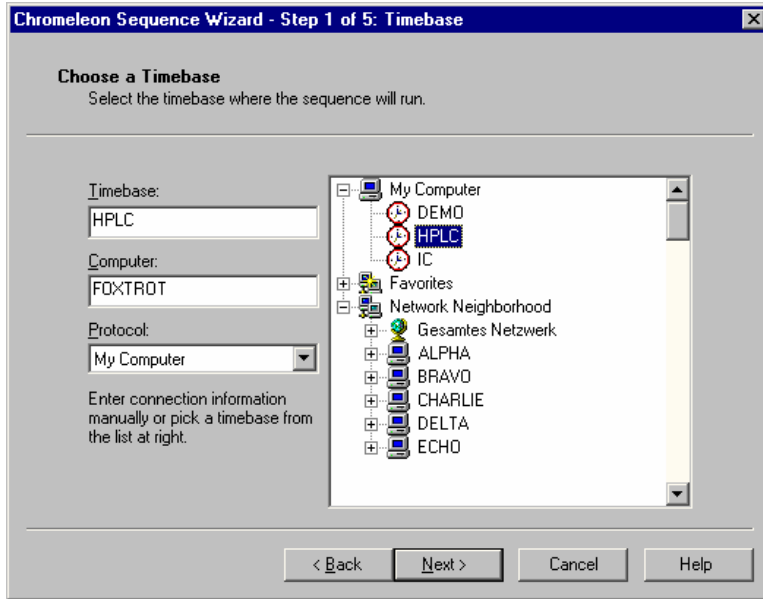
Step 2

Create all unknown samples to be processed.
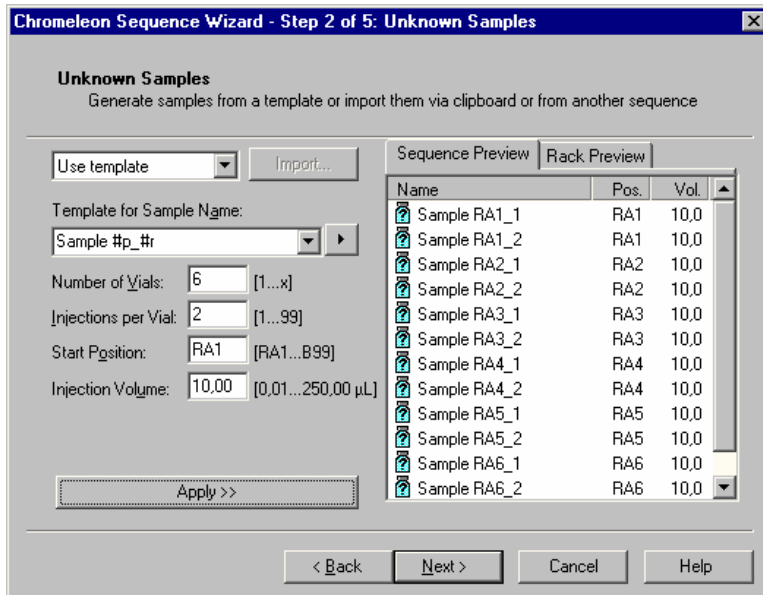
- Select **Use Template**.

- Enter a sample name and complete the name using the arrow key to add, for example, #n. This is to number the samples consecutively, for example, sample#n. If you want to inject several times from the same vial, it may make sense to use the position number (#p) and the injection number (#r) as well (see the example above).

- Enter the number of injections (**Injections per Vial**).

- Specify the position of the first sample (**Start position**).

- Specify the sample volume to inject (**Injection Volume**).

- Click **Apply** to display the result of your input in the sample list (**Sequence Preview**). If the result requires editing, for example, because the injection volume should vary or individual vials are not positioned consecutively, you can change the information manually in the Browser after closing the Sequence Wizard. The **Rack Preview** displays the positions of the samples in the rack.

Step 3

Create all standard samples to process.

- Proceed as described in step 2. In addition, specify any number of standards (**Variation**:) that shall be analyzed after a specified number of samples (**after each**).

Step 4

Determine how to process, evaluate, and print the sequence.

- Enter the name and the directory of the ➔*PGM File* and the ➔*QNT File* to be used in the **Program** and **Quantification Method** fields. Or else, click **Browse...** and navigate to the desired file. If you have not generated a new PGM File or QNT File before, you can leave these fields empty for now.

- For ion chromatography systems, you can select an application template instead. This template provides the appropriate PGM and QNT files. First, specify the suppressor type you are using:

- For HPLC, IC, and GC sequences, select the **DEFAULT.RDF** report template in the **Preferred Report** field. The template is provided in the **REPORT** directory of the local database. The **Preferred Channel** field determines the channel for which data are displayed.

Note:

*You can leave the **Quantification Method**, **Preferred Report**, and **Preferred Channel** fields empty here. Any input is optional only.*

Step 5

Specify the name for the sequence to create and where to save it.

- Enter any name in the **Sequence Name** field and select, for example, the local data <USERNAME_LOCAL> from the **Datasource** field. Generate a separate sequence directory by specifying a directory name in the **Directory** field (you can also create a new directory).

☞ *Tip:*

Do not use special characters (such as an umlaut) in sequence and directory names as this may cause problems in Novell networks!

- On the last Wizard page, click **Done** to complete your input.

1. b) Editing an Existing Sequence

After you have clicked **Done**, Chromeleon generates a sequence based on the entered information. The sequence is displayed in the Browser. As mentioned before, the sequence is a basic structure that needs to be adjusted to special requirements.

For example, if you wish to inject different volumes, you can edit the sequence later accordingly. Or else, if you have not specified the PGM and/or QNT file in step 4 of the Sequence Wizard, you can select this file later. Edit the sequence afterward in the Browser.

- In the sample list, change individual fields directly via the keyboard or open an edit box by pressing the F8 key.

- You may add additional samples. (Select **Append Sample** or **Insert Sample** on the context menu. Or else, place the cursor in the last line, and then press the arrow down key.)

- Of course, you can also copy and paste or delete samples via the corresponding commands on the context menu.

 Note:

*If you did not specify a control file when you created a sequence (Step 4 of the Sequence Wizard), the **Program** column cells on the sample list are empty! In this case, copy the required PGM File to the sequence directory. For each sample, select the PGM File to be used for processing.*

2. Starting the Analysis

Analyzing one sample can be performed manually. For several samples, automatic sample processing is recommended. This requires a ➔*PGM File*, a ➔*Sequence*, and a ➔*Quantification Method* (if possible).

Automatic Sample Processing

You can start automatic sample processing as soon as the chromatographic conditions, the samples to process, and their order have been defined during sequence creation.

Automatic sample processing is also called **Online Batch** or **Batch Processing**.

- Open a Control Panel. Select **Edit** on the **Batch** menu. Or else, click the

  following icon on the **Online** toolbar:
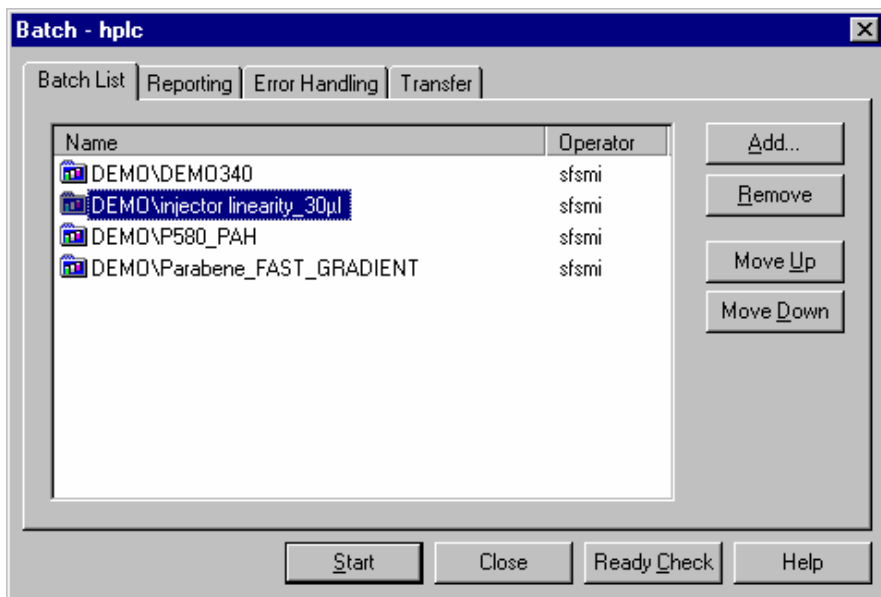
- On **Batch List** tab page, click **Add** to open the **Browse** dialog box. Under Object of Type, select Sequence from the drop-down list box. Select a sequence in your datasource and click **Open**.

This action enters the path and the name of the sequence in the Batch dialog box. If a sequence contains a sufficient number of samples, sample processing is possible around the clock. Instead of listing all samples in one sequence, you can create several sequences. In this case, enter all required sequence names in the Batch List.

- Repeat adding sequences until all required sequences are listed in the display field.

The order of the listed sequences determines the order of sample processing: When starting a batch, samples 1 to n of the first sequence are analyzed, followed by samples 1 to n of the second sequence, etc.

- Select a sequence and change the processing order by clicking the **Move Up** and **Move Down** buttons.

- Click **Ready Check** to check whether automatic sample processing (batch) is possible; i.e., Chromeleon checks whether all devices to be used are ready for use (turned on, connected, lamp switched on, etc.) In addition, this action verifies that all data are available and the memory capacity is sufficient.

- Click **Start** to start the analysis.

As soon as the online batch starts, all samples of the sequence(s) that have the status **Single** and **Multiple** (depending on the setting those with the status **Interrupted** as well) are analyzed in the listed order. During a running batch, the currently processed sequence is labeled with a green triangle in the batch list.

VI. Quantification Method (QNT File)

Objectives of this chapter:

To define a method for peak detection and recognition, quantification, and evaluation

To use this method for many samples and sequence and thus, save time

Introduction

After a chromatogram has been recorded, you need to integrate and assign the peaks first before you can quantify them. It would be a time-consuming task to do this in the report for each single sample. That is why all these steps are combined and saved in the QNT Method. Nevertheless, it is possible to edit a single sample in the report later. However, the better the QNT Method evaluates your samples, the less effort will be necessary for reprocessing the samples.

Strictly speaking, the QNT File is required after the analysis only. However, we recommend creating the file earlier. This allows you to follow and evaluate the course of the analysis.

Creating a Quantification Method

- Select **New** on the **File** menu.

- Select **Method File** as file type and click **OK** to create a new method.
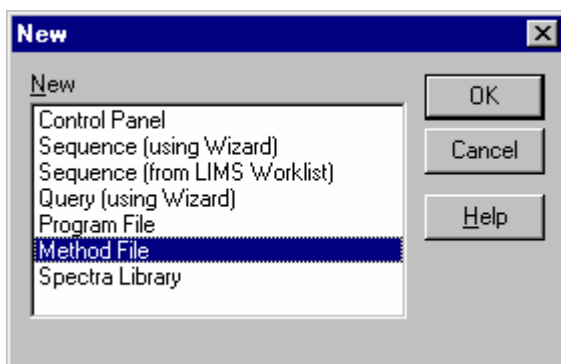
The QNT File Window

The QNT File window contains various worksheets (**General**, **Detection**, **Peak Table**, **Amount Table**, **Peak Tracking**, **Calibration**, **Spectra Library Screening, SST,** and **UV**). Open the required worksheet by clicking the respective tab on the lower window bar. Additionally, the worksheets **MS Tracking** and **MS** are displayed when XCalibur is installed when you are creating a new QNT file.
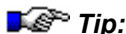
📖☞ *Tip:*

*Some worksheets may not be displayed. Select the **View** menu and then **Select Sheets** to change the display settings.*
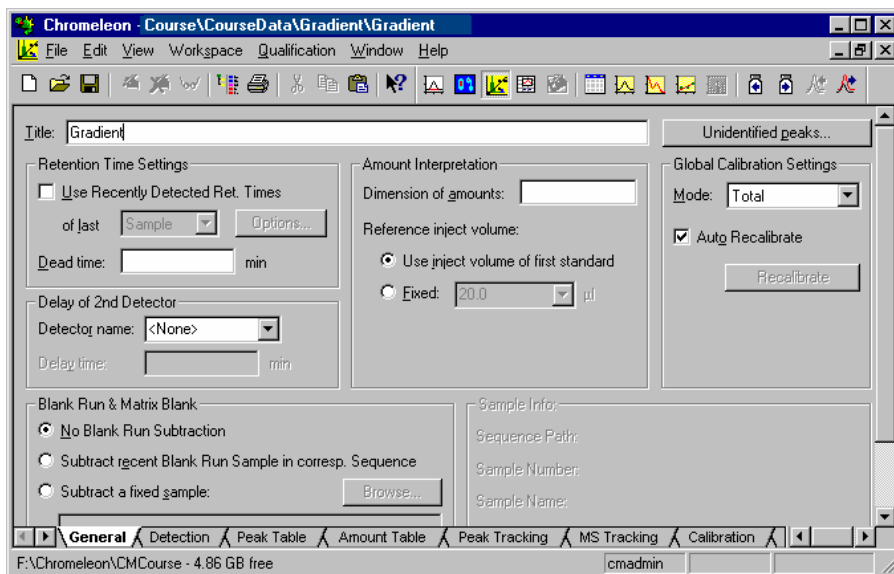
To create a simple evaluation method, it is sufficient to enter the **Peak Table** settings.

Creating a Peak Table

The peak table allows you to recognize peaks (= ➔*Detecting Peaks*), assigning names to the peaks in a chromatogram (= ➔*Identifying Substances*), and converting the determined peak areas into substance amounts (= ➔*Quantifying Substances* and ➔*Defining the Calibration Mode and Calibration Type*). The required information is usually entered before the analysis.

Saving the QNT File

Use the **Save as** command to save the quantification method under a separate name.

Editing the QNT File

When the QNT File is edited, all changes will be implemented immediately in all involved components (only if the **Auto Recalibrate** check box has been selected on the **General** tab page).

If you have opened a channel of a specific sample (see: ➔ *The Browser - Appearance and Functions (Part b)*) and you see the corresponding chromatogram, this representation is updated immediately after changing the QNT File. The same applies to the representation of numerical values as they appear in any report.

⚠️ *Caution:*

*If you have not specified a quantification method when creating a sequence (Step 4 of the ➔ Sequence Wizard), the **Method** column cells in the sample list will be empty! Enter the name of the desired QNT File for each of the samples. To evaluate all samples of a sequence with the same QNT File, select the QNT File for the first sample, and then copy the entry for all entries using the F9 key.*

1. Detecting Peaks (Detection Tab)

Before peaks can be identified and quantified, they have to be detected. Based on default values for the peak recognition algorithm, Chromeleon is able to detect even the smallest peaks. Normally, however, you will not be interested in these small peaks so that you may want to include certain filters to exclude them from being displayed. This and other detection tasks are defined on the **Detection** tab page.

Double-click the default parameter (Minimum Area = 0.001 {Signal}*min) to open the **Edit detection parameter** dialog box:
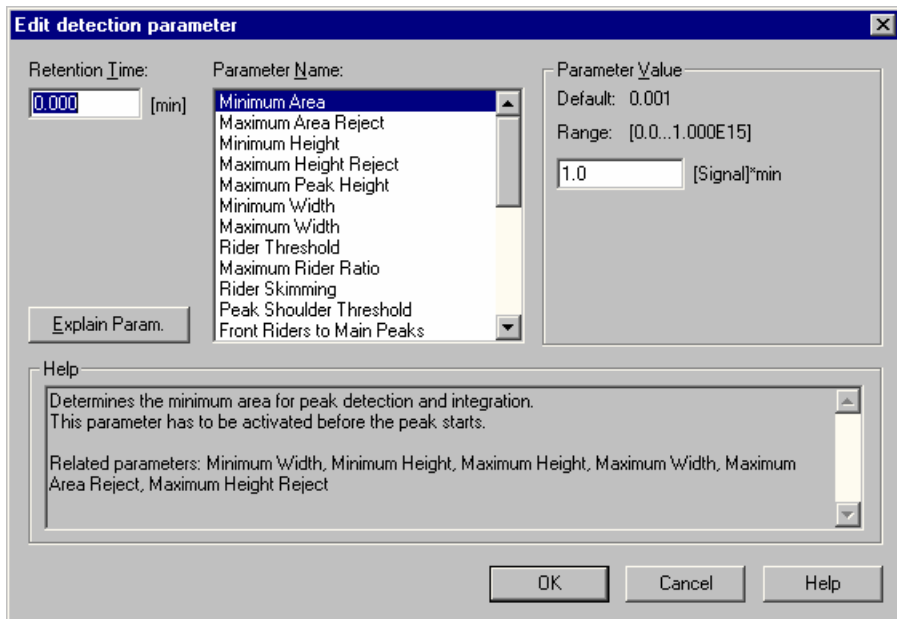
Frequently used Detection Parameter

Minimum Area

Usually, the **Minimum Area** default parameter will be sufficient as filter. **Minimum Area** defines the area threshold, below which peaks are not detected during peak detection or integration. In HPLC-UV, 1.000 mAU*min usually is an appropriate value. Set the parameter to the desired value and click **OK** to accept the changes. This action usually suppresses the small peaks that are of no interest:
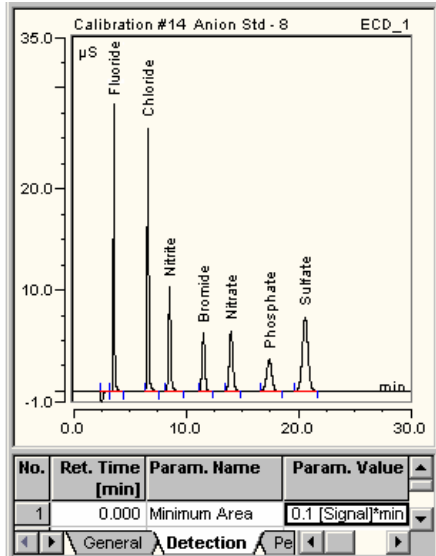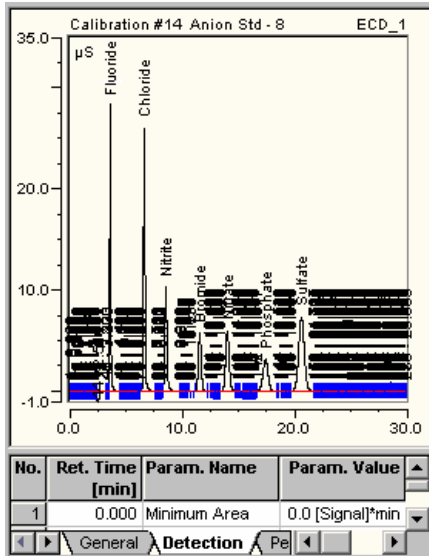
To add more detection parameters, select the **Lines** command, and then select **Append Lines**. You can also use the **Minimum Height**, **Minimum Width**, **Maximum** Peak **Height,** and **Maximum Width** detection parameters as additional filters.

☞ *Tip:*

*Changing the **Minimum Area**, **Minimum Height**, or **Minimum Width** parameters influences the baseline; changing the **Maximum Area Reject** or **Maximum Height Reject** parameter does not. If you change the **Maximum Area Reject** or **Maximum Height Reject** parameters, it may happen that the corresponding peaks are no longer displayed.*

Inhibit Integration and Void Volume Treatment

Use the **Inhibit Integration** detection parameter to disable peak integration. If the parameter is set to **On**, peak detection is disabled. This may be useful for disabling the integration of injection peaks. For example, this function can be used to inhibit the injection peak by enabling the parameter at the start time of the chromatogram and by disabling it shortly after the void time. In the example below, the retention time of the negative peak is 2.513 min.:
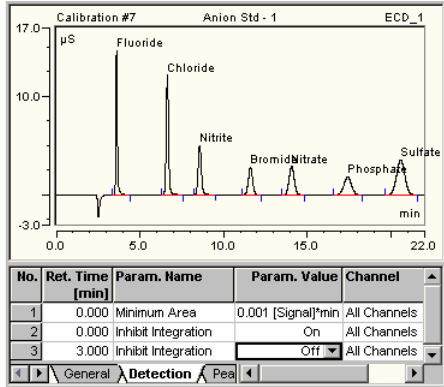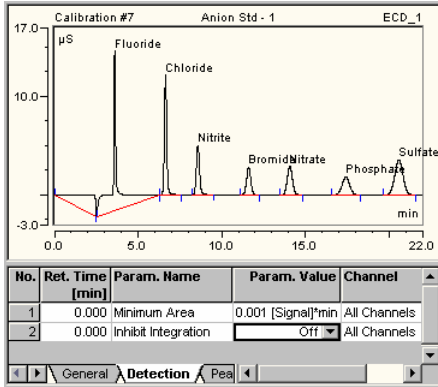
If the value is set to **On** before the first peak to be inhibited, peak detection is disabled until the parameter is disabled (**Off**), i.e., no peaks are recognized. The chromatogram is drawn on the screen but it is not integrated in this area.

If the first peak follows shortly after the negative water peak and if the retention times of the separate chromatograms show considerable fluctuations it may be difficult to set the end time for Inhibit Integration exactly. If the end time is set too early or too late, the first peak may not be integrated as desired.

In this case, use the **Void Volume Treatment** parameter instead. If the peak start is still set too early, use the **Fronting Sensitivity** parameter to remedy the situation.
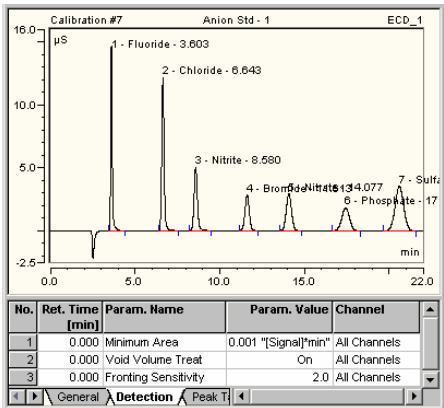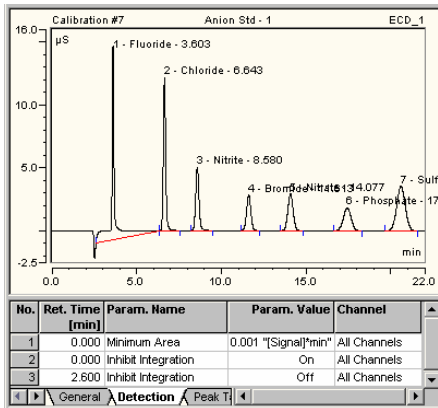
![icon] *Note:*

The Void Volume Treatment parameter only inhibits integration of the negative peak with the lowest signal value.

Detect Negative Peaks

The default setting is that negative peaks are not detected. Negative peaks, for example, the negative water peak in ion chromatography, may cause an undesired baseline. Enable the **Detect Negative Peaks** detection parameter to avoid this:
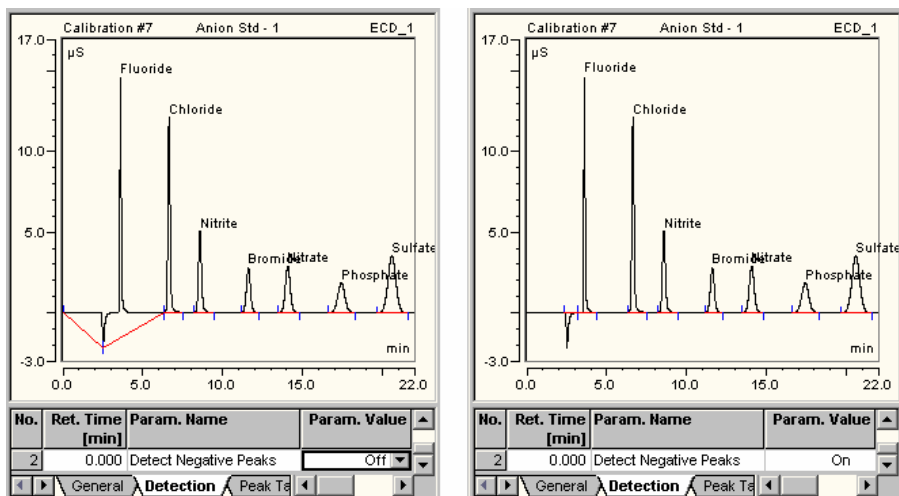
To detect a negative peak this switch must be activated before the peak start. To correct the baseline without labeling the peaks or including peaks in the peak list, select **Don't label**.

![icon] *Note:*

In the result report, the area of negative peaks is indicated as a positive value.

More Detection Parameters

Use the **Rider Threshold** and **Maximum Rider Ratio** detection parameters to define the peaks that shall be classified as riders. Use **Rider Skimming** to define how to divide a rider peak from the main peak.

Besides, you can use the **Valley to Valley** parameter to enforce valley-to-valley integration, and the **Fronting Sensitivity Factor** or **Tailing Sensitivity Factor parameters** to influence the determination of the peak start and peak end.

Graphical Input of Detection Parameters

Click the detection parameter tool to display the position of the detection parameters in the chromatogram.

This is especially useful if you want to change the position of detection parameters or graphically insert additional parameters. Use the **Detection Parameter Tool** to change the position of detection parameters. Open the tool by selecting the respective command on the context menu or by clicking the icon. In the chromatogram, the parameters are marked by a dotted line at which the abbreviation for the parameter and the respective parameter value is indicated. For example, the figure below shows the values for the Rider Threshold (in short: RidThd), Rider Skimming (RidSki), and Maximum Rider Ratio (RidRat) at 1.000 min. Position the mouse on a detection parameter in the chromatogram to activate the detection parameter tool so that you can move this parameter via the left mouse button.

To insert a detection parameter at the position of the mouse cursor, select
Detection Parameters on the context menu:
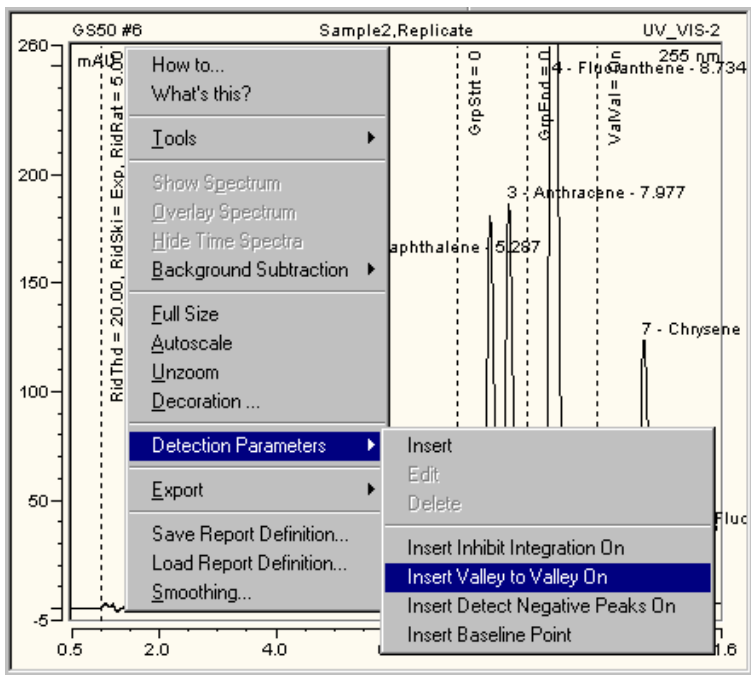
For example, this setting enforces valley-to-valley integration as from the
selected time.

You can also enter detection parameters and some other settings that
influence peak calculation graphically by holding down the right mouse
button and selecting a chromatogram area. The context menu provides the
following options:

- Set Averaged Baseline Start and Set Average Baseline End

- Set Background Subtraction Range

- Set Minimum Area

- Set Minimum Height

- Set Minimum Width

- Set Peak Slice & Sensitivity

- Set Inhibit Integration Range
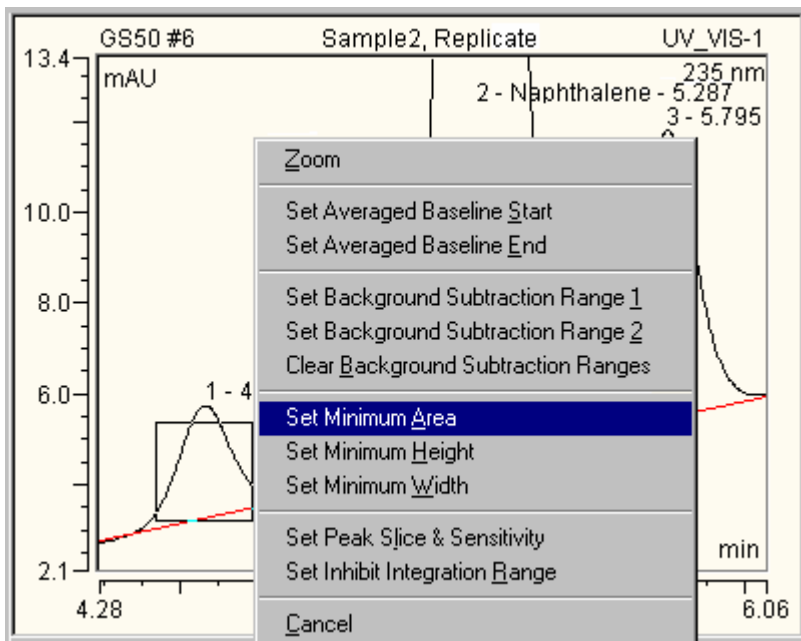
📧☞ *Tip:*

You can undo the graphical input of detection parameters. Click one of the QNT editor tables and select **Undo** *on the* **Edit** *menu. (In the chromatogram itself, you can only undo changes that were made in currently open chromatograms.) In the* **Detection** *table of the QNT Editor, you may just as well delete the parameters that are no longer required.*

2. Identifying Peaks (Peak Table Tab)

The Peak Table

The Peak Table is used to assign peak names to all peaks of interest in a chromatogram. Peaks are typically identified by the retention time. However, it is also possible to identify peaks by the corresponding spectra.

- Select the **Peak Table** tab to open the peak table.

- Enter the names of **all** peaks to be identified in the **Peak Name** column. Assign the expected retention time to each peak in the **Ret.Time** column (= nominal retention time (Tret)). *(You can add additional lines using the **Lines .../ Append Line** commands on the context menu)*.
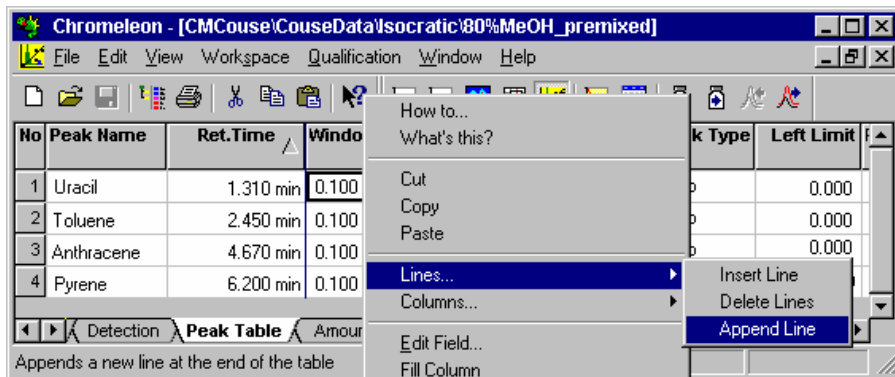
If a peak is detected at the specified time, the name is assigned automatically (fig. a). An additional tolerance range for peak detection is defined in the **Window** column (fig. b). The width corresponds to the double **Window** value. To determine the retention time area, this value is added or subtracted from the retention time. If a peak is detected in this range, it is identified even if the actual retention time does not correspond exactly to the entered nominal retention time (fig. c). If several peaks are detected within this range, the Chromeleon identifies the greatest, the first, or the nearest peak to the retention time (fig. d), depending on the extension of the **Window** parameter.

a)   b)   c)   d)
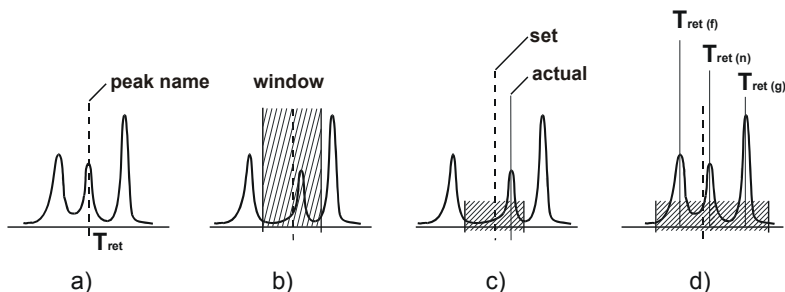
- In the **Window** column, enter, for example, **0.25 AG**, for each peak to identify the largest peak within a 30-second window. Time input is in industry minutes. For example, the value 1.00 corresponds to 60 s. The value 0.25 thus corresponds to 15 s, which means a window range of ±15 s. The setting **0.25AF** identifies the first peak; the setting **0.25AN** identifies the nearest peak to the set retention time.

Automatically Generating the Peak Table

You can create the peak table manually by entering the single peak names and retention times. However, generating the peak table automatically by using the **Autogenerate Peak Table** command considerably facilitates this task.

Select **Autogenerate Peak Table** on the context menu. Chromeleon automatically generates the peak table and enters the retention times of all peaks detected in the currently open chromatogram as set times in the QNT Method. You only have to complete the table by entering component names and altering the default window and other values as necessary. Peaks, which are not of interest, can be deleted from the table. These will consequently be excluded from the report if the **Including all unidentified peaks of the peak table** parameter in the **Integration Report Properties** has been disabled. (To change the setting of this parameter, select **Table Properties** on the **Edit** or context menu.)

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Peak Type	Comment
1	Anion Qnt-1	3.603 min	0.145 AG	External	Area	Lin	Auto	Autogenerated
2	Anion Qnt-2	6.643 min	0.160 AG	External	Area	Lin	Auto	Autogenerated
3	Anion Qnt-3	8.580 min	0.187 AG	External	Area	Lin	Auto	Autogenerated
4	Anion Qnt-4	11.613 min	0.242 AG	External	Area	Lin	Auto	Autogenerated
5	Anion Qnt-5	14.077 min	0.305 AG	External	Area	Lin	Auto	Autogenerated
6	Anion Qnt-6	17.430 min	0.413 AG	External	Area	Lin	Auto	Autogenerated
7	Anion Qnt-7	20.603 min	0.483 AG	External	Area	Lin	Auto	Autogenerated

General ╱ Detection ╱ **Peak Table** ╱ Amou

During automatic table generation, the variables described below are set as follows:

- The **Peak Names** are constructed from the name of the QNT Method, for example, in the Anion Qnt method, detected peaks will be assigned the names Anion Qnt -1, Anion Qnt -2, Anion Qnt -3, etc.

- The **Window** values are entered as absolute values (indicated by the suffix A).

- No **Group**.

- The **Amount** values and the **Response** Factors are set to 1.0.

- The **Peak Type** is Auto (i.e., based on the peak, it is determined by Chromeleon).

- The entry in the **Comment** column is **Autogenerated**.

When you generate peak tables via the **Autogenerate Peak Table** from the **Edit** menu, all peaks in the chromatogram are usually enumerated (type Enumerate peaks of current chromatogram). However, you can also use the results of the spectra library screening (see ➜ *Spectra Library Screening*) for peak naming (type: Use spectra library screening results).

3. Quantifying Substances (Amount Table and Peak Table Tabs)

You can use standard samples (sample type: Standard) to quantify unknown samples. To do so, you have to enter the known substance amount of these standard samples in the amount table together with the standard method. Enter this information in the **Amount** and **Standard** columns.

Amount Column

- Search the amount table for the name of the standard substance(s). If the required names are not listed, enter the names and the retention times as described ➜ *Identifying Peaks*.

- Move along the line to the **Amount** column.

- Enter the amount values of the substances (Substance A, Substance B, etc.).

No.	Peak Name	Ret.Time	Standard	Int.Type	Cal.Type	Amount
1	Substance A	1.500 min	External	Area	Lin	12.00000
2	Substance B	2.600 min	External	Area	Lin	17.00000

Enter a concentration value, such as, µg/µl or an absolute value, such as, µg. (Enter the used dimension in the **Amount Interpretation** section on the **General** tab.)

Multi-Point Calibration

Multi-point calibration can be achieved by

- Using different standards with different concentrations
- Injecting different volumes from a single sample vial

Different standards with different concentrations

If standards are available in various concentrations, i.e., in several vials, enter the concentrations of all substances to be calibrated in a separate **Amount** column. Do this for each vial from which injection is performed.

- To add additional **Amount** columns, select **Columns.../Edit Amount Columns...** on the context menu:
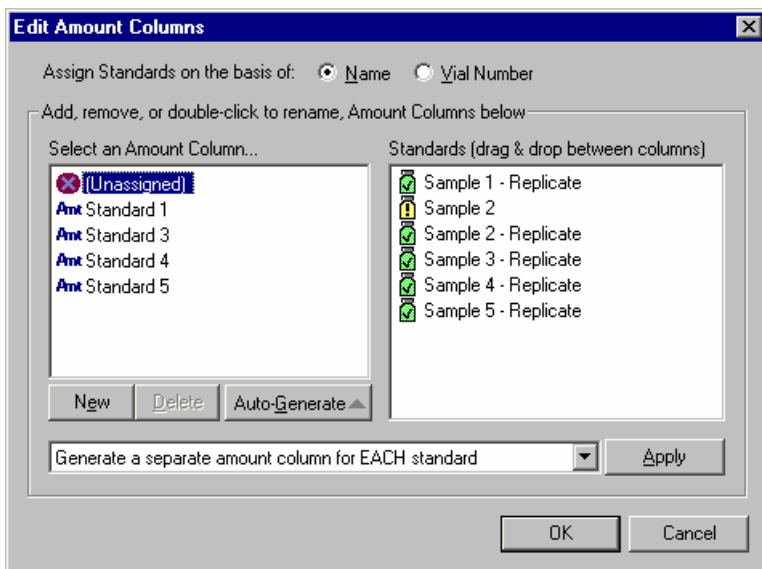
- Standard samples, validation samples, and spiked samples are available for this.

- Click **New** to create a new amount column. Enter any name of your choice.

- Select **Unassigned** from the left list box and then move the desired sample to the new column. (For example, Sample 2 in the above image.)

- Enter the concentration value of the second vial in the new **Amount** column.

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount Standard 1	Amount Standard 2
1	Substance A	1.500 min	0.400 AG	External	Area	Lin	12.00000	48.00000
2	Substance B	2.600 min	0.400 AG	External	Area	Lin	17.00000	56.00000

Injecting different volumes from a single sample vial

If the range to calibrate does not contain several powers of ten, you can inject different volumes from the same vial. Chromeleon automatically considers the different volumes. In this case, you only need one Amount column, as the concentration is constant.

For this type of multi-point calibration, the decisive column of the sample list in the Browser is the **Inj. Vol.** column. The column could look as follows:

No.	Name	Type	Pos	Inj. Vol.
1	Standard 1	Standard	R99	5.00
2	Standard 2	Standard	R99	10.00
3	Standard 3	Standard	R99	20.00
4	Standard 4	Standard	R99	40.00
5	Sample 1	Unknown	RA1	10.00
6	Sample 2	Unknown	RA2	10.00

If you enter the Amount as amount value (and not as concentration value), make sure that the amount corresponding to the reference injection volume is entered in the **Amount** column. The reference injection volume does not have to equal the actual injection volume, i.e., the injection volume specified in the sample list of the Browser. Enter the reference injection volume on the **General** tab page of the QNT Editor:
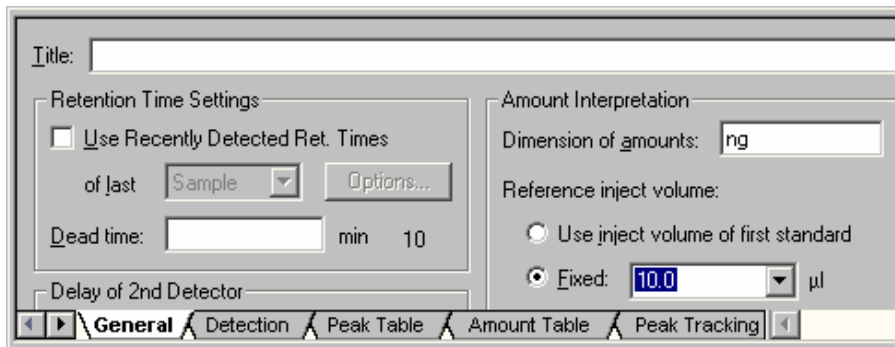
"Standard" Column

The standard method determines how a calibration is performed. A general distinction is made between calibrations based on *external* or *internal* standards.

An *external* standard refers to a calibration that is performed based on one or several standard samples (normal case). An *internal* standard is the known amount of a standard substance that is added to the unknown sample. (The Tutorial provides only a short description for this special case. For more information, see ➔ *Internal and External Standard* in the **Special Functions** section.)

- Make sure that the correct standard method for each peak to calibrate is entered in the **Standard** column (= **External** = default value).

For information about further calibration options (Internal or Internal/External), see the online Help or the User Manual (for example, in the **Calibration** section).

Further Columns

All other peak table columns determine how the determined area values are converted into the resulting substance amount values. Generally, the standard settings can be used. N*o changes* are required.

Verify that the following default settings in your QNT Method are correct for your application:

- **Calibration Type = Lin**
- **Integr. Type = Area**
- **Response Factor = 1,000**

4. Defining the Calibration Mode and Calibration Type

The ➔*Quantifying Substances* topic already described the basic elements of the calibration with Chromeleon. However, Chromeleon provides many more calibration functions. The complete description of these functions would go beyond the scope of this Tutorial. Nevertheless, the most important functions are described below.

Calibration Mode

The calibration mode allows you to define which standard samples shall be used for calibrating which unknown samples. Define the calibration mode on the **General** tab page. The following options are available:
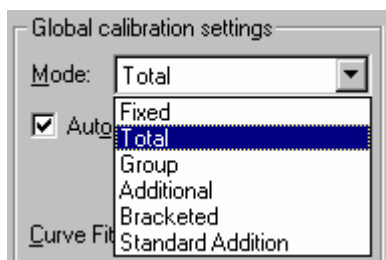
Select the **Total** mode to use all standard samples for calibrating all unknown samples. Use the **Fixed** mode to use certain standards (also from previous sequences) for calibrating all unknown samples. Select the **Standard Addition** mode to analyze unknown spiked or unspiked samples. For more information about the **Group**, **Additional** and **Bracketed** modes, see below:

Calibration Type

The calibration type defines and weighs the calibration function to be used as necessary. Enter the calibration type on the **Peak Table** tab page of the QNT Editor:

- Press the F8 key in the **Cal.Type** column to open the **Calibration Type for <Peakname>** dialog box.
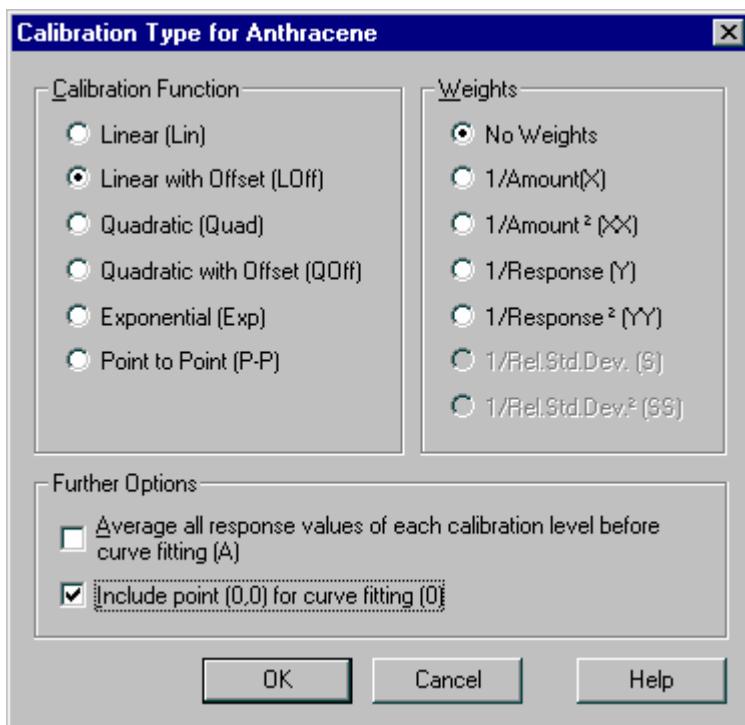
- In the **Calibration Function** section, select a calibration function for the current peak. This is usually the Linear or Linear with Offset function (if the calibration function does not pass the origin). However, quadratic and exponential functions and a polygon (Point-to-Point) through the calibration points are available as well.

- In the **Weights** section, define the weights for the single calibration points. If you selected No Weights, higher amounts/signal values are weighted more. Select **1/Amount** or **1/Response** for proportionate weighting of small and high amounts/signal values. Select **1/Amount^2** and **1/Response^2** to weight smaller values more than high values.

- In the **Further Options** section, more options are available. Select **Average all response values for each calibration level before curve fitting** to average the calibration points of each calibration level before calculating the calibration curve. Select **Include point (0.0) for curve fitting** to include the origin as calibration point into the calibration if you selected a calibration function with offset. However, this does not mean that the calibration curve runs through the origin.

- Special rules apply to calibrations that use the Standard Addition function. For more information, refer to the corresponding sections in the Chromeleon online Help.

VII. Data Analysis

Objectives of this chapter:

- To describe the chromatogram, calibration curve, and spectra views

- To display the sample results in report tables

- To evaluate and edit single chromatograms

Introduction

Chromeleon allows you to display sample data under various aspects. You can:

- Display a single chromatogram.

- Compare several chromatograms.

- Check the peak purity.

- Display calibration curves.

- Search single spectra in a spectra library.

Each action is performed in a separate window or pane. Each pane is intended for a specific task and has its own window arrangement and menu structure.

Data Representation

To display data, select the corresponding data first:

- In the Browser, click the sequence of interest.

- Open a sample in the sequence by double-clicking the sample name.

- The sample opens in the **Integration** window. The **Integration** window usually displays the chromatogram and the report table. Sometimes, only the chromatogram is opened. This depends on the report definition file (RDF):
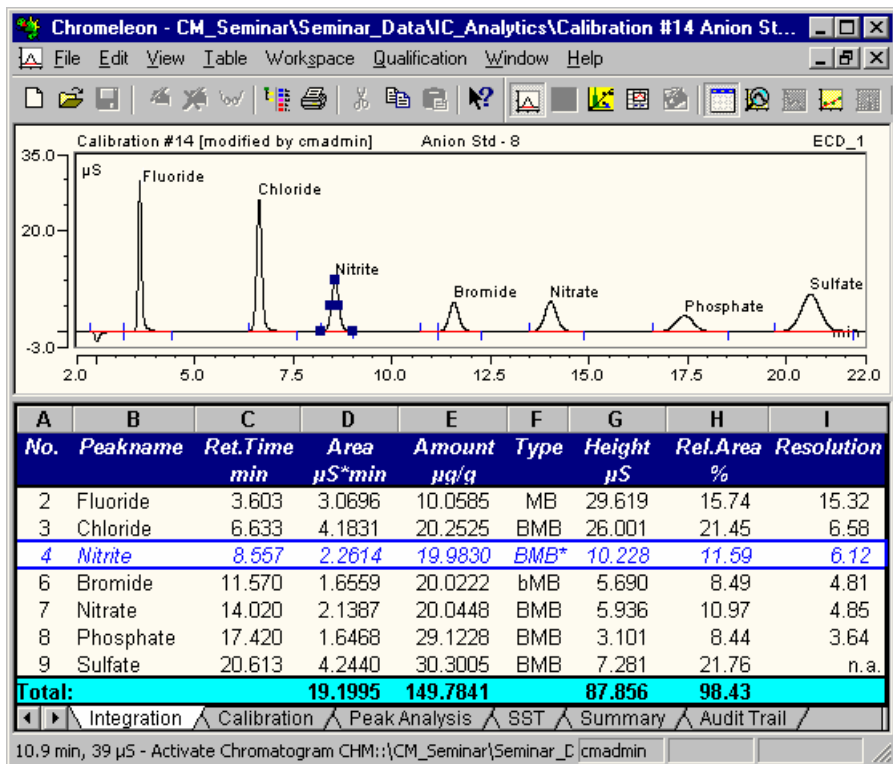
Use the icons on the **Method** toolbar to quickly change from one view to another:
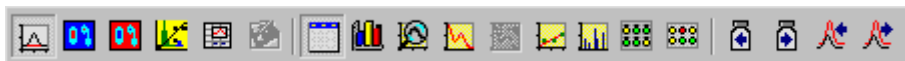
The first five icons open a separate window each:

- **Integration** (opens the screen report and displays the results of the analysis)

- **PPA** (opens a window that displays the data of a diode array detector)

- **QNT-Editor** (opens the view for editing the quantification method)

- **Printer Layout** (opens the view for preparing the printout)

- **Signed Results** (opens the signed results for the current sample)

The following icons open an additional part window:

- **Show Report** (displays the report table)

- **Show Trend** (displays the trend plot--changes of variables are graphically displayed from sample to sample)

- **Show Chromatogram/Split Zoom** (displays the chromatogram in two panes: complete view and enlarged view)

- **Show Spectra** (displays the UV spectrum)

- **Show Calibration Curve** (displays the calibration curve)

- **Show Mass Spectra** (displays the mass spectrum)

Click the four icons on the very right to:

- Display the **Previous Chromatogram** or the **Next Chromatogram**

- Display the **Previous Channel** or the **Next Channel**

The **Integration** window is the most important window for data representation. For more information, see ➔*The Integration Window* below. For information about the ➔*QNT Editor* and the ➔*Printer Layout* windows, see the associated sections in this Tutorial.

1. The Integration Window

- To open a sample chromatogram, double-click the sample name in the Browser. In the easiest case, you will only see the chromatogram of the sample. For example:
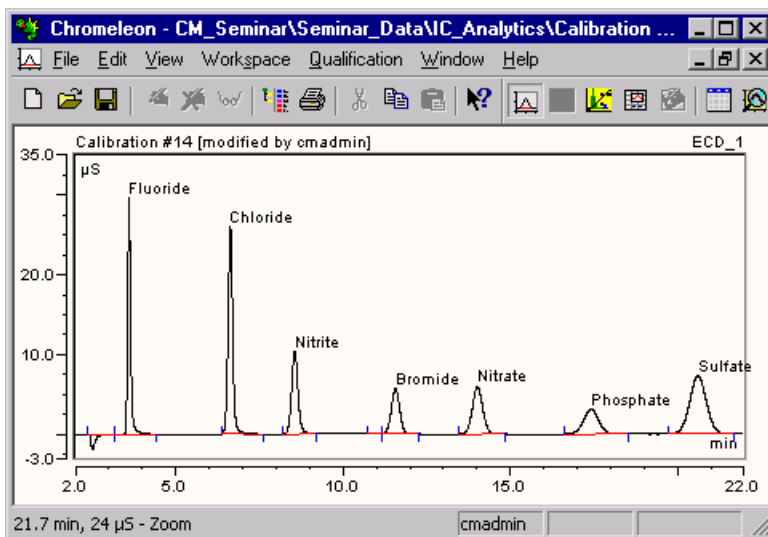
- Select **Decorations** on the context menu to change the appearance of the chromatogram. You can change the captions, axis ratios, and coloring as well as the actual chromatogram representation (display of peak heights, additional chromatograms, or grid).

- Double-click an individual peak to display its properties:
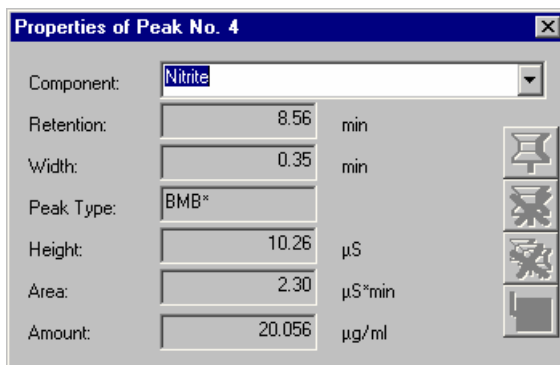
- Select a peak and click on the corresponding icons to display the **Spectra Plot** and the **Report**.
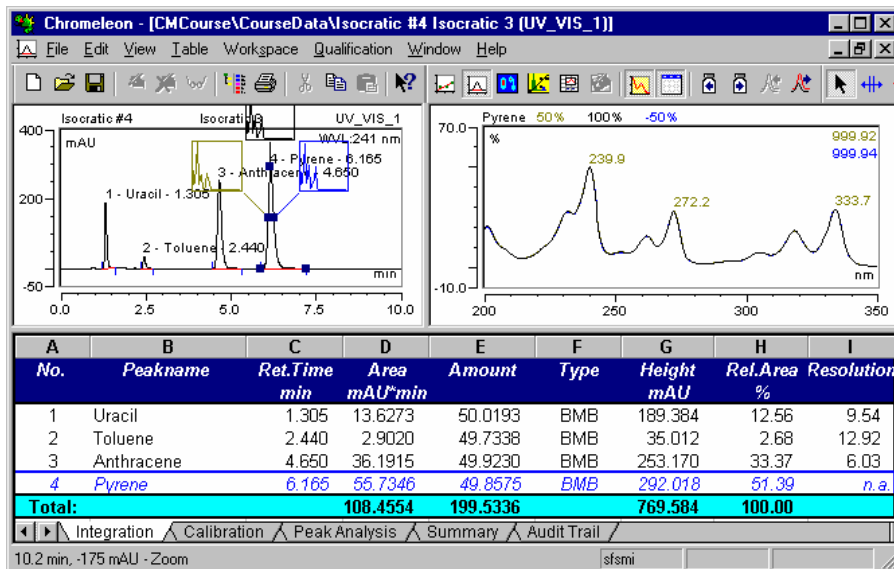
If the analysis was performed with a diode array detector, the spectrum of the selected peak, for example, anthracene, is displayed on the top right; the lower section displays part of the integration report (four lines, the line with the currently selected peak is highlighted by a different background color). You can change the Spectra Plot window via the **Decorations** command as described above. Select **Decorations** to display the available options. This command also minimizes the spectrum in the chromatogram.

The displayed default report contains information about various parameters. Each worksheet has a number of default variables that are an integral part of the specific report type. For example, the integration report contains the **Ret.Time**, **Area** and **Amount** columns; the calibration report includes the **Offset (c0)**, **Slope (c1)**, and **Curve (c2)** columns, etc.

Double-click a peak name in a report window to change the selection of the current peak. Instead of anthracene, pyrene is highlighted by a different color. The spectra representation is updated as well.

2. Modifying the Chromatogram

In some cases, it may be useful to change the sample integration that was performed manually, for example, by moving peak delimiters. To do this, use the icons on the **Integration** toolbar:

Use the **Automatic Tool** to perform the most important actions. The shape of the mouse cursor indicates the performed action.

⅃⅃ ꓔ	Move left/right peak delimiter
𐐒 𐐒 𐐒	Change baseline point (left/right/center)
⇕ ⇕ ⇕	Move baseline point (left/right/center)
⇌⇌	Move perpendicular line
⇄⇄	Move baseline segment
⇔	Change retention window
⇔	Move detection parameter
⋀	Insert peak
✎	Display spectra
+	Zoom out an area
⊘	Action not possible

The action that can be performed at the current position is indicated on the status bar.

Example:

The mouse cursor changes its shape when it is near the blue peak delimiters. ⅃⅃ indicates the peak start, while ꓔ marks the peak end.

- Left-click to change the integration limits of a peak by moving the peak delimiters.

It is not possible to "run over" another peak delimiter. After moving a peak delimiter, Chromeleon draws a new baseline between the peak start and the peak end. All peak properties such as area, width, substance amount etc. are recalculated. The integration report is immediately updated.

- Select **Save Manipulations** on the context or **Edit** menu to save the reprocessed results.

Similar to moving peak delimiters, you can perform other actions. For example, you can insert or delete peaks or change the baseline.

3. Report Tables

The default report (default.rdf) contains five tabs. The **Integration** tab mentioned above describes the general characteristics of the respective peaks in the current sample such as the **Ret. Time**, **Area**, and **Amount** columns or the concentration of the respective substance. The **Calibration** tab displays the calibration data of the current sample:

A	B	C	D	E	F	G	H	I
No.	Peak Name	Cal.Type	#Points	Rel.Std.Dev. %	Corr.Coeff. %	Offset	Slope	Curve
2	Fluoride	Lin	5	2.782	99.989	0.000	0.305	0.000
3	Chloride	Lin	5	4.456	99.968	0.000	0.207	0.000
4	Nitrite	Lin	5	2.177	99.995	0.000	0.115	0.000
5	Bromide	Lin	5	0.870	99.999	0.000	0.083	0.000
6	Nitrate	Lin	5	1.008	99.999	0.000	0.107	0.000
7	Phosphate	Lin	5	14.515	99.212	0.000	0.057	0.000
8	Sulfate	Lin	5	7.803	99.860	0.000	0.140	0.000
Average:			5	4.802	99.860	0.000	0.145	0.000

◄ ► \ Integration ∧ Calibration ∧ Peak Analysis ∧ Summary ∧ Audit Trail /

The **Calibration** tab page includes by default:

The calibration type and the number of calibration points in the calibration curve

The relative standard deviation (of the points from the calibration curve)

The correlation coefficient (independent of the calibration curve)

The calibration curve parameters (Offset, Slope, and Curve)

The **Peak Analysis** tab provides a summary of the characteristics of the single peaks such as the peak width, peak height, peak type, resolution, asymmetry, and the number of theoretical plates.

In contrast to the first three tabs, the summary includes all samples in the sequence:

A	B	C	D	E	F	G	H
Sample No.	**Sample Name**	**Ret.Time min Sulfate ECD_1**	**Area µS*min Sulfate ECD_1**	**Height µS Sulfate ECD_1**	**Amount µg/ml Sulfate ECD_1**	**Type Sulfate ECD_1**	**Plates (EP) Sulfate ECD_1**
15	FIN010608A	20.427	22.312	39.736	159.30	BMB	8587
16	RAW010608A	20.603	4.314	7.427	30.80	BMB	8070
17	*PCE010608A*	*20.623*	*4.137*	*7.103*	*29.53*	*BMB*	*8041*
18	PCI010608-1	20.573	4.279	7.363	30.55	BMB	8031
19	PCI010608-2	20.640	4.244	7.295	30.30	BMB	8074
20	PCI010608-3	20.587	4.057	6.962	28.96	BMB	7988
21	PCI010608-4	20.580	4.133	7.113	29.50	BMB	8047
22	FIN010608B	20.617	4.159	7.152	29.69	BMB	8060
23	RAW010608B	20.597	4.400	7.568	31.41	BMB	8055
Average:		20.623	3.047	5.300	21.76		7914
Rel.Std.Dev:		0.283 %	158.7%	162.1 %	158.7 %		3.61%

◀ ▶ \ Integration ∧ Calibration ∧ Peak Analysis ∧ Summary ∧ Audit Trail /

The **Summary** always refers to the current peak. To display the summary for any other peak, click the corresponding peak in the chromatogram.

The last report tab (**Audit Trail**) displays the corresponding sample protocol:

A	B	C
Day Time	**Ret.Time**	**Command/Message**
10:01:32	-2.400	Suppressor_Type = AAES
10:01:32	-2.400	Suppressor_Current = 60
10:01:32	-2.400	Flow = 1.00
10:01:32	-2.400	%B = 0.0
10:01:32	-2.400	%C = 0.0
10:01:32	-2.400	%D = 0.0
10:01:32	-2.400	Curve = 5
10:01:32	-2.400	Pump_TTL_1.0v Duration = 5.00
10:03:56	0.000	UV.Autozero
10:03:56	0.000	ECD.Autozero
10:03:56	0.000	InjectPosition Duration = 30.00
10:03:56	0.000	UV_VIS_1.AcqOn
10:03:56	0.000	ECD_1.AcqOn
10:33:56	30.000	UV_VIS_1.AcqOff
10:33:56	30.000	ECD_1.AcqOff
10:34:02		End of sample "PCE010608A".

◀ ▶ \ Peak Analysis ∧ Summary ∧ Audit Trail / ◀ ▶

It lists all commands sent to the corresponding timebase during the analysis including time and retention time.

4. Modifying a Report Table

The individual report sheets are designed such that they contain the most important peak and sample characteristics for most applications. Nevertheless, it may be possible that you do not need an existing column or that you want to add a new one.

- Double-click the column to be changed. The **Properties Report Column** dialog box is opened:
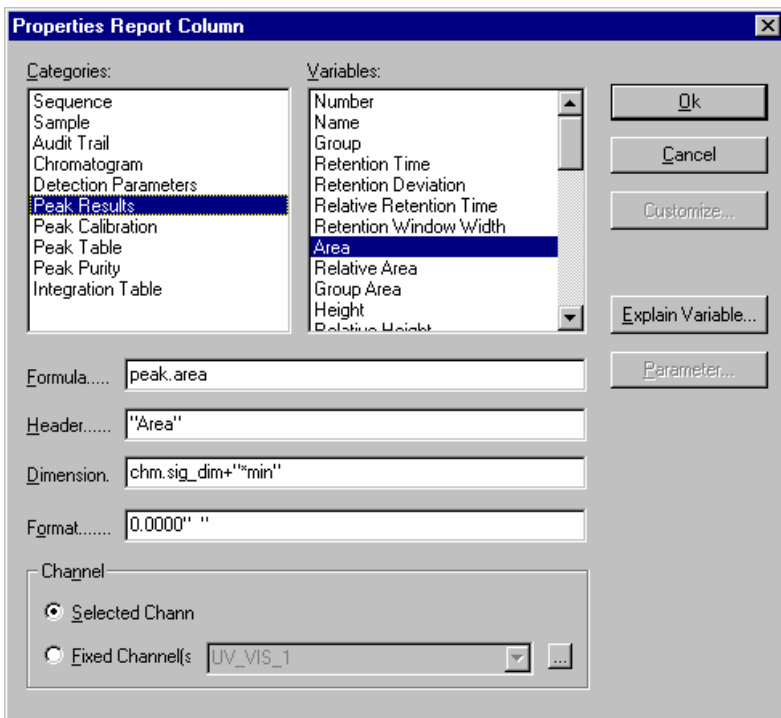
The report columns are listed under **Categories**. Under **Variables**, all result variables calculated and evaluated by Chromeleon are listed. Select a variable to display its properties (Formula, Header, Dimension, Format).

Header shows the text of the column header. **Dimension** shows the dimension name. **Format** defines the number of decimal places. Select the **Selected Channel** option to display the values of the current channel. Or else, select **Fixed Channel** to display the data of a specific channel independently of the representation in the integration window.

- Click **OK** to replace the selected column with the newly defined column.

- Select a column, and then select **Delete Column(s)** to remove the column.

🗒️ *Note:*

These report options influence the on-screen representation only. In the **Printer Layout***, define the appearance of the printout!*

5. Saving Changes

Select **Save Report Definition..** to save all changes within a report as a separate report definition file (RDF). The arrangement and the appearance of the individual report window sections are saved plus the variables to be displayed.

With each new start of Chromeleon, the report opens just as it was saved last. If you have created your own Report Definition Files (RDF), you can select a different appearance via **Load Report Definition.**

Keep in mind that each sequence is saved with a preferred profile. Upon opening a sample of the corresponding sequence, it is not the appearance of the window saved last that is used but the appearance of a specific report definition file (RDF). Proceed as follows to define a sequence for a preferred report:

- Select a sequence in the Browser and then select **Properties** on the context menu.

- Under **Preferred RDF File**, enter the name of the report definition to load.

VIII. Printing Results

1. Creating a Print Template (Printer Layout)

The **Printer Layout** window displays custom print templates. To open a print template for a specific sample:

- Double-click a sample name in the **Browser**. Chromeleon displays the chromatogram in the **Integration** plot window.
- Select **Printer Layout** on the **View** menu to change from the **Integration** plot to the **Printer Layout**.

☞ *Tip:*

*The **Method** toolbar allows you to quickly change from one view to another (for more information, see* ➔*Data Analysis). Place the mouse cursor on an icon to read its label in the quick info field.*

The image shows part of a report based on the default report template (DEFAULT.RDF):
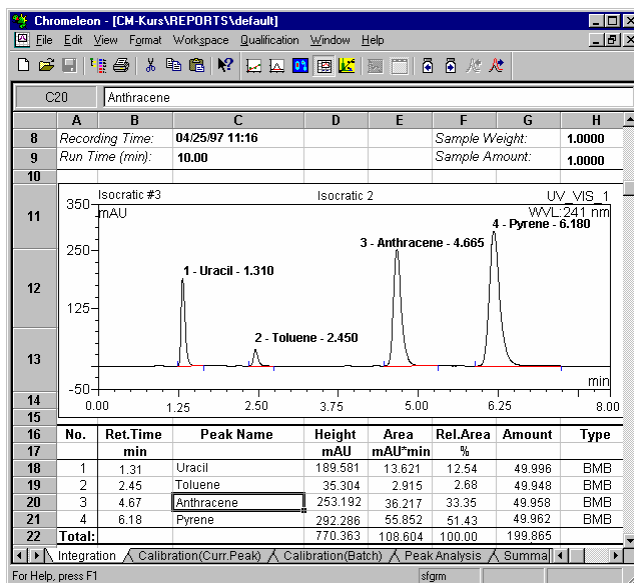
The **Integration**, **Calibration (Curr. Peak)**, **Calibration (Batch)**, **Peak Analysis**, **SST**, **Summary**, and **Audit Trail** worksheets are part of the DEFAULT.RDF report template.

- Select one of the tabs on the window bottom to open the corresponding sheet of the report.

The appearance and the structure of the individual worksheets are very similar to **Microsoft Excel** spreadsheets. Each sheet consists of a large number of columns (256) and lines (16000) and thus is much larger than a single printed page. A worksheet can consist of many horizontally or vertically arranged printed pages. Chromatograms or tables that exceed one printed page are automatically printed on two or more pages. Define the order in which the pages are printed in the **Page Setup** (on the **File** menu).

Top To Bottom   **Left To Right**

Editing an Existing DEFAULT.RDF Template

It is less effort to change an existing report template than to create a completely new one. Different templates, such as the DEFAULT.RDF and the DEFLTDAD.RDF templates, are available on the Chromeleon software CD. Copy any template you wish to use as the basis for a new template to a different and writable directory.

- Select **Save Report Definition** on the context or **Workspace** menu and save the template to a different and writable directory.

You can now edit and save the copied template. For information about how to proceed and the available options, see below. For more information about the individual steps, refer to **How to ...: Actions in the Window Printer Layout** section in the User Manual or the online Help.

- Select **Layout Mode** on the **Edit** menu.

- Select the worksheet you wish to edit or add an additional (empty) worksheet by selecting **Insert Sheet** on the **Edit** menu.

- Select **Delete Sheet** on the **Edit** menu to delete an unnecessary worksheet.

- Double-click a tab page to change its name, for example, **Integration Special**.

- Select **Insert Row(s)/Column(s)** or **Delete Row(s)/Column(s)** to add or remove single rows or columns on the worksheet.

- Select a field, a column, or an area, and select then **Clear ...** on the context menu to remove unnecessary information from the worksheet.

Having removed all unnecessary parts, you can fill cells, columns, or areas of the worksheet with new contents. You can either use the Windows clipboard (**copy** & **paste**) or the **Insert ...** command from the context menu. Follow the steps below:

- Select **Insert ...** to further specify the desired command, for example:

  - **Insert ... Chromatogram** inserts a chromatogram.

  - **Insert ... Calibration Plot** inserts a calibration curve.

  - **Insert ... Spectra Plot** inserts a UV spectrum, etc.

- Use the small cross to mark the area where to insert the desired element. The element is automatically inserted at the desired position.

- If you want to insert a report table or a single report variable, mark an empty field first. Then, use the corresponding command to insert the table or variable at the selected position.

- Report columns can be inserted only in existing tables.

- Select **Save Report Definition** on the context menu or the **Workspace** menu and save your changes.

2. Printing

Printing in Automatic Batch Operation

You can start printing the results during automatic sample processing (**Online Batch**). Make the corresponding settings in the **Batch** dialog box.

- Select **Reporting** on the **Batch** menu in the Browser or on a control panel.

- Enable the **Print/Export Report** check box to print or export the sample processing results.

- Select **Print each sample immediately** to start printing immediately after the sample has been analyzed. Select **Print when the entire batch has finished** to print all sample results after the entire sequence is completed.
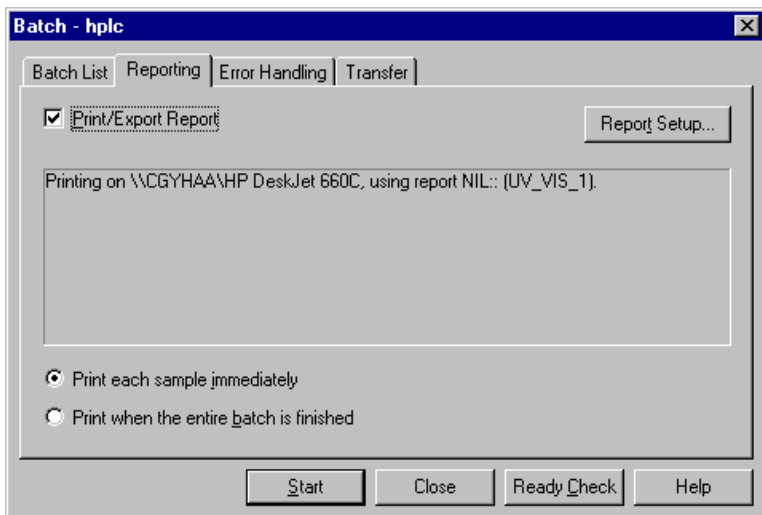
- To specify the report type and the extent of the printed output, click the **Report Setup** button. The **Batch Report** dialog box is opened:
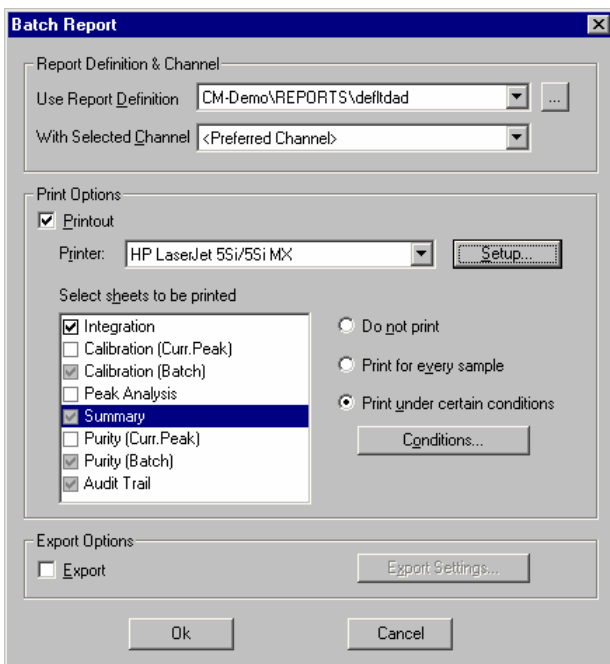
Select a report template in the **Use Report Definition** field. The default report Chromeleon templates, i.e., DEFAULT.RDF and DEFLTDAD.RDF, are available in the **Dionex Templates > Reports** directory.

- From the **With Selected Channel** field, select the channel to print, for example, UV_VIS_1. If the field remains empty, the preferred channel is printed (see ➔ *The Browser: Appearance and Function (Part b)*).

- Select the **Printout** option to print the sheets.

- The **Printer** field shows the name of the previously selected default printer. Select **Setup** to select another printer.

You have now specified the channel, the printer, and the report definition for the printed output. The Printer Layout allows you to determine the appearance of the report and to specify the information to be printed.

As described in detail in the ➔ *Creating a Print Template (Printer Layout)* topic, a report template contains several special sheets for different print data. The sheets included in the selected report template are listed in the **Selected sheets to be printed** field. The **Integration**, **Calibration (Curr.Peak), Calibration (Batch)**, **Peak Analysis**, **Summary**, and **Audit Trail** sheets are included in the default report template.

- Under **Selected sheets to be printed**, select the worksheets of the report template you wish to print.

- Determine for each worksheet whether all samples or only samples of a specific type shall be printed.

- Select **Print for every sample** to print the results for each sample.

- Select **Print under certain conditions** to print the results only when a certain condition is met (for example, only for a specific sample type).

For example, if you want to print the **Calibration** (Batch) worksheet for the last standard sample only, select the **Print under certain conditions** option and then click **Conditions**. In the dialog box that opens, enable the **Last Sample in a List of Standards** check box.
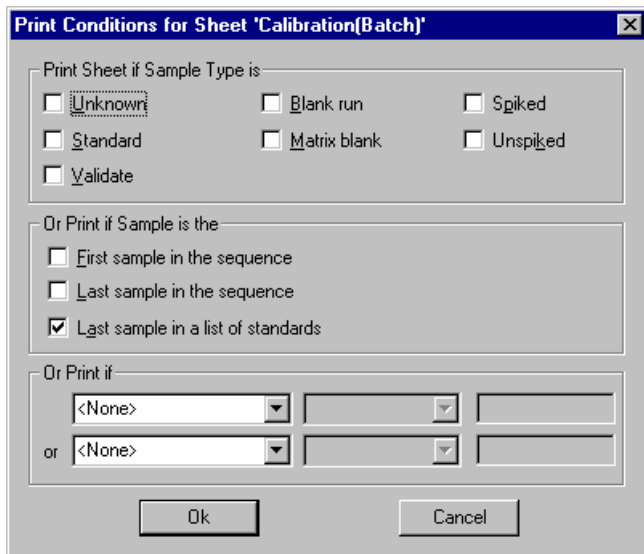
- Click **OK** to return to the **Batch Report** dialog box and define the other worksheets.

- Click **Start** as described in ➔*Starting the Analysis* to start sample processing.

Printing Samples

Of course, sample-processing results can also be printed independently of the online batch. This applies to all samples for which raw data are available. Chromeleon assigns **Finished** as sample status in the sample list. Proceed as follows to print the results of any samples.

- Open the **Browser** and select **Print Setup** on the **File** menu. Select a printer for printing the results and exit the dialog box by clicking OK.

- Select the name of a sequence to print the results of all included samples.

- If you only want to print specific samples of a sequence, select them one by one with the mouse. Simultaneously press the CTRL key and left-click to select several samples.

- Select **Batch Report...** on the **File** or context menu and determine how to print the report (see **Printing in Automatic Batch Operation**).

IX. Special Functions

In the previous chapters, the Tutorial described the basic Chromeleon functions. Most users for routine operation use these functions. In addition, Chromeleon provides many special functions that can save time for special operations. However, these functions will certainly not be relevant for all users.

Below please find a short introduction to the most important special functions and the actions they perform:

In the Browser:

➔ *Installing Datasources*

➔ *Finding Samples (Query)*

➔ *Backup and Restore*

➔ *Signing Sequences Electronically*

On the Control Panel:

➔ *Modifying a Control Panel*

In the QNT Editor:

➔ *Internal and External Standards*

➔ *Spectra Library Screening*

In addition:

➔ *Defining the Workspace*

1. Installing Datasources

Datasources represent the top level in the Browser. Each user can access at least one datasource that is the default datasource created during installation (<PC-NAME_LOCAL>).

Creating additional datasources or accessing datasources on other computer in the network is often useful. Therefore, these options are supported as well.

The section below describes how to create links to other datasources (For more information about how to create a new datasource, refer to **How to ...: Actions in the Browser/Setting up a Datasource** in the User Manual and the online Help).

All steps required for setting up datasources start with the **Datasources** command on the **File** menu.

- Open the Browser and select **Datasources**.

The **Datasource Manager** shows all datasources that the client can currently access.
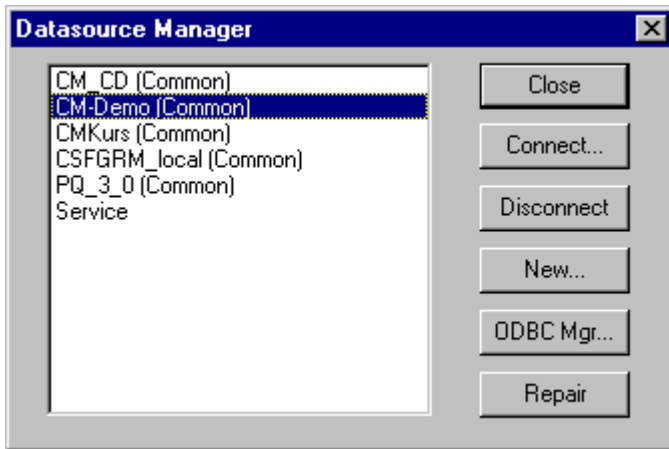
Connecting an Existing Datasource ("Connect")

If you know the directory in which the other datasource is located, you can connect to this datasource.

- Click the **Connect** button of the Datasource Manager to establish the connection.
- Select the **format** of the underlying database.
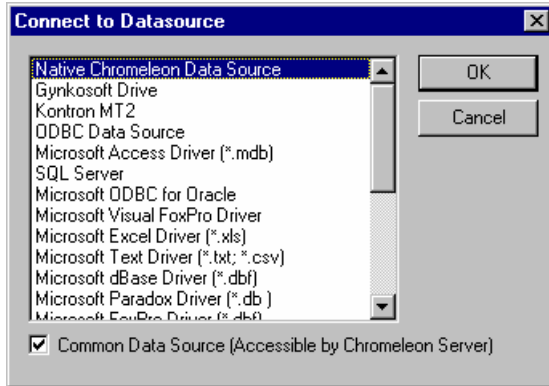
Usually, you will select Chromeleon datasources (**Native Chromeleon Data Source**). Other supported formats include default Microsoft Access databases (**mdb container**), SQL server, and Oracle databases.

- Select the appropriate datasource type and click **OK**.

- In the following dialog box, navigate to the network folder that houses the datasource.
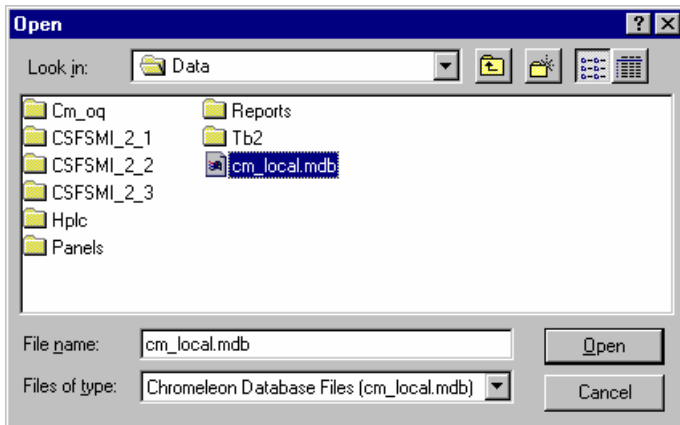
- Select the folder and click **Open**.

📙 *Note:*

If the folder and the datasource are located on a remote computer, the user of that computer must share the corresponding folder! If (s)he does, you can connect to the selected datasource. The name and the type icon of the datasource will appear in the Browser.

2. Finding Samples (Query)

You will often have to find samples according to defined problems. Instead of endlessly poring over old folders, you can use the query function of the Browser to do so easily, fast, and purposefully. Select **New** on the **File** menu to start the Query Wizard:
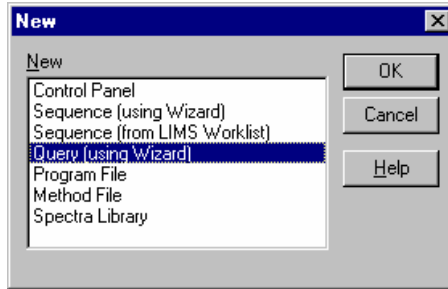
On the first query page, define whether to perform the query in the current datasource or in a fixed one. Also, define whether to search for sequence properties, sample properties, or any other properties of samples.

On the next pages, define in the **Data Field** the variable whose properties you want to find. Select an operator and define the value. The combo box next to the respective line allows you to enter a logical connective with another query property. Only after having entered **AND** or **OR**, you can enter another property:

The following query searches for all samples with

- Sample type = **Matrix Blank** and

- Whose comments start with **Charge 123456** or

- An injection date between 2/2/2000 and 2/3/2000.

On the next page, enter the following settings in order to restrict the query to samples

- Containing an Anthracene peak or

- Containing more than 10 calibration points
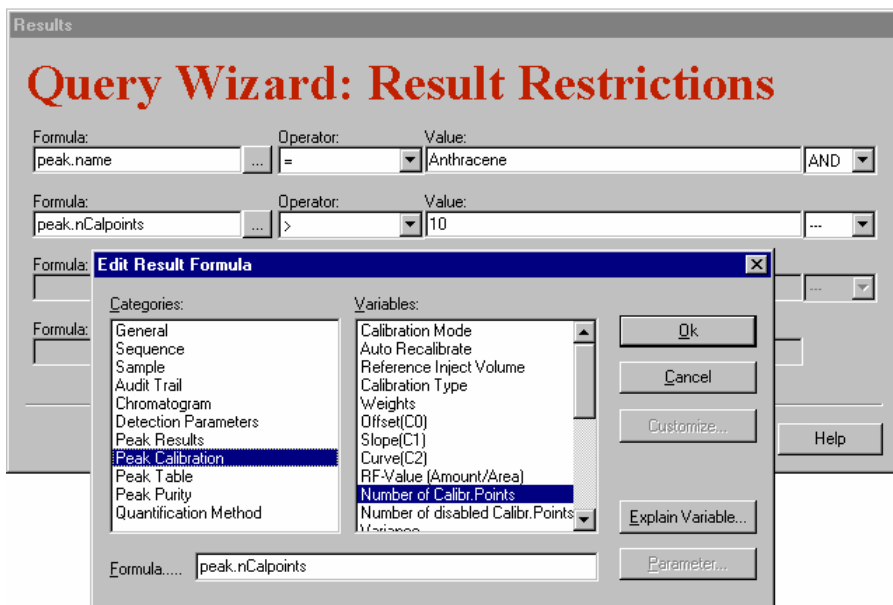
3. Backup and Restore

In the course of time, large amounts of data will result, especially when PDA detectors are used, but also when a great number of sequences is recorded. To ensure that you can continue to store data on your hard disc, you have to delete some of your data from time to time. However, do not destroy these data permanently. They still have to be available for future use. Of course, you can save the respective sequences and datasource on your network as well.

However, Chromeleon provides the possibility to backup sequences, directories, or entire datasources and save these backup files (which can also be compressed), for example, on CD. Proceed as follows:

- Select the object to save.

- Select **Export/Backup** on the File menu and then, select **Backup**.

- Indicate where to save the backup file and define all further backup options:
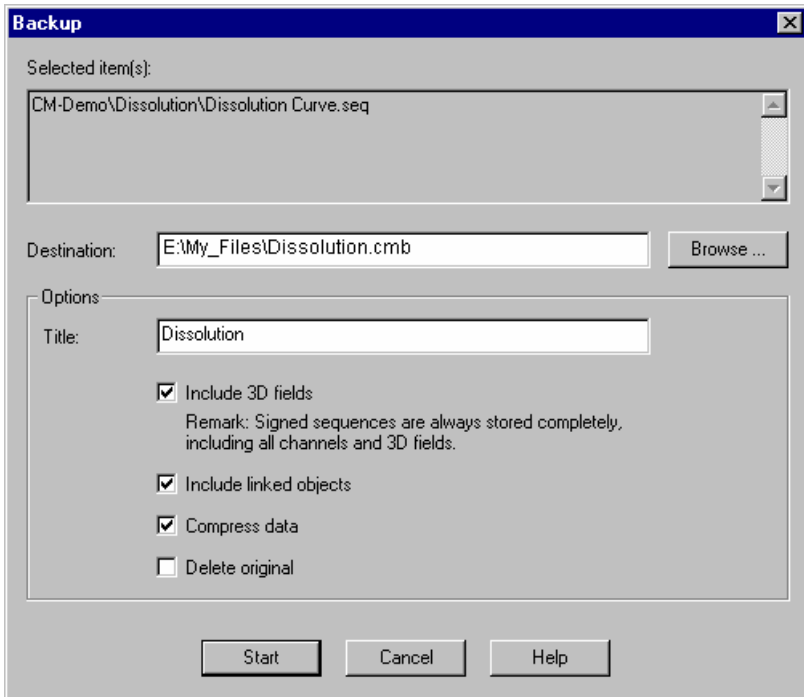
- Start the backup to create the backup file. The extension of the backup file is **cmb**.

- You can then, for example, burn the backup file to a CD or attach it to an Email.

Data that were saved in this way can be used again via the **Restore** function. Select **Import/Restore** on the **File** menu, and then select **Restore**. Find the respective backup file and indicate where to restore it.

The Backup and Restore functions are an easy way to save large amounts of data in such a way that they can be quickly accessed later. Structure and cross-references remain intact. In addition, these functions facilitate exchanging data via E-mail.

4. Signing Sequences Electronically

Electronic Signature allows you to sign the results that have been generated from your ➔ *Raw Data*. This function is important aspect for quality assurance and GLP. When User Mode is enabled, you can sign and protect ➔ *Sequence* reports that have been accepted as correct. In this way, it is possible to review and reproduce the results at any time later.

Electronic Signature includes three steps:

- Submit
- Review
- Approve

Typically, the user who created the report signs and **submits** it. Afterward, for example, the laboratory manager **reviews** the report and signs it as well. Finally, the quality assurance manager approves the results.

 Note:

Enable User Mode. If User Mode is disabled, electronic signature will not be possible. Besides, electronic signature is available only for user databases that have been created with a CmUser program version 6.10 or higher. Update your database if an error message notifies you that electronic signature will not be possible.

To sign a sequence proceed as follows:

- Click the sequence and select **Submit Results** (first signature step) on the context menu.

- Select the Report Definition file (RDF) to be used for displaying the sequence, select the channel for which you want to show the results, and select the report sheets to be signed:

- The **Check Signed Results** dialog box appears. This dialog box allows you to check the report to be signed. Use the arrow keys to toggle between different sequence samples. For each sample, the tab pages for the selected report sheets are displayed. Click **OK** when you have finished checking the report.

- The **Submit Signature** dialog box is opened. To sign the SOR file, enter your User ID and the signature password:

- The extension of the newly created file is .SOR. The SOR file is stored in the signed sequence.

- In this way, the sequence and its samples are protected against changes.

- Select **Electronic Signature** on the **File** menu and then select **Verify** to have Chromeleon check the signed sequence report once again, i.e., the sequence, the corresponding files (pgm, qnt, rdf, sor), and the individual samples.

To view the signature status of a sequence, select the sequence and select **Properties** on the context menu. The **Signature** tab page indicates the status of the selected sequence:

Properties of Sequence "Isocratic"   ☒

General | Statistics | Signature | Report Columns |

Status: Reviewed

Submit
Authorized users: User1;User2;Lab Manager   Edit...
Submitted: 25/07/2002 14:06:57 by User2
User name: User2   Details...

Review
Authorized users: Lab Manager   Edit...
Reviewed: 25/07/2002 14:20:45 by Lab Manager Details...
User name: Lab Manager

Approve
Authorized users: Quality Officer   Edit...
Approved: Details...
User name:

OK   Cancel   Apply   Help

You can only remove a signature if you are authorized by the corresponding privileges. Removing the signature also deletes the corresponding SOR File.

5. Modifying a Control Panel

Chromeleon includes more than a hundred default control panels for different devices and functions. If you do not find a control panel for your requirements, we recommend modifying the panel that is closest to your requirements. You can add further controls or change the functionality and appearance of existing ones.

 Note:

If you cannot perform the following steps, you do not have the user privileges required for these actions. In this case, contact your system administrator.

Enabling the Layout Mode

- Enable Layout Mode on the context menu. You can modify the control panel only in the Layout Mode.

Adding a control

- Move the cursor on the layout toolbar.

- The Quick Info provides a brief description for the control on which the cursor is currently positioned.

- Select a control by clicking. The corresponding object is now attached to the cursor.

- Place the cursor on the location where to add the new object and left-click.

Functionality and appearance of the control

- Right-click the control for which you want to change the functionality and/or appearance.

- Select **Properties** to open the **Properties** dialog box.

Depending on the selected control, the **Properties** dialog box includes various tabs. For a slider, the **Style** tab looks as follows:

Gauge/Slider Properties

General | Link | Autosize | Color | **Style**

Scale: [........] ▾   ☑ Gray

Range: ⦿ Auto   ○ [0]   to [400]

Slider: [———] ▾

Slider Box: [◁] ▾   ☑ Colored   ☐ Snap To Scale

[OK]   [Cancel]   [Apply]   [Help]
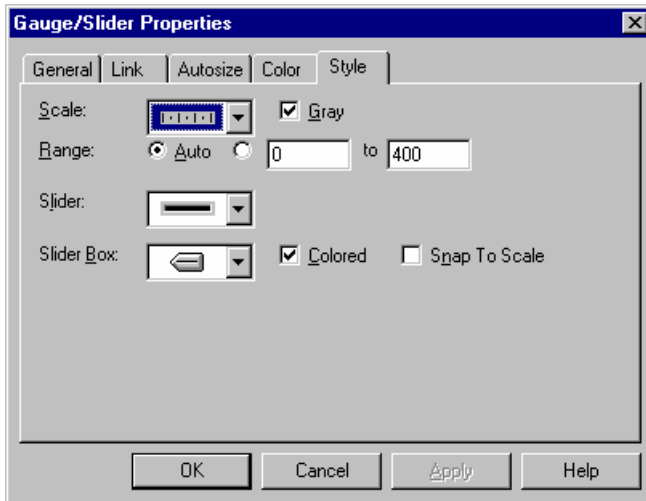
- Select the **General**, **Color**, or **Style** tab to change the appearance such as the color, the shape, or the caption of the control.

- Select the **Link** tab to determine the function and the instrument to be operated via the control.

Gauge/Slider Properties

General | **Link** | Autosize | Color | Style

Object:

Pump

| NextSample |
| P580_MotorValve |
| P580_OperableOut |
| P580_Relay1 |
| P580_Relay2 |
| PrevSample |
| PrevStandard |
| Pump |
| Relay_1 |

Total flow through the pump.

Object Property:

Flow

| %A |
| %B |
| %C |
| %D |
| Connected |
| CumulatedWorkLoad |
| Flow |
| Leak |
| Pressure |

[OK]   [Cancel]   [Apply]   [Help]
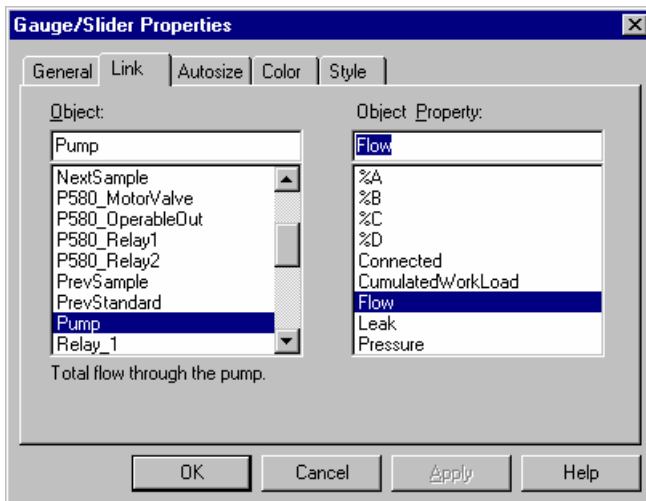
- From the **Object** list, select the instrument itself. From the **Object Property** list, select the instrument's function. Instrument and function selection depend on the components installed with the timebase.

With the settings shown above, you can use a slider to control the pump flow rate.

6. Internal and External Standards

Substances in unknown samples are usually quantified using external standard samples. However, it is also possible to add a known amount of a standard substance (= internal standard, ISTD) to an unknown sample and then use this standard for calibration. Use the **Standard** column on the **Peak Table** tab page to specify whether you want to calibrate a substance using an external or internal standard.

- Press the F8 key to open the **Standard Method for <Peakname>** dialog box.
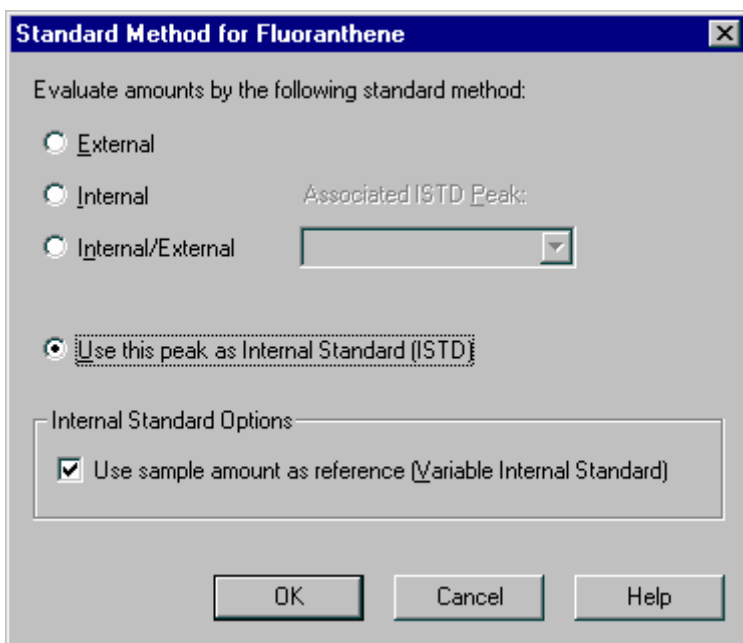
- Select **External** to use external standard samples for calibration.
- Select **Internal** to use internal standard samples for calibration.
- If you use several sample preparation steps during which substance may be lost, correct this using the **Internal/External** method: Add to your unknown sample a known amount of a substance that originally was not part of the sample (= ISTD). Be sure that the chemical behavior of the substance is similar to the behavior of the substances to quantify. During sample preparation, a corresponding amount of this standard substance will be lost as is for the substances to quantify.

In this case, you can use the known internal standard in the **Internal/External** method to correct the external calibration. First, define a substance as internal standard (**Use this peak as Internal Standard**) and then use this substance as **Associated ISTD Peak**.

- If you did not add exactly the same amount of internal standard to all samples, select the **Use sample amount as reference** option. In this case, you have to enter the amount of internal standard that was added to the sample in the sample list of the Browser. This entry is then used for the calculation (instead of the entry made in the **Amount** column of the QNT editor).

For an example of the possible settings in the Standard column, see the image below:

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Peak Type
1	Naphthalene	5.327 min	0.090 AG	ISTD Internal	Area	Lin	Auto
2	*Phenanthrene*	*7.682 min*	*0.092 AG*	*Internal Naphthalene*	*Area*	*Lin*	*Auto*
3	Anthracene	7.999 min	0.070 AG	Internal Naphthalene	Area	Lin	Auto
4	Fluoranthene	8.751 min	0.102 AG	ISTD Var.Int/Ext	Area	Lin	Auto
5	Pyrene	9.144 min	0.065 AG	Var.Int/Ext Fluoranthe	Area	LOff	Rider
6	Dimethyl-Fluoranthene	9.772 min	0.078 AG	External	Area	Lin	Auto
7	Chrysene	10.294 min	0.090 AG	External	Area	Lin	Auto

\\ General Λ Detection \Peak Table Λ Amount Λ

Dimethylfluoranthene and *Chrysene* are calibrated, using external standards. *Naphthalene* and *Fluoranthene* are used as internal standards with Naphthalene being used for internal calibrations and Fluoranthene for internal/external calibrations. Phenanthrene and Anthracene are calibrated, using Naphthalene as internal standard, whereas the internal/external method is used with Fluoranthene as internal standard to quantify Pyrene.

7. Spectra Library Screening

The **Spectra Library Screening** tab page of the QNT editor allows searching spectra libraries for reference spectra for the different peaks of the current chromatogram. As the chromatographic conditions considerably influence the spectra, we recommend filling your spectra library with spectra from chromatograms that were recorded under the same conditions as the current chromatogram.

📖 *Note:*

*To create a new library, select **New** on the **File** menu in the Browser and then copy the desired spectra in the spectra plot (Ctrl + C) and paste them into the library (Strg + V).*

On the **Spectra Library Screening** tab page, select the spectra library in which to search for the reference spectra. In addition, enter the comparison criteria for the spectra search. Normally, the default values of the **Match Criterion** (Least Squares) and **Hit Threshold** (950) will be sufficient. However, you can also specify many additional search criteria.
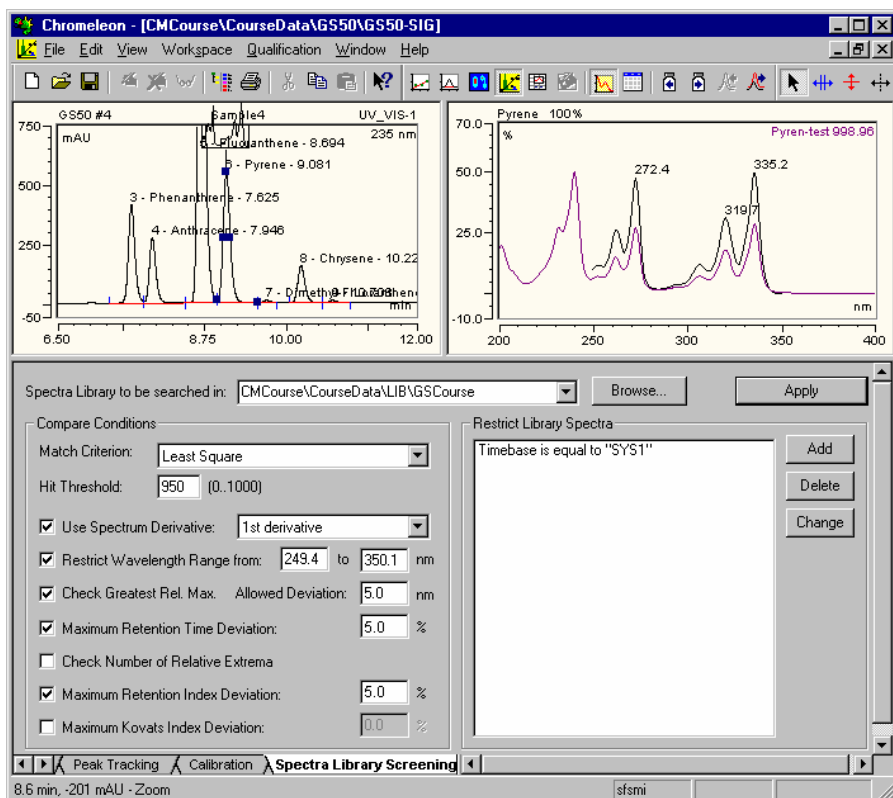
Click **Apply** to start the spectra search. The retention spectrum of the actual peaks is compared to the reference spectrum that was found. To the top right of the spectra plot, the corresponding match factor is displayed. The match factor indicates the correspondence of the two spectra.

A value of 1000 indicates that the spectra are identical. If the match factor were 0, they would be completely different. If no corresponding spectrum is found based on the selected criteria, the following message appears **No spectra library hits found!**

 Note:

You can use the spectra library screening results for automatic peak table creation (see ➔ *Automatically Generating the Peak Table).*

8. Defining the Workspace

Chromeleon allows you to save the window arrangement of any work situation, i.e., the combination of different windows, in a workspace.

This facilitates opening single files or windows and allows you to work in whatever work environment you prefer.

The information about the windows involved is stored in a WSP file. If you want to start working using a specific workspace, open the corresponding WSP file or generate it by storing the screen contents. The following commands are available on the **Workspace** menu:

- Select **Open Workspace** to open an existing workspace.
- Select **Save Workspace** to save the current workspace.
- Select **Save Workspace** as to save the current workspace with a new name.
- Select **Autosave Workspace** to always save the most recent workspace as the default workspace.

There is no restriction on the number of windows that can be saved with each workspace

A useful workspace arrangement might combine, for example, the report, a control panel, and the Browser (refer to the picture).

☞ *Tip:*

When Chromeleon is started, the most recently used workspace is loaded.

Addendum: Keyboard Shortcuts + Short Glossary

Quick Access Keys (Shortcut Keys)

Quick access keys and/or shortcut keys are provided for many operations, especially in online control:

	Action	Where	Description
General	ESC or right-click		Aborts the drag/move action.
	F1		Opens the context-sensitive Help;
	F2		Enables the Edit mode.
	F3		After the Find command: Find Next.
	F4	Chromatogram	Takes you to the next sample.
	SHIFT+F4		Takes you to the previous sample.
	Alt+F4		Exits Chromeleon.
	F5		Updates all windows.
	F6		Takes you to the next partial window.
	SHIFT+F6		Takes you to the previous partial window.
	F10		Takes you to the next channel.
	SHIFT+F10		Takes you to the previous channel.
	F7	Browser (F7 key only) +	Optimizes the column width.
	SHIFT+F7	QNT Editor	Optimizes the line height.
	F8		Opens an edit dialog box for the respective field.
	F10 or ALT		Shows the hotkey underlines.
	SHIFT+F5	Browser	Displays the chromatogram.
	ALT+ENTER	Browser + Control Panels	Opens the associated **Properties**.
	CTRL+TAB		Toggles between open windows.
	CTRL	Signal plot in the report and in the QNT Editor	Allows zooming (the cursor becomes a zoom cursor).

	Action	Where	Description
General (Cont'd)	CTRL+N		Creates a new file.
	CTRL+O		Opens the file.
	CTRL+S		Saves the file.
	CTRL+R		Opens the batch report.
	CTRL+P	Report + Printer Layout	Prints the selected object(s).
Edit	CTRL+Z		Undoes the previous action.
	CTRL+Y		Repeats the previous action.
	CTRL+X		Cuts the selected object(s).
	CTRL+C		Copies the selected object(s).
	CTRL+V		Pastes the selected object(s).
	CTRL+F		Finds a string of characters.
	F3		Finds the next string of characters.
	CTRL+H		Replaces the entry in the field.
	F9		Fills the column and/or selected cell(s) with the first value of the selection.
	INSERT		Inserts the selected object(s).
	DELETE		Deletes the selected object(s).
Sample List	CTRL+I		Inserts a sample.
	CTRL+D		Deletes a sample.
	CTRL+A		Adds a sample.
Control	CTRL+F		Changes the pump's settings
	CTRL+I		Injects
	CTRL+Break		Stops the pump.
	Break		Turns on the Hold mode.
	Ctrl+A		Turns on data acquisition.
	Ctrl+B		Edits the batch.
Signal plot	Double-click...	- Overview window	Unzooms.
		- Time axis	Auto Plot Speed
		- Signal axis	Autoscale
		- Plot range	Signals...
		(- or else:…)	Axis/decoration
	SHIFT	- when zooming	Retains the scale ratio between signal and value axis. (The shape of chromatograms is maintained.)

	Action	Where	Description
Gauge/Slider	CTRL key	Press when dragging	Toggles the **Snap To Scale** option.
Script Button	Click	Button	Indicates whether the program is still running. Stops the program upon confirmation.
Edit Field	TAB/ENTER		Sends the new value.
	ESC		Aborts the input.
Layout Mode	ALT + Click	On the selected panel	Draws a selection frame on the control panel.
	ALT + Drag		Temporarily toggles the **Snap To Grid** option.
	ESC		Deselects all.
	ARROW keys		Drags the selection pixel by pixel.
	SHIFT + ARROW keys		Increases/reduces the selection pixel by pixel.
	SHIFT+SELECT		Extends the selection.
	CTRL+SELECT		Highlights the selected control (the highlighted control is used in **Align**).
	CTRL+Drag		Copies controls.
	Double-clicking	On the online signal plot on the control panel	Opens the **Properties** dialog box.

Datasource and Database

A database that is mounted to the Chromeleon client is referred to as a datasource. To mount a datasource, select **Mount Datasource** on the **File** menu in the Browser and then specify the format and location of the database.

During the initial installation of Chromeleon, a local default datasource is created on each client PC. In network operation, the datasource name is composed of the computer name and the extension **LOCAL** (<PC name>_local). On a local station that is not available on a network, the name stated under **Control Panel > Network > Identification** in the operating system is used. If no identification is entered, the datasource is named DEFAULT_LOCAL.

Chromeleon data is usually stored in an Access database, that is, in an MDB (**Microsoft Data Base**) container. The default datasource is based on an Access database, too. The ODBC capability of Chromeleon allows you to use various other formats (SQL, Oracle, etc.) as well. Both, **Sample data** and Sequence data are saved in a datasource, independently of the chosen format. You can save a database on a local hard disk or any other mass storage device.

Select **New Directory** on the **File** menu in the Browser to create individual subdirectories under a datasource. You can then use these directories to save ➔ *Sequences* and the corresponding data and programs.

If the datasource is located on a centralized network PC, all clients with the appropriate access rights, which have been assigned by the system administrator, can access the database. If the datasource is stored on a local hard disk, the corresponding client grants database access via the Windows **File Sharing** option. Chromeleon also allows you to lock datasources, directories, or sequences.

Raw Data

Raw data refers to all analog and digital data points that are digitally stored on a PC. That is why raw data exists for signals or channels only selected by the user before data acquisition.

The scope and accuracy of the stored raw data depends on the selected **Sampling Rate**, the **Step**, and the resolution of the detector signal.

Sequence

A sequence combines samples that belong together due to their origin or processing. The names of all samples belonging to one sequence are entered in the sample list (also called sequence table). Thus, a sequence is a container for various samples.

Theoretically, the number of samples is not limited, but use more than 100 sample entries in exceptions only. Create additional sequences to reduce the number of samples included in one sequence and thus to accelerate access to the individual samples. In this way, it is also easier for you to keep track of the processed samples.

The sequence table also defines how to process a sample. It includes information about the sample itself (name, injection volume, position, sample weight, dilution factor, etc.) and references chromatographic methods that specify the program (PGM File) to be performed for the analysis and the evaluation parameters to be used (QNT Method).

The entire data collected in connection with creating and processing a sequence is saved in the associated sequence. This also includes the raw data and protocol data recorded during the analysis.

Similar to ➔*Datasources*, sequences can be "locked." In this status, data and results are read-only. You cannot modify or extend them.

(Chromatography) Server

PCs connected to the components of a system via interfaces can be used as chromatography servers.

A chromatography server is automatically installed on the PC during installation. The chromatography server controls the data exchange between the chromatographic system and the PC. Upon starting, each server is capable of serving up to six controlled or 16 non-controlled systems (➔*Timebases*). The server receives the commands that have been entered on the control panel of a client PC and executes them at the specified time, for example, by transmitting them to the corresponding device driver of the HPLC system. The server also assumes this function in the opposite direction. Thus, the raw data of each system is stored at the location specified by the client and the entire system-relevant data is forwarded to the client.

Configure the server and install the timebase(s) in the Server Configuration program.

If the Chromeleon server and client software are located on the same PC, the installation is called a local installation or referred to as workstation. If they are not, the installation is called network installation.

🖐 *Tip:*

In case of manual data acquisition, ➔Raw Data is always stored in the manual sequence of the timebase directory (if not otherwise defined). This directory is available only in the local datasource of a local installation. In the case of a batch, the user can decide where to store the data.

Timebase

All components that are combined in a chromatography system to enable the chromatographic separation and related in a time context with each other are assigned to the same timebase.

A timebase can be a very complex system. For example, it can consist of two pumps, one autosampler, one column oven, two detectors switched in series, and one fraction collector. However, an isolated gas chromatograph can also represent a timebase.

Any other system that is completely independent from the first one represents a new timebase. Administration of different timebases is on one or several ➔ *Chromatography Servers*.

Create your timebase(s) in the Server Configuration program.

Index

A

B

C

D

E

F

CHROMELEON®

Chromatography Management System

User Manual

Contents

Device Control...749

Commands and Tips for Third-Party Devices.........881

General Information

Using the User Help and Manual

Although the User Help section of the Chromeleon online Help and the User Manual differ only slightly in content, they are intended for different situations.

- Refer to the User Help section in the Chromeleon online Help to look up the meaning of unknown terms or to learn how to use the application in a specific situation on-screen. The easiest and quickest way to access the online Help is to press the ➤*F1 Key*. For more information, refer to **Activating Online Help**.

- Look up information in the User Manual to become familiar with the ***fundamental features*** and operating procedures of the system. Dionex recommends that you read the first two sections and look up unfamiliar terms in the glossary.

Note:

For more information about the contents and structure of the User Help and/or Manual, refer to **The User Help (Overview)**.

In addition to the User Help section, the Chromeleon online Help includes an Administrator Help section. For more information, refer to **The Administrator Help**.

The Administrator Help

In addition to the User Help section, the Chromeleon online Help includes an Administrator Help section. The User Help section provides useful information for the Chromeleon end user. The Administrator Help section is intended for system administrators and Dionex Service.

For an overview of the topics described in the User Help section, refer to the Table of Contents.

The Administrator Help section provides information about the different Chromeleon management tools and the hardware installation procedures:

Software Installation and Communication

Validation and Qualification

Working with Files, Databases, and Networks

Installing and Configuring Mass Spectrometers

Configuring the Chromeleon Server

Starting and Monitoring the Server, Setting Up the Server for Network Access

21 CFR Part 11 and Electronic Signature

Chromeleon User Management (covering the ➢*User Manager (CmUser) and Security Activation Tool (CmSecure)*)

Hardware Installation. This section includes among others the following topics:

   Installing Dionex Devices

   Installing and Controlling Third-Party Devices

The Online Help (Overview)

As with most Windows programs, the online Help features various windows and levels. These largely correspond to the various parts of the User Manual, such as the table of contents, main section, appendix, and index. In addition to the manual, the online Help provides many context-sensitive tips that open only from the respective program window.

The **Contents** tab provides an overview of the different Help sections. This information corresponds to the table of contents in the User Manual:
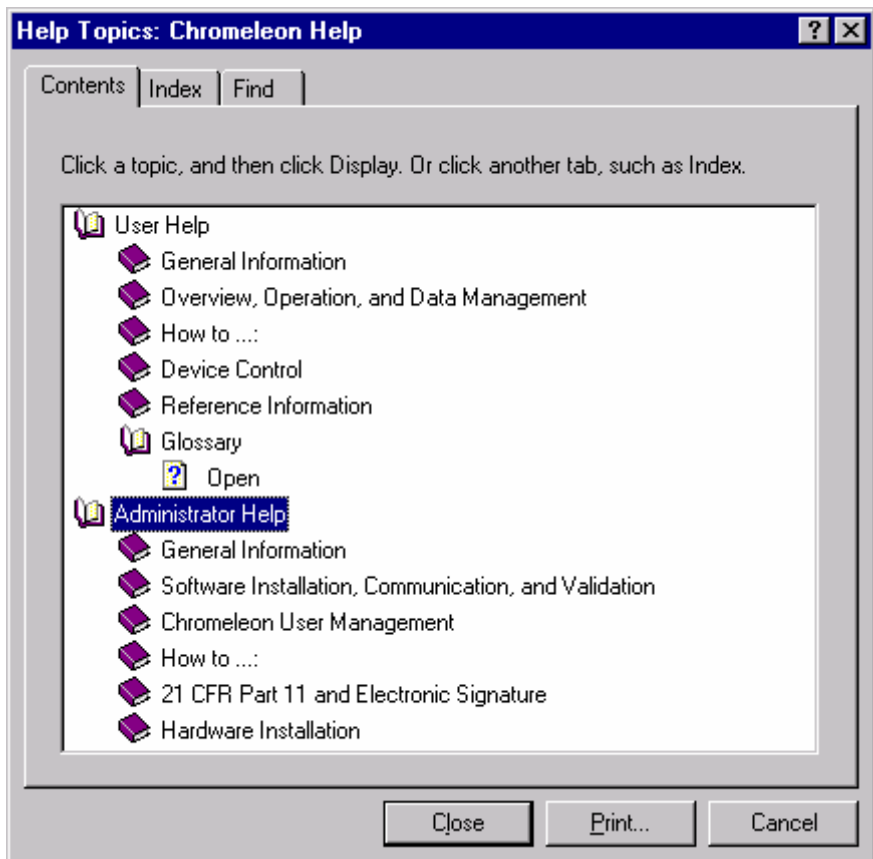
- Double-click a **book symbol** to display the topic titles.

- Double-click a **question mark symbol** to open the corresponding topic.

- The **Index** tab page enables you to find a specific term by searching the index entries.

- The **Find** tab page enables you to search for specific words and phrases in the Help topics by searching the entire text of the Help system.

The selected topic appears in a separate Help window. The window has a white background if the topic deals with questions regarding theory, installation, and operation. The background color is yellow if the topic provides tips that are more practical (**How to:**). Both windows can be displayed simultaneously.

👉 *Tips:*

Online Help is automatically displayed in the language (German or English) of the active language setting (see Windows Control Panel > Regional Options). The English version is loaded by default.

Occasionally, the Help may not include information about a last-minute feature supported by your Chromeleon version. In this case, please refer to the Release Notes for a detailed description of the new feature.

The User Help (Overview)

📋 *Note:*

The structure of the User Manual mainly corresponds to the structure of the User Help section.

The User Help section in the Chromeleon online Help is divided into different sections. The **Table of Contents** provides an overview of the various topics.

When appropriate, references to related topics are provided. These references are indicated as follows:

➢	*Jump term*	References technical terms in the **Glossary**.
⇒	*Jump term*	References topics in the **Reference Information** section.
🔲	**Shortcut link**	References topics in the **Overview, Operation, and Data Management** and **How to:** sections.
		In addition, this link references related topics in the Administrator Help section.
➡	**CmUser link**	References topics in the **Chromeleon User Management** section of the Administrator Help section.
🔵	**Installation link**	References topics in the **Hardware Installation** of the Administrator Help section.

1. Section: General Information, Overview, Operation, and Data Management

This section describes the structure and functions of Chromeleon, as well as basic chromatographic facts and methods.

2. Section: How to:

The **How to** section provides helpful answers to frequently asked questions, such as:

"How do I perform external calibrations?"

"How do I generate program files?"

"How do I re-integrate chromatograms manually?"

3. Section: Device Control

This section provides information about the commands supported for controlling Dionex and/or third-party devices, as well as practical tips for device control.

4. Section: Reference Information

This section describes commands, parameters, variables, and report categories in table form.

5. Section: Glossary and Index

Glossary

The glossary is an alphabetical list of chromatographic and program-related terms. Consult the glossary for the exact definition of

- A technical term; for example, Blank Run Subtraction.

- A command; for example, **Draw**.

- A parameter; for example, **Skewness**.

Index

Refer to the Chromeleon Help index to locate information about a specific term. In the User Manual, ordinary page numbers refer to the first four manual sections, while page numbers preceded by the letter **A** refer to the glossary section; for example:

Activating Online Help

There are several ways how you can access online Help:

➢*F1 Key*   Press the F1 key to display context-sensitive information.

How to...
What's this?

Undo   Ctrl+Z
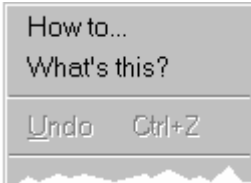
                                             Select **How to** on the context menu to display detailed information about how to perform a particular task. Select **What's this?** to display a short description of the corresponding control or active window.

Help menu   Select ➢*Index* on the **Help** menu to open the Chromeleon online Help. The **Contents** tab provides an overview of the different Help sections and topics. The **Index** tab displays an alphabetical list of terms that are explained in the Help. To find a specific term, enter the term of interest in the input field. The **Find** tab enables you to search for specific words and phrases in the Help topics by searching the entire text of the Help

                                             Select **Using Help** on the **Help** menu for more information about how to use the online Help.

▶?   Clicking this icon on the standard toolbar changes the appearance of the mouse pointer: a question mark is appended to the pointer. To display information about an on-screen item, click this icon, and then click the item of interest.
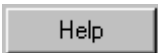
Help   Click **Help** in a dialog box for more information about the dialog box.

Online Help topics often include links that jump to other Help topics. To ➢*jump to another Help topic*, click the green underlined hyperlink or click a shortcut symbol.

Overview, Operation, and Data Management

Chromeleon (Overview)

Chromeleon (Overview)

Chromeleon is a modern **Chromatography Management System** that allows you to control and monitor chromatography installations and to backup, evaluate, and reprocess data. Chromeleon provides various sub-programs, making it very flexible and ➢*GLP* conforming, and offering you numerous options for operating effectively and productively:
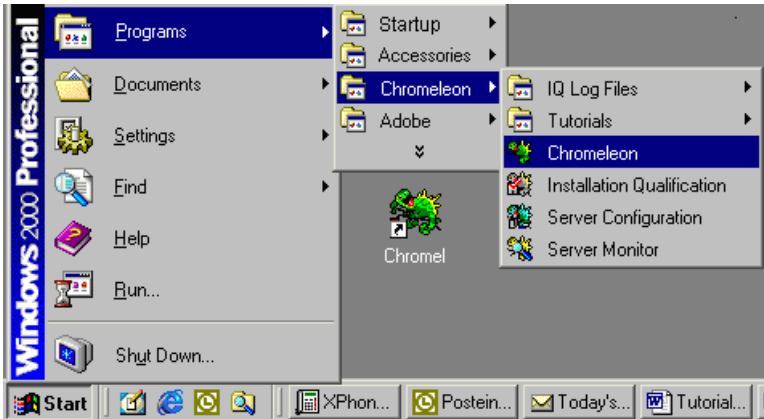
The sub-programs support the following functions:

Icon	Description	Function
🐸	Chromeleon (Client)	Device control, data backup, reprocessing and evaluation, validation (user)
🐸	Installation Qualification	Verification and documentation of the installation (administrator)
🐸	➢*Server Configuration*	Device configuration (administrator)
🐸	➢*Server Monitor*	Interface between the installation and the Chromeleon Client (administrator)

The Server Configuration and Server Monitor programs allow you to create a client/server structure, thus adding to the networking capability of Chromeleon.

In addition, there are two User Management programs available to the administrator in the Chromel\CmUser directory:

Program	Description
CmSecure.exe	Enabling and disabling User Management (administrator)
CmUser.exe	Installing User Management (administrator)

As a user, you will almost exclusively work with the Chromeleon Client program. The different windows support all required functions.

Also, refer to:

Chromeleon Windows

Chromeleon Features

Chromeleon Licenses

Installing Chromeleon

Chromeleon Windows

You can open the various windows of the Chromeleon **Client Program** via the associated icons or by double-clicking the respective directory:

Click an icon to open one of the following windows:

Symbol	Description	Function
	➢*Browser*	Data administration and storage
	➢*Panel Tabset*	Control of all configured devices
	Integration	Chromatogram display and reprocessing
	➢*PPA*	Peak Purity Analysis: 3D Field (only for PDA data)
	➢*3D Amperometry*	3D amperometry data display and evaluation
	QNT Editor	Quantification Method editing
	➢*Printer Layout*	Printer Layout creation and/or modification
	Signed Results	Electronically signed sequence (➢*SOR File*)

Double-click the associated file to open these windows:

Symbol	Description	Function
🗔	➢*Panel*	Control of a specific device or system
🔧	PGM Editor	Processing of a control file
🔲	➢*Panel Tabset*	Control of all configured devices

The **Integration** window and **QNT Editor** support different panes; click the associated icon on the Method toolbar:

Symbol	Description	Function
📅	Report	Display of various report tables
📊	➢*Trend Plot*	Display of trends in data series
📈	Spectrum	UV spectrum (only for PDA data)
📉	Calibration Curve	Calibration curve
📊	MS Spectrum	➢*Mass Spectrum*

For more information about the different windows, refer to:

🔲 **The Browser**
🔲 **The Control Panel**
🔲 **The Panel Tabset**
🔲 **The QNT Editor**
🔲 **The PGM Editor**
🔲 **The Integration Window**
🔲 **The Printer Layout**

▪ Chromeleon Features

Chromeleon supports a wide variety of features, including:

- Modern control technology via client/server architecture and Windows RPC technology. (For more information, refer to 🔳 **The Network**.)

- Complete 🔳 **Network Installation**.

- Separate ➢*User Manager* (CmUser program) for user management.

- Query-based data access via an integrated ➢*Database*.

- Connection to standard ➢*ODBC* and/or SQL databases.

- Tele-service (remote maintenance).

- Freely definable workspace on the 🔳 **Control Panel**.

- ➢*Mass Spectrometer* control and MS data evaluation.

- ➢*Sample-Oriented Operation*.

- ➢*Electronic Signature* of sequences.

- Compliance with GLP through automated ➢*Instrument Qualification (IQ)* and ➢*Operational Qualification (OQ)*, as well as Instrument OQ and ➢*Performance Qualification (PQ)*.

The user interface and operation correspond to the standard Windows requirements:

- Comprehensive ➢*Online Help*.

- Easy operation based on ➢*Toolbars* and assisting ➢*Wizards*.

- Comprehensive context menus via the ➢*Right Mouse Button*.

- **Drag & Drop** functionality (refer to **How to …: Creating and Managing Files and Data** 🔳 **Moving and Copying Elements**).

- Real ➢*Multi-Tasking* and ➢*Multi-Threading*.

In addition, the chromatographic interface features these special enhancements:

- Optical representation of the gradient profile.

- Online zooming beyond the current time.

- Grid in online window.

- Additional peak variables.

- Determination of reference wavelength for individual channels.

- Wavelength compensation via holmium oxide filter.

- Enhanced data compression and restoration of old data.

- Baseline subtraction can be undone at any time.

- Base area correction/base area recognition.

◾ **Chromeleon Licenses**

Chromeleon can be configured to satisfy the requirements of many different applications. In addition to the basic software package, various options are available from Dionex.

The set of licensed capabilities for a Chromeleon station is controlled by means of a serial number and the corresponding license key. The serial number is coded on a software protection device that is installed locally, together with the license key, or managed by a ➢*License Server*. (For more information, refer to **Software Installation and Communication** ◾ **The Software License** in the **Administrator Help** section.) Chromeleon can only be started with the full range of features if a protection device (such as a ➢*Dongle* on the parallel or USB PC interface or a ➢*PAL* on the A/D converter card) is detected, or if a license provided by the license server is detected.

For information about which Chromeleon licenses are available from Dionex, see the table below.

☞ *Tip:*

*To check the licenses after the installation, select **About Chromeleon** on the **Help** menu.*

Server Features

Server License:	The base server license enables control and data acquisition for instruments attached directly to the PC that has the license. The server must always be configured with one or more timebases; other server features are optional.
Timebases Class 1 to 3:	Each ➢*Timebase* license enables control of one chromatography system (defined as a set of instrument components that operate according to the same elapsed-time clock).

The timebase class determines which instruments can be controlled:

🔲 **Timebase Class 1 License: Supported Devices**

🔲 **Timebase Class 2 License: Supported Devices**

🔲 **Timebase Class 3 License: Supported Devices**

👉 *Tip:*

All Dionex and LC Packings instruments are controlled by a Timebase Class 1 license.

The Timebase Class 2 and Timebase Class 3 licenses allow you to control additional chromatography instruments that are not supported by the Timebase Class 1 license. Please note:

License	**Also Supports Devices of**
Timebase Class 2	Timebase Class 1
Timebase Class 3	Timebase Classes 1 and 2

Up to six timebases can be configured on one server PC; a maximum of four of these timebases can be fully-controlled Dionex LC or IC devices. However, only two Timebase Class 2 or Timebase Class 3 licenses are allowed.

Multiple Network Control:	This license enables the server's timebases to be controlled by other Chromeleon client stations across a network. In addition, this license is required for the ➢*Online Transfer Agent (OTA)* and ➢*Network Failure Protection* (= NFP). One separate license is required for each installed chromatography ➢*Server*.

MS Control: Additional license for a ➢*Mass Spectrometer* on a chromatography server: Enables MS control, digital data acquisition of a three-dimensional data field, and analysis of MS data. The MS Control license is required for spectra and channel acquisition. One license is needed on each chromatography server.

IC Control SE: Special low-cost control license for operating a single DX-120, ICS-90, or ICS-1000 stand-alone ion chromatography system. This license also supports the following modules: AD25, AS50, AS50 (USB), AS, UCI-50, UCI-100, and UI20. In addition, the following drivers are supported: Integrator, Remote Inject, Shared Relays and Inputs, and Virtual Channel.

3-D Data Acquisition   Additional license that enables a chromatography server to acquire digital 3-D data from ➢*Photodiode Array Detectors* and the ICS-3000 Electrochemical Detector (ED). For details, see the Additional Notes at the end of this topic.

 Note:

This license does not enable collection of mass spectrometry data. The MS Control license is required for acquisition and analysis of MS data.

ICS-2000 Gradient Generation   Additional license that enables electrolytic eluent gradient generation with the ICS-2000 Ion Chromatography System.

Fraction Collection   Additional license that enables intelligent fraction collection based on up to two detector signals, with wizard-based program setup and complete fraction reporting.

Purification   Additional license for ➢*Autopurification*. (The Purification license includes the **Fraction** license.) The license supports control of automatic sample purification via the associated ➢*Post-Acquisition Steps*, color-coded sample and fraction assignment, special chromatogram views, and sophisticated fraction collection algorithms with peak shoulder detection. More than two detection channels are allowed.

Control-Only This license is for Chromeleon Xpress only. It enables full instrument control and monitoring, but does not allow storage of data channels.

Client Features

Client License: License for data reprocessing on a network PC *without* control. The license supports multitasking and reports from single- and multiple-point calibration with various fit models, integration, ratio test, user programs, etc.

Server Control: License that enables the Client to connect to local or remote Chromeleon servers for real-time system control and status monitoring. For control across networks, the Chromeleon servers must have the Multiple Network Control license.

Note:

This license is available only with a Client License.

Concurrent Clients: Number of client sessions that can be used concurrently on a PC. A maximum of three Concurrent Clients can be established and licensed on one PC. For a ➤*License Server*, the number of floating licenses allowed on one PC depends on the INI file (see **Hardware Installation** 🌐 **Installing the License Server** in the **Administrator Help** section).

Report Publisher: This license extends Chromeleon's reporting capabilities by enabling use of custom formulas (written in standard Excel syntax) and custom charting. For more information, see ➤*Additional Functions*.

GLP Compliance: This license supports additional capabilities required for ➤*GLP* features:
- ➤*File History*

- ➤*Electronic Signature*

- User Management (see ➤*User Management: User Manager and Security Activation Tool*)

Virtual Column	Additional license options for the ➢ *Virtual Column* IC Separation Simulator tool. The Isocratic license enables modeling of isocratic separations only; the Linear Gradient license enables modeling of isocratic and linear gradient separations. (The Linear Gradient license is sold only in a Virtual Column Complete package that also includes the Isocratic license.)
Xpress mode	Provides a simplified interface for operating and monitoring instruments. Does not provide any data review or data management functions.

➢*SDK* Features

ASAP:	Additional license for the ASAP open-access option (ASAP = Automatic Switching and Processing).
Analyzer:	Additional license for controlling the DX-800 process analyzer.

Additional Notes about the 3-D Data Acquisition License

Each 3-D license applies to one chromatography server. A maximum of one 3-D photodiode array channel and one 3-D amperometry channel can be configured on a timebase. The total number of 3-D data channels one server can acquire simultaneously is limited by the computer performance. (No special client license is required for reviewing and processing acquired 3-D data.)

With photodiode array detectors, 3-D data provides spectral information that can be used for peak purity analysis and compound identification using spectral library matching. With the ICS-3000 ED, the 3-D option enables capabilities such as cyclic voltammetry and post-run adjustment of the waveform integration period. These types of 3-D data can be viewed in wireframe or iso-response projections, and chromatograms can be extracted from the 3-D data set.

For more information about 3-D amperometry data and the ICS-3000 ED, refer to **How to …: 🔖 Analyzing 3-D Amperometry Data**.

▣ Timebase Class 1 License: Supported Devices

☞ *Tip:*

To control the Dionex and/or LC Packings devices a Timebase Class 1 License is required.

Manufacturer (See the **Administrator Help** section.)	Device (Driver Name)
🔵 **Agilent** (formerly **HP**)	5890 Gas Chromatograph
	6850 Gas Chromatograph
	6890 Gas Chromatograph
	7673A GC Autosampler
	7683 GC Autosampler
	1050 Autosampler
	1050 Gradient Pump
	1050 UV VWD Detector
🔵 **CTC Analytics**	CTC PAL Sampler for GC CTC_A200S_Sampler
🔵 **Dionex**	All devices
🔵 **Dostmann**	Thermometer Series P500/P600
🔵 **Fisons**	AS800 Autosampler
	8000 Gas Chromatograph (+ Mega 2 GC)
	A200S: see CTC_A200S_Sampler
🔵 **Isco**	Foxi 200 Fraction Collector
	Foxy Jr. Fraction Collector
LC Packings (see 🔵 **Dionex**)	All devices
🔵 **Perkin Elmer**	Autosystem(XL) Gas Chromatograph
	TurboMatrix Headspace Sampler
🔵 **Rheodyne**	LabPro Valve
	RV/EV Valve
🔵 **Shimadzu**	GC-2010 GC System
🔵 **Shodex**	RI-101/102/104 RI Detector
🔵 **ThermoFinnigan/ ThermoQuest/TSP**	ThermoFinnigan aQA MS
	ThermoFinnigan MSQ MS
	Thermoquest_AS2000
	TQ Trace 2000 Gas Chromatograph
	TSP AS3000/AS3500 Autosampler

Manufacturer (See the **Administrator Help** section.)	Device (Driver Name)
Valco	Multi-Position Valve
	Two-Position Valve
Varian	3400 Gas Chromatograph
	3600 Gas Chromatograph
	3800 Gas Chromatograph
Waters	717 Plus Autosampler

Timebase Class 2 License: Supported Devices

Tip:

A Timebase Class 2 License allows you to control the devices listed below in addition to the devices of a Timebase Class 1 License (including all Dionex and/or LC Packings devices).

Manufacturer (See the **Administrator Help** section.)	Device (Driver Name)
ABI	ABI_785A_UV_VIS_Detector
Agilent (formerly **HP**)	1100 HPLC System
Berthold	Berthold_LB_507_509
Bio-Rad	AS 100 Sampler
CTC Analytics	A200S Autosampler
Jetstream	Jetstream_2_Column_Oven
Knauer	2600 UV Detector
Kontron	360/560 Autosampler
	420 Pump (also for 422 Pump)
	425 Gradient Former
	460 Autosampler
	465 Autosampler
	565 Autosampler
	Pumps 32X and 52X
	UV Detector 430
	UV Detector 432 (also for 332 UV Detector)
	UV Detector 535
	SFM 25 UV Detector
	CO200: seeJetstream_2_Column_Oven

Manufacturer (See the **Administrator Help** section.)	**Device (Driver Name)**
Kratos	Spectroflow 783 UV Detector
Linear Instruments	Linear 205 UV-Det.: see TSP UV2000/UVIS205 Detector
(see **ThermoFinnigan/** **ThermoQuest/TSP)**	Linear 206 UV-Det.: see TSP UV3000 Detector
LKB	2150 Pump
(see **Pharmacia)**	Superrac 2211 Fraction Collector
Nelson/Perkin Elmer	941 Interface (also, for the 901 Interface)
	950/960/970 Interface
Pharmacia	2150 Pump
	Superrac 2211 Fraction Collector
Soma	3710-178 Detector
	3710 Detector (new)
ThermoFinnigan/ **ThermoQuest/TSP**	TSP FL2000 Fluorescence Detector
	TSP FL3000 Fluorescence Detector
	TSP P2000 Pump
	TSP P4000 Pump
	TSP UV1000 Detector
	TSP UV2000/UVIS205 Detector
	TSP UV3000 Detector
	TSP UV6000 PDA
Waters	2487 Detector
	2690/2695 Separation Module
	2790/2795 Separation Module
	996/2996 PDA

▣ Timebase Class 3 License: Supported Devices

☞ *Tip:*

A Timebase Class 3 License allows you to control the devices listed below in addition to the devices of the Timebase Class 1 and Timebase Class 2 Licenses (including all Dionex and/or LC Packings devices).

Manufacturer (See the **Administrator Help** section.	Device (Driver Name)
🔵 **Antec**	Decade ECD
🔵 **CTC Analytics**	CTC PAL Sampler for LC
🔵 **ESA**	CouloChem II ECD
🔵 **Gilson**	116 UV Detector
	117/118 UV Detector
	119 UV Detector
	152 UV Detector
	201/202 Fraction Collector
	202 Fraction Collector (ext.)
	204 Fraction Collector
	206 Fraction Collector
	215 Liquid Handler
	231 Autosampler (also used for the 231A autosampler)
	231XL Autosampler
	232 Bio Autosampler
	234 Autoinjector
	235 Autoinjector
	302 Pump (also for 303 Pump)
	305/306/307 Pump
	333/334 Pump
	Valve Modules: 817, 819, UVSM, and ValveMate
	XL Sampler Series (Extended): 221XL, 222XL, 231XL, 232XL, 233XL, and ASPEC XL
🔵 **Jasco**	980/1580 Pump
	1520_Fluorescence_Detector
	920_Fluorescence_Detector
	970_UV_VIS_Detector
	950/1555_Sampler
	975/1575_UV_VIS_Detector
	970_UV_VIS_Detector

Merck Hitachi

AS2000 Autosampler
AS4000 Autosampler
L4250 Detector
L6200/L6210 Pump
L6250 Pump
L7250 Autosampler
LaChrom HPLC System

Shimadzu

LC 10A HPLC System
LC 2010 HPLC System
SPD-M10Avp/M20A PDA Detector

Varian

ProStar215/218
SD-1 Pump
SD-200/SD-300 Pump

Installing Chromeleon

Usually, Dionex Service or a network administrator installs the Chromeleon software. Therefore, refer to **Software Installation and Communication Software Installation and Communication** in the **Administrator Help** section for a more detailed description of the installation procedure.

Components of a Chromatography System

Components of a Chromatography System

Modern HPLC (high pressure/performance liquid chromatography) or IC (ion chromatography) systems consist of the following components:
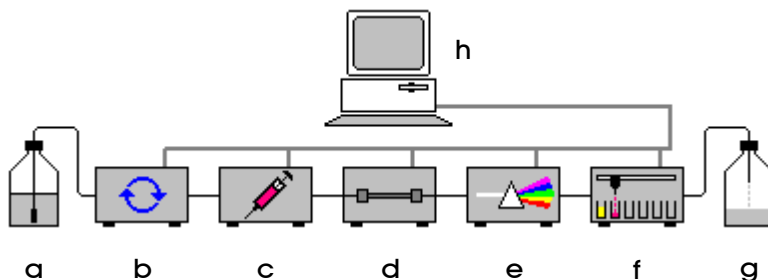
The pump (**b**) draws up to four solvents from one or several reservoirs (**a**), mixes them as defined, and then directs this mixture through the system. The solution of interest (the sample) is injected into this flow via an ➤*Autosampler* (**c**) and separated into its individual fractions or substances on the column (**d**). A thermostatted column compartment may be used to optimize the separation process. When a substance reaches the detector flow cell (**e**), a signal is produced that is proportional to the concentration of the substance. A signal with a profile that corresponds to a Gaussian distribution is referred to as a peak. The exact quantity of each substance can be calculated by determining the peak area and by means of a previously acquired calibration curve (quantitative analysis).

In suppressed conductivity mode IC, a ➤*Suppressor* is installed before the detector. As an option, a fraction collector (**f**) can be installed after the detector to distribute individual substances or fractions to different containers (**g**).

The peak area is determined by the chromatography data system that is installed on a PC (**h**). In addition, the data system

- Controls and monitors all connected chromatography instruments.

- Collects data and displays status messages.

- Enables quantitative and qualitative evaluation of the data, using ➢*Photodiode Array Detectors* or ➢*Mass Spectrometers*.

The chromatography instruments communicate with the computer and the data system via special interfaces, such as the ➢*UCI Universal Chromatography Interface*, or via serial interfaces.

If several PCs are connected via a network, the systems can be controlled from remote locations on the network. In addition, data can be managed centrally and/or retrieved from any workstation.

For more information about the components of a chromatography system, refer to:

🔲 **Chromatography Instruments**

🔲 **The Chromatography Data System**

🔲 **The PC**

🔲 **The Operating System**

🔲 **The Network**

Chromatography Instruments

Chromeleon is especially designed to control and monitor the following Dionex devices:

Eluent Generators	EG, EG40, EG50
Micro Pump:	LPG-3x00
HPLC Pumps	DGP-3600, HPG-3x00, ISO-3100A, LPG-3x00, P680
IC Pumps	AXP, DP, GP40, GP50, GS50, IP20, IP25, IS25, SP
Flow Managers	FLM-3x00
HPLC Autosamplers	ASI-100, WPS-3000, WPS-3000SL (temperature control option available for both)
IC Autosamplers	AS, AS-HV, AS50, AS3500
Autosampler and Fraction Collector	SFM Sample and Fraction Manager
Column Thermostats	AS50 Thermal Compartment, DC, LC25, LC30, TCC-100, TCC-3x00

UV/VIS Detectors	
Single-wavelength	AD20, AD25, VWD-3100
Multiple-wavelength	UVD 170U, VWD-3400
Full-spectrum (PDA)	PDA-100, PDA-3000, UVD-3000, UVD 340U
Electrochemical Detectors	CD, CD20, CD25, CD25A, ED, ED40, ED50, ED50A
Fluorescence Detector	RF2000
Mass Spectrometer	(Thermo Finnigan MSQ (also included in the Dionex APS) and aQa)
Refractive Index Detector	Shodex RI-101
Instrument/PC Interfaces	UI20 Universal Interface, UCI Universal Chromatography Interface
System Modules	DX-120, IC20, IC25, IC25A, ICS-90, ICS-1000, ICS-1500, ICS-2000

The HPLC instruments listed above are part of the ➢*UltiMate 3000*, ➢*Summit*, and ➢*APS* (autopurification) system series. (Appropriate instrument control options may need to be purchased and installed in order to control these instruments.)

Third-party analytical instruments are also supported. See the following examples:

Gas Chromatographs	Such as, the Agilent (or HP) 6890 GC
HPLC Systems	Such as, the Agilent (or HP) 1100 HPLC System
HPLC Modules	Such as, the Waters Alliance 2690 HPLC Module
Radioactivity Detectors	Such as, the Berthold LB507A detector

Appropriate instrument control options may need to be purchased and installed in order to control these instruments.

For more installation information, refer to **Hardware Installation** 🔵 **Installing Dionex Devices** in the **Administrator Help** section.

The Chromatography Data System

The data system is the control center of a modern chromatography system.

Tasks of a Data System

The data system

- Converts user input into time-precise control commands.

- Monitors the state of the connected chromatography instruments.

- Logs all user entries and modifications to the system.

- Saves and archives all data.

- Graphically represents data and allows you to check the system status and system results.

- Allows you to thoroughly check and evaluate data.

Components

- The basis for precise control and key component of the system is an **Operating System** that is capable of performing all operations in real time.

- The components of the chromatography system are installed and configured in the ➢ *Server Configuration Program*.

- **Device Drivers** and other drivers, for example, **Virtual Channel Drivers**, enable communication with the different instruments and device types. (For more information, refer to the related sections in **Special Drivers** in the **Administrator Help** section.)

- Each event (for example, execution of a command or appearance of an error message) is logged in the **Audit Trail**.

- Depending on the required scope of performance, Chromeleon supports various options. (For more information, refer to **Chromeleon (Overview) Chromeleon Licenses**.)

Chromeleon User Interface

- Chromeleon supports all typical Windows properties, such as menu structure, screen elements, and operation. A homogeneous, situation-related menu structure is as evident as the Windows technology, toolbars, and context-sensitive use of the right mouse button. (For more information, refer to **The Client User Interface**.)

- Each user can save and activate a "personal" screen. (For more information, refer to **User Profiles (Workspaces)**.)

- The user can modify the graphical representation of the online control windows to suit his(her) specific needs. (For more information, refer to **Control** **The Control Panel**.)

Operation

- Before starting Chromeleon (see **Starting the Program**), verify that the hardware configuration is correct and that the PC is connected with the system and a datasource.

- In network operation (see **The Network)**, the client PC can control systems and datasources that are not directly connected with the PC.

- Various assistants, the ➢ *Wizards*, facilitate operation of Chromeleon.

The PC

Overview of the minimum PC requirements

	Windows 2000	Windows XP
Service Pack	SP2, 3, or 4 (recommended: **SP4**)	SP1 or 2 (recommended: **SP2**)
CPU	Pentium III 800 MHz	
RAM	256 MB	
Hard Disk	20 GB 40 GB for systems with mass spectrometer, diode array or 3D amperometry detectors	
Display	1024 x 768 x, minimum 256 colors	
Ports	At least one available USB port for license device (dongle)	
Expansion Slots	One PCI slot for each DX-LAN, RS-232, or GPIB card	

For better software performance, Dionex recommends a Pentium 4 with a CPU of at least 1 GHz and 512 MB RAM.

Dionex recommends a higher screen resolution for applications, such as mass spectrometry, in which several windows and control panels are displayed.

The Operating System

Chromeleon supports the following operating systems:

- Windows XP SP1 or SP2

- Windows 2000 SP2, 3, or 4

These operating systems are 32-bit ➢*Multi-Tasking* operating systems, which means that several programs can be executed simultaneously. This is especially important for instrument control, data acquisition, and comfortable printer support.

🖝☞ *Tip:*

For more information, refer to **Software Installation and Communication** 🔲 **Chromeleon and Windows Operating Systems** *in the* **Administrator Help** *section.*

The Network

The network capability of Chromeleon allows you to operate the data system within local and global networks, also referred to as LAN (Local Area Network) or WAN (Wide Area Network).

Chromeleon can be operated either locally in a single-user installation or on a network. This includes data transfer and remote operation via ISDN. The Chromeleon stations can be linked around the world (Wide Area Network).

To use all advantages and possibilities provided by network operation, such as centralized data storage, backup, and administration, shared access to methods and worldwide availability, safe and fast data exchange is essential.

Sometimes, even state-of-the-art networks have difficulty coping with the enormous amount of data. That is why client/server systems provide decisive advantages by specifically selecting transferred data.

Client/Server System

On a decentralized PC (client), ➢*RPC* commands (Remote Procedure Calls) are used to start sub-programs on a central computer (➢*Server* or Chromatography Server). The server performs the actual "work." For example, the server searches for data in a database or runs an application. The client only receives the search result or the status of the application. This means that the client provides the user interface while the actual operation is remotely performed on the server. Chromeleon, too, uses this "division of labor."

After starting Chromeleon on a local PC, the ➢*Client*, the user can perform all server-independent tasks; for example, re-process raw data, create sequences, or search for individual spectra in a library.

If a chromatography ➢*Server* is running, it is also possible to control and monitor the chromatography instruments connected to this server. Theoretically, each client connected to a running server via a ➢*Control Panel* can do this. In practice, only the first client is allowed to control the instruments connected to the timebase. All other Chromeleon users can only monitor the system status.

Via device drivers, the server converts the control commands entered on the first client PC for the analytical instruments. Inversely, the server receives information from the system and forwards it to the appropriate locations. Thus, status information, such as the current flow rate, appears on all client PCs connected with this chromatography system. Raw data is automatically stored in the directory of a ➢*Datasource* and the underlying database.

The installation type determines whether the client and the server are located on the same PC (local client/server installation) or on different PCs in a network (network installation). For more information, refer to:

🔲 **Local Client/Server Installation**
🔲 **Network Installation**

▪ Local Client/Server Installation

The ➢*Client* and the (Chromatography) ➢*Server* are located on the same PC. They must be started separately.

The controlled instruments in the chromatography system can be connected with the PC, e.g., via RS-232 ports, a ➢*DX-LAN*, or a USB (Universal Serial Bus) connection. Additional interface cards can supply a sufficient number of ports. Each chromatography server can control a maximum of six controlled ➢*Timebases* (chromatography systems).

The data is saved on the local PC. During initial installation of Chromeleon, a local datasource is installed on each local computer.

If the PC is part of a local network (LAN = **L**ocal **A**rea **N**etwork or WAN = **W**ide **A**rea **N**etwork), data can also be saved externally. In the same way, external data can be used for data editing.
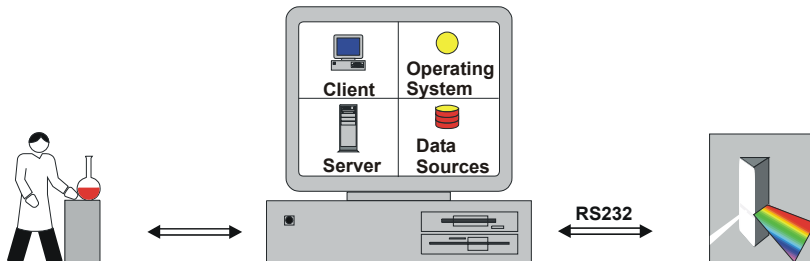
If the local PC is connected to a network, all options of a ▪ **Network Installation** are available.

▪ Network Installation

The ➢*Client*, the Chromatography ➢*Server*, and the ➢*Datasource* can be installed on different computers. They are connected via the network and the corresponding network server. Each chromatography server can operate up to six chromatography systems (➢*Timebases*).

The client, server, and datasource are independent units on the network.
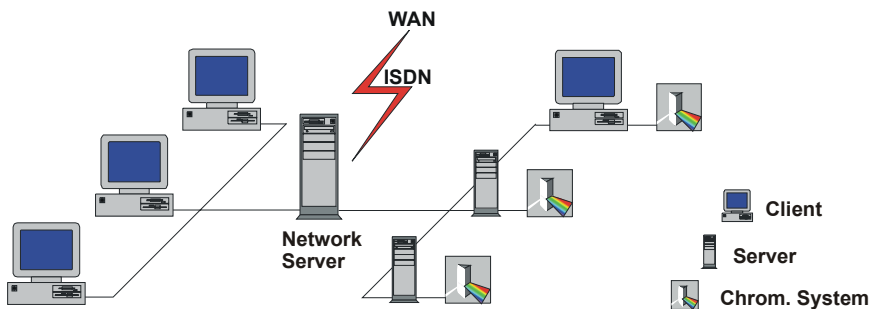
On each PC, a server, client, and local datasource are set up during the installation of Chromeleon. Independently of this, each PC can act as "server only" or "client only." Theoretically, each client can access each datasource and each server. In practice, this may not be desirable for safety reasons. Therefore, various options are available to restrict user access:

- The ➢*Server Configuration* program allows you to define the extent of network operation for each ➢*Server* and each timebase. A server can be made available for the entire network or for certain ➢*Access Groups* only. The server can be locked either partly or completely. If the server is partly locked, for example, it is still possible to monitor the server, but control of the connected instruments is disabled. In this case, control is reserved for the local client. This distinction also applies to the timebases. If three timebases (TIME1, TIME2, and TIME3) are installed on a server, TIME1 could be completely shared, TIME2 could be excluded from network operation, and TIME3 could be shared for monitoring only.

- Datasources and/or their subdirectories can also be protected from undesired access. Depending on the location of the corresponding database (on the local hard disk or a network PC), the respective user or the network administrator decides (by "sharing" a directory) which data can be accessed and by whom. In addition, datasources shared in Windows can be locked in Chromeleon.

- In addition, access can be restricted via ➢*Passwords* or the Chromeleon User Management. If the administrator has enabled access control (in the Security Activation Tool (CmSecure program)), the user must enter a password before being allowed to perform specific operations. The system administrator determines these operations in the User Manager (CmUser program). In this way, the administrator can deny controlling rights or prohibit "locking" of datasources.

- Besides, access is not possible if the appropriate license is missing. For example, if a server does not have a **Multiple Network Control** license and/or if the client does not have a **Server Control** license, the server cannot be controlled on the network.

Important data are often stored on central data server PCs. If, during data acquisition, the network connection is interrupted or the data server PC crashes, data acquisition should continue, nevertheless. All data that are relevant for the Chromeleon server are locally stored on the server's hard disk, thus ensuring that data acquisition will not be interrupted in case of a network failure (see ➢*Network Failure Protection*).

In addition to allowing data exchange within a local area on a Windows, Novell, DEC, or UNIX network (LAN), it is possible to transfer data across huge distances (WAN) via ISDN. The basic requirement for any type of network operation is the availability of the corresponding network drivers for Windows 2000/XP.

☞ *Tip:*

*The **Administrator Help** section provides more information; refer to **How to …: Working with Files, Databases, and Networks:***

🔼 **Sharing the Local Datasource on the Network**

🔼 **Creating a Network Datasource**

🔼 **Saving Chromatography Data on the Network**

Basic Operation

Operation via the Keyboard

All commands and menu options are accessible from the keyboard. Press the **ALT** key to display the underlined access letters. To open a menu from the keyboard, press **ALT** and the underlined letter. To select a command or option on the menu, press another underlined letter. Alternatively, use the arrow keys to move to an option and then confirm your selection by pressing the **Enter** key.

Example: To left align several objects on a control panel, select **Align** on the **Edit** menu, and then select **Left**. Alternatively, hold down the **ALT** key and press **A+L**. Else, press **ALT**+**E**, and then select an option, using the ↓ and ➜ arrow keys. Confirm your selection by pressing <Enter>.

Important commands can also be accessed via shortcuts. The shortcuts are displayed next to commands and menu options. Enter the key combination to perform the corresponding function directly.

Example: Hold down the **Control** key and then press **C** (CTRL+C) to copy a selected item.

For a list of available shortcuts, refer to **Operation** **Quick Access Keys (Shortcuts)**.

Quick Access Keys (Shortcut Keys)

Quick access keys and/or shortcut keys are provided for many operations, especially in online control:

	Action	Where	Description
General	ESC or right-click		Aborts the drag/move action.
	F1		Opens the context-sensitive Help;
	F2		Enables the Edit mode.
	F3		After the Find command: Find Next.
	F4	Chromatogram	Takes you to the next sample.
	SHIFT+F4		Takes you to the previous sample.
	Alt+F4		Exits Chromeleon.
	F5		Updates all windows.
	F6		Takes you to the next partial window.
	SHIFT+F6		Takes you to the previous partial window.
	F10		Takes you to the next channel.
	SHIFT+F10		Takes you to the previous channel.
	F7	Browser (F7 key only) +	Optimizes the column width.
	SHIFT+F7	QNT Editor	Optimizes the line height.
	F8		Opens an edit dialog box for the respective field.
	F10 or ALT		Shows the hotkey underlines.
	SHIFT+F5	Browser	Displays the chromatogram.
	ALT+ENTER	Browser + ➢*Control Panels*	Opens the associated **Properties**.
	CTRL+TAB		Toggles between open windows.
	CTRL	Signal plot in the ➢*Report* and in the QNT Editor	Allows zooming (the cursor becomes a zoom cursor).

	Action	Where	Description
	CTRL+N		Creates a new file.
	CTRL+O		Opens the file.
	CTRL+S		Saves the file.
	CTRL+R		Opens the batch report.
	CTRL+P	Report + ➤*Printer* *Layout*	Prints the selected object(s).
Edit	CTRL+Z		Undoes the previous action.
	CTRL+Y		Repeats the previous action.
	CTRL+X		Cuts the selected object(s).
	CTRL+C		Copies the selected object(s).
	CTRL+V		Pastes the selected object(s).
	CTRL+F		Finds a string of characters.
	F3		Finds the next string of characters.
	CTRL+H		Replaces the entry in the field.
	F9		Fills the column and/or selected cell(s) with the first value of the selection.
	INSERT		Inserts the selected object(s).
	DELETE		Deletes the selected object(s).
Sample List	CTRL+I		Inserts a sample.
	CTRL+D		Deletes a sample.
	CTRL+A		Adds a sample.
Control	CTRL+F		Changes the pump's settings
	CTRL+I		Injects
	CTRL+Break		Stops the pump.
	Break		Turns on the Hold mode.
	Ctrl+A		Turns on data acquisition.
	Ctrl+B		Edits the batch.
Signal plot	Double-click...	- Overview window	Unzooms.
		- Time axis	Auto Plot Speed
		- Signal axis	Autoscale
		- Plot range	Signals...
		(- or else:…)	Axis/decoration
	SHIFT	- when zooming	Retains the scale ratio between signal and value axis. (The shape of chromatograms is maintained.)

	Action	Where	Description
Gauge/Slider	CTRL key	Press when dragging	Toggles the **Snap To Scale** option.
Script Button	Click	Button	Indicates whether the program is still running. Stops the program upon confirmation.
Edit Field	TAB/ENTER		Sends the new value.
	ESC		Aborts the input.
Layout Mode	ALT + Click	On the selected panel	Draws a selection frame on the control panel.
	ALT + Drag		Temporarily toggles the **Snap To Grid** option.
	ESC		Deselects all.
	ARROW keys		Drags the selection pixel by pixel.
	SHIFT + ARROW keys		Increases/reduces the selection pixel by pixel.
	SHIFT+SELECT		Extends the selection.
	CTRL+SELECT		Highlights the selected control (the highlighted control is used in Align..).
	CTRL+Drag		Copies controls.
	Double-clicking	On the online signal plot on the control panel	Opens the **Properties** dialog box.

Especially in the chromatogram, many additional keys and key combinations are available. For more information, refer to **How to …: Working with Chromatograms** **Using Keyboard Keys in the Chromatogram**.

Operation via the Mouse

Chromeleon supports all Windows-typical properties and capabilities of a two-button mouse.

The **left mouse button** allows you to select menus, commands, and icons. It also allows you to operate display and control elements and to modify windows (increase or reduce the window size, zoom, modify display and control elements, etc). Double-click the left mouse button (= left-click) to execute the selected function.

Hold down the SHIFT key and left-click to select several cells in a table. Left-click in the first cell, hold down the SHIFT key, and then select a new cell. All cells between these two cells are selected.

Hold down the CTRL key and left-click to select non-adjacent cells in a table. Left-click in the first cell, hold down the CTRL key, and then select a new cell. Hold down the CTRL key and repeat left-clicking until all required cells are selected.

Left-click an object and drag it to the desired position (**Drag and Drop**). This action allows you to move selected text or samples. To copy the corresponding object, hold down the CTRL key while performing the Drag and Drop operation.

The **right mouse button** (= right-click) allows you to open context-sensitive menus. Context-sensitive menus provide functions that are required or allowed in the current situation.

For more information, refer to your Windows manual.

Undo and Redo Commands

Chromeleon "remembers" the user's last modification. Select **Undo** on the **Edit** menu to cancel the last operation. Select **Redo** to cancel the Undo operation.

Starting the Program

Chromeleon runs on client PCs with Windows 2000 or Windows XP operating systems. To start Chromeleon:

- Click **Start** to open the **Start** menu. Point to **Programs** and wait until the submenus appear.

- Point to **Chromeleon**. Another submenu appears:
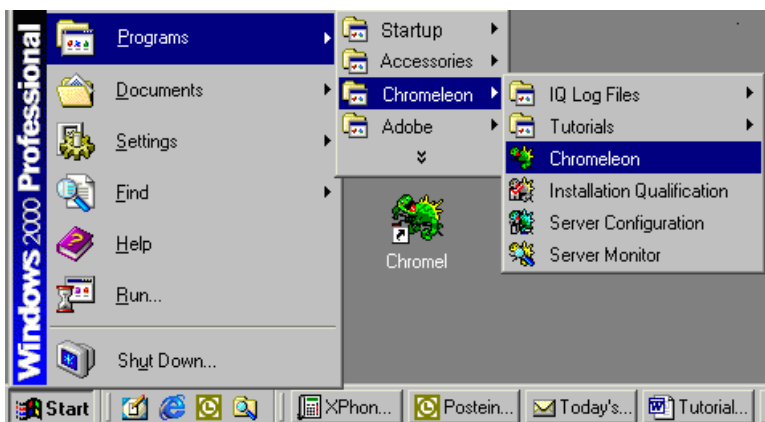
Client

- Click **Chromeleon** to start the Chromeleon software so that you can begin with data evaluation.

- If Chromeleon has been installed with integrated access control, you must enter your user name and password for identification. If you have any questions regarding system access, please contact your system administrator.

☞ *Tip:*

To control a chromatography system with Chromeleon, you must also start the Chromeleon server.

Server (Server Monitor)

🖐☞ *Tip:*

During installation, Chromeleon usually includes a link to the Server Monitor program in the Autostart group. Thus, the program is started when you start your computer. The Server Monitor icon appears on the taskbar.

- If the icon does not appear on the taskbar, click **Start**, point to **Programs** > **Chromeleon**, and select **Server Monitor** to start the ➢*Server Monitor Program*. The Chromeleon icon will appear next to the Windows system clock on the Windows taskbar.

- Point to the icon. The **Chromeleon Server is not running** quick info message appears.

- Select **Start Server** on the context menu (or double-click the icon and then click **Start**). The color of the icon indicates the status of the server monitor. Gray coloring indicates that the server is running (the quick-info message reads: **Chromeleon Server is running idle**). You can now open a control panel and directly access the devices installed in the selected timebase. If the devices are installed correctly, you can operate them online.

🖐☞ *Tip:*

Any problems occurring when controlling single instruments may be caused by an incorrect server configuration. Start the ➢Server Configuration program to check the configuration.

The Client User Interface

The Chromeleon user interface supports all known Windows elements, such as menu bar, ➢*Toolbar*, and ➢*Status Bar*.
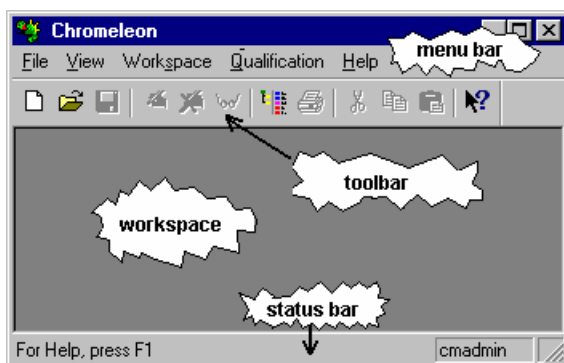
To display the toolbars listed below, select them on the **View > Toolbars** menu:

Standard Toolbar for various standard functions

Online Tools for using the control panel

Layout Tools for designing the control panel

Method Tools for selecting a method window

Integration Tools for the most important operations in the **Chromatogram**

For descriptive information about a toolbar, point to the related icon.

The space between the task bar and the status bar represents the workspace, allowing you to display the different **Windows** supported by Chromeleon.

The Windows

The appearance of the different windows can vary considerably. A window can have various window sections (panes) whose borders can be moved (a). If the window section is too small to display the entire information, use the scroll arrows or scroll bars to view the hidden area (b).
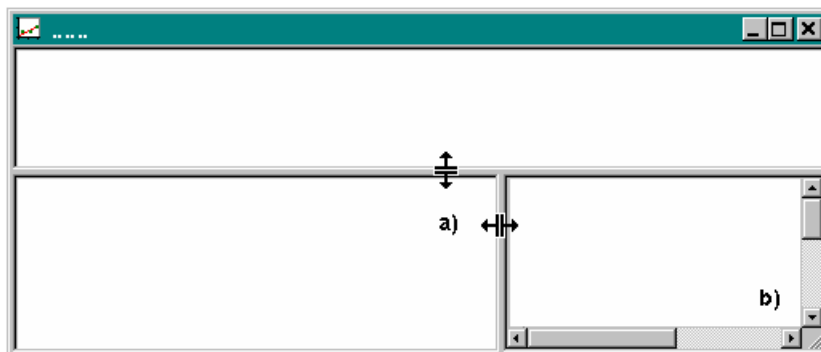
The window content depends on the type of the represented data. It is also possible to integrate table editors as a window section in a method window. For window sections with graphical representation, the coordinates of the mouse pointer appear on the status bar.

Window Types

In addition to the Browser and the control panels, Chromeleon supports the following method windows:

- QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor**)

- Integration

- ➢*Peak-Purity-Analysis (PPA)*

- ➢*3D Amperometry*

- ➢*Printer Layout*

- ➢*Signed Results*

- ➢*Virtual Column*

Only one method window is enabled by default. To use several method windows simultaneously, select **Preferences** on the **File** menu of the Browser. On the **Browser** tab page, clear the **Open only one method window** check box. This allows you to open each window any number of times.

The Table Editor

Table editors serve to enter and represent various parameters and variables. For example, one table editor is available in the Browser. This editor allows you to list or edit single samples or sample data, or to display numerical results in a report. It also serves to determine integration, calibration, and peak table parameters.

- Appearance and use of all tables correspond to the Windows standard. Editing is by cell, column, or line.

- It is possible to move or hide selected columns and to make them visible again at the active cursor position. Adjust the column width by moving the left or right delimiter. Or else, select **Optimum column width,** e.g., on the **View** menu in the Browser.

- You can add additional lines or remove selected lines anywhere in the table.

- Most of the described functions are available on the context menu.

- You can change the font size. For example, select a smaller size to display more on-screen information.

- Press the F1 key for online Help information about a specific column.

- Press the F8 key to open an edit dialog box for a specific field. This prevents input of incorrect or invalid values and names.

- Press the F9 key to fill an entire column with the value of the current field. In this way, you can also extend logical rows of numbers. For example, if the input in the first three fields is 2, 4, and 6, the row is continued with 8, 10, 12, etc.

Working with Several Windows

Chromeleon not only supports working efficiently with several open windows, as is typical in Windows programs, but it also introduces what has become possible with object-oriented programming of applications and was not common before:

Data representation is always updated in all windows.

For the examples below, the sample chromatogram was integrated and analyzed, using specific detection parameters.

Example 1: If you correct the ⇒*Minimum Area* detection parameter in the QNT Method by entering a smaller value, the related chromatogram in the Integration window is immediately updated. Peaks with an area smaller than the minimum area are not considered for integration.

Example 2: Similarly, the result of a baseline that has been modified manually is displayed immediately in the Integration report window.

User Profiles (Workspaces)

Chromeleon allows you to save the window arrangement of any work situation, i.e., a combination of the different windows, in a workspace.

This facilitates opening single files or windows and allows you to work in whatever work environment you prefer.

The information about the windows is stored in a WSP file. If you want to use a specific workspace when you start working, open the corresponding WSP file, or generate a new WSP file by storing the screen contents. The following commands are available on the **Workspace** menu:

- Select **Open Workspace** to open an existing workspace.

- Select **Save Workspace** to save the current workspace.

- Select **Save Workspace as** to save the current workspace with a new name.

- Select **Autosave Workspace** to always save the most recent workspace as the default workspace.

There is no restriction as to the number of windows that can be saved with each workspace. (Also, see **Basic Operation** **The Windows**.)

A useful workspace arrangement might combine, for example, the report, a control panel, and the Browser:

The appearance of each window is stored in the related file. For example, the appearance of the on-screen report and the Printer Layout is stored in the 🔳 **Report Definition** file.

☞ *Tip:*

When Chromeleon is started, the most recently used workspace is loaded.

Report Definitions

The ➢*Report Definition File (RDF)* comprises the current settings, such as, the names and scaling of axes, representation of chromatograms and spectra, display of additional information or auxiliary lines, font settings, styles, and sizes, as well as the column arrangement in a table. The report definition also determines how the screen contents (hardcopy) or online batch results are printed. (For more information, refer to **Basic Operation** 🔳 **Printing**.)

Unlike a workspace, a report definition can be used for individual windows. If no workspace is loaded, each new window is opened based on the most recently used report definition. If you have not yet stored a report definition file, the default Chromeleon RDF is used. The default report definition file (DEFAULT.RDF) is available in the **Report** directory.

- Select **Save Report Definition** on either the context or **View** menu to save the current settings.

- Select **Load Report Definition** on either the context or **View** menu to open a previously saved Report Definition File.

Create or Open Files, Windows, and Templates

There are several ways to open or create files, windows, and templates. Frequently, additional information is required, also.

For example, when you open a control panel, the control panel searches "its" timebase, i.e., the correct link between the client PC and a certain chromatography system (➤*Timebase*) and each method window searches data from a specific ➤*Datasource*.

Tips:

If problems arise, they mainly occur because timebases or datasources were renamed that were correctly installed before by Dionex Service.

Similar problems may occur if databases are located on a network PC to which you currently cannot connect or for which you do not have share authorization.

In this case, refer to **How to …: Working with Files, Databases, and Networks** *Connecting a Datasource* *in the* **Administrator Help** *section.*

Automatically Loading the Most Recent User Profile

Chromeleon automatically loads the most recently used workspace. If this is not possible, Chromeleon opens the Browser.

Opening Existing File Types and Windows

Select **Open** on the **File** menu, and then select the datasource and the directory containing the file to be opened and the file type. Refer to the online Help for more information about this dialog box.

Or else, double-click to open a file in the ➢*Browser*.

Open Most Recent Files and Templates

The lower section of the **File** menu and the **Workspace** menu list the most recently used templates and files. Click to open a template or file. This is the simplest and quickest way to continue an interrupted task.

New

Select **New** on the **File** menu to receive a list of all possible file types and chromatographic operations.

- Select **Control Panel** to open a window from which you can control the chromatography devices of a specific timebase.

- Select **Sequence File (using Wizard)** to start the 🔺 **Sequence Wizard**. The wizard guides you through the creation of a ➢*Sequence*.

- Select **Sequence (from LIMS Worklist)** to include the data and ➢*Sequence* of a ➢*LIMS* in the ➢*Worklist* format.

- Select **Program File** to start the 🔺 **Program Wizard**. The wizard guides through the creation of a 🔺 **Control Program** (PGM File). A ➢*PGM File* includes all ⇒*Control Commands* that must be communicated to the different chromatography devices so that they can process a sample or a series of samples.

- Select **Method File** to create a new quantification method.

- Select **Spectra Library** to create a new spectra library.

Printing

The Chromeleon ➤*Printer Layout* provides numerous options for the presentation of the results. Similar to a word processor, you can choose between printing the on-screen contents and printing based on defined templates.

Printing from the Browser

If you have selected one or several samples or sequences in the Browser, you can select **Batch Report** on the **File** menu to start the printout. Use the **Batch Report** dialog box to determine which ➤*Report Definition File (RDF)* is used, which pages of the printer layout are printed, and for which sample type and channel the printout is performed. The single pages are created in the Printer Layout. (See **Data Representation and Reprocessing** 🔲 **The Printer Layout** for more information.)

🔲 *Note:*

*Click **Setup** in the Printer Layout to define the page format. (For more information, refer to **How to …: Preparing the Printout** 🔲 **Changing the Page Format**.)*

Printing from an Online Batch

If several samples are processed in an automatic sample batch (➤*Batch Processing*), you can determine which pages of the Printer Layout are printed. Printing can start either immediately after a sample has been processed or after the entire sequence has been processed.

🔲 *Note:*

The report template used for printing is stored with other settings (such as screen settings) in the Report Definition File (RDF). Future Chromeleon versions will save the screen and the print settings separately.

Printing the Screen Contents

To print the contents of the active window, select the related **Print** command. Select **Print Sequence** to print the current sample list from the Browser, with either the corresponding PGM File or QNT File. If a ➢*PGM File* is open, you can print the required views by selecting the **Print** command. In the same way, you can print the selected pages of a ➢*Quantification Method (QNT Method)* from the QNT Editor by selecting **Print QNT Method**.

Control

Control (Overview)

Chromeleon supports controlling instruments from different HPLC, IC, and GC device manufacturers. The prerequisite is that the instrument to be controlled is installed and configured in Chromeleon. For more information, refer to **Control Requirements**.

In principle, there are two ways a sample is analyzed:

1. Automatic analysis of many samples in a sequence
2. Manual analysis of single samples

Automatically analyzing many samples in a sequence

If you want to process many samples one after the other, you have to determine in a **Control Program** how each sample is to be analyzed.

First, use the **SmartStartup Wizard** to create an equilibration program for preparing a Dionex Summit system for sample analysis.

The **Program Wizard** assists you in creating typical control programs. If you want to edit the program later, use the **PGM Editor**.

In addition, you have to determine in a ➢*Sequence* in which order the samples are analyzed and which program is used. For more information, refer to **The Sample List (Sequence)**.

Manually analyzing single samples

To create a new chromatographic method, you can control the instrument directly from the ➢*Control Panel*, which means that individual devices and device functions are controlled directly and interactively from the control panel. (Also, see **Basic Operation** **The Control Panel**.)

Control Requirements

To control devices, make sure that

- The appropriate 🔝 **Chromeleon License** is available.

- The controlled instruments are correctly installed and configured in the ➤*Server Configuration* program.

  ### 👉 *Tips:*

  *Dionex Service or a network administrator usually performs installation. The **Administrator Help** section provides more information; refer to **Hardware Installation:***

  🔵 *Installing Dionex Devices*

  🔵 *Installing and Controlling Third Party Devices*

- A connection exists between the PC and the ➤*Chromatography Server*.

- A connection has been established between the chromatography server and the chromatography system via a serial interface, a DX-LAN, or any other interface (TCP/IP, GPIB, USB). (For more information, refer to **Software Installation, Communication, and Validation** 🔝 **The Serial (RS-232) Interface** and/or 🔝 **The DX-LAN** in the **Administrator Help** section.)

The Control Program

Modern analytical laboratories usually analyze many samples. These samples are grouped in ➤*Sequences* and processed with a chromatographic control ➤*Program*. The control program is part of the PGM File. When creating a program, you include a list of commands and the times when the commands will be executed (relative to the time of injection). Chromeleon automatically adds the ⇒*Inject* and ⇒*End* commands. The ➤*Program* is displayed in the **Commands** view of the PGM Editor:

📖☞ *Tip:*

*Dionex recommends always editing programs in the associated **Device** view. The **Device** view is easy to use and ensures correct command syntax. Use the **Commands** view only if the desired parameters are unavailable in the **Device** view.*

When starting the program, all commands are executed precisely at the defined time.

For more information, refer to:

The SmartStartup Wizard

The Control Program (Details)

The Program Wizard

The PGM Editor

The Program Syntax

The SmartStartup Wizard

Dionex recommends that you equilibrate the chromatography system with a special equilibration program before starting sample analysis. The ➢*SmartStartup* Wizard assists you in creating such a program for the ➢*Summit* and ➢*UltiMate 3000* (excluding nano and capillary modules) HPLC systems and allows you to define the necessary equilibration steps. You may extract these steps from an existing program or use the current instrument settings, instead. The wizard includes the following steps:

Step 1: Select a ➢*Timebase* (if no timebase is selected in the current window)

Step 2: Extract the equilibration conditions

Step 3: Edit the equilibration conditions (optional)

Step 4: Start equilibration

After you exit the wizard, the following events occur:

- Chromeleon generates an equilibration program and sample.

- A ➢*Control Panel* opens. The control panel, which contains one stripe for each device, displays the status of the equilibration.

- The **Start Batch on …?** dialog box opens. The equilibration sequence appears at the top of the ➢*Batch* list.

- When you start the batch, system equilibration begins. When the equilibration is successfully completed, Chromeleon begins processing any other sequences in the batch.

However, if a batch is currently running, the equilibration sequence cannot run immediately, and thus the equilibration panel is not opened automatically. Instead, the following events occur:

- Chromeleon adds the equilibration sequence at the end of the batch list.

- After you have created the equilibration sequence, the **Delay Equilibration Sequence?** dialog box opens. Specify the date and time for the equilibration procedure.

For more information about how to equilibrate the Dionex chromatography system with Chromeleon, refer to **How to ...:** **Equilibrating the Chromatography System**.

The SmartShutdown Wizard

Chromeleon provides a shutdown and standby process to automatically shut down the chromatography system or set it to standby mode. The ➢*SmartShutdown* Wizard is an easy and intuitive way to create the shutdown or standby program for an ➢*UltiMate 3000* or ➢*Summit* HPLC system (excluding nano capillary modules).

A shutdown process shuts down the chromatography system by stopping the flow automatically and turning off the detector lamps. In addition, it turns off the column thermostat's temperature.

The standby mode allows you to quickly re-activate your chromatography system from standby. A typical standby process comprises steps such as reducing the flow and turning off the column thermostat's temperature. In combination with the ➢*SmartStartup* process and an ➢*UltiMate 3000 x2 Dual-Gradient System*, the standby process enables you to quickly prepare the instrument for a different method of analysis.

Both processes maintain the equilibrated status of an instrument for a later time-controlled system restart.

The *SmartShutdown Wizard* assist you in creating the shutdown or standby program step by step and helps you to automate repeated routines and prevent typical errors, such as stopping the flow while a buffer is in the mobile phase or interrupting the flow at high column temperatures, which may damage the column.

The wizard comprises the following steps:

Step 1: Selecting the ⊳*Timebase* (if no timebase is selected in the active window)

Step 2: Selecting the mode

Step 3: Determining the device settings

Step 4: Finishing the shutdown or standby program

When the wizard was completed, the PGM file is added to the end of the batch list and, if not already open, the **Start Batch** dialog box opens (or the **Batch edit** dialog box if a sequence is running).

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to **How to ...:** 🔲 **Shutting down the Chromatography System (SmartShutdown)**.

For an overview of how to equilibrate the HPLC system with SmartStartup, refer to **How to ...:** 🔲 **Equilibrating the Chromatography System (SmartStartup)**.

🔲 The Control Program (Details)

The control program, which is often referred to as the ⊳*Program* or PGM File, includes the user's list of time-precise ⇒*Control Commands*. The purpose of a control program is the automatic and repeated execution of specific routine tasks, such as processing samples automatically or conditioning a column by rinsing with various solvents. In addition, the control program allows monitoring certain parameters or limits, or triggering reactions when these limits are exceeded.

A ⊳*Wizard* (see 🔲 **Program Wizard**) assists you in creating a program by automatically converting your entries into the appropriate Chromeleon program commands. In this way, you can create a program even if you do not know the command syntax.

To edit an existing program, double-click the program name in the Browser to open the program in the most recently used view of the PGM Editor (see **Control** 🔲 **The PGM Editor**).

To edit the control settings for a device, click the associated device icon on the left pane. This view provides the corresponding pages of the **Program Wizard**. Enter new parameters or change existing parameters according to your requirements.

Tip:

*Dionex recommends always editing programs in the associated **Device** view. The **Device** view is easy to use and ensures correct command syntax. Use the **Commands** view only if the desired parameters are unavailable in the device view.*

Tip:

*Similar to a control panel, you can edit a PGM File only if it is connected with a ➢Timebase while the server is running. Therefore, verify that the server is running. Start the server in the Chromeleon ➢Monitor Program if necessary. To connect the PGM File with a timebase, select **Connect to Timebase** on the **Control** menu.*

*If the PGM File is correctly connected with a timebase, icons for all devices in that timebase are provided, allowing you to access the associated **PGM Wizard** pages.*

For more information about how to create a program, refer to **The Program Wizard**.

For information about how to edit a program with the PGM Editor, refer to **The PGM Editor**.

For practical tips, refer to:

How to …: Creating and Modifying Programs Creating a Program

Device Control Practical Tips for Device Control (Overview)

The Program Wizard

The **Program Wizard** guides you through ➢*Program* creation. To start the Program Wizard, select **New** on the **File** menu, and then select **Program File**.

The Program Wizard systematically collects all information required to generate a basic program. Each step provides a template, allowing you to enter or select data. It depends on the installation which steps are required.

For a typical HPLC or IC timebase, the Program Wizard includes the following steps:

Step 1: Select a ➢*Timebase*

Step 2: Select the tandem program (if a tandem timebase is installed)

Step 3: Select the temperature settings (if supported)

Step 4: Define a flow system

Step 5: Determine a gradient or ramp profile (optional)

Step 6: Define the tandem operation (if a tandem timebase is installed)

Step 7: Enter the autosampler settings (if supported)

Step 8: Determine channels and the duration of data acquisition

Step 9: Determine signal parameters for the individual channels

Step 10: Define the peak detection parameters for fraction collection (optional)

Step 11: Complete the Program Wizard

Chromeleon completes the information entered in steps 1 through 10 by adding the ⇒*Inject* and ⇒*End* commands, thus creating an operable program. The program is displayed in the **Commands** view of the PGM Editor. (For more information about the PGM Editor, refer to 🔊 **The PGM Editor**.)

To edit the basic program, use the 🔊 **Device Views**.
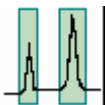
The PGM Editor (Overview)

The PGM Editor allows you to edit the control programs (➤*PGM Files*). To open the different views of the PGM Editor, click the icons on the left pane (the shortcut bar):

View*	Icon	Description
Commands		Displays the program (see **The Commands View**)
Post-acquisition steps		Determine the steps for data reprocessing (see **The Post-Acquisition Steps View**)

If the respective instrument is installed, the associated icon is available:

Surveyor MSQ *or* **Finnigan AQA**		Enter or edit MSQ or AQA parameters (see **Surveyor MSQ or Finnigan AQA Views**)
ColumnOven		Enter or edit thermostat or GC oven parameters (see **Device Views**)
Pump		Enter or edit pump parameters (see **Device Views**)
x2 Tandem Operations		Enter or edit ➤*Tandem Operation* parameters (see Device Views)
GC		Enter or edit GC parameters (see **Device Views**)
Sampler		Enter or edit autosampler or GC injector parameters (see **Device Views**)

View*	Icon	Description
UV		Enter or edit UV or GC detector parameters (see **Device Views**)
RI		Enter or edit refractive index detector parameters (see **Device Views**)
Emission		Enter or edit fluorescence detector parameters (see **Device Views**)
Fraction Collection (FCA_Multi)		Enter or edit fraction collection parameters (see **How to ...: Collecting Fractions**).
Relay and State Devices		Enter or edit relay and condition parameters (see **Device Views**)

* *Note:*

The Device Name used in the Server Configuration program determines the Device Views name. For example, if you have named your pump HPLC Pump, the Device View is named HPLC Pump, also. (It is not named Pump, as stated in the above table.) The names in the table refer to the default names for Dionex devices, mass spectrometers, and GCs in the Server Configuration program.

The Device Views

The different device views in the **PGM Editor** provide a user-friendly way to edit an existing program at any time according to your requirements.

The device views contain the related pages of **The Program Wizard**. To modify existing commands or to enter new commands for a device, click the device icon on the left pane. In the device view, the respective page of the Program Wizard is re-opened.

Neither the device view nor the Program Wizard supports some of the special commands. Only use the **Commands** view to define these commands. For more information, refer to **The Commands View**.

☞ *Tip:*

*Dionex recommends always editing programs in the associated **Device** view. The **Device** view is easy to use and ensures correct command syntax. Use the **Commands** view only if the desired parameters are unavailable in the device view.*

▪ **The Surveyor MSQ or Finnigan AQA Views**

These views of the ▪ **PGM Editor** are part of ➢*Xcalibur*. They allow you to specify the method used by the Surveyor MSQ or aQa mass spectrometer.

AQA: On the **Ionization Mode** tab page, specify the mode of ionization (Electrospray/APCI). On the **Analysis** tab page, specify the sensitivity and fine-tune the mass spectrometer.

⚠ *Caution:*

*When using the Xcalibur method editor, disregard the **Other detectors** section and perform data acquisition as usual. Do not use this section for data acquisition with other detectors (such as the UV detector)!*

On the **Acquisition** tab page, set the aQa-specific signal parameters for ➢*Mass Spectra* acquisition.

Surveyor MSQ: The view displays the selected chromatogram, if available. Specify the **Ionization Mode** and, in the **Full/SIM Scan Events** section, specify the device parameters for mass spectra acquisition.

☞ *Tip:*

*These views of the PGM Editor are part of the Xcalibur program. The Xcalibur Help system provides detailed information about mass spectra acquisition. If you use an aQa mass spectrometer, open the Xcalibur Help system via the **Help** menu or by clicking **Help**. If you use the MSQ, first click the question mark (at the top right) and then click the option of interest to open the corresponding Help topic.*

For more information about how to create an MS method, refer to **How to …: Using Mass Spectrometers:**

▪ **Creating a PGM File for the aQa MS** and/or

▪ **Creating a PGM File for the MSQ**

◼ The Commands View

The **Commands** view in the ⬛ **PGM Editor** shows the actual program, listing the various commands in chronological order. To facilitate orientation within a program, control commands are in **black**, comments in **green**, and ⇒*Triggers* and ⇒*If Constructs* in **blue**.

🖝 *Tip:*

*Dionex recommends always editing programs in the associated **Device** view (see **Control/The PGM Editor** ⬛ **The Device Views**). The Device view is easy to use and ensures correct command syntax. Use the **Commands** view only if the desired parameters are unavailable in the **Device** view.*

A **control command** starts with the time at which the command is executed. The time appears in ➤*Decimal Minutes*, i.e., "2.500" means "2 minutes, 30 seconds". The actual program instruction, such as ⇒*AcqOn/Off*, appears after the time.

📝 *Note:*

*You can integrate control commands into a program or perform them separately either from the **Online** toolbar or via the script buttons on the control panels.*

If the program instruction requires **parameter** input, enter the parameter(s) next in the text line. Use a point ("**.**") to separate the parameter from the instruction. If several parameters are required, specify them in the following lines. Each parameter is identified by its parameter name, followed by "=" and the parameter value. Example:

```
2.500 Pressure.LowerLimit =  10
      Pressure.UpperLimit =  250
```

You may include any number of **comment lines** between the instruction lines. Start each comment line with a semicolon.

📋 *Note:*

*If you want to edit your program later in the **Device** views, Dionex recommends entering all comments right at the beginning of the program. In some cases, Chromeleon cannot assign comments to the associated commands when re-sorting. Thus, it may happen that some comments appear at the wrong position after you have edited the program in the **Device** views.*

👉 *Tip:*

*By definition, the injection, i.e., the **Inject** command, is performed at retention time = 0. All preceding commands have negative times; all following instructions have positive times.*

*The advantage is that the PGM time corresponds to the **retention time** of data reduction, e.g., integration, and thus, avoids errors such as falsely interpreted gradient profiles. The times of the **AcqOn** and **AcqOff** commands similarly coincide with the time axis of the reduction method.*

Select **Command** on the **Control** menu or press the F8 key to open a dialog box that assists you in editing the control commands. You can enter all instruction components (time, instruction, instrument, parameter, comment) separately. The system configuration determines which instructions and instruments are listed in the dialog box:

🔖 *Note:*

*If the PGM File and the timebase are not connected correctly, the F8 box is not available and the program lines are displayed in **gray**.*

The input procedure via the respective device icons or the F8 key prevents entering invalid command syntax. If Chromeleon finds a command with unknown or incorrect syntax, the corresponding line appears **red**.

• Press <F4> or <SHIFT> + <F4> to browse through the errors and correct any invalid input.

When you edit a program file in the PGM Editor, the editor checks the validity of the instructions, also. You only have to enter the commands and their parameters and Chromeleon takes care of the rest. This means that Chromeleon:

• Arranges the lines in chronological order. (Select **Sort by Retention Time** on the **Control** menu.)

• Checks whether the input is valid. (Select **Check** on the **Control** menu.) For example:

  • Does the file contain at least one ⇒*Inject* command?

  • Is the first **Inject** command at time 0.000?

  • Does the file contain at least one **Acquisition On** command after the first inject? (If it does not, a warning appears)

  • Is a **Acquisition Off** command present for each **Acquisition On** command? (This input is compulsory.)

  • Are total and partial flow rates set at the beginning of the file (required for clearly defined gradients)?

  • Are the execution times of preceding instructions considered in the start times of program instructions? (If they are not considered, the start times in the program file do not coincide with the actual start time.)

  • Is the final instruction an **end** command?

Before a program is started, Chromeleon performs a Ready Check to check the program for validity. In case of logical errors, for example, when Pressure.LowerLimit exceeds Pressure.UpperLimit, the program cannot start and a message appears, such as:
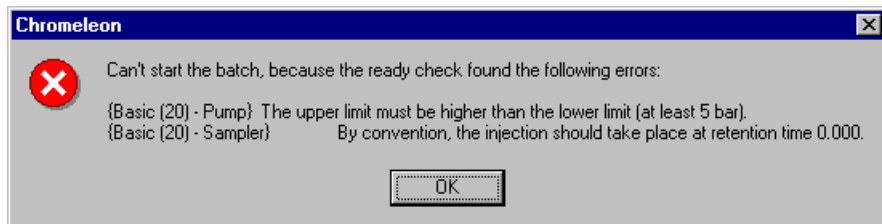
In case of errors, which may affect the course of the program without being critical, a warning appears. In this case, you can start the batch, nevertheless:
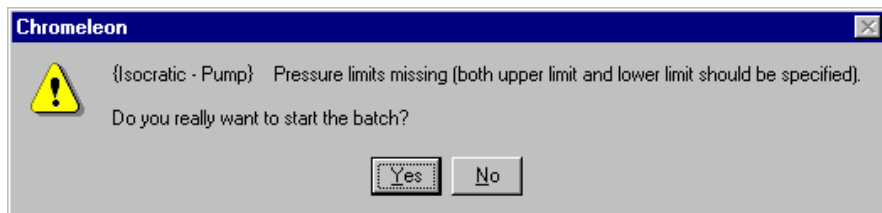
If you are familiar with the 🔲 **Program Syntax**, you can change or extend your program directly from the keyboard. After editing, save the ➢*PGM File* by selecting **Save** on the **File Menu**.

For more information about how to create and/or edit a PGM File, refer to **Control**:

🔲 **The Control Program**

🔲 **The Program Syntax**

🔲 **The Program Wizard**

For information about the different ⟹*Control Commands*, refer to:

How to …: Creating and Modifying Programs 🔲 **Creating a Program**

Device Control 🔲 **Practical Tips for Device Control (Overview)**

◼ The Post-Acquisition Steps View

Use the **Post-acquisition steps** view of the ◘ **PGM Editor** to define extraction and smoothing steps that are performed by the PGM File after data acquisition. In addition, you can copy existing channels or combine them using arithmetic operations. A new data channel is created for each step. The individual steps can be performed online after data acquisition, or offline of the chromatogram, UV spectrum, or mass spectrum.

Therefore, the **Post-acquisition steps** view is one of many data reprocessing tools in Chromeleon, but it is not part of the actual control. For more information, refer to ◘ **Post-Acquisition Steps**.

For more information about post-acquisition, refer to **How to …: Creating and Modifying Programs** ◘ **Adding Post-Acquisition Steps**.

◼ The Program Syntax *(Experts Only)*

◼☞ *Tip:*

*Dionex recommends always editing programs in the associated **Device** view (see **Control/The PGM Editor** ◘ **The Device Views**). The **Device** view is easy to use and ensures correct command syntax. Use the **Commands** view only if the desired parameters are unavailable in the device view. To enter the commands, use the F8 dialog box as described in the Commands view (see **Control/The PGM Editor** ◘ **Commands View**). Enter commands directly only if you know the correct syntax.*

For uniform operation by different users, ⇒*Control Commands* are in English. The syntax for the ➤*Program* commands is as follows:

```
Retention Time     DeviceName.Command
```
or
```
Retention Time     DeviceName.Property      =     Value
```

If you are not familiar with the syntax, use the ◘ **Program Wizard** and the different **Device Views** that guide you through program creation.

For a list of the general commands and the commands supported for Dionex and third-party devices, refer to ◘ **Device Control (Overview)**.

Time (Retention Time)

The time appears at the beginning of the control command and determines when the command will be executed. The time is in ➢*Decimal Minutes*, for example,

```
2.500
```

This input is optional. If no time is entered, the time specified in the previous program line will be used.

Device

Devices are all instruments, channels, relays, or remote inputs that are available in the active timebase. You can identify them in the F8 dialog box by the device icon (⊞ 🖳). Each **Device** has a number of commands and/or properties.

Various instruments can have the same commands or properties. Therefore, add the device name in front of the command to identify the instrument. The syntax is as follows:

```
Retention Time      DeviceName.Command
```
or
```
Retention Time      DeviceName.Property           =    Value
```

If no confusion occurs with other commands or properties, the device name can be omitted. For example, you can omit the device name in the **Flow** command when only one pump is installed. The syntax is as follows:

```
Retention Time      Flow                           =    Value
```

Command

In the F8 edit box, commands are marked by an exclamation mark (🔧). If a command exists for only one instrument, the name is sufficient for identification:

```
2.500    NeedleUp
```

The ⇒*NeedleUp* command exists only for the Dionex Autosampler GINA 50. In this case, the device name can be omitted. This is in contrast to:

```
2.500    UV_VIS_1.AcqOn
```

The ⇒*AcqOn* command is not unique if there is more than one channel in the system. To address a specific channel, you have to add the channel name to the command.

In addition, commands can be extended by additional parameters; for example:

```
2.500    ⇒Inject              Position = 20, Volume = 30   or
2.500    Relay1.On            Duration = 20
```

The possible command extensions and their order are predefined. They are listed in the F8 edit box. As confusion is impossible, you can also use the following syntax:

```
2.500    Inject               20, 30                       or
2.500    Relay1.On            20
```

Device-Independent Control Commands

Commands that are independent from a device appear as main entries in the F8 box. These are, for example 📢 ⇒*Branch*, ⇒*If*, ⇒*ElseIf*, ⇒*Else*, ⇒*EndIf*, ⇒*Log*, ⇒*Message*, ⇒*Protocol*, ⇒*Wait*, ⇒*Delay*, ⇒*Trigger*, ⇒*EndTrigger*, and ⇒*End*.

For more information, refer to **Practical Tips for Device Control**

🔼 **Trigger Commands**

🔼 **Mixed Commands**

Property

Properties are distinguished by their value. An I/0 icon (🔴) in the F8 edit box indicates that the value is predefined by the system. The 🔢 icon indicates that the values are freely selectable. A command string is also considered a property (🔤). For example:

```
2.500    UV.Lamp                 = On              or
2.500    UV_VIS_1.Wavelength     = 300
2.500    %A.Equate               = "%A"
```

If a property reports an actual value (for example, Pressure (bar), %A (%), Signal (mAU), etc.), the icon looks as follows: (📊). Properties related to the actual value are subordinate to it.

For example, Chromeleon enables the output of the current system pressure (⊞ pressure) and the definition of an upper and lower pressure limit (🔢 UpperLimit and 🔢 LowerLimit). Solvent names are assigned in the same way. (⊞ %B, 🔢 Value, Equate). The syntax is as follows:

```
2.500    pressure.UpperLimit    = 350
2.500    pressure.LowerLimit    = 20
2.500    %B.Value               = 30
2.500    %B.Equate              = "Methanol"
```

If the syntax is not clear, the device name must precede the command or property. For example:

```
2.500    UV_VIS_1.Signal.UpperLimit    = 500
```

Text, Names

Comments on the program or individual commands can be included before, after, or between individual commands. Comment lines always start with a semicolon "**;**" and appear green:

```
;        The following program ...
```

Text that appears on the screen due to a command (as with the ⇒*Protocol* and ⇒*Message* commands) and that is logged in the Audit Trail, must be entered in quotation marks:

```
2.500    Protocol      "Test program"
```

When you enter these commands in the F8 edit box, the quotation marks are added automatically.

The Control Panel

You can control the different devices of a timebase directly from a ➤*Control Panel* (in short: panel; also referred to as online plot or online window). A control panel controls and monitors the chromatography instruments configured in a ➤*Timebase*. With regard to appearance and function, it is a special type of window. In accordance with the Chromeleon philosophy, you are free to design its appearance in any way to meet your requirements.

For more information, refer to:

🔲 **Control Panel: Appearance**

🔲 **Control Panel: Function**

🔲 **Control Panel: The Signal Plot**

🔲 **Control Panel: The Audit Trail**

🔲 **Control Panel: The Trend Plot**

🔲 Control Panel: Appearance

➤*Control Panels* do not have a uniform user interface. A combination of various default controls determines the appearance of the control panel.

Control panels can include, for example:

- A slider to change the pump flow or any other variable parameter.

- A separate field to display status information, such as the running retention time.

- A "screen" LED to indicate whether the detector lamp is burning.

- A script button to execute the inject command.

- A signal plot to monitor the detector signal.

- The current ➤*3D Field* and ➤*Mass Spectrum*.

- The current ➤*Trend Plot* and ➤*3D_Amp* plot.

- The Audit Trail to track the execution of an operation.

You are free to determine the number of controls and their functionality to suit your needs. The functionality of the analytical instruments installed in the timebase determines which functions are available.

For example, if the system includes a controllable column oven, the oven temperature can be controlled.

The system administrator assigns user-specific ➤*Privileges* in the ➤*User Manager* (CmUser program) to determine whether a user is authorized to create his or her personal user interface. The organization of the panel allows locking certain functions on the user interface or hiding irrelevant information.

You can save a new user interface as a separate file (*.pan). Each user who can access the directory containing the file can select and use the file by clicking **Open** on the **File** menu.

In addition, Chromeleon provides many default control panels, which cannot be modified by the user. These panels do not only cover all standard control functions but they can also be used easily and intuitively.

For more information about how to create a control panel, refer to **How to …:** **Controlling Devices from the Control Panel**.

Control Panel: Function

➤*Control Panels* allow you to control and monitor the different ➤*Timebases*. When you create a control panel, you specify which timebase shall be controlled from this panel. When you open the control panel, Chromeleon tries to connect to the specified timebase. A message appears if Chromeleon cannot connect to the timebase, for example, because it was renamed or because the related chromatography ➤*Server* is not running. In this case, change the assignment manually:

- Open the control panel by selecting **Open** on the **File** menu.

- Select **Connect to Timebase** on the **Control** menu. Enter the name of the timebase to be connected with the control panel or select a name from the list.

The new assignment is valid until you close the window. Save the window to have the current assignment available when you reopen the window the next time.

The status bar indicates the selected timebase.

Tip:

*Select **Integrate** on the **View** menu to display the report for the running sample from the control panel.*

▣ Control Panel: The Signal Plot

The signal plot is an essential part of the ▣ **Control Panel**. The ➢*Signals* of the channels, which have been selected by the user, appear online, i.e., during data acquisition.

Different commands are available on the context menu (right-click). They allow you to define how the signals are displayed:

Autoscale	Each time when performed, ➢*Autoscale* adjusts the scaling of the signal axis exactly to the open chromatogram or to a section thereof. You can perform the command either from the context menu or by double-clicking the signal axis.
Auto Autoscale	➢*Auto Autoscale* automatically adjusts the scaling of the signal axis exactly to the open chromatogram or to a section thereof whenever the signal leaves the signal plot.
Auto Plot Speed	**Auto Plot Speed** prolongs the time axis automatically by the period defined on the **Axis/Decoration** tab page when the end of the signal plot is reached.
Replot from Beginning	When the signal reaches the right border of the signal plot, ➢*Replot from Beginning* enlarges the window by the period defined on the Axis/Decoration tab page. Thus, the entire chromatogram is displayed, always.

▣ Control Panel: The Audit Trail

The **Audit Trail** on a ➢*Control Panel* logs all commands performed during sample processing and saves information regarding the entire system. This includes graphical and text information. Chromeleon classifies ⇒*Control Commands*, status information, and error messages.

When data acquisition is started, the Audit Trail reports the start time and all commands that are performed afterward (⇒*AcqOn/Off*, ⇒*Inject*, etc.).

Each event that appears in the Audit Trail is stored. Storage is very precise and comprehensive, allowing you to track how a sample was processed and which events occurred during sample processing. For more information, refer to **Data Management** ▣ **Audit Trails**.

For documentation purposes, the **Sample Audit Trail** is included in a report, by default. For more information, refer to **How to …: Creating and Using Report Tables** **Displaying an Audit Trail**.

Control Panel: The Trend Plot

When included in a **Control Panel**, the ➢*Trend Plot* provides the ability to monitor impending problems by viewing a plot of module-specific data.

Tip:

*The **Dionex Templates > Panels > Wellness** directory of the local ➢Datasource includes several examples of control panels with trend plots of module-specific data.*

The following commands are available via the context menu (right-click to select a command). The commands allow you to define the appearance of the trend plot.

Full Size	Displays the full-size plot of all trend data.
➢*Autoscale*	Scales the trend plot for optimal fit of the *y* values.
➢*Auto Autoscale*	Automatically adjusts the scaling of the trend plot for optimal fit of the *y* values.
Unzoom	Restores the previous zoom position.
Flow Change Marks	Displays vertical solid lines on the trend plot to indicate a change in flow rate.
Eluent Change Marks	Displays vertical solid lines on the trend plot to indicate a change in eluent.
Module Change Marks	Displays vertical solid lines on the trend plot to indicate that different serial numbers were found in sample sets with the same device name.
Consumable Change Marks	Displays vertical solid lines on the trend plot to indicate a change in a consumable part (for example, a column or suppressor).
Calibration Marks	Displays vertical solid lines on the trend plot to indicate that calibration was performed.

Statistics From	Defines how statistics are calculated on the trend plot. Select one of these options:

- **All Data:** Statistics are calculated from all data points.

- **Data in Viewed Range:** Statistics are recalculated as soon as the currently viewed time axis changes.

- **Control Chart:** Statistics are calculated from the target and 1s values entered on the **Statistics** tab page in the Trend Properties dialog box.

Trend Variable	Opens a dialog box in which you can select the properties of the plot, including the events, statistics, and data to display.

For information about how to add a trend plot to a control panel, refer to **How to …:** **Modifying a Control Panel**.

 Tip:

You can view post-run trend data, including sample result data such as retention time, area, peak height, etc. in the integration window, see Integration: *The Trend Plot.*

The Panel Tabset

A panel tabset provides a convenient interface for controlling and monitoring a ➢*Timebase*. A panel tabset provides (on one window) a set of Chromeleon ➢*Control Panels* for controlling the individual devices in the timebase and for performing system-wide functions (for example, creating and running sequences). To move from one control panel to another you click the corresponding tab. If multiple timebases are connected to a chromatography ➢*Server*, the window is divided into panes, with each pane containing a panel tabset for one timebase.

The default set of control panels in a panel tabset typically includes one panel for each device in the timebase and one or more panels for system-wide controls.

You can use these panels to perform the following tasks:

- Operate system instruments manually (direct control)

- Operate system instruments automatically (programmed control)

- Monitor instrument status

- View real-time data

- Collect and view the system event log (➤*Audit Trail*)

- Perform calibration and diagnostic functions (➤*System Wellness*)

Additional characteristics of the panel tabset:

- Clicking a panel highlights its border and makes it the active panel.

- Commands selected on the Chromeleon main menu apply to the active panel.

- You can expand a panel tabset (and thus hide the other tabsets), split the window to view all tabsets at one time, or change the width of a panel.

- You can add, delete, or rename individual panels within a panel tabset.

☞ *Tip:*

To open a default panel tabset, click the 🔲 *icon on the standard toolbar.*

Also, refer to **How to …:** 🔲 **Controlling Devices from the Panel Tabset**.

Data Management

Data (Overview)

Input Data

For the analysis of a sample and for documentation and archiving purposes, various types of input data are required, which you have to determine or enter before you can start the analysis.

The required input includes, for example, the sample name, weight, injection volume, chromatographic conditions (solvent, flow, detection wavelength, connected devices, etc.), as well as the run time. Distinguish between:

* Data describing a sample (➢*Sample Data*),

* Data describing a sequence (➢*Sequence Data*, usually entered automatically), and

* Data describing the chromatographic treatment of a sample (➢*Chromatographic Methods*).

The user input is the basis for the analytical process.

Sample Data, Chromatographic Method	→	**Analysis**	→	Raw Data, Protocol Data
Input Data				*Output Data*
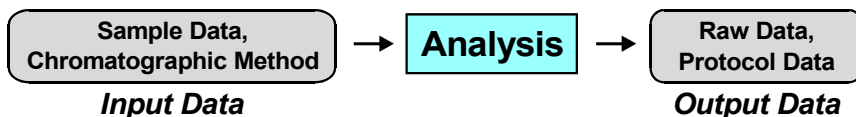
Output Data

The data recorded during analysis is referred to as output data. Output data includes:

* Data provided by the analysis (analysis and/or ➢*Raw Data*)

* Protocol data on the analysis (➢*Audit Trail*).

Due to this variety of data types, systematic data organization and storage is especially important (see **Data Management 🔲 Data Storage**).
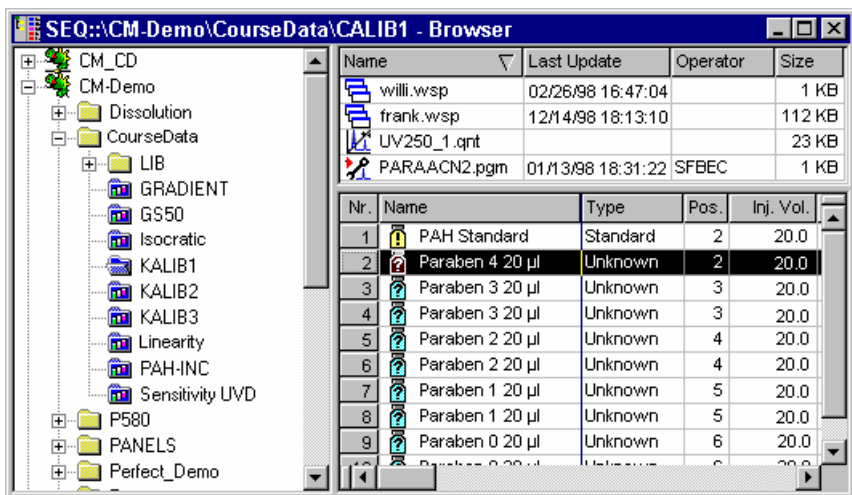
The Browser

The ➤*Browser*, Chromeleon's main tool for file management, lists all directories containing chromatographic data that you can access.

In the Browser, you can open, move, and/or delete chromatographic data. In addition, you can search various databases for specific data. To open and view a file in a separate window, select the file by its name.

⚠ *Caution:*

*Browser functions and structure are similar to the Windows Explorer. However, do not confuse the Browser with the Windows Explorer. Do **not** use the Windows Explorer for operations within Chromeleon ➤Datasources. Administrators can prevent these operations by selecting the **Protect Datasource Directory** option on the **General** tab page (via the **Properties** option on the datasource context menu).*

For more information, refer to:

🔼 **Common Features with the Windows Explorer**

🔼 **Differences from the Windows Explorer**

🔼 **Function**

◾ Common Features with the Windows Explorer

Similar to the Windows Explorer, the Browser window has two separate panes: The left pane shows the directory structure in which the directories and folders are arranged. Detailed information about the files and ➢*Sequences* appears on the right. You can handle the Brower items in the same way as you handle the items in the Windows Explorer:

- On the left pane: Click the "+" sign beside a directory name to display the items underneath. Click the "-" sign to hide the items.

- On the left pane: Click a directory to view its contents on the right pane. Files and samples appear on the right pane, as well.

- On the right pane: Double-click a file open the appropriate editor for the file. Double-click a sample to open the chromatogram.

A type-specific icon allows easy identification of the file types (sequence, ➢*PGM File*, ➢*Quantification Method*, etc.). Chromeleon automatically recognizes the ⇒*Type (Sample Type)* (standard sample, unknown sample) and/or the data format (e.g., 3D field) and displays the data in the appropriate chromatographic environment.

- To drag a subdirectory, sequence, or file to a different directory, select the item. When you drag the selected item while holding down the right mouse key, a context menu opens. Select an option to copy or move the item to the new location. When you drag the selected item while holding down the left mouse key, the action, which was defined as the default action (**Ask**, **Copy**, or **Move**), is performed.

◾ Differences from the Windows Explorer

Structure

Only chromatographic data that is part of a ➢*Datasource* is presented in the Browser. (For more information, refer to the **Data Management** section.)

The top-level item in the Browser hierarchy is the datasource. A datasource can be created only in the Browser; it is not a standard subdirectory in the Windows Explorer. A small icon indicates the type of data on which the datasource is based (e.g., Chromeleon data or GynkoSoft data). You can see only those datasources for which you have the appropriate access rights.

The directory structure underneath the datasource is similar to the directory structure in the Windows Explorer (yellow file icons), allowing you to organize the different ➢*Sequences* in the datasource (identified by blue file icons). It is not possible to create a subdirectory underneath a sequence.

Operation

- Select a datasource, subdirectory, or sequence to display its contents on the right Browser pane.

For a datasource or subdirectory, you see the usual list of all directories and files. If you select a sequence, the right pane is divided horizontally into two panes, providing information about the general properties of the selected sequence (header) and listing all analysis and standard samples and their sample data (**Sequence Editor**).

⚠️ *Caution:*

*It is possible to edit the sample data right here. For GynkoSoft users, this corresponds to the **Sample (SMP) File**. You can add additional samples to the sequence, modify existing sample data, or remove old samples. For more information about the sequence editor, refer to the **Samples and Sequences** section.*

- To search for specific sequences, data, or samples, select **Query** on the context menu. It is possible to search several datasources simultaneously. A ➢*Query* allows you to search both across several datasources and for specific properties, for example, "all samples starting with PAK."

Moving, deleting, or copying directories, data, and/or files is very similar to these actions in the Windows Explorer. (For more information, refer to **The Browser** 🔲 **Common Features with the Windows Explorer**.) However, keep the following in mind:

⚠️ *Caution:*

*Move, delete, or open chromatographic files **only** in the Browser. The reason is that, except for the visible results, processes are performed below the surface.*

▪ Function

In the Browser, you can:

- Set up ➢*Datasources*, create subdirectories, copy or move files via **Drag & Drop**, or remove files with the **Cut** command.

- Select a ➢*Sequence* on the left pane (blue folder icon) to display the contents, header, and sequence editor on the right pane.

- Double-click a sample name to display the associated chromatogram.

- Double-click a ➢*PGM File* name to open the PGM Editor.

- Select a file and right-click for more functions.

- Select several files (as in the Windows Explorer) and right-click to perform a command for several files simultaneously.

- Select a file, right-click, and start a ➢*Query* for several sequences and/or datasources. The result of the query, which means the different files or samples with at least one common feature, is displayed on the right pane.

The Datasource

Top-level directories in the directory structure of the ➢*Browser* are referred to as ➢*Datasources*. The tool for datasource handling is the Browser. Each datasource is based on a separate database. When setting up a datasource, a path to an existing database is entered or a new database is created.

- Select **Datasources** on the **File** menu to set up a datasource. For more information about the required steps, refer to **How to …: Working with Files, Databases, and Networks** ▪ **Setting up a Datasource** in the **Administrator Help** section.

The Browser indicates only the name of the *datasource*, but not the name of the underlying *database*. The type and number of the datasources visible to the user determine which data that can be accessed by the user.

The advantages are as follows:

- Users access all data in the same way. They do neither have to worry about the data's actual storage location on the network nor do they have to enter the entire path. The location is specified when the datasource is created.

- Each user can take advantage of a database without having to deal with special database programs.

Database Formats of a Datasource

Chromeleon supports several database formats. In addition to the most frequently used Access database format (mdb container), Chromeleon's ➢*ODBC Capability* allows handling SQL ("Structured Query Language") and database formats, such as Oracle and SQL servers. "Old" GynkoSoft directories ("drives") and third-party data can be displayed as if they were datasources with an underlying database. Different icons allow easy identification of GynkoSoft and Chromeleon:
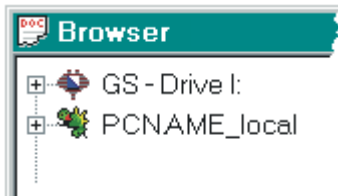
⊞ ◆ GS - Drive I:	**GynkoSoft - Datasource**
⊞ ✲ PCNAME_local	**local CHROMELEON datasource**

When Chromeleon is installed, a default datasource is automatically created on each client PC. The datasource name is the computer name (assigned during the installation under Windows) plus **_Local**. In this way, each user has a separate datasource in which (s)he can store his(her) "personal" results and data. For single systems and for users who do not have additional access rights on a network, this is the only way to store their data. The raw data of manually performed analysises are stored in this datasource, also. Therefore, do not delete the <PC NAME_Local> datasource.

Directory Structure of a Datasource

Each datasource can have any number of subdirectories organized in a hierarchical structure. To create a subdirectory, select the datasource in the Browser, and then select **New Directory** on the **File** menu. The data tree structure is similar to the MSDOS data tree.

⚠️ *Caution:*

Do not use special characters (such as the umlaut) for new directory names or sequences. This may cause problems in Novell networks!

Actions performed in the Browser, for example, creating datasources or directories, require complex operations below the user interface. They are different from actions in the Windows Explorer and therefore, they should not be performed from the Windows Explorer. The representation of directories and data differs considerably from the Windows Explorer, also.

See the image below for examples of datasources and their different directory structures:
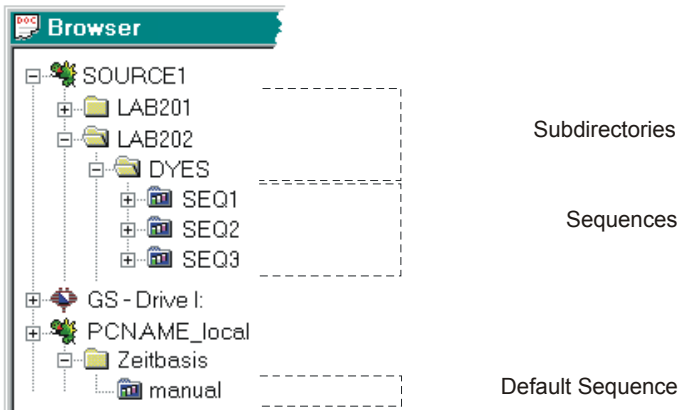
The **SOURCE1** datasource has three subdirectories: LAB201, LAB202, and DYES. The **DYES** directory contains three ➤*Sequences*: **SEQ1**, **SEQ2**, and **SEQ3**. The default **PC Name_local** datasource has one subdirectory. The name of the subdirectory corresponds to the name of the timebase installed on this computer. The default sequence (named **manual**) is located in this subdirectory.

When a sequence is selected, its "inner life" is visible on the right Browser pane: file structure (control ➤*Program* (🔧), ➤*Quantification Method (QNT Method)* (📈), ➤*Report Definition Files (RDFs)*, etc.), sequence information, and samples (standard samples (🔒), and unknown samples (❓), etc.). For information, refer to **Data Management** 🔲 **The Browser**. For information about the functions and significance of sequences, refer to the **Samples and Sequences** section; especially, refer to 🔲 **The Sample List (Sequence)**.
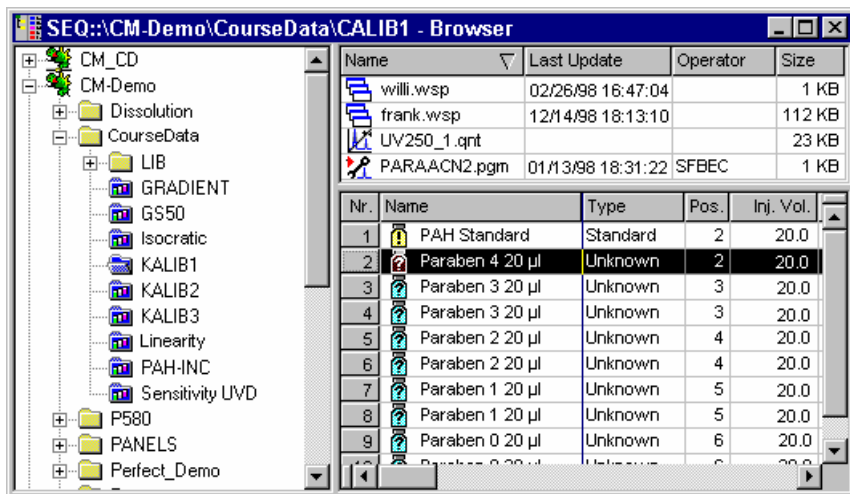
Drag & Drop allows you to move subdirectories, sequences, and chromatographic methods both in a datasource and between datasources of different types. Note that the items are copied and that this may change the underlying database. If you want to actually move a sample, first copy the sample to the new location, and then delete the original sample in the Browser.

⚠️ *Caution:*

*Perform **drag & drop** operations only in the ➢Browser. Performing these operations outside Chromeleon, for example, in the Windows Explorer, will result in the loss of data.*

Locking Datasources, **Directories**, **and Sequences**

To protect data and results, you can lock datasources, directories, or sequences. It is not possible to modify **Locked** objects or any object under the locked one. For example, if a datasource is locked, all subdirectories and all sequences therein are locked as well. Locked objects are identified in the Browser by the red lock on the corresponding icon (🐾/📁/📋).

How To

- Select the object in the Browser.

- Select **Properties** on the context menu.

- Select the **Locked** check box.

To remove the lock, return to the **Properties** dialog box and clear the **Locked** check box.

Note:

*Locking and sharing objects is subject to access control. (The **Administrator Help** section provides more information; refer to **Software Installation and Communication** **Access Control**.) Only users who have the corresponding privilege can lock and/or share objects.*

In addition to locking datasources and directories by the **Locked** check box, access to these items can be controlled by adding them to ➢*Access Groups* or removing them.

- Select the datasource or directory in the Browser.

- Select **Properties** on the context menu. On the **Access Control** tab page, define the Access Group assignment, using the **Add** and **Remove** buttons.

Only users who are members of an Access Group listed in the **Access Groups** are authorized to access datasources and directories.

Data Acquisition

Even the best method of **Data Storage** is only as good as the quality of the stored data. Therefore, data acquisition is a very important process, from the quality of the detector through all components participating in the data flow to data processing in Chromeleon.

A distinction has to be made between detectors supplying digital data and detectors supplying analog data. The best results are obtained with detectors that are capable of communicating digital signals via a serial interface. (The **Administrator Help** section provides more information; refer to **Software Installation and Communication** **The Serial (RS-232) Interface**.)

Detectors that supply analog signals have to rely on precise conversion of the signals. The product range available from Dionex includes an extremely sensitive and low-noise ➢*UCI Universal Chromatography Interface* for converting analog signals to digital signals. The UCI guarantees highest precision with minimum noise.

The following terms are important for data acquisition: ➢*Data Collection Rate*, ➢*Sampling Rate*, and ➢*Step*. However, sometimes it may be difficult to understand the relationship between these terms.

If the detector directly supplies digital data to the PC, this is referred to as **Data Collection Rate**. In contrast, the term **Sampling Rate** is used if an A/D converter, such as a UCI Universal Chromatography Interface, supplies the data. Both terms describe the number of data recorded per second.

Step describes the time interval between two data points. By default, the step is the reciprocal value of the data collection rate or the sampling rate. Nevertheless, it is possible to select a different step. There is nothing particular you need to observe if an A/D converter supplies the data. However, for detectors supplying digital data make sure to define the **Step** command after the **Data Collection Rate** command.

Data Acquisition with Detectors without Separate Drivers

In addition to the ➢*Device Drivers* for controlling Dionex detectors, Chromeleon supports many drivers to control third-party detectors. For an overview of the different manufacturers whose devices can be controlled by Chromeleon, refer to **Hardware Installation 🌐 Installing and Controlling Third-Party Devices** in the **Administrator Help** section.

In addition, it is also possible to acquire data using detectors for which separate device drivers are not available. In this case, install the ➢*Integrator Driver*.

For information about how to install the device drivers, refer to **How to …: Configuring the Chromeleon Server 🔖 Adding, Configuring, or Deleting Components** in the **Administrator Help** section.

Data Storage

Intelligent data storage and organization is a prerequisite for fast and efficient access to specific data. That is why Chromeleon stores data and files in different locations. Databases and sequence directories, which are both part of the 🔲 **Datasource**, are available for this.

Storing data in a database

Data that can be compared across sequences is stored and managed in a relational, ➤*ODBC*-capable database. This applies to all ➤*Sample* and ➤*Sequence Data*. The advantages of this type of data management are comfortable integration in other applications, such as Excel, Access, dBase, etc., and efficient searching and sorting capabilities.

Therefore, you can perform, for example, a ➤*Query* to find all samples processed on a certain day, created by a certain user, and/or having a certain name.

Storing data together with the sequence

The entire data describing the chromatographic treatment of a sample or recorded during the analysis is stored in a ➤*Sequence*, including control files (➤*PGM File*), evaluation parameters (QNT Method), and the entire raw and protocol data (see **Data Management 🔲 Raw Data Storage**).

History (Modification History)

For the datasources, you can enable the modification history (in short: ➤*History*). This allows you to document all modifications together with the user name and the object name.

Objects can be samples, sequences or datasources, ➤*Control Panels*, ➤*Report Definition Files* (RDFs), ➤*PGM Files* and/or ➤*QNT* files, and modified chromatograms.

For more information, refer to **How to …: Working with Files, Databases, and Networks 🔲 Tracking File Modifications (History)** in the **Administrator Help** section.

Data Export

Chromeleon supports various data export options for communication with other programs:

1. You can export report pages from the ➢*Browser*, by selecting **Batch Report** on the **File** menu. In the **Export options** section, select the **Export** check box. The **Export Wizard** is opened automatically (otherwise, click **Export Settings** to open the **Export Wizard** manually). Chromeleon supports the following formats for data export:

    a) ANDI/Chromatography - ➢*AIA* (*.cdf)

    b) ASCII text format (*.txt)

    c) Excel file format (*.xls)

    d) Adobe Acrobat file format (*.pdf)

    e) Chromeleon Archive format (*.cmb = ➢*Backup* files)

    For more information, refer to **How to …: Creating and Managing Files and Data 🔹 Exporting Data During or After a Batch**.

2. In the ➢*Printer Layout*, you can open a dialog box that similar to the Export Wizard. First, enable the **Layout Mode** on the **Edit** menu. Then, select **Batch Report Setup** on the **File** menu to open the **Batch Report Setup** dialog box. Via the Printer Layout, you can export data from different samples in a single data file (refer to **How to …: Creating and Managing Files and Data 🔹 Exporting Data from Different Samples to a Single File**).

3. You can also open the Export Wizard from a ➢*Control Panel*. Select **Reporting** on the **Batch** menu and select the **Print/Export Report** check box.

4. In addition, you can export raw data in the AIA format. To do so, select **Export/Backup** on the **File** menu in the Browser, and then select **ANDI/Chromatography (AIA)**. (Also, refer to **Data Management 🔹 Raw Data Export**.)

5. If you wish to send Chromeleon data to another laboratory, for example, via e-mail, Dionex recommends that you perform a 🔹 **Backup** first and then transmit the compressed data as *.cmb file, which is the Chromeleon archive format.

You can also start the Chromeleon export function from a separate program, using the command line under **Start > Run** or a DOS command prompt. For more information, refer to **How to …: Working with Files, Databases, and Networks** 🔼 **Using Chromeleon Data in an External Program** in the **Administrator Help** section.

Backup

To avoid loss of data (for example, due to a defective hard disk), Dionex recommends that you back up your data to a different data medium at regular intervals, using the ➢*Backup* command on the **File** menu in the ➢*Browser*.

Backup data is compressed, which means that the data is "packed" and stored in a different location. For security reasons (GLP does not allow modification of backup data), direct access to the data is not possible. To unpack the data, select the ➢*Restore* command on the **File** menu in the Browser.

The backup logs the files that are copied and issues warnings if an error occurs. The directory structure is maintained.

For more information, refer to **How to …: Creating and Managing Files and Data:**

🔼 **Creating Backup Files**

🔼 **Restoring Backup Files**

Raw Data

Data generated by the system is referred to as raw data. Data entered by the user is referred to as user data. The user is not allowed to change the raw data, e.g.:

- Sample data recorded on different channels (see ➢*Signal*)

- 🔼 **Audit Trails**

- Injection times (see ⇒*Inj. Date/Time*)

- ➢*History*

In a narrower sense, all analog or digital values that are measured by a detector and stored digitally on the PC are referred to as raw data. Raw data exists only for signals or channels that were selected by the user before data acquisition.

The selected ➤*Sampling Rate* and/or the ⇒*Step* determine the extent and precision of the stored raw data.

For more information, refer to:

🔺 **Raw Data Storage**
🔺 **Raw Data Compression**

Raw Data Storage

Saving detector signals in digital form is referred to as raw data storage. However, raw data storage also includes other important data, such as the analysis time, signal unit, number of data points, etc.

If a detector is equipped only with an analog output, the data must be converted into digital signals--a task performed by the A/D converter card.

Storage Procedure

With conventional data systems, an analog value is digitized at a fixed time interval, for example, every second, and stored with a defined accuracy. The number of values stored per second is referred to as the ➤*Sampling Rate*. The inverse of the sampling rate (the time interval between two data points) is referred to as ⇒*Step*.

The higher the sampling rate is (i.e., the smaller the step is), the more data points are stored and the more exactly the original signal can be restored from the stored data. However, a higher sampling rate has higher memory requirements. Therefore, Chromeleon supports the **Step=Auto** setting. It is true that this setting requires high algorithmic resources in real time, but it provides the following advantages:

- Raw data files are as small as possible. Fewer data points would result in a loss of precision. If an analysis requires a conventional step width of 0.5 seconds, Chromeleon can typically acquire such chromatograms with an *average* step width (= chromatogram length divided by the number of data points) of 2 seconds. Thus, the compression factor is 4:1, making optimum use of the available storage capacity.

- Despite the minimal file size, maximum integration accuracy is ensured for the given chromatographic conditions, as the continuous signal is approximated to the optimum. Generally, each peak includes more data points than with conventional acquisition methods.

- The processing speed, for example, for peak detection, re-integration, graphical output, etc., is significantly higher due to the reduced number of data points.

Storage Location

Raw data is stored in the directory of the current sequence. Chromeleon creates a separate subdirectory for each channel specified during installation of the program. An Audit Trail directory is created, also. The directories are not visible in the Browser.

The reason is that Chromeleon manages raw data automatically. The user does not need to access the raw data directly at any time.

You can view this type of data only in the Windows Explorer, but this is possible only if the datasources are not locked. Besides, the datasource names are not displayed in the Explorer. Therefore, you must follow the path to the corresponding sequence directory (see figure).
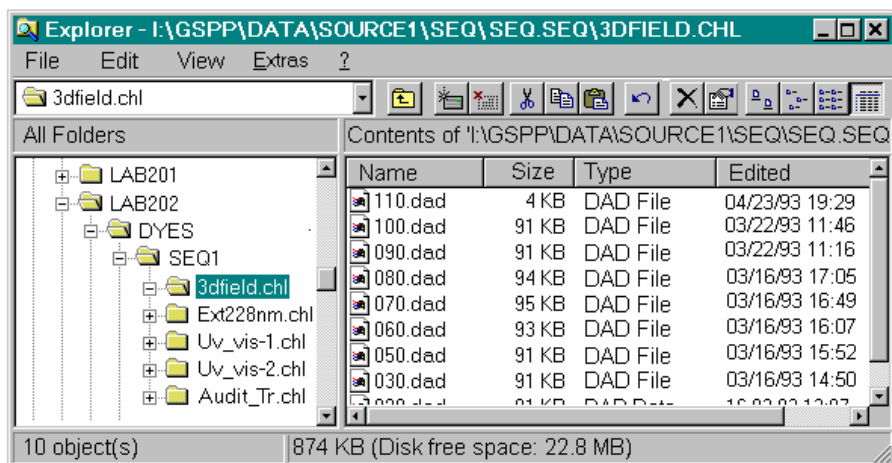
In the example, the **3dfield**, **Ext228nm**, **Uv_vis-1**, and **Uv_vis-2** channels were defined. An Audit Trail directory (**Audit_Tr**) is available, also. Click the "Plus" sign (+) beside a directory name to display the raw data of the corresponding channel.

A separate raw data file is created for each sample in a sequence, for which raw data of a specific channel was recorded.

⚠️ *Caution:*

*Do not modify these directories! Operations outside Chromeleon are not permitted. Dionex recommends that you protect your datasources to prevent access from the Windows Explorer. Select **Properties** on the context or **File** menu of the datasource and then select the **Protect Datasource Directory** check box.*

Raw Data Compression

The data is compressed automatically when the raw data is stored. For the signal value, data compression is achieved by storing the difference to the next data point instead of storing each data point separately. The actual value is stored only at intervals. In this way, the compression can be increased by 50%. This effect is especially noticeable with ➢*3D Fields*.

The size of the raw data file for a 3D field increases with the number of recorded data points. These depend on the ➢*Optical Resolution* of the detector, field size (area between the upper and lower limit of the 3D field), and selected ➢*Sampling Rate*.

For example, at a sampling rate of two spectra per second (step = 0.5) and an optical resolution of 2nm, this means that 2 x 60 x 70 = 8400 data points per minute must be recorded for the UV range from 200 to 340 nm. As each absorption value is recorded with an accuracy of 25 bits, a hard disk storage capacity of m x (N+1) x 4 = 70 x ((2x60)+1) x 4 = 33.88 kByte per minute is required. Thus, a storage capacity of 0.678 Mbyte is required for the 3D field of a "normal" (20-minute) chromatogram.

However, special data compression procedures allow reducing the required storage capacity by approximately 50-60% by storing only each eighth spectrum completely. For all other spectra, only the difference to the previous one is stored and re-calculated when needed. This procedure is a good compromise between optimum data compression and the required time for restoring a 3D field.

 Note:

The compression procedure is not destructive, that is, the complete data is stored. Thus, the 3D field contains the complete information provided by the detector. The data can be restored at any time.

There are three additional ways how you can minimize storage capacity requirements:

• Limit the wavelength range to the necessary range.

• Reduce the sampling rate (step) in such a way that the number of spectra that are below the narrowest peak is limited to 10 to 20. As an alternative, select an automatic sampling rate (step).

• Use the possibilities of ⇒*Diode Bunching*.

Restoring a Chromatogram from Raw Data

When you restore a chromatogram from the raw data, Chromeleon connects equidistant data points with straight lines, thus creating a diagram "resembling" the recorded analog signal.

Clearly, the resemblance (and thus the precision of integration) is increased with an increasing sampling rate. However, a higher sampling rate requires more storage capacity. To integrate the narrowest peaks, which are usually the earliest peaks, with the same precision as the widest peaks, the sampling rate must be set in such a way that a minimum of 10 datapoints is stored for the narrowest peak. However, this results in huge data volumes, especially for wide peaks and long baseline sections.

Using a dynamic sampling rate can solve this problem. Chromeleon is capable of continuously optimizing the sampling rate during an analysis. This means that Chromeleon stores few data points during baseline sections, but stores many datapoints below peaks. The local sampling rate is set according to the actual information volume so that the deviation between the resulting diagram and the actual analog signal is neither greater nor smaller than the actual noise component of the signal. This method ensures that neither too many nor too few datapoints are stored, but always the optimum. The ⇒*Step* values vary between 0.01 and 5 seconds (sampling rate: 0.2 to 100 Hz).

Raw Data Export

Chromeleon supports exporting raw data by conversion to the ➢*AIA* and ASCII formats. In addition, you can export raw data to a ➢*Backup* file (*.cmb).

To export raw data as an AIA or backup file, select **Export/Backup** on the **File** menu in the Browser.

Format	Description
ANDI Cat. 2	Samples and peak variables are stored in the AIA format.
ANDII Cat. 1+2	In addition to samples and peak variables, the raw data of a chromatogram (each stored data point) is stored in the ANDI format.
Chromeleon backup (*.cmb):	All data of a ➢*Sequence* or a ➢*Datasource* are stored in the *.cmb format.

To export raw data in the ASCII format, select **Batch Report** on the **File** menu or context menu.

Format	Description
ASCII	In the Batch Report dialog box, select the **Export** check box. This opens another dialog box; select the **ASCII text format** check box in the new dialog box. All raw data from the channels selected for the actual sequence are stored in the ASCII format.

🖙 *Tip:*

If you intend to export raw data to other applications or other computers, it may be necessary to use a fixed ⇒Step (= equidistant raw data storage) instead of a dynamic step.

It is also possible to export report data sheets in different formats (see **Data Management 🔲 Data Export**).

Raw Data Import

Chromeleon supports importing the following raw data:

- ANDI data (*.cdf) (see ➤*AIA*)

- Agilent/HP ChemStation

- HP1100 LAS data

- ➤*LIMS*/➤*Worklist* data (*.wle)

- PeakNet (up to version 5.2) data

- Files in the Chromeleon archive format (*.cmb)

In the Browser, select **Import/Restore** and an option on the **File** menu. To import Chromeleon ➤*Backup* data, select **Import/Restore** and ➤*Restore*.

In addition, you can install databases from third-party chromatography data systems.

How To

- Open the Browser and select **Datasource** on the **File** menu.

- Follow the instructions described in **How to …: Working with Files, Databases, and Networks** 🔲 **Connecting a Datasource** in the **Administrator Help** section.

For details on importing PeakNet 5.2 or earlier data files, refer to **How to …: Creating and Managing Files and Data** 🔲 **Importing PeakNet (Release 4.5 through 5.2) Data Files**.

Raw Data Storage in Case of Power Failure

The raw data of a sample interrupted by a power failure is not lost because raw data autosave is performed continuously during sample processing. Autosave ensures that the raw data is stored on the hard disk at short intervals specified by the user (the shortest possible interval is approximately 30 seconds). If a power failure occurs, only data collected since the last autosave is lost.

If a power failure interrupts the automatic sample batch, ➤*Power Failure Protection* and power failure handling ensure that processing is continued at the same position when the system is restarted. In addition, you can run a power failure program to reset the system to a defined state.

If processing is interrupted by warnings or error messages, you can run an appropriate ➤*Emergency Program*. This sets the system to a defined state that is recorded in the ➤*Audit Trail*.

For more information, refer to:

How to …: Creating and Modifying Programs:

🔳 **Specifying an Emergency Program**

🔳 **Creating a Power Failure Program**

Audit Trails

Chromeleon maintains an event log called the Audit Trail. The Audit Trail includes the following information:

Audit Trail entries for ➤*Preconditions*:

📷   Device settings before a sample run, such as the column oven temperature (settings are displayed against a light blue background)

📑 *Note:*

These entries are displayed in the Daily Audit Trail and Sample Audit Trail only.

Audit Trail entries for a sample run:

ℹ️   System messages, such as:
- Restart of Chromeleon after booting the computer
- Start of sample processing

⚠️   Warning

⚠️   Error

🛑   Abort Error (batch or program is aborted)

▶️   Next command in the batch

◻ Instruction performed. The color indicates the filter level. The message is displayed from this level on:

▬ Green: Normal

▭ Yellow: Advanced

▬ Red: Expert

Color-coding is not yet supported for manual and triggered commands.

🔧 Command performed manually from the control panel or F8 dialog box

🔧 Triggered command

✳ Fulfilled ⇒*Trigger* condition

◆ ⇒*IF* construct or ⇒*Branch* command performed manually from the control panel or F8 dialog box

📒 ⇒*Protocol*; comments about program steps or descriptions of chromatographic conditions

💬 ⇒*Message* on the screen that the user must confirm

📕📘 ⇒*Log* command performed by either the user or the ➢*Device Driver*

👉 *Tip:*

*When samples overlap, entries relating to the current sample and at least one sample in preparation have a yellow background. (For more information about sample overlapping, refer to **Device Control/Practical Tips for Device Control/Autosampler Control** ⬛ **Overlapping Samples**.)*

- Each item is stored with the current time of the Chromeleon server PC. Information gathered during data acquisition or sample batch processing also includes the associated retention time.

- The Audit Trail information is stored continuously both over the entire workday and for the processing time of a single sample. Chromeleon supports the following Audit Trails:

⬛ The Daily Audit Trail
⬛ The Sample Audit Trail

Audit trails can be displayed in the Browser (as Daily or Sample Audit Trails), on a control panel, or in a report or Printer Layout (as a sample log only).

To define the extent and type of the Audit Trail display, select the display filter (**Normal**, **Advanced**, **Expert**, or **Error and Warning**) on the context menu. The **Advanced** and **Expert** options are not available for Audit Trails created with Chromeleon 4.0 or lower.

In addition, you can select one of the following options on the context menu:

- **Preconditions only** displays only the conditions before a sample run

- **Preconditions and Run** displays all entries

- **Run only** displays only the entries for the sample run

▪ The Daily Audit Trail

The Daily 🔳 **Audit Trail** records all events related to a specific timebase, thus facilitating compliance with GLP (Good Laboratory Practice). The information is displayed in the Audit Trail on the control panel (refer to **The Control Panel 🔳 The Audit Trail**). The Daily Audit Trail also records the preconditions before the sample run.

When the Chromeleon ➤*Server* is started, Chromeleon creates an **AUDIT** directory for the timebase in the server's standard datasource. The server saves the Daily Audit Trail for the timebase to this directory. The name under which the file is saved is the current date. For example, the Audit Trail file for March 5, 2005, is named 20050305.slg. A separate file is created for each day.

In the Browser, the right pane lists the Daily Audit Trails by name, date, and time of the last update. Double-click to open an Audit Trail in a separate window. The information that is displayed corresponds to the information displayed in the Audit Trail section on the control panel. However, please note that the file also includes the preconditions. You can print the open Daily Audit Trail.

Copy, move, rename, and delete Daily Audit Trails in the Browser. The user rights required to perform these actions are defined in the ➤*User Manager* (CmUser program).

📝 *Note:*

Chromeleon generates a new file for each Daily Audit Trail. Therefore, periodically transfer files that are no longer required to an external storage device.

☞ *Tip:*

You can also open a Daily Audit Trail from the panel tabset (refer to ***Control 🔺 The Panel Tabset****). Go to the* ***Sequence Control*** *panel and click* ***Daily Audit Trail*** *under* ***System Maintenance****.*

In addition to the Daily Audit Trail, a 🔺 **Sample Audit Trail** exists for each sample.

🔳 The Sample Audit Trail

The Sample Audit Trail contains all information that was recorded for the specific sample in the Daily Audit Trail (see 🔺 **Audit Trails**). The Sample Audit Trail is part of the default reports. For more information, refer to **How to …: Creating and Using Report Tables 🔺 Displaying an Audit Trail**.

To display a Sample Audit Trail, select one of the following options:

- To view the Audit Trail information for the current sample, display a report in any method window and select the **Audit Trail** worksheet.

- To view the Audit Trail information for a specific sample, right-click the sample of interest in the **Browser** and select **Audit Trail** on the context menu.

If you do not find a specific entry in the Sample Audit Trail, check the 🔺 **Daily Audit Trail**.

Samples and Sequences

Sample Preparation

Sample preparation is a major part of any chromatographic analysis. It can include simple procedures such as weighing, dissolving, and diluting a sample, but also more complicated physical (filtration, centrifugation, etc.) and chemical separation procedures (liquid-liquid-extraction, fixed phase extraction). Careful preparation substantially contributes to the quality and reproducibility of chromatographic separations.

In addition, Chromeleon supports two correction factors: ➢*Sample Weight Factor (Weight)* and ➢*Dilution Factor*. They allow you to use the "approximate weight" and to define dilution steps. Thus, they can be used to consider sample preparation during data evaluation.

Sample Processing

Sample processing includes three major steps:

- Sample definition (single samples and ➢*Sequence* and/or ➢*Batch*)

- Analytical procedure (manual or automatic control (➢*PGM File*))

- Evaluation (➢*QNT Methods* and reports)

The performance of each step depends on the methods that are used and on the available instruments. The working environment could range from a fully automatic sample laboratory with large quantities of samples to single-user applications in a research lab. Easy and quick analysis procedures may be the focus in the first case, while special methods and parameters for peak detection may have priority in the second case. Thus, it is not surprising that functions crucial to one group of users may be irrelevant to others.

Keep this in mind when you read the information in the following sections.

Sample Definition

In Chromeleon, the term **sample** is defined as follows: **Each injection is an individual sample**. Thus, multiple injections from the same sample vial under similar conditions are several samples.

Sample definition is the process of determining how much of a substance is injected, from which vial and under which conditions it is injected, and which evaluation parameters are used. Distinguish between a single sample and a series of samples (= sequence).

Single Sample

To enter a single sample, you can perform all required information and user commands via the keyboard or mouse.

For manual injections, click the **Inject** icon on the **Online** toolbar, enter the injection volume, and perform the injection via a hand-operated valve. If an ➤*Autosampler* is available, determine the sample position via the ⇒*Inject* command. Afterward, start data acquisition via the ⇒*AcqOn/Off* command. When the end of the sample is reached, end data acquisition by clicking the **Acq Off** icon on the **Online** toolbar.

The recorded data is temporarily saved to the **manual** sequence of the default ➤*Datasource* of the system. In network operation, this datasource is identified by the name of the computer from which the user logged on to the 🔼 **Network**.

When data acquisition is complete, Chromeleon prompts you to enter the final storage location for the temporarily saved data.

Sequence/Batch

If several samples are to be processed successively, they are included in a sample list (sequence), together with the instrument control and evaluation information.
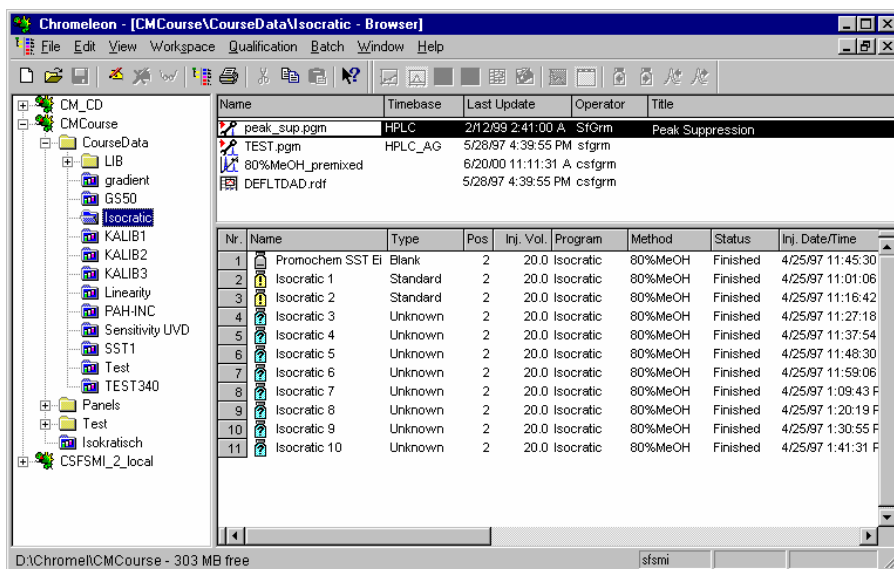
The samples are processed automatically. (For more information, refer to ➤*Batch*.)

For more information about how to create a sample list, refer to 🔼 **The Sample List (Sequence)**.

The Sample List (Sequence)

The sample list is part of the Browser. (For information about the Browser, refer to **Data Management** 📕 **The Browser**). When you select a ➤*Sequence*, the sample list is displayed on the lower right of the Browser window. One line corresponds to one sample. During the chromatographic analysis, the samples to be analyzed are processed from the top to the bottom of the list. Thus, the sample list also determines the order (= sequence) in which the analysis is performed.

The entire data collected for creating and processing a sequence is saved in the sequence directory of a ➤*Datasource* or in the underlying database. This also includes the raw and protocol data recorded during the analysis:

The sample list contains various columns to describe the sample. You may add or delete columns or change their order. In addition, Chromeleon supports ➤*User-defined Columns* and ➤*Sequence Report Columns*, allowing you to adapt the appearance of the list according to your specific requirements. The layout is saved as well. Therefore, if you reedit the sequence later, the appearance will correspond to the one of your last access. The samples are processed in the order in which they appear in the list.

The entries are managed in a database; they are referred to as ➤*Sample Data*. For a brief explanation of the columns, press the F1 key. For more information about the columns, refer to ⇒*Sample Variables*.

Before each new analysis, you have to enter all samples to be processed and the characteristic sample data into the sample list. There are two options:

1. Manually change an existing sequence and save it under a new name.

2. Have Chromeleon create a new sequence automatically via the **Sequence Wizard**.

For more information, refer to **How to …: Creating and Managing Files and Data** **Creating a Sample List**.

In addition to "real" sample data (such as, the sample name, injection volume, vial, and sample type (**unknown** or **standard**)), the sample list also contains the **Program File** and **Method** columns. The entries made in these columns refer to specific ➤*Chromatographic Methods* determining the performance of the analysis. They include the control program (see **Control** **The Control Program**) required for fully automatic control of analytical instruments as well as evaluation instructions (see **The QNT Editor** **The Quantification Method (QNT Editor)**) determining the integration and calibration. In addition, the sample list can contain ➤*User-defined Columns* and ➤*Sequence Report Columns*.

The number of samples per sequence is virtually unlimited, but more than 100 samples should be an exception. The fewer samples a sequence contains, i.e., the more sequences you create instead of including all sample in one sequence, the faster single samples can be accessed and the easier it is for the user to keep track of the processed samples. Criteria for combining several samples in one sequence could be, for example, the same analysis conditions, the same origin, the samples of the same day, etc.

The Sequence Wizard

The Sequence ➢*Wizard* helps you to quickly create a basic sample list consisting of analysis and standard samples. To open the **Sequence Wizard**, select **New** on the **File** menu in the Browser. Follow the steps below to create a ➢*Sequence*:

- **Timebase**: Select the timebase.

- **Unknown Samples**: Generate the analysis samples.

- **Standard Samples**: Generate the standard samples.

- **Methods and Reporting**: Determine the ➢*PGM File* and the analysis method.

- **Saving the Sequence**: Name and save the sequence.

A separate wizard page is available for each step. Clicking **<Back** or **Next>** takes you to the previous or next page.

In the last step, click **<Finish>** to save the sequence and close the wizard.

⚠️ *Caution:*

Do not use special characters (such as the umlaut) for new directory names or sequences, as this may cause problems on Novell networks.

📥 *Note:*

The Timebase step is not included in ➢Chromeleon Xpress. In addition, in the Methods and Reporting step, only a PGM file is selected.

📥 *Note:*

*In future Chromeleon versions, it will be possible to include validation and/or blank run samples with the **Sequence Wizard**.*

For information about how to create a sample list, refer to **How to …: Creating and Managing Files and Data** 📄 **Creating a Sample List**.

The Application Wizard

The Application ➢*Wizard* helps you to quickly generate a ➢*PGM File* and a ➢*QNT Method* for a ➢*Timebase*, using parameters from either an application template or a ➢*Virtual Column* template. You can then use the wizard to create a new ➢*Sequence*.

To open the wizard, select **Application Wizard** on the **Tools** menu in the Browser. The Application Wizard includes the following steps:

- **Step 1**: Choose a timebase

- **Step 2**: Choose an application tool

- **Step 3**: Choose how program and method files are generated

- **Step 4**: Generate and store the files for the application

A separate wizard page is available for each step. Clicking **<Back** or **Next>** takes you to the previous or next page.

🗋 *Note:*

Steps 1 and 4 are not included in ➢Chromeleon Xpress. In addition, method files are not created in Chromeleon Xpress.

Automatic Batch Processing

The technical complexity of modern chromatography systems, the resulting high purchasing costs, and the constantly increasing number of samples in analysis laboratories make continuous operation a necessity. Thanks to ➢*Autosamplers*, very efficient PCs, and modern data systems, this has become routine. The user merely provides "replenishment."

When the actual sample preparation is completed, the chromatographic conditions for processing, the samples to be processed, and the processing order must be communicated to Chromeleon. This information is determined in the sample list and stored as a ➢*Sequence*.

Independently processing of one or several sequences is referred to as ➢*Batch* processing or batch.

Starting the Automatic Batch

To start processing, the following steps are required:

- Open a control panel and select **Edit** on the **Batch** menu. (This command is also available on the **Batch** menu in the Browser.)

- Enter the names of the sequences containing the samples to be analyzed.

- Perform a ➢*Ready Check*.

- Start the analysis by clicking **Start**.

Online Batch: Chromatographic analysis of the batch

When the online batch has been started, all samples of the sequence with the status **single** or **multiple** are analyzed successively. If a sequence contains a sufficient number of samples, sample processing "around the clock" is possible.

Instead of including all samples in one sequence, they can be distributed among several sequences. In this case, enter all sequences in the batch dialog.

The sequence list is considered a batch; it is also referred to as online sample batch or online batch. The order of the sequences determines the order of processing: After the batch has been started, samples 1 to n of the first sequence are analyzed, then samples 1 to n of the second sequence, etc.

Offline Batch: Actions after the chromatographic analysis

The data acquisition results of the batch are saved with the individual samples. When the results are processed offline after data acquisition, e.g., when they are printed, exported, signed, etc., the batch is referred to as offline batch.

Sample Evaluation

In spite of largely automated work processes and intelligent pre-settings, it is the responsibility of each user to set the framework conditions for sample evaluation.

Calculations

Sample processing is performed based on a ➢ *PGM File* that was previously created and included in the sample list. Calculation of the analysis results is based on the evaluation method indicated in the sample list. The method itself is created in the QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor**). For more information, refer to **How to …:** 🔲 **Integrating Chromatograms and Identifying Peaks**.

Result Output

The result of sample processing can be represented in graphics and tables, either on the screen or in a printout. Chromeleon provides method windows for generating this output. Use the **Printer Layout** to define templates (standardized presentations of the sample results). For more information, refer to **How to …:** 🔲 **Creating and Using Report Tables** and 🔲 **Preparing the Printout**.

Overview of the Most Important Results in the Browser

For an overview of the sequence results already in the sample list, add the desired ➢ *Sequence Report Columns* to the list. For more information, refer to **How to …: Creating and Managing Files and Data** 🔲 **Creating a Sequence Report Column**.

For more information about the available method windows, refer to 🔲 **Data Representation and Reprocessing**.

Electronic Signature

During the last decades, quality assurance and ➤*GLP* have become increasingly important. Data verification is one of the key aspects. That is why it is especially important to ensure that ➤*Raw Data* is not modified later. In addition, the results generated from this raw data must not be modified without authorization once they have been accepted. Contrary to a data system, printed records can ensure this in part only.

If ➤*User Mode* is enabled, Chromeleon allows you to electronically sign the results generated from the raw data. This is an important aspect for quality assurance and GLP. Electronic Signature allows you to sign and to protect ➤*Sequence* reports that have been accepted as correct and thus, to 'freeze' the current state of the results.

Note:

Electronic signature is only available for user databases created with a User Manager (CmUser) program version 6.10 or higher. Update your database if an error message notifies you that electronic signature is not supported.

Electronic signature includes three steps:

- Submit

- Review

- Approve

Typically, the user who created the report signs and **submits** it. Afterward, for example, the laboratory manager **reviews** the report and signs it as well. Finally, the quality assurance manager **approves** the results.

For information about how to sign reports electronically, refer to **How to …: Creating and Managing Files and Data** Signing Sequences Electronically**.

Theory of Calibration

Calibration (Overview)

If the signal of a chromatography detector is proportional to the concentration of a substance in the flow cell, it is suitable for quantitative determination. This is a characteristic, for example, of the absorption supplied by a UV detector in the scope of the Lambert-Beer law.

The proportionality constant depends on the chemical quality of the substance of interest and on the physical properties of the used detector. For UV detectors, these are mainly the optical wavelength and the spectral bandwidth. As integration programs can determine only the area (and height, respectively) below a peak, conversion into absolute amount or concentration units is possible only if *calibration* was executed before the analysis.

For more information, refer to:

 Calibration Principle

 Calibration Types (Linear)

 Calibration Types (Non-linear)

 Using the Calibration Curve

 Calculating the Calibration Curve

 Standard Methods

 Evaluation with Various Standard Methods

 Evaluation with the Standard Addition Method

 Implementation

Using the available calibration points, Chromeleon creates calibration curves for each calibrated substance at the end of the calibration. The curves are represented and evaluated in the calibration curve method window (see **Data Representation and Reprocessing** **The Calibration Curve**).

▣ Calibration Principle

Calibration means that one or several samples of known composition are chromatographically analyzed and that an amount (or concentration) / area conversion factor is calculated from the areas detected below the single peaks and the known amounts or concentrations. This conversion factor can then be used to multiply the area of the respective peak of an unknown sample. The result is the corresponding amount (or concentration) of the substance. However, this simple method will work only,

- If the relation between amount and area is strictly linear, i.e., if the Lambert Beer Law is applicable for UV detectors.

- If the area zero equals the amount zero, i.e., if the calibration line leads through the origin (no offset) and

- If matrix effects can be neglected.

If the detector signal (S) is proportional to the concentration (K) of a dissolved substance, the proportionality factor c1 applies:

$$S = c1 * K$$

Under certain conditions, the area F(x) corresponding to a certain amount (x) is proportional to the contained amount.

$$F(x) = c1 * x$$

If a sample of the substance A of known concentration (the standard or calibration sample) is analyzed chromatographically, the result is a specific ratio between the injected amount and the determined area value. The result can be graphically presented by entering the value pair in an amount/area diagram. In this diagram, each injection corresponds to one ➢*Calibration Point*.

Ideally, all calibration points are located on a straight line and a direct ratio between the amount and the determined area exists. The conversion factor corresponds to the slope of the calibration line (left fig. "ideal").

Determined Area Value
e.g. mAU x min
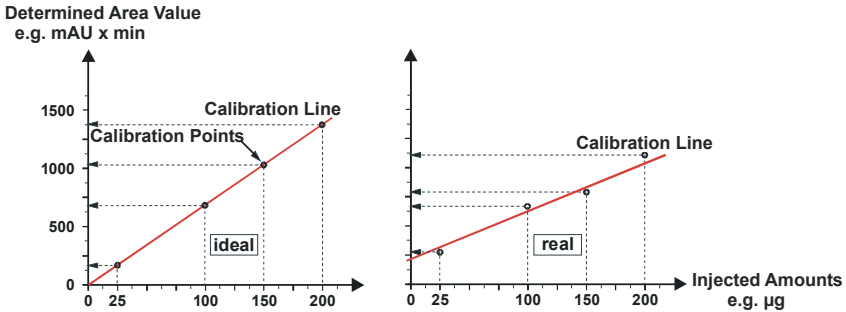
During each calibration, deviations from the ideal behavior might occur. These deviations, which are mainly caused by weight and/or dilution errors, cause scattering of calibration points. Therefore, the Gaussian method of the least squares (see 🔳 **Calculating the Calibration Curve**) is used to calculate a regression line. This line is defined as the best approximation to the existing calibration points. Usually, it does not lead through the origin (right fig. "real").

If the various calibration points are not located on a straight line, but show a parabola or exponential shape, the slope of the curve and the distance to the zero point (**offset**) describe the corresponding (approximate) curve (**calibration curve**). The basic mathematical function is referred to as ➤*Calibration Function*; the coefficients are the calibration coefficients.

By selecting the ⇒*Calibration Type* peak table variable, the user determines whether a linear or non-linear calibration curve is calculated from the existing calibration points. Distinguish between the following calibration types:

🔳 **Calibration Types (Linear)**
🔳 **Calibration Types (Non-linear)**

🔳 Calibration Types (Linear)

If only one calibration sample of a single standard substance is analyzed for the calibration, you have to enter only one concentration value in the first ⇒*Amount* column of the amount table in the QNT Editor. (For more information about the editor, refer to **Data Representation and Reprocessing** 🔳 **The QNT Editor**.) The result is exactly one ➤*Calibration Point*. Connecting the calibration point with the origin forms the calibration curve. It is described by the function derived from the Lambert Beer law:

$$F(x) = c_1 * x$$

The slope of the line corresponds to the proportionality factor c1 (leftmost image); c1 is also called RF value.
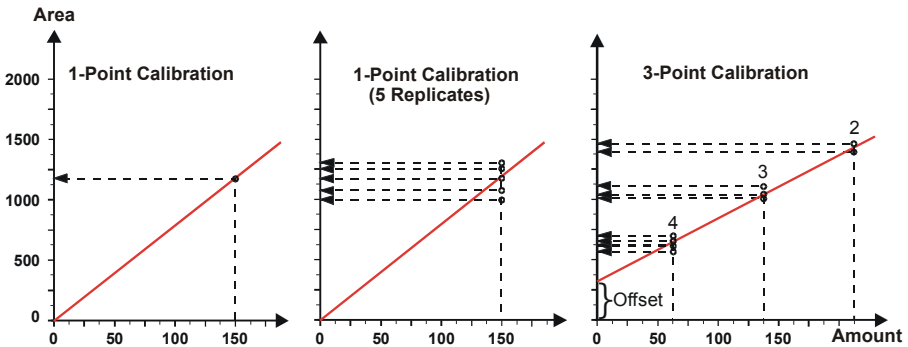
If one calibration sample is analyzed several times, several points can be entered in the amount/area diagram. The points of one concentration are called **replicates**. With an increasing number of replicates, the impact of imprecision decreases after averaging. Although several replicates exist, only one amount/area ratio is determined. This is referred to as **multi-point calibration on one calibration level** (center image).

The result is more reliable if several concentrations are measured. Of course, several replicates can be used per concentration. As a result, calibration points at different concentrations are received in addition to the replicates of one concentration. This is called a **multiple point calibration on several levels** (for example, 3-level calibration (see the rightmost image)). The calibration curve does not necessarily have to go through the origin. The linear ➢ *Calibration Function* is therefore corrected by an offset.

$$F(x) = c_0 + c_1 * x$$

⚠️ *Caution:*

*The decision whether it is physically sensible to use a calibration type other than the **linear** default is the responsibility of the user. (With a calibration type that is physically not sensible, no y values may appear for the actually measured values. For example, if, for the Quadratic calibration type, the calibration function is a bottom-down parabola-shaped curve, the values measured above the summit cannot be evaluated. In this case, Chromeleon reports "n.a.")*

🗔 *Note:*

*If you calibrate using the ➢Standard Addition method and if no calibration points with Amount = 0 are available (**Spiked** samples only), Chromeleon calibrates with an offset.*

*If calibration points with Amount = 0 are available (**Unspiked** samples, also) and if you calibrate using the **Linear** calibration method, the calibration curve does not go through the origin. Instead, it is forced through the mean of all samples for which Amount = 0; i.e., all unspiked samples for this substance. Please note that the results may be different from those obtained by calibrating with the **Linear with Offset** calibration method.*

🔲 Calibration Types (Non-linear)

Non-linear calibration is more general. In this case, more terms are added to the linear ➢Calibration Function.

Parabola-shaped curves are described as follows:

$$F(x) = c_1 * x + c_2 * x^2$$ (Quadratic)

$$F(x) = c_0 + c_1 * x + c_2 * x^2$$ (Quadratic with offset)

In some cases, especially in gas chromatography, there is cubic relationship:

$$F(x) = c_1 * x + c_2 * x^2 + c_3 * x^3$$ (Cubic)

$$F(x) = c_0 + c_1 * x + c_2 * x^2 + c_3 * x^3$$ (Cubic with offset)

⚠️ *Caution:*

*The decision whether it is physically sensible to use a calibration type other than the **linear** default is the responsibility of the user. (With a calibration type that is physically not sensible, no y values may appear for the actually measured values. For example, if, for the Quadratic calibration type, the calibration function is a bottom-down parabola-shaped curve, the values measured above the summit cannot be evaluated. In this case, Chromeleon reports "n.a.")*

To calculate curves of this type, a minimum of two (quadratic), three (quadratic with offset or cubic), or four (cubic with offset) calibration samples must be available (for the quadratic relationship, see the leftmost picture).
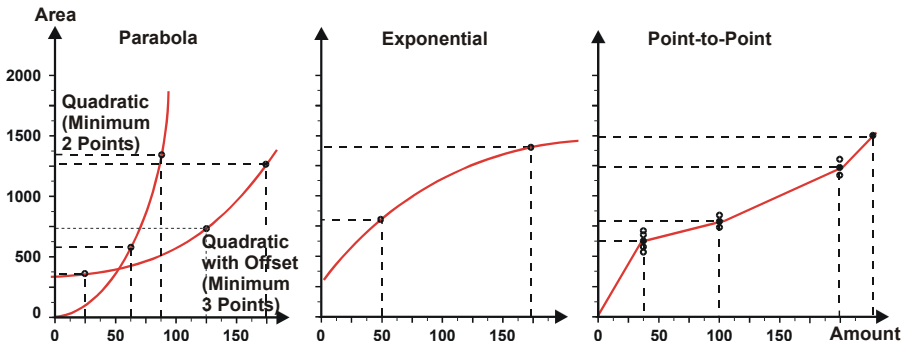
🗋 *Note:*

*If you calibrate using the ➢Standard Addition method and if no calibration points with Amount = 0 are available (**Spiked** samples only), Chromeleon also calibrates with an offset when the **Quadratic** or **Cubic** calibration method is used.*

*If calibration points with Amount = 0 are available (**Unspiked** samples, also) and if you calibrate using the **Quadratic** or **Cubic** calibration method, the calibration curve does not go through the origin. Instead, it is forced through the mean of all samples for which Amount = 0; i.e., all unspiked samples for this substance. Please note that the results may be different from those obtained by calibrating with the **Quadratic with Offset** or **Cubic with Offset** calibration method.*

The power function is described as follows:

$$F(x) = c_0 * x^{c_1}$$

To calculate curves of this type, a minimum of two calibration samples must be available (see the image in the center).

🗒️ *Note:*

If you calibrate using the ➢Standard Addition method and if you use the **Exponential** *calibration function, the expected offset value is subtracted from all calibration points with positive amount values (i.e., from all ➢Spiked Samples). Thus, the following formula applies:*

$$F(x) = \widetilde{c} + c_0 * x^{c_1}$$

Distinguish between the following cases:

*a) If no calibration points with Amount = 0 are available (**Spiked** samples only), the expected offset is calculated using the **Linear with Offset** method, i.e.:*

$$\widetilde{c} = c_0$$

*b) If calibration points with Amount = 0 are available (**Unspiked** samples, also), the expected offset is the average of all samples with Amount = 0.*

If none of the above functions can be applied to the available ➢Calibration Points, the calibration curve can be described as a polygon, i.e., a linear interpolation between two adjacent calibration points (**Point to Point**). If several replicates of one calibration level are available, these are averaged before interpolation.

⚠️ *Caution:*

A large number of replicates increases the precision and the reliability of the curve at this point (on the calibration level), but is not decisive for the entire curve. The more calibration levels are examined, i.e., the more standards of different concentrations are measured, and the more precise is the area/amount allocation for a larger range. To be exact, the calibration is valid only for the range of the calibration samples and not beyond it.

▪ Using the Calibration Curve

If you know the calibration coefficients, you can calculate the amount value for any area value by inserting the coefficients in the formula of the respective calibration type.

Within the range of the curve covered by the calibration points, it is possible to convert any peak area into the corresponding amount (see the example below).

Standard Sample

Enters the amounts (x1 to x4) of the different standard samples and determine the ➤ *Calibration Function* by selecting the calibration type (here: linear with offset). Depending on the selected integration type, area values (F1 - F4) are established from the detected peaks. One area and one substance amount value form one calibration point. The positions of the calibration points determine the curve that Chromeleon calculates with an approximate method. The final course of the calibration curve is determined by the calibration coefficients (here: c0, c1).
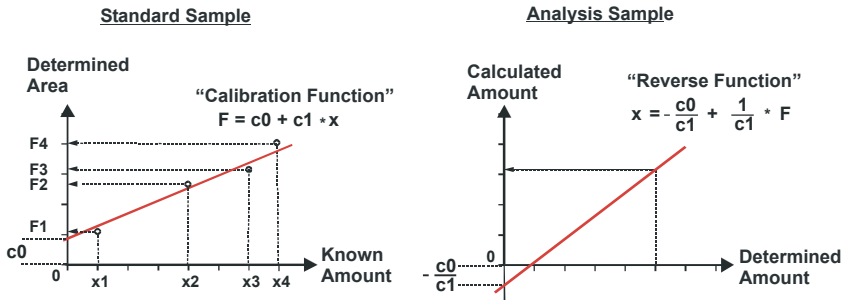
Unknown Sample (Analysis Sample)

For an unknown sample, the previously calculated calibration coefficient and one or several area values are known. The area value is a known parameter and is on the x-axis. This means that the two axes are interchanged in the diagram. As a result, the calibration function must be converted in its inverse function. Chromeleon performs this, also. You can now calculate the amount by inserting the calibration coefficients and area values.

Alternative ways of calculating the calibration curve include the exclusion of outliers, the different weighting of calibration points, and the formation of "averaged" calibration points from one calibration level.

◼ Calculating the Calibration Curve

Calculation of the calibration curve (➢*Calibration Function*) is based on the method of least squares. With a given calibration type (linear, linear with offset, etc.), the parameters of the calibration curve F(a), i.e., c0, c1, c2, and c3, are determined so that the sum of the squared distances of all measured points becomes negligible. For this purpose, the following optimization problem is solved:

$$\sum_{i=1}^{n} w_i * (x_i - F(a_i))^2 \rightarrow \min$$

(xi- F(ai)) refers to the distance of the xi-value from the calibration curve F(a), wi is the selected weighting (see ➢*Weights*, for example, wi=1, wi=1/Amount or wi=1/Amount^2) and xi is the actual value.

The formula used for calculating the calibration curve depends on the ◼ **Standard Method (External/Internal/…)**. For information about these formulas, refer to ◼ **Evaluation with Various Standard Methods**.

◼ Standard Methods

Calibrations can be based on external or ➢*Internal Standards ("ISTD")*. **External standard** means that there is a separate standard sample. Using an **internal standard** means adding the standard to the unknown sample. This can be either before (**External/Internal**) or after (**Internal**) sample preparation.

Standard	Description
external	= Default setting. Calibration is via one or several standard samples. Via the amount values entered in the **Amount** column, a ratio is established between the area and the amount. On this basis, the amount in samples of unknown concentration is determined via the peak area.
	An increasing number of different amounts allows determining the area/amount ratio (=calibration curve) more exactly. If different amounts are obtained by diluting the original substance, a ➢*Dilution Series* is resulting. The amount of each concentration appears in a separate amount column in the corresponding line of the amount table.
	If calibration is performed with a single standard sample by injecting different volumes (**Var.InjectVol.**), only the amount of the original sample is stored in an amount column. Chromeleon calculates the remaining amount values (for the different injection volumes).

internal/ external	Choose a substance as ➤*Internal Standard ("ISTD")* whose retention time behavior is similar to the behavior of the substances to be analyzed. Before the sample is prepared, add exactly the same amount of internal standard to all samples (unknown and standard samples) so that its concentration is identical in all samples. For example, diluting the sample or performing a pre-column derivatization later will change the concentration of the internal standard. During calibration, the internal standard and the substances to be determined are calibrated.
internal	Instead of using absolute areas and amounts, area and amount ratios are used for calibration. This procedure requires that a constant amount of the ➤*Internal Standard ("ISTD")* is injected, i.e., that the standard is added before sample preparation, but that, for example, it is not diluted in a dilution series (**Const. Internal Standard**). Due to the equivalent amount of added internal standard, the same ISTD result should be resulting for all samples. Forming the ratio of ISTD values allows you to draw conclusions about the precision of the analysis and to calculate the actual result.
	Due to the intense experimental procedure, this type of calibration is rarely used in HPLC.

For examples of the different standard methods, refer to **How to …: ⬛ Calibrating**.

⬛ Evaluation with Various Standard Methods

Below please find a description of how Chromeleon calculates calibration points, which form the basis for any calibration function **F**. Note that the formula used for the calculation depends on the selected ⬛ **Standard Methods** (External, Internal/External (with/without var. ISTD), Internal (with/without var. ISTD)).

For evaluating unknown samples, i.e., calculating the ⇒*Amount* values, the inverted form of the corresponding ➤*Calibration Function* **F** is used (=inverted function **A**). This means that the c0, c1, c2, and c3 calibration coefficients form function **A** that is inverted for amount calculation. The result is **F**(x).

🖙 *Tip:*

For the formulas for the Standard Addition method, refer to ⬛ *Evaluation with the Standard Addition Method.*

'External' Evaluation:

Calibration:

- Y(i,k) = RESPONSE(i,k)

- X(i,k) = AMOUNT_NOMINAL(i,k) $*$ WEIGHT(k)/DILFAC(k) $*$ INJECTVOL(k)/REFINJECT

Evaluation: Calculation of the Amount peak variable for peak i in the sample x

- AMOUNT(i,x) = DILFAC(x)/WEIGHT(x). RSP-FACTOR(i) * F[RESPONSE(i,x)]

   Description: see below.

'Internal' Evaluation:

Calibration:

- Y(i,k) = 100. RESPONSE(i,k)/RESPONSE(ISTD,k)

- X(i,k) = AMOUNT_NOMINAL(i,k). WEIGHT(k)/DILFAC(k)

Evaluation: Calculation of the Amount peak variable for peak i in the sample x

- AMOUNT(i,x) = DILFAC(x)/WEIGHT(x). RSP-FACTOR(i).
                    F[100. (RESPONSE(i,x)/RESPONSE(ISTD,x)]

   The ISTD peak itself will not be evaluated!

   Description: see below.

'Internal' Evaluation (with variable ISTD):

Calibration:

- Y(i,k) = AMOUNT_NOMINAL(ISTD). RESPONSE(i,k)/RESPONSE(ISTD,k)

- X(i,k) = AMOUNT_NOMINAL(i,k). WEIGHT(k)/ DILFAC(k). INJECTVOL(k)/REFINJECT

Evaluation: Calculation of the Amount peak variable for peak i in sample x

- AMOUNT(i,x) = DILFAC(x)/WEIGHT(x). RSP-FACTOR(i). F[AMOUNT_SOLL(ISTD).
                    (RESPONSE(i,x)/RESPONSE(ISTD,x)]

   The ➢*Internal Standard ("ISTD")* peak itself is not evaluated!

   Description: see below.

'Internal/External' Evaluation:

Calibration:

'External' calibration, including the ISTD peak! (Also, refer to 'External')

Evaluation: Calculation of the Amount peak variable for peak i in the sample x

- The ISTD peak itself is evaluated 'Externally'!

- $FACTOR\_IS(x) = AMOUNT\_NOMINAL(ISTD)/AMOUNT(ISTD,x)$

- $AMOUNT(i,x) = DILFAC(x)/WEIGHT(x)$. $RSP\text{-}FACTOR(i)$. $FACTOR\_IS(x)$. $F[RESPONSE(i,x)]$

  Description: see below.

'Internal/External' Evaluation (with variable ISTD):

Calibration:

Calibration is 'External', including the ISTD peak! However, the nominal amount for the ISTD peak from the sample list (ISTD Amount) is used. The sample weight of the ISTD peak is not considered.

- $Y(i,k) = RESPONSE(i,k)$

- $X(i,k) = AMOUNT\_NOMINAL(i,k)$. $WEIGHT(k)/DILFAC(k)$. $INJECTVOL(k)/REFINJECT$

- $X(ISTD,k) = AMOUNT\_NOMINAL(ISTD,k)$. $1/DILFAC(k)$. $INJECTVOL(k)/REFINJECT$

Evaluation: Calculation of the Amount peak variable for peak i in the sample x

- The ISTD peak itself is evaluated 'Externally', but without Weight correction.

- AMOUNT(ISTD,x) = DILFAC(x). RSP-FACTOR(ISTD) F[RESPONSE(ISTD,x)]

- FACTOR_IS(x) = AMOUNT_NOMINAL(ISTD,x)/AMOUNT(ISTD,x)

- AMOUNT(i,x) = DILFAC(x)/WEIGHT(x). RSP-FACTOR(i). FACTOR_IS(x). F[RESPONSE(i,x)]

Description:

F:	Calibration function
X(i,k):	X-coordinate of a calibration point for peak i for the standard sample k
Y(i,k):	Y-coordinate of a calibration point for peak i for the standard sample k
k:	Calibration sample (standard)
x:	Unknown sample (analysis sample)
RESPONSE(i):	Reference variable (Int.Type; that is, Area, Height, CE-Area) of peak i in a sample
RESPONSE(ISTD):	Reference variable (Int.Type; that is, Area, Height, CE-Area) of the corresponding ISTD peak of a sample
AMOUNT_NOMINAL(i):	Amount of peak i from the amount table for the standard sample k
AMOUNT_NOMINAL(ISTD):	⇒*ISTD Amount* for the sample k from the sample list
AMOUNT(ik):	Calculated amount of the peak i for the sample k
WEIGHT:	⇒*Weight (Sample Weight Factor)* for a sample
DILFAC:	⇒*Dil. Factor (Dilution Factor)* for a sample
INJECTVOL:	⇒*Inj. Vol. (Injection Volume)*
REFINJECT:	Injection volume of the first sample in a calibration series
RSP-FACTOR(i):	⇒*Response Factor* of the peak i from the QNT amount table

◩ Evaluation with the Standard Addition Method

For the ➢*Standard Addition* method, calibration and evaluation are slightly different from "normal" calibration. One reason is that the original sample has a spiked amount = 0. Therefore, multiplication with factors it not possible.

⚠️ *Caution:*

Only if the calibration curve is forced through the origin (i.e., in this case, through the calibration point of the original, unspiked sample), the amount of this sample corresponds to the negative intercept on the x-axis, i.e., to the average of all values based on this sample. For all other calibration types, the calculated amount of the original, unspiked sample may deviate from the negative intercept on the x-axis of the calibration curve.

'External' and **'Internal/External' Evaluation:**

Calibration:
- $Y(i,k) = RESPONSE(i,k) / DILFAC(x)/WEIGHT(x)_{\cdot} INJECTVOL(k)/REFINJECT)$
- $X(i,k) = AMOUNT\_NOMINAL(i,k)$

Evaluation: Calculation of the Amount peak variable for peak i in the sample x
- $AMOUNT(i,x) = RSP\text{-}FACTOR(i)_{\cdot} F[RESPONSE(i,x)_{\cdot} DILFAC(x)/WEIGHT(x)]$

Description: see ◫ *Evaluation with Various Standard Methods.*

'Internal' Evaluation:

Calibration:
- $Y(i,k) = 100_{\cdot} RESPONSE(i,k)/RESPONSE(ISTD,k)$
- $X(i,k) = AMOUNT\_NOMINAL(i,k)_{\cdot} WEIGHT(k)/DILFAC(k)_{\cdot} DILFAC(x)/WEIGHT(x)$

Evaluation: Calculation of the Amount peak variable for peak i in the sample x
- $AMOUNT(i,x) = RSP\text{-}FACTOR(i)_{\cdot} F[100_{\cdot} RESPONSE(i,x)/RESPONSE(ISTD,x)_{\cdot} DILFAC(x)/WEIGHT(x)]$

  The ISTD peak itself will not be evaluated!

'Internal' Evaluation (with variable ISTD):

Calibration:

- $Y(i,k)$ = AMOUNT_NOMINAL(ISTD). RESPONSE(i,k)/RESPONSE(ISTD,k) / DILFAC(x)/WEIGHT(x). (NJECTVOL(k)/REFINJECT)

- $X(i,k)$ = AMOUNT_NOMINAL(i,k)

Evaluation: Calculation of the Amount peak variable for peak i in sample x

- AMOUNT(i,x) = RSP-FACTOR(i). F[AMOUNT_SOLL(ISTD). RESPONSE(i,x)/RESPONSE(ISTD,x). DILFAC(x)/WEIGHT(x)]

  The ➢*Internal Standard ("ISTD")* peak itself is not evaluated!

'Internal/External' Evaluation (with variable ISTD):

Calibration:

Calibration is 'External', including the ISTD peak! However, the nominal amount for the ISTD peak from the sample list (ISTD Amount) is used. The sample weight of the ISTD peak is not considered.

- $Y(i,k)$ = RESPONSE(i,k). DILFAC(k)/WEIGHT(k). REFINJECT/INJECTVOL(k)

- $X(i,k)$ = AMOUNT_NOMINAL(i,k)

- $Y(ISTD,k)$ = RESPONSE(i,k). DILFAC(k). REFINJECT/INJECTVOL(k)

- $X(ISTD,k)$ = AMOUNT_NOMINAL(ISTD,k)

Evaluation: Calculation of the Amount peak variable for peak i in the sample x

- The ISTD peak itself is evaluated 'Externally', but without Weight correction.

- AMOUNT(ISTD,x) = RSP-FACTOR(ISTD). F[RESPONSE(ISTD,x). DILFAC(x)]

- FACTOR_IS(x) = AMOUNT_NOMINAL(ISTD,x)/AMOUNT(ISTD,x)

- AMOUNT(i,x) = RSP-FACTOR(i). FACTOR_IS(x). F[RESPONSE(i,x). DILFAC(x)/WEIGHT(x)]

▣ Implementation

Follow the description below to implement and perform calibration:

Sample List

- Enter the available standard samples in the ▣ **Sample List (Sequence)**, similar to unknown samples.

- To facilitate and automate the input, use the ▣ **Sequence Wizard**.

- Each sample in the sample list can be converted into a standard sample by assigning the ➢*Sample Type* **STD**. Note that the position (line number) in the sample list determines the order of processing. If a standard sample is to be injected several times, a separate line is created in the sample list for each injection.

- In the **Method** column, specify the quantification method to be used for evaluating the sample.

- Input in the remaining fields of the sample list is analog to each unknown sample (**Position**, **Injection Volume**, etc.).

- For a detailed description of the procedure, refer to **How to …: Creating and Managing Files and Data** ▣ **Creating a Sample List**.

Quantification Method (QNT Editor)

- Go to the **General** tab page and check the current settings. Define the calibration⟹ *Mode* to be used.

- On the **Peak Table** tab page, enter the names and the retention times of the peaks to be determined. Select the **Autogenerate Peak Table** command if a processed sample refers to a QNT File with an "empty" peak table. This command fills the peak table automatically with the retention times of the integrated peaks after the analysis. Each peak has a default name extended by a successive number.

- Enter the amount values of the standard substances in the amount columns.

- Determine the standard method, calibration type, and integration type to be used for the calibration.

- For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** ▣ **Creating a Peak Table**.

Validation, AutoQ and System Wellness

Validation and Qualification

Analysis data from various workstations or laboratories can be compared only if it is possible to determine the quality of the results produced with a chromatography system.

Validation (Definition)

The process of ensuring that a system and its analysis procedures supply reproducible and reliable results is referred to as validation. This includes above all procedures regarding the planning, implementation, and documentation of an analytical method. Thus, validation is an integral part of ➤ *GLP ("Good Laboratory Practice")*.

Qualification (Definition)

First, the manufacturer validates the single devices and the data system. During qualification, the user checks whether a device or data system works according to its specification. This includes procedures guaranteeing the optimum technical condition of instruments (hardware and firmware) and of computers (hardware and software). Thus, qualification, too, is an integral part of Good Laboratory Practice.

When is qualification necessary?

Instruments should be qualified before setting them into operation and at regular intervals thereafter; especially after exchanging worn-out parts, performing repair work, or replacing an instrument by a new one. Also, perform system qualification procedures after you have updated the software of your data system.

In addition, the data system itself should be qualified at least after an update. ➤ *Installation Qualification* and ➤ *Operational Qualification* are available for this. In addition, you can check the datasource performance (= datasource ➤ *Performance Qualification (PQ)*). Performing the different qualifications is usually the task of the system administrator.

For more information, refer to 🔲 **Validation and Qualification** in the **Administrator Help** section.

Many instruments perform an automatic self-test upon startup to ensure optimum function. For example, for the Dionex UVD 340U ➢*Photodiode Array Detector*, spectra calibration is performed automatically via the ➢*Holmium Oxide Filter* whenever the detector is started.

When is validation necessary?

The analytical method and the ➢*PGM File* should be validated before they are used in daily laboratory procedures. As modifying single parameters can already be of great importance, validation is also necessary in the daily routine. On the **SST** tab page of the QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor**), define ➢*System Suitability Tests* to check whether your analytical method and your program file are suitable for analyzing special samples.

How is validation performed?

The focus of an analysis procedure is on Calibration (see **Theory of Calibration** 🔲 **Calibration (Overview)**). Within the scope of validation, it is then important to check whether calibration has been performed correctly. The precision, ➢*Limit of Detection*, dynamic work range, and robustness of a procedure and the involved components have to be determined. The following features are available for this: ➢*Validation Samples*, ➢*Blank Run Samples*, ➢*Matrix Blank Samples*, ➢*Confidence Interval/Range*, averaging, normal distribution, outlier tests, detection of statistical and systematic errors, ➢*Correlation Coefficient*, ➢*Standard Deviation*, ➢*Relative Standard Deviation*, etc.

Chromeleon provides numerous options to meet all GLP, qualification, and validation requirements. For more information, refer to:

🔲 **AutoQ Equipment Qualification**

🔲 **The System Suitability Test (SST)**

🔲 **System Wellness for IC Devices (Overview)**

🔲 **System Wellness for Summit HPLC Devices**

🔲 **System Wellness and Predictive Performance for UltiMate 3000 Devices (Overview)**

◼ AutoQ Equipment Qualification

Dionex AutoQ is a comprehensive range of user-friendly Chromeleon qualification tools. These tools help you quickly and easily perform qualification tasks that would otherwise be troublesome and time-consuming. In addition, they simplify compliance with qualification standards and qualification rules. AutoQ qualification tests are available for Chromeleon software and for several HPLC and IC systems. AutoQ test procedures can automate the following tasks.

For Chromeleon software:

- ➢*Installation Qualification* (IQ)

- ➢*Operational Qualification* (OQ)

For instruments:

- Installation Qualification (IQ)

- Operational Qualification (OQ)

- ➢*Performance Qualification* (PQ)

What is unique about Dionex AutoQ?

Dionex AutoQ is a comprehensive suite of qualification procedures for instruments from several manufacturers. AutoQ is available for the following systems:

- **Dionex** IC modules and **Summit** HPLC modules

- **Agilent 1100 HPLC System** modules

- **Shimadzu LC10 and LC2010 HPLC** instruments

- **Shodex RI-101 HPLC Refraction Index Detector**

- **TSP HPLC modules**

- **Waters** HPLC modules (including the **Alliance 2690/2695** Systems and the 996/2996 PDAs)

Which standards and regulations does Dionex AutoQ help you to meet?

The following standards and regulations are important in a validated environment:

- Good Laboratory Practice (➢*GLP*)

- Current Good Manufacturing Practice (cGMP)

- 21 CFR Part 11

- ISO 9000

What are the benefits of AutoQ?

- Most AutoQ tests run automatically most of the time. Therefore, they require very little operator time. For example, it only takes about 30 minutes to prepare the Instrument OQ, after which Chromeleon runs the test automatically. In comparison, the conventional test routines used in many validated laboratories typically require a full day of the analyst's time.

- The high level of automation reduces the risk of errors and ensures comparable results.

- Chromeleon automatically documents the results. The reports created by the system include charts, calculations, and the single results (**Passed/Failed**).

- AutoQ is virtually identical for all instruments, regardless of the manufacturer. This means:

    - Considerable time savings for the creation and maintenance of SOPs (Standard Operating Procedures) for laboratories using instruments from different manufacturers.

    - Only one test procedure to learn and work with.

    - Test reports have the same format for all instruments.

    - All reports can be easily managed, saved, and stored as electronic documents, using Chromeleon electronic reports and ➢*Electronic Signatures*.

- AutoQ instrument qualification tests can be adapted for use with instruments from other manufacturers.

Tip:

> *This requires advanced knowledge of report creation in Chromeleon.*

- AutoQ qualification procedures are included in every Chromeleon software package.

Note:

> *Certified standard solutions, which are available from Dionex, are needed to run AutoQ qualification tests.*

Instruments Operational and Performance Qualification

After the instruments of a chromatography system have been installed, Installation Qualification should be performed. Usually, the system administrator performs this check. Therefore, refer to **Validation and Qualification** **Instruments Installation Qualification** in the **Administrator Help** section for more information. Afterward, perform ➢*Operational* and/or ➢*Performance Qualification* for the instruments.

The **Qualification** menu in the Chromeleon Browser provides the following options: **Instruments PQ** and **PQ Setup** and **Instruments OQ** and **OQ Setup**. Select **Instruments PQ** (or **Instruments OQ**) to perform the performance (operational) qualification. Select **PQ Setup** (or **OQ** Setup) to create the templates required for performing the performance (operational) qualification. Usually, this is necessary only during the initial installation of the system or if the configuration has been changed.

Note:

Only qualified Dionex service personnel should perform the Performance and Operational Qualification checks. For more information, please contact Dionex Service.

For information about performance qualification, refer to **Operational and Performance Qualification for HPLC Systems** and/or **Performance Qualification (PQ) for IC Systems**.

In addition, you can perform operational qualification for the ➢*UCI Universal Chromatography Interface*. For more information, refer to the *UCI Operational Qualification Operating Instructions*.

◾ Operational and Performance Qualification for HPLC Systems

Chromeleon provides a datasource with a master template in the Template directory on the Chromeleon software CD. This template is designed for Performance (Operational) Qualification in a standard HPLC configuration. In addition to various sequences, the template contains a ➤*Report Definition File (RDF)* for OQ and PQ, providing the following pages:

SPECIFICATION: On the **SPECIFICATION** page, enter the system specifications (instruments, fluidics, and limits).

COLUMN OVEN: The **COLUMN OVEN** page indicates whether the column oven temperature corresponds to the selected temperature (within the specified limits).

INJ_REPRO_AND_RET_REPRO and **ASI_100_REPRO_AND_RET_REPRO**, respectively: On the **INJ_REPRO_AND_RET_REPRO** page, serves for checking the reproducibility of the injector and the retention time. If the result is within the specified limits, the **Result** column indicates **OK**.

INJ_CARRY_OVER: The **INJ_CARRY_OVER** page supplies a measure for the carry over in your system. If the result is within the specified limits, the **Result** column indicates **OK**.

INJ_LINEARITY: The **INJ_LINEARITY** page supplies a measure for the linearity of injection volume and peak area.

PUMP_GRADIENT: On the **PUMP_GRADIENT** page serves for checking the gradient precision. The limits of the **Specification** page are included. If the values are within the specified limits, the last column (**Result**) indicates **OK**. The **Result of all tests** column indicates **Test passed**.

PUMP_GRADIENT_REPRO: The **PUMP_GRADIENT_REPRO** page serves for checking the gradient reproducibility (with 3 repetitions in this example). The limits of the **Specification** page are included. If the values are within the specified limits, the last column (**Result**) indicates **OK**. The **Result of all tests** column indicates **Test passed**.

RI_NOISE_AND_DRIFT and **RI_LINEARITY**: These pages serve for evaluating the noise, drift, and linearity of the Shodex Refractive Index Detector.

DET_NOISE_AND_DRIFT: The **DET_NOISE_AND_DRIFT** page serves for checking whether noise and drift in your system correspond to the limits on the **SPECIFICATION** page. If the values are within the specified limits, the **Result column** indicates **OK**.

DET_WAVELENGTH: The **DET_WAVELENGTH** page supplies a measure for the wavelength precision of the corresponding detector.

DET_LINEARITY: The **DET_LINEARITY** page serves for checking the detector linearity. From five different injections at different concentrations, the correlation coefficient (supplied in %) between the peak height and the concentration is determined. If the value is above the specified limit, **Test passed** is returned as the result.

RF_DET_NOISE and **RF_DET_WAVE**: These pages evaluate the noise and the wavelength precision of the fluorescence detector.

Audit Trail: The **Audit Trail** page displays the Audit Trail for the analyzed sample.

⚠️ *Caution:*

Do not edit the report pages (except the Specification page), even if editing is possible. Chromeleon automatically reads the corresponding values and often references individual data sheets in the report. If you insert or delete lines and columns, these references will be lost. Thus, the calculations will be wrong.

*The report **must** be printed as **Batch Report** from the browser. This ensures that Chromeleon reads and processes the data correctly in the report. Select the sequence for which you want to print the report. **Make sure that no sample is selected**. Select **Batch Report** on the **File** menu and click **OK** to start printing.*

👉 *Tip:*

Enter the actual concentrations of the used standards in the Amount columns of the QNT File for evaluating the detector linearity.

For more information about Operational and Performance Qualification, refer to the **Operational Qualification/Performance Qualification** Operating Manual that is available from Dionex Service.

◾ Performance Qualification (PQ) for IC Systems

Validation is becoming increasingly important to analytical laboratories. Documented evidence must be provided to demonstrate the integrity of data collected and validate the results obtained on laboratory instrumentation.

The **Qualification** menu in the Chromeleon Browser includes options for **Instruments PQ** and **PQ Setup**. **Instruments PQ** is used to perform the performance qualification. **PQ Setup** is used to generate the templates required for performing the performance qualification. (This is generally necessary only after a new installation or after changes to the configuration.) The Chromeleon CD provides a datasource with a master template in the PQ\Templates\PQ directory.

PQ should be performed at regular intervals after the initial installation and Operational Qualification (OQ). Dionex recommends performing PQ every six months. A qualified Dionex Service Representative should perform all tests, in accordance with the instructions in the *IC System Operational and Performance Qualification User's Guide*. The user's guide is included in the IC OQ/PQ Kit with Test Cells (P/N 057599) and the IC OQ/PQ Basic/Refill Kit (P/N 057608).

The PQ procedure used to qualify Dionex Ion Chromatography Systems meets the requirements established by the National Institute of Standards and Technology (NIST) and the American Society for Testing and Materials (ASTM). This PQ procedure provides qualification testing for **ICS-3000**, **ICS-2500, ICS-2000, ICS-1500, ICS-1000, DX-600, DX-500, DX-320, DX-120,** and **BioLC** systems.

◾ The System Suitability Test (SST)

On the **SST** tab page in the QNT Editor (see **Data Representation and Reprocessing** ◪ **The QNT Editor**), define a ➢*System Suitability Test (SST)* to check whether the quantification method and the ➢*PGM File* are suitable for analyzing special samples. (For more information, refer to **How to …: Performing Validation and Qualification** ◪ **Defining System Suitability Tests**.)

You can perform the System Suitability Test while Chromeleon records the chromatogram. The related QNT Method needs to be available in the sample list (in the Browser). As **Fail Action**, select **Abort Batch** to automatically abort the sample ➢*Batch* if a test condition is not met.

System Wellness for IC Devices (Overview)

What is System Wellness?

System Wellness monitors the overall "health" of a chromatographic system. It provides built-in diagnostic and calibration features that help prevent unscheduled system shutdowns and assure reliable operation of system devices. Calibration and diagnostic commands are available from Wellness control panels and Help topics are provided for performing the various tasks.

Supported Devices

For System Wellness support, a device must have a version of ➢*Moduleware* installed that supports System Wellness. The following devices are supported:

Device	Moduleware Version Required
IC Pumps	
GP40/IP20	3.46 (or higher)
GP50/IP25	3.46 (or higher)
GS50/IS25	1.00 (or higher)
Detectors	
AD25	1.02 (or higher)
CD	1.0.0 (or higher)
CD20/ED40	3.05 (or higher)
CD25/ED50	1.05 (or higher)
CD25A/ED50A	1.00 (or higher)
ED	1.0.0 (or higher)
PDA-100 (DX-LAN)	1.04 (or higher)
PDA-100 (USB)	1.0.0 (or higher)
➢*Autosampler*	
AS	2.0.0 (or higher)
AS50 (DX-LAN)	1.05 (or higher)
AS50 (USB)	1.00 (or higher)
➢*Eluent Generator*	
EG40	2.23 (or higher)
EG50	2.26 (or higher)
System Modules	
IC20	3.08 (or higher)

IC25	1.04 (or higher)
IC25A	1.00 (or higher)
ICS-90	1.00 (or higher)
ICS-1000	1.1.0 (or higher)
ICS-1500	1.1.0 (or higher)
ICS-2000	1.1.0 (or higher)

System Wellness Features

- System Wellness control panels, which allow easy access to diagnostic and calibration commands and data

- Download of current, previous, or factory calibration data

- Leak detector testing and calibration

- Wavelength verification and calibration for UV and PDA detectors

- Cell calibration for conductivity detectors

- pH calibration for amperometry detectors

- Warning when Flow = 0 while the detector lamp is burning. This is to prevent damage resulting from running the flow cell dry.

- Pressure offset and degas calibration for pumps

- Flow rate calibration for pumps

- Solvent and waste level monitoring

For instructions on setting up and using System Wellness features, refer to **How to …: Performing Validation and Qualification** **Ensuring System Wellness**.

System Wellness for Summit HPLC Devices (Overview)

Chromeleon provides several system wellness features for the Summit HPLC modules.

Tip:

The user does not have to calibrate these modules because either they have been calibrated in the factory (e.g., the pump flow) or calibration is performed automatically (for example, for the UV detector via a ➢*Holmium Oxide Filter).*

Device	Feature	Description
P680	Leak detector	Reliable operation
	Monitoring of piston seal tightness	Reliable operation
	Pressure limits	Prolongs the life of HPLC columns, reliable operation
	Total workload monitoring	Allows you to schedule the next service date.
	Degasser vacuum level monitoring	Reliable operation
	Active rear-seal wash system	Prolongs the life of the consumable parts
	Monitoring of the liquid level for rear-seal washing	Reliable operation
	Solvent and waste level monitoring	Reliable operation
ASI-100	Leak detector	Reliable operation
	Injection counter	Allows you to schedule the next service date.
	Needle seal wear monitoring	Allows you to schedule the next service date.
	Rotor wear and stator wear monitoring for the internal motorized switching valve	Allows you to schedule the next service date.
	Needle port wear monitoring	Allows you to schedule the next service date.
	Synchronization of injection and pump cycle	Injection reproducibility
TCC-100	Gas and leak detectors	Reliable operation
	Column identification features	Tracking and troubleshooting
	Temperature limit monitoring	Tracking and troubleshooting

UVD340U	Lamp ignition counter	Allows you to schedule the next replacement date
	Lamp age monitoring	Allows you to schedule the next replacement date
	Minimum lamp intensity monitoring	Reliable operation
	Warning when Flow = 0 when the detector lamp is burning.	Avoids damage resulting from running the flow cell dry.
System	Documentation when new wear parts have been installed.	Tracking and troubleshooting
	Documentation when the solvent has been changed	Tracking and troubleshooting

For information about the System Wellness and Predictive Performance features of the UltiMate 3000 modules, refer to 🔳 **System Wellness and Predictive Performance for UltiMate 3000 Devices (Overview)**

🔳 System Wellness and Predictive Performance for UltiMate 3000 Devices (Overview)

The UltiMate 3000 modules provide various integrated functions for estimating the lifetime of wear parts and monitoring and recording service and (re)qualification information. These are referred to as *System Wellness* and *Predictive Performance*.

The related commands are available on special panels or in the **Commands** dialog box.

Supported devices

For predictive performance support, the device must have a version of ➢*Firmware* installed that supports predictive performance. The following devices are supported:

Device	Firmware Version Required
UltiMate 3000 pumps	
DGP-3600	2.79.07 (or later)
ISO-3100A,	2.79.07 (or later)
HPG-3x00	2.79.07 (or later)
LPG-3x00	2.79.07 (or later)
UltiMate 3000 thermostatted column compartments	
TCC-3x00	1.05.18 (or later)

UltiMate 3000 detectors
    VWD-3x00   1.1.9 (or later)
    PDA-3000   2.2.0 (or later)

UltiMate 3000 autosamplers
    WPS-3000(T)SL   1.29.17 (or later)

System wellness and predictive performance features

- Lifetime forecast for wear parts

- Integrated counters for monitoring several devices and module parameters

- Reminder function for maintaining service and qualification periods

- Warning messages when limits are exceeded

- Graphical display for monitoring the service and qualification status

- Storage of parameter data in the device firmware

For more information, refer to **How to …**: **Validation and Qualification** ⬛ **Predictive Performance**.

Chromeleon supports specific system wellness and predictive performance commands. For a list of available commands, refer to:

⬛ **Dionex UltiMate 3000 Series - Pumps**

⬛ **Dionex UltiMate 3000 Series - Autosamplers**

⬛ **Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments**

⬛ **Dionex UltiMate 3000 Series - Variable Wavelength Detectors**

⬛ **Dionex PDA-100/PDA-3000 Photodiode Array Detectors**

Data Representation and Reprocessing

Data Representation and Reprocessing

Chromeleon allows you to represent your raw data under various aspects. It depends on the aspect of interest and on the type of data reprocessing you wish to perform, which window or pane is most appropriate. For example, you can reintegrate a chromatogram manually, compare several chromatograms, analyze the peak purity, calculate calibration curves, or search single spectra in a spectra library.

Each operation appears in a separate window. Each window is intended for one specific task and has its own window arrangement and menu structure.

Immediately after data acquisition, you can perform certain steps for data reprocessing. You can define these steps in the ➢*Program* in the PGM Editor; see **Post-Acquisition Steps**.

You can perform all other steps of data reprocessing at any time. Use the following windows (or panes):

The Quantification Method

Integration

Report Tables

Calibration (Overview)

The Printer Layout

PPA: Peak Purity Analysis

Spectra Libraries

After the raw data has been acquired, the Quantification Method determines how the data is evaluated. Thus, the QNT Editor with the Integration window is the most important window for data reprocessing.

If, after the analysis, the detection and peak table parameters chosen in the QNT Method prove to be inappropriate for the sample and/or sequence, you can change them in the QNT Editor at any time. You do not need to analyze the corresponding sample again.

All modifications are immediately and globally effective. All modified variables are immediately re-calculated and the new values are displayed on the screen. The modifications are finally accepted by selecting the **Save** or **Save Manipulations** command.

If the modified quantification method is saved, the results of all samples evaluated by this method will be adjusted.

Post-Acquisition Steps (PGM Editor)

Immediately after data acquisition, you can perform certain data reprocessing steps. You can use the **Post-acquisition steps** view to define these steps in the ➢*Program* via the PGM Editor. (Also, refer to **Control 🔳 The PGM Editor**.)

The following data reprocessing steps are available in the Post-acquisition view:

- **Arithmetic combination of channels** (arithmetic combination of 2D channels - see 🔳 **Combining Channels via Arithmetic Operations**)

- **Copy Channel** (see 🔳 **Copying a Channel**)

- **Create Fraction Analysis Samples** (see **How to …: Collecting Fractions Automatically (Autopurification) 🔳 Creating Fraction-Type Samples**)

- **Create Purification Samples** (see **How to …: Collecting Fractions Automatically (Autopurification) 🔳 Creating Preparation-Type Samples**)

🔳☞ *Tip:*

*The **Create Fraction Analysis Samples** and **Create Purification Samples** options are part of ➢Autopurification. They are visible only if the **Purification** license is installed and if a connection to the server exists. A connection exists if a PGM File is associated with a timebase, the server on which this timebase resides is running, and the client is connected to this server.*

- **Extract ED channel** (for extracting an ED channel – only possible if ➢*3D_Amp* data is available)

- **Extract MS channel** (for extracting a ➢*Mass Trace* - only possible if MS data is available; see **How to …: Using Mass Spectrometers** **Extracting Mass Traces Afterward**)

- **Extract optimum integration path** (for extracting the ➢*Optimum Integration Path* - only possible if a 3D field is available; see **How to …: Displaying and Using Spectra Libraries** **Selecting the Optimum Integration Path**)

- **Extract UV channel** (for extracting a UV channel - only possible if a 3D field is available; the procedure is similar to selecting the optimum integration path)

- **Smooth data** (for chromatogram ➢*Smoothing* - see **How to …: Working with Chromatograms** **Performing Data Smoothing**)

For more information, refer to **How to …: Creating and Modifying Programs** **Adding Post-Acquisition Steps**.

The QNT Editor

The Quantification Method

In Chromeleon, all instructions and parameters representing the basis of calculation for the sample evaluation are included in the Quantification Method (QNT File). This refers for example to the following questions:

- Below which height, width, or area a peak will be ignored?

- Which course has the baseline?

- Which peaks are classified as ➤*Riders*?

- What is the ⇒*Amount* of the standard samples?

- Which ➤*Calibration Function* is used for creating a calibration curve?

- Which peaks are identified by name?

Set the evaluation parameters in the quantification method (QNT File) before the analysis:

- Select **File > New > Method File** to generate a new QNT File.

- Alternatively, select a sequence in the Browser and double-click to open the related QNT File.

- To open the QNT Editor from a method file, click the **QNT Editor** icon on the toolbar. In this way, the data of the QNT File are displayed for the current sample.

For more information about the QNT Editor, refer to **The QNT Editor**.

The QNT Editor

The **QNT Editor** allows you to create a method for evaluating chromatographic results of different samples. The QNT Editor usually provides:

- The chromatogram of the current sample

- The calibration curve of the current peak

- Various tab pages
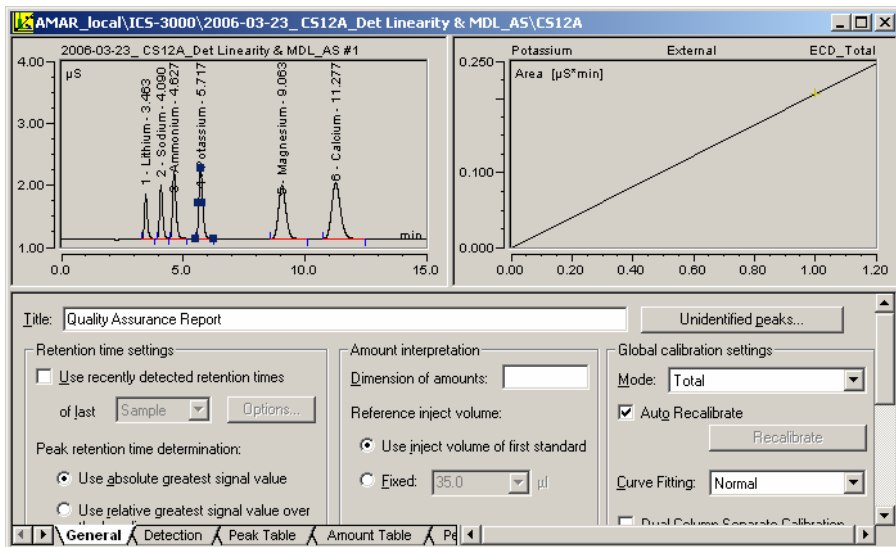
Instead of the calibration curve, you can display the following items:

- A ➤*Trend Plot* of the current peak

- UV spectrum (if available)

- ➤*Mass Spectrum* (if available)

- ➤*I-t Plot* (if available)

In addition, you can include report tables in the tab pages.

The QNT Editor consists of two panes. The upper pane is the information pane. The lower pane is used to create a QNT File.

Upper Pane

The upper pane displays additional information (plots):

- Select **Show Chromatogram** or **Show Calibration Curve** on the **View** menu to display or hide the chromatogram and/or calibration curve.

- Press F4 or SHIFT+F4 to toggle between the chromatograms of the individual samples. As an alternative, select **Next Chromatogram** or **Previous Chromatogram** on the **File** menu.

- Select **Show Spectra** to display or hide the spectrum of a single peak.

- Select **Show I-t Plots** on the **View** menu to display or hide I-t plots.

Lower Pane

On the lower pane, specify the evaluation parameters:

- Click a specific tab to open a worksheet, similar to Microsoft Excel.

- The worksheets are assigned default names. To rename a worksheet, double-click a name and enter a new name in the edit dialog box.

For more information, refer to **Function of the QNT Editor**.

The QNT Editor provides the following tabs:

General (see **The General Tab Page**)

Detection (see **Detection Parameters**)

 Peak Table, Amount Table, Peak Tracking, and MS Tracking

Calibration (see **Calibration Settings**)

 Spectra Library Screening

SST (see **System Suitability Test**)

MS (see **Mass Spectra**)

UV (see **UV Spectra**)

Except for the **UV** tab, all tabs are displayed by default. To show the **UV** tab, choose **Select Sheets** on the **View** menu. In the **Select Sheets** dialog box, select the **UV** check box and click **OK**. To hide a tab, clear the tab's check box in the **Select Sheets** dialog box. To rename a tab page, double-click the tab and enter a new name.

🖝☞ *Tip:*

*These topics describe the structure and functions of the individual worksheets. For detailed information about how to enter data, refer to **How to …:** 🔳 Integrating Chromatograms and Identifying Peaks.*

🔳 Function of the QNT Editor

The QNT Editor allows you to define the quantification method (also referred to as QNT Method or method). The quantification method defines parameters for identifying the peaks within a chromatogram (assigning substances) and for calculating the amount/concentration from the recorded peak areas (calibration).

Peak Identification

Chromeleon supports two ways for identifying detected peaks, i.e., for assigning substance names.

Peaks are usually identified by their ⇒*Retention Times*. If a peak elutes within a defined time window, it is assigned the corresponding name, calibration function, etc.

If many peaks elute in rapid succession or if their order is changed by varying chromatographic conditions (for example, pH value), identification by the **UV spectrum** or the ➢*Mass Spectrum* is more reliable.

📝 *Note:*

Peak identification by UV spectra requires use of a ➢Photodiode Array Detector, such as the Dionex UVD 340, PDA-100 or PDA-3000, and the appropriate software configuration.

For mass spectra acquisition, a ➢Mass Spectrometer and the ➢Xcalibur software are required in addition to the Chromeleon software.

Calibration

The peak table provides all information for the calibration. The **Amount** columns and the recorded peak areas are used to calculate the offset, slope, and curve parameters.
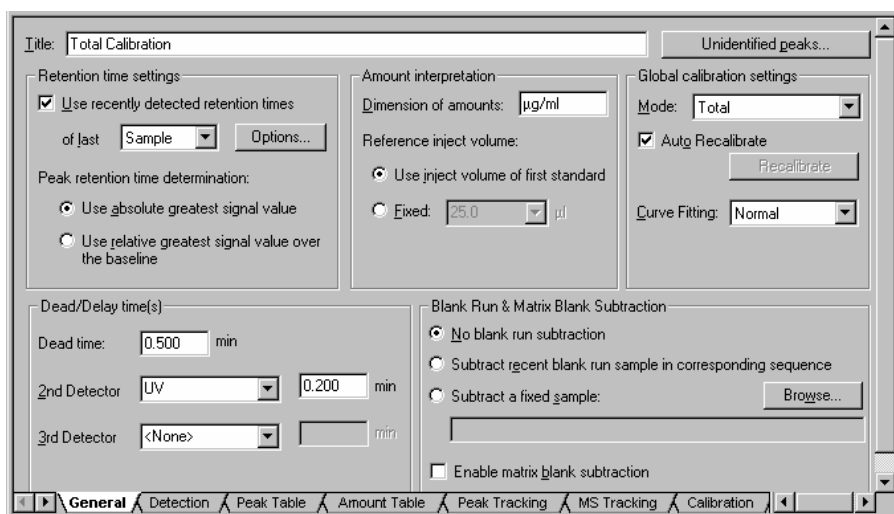
☞ *Tip:*

With each new calibration, the results are automatically recalculated for the report as the integration programs update the peak table with the calibration constants and the recorded integration values.

To save you from having to determine the retention time of each peak manually, Chromeleon peak tables can be generated automatically. (For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks ⬛ Autogenerating the Peak Table**.)

⬛ The General Tab Page

The **General** tab page contains global settings for the following worksheets: ⬛ **Peak Table** ("Retention Time Settings"), **Amount Table** ("Amount Interpretation"), and ⬛ **Calibration** ("Global Calibration Settings"). Enter a name for the current QNT File in the **Title** field. The name appears in the Browser and can be included in a Report when printing data of the QNT Editor. Click **Unidentified Peaks** to determine how Chromeleon quantifies unidentified peaks.

Retention Time Settings

- Select the **Use Recently Detected Ret. Times** check box to use the ⇒*Retention Time* of the preceding sample to identify a peak via a retention time window (⇒*Window*). You can also use the retention time of the last standard. Select Standard from the **of last** drop-down list. Click **Options…** to display more options. (For more information, refer to ⇒*Use Recently Detected Retention Time*.) This function allows the system to automatically react to changing retention times that are, for example, due to column trends. If the option is disabled, the actually determined retention time listed in the peak table is used for identifying the peak.

- In the **Peak retention time determination** section, specify how the retention time of peaks are determined: Select **Use absolute greatest signal value** to use the retention time of the greatest absolute signal value. Select **Use relative greatest signal value over the baseline** to use the retention time of the greatest relative signal value, i.e., of the largest distance to the baseline.

Dead/Delay time(s)

- Enter the ⇒*Dead Time* in the **Dead Time** field. The dead time is used for calculating the ➤*Capacity Factor* k' and the ⇒*Kovats Indexes*.

- Select the name of any further detector from the **2nd Detector and/or 3rd Detector** drop-down list and enter the ⇒*Delay Time* in the **min** field. (Also, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔖 **Defining the QNT Method for Several Detectors**.)

Amount Interpretation

- For documentation purposes (exclusively), the physical dimension (amount or concentration) that is used for the amount values can be included in the field ⇒*Dimension of Amounts*. Amount values are not automatically converted into concentration values or vice versa.

- In addition, the reference injection volume is defined on this page. Either select the injection volume of the first standard (**Use inject volume of first standard**) or enter any volume via **Fixed**.

Global Calibration Settings

- Use the **Mode** field to determine how the samples of a sequence are calibrated and on which ⇒*Calibration Mode* the calibration is based. For example, this allows you to calibrate certain samples as a group or to include calibration samples for samples that are analyzed later. For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Calibrating**.

- When **Auto Recalibrate** is enabled, each modification within a chromatogram such as moving peak delimiters results in automatic recalculation of the ➤*Calibration Coefficient* and all derived calibration data. Disable **Auto Recalibrate** in the peak table to include the c0, c1, c2, and/or c3 columns. Perform recalibration by clicking **Recalibrate**. You may enter the corresponding values manually, as well.

- **Curve Fitting** allows you to determine dependent and independent variables for calibration: Select **Normal** to accept normal evaluation and the axis settings of the calibration curve (x-axis = amount, y-axis = measured value). Select **Inverted** to use inverted evaluation and to invert the axes (x-axis = measured value, y-axis = amount). For more information, refer to **How to …: Calibrating** **Inverting Dependent and Independent Variables**.

Blank Run & Matrix Blank Subtraction

In the **Blank Run & Matrix Blank Subtraction** section, determine whether the absorption values of a ➤*Blank Run Sample* are considered (= subtracted) in the sample evaluation (= ➤*Blank Run Subtraction*). You can also subtract the results of a single ➤*Matrix Blank Sample*.

- Select **No Blank Run Subtraction** if no correction is to be performed.

- Select **Subtract Recent Blank Run Sample in Corresponding Sequence** to use a finished blank run sample of the current sequence for the subtraction. Chromeleon subtracts the chromatogram of the blank run sample from the active chromatogram, point by point.

- Select **Subtract a Fixed Sample** to perform the correction with any sample. Click **Browse** to search for the sample.

- Select **Enable Matrix Blank Subtraction** to enable the subtraction of matrix blank samples. Contrary to the other options, the resulting peak areas or peak heights are subtracted.

Also, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Subtracting a Blank Run Sample**.

Detection Parameters (Detection)
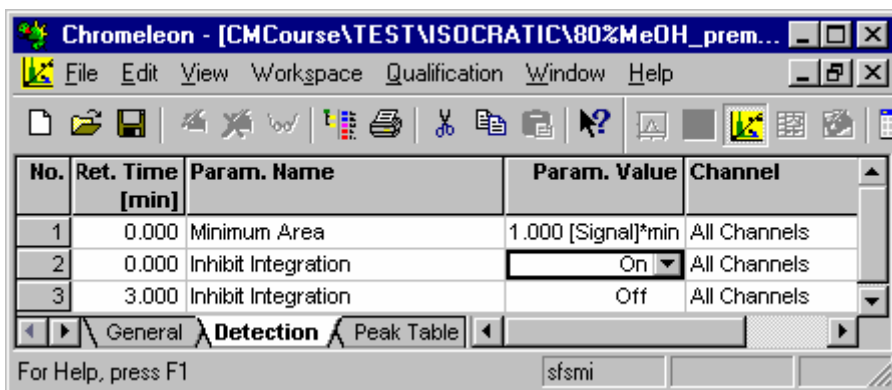
Detection parameters serve, for example, to recognize, classify, and suppress peaks as well as to determine the baseline.

The default values are normally suitable for optimum integration of 90% of all recorded chromatograms.

In critical cases (for example, with wavelength switching), the user can improve the integration results by modifying the parameters.

Detection parameters are time-dependent. At a specific time, each parameter can be enabled or disabled and/or its value can be changed. Enter the time at which the parameter changes, as well as the parameter name and value in the related column in the first line. It is possible to change a parameter several times in succession.

Detection parameters are defined in the QNT Editor. You can either enter them in the table of the **Detection** tab page or define them graphically in the chromatogram.

In the example above, integration is inhibited ($\Rightarrow$*Inhibit Integration* command) at the time t = 0.000 (**On**). After three minutes (t = 3.000), integration is enabled again (**Off**). It is also stipulated that peaks with a minimum area of 1 x [Signal] *min only are recognized as peaks.

The last defined value of each parameter is valid until the sample run is completed. After that, the parameters assume their preset (default) values.

If a parameter value changes at a specified time, this is referred to as **event**. You can have Chromeleon list these events in the report at the time of the change with a short description and the numerical value.

You are not required to enter the events in chronological order. Chromeleon sorts them in ascending order before saving them.

The meaning of the parameter table columns is as follows:

No.	Line number: The number is read-only.
Ret. Time [min]	Retention time in minutes. The allowed range is 0.000 - 999.999 minutes.
Param. Name	**Parameter name**: The dialog box lists all available parameters. To open the dialog box, press the F8 key or double-click the column.
Param. Value	**Parameter value:** The dialog box provides exact information about which values are allowed for the selected parameter.
Channel	**Channel**: Click the arrow or press the F8 to open the dialog box and select an option, i.e., either **All Channels** or one of the recorded channels.

Usually, the chromatogram and the report table are immediately updated when the detection parameters have been changed. However, you may disable this function by deselecting **Autom. Re-Integerate** on the **View** menu of the QNT Editor. If this option is disabled, save either the QNT Method or select **Start Integration** on the **View** menu to start re-integration with the new integration parameters.

📰 *Tip:*

The setting for this option is saved in the corresponding ≻Report Definition File.

For more information and examples, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔴 **Defining Detection Parameters** and ⇒*Detection Parameters (Overview)*.

In many cases, it is easier to define the detection parameters graphically in the chromatogram. For more information, refer to **How to …: Working with Chromatograms** 🔴 **Defining Detection Parameters Graphically**.

🔳 Peak Table, Amount Table, Peak Tracking, and MS Tracking

The peak table contains all parameters required to identify a peak and to determine the amounts of the substances serving as standards. The peak table contains a minimum of 26 columns (the **Amount**, **C0**, **C1**, and **C2** columns are duplicated as required, depending on the number of different standards). By default, the peak table consists of four tabs: **Peak Table**, **Amount Table**, **Peak Tracking**, and **MS Tracking**. You can show and hide columns and entire pages.

Usually, the peak table is created manually; i.e., the user enters the names and retention times of the expected peaks. In addition, (s)he labels peaks serving as standard peaks correspondingly in the ⇒*Standard* column. The (known) concentration of a standard is indicated as the ⇒*Amount* in the **Amount** column. These values form the basis for any type of calibration.

Select **Autogenerate Peak table** on the **Edit** menu to automate peak table creation. There are two ways how to create a peak table:

- Select **Enumerate peaks of current chromatogram** to include all peaks integrated in the current sample in the peak table. The peak name is the sequence name plus a consecutive number.

- Select **Use Spectra Library Screening results** to use reference spectra found via 🔴 **Spectra Library Screening** for automatic peak table creation.

In both cases, Chromeleon recalculates the values in the **Retention Time** and **Window** columns and replaces all other entries with the default settings (see **How to …: Integrating Chromatograms and Identifying Peaks** 🔴 **Autogenerating the Peak Table**).

⚠️ *Caution:*

All previous entries are overwritten. Thus, they are lost when you save the peak table or the QNT File.

For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Creating a Peak Table**

Peak Identification/Peak Tracking and MS Tracking

Peak identification refers to the following procedure: A previously unknown peak is identified due to peak-specific properties, such as the retention time or UV spectrum, and labeled with a characteristic substance name.

Peaks are usually identified by their **retention times**. If you know the exact retention time of a peak, enter the retention time and the **Substance name** in the corresponding columns of the peak table. If a peak is detected at the specified time, the name is automatically assigned (Fig. a).

To ensure peak identification even if there are retention time fluctuations or neighboring peaks, use the ⇒*Window* peak table parameter to define a tolerance range (fig. b). If a peak is detected within the tolerance range, it is identified, even if the *set* (nominal) and *actual* retention time do not match exactly (fig. c). If several peaks are detected within this range, Chromeleon identifies the "*greatest,*" the "*first,*" or the "*nearest*" peak, depending on the selected extension for the **window** parameter (fig. d).

a)   b)   c)   d)
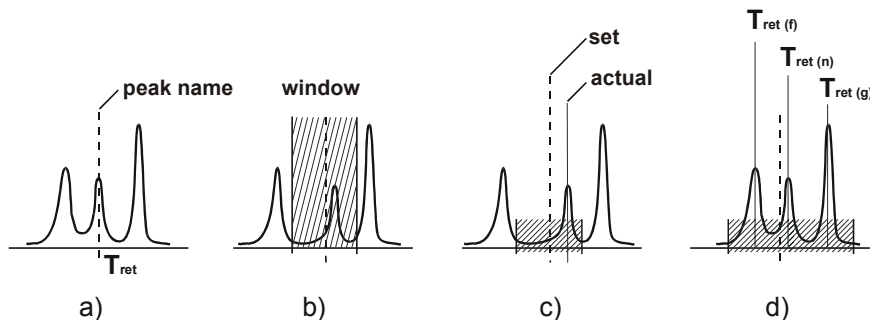
The retention time value previously entered in the peak table is corrected accordingly!

Peaks can be identified by the **UV spectrum**, too. You can use the **Spectrum** or the spectrum and retention time (**Spectrum and time**). This method requires a ➤*Photodiode Array Detector*, such as the Dionex UVD 340U. For each peak, Chromeleon records the substance spectrum and compares it to the library spectra, using certain criteria (see **How to …: Integrating Chromatograms and Identifying Peaks** **Peak Tracking**). If the spectra match, the peak is identified. This method is very reliable.

If you use a ➢*Mass Spectrometer*, peak identification can be based on ➢*Mass Spectra*. Use the **MS Tracking** tab page to select different options (see **How to …: Integrating Chromatograms and Identifying Peaks** **Identifying Peaks via Their Mass Spectra (MS Tracking)**). This method is also very reliable.

Calibration/Amount Table

During calibration, the peak area of a known standard amount is determined. The result is used for calculating the amount of unknown samples via the area to amount ratio.

Enter the amount contained in the different substances. In addition, determine the calibration function and specify whether external and/or internal standards will be used.

This is performed via the **Amount**, **Standard**, **Cal.Type**, and **Integration Type** columns in the **Amount Table** and/or **Peak Table**. For a description of the columns and for details on the required column input, refer to **How to …: Calibrating:**

 Entering Amount Values (Amount Column)

 Selecting the Standard Method (Standard Column)

 Selecting the Calibration Function (Cal. Type and Int. Type Columns)

 Tip:

For background information about calibration, refer to **Theory of Calibration** **Calibration (Overview)**. *For information about how to perform calibration, refer to* **How to …: Integrating Chromatograms and Identifying Peaks** **Calibrating**.

Calculating the Amount of Samples with Several Channels

If no retention time is defined in the QNT Editor for one of the channels, a special procedure is required for calculating the amount of samples with several channels (e.g., SIM or UV channels). For a more detailed description, refer to **Calibrating Samples with Several Channels with an Internal Standard Substance** in **How to …: Calibrating** **Calibrating with an Internal Standard Substance**.

◼ Calibration Settings (Calibration)

In the simplest case of a chromatographic analysis, first one or several standard samples and then the unknown samples are analyzed. All samples of a sequence are evaluated based on the same standard samples. If the quality of the column changes between processing the first and the last sample, this will not be considered.

That is why Chromeleon allows sample evaluation via specific patterns or based on any selected standard samples. The settings are determined via the ⇒*Calibration Mode* on the **General** tab page of the **QNT Editor**.

The **Calibration** tab page indicates which standard samples are used for calibrating the current sample. When you change from the current sample to another sample by pressing the F4 or SHIFT+F4 keys, the list of the displayed standard samples is updated.

If you notice that a specific standard sample falsifies your calibration results, you can disable this sample in the **Enabled** column: The standard sample is excluded from the calculation. The associated calibration points appear in a different color in the curve.

For more information, refer to **How to …: Calibrating 🔼 Disabling Calibration Samples**.

◼ Spectra Library Screening

To identify substances, spectra can be compared to library spectra. To perform a spectra search, open the **Spectra Library Screening** sheet of the **QNT Editor**.

For more information about Spectra Library Screening, refer to **How to …: Displaying and Using UV Spectra 🔼 Searching Reference Spectra**.

▣ The System Suitability Test (SST)

The aim and objective of the ➢*System Suitability Test (SST)* is to ensure that the operational conditions required for a specific measurement are achieved. Specify the conditions for the SST on the **SST** tab page in the QNT Editor.

For more information about System Suitability Testing, refer to **How to …: Integrating Chromatograms and Identifying Peaks 🔼 Defining System Suitability Tests**.

▣ Mass Spectra (MS, MS Tracking)

If you have a Thermo Finnigan ➢*Mass Spectrometer* installed, you can acquire mass spectra using Chromeleon. For more information about how to install the spectrometer and acquire data, refer to **How to …: 🔼 Using Mass Spectrometers**.

In HPLC MS, mass spectra especially serve for peak identification. Using mass spectra for identifying substances is via the **MS Tracking** tab page. For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks 🔼 Identifying Peaks via Their Mass Spectra**.

As MS chromatograms normally show increased noise, you have to process ➢*Mass Spectra* before you can use them. On the **MS** tab page, define the number of single peak spectra to be averaged. Also, define how many background spectra shall be aggregated into a total background spectrum that is then subtracted from the averaged peak spectrum.

For more information, refer to **How to …: Using Mass Spectrometers 🔼 Processing Mass Spectra**.

▣ UV Spectra (UV)

On the **UV** tab page, you can process UV spectra before you use them. Define the number of single peak spectra to be averaged. Also, define how many background spectra are aggregated into a total background spectrum that is subtracted from the averaged peak spectrum afterward.

🗋 *Note:*

*The **UV** tab is hidden by default. To show the **UV** tab, choose **Select Sheets** on the **View** menu. In the **Select Sheets** dialog box, select the **UV** check box and click **OK**.*

For more information, refer to **How to …: Displaying and Using UV Spectra 🔼 Processing UV Spectra**.

Integration

Integration (Overview)

The chromatographic process of converting peak areas below peaks in amount or concentration values is referred to as integration.

Amount and concentration values are calculated based on the calibration, which supplies the calibration curve and the ➤*Calibration Coefficient*. (For more information about calibration, refer to **Theory of Calibration** 🔲 **Calibration (Overview)**.)

To calculate the amount concentration for a single peak area, the calibration coefficients are inserted in the ➤*Formula for Amount Calculation*. Chromeleon automatically performs this process for all peaks found.
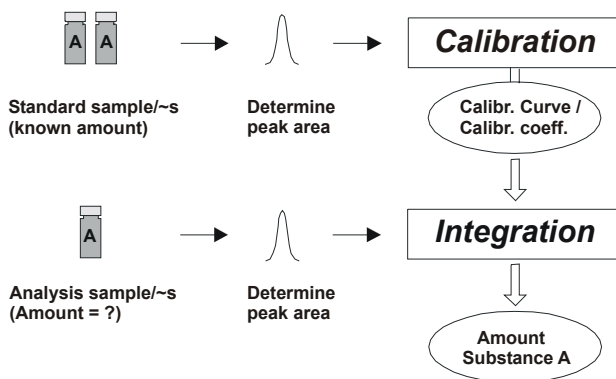
It depends on the 🔲 **Quantification Method (QNT File)** specified for each sample before the analysis how many peaks are detected and whether at least part of these peaks can be identified. The results of the area calculation and peak identification processes are represented graphically in the **Integration** window (chromatogram) and as a table (Report) on the Integration plot.

For more information, refer to:

🔲 **The Integration Window**

🔲 **Report Tables (Overview))**

🔲 **The Calibration Curve**

The Integration Window

Chromatographic results appear in the **Integration** window. In addition, you can use this window to reprocess single chromatograms. Usually, the following items are displayed by default:

- The chromatogram of the current sample

- Various report tables
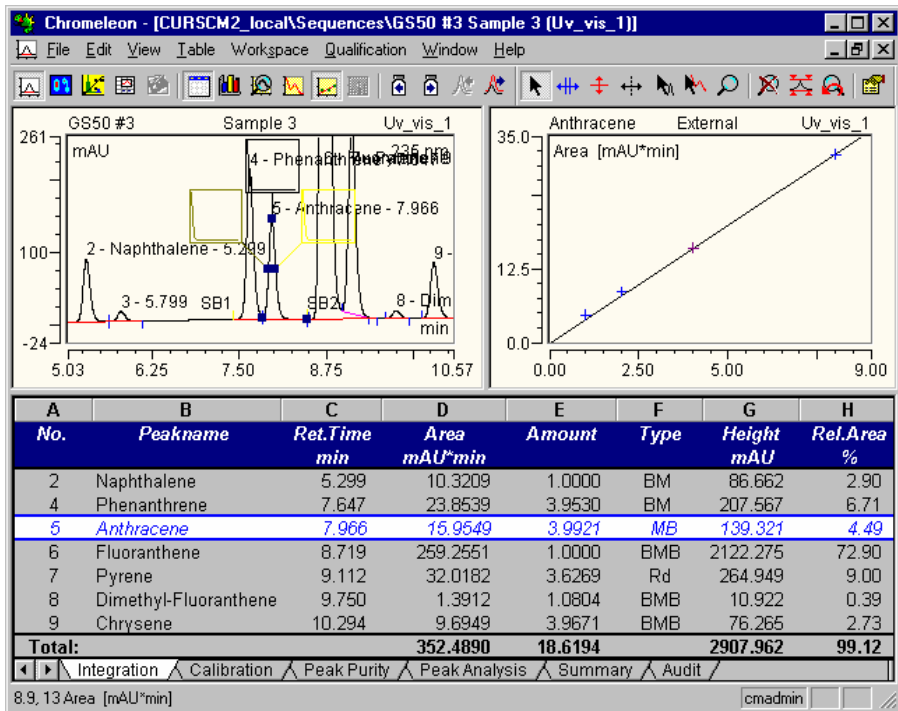
In addition, you may display:

- The calibration curve of the current peak (see the image above)

- A ➢*Trend Plot* of the current peak

- UV spectrum (if available)

- ➢*Mass Spectrum* (if available)

- ➢*I-t Plot* (if available)

Settings in the **Integration** window are saved together with the 🔳 **Printer Layout** in the ➤*Report Definition File (RDF)*.

For more information, refer to:

🔳 **Opening a Sample**

🔳 **Operation**

🔳 **Manual Re-Integration**

🔳 **Chromatogram Comparison**

🔳 **Data Smoothing**

🔳 **Peak Ratio**

🔳 **The Trend Plot**

🔳 **The UV Spectra Plot**

🔳 **The Mass Spectra Plot**

🔳 **The I-t Plot**

🔳 **Opening a Sample**

When you open a sample, this action automatically opens the integration method window and displays the sample chromatogram. There are three options:

- Select a sample and select **Open** on the **File** menu.

--or--

- Double-click a sample of a specific sequence in the ➤*Browser*.

--or--

- Select a sample in the Browser, right-click to open the context menu, select **Open**, and then select a channel of the sample.

Usually, samples are opened after being processed. However, if a certain amount of data has already been acquired, you may open a sample during the analysis (sample ⇒*Status* = **Running**), also. Depending on the ⇒*Step* and other detector settings, the sample must have run for several minutes. To open a running sample:

- To open the sample in the Browser, use one of the options described above.

--or--

- To open the sample from a ➤*Control Panel*, select **Integrate** on the **View** menu.

◾ Operation

Modifying the Appearance

You can change the appearance of each chromatogram by enabling and disabling the display of various elements and by modifying them.

- Double-click a peak to display the **Peak Properties**.

- Draw a frame around the chromatogram section to be enlarged.

- Select **Decoration** on the **View** menu to modify the window background, the different axes, the layout of individual peaks as well as the color, shape, and size of their captions.

- If spectra or ➢*3D_Amp* data is available for the chromatogram, you can display a spectrum or I-t plot from the chromatogram at a selected retention time. Select **Tools>Spectra/I-t Plots Tool** on the context menu (or click the corresponding icon on the **Integration Toolbar**. The spectrum/I-t plot symbol is added to the mouse pointer. Click anywhere to extract and display the selected spectrum or I-t plot in a pane below the chromatogram. A thumbnail of the spectrum or I-t plot is displayed in the chromatogram. To overlay single spectra or I-t plots, hold down the SHIFT key and repeat the operation. Alternatively, select **Overlay Spectra** or **Overlay I-t Plots** on the context menu. To remove the overlaid spectra or I-t plots, select **Hide Time Spectra** or **Hide Time I-t Plots**.

Opening Additional Window Sections

Select one of the following commands to display more window sections:
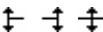
- Select **Split Zoom** on the **View** menu to split the window. By drawing a frame, you can zoom a window section. The left window shows the entire chromatogram and the frame; the right window displays the zoomed section. Use the mouse to move and position the frame in the left window section. The shape of the mouse pointer (✛) indicates that this mode is enabled. You can also draw a new frame at any time.

- Select **Report** on the **View** menu to display the integration report (see **Report Tables** 🔳 **The Integration Report**).

- Select **Show Spectra** on the **View** menu to display the peak spectrum of the currently selected peak (see 🔳 **The Spectra Plot**).

- Select **Show I-t Plots** on the **View** menu to display the peak I-t plots for the currently selected peak (see **Displaying Peak I-t Plots**).

- Compare two chromatograms by enabling the display of an additional chromatogram (see **Chromatogram Comparison**).

Manual Re-Integration

Chromeleon largely automates sample integration. However, in special cases, the user may prefer to perform the changes manually. Chromeleon allows you to move peak delimiters manually, to insert and delete peaks, or to modify the baseline, etc.

Select the **Automatic Tool** on the context menu to perform these changes directly in the chromatogram. The shape of the mouse pointer indicates which operation can be performed.

⊥ ⊤	Move left or right peak delimiter
↗ ↑ ↖	Change baseline point (left/right/center)
↨ ↨ ↨	Move baseline point (left/right/center)
↔	Move
↔	Move baseline segment
↔	Move ⇒*Detection Parameter*
∧	Insert peak
+	Zoom out an area
✎	Display UV spectrum
⊘	Operation not possible

Select the individual commands such as **Baseline Tool**, **Insert Peak Tool**, or **Zoom Tool**, if you only need a specific scope of functions. For more information about the operations that can be performed, refer to **How to …: Working with Chromatograms** **Manual Re-Integration**.

 Tip:

If manual modifications are performed, display the integration report to see the numerical results (see Report Tables *The Integration Report).*

◼ Chromatogram Comparison

The most exact method to compare two or more samples or chromatograms is to compare their numerical results. However, in many cases, it may be sufficient to overlay the chromatograms. This is referred to as a chromatogram comparison.

What can be compared?

Chromatogram comparison always compares single channels. It is irrelevant whether these channels are from the same sample or from different samples. Theoretically, Chromeleon can display an unlimited number of chromatograms simultaneously.

However, the presentation may be confusing when many chromatograms are displayed. That is why Chromeleon prompts you to confirm that you want to view more than 20 chromatograms. This is to avoid that too many chromatograms are displayed due to an operator error. Displaying many chromatograms at a time may decrease the system performance.

How are chromatograms compared and displayed?

To compare chromatograms, display them in the **Integration** window. One chromatogram is the active chromatogram. To make a chromatogram the active chromatogram, select it with the mouse. Only for the active chromatogram, additional information, such as the decoration, etc. is displayed. The names of all chromatograms displayed are indicated above the **Integration** window. The name of the active chromatogram is written in a different color. Click the name of any chromatogram to select it as the active chromatogram.

Select **Decoration** on the context menu to edit the appearance of the window and the active chromatogram.

How do I select the chromatograms or channels to be compared?

- Select one or several samples in the Browser and drag them into the open Integration window. Chromeleon automatically attempts to load the ➢*Channel* of the current sample. If this is not possible, for example, because the channel does not exist, the system loads the default channel. The default channel is the first channel that appears in the list when you open a sample. (Select **Open** on the context menu in the Browser.)

- Select **Add Overlay** on the **File** menu to display a specific channel of any sample in an opened integration window.

- Select **Open > All Channels** to compare all channels of a single sample.

- Select **Compare** to compare a specific channel in several selected samples.

- Perform a query to compare samples with certain properties.

- Hold down the CTRL key and click the **Next Chromatogram** icon ( ) to display the chromatogram of the next sample in addition.

- Hold down the CTRL key and click the **Previous Chromatogram** icon ( ) to display the chromatogram of the previous sample in addition.

- Hold down the CTRL key and click the **Next Channel** icon ( ) to display the next channel of the same sample in addition.

- Hold down the CTRL key and click the **Previous Channel** icon ( ) to display the previous channel of the same sample in addition.

For more information, refer to **How to …: Working with Chromatograms Comparing Chromatograms**.

Placing chromatograms and channels in relation to each other

External factors such as the flow rate, solvent, column quality, detector amplification, etc. considerably influence the appearance of the chromatogram.

As you cannot modify these conditions later, you must use other ways to perform the comparison as exactly as possible. Adapt the position and the size of a chromatogram to match another. Chromeleon provides several options. You can:

- Assign chromatograms an offset in x- and y-direction.

- Normalize the chromatogram time, i.e. you can overlay the chromatograms at a specific retention time.

- Normalize the peak height, i.e., you can adjust the height of a specific peak.

- Stretch and compress chromatograms.

- Subtract chromatograms from each other.

Select **Decoration** on the context menu of the active chromatogram and enter the settings in the **Comparison** tab page.

For more information, refer to **How to …: Working with Chromatograms** **Comparing Chromatograms**.

Data Smoothing

Data smoothing applies a digital filter to sample data to reduce signal noise and helps improve chromatogram appearance and reproducibility of peak baselines. Data smoothing is performed in the integration window. (For MS chromatograms, data smoothing is defined in the ➢*PGM File* or during ➢*Mass Trace* extraction.) After smoothing, the smoothed chromatogram is displayed overlaid over the original chromatogram. The original sample data file is **not** altered and the smoothed data file is stored separately.

Filter Types

The **Savitzky-Golay** filter smoothes to least-squares fit, using a weighting function based on second-degree and third-degree polynomials. Savitzky-Golay smoothing is useful for reducing high-frequency noise of a data set that is continuous (such as a chromatogram) without significantly degrading the underlying signal.

The **Moving Average** (= **Boxcar**) filter is a simple algorithm that produces a set of output values in which each output value is equal to the average of n points centered around the corresponding input value, where n represents the filter size. Because the Moving Average filter equally weights each point, its ability to discriminate between noise and signal is limited.

The **Olympic** filter is very similar to the Moving Average filter, except that the maximum and minimum points of each input data set are rejected before the average is calculated. This provides better rejection of impulse noise (spikes) than the moving average filter.

In addition, the **Gaussian** filter is available for acquiring MS chromatograms and extracting a mass trace (in the Mass Spectra window). This filter applies the Gaussian distribution for chromatogram smoothing.

Filter Size

Filter size is the number of input data points used to generate each output data point. The filter size is an odd number between 5 and 999. Use a narrow filter size if the desired peaks are narrow, and a wider filter size for wider peaks. As a rule of thumb, select a filter size that approximately equals the peak's half width. Note that too narrow a filter results in insufficient smoothing while too wide a filter can lead to distorted data.

Tip:

Distortion of data during data smoothing mainly affects the peak height. Therefore, it is generally better to evaluate smoothed chromatograms by area rather than height.

Iterations

If a filter is applied several times, by far the highest smoothing result (>95%) is achieved when the filter is applied the first time. Thus, normally a single smoothing step is sufficient. However, applying a narrower filter multiple times often provides improved noise reduction without the signal degradation that can occur when using a wider filter size. This requires additional processing time, however, so a wider filter size may be preferable if its results are acceptable.

For additional details, refer to **How to …: Working with Chromatograms Performing Data Smoothing**.

For MS Chromatograms, refer to **How to …: Using Mass Spectrometers Extracting Mass Traces afterward**.

Combination of Channels

Chromeleon allows you to combine two 2D channels using arithmetic operations. Each data point is created by combining the associated two data points from the existing channels (i.e., the data points at the corresponding time), using the selected operation. The resulting channel is a 2D channel, too.

Note:

If there is a data point for channel A at a specific time but not for channel B, the missing data point is calculated by linear interpolation. That is why the resulting chromatogram may be shorter than the two original chromatograms.

You can combine channels:

- In the chromatogram: Chromeleon calculates the resulting channel immediately.

- As ➢*Post-Acquisition-Step* in the PGM Editor: Chromeleon calculates the resulting channel immediately after data acquisition is complete.

Arithmetic combinations are not restricted to chromatograms in the strict sense. You can use them for all 2D channels, except for temporary channels.

▣ Peak Ratio

If the baseline-corrected signals of two channels (of the same sample!) are related to each other, a rectangular curve results. This curve is referred to as peak ratio. It is based on the observation that the ratio between two detector signals must be constant, as according to the Lambert-Beer law the detector signal (S) is always be proportional (c1) to the concentration (K) of a dissolved substance.

$$S = c1 x K$$

If the quotient q is formed of the two channels, the substance concentration K is reduced. The quotient now only depends on the ratio of the two wavelengths, and not of the time.

$$q = \frac{S1}{S2} = \frac{c1(\lambda 1)}{c2(\lambda 2)}$$

If q is entered against the time, the (theoretical) result for each peak of the sample is a horizontal line of the height c1(λ1)/c1(λ2).

The baseline and the ⇒*Peak Purity Threshold* parameter determine the width of the rectangle. The ratio is only formed where both (!) peaks have a baseline and where the intensity of both (!) peaks is above the defined peak purity threshold. Thus, the range to be actually overlaid (= width of the rectangle) is the intersection of the baseline and Peak Purity Threshold condition. The default Peak Purity Threshold value is 10% of the peak maximum. You can change this value in the QNT Editor.

The rectangle heights of two adjacent peaks differ if the corresponding peaks have different spectra and if the two wavelengths are selected so that the absorption quotient is significantly different.

Consider the following limitations and requirements:

- The detector may not drift.
- The correlation only applies to the linear range of the Lambert-Beer law (<2000 mAU).
- The solvent composition may not be altered (isocratic conditions).
- The solvent only slightly contributes to the absorption. Baseline correction allows you to eliminate the solvent absorption.

🗒 *Note:*

A peak ratio can only be formed with the signals of the same detector.

Forming the Peak Ratio

- In the **Integration** window, overlay two channels of the same sample, for example, by simultaneously clicking the **Next/previous channel** icon while pressing the CTRL key.
- Select **Decoration** on the context menu to open the **Chromatogram Decoration** dialog.
- Under **Peak Decoration**, select the **Peak Ratio** check box.

In addition to the two chromatograms, a rectangle curve should appear in a different color should appear in any place in the Integration window where two peaks are overlaid.
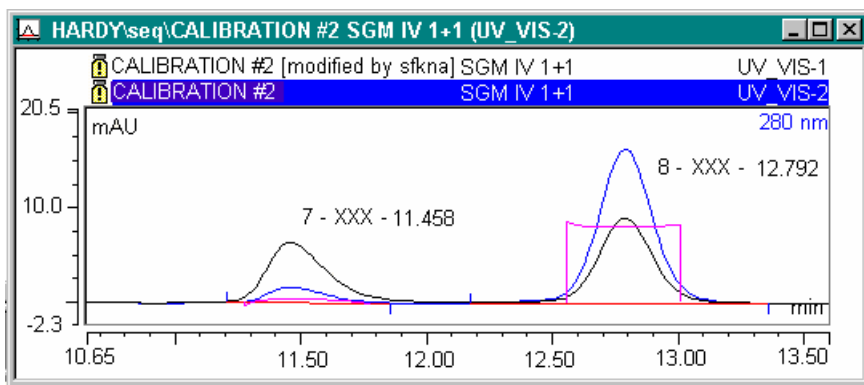
Result

A regular rectangle shape, as shown at 12.70min in the illustration, *can* serve as a criterion for evaluating the peak purity. The more the curve deviates from the rectangle shape, the higher is the probability that the overlaid peaks do not originate from the same substance. If the rectangle slightly overshoots on the right or on the left, this is tolerable due to the lower signal intensity at the peak start and the peak end.

Select the **Peak Ratio Mean Value** and **RSD Peak Ratio** report variables from the ⇒*Peak Purity and Identification* category to numerically express the result of the peak ratio.

▣ **The Trend Plot**

The ➤*Trend Plot* provides a graphical representation of specific data acquired from a set of samples. You can view a trend plot from samples in an existing ➤*Sequence* or perform a ➤*Query* to include samples from multiple sequences.

When viewed in the Integration window, two types of trend data variables can be plotted:

• Instrument-specific variables, such as pump pressure and background signal

• Result variables, such as retention time, area, and peak height

To open the trend plot, first open a sample to display the Integration window, and then select **Show Trend** on the **View** menu or click the following icon ![icon] on the **Method** toolbar.

The initial trend plot displays the trend variable and other plot properties defined by the settings in the current ➤*Report Definition File*.

• To plot a different trend variable, right-click on the plot and select **Trend Variable** from the context menu.

• To change the plot appearance or other plot properties, right-click and select **Decoration** from the context menu.

• An asterisk (∗) indicates the data point of the sample currently displayed in the Integration window. To display the chromatogram of a different sample, double-click the sample's data point.

The following example shows a chromatogram in the Integration window with a trend plot of sodium retention times below.
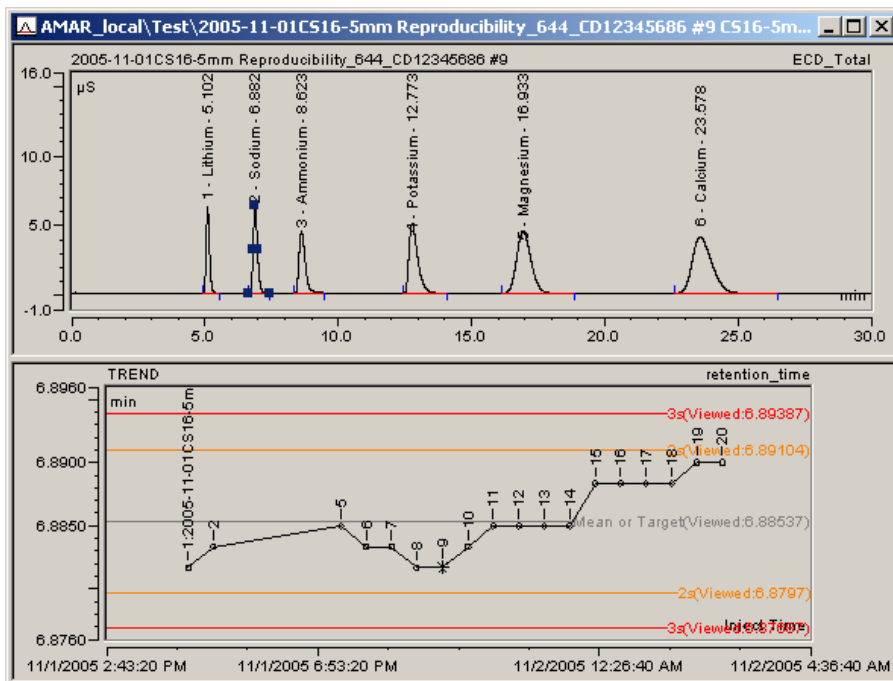
In addition to viewing trend data in the Integration window, a trend plot can be included in the printer layout (see **Preparing the Printout** 🔲 **Inserting a Plot**) and on a control panel (see **Control Panel** 🔲 **The Trend Plot**).

🔳 The UV Spectra Plot

Overview

The **Spectra Plot** enables the display of UV spectra. The prerequisite for the spectra plot is that the corresponding raw data is available. Raw data is generated by recording a 3D field, using a ➢*Photodiode Array Detector*. Open the spectra plot from the **Integration** method.

- To open the spectra plot window, select **Show Spectra** on the **View** menu or click the following icon: .

The representation of a spectrum in the **Spectra Plot** is usually (height) normalized: The height of the spectrum is represented in percent. Thus, it is independent of the concentration (also, refer to ➤ *Normalization*). As a default, normalization is by the greatest relative maximum within the spectrum.

Normalization allows objectively comparing two spectra of different concentrations. If spectra of the same peak but different peak heights are overlaid, these will generally coincide despite the differences in concentration.

- Select **Decorations** on the **View** or context menu. On the **Frame & Axes** tab page, select which type of normalization is applied to the spectrum.

Normalized spectra representation allows you to perform the following tasks:

- Compare two spectra such as a standard and a sample

- Determine the number and position of minima and maxima, even with less distinct spectra at low concentrations

- Select an appropriate type of normalization

- Enable or disable baseline correction

- Determine exact integration limits by checking peak purity at various wavelengths and peak heights

- Identify components

Displaying Spectra of one Peak

- In the chromatogram plot, select the peak for which to display the peak spectrum (= spectrum in the peak maximum).

- If several peak heights were enabled on the **Peak Spectra** tab page in the **Decoration** dialog box of the spectra plot, the spectra of different peak heights are simultaneously displayed when you click the peak.

Displaying any Spectra of a Chromatogram

To extract any spectra of a chromatogram via mouse-click, follow the steps below:

- Select **Tools** on the context menu and select **Spectra/I-t Plots Tool** or click the corresponding icon on the **Integration Toolbar**. A spectra symbol is added to the mouse pointer, indicating that the mode was changed.

- Click anywhere in the chromatogram to display the corresponding spectrum.

- Repeat the operation while pressing the SHIFT key to overlay several spectra.

Displaying Spectra of Different Samples

To objectively compare spectra of different samples:

- Select **Decoration** on the context menu of the spectrum to open the **Decoration** dialog box and select the **Peak Spectra** tab page.

- Select the **Retention time spectrum of a fixed sample** option and click **Browse** to navigate to the sample of interest.

- Alternatively, you may also use the retention time spectrum of the last standard (= **Retention time spectrum of recent standard**), the reference spectrum of the peak table (= **Reference spectrum in corresponding peak table**), or any spectra that was found during library screening (**Spectra library screening result**).

Match Factor, Difference Spectra, and 1st and 2nd Derivative of Spectra

As soon as two or more spectra are represented on the spectra plot, a frequent question is the similarity between the various spectra.

The similarity is indicated by the Match Factor, the formation of difference spectra or by representing the first or second derivative of a spectrum.

- Select **Decorations** on the **View** or context menu of the spectrum, and then select the **Show match** check box on the **Label** tab page. Chromeleon returns a value for each represented spectrum specifying the match degree relative to the main spectrum (0 = no match; 1000 = perfect match).

- On **Analysis** tab page, select whether the difference spectrum or the first or second derivative of a spectrum shall be displayed in a second window in addition to the actual spectra.

In the case of the match factor and the difference spectrum, the question which spectrum is considered a main spectrum is especially important, as this is the basis for the comparison and for all calculations.

The main spectrum is usually the peak spectrum extracted at the retention time. If there is no peak spectrum, distinguish the following cases: If you used the **Spectra/I-t Plots Tool** to extract the single spectra from the chromatogram, the spectrum that was extracted first is the main spectrum. If the spectra were automatically extracted at different peak heights (see **Displaying Spectra of one Peak**), the spectrum with the "oldest" retention time is considered the main spectrum. When representing difference spectra, the **Difference to** entry indicates the basis for calculation.

Comparing a Spectrum with Spectra of an Existing Spectra Library

To clearly identify a spectrum, compare it to a reference spectrum stored in a ➢ *Spectra Library*.

- On the context menu, select **Library Search** to start the comparison. The 🔳 **Spectra Library** window lists all library spectra with a minimum similarity to the (normalized) spectrum. For more information about how to perform library screening, refer to **How to …: Displaying and Using UV Spectra 🔳 Searching Single Reference Spectra**.

If the spectra plot contains more than one starting spectrum, Chromeleon always uses the spectrum displayed first and then compares it to library spectra.

🔳 The Mass Spectra Plot

General

The Mass Spectra Plot enables the display of ➢ *Mass Spectra*. The prerequisite for opening the mass spectra plot is that the corresponding raw data recorded by a ➢ *Mass Spectrometer* are available. Open the mass spectra from the **Integration** method.

- To open the spectra plot, select **Show Mass Spectra** on the **View** menu or click the following icon: 📊.

The representation of a mass spectrum is height normalized: The height of the spectrum is represented in percent and thus independent of the concentration (also, refer to ➢*Normalization*). As a default, this normalization is by the ➢*Base Peak* of the spectrum.

Normalization allows you to objectively compare two spectra of different concentrations. If mass spectra of the same peak, but from different peak heights are overlaid, these will generally coincide despite the differences in concentration. Due to their higher information density, mass spectra are displayed in Chromeleon below each other, which is contrary to the display of UV spectra that are overlaid.

Displaying Mass Spectra of one Peak

- In the chromatogram plot, select the peak for which to display the mass spectrum (= spectrum in the peak maximum).

- If several peak heights were enabled on the **Peak Spectra** tab page in the **Decoration** dialog box of the mass spectra plot, the mass spectra from different peak heights are displayed simultaneously when you click a peak.

Displaying Mass Spectra of Different Samples

To compare mass spectra of different samples objectively:

- Select **Decoration** on the context menu of the spectrum to open the **Decoration** dialog box and select the **Peak Spectra** tab page.

- Select the **Retention time spectrum of fixed sample** option and click **Browse** to navigate to the sample of interest.

- Alternatively, you may also use the retention time spectrum of the last standard (= **Retention time spectrum of recent standard**), the reference spectrum of the peak table (= **Reference spectrum in corresponding peak table**), or any spectra that was found during library screening (**Spectra library screening result**).

◾ The I-t Plot

Overview

An I-t plot is a graphical display of current (I) vs. waveform time (t) of ➢*3D_Amp* data. When 3D amperometry data is collected for a sample, the I-t plot can be opened from the Integration window (see 🔖 **Displaying and Using I-t Plots**).

☞ *Tip:*

3D amperometry data collection is available only with the ED electrochemical detector running in integrated amperometry mode. The Chromeleon PC must also have the 3D Data Acquisition license. To turn on collection of 3D amperometry data, enable the ➢3D_Amp channel on the ED Options tab page of the Program Wizard or PGM Editor.

Plot Types

Two types of I-t plots can be displayed:

- Peak I-t plots are I-t plots at the peak apex or at a defined peak height. Peak I-t plots at the peak apex are also called retention time I-t plots.

- Time I-t plots are I-t plots selected at any retention time in the chromatogram. They are not defined by a peak height.

Plot Normalization

The I-t plot can be (height) ➢*Normalized*. When normalized, the height of the I-t plot is represented in percent. Thus, it is independent of the concentration.

Normalization allows objectively comparing two I-t plots of different concentrations. I-t plots of the same peak but different peak heights will generally coincide when overlaid, despite the differences in concentration.

- To select the type of normalization applied to the I-t plot, select **Decorations** on the **View** or context menu and select the **Frame & Axes** tab. Normalization is off by default.

The following example shows a chromatogram in the Integration window with an I-t plot window below. The I-t plot window shows five overlaid peak I-t plots (identified by the five thumbnail images on the chromatogram).
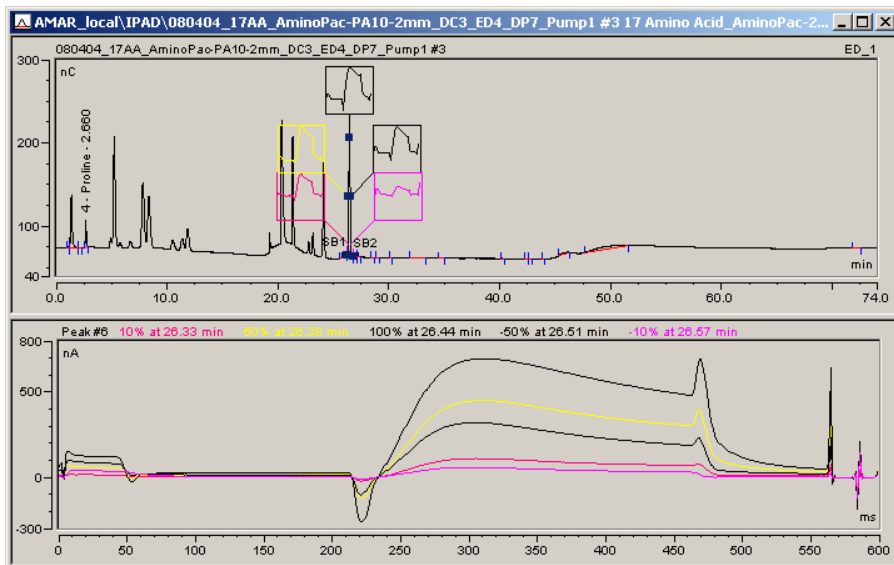
Other features available for I-t plots in the Integration window include:

- Overlaying a reference I-t plot.

- Comparing I-t plots from different samples.

- Displaying a difference plot or 1st or 2nd derivative plots.

For more information, refer to:

Displaying Peak I-t Plots

Displaying and Overlaying Time I-t Plots

Displaying Difference, 1st Derivative, or 2nd Derivative I-t Plots

I-t plots can also be displayed in the following locations:

- Printer layout (see **Preparing the Printout Inserting a Plot**)

- QNT Editor (see **The QNT Editor**)

- 3D Amperometry Window (see **The 3D Amperometry Window**)

◼ The Autopurification Tray View

Chromeleon supports two ➤*Autopurification* tray views: **Inject Trays** and **Fraction Racks**. To open the associated view and display the different samples and/or fractions, click the related button:

⬛ opens the **Inject Trays** view, showing the analytical and preparative samples.

⬛ opens **Fraction Racks** view, showing the tubes of the fractionated samples.

The default setting is that an overview of the single racks is displayed below the title line, also showing the racks that do not contain samples and/or fractions. The racks and their samples are displayed below this general overview. The samples and/or fractions are color-coded, based on their ⇒*Type (Sample Type)* and ⇒*Status*.

Report Tables

Report Tables (Overview)

The report (or on-screen *report*, to distinguish it from the ➤*Printer Layout* that defines the printout) includes several graphics and tables for displaying all relevant sample data on the screen. The report is saved in the ➤*Report Definition File (RDF)*, together with the printer layout.

To open the report tables, either select **Show Report** on the **View** menu or click the following icon: 🗓. The report is usually displayed in the **Integration** window. However, you can also have the report tables displayed in the QNT Editor to directly check the effects of the changes made in the QNT Method. (For more information about the editor, refer to **Data Representation and Reprocessing** 🔳 **The QNT Editor**.)

You can add, edit, or extend the individual report tables as required. Chromeleon provides the following tables when you select **Insert Report** on the **Table** menu:

Result Tables

Peak Results	These reports combine data of the current sample.
Calibration Report	Displays all variables required for creating a calibration report.
Integration Report	Displays all variables required for creating an integration report.
Sample Results	These reports combine data of the entire sequence.
Calibration History	Displays all variables documenting the course of the calibration.
Peak Summary	Displays those variables for the entire sequence that are required for creating a peak summary.
SST Summary Report	Documents the results of the ➤*System Suitability Test* for the entire sequence.

Audit Trails

Audit Trail (Commands, SST, ...)	Displays the Audit Trail of the current sample. (Also, see **Data Management 🔳 Audit Trails**).
MS Instrument Info Report	Displays information about the ➢*Mass Spectrometer*.
MS Status Log Report	Displays the mass spectrometer settings.
MS Tune Data Report	Displays the tune data of the ➢*Xcalibur* raw data file.
MS Instr. Method Report (XRaw)	Indicates the MS method.

Spectra Results

MS Raw Report	Displays the raw data of the current ➢*Mass Spectrum*.

Fraction Collection Results

Fraction Report	Displays all variables required to create a fraction report.
Tube Report	Displays the different variables describing the single fraction collection tubes. This also includes the fraction report variables. However, please note that these variables are not selected by default.

QNT Tables	These reports combine data of the current sample.
Detection Parameter	Displays all ⇒*Detection Parameters* of the current sample.
Peak Table	Displays all variables that are required for displaying the peak table of the QNT Editor.
SST Table	Lists variables of the ➢*System Suitability Test* for the current sample.

PGM Tables

Commands	Displays the ➢*Program* for the current sample.
Post Acq. Processing	Displays the ➢*Post-Acquisition Steps* for the current sample.
MS Method	Displays information about the current method of the ➢*Mass Spectrometer*

Miscellaneous Tables

Database Query	Inserts any kind of database queries into the report.
History Report	Displays all variables required for creating a ➢*History* report.

📑☞*Tip:*

*Select **Print** on the **File** menu to print chromatograms, spectra, report tables, etc. at any time. The pages defined in the Printer Layout are printed. This also applies when you print from an on-screen report.*

For more information about some of the reports mentioned above, refer to:

🔲 **The Integration Report**

🔲 **The Calibration Report**

🔲 **The Peak Summary Report**

🔲 **The History Report**

🔲 **Special Report Tables**

For information about how to create a report, refer to **How to …:** 🔲 **Creating and Using Report Tables**.

▪ **The Integration Report**

The Integration Report (or more precise, the Integration Report Table) is usually the first table of a report. For example, the **defltdad** ➢*Report Definition File (RDF)* includes the following integration report tables:

- Integration
- Peak Purity
- Peak Analysis

Contents

- The integration report contains all relevant numeric data for the active sample, such as the retention time, peak area and height, amount, peak type, and other ⇒*Peak Variables*.

- The individual columns contain variables of the ⇒*Peak Results* report category. Nevertheless, you can add more columns from other ⇒*Report Categories*.

- If the chromatogram is modified, for example, by manual re-integration of a peak, this is reflected immediately by the changed report data.

- Via the Windows clipboard, data of the report table can be integrated directly in other Windows applications.

- In the report, the values of different cells are added, subtracted, multiplied, or divided in the same way as in MS Excel. Enable **Layout Mode** on the **Table** menu.

Formatting

You can adapt the integration report to your requirements by selecting the corresponding format commands on the context or **Table** menu. (The format commands are only available after you have enabled **Layout Mode** on the **Table** menu.)

For more information, refer to **How to …:** **Creating and Using Report Tables**.

The Calibration Report

The Calibration Report (or more precisely, the Calibration Report Table) is very similar to the **Integration Report**:

- Similar to the integration report, the calibration report displays data of the current sample. However, in the calibration report table, this data refers to the calibration of the current sample.

- The individual columns contain variables of the ⇒*Peak Calibration* report category. Nevertheless, you can add more columns from other ⇒*Report Categories*.

- The calibration report table allows you to understand changes made during the calibration (in the QNT Method).

For more information, refer to **How to …:** **Creating and Using Report Tables**.

▣ The Peak Summary Report

Commonly used reports such as the ⬛ **Integration Report** and the ⬛ **Calibration Report** contain data of one sample only. In case of a chromatogram comparison, only the data of the selected (active) chromatogram is displayed.

Comparing peak data from different samples is possible via the (peak) **Summary** report table.

Sample Selection

- If **one sample** is selected and opened from the Browser, the peak summary is based on the corresponding ➢ *Sequence*.

- If **several samples** are selected in the Browser, these selected samples only will be part of the **Summary** report table.

- If a ➢ *Query* is started in the Browser, the search result forms the basis for the Summary. In this way, it is possible to compare peaks from different sequences.

Peak Selection

- Normally, **no peak** is selected in the chromatogram of the sample. The Peak Summary Report therefore contains **no entries**. All fields are marked **n.a.** (not available) or **Div/0** (Division/0)., The Peak Summary Table receives entries only when a peak is selected manually within a chromatogram. As soon as this is performed, this peak is searched in all previously selected samples. The search result is displayed in the **Summary Report**.

- For each Peak Summary column, it is possible to define whether the values of the currently selected (**Selected Peak**) or one specific peak (**Fixed Peak**) are displayed. Access these options by selecting **Column Properties** on the context menu.

- If the currently selected peak is an **identified** peak, that is, if the peak is labeled with its name in the peak table, all involved samples are searched for this peak name. The corresponding values of all found peaks are displayed in the Summary.

- If the currently selected peak is a **non-identified** peak, that is, if the peak is not labeled with its name in the peak table, a "fixed time window" ($\pm$ 2.5% of the detected retention time) is calculated for this peak. This calculated time window appears in the header instead of the peak name. If two or more peaks are detected in the time window, the peak that is nearest to the specified retention time will be selected.

Creating a Summary

- Double-click one of the samples to open the Integration window.

- Display the Report and select the default **Summary** worksheet available in Chromeleon. It contains a selection of the most important default ⇒*Peak Variables*. For each sample that is part of the Summary, a separate line is reserved in the report.

- If the default Summary Report is not available, a new worksheet can be defined. Proceed as described in: **How to …: Creating and Using Report Tables** ![icon] **Adding and/or Renaming a Worksheet.**

The sample that is currently selected in the chromatogram window is highlighted in the Summary Table.

![icon] The History Report

The standard ➤*Report Definition Files (RDFs)* include both, an ![icon] **Integration Report** and a ![icon] **Calibration Report** but no history report table. However, you can easily include the History Report table (see **How to …: Creating and Using Report Tables** ![icon] **Adding and/or Renaming a Worksheet**).

Contents

- By default, the history report displays the ➤*History* of the current sample. In order to display the histories of other objects, select **Table Properties** on the context menu and make your selection on the **History Objects** tab page.

- In addition, you can limit the displayed history as to time and/or certain actions and/or users.

Formatting

- You can sort the history report entries according to specific details, such as Time, Name, Version, etc.

- The history mode includes a special ➤*Layout Mode*. Enable this mode on the **Layout** tab page by selecting the **Design template mode** check box.

▪ Special Report Tables

The standard ➢*Report Definition Files (RDFs)* include both an 🔳 **Integration Report** and a 🔳 **Calibration Report**. In addition to the two standard reports, you can include the special reports listed below (see **How to …: Creating and Using Report Tables** 🔳 **Adding and/or Renaming a Worksheet)**.

MS Reports

MS Instrument Info Report	Information about the ➢*Mass Spectrometer*.
MS Instrument Method Report	MS method.
MS Raw Report	Raw data of the current ➢*Mass Spectrum*.
MS Status Log Report	Mass spectrometer settings.
MS Tune Data Report	Tune data of the ➢*Xcalibur* raw data file.

📋 *Note:*

*If you do not have the **MS Control** option enabled on your PC, MS reports will not be displayed in the **Insert Report** dialog.*

Other Special Report Tables

Database Query	Results of a database query.
Detection Parameter Report	Indicates the⇒ *Detection Parameters*.
Program Report	➢*Program* of the current sample.
SST Report	➢*System Suitability Test*.

📋 *Note:*

*All MS report tables as well as the **Database Query, Detection Parameter**, and **Program** report tables comprise only the default columns. It is not possible to add more columns.*

The Calibration Curve

The Calibration Curve

The **Calibration Curve** method window allows you to evaluate the calibration. For more information about the calibration, refer to **Theory of Calibration** ▣ **Calibration (Overview)**.

- Open the window from a different method, for example, from the **Integration** window, by selecting **Show Calibration Curve** on the **View** menu.

- Or, click the corresponding icon on the toolbar.

As described for the **QNT Editor** and **Integration** methods, various window sections can be displayed. The window arrangement usually comprises the **Chromatogram**, **Calibration curve**, and **Report** windows.
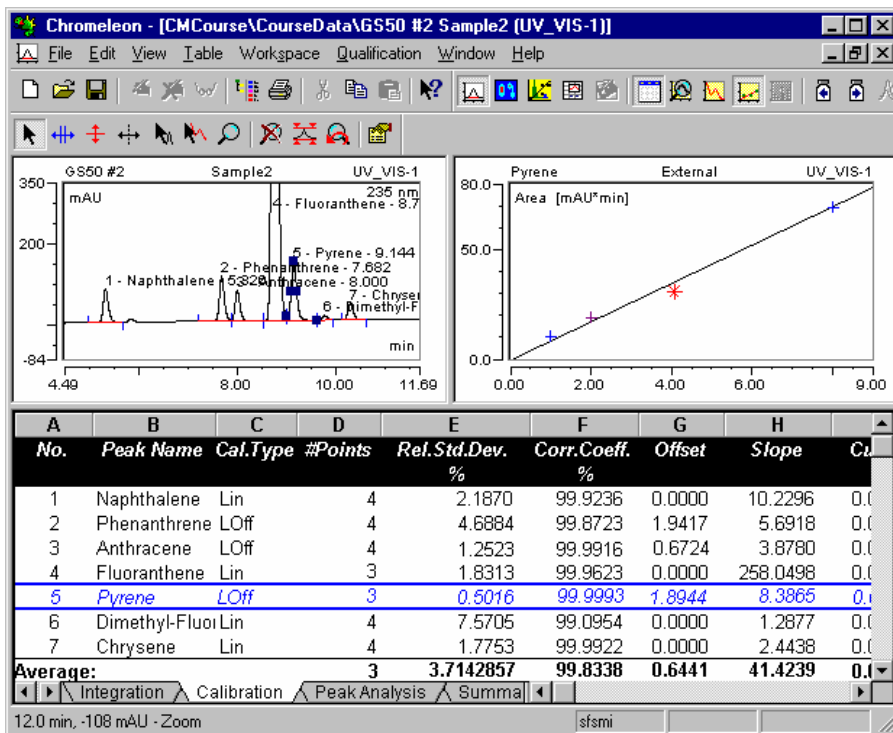
- Press F4 or SHIFT+F4 to change to the chromatogram of a different sample. Generally, all chromatograms of a sequence can be displayed on after the other.

- In the chromatogram, select the peak for which you want to display the calibration curve. The peak's background color changes.

- The **Calibration** tab page of the **Report** window shows the most important calibration data, such as ⇒*Calibration Type*, number of calibration points, ➢*Relative Standard Deviation* (Rel.Std.Dev), ➢*Correlation Coefficients* as well as the following calibration coefficients: ➢*Offset (c0)*, ➢*Slope (c1)*, ➢*Curve (c2)*, and ➢*Cubic Coefficient (C3)*. In addition, you can display, for example, the ➢*Variance* or ➢*Standard Deviation* (Std.Dev).

- Select **Decoration** on the context menu to change the colors and the captions of various window elements.

- Select **Column Properties** or **Table Properties** on the context menu to change the contents and the layout of the table. For more information, refer to **How to …:** **Creating and Using Report Tables**.

For more information about the calibration curve, refer to **How to …:** **Displaying Calibration Curves**.

 Note:

We would like to point out that the default use of the report variables in the Integration, Calibration, and Peak Summary reports is not binding in Chromeleon. You can freely configure each of the three reports, use any variables, or rename the report as desired.

The Printer Layout

The Printer Layout (Overview)

The increasing number of samples makes printing analytical results a complex and time-consuming procedure, especially because results are not only required on hardcopy but in all types of presentations.

Chromeleon considers this and emphasizes flexible report generation that meets all your requirements. The Printer Layout is the appropriate tool for this.

With each Chromeleon installation, different ➢*Report Definition Files (RDFs)* are stored in the **Dionex Templates** > **Reports** directory of the client PC.

- Click the 🖼 icon on the **Method** toolbar to open the **Printer Layout**.

📒 *Note:*

*For more information about the printing options, refer to **Basic Operation*** 🔲 ***Printing***.

The ➢*Printer Layout* represents a type of folder for various worksheets. Each sheet describes one or several printed pages (size of the chromatogram, where to position it, columns included in the numerical report, results represented in a chart, what is included in headers and footers).

The Printer Layout is saved together with the (on-screen) report of the 🔲 **Integration Window** in the Report Definition File. With an increasing number of worksheets in the Printer Layout of a Report Definition File, the printing possibilities increase as well. For example, if you want to print all samples of one sequence, it may be a good idea to present the results of a calibration sample differently from the unknown samples, and the lines of a ➢*PGM File* or summary report in yet another way. Specify which sample or file type should be printed and the worksheet to be used.

The Printer Layout of the **default.rdf** report definition file contains, for example, the **Integration**, **Calibration (Curr.Peak), Calibration (Batch), Peak Analysis**, **Summary**, and **Audit Trail** worksheets. Thus, it covers all default requirements.

For more information, refer to:

⬛ **Appearance and Function**

⬛ **Creating Your Own Templates and Worksheets**

⬛ **Printing**

For information about how to create a printer layout, refer to **How to …: ⬛ Preparing the Printout**. Also, refer to ⬛ **Specifying the Printout** for printing your results.

▪ Appearance and Function

The most important features of the Printer Layout are:

- The appearance and structure, as well as the functions are similar to Microsoft Excel. To enter formulas and create diagrams, use the ➢*Report Publisher*, which is an add-on product to Chromeleon.

- Toggle between the individual sheets by clicking the tabs provided at the bottom of the window.

- Double-click a tab to change the name, for example, to change **Integration** to **Integration Special**.

- Each sheet consists of a large number of columns (256) and lines (16000).

- Select **Insert Row(s)/Column(s)** or **Delete Row(s)/Column(s)** to insert columns or to delete columns or lines on the worksheet.

- Single areas or cells of a worksheet can be "filled" using the Windows clipboard (**Copy** and **Paste** commands) or via the **Insert** command on the context menu. (For more information, refer to **The Printer Layout ⬛ Creating Templates and Worksheets**.)
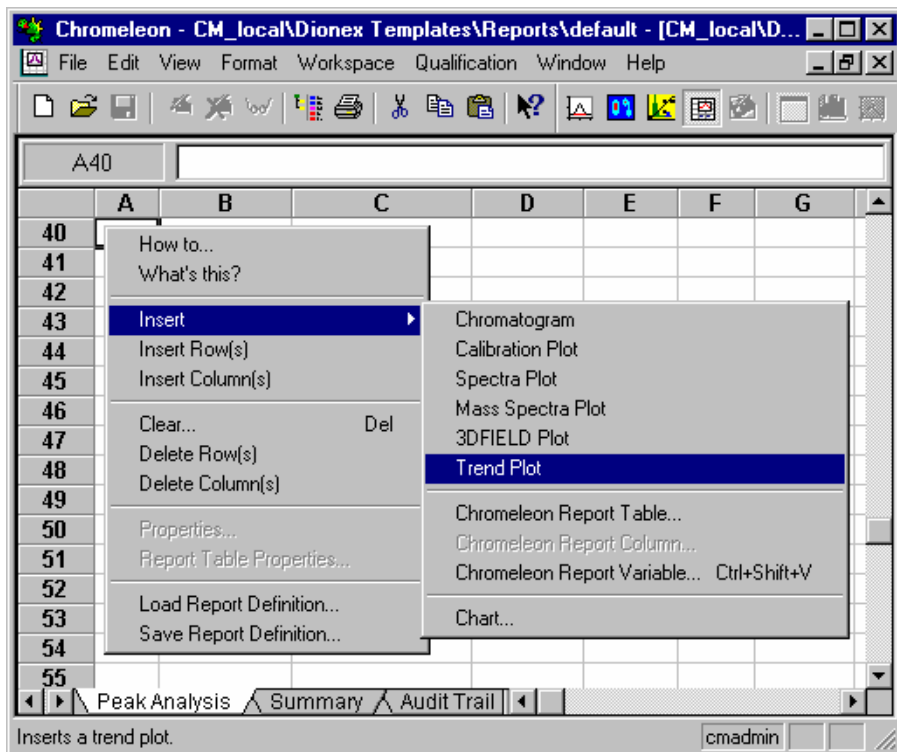
- If the ➤*Layout Mode* is enabled on the **Edit** menu, Chromeleon objects are marked by a red triangle. Chromatograms, tables, calibration curves, etc., have a red triangle in all four corners. For cells with single variables, the red triangle appears only in the upper right corner of the cell.

☞ *Tip:*

Note that you can place several report tables on a worksheet. The tables, however, must be positioned one below the other, not next to each other!

The worksheets that are virtually unrestricted in horizontal and vertical direction, allow creating page layouts larger that one printed page. Depending on the selected page size on the printer, one or several pages are required for printing this type of page layout. The worksheet is divided in many invisible horizontally and/or vertically positioned print pages.

If the user, for example, inserts a chromatogram that does not fit on one page, the rest is printed on a new page.

- Select **Page Setup** on the **File** menu to specify the order ("from left to right" or "top to bottom") in which to print the printed pages of a worksheet.

Top To Bottom   **Left To Right**

▪ Creating Templates and Worksheets

In addition to the default report templates provided in the **Dionex Templates > REPORTS** directory, Chromeleon allows you to create your own templates:

- Click the 🔲 icon on the **Method** toolbar to open the **Printer Layout** from your currently selected view. Chromeleon automatically opens the report template of the current sequence. Select the **Load Report Definition** command on the context menu to open a specific report template.

📝 *Note:*

*If only the default report templates are available in your system, save it under a new name via the **Save Report Definition** command. Afterward, you can edit the duplicate as follows:*

- Enable **Layout Mode** on the **Edit** menu to perform manual changes on the report template.

- Select the worksheet you wish to edit, or insert an additional (empty) worksheet via the **Insert Sheet** command (**Edit** menu).

- Select **Delete Sheet** on the **Edit** menu to delete a superfluous worksheet.

- Select **Insert** on the context menu to insert chromatograms, tables, diagrams, report variables, or other elements.

⚠️ *Caution:*

Note that you can place several report tables on a worksheet. The tables, however, must be positioned one below the other, not next to each other!

Each element inserted in this way reserves a specific area on the worksheet. If a report template or a single worksheet serves as a print template, these areas are "filled" with the values or graphics of the current sample. It is also possible to determine whether the printed output includes data and chromatograms of all channels of a sample or only of one channel.

📝 *Note:*

In addition to report variables that can be combined to form new functions via the four basic arithmetical operations (plus powers), it is possible to calculate additional functions known from Microsoft Excel. For a list of supported functions, refer to ➢Additional Functions in the Glossary.

For more information about how to create worksheets and report templates, refer to **How to …:** 🔳 **Preparing the Printout**.

🔳 Printing

From the Printer Layout

The Printer Layout allows you to print the created report template at any time.

- Select **Print** on the **File** menu to start printing.

- Click **Preview** in the **Print** dialog box to preview the layout of the printed output.

The printed output uses the data of the current sample with the layout of the currently opened sheet.

From the Browser

You can print larger quantities of data, either single samples or even entire sample series, from the Browser:

- Use the mouse to select the samples to be printed. If you want to print all samples of one or several sequences, select the associated sequence(s).

- To print samples from several sequences, perform a ➢*Query*

- Afterward, select **Batch Report** on the context menu and select the **Report Definition** to be used for the printed output. In addition, select the channel to be printed and specify the printer.

In addition to the results, it is possible to print sample data, that is, the information in the sample list.

- Open a sample list and select **Print Table** on the context menu.

Peak Purity Analysis

PPA: Peak Purity Analysis

⚠️ *Caution:*

The basic requirement for using the PPA method is the availability of a Dionex ➢Photodiode Array Detector. In this case, the data supplied by a detector can be "read" and viewed on any client PC. The most common method to relate chromatograms to spectra is the representation of data in a ➢3D Field in the method PPA. The 3D field is the default view in the iso-pixel plot representation.

Use one of the following methods to display a sample (for which you have the corresponding 3D raw data) in the method PPA:

- Select the sample in the Browser, and then select **Open > 3DFIELD** on the context menu.

--Or--

- Change from a different method to the PPA method. Click the PPA icon or select **PPA** on the **View** menu.

View and Operation

In the default view, the method window is divided in four sections. The 3D field window (A3) is the most important section. Select either **Iso Pixel Plot** or **3D plot** (see ➢*3D Field Presentation Modes*).

The cross-wires that are freely positioned via the left mouse button or the arrow keys "extract" a chromatogram at a specific wavelength in horizontal orientation from the plot representation. In vertical orientation, a spectrum is extracted at the time t and is displayed above (A1) or next (A2) to the 3D field (A3). Select the ➢*Zoom* function for a more detailed view.
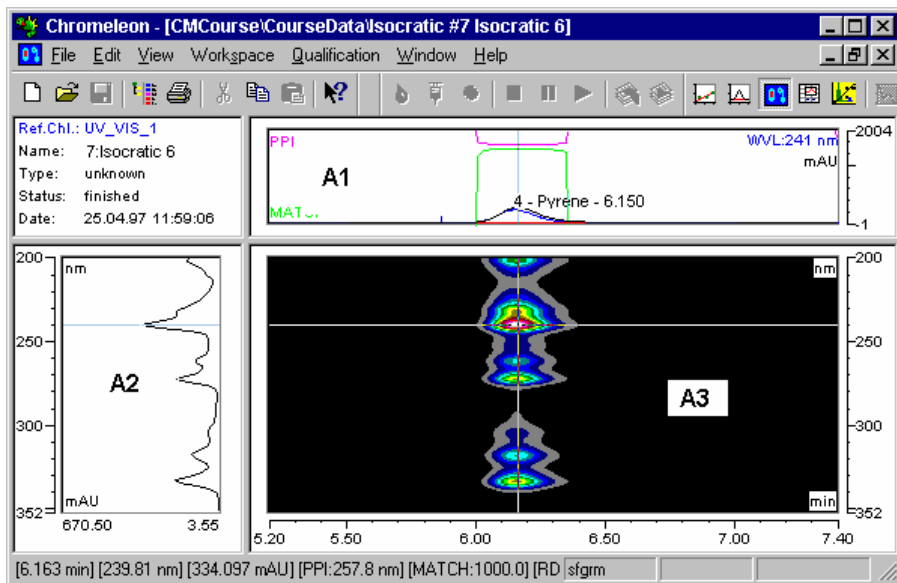
To show or hide the window sections A1 and A2, select **Show Chromatogram** or **Show Spectra**. As an option, a numeric report (PPA report) can be displayed in addition (**Show Report**). The line of the currently selected peak is marked by a different color.

In addition to the context menu commands that allow undoing a **Zoom** operation, restoring the original 3D field, selecting a different 3D representation, or displaying additional information such as the ➢*PPI Index*, ➢*Match Factor*, etc., there are additional operations that can be executed via the keyboard or a mouse-click.

- Double-click to open the **3D field Decorations** dialog box.

- Right-click while the 3D field is redrawn to stop redrawing. This option is useful, e.g., when you chose the wrong zoom area.

- Double-click the time axis of the window to perform the **Full Size** command. In the wavelength scaling, the same operation performs the **Autoscale** command.

- Press the CTRL key to make the currently displayed spectrum in the spectra window "permanent." In this way, it remains on the screen even when you move the cross-wires. The spectrum extracted via the cross-wires is displayed in addition. Perform this operation several times if you want to display several spectra in the spectra window.

- It is also possible to combine pressing the CTRL key and moving the cross-wires: Position the x-axis of the cross-wires on the required wavelength and press the CTRL key. Then move the y-axis over a peak in the chromatogram. The spectra window now shows all spectra within the covered range. You can perform this procedure for various peaks in a chromatogram. Release the CTRL key between the individual peaks to represent each peak in a different color. For each peak, a number of spectra can be displayed.

- If the y-axis of the cross-wires is located on a peak, the peak spectrum (= spectrum in the peak maximum) can be copied to the Windows clipboard via the **Copy** command. From there, the spectrum can be included in the spectra library. For this operation, approximately positioning the y-axis is sufficient.

- Select one of the **Extract:** commands to extract the active chromatogram, the optimum integration path, the current spectrum or the 3D field data and save the item under a separate name. For a description of the required steps, refer to **How to …: Displaying and Using UV Spectra** **Extracting Spectra, Chromatograms**, **3D Field Data** and **Selecting the Optimum Integration Path**.

 Note:

Copying or printing a 3D plot is more time-consuming than copying or printing the Iso plot!

- Select **Library Search** on the context menu to search the spectra library for the displayed spectrum. For more information, refer to **How to …: Displaying and Using UV Spectra** **Searching Single Reference Spectra**.

Function

Use the PPA method:

- To analyze the peak purity (see **How to …: Displaying and Using UV Spectra** **Analyzing the Peak Purity**).

- To assign peaks interactively via the spectrum.

- To extract chromatograms, spectra, and the optimum integration path.

- To visualize chromatograms for presentation and archiving purposes.

- To evaluate baseline effects.

- To check the Lambert-Beer linearity range.

- To perform quantitative analysis of overlapping peaks.

To complete these methods, Chromeleon supports various procedures and calculations for result interpretation and/or comparison. These include:

➢*Baseline Correction of Spectra*

➢*Blank Run Subtraction*

➢*Normalization*

➢*PPI: Peak Purity Index*

➢*PPI: Match Factor*

Spectra Libraries

Spectra Libraries

The **Spectra Library** pane allows you to compare ➢*Normalized* and single ➢*baseline-corrected* spectra with spectra from various libraries. There are three sections:

Spectra Table

The upper section lists all spectra that are contained in a ➢*Spectra Library*, as well as their data.
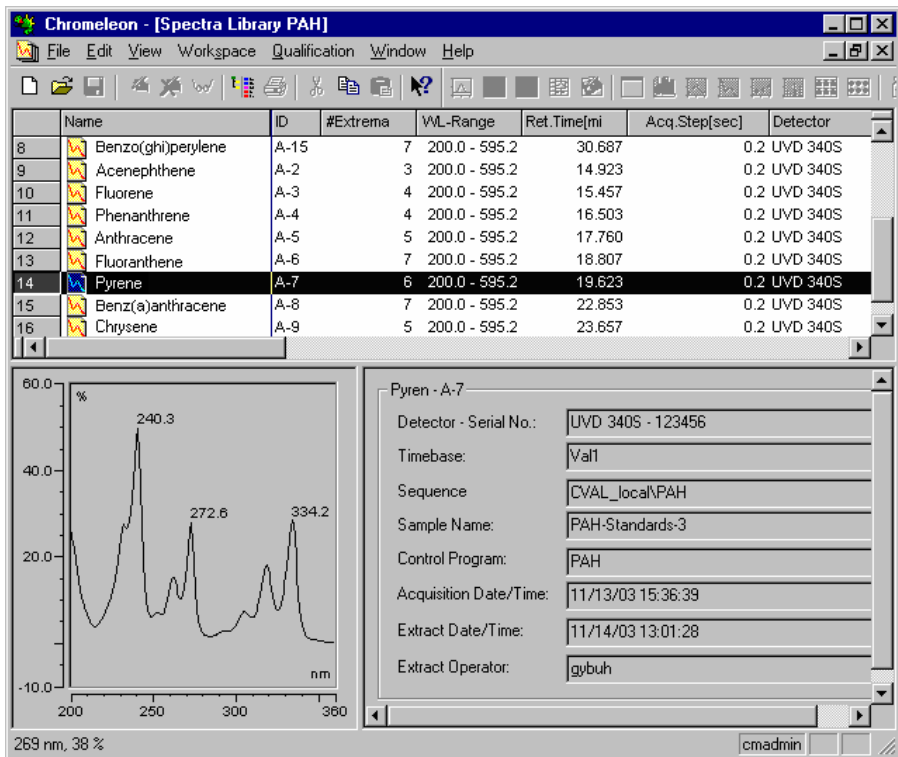
Spectra Plot

The spectra plot is displayed at the bottom left, underneath the table, showing the spectra of all substances selected in the spectra table.

Data Window

The bottom right section is reserved for data representation, showing important data related to the spectrum that is selected in the spectra table.

For more information about Spectra Libraries, refer to **How to …: Displaying and Using UV Spectra:**

 Creating and Using Spectra Libraries

 Creating a New Library

 Comparing Spectra

3D Amperometry

The 3D Amperometry Window

The 3D Amperometry window displays a sample's ➤*3D_Amp* data, which is collected using an ICS-3000 ED electrochemical detector in integrated amperometry mode.

Use one of the following methods to display 3D amperometry data:

- Select the sample in the Browser and then select **Open>3D_Amp** from the context menu.

- If the sample is already open in another window (for example, the Integration window), select **3D Amperometry** from the **View** menu or click the following icon on the **Method** toolbar .

Tip:

*A real-time plot of the 3D amperometry data can be displayed online during a run. See **How to …:** Controlling Devices from the Control Panel for more information.*

The window is divided into several display areas. The main area in the lower right displays the raw 3D amperometry data. The area above the 3D data displays a chromatogram of selected data. The area to the left of the 3D data displays an I-t plot (current vs. waveform time) of selected data.
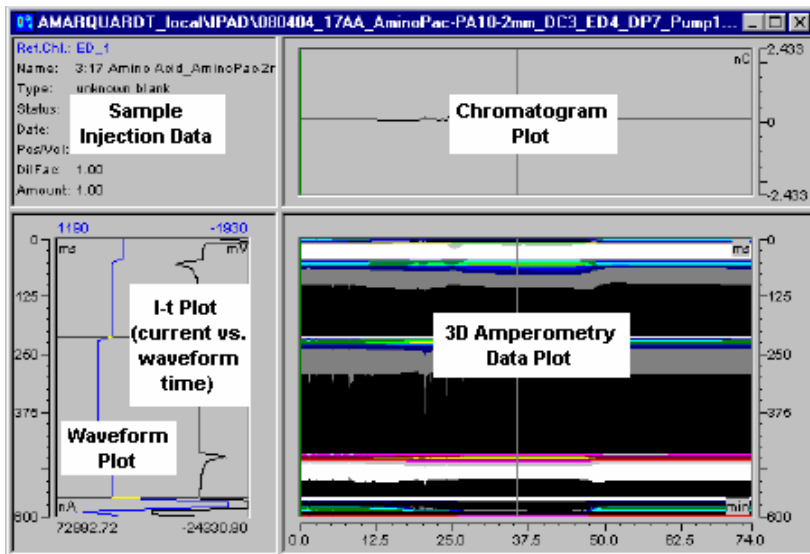
You can use several methods to select the data displayed on the plots:

- Drag the horizontal and vertical line cursors.

- Use the mouse to zoom into a selected area.

- Select a command (for example, **Zoom to Integration Interval**) from the **View** or context menu.

To select various plot options (for example, to switch from integrated to raw data view), select **Decoration** from the **View** or context menu.

For more information, refer to 🔲 **Display Areas** and 🔲 **Data Selection**.

Also, refer to **How to …:** 🔲 **Analyzing 3D Amperometry Data**.

🔲 Display Areas

The 3D Amperometry window displays three plots of the ➤*3D_Amp* data:

Chromatogram Plot

The chromatogram plot is generated either by retrieving one raw data point per ➤*Waveform* from the selected 3D data (if raw data is displayed) or by calculating one integrated data point per waveform cycle from the selected 3D data (if integrated data is displayed).

The x-axis is the retention time (min) and the y-axis is the response (nA for raw data or nC for integrated data).
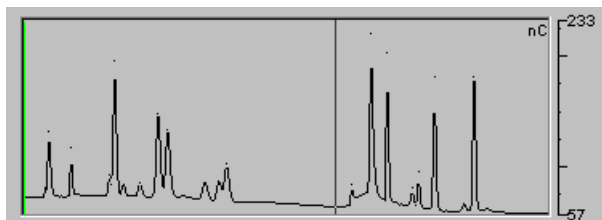
Raw 3D Data Plot

The raw 3D amperometry data plot can be viewed as either an isoamperometric or a 3D (wire frame) plot. For both plot types, colors are used to represent the ranges of response values.

The isoamperometric view is the default view for the 3D raw data. This is a top-down view of the data (imagine you are looking down on the data from above the plot). The x-axis is the retention time (min) and the y-axis is the ➢*Waveform Period* (ms). The z-axis, which is not visible on the plot, is the response (nA). Different levels of response are represented by different colors.
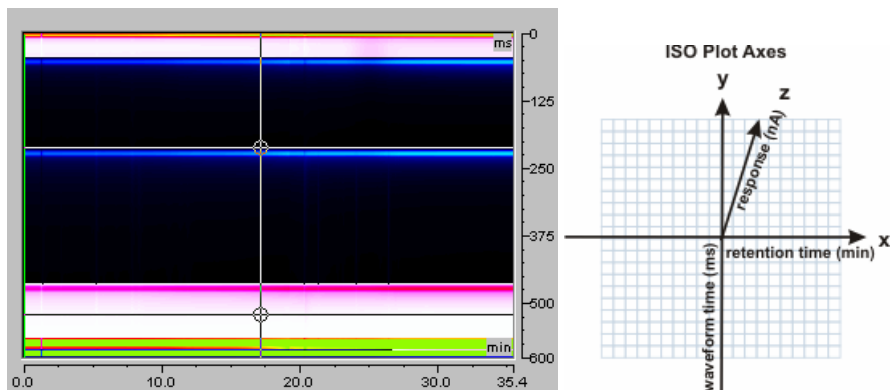
The 3D view projects the response values in the third-dimension, which allows you to see the height of responses and well as the color mappings. For this view, imagine you are standing in front and slightly to the left of the plot.
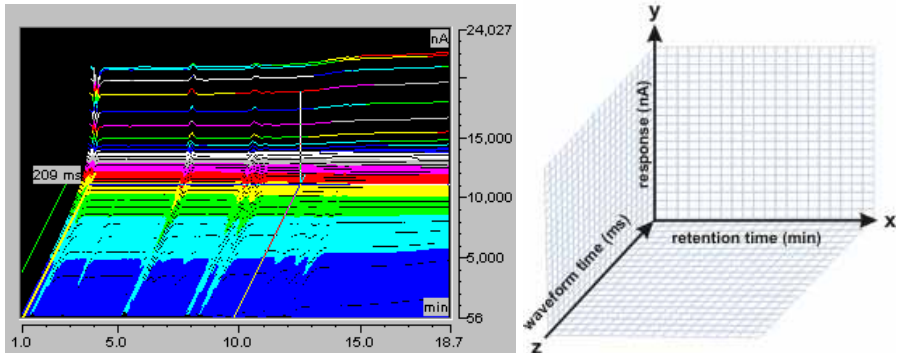
I-t Plot

The I-t plot is a plot of current (I) vs. waveform time (t). To better visualize this plot, imagine that a vertical slice of the 3D amperometry data is taken at retention time (T) and then the slice is laid flat. The left axis is the waveform period (ms) and the bottom axis is the current (nA). A waveform can optionally be overlaid on the plot. When the waveform is displayed, the top axis indicates the applied voltage (mV).
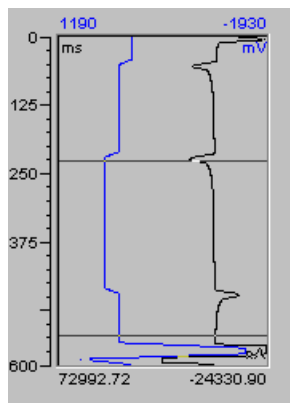
Also, refer to:

Data Selection

How to …: Analyzing 3D Amperometry Data

■ Data Selection

The 3D Amperometry window provides the following data selection features:

- If integration is enabled, parallel horizontal line cursors on the 3D and I-t plots select the ➤*Integration Interval*. You can move the integration interval by dragging the top line on either the 3D or I-t plot. You can also increase or decrease the width of the interval by dragging the bottom line. Changing the integration interval selects new data to be integrated. This generates a new chromatogram, which is displayed on the chromatogram plot.

■☞ *Tip:*

If multiple integration intervals are present, select the interval that you want to change by dragging the vertical retention time line cursor into the interval's time range.
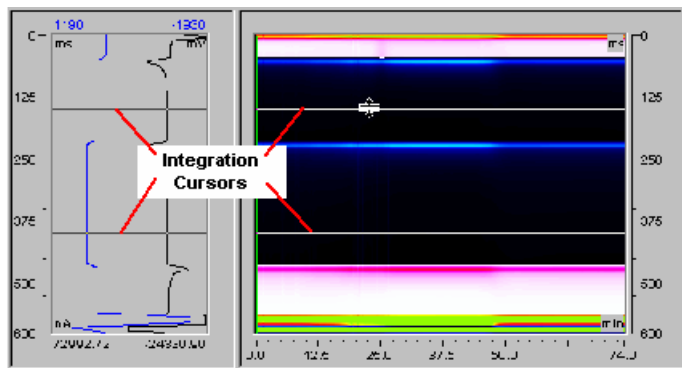
- If integration is disabled, a single horizontal line cursor on the 3D and I-t plots selects a waveform time slice. The selected waveform time determines which data points are used to plot the chromatogram. You can drag the cursor to select a different waveform time and generate a new chromatogram.
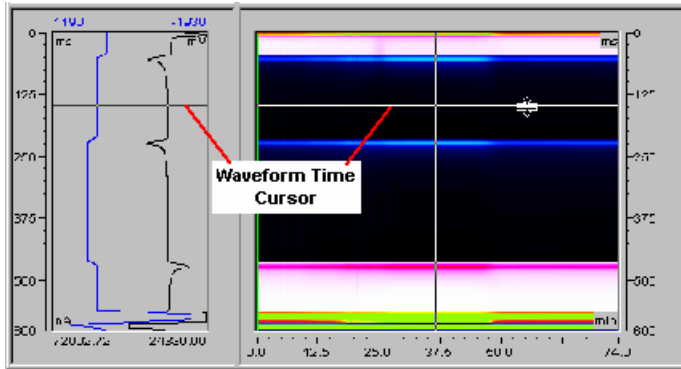
- The vertical line cursor on the 3D and chromatogram plots selects a retention time slice. The selected retention time determines which data points are used to create the I-t plot. You can drag the cursor to select a different retention time and thus generate the corresponding I-t plot.
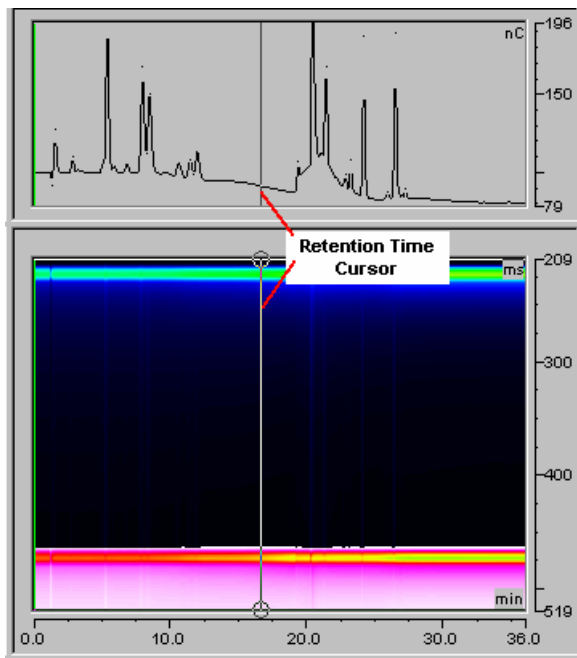
- The mouse can be used to zoom into an area of a plot. Point and drag to draw a frame around the area that you want to zoom into.

- The **View** and context menus also provide commands for selecting data.

📑 *Tip:*

If multiple waveforms were applied during the run, or if additional integration intervals were added post-run, non-movable vertical markers (in green) designate where each waveform change occurs.
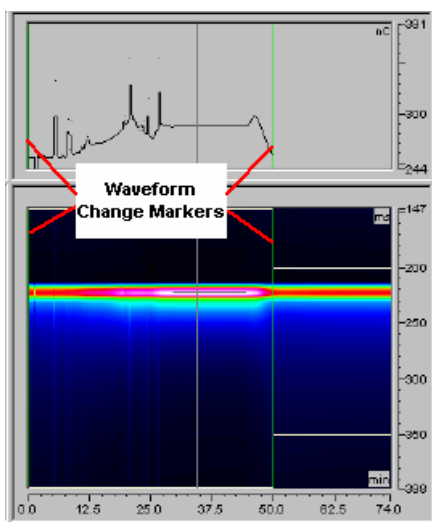
Also, refer to **How to …:** 🔲 **Analyzing 3D Amperometry Data**.

How to …:

Creating and Managing Files

Chromeleon allows you to record a huge number of chromatograms. To manage, copy, move, and/or delete the different files that are required for this task, Chromeleon provides a central tool for file management, the Browser (also, see **Data Management** **The Browser**). In the Browser, you can, e.g., create sample lists, save data in a datasource, search for this data later, and import data from other programs. For information about which actions you can perform in the Browser, refer to:

 Creating a Sample List (Sequence)

 Handling Files and Datasources

 Signing Sequences Electronically

 Performing a Query

 Importing PeakNet Method Files (Release 4.5 Through 5.2)

 Importing Agilent/HP ChemStation Data Files

For information about the actions that administrators can perform in the Browser, refer to **How to …:** **Working with Files, Databases, and Networks** in the **Administrator Help** section.

 Caution:

Browser functions and structure are similar to the Windows Explorer. However, do not confuse the Browser with the Windows Explorer! Do not use the Windows Explorer for operations within Chromeleon datasources.

Creating a Sample List (Sequence)

There are two basic ways how to create a sample list. (For more information about the sample list, refer to **Samples and Sequences** **The Sample List (Sequence)**):

1. Manually edit an existing sequence and save it under a different name.

2. Automatically create a sequence using the **Sequence Wizard**. (For information about the wizard, refer to **Samples and Sequences** **The Sequence Wizard**).

Manually creating a sample list

- In the Browser (see **Data Management** 🔼 **The Browser**), select the sequence whose QNT and ➢*PGM Files* you want to use for the new sequence, probably after having edited them. Select **Save as** on the **File** menu to save the sequence under a different name. Edit the new sequence as necessary.

- In the lower right Browser section, enter the names and the properties of the standard samples and the unknown samples to be analyzed:

- Click a cell in the table and enter the new value or the name via the keyboard. To open a dialog box for assistance, press the F8 key.

- In the ⇒*Name* column, enter a name for each sample to be analyzed.

- In the ⇒*Type* column, select the sample type from the drop-down list (Unknown, Blank, Validate, Standard, Spiked, or Unspiked).

- In the ⇒*Pos.* (sample position) and ⇒*Inj. Vol.* (injection volume) columns, type the autosampler position from which to inject and the substance volume.

- In the ⇒*Program* and ⇒*Method* columns, determine the ➢*Chromatographic Methods* to be used for the analysis.

- In the ⇒*Status* (sample status) column, define how often a sample shall be processed. Select **Single** to process the sample only once. Select **Multiple** to process the sample several times.

- To analyze two unknown samples (Sample 1 and 2) on the basis of a 2-point calibration, the input can be as follows:

No.	Name	Type	Pos.	Inj. Vol.	Program	Method	Status
1	Std - 1. Inj.	Standard	R99	10.00	Control 1 ▼	QNT 1	Single
2	Std - 2. Inj.	Standard	R99	20.00	Control 1	QNT 1	Single
3	Sample 1	Unknown	RA1	10.00	Control 1	QNT 1	Single
4	Sample 2	Unknown	RA2	10.00	Control 1	QNT 1	Single

- It is not imperative that you fill the other columns, e.g., ⇒*Weight* (Sample Weight Factor), ⇒*Dil. Factor* (dilution factor) and ⇒*Inj. Date/Time*. Either they contain special parameters or the system fills them automatically after the analysis.

- If you are not sure which values or names to enter, select the cell and press the **F8** key. An edit dialog box appears listing the allowed values and/or options. Enter a value or select an option. Clicking OK automatically updates the cell.

- Press the **F1** key to display more information about the individual columns.

- Press the **F9** key to fill all subsequent cells of a column with the same input.

- You can have Chromeleon generate a unique ID for each sample (see 🔧 **Using Globally Unique Sample Identifiers**).

- To enter special values, create ➢*User-defined Columns* (see 🔧 **Creating User-defined Columns**).

- To take an overview of the most important results in the sample list after the samples have been analyzed, add ➢*Sequence Report Columns* (see 🔧 **Creating a Sequence Report Column**).

- To save the sequence under a new name, select **Save As** on the **File** menu. To save the sequence under the existing name, select **Save**.

- Check the sequence properties by pressing <ALT**>** + <ENTER**>**. Or else, select the sequence, and then select **Properties** on the context menu.

Automatically creating a sample list

- Use one of the following methods to open the Sequence Wizard:

  In the Browser, select **New** on the **File** menu. Select **Sequence (using Wizard)** from the list and click **OK**.

  In a panel tabset, click the **Sequence Control** tab. Under **Create Application**, click **Create Sequence**.

- Follow the instructions to create a basic sequence structure.

- Press the F1 key for more help.

For more information about the appearance of the sample list, refer to 🔧 **Displaying Sequence Columns**.

For information about the wizard, refer to **Samples and Sequences** 🔧 **The Sequence Wizard**.

▣ Using Globally Unique Sample Identifiers

Chromeleon supports the generation of unique 128-bit character strings, allowing you to clearly identify each sample. For new datasources, Chromeleon always generates a globally unique identifier (➢*GUID*). To use globally unique sample identifiers for existing datasources, follow the steps below:

- Select the datasource for which you want to enable unique sample identification.

- Select **Properties** on the context menu.

- On the **General** tab page, select the **Use Globally Unique Sample IDs** check box.

📝 *Note:*

*You can select the check box only if you have the **CustomizeDS CmUser** ➢Privilege.*

📨 *Tip:*
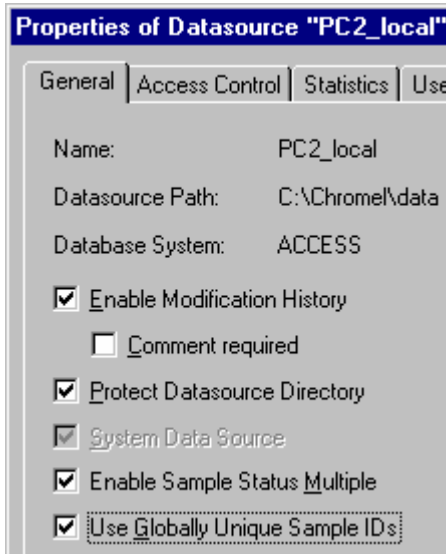
It is not possible to disable this feature once you have enabled it.

- Chromeleon generates a GUID number for each sample in the datasource when the analysis is started:

Status	Inj. Date/Time	GUID
Finished	22.03.2005 15:34:26	92f53a3e-9ade-11d9-bbc2-aee437656b2a
Finished	22.03.2005 15:38:42	0c8b8bea-9ae0-11d9-bbc2-aee437656b2a
Finished	22.03.2005 15:42:58	a547fe46-9ae0-11d9-bbc2-aee437656b2a
Finished	22.03.2005 15:47:14	3e0d9bda-9ae1-11d9-bbc2-aee437656b2a
Finished	22.03.2005 15:51:31	d6c5789a-9ae1-11d9-bbc2-aee437656b2a
Finished	22.03.2005 15:55:47	6f75b156-9ae2-11d9-bbc2-aee437656b2a
Finished	22.03.2005 16:00:03	0866189a-9ae3-11d9-bbc2-aee437656b2a
Finished	22.03.2005 16:04:20	a144296e-9ae3-11d9-bbc2-aee437656b2a
Finished	22.03.2005 16:08:36	39f77092-9ae4-11d9-bbc2-aee437656b2a
Running	22.03.2005 16:12:37	d2be955a-9ae4-11d9-bbc2-aee437656b2a
Single		

The column is hidden by default. To add the column to the sample list, select **Display Column** on the context menu. The related dialog box is opened. Select **GUID** on the **Hidden columns** list and click **Add** (or **Add to End**) to add the column to the **Visible columns** list.

📖 *Tips:*

GUIDs are not generated during manual sample acquisition.

*When you rerun a sample later, e.g., a sample with the status **Multiple**, Chromeleon generates a new GUID, overwriting the previous one.*

▣ Creating User-defined Columns

New columns can be created either in the sample list of the Browser (see **Data Management** 🔼 **The Browser**) or in the peak table of the QNT Editor:

1. a) In the sample list of the Browser: Click the datasource for the sequence for which you want to create a new column. Select **Properties** on the context menu, and then click the **User-defined Columns** tab page.

   b) In the peak table of the QNT Editor: On the context menu or **Edit** menu, select **Columns**, and then select **Display User-defined Columns**.

2. Select **<New user-defined column>** from the **Columns** list to enable the **Properties** section.

3. Type a caption for the new column in the **Name** input field.

 Note:

When you enter the name, keep the following restrictions in mind:

- *Use only characters, numbers, and the underscore. The first character must be a letter.*

- *Do not use the German "Umlaute" or "ß."*

- *Names starting with **SEQ_** or **SMP_** are not permitted.*

- *If the name is also an SQL keyword, Dionex recommends preceding it with an abbreviation of your company name (or some other agreed-upon text). For example, if the new caption is INTEGER, you might enter it as DX_INTEGER, where DX stands for Dionex. This will prevent collisions with SQL keywords and help you instantly recognize user-defined columns.*

- *Several protected names, such as, Retention, Wavelength, Flow, etc., cannot be used as names for user-defined columns unless a prefix or suffix is added. Since it would be very time-consuming to check long lists of protected names before assigning a name, it is recommended in this case, also, that you use a company abbreviation as a prefix. For example, instead of "Flow," assign the name 'DX_Flow' to a user-defined column.*

- *Names of columns that have been deleted in the current 'dialog session' cannot be entered again. You must close and then re-open the dialog box before re-entering these names.*

4. Specify the **Value Type** for the new column by selecting the corresponding option from the drop-down list:

- Integer (whole numbers)
- Floating point (numbers with a defined number of decimal places)
- Date
- Time
- Date and Time
- Enumeration (open the combo box to select an item from the list)
- String (any sequence of characters. Please note: Spaces at the end of the string are deleted.)

5. Type the **Dimension** for the values in the new column in the corresponding input field.

6. More specification fields may be displayed, depending on the selected value type.

Note:

   The operating system restricts date input to the period of 1/2/1970 - 12/30/2037.

7. a) When creating a user-defined column in the sample list of the Browser, click **Append Column** to add the new column to the **Columns** list. Click **OK** to append the new column to the sample list.
    b) When creating a user-defined column in the peak table of the QNT Editor, click **Append Column** to append the new column to the peak table.

Any new column created in the sample list of the Browser applies to the entire ➢*Datasource*; i.e., it becomes part of all ➢*Sequences* in the datasource.

Tips:

Be careful when creating user-defined columns in the sample list of the Browser. Be sure not to assign the same column name twice in different datasources or on different computers that may communicate with each other. Otherwise, unless the column definitions are identical, problems may occur when you copy sequences or when you **Restore** *backup files.*

When you have entered or changed user-defined columns in the sample list, shut down and restart the ➢Server to use the new and/or changed columns in the ➢PGM File.

There are some restrictions on the deletion of user-defined columns. Chromeleon cannot delete user-defined columns unless they are empty. In addition, Chromeleon cannot delete user-defined columns in some database configurations, e.g., this is not allowed in Oracle, version 8.0, or MS SQL Server, version 6.5. In this case, use the Oracle or MS SQL Server database tools to delete the column. For the corresponding database columns, refer to the SAMPLES database table. The column header corresponds to the name of the user-defined column in Chromeleon (without the preceding asterisk). Please note: Deleting a user-defined column in Oracle or MS SQL invalidates ➢Electronic Signature for all sequences containing this column.

Note:

In a typical HPLC or IC configuration, it is possible to process at least 16 user-defined sample list columns in the program.

For two examples of how to use user-defined columns, refer to **How to …: Creating and Using Report Tables** ◪ **Calculating the Concentration Percentage (in Relation to the Total Concentration)**.

New columns are added on the right-hand side of the table. However, you can also change the order of the columns afterward. For more information, refer to ◪ **Displaying Sequence Columns**.

If you still cannot display all the values you need, see ◪ **Creating a Sequence Report Column**.

◪ Creating a Sequence Report Column

It often makes sense to have some sample results displayed in the sample list of a sequence. To do so, add sequence report columns to the sample list, in addition to the default columns and the ➢*User-defined Columns*. Thus, the sequence report columns can provide an overview of the most important results of the single samples, already in the Browser.

Note:

*You need the **CustomizeColLayoutSEQ** privilege to add sequence report columns while the ➢User Mode is enabled.*

To add a sequence report column:

- Click the sequence, and then select **Properties** on the context or **File** menu. The **Properties** dialog box appears.

- On the **Report Columns** tab page, the **Report Columns** list contains all existing sequence report columns:

- Click **New** to open the **Create Sequence Report Columns** dialog box.

☞ *Tip:*

You can also open this dialog box from the sample list. Select either **Report Columns** *on the context menu or* **Sequence Report Columns** *on the* **View** *menu, and then select* **New Report Column***.*

- First, select a report variable. All report variables of the different ⇒*Report Categories* are available for selection. Click the '**...**' button, and then select a variable from the **Categories** and **Variables** lists.

- Change the column name (**Identifier**) and the column header. (It is usually not necessary to change the unit (**Dimension**)).

- Having selected a peak-specific variable, define the peak to which it refers. Select a **Peak** option.

- In the same way, if you have selected a channel-specific variable, define the channel under **Channel**.

- In addition, you can display the statistical values for the corresponding column. Select an option: **Sum, Average Value**, or **Relative Standard Deviation**.

In this way, you can create different sequence report columns and thus, display all required values in the sample list:

No.	Name	SST Result	Modified?	#Peaks	RelArea_N [%] Naphthalene	RelArea_N_2 [%] Naphthalene	RelArea_N_3 [%] Naphthalene	Ret.Time (det) [min] Pyrene	Area_P [mAU*min] Pyrene	Amount_P [µg/ml] Pyrene
		Uv_vis_1	Uv_vis_1	Uv_vis_1	Uv_vis_1	Uv_vis_2	Uv_vis_3	Uv_vis_1	Uv_vis_1	Uv_vis_1
1	Sample 1	Passed	Yes	10	3.581	18.642	24.807	9.146	8.590	1.035
2	Sample 2	Passed	No	7	3.167	14.187	23.350	9.144	16.542	2.014
3	Sample 3	Passed	No	9	2.902	10.616	22.632	9.112	32.018	3.918
4	Sample 4	Passed	No	9	2.303	6.564	19.340	9.081	65.447	8.033
5	Sample 1 (Replicate)	Failed	No	7	3.167	16.864	23.842	9.121	8.128	0.978
6	Sample 2 (Replicate)	Passed	No	8	3.005	13.782	22.723	9.126	16.059	1.954
7	Sample 3 (Replicate)	Passed	No	8	2.639	9.806	6.292	9.114	31.928	3.907
8	Sample 4 (Replicate)	Passed	No	10	2.292	6.726	19.781	9.095	63.018	7.734
9	Sample 1 (Replicate 2)	Passed	No	7	3.315	16.904	24.525	9.087	8.672	1.045
10	Sample 2 (Replicate 2)	Passed	No	8	3.091	13.599	22.989	9.113	16.260	1.979
11	Sample 3 (Replicate 2)	Failed	No	8	2.812	10.017	22.134	9.095	31.411	3.844
12	Sample 4 (Replicate 2)	Passed	No	10	2.236	6.491	19.321	9.080	63.154	7.751
Σ	Sum									44.192
X̄	Average Value	n.a.						9.109		
σ	Rel. Std. Dev.	n.a.						0.247 %		

New columns are added on the right-hand side of the sample list. However, you can change the order of the columns afterward or hide any columns that are currently not required. For more information, refer to 🔲 **Displaying Sequence Columns**.

Editing Sequence Report Columns

To edit report columns afterward:

- Select the column by clicking the column header.

- Select **Report Columns** on the context menu or select **Sequence Report Columns** on the **View** menu. Then, select **Modify Report Column**.

- The **Modify Sequence Report Columns** dialog box appears. The dialog box corresponds to the **Create Sequence Report Columns** (see above). Make your settings as required.

☞ *Tip:*

You can also open this dialog box from the sample list by double-clicking the column header of the selected column.

📖 *Note:*

*Or else, you can also select the sequence, and then select **Properties** on the context menu. Click **Modify** on the **Report Columns** tab page or double-click an entry on the list to open the **Modify Sequence Report Columns** dialog box.*

Deleting a Sequence Report Column

To delete a sequence report column:

- Select the sequence by clicking the column header.

- Select **Report Columns** on the context menu or select **Sequence Report Columns** on the **View** menu, and then select **Delete Report Column**.

☞ *Tip:*

You can also select the entire column by clicking the column header and then pressing <Del>.

📝 *Note:*

*Or else, you can select the sequence and then select **Properties** on the context menu. On the **Report Columns** tab, select the column from the Report Columns list, and then click **Delete**. Alternatively, select an entry and press <Del>.*

Undoing Changes

There are different ways to undo changes made for a sequence report column:

- On the sample list: Press <CTRL> + <Z> or select **Undo** on the **Edit** menu.

- On the **Report Columns** tab page of the **Properties of Sequence** dialog box: Click **Undo Changes**.

If the sequence report columns are not sufficient to display all the values you need, also see 🔳 **Creating User-defined Columns**.

◼ Displaying Sequence Columns

Chromeleon provides different options for displaying the single columns in the sample list. Use these options to create the sample list according to your requirements.

Displaying or Hiding Columns

Often, it is often not necessary to display all columns in the sample list. Therefore, you can hide any columns that are currently not required:

- In the sample list, select **Display Columns** on the context or **View** menu. In the **Display Columns** dialog box, select the columns you want to display and determine the order in which they shall appear:
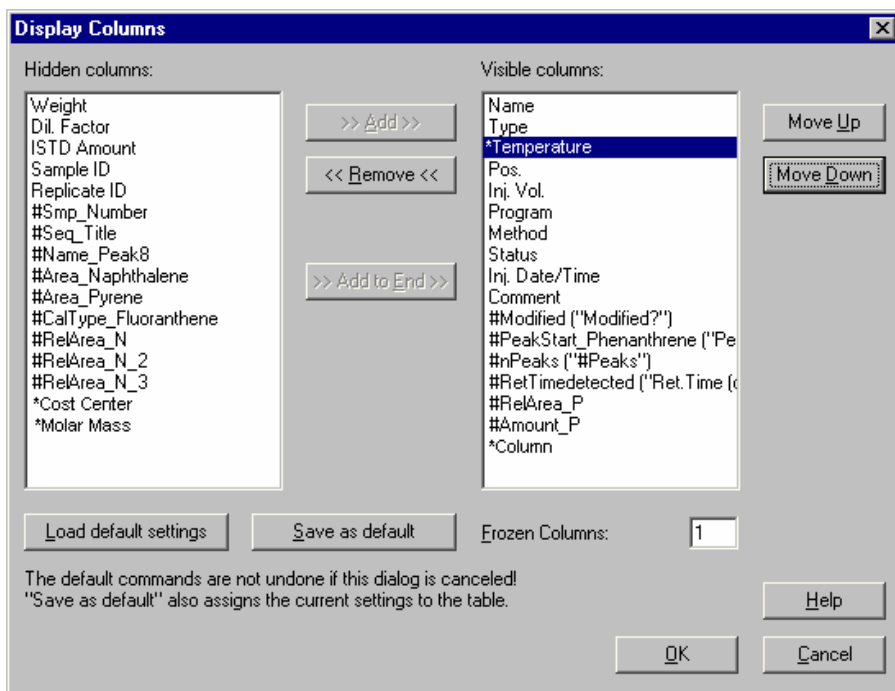
Under **Visible Columns**, all columns are listed that are visible in the sample list. All columns that are currently hidden are listed under **Hidden Columns**.

A '#' character in front of the column name, e.g., #Area_Pyrene indicates that the column is a report column. If the name of the report column, i.e., its identifier, is different from the column header, the column header appears in parenthesis and brackets after the column name, e.g., #nPeaks ("#Peaks"). An asterisk (*) in front of the column name, e.g., *Temperature, indicates that this column is a ➢*User-defined Column*.

- Under **Visible Columns**, select the columns you want to hide.

- Click **<<Remove<<** to remove these columns from the **Visible Columns** list.

In the same way, you can later add them again to the sample list. Select them in the **Hidden Columns** list, and then click **>>Add>>** or **>>Add to End>>**.

 Note:

It is also possible to hide columns interactively. Left-click the right column separator. While clicking, push the column together completely. To display the column again, move the mouse pointer to the right of the column separator until the pointer changes it appearance. Left-click and drag the column separator to the right.

Changing the Order

You can also change the order in which the columns appear in the sample list:

- Select the column(s) to want to move.

- Move the column(s) to the desired position via the **Move Up** and **Move Down** buttons.

 Note:

It is also possible to change the order interactively. Left-click and draw the column to the desired position.

Displaying Columns by Default

If the sample list includes many columns, not all of them can be displayed on the screen at the same time. Use the scroll bar to move to the left or right. However, you might want to see at least the most important columns by default. Under **Frozen Columns**, define the number of leftmost columns to be displayed even if the scroll bar is at the utmost right.

Also, refer to:

 Creating a Sample List (Sequence)

 Creating User-Defined Columns

 Creating a Sequence Report Column

Handling Files and Datasources

Handling files and datasources includes managing, copying, moving, and/or deleting chromatographic data and files (see **Moving and Copying Elements**). Chromeleon provides several ways to handle, store, and save the data collected during operation:

 Datasources on Removable Media

 Opening Audit Trails

 Creating Backup Files

 Restoring Backup Files

 Exporting Files

Moving and Copying Elements

Use Drag & Drop to move or copy various elements in the Browser; for example, directories, ➢*Sequences*, samples, and individual files, such as ➢*PGM Files*, ➢*QNT Methods*, ➢*Audit Trails*, etc.

Drag & Drop via the left mouse button

To move or copy an element, hold down the left mouse button and move the element to the target directory. To define the Drag & Drop behavior, select **Preferences** on the **File** menu, and then select a **When dragging item between folders** option:

Ask	For each Drag & Drop action within a datasource, you are prompted to click a button. Click **Copy** or click **Move**.
Copy	For simple Drag & Drop actions, i.e., when you do not hold down any other key, the selected element is copied to the new position without being deleted at the original position.
Move (As in the Windows Explorer)	There are two different cases:
	Within a datasource: For simple Drag & Drop actions, the selected element is moved to the new position and deleted from the original position.
	To a different datasource: The selected element is copied to the target position, but is not deleted from the original location.

Independent of the selected setting, you can force the desired behavior for each Drag & Drop action by pressing an additional key:

<CTRL>	Copies the element.
<SHIFT>	Moves the element.

Tips:

If network failure occurs while a Move action is in progress, the elements either in the target directory or in the original directory will be incomplete. After rebooting your computer, check whether the elements were moved correctly. If they were not, complete the action as required.

If you cancel a Move action, all data copied so far will be deleted. This may take some time.

Sequences that are locked and signed can only be moved as a whole. Locked directories cannot be moved at all.

Drag & Drop via the right mouse button

Hold down the right mouse button and move an element to the target directory. When you release the mouse button, the context menu is opened:

Copy
Move
Cancel

Select an action (Copy, Move, or Cancel).

Copy elements via Copy & Paste

To copy the selected element, you can also select the **Copy** and **Paste** commands on the **Edit** or context menu or click the corresponding icons on the **Standard** toolbar. First, copy the element, and then paste it at the desired location. Or else, use the corresponding shortcuts:

<CTRL> <C> = Copy

<CTRL> <V> = Paste

Move elements via Cut & Paste

To move the selected element, you can also select the **Cut** and **Paste** commands on the **Edit** or context menu or click the corresponding icons on the **Standard** toolbar. First, cut the element and then paste it at the desired location. Or else, use the corresponding shortcuts:

<CTRL> <X> = Cut

<CTRL> <V> = Paste

Behavior if a file already exists in the target directory

If you move a file to a target directory in which a file with the same name already exists, a dialog appears. You are prompted to confirm overwriting the existing file. Click either **Yes** or **Yes to all** to confirm the action. Click **No** or **No to all** to cancel the move.

Tips:

If the Move action is later cancelled by the user or terminated due to an unforeseeable error, all data overwritten so far will be lost.

To overwrite a file, you need the corresponding Delete ➢*Privilege. For example, to overwrite a sequence, you need the* **DeleteSEQ** *privilege. The administrator can assign you this privilege in the* ➢*User Manager (CmUser program). A Delete privilege is not required if you move a file (the file is no longer available at the original location after the move, i.e., it is "deleted" there).*

◼ **Datasources on Removable Media**

➢*Datasources* can be installed not only on the hard disk of the local PC or a network PC, but also on removable media, such as a disk drive, USB stick, or CD-ROM.

- Select **Mount Datasource** on the **File** menu. Chromeleon automatically displays the Windows drive letters of all removable media that are currently available.

- Select a drive. Or else, select **Browse** to navigate to the datasource of interest.

- If Chromeleon finds an existing datasource on the selected media, the system automatically connects to this datasource, displaying it in the Browser in the same way as any other datasource.

- If Chromeleon does not find a datasource on the selected media, the user can install a new one on this drive. The datasource will receive the name of the drive.

📔 *Note:*

You can easily copy datasources in Microsoft Access database format to a removable medium. Copy the DATA directory of the local datasource or the desired subdirectory of a network datasource to the corresponding removable medium.

⚠️ *Caution:*

If you connected to a datasource on a removable medium via the **Mount Datasource** *command, do not remove the medium until you have closed the connection. To do so, select the* **Dismount Datasource** *on the context menu.* **If you do not close the connection correctly, data may be lost**.

Tip:

When storing access datasources on CD, DVD, or other read-only media, make sure that no Chromeleon server, Chromeleon client, or ➢Online Transfer Agent (OTA) is connected to the datasource. In addition, remove any existing CM_local.ldb-File from the root directory of the datasource.

Opening Audit Trails

In the Browser, you can open two types of event logs (see **Data Management** **The Audit Trail**):

Daily Audit Trails

Sample Audit Trails

In the ➢*Standard Datasource*, the Sample Audit Trails are listed in the AUDIT directory for the corresponding ➢*Timebase*. Double-click to open a Sample Audit Trail.

To display a Sample Audit Trail, select the related sample. Select **Open** on the context menu, and then select **Audit Trail**. You can also display Sample Audit Trails in the **Report**. Double-click to open the related sample and click the **Audit Trail** tab.

Creating Backup Files

The Chromeleon ➢*Backup* program allows you to create backup files, including all linked objects, for data storage and exchange.

In the ➢*Browser*, select the objects to be archived, and then select **Export/Backup** on the **File** menu.

You can back up the following objects:

Select a	To backup
Directory	All subdirectories (including all files, sequences, etc.)
Sequence	All samples and files included in the sequence (QNT File and ➢*Program File (PGM File)*)
Sample	The sequence information (name, directory, title, etc.) and selected samples, as well as the ➢*PGM File* and ➢*QNT File* for these samples
Other files	Related files

To save samples found in a ➢*Query*, select one of the following options:

Select a	To save
Sample (on the sample list in the Browser)	The sequence information (name, directory, title, etc.) and selected samples, as well as the PGM and QNT Files for these samples
Sequence (on the sequence list in the Browser)	All samples of the selected sequence that are contained in the query
Query (in the Browser)	All samples contained in the query

⚠ *Caution:*

When performing a backup for a query, back up the entire sequence and not only the samples found during the query if you want to delete the original data after the backup. Before deleting the original data, verify that the backup file contains all samples.

The following objects are linked to the associated files, and are also copied for the backup:

Object	Linked Object
Sequence	Preferred ➢*Report Definition* file (RDF)
Peak table	Calibration list for calibration mode = Fixed (**General** page of the QNT Editor) Blank run sample for Blank Run Subtraction = Subtract a fixed sample (**General** page of the QNT Editor) and/or If selected, spectra library for spectra library screening (**Spectra Library Screening** page of the QNT Editor)
Query	Preferred report definition file (RDF)
Samples	QNT File PGM File

If a linked object is included in the selected objects list, it is treated as a normal object.

Select **Backup** to open the **Backup** dialog box:
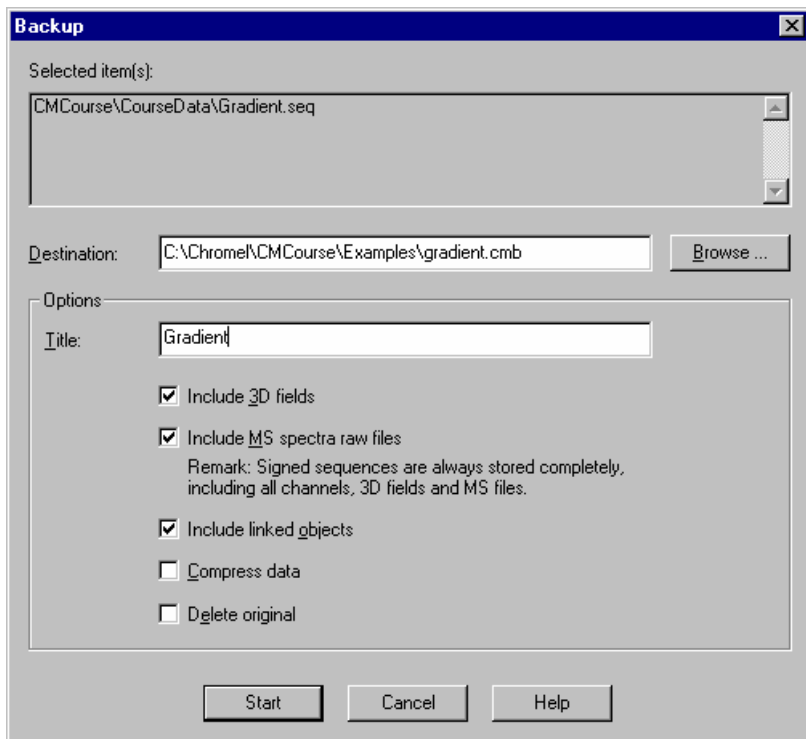
For a description of the individual fields, refer to the online Help for the **Backup** dialog box.

Before starting the backup, Chromeleon determines the amount of storage space required. For hard disks and network drivers, Chromeleon checks whether sufficient storage capacity is available. If there is not enough space, an error message appears and you can cancel the backup procedure.

If you use removable media, Chromeleon displays the expected number of media. If you use data compression, the number of actually required media may differ from the displayed number.

You can monitor the procedure on the screen via a status bar that indicates the percentage of stored data already copied to the medium:
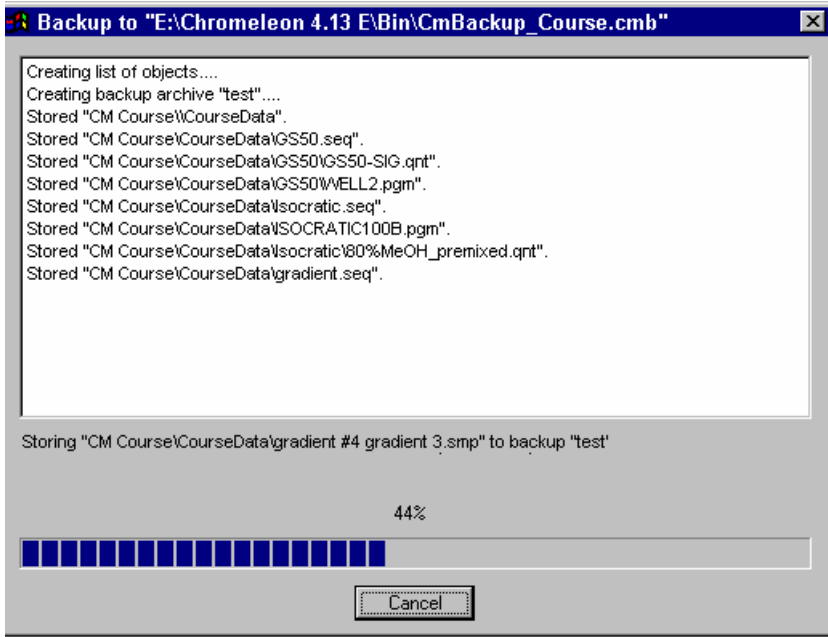
If the destination is a removable medium, you will be prompted when to change the medium.

To perform a backup, you must have the **Backup** privilege (see ➢*Privileges (User Rights)*). The backup is logged in the file history. To stop the backup, click **Cancel**. The backup contains all objects stored up to this point.

You can delete the original objects after the backup has been performed. A dialog box appears and prompts you to confirm this action. If History Mode is enabled, a dialog box appears in which you can enter a comment for the history.

Backup files have the extension **.cmb**. For information about how to restore backup files, refer to **How to …: Creating and Managing Files and Data Restoring Backup Files**.

◼ Restoring Backup Files

To restore backup files created via the ➢*Backup* command, select **Import/Restore** on the **File** menu in the Browser, and then select ➢*Restore* (also, refer to **How to …: Creating and Managing Files and Data 🔺 Creating Backup Files**).

☞ *Tip:*

As an alternative, you can select the backup file by double-clicking the file name in the Windows Explorer. Backup files are identified by their extension (.cmb).*

After having selected **Import/Restore > Restore** on the **File** menu, select the backup file you want to restore in the Windows **Open** dialog box. Chromeleon recognizes whether the file is stored on a removable medium, a hard disk, or a network drive.

If the backup file is stored on removable media, you have to insert the first medium first because it includes the list of contents. Else, an error message appears Chromeleon displays the contents in the following dialog box:
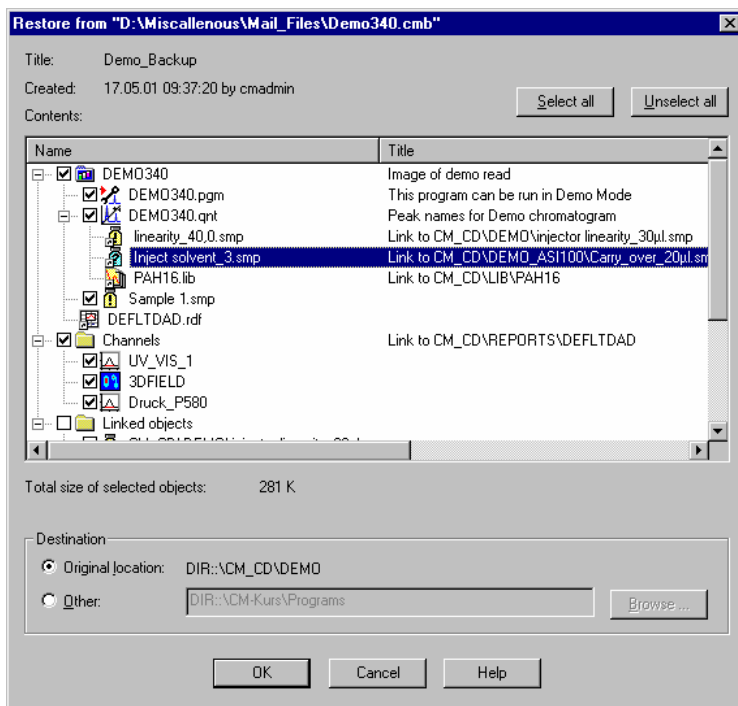
For a description of the individual fields, refer to the online Help for the **Restore** dialog box.

The ➢*Channels* and ➢*Linked Objects* that are shared by all samples are listed in separate groups. The linked objects appear under the corresponding objects.

Select the channels and links to be restored from these groups. The default setting is that all channels are restored, but not the links.

The selected objects are restored, together with the raw data files of the selected channels, the Audit Trail files, and the history files. A dialog box similar to the Backup dialog box appears. If the backup is located on several removable media, you will be prompted when to insert the next medium.

If the directory for an object to be restored is missing, Chromeleon creates the directory. Links are always restored to the original location. Note that the related sequence and datasource must exist. However, missing directories will be created if necessary.

If the destination object already exists, you are prompted whether to overwrite it. Select one of the following options: **Yes**, **Yes to all**, **No**, **No to all**, and **Cancel**. For each restored object, the corresponding entry is logged in the history.

To restore an object, you must have the **Restore** privilege (see ➢*Privileges (User Rights)*). To overwrite an existing object, you must have both the **Restore** and **Copy** privileges for the object. To stop restoration, click **Cancel**.

🔖 *Tip:*

If you have created a backup file that contains a new feature, keep in mind that you cannot read this file with a Chromeleon version that does not support this feature. For example, this refers to:

- *➢Sequence Report Columns (available since Chromeleon 6.50)*

- *⇒Std. Add. Group and ⇒Ref. Amount Set sample columns (available since Chromeleon 6.60)*

- *The ➢Trend Plot (available since Chromeleon 6.50)*

▪ Exporting and Transferring Data

Chromeleon provides various options for exporting data (see **Data Management** 🔳 **Data Export**):
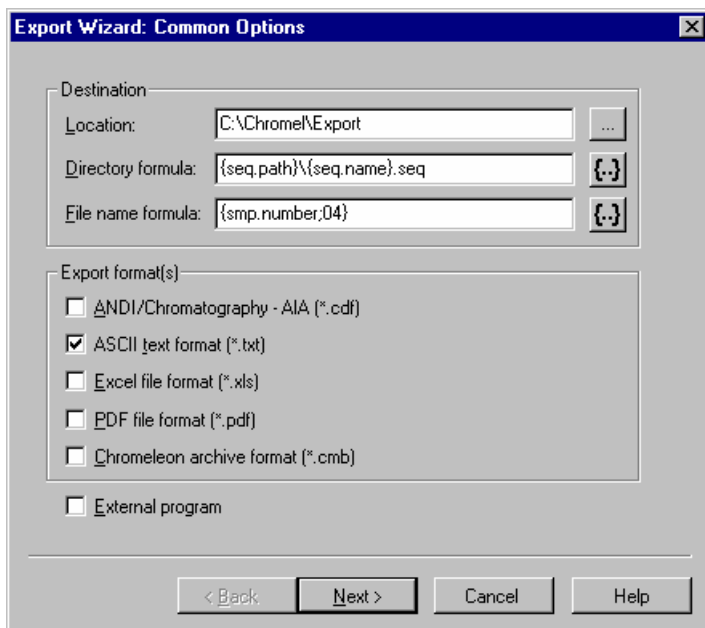
1. Data can be exported during or after a ➢*Batch*, using the **Export Wizard** of the **Batch Report**.

2. Sequences can be transferred at a specified time, using the ➢*Online Transfer Agent (OTA)*.

For more information, refer to 🔳 **Exporting Data during or after a Batch** and 🔳 **Transferring Sequences Automatically**.

▪ Exporting Data during or after a Batch

Select the sample(s) or sequence(s) to be exported:

- Select **Batch Report** on the **File** menu to open the **Batch Report** dialog box.

- To open the **Export Wizard**, select the **Export** check box in the **Export** options section. (If the check box is already selected, click **Export Settings**.)

- Click the "**...**" button in the **Location** field to specify where data is stored.

- Use the **Directory formula** field to automatically create additional folders under the existing location folders in the Windows Explorer. To facilitate finding your files, Dionex recommends using the same structure as in the **Browser**. Use the default {seq.path}\{seq.name}.seq formula to create the same structure.

  If you do not know the syntax, click the **{...}** button to enter the formula. For the {seq.path}\{seq.name} formula, select **Sequence** from the **Categories** list, and then select **Directory** from the **Variables** list. Click **OK** to confirm your selection. Enter a backslash and return to the previous dialog box by clicking the **{...}** button again. Select **Sequence** from the **Categories** list and **Name** from the **Variables** list. Confirm your input by clicking **OK**.

- Use the **File name formula** field to enter a formula for the file name. Individual files are usually created for the corresponding samples. Therefore, **{smp.name}**, i.e., the sample name, is an appropriate entry if every sample has a specific name.

  If you do not know the syntax, click the **{...}** button to enter the formula. For the **{smp.name}** formula, select **Sample** from the **Categories** list and **Sample Name** from the **Variables** list, and then click **OK** to confirm your selection.

  The default entry is **{smp.number;04}**. The number of the corresponding sample is used for the file name. 04 indicates a four-digit number; i.e., this entry creates file names such as **0001.txt** or **0053.txt**.

- Under **Export format(s)**, select an export format.

- Click **Next** to go to the next wizard page. Select the export options. Please note: The selected export option determines which wizard pages appear. For example, for the ASCII format, if you want to export raw data with the report, select the channel(s) first and then select the sheets to be exported.

👉 *Tip:*

*Before exporting a **Summary** page, verify (in the Printer Layout) that a peak was selected in the individual columns. If the setting is **Selected Peak**, no data will be exported because you did not select a peak in the Browser. Double-click the column to open the **Properties Report Column** dialog box. If necessary, select **Fixed Peak(s)** and specify the peak for which you want to export the data from the column.*

- Click **Finish** to complete the **Export Wizard**, and then click **OK** to start exporting.

For more information, refer to:

🔃 **Exporting Data from Different Samples to a Single File**

🔃 **Transferring Sequences Automatically**

🔳 Exporting Data from Different Samples to a Single File

Use one page of the ➤*Printer Layout* containing a peak summary table (which is usually the **Summary** page) to export data for a specified peak from different samples of a sequence to just one file. First, define the columns of interest:

- Enable ➤*Layout Mode* on the **Edit** menu.

- Double-click the header of the column of interest to open the **Report Column Properties** dialog box. Select a peak, and then select **Save Report Definition…** on the **Workspace** menu to save the Report Definition File (RDF).

👉 *Tip:*

*If the setting in the dialog box is **Selected Peak**, it is not possible to export any data. Although data is always exported from the Browser, it is not possible to select the peak there.*

- Change to the Browser and follow the description in 🔃 **Exporting Data during or after a Batch**. Use the following settings:

  - In the **File name formula** field, enter **{seq.name}**, i.e., the formula**,** for the sequence name; this generates a single file for the sequence without a superior directory.

  - Select the export format: **ASCII**, **Excel**, or **PDF**.

  - Select **Summary** or the corresponding page of the peak summary table as the report page to be exported.

  - Click **Finish** to complete the **Export Wizard**, and then click **OK** to start exporting.

For more information, refer to 🔃 **Transferring Sequences Automatically**.

▣ Transferring Sequences Automatically

Sequences found with a ➢*Query* can be transferred at specified times, using the ➢*Online Transfer Agent (OTA)*.

▪☞ *Tip:*

When the ➢User Mode is enabled, you need administrator rights to be able to transfer sequences automatically.

In the Browser, select **Preferences** on the **File** menu. The **Preferences** dialog box is opened. The **Online Transfer Agent** tab page shows a Log File displaying errors, warnings, and information, as well as a list of the tasks to be executed.
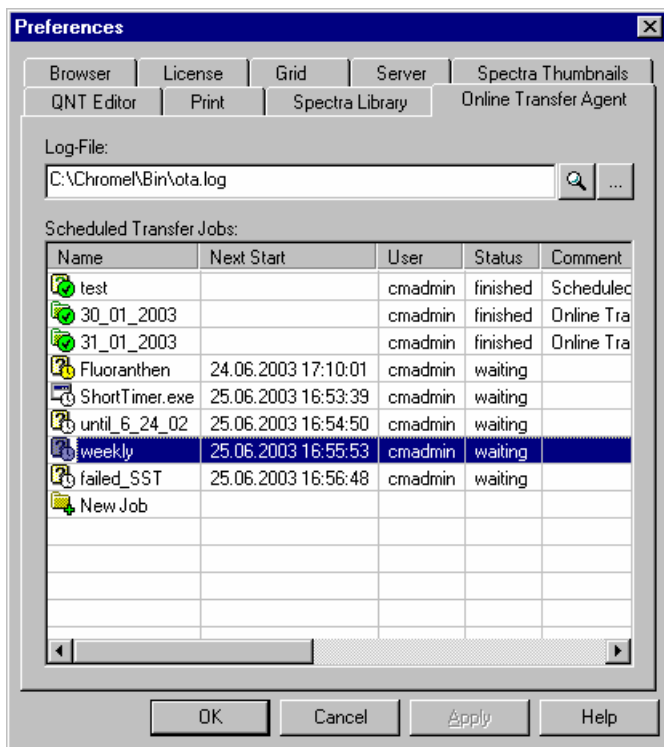
- Double-click the **New Job** entry at the bottom of the list to enter a new job. The **Scheduler Job Type** dialog box is opened.

- Select **Transfer sequences selected by a query** to open the **New Scheduler Job** dialog box. The dialog box contains two tabs:

  - On the **Time Plan** tab page, define when, and how often, data will be transferred.

  - On the **Transfer** tab page, select the source and target of the transfer job to be executed by the **OTA**.

Tips:

During batch transfer, sequences in the destination directory may be overwritten if they have the same name as the sequences to be transferred. However, it is not possible to overwrite locked data, signed data, or sequences containing raw data. (Example: If you appended samples to a sequence that was already transferred, you have to delete the existing sequence in the destination directory manually before you can transfer the extended sequence.)

Sequences not containing raw data are overwritten; a warning does not appear before this operation nor are the ➢Privileges (e.g., DeleteSEQ) checked.

For more information, refer to:

Exporting Data during or after a Batch

Exporting Data from Different Samples to a Single File
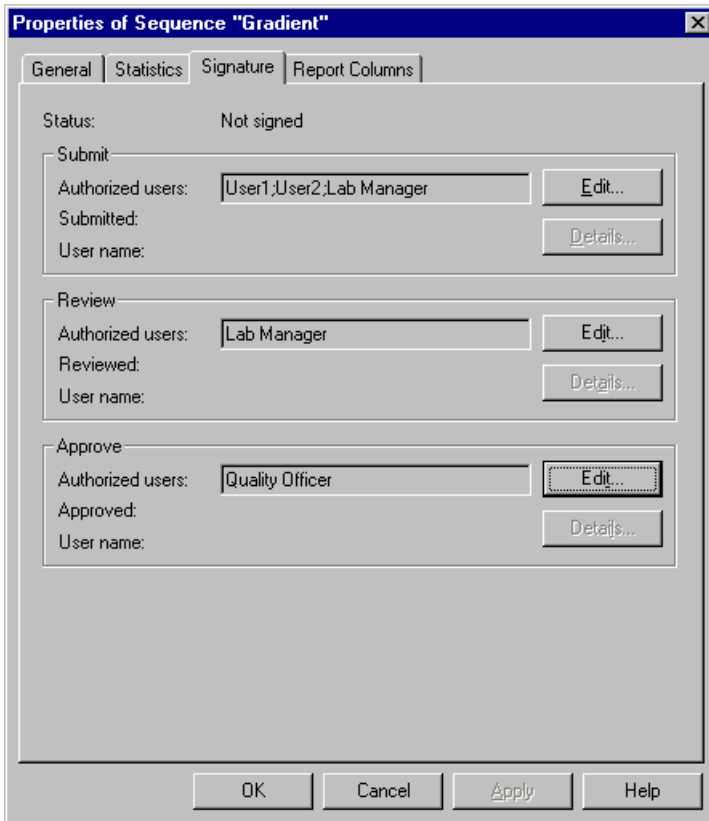
Signing Sequences Electronically

Electronic signature is an important tool for securing data within the scope of quality assurance and ➢*GLP*. (For general information about electronic signature, see **Samples and Sequences Electronic Signature**.) In order to sign sequences electronically, the ➢*User Mode* must be enabled and the logged-on user must have the corresponding signature ➢*Privileges*; for example, **SignResults**. For more information, refer to **Chromeleon User Management Signature Privileges** in the **Administrator Help** section.

☞ *Tip:*

➢*Electronic Signature is available only for user databases that were created with a User Manager (CmUser) program version 6.10 or higher. If an error message notifies you that electronic signature is not possible, update your database.*

You can sign a specific sequence only if you are authorized to do so. To check and/or edit authorization, open the **Properties** dialog box of the sequence from the context menu, and then click the **Signature** tab page:

If you have been authorized in the CmUser database to modify the signature requirements (**ModifySignRequirements** privilege), click **Edit** to edit the list of users authorized for each signature level.

If these conditions are met, ➢*Sequences* can be signed in three steps:

- Submit

- Review

- Approve

The sequence's signature status determines which options are available on the context menu:

- **Submit Results** (for unsigned sequences)

- **Review Results** (for submitted sequences)

- **Approve Results** (for reviewed sequences)

You can also access these options by selecting **Electronic Signature** on the **File** menu or by clicking the following icon on the standard ➢*Toolbar*:

The function of the button depends on the signature status.

For more information, refer to:

Individual Steps of Electronic Signature
Checking the Signature Status and Undoing the Signature

▪ Individual Steps of Electronic Signature

The first step of electronic signature is the **Submit** process. (For general information about electronic signature, see **Samples and Sequences** ▪ **Electronic Signature**.) Click a ➢ *Sequence* that has not yet been signed, and then select **Submit Results** as described in ▪ **Signing Sequences Electronically**.
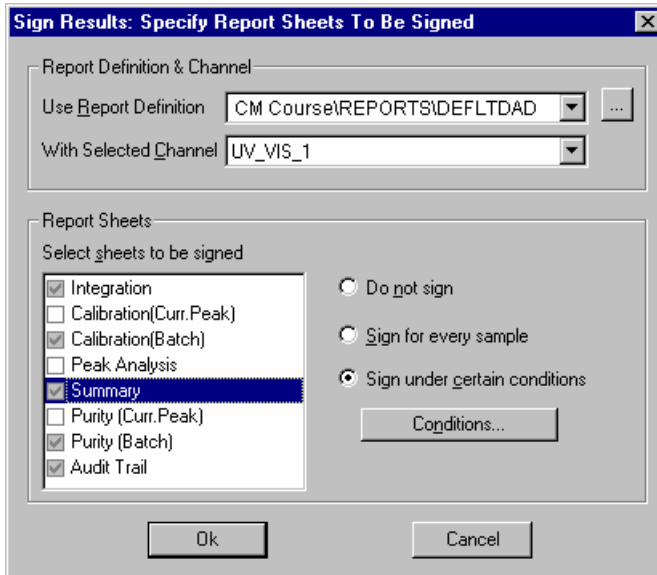
Select the ➢ *Report Definition File (RDF)* to be used for displaying the sequence, select the channel for which you want to show the results, and select the report sheets to be signed.

▪☞ *Tip:*

The settings saved in the Report Definition File are used as defaults for the worksheets to be signed and for the respective conditions. For more information, refer to **How to …: Preparing the Printout** ▪ *Specifying the* **Pages to Be Printed***.*

After you have selected the report definition file, the system writes the results to an ➢ *SOR-File (Signed Off Results)*. You can monitor the procedure on the screen via a status bar that indicates the percentage of results already copied to the file.

Simultaneously, the pages of the report for the individual samples are frozen. Afterward, the **Check Signed Results** dialog box appears:

This dialog box allows you to check the report to be signed. Use the arrow keys to toggle between different sequence samples. For each sample, the tab pages for the selected report sheets are displayed. Click **OK** when you have finished checking the report. The **Submit Signature** dialog box appears.

To sign the SOR file, enter your User ID and the signature password:

In the **Comment** field, you can enter additional information, such as any critical or doubtful points you noticed while creating the report.

When a sequence is open, the SOR file is displayed under the other sequence files in the top right Browser section. Double-click to open the SOR file:
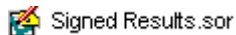
To review sequences that have been signed and submitted, select **Review Results**. You can monitor the process on the screen. The files and samples of the sequence that have already been reviewed are listed, as well as the result of the review. For the file or sample under review, the status bar indicates the percentage of completion:
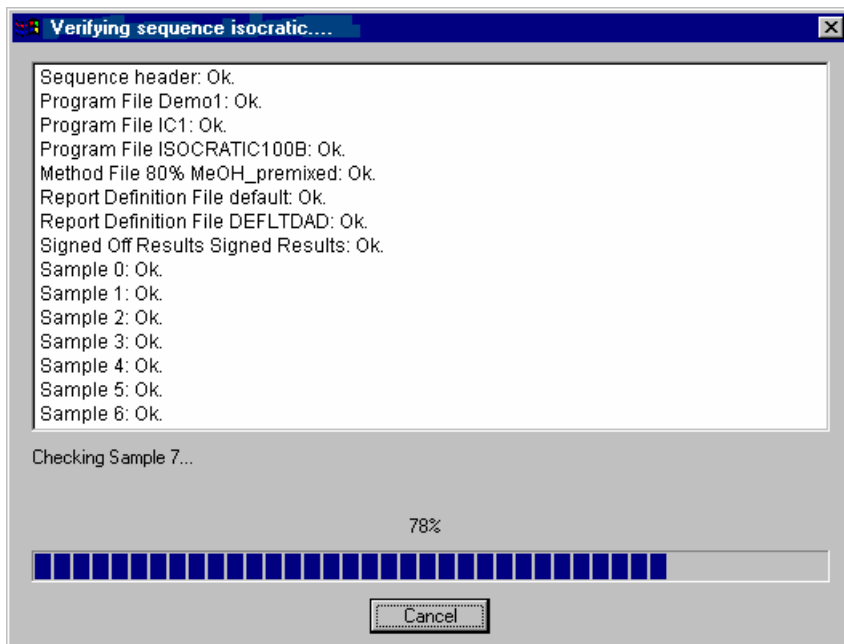
(Reviewing the results is much faster than submitting the signature. Thus, the above dialog box may be visible only for a few seconds, especially with short sequences.) To **approve** a sequence, proceed in the same way.

▣ Checking the Signature Status and Undoing the Signature

To check the signature status of a ➤*Sequence*, select the sequence, and then select **Properties** on the context menu. The **Properties** dialog box is opened. Click the **Signature** tab page for information about the users authorized to sign, review, and/or approve the sequence. (For general information about electronic signature, refer to **Samples and Sequences** ⬆ **Electronic Signature**.)

To check the sequence signature, select **Electronic Signature > Verify** on the **File** menu or click the following icon on the standard ➢*Toolbar*:

∖σσ∕

📖 *Tip:*

*If you use the sample ⇒Types **Spiked** and/or **Unspiked**, please keep in mind that*

- *An ➢Electronic Signature created with Chromeleon 6.50 or earlier is invalid in Chromeleon 6.60 or higher.*

- *An electronic signature created with Chromeleon 6.60 or later is invalid in Chromeleon 6.50 or earlier.*

Manipulations attempted on the signed sequence or errors in the signature are identified during verification. To check the reports once again in such a case, you have to remove the signature first. To remove the signature you must have the **UndoSignResults**➢ *Privilege*. If you have this privilege, you can select **Electronic Signature > Undo Signature** on the **File** menu or click the following icon:

The corresponding ➢*SOR-File (Signed Off Results)* is deleted as well.

Performing a Query

The term ➢*Query* refers to the search for data based on specific search criteria. In Chromeleon, you can search for samples and the corresponding sequences based on freely selectable parameters. Either you can use very detailed criteria to search for one specific sample, or you can use criteria that are more general to search for a specific series of samples with the same properties.

⚠️ *Caution:*

Make sure that you back up the entire sequence and not only the samples found during the query if you want to delete the original data after the backup. Before deleting the original data, verify that the backup file contains all samples.

How To

- In the ➢*Browser*, select **Query** on the context menu. Or else, select **New > Query (using the Wizard)** on the **File** menu. Either way, a ➢*Wizard* guides in entering the required conditions.

- On the first wizard page, determine the ➢*Datasource* in which the query is performed. Also, determine whether the query is performed for ➢*Sequence* properties (**Sequences**) and/or sample properties (**Samples**), and/or other conditions (**Results**).

- Clicking **Next>** takes you to the next wizard page(s). The pages that are opened depend on the properties selected on the first wizard page. Determine the search criteria.

Click **Finish** to open the **New Query Properties** dialog box, which provides four tab pages:

- Use the **General** tab page to select the datasource in which the query is performed.

- Use the **Native SQL** tab page to edit the SQL statements directly in the ➤*SQL* syntax of the corresponding ➤*ODBC* driver (contains translated Chromeleon statements).

- Use the **SQL** tab page to edit the search statement in an entry dialog box.

- Use the **Result Restrictions** tab page to limit the resulting sample list by more result queries.

For more information, refer to:

🔲 **Entering the Sample Query Using the Wizard**

🔲 **Selecting Search Criteria for Samples and/or Sequences**

🔲 **Examples (Wizard)**

🔲 **Specifying the Sample Query Using the Dialog Box**

🔲 **Examples (Dialog Box)**

🔲 **Editing a Query in SQL Syntax**

🔲 **Saving and Performing a Query**

▪ Entering the Sample Query Using the Wizard

On the first wizard page, determine the ➤*Datasource* in which the ➤*Query* is performed. Select either **Selected datasource** (the name of the currently selected datasource is indicated in brackets) or **Fixed datasource**, and then select a datasource from the drop-down list.

In addition, determine the **Field Type** for which the query is performed. Select:

- ➤*Sequences* and/or

- Samples and/or

- Results (variables from the different ⇒*Report Categories*)

On the **Samples** and/or **Sequences** wizard page, click the **Data Field** input field and select the variable for which you want to perform the query from list. On the **Results** wizard page, either enter the formula manually or click the "**…**" button, and then select a formula from the **Edit Result Formula** dialog box.

👉 *Tip:*

*On the **Results** wizard page, you cannot access ➢User-defined Columns directly by clicking the '...' button. For user-defined columns from the sample list of the Browser, select the **Samples** field type, and then select an entry from the **Data Field** field on the **Samples** wizard page. For user-defined columns from the peak table of the QNT Editor, select the **Results** field type, and then enter the formula directly in the **Formula** field on the **Results** wizard page. The syntax of the formula is as follows:*

> *peak_tab.user_x*

where x is the name of the user-defined column.

You can also search for samples with specified ➢Audit Trail properties. In this case, enter the formula for the selected Audit Trail variable. For example, to search samples that were recorded at a ⇒LampIntensity > 500.000 counts/s, use the formula below:

> *AUDIT.LampIntensity(0.0,"forward")>500000.*

Select an operator from the **Operator** field and enter a value in the **Value** field. For information about the operators, refer to 🔳 **Selecting Search Criteria for Samples and/or Sequences**.

To connect one search criterion with another search criterion, select a logic connective from the rightmost combo box. The next entry line is enabled only after you selected either **AND** or **OR**.

Click **Finish** to complete the entries. The **New Query Properties** dialog box is opened. Use this dialog box to specify your query further (see 🔳 **Specifying the Sample Query Using the Dialog Box**).

For examples of how to enter the query using the wizard, refer to 🔳 **Examples (Wizard)**.

◧ Selecting Search Criteria for Samples and/or Sequences

A ➤*Query* allows you to search for samples and sequences, using a variety of sample or sequence properties. Enter the search criteria (see table) on the **Query Wizard: Sequences** and/or **Samples** pages. To further restrict the query, select the **SQL** tab page of the **New Query Properties** dialog box. This dialog box is opened automatically after you have clicked **Finish**. (You can also use this dialog box to edit an existing query. To reopen the dialog box (then called **Properties of Query "xyz"**), select the query in the Browser, and then select **Properties** on the context menu.) Click **Edit/Insert** to open the **Edit Conditions** dialog box. Select the criteria from the **Field** list box.

Name	Search Parameter
Sample Name	⇒*Name*
Sample Number	⇒*No.*
Sample Type	⇒*Type*
Sample Status	⇒*Status*
Sample Comment	⇒*Comment*
Sample Replicate ID	⇒*Replicate ID*
Sample Inject Volume	⇒*Inj. Vol.*
Sample Inject Time	⇒*Inj. Date/Time*
Sample Dilution Factor	⇒*Dil. Factor*
Sample Weight	⇒*Weight* (Sample Weight Factor)
Sample Amount	⇒*ISTD Amount*
Sample Raw Data ID	Chromeleon sample ID.
Sample ID	Sample ID assigned by the user
Sample Program Name	⇒*Program* used for sample processing
Sample Quantification Method	⇒*Method* used for sample evaluation
Sample Standard Addition Group	➤*Standard Addition* group for assigning ➤*Spiked Samples* to unspiked unknown samples (see ⇒*Std. Add. Group*)
Sample Reference Amount Set	Reference amount ID for assigning standards, ➤*Validiation Samples* and/or spiked samples (see ⇒*Ref. Amount Set*)
Sample Auto Purification Type	➤*Autopurification* type (also, see ⇒*Auto Purif. Type*)
Sample Auto Purification Reference	Autopurification ID assigned by Chromeleon (see ⇒*Auto Purif. Ref*)
Sample Auto Purification Fraction	Fraction ID assigned by Chromeleon (see ⇒*Auto Purif. Frac*)

Name	Search Parameter
Sample Tray	
Sample GUID	Globally unique sample ID (➢*GUID*)
***Sample x**	➢*User-defined Column* (column name)
Sequence Name	
Sequence Title	➢*Sequence*
Sequence Preferred Channel	
Sequence Preferred Report	
Sequence Directory	
Sequence Timebase	➢*Timebase*
Sequence Creation Date	
Sequence Creation Operator	
Sequence Last Update	
Sequence Last Update Operator	
Sequence Sign Status	
Sequence Authorized Submit Users	Users authorized to submit the signed sequence
Sequence Submit User	User who submitted the signed sequence
Sequence Submit Date	Date when the signed sequence was submitted.
Sequence Authorized Review Users	Users authorized to review the signed sequence.
Sequence Review User	User who reviewed the signed sequence.
Sequence Review Date	Date when the signed sequence was reviewed.
Sequence Authorized Approved Users	Users authorized to approve the signed sequence.
Sequence Approve User	User who approved the signed sequence
Sequence Approve Date	Date when the signed sequence was approved

There are four different groups of search criteria:

1. Text variables, such as, **Sample** or **Sequence Name**, **Sample ID**, etc.

2. Numerical variables, such as, **Sample Number**, etc.

3. Variables with specific values, such as, **Sample Type** or **Sample Status**, etc.

4. Time variables, such as, **Sample Inject Time**, **Sequence Creation Date**, etc.

These search parameters must be further specified. Different operators are available to link the search criterion with a comparative value. The search criterion determines which operators are available:

Operator	Searches all samples...	Available for group no.
=	whose property is identical to the specified character string.	1, 2, 3, 4
< >	whose property is not identical to the specified character string.	1, 2, 3, 4
>	whose property is larger than the specified character string.	1, 2, 4
<	whose property is smaller than the specified character string.	1, 2, 4
>=	whose property is larger than or equal to the specified character string.	1, 2, 4
<=	whose property is smaller than or equal to the specified character string.	1, 2, 4
contains	whose property contains the specified character string.	1
contains not	whose property does not contain the specified character string.	1
starts with	whose property starts with the specified character string.	1
does not start with	whose property does not start with the specified character string.	1
ends with	whose property ends with the specified character string.	1
does not end with	whose property does not end with the specified character string.	1
is like	whose property fulfills the specified wildcard condition.	1
is not like	whose property does not fulfill the specified wildcard condition.	1
is between	whose property is between two values.	1, 2, 4
is not between	whose property is not between two values.	1, 2, 4
during the previous	whose property was created during a specified time before the query.	4
is null	in which the variable does not exist.	1, 2, 3, 4
is not null	in which the variable exists.	1, 2, 3, 4

If the operators >, <, >=, or <= are used for text variables, the alphabetical order is considered; for example: A<B.

Note:

*All SQL-time queries containing a relative reference (= **during the previous** operator) are recalculated for each query.*

Wildcards represent character strings. The following wildcards can be used for text variables when the operator is either "is like" or "is (not) like":

Wildcard	Description
%	Represents any character string with 0 or more characters.
_(underscore)	Represents any single character.
[ ]	Represents a single character in a specified range; for example, [a-f].
[^]	Represents any character except the specified range.

When you have completed your entries, click **Apply** to start the query.

To restrict the query further, you can enter the restrictions on the **Result Restrictions** page of the **Query Wizard**. Else, you can select the **Result Restrictions** tab page of the **New Query Properties** (or **Properties of Query "xyz"**) dialog box. Click **Edit/Insert** to open the **Edit Conditions** dialog box. Click the "**…**" button to open the **Edit Result Formula** dialog box and select a report variable (see ⇒*Report Categories*). The available logical operations depend on the selected sample and sequence properties.

Note:

Depending on the number of samples resulting from the SQL query, a result-type query may take some time. After approximately 3 seconds, a window is opened indicating the status of the result-type query (in percent of the samples to be tested). The user can use this window to stop the query. In this case, the result of the query includes only those samples of the SQL query that have passed the result test until then.

For examples of how to enter the query using the wizard, refer to **Examples (Wizard)**.

▣ Examples (Wizard)

Use the following SQL query to search for all samples for which

- The sample type is ➤*Matrix Blank* **and**
- The sample comment starts with **Charge 123456** *or*
- The sample inject time is between 6/12/2003 and 7/12/2003:

Use the following settings to restrict the ➤*Query* to samples that contain

- An Anthracene peak *or*
- More than 10 calibration points:

For more examples, refer to ▣ **Examples (Dialog Box)**.

◼ Specifying the Sample Query Using the Dialog Box

After you specified the ➢*Query* in the Query ➢*Wizard* and completed your entries by clicking **Finish**, the **New Query Properties** dialog box is opened. You can use this dialog box to specify the query further. You can also use this dialog box to edit an existing query later. In this case, the dialog box is named **Properties of Query** "**xyz**." To open the dialog box, select the query, and then select **Properties** on the context or **File** menu.

The dialog box provides the four tab pages:

- **General** tab page: Enter or edit the query title in the **Title** field. From the **Datasource** drop-down list, select the datasource for which the query is performed. If this field remains empty (default), the query is performed for the datasource in which the query is saved. The **Preferred RDF File** and **Preferred Channel** settings are used when a sample is opened or a batch report is started.

- **Native SQL** tab page: This tab page is read-only by default, providing information about the SQL statement sent to the ODBC driver. (Please note that the SQL statement is translated into the ➢*SQL* syntax of the corresponding ODBC driver.) To edit the SQL statement manually, select the **Always use native SQL** check box.

⚠️ *Caution:*

*This edited SQL statement is used whenever the query is performed, as long as the **Always use native SQL** check box remains selected.*

- **SQL** tab page: Use this tab page to edit the SQL condition(s) that the samples must fulfill. To open the **Edit Condition** dialog box, click **Edit > Insert**. Or else, place the mouse pointer in the list field and double-click the left mouse button or select **Edit Condition** on the context menu. Use this dialog box to add SQL conditions (select **Restrict Condition (AND)** or **Expand Condition (OR)**) or to change the existing condition (select **Change Condition**).

- **Result Restrictions** tab page: Use this tab page to specify the query further. You can change, restrict, or expand the query. Open the **Edit Condition** dialog box by clicking **Edit/Insert** and select **Change Condition**, **Restrict Condition (AND)** or **Expand Condition (OR)**.

Enter a report variable in the **Formula** field. Click the '**...**' button to open the **Edit Result Formula** dialog box and select a formula. (For more information about the report variables and their categories, refer to ⇒*Report Categories*). Enter an operator. (For information about which SQL operators are supported, refer to **How to …: Creating and Managing Files and Data** 🔳 **Selecting Search Criteria for Samples and/or Sequences**.) Finally, type a reference value in the **Value** field.

☞ *Tip:*

It is not possible to access ➢*User-defined Columns by clicking the '...' button. Enter the formula for the user-defined column directly in the* ***Formula*** *field:*

For user-defined columns from the sample list of the Browser, enter:

> *smp.x*

where x is the name of the user-defined column.

For user-defined columns from the peak table of the QNT Editor, enter:

> *peak_tab.user_x*
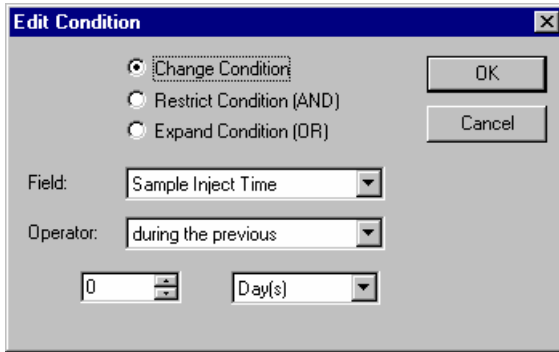
where x is the name of the user-defined column.

- After you have made the necessary entries, click **Apply** to start the search.

For examples of how to enter the query using the dialog box, refer to 🔳 **Examples (Dialog Box)**.

◾ **Examples (Dialog Box)**

To search for all samples of the current day, set up the following ➢*SQL* query in the **Edit Condition** dialog box of the **SQL** tab page. (For information about how to open this dialog box, refer to 🔰 **Specifying the Sample Query Using the Dialog Box**.)

With these settings, the following SQL statement is displayed on the **SQL** tab page:

   Samples.smp_inject_time >= CURRENT_INTERVAL '0' DAY.

To restrict the above search to the standard samples of the current day, reopen the **Edit Condition** dialog box and make the following settings:

The following SQL statements are listed on the **SQL** tab page:

Samples.smp_inject_time >= CURRENT_INTERVAL '0' DAY

AND Samples.smp_type = 'S'

To search for all samples in a ➢ *Sequence* named **S7709**, for which the **Benzene** peak or a peak of the **PAK** (PAH) peak group was identified, use the following SQL query:

Open the **Edit Condition** dialog box of the **SQL** tab page and enter the condition for the sequence name:
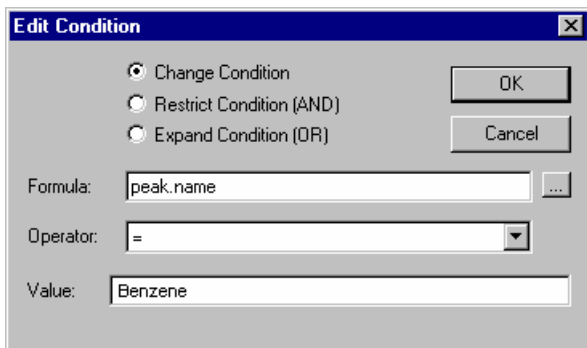
Change to the **Result Restrictions** tab page and click **Edit/Insert** to open the **Edit Condition** dialog box. Enter the condition for the Benzene peak:
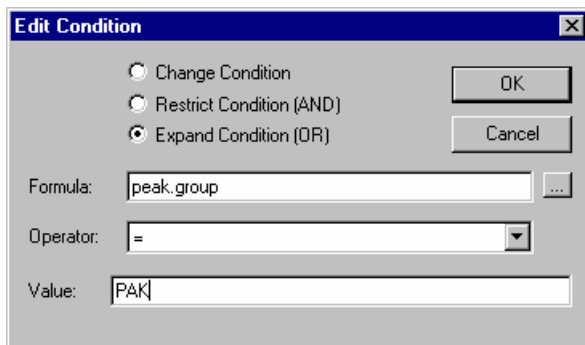
Return to the **Edit Condition** dialog box and enter the condition for the peak of the **PAK** (PAH) group:

With these settings, the following SQL statements are displayed:

On the **SQL** tab page:

Sequences.seq_name = 'S7709'

On the **Result Restrictions** tab page:

peak.name = 'Benzene'

OR peak.group = 'PAK'.

For examples about how to enter the query using the wizard, refer to **Examples (Wizard)**.

Editing a Query in SQL Syntax

On the **Native SQL** tab page, the query created by Chromeleon is displayed in SQL syntax. (For information about how to open these tab pages, refer to **Specifying the Sample Query Using the Dialog Box**.)

If you have a good knowledge of SQL, you can use the **Native SQL** tab page to specify your ➢ *Query* further. Select the **Always use native SQL** check box and modify the statement via the keyboard.

🖙 *Tip:*

*If you have edited the SQL statement on the **Native SQL** tab page, this edited SQL statement is used whenever the query is performed, as long as the **Always use native SQL** check box remains selected. When you clear the check box, the current entries from the **SQL** and **Result Restrictions** tab pages are used, overwriting your SQL entries on the **Native SQL** tab page.*

▪ Saving and Performing a Query

Queries can be saved as a file in the datasource, similar to ➢*Sequence*.

To create a query, select **New** on the **File** menu. In the **New** dialog box, select **Query (using Wizard)** from the list box to open the ➢*Query* Wizard. After you have entered all conditions for the query, click **Finish**. The **New Query Properties** dialog box is opened. Click **Save** to save the conditions. The **Save as** dialog box is opened, indicating that the **Object of type** is **Query**. Enter the name under which the query is saved.

In the Browser, the ➢*Query* is indicated by the following symbol: 🗌.

If you select a query with the mouse, Chromeleon behaves in the same way as for a sequence: If you select the query in the left ➢*Browser* pane, the query is performed immediately and the results are displayed in the right Browser pane. If you select the query in the right Browser pane, click **Return** or double-click the file to perform the query and to display the results.

To edit an existing query, select **Properties** on the **File** or context menu and edit the query as desired (see 🗒 **Specifying the Sample Query Using the Dialog Box**).

Answering Frequently Asked Questions on the Browser

Question: What can I do when I can no longer copy data to the network datasource?

Answer: The reason is probably a communication error. Verify that the network connection still available. In rare cases, there might be a problem with the datasource. In this case, first disconnect the datasource using the **Disconnect** command in Chromeleon, and then reconnect the datasource. The **Administrator Help** section provides more information; refer to **How to …: Working with Files, Databases, and Networks**:

🔼 **Disconnecting a Datasource**

🔼 **Connecting a Datasource**

Question: I appended a sample to a running sequence. How can I make sure that the last sample is processed, too?

Answer: After appending the sample save the sequence by selecting **Save** on the **File** menu. Only then, the sample is part of the sequence and can be processed.

Question: Can I start a single sample from the *Browser* or the *Control Panel*?

Answer: No, you cannot. You have to start a batch. However, the batch may contain only one sequence with only one sample.

Question: Can I change the injection volume after the analysis is finished?

Answer: Yes, you can change the injection volume. But you must have the **ModifyFinishedSample** ➢*Privilege*. (The system administrator assigns this privilege in the ➢*User Manager* (CmUser program).)

👉 *Tip:*

Usually, you should not change the injection volume after the analysis. This would falsify the analysis result when an autosampler injected the sample(s). You should only change the injection volume after the analysis when you entered the wrong volume in case of manual injection of the sample.

Question: Where are programs (PGM Files), QNT Methods, and Report Definition Files (RDF) saved? Are they copied automatically to the sequence?

Answer: These files are not saved at a default location. When creating the files you have to determine where they should be saved. Programs and QNT Methods must also be saved to the sequence in which you want to use them. When saving an old sequence under a new name, using the **Save as…** command on the **File** menu, all files saved in the old sequence are also saved in the new sequence. The ➢*Status* of the single samples is set to **Single**, i.e., the raw data is not saved in the new sequence.

 Notes:

*It may make sense to additionally save all programs to a **Programs** folder. Save the QNT Methods and RDF's in the same way.*

When saving a file to more than one location, make sure that the content of the file is identical, in both locations.

Question: What happens with the files saved in a sequence when I copy the sequence using the Drag&Drop command?

Answer: The Drag&Drop operation corresponds to the **Save as…** command on the **File** menu. All files are copied together with the sequence.

Question: Is an Audit Trail recoded even if I lock the AUDIT directory?

Answer: Select the directory, and then select **Properties** on the context menu. On the **Access Control** tab page, select the **Locked** check box. In this way, the Audit Trail is still saved and a new Audit Trail will be created the next day, too. However, it is no longer possible to change the files in this directory or to copy files into this directory.

Question: How can I reduce the number of ➢*Workspaces* displayed on the **Workspace** menu?

Answer: This operation is quite complex. Contact your system administrator for assistance. (Close the Chromeleon client and edit the information in the registry, using the *RegEdit* program. The path for accessing the information is: HKEY_CURRENT_USER\Software\Dionex\Chromeleon\Recent Workspaces.)

For tips to solve similar questions, also refer to **How to …:** **Creating and Managing Files and Data** and the related subtopics.

Importing PeakNet (Releases 4.3 Through 5.2) Method Files

Overview

In order to use Method files from PeakNet (releases 4.3 through 5.2) with Chromeleon, they must first be imported into Chromeleon.

When imported, the PeakNet 5 Method file is converted into a Chromeleon ➢*PGM File* and/or ➢*Quantification Method (QNT Method)*. Converted PGM Files contain timed events and setup parameters for all modules included in the original PeakNet 5 Method. Converted QNT Files contain component names, retention times, reference peaks, tolerance, calibration options, groups, and level amounts for each detector in the PeakNet 5 Method.

PeakNet 5 Methods from the following modules can be imported: AS50, GP40/GP50, IP20/IP25, AD20, AD25, CD20/CD25, ED40/ED50, IC20/IC25, DX-120, and UI20. For Methods that were created with AI-450 software, only the QNT portion of the Method is converted.

Because of differences in the way that PeakNet 5 and Chromeleon function, some features available in a PeakNet 5 Method file are not imported into Chromeleon. These include:

- Replicate calibration information

- CE Method parameters

- High/low limit values

- Outlier rejection

- Linear weighting options

In addition, Chromeleon allows only a single component table per injection, whereas PeakNet 5 allows one table per detector. When the PeakNet 5 Method contains multiple detectors, the first detector is converted entirely; that is, information about every component and unknown peak is added to the peak table. Then, for the other detector(s), only unique components and unique unknown groups not present in the first detector's data are added to the table. This means that any reference component information for detectors other than the first detector may be lost.

How To

1. In the ➢*Browser*, select a ➢*Datasource* folder (not a sequence name).

2. Select **Import/Restore** on the **File** menu and then select **PeakNet 5** and **Method Files**.

3. In the Import PeakNet 5 Method Files dialog box, navigate to the folder that contains the PeakNet 5 Method file(s) to be imported. These files are named with the .MET extension.

4. Select one or more of the Method files and click **Add**. The files appear in the **Selected files** list.

5. To convert the PeakNet 5 Method into a PGM File for Chromeleon, select the **Program** box. The PGM File is created in the folder shown next to the **Program** box. The default is the datasource folder currently selected in the Browser. To choose a different location, click the **Browse** button and select a location.

6. To convert the PeakNet 5 Method component table and calibration parameters into a Chromeleon QNT Method, select the **QNT** box. The QNT File is created in the folder shown next to the **QNT** box. The default is the datasource folder currently selected in the Browser. To choose a different location, click the **Browse** button and select a location.

7. Select the **Inverted Curve Fitting** check box to use inverted calibration (concentration or amount is plotted against measured values). Clear the check box for "normal" curve fitting (measured values are plotted against concentration or amount). See **How to …: Calibrating** 🔝 **Inverting Dependent and Independent Variables** for details.

8. Click **Import**. The selected PGM and/or QNT Files are created.

   The new files have the same name as the PeakNet 5 Method file, but with PN5 appended and with PGM and QNT extensions. Once a Method file is converted, the file name is removed from the **Selected files** list.

👉 *Tip:*

If the QNT option is selected, but the PeakNet 5 Method does not contain a detector module, an empty QNT Method file is created.

Also, refer to 🔝 **Importing PeakNet (Releases 4.3 Through 5.2) Data Files**.

Importing PeakNet (Releases 4.3 Through 5.2) Data Files

Overview

In order to use data files from PeakNet (releases 4.3 through 5.2) with Chromeleon, the files must first be imported into Chromeleon.

When the PeakNet 5 data file is imported, a ➢*Sequence* for Chromeleon is created. The sequence includes the raw data from the data file, the embedded PeakNet 5 Method (converted to a Chromeleon ➢*PGM File* and ➢*Quantification Method (QNT Method)*, and additional information required to complete the sequence. If multiple data files are selected for import, they are grouped into one sequence.

If the PeakNet 5 data file contains multiple detectors, a separate sample line in the sequence is created for each detector (up to four).

How To

1. In the ➢*Browser*, select a ➢*Datasource* folder (not a sequence name).

2. Select **Import/Restore** on the **File** menu and then select **PeakNet 5** and **Data Files**.

3. In the Import PeakNet 5 Data Files dialog box, navigate to the folder that contains the PeakNet 5 data file(s) to be imported. These files are named with a .DXD or .Dxx extension (where xx is 01 - 99).

4. Select one or more data files and click **Add**. The files appear in the **Selected files** list.

5. The selected data files will be imported into the folder shown in the **PeakNet Folder** box. The default is the datasource folder currently selected in the Browser. To import the data file(s) into a different location, click the **Browse** button and select a location.

6. In the **Sequence Name** box, enter a name for the sequence to be created from the imported data files.

7. Select the **Inverted Curve Fitting** check box to use inverted calibration (concentration or amount is plotted against measured values). Clear the check box for "normal" curve fitting (measured values are plotted against concentration or amount). See **How to …: Calibrating** 🔲 **Inverting Dependent and Independent Variables** for details.

8. Click **Import**. The selected PeakNet 5 data files are imported into Chromeleon.

The following files are created:

- One sequence, which contains a sample line for each PeakNet 5 data file selected for import. If any of the data files contained data from multiple detectors, separate sample lines are created for each detector's data.

- One PGM File and one QNT File for each sample in the sequence. The PGM and QNT Files are created from the PeakNet 5 Method embedded in the PeakNet 5 data file.

The PGM and QNT Files are named as follows: the Method file name from the PeakNet 5 data file is used, followed by a three-digit identifier that corresponds to the detector's position in the sequence, and then a pgm or qnt extension.

Example

In the Import PeakNet 5 Data Files dialog box, three data files are selected for import.
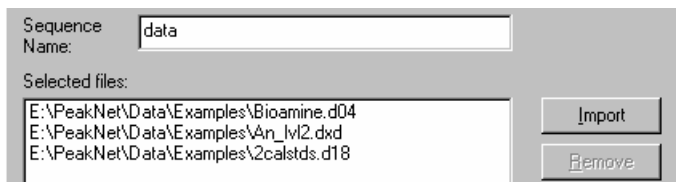
After importing, the following sequence is created. Notice that, because the Bioamine.d04 data file contained data from two detectors, two sample lines were created in the sequence:

The following PGM and QNT Files were also created. These correspond to the programs and methods listed in the sequence:

Also, refer to **Importing PeakNet (Releases 4.3 Through 5.2) Method Files**

PeakNet (Releases 4.3 Through 5.2) Translation Tables

See the following topics for an explanation of how PeakNet (releases 4.3 through 5.2) setup and timed event Method parameters are translated to Chromeleon PGM File commands.

 AS50 Autosampler

 Pump Modules and Eluent Generator

 AD20 Detector

 AD25 Detector

 CD20/CD25 Detectors

 ED40/ED50 Detectors

 IC20/IC25 Systems

 DX-120 System

 UI20 Module

See **Detector Component Table Translation** for how PeakNet (releases 4.3 through 5.2) component table parameters are translated to Chromeleon QNT Method parameters.

▪ AS50 Autosampler Setup and Timed Event Parameter Translation

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
TTL 1 (checked/unchecked)	Sampler_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	Sampler_TTL_2.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	Sampler_Relay_1.State (=Closed/Open)	Setup & timed portions
Relay 2 (checked/unchecked)	Sampler_Relay_2.State (=Closed/Open)	Setup & timed portions
CSV (A/B)	ColumnValve.State(=Col_A/Col_B)	Setup & timed portions
Sample NeedleHeight	NeedleHeight	Setup portion
ColumnTemperature	ColumnTemperature	Setup portion
TrayTemperature	TrayTemperature	Setup portion
Cycle Time	Cycle	Setup portion
Valve (Load/Inject)	Load/Inject	Setup & timed portions
	If Inject, "Wait InjectState" command is added	
Pipet function (Source/Volume/Destination)	Pipet (SourceVial/Volume/ DestinationVial)	Setup portion
Mix function	Mix	Setup portion
(Vial/Volume/Cycles)	(SourceVial/Volume/NumberOfTimes)	
Flush function (Volume)	FlushSP (Volume)	Setup portion
Delay function (Delay Time)	DelaySP (Time)	Setup portion
Needle function (Height)	SetNeedleHeight (Height)	Setup portion
Dilute function	Dilute	Setup portion
(Concentrate Source/ Concentrate Volume/	(SourceVial/ ConcentrateVolume SourceReservoir/ DiluentVolume/ DestinationVial)	
Diluent Source/		
Diluent Volume/		
Destination)		
Dispense function	Dispense (SourceReservoir/Volume/ DestinationVial)	Setup portion
(Source/Volume/Destination)		
Wait function	Wait (SamplePrep)	Setup portion

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Wait for stable temperature (checked/unchecked)	WaitForTemperature (=True/False)	Setup portion
	In any case 2 commands are added: 1."Flush Volume=100" 2."Wait FlushState"	Setup portion
	Syringe Speed always set to default value = 3	Setup portion
	CutSegmentVolume always set to default value = 0	Setup portion
Output Labels	Not converted	
Comments	Comments	
Description	Comments	

Pump and Eluent Generator Setup and Timed Event Parameter Translation

Pump Modules Translation Table

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Oven Temperature	Temperature	Setup portion
High Pressure Limit	Pressure.UpperLimit	Setup portion
Low Pressure Limit	Pressure.LowerLimit	Setup portion
Eluent Label (A/B/C/D)	(%A/%B/%C/%D).Equate	Setup portion
Eluent percentage (B/C/D)	%B/%C/%D	Timed portion
Inject (checked/unchecked)	InjectValve.State (=InjectPosition/LoadPosition)	Setup & timed portions
Column (checked/unchecked)	ColumnValve.State (=Col_B/Col_A)	Setup & timed portions
TTL 1 (checked/unchecked)	Pump_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	Pump_TTL_2.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	Pump_Relay_1.State (=Closed/Open)	Setup & timed portions
Relay 2 (checked/unchecked)	Pump_Relay_2.State (=Closed/Open)	Setup & timed portions
Flow	Flow	Setup & timed portions

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Eluent percentage (%A)	Not converted	Calculated from %B, %C, and %D
Pump (On/Off)	Not converted	
Curve	Curve	
Comment	Comments	
Piston Size	Not converted	Set in Server Configuration instead
Pressure Units	Not converted	Set in Server Configuration instead
Description	Comments	

Eluent Generator Translation Table

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Eluent Concentration	Concentration	Timed portion
TTL 1 (checked/unchecked)	EluentGenerator_TTL_1.State (=0v/5v)	Timed portion
Offset Volume	Not converted	Set in Server Configuration instead
Curve	Curve	
Eluent Label	Not converted	
TTL 1 Output	Not converted	

◪ AD20 Setup and Timed Event Parameter Translation

PeakNet (Release 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Rate	Data_Collection_Rate	Setup portion
Time	UV_VIS_1.AcqOff (at time=time of AcqOn + Time)	Timed portion
TTL 1 (checked/unchecked)	UV_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	UV_TTL_2.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	UV_Relay_1.State (=Closed/Open)	Setup & timed portions

PeakNet (Release 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Relay 2 (checked/unchecked)	UV_Relay_2.State (=Closed/Open)	Setup & timed portions
Offset (checked)	Autozero	Setup & timed portions
Mark (checked)	UV_Analog_out.Mark	Setup & timed portions
Range	UV_Analog_out.Recorder_Range	Setup & timed portions
Wavelength	Wavelength	Setup & timed portions
Collection Begin (checked)	UV_VIS_1.AcqOn	Setup or timed portion
UV Lamp (Off/Low/High)	UV_Lamp (=Off/Low/High) If =Low/High, command "Wait UV_Lamp_Ready" is added	Setup portion
Visible Lamp (Off/Low/High)	Visible_Lamp (=Off/Low/High)	Setup portion
Plot Scales (Minimum/Maximum)	Not converted	
Detector Units	Not converted	
X-Y Data	Not converted	

◾ AD25 Setup and Timed Event Parameter Translation

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Rate	Data_Collection_Rate	Setup portion
Time	UV_VIS_1.AcqOff (at time=time of AcqOn + Time)	Timed portion
TTL 1 (checked/unchecked)	UV_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	UV_TTL_2.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	UV_Relay_1.State (=Closed/Open)	Setup & timed portions
Relay 2 (checked/unchecked)	UV_Relay_2.State (=Closed/Open)	Setup & timed portions
Offset (checked)	Autozero	Setup & timed portions

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Mark (checked)	UV_Analog_out.Mark	Setup & timed portions
Range	UV_Analog_out.Recorder_Range	Setup & timed portions
Wavelength	Wavelength	Setup & timed portions
Collection Begin (checked)	UV_VIS_1.AcqOn	Setup or timed portion
UV Lamp (On/Off)	UV_Lamp (=On/Off) If =On, command "Wait UV_Lamp_Ready" is added	Setup portion
Visible Lamp (On/Off)	Visible_Lamp (=On/Off)	Setup portion
Offset Level	UV_Analog_out.Offset_Level	Setup portion
Calibration (Off/Zero/Full Scale)	Recorder_Calibration (AU/Zero/Full_Scale)	Setup portion
Polarity (Negative/Positive)	UV_Analog_Out.Polarity (=Negative/Positive)	Setup portion
Rise Time	Rise_Time	Setup portion
Comment	Comments	
Description	Comments	
Labels	Not converted	
Plot Scales (Minimum/Maximum)	Not converted	
Detector Units	Not converted	
X-Y Data	Not converted	

◢ CD20/CD25 Setup and Timed Event Parameter Translation

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Rate	Data_Collection_Rate	Setup portion
Time	AcqOff (at time=time of AcqOn + Time)	Timed portion
Range (μS)	ECD_Analog_Out.Recorder_Range	Setup & timed portions
TTL 1 (checked/unchecked)	ECD_TTL_1.State (=0v/5v)	Setup & timed portions

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
TTL 2 (checked/unchecked)	ECD_TTL_2.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	ECD_Relay_1.State (=Closed/Open)	Setup & timed portions
Relay 2 (checked/unchecked)	ECD_Relay_2.State (=Closed/Open)	Setup & timed portions
Offset (checked)	Autozero	Setup & timed portions
Mark (checked)	ECD_Analog_Out.Mark	Setup & timed portions
Temp. Comp.	Temperature_Compensation	Setup portion
Cell Temp.	DS3_Temperature	Setup portion
SRS Current (Off/50/100/300/500 mA)	SRS_Current (=Off/50/100/300/500)	Setup portion
Collection Begin (checked)	AcqOn	Setup or timed portion
Plot Scales (Minimum/Maximum)	Not converted	
Detector Units	Not converted	
X-Y Data	Not converted	

ED40/ED50 Setup and Timed Event Parameter Translation

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command of	Comment
Rate	Data_Collection_Rate	Setup portion
Time	AcqOff (at time=time of AcqOn + Time)	Timed portion
Range (μS)	ECD_Analog_Out.Recorder_Range	Setup & timed portions
TTL 1 (checked/unchecked)	ECD_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	ECD_TTL_2.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	ECD_Relay_1.State (=Closed/Open)	Setup & timed portions
Relay 2 (checked/unchecked)	ECD_Relay_2.State (=Closed/Open)	Setup & timed portions

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command of	Comment
Offset (checked)	Autozero	Setup & timed portions
Mark (checked)	ECD_Analog_Out.Mark	Setup & timed portions
Temp. Comp.	Temperature_Compensation	Setup portion.
		Only for ED40/ED50c (conductivity)
Cell Temp.	DS3_Temperature	Setup portion.
		Only for ED40/ED50c
SRS Current	SRS_Current	Setup portion.
(Off/50/100/300/500 mA)	(=Off/50/100/300/500)	Only for ED40/ED50c
Amperometry Cell (On/Off)	Cell (=On/Off)	Setup portion.
		Only for
		ED40/ED50d (DC amperometry) & ED40/ED50i (integrated amperometry)
Oven Temperature (enabled)	Oven_Temperature	Setup portion.
		Only for
		ED40/ED50d & ED40/ED50i
Voltage	DC_Voltage	Setup portion.
		Only for ED40/ED50d
Collection Begin (checked)	AcqOn	Setup or timed portion
Plot Scales (Minimum/Maximum)	Not converted	
Detector Units	Not converted	
X-Y Data	Not converted	

▣ IC20/IC25 Setup and Timed Event Parameter Translation

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Rate	Data_Collection_Rate	Setup portion
Time	ECD_1.AcqOff (at time=time of AcqOn + Time)	Timed portion
High Pressure Limit	Pressure.UpperLimit	Setup portion
Low Pressure Limit	Pressure.LowerLimit	Setup portion
SRS Current (Off/50/100/300/500 mA)	SRS_Current (=Off/50/100/300/500)	Setup portion
Oven Temp.	DS3_Temperature	Setup portion
Temp. Comp.	Temperature_Compensation	Setup portion
Inject (checked/unchecked)	Pump_InjectValve.State (InjectPosition/LoadPosition)	Setup & timed portions
TTL 1 (checked/unchecked)	Pump_ECD_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	Pump_ECD_TTL_2.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	Pump_ECD_Relay_1.State (=Closed/Open)	Setup & timed portions
Relay 2 (checked/unchecked)	Pump_ECD_Relay_2.State (=Closed/Open)	Setup & timed portions
Offset (checked)	Autozero	Setup & timed portions
Mark (checked)	Mark	Setup & timed portions
Flow	Pump_ECD.Flow	Timed portion
Range	Recorder_Range	Setup & timed portions
Eluent (A/B)	Pump_ColumnValve.State (=Col_A/Col_B)	Setup & timed portions
Collection Begin (checked)	ECD_1.AcqOn	Setup or timed portion
Pressure Units	Not converted	Set in Server Configuration instead
Piston Size	Not converted	Set in Server Configuration instead
Pump (On/Off)	Not converted	

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Comment	Comments	
Description	Comments	
Labels	Not converted	
Plot Scales (Minimum/Maximum)	Not converted	
Detector Units	Not converted	
X-Y Data	Not converted	

◼ DX-120 Setup and Timed Event Parameter Translation

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Rate	Data_Collection_Rate	Setup portion
Time	ECD_1.AcqOff (at time=time of AcqOn + Time)	Timed portion
Pump (On/Off)	Pump (=On/Off)	Setup portion
Column (A/B)	Column (=A/B)	Setup portion
	Wait RinseComplete command added	Only if System Mode is Column
Pressure (psi/MPa)	PressureUnit (=psi/MPa)	Setup portion
SRS/Cell (On/Off)	SRS (=On/Off)	Setup portion
Eluent Pressure (On/Off)	EluentPressure (=On/Off)	Setup portion
Offset (checked)	Autozero	Setup & timed portions
Inject (checked/unchecked)	Pump_InjectValve.State (InjectPosition/LoadPosition)	Setup & timed portions
TTL 1 (checked/unchecked)	ECD_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	ECD_TTL_2.State (=0v/5v)	Setup & timed portions
Controlled AC (checked/unchecked)	ControlledAC (=On/Off)	Setup & timed portions
Eluent (A/B)	Eluent (=A/B)	Setup & timed portions
		Only if System Mode is Eluent
Collection Begin (checked)	ECD_1.AcqOn	Setup or timed portion

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
System Mode	Not converted	
Comment	Comments	
TTL Output Labels	Not converted	
Plot Scales (Minimum/Maximum)	Not converted	
Detector Units	Not converted	
X-Y Data	Not converted	

■ UI20 Setup and Timed Event Parameter Translation

PeakNet (Releases 4.3 Through 5.2) Method Parameter	Chromeleon PGM Command	Comment
Rate (A&B)	Data_Collection_Rate	Setup portion
Time (A&B)	Channel_A.AcqOff for Channel A Channel_B.AcqOff for Channel B (at time=time of AcqOn + Time)	Timed portion
TTL 1 (checked/unchecked)	Interface_TTL_1.State (=0v/5v)	Setup & timed portions
TTL 2 (checked/unchecked)	Interface_TTL_2.State (=0v/5v)	Setup & timed portions
TTL 3 (checked/unchecked)	Interface_TTL_3.State (=0v/5v)	Setup & timed portions
TTL 4 (checked/unchecked)	Interface_TTL_4.State (=0v/5v)	Setup & timed portions
Relay 1 (checked/unchecked)	Interface_Relay_1.State (=Closed/Open)	Setup & timed portions
Relay 2 (checked/unchecked)	Interface_Relay_2.State (=Closed/Open)	Setup & timed portions
Full Scale Voltage (mV) (10/100/1000/10000)	Full_Scale_Voltage (0.011/ 0.110/ 1.100/ 11.000)	Setup portion
Collection Begin (checked)	Channel_A.AcqOn for Channel A Channel_B.AcqOn for Channel B	Setup or timed portion
Comment	Comments	
Labels	Not converted	
Plot Scales (Minimum/Maximum)	Not converted	
Units	Not converted	
X-Y Data	Not converted	
TTL Inputs	Not converted	
Trigger	Not converted	

▣ Detector Component Table Parameter Translation

PeakNet Releases 4.3 Through 5.2 Component Table Parameter	Chromeleon QNT Method Parameter	Comment
Component Name	Peak Name	
Retention Time	Retention Time	If peak Reference Component column = None then Retention Time options are set to Absolute.
		If peak Reference Component = <Component> then Retention Time options are set to Time Distance to Reference Peak and Reference Peak = <Component> is assigned
Tolerance	Window	If Tolerance = time then Window options are set to Absolute and Greatest.
		If Tolerance = % then Window options are set to Relative and Greatest.
Reference Component	Retention Time Options	See Retention Time comments for rules.
Internal Standard Component	Standard	If global PN5 calibration options = External then all Chromeleon Standards = External
		- If Internal Standard Component = Internal Standard then "Use this peak as Internal Standard" option is set.
		- If Internal Standard Component = <Component> then Internal Standard is set and Associated ISTD Peak is assigned.
Calibration Standards Level [1...32] Amounts	Amount column for each level	An amount column for each level labeled Std1, Std2, … Stdn is inserted where n = total levels. The amounts for each peak in the table are filled in.
Check Standards Level [1...32] Amounts	Amount column for each level	An amount column for each level labeled CStd1, CStd2, … CStdn is inserted where n = total levels. The amounts for each peak in the table are filled in.

PeakNet Releases 4.3 Through 5.2 Component Table Parameter	Chromeleon QNT Method Parameter	Comment
Curve Fit Type	Calibration Type	If Cubic in PN5 then set to quadratic.
		- PN5 Fit Linear/quadratic with Origin = Force is Linear/quadratic in Chromeleon.
		- PN5 Fit Linear/quadratic with Origin = Ignore is Linear/quadratic with Offset in Chromeleon.
		- PN5 Fit Linear/quadratic with Origin = Include is Linear/quadratic with Offset and Include point (0,0) options in Chromeleon.
		- All points are set to No Weight in Chromeleon.
		- No Average Response Factor in Chromeleon, so set to Linear.
Origin	Calibration Type	See Curve Fit Type for rules.
Calibrate By	Calibrate By	- Area or Height
		- If Relative then set to Relative Area for Identified Peaks Only.
Relative Response Component	Response Factor	Relative to Peak is set and correct peak is assigned.
Relative Factor	Response Factor	Value for factor is assigned.
Groups	Groups	- For each peak in a group, the Chromeleon group item is set to that name.
		- For each time range in a group, the range is added to Unidentified Peaks list.
Standardization	External/Internal	See handling in Quantification section above.
Calibration Standard Volume	Reference Inject Volume	
Amount Units	Dimension of Amounts	
Replace Retention Time	Use recently detected retention times check box is checked	Last Standard Options = Last Value
Low Limit Amount	Not Available (N.A.)	
High Limit Amount	N.A.	
Total Levels for Calibration Standards	N.A.	
Total Levels for Check Standards	N.A.	

PeakNet Releases 4.3 Through 5.2 Component Table Parameter	Chromeleon QNT Method Parameter	Comment
Replicates Table	N.A.	No replicate information will be converted from PN5.
Replicates	N.A.	
Rejection of Outlier	N.A.	
Linear Weighting	N.A.	
Replace/Average Response	N.A.	
Sample Volume Default	N.A.	
Sample Weight	N.A.	
Dilution Factor	N.A.	
Internal Standard Amount	N.A.	
Unknown Response Factor	N.A.	
Response for Unknowns	N.A.	
CE Information	N.A.	If CE information is contained, it will not be converted in Chromeleon.

Importing Agilent/HP ChemStation Data Files

In order to use Agilent/HP ChemStation data files with Chromeleon, you must first import the files into Chromeleon. However, keep in mind that the structure of the two systems is different:

■👉 *Tip:*

Due to incompatible data formats, it is not possible to import data that were recorded with a 32-bit version of the Agilent ChemStation (version B.01.01 and later) using the Chromeleon Agilent ChemStation Import command. .

• **Agilent/HP ChemStation** contains single files.

• **Chromeleon** is a database-aided system.

Select the type of data you want to import in the wizard that guides you through the process (see below):

Agilent/HP ChemStation	Chromeleon
Batch	One ➤*Sequence* that has already been analyzed (<u>not</u> a ➤*Batch*!)
Sequence	A sequence that has not yet been processed.
Method	Contains elements of the ➤*PGM File*, the ➤*QNT Method*, and the ➤*Report Definition File (RDF)* ■👉 *Tip:* *Only the transferable QNT part of each file is imported.*
Sample	Single sample
Spectra Library	➤*Spectra Library*

Depending on your selection, the following Agilent/HP ChemStation data are imported together with the selected data type:

Agilent/HP ChemStation	Chromeleon
2D/3D raw data file	The corresponding channels; for example, UV and 3D channels.
Instrument data such as pressure, flow, temperature, and gradient composition	
Sample LOG File	➤*Audit Trail*

How To

- In the Browser, select **Import/Restore** on the **File** menu, and then select **Agilent/HP ChemStation**. The **Agilent/HP ChemStation Data Import Wizard** is opened.

- On the first page, select the data type to be imported. Clicking **Next>** takes you to the next wizard page.

- Click **Add**. In the **Select Agilent/HP ChemStation …** dialog box, navigate to the folder in the Windows Explorer that contains the file(s) to be imported. (The picture refers to importing a sample.)
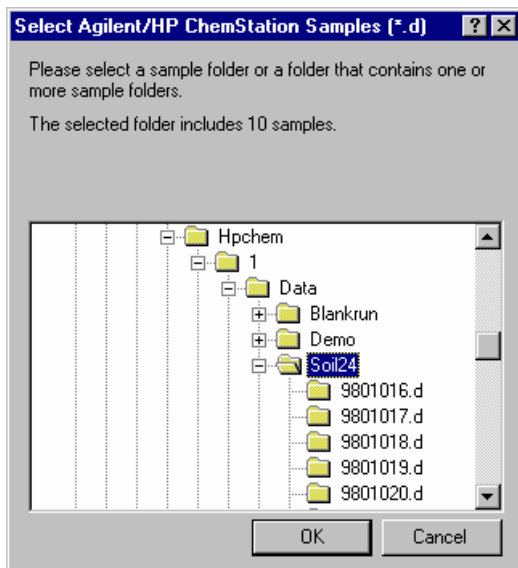
- Select the first file to be imported. Click **OK** to confirm your selection. This action automatically returns you to the wizard. (When you import a batch, a sequence, or a spectra library, the appearance of the dialog box is slightly different. In this case, click **Open** to confirm your selection.)

- Repeat these steps until all files of interest appear in the selected files list. Clicking **Next>** takes you to the next wizard page.

- Determine the destination directory. (Depending on the data type to be imported, you may have to determine the destination sequence and the method to be used):

- Select **Import instrument signals** by the check box if you want to include the instrument signals into the import. Clicking **Next>** takes you to the next wizard page.

- All files selected for import and the desired location are listed.

- Click **Finish**. The selected files are imported into Chromeleon.

For more information about how to import ChemStation data files, refer to **ChemStation Translation Tables**.

ChemStation Translation Tables

For information about how ChemStation data are translated to Chromeleon data, refer to:

Translating ChemStation Sequences and Batches

Translating ChemStation Samples

Translating ChemStation Methods

Translating ChemStation Spectra Libraries

Translating ChemStation Sequences and Batches

ChemStation (CS) sequences and batches correspond to the ➤*Sequences* in Chromeleon. Chromeleon uses all information available in the ChemStation sequence or in the ChemStation batch to create a new sequence with the same name. A message appears if this sequence already exists. You can either overwrite the old sequence or cancel the import.

The table below lists the parameters that are available for a sequence and/or batch in ChemStation and their equivalents in Chromeleon:

ChemStation (CS)	Chromeleon
Comment (of a CS sequence)	Title
Instrument	Timebase
Operator Name (of a CS sequence)	Created by
Last modification time (of a CS sequence)	Created
Current CM user (user who imported the data)	Last Update by
Date/time of import	Last Update
-	Locked
-	Preferred RDF file
-	Preferred channel

Some ChemStation sequence and/or batch settings cannot be mapped directly to Chromeleon settings:

- <u>Cal Level</u>: Used for ChemStation calibration; not used in Chromeleon. In Chromeleon, the corresponding information is available in the ⇒*Amount* column of the ➤*QNT Method*.

- Inj/Location: Indicates the number of replicates. The corresponding number of samples will be generated for the Chromeleon sequence.

- Interval: If the interval value for one of the standards is > 0, the ChemStation sequence runs in a special mode: The standard samples are injected first; then the unknown samples are injected. After x unknown samples have been injected (where x is defined by Interval), one or more standards are injected again. After importing, a sequence is created in Chromeleon in which standards are injected again after x unknown samples.

Translation table for generating a sample list:

ChemStation	Chromeleon
Sample Name	Name REFERENCE_SAMPLE_NAME> referenc (sample name)
Sample Type	⇒*Type* (sample type)
Sample	Unknown,
Calibration	Standard,
Control Sample	Validation.
Location	⇒*Pos.* (sample position)
Inj Volume	⇒*Inj. Vol.* (injection volume)
-	⇒*Program* (➢*PGM File*)
Method Name	⇒*Method* (quantification method)
	⇒*Status* (sample status)
Date/Time *of injection (evaluated from CS log file)*	⇒*Inj. Date/Time* (injection date and time)
Product of Sample Amount *and* Multiplier	⇒*Weight* (Sample Weight Factor)
Dilution	⇒*Dil. Factor* (Dilution Factor)
ISTD Amount	⇒*ISTD Amount* (Amount of the ➢*Internal Standard*)
Datafile *(standards are named automatically - see below)*	⇒*Sample ID*
-	⇒*Replicate ID*
Sample Info	⇒*Comment*

The ChemStation does not name the calibration standards; the names are automatically generated with a prefix and counter. Chromeleon needs the exact file name for data import and thus, uses the same algorithm.

◨ Translating ChemStation Samples

Chromeleon uses all information available for the ChemStation (CS) sample to create a new sample with the same name. If the new sample is imported into an existing sequence, the sample is appended to the sequence. Existing samples will <u>not</u> be overwritten.

Unlike Chromeleon, ChemStation does not provide a sequence context for the single samples. That is why the sample information is gathered from raw data, instrument diagnosis, macros, and the LOG files.

The table below lists the properties that are available for a ChemStation sample and their equivalents in Chromeleon:

ChemStation	Chromeleon
Sample name information from a 2D/3D raw data file	⇒*Name* (sample name)
Cannot be evaluated from the CS sample data; set to "unknown"	⇒*Type* (sample type)
Vial position information from a 2D/3D raw data file	⇒*Pos.* (sample position)
Inj Volume from the Lcdiag.reg *file*	⇒*Inj. Vol.* (injection volume)
Not specified, no entry.	⇒*Program* (➢*PGM File*)
Method information from a 2D/3D raw data file	⇒*Method* (quantification method)
Set to "Finished"	⇒*Status* (sample status)
Date/time of injection (from the LOG file*)*	⇒*Inj. Date/Time* (injection date and time)
Cannot be evaluated from the CS sample data, set to 1.0	⇒*Weight* (Sample Weight Factor)
Cannot be evaluated from the CS sample data, set to 1.0	⇒*Dil. Factor* (dilution factor)
Cannot be evaluated from the CS sample data, set to 1.0	⇒*ISTD Amount* (Amount of the ➢*Internal Standard*)

ChemStation	Chromeleon
Sample name (*.d)	$\Rightarrow$*Sample ID*
-	$\Rightarrow$*Replicate ID*
Sample information from the "sample.mac" file	$\Rightarrow$*Comment*

If you did not specify the ChemStation method to be used, all methods used for the imported samples will be imported. If one of these methods does not exist, Chromeleon generates a standard QNT Method.

Translating ChemStation Methods

The ChemStation (CS) method contains parts of the Chromeleon ➢*PGM File*, the ➢*QNT Method*, and the ➢*Report Definition File (RDF)*. Chromeleon uses only the relevant information of a ChemStation method (i.e., Peak Table and Integration Events) to create a new QNT Method with the same name. A message appears if this method already exists. You can either overwrite the old method or cancel the import.

The table below lists the Integration Events of a ChemStation method and the equivalent detection parameters in Chromeleon:

ChemStation	Chromeleon	Comment
Slope Sensitivity	Sensitivity	Translation factor: 20
Peak Width	Peak Slice	Translation factor: 0.8
Area Reject	Minimum Area	Translation factor: 1/60
Height Reject	Minimum Height	
Shoulders	Peak Shoulder Threshold	Off = 0ff, On = 0.1
Baseline Now	Lock Baseline	On
Baseline at Valleys	Valley to Valley	Off = Off, On = On
Baseline Hold	Lock Baseline	On = At Current Level, Off = Off
Tail Tangent Skim	-	
Tangent Skim Mode	-	
Area Sum	Peak Group Start	On
	Peak Group End	Off
Integration	Inhibit Integration	Off = On, On = Off
Negative Peak	Detect Negative Peaks	Off = Off, On = On
Split Peak	-	

ChemStation	Chromeleon	Comment
Fixed Peak Width	Peak Slice	
Auto Peak Width	-	
Detect Shoulders	Peak Shoulder Threshold	Off = 0ff, On = 0.1
Shoulder Mode	-	-
Solvent Peak	-	
Baseline Backwards	-	
Baseline Next Valley	-	
Unassigned Peaks	-	

Chromeleon provides equivalents in the QNT Method for the following peak table parameters of a ChemStation method:

ChemStation	Chromeleon	Comment
Compound	Peak Name	
RT	Ret. Time	
RT Window Low	Window	Calculated from the difference: RT High - RT Low
RT Window High		

◾ Translating ChemStation Spectra Libraries

When importing a ChemStation (CS) spectra library into Chromeleon, the library name is the same in Chromeleon and in ChemStation. All spectra of the ChemStation library are imported into the Chromeleon library. Only the unmodified spectra are imported, all specific wavelength entries are ignored.

The table below lists the information available in a ChemStation spectra library and the equivalent parameters in Chromeleon:

ChemStation	Chromeleon
File name of the imported library + hint "Imported CS library"	Name
User who imported the spectra library	Created by
Creation date of the imported spectra library	Created
Current user	Last Update by
Date/time of the import	Last Update
Information on the imported library	Title

Equilibrating the Chromatography System (SmartStartup)

Before you can start analyzing your samples, you have to equilibrate the chromatography system. (This is especially important if the system has not been used for an extended period of time or if the analysis conditions have changed.) System equilibration typically includes the following events:

- The entire system, including the column, is flushed with the starting solvent until the system is free of any other liquid compositions.

- The column thermostat and the thermostatted autosampler, if installed, are warmed or cooled to the starting temperature.

- Chromeleon checks the lamp for stability (to prevent increased ➤*Drift* or ➤*Signal Noise*).

- The pressure ripple is checked.

- The wavelength is calibrated.

The **SmartStartup Wizard** assists you in automating equilibration. The SmartStartup feature is supported for the ➤*Summit* and ➤*UltiMate 3000* HPLC systems (excluding nano and capillary modules). To start the wizard, select **SmartStartup** on the ➤*Batch* menu:
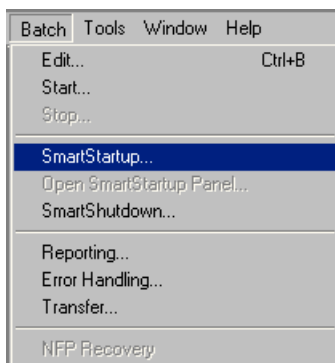
Or, click the following icon on the **Online** ➤*Toolbar*:

The SmartStartup Wizard guides you through the following steps:

🔳 **Selecting the Timebase**

🔳 **Extracting the Equilibration Conditions**

🔳 **Editing the Equilibration Conditions**

🔳 **Starting Equilibration**

▣ Selecting the Timebase

In the first step of the SmartStartup Wizard, select the ➢*Timebase* to be equilibrated. To do so, SmartStartup Wizard support must be available for at least one device in the timebase. The SmartStartup Wizard currently supports the following Dionex devices:

- P680, ISO-3100A, LPG-3x00A, HPG-3x00A/P, and LPG-3x00M and DGP-3600 HPLC pumps

- ASI-100 and WPS-3000T(SL) HPLC autosamplers

- TCC-100 and TCC-3x00 HPLC thermostatted column compartments

- UVD 170U, UVD 340U, UVD 170S, and UVD 340S, as well as PDA-100, PDA-3000, VWD-3x00, RF2000, and RI-101 HPLC detectors

🖙 *Tip:*

Chromeleon does not support SmartStartup equilibration of a timebase that includes a flow manager. Therefore, an error message appears if you select a timebase in which a flow manager is configured.

If a timebase is already selected in the current window, this timebase is used and the related wizard step is omitted. The right-hand pane lists various computers, timebases, and networks. The access right(s) of the user determine which items are displayed.

Clicking **Next>** takes you to the next wizard page.

For an overview of how to equilibrate the HPLC system with Chromeleon, refer to **How to …:** 🔲 **Equilibrating the Chromatography System**.

🔳 Extracting the Equilibration Conditions

Use the **Extract Equilibration Conditions** page of the SmartStartup Wizard to determine the source of the equilibration conditions:

- Select **<Choose Program...>** to use an existing program.

- Select **<Manual Input>** to use the current device settings.

- Any existing equilibration programs are listed and available for selection, also.

After making your selection, click **Next>** to continue:

If you selected **<Choose Program...>**, clicking **Next>** opens the **Browse** dialog box. Select a program from the list and click **Open**. This takes you to the next wizard page.

If you selected **<Manual Input>**, clicking **Next>** takes you to the next wizard page. Use this page to 🔲 **Editing the Equilibration Conditions**.
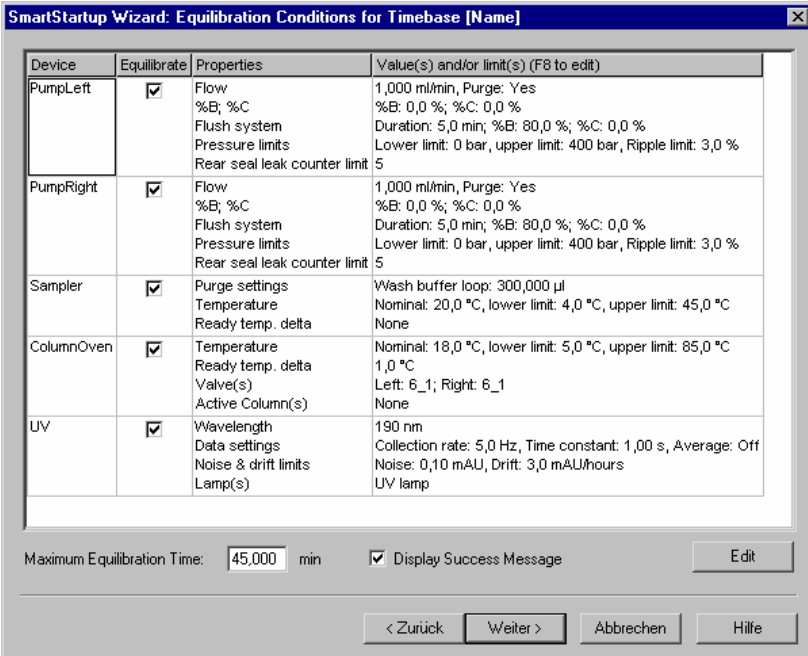
If you selected an existing equilibration program, click **Next>** and extract the equilibration conditions on the next wizard page.

For an overview of how to equilibrate the HPLC system with Chromeleon, refer to **How to …: ⬛ Equilibrating the Chromatography System (SmartStartup)**.

⬛ Editing the Equilibration Conditions

The **Equilibration Conditions** page of the SmartStartup Wizard provides an overview of the equilibration conditions, including a brief description of the parameter settings for each device:

If you want to exclude a device from equilibration, clear the **Equilibrate** check box. To edit the parameters for a device, open the related dialog box by double-clicking the device name in the **Devices** column. As an alternative, select the device and click **Edit** or press the **F8** key.

For example, double-click **PumpLeft** to open the following dialog box for the left pump of the DGP-3600:

Modify the equilibration conditions as required. For example, for the pump, change the flow rate, pressure limits, and the maximum allowed ripple.

Clicking **Next>** takes you to the next wizard page.

For an overview of how to equilibrate the HPLC system with Chromeleon, refer to **How to …: Equilibrating the Chromatography System (SmartStartup)**.

Starting Equilibration

The **Start Equilibration** page is the last page of the SmartStartup Wizard. Click **Finish** to start equilibration. The **Equilibration Start Log** section will display a list of steps that Chromeleon automatically performs during equilibration. Before performing the first step (**Preparing Equilibration Start**), Chromeleon displays a prompt asking whether you want to save the equilibration program for later use (this requires the *Create PGM* privilege).

To save the program, click **Yes** to open the **Save Startup Conditions** dialog box:
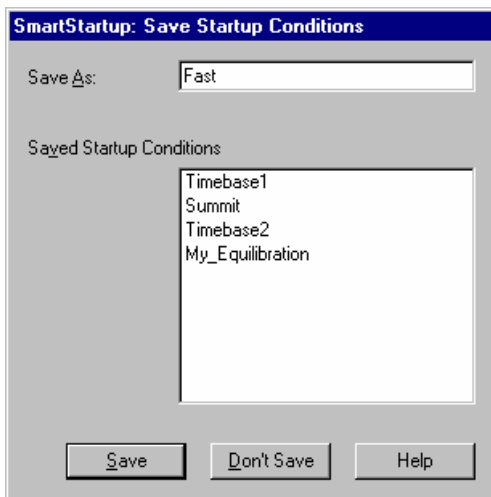
Type a program name in the **Save As** field. The **Saved Startup Conditions** field lists all existing equilibration programs.

Click **Save** to save the equilibration program for later use. Chromeleon creates an **Equilibration** sample (⇒*Type* = **Blank**) and saves it to the **Equilibration** sequence in the timebase's directory located in the server's standard datasource. The **Equilibration** sequence also contains the new equilibration program (see ➢*PGM File*).

When you have saved the equilibration program, Chromeleon closes the SmartStartup Wizard and opens the appropriate equilibration panel for your timebase.

☞ *Tip:*

*Do not modify the default equilibration panels in the **Dionex Templates/Panels/Equilibration** folder. Chromeleon uses these panels when automatically creating equilibration panels.*

Chromeleon automatically connects the panel to the related timebase. The panel contains a stripe for each device in the timebase that is supported by the SmartStartup Wizard.

In addition, the **Start Batch** dialog box opens. The dialog box contains the **Equilibration** sequence with the new sample (first position on the list).

Click **Start** to start the ➢*Batch* and have Chromeleon automatically equilibrate the system. If all devices reach the specified settings at the end of the program, equilibration is successful and Chromeleon goes on to process any other sequences in the batch. If equilibration fails, the batch is aborted after the maximum time.

Starting Equilibration While a Batch Is Running

If you start the equilibration sequence while a batch is running, the equilibration sequence cannot run immediately, and thus the equilibration panel is not opened automatically. Instead of the **Start Batch** dialog box, the **Batch** dialog box opens:

- Click **Start** to open the **Delay Equilibration Sequence?** dialog box. Specify a date and time for the equilibration procedure; for example, the morning of the next day.

- After you save the equilibration sequence, Chromeleon adds the sequence at the end of the batch list (not in the first position).

For an overview of how to equilibrate the HPLC system with Chromeleon, refer to **How to …:** **Equilibrating the Chromatography System (SmartStartup)**.

Shutting down the Chromatography System (SmartShutdown)

Chromeleon offers a shutdown or standby program to automatically shut down the chromatography system after a run or to set it to standby mode. The shutdown or standby process can be performed automatically in Chromeleon for the ➤*UltiMate 3000* and ➤*Summit* HPLC systems (not including the nano and cap modules) and is referred to as *SmartShutdown.*

A shutdown process shuts down the chromatography system by stopping the flow automatically and turning off the detector lamps. In addition, it turns off the column thermostat's temperature (and if available the thermostatted autosampler's temperature).

The standby process prepares the chromatography system for a later time-controlled system start. In combination with the SmartStartup process and an ➤*UltiMate 3000 x2 Dual-Gradient System*, the standby process enables you to quickly prepare the instrument for a different method of analysis. A typical standby process comprises steps such as reducing the flow and turning off the column thermostat's temperature. As opposed to the shutdown program, the detector lamp(s) remain(s) on.

The *SmartShutdown Wizard* assist you in creating the shutdown or standby program step by step and helps you to prevent typical errors, such as stopping the flow while a buffer is in the mobile phase or interrupting the flow at high column temperatures, which may damage the column.

You can create a shutdown or standby program for the Dionex Summit or UltiMate 3000 HPLC systems using ⬛ **SmartShutdown**. To do so, perform the **SmartShutdown** command on the ➢*Batch* menu:

Batch	Tools	Window	Help
Edit...			Ctrl+B
Start...			
Stop...			
SmartStartup...			
Open SmartStartup Panel...			
SmartShutdown...			
Reporting...			
Error Handling...			
Transfer...			
NFP Recovery			
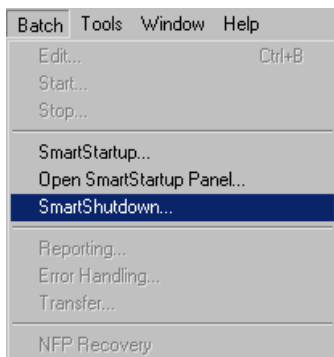
The SmartShutdown Wizard is opened and assists you in:

⬛ **Selecting the Timebase**

⬛ **Selecting the Mode**

⬛ **Determining the Device Settings**

⬛ **Finishing the Shutdown or Standby Program**

⬛ Selecting the Timebase

In the first step of the SmartShutdown Wizard, select the ➢*Timebase* to be equilibrated. To do so, SmartShutdown Wizard support must be available for at least one pump in the timebase for which you want to create a shutdown or standby program. The SmartShutdown Wizard currently supports the following Dionex pumps:

- DGP-3600, ISO-3100A, HPG-3x00A/P, LPG-3x00A, LPG-3x00M and P680 HPLC pumps

In addition, the SmartShutdown Wizard supports the following Dionex devices:

- WPS-3000T(SL) and ASI-100 HPLC autosamplers

- TCC-3x00 and TCC-100 HPLC thermostatted column compartments

- VWD-3x00, PDA-100, PDA-3000, UVD 170U, UVD 340U, UVD 170S, and UVD 340S, as well as RF2000, and RI-101 HPLC detectors

☞ *Tip:*

Chromeleon does not support SmartShutdown for a timebase that includes a flow manager. Therefore, an error message appears if you select a timebase in which a flow manager is configured.

If a timebase is already selected in the current window, this timebase is used and the related wizard step is omitted. The right-hand pane lists various computers, timebases, and networks. The access right(s) of the user determine which items are displayed.

Clicking **Next>** takes you to the next wizard page.

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to **How to …:** ⬛ **Shutting down the Chromatography System (SmartShutdown)**.

◾ Selecting the Mode

The next wizard step takes you to the **Select Mode for Timebase <name>** page. This page allows you to select the mode (i.e., whether you want to **Shutdown the system** or **Set the system into standby mode**.

If you want to shut down the system, you must specify whether your mobile phase includes a buffer under Mobile Phase includes Buffer. If you select **Yes**, Chromeleon issues the warning *You cannot shutdown a system containing buffers in the mobile phase!* and aborts the process to prevent damage to the columns through salts that crystallize out when the flow is interrupted. Make sure that the mobile phase no longer contains a buffer before you continue configuration of the shutdown program. To continue the shutdown process, select **No**.

Clicking **Next>** takes you to the next wizard page.

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to **How to …:** 🔲 **Shutting down the Chromatography System (SmartShutdown)**.

◾ Determining the Device Settings

After selecting the mode, you can now configure the required device settings (**Pump Settings**, **Sampler Settings**, **Column Oven Settings** and **Detector (UV)-Settings**) for a shutdown or standby program on the following wizard pages. The available device settings may vary depending on which mode you have selected.

Determine the device settings in each dialog box. For example, specify the **Upper / Lower Pressure Limit**, the **Final Flow** or a user-defined flow gradient profile (**Define Flow Gradient**) for the pump.

�’ *Tip:*

If both sub pumps of a DGP-3600 are installed in the same timebase, you can configure the shutdown process for each pump separately.

Clicking **Next>** takes you to the next wizard page.

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to **How to …:** 🔲 **Shutting down the Chromatography System (SmartShutdown)**.

◼ Finishing the Shutdown or Standby Program

The last page of the SmartShutdown Wizard completes creation of the shutdown or standby program. Specify the program name and then click **Finish** to create the program. The program will be saved to the **Shutdown Programs** subdirectory in the main directory of the timebase. Additionally, it is added to the Batch List. If not already open, the **Start Batch** dialog box opens (or the **Batch edit** dialog box if a sequence is running).

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to **How to …:** 🔽 **Shutting down the Chromatography System (SmartShutdown)**.

300 Shutting down the Chromatography System (SmartShutdown)

Controlling Devices from the Control Panel

Chromeleon provides different control panels for device control. Using an appropriate control panel, you can connect and control your chromatography instruments. In addition, you can modify the control panel controls and/or the timebase assignment. (For general information about control panels, refer to **Control** **The Control Panel**.)

For more information, refer to:

 Opening a Control Panel

 Connecting a Control Panel to a Chromatography Device

 Creating and Starting Sample Batches

 Modifying a Control Panel

 Displaying Sample and Sequence Information

As an alternative to recording real data, you may simulate data acquisition, also. Refer to **Using and/or Recording Demo Data** for more information.

Also, refer to **Answering Frequently Asked Questions**.

Device control also includes System Wellness (see **Performing Validation and Qualification** **Ensuring System Wellness**).

Opening a Control Panel

To process unknown samples or to control an instrument with Chromeleon, open a ➢*Control Panel*, either manually or automatically.

Chromeleon is shipped with more than 100 default control panels that provide all standard functions required for operation. The default panels are usually stored in the **Dionex Templates > Panels** directory of the local ➢*Datasource*.

Open a control panel manually

- Select **Open** on the **File** menu. In the **Open** dialog box, select **Control Panel** from the **Object of type** drop-down list and navigate to the folder that contains the control panels. All panels available in the selected folder are listed. Type the panel name in the **Name** field. Or else, click the name in the list. In this case, the panel name is automatically written into the **Name** field. Click **Open** to open the panel.

- Alternatively, you can select the panel directly from the ➢*Browser*. Double-click the panel name to open the panel.

Each control panel is connected to a ➢*Timebase*. When you open a control panel, Chromeleon automatically connects to the timebase saved last. When installing the software, Dionex Service usually specifies the timebase to which Chromeleon connects automatically. Of course, you can connect the control panel to any other timebase whenever you want (see **How to …: Controlling Devices** 🔳 **Connecting a Control Panel to a Chromatography Device**).

🔳☞ *Tip:*

To change the automatic timebase assignment for a control panel:

*Select **Properties** on the **Edit** menu. On the **Timebase** tab page, delete the entry in the **Timebase** field and click **OK**. When you open the control panel again, no timebase will be assigned. Return to the **Timebase** tab page, and select a timebase. Click **OK** to save the new assignment.*

Open a control panel automatically

When started, Chromeleon opens the most recently used ➢*Workspace*. If the workspace contains a control panel, the corresponding control panel is opened as well.

If you cannot open a workspace, for example, because you have not yet created such a view, open the control panel manually.

🔳☞ *Tip:*

*Instead of opening a single control panel, you can open a panel tabset, which provides (on one window) a group of control panels for controlling and monitoring a timebase (see **How to …:** 🔳 **Controlling Devices from the Panel Tabset**).*

◾ Connecting a Control Panel to a Chromatography Device

When you open a ➢*Control Panel*, it is automatically connected to the assigned ➢*Timebase*, i.e., to the chromatography devices in this timebase. You can modify the timebase assignment any time:

- Open a control panel. Select **Connect to Timebase** on the **Control** menu.

The **Timebase** field on the left reports the name of the currently selected timebase, the **Computer** field reports the name of the computer on which the corresponding ➢*Server* is running, and the **Protocol** field reports the used communication protocol. The list box on the right shows an icon for the local computer (**My Computer**). If the computer is connected to a network, an icon for the network neighborhood appears, also (see **Chromatography Components: Hardware and Software** 🔝 **Network Installation**.)

- Click the Plus sign (**+**) beside an icon to display the items underneath.

- If the server is running, all timebases configured for the local server are listed under **My Computer**. If the server is not active, the entry is **The server is not running**.

- All PCs available on the network are listed under **Network Neighborhood**. Click the "+" sign beside a computer name to display the Chromeleon servers underneath. If there is no active Chromeleon server, the entry is **Server not found**.

- Select a timebase and click **OK** to confirm your selection. The name of the timebase is automatically written to the **Timebase** field on the left.

- If you want to connect to a different timebase whenever you open the control panel, delete the entry in the **Timebase** field. The panel is no longer assigned to a specific timebase and the message stating that the former timebase is not found does not appear.

- Click **OK** to have Chromeleon connect the control panel to the selected timebase. If the connection is successful, you can control the connected instruments, using the control elements on the control panel.

📖☞ *Tip:*

If you want to connect automatically to the currently timebase when you reopen the control panel later, save the panel with the current timebase assignment.

Problems when Connecting to a Timebase

It depends on the selected network protocol whether a non-local server is displayed or can be accessed. The system needs several seconds to check whether the selected protocol can be used. If the connection to a specific timebase is not successful, this can be due to several reasons.

• First, check whether the corresponding server is running.

• If the server is running, try a different network protocol. Chromeleon can communicate via various network protocols, such as IPX, TCP/IP, or NetBEUI.

Tip:

*Communication between two stations is possible only if the same network protocol is installed and selected on both stations. It is usually sufficient if you have installed the corresponding network protocols, e.g., IPX/SPX compatible protocol; NetBEUI Protocol, or Internet Protocol (TCP/IP). (Click **Settings > Control Panel > Network and Dial-up Connections > Local Area Connection**, and then select **Properties** on the context menu.) The current network installation determines which protocol is actually used. Please contact your network administrator. To connect to a Windows 2000 or Windows XP computer, use the **Named Pipes** protocol. Usually, the connection is successful when this protocol is used.*

• It may happen that the required timebase exists (**Server is running**) but that it is not displayed. In this case, return to the **Connect to Timebase** dialog box. The timebase is usually displayed then.
This may also happen when you connect two computers using an ISDN connection. Usually, the reason is that the network installation is not 100 per cent correct.

• If you use an **IPX/SPX compatible protocol**, verify that **NWLink IPS/SPX/NetBIOS Compatible Transport Protocols** is installed and that the check box is selected. To check this, click **Settings > Control Panel > Network and Dial-up Connections > Configuration** and select **Properties** on the context menu. If necessary, install the protocol via the **Install** button.

If the connection is still not successful, contact Dionex Service.

Problems when Controlling Instruments

In addition to the control mode, Chromeleon also provides a **Monitor Only** mode. If you connect to a timebase that is only available on a network, you can only monitor this timebase.

The **Monitor Only** status is also assigned when you attempt to access a local timebase that is currently controlled by an external user.

- Deselect **Monitor Only** on the **Control** menu to enable complete control over the timebase.

◼ Creating and Starting Sample Batches

Chromatography systems are often operated day and night to ensure the best possible rate of utilization. To facilitate processing, Chromeleon allows you to group different sequences in a ➤*Batch*:

- Select **Edit** on the **Batch** menu. The **Batch** dialog box is opened.

- On the **Batch List** tab page, click **Add** to add the sequences to be processed. (In the **Browse** dialog box, select **Sequence** as **Object of type** and navigate to the sequence(s) of interest. Click **Open** to confirm your selection.) If necessary, click **Remove** to remove any unwanted sequences. To delay the start of a sequence, select the sequence and click **Set Delay**. A dialog box is opened. Enter the date and time to start the sequence and click **OK**.
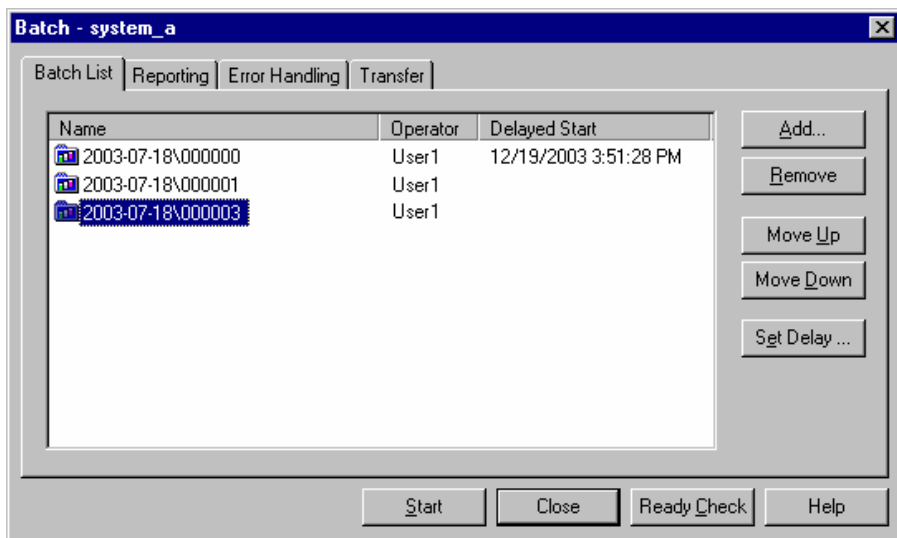
- Use the **Reporting** tab page to determine the print and report options. Select the **Print/Export Report** check box to open the **Batch Report** dialog box. Use this dialog box to specify which pages of the ➤*Printer Layout* you want to print or export and for which samples you want to print/export the selected pages. Confirm your settings by clicking **OK**. On the **Reporting** tab page, also determine whether the selected pages shall be printed or exported immediately after the corresponding sample has been processed or when the entire batch has been finished. (For more information, refer to **How to …: Preparing the Printout 🔺 Printing the Results of a Sequence or a Sample Batch**.)

- On the **Error Handling** tab page, determine the behavior of Chromeleon if errors occur during batch processing.
  Under **Emergency Program**, select the program to be run if an Abort error occurs. Select the program from the drop-down list or click **Browse** to navigate to the file of interest. (For more information, refer to **How to …: Creating and Modifying Programs 🔺 Specifying an Emergency Program**).
  Under **Power Failure Handling**, determine the program to be run when the server restarts after a power failure. Select the program from the drop-down list or click **Browse** to navigate to the file of interest. (For more information, refer to **How to …: Creating and Modifying Programs 🔺 Creating a Power Failure Program**). In addition, determine how the system shall proceed after running the power failure program. Select an action from the drop-down list.

- On the **Transfer** tab page, determine whether the data shall be copied when the batch has been processed. Select the **Copy data to the following location** check box, and then click **Browse** to navigate to the desired location. The options provided in the **Options** section depend on whether the ➤*Online Transfer Agent* (= OTA) is installed on your computer. If the Online Transfer Agent is installed, the following options are available:
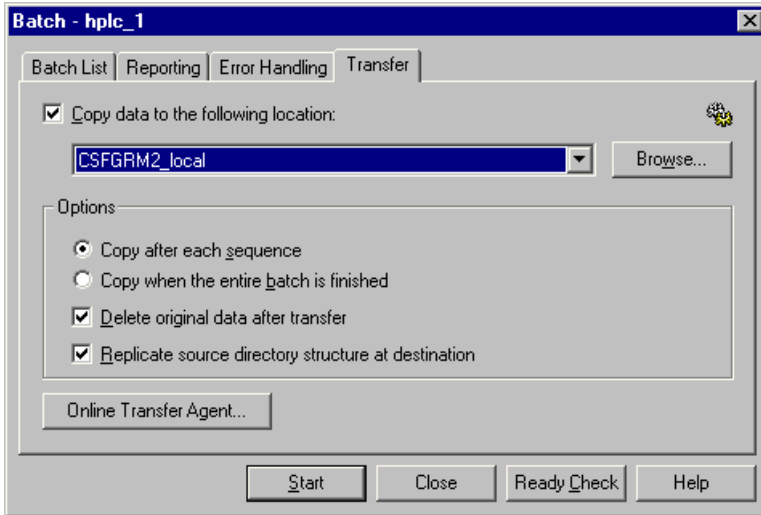
If the Online Transfer Agent is not installed on your computer, the Chromeleon client transfers the data. If the OTA is installed, data transfer is performed by the OTA service. In this case, it is possible to start the batch even if the client is not running and to have data transferred to network datasources that you are not allowed to access.

Tip:

*To use the Online Transfer Agent, make sure that the **Multiple Network Control** license is available on your PC (see **Chromeleon Licenses**).*

For information about how to install the Online Transfer Agent, refer to **How to …: Starting and Monitoring the Server, Setting Up the Server for Network Access Setting Up the Online Transfer Agent for Network Access** in the **Administrator Help** section.

▣ Modifying a Control Panel

Open the ➢*Control Panel* you intend to modify (see **How to …: Controlling Devices from the Control Panel** ▣ **Opening a Control Panel**).

Saving the Panel under a Different Name

Dionex recommends that you do not overwrite any panels from the Dionex Templates > Panels directory. Also, before modifying an existing panel, select **Save as** on the **File** menu and save the panel under a different name.

Enabling Layout Mode

- Enable ➢*Layout Mode* on the **Edit** or context menu.

Adding a Control

- Move the mouse pointer over the ➢*Layout Toolbar*:

  The **quick info** box provides a brief description of the ➢*Control* to which you are currently pointing.

- Left-click to select and append the selected control to the pointer.

- Point to a position. Left-click to position the new control.

Removing a Control

- Left-click the control to be removed. A control frame marks the selected control.

- Press the **Del** key to remove the control. Or, select **Delete** on the **Edit** menu.

Modifying the Size and Position of Controls

- To resize left-click the control and draw its ➢*Control Frame* to the desired size.

- To move a control left-click and drag the control to the new position, holding down the left mouse button. To select several controls simultaneously, press the **SHIFT** key and left-click the selected controls.

- To align several controls, press the **SHIFT** key and left-click the selected controls. Select ➤*Align* on the **Edit** menu, and then select an option. The control frame of the control selected first is used as the reference point and reference size for the other controls.

Also, refer to:

Modifying a Control

Linking a Control to a Device

Creating a Script Button

Linking a Control to a Command

Creating a Device Command Button

Creating a Timebase Command Button

Creating Hidden Windows

Modifying a Control

In Online Mode

- Right-click the ➤*Control* (= object) to be modified.

- Select **Properties** on the context menu to open the **Properties** dialog box for the selected control.

In Layout Mode

- Select the control to be modified.

- Select **Properties** on the context menu to open the **Properties** dialog box.

Properties Dialog Box

The tab pages provided in the **Properties** dialog box vary, depending on the selected control:

Name		Function
1.	General	Determine the caption, font, and text position and size for the control.
2.	Link	Link to the device for which the property is displayed.
3.	Autosize	Determine how the size of the control changes when the entire panel is increased or reduced in size.
4.	Color	Select the colors for the individual components of the control.
5.	Style	Select the shape of the control.
6.	Button	Specify whether and which additional ➢*Control Panel(s)* are opened when the corresponding Script button is clicked (also, see **How to …: Controlling Devices from the Control Panel** **Creating Hidden Windows**).
7.	Command	Type the command or program to be executed when the Script button is clicked.
8.	Axis/ Decoration	Determine the scaling of the axes and the representation of the online signals and 3D plot.
9.	Signals	Determine which signals are displayed and which offset is used.
10.	Chrom.	Determine whether and how chromatograms overlap on the signal plot.
11.	Device	Select the pump(s) for which gradient profiles are displayed.
12.	Timebase	Connect to the selected ➢*Timebase*.
13.	Default Parameters	Specify the default parameters for a command that is linked to a Device Command button.
14.	Events	Select the events to be marked on the ➢*Trend Plot*.
15.	Statistics	Select the statistics to be marked on the trend plot and define how they are calculated.
16.	Data Labels	Specify which data points on the trend plot are labeled, and the text of the label.
17.	Data	Select the trend variable to be plotted, the datasource from which trend data is retrieved, and the time period of the trend data.
18.	Function	Select the function to assign to the timebase command button.

These tab pages are available for the following controls:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Form	X			X	X							X						
Check Box	X	X	X															
Color Box	X	X	X	X														
String Display	X	X	X															
Gauge Indicator	X	X	X	X	X													
Script Button	X		X			X	X											
Online Signal Plot	X		X	X				X	X	X								
Group Box	X		X		X								X					
Gauge/ Slider	X	X	X	X	X									X	X	X	X	
Lamp	X	X	X	X	X													
Switch	X	X	X		X													
Edit Field	X	X	X	X	X													
Audit Trail	X		X															
Gradient Display	X		X	X							X	X						
3D Plot	X		X	X				X										
3D Amp Plot	X		X	X				X			X							
Rack Display	X	X	X															
Online Mass Spectrum Plot	X		X	X														
Device Command Button	X	X	X										X					
Trend Plot	X		X	X										X	X	X	X	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Timebase Command Button	X		X															X
Sample List	X		X															
Wellness Gauge	X	X	X	X	X													

For information about how to specify the property of a control, refer to **How to …: Controlling Devices from the Control Panel** ▣ **Linking a Control to a Device**.

▣ Linking a Control to a Device

To have a control display the status or the parameter of a device, you have to link the element to the device:

- Right-click the corresponding ➢*Control* and select **Properties** on the context menu.

- Open the **Link** tab page.

- From the **Object** list box, select the object to which you want to link a property or parameter. For example, select an instrument, a function (relay), a ➢*Channel*, or a system. Select a property from the **Object Property** list box. Click **OK** to link the control to the object and the property.

Example

To have a color box indicate whether the pump is connected to Chromeleon, select **Pump** from the **Object** list box and **Connected** from the **Object Property** list box:
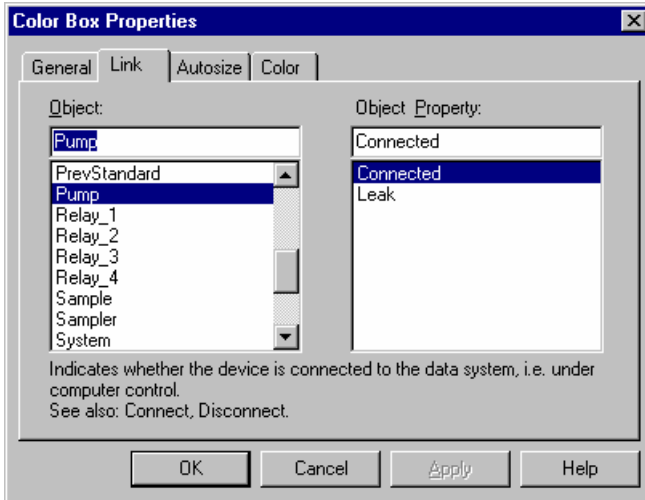
Tip:

*Not every control is an ideal choice for representing a certain function or parameter. The functions and properties that appear in the **Object Property** list box depend on the selected control.*

Note:

*The **Link** tab page is not available for the signal plot, the gradient profile, and the 3D plot. Use the **Signals** tab page for the signal plot and the **Device** tab page for the gradient profile instead. You cannot change the parameters for the 3D plot.*

▣ Creating a Script Button

Chromeleon lets you assign a command or an entire sequence of commands to a **Script Button**. In this way, you only need to click the corresponding button to have Chromeleon execute the command or program. To create script buttons, you must have the corresponding authorization.

- Right-click the script button, select **Properties** on the context menu, and then select the **Command** tab page.

- Type all commands to be executed successively into the list box. The syntax corresponds to the syntax used when creating a ➢*Program*.

When you have entered the commands, click **Check** to check the syntax. Click **Try it now** to test the operation before you complete programming.

- You can also use a script to start a previously written program: Copy the entire program text and paste it into the list box on the **Command** tab page. Or else, use the ⇒*Branch* command. Enter the **Branch** command and then enter the path and name for the program to be started, e.g.:

```
Branch „CM_Seminar\\Programs\\Equilibration"
```

This button allows you to start a column equilibration program that is stored on the **CM_Seminar** datasource in the **Programs** directory.

For more information, refer to **Control** 🔲 **The Program Syntax**.

For practical tips, refer to:

How to …: 🔲 **Creating and Modifying Programs** and **Device Control** 🔲**Practical Tips for Device Control (Overview)**.

🔲 Linking a Control to a Command

To have a control execute a specific device command, you have to link the control to both the device and the command:

- Right-click the corresponding ➤*Control* and select **Properties** on the context menu.

- Open the **Link** tab page.

- From the **Object** list box, select the object to which you want to link a property from the **Object** list box. For example, select an instrument, a function (relay), a ➤*Channel*, or a system. Select a property from the **Object Property** list box. Click **OK** to link the control to the object and the property.

▣ Creating a Device Command Button

Chromeleon lets you assign a command to a device command button so that you can execute the command by clicking the button. To create a device command button, you must have the appropriate authorization.

- Right-click the corresponding button and select **Properties** on the context menu.

- Open the **Link** tab page.

- From the **Object** list box, select the object to which you want to link the command. From the **Command** list box, select the command to be linked to the selected object.

- Select the **Default Parameters** tab page.

- Specify the default setting for the linked command and enter any comment text you want to have associated with the command.

☞ *Tip:*

Device command buttons provide commands for controlling a specific device (for example, performing a detector autozero). Timebase command buttons provide commands for controlling a timebase (for example, creating a program).

Also, refer to ▣ **Creating a Timebase Command Button**.

▣ Creating a Timebase Command Button

Chromeleon lets you assign a command to a button so that you can execute the command by clicking the button. To create a timebase command button, you must have the appropriate CmUser rights.

- If you have not already done so, add a timebase command button to the control panel (see **How to …:** ▣ **Modifying a Control Panel**).

- Right-click the button and select **Properties** on the context menu.

- On the **General** tab page, enter a caption to identify the function to be assigned to the button. To include a variable in the name, click the **{..}** button.

- On the **Timebase Command** tab page, select the command to assign to the button and click **OK**.

 Tip:

Timebase command buttons provide commands for controlling a timebase (for example, creating a program). Device command buttons provide commands for controlling a specific device (for example, priming an autosampler).

Also, refer to **Creating a Device Command Button**.

Creating Hidden Windows

In some cases, a ➢*Control Panel* has to fulfill so many different functions that they do not fit in one window. For example, there may be only a small space left for the signal plot. In this case, create a **Script Button** to open an additional window for the remaining controls or, for example, a large signal plot:

- First, create an additional window: Enable **Layout Mode** on the **Edit** or context menu.

- Select **New Window** on the **Window** menu.

- Click the **Online Signal Plot** icon on the toolbar. Add the signal plot to the window by clicking inside the window, and then determine the size of plot. Right-click the signal plot to open the **Online Signal Plot Properties** dialog box. Use this dialog box to determine the properties for the signal plot.

- Right-click the background of the new window, i.e., right-click outside beside the signal plot. Select **Properties** on the context menu to open the **Form Properties** dialog box.

- On the **General** tab page, enter a name, e.g., **Signal**. On the **Style** tab page, clear the **Initially Visible** check box.

- Return to the original window by clicking the little cross on the top right of the new window.

- Select the **Script Button** icon on the toolbar and add a new script button to the original window. Right-click the button and select **Properties** on the context menu. The **Script Button Properties** dialog box is opened.

- The **Button** tab page indicates the names and captions of all windows assigned to this panel. Select the new window and select the **Show Form** check box.
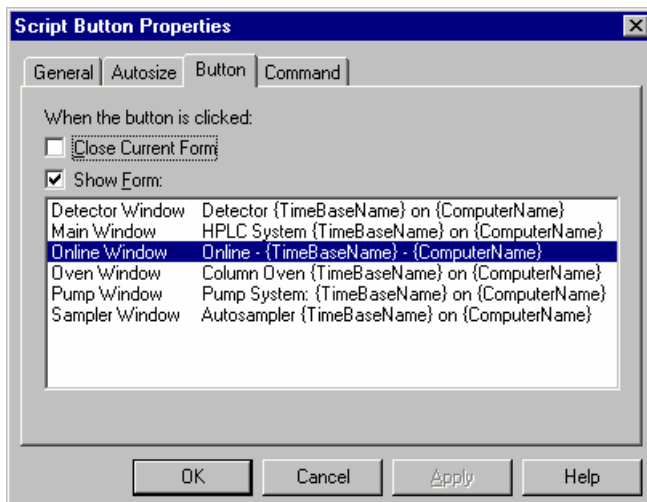
After disabling Layout Mode, you can open the signal plot by clicking this button.

Displaying Sample and Sequence Information

In addition to the current analysis and status values, information specific to sequences and/or samples can be displayed on a ➢*Control Panel*. Thus, you can read from the control panel which sample is processed, to which ➢*Sequence* and ➢*Datasource* the sample belongs, which status the sample has, and so on.

There are two ways to display this type of information:

- Display individual properties for a sample or sequence by linking a ➢*Control* (alphanumeric display) to one of the following functions (**Object Properties**):

Comment	Datasource	Program
Name	Sequence	ProgramMoniker
Number	SequencePath	Moniker
Type	SequenceMoniker	ID

- Display information about all sample injections in the current batch by adding a **Sample List** control to the control panel.

For more information about controls, refer to the topics in **How to …: Controlling Devices from the Control Panel**.

◾ Using and/or Recording Demo Data

Simulating Data Acquisition

➢*Demo (or Virtual) Mode* lets you simulate data acquisition by loading a pre-recorded demo file and displaying the data from the demo file on the ➢*Control Panel's* signal plot. The demo file is "read back" as though the data were being acquired in real time.

1. To select **Demo** or **Virtual Mode** for a device, start the **Server Configuration** program, and then select a device in the corresponding ➢*Timebase*.

2. Select **Properties** on the **Edit** or context menu.

3. For detectors that use demo mode, select **Read** under **Demo Mode** on the **General** tab page, and then select an existing demo file from the **Demo File Name** drop-down list.

   For other detectors, select **Virtual Mode** on the **General** tab page. Then, select the **Demo Chromatogram** tab, select the **Read demo chromatogram from:** check box, and select a demo file from the drop-down list.

   For the VWD-3x00 detectors of the UltiMate 3000 series, select **Read** under **Virtual Mode** to read and display an existing demo file instead of real data. Select a file under **Virtual Mode File Name**.

4. (Optional) Enable the **Demo** or **Virtual Mode** for all other devices of the timebase on the corresponding **General** tab pages.

5. To run the demo file, open the control panel for the device, connect to the timebase, and then enable data acquisition. The **Data Acquisition** dialog box appears.

6. If the demo file contains more than one channel, select the channel(s) and click **OK**. The demo file begins running. It runs continuously (repeats) until data acquisition is disabled.

Recording Demo Data

1. To create a demo file for an installed detector, open the **Properties** dialog box in the **Server Configuration**.

2. For detectors that use demo mode, select **Write** under **Demo Mode** on the **General** tab page.

For other detectors, select **Live Mode** on the **General** tab page. Then, select the **Demo Chromatogram** tab page and select the **Write demo chromatogram to:** check box.

For the VWD-3x00 detectors of the UltiMate 3000 series, select **Write** under **Virtual Mode** to save the data currently provided by the detector to a demo file. Enter a name for the demo file under **Virtual Mode File Name** or select a name from the list.

3. Enter a name for the demo file. If the device is connected via the Dionex DX-LAN, select the **Device ID** under **Communication**.

4. To write the demo file, open the control panel for the device and enable data acquisition. The data acquired from the detector is recorded in the demo file.

5. To stop recording data, disable data acquisition. The demo file is then complete. Alternatively, instead of starting and stopping data acquisition manually, you can run a ➢*PGM File* that turns acquisition on and off.

◾ Answering Frequently Asked Questions

Question: Why does an error message appear in Chromeleon when I shut down a device?

Answer: The Chromeleon server reports an error because the device is no longer connected. Stop the Chromeleon server. Right-click the ➢*Chromeleon Monitor* icon and click **Stop Server**.

📖 *Note:*

Keep in mind that you have to restart the Chromeleon server before you can record another chromatogram.

Question: How can I learn from a control panel which program is being sent to the device and which commands are being executed?

Answer: The program used for analyzing the sample is displayed in the Audit Trail section on the control panel. Under the program, the times at which the different commands were executed by the device are listed.

Question: What is the function of the **Inject** command on the **Control** menu? Does it also start data acquisition?

Answer: Only select this command to perform a manual injection. If you select the command while a program is running, the program will be interrupted. Data acquisition will not be started. To start data acquisition, select **Acquisition On** on the **Control** menu.

Question: Several control panels are listed on the **Window** menu. How can I delete an entry?

Answer: Select the control panel that is not used any longer. Close the panel by either clicking the left-most or right-most button on the top of the panel or by selecting **Close** on the **File** menu. If you have modified the panel, a dialog box appears in which you can confirm to save the modifications.

For tips to solve similar questions, also refer to **How to …:** **Controlling Devices from the Control Panel**.

Controlling Devices from the Panel Tabset

A panel tabset provides (on one window) a set of ➤ *Control Panels* for controlling and monitoring a timebase. If more than one timebase is connected to the chromatography ➤ *Server*, the window provides a panel tabset for each timebase. For general information, refer to **Control** 🔲 **The Panel Tabset**.

Chromeleon provides a default panel tabset, which you can use as is, or modify to suit your needs. You can also create new panel tabsets.

For more information, refer to:

🔲 **Opening a Default or Saved Panel Tabset**

🔲 **Modifying a Panel Tabset**

🔲 **Creating a New Panel Tabset**

🔲 **Expanding, Splitting, or Resizing a Panel Tabset**

🔲 **Using the Sequence Control Panel**

🔲 Opening a Default or Saved Panel Tabset

To open a default panel tabset:

1. Select **Default Panel Tabset** from the **View** menu or click the 🔲 icon on the standard toolbar. The **Connect to Chromeleon Server** dialog box opens.

2. Select the chromatography ➤ *Server* on which the timebase you want to control is configured and click **OK**. The panel tabset opens.

   The panel tabset consists of a set of default ➤ *Control Panels*, organized on tab pages. The timebase is automatically connected to the panels. If there are multiple timebases connected to the selected server, the window displays a panel tabset for each timebase.

🖙 *Tip:*

The number and type of control panels included in a default panel tabset depend on the timebase configuration. After opening the default tabset, you can add or delete panels.

To open a saved panel tabset:

1. Select **Open** on the **File** menu.

2. In the **Open** dialog box, select **Panel Tabset** from the **Object of type** drop-down list.

3. Navigate to the folder that contains the panel tabset. Click the name in the list and click **Open**.

🖙 *Tip:*

You can also select a panel tabset directly from the ➢Browser. Double-click the name (*.PTS) to open the panel tabset.

For more information, refer to:

Control: 🔳 The Panel Tabset

🔳 Modifying a Panel Tabset

🔳 Creating a New Panel Tabset

🔳 Expanding, Splitting, or Resizing a Panel Tabset

🔳 Using the Sequence Control Panel

▪ Modifying a Panel Tabset

After opening a panel tabset, you can customize it to suit your needs. You can:

• Add panels

• Delete panels

• Edit panel tab labels

After modifying a panel tabset, select **Save** from the **File** menu to save it under the same name, or **Save As** to save it under a different name and keep the original unchanged.

 Tip:

To change the controls found on individual control panels on a panel tabset, open the control panel file (.PAN) directly from the **Browser** (see **How to …:** **Modifying a Control Panel**).*

To add a panel:

1. Click the panel tabset. Its border changes to bold, indicating it is active.

2. Select **Add Panel** on the **Edit** or context menu.

3. In the **Browse** dialog box, navigate to the folder that contains the control panel to be added.

4. Click the panel name and click **Open**. Chromeleon automatically connects the new panel to the timebase.

To delete a panel:

1. Select the tab for the panel to be deleted.

2. Select **Remove Current Panel** on the **Edit** or context menu.

To edit tab text:

1. Click the tab to be edited.

2. Select **Edit Panel Tab Text** on the **Edit** or context menu.

3. Type the new tab label. After 16 characters are displayed on the tab, ellipses are used to represent any additional text.

For more information, refer to:

Control: **The Panel Tabset**

 Opening a Panel Tabset

 Creating a New Panel Tabset

 Expanding, Splitting, or Resizing a Panel Tabset

 Using the Sequence Control Panel

⬛ Creating a New Panel Tabset

1. In the **Browser**, select **New** on the **File** menu.

2. Select **Panel Tabset** and click **OK**. The **Connect to Chromeleon Server** dialog box opens.

3. Select the chromatography ➢ *Server* to which the timebase(s) that you want to control are connected and click **OK**. An empty panel tabset opens.

4. The window is divided into panes (one for each timebase). On each pane there are two choices for adding ➢ *Control Panels*:

   Add Panel: Click this option to choose a panel from a **Browse** dialog box. After adding the first panel, you can add additional panels by selecting **Add Panel** from the **Edit** or context menu.

   Add Default Panels: Click this option to have Chromeleon add a set of default control panels. The control panels added for each timebase depend on its configuration. Typically, there is a panel for each instrument (pump, detector, autosampler, etc.), a panel for sequence control functions (creating programs and sequences, loading sequences, starting and stopping a batch, etc.), and a "home" panel that provides basic control functions and status for the system.

5. Select **Save** from the **File** menu to save the panel tabset. Panel tabsets are saved as PTS (*.pts) files.

For more information, refer to:

Control: 🔺 The Panel Tabset

🔺 Opening a Panel Tabset

🔺 Modifying a Panel Tabset

🔺 Expanding, Splitting, or Resizing a Panel Tabset

🔺 Using the Sequence Control Panel

◢ Expanding, Splitting, or Resizing a Panel Tabset

If a panel tabset window contains multiple timebases, you can switch between two display options:

- Click ⛶ to expand the selected panel tabset and hide the other panel tabsets.

- Click ⁕ to view panel tabsets for each timebase simultaneously (split-window view).

In the split-window view, you can also change the width of the panels.

To do this, point to the vertical bar between two panels and drag left or right. Increasing the width of one panel decreases the widths of the other panels.

For example, the panel tabset window below is comprised of two panel tabsets.
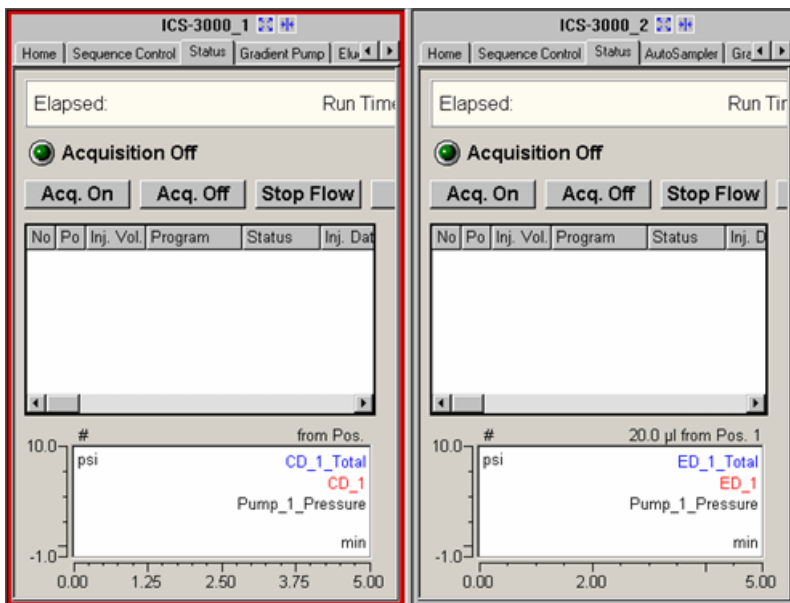
Drag the vertical bar to the left to increase the width of the ICS-3000 panel tabset.
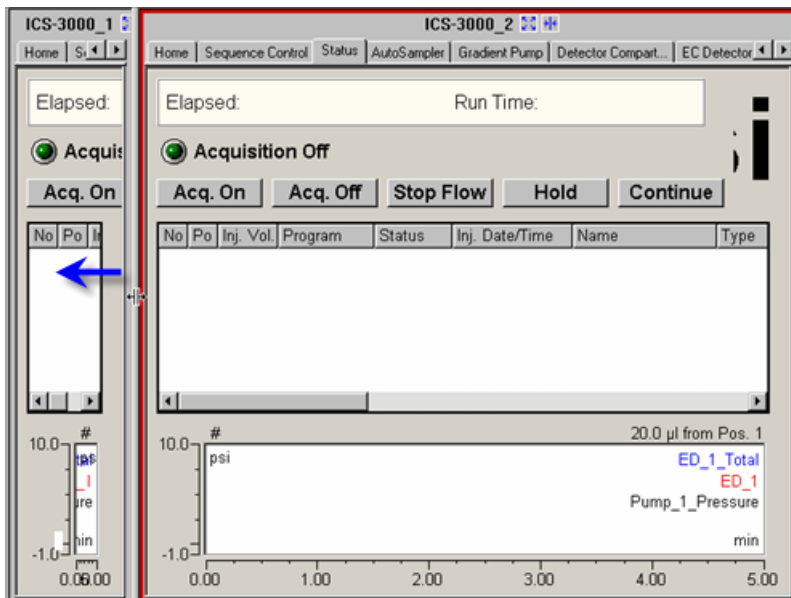
To return to the default split-window view, click ⧏⧐.

For more information, refer to:

Control: 🔙 **The Panel Tabset**

🔙 **Opening a Panel Tabset**

🔙 **Modifying a Panel Tabset**

🔙 **Creating a New Panel Tabset**

🔙 **Using the Sequence Control Panel**

Using the Sequence Control Panel

Each default panel tabset includes a **Sequence Control** panel that provides a central location from which you can develop applications, run samples, view sample status, and record system maintenance events.

Developing an Application

Two approaches are available:

- Use the Program and Sequence Wizards. Click **Create Program** under **1. Create Application**. The Program Wizard opens and guides you through creating and saving a program. After creating the program, click **Create Sequence**. The Sequence Wizard opens and guides you through creating and saving a sequence.

- Alternatively, use the Application Wizard. Click **Application Wizard** under **2. Prepare System**. The wizard guides you through creating a program, quantification method, and sequence.

Running Samples Manually

After injecting the sample, click **Acq On** under **2. Prepare System** to start data acquisition and **Acq Off** to stop acquisition.

Running Samples Automatically

1. Click **Load Sequence** under **2. Prepare System**. Select the sequence to be run and click **Open**.

   - If the system is idle (not currently running a ➢*Batch*), the injections in the selected sequence are displayed in the **Sequence** list. If a sequence is already loaded you can append the new sequence to the list or clear the list first.

   - If the system is running when you load a sequence, the new sequence is automatically appended to the existing list.

2. After loading the sequence, you can edit ➢*Sample Variables* in the **Sequence** list if desired.

3. To run the samples, click **Start Batch**.

4. To hold, continue, or abort the batch, click the corresponding button under **3. Execute Application**.

Viewing the Daily Audit Trail

To view an Audit Trail for a previous day, click **Daily Audit Trail** under **System Maintenance**. The **View Daily Audit Trail** window opens. Select a date to see the Audit Trail for that day.

 Tip:

Point to the Audit Trail pane and right-click to open the context menu. The menu includes commands for filtering the Audit Trail, and for selecting, copying, and searching entries. Refer to *Audit Trails for additional information.*

Recording Consumable and Eluent Changes

After changing a consumable (for example, a column) or changing eluent, click the corresponding button under System Maintenance. The change is recorded in the Audit Trail.

 Tip:

In ≻Trend Plots, the recorded consumable and eluent changes show up as events. This allows a change in a parameter, such as signal background, to be correlated with a consumable or eluent change.

For more information, refer to:

Control: **The Panel Tabset**

 Opening a Default or Saved Panel Tabset

 Modifying a Panel Tabset

 Creating a New Panel Tabset

 Expanding, Splitting, or Resizing a Panel Tabset

 Using the Sequence Control Panel

Creating and Modifying Programs

A ⮞*Program* is used to define precisely timed start conditions and ⇒*Control Commands*. Programs are usually created with the 🔳 **Program Wizard** and later edited, if necessary, in the PGM Editor (see **Control** 🔳 **The PGM Editor**). In order to be valid, every program must contain the following information:

- Signals to be recorded and their parameters

- ⇒*Flow* rate and solvent composition (⇒*%B, %C, %D* - for controlled pumps only)

- ⇒*Inject* command

- Start of data acquisition (⇒*AcqOn*)

- End of data acquisition (AcqOff)

- End of the program (⇒*End*)

Verify that all relevant parameters are defined in the program. If a parameter is not explicitly defined, Chromeleon will use the setting in the last sequence.

Dionex recommends creating the program automatically (see 🔳 **Automatically Creating a Program**), and then editing the file either in the PGM Editor (see 🔳 **Editing PGM Files in the Device Views of the PGM Editor**) or manually (see 🔳 **Manually Editing a PGM File in the Commands View**).

With respect to ⮞*GLP (Good Laboratory Practice)*, Dionex recommends adding comments about all chromatographic settings (e.g., for the column, detector, pump, and the sample components), as well as explanatory notes, at the beginning of the program. However, this is possible only in the **Commands** view.

To see the structure of a simple program, refer to 🔳 **Creating a Program Example**.

▪ **Automatically Creating a Program**

The **Program Wizard** helps you create the basic structure of a ➢*Program*. (For general information about the wizard, refer to **Control** 🔼 **The Program Wizard**.)

- Select **New** on the **File** menu, and then select **Program File** from the list box. The Program Wizard guides you through program creation.

- Enter the required information in the input fields on the corresponding wizard pages. Clicking **Next>** takes you to the next wizard page.

- If you need help, press the F1 key.

Click **Finish** when the input is complete. Chromeleon creates the program based on your input. You are not required to pay attention to the syntax or other programming details.

Refer to the Tutorial 🔼 **Program Wizard** for basic information about the wizard. For information about special applications, refer to:

🔼 **Defining Tandem Gradients (UltiMate 3000 x2/Summit x2)**

🔼 **Setting the Bypass Mode Options**

You can modify and/or extend the basic program later, as described in 🔼 **Editing PGM Files in the Device Views of the PGM Editor**.

For a simple program example, refer to **How to …: Creating and Modifying Programs** 🔼 **Creating a Program Example**.

For examples for special programs, refer to **How to …: Creating and Modifying Programs**.

🔼 **Specifying an Emergency Program**

🔼 **Creating a Power Failure Program**

For an overview of the numerous programming capabilities, refer to **Device Control** 🔼 **Practical Tips for Device Control (Overview)**.

◾ Defining Tandem Gradients (UltiMate 3000 x2/ Summit x2)

The➤ *UltiMate 3000 x2 Dual-Gradient HPLC-System* as well as the ➤*Summit x2 Dual-Gradient HPLC System* feature ➤*Tandem Operation*. Running the system with two separate pumps that deliver at the same flow rate or a dual-gradient pump, a second column, and a 10-port valve allows a significant increase in sample throughput (an increase of 50% to 100% is typical). After the active gradient during which all important substances eluted from the column, the column is excluded from the chromatography flow and reconditioned offline. The re-equilibration time of the column is used to analyze the next sample on the second column.

The Program Wizard supports tandem operation only if either

the selected timebase includes an UltiMate 3000 x2 Dual Gradient System and if a 10-port valve is selected on the **Components** tab page for the TCC-3x00 in the Server Configuration, or

the selected timebase includes a Summit x2 Dual-Gradient HPLC System and if the motorized switching valve (MSV) is selected for the TCC-100 on the **Configuration** page in the Server Configuration program.

For more information about the wizard, refer to **The Control Program** ◾ **The Program Wizard**. On the **Wizard Options** page, select **Program for x2 Tandem Operation** and follow the instructions in the wizard to create the program.

On the **Tandem Gradient Options** page, determine the (virtual) profile, assuming that only one pump is installed. The picture graphically represents this virtual profile.
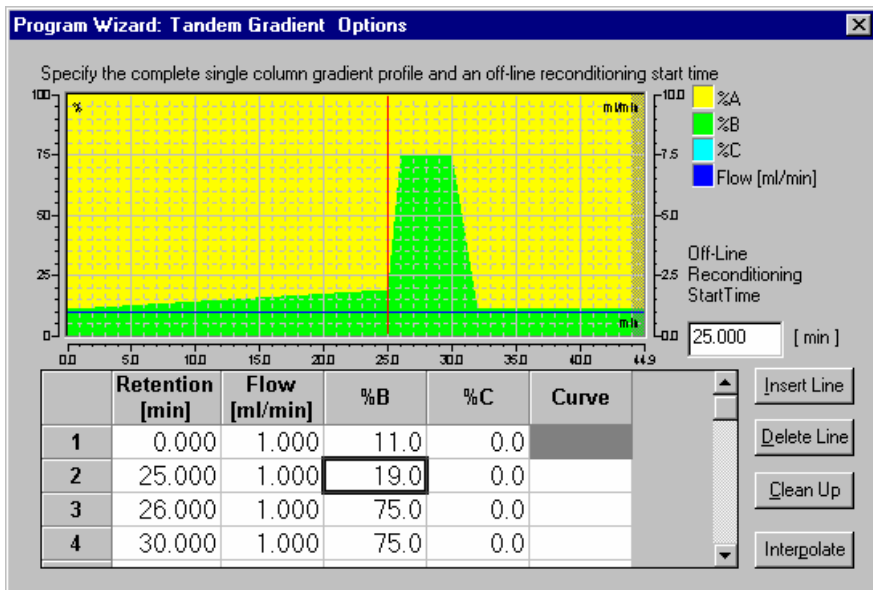
Determining the start for off-line reconditioning

To determine when off-line reconditioning begins, enter a time value in the **Off-line Reconditioning Start Time** input field.

📝 *Note:*

Starting off-line reconditioning does not mean that the column switching valve immediately switches to the other column. Before the valve can switch, the solvent lines between the pump and the column switching valve must be purged. This ensures that only solvent of the starting composition is transported to the second column. In this way, the second column is optimally conditioned when the next sample is injected.

Set the start of the off-line reconditioning to a retention time at which all peaks have eluted from the column. Alternatively, draw the red line in the graphical representation to the desired gradient position. Note that you can draw the line only to certain values, e.g., the basic points of the gradient. In the input field, you can enter the time with three places after the decimal point.

The pictures on the **Tandem Operation Options** page represent the gradients actually delivered by the two pumps.
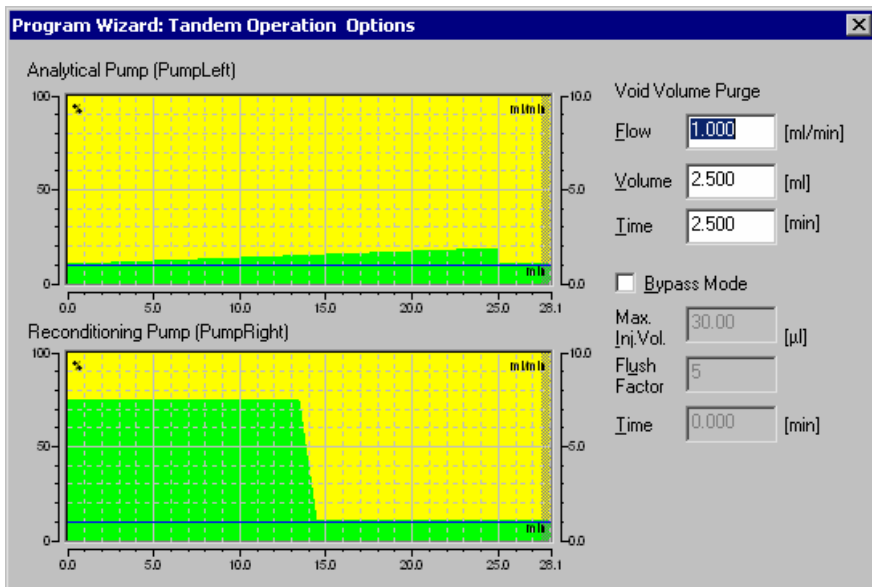
☞ *Tip:*

*The virtual gradient on the **Tandem Gradient Options** page may appear to be shorter than the actual gradients on the **Tandem Operation Options** page. This is because the time required to purge the void volume is not considered for the virtual gradient.*

The (virtual) gradient (defined in the table) is delivered by the analytical pump until off-line reconditioning starts. Then, the analytical pump flushes the solvent lines between the pump and the column switching valve. The column switching valve cannot switch to the other column until after the solvent lines have been completely purged. Afterward, the reconditioning pump delivers the remaining (virtual) gradient that has not yet been delivered by the analytical pump, starting with the composition that contains a minimum of solvent A. If the second part of the original virtual profile is shorter than the first part, the time for the minimum %A portion is prolonged to ensure that the other times of the actual reconditioning profile correspond to the original virtual profile.

For program examples, refer to:

🔼 **Creating a Tandem Program (including Bypass Mode) for an UltiMate 3000 HPLC System**

🔼 **Creating a Tandem Program (including Bypass Mode) for a Summit HPLC System**

▣ Setting the Bypass Mode Options

The Dionex autosamplers with split-loop injection (for example, the ASI-100 or WPS-3000SL) feature a bypass mode that reduces the gradient delay time and minimizes the flush time. The Program Wizard (see **The Control Program** 🔲 **The Program Wizard**) supports the bypass mode for the ➢*Summit x2 Dual-Gradient HPLC System* and the UltiMate 3000 HPLC system.

When the Bypass Mode is enabled, the chromatographic flow bypasses the sample loop of the autosampler. For this purpose, the injection valve is switched into the **Load** position with the related autosampler command. The solvent flows directly onto the separator column.

👉 *Tip:*

Make sure that the system runs under isocratic conditions until switching to the bypass mode.

To avoid carry-over, bypass the sample loop only after the entire sample has left the sample loop. To ensure this, purge the loop volume and the void volume between the injection needle and the MSV at least five times.

The bypass time, i.e., the time after which the injection valve is switched, is calculated as follows:

Time = FlushFactor * (Max. Inj. Vol. + void vol. between injection needle and MSV) / Flow

The void volume between the injection needle and the MSV is 20 µl. Set all other parameters in the Program Wizard. On the **Wizard Options** page, select **Program for x2 Tandem Operation**, and create the program on the next wizard pages. To enable the Bypass Mode, select the related check box on the **Tandem Operation Options** page:
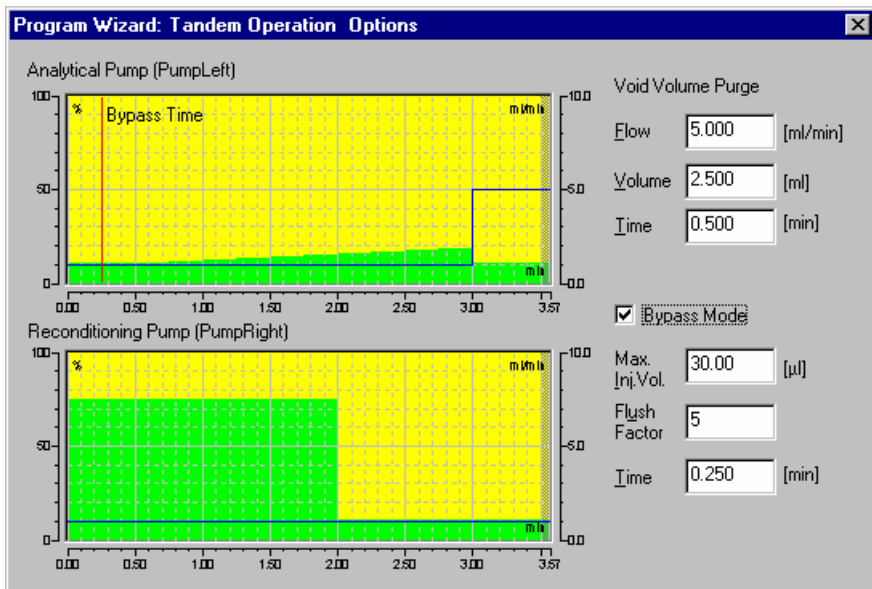
Select the following settings:

Max Inj. Vol.	Enter the maximum injection volume for the application.
Flush Factor/Flush Out Factor (WPS-3000SL)	Enter a factor for calculating the purge volume. 📖 *Tip:* *Dionex recommends using a factor of = 5.*
Time	Usually, the time is calculated from the above settings: Time = FlushFactor * (Max. Inj. Vol. + void volume between needle and MSV) / Flow Enter a time if you want to limit the time until bypass mode is enabled. In this case, the flow is recalculated as follows: Flow = FlushFactor * (Max Inj. Vol. + void vol. between injection needle and MSV) / Time.

At the bypass time, the `MsvToLoad` (ASI-100) or `InjectValveToLoad` (WPS-3000SL) command is added to the program file. The chromatographic flow bypasses the sample loop of the autosampler.

 Tip:

In addition, enter the `PrepareNextSample` command about 1 minute before injection of the next sample to load this sample into the loop and inject it immediately after column valve switching.

For program examples, refer to:

 Creating a Tandem Program (including Bypass Mode) for an UltiMate 3000 HPLC System

 Creating a Tandem Program (including Bypass Mode) for a Summit HPLC System

Editing PGM Files in the Device Views of the PGM Editor

The different device views in the PGM Editor offer a user-friendly way to edit an existing program at any time according to your requirements. (For general information about the PGM Editor, refer to Control **The PGM Editor**.)

- Click a device icon on the left pane in the PGM Editor to change the parameters and/or to enter new parameters for the associated device.

- The associated page(s) of the **Program Wizard** are opened.

- Enter the parameters or changes.

- If you want to change and/or enter parameters for other devices, click the respective icon and follow the description above.

The wizard pages or the PGM Editor provide only the most important commands for the devices. The **Commands** view allows you to enter additional commands manually (see **Manually Editing a PGM File in the Commands View**).

 Note:

*If your program already contains many comments, it may make sense to edit the PGM File in the **Commands** view and not in the Device Views. If you edit such a program in the Device Views, it may happen in some configurations that the single commands appear at the "wrong" position. Please note that comments that were entered at the end of a line are removed. Therefore, please check the program before saving the changes.*

▣ Manually Editing a PGM File in the Commands View

If you want to modify and save an existing ➢*Program*, follow the steps below. (For information about how to create a new program, refer to ▣ **Automatically Creating a Program**. Modify the program in the device view as described in ▣ **Editing PGM Files in the Device Views of the PGM Editor**.)

General Information

- Select **Open**… on the **File** menu, and then select an existing PGM File from the **Open** dialog box.

- Verify that the server is running. If the server is not yet running, start the server in the ➢*Server Monitor Program*.

- Select **Connect to Timebase** on the **Control** menu, and then select the timebase in which you want to use the modified PGM File.

- To open the **Commands** view, click the following icon on the icon bar:

- Place the cursor on the position you wish to modify or press the **Enter** key to insert a new program line.

Entering commands or properties

- Press the F8 key to open the **Commands** dialog box.

- Select the instrument (called **Device** below) for which you want to edit an instruction. A device can be any of the following: an instrument in a timebase, an installed channel, a relay, a remote input, or the system itself. Devices are marked by the following icon ⊞▦. Click the **"+"** character beside the device name to display the items underneath.

- Each device has its own **commands** (📢) and/or **properties** (▣, ▣, ▣, ▣).

- When you select a command or a property, additional input boxes appear under the **Retention Time** input box. The **Help** section displays a brief description of the selected command or property.

- Enter the retention time at which the command will be executed or when the status of a property will be changed.

- Assign either the required value (e.g., a number) or a new status (e.g., **On**) to the command or property.

- Click **OK** to complete the input. Click **OK & Prev** or **OK & Next** to go to the previous or next program line, respectively.

- Repeat the input procedure until you have changed all commands and/or properties.

Adding command/property values to the Audit Trail

- Press the F8 key to open the **Commands** dialog box.

- Select the ⇒*Log* command from the commands list. The devices listed in the left window are displayed a second time, in the **Property** box on the right. The structure in which the devices are displayed is the same as described above. The **Property** box provides the commands and properties in the left window; several read-only variables are available, also.

- Select the command or the property for which you want to write the value at the time specified in the **Retention Time** field to the Audit Trail. The **Help** section displays a brief description of the selected command or property.

- Click **OK** to enter the selected **Log** command into the program.

Adding comments to the Audit Trail

- Press the F8 key to open the **Commands** dialog box.

- Select the ⇒*Protocol* command from the commands list. Enter a comment into the **Text** input box that appears on the right.

- Click **OK** to enter the **Protocol** command into the program.

Checks during command input

- When you enter commands, Chromeleon performs different checks (see ➢*Check Command*):

  Syntax check: Does the entry correspond to the formal rules of the program language?

  Semantics check: Does the program make sense with regard to chromatography?

- If an error is detected, the corresponding line is displayed in red. Check and correct the entry. If necessary, repeat this procedure, using the F8 box.

Adding comments

To enter a comment, place the cursor at the position. Type the text, starting with a semicolon. Alternatively, press the F8 key to open the **Commands** dialog box and enter the comment in the **Comment** field. Click **OK** to add the comment to the program.

Comments are displayed in green. Any comment entered in this way does not appear in the Audit Trail. The purpose of entering a comment is to provide details about a program so that you can, for example, easily recall and understand the various steps later.

 Note:

*If you want to edit your program later in the Device views (see **Control/The PGM Editor** **The Device Views**), Dionex recommends entering all comments right at the beginning of the program. In some cases, Chromeleon cannot assign comments to the associated commands when re-sorting. Thus, it may happen that some comments appear at the wrong position after you have edited the program in the Device views.*

For information about how to perform calculations in a program, refer to **Calculating in a Program**.

Save the result as a new PGM File, using the **Save as** command.

 Note:

*Experienced users can enter these commands directly, that is, without opening the F8 box. However, this requires profound knowledge of the program syntax (see **Control** **The Program Syntax**).*

 Tip:

*Try to avoid editing programs for ➢Tandem Operation. If editing is necessary, keep in mind that you have the make the corresponding changes also for the other pump. Any inconsistencies that might occur are displayed on the **Consistency Check** page. (When Chromeleon detects inconsistencies, this page opens automatically when you exit the PGM Editor and specify that you want to solve the problem(s). As an alternative, you may open this open by clicking the **x2 Tandem Operation** icon.)*

For a simple program example, refer to **Creating a Program Example**.

For special program examples, refer to **How to …: Creating and Modifying Programs**:

🔼 **Creating an Equilibration Program**

🔼 **Creating a Shutdown Program**

🔼 **Specifying an Emergency Program**

🔼 **Creating a Power Failure Program**

For program examples for special applications, refer to **How to …: Creating and Modifying Programs**

🔼 **Creating a Tandem Program (including Bypass Mode)**

🔼 **Using User-defined Columns in a Program**

For an overview of the numerous programming capabilities, refer to **Device Control** 🔼 **Practical Tips for Device Control (Overview)**.

▪ Calculating in a Program

To use different values and signals in one program, Chromeleon lets you use the four fundamental operations of arithmetic and powers. See below for some examples:

Addition or Subtraction: Relative Sample Position

The two examples below refer to the ASI-100 autosampler. (For general information about this autosampler, refer to **Hardware Installation** 🌐 **ASI-100 HPLC Autosampler Series** in the **Administrator Help** section.)

1. To determine that the vial that is five incremented positions away from the current position is always used as the sample preparation vial, use the following command line:

```
0.000   Sampler.PrepVial   Position + 5
```

For example, if you are currently processing the sample from position RB3, the vial at position RB8 is used for sample preparation.

2. If the autosampler is currently at a position in the RA ring, the following line takes you to the corresponding position in the RB ring:

```
0.000   Position    Position + RB1 - RA1
```

Multiplication: Calculating the Volume

3. To record the solvent volume, which is transported through the column while the current sample is processed, as a virtual channel use the following line:

```
VirtualChannel_01.Formula Formula=pump.flow*system.retention
```

In addition, you have to define: `VirtualChannel_01.FormulaMin` and `VirtualChannel_01.FormulaMax`.

👉 *Tip:*

Always set FormulaMin and FormulaMax after you have defined the formula.

Division: Ratio

4. To determine the ratio of two channels, record the quotient of the two channels as virtual channel:

```
VirtualChannel_01.Formula Formula=UV_VIS_1/UV_VIS_2
```

Powers: Calculating the radioactive decay

5. Use the following formula to record a channel for a radioactive substance as if this substance would not decay:

```
VirtualChannel_01.Formula    UV_VIS_1 / 2.718**
                             (-0.69314718*System.Retention/t½)
```

📋 *Note:*

*For detailed examples of the ➢Virtual Channel Driver, refer to **Practical Tips for Device Control Special Commands, Relays and Others** 🔲 **Program Examples for Virtual Channels**.*

Relational and logical operators: ➢*Trigger Commands*

6. Use the following condition to combine, for example, fraction collection:

```
Trigger FRACTION   UV_VIS_1 OR UV_VIS_2 > 100
```

In the above example, fraction collection could start if a signal with a peak area larger than 100 mAU is recorded on either the UV_VIS_1 or the UV_VIS_2 channel.

The following operators are available for trigger commands:

Operator	Description
>	Greater than
<	Less than
=	Equals
AND	All parameters must fulfill a condition.
OR	Only one parameter must fulfill a condition.
NOT	Verifies that a parameter has no specified value.
XOR (=^)	Verifies that the values are different.

Dionex recommends using the **Fraction Collection** device driver to control fraction collection. For more information, refer to **How to …:** 🔲 **Collecting Fractions**.

🔲 Creating a Program Example

A ➢*Program* for a 20-minute chromatogram (flow rate: 1 ml/min, components: A (60%) and B (40%), signals: UV_VIS_1 (256 nm), UV_VIS_2 (300 nm), and 3D field: recorded in the 200 to 360 nm range at a step of 0.5 seconds) could have the following appearance:

```
-10.000  Lamp=On
         Pressure.LowerLimit =        5
         Pressure.UpperLimit =        250
         %A.Equate =                  "%A"
         %B.Equate =                  "%B"
         %C.Equate =                  "%C"

         3DFIELD.MaxWavelength =      360.0
         3DFIELD.MinWavelength =      200.0
         3DFIELD.BunchWidth =         1.9
```

```
       3DFIELD.Step =                0.5
       3DFIELD.RefWavelength =       600.0
       3DFIELD.RefBandwidth =        1.9

       UV_VIS_1.Wavelength =         256
       UV_VIS_1.Bandwidth =          1
       UV_VIS_1.Step =               2
       UV_VIS_1.Average =            On
       UV_VIS_1.RefWavelength =      600
       UV_VIS_1.RefBandwidth =       1

       UV_VIS_2.Wavelength =         300
       UV_VIS_2.Bandwidth =          1
       UV_VIS_2.Step =               2
       UV_VIS_2.Average =            On
       UV_VIS_2.RefWavelength =      600
       UV_VIS_2.RefBandwidth =       1

       Flow =                        1.000
       %B =                          60

 0.000 UV.Autozero
       Inject
       3DFIELD.AcqOn
       UV_VIS_1.AcqOn
       UV_VIS_2.AcqOn

20.000 3DFIELD.AcqOff
       UV_VIS_1.AcqOff
       UV_VIS_2.AcqOff

       End
```

Notes:

- A program for processing a sample batch must contain the ⇒*Inject*, ⇒*AcqOn/Off*, and ⇒*End* commands.

- It is possible but it is not necessary to include the commands for controlling the pump and the detector. Instead of entering the ⇒*Flow* rate, eluent composition (⇒*%B, %C, %D*), and signal parameters directly on the instruments, you can set these values in the program, as shown in the example. This is only possible for controllable chromatography instruments connected to Chromeleon via an RS-232 interface.

- By definition, the injection time is t = 0.000. For all commands that are to be executed before the injection time, the time entry is t < =0.000; for example, the detector lamp on command, here -10.000 min.

- You can omit the ⇒*Lamp*=On command if you allow sufficient time for the lamp to reach the operating temperature.

- Any PDA-100 or PDA-3000 Photodiode Detector command that triggers an Autozero or Auto Offset command must be followed by either a Wait_AZ command or a delay of at least 30 seconds until the next command. The following commands trigger Autozero or Auto Offset: UV Lamp, Visible Lamp, Wavelength, Bandwidth, RefWavelength, RefBandwidth, Reference Mode, Rise Time, MinWavelength, and MaxWavelength.

- For commands that are not listed but are automatically considered, such as, the ⇒*Step* parameters of the UV_VIS_1 channel, either the default values or the values that were last used are used; for example, Step = 0.25. The decision which value is considered depends on the respective device driver.

- In your programs, keep in mind that the instruments need a certain time to execute the different processes. If you use an HP autosampler, for example, the program files must be at least 2 min long to allow the ➤*Autosampler* sufficient time until the next injection.

For special program examples, refer to **How to …: Creating and Modifying Programs**:

🔽 **Creating an Equilibration Program**

🔽 **Creating a Shutdown Program**

🔽 **Specifying an Emergency Program**

🔽 **Creating a Power Failure Program**

🔽 **Creating a Tandem Program (including Bypass Mode)**

🔽 **Program with Variable Run Time**

◨ Creating an Equilibration Program

An equilibration program of a ➢*Summit HPLC System* (consisting of a P680A LPG, ASI-100 with temperature control, TCC-100 with motorized switching valve (MSV), and UVD 340U) could look as follows:

```
-12.800  %B =  0.0 [%]
         %C =  0.0 [%]
         %D =  0.0 [%]
         Pressure.LowerLimit =              0 [bar]
         Pressure.UpperLimit =              400 [bar]
         Pump_Pressure.Ripple.UpperLimit =  3.0 [%]
         RearSealLeakLimit =                5
         MsvPosition =                      A
         ActiveColumn =                     Column_A

         MaximumFlowRamp =                  10000.00 [ml/min²]
         Flow =                             0.000 [ml/min]
         Message "Please   carefully   observe   the   following
                 instructions. Please note that disregarding the
                 instructions  might  cause  erroneous  results  or
                 damage parts of your system."
         Message "Open the purge valve screw(s)."
         Message "CHECK!!! Make sure that the purge valve screw(s)
                 is(are) open!!!"
         Flow =  0.000 [ml/min]

-12.700  Flow =  5.000 [ml/min]
         Message "Wait at least 5 minutes while the pump purges with
                 a flow of 5.000 ml/min before clicking the 'OK'
                 button; take care that no air bubbles occur in the
                 corresponding channel(s)."

-12.600  Flow =  5.000 [ml/min]

-12.500  Flow =  0.000 [ml/min]
         Message "Close the purge valve screw(s)."

         WashSpeed =  10.00 [µl/s]
         DispSpeed =  10.00 [µl/s]
         Message "Verify that you have placed the 4 mL vials in the
                 correct positions: Solvent position: R99, eluent
                 position: B99. Make sure to fill the vials only to
                 half the vial height."
         WashVial =    R99
         PrimeSyringe
         Wait          Sampler.Ready
         WashVial =    B99
         PrimeSyringe
```

```
          Wait            Sampler.Ready
          MsvToInject

-12.000   Flow =   0.000 [ml/min]

-11.000   Flow =   1.000 [ml/min]
          Pressure.LowerLimit =   0 [bar]
          Pressure.UpperLimit =   400 [bar]

          Lamp =    On

-9.000    Sampler.TempCtrl =                      On
          Sampler.Temperature.Nominal =          20 [°C]
          Sampler.Temperature.LowerLimit =       4 [°C]
          Sampler.Temperature.UpperLimit =       45 [°C]
          ColumnOven.TempCtrl =                  On
          ColumnOven.Temperature.Nominal =       18.0 [°C]
          ColumnOven.Temperature.LowerLimit =    5.0 [°C]
          ColumnOven.Temperature.UpperLimit =    85.0 [°C]
          ReadyTempDelta =                       1.0 [°C]

-7.000    %B =  0.0 [%]
          %C =  0.0 [%]
          %D =  0.0 [%]

-6.500    %B =  80.0 [%]
          %C =  0.0 [%]
          %D =  0.0 [%]

-1.500    %B =  80.0 [%]
          %C =  0.0 [%]
          %D =  0.0 [%]

-1.000    %B =  0.0 [%]
          %C =  0.0 [%]
          %D =  0.0 [%]

          UV.Disconnect
          Delay 10.0
          UV.Connect

          3DFIELD.Step =             0.5 [s]
          UV_VIS_1.Wavelength =      225 [nm]
          UV_VIS_1.Bandwidth =       1 [nm]
          UV_VIS_1.RefWavelength =   600 [nm]
          UV_VIS_1.RefBandwidth =    1 [nm]
          UV_VIS_1.Step =            Auto
          UV_VIS_1.Average =         On
          UV_VIS_1.Noise.UpperLimit =   0.10 [mAU]
          UV_VIS_1.Drift.UpperLimit =   3.0 [mAU/hours]
```

```
            MinLampIntensity =              250000 [counts/s]

 0.000   UV.Autozero

         Inject

         Pump_Pressure.AcqOn
         ColumnOven_Temp.AcqOn
         UV_VIS_1.AcqOn

         Pump_Pressure.StartEquilibration
         UV_VIS_1.StartEquilibration

Wait     Pump_Pressure.Equilibration        AND        15        <=
         Sampler.Temperature.Value AND Sampler.Temperature.Value <=
         25  AND  ColumnOven.Ready  AND  UV_VIS_1.Equilibration  AND
         LampIntensity >= 250000, Continue, Timeout = 45.000

         Pump_Pressure.AcqOff
         ColumnOven_Temp.AcqOff
         UV_VIS_1.AcqOff

         Message   "The system was equilibrated successfully."
         End
```

Creating a Shutdown Program

When creating a shutdown program, keep the following aspects in mind:

- Reduce the flow to make sure that as little solvent as possible is used. However, do not reduce the flow to zero because this may result in crystallization of salts in ion chromatography, for example.

- Therefore, set the lower pressure limit (Pressure.LowerLimit) to 1. This setting avoids that the pressure falls below the lower pressure limit when the flow is reduced and that the pump is shut down.

- In addition, the detector lamp(s) should be turned off.

Tip:

Dionex does not recommend performing data acquisition in the shutdown program, i.e., do not use an AcqOn command.

An example program could look as follows:

```
       Pressure.LowerLimit = 1
       Pressure.UpperLimit = 350

0.000  Flow = 0.500

0.500  UV_Lamp = Off
       Flow = 0.100
       End
```

☞ *Tip:*

A simple and comfortable way of creating a shutdown or standby program for a Dionex UltiMate 3000 or Summit Chromatography system is the Chromeleon SmartShutdown Wizard.

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to 🔁 **Shutting down the Chromatography System (SmartShutdown)**.

Also, refer to:

🔁 **Specifying an Emergency Program**

🔁 **Creating a Power Failure Program**

▪ Specifying an Emergency Program

If a ➤*Batch* was automatically aborted due to a severe or serious error, an ➤*Emergency Program* can be started. Determine which ➤*Program* is executed by default.

Select **Error Handling…** on the **Batch** menu to open the **Batch - <Timebase>** dialog box. On the **Error Handling** tab page, click **Browse** in the **Emergency Program** section and navigate to the emergency program to be used.
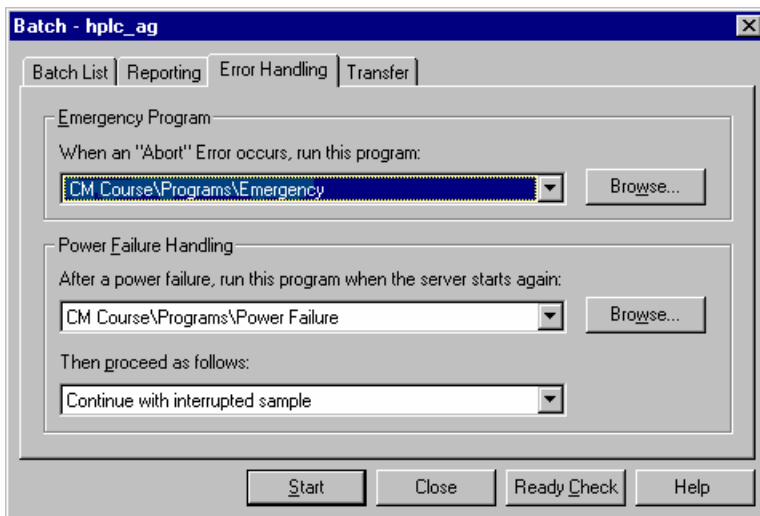
Example 1:

Failure of the detector lamp is a severe error because data acquisition is no longer possible. However, it is not necessary to stop the flow immediately. Instead, the column should be reconditioned after the batch is aborted. You may use, for example, the following emergency program:

```
;       Program: Emergency.PGM
        Recondition column after abort.
0.000   Flow                 = 1
        %A.Value             = 0
        %B.Value             = 100
        %C.Value             = 0
10.000  Flow                 = 1
10.500  End
```

Example 2:

Power failure of an ➢*Autosampler* is a serious error. In this case, the entire system should be stopped immediately to prevent more damage. The **Abort.PGM** emergency program stops the pump flow and turns off the detector lamp.

```
;       Program: Abort.PGM
        The detector lamp and the pump flow are turned off.
0.000   Flow      = 0
        Lamp      = Off
0.500   End
```

For a program example for the 1100 HPLC System, refer to **Hardware Installation** 🌐 **Agilent 1100 HPLC System: Program Tips** in the **Administrator Help** section.

Also, refer to:

🔺 **Creating a Shutdown Program**

🔺 **Creating a Power Failure Program**

▪ Creating a Power Failure Program

You can determine how Chromeleon continues operation after a power failure (see ➢*Power Failure Protection*). After booting the server, you may run a power failure program first.

Select **Error Handling…** on the **Batch** menu to open the **Batch <Timebase>** dialog box. On the **Error Handling** tab page, click the **Browse** button in the **Power Failure Handling** section and navigate to the power failure program to be used.

If a power failure program is not yet available, create a program for the timebase on which you want to use it. Make sure that all instruments are reconnected to Chromeleon first. Very often self-tests have to be performed during which the devices are not ready for access. Therefore, issue the ⇒*Connect* commands 1-2 minutes before the first ⇒*Control Commands* are sent to the instruments.

Example:

Use the following program example, for example, for a ➢*Timebase* that contains an ASI-100 ➢*Autosampler*, a UVD 340U ➢*Photodiode Array Detector*, and a P580 pump:

```
-2.000 UV.Connect
    Sampler.Connect
    Pump.Connect
-1.500 Lamp =             On

; After the UVD 340U has been turned on, the detector needs some time
; for spectra; calibration. That is why you have to issue this
; command, too, some minutes before data acquisition is started.

 0.000 Flow =             0.300
1.000  Flow =             1.000
       End
```

Also, refer to:

🔺 **Creating a Shutdown Program**

🔺 **Specifying an Emergency Program**

🔲 Creating a Program with Variable Run Time

To be able to create a program with variable run time for method development, you need a generic device driver. This driver is not included on the Chromeleon software CD. However, you may use the example driver described in the **Administrator Help** section (see **Hardware Installation: Special Drivers: The Generic Device Driver** 🔵 **Example**), and then generate the following program for this driver:

```
(…)
          Flow =   1.000
          %B = 60

  0.000   UV.Autozero
          Inject
          3DFIELD.AcqOn
          UV_VIS_1.AcqOn
          UV_VIS_2.AcqOn
          Stop_Device.Time = 15.000

          Trigger EndTime System.Retention>Stop_Device.Time
              3DFIELD.AcqOff
              UV_VIS_1.AcqOff
              UV_VIS_2.AcqOff
              End
          EndTrigger

999.000   3DFIELD.AcqOff
          UV_VIS_1.AcqOff
          UV_VIS_2.AcqOff

          End
```

The program ends when the time defined for the stop device (here: 15.000 minutes) has been reached. You can change this time from the panel during the program run. If the Stop-Device.Time is not reached, the above program runs 999 minutes.

To change the run time quickly, create, e.g., an edit field on the control panel, following the instructions in **How to …: Controlling Devices from the Control Panel** 🔲 **Modifying a Control Panel**. On the **Link** tab page, link the edit field to the **Time** object property of the Stop_Device. You can then use this edit field to set the run time to the desired value.

In addition, to identify the edit field, create a **Color** box above the field:
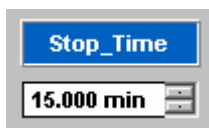
🔳 Creating a Tandem Program (including Bypass Mode) for an UltiMate 3000 HPLC System

A program for operating an ➤*UltiMate 3000 x2 Dual-Gradient HPLC System* (consisting of a DGP-3600, WPS-3000SL, TCC-3200 and VWD-3400) in ➤*Tandem Operation* mode with enabled ➤*Bypass Mode* could look as follows:

```
TempCtrl =                  On
ColumnOven.Temperature.Nominal =    18.0 [°C]
ColumnOven.Temperature.LowerLimit =     5.0 [°C]
ColumnOven.Temperature.UpperLimit =    85.0 [°C]
EquilibrationTime =      0.5 [min]
ReadyTempDelta =         1.0 [°C]
PumpLeft.Pressure.LowerLimit =  1 [bar]
PumpLeft.Pressure.UpperLimit =  400 [bar]
PumpRight.Pressure.LowerLimit = 1 [bar]
PumpRight.Pressure.UpperLimit = 400 [bar]
PumpLeft.%A.Equate =     "%A"
PumpRight.%A.Equate =    "%A"
PumpLeft.%B.Equate =     "%B"
PumpRight.%B.Equate =    "%B"
PumpLeft.%C.Equate =     "%C"
PumpRight.%C.Equate =    "%C"
PumpLeft.MaximumFlowRampDown =  3.000 [ml/min²]
```

```
      PumpRight.MaximumFlowRampDown = 3.000 [ml/min²]
      PumpLeft.MaximumFlowRampUp = 3.000 [ml/min²]
      PumpRight.MaximumFlowRampUp =   3.000 [ml/min²]
      AnalyticalPump =         "PumpLeft"
      ReconditionPump =        "PumpRight"
      DrawSpeed =              5.000 [µl/s]
      DrawDelay =              2000 [ms]
      DispSpeed =              2.000 [µl/s]
      DispenseDelay =          2000 [ms]
      WasteSpeed =             4.000 [µl/s]
      SampleHeight =           2.000 [mm]
      InjectWash =             NoWash
      PunctureOffset =         0.0 [mm]
      PumpDevice =             "PumpLeft"
      InjectMode =             Normal
      FlushOutFactor =         2.0
      MaxInjectVolume =        100.000 [µl]
      LoopWashFactor =         1.000
      Data_Collection_Rate =   2.5 [Hz]
      TimeConstant =           0.60 [s]
      UV_VIS_1.Wavelength =    254 [nm]
      UV_VIS_2.Wavelength =    272 [nm]


0.000   Autozero
      PumpRight.Flow =         2.000 [ml/min]
      PumpRight.%B =           50.0 [%]
      PumpRight.%C =           0.0 [%]
      PumpLeft.Flow =          1.000 [ml/min]
      PumpLeft.%B =            0.0 [%]
      PumpLeft.%C =            0.0 [%]
      Wait                     UV.Ready  and  ColumnOven.Ready  and
Sampler.Ready
      Inject
      UV_VIS_1.AcqOn
      UV_VIS_2.AcqOn
      PumpRight.Flow =         2.000 [ml/min]
      PumpRight.%B =           50.0 [%]
      PumpRight.%C =           0.0 [%]
      PumpLeft.Flow =          1.000 [ml/min]
      PumpLeft.%B =            0.0 [%]
      PumpLeft.%C =            0.0 [%]
```

```
 0.875    InjectValveToLoad
; Sets that the chromatographic flow bypasses the sample loop
; (= bypass mode).

 1.000    PumpLeft.Flow =           1.000 [ml/min]
          PumpLeft.%B =             0.0 [%]
          PumpLeft.%C =             0.0 [%]


 2.000    PumpLeft.Flow =           2.000 [ml/min]
          PumpLeft.%B =             50.0 [%]
          PumpLeft.%C =             0.0 [%]


 2.500    UV_VIS_1.AcqOff
          UV_VIS_2.AcqOff
          PumpLeft.Flow =           2.000 [ml/min]
          PumpLeft.%B =             50.0 [%]
          PumpLeft.%C =             0.0 [%]
          PumpLeft.%B =             0.0 [%]


 2.600    PumpRight.Flow =          2.000 [ml/min]
          PumpRight.%B =            50.0 [%]
          PumpRight.%C =            0.0 [%]


 3.100    PumpRight.Flow =          2.000 [ml/min]
          PumpRight.%B =            0.0 [%]
          PumpRight.%C =            0.0 [%]
          PumpLeft.Flow =           2.000 [ml/min]
          PumpLeft.%B =             0.0 [%]
          PumpLeft.%C =             0.0 [%]
          NextColumn
; Switches the column switching valve to the next column.


                         End
```

■ Creating a Tandem Program (including Bypass Mode) for a Summit HPLC System

A program for operating a ➤*Summit x2 Dual-Gradient HPLC System* (consisting of a P680-DGP, ASI-100, TCC-100, UVD 340U) in ➤*Tandem Operation* mode with enabled ➤*Bypass Mode* could look as follows:

```
TempCtrl =                  On
ColumnOven.Temperature.Nominal =    18.0 [°C]
ColumnOven.Temperature.LowerLimit =     5.0 [°C]
ColumnOven.Temperature.UpperLimit =    85.0 [°C]
EquilibrationTime =      0.5 [min]
ReadyTempDelta =         1.0 [°C]
HumidityLeakSensor =     Standard
GasLeakSensor =          Standard
PumpLeft.Pressure.LowerLimit =  1 [bar]
PumpLeft.Pressure.UpperLimit =  400 [bar]
PumpRight.Pressure.LowerLimit = 1 [bar]
PumpRight.Pressure.UpperLimit = 400 [bar]
PumpLeft.%A.Equate =     "%A"
PumpRight.%A.Equate =    "%A"
PumpLeft.%B.Equate =     "%B"
PumpRight.%B.Equate =    "%B"
PumpLeft.%C.Equate =     "%C"
PumpRight.%C.Equate =    "%C"
PumpLeft.MaximumFlowRamp =  3.00 [ml/min²]
PumpRight.MaximumFlowRamp = 3.00 [ml/min²]
AnalyticalPump =         "PumpLeft"
ReconditionPump =        "PumpRight"
DispSpeed =              10.00 [µl/s]
DrawSpeed =              10.00 [µl/s]
SampleHeight =           0.50 [mm]
SyringeDelay =           5 [s]
UpSpeed =                10.00 [mm/s]
DownSpeed =              10.00 [mm/s]
RadialSpeed =            20.00 [mm/s]
UV_VIS_1.Wavelength =    254 [nm]
UV_VIS_1.Bandwidth =     1 [nm]
UV_VIS_1.RefWavelength = 600 [nm]
UV_VIS_1.RefBandwidth =  1 [nm]
UV_VIS_1.Step =          Auto
```

```
        UV_VIS_1.Average =       On
        UV_VIS_2.Wavelength =    272 [nm]
        UV_VIS_2.Bandwidth =     1 [nm]
        UV_VIS_2.RefWavelength = 600 [nm]
        UV_VIS_2.RefBandwidth =  1 [nm]
        UV_VIS_2.Step =          Auto
        UV_VIS_2.Average =       On
        3DFIELD.RefWavelength =  600.0 [nm]
        3DFIELD.RefBandwidth =   1.9 [nm]
        3DFIELD.Step =           0.5 [s]
        3DFIELD.MinWavelength =  200.0 [nm]
        3DFIELD.MaxWavelength =  595.2 [nm]
        3DFIELD.BunchWidth =     1.9 [nm]


0.000   Autozero
        PumpRight.Flow =         2.000 [ml/min]
        PumpRight.%B =           50.0 [%]
        PumpRight.%C =           0.0 [%]
        PumpLeft.Flow =          1.000 [ml/min]
        PumpLeft.%B =            0.0 [%]
        PumpLeft.%C =            0.0 [%]
        Wait                     ColumnOven.Ready and Sampler.Ready
        Inject
        UV_VIS_1.AcqOn
        UV_VIS_2.AcqOn
        3DFIELD.AcqOn
        PumpRight.Flow =         2.000 [ml/min]
        PumpRight.%B =           50.0 [%]
        PumpRight.%C =           0.0 [%]
        PumpLeft.Flow =          1.000 [ml/min]
        PumpLeft.%B =            0.0 [%]
        PumpLeft.%C =            0.0 [%]


0.240   MsvToLoad
; Sets that the chromatographic flow bypasses the sample loop
; (= bypass mode).


1.000   PumpLeft.Flow =          1.000 [ml/min]
        PumpLeft.%B =            0.0 [%]
        PumpLeft.%C =            0.0 [%]
```

```
2.000   PumpLeft.Flow =          2.000 [ml/min]
        PumpLeft.%B =            50.0 [%]
        PumpLeft.%C =            0.0 [%]


2.500   UV_VIS_1.AcqOff
        UV_VIS_2.AcqOff
        3DFIELD.AcqOff
        PumpLeft.Flow =          2.000 [ml/min]
        PumpLeft.%B =            50.0 [%]
        PumpLeft.%C =            0.0 [%]
        PumpLeft.%B =            0.0 [%]


2.600   PumpRight.Flow =         2.000 [ml/min]
        PumpRight.%B =           50.0 [%]
        PumpRight.%C =           0.0 [%]


3.100   PumpRight.Flow =         2.000 [ml/min]
        PumpRight.%B =           0.0 [%]
        PumpRight.%C =           0.0 [%]
        PumpLeft.Flow =          2.000 [ml/min]
        PumpLeft.%B =            0.0 [%]
        PumpLeft.%C =            0.0 [%]
        NextColumn
; Switches the column switching valve to the next column.


        End
```

◼ Converting Units

Chromeleon allows you to convert the registered units. Use the following command, observing the correct syntax:

<Parameter> = <Value> [<Unit>]

👉 *Tip:*

*Chromeleon can only convert registered units, e.g., the pressure units bar, psi, and MPa. Conversion is case-sensitive, i.e., **Bar** is not registered, and thus, it would not be converted.*

Example

If the pressure unit set on a GP50 pump is **psi**, you can nevertheless enter the pressure limits as follows:

```
Pressure.LowerLimit = 15 [bar]
Pressure.UpperLimit = 200 [bar]
```

When the program is started, the pressure limits are converted into psi. Chromeleon automatically converts the values and communicates the following commands to the pump:

```
Pressure.LowerLimit = 218 [psi]
Pressure.UpperLimit = 2901 [psi]
```

This feature is important for programs that run on different systems. Even if the pressure units are different for these systems, you can use the same PGM File without adapting the values.

Chromeleon can convert the following units:

Value	Registered Units			
Absorption	AU	mAU	µAU	
Charge	C	µC	nC	
Conductivity	S	mS	µS	nS
Current	A	mA	µA	nA	pA
Density	kg/m³	g/ml		
Flow rate	m³/s	ml/min	µl/min	µl/s
Flow acceleration	m³/s²	ml/min²		
Fraction	*None*	%		

Value	Registered Units				
Frequency	Hz	kHz	MHz		
Inverse pressure	1/Pa	1/Mbar			
Inverse temperature	1/K	%/K			
Length	m	mm	µm	nm	Å
Mass	kg	g	amu		
Molar concentration	mol/m³	mM			
Pressure	Pa	bar	psi	MPa	kPa
Refractive index	RIU	µRIU	nRIU		
Refractive index/voltage	RIU/V	µRIU/V			
Refractive index/time	RIU/s	nRIU/h			
Temperature	K	°C	°F		
Time	s	h	min	ms	µs
Velocity	m/s	mm/s			
Voltage	V	mV	µV		
Volume	m³	l	ml	µl	

🗒 *Note:*

Concentration units, such as mg/ml and µg/ml, are not available in a PGM File.

▪ Using User-defined Columns in a Program

You may include ➢*User-defined Columns* from the sample list of the Browser in a program, e.g., for special applications. However, you can only use user-defined columns of the server's ➢*Standard Datasource* for which the **Value type** is neither **Time** nor **Date and time**.

⚠ *Caution:*

Only experienced users should include and program user-defined columns in a program. Errors in the control program may abort processing of the single program steps, and thus the control program.

If you use user-defined columns in PGM files, verify that the column does not contain empty fields. Otherwise, it may happen that the program is not executed.

🖝 *Tip:*

You cannot use user-defined columns with a date or time format in PGM files.

When user-defined columns are used in a program, the entry of the current sample is usually used. The syntax is as follows:

```
<Command>   Sample.<UDC Name>
```

🖝 *Tip:*

You can also use the values of the user-defined column of the next or previous sample (`NextSample` / `PrevSample.<UDC-Name>`) or of the previous standard (`PrevStandard<UDC-Name>`). The standard may not be required for the current sample.

Example 1: Defining the wavelength

In a control program, you can use user-defined columns, e.g., to define the wavelength of a certain channel separately in the sample list for each sample:

- Create a user-defined column in the ➢*Standard Datasource* of the server (see **Creating and Managing Files: Creating a Sample List 🔲 Creating User-defined Columns**).

- Name the column, e.g., WL (for wavelength). To avoid confusion, do not use a property that is already available in Chromeleon. That is why you should not use, e.g., Wavelength. (Instead of WL, any other agreed-upon abbreviation is acceptable.)
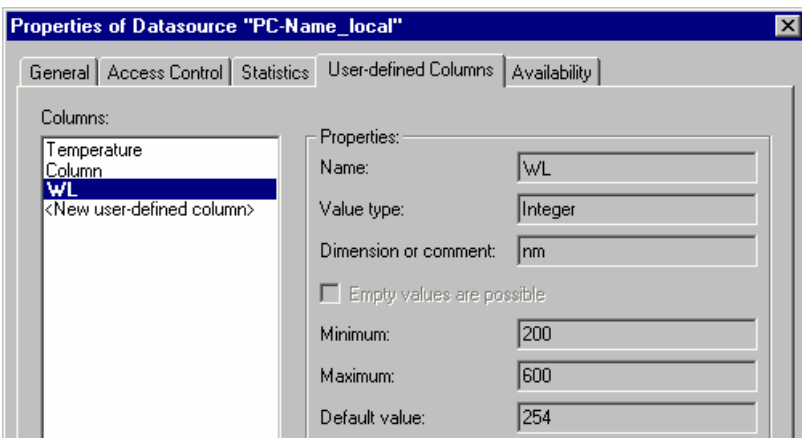
🖐️ *Tip:*

Do not use a German "Umlaut" or "ß" in the name.

Restart the server.

- In the program, enter, e.g., UV_VIS_1 for the channel:

```
UV_VIS_1.Wavelength = Sample.WL
```

This entry means: For each sample, the wavelength used for the UV_VIS_1 channel is the wavelength defined in the corresponding cell of the user-defined column called WL.

Example 2: Starting and stopping fraction collection

You can use the **Program Wizard** to create user-defined columns to define the fraction collection start and the end. (For information about the wizard, refer to **Control 🔽 The Program Wizard**). For more information, refer to **How to …: Collecting Fractions 🔽 PGM Wizard: Fraction Collection - General Options**.

Restrictions when using user-defined columns in a program

You cannot use user-defined columns if the value that the parameter shall reach must be known in advance. This applies to all gradient commands, for example:

- ⇒*Flow*

- ⇒*%B, %C, %D* in % gradients

- GC.Temperature in temperature gradients

- Concentration

- Pump.Curve and EluentGenerator.Curve (see ➤*Gradient Curves*)

📝 *Note:*

Only if you issue these commands before you enter the retention time, you can determine the corresponding values using user-defined columns.

For some instruments, the entire control program is downloaded before sample processing, e.g., for the 1100 HPLC System. In this case, you cannot make changes during the analysis.

Adding Post-Acquisition Steps

Use the **Post-acquisition steps** view of the PGM Editor to define extraction and data smoothing steps to be performed after data acquisition. (For general information about the PGM Editor, refer to **Control ⬛ The PGM Editor**.) In addition, you can copy existing channels or combine them, using arithmetic operations. A new data channel is created for each of these steps. If a Purification license is installed on your PC, Chromeleon also supports automatic creation of samples for fractionation. The single data reprocessing steps are performed directly after either data acquisition on all channels is finished or the program is completely executed.

To open the **Post-acquisition steps** view, click the following icon on the shortcut bar:

Click the bottom line to add a new post-acquisition step. In the dialog box, select a step:

- **Arithmetic combination of channels** (arithmetic combination of 2D channels - see ⬛ **Combining Channels via Arithmetic Operations**)

- **Copy Channel** (see ⬛ **Copying a Channel**)

- **Create Fraction Analysis Samples** (see **How to …: Collecting Fractions Automatically (Autopurification)** ⬛ **Creating Fraction-Type Samples**)

- **Create Purification Samples** (see **How to …: Collecting Fractions Automatically (Autopurification)** ⬛ **Creating Preparation-Type Samples**)

👉 *Tip:*
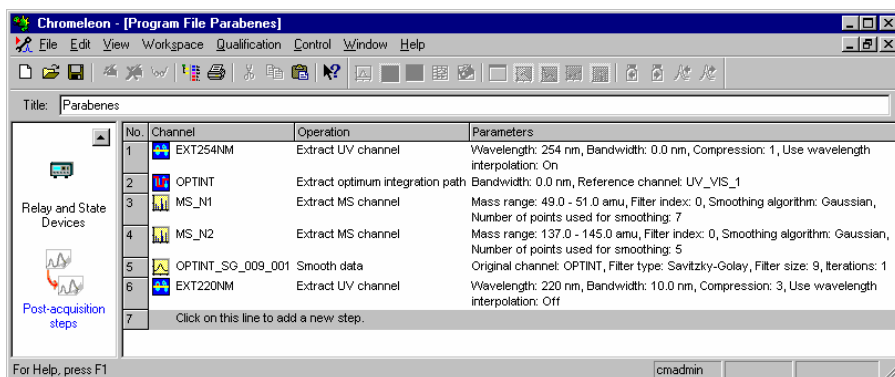
   *The **Create Fraction Analysis Samples** and **Create Purification Samples** options are visible only if a Purification license is installed and if a connection to the server exists. A connection exists if a PGM File is associated with a timebase, the server on which this timebase resides is running, and the client is connected to this server.*

- **Extract ED channel** (for extracting an ED channel – only possible if ➢*3D_Amp* data is available)

- **Extract MS channel** (for extracting a ➢*Mass Trace* - only if MS data are available; see **How to …: Using Mass Spectrometers** 🔳 **Extracting Mass Traces Afterward**)

- **Extract optimum integration path** (for extracting the ➢*Optimum Integration Path* - only possible if a 3D field is available; see **How to …: Creating and Using Spectra Libraries** 🔳 **Selecting the Optimum Integration Path**)

- **Extract UV channel** (for extracting a UV channel extraction - only possible if a 3D field is available; the procedure is similar to selecting the optimum integration path)

- **Smooth data** (for chromatogram ➢*Smoothing* - see **How to …: Working with Chromatograms** 🔳 **Performing Data Smoothing**)

For example, the following steps can be added:

In steps 1 and 6, two UV channels are extracted at 254 nm and 220 nm, respectively. The EXT254NM channel does not use compression; only data acquired at 254 nm are used. For the EXT220NM, a range of 10 nm was selected, i.e., the wavelengths from 215 to 225 nm are used. In addition, compression is used during channel extraction. Compression = 3 means that only every third data point is saved.

In steps 3 and 4, two MS channels (MS_1 and MS_2) are extracted. The first mass trace covers the mass range 49.0 to 51.0 m/z, while the second trace covers masses from 137.0 to 145.0 m/z. For both channels, the Gaussian algorithm is used for data smoothing. Seven points are used for data smoothing when the MS_N1 channel is extracted; five points are used when the MS_N2 channel is extracted.

In step 2, the ➢*Optimum Integration Path* is saved as OPTINT channel. In step 5, data smoothing is performed for this new channel, using the Savitzky-Golay ➢*Filter*. The channel is then saved again as OPTINT_SG_009_001 channel. **Filter size: 9** indicates that 9 input data points are used to create one output data point.

Use the bottom line to add additional steps. Alternatively, select **Insert Line** or **Append Line** on the context menu.

The server performs the post-acquisition steps when the program is completed and before the ➢*System Suitability Test (SST)* is started. If there is a network failure (see ➢*Network Failure Protection*), execution of the post-acquisition steps is delayed until the network source is available again.

Manual Injections with Templates

If no autosampler is found in the server configuration, Chromeleon assumes that an AS40 Autosampler is connected to the Pump's Relay 1. If, however, sample is being loaded and injected manually instead of via an AS40, and the program is being created by either an application template or a ➢*Virtual Column* template selected in the Application ➢*Wizard*, you must modify the ➢*PGM File* to support this. The PGM File created by the application template is saved in the sequence created by the **Sequence Wizard**. The PGM File created by the Virtual Column template can be saved to a new sequence or an existing sequence.

Open the PGM File and locate the AS40 load command. The command typically occurs at the beginning of the timed events, at time -2.300. Below is an example AS40 load command; note that "`Pump_Relay_1.Closed`" closes the AS40 relay, thereby loading the sample into the loop.

```
-2.300 Pump_Relay_1.Closed   Duration=130.00   ; For AS40 injection.
Replace this line with the following for manual injection
```

A manual injection line has been added to the PGM File immediately after the load command:

```
;-0.100 Message "Load the sample into the injection loop, press OK to
continue"
```

The semicolon at the beginning of the line deactivates (comments out) the manual injection ⇒*Message*. To reactivate it, remove the semicolon at the beginning of the manual injection message and add one to the beginning of the AS40 load command. Do not change the times preceding the commands, even if they differ from the above examples.

```
;-2.300 Pump_Relay_1.Closed  Duration=130.00   ; For AS40 injection.
Replace this line with the following for manual injection
```

```
-0.100 Message "Load the sample into the injection loop, press OK to
continue"
```

Save and close the PGM File.

Performing Validation and Qualification

To ensure the quality of analytical results, **Validation and Qualification** have become increasingly important in modern chromatography laboratories.

Chromeleon supports ➢*Installation Qualification* and ➢*Operational Qualification* for qualification of Chromeleon software. The system administrator usually performs both installation and operational qualification. The **Administrator Help** section provides more information; refer to:

 Chromeleon Installation Qualification

 Chromeleon Operational Qualification (OQ)

How to …: Working with Files, Databases, and Networks
 Performing Chromeleon OQ.

Chromeleon also supports instrument qualification, which is usually performed by the system administrator, too. For more information, refer to **Instrument Installation Qualification** in the **Administrator Help** section. Dionex recommends performing ➢*Operational Qualification* and ➢*Performance Qualification* for devices, as well. For details, refer to *Operating Instructions for Operational Qualification/Performance Qualification for HPLC Instruments*, available from Dionex Service.

In addition, Chromeleon provides several System Wellness features that help ensure the functionality of individual devices and, thus, the wellness of the entire system. For more information, refer to **Ensuring System Wellness**.

To ensure that your chromatography method is appropriate for the current analysis, Chromeleon supports ➢*System Suitability Tests (SSTs)*. For more information, refer to **Defining System Suitability Tests**.

Ensuring System Wellness

System Wellness provides built-in diagnostic and calibration features that help prevent unscheduled system shutdowns and assure reliable operation of system devices. System Wellness features are available for IC devices that have a version of ➢*Moduleware* installed that supports System Wellness. Wellness features are available for devices in the Summit HPLC product line, also.

For an overview of System Wellness features and a list of supported devices, refer to:

🔲 **System Wellness for IC Devices (Overview)**

🔲 **System Wellness for Summit HPLC Devices (Overview)**

🔲 **System Wellness and Predictive Performance for UltiMate 3000 Devices (Overview)**

System Wellness tasks for IC devices are performed from the control panel. For more information, refer to:

🔲 **Opening a Wellness Control Panel**

🔲 **Viewing and Restoring Calibration Data**

🔲 **Uploading ICS-1500/2000 Calibration Data**

🔲 **Performing Device Calibrations**

🔲 **Performing Device Diagnostics**

🔲 **Entering Device Parameters**

Also, refer to **How to …: Configuring the Chromeleon Server** 🔲 **Enabling and Disabling System Wellness Functions** for IC Devices in the **Administrator Help** section.

▪ **Opening a Wellness Control Panel**

➢*Control Panels* for performing System Wellness functions are provided for various system configurations.

📙 *Note:*

System Wellness control panels that include "Service." as part of the name are reserved for use by Dionex Service Representatives.

To open a Wellness panel from the Browser:

1. In the ➢*Browser* under the local ➢*Datasource*, open the **Panels** folder, and then open the **Wellness** sub-folder.

2. Double-click the panel name that corresponds to your ➢*Timebase* configuration.

3. The control panel is opened; then Chromeleon attempts to connect to the timebase assigned to the panel.

4. If an error message appears stating that the timebase was not found, close the message, and then select **Connect to Timebase** on the **Control** menu. Select the timebase to be connected to the panel.

5. After communication is established with the timebase, the various calibration and diagnostic controls on the panel are enabled.

To open a Wellness panel for a CD or ED from the panel tabset:

1. In the panel tabset, click the **Cond. Detector** or **EC Detecto**r tab.

2. On the instrument control panel, click **Calibration**.

3. The Wellness panel opens.

▪ **Viewing and Restoring Calibration Data**

Calibration data for IC device is displayed on the Wellness ➢*Control Panel* (see ▪ **Opening a Wellness Control Panel**). Chromeleon stores three sets of calibration data for each System Wellness supported IC device.

- **Current** is the data most recently sent (uploaded) from the device. It is the data currently stored in the device memory.

- **Previous** is the data from the previous time calibration data was uploaded from the device.

- **Factory** is the data obtained (uploaded) from the device when it was initially configured in the System Configuration program (see **How to …: Configuring the Chromeleon Server** 🔼 **Enabling and Disabling System Wellness Functions** in the **Administrator Help** section).

The Wellness control panel displays the current data and the date the current calibration function was performed. If the factory value is the current data, the date field displays "---."

- To display previous data or factory data or download calibration data from Chromeleon to the device, click **Detail**.

  A **Calibration Detail** dialog box appears that displays the three sets of calibration data values and their corresponding dates. The dates indicate when the values were uploaded from the device to Chromeleon.

- To download calibration data to the device, select **Current**, **Previous**, or **Factory** from the list next to the **Download** button. Then, select **Download**.

▪ Uploading ICS-1500/2000 Calibration Data

After performing a calibration procedure or changing a calibration variable from the ICS-1500 or ICS-2000 touch screen, use the procedure below to update the System Wellness database with the new calibration data.

📒 *Note:*

Do not open a System Wellness ➢Control Panel if the name includes "Service.pan." These Wellness panels are reserved for use by Dionex Service Representatives.

1. Open the System Wellness Control Panel for the ICS-1500/2000 (see 🔼 **Opening a Wellness Control Panel**).

2. Locate the **Update Wellness Database** controls. If desired, click **Instructions** for an overview of the procedure.

3. Click **Upload** to begin uploading (sending) calibration data to Chromeleon.

▣ **Performing Device Calibrations**

Wellness ➤*Control Panels*, which are supplied by Chromeleon, display calibration data and provide script buttons for performing IC device calibrations. After a calibration is performed, the device uploads the new calibration data to Chromeleon.

☞ *Tip:*

Many calibrations require setup steps before the actual calibration command is performed. Before selecting a calibration script button on a control panel, refer to the topics below for details about the particular calibration task you are performing.

Devices	Calibration Procedures
ICS-1000/1500/2000 systems	Flow Rate
	Degas Pump
	Conductivity Cell
ICS-90 system	Pressure Transducer
	Conductivity Cell
Autosamplers, Detectors, Pumps	Leak Detector
AS/AS50 Autosamplers	Inject Port Volume
Pumps	Flow Rate
	Pressure Transducer Offset
	Degas
Detectors	Wavelength (AD25/PDA-100)
	Conductivity Cell (CD20/25/25A, ED40/50/50A, IC20/25/25A)
	pH Reference Electrode (ED40/50/50A Amperometry Mode)
	ICS-3000 Conductivity Cell
	ICS-3000 pH Reference Electrode

◾ Calibrating the Leak Detector

When to Calibrate:	After installing a new leak detector
	If the leak detector diagnostic test fails
	Every 6 months

Many System Wellness supported devices are equipped with leak detectors. The calibration procedure for all of the sensors of IC devices is the same.

1. Thoroughly dry the sensor.

2. Open the System Wellness ➢*Control Panel* for the device (see ◾ **Opening a Wellness Control Panel**.)

3. Under **Calibration**, **leak detector**, click the **internal** script button to calibrate the sensor installed in the device itself. Click the **external** script button to calibrate the sensor on a controlled device. For example, for a pump, internal refers to the sensor in the pump and external refers to the sensor in a chromatography oven controlled by the pump.

4. The device calibrates the sensor and uploads the new value to Chromeleon. Chromeleon stores this new calibration value as the current value.

◾ Calibrating the Pump Flow Rate

When to Calibrate:	Every 6 months
Items Needed:	Backpressure tubing to create 14 MPa $\pm$ 2 MPa (2000 psi $\pm$ 300 psi). Use 0.076 mm (0.003 in) ID yellow PEEK tubing (P/N 049715)
	Deionized water
	Tared beaker

1. Verify that there is about 14 MPa (2000 psi) of backpressure.

2 Pump deionized water at 1.0 ml/min.

3. Allow the pump to stabilize for at least 5 minutes.

4. Collect water into a tared beaker for *exactly* 5 minutes.

5. Open the System Wellness ➢*Control Panel* for the pump (see 🔳 **Opening a Wellness Control Panel**).

6. Enter the weight of the water into the **weight** entry field under **flow rate** and press <Enter**>**. Chromeleon downloads the value to the pump and stores this calibration value as the current value.

🔳 **Calibrating the ICS-1000/1500/2000 Flow Rate**

When to Calibrate:	Every 6 months
	If the OQ/PQ flow rate accuracy and precision test fails
Items Needed:	Backpressure tubing to create 14 ± 1.4 MPa (2000 ± 200 psi); use 0.076-mm (0.003-in) ID yellow PEEK tubing (P/N 049715)
	Deionized water
	Tared beaker

📝 *Note:*

Do not open a System Wellness ➢Control Panel if the name includes "Service.pan." These Wellness panels are reserved for use by Dionex Service Representatives.

1. Open the System Wellness Control Panel for the ICS-1000/1500/2000 (see 🔳 **Opening a Wellness Control Panel**).

2. Locate the **Pump Flow Rate Calibration** controls. If desired, click **Instructions** for an overview of the calibration procedure.

3. Click **Reset Cal** to reset the flow rate calibration value.

4. Click **1.00 ml/min** to set the pump flow rate.

5. Allow the **Current Pump Pressure** reading to stabilize at 14 ± 1.4 MPa (2000 ± 200 psi) for 20 minutes.

6. Click **5.00 min** to start the timer and *immediately* start pumping deionized water at 1.00 ml/min.

7. Collect water into a tared beaker for exactly 5 minutes.

8. Enter the weight of the deionized water (in grams) in the **Enter new Flow Rate Cal** field.

9. Click **Calibrate** to begin the calibration. When the calibration is complete, Chromeleon will download the new calibration value to the ICS-1000/1500/2000 and will store it as the current calibration value.

10. Click **Log** to record the new calibration value in the Audit Trail. Chromeleon will automatically update the **Current Calibration Date** and **Last Calibration Date** fields.

11. Wait at least 15 minutes, and then recheck the flow rate to verify that the calibration was successful.

▣ Calibrating the Pressure Transducer Offset

When to Calibrate: Every 6 months

1. Turn off the pump flow.

2. Open the waste valve.

   - For GP40/50, IP20/25 pumps: The waste valve is on the pressure transducer. To open the valve, turn the knob about two turns counterclockwise.

   - For GS50/IS25 pumps: The waste valve is on the secondary pump head. To open the valve, turn the knob one-quarter to one-half turn counterclockwise.

3. Open the System Wellness ➢*Control Panel* for the pump (see ▤ **Opening a Wellness Control Panel**).

4. Click the **offset** script button under **pressure transducer**. The pump calibrates the offset and uploads the new value to Chromeleon. Chromeleon stores this new offset as the current value.

5. Close the waste valve.

▣ Calibrating the ICS-90 Pressure Transducer

When to Calibrate: Every 6 months

About 10 minutes before starting the calibration, toggle the injection valve position a few times by clicking the valve **Load** and **Inject** buttons on the ICS-90 Control Panel. This removes any air or contaminant buildup in the injection valve loop.

1. Turn off the pump flow.

2. Open the waste valve on the front of the pump head by turning the knob counterclockwise two turns.

3. Open the ICS-90 System Wellness ➢*Control Panel* (see 🔳 **Opening a Wellness Control Panel**).

4. Click the **Calibrate Offset** script button under **pressure transducer.** The pump calibrates the transducer and uploads the new value to Chromeleon. The new offset is stored as the current value.

5. Close the waste valve.

6. Connect a pressure gauge between the pump outlet and the pressure transducer. Turn on the pump and let the system stabilize. Note the average pressure reading on the gauge, and enter this reading in the **measured** field.

7. Click the **Calibrate Slope** script button under **pressure transducer**. The pump calibrates the slope and uploads the new value to Chromeleon. The new slope is stored as the current value.

8. Turn off the pump.

9. Disconnect the pressure gauge. Reconnect the pressure transducer to the pump.

🔳 Degas Calibration

When to Calibrate: Every 6 months

1. Open the System Wellness ➢*Control Panel* for the pump (see 🔳 **Opening a Wellness Control Panel**).

2. Select the **degas** script button. The pump performs the degas calibration and uploads the new calibration value to Chromeleon. Chromeleon stores this new value as the current degas value.

🔳 Calibrating the ICS-1000/1500/2000 Degas Pump

When to Calibrate: Every 6 months

📋 *Note:*

Do not open a System Wellness ➢Control Panel if the name includes "Service.pan." These Wellness panels are reserved for use by Dionex Service Representatives.

1. Open the System Wellness Control Panel for the ICS-1000/1500/2000 (see **Opening a Wellness Control Panel**).

2. Locate the **Degas Calibration** controls. If desired, click **Instructions** for an overview of the calibration procedure.

3. Click **Calibrate** to begin the calibration. When the calibration is complete, the ICS-1000/1500/2000 will upload the new calibration value to Chromeleon. Chromeleon will store the new calibration value as the current calibration value.

4. Click **Log** to record the new calibration value and degas pressure in the Audit Trail. Chromeleon will automatically update the **Current Cal. Date** and **Last Calibration Date** fields.

Wavelength Calibration

Use this manual wavelength calibration procedure for the Dionex AD25 and PDA-100 detectors.

When the AD25 power is turned on, the wavelength is automatically calibrated. The PDA-100 wavelength is automatically checked under certain conditions, but is not automatically calibrated.

Manually calibrate the wavelength at the following time:

When to Calibrate:	After a failed wavelength calibration check
	After a failed wavelength calibration

1. Verify that there is solvent flowing through the cell, the background absorbance is low, and there are no bubbles in the light path.

2. Open the System Wellness ➢*Control Panel* for the detector (see **Opening a Wellness Control Panel**).

3. Click the **wavelength** script button under **Calibration**. The detector performs the wavelength calibration routine and uploads the results (Pass or Fail) to Chromeleon.

If wavelength calibration fails, refer to the troubleshooting section of the detector operator's manual.

▪ Calibrating the Conductivity Cell (CD20/25/25A, ED40/50/50A, IC20/25/25A)

When to Calibrate:	After installing a new cell (use Method A)
	Every 6 months (use Method B)
Items Needed (Method B Only):	1.0 mM KCl solution: Prepare by dissolving 0.07456 g of reagent grade KCl in one liter of 18 megohm deionized water
	Backpressure tubing to provide at least 7 MPa (1000 psi). Use 0.076 mm (0.003 in) ID yellow PEEK tubing (P/N 049715)

Method A: For Calibrating New or Replacement Cells

1. Open the System Wellness ➢ *Control Panel* for the detector (see ⬛ **Opening a Wellness Control Panel**).

2. In the **conductivity cell calibration** entry field, type the cell calibration constant (written on a tag on the conductivity cell's cable) and press <Enter>. Chromeleon downloads the value to the detector and stores the value as the current cell calibration constant.

Method B: For Calibrating After Every 6 Months of Use

1. Disconnect the pump output line from the injection valve.

2. Connect the pump output line directly to the inlet of the DS3 or cell.

3. Verify that there is a minimum of 7 MPa (1000 psi) of backpressure.

4. Pump 1.0 mM KCl through the cell at 1.0 ml/min.

5. If using a DS3, set the DS3 temperature to the intended operating point and allow it to reach this temperature.

6. Allow the conductivity to stabilize for about 5 minutes.

7. Open the System Wellness control panel for the detector (see ⬛ **Opening a Wellness Control Panel**).

8. Click the **calibrate** script button under **conductivity cell**. The detector calibrates the cell and uploads a new cell calibration constant to Chromeleon. Chromeleon stores this value as the current cell calibration constant.

   After calibration, the conductivity reading should be 147.00 ± 2 μS/cm and the cell calibration constant should be between 130 and 190. If this is not the case, refer to the troubleshooting section of your detector operator's manual.

9. Flush the KCl solution from the system by pumping deionized water through the cell. When the conductivity drops to less than 1 μS/cm, stop the pump.

10. Reconnect the pump to the injection valve and reconnect the line from the suppressor to the cell inlet.

Calibrating the ICS-90 Conductivity Cell

When to Calibrate:	After installing a new cell
	Every 6 months
Items Needed:	1.0 mM KCl solution: Prepare by dissolving 0.07456 g of reagent-grade KCl in one liter of 18 megohm deionized water
	Backpressure tubing to provide at least 7 MPa (1000 psi). Use 0.076 mm (0.003 in) ID yellow PEEK tubing (P/N 049715)

1. Open the ICS-90 System Wellness ➤*Control Panel* (see **Opening a Wellness Control Panel**).

2. Use an Allen wrench to remove the two screws securing the DS5 Detection Stabilizer (which holds the cell) to the ICS-90 component mounting panel.

3. Pull the DS5 straight out from the component mounting panel to unplug the cell from its electronics. Let the DS5 hang by the tubing.

4. Allow the conductivity to stabilize for 5 to 10 minutes.

5. Click the **Calibrate Offset** script button under **conductivity cell**.

6. Line up the 9-pin connectors on the DS5 and the component mounting panel and plug the DS5 back into the electronics. Replace the screws and tighten.

7. Disconnect the pump outlet line from port P (2) on the injection valve; connect the line directly to the cell inlet using the yellow PEEK backpressure tubing.

8. Fill an eluent bottle with the 1.0 mM KCl solution and connect it to the eluent out line. Prime the pump and then turn on the pump.

9. Verify that there is a minimum of 7 MPa (1000 psi) of backpressure.

10. Allow the conductivity to stabilize for 5 to 10 minutes.

11. Click the **Calibrate Slope** script button under **conductivity cell**. The cell is calibrated and a new cell calibration constant is uploaded to Chromeleon. The new value is stored as the current cell calibration constant.

12. After calibration, the conductivity reading should be 147.00 ± 2 μS/cm. If this is not the case, call Dionex for assistance.

13. Flush the KCl solution from the system by pumping DI water through the cell. When the conductivity drops to less than 1 μS/cm, stop the pump flow.

14. Reconnect the pump to the injection valve and reconnect the line from the suppressor to the cell inlet.

◾ Calibrating the ICS-1000/1500/2000 Conductivity Cell

When to Calibrate:	(Optional) After installing a new cell (use Method A)
	Every 6 months (use Method B)
Items Needed (Method B Only):	1.0 mM KCl solution: Prepare by dissolving 0.07456 g of reagent-grade KCl in one liter of 18 megohm deionized water.
	Backpressure tubing to provide at least 7 MPa (1000 psi); use 0.076-mm (0.003-in) ID yellow PEEK tubing (P/N 049715).

📝 *Note:*

Do not open a System Wellness ➤Control Panel if the name includes "Service.pan." These Wellness panels are reserved for use by Dionex Service Representatives.

Method A: For Calibrating New or Replacement Cells (Optional)

1. Open the System Wellness Control Panel for the ICS-1000/1500/2000 (see 🔲 **Opening a Wellness Control Panel**).

2. Locate the **System Status** controls.

3. Click **Details** to open the **Calibration Details** dialog box.

4. Locate the **Conductivity Cell Cal Details** controls.

5. Enter the cell calibration constant (written on the front of the cell) in the **Conductivity Cell Constant** field and press <Enter>. Chromeleon will download the new value to the ICS-1000/1500/2000 and will store it as the current cell calibration constant.

6. Click **Close** to exit the dialog box.

Method B: For Calibrating After Every 6 Months of Use

1. Open the System Wellness Control Panel for the ICS-1000/1500/2000.

2. Locate the **Electric Conductivity Cell Calibration** controls. If desired, click **Instructions** for an overview of the calibration procedure.

3. Click **Offset Cal** to begin the offset calibration.

4. When the offset calibration is complete, click **Slope Cal** to begin the slope calibration. When the slope calibration is complete, Chromeleon will retrieve the new calibration value from the ICS-1000/1500/2000 and will store it as the current calibration value. Chromeleon will automatically update the **Current Calibration Date** and **Last Calibration Date** fields.

5. Disconnect the pump output line from the injection valve.

6. Connect the pump output line directly to the inlet of the conductivity cell.

7. Verify that there is a minimum of 7 MPa (1000 psi) of backpressure.

8. Locate the **Conductivity Cell Calibration** controls. If desired, click **Instructions** for an overview of the calibration procedure.

9. Click **Cell 35 C** to select the cell heater temperature. Monitor the cell during the warm-up period:

    *   If you have an ICS-1000, monitor the cell temperature on a standard ICS-1000 control panel. When the temperature reaches 35 °C, wait an additional 5 minutes and then go on to Step 11.

    *   If you have an ICS-1500 or ICS-2000, monitor the cell temperature on the **HOME** page on the instrument front panel. When the "=" symbol is displayed next to the **Cell Heater** control, go on to Step 11.

10. Click **1.00 ml/min** to set the pump flow rate. Begin pumping 1.0 mM KCl through the cell.

11. Wait until the **Total Conductivity** reading stabilizes (in approximately 15 minutes), and then click **Calibrate**. When the calibration procedure is complete, Chromeleon will retrieve the new calibration value from the ICS-1000/1500/2000 and store it as the current calibration value. After calibration, the conductivity reading should be 147.00 ± 2 μS/cm and the cell calibration constant should be between 130 and 190. If this is not the case, refer to the troubleshooting section of your ICS-1000/1500/2000 operator's manual.

12. Click **Log** to record the new calibration value in the Audit Trail. Chromeleon will automatically update the **Current Calibration Date** and **Last Calibration Date** fields.

13. Flush the KCl solution from the system by pumping deionized water through the cell. When the conductivity drops to less than 1 μS/cm, stop the pump flow.

14. Reconnect the pump to the injection valve and reconnect the line from the suppressor to the cell inlet.

◢ Calibrating the ICS-3000 Conductivity Detector Cell

When to Calibrate:	Every 6 months
Items Needed	1.00 mM KCl solution: Prepare by dissolving 0.07456 g of reagent-grade KCl in 1.000 liter of 18 megohm deionized water.
	Backpressure tubing to provide at least 7 MPa (1000 psi); use 0.076-mm (0.003-in) ID yellow PEEK tubing (P/N 049715).

1. In the panel tabset, click the **Cond. Detector** tab.

2. Under **Conductivity Detector Settings**, click **Calibration**.

   The **Conductivity Detector Wellness** panel opens.

3. Locate the **External Conductivity Cell Calibration** controls. If desired, click **Instructions** for an overview of the calibration procedure.

4. Disconnect the pump output line from the injection valve.

5. Disconnect the line from the suppressor **ELUENT OUT** port to the cell inlet and connect the pump output line directly to the inlet of the conductivity cell.

6. Verify that there is a minimum of 7 MPa (1000 psi) of backpressure.

7. Under **External Conductivity Cell Calibration**, click **Cell 35 C** to select the cell heater temperature. Allow the cell to reach this temperature and then wait an additional 5 minutes to let it stabilize.

8. Click **1.00 ml/min** to set the pump flow rate and begin pumping 1.00 mM KCl through the cell.

9. Wait until the **Total Conductivity** reading stabilizes (in approximately 15 minutes), and then click **Calibrate**. After calibration, the conductivity reading should be 147.00 ± 2 μS/cm. If this is not the case, contact Dionex for help.

9. Click **Log** to record the new calibration value in the Audit Trail.

10. Flush the KCl solution from the system by pumping deionized water through the cell. When the conductivity drops to less than 1 μS/cm, stop the pump flow.

11. Reconnect the pump to the injection valve and reconnect the line from the suppressor to the cell inlet.

◾ Calibrating the ED40/50/50A pH Reference Electrode

When to Calibrate: After installation of a new reference electrode

Items Needed: pH 7 buffer

A second buffer of known pH (usually a calibration buffer that most closely matches the pH of the eluent used in your application)

1. Carefully remove the combination pH/Ag-Ag/Cl reference electrode from the amperometry cell, making sure to leave the electrode leads connected to the cell.

2. Place the electrode into a pH 7 buffer.

3. Wait for the pH reading to stabilize (about 1 minute).

4. Open the System Wellness ➤*Control Panel* for the detector (see ◾ **Opening a Wellness Control Panel**).

5. Click the **pH 7** script button under **pH electrode**.

6. Remove the electrode from the pH buffer, rinse, and then dry it.

7. Place the electrode in the second buffer.

8. Wait for the pH reading to stabilize.

9. Enter the pH of the second buffer into the edit field above the **2nd buffer** script button.

10. Click the **2nd buffer** script button.

▣ Calibrating the ICS-3000 ED pH Reference Electrode

When to Calibrate:	After installation of a new reference electrode
Items Needed:	pH 7 buffer
	A second buffer of known pH (usually a calibration buffer that most closely matches the pH of the eluent used in your application)

1. Carefully remove the combination pH/Ag-Ag/Cl reference electrode from the amperometry cell, making sure to leave the electrode leads connected to the cell.

2. Place the electrode into a pH 7 buffer.

3. Wait for the pH reading to stabilize (about 1 minute).

4. In the panel tabset, click the **EC Detector** tab.

5. Under **Electrochemical Detector Settings**, click **Calibration**.

   The **Electrochemical Detector Wellness** panel opens.

6. Under **pH Electrode Calibration**, click **pH Offset Cal.**

7. Remove the electrode from the pH buffer, rinse, and then dry it.

8. Place the electrode in the second buffer.

9. Wait for the pH reading to stabilize.

10. Enter the pH of the second buffer into the **pH Slope Buffer Value** field.

11. Click **pH Slope Cal.**

◼ Entering the AS/AS50 Inject Port Volume

The inject port volume is the volume of tubing between the AS or AS50 inject port and the injection valve. The autosampler uses this information to determine how much fluid to push through the line in order to position the sample correctly in the loop for precision injections.

When to enter a new inject port volume: After recalibrating the inject port volume on a new or existing needle seal assembly.

For detailed instructions on replacing a needle seal assembly or calibrating the inject port, refer to the AS or AS50 operator's manual.

1. Open the System Wellness ➤*Control Panel* for the autosampler (see 🔲 **Opening a Wellness Control Panel**).

2. In the **Inject Port Volume** field, enter the volume determined during recalibration of the inject port volume on a new or existing needle seal assembly.

3. Press <Enter>. Chromeleon downloads the value to the autosampler and stores this calibration value as the current value.

◼ Performing Device Diagnostics

Wellness control panels, which are supplied with Chromeleon, display diagnostic test results and provide script buttons for performing the tests.

The following tests are available; see:

🔲 **Leak Detector (for any IC device equipped with a leak detector)**

🔲 **Wavelength Verification (for AD25 and PDA-100 detectors)**

🔲 **Pressure and Conductivity Diagnostics (for ICS-90 Ion Chromatography System)**

🔲 **Conductivity Diagnostics (for ICS-1000/1500/2000 Ion Chromatography Systems)**

◾ Testing the Leak Detector

Many System Wellness supported devices are equipped with leak detectors. The test procedure for all of the sensors of IC devices is the same.

1. Thoroughly dry the sensor.

2. Open the System Wellness ➢*Control Panel* for the device (see ◾ **Opening a Wellness Control Panel**).

3. Under **Diagnostic Tests**, **leak detector**, select the **internal** script button to test the sensor installed in the device itself. Select **external** to test the sensor on a controlled device. For example, for a pump, internal refers to the sensor in the pump and external refers to the sensor in a chromatography oven controlled by the pump.

4. The device tests the sensor and reports the results to Chromeleon. Possible test results are:

   - Passed (Dry)

   - Failed (Wet)

   - Failed (Open circuit): The sensor may be disconnected. Check the connection.

   - Failed (Short circuit): The sensor may need replacing. Contact Dionex for assistance.

   - Failed (Out-of-calibration): Calibrate the sensor (see ◾ **Calibrating the Leak Detector**) and retest. If the test still fails, the sensor may need replacing. Contact Dionex for assistance.

◾ Wavelength Verification

Use this procedure for the Dionex AD25 and PDA-100 detectors. The **Wavelength Verification** test verifies the wavelength accuracy of the AD25 or PDA-100 detectors. When this test is run, a holmium oxide filter is placed in the light path and measured wavelengths are compared to theoretical wavelengths for holmium oxide.

1. Verify that there is solvent flowing through the cell, the background absorbance is low, and there are no bubbles in the light path.

2. Open the System Wellness ➢*Control Panel* for the detector (see ◾ **Opening a Wellness Control Panel**).

3. Under **Diagnostic Tests**, **wavelength verification**, click the **Verify** script button. The detector runs the test and then reports the results to Chromeleon. The overall results (**Passed** or **Failed**) are reported and the theoretical and measured values for three peaks in the holmium oxide spectrum.

If the test fails, run the wavelength calibration (see 🔳 **Wavelength Calibration**) and then rerun the verification test.

🔳 ICS-90 Pressure and Conductivity Diagnostics

Use this procedure to test the variance in pressure and conductivity readings for the Dionex ICS-90 Ion Chromatography System.

1. Verify that the ICS-90 pump is on.

2. Open the ICS-90 System Wellness ➢*Control Panel* (see 🔳 **Opening a Wellness Control Panel**).

3. Under **Diagnostic**, click the **Start** script button. The ICS-90 begins collecting pressure and conductivity readings. To stop the test, click the **Stop** script button. The ICS-90 reports the minimum, maximum, and variance results for both pressure and conductivity.

   To test the conductivity variance on the electronics alone, select the **Dummy Cell** check box before running the test.

🔳 Conductivity Verification (ICS-1000/1500/2000)

Use this procedure to test the conductivity variance on the DS6 conductivity cell electronics.

📭 *Note:*

Do not open a System Wellness ➢Control Panel if the name includes "Service.pan." These Wellness panels are reserved for use by Dionex Service Representatives.

1. Open the System Wellness Control Panel for the ICS-1000/1500/2000 (see 🔳 **Opening a Wellness Control Panel**).

2. Locate the Dummy Cell Test controls. Click **Instructions** for an overview of the test procedure.

3. Click **On** to begin the test.

4. When the test is complete, click **Log** to record the new dummy cell value in the Audit Trail. The new dummy cell value should be 21 μS; if it is not, the CPU card may be defective. Contact Dionex for assistance.

◾ Entering Device Parameters

For some devices, System Wellness ➤*Control Panels* include fields that let you enter various device parameters. Also, see:

🔲   **Applying a Sodium Correction (for ED40/ED50/ED50A Amperometry detectors**)

◾ Applying a Sodium Correction

If you are using a NaCl reference electrode with an ED40, ED50, or ED50A detector in amperometry mode, turn on the sodium correction parameter. This adjusts the detector's signal response for the NaCl electrode, instead of the default AgCl reference electrode.

1. Open the System Wellness ➤*Control Panel* for the detector (see 🔲 **Opening a Wellness Control Panel**).

2. Under **Device Parameters**, **sodium correction**, select **On**.

Defining System Suitability Tests

Use the **SST** page (see ➤*System Suitability Test (SST)*) of the QNT Editor (see **Data Representation and Reprocessing 🔲 The QNT Editor**) to check your system's performance for individual samples. The number of tests is limited only by the capacity of your computer.

Each test is defined on a separate line. To append additional lines to the table and open the SST Wizard, press the DOWN ARROW key. The SST Wizard will guide you through all additional steps.

🔲☞ *Tip:*

*In order to perform a System Suitability Test, enter the QNT File into the sample list before you start the analysis. If you start the analysis before the QNT File has been entered, the test is not performed during the batch run. Thus, the ➤Batch cannot be aborted in case of **Fail Action** - **Abort Batch**.*

For more information, refer to:

🔺 **SST Wizard: Overview and Start Conditions**

🔺 **SST Wizard: Sample and Test Conditions**

🔺 **SST Wizard: Other Wizard Pages**

🔺 **Modifying the System Suitability Test**

🔺 **SST Example: Is the Amount in the Calibrated Range?**

🔺 **SST Example: Amount Deviation on Reinjection**

🔺 **Inserting SST Results in the Printer Layout**

🔹 SST Wizard: Overview and Start Conditions

The SST Wizard guides you through the creation of a new ➤*System Suitability Test (SST)*. Use any of the following methods to open the SST Wizard:

- Double-click a cell.

- Press the F8 key.

- Select **Lines… Append Line** or **Insert Line** on the context menu.

- Position the cursor on the last line of the table and press the DOWN ARROW key.

The SST Wizard includes the following pages:

- **Start**

- **Sample Condition**

- **Test Condition**

- **Aggregate (optional)**

- **Peak & Channel Condition (optional)**

- **N.A. & Fail Action**

On the first wizard page, select a test from the **Predefined Tests** list:

All predefined values on the following pages depend on this selection.

To copy the previous test, select **<Copy Previous Test>** from the list. If there is no previous test, i.e., if the test is the first on the list, this entry is not available.

To continue checking and editing predefined conditions, click **Next**. If no other conditions are required, click **Finish** to close the wizard.

For more information, refer to:

SST Wizard: Sample and Test Conditions

SST Wizard: Other Wizard Pages

◾ SST Wizard: Sample and Test Conditions

Sample Condition

Use the **Sample Condition** page to specify for which sample(s) the test is performed. Select an option:

- Select **Apply on all Samples** to perform the test for each sample.

- Select **Sample Type** to perform the test for a certain sample type only.

- In the **Sample Number(s)** and/or **Vial Number(s)** input fields, enter the sample or vial number(s) for which to perform the test; for example 1,3,7-10. (Note: To separate the entries, you can use either a comma or a semicolon.)

- Select **Sample Property** to perform the test only for samples with the specified property. Enter the property in the corresponding input fields.

- The **User defined condition** option is reserved for advanced users. Select this option to enter any kind of formula in the report format. The test is performed only for those samples for which this condition is true.

Test Condition

Use the **Test Condition** tab page to enter the basic conditions for each system suitability test:

First, enter a unique name in the **Test Name** input field. Specify the **Test Condition** by clicking the "**...**" (Browse) button. Select a variable from the **Edit Result Formula** dialog box. Select the **Operator** from the drop-down list, and then enter the compare value in the **Value** input field. The following operators are available:

Operator:	Description:
	Sample Condition: The SST if performed for all samples for which the property .....
	Test Condition: The SST checks whether the sample property ....
=	*equals* the entered string of characters.
< >	*does not equal* the entered string of characters.
>	*is larger* than the entered string of characters.
<	*is smaller* than the entered string of characters.
>=	*is larger than or equals* the entered string of characters.
<=	*is smaller than or equals* the entered string of characters.

Operator:	Description:
	Sample Condition: The SST if performed for all samples for which the property .....
	Test Condition: The SST checks whether the sample property ....
contains:	*contains* the entered string of characters.
does not contain:	*does not contain* the entered string of characters.
starts with:	*starts with* the entered string of characters.
does not start with:	*does not start with* the entered string of characters.
ends with:	*ends with* the entered string of characters.
does not end with:	*does not end with* the entered string of characters.

 Note:

If the operators >, <, >=, or <= apply to text variables, the lexicographical order is observed; for example, A<B.

The **Compare Value** can be either a numerical value or a report variable or formula. The **Round "Test Condition" and "Compare Value" to x decimals before comparing them** option allows you to round the values for **Test Condition** and **Compare Value** before comparing them by selecting the number of digits to the right of the decimal separator. Then click in the **Value** field to update the value. Select the **Use aggregation** check box to aggregate the test conditions over several samples. Selecting this check box takes you to the **Aggregate** page.

For more information, refer to:

 SST Wizard: Overview and Start Conditions

 SST Wizard: Other Wizard Pages

SST Wizard: Other Wizard Pages

Aggregate

Use this page to define the function, sample(s), and condition for sample aggregation.

Peak & Channel Condition

This page is optional and appears only if the test condition requires that you enter a peak or channel.

N.A. & Fail Action

Use this page to determine the test result if the test cannot be performed (**N.A.**). Also, determine what action is taken if the test fails (**Fail Action**).

For more information, refer to:

🔲 **SST Wizard: Overview and Start Conditions**

🔲 **SST Wizard: Sample and Test Conditions**

▪ Modifying the System Suitability Test

To modify an existing ➢*System Suitability Test (SST)*, double-click a cell or press the F8 key. The **SST Properties** dialog box includes the following tab pages:

- **Sample Condition**
- **Test Condition**
- **Aggregate**
- **Peak & Channel Condition**
- **N.A. & Fail Action**

These pages correspond to the SST Wizard pages. For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **SST Wizard: Overview and Start Conditions**.
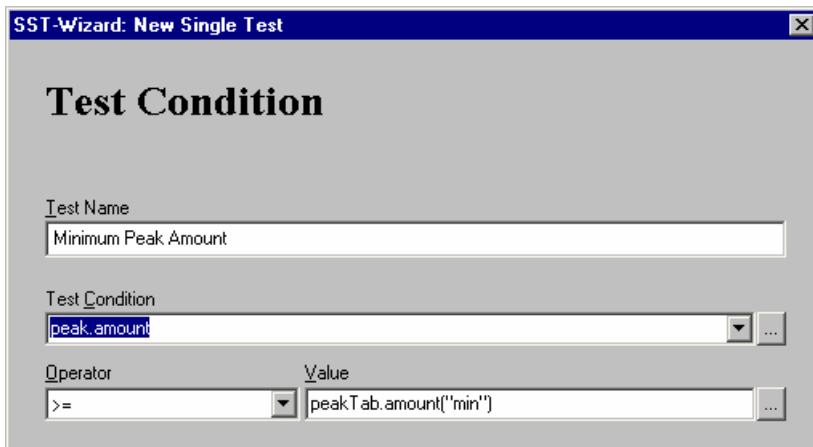
▪ SST Example: Is the Amount in the Calibrated Range?

It is only possible to determine the amount of an unknown sample if it is in the calibrated range, i.e., between the smallest and largest Amounts of the standard samples.

To create a test that checks whether the current Amount is equal to or larger than the smallest Amount of all standard samples, follow these steps:

1. If no System Suitability Test has been created yet, double-click the empty line on the **SST** tab page to open the SST Wizard. If a System Suitability Test already exists, select the bottom line of the test and press the DOWN ARROW key to open the wizard.

2. From the **Predefined Tests** list, select the **Minimum Peak Amount** test.

3. On the **Sample Condition** page, specify for which samples the test is performed. The default setting is **Apply on all Samples**.

4. You can accept the settings on the **Test Condition** page:

5. Use the next wizard page to determine the peak(s) and the channel for which the test is performed.

6. Finally, determine the test result if the test cannot be performed and what action is taken if the test fails.

Repeat the procedure above to create a second test that checks whether the current Amount is equal to or smaller than the largest Amount of all standard samples. In this case, select the **Maximum Peak Amount** test from the **Predefined Tests** list.

▣ SST Example: Amount Deviation on Reinjection

For reinjections from the same vial, the relative standard deviation of the determined Amount values should be as small as possible. To check whether the standard deviation is below a specified minimum value, follow the steps below.

1. If no System Suitability Test has been created yet, double-click the empty line on the **SST** tab page to open the SST Wizard. If a System Suitability Test already exists, select the bottom line of the test and press the DOWN ARROW key to open the wizard. The wizard guides you through test creation. Clicking **Next>** takes you to the next wizard page.

2. On the **Sample Condition** page, specify for which samples the test is performed. The default setting is **Apply on all Samples**.

3. On the **Test Condition** page, enter an appropriate name for the new System Suitability Test, e.g., Amount Deviation.

4. Determine the **Test Condition**, e.g., **peak.amount >= 1**. (In this case, 1 is the maximum allowed deviation expressed as a percentage.)

5. Click the "**…**" button to the right of the **Test Condition** input field.

   a. In the dialog box, select ⇒*Peak Results* from the **Categories** list, and then select **Amount** from the **Variables** list.
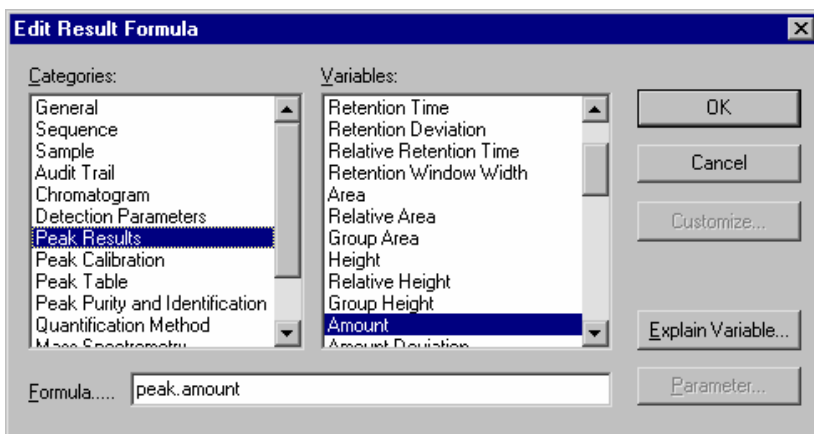
   b. Click **OK** to confirm your selection and return to the SST Wizard.

6. In the **Operator** input field, select **>=**. In the **Value** input field, enter **1**.

7. Select the **Use aggregation** check box. Clicking **Next>** takes you to the **Aggregate** page.

8. On the **Aggregate** page, select **RSD%** from the **Aggregate Function** drop-down list.

9. Enter the **Maximum number of samples to aggregate**, e.g., **5**.

10. For **Only aggregate samples with**, determine **Replicate ID = smp.replicate**. This searches for a maximum of four previous samples for which the replicate ID matches the replicate ID of the current sample. The relative standard deviation of the Amount value is determined for a maximum of five samples--for the current sample and up to four others.

11. Use the next wizard page to determine the peak(s) and the channel for which the test is performed.

12. Finally, determine the test result if the test cannot be performed and what action is taken if the test fails.

◾ Inserting SST Results in the Printer Layout

If you save the ➤*System Suitability Test (SST)* parameters in the QNT File, you can use the test results for peak labels or as individual variables in the ➤*Printer Layout* (not in tables).

Use the **Report** category **System Suitability Test** to display the SST results in the Printer Layout. The following variables are available:

Variable	Description
Number	Number of the SST test
Name	Name of the SST text
Sample Condition	Sample condition of the SST test
Test Condition	
Aggregate Condition	
Min. Aggregation Sample Count	Minimum number of samples to be aggregated
Max. Aggregation Sample Count	Maximum number of samples to be aggregated
Operator	
Value	
Round Digits	
Peak Condition	Conditions for selecting peaks
Channel	
N.A.	User-defined test result if the test is not performed
Fail Action	
Sample Condition Result	
Sample Result	Result of the test condition for the current peak and current sample
Result of Test Condition or Aggregate	
Result of Compare Value	
Peak Valuation	
Test Results	
Aggregated Sample Count	
Aggregation: Average	
Aggregation: Standard Deviation	Standard deviation of the aggregated samples
Aggregation: Rel. Standard Deviation	Relative standard deviation of the aggregated samples in percent
Aggregation: Range	Result range of the aggregated samples
Aggregation: Rel. Range	Relative result range of the aggregated samples in percent

The **Formula** field indicates your selection in short; for example, **sst.test_condition** for the test condition.

Monitoring the Device Status with Predictive Performance

Predictive Performance provides various functions for estimating the lifetime of replaceable parts, and for planning and recording service and qualification information. It helps users to define intervals for regular service and qualification dates and replace parts on a timely basis, as well as to document these measures.

The Predictive Performance functions can be selected on a panel or in the **Commands** dialog box. Colored bars on the panels provide visual indicators of qualification and service periods, making it easy for users to get a quick overview and monitor the device status. The colors used in the panels have the following significance:

- Green: The device or module works properly.

- Yellow: A value will soon reach the specified limit, or the related part should soon be serviced or replaced.

- Orange: (Only gauges for monitoring a *Qualification* property.) A value has reached the limit, but there is a Grace Period during which the device or module may be operated.

- Red: A value has reached the limit. Replacement, servicing, or qualification of the device or module is overdue. The device or module can no longer be used and it is not possible to start a batch. (The *OverrideQualification* privilege deactivates this setting).

To create a Predictive Performance gauge for a module on a panel, follow these steps:

1. Add a **Wellness Gauge** control to the panel by selecting the wellness gauge symbol on the ➢*Layout Toolbar*.

2. Select **Properties…** on the context menu.

3. On the **Link** tab page, select the device or module ("*Object*") and assign it a property ("*Object Property*").

4. Define the gauge design, color, and style on the other tab pages.

5. Select **Set Limits** on the context menu to modify the device-specific limits.

For details about how to add the control to the panel, also refer to **Controlling Devices from the Control Panel** **Modifying a Control Panel**.

🗒 *Note:*

*These settings require the Predictive Performance, ChangePerformanceLimit and ChangeQualificationInterval ➢Privileges. The privileges can be defined in the CM Server Control category of the CmUser Program. For more information, refer to the Administrator Online Help: **Chromeleon User Management** ➡ **Privileges: CM Server Control**.*

The following UltiMate 3000 modules support Predictive Performance:

- DGP-3600, ISO-3100A, HPG-3x00, and LPG-3x00 HPLC pumps

- WPS-3000 (T)SL HPLC autosamplers

- TCC-3x00 HPLC thermostatted column compartments

- VWD-3x00 HPLC detectors

- PDA-3000 Photodiode Array Detector

🗒 *Note:*

The FLM-3x00 does not support Predictive Performance.

The following Dionex module also supports Predictive Performance:

- PDA-100 (USB) Photodiode Array Detector

For a list of Predictive Performance commands available for these modules, refer to:

🔗 **Dionex UltiMate 3000 Series - Pumps**

🔗 **Dionex UltiMate 3000 Series - Autosamplers**

🔗 **Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments**

🔗 **Dionex UltiMate 3000 Series - Variable Wavelength Detectors**

🔗 **Dionex PDA-100/PDA-3000 Photodiode Array Detectors**

Integrating Chromatograms and Identifying Peaks

Chromeleon supports various ⇒*QNT Parameters*, such as calibration and peak table parameters, as well as ⇒*Detection Parameters*. Specify in the ➢*Quantification Method (QNT Method)* which parameters are used for evaluating chromatographic raw data.

For more information, refer to:

 Creating a Peak Table

 Defining Detection Parameters

 Grouping Peaks

 Subtracting a Blank Run Sample

For information about how to perform calibration and enter the calibration parameters in the QNT Editor, refer to **Calibrating**.

Creating a Peak Table

The peak table contains data for

- Peak identification

- Amount calculation

Specific peaks of a chromatogram are identified by a name. The determined peak areas are converted into amount values (= ➢*Formula for Amount Calculation*). The peak table contains the amount values for all standard samples. Hence, it is the basis for each calibration. Usually, all the information is entered into the peak table before the analysis is started.

The peak table contains more columns than can be displayed clearly on the screen. Thus, the columns are distributed on the following tabs:

- **Peak Table** (general peak table)

- **Amount Table** (parameters to determine the amount)

- **Peak Tracking** (parameters to assign peaks using reference spectra)

- **MS Tracking** (parameters to assign peaks using ➢*Mass Spectra*)

To determine which columns are displayed on the tab pages, select **Display Columns** on the **View** menu or select **Columns > Display Column** on the context menu. In this way, you can adjust the tab pages to your individual requirements.

For more information, refer to:

🔲 **Identifying Peaks**

🔲 **Identifying Peaks via Their UV Spectra (Peak Tracking)**

🔲 **Identifying Peaks via Their Mass Spectra (MS Tracking)**

🔲 **Defining Retention Times and Reference Peaks**

🔲 **Defining the Retention Index and the Kovats Index**

🔲 **Defining the QNT Method for Several Detectors**

🔲 **Entering Reference Spectra**

🔲 **Autogenerating the Peak Table**

Also, refer to **How to …: Calibrating:**

🔲 **Selecting the Standard Method (Standard Column)**

🔲 **Selecting the Calibration Function (Cal. Type and Int. Type Columns)**

🔲 **Entering Amount Values (Amount Column)**

and

How to …: Creating and Managing Files and Data 🔲 **Creating User-defined Columns**

▪ **Identifying Peaks**

(Peak Name, Retention Time, **and Window columns)**

Most frequently, peaks are identified by their retention time.

- Enter the names of all peaks to be identified in the ⇒*Peak Name* column, line by line. Assign a nominal retention time to each peak by entering a retention time value in the **Ret. Time** peak table column (= manually creating a peak table). Or else:

- Select 🔲 **Autogenerate Peaktable** on the **Edit** menu to generate the peak table automatically, based on the current sample. In this case, Chromeleon includes all integrated peaks of the current sample into the peak table. The assigned peak names consist of the sequence name and a consecutive number. Chromeleon uses the maximum values of the single peaks to recalculate the values to be entered in the ⇒*Retention Time* and ⇒*Window* columns. All other entries are replaced by the default values (= automatically creating a peak table).

If a peak is detected at the specified time in an unknown sample, the peak is automatically assigned a name (fig. a).

Identification is possible even if the retention times deviate or if neighboring peaks are very close. To allow this, use the **Window** (fig. b) peak table parameter to define a tolerance range. If a peak is detected in this range, it is identified even if the *nominal* and the *actual* retention times do not coincide exactly (fig. c). If several peaks are detected in this range, Chromeleon identifies the greatest peak, the first peak, or the peak nearest to the retention time (fig. d), depending on the selected option.

a)   b)   c)   d)
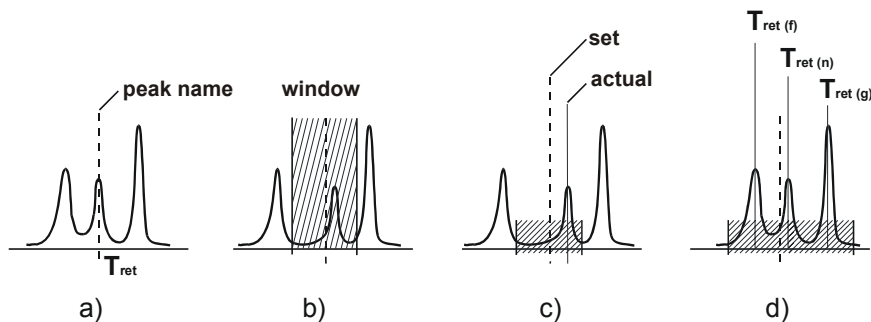
To open the **Peak Window** dialog box, mark the **Window** column cell of the peak of interest, and then either press the F8 key or double-click in the cell. Determine the Window Interpretation: Select **Absolute** to enter the window width in minutes. Select **Relative** to define the window width in percent.

In the **Peak Match** section, select the criterion for peak identification. If you use a ➢*Photodiode Array Detector*, you can also identify the peaks by the spectrum or by the spectrum and the retention time. For more information, refer to 🔲 **Identifying Peaks via Their UV Spectra (Peak Tracking)**. If you use a ➢*Mass Spectrometer* for data acquisition, you can identify the peaks by their mass spectra. (For more information, refer to 🔲 **Identifying Peaks via Their Mass Spectra (MS Tracking)**.)

The **Window** column in the peak table indicates the window width in ➢*Decimal Minutes*; for example, 0.25, followed by the abbreviation for the selected window interpretation and the peak match criterion. To identify the greatest peak in a 30-second window, the entry in the column must be as follows: 0.25 AG (0.25 min or 15 seconds to the left and right of the retention time). If the entry is 0.25 AN, the peak that is nearest to the nominal retention time is identified.

 Notes:

The retention times and window values indicated in the peak table are intended for peak identification only. The retention times indicated in a ➢Report are the actual retention times (i.e., the retention time in the peak maximum).

*If the retention time of the same peak shifts from sample to sample due to a column trend, it is possible for the peak to leave the retention time window at some point. In this case, peak identification is no longer possible. However, Chromeleon provides a method to reliably identify peaks even then: Select the ⇒Use Recently Detected Retention Time check box on the **General** tab page of the QNT Editor.*

In addition to peak identification by the nominal retention time, it is possible to identify compounds by their substance spectrum.

Identifying Peaks via Their UV Spectra (Peak Tracking)

Chromeleon allows you to perform peak tracking. Peak tracking means identifying peaks by comparing spectra. In addition to the **Reference Spectrum** column, other peak table columns also allow you to influence spectra comparison: **Match Criterion**, **Check Derivative**, **Min. WL**, **Max. WL**, **Threshold**, **Rel. Max. Deviation**, and **Check Extrema**. The values entered in these columns have the same meaning as described in **How to …: Displaying and Using UV Spectra** **Entering Criteria for the Spectra Library Screening**.

 Tip:

*Unless you set the minimum wavelength (**Min. WL**) or maximum wavelength (**Max. WL**), the comparison is performed for the entire wavelength range of the reference spectrum.*

To enable peak tracking, press the **F8** key in any cell in the **Window** column (or double-click). In the **Commands** dialog box, select **Spectrum** or **Spectrum and time** under **Peak Match**.
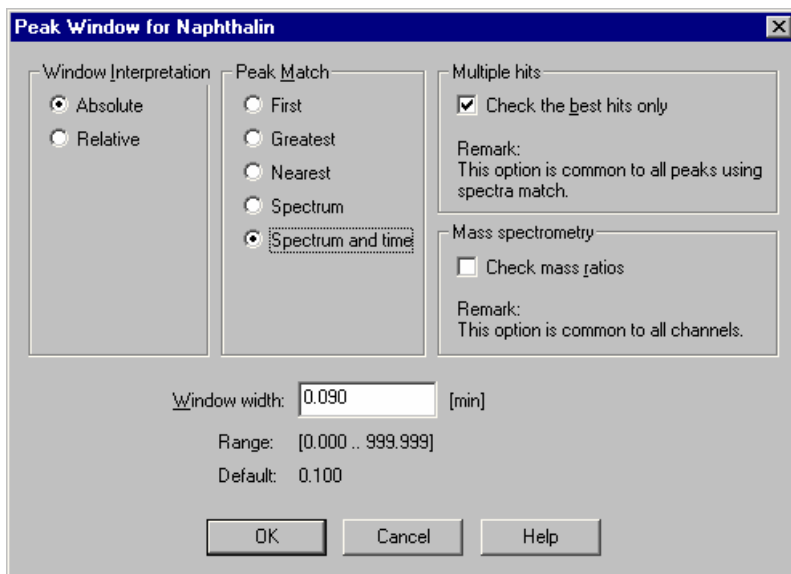
The algorithm for peak identification by comparing spectra can be described as follows: If, in the **Window** column of the peak table, the **Peak Match** criterion for a peak is **Spectrum** or **Spectrum and time**, peak tracking uses the reference spectra in the corresponding column. If **Spectrum and time** is selected, the spectra comparison is limited to the specified time window. Peak tracking generates a list of spectra sorted by the match factor; a peak hit list is not displayed.

☞ *Tips:*

Peak tracking is performed using the parameters (match criteria, etc.) specified for the peak in the peak table.

The peak hit list includes only peaks with a match factor above the threshold specified for this peak.

An empty peak hit list indicates that no spectrum fulfilling the match criteria was found. This could be due to a very high threshold.

The peak hit list is calculated for all peaks in the peak table, for which peak tracking was enabled. After calculation, the peak hit lists are checked for multiple hits. If the **Check the best hits only** option is enabled, only the best hits are compared for the individual peaks.

In case of multiple hits, the peak with the best match factor receives the name of the reference substance.

Usually, multiple hits are not found if you select **Spectrum and time** as match criterion.

All other peaks in the peak table, i.e., peaks for which the peak tracking function is disabled, are identified via the window assignment (**First**, **Nearest**, **Greatest**) as described in 🔲 **Identifying Peaks**.

If you acquire data with a ➢*Mass Spectrometer*, you can identify peaks via their mass spectra. (For more information, refer to 🔲 **Identifying Peaks via Their Mass Spectra (MS Tracking)**).

▣ Identifying Peaks via Their Mass Spectra (MS Tracking)

(Mass peak x, MS threshold, MS filter conditions, and Check MS ret. times columns)

➢*Mass Spectra* present a very reliable method for peak identification. For the available parameters, refer to the **MS Tracking** tab page.

No.	Peak Name	Ret.Time	Mass peak 1	MS threshold	MS filter conditions	Check MS ret. times
1	Coffein	3.077 min	195.0 amu @ 100.0 %	5.0	Automatic	Off
2	Methylparabene	3.630 min	151.0 amu @ 100.0 %	5.0	Automatic	Off
3	*Ethylparabene*	*4.200 min*	*165.0 amu @ 100.0 %*	*5.0*	*Automatic*	*Off*
4	Propylparabene	5.200 min	179.0 amu @ 100.0 %	5.0	Automatic	Off
5	Butylparabene	6.900 min	193.0 amu @ 100.0 %	5.0	Automatic	Off

◀ ▶ Ⓚ Amount Table ⓀPeak Tracking ⋏ MS Tracking Ⓚ ◀ ▶

4.1 min, 45 mAU - Select Peak 3 - Ethylparabene - 4.083   SfGrm

Double-click the respective cell or press the **F8** key to open the **Mass spectrometry conditions for** dialog box:

If you have entered the mass of at least one mass peak, you can enable and disable peak identification via mass spectra by selecting the **Check mass ratios** check box.

Under **Mass Peak 1**, enter the mass of the substance (range: 1.0 to 2000.0 m/z) that is expected at this peak. If you expect fragmentation and can estimate the fragments, you can enter two of them, with their expected intensities, under **Mass Peak 2** and **Mass Peak 3**.

Select the **MS Threshold** option to filter the noise. A mass is detected in the mass spectrum only if its relative intensity compared to the ➤*Base Peak* (largest peak) is higher than the threshold value.

The filter settings limit the mass spectra used for peak identification. This option is required if you modify the polarity and/or use a different maximum voltage for ionization of the sample.

Select the **Check retention time of mass spectra** check box to use the retention times of all masses for peak assignment. All retention times must be within one scan.
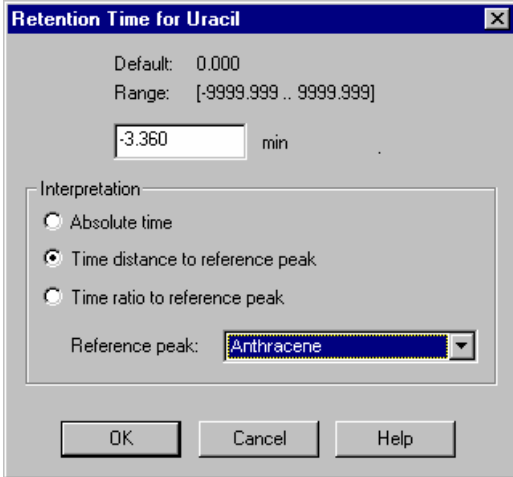
The settings selected in the **Mass spectrometry conditions for** dialog box are not active immediately. You can activate them later from the **Peak Table** tab page: Press the F8 key in the **Window** column or double-click to open the **Peak Window for** dialog box. Select the **Check mass ratios** check box.

If you acquire data with a ➢*Photodiode Array Detector*, you can identify peaks via their UV spectra. (For more information, refer to 🔳 **Identifying Peaks via Their UV Spectra (Peak Tracking)**).

🔳 Defining Retention Times and Reference Peaks

(Retention Time column)

The **Retention Time** column allows you to select reference peaks for relative retention times and to determine how the ⇒*Retention Time* is interpreted for the respective peak. Open the **Retention Time for** dialog box by double-clicking the corresponding peaks or pressing the F8 key in the **Ret. Time** column.

In the **Interpretation** section, determine the display of the retention time (relative or absolute time) individually for each peak. Note that relative times can be displayed either as difference or as percentage ratio to the retention time of a reference peak.

Use the **Reference peak** drop-down list box to define one (or several) of the other peaks as reference peaks. Note that only peaks with absolute reference times (indicated with a light blue background can be selected as reference peaks. Reference peaks can only be deleted from the **Retention Time for** dialog box.

The retention time is re-calculated automatically when the retention time interpretation is changed or when a different reference peak is selected.

You can enter the retention time directly in the input field, also (**Peak Table** page in the **Ret. Time** column):

[<Reference Peak>] <Ret. Time> [<Unit>]

You can enter the fields in any order.

If you do not enter a name for the reference peak, the time is interpreted as absolute time.

Select **min** or **%** as the unit. **min** is the default if no entry is made. The unit determines whether the time is indicated as the difference (**min**) or the ratio (**%**) to the time of the reference peak. For absolute times, only **min** is permitted.

◾ Defining the Retention Index and the Kovats Index

(Ret. Index and Kovats Index columns)

Retention indexes can be used to generate generally comparable retention times. Determine one or several marker peaks in the ⇒*Ret. Index* (= RI) or ⇒*Kovats Index* (= KI) column.

Enter a value between 0 and 99.999. Each peak for which a value is entered is used as marker peak. Increment the values that you enter, i.e., either leave the field empty or enter any value above the value for the predecessor peak. The column is 'empty' by default.

The retention index and the Kovats index are calculated based on these variables. Both indexes are mainly used in GC to correct retention time variations. Therefore, the peak table must contain certain marker peaks, which usually have the following RI or KI values assigned:

Retention Index: 100, 200, 300, etc.

Kovats Index: 100*number of carbon atoms of the alcane.

The RI or KI values of all other peaks remain empty; they are calculated by means of interpolation.

Select **Columns > Duplicate Column** on the **Edit** or the context menu to duplicate the column and thus, allow different values for the individual channels.

☞ *Tip:*

*The Kovats index is calculated using the adjusted retention times. Therefore, to enable calculation, enter a ⇒Dead Time on the **General** tab page of the QNT Editor.*

■ Defining the QNT Method for Several Detectors

1. If two detectors are connected in series, the retention times are delayed against each other. As the flow normally remains unchanged, the shift of the retention times is constant. Select the ⇒*Delay Time* parameter to consider this fact.

On the **General** tab page of the QNT Editor, click the arrow next to the **2nd Detector** and/or **3rd Detector** input field and select the name of the detector from the list. Enter the measured delay time in the next field.

2. For some applications, it might be necessary to modify the QNT Method for a second detector or another channel. For example, you cannot use the **Delay Time** parameter when you are working with ➢*Flow Gradients*. Instead, duplicate the following columns and assign the duplicate to one or several channels:

⇒*Calibration Type*

⇒*Integration Type*

⇒*Kovats Index*

⇒*Left/Right Limit*

⇒*Peak Type*

⇒*Response Factor*

⇒*Retention Index*

⇒*Retention Time*

⇒*Window*

Select the column to duplicate in the Peak Table (Amount Table, Peak Tracking table, respectively) and select **Columns** on the context or **Edit** menu. Select **Duplicate Column** to open the **Duplicate Column** dialog box. Click **Add Channel** to define the channel(s) for the new column.

If an individual peak is not detected in a channel, the corresponding cell in the **Retention Time** column can be left empty. Thus, this channel will not identify this peak. If you do not wish to include a detected peak into the report, just leave the corresponding cell in the **Retention Time** column empty.

In addition, you can define more detection parameters for the respective channel(s) in the **Channel** column on the **Detection** tab page. Also, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Detection Parameters**.

Entering Reference Spectra (Reference Spectrum Column)

You can enter a reference spectrum for each peak in the peak table of the QNT Editor:

Position the cursor in the **Reference Spectrum** column, press the **F8** key or left-click twice. In the dialog box, select the reference spectrum of the peak. Click **Browse** to load any samples or spectra libraries for selecting the reference spectrum. If samples are loaded, a list of all peaks in the corresponding chromatogram is displayed. For spectra libraries, the list displays the spectra included in the spectra library (see figure). The selected reference spectrum is displayed in the right window. If the relevant peak of the peak table is identified in the active chromatogram, the spectrum is included as overlay. The corresponding match factor is displayed in the upper corner of the plot frame.
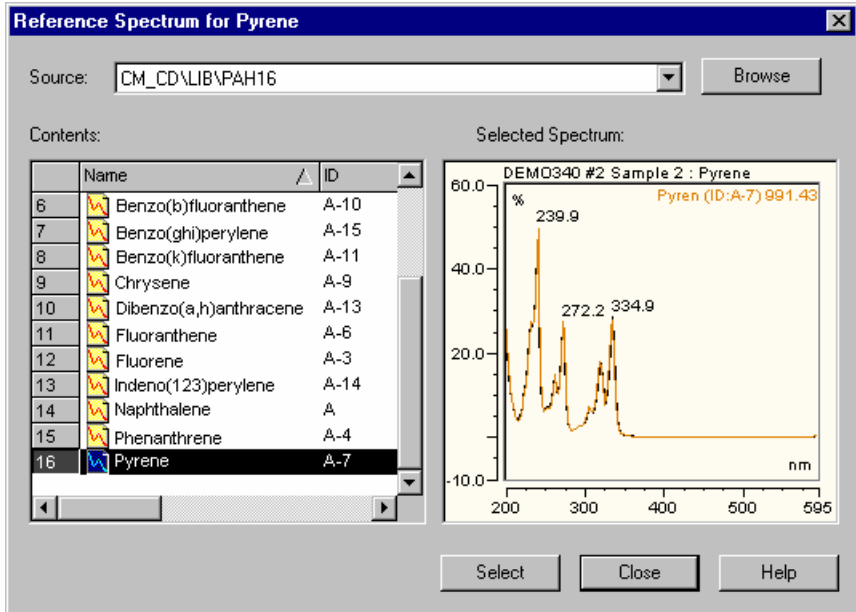
Click **Select** to copy the selected reference spectrum to the corresponding line of the peak table.

Clipboard: Spectra can copied to the clipboard from the spectra plot window or the spectra library by simultaneously pressing the **CTRL**+**C** keys or by selecting **Copy** on the **Edit** menu. In the QNT Editor, either simultaneously press the **CTRL+V** keys or select **Paste** on the **Edit** menu to insert the copied spectrum as reference spectrum into the **Reference Spectra** column.

Copy Peak Spectra as Reference: If one or several lines are selected in the **Reference Spectrum** column, select **Copy Peak Spectra as Reference** on the **Edit** or context menu to use the spectra of the active chromatogram as the reference spectra.

Overlay of the Reference Spectrum on the Spectra Plot

To include the current reference spectrum from the peak table as **Overlay** on the Spectra plot, place the cursor in the spectrum and right-click. Select **Decoration** on the context menu. On the **Peak Spectra** tab page, click **Reference Spectrum in corresponding peak table**. Click **OK** to receive the spectrum from the **Reference Spectrum** column in addition to the current one.

▣ Autogenerating the Peak Table

To save the user from having to determine the retention time of each peak, peak tables can be created automatically by selecting **Autogenerate Peak Table** on the **Edit** or context menu.

Chromeleon automatically generates a peak table, entering the retention times of all detected peaks of the currently open chromatogram as set times. The user need only complete the table by entering component names and altering the default window and other values as necessary. Peaks, which are not of interest, can be deleted from the table. These will consequently be excluded from the report, provided that the **Including all not detected peaks of the peak table** parameter in the **Integration Report Properties** (**Table Properties** command on the **Edit** or context menus) is turned to OFF.

During automatic table generation, the variables described below are set as follows:

Autogenerated Peak Table is entered as ⇒*Comment*.

The peak names (⇒*Name*) are constructed from the name of the QNT Method; for example, in the **Test** peak table, detected peaks will be assigned the names Test-1, Test-2, Test-3, etc.

The ⇒*Window* values are entered as absolute values; they represent one-third of the distance from the nearest peak.

No ⇒*Group*.

The ⇒*Amount* values and the ⇒*Response Factor* are set to 1.0.

The peak type (⇒*Sample Type*) is Auto; that is, Chromeleon determines the peak type.

If you select **Autogenerate Peak Table** on the **Edit** menu to generate peak tables, it is possible to use the results of the spectra library screening (type: **Use spectra library screening results**) instead of enumerating all peaks in the chromatogram (type: **Enumerate peaks of current chromatogram**).

In this case, library screening is performed based on the parameters entered in the QNT File. The peak table will include all peaks for which at least one reference spectrum was found and the name of the best hit.

The following options can also be used:

- **Apply only to peaks with**: With this option, only peaks exceeding a selectable absolute or relative area or height will be included in the peak table.

- **Apply only to peaks in current time interval**: Click this button to include only peaks in the currently displayed section of the chromatogram; for example, in the zoomed chromatogram section.

- **Copy reference spectrum from**: This option simultaneously includes the reference spectrum for each peak. Either the current peak spectrum from the displayed chromatogram or the library spectrum of the best hit from library screening can be used. If you want to use the library spectrum of the best hit, make sure that the **Use spectra library screening results** autogeneration option is enabled in the upper window section.

- Select **Enable peak tracking using peak match type** to enable peak tracking for each included peak. Select **Spectrum** or **Spectrum and time** as match type (see **How to …: Integrating Chromatograms and Identifying Peaks** 🔳 **Peak Tracking**).

- **Allow multiple best hits** or **Unique identification**: The results of the spectra library screening will produce a hit list of library spectra for each peak in the chromatogram. If **Unique Identification** is selected, these hit lists are handled as in Peak Tracking; that is, multiple identical hits with smaller match factors are eliminated. In the case of **Allow multiple best hits**, each peak in the chromatogram is copied to the peak table with the best hit. If the best hits are identical, this can result in identical entries in the peak table. In this case, adding a number to the name generates a unique peak; for example 2-, -3.

If you have generated a new peak table via **Autogenerate Peak Table**, a note is automatically included in the **Comment** column of the peak table:

- If you have selected **Enumerate peaks of current chromatogram**, the comment is: **Autogenerated**.

- If you have selected **Use spectra library screening results**, the comment is: **Autogenerated. Spectrum:** *Name of reference spectrum*, **Match:** *Match factor*.

🔳☞ *Tip:*

*If **Use spectra library screening results** is enabled, the settings made on the **Spectra Library Screening** tab page are automatically entered in the peak table (**Peak Tracking** tab page).*

Defining Detection Parameters

The ⇒*Detection Parameters* define how the chromatograms are integrated, in which areas peaks are suppressed, how peak starts and peak ends are detected, etc. Thus, the detection parameters allow you to adapt the integration to your requirements and to minimize the re-integration effort for individual chromatograms.

You can either enter the detection parameters in the table on the **Detection** tab page of the QNT Editor or define them graphically in a chromatogram.

The detection parameters influence the integration of all chromatograms to which the selected QNT Method applies. They can assume new values at any time. Only the value that was defined last will take effect, however, only for the time of the chromatogram.

The parameters are reset to their default values afterward. If a parameter assumes a new value at a specified time (**Retention Time** column), this is called an **Event**. You do not have to enter these events in chronological order. They be sorted automatically when being saved. The **Channel** column allows you to define whether this applies to an individual channel only or to all channels (default) of a sample.

Each detection parameter has a default value assigned. Using this value, the system automatically integrates about 90% of all chromatograms correctly. However, for critical applications, such as **wavelength switching**, the user can influence the baseline or the peak type, for example, rider peak or main peak, or disable detection for defined periods.

Parameter tables can include up to 100 lines. For an example of how to enter detection parameters in the table of the **Detection** tab page, refer to the following image:

No.	Ret. Time [min]	Param. Name	Param. Value	Channel
1	0.000	Minimum Area	1.000 [Signal]*min	All Channels
2	1.000	Rider Threshold	20.00 %	All Channels
3	1.000	Rider Skimming	Exponential	All Channels
4	1.000	Maximum Rider Ra	5.00 %	All Channels
5	7.100	Peak Group Start	Auto	All Channels
6	8.300	Peak Group End	Auto	All Channels
7	9.500	Valley to Valley	On	All Channels

\ General \ **Detection** \ Peak

In many cases, it may be easier to enter the detection parameters graphically in the chromatogram (see **How to …: Working with Chromatograms** ▣ **Defining Detection Parameters Graphically**).

Usually, the chromatogram and the report table are immediately updated when the detection parameters have been changed. However, you may disable this function by deselecting **Autom. Re-Integerate** on the **View** menu of the QNT Editor. If this option is disabled, save the QNT Method or select **Start Integration** on the **View** menu to start re-integration with the new integration parameters.

☞ *Tip:*

Save the setting for this option in the corresponding ➢*Report Definition File (Select **Save Report Definition** on the **Workspace** menu.)*

In addition, the QNT Editor provides more detection parameters. The following sections describe how to edit the default values together with the respective possibilities and advantages. Use the detection parameters for:

Reducing the Number of Evaluated Peaks

Excluding Certain Peaks

Inhibiting Peak Integration

Modifying the Baseline

Modifying the Peak Recognition Algorithm

Defining the Peak Start/End

Defining Rider Peaks

Defining the Area for PPA

Reducing the Number of Evaluated Peaks

To limit the integration report to a manageable size, only the most important peaks (i.e., the largest peaks) should be included in the evaluation. Define either the ⇒*Minimum Height* or the ⇒*Minimum Area*.

How To

The following example refers to the minimum peak height:

- Open a QNT File (select **Open** on the **File** menu) and click the **Detection** tab page.

- Look at the chromatogram and determine the minimum peak height to be evaluated. You may also click the smallest peak to be integrated. Then open the corresponding **Peak Properties** via the context menu. The dialog box then indicates the width, height, and area of the peak.

- Enter the name of the parameter to modify; for example, the ⇒*Minimum Height* in the **Parameter Name** column of the quantification method.

- Assign the smallest possible signal value, for example, in mAU, as the new parameter value in the **Parameter Value** column.

- In the **Time** column, determine from which time (relative to injection time) the parameter becomes valid.

- Alternatively, you can open an edit dialog box (press the **F8** key) to enter the parameter name, value, and time.

All peaks with a height lower than the indicated value will not be displayed.

As the parameters affect peak recognition and thus the baseline, you must consider a tolerance of approximately 5% to ensure that all peaks of interest are recognized.

Another way to define, for example, the minimum area, is to graphically define the corresponding parameter. For example, follow the steps below:

- In the chromatogram, find the smallest peak just no longer to be displayed.

- Enlarge this peak to be clearly visible.

- In the chromatogram, right-click to select an area whose area is a slightly smaller than that of the selected peak.

- Select **Set Minimum Area** on the context menu.
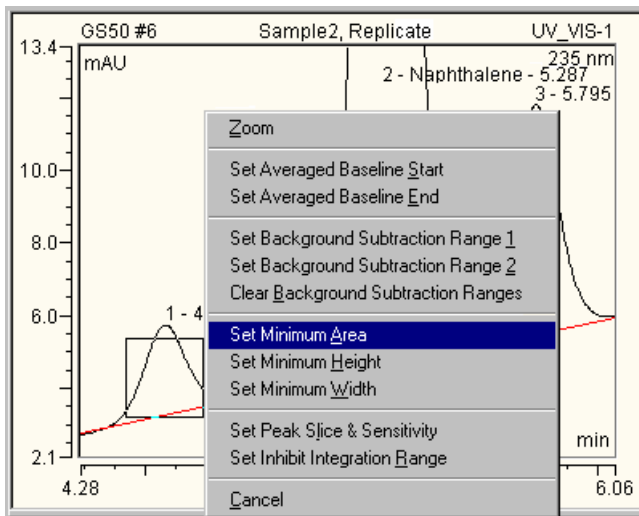
This action enters the selected area as minimum area together with the time of its left edge into the QNT Method. Enter the 0.000 as time into the **Detection** tab page or move the parameter in the chromatogram with the **Detection Parameter Tool** so that the minimum area is valid for the entire chromatogram.

Note:

*You can undo the graphical input of detection parameters. Click one of the tables of the QNT Editor and select **Undo** on the **Edit** menu. (In the chromatogram itself, you can only undo the modifications of the currently open chromatogram.)*

Tip:

To make the report clearer:

- *Select any cell in the report (integration tab page) and select **Table Properties** on the **Table** menu.*

- *Select the **Reject peaks with smaller area than ...%** check box and specify below which size not to include a peak in the report.*

Excluding Certain Peaks

Spikes

Sometimes, very narrow peaks (spikes) occur in chromatograms due to, for example, gas bubbles in the HPLC system. In such a case, the problem (= the gas bubbles in the HPLC system) should be solved first, of course. However, to use the chromatogram, identification of these narrow peaks can be suppressed with the QNT Method.

Select the ⇒*Minimum Width* parameter to define the minimum width for the peaks to be integrated. Proceed as when entering the minimum height (see **Reducing the Number of Evaluated Peaks**).

Peaks above the Detector Maximum

Sometimes peaks occur whose absorption maximum is above the detector maximum so that integration does not make sense. You can then include these peaks as unidentified peaks into the peak table by using the ⇒*Maximum Peak Height* parameter.

Wide Peaks

If an unusually wide peak occurs in the chromatogram, it may be from a previous sample. Select the ⇒*Maximum Width* parameter to define this peak as being unidentified.

◨ Inhibiting Peak Integration

The ⇒*Inhibit Integration* parameter can be enabled at the time t1 and can be disabled at the time t2. The peaks within the time window t1 - t2 are not integrated. Whether this applies to all channels or to only one channel is specified via the **Channel** column.

To prevent the integration of all peaks in the range from 0 to 1.0min (especially the injection peak), the following input is necessary in the quantification method.

No.	Ret. Time [min]	Param. Name	Param. Value	Channel	
1	0.000	Inhibit Integration	On	All Channels	
2	1.000	Inhibit Integration	Off	All Channels	

◀ ▶ \ General ⋏ **Detection** ⋏ Peak Table ⋏ Amount Table ◀ | | ▶

Peaks detected in this time span will not be integrated and will not be included in an integration report.

◨ Modifying the Baseline

It may be necessary to modify the baseline, especially for non-resolved peaks. The baseline is usually defined via a mathematical procedure. For calculating the individual peak areas, a perpendicular is dropped to the baseline from each local minimum (standard).

If you think that integration starts too early or that the peak end is delayed, you can force a better peak start or end by inserting a ⇒*Baseline Point*.

If a series of non-resolved peaks is piled on an "absorption mount", for example, due to increased solvent absorption, the ⇒*Valley to Valley* detection parameter can be used to force the baseline from minimum to minimum via.

Move baseline point ◀——— **Default** ———▶ **Valley to Valley**
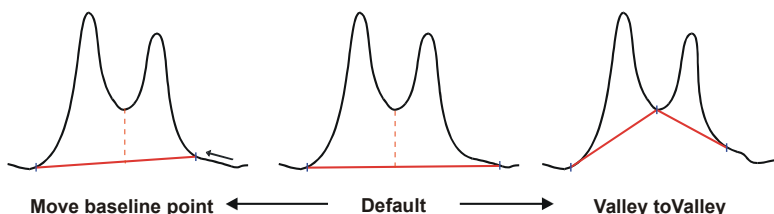
Single peaks that are piled on a recognizable "absorption mount" are integrated individually. To integrate the entire area, the baseline can be fixed. Setting the ⇒*Lock Baseline* parameter to **At Current Level** extrapolates the baseline horizontally to the intersection with the signal curve. Setting the parameter to **At Global Minimum** searches for the absolute minimum in the direction of the peak end or until the next **Lock Baseline**. Both parameters disable the **Valley to Valley** parameter.

| Lock Baseline -
current level | ← Default → | Lock Baseline -
global minimum |
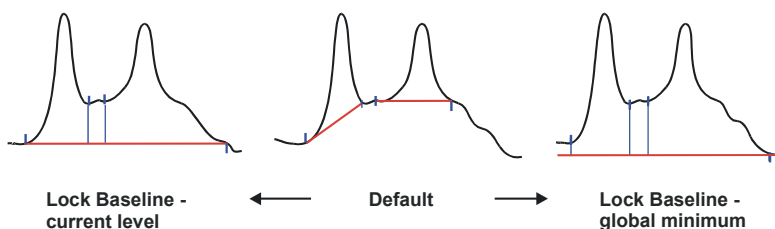
📖 *Tip:*

*In all these actions, the **Peak Type** classification criterion of the peak table has priority. For a **Baseline-Main-Baseline** type peak, the peak limits always have baseline contact.*

As an alternative, you may modify the baseline manually (refer to **Working with Chromatograms/Manual Re-Integration** 🔲 **Modifying the Baseline Manually**.)

🔲 Modifying the Peak Recognition Algorithm

Whether signal variations are interpreted as peaks or not, is usually set automatically. Manual modification of this "recognition sensitivity" is possible via the combination of the ⇒*Peak Slice* and ⇒*Sensitivity* detection parameters. Changing the parameters is required, for example, in chromatograms with unusually wide (many minutes) or very narrow (< 0.1sec) peaks.

Another way to define the peak recognition algorithm is to define both parameters graphically.

How To

- Enlarge a baseline section so that the noise is clearly visible.

- In the chromatogram, right-click to select an area from which the baseline runs out neither at the top nor at the bottom.

- Select **Set Peak Slice & Sensitivity** on the context menu.
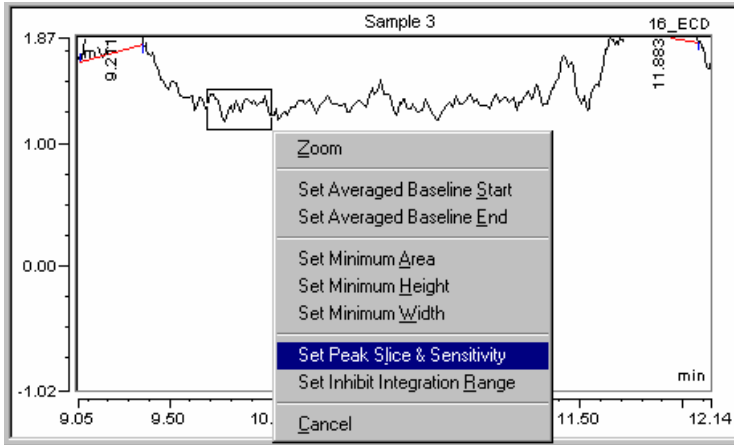
The width of the selected area is entered into the QNT Method as peak slice and the height is entered as sensitivity at the time of the left edge of the area. Enter 0.000 as time onto the **Detection** tab page or move the parameters in the chromatogram with the **Detection Parameter Tool** so that this peak recognition algorithm applies to the entire chromatogram.

📝 *Note:*

*You can undo the graphical input of detection parameters. Click one of the tables of the QNT Editor and select **Undo** on the **Edit** menu. (In the chromatogram itself, you can only undo the modifications of the currently open chromatogram.)*

The peak recognition algorithm considers signal variations only beyond the adjusted sensitivity values. Peaks below this threshold are interpreted as noise.

Both parameters affect peak recognition only, not integration! The area calculation (integration) is not affected.

◾ Defining Peak Start or Peak End

Depending on the chromatogram type, the peak start or the peak end can be detected too early or too late. There are several ways to prevent this:

If you think that the integration is started too early or that the peak end is delayed too much, select the **Fronting Sensitivity Factor** parameter for the peak start and the ⇒*Tailing Sensitivity Factor* for the peak end. The entered value multiplied with the left or right peak width determines the peak start or the peak end. Depending on the chromatogram type, different values may make sense. Test which value is best for your chromatograms. A value of **2** is often an appropriate starting point for finding the best **Fronting/Tailing Sensitivity Factor**.

You can also set a new ⇒*Baseline Point* to force the peak to start later or to end earlier.

⚠ *Caution:*

However, when setting a baseline point keep in mind that this point will be valid for all chromatograms, which are evaluated with the respective QNT Method. If in one of these chromatograms a peak maximum occurs by coincidence at the time of your hard entered baseline point, the peak maximum will be defined as base point and the peak will not be detected.

Correct too late a peak start or too early a peak end (the latter can occur, for example, with increased baseline noise as follows:

1. In recorded chromatograms: Select a higher ⇒*Peak Slice* (= about 20% of the smallest peak width) and, in addition, a higher ⇒*Sensitivity*, if necessary.

2. For samples that have not been processed yet: Change the data acquisition ⇒*Step* in the program file. Select the step so that only about 20 data points are recorded for the smallest peak.

◾ Defining Rider Peaks

The detection parameters ⇒*Rider Threshold* and ⇒*Maximum Rider Ratio* allow you to define which peaks shall be detected as ➢*Rider Peaks* and which shall be detected as main peaks. The following applies:

The smaller the rider threshold is, the smaller are the peaks that can be detected as rider peaks. (Peaks below the rider threshold are always regarded as main peaks.

For peaks above the rider threshold, the Maximum Rider Ratio defines whether a peak is a rider peak or a main peak.) The larger the maximum rider ratio is, the larger the peaks that can be defined as rider peaks.

🖐☞ *Tip:*

*When defining rider peaks with these parameters please keep in mind that the same peak may be detected in two different chromatograms as main peak and as rider peak. This would result in considerable deviations; for example, in the calibration. To prevent this, select the peak type **Rider (or Main)** for the respective peak in the peak table. Thus, the peak is a rider peak, if possible (or always a main peak).*

In addition, you can define how to skim rider peaks by using the ⇒*Rider Skimming* parameter. With the two options **Tangential at lower peak end** and **Tangential at both peak ends** the peak is skimmed by a tangent. Usually, there is hardly any difference between the results of the two options.

With the **Exponential** option, the course of the baseline is approximated by an exponential function; that is, the peak is skimmed by the exponential function. This option clearly distinguishes from the two others. In most of the cases, **Exponential** maps the actual baseline course very accurate. With this option, the rider peak will usually receive a more realistic larger area. To be able to use this option, make sure that a sufficient number of data points is available.

🖐☞ *Tips:*

You can move the baseline in the chromatogram with the mouse for tangentially skimmed riders, but you cannot for exponentially skimmed riders.

***Split Peak** on the context menu allows splitting one rider peak into two peaks.*

▣ Defining the Area for PPA

When using ➤*Photodiode Array Detectors* and recording ➤*3D Fields*, you may check the peak purity with the ➤*Peak Purity Index (PPI)*.

Define the most expressive area of you spectrum by means of the ⇒*Peak Purity Start/End Wavelength* parameters and limit the examination to this area only.

The detection limit becomes especially apparent with very small peaks. The influence (that is the noise, drift and limits of the measuring method) on the peak spectrum is strongly developed at the peak start and peak end because, in these areas, the concentration of the peak substance in the flow cell is very low. Set the ⇒*Peak Purity Threshold* parameter to reduce the influence on the PPI and the match factor and, thus, to prevent that contaminated substances are indicated falsely. The PP threshold value defines the percentage of the peak height starting as of which the spectra will be considered for the respective purity examination.

If you work close to the detection limit, use higher PP threshold values to reduce the influence of the detection limit on the purity examinations. If you are mainly interested in the purity of large peaks, use lower PP threshold values to examine the purity over the largest possible area of the peaks.

Grouping Peaks

If you are interested in the sum parameters for two or more peaks, you can

1. Define these peaks as a peak group (that is treated as one single peak).

2. Define them as a group of peaks.

1. If the peaks lie close together and are not baseline separated, for example, with overloaded columns, define the peak group start and the peak group end via the ⇒*Peak Group Start/End* parameters on the **Detection** tab page. The baseline will then be drawn from the start of the peak group to its end. Such a peak group is treated as one single peak.

2. To define a peak group in which the peaks do not necessarily succeed one another, for example, to determine the amount/concentration of an entire class of substances, take the following steps:

- **Identified peaks**: Select **Column** > **Display Column** on the context menu to insert the ⇒*Group* column into the peak table if the column is not yet displayed. For those peaks that should belong to this group, type a group name into this column.

- **Unidentified peaks**: Click **Unidentified peaks…** on the **General** tab page to define the period for which this group shall be valid. The baseline will be drawn in the same way as for individual peaks.

- Add the **Group Amount** column to the **Report** and the ➢*Printer Layout*. This column shows the desired ⇒*Amount* value. In the report, open the dialog box **Insert/Add Report Column** via the **Insert Column** or **Add Column** commands on the context menu. Select the **Peak Results** category and then choose **Group Amount** as variable.

🖑 *Tip:*

*If the selected calibration type is, for example, **LOff** instead of **Lin**, the value in the **Group Amount** column will not be identical to the sum of the amount values of the individual peaks even if the group includes all peaks of the chromatogram.*

Subtracting a Blank Run Sample

On the **General** tab page of the QNT Editor, use the **Blank Run & Matrix Blank** section to consider absorption values of a ➤*Blank Run Sample*, a ➤*Matrix Blank Sample*, or any other sample (➤*Blank Run Subtraction*).

• Select **No Blank Run Subtraction** if no correction is to be performed.

If the absorption values of a specific sample are to be considered for sample evaluation, determine which sample is to be used as Blank Run Sample.

• Select **Subtract Recent Blank Run Sample in Corresponding Sequence** if the Blank Run Sample (sample type: **Blank**) that was processed last in the current sequence before the current sample is to be used.

• Select **Subtract a Fixed Sample** to perform the correction with a specific Blank Run Sample. Click **Browse** to search for the sample.

   When subtracting a blank run sample, the chromatogram of the blank sample is subtracted point by point from the active chromatogram. If the current sample is a standard sample, the difference between the two chromatograms is used for the calibration.

• **Enable Matrix Blank Subtraction** enables the subtraction of matrix blank samples. Contrary to the other options, the resulting peak areas or peak heights are subtracted for peaks that have been identified for the matrix blank sample and for the unknown sample.

🖑 *Tip:*

Although sample types other than "blank" can be subtracted as well, this usually does not make sense because often-negative peaks would occur in the resulting chromatogram.

Calibrating

In calibration, a functional relationship is determined between the peak area or peak height and the concentration of a substance (in HPLC/IC; in GC, usually the injected amount of a substance). To allow this you have to enter certain values or make other settings in Chromeleon. The required input is listed below. The order corresponds to the order in which the input is usually made.

Sample List (Browser)

- Injection volume ($\Rightarrow$*Inj. Vol.*)

- Sample weight factor ($\Rightarrow$*Weight*)

- Dilution factor ($\Rightarrow$*Dil. Factor*)

- Amount of the $\triangleright$*Internal Standard* ($\Rightarrow$*ISTD Amount*)

☞ *Tip:*

In many cases, it is sufficient to enter the injection volume in the sample list. Usually, you enter the injection volume in the Sequence Wizard (unless you copy an existing sequence).

QNT Editor: General Tab

- $\Rightarrow$*Dimension of Amounts*

- $\Rightarrow$*Calibration Mode*

QNT Editor: Peak Table Tab

- $\Rightarrow$*Standard*; also, see 🔲 **Selecting the Standard Method (Standard Column)**

- $\Rightarrow$*Integration Type*; also, see 🔲 **Selecting the Calibration Function (Cal Type and Int. Type Columns)** and 🔲 **Weighting and Averaging Calibration Points**

- $\Rightarrow$*Calibration Type*

QNT Editor: Amount Table Tab (The amount table is part of the peak table.)

- $\Rightarrow$*Amount*; also, see **Entering Amount Values (Amount Column)**

QNT Editor: Calibration Tab

- See **Disabling the Calibration Sample**

For calibration examples, refer to **Calibration Examples**.

For information about how to display calibration curves, refer to **Displaying Calibration Curves**.

Selecting the Standard Method (Standard Column)

Use the Standard method to determine how calibration is performed. Generally, a distinction is made between a calibration based on an *internal* or an *external* standard.

External means that the calibration is performed based on one or several standard samples. (This is the default.)

Using an **internal standard** substance means adding a standard to the unknown sample. This can be either before (**External/Internal**) or after (**Internal**) sample preparation. Either the standard (= ➢*Internal Standard*) can be added to all samples or it can serve as a basis for a relative area calculation. In this case, results are displayed only in relation to the amount or area of the internal standard. (For more information, refer to **Theory of Calibration**, **Standard Methods**.)

- In the **Standard** column, use the **Standard Method for <Peak Name>** dialog box to determine the standard method (**External**, **Internal/External**, **Internal**) for each peak to be calibrated. To open the dialog box, press the F8 key in the corresponding cell.

- For the last two options, at least one peak of the peak table must be used as the internal standard: In the **Standard Method for <Peak Name>** dialog box for the peak to be used, select **Use this peak as Internal Standard (ISTD)**.

- Press the F8 key to enter the standard method assignment via an edit dialog box.

⬛ Selecting the Calibration Function (Cal.Type and Int. Type Columns)

Use the **Int. Type** ($\Rightarrow$*Integration Type*) column to define how the individual peaks are evaluated.

Press the F8 key or double-click a cell in the **Int. Type** column. The following dialog box appears:
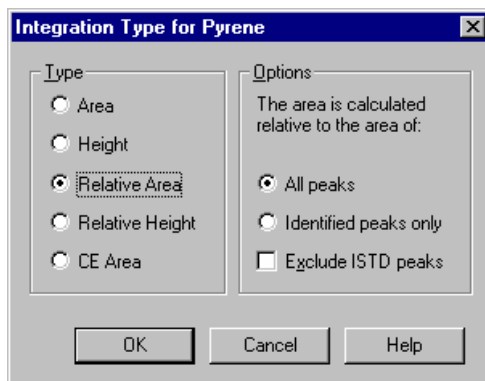
In addition to **Area**, **Height**, and **CE-Area**, you can select the relative area or the relative height as the reference for the evaluation (Integration Type).

If you select **Area**, all amount calculations refer to the area of one peak. This **Peak Area Integration** is the default setting. The peak height integration is only used in exceptional cases.

⚠️ *Caution:*

Only the different area integration methods are physically sensible.

The relative height is either calculated relating to all peaks or all identified peaks. ISTD peaks can be considered for the calculation of the total area (height).

There are several peak table columns, which define the conversion of the determined area values into the calculated amount values. A separate calibration and integration type must be assigned to each calibrated peak in the peak table.

Enter a calibration function in the **Cal. Type** column. Press the F8 key to receive a list of available functions and options. Apart from few exceptions, the calibration types **Linear** or **Linear with Offset** are used.

The ⇒*Calibration Type* determines which ➤*Calibration Function* is used for deriving a valid amount/area assignment for a larger range from the calibration points of the standard samples.

⚠ *Caution:*

*The decision whether it is physically sensible to use a calibration type other than the **linear** default is the responsibility of the user. (With a calibration type that is physically not sensible, no y values may appear for the actually measured values. For example, if, for the Quadratic calibration type, the calibration function is a bottom-down parabola-shaped curve, the values measured above the summit cannot be evaluated. In this case, Chromeleon reports "n.a.")*

For more information about linear and non-linear calibration types, refer to **Theory of Calibration ▣ Calibration Types (Linear),** or ▣ **Calibration Types (Non-linear)**.

Via the calibration type, you can also define the weighting and averaging of calibration values (see **How to …: Calibrating ▣ Weighting and Averaging Calibration Points**.

Either the calibration curve leads through the origin, for example, with the **Lin** and **Quad** calibration types, or the origin is not considered, for example, with the **LOff** and **QOff** calibration types. For calibration types with offset, the origin can be treated as a calibration point, using the **Add zero point (0,0) for curve fitting (0)** option. In this case, the calibration curve will not be forced through the origin, but the origin will be considered nevertheless.

Usually, all other columns of the peak table can be used with the default settings. Press the F1 key to display more information. Press the F8 key to open an edit box.

■ Weighting and Averaging Calibration Points

Weighting

With the default settings, Chromeleon weights calibration points of higher concentrations more strongly than lower concentrations, that is, the course of the calibration curve is oriented towards the calibration points of higher concentration. This makes sense as smaller concentrations also cause a stronger dispersion of the determined area values, which would distort the result beyond proportion.

To undo or even reverse this type of weighting, four additional weighting functions have been introduced:

The weighting **1/Amount** (or **1/Response**) virtually undoes the "normal" weighting described above; i.e., low and high concentrations are weighted similarly. The weighting **1/Amount²** (or **1/Response²***)* results in an over-proportional weighting of smaller amounts.

By variation of the ➢*Number of Replicates*, this weighting can be avoided. Smaller concentrations are injected more frequently than larger concentrations, more calibration points in the low concentration range support the calibration curve. **Outliers** are then less relevant.

☞ *Tips:*

*The stronger weighting of higher concentrations is valid in all ➢Calibration Functions, with the exception of **Point-to-Point**.*

*Outliers can be explicitly "disabled" by excluding a specific standard sample from the calculation. Exclude the sample on the **Calibration** tab page of the quantification method.*

*When calculating the calibration values **Variance**, **Var.Coeff**, **Std.Dev**, **Rel.Std.Dev**, and **Corr.Coeff**, averaging is not considered! Weighting only influences the course of the calibration curve, the values are a measure for the quality of the calibration.*

Averaging

To determine the calibration curve, all available ➢*Calibration Points* are normally used. As dispersion is stronger for the lower calibration levels, many users verify the results by using a large number of calibration points. The calibration curve is thus determined by a larger number of points on the lower than on the higher level.

If all points of a ➢*Calibration Level* are averaged before calculating the calibration curve, and the subsequent calibration is performed based on these average values only; the calibration curve is based on one point of each calibration level only.

Entering Amount Values (Amount Column)

Standard substances are labeled by user input in the **Amount** column.

- Search the peak table for the substance name of the standard substance(s), or

- Enter the name and the retention time as described in **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Identifying Peaks**.

- Type the amount value of the standard into the first **Amount** column. This can be a concentration value (such as µg/ml) or the absolute value (such as µg). If a standard is available in different concentrations, enter the concentration of each vial in a separate ⇒*Amount* column. For example, two concentrations result in two **Amount** column entries:

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount1	Amount2
1	Subst. A	4.400 min	0.100 AG	External	Area	Lin	50.000000	100.000000

If injection is performed several times from the same sample vial (multiple injection), one amount value is sufficient, even if a different volume and thus, a different amount, are injected. Chromeleon considers this automatically.

- Repeat the procedure for each substance serving as a standard.

Inserting new Amount columns

- Double-click the header of an existing **Amount** column (or select **Columns > Edit Amount Columns** on the **Edit** or context menu) to open the **Edit Amount Columns** dialog box.

- From the **Assign Standards on the basis of** list, select the sample variable (e.g., ⇒*Name* for the sample name, ⇒*No.* for the sample (vial) number, or ⇒*Ref. Amount Set*, etc.) that shall be used to identify and assign standards to the amount column.

- Use one of the following methods to create new **Amount** columns:

  Click **New**, type a unique column name in the edit field that appears in the **Amount Column** window, and press **Enter**. Select an **Amount** column (or **Unassigned**) in the **Amount Column** window to display the associated standards. Drag the selected sample(s) (= standard sample; = ⇒*Validation Sample* or = ⇒*Spiked Sample*) from the **Standards** window to the new column.

  -o*r*-

  Click **Auto-Generate**. Select the preferred option from the drop-down list and click **Apply**. The following options are available: Select **Generate a separate column for EACH standard** to generate a separate column for all samples with the same value for the selected sample variable. Or else, select **Generate a single column to apply to ALL standards** to generate only one common column for all standards.

 Note:

*The selected option will apply to all sequences that use the current QNT File. For example: You select the **Vial Number** option, and then assign vial number 5 to **Amount** column B. From now on, vial number 5 (regardless of its contents) will be assigned to **Amount** column B in every sequence that uses this QNT File.*

- Click **OK** to close the dialog box and return to the peak table.

Disabling Calibration Samples

Use the **Calibration** tab page to determine which sample shall be used.

It depends on the ⇒*Calibration Mode* which sample ⇒*Types* available (for the sample type, refer to the symbol in the **Name** column):

Usually standard samples are used for calibration.

Only in the **Standard Addition** mode, i.e., if you use the ➢*Standard Addition* method, you can use the sample types **Unspiked** and **Spiked** (see ➢*Spiked Sample*) for calibration.

Click the box in the **Enabled** column to open the **Disable (Enable) Standard xyz** dialog box and define whether the respective sample shall be used for calibration purposes. You can exclude the respective calibration sample from calibration either for all peaks and all channels (default setting) or only for the selected peak and/or channel. For more information about how to use this option, refer to the following examples:

The calibration sample was contaminated

Exclude the sample from the calibration. Accept the default setting: Disable the sample for **All Peaks** and **All Channels**.

By mistake, too much of a substance was added to the calibration sample

Do not exclude the sample completely from calibration, but disable the calibration for this substance, instead. Click the peak and select **Selected Peaks** and **All Channels** in the **Disable (Enable) Standard xyz** dialog box.

Contamination in the calibration sample that is detected in one channel only

You do not need to exclude the sample completely from calibration. Select the channel that detects the contamination and select **All Peaks** and **Selected Channel** in the **Disable (Enable) Standard xyz** dialog box.

For more information about calibration, refer to **How to …:** **Calibrating**.

Calibration Examples

The following topics provide a detailed description of the theory and practical use of the available calibration possibilities. The first examples describe the different applications:

Introduction and Example: 1 Standard and 1 Substance

Several Standards with Several Substances Each

Multiple-Point Calibration Using One Single Standard

Calibrating Using Standards of an Old Sequence

Standard Addition

Calibrating Unstable Substances

No Pure Substance Available - Known Relative Extinction Coefficient

For an overview of the different calibration options for which an ➢*Internal Standard (ISTD)* is used, refer to **Calibrating with an Internal Standard Substance**.

The ⇒*Calibration Mode* determines the standard samples that are used for calibrating specific unknown samples. For an overview of the different calibration modes and the topics that provide more information, refer to **Calibration Modes for External Calibration**.

For more information about validation, refer to:

Entering the Concentration/Amount of the Validation Sample

Validating the Calibration Curve

For a better compatibility with PeakNet 5, Chromeleon also supports *Inverting Dependent and Independent Variables*.

Note:

*To apply an existing calibration to a new sequence that consists of one or several **Unknown** samples, set the ⇒Calibration Mode to **Fixed**. Always perform calibration manually (**Calibrate**). The **Auto-Recalibrate** option is not available.*

◼ Introduction and Example: 1 Standard and 1 Substance

Most calibrations in HPLC and IC are performed using external standard samples. In the simplest case, the corresponding peak area for a known amount of a substance is determined for one standard sample only. Chromeleon then calculates the slope (c1) of the ➤*Calibration Function* from the ratio of the amount and the peak area. (In this case, you can only select the Linear without Offset calibration type). Chromeleon uses this slope to calculate the ⇒*Amount* of this substance in unknown samples.

Example:

You want to determine the amount of substance A in two samples, Sample 1 and Sample 2. A standard sample (Standard) is available. For each sample, an injection volume of 20 µl is injected by an autosampler. The samples are located at the autosampler positions 1 (standard), 2, and 3 (unknown samples).

Sample List

In the Browser, create the following sequence using the Sequence Wizard:

No.	Name	Type	Pos	Inj. Vol.
1	🔶 Standard	Standard	1	20.0
2	❓ Sample 1	Unknown	2	20.0
3	❓ Sample 2	Unknown	3	20.0

(For information about the Browser, refer to **Data Management** 🔲 **The Browser**.)

📒 *Note:*

*For more information about how to create a sample list, refer to **How to …: Creating and Managing Files and Data** 🔲 **Creating a Sample List (Sequence)**.*

QNT Method/General Tab

The concentration of substance A in the standard sample is 12 mg/l. The calibration curve for substance A shall be linear (calibration type: Lin) and run through the origin. After processing this sequence, you have to create the QNT Method for performing the calibration.

On the **General** tab page of the QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor**), enter the unit for all other entries in the ⇒*Dimension of Amounts* field. (For this example, enter "mg/l"). In the **Global Calibration Settings** section, keep the setting for the ⇒*Calibration Mode*, i.e., **Total**.

QNT Method/Peak Table Tab

Do not change the defaults in the **Standard** (External) and ⇒*Calibration Type* (Lin) columns on the **Peak Table** tab page either. Chromeleon automatically creates a "default" amount column. If only one standard sample is available (as is in this case), you can keep this standard sample as well. Enter the concentration in the **Amount** column:

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount
1	Subst. A	5.342 min	0.100 AG	External	Area	Lin	12.000000

📋 *Note:*

*If more than one substance is available in the samples, append a new line to the table using the **Lines > Append Line** commands on the context menu. Enter the concentration in the corresponding cell of the **Amount** column.*

Chromeleon now automatically calculates the amount for the two unknown samples. You can display the results afterwards in the report on the **Integration** tab page:

A	B	C	D	E
No.	Peakname	Ret.Time min	Area mAU*min	Amount
1	Subst. A	5.287	9.6593	11.3217

◄ ► \ Integration ∧ Calibration ∧ Peak Purity ∧ Peak Analysis ∧ Sum

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** 🔲 **Calibrating**.

▣ Several Standards with Several Substances Each

Calibrations are usually performed using several standards. They often contain more than one substance to be calibrated. The simplest case is two standard samples that contain two substances each. In this case, you can determine the slope and the offset and/or curvature of the calibration curve (depending on the number of acquired data points).

Example:

You want to determine the amount of substances A and B in two samples, Sample I and Sample II. Two standard samples, containing different levels of Standard 1 and Standard 2, are available. Both standard samples contain substance A and substance B. For each sample, an injection volume of 20 µl is injected by an autosampler. The samples are located at the autosampler positions 1 (Standard 1), 2 (Standard 2), 3 (Sample I), and 4 (Sample II).

Sample List

The sequence appears as follows (for more information, refer to **How to …: Creating and Managing Files and Data** ▣ **Creating a Sample List (Sequence)**):

No.	Name	Pos	Inj. Vol.
1	⚠ Standard 1	1	20.0
2	⚠ Standard 2	2	20.0
3	❓ Sample 1	3	20.0
4	❓ Sample 2	4	20.0

QNT Method/Peak Table Tab

In Standard 1, for example, the concentration of substance A is 10.2 mg/l and the concentration of substance B is 20.1 mg/l. Standard 2 contains 30.5 mg/l of substance A and 49.7 mg/l of substance B. Thus, you have to create the following peak table in the QNT Editor (For more information about the editor, refer to **Data Representation and Reprocessing** ▣ **The QNT Editor**.)

No.	Peak Name	Ret.Time	Window	Cal.Type	Amount 1	Amount 2
1	*Substance A*	*1.310 min*	*0.100 AG*	*Lin*	*10.200000*	*30.500000*
2	Substance B	2.450 min	0.100 AG	Lin	20.100000	49.700000

Remove the default ⇒*Amount* column and add two new **Amount** columns (one for each standard level):

- Double-click the header of an existing **Amount** column (or select **Columns > Edit Amount Columns** on the **Edit** or context menu) to open the **Edit Amount Columns** dialog box.

- From the **Assign Standards on the basis of** list, select the criterion (**Name**, **Vial Number**, **Sample ID**, etc.) to use to identify and assign standards to the amount columns.

🗔 *Note:*

*The selected option will apply to all sequences that use the current QNT File. For example: You select the **Vial Number** option, and then assign vial number 5 to **Amount** column B. From now on, vial number 5 (regardless of its contents) will be assigned to **Amount** column B in every sequence that uses this QNT File.*

- Click **Auto-Generate**. Select **Generate a separate column for EACH standard** from the drop-down list and click **Apply**. Two new columns will appear in the **Amount Column** window. Double-click the columns to rename them, if desired.

- Click **OK** to close the dialog box and return to the peak table.

🗔 *Note:*

*If one of the substances is not available in the standard sample, the corresponding cell in the **Amount** column should be left empty.*

If you are using several standards for calibration, you may prefer to select a ⇒*Calibration Type* other than Lin (Linear without Offset).

Using these settings, Chromeleon automatically calculates the concentrations of substance A and substance B in the two unknown samples.

For an overview of the calibration options provided by Chromeleon, refer to **How to …:** 🔳 **Calibrating**.

▣ Multiple-Point Calibration Using 1 Single Standard

If you want to perform a multiple-point calibration (see ➤*Single-Point and Multiple-Point Calibration*) using only one standard sample, you can inject different injection volumes (= quasi ➤*Dilution Series*).

Sample List

For example, if you inject 10, 20, and 40 µl of just one standard, the sample list will appear as follows:

No.	Name	Type	Pos	Inj. Vol.
1	🔶 Standard1_1	Standard	1	10.0
2	🔶 Standard1_2	Standard	1	20.0
3	🔶 Standard1_3	Standard	1	40.0
4	❓ Sample1	Unknown	2	10.0
5	❓ Sample2	Unknown	3	10.0

For Chromeleon, each injection is an individual sample. To distinguish between different injections made from the same standard sample vial, you may append the sample number and the injection number (see example above).

QNT Method/General Tab

The standard sample contains, for example, 10 ml/l Uracil. As all injections of a dilution series are made from the same sample vial with the same concentration, you cannot represent the concentration in the calibration curve. Instead, enter the actually injected amount in the ⇒*Amount* column and enter a ⇒*Dimension of Amounts*. For example, enter **ng** on the **General** tab page of the QNT Editor. (For more information about the editor, refer to **Data Representation and Reprocessing** 🔲 **The QNT Editor**).

QNT Method/Peak Table Tab

As in the introduction example (see 🔲 **Introduction and Example),** you do not need to change the default **Amount** column setting on the **Peak Table** tab page.

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount
1	*Uracil*	*1.323 min*	*0,040 AG*	*External*	*Area*	*LOff*	*100.000000*

If the calibration line does not run through the origin as in the example below, select **Linear with Offset** (= LOff) as ⇒*Calibration Type*.

These settings will then result in the following calibration line:
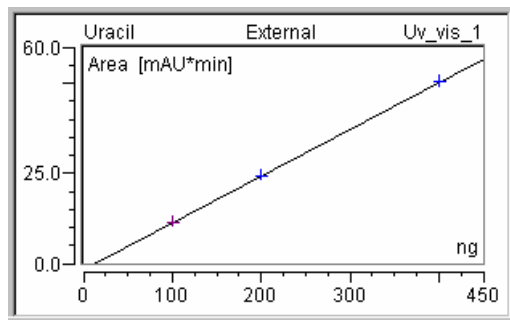
For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** **Calibrating**.

Calibrating Using Standards of an Old Sequence

Calibration standards are often quite expensive. Thus, if the calibration curve remains constant for weeks or months, the standards of a sequence can be used for calibration for several weeks before the calibration constancy needs to be checked again.

Sample List

In this case, the new sequence will neither contain standards nor ➤*Validation Samples* but unknown samples only (perhaps plus ➤*Blank Run Samples* and/or ➤*Matrix Blank Samples*).

QNT Method/General Tab

Select **Fixed** as ⇒*Calibration Mode* in the **Global Calibration Settings** section:
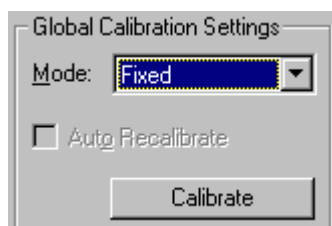
In **Fixed** mode, manual calibration is possible only. That is, you have to add standard samples on the Calibration tab page (see below). Then, click **Calibrate** on the **General** tab page to include the newly entered standards in the calibration.

QNT Method/Calibration Tab

If the **Fixed** calibration mode is selected, the **Calibration** table is empty at first. Select **Append Standard** on the context menu to add the standard(s). The **Browse** dialog box is opened. Select a standard sample from any sequence:
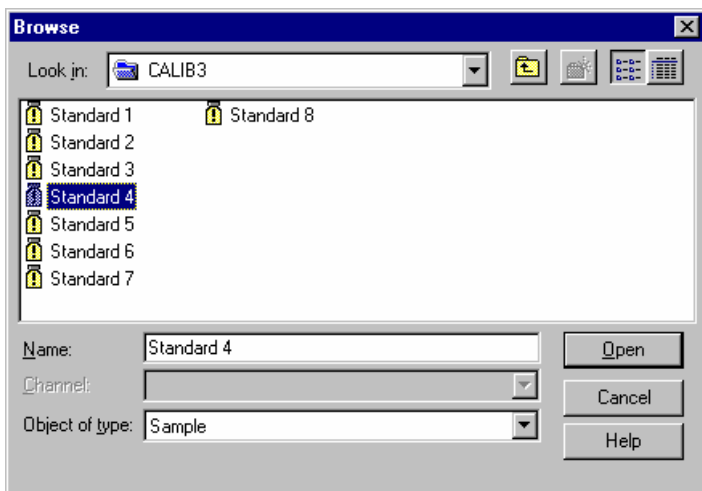
The **Calibration** tab page then lists the standards:

No.	Enabled		Name	Sequence	Smp.No.	Pos.	Inj. Vol.	Inj. Date/Time
1	✓		Standard 1	Parabenes\CALIB3.SEQ	1	6	4.0	04.12.00 12:51:27
2	✓		Standard 2	Parabenes\CALIB3.SEQ	2	6	8.0	04.12.00 13:03:25
3	✓		Standard 3	Parabenes\CALIB3.SEQ	3	6	12.0	04.12.00 13:15:24
4	✓		Standard 4	Parabenes\CALIB3.SEQ	4	6	16.0	04.12.00 13:27:24
5	✓		Standard 5	Parabenes\CALIB3.SEQ	5	6	20.0	04.12.00 13:39:23
6	✓		Standard 6	Parabenes\CALIB3.SEQ	6	6	24.0	04.12.00 13:51:24
7	✓		Standard 7	Parabenes\CALIB3.SEQ	7	6	28.0	04.12.00 14:03:22
8	✓		Standard 8	Parabenes\CALIB3.SEQ	8	6	32.0	04.12.00 14:15:24

After you have entered all standards, click **Calibrate** on the **General** page to perform calibration with those standards. After each change, for example, if you exclude a standard by disabling the **Enabled** check box or if you correct the injection volume in the sample list, you have to click **Calibrate** again.

 Note:

*If you later edit the name of the sequence, from which the standards were added or if you change the name of the directory that houses the sequence, the reference becomes invalid. Then, you have to add the standards again on the **Calibration** page.*

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** **Calibrating**.

Standard Addition

The sample matrix can considerably influence sample analysis. To consider this, a known amount of one or more substances is added to the unknown sample, particularly in ion and gas chromatography. In this way, the concentration of these substances is increased by a value that is exactly known. Afterward, the original and the ➢*Spiked Sample* are analyzed, using ➢*Standard Addition*.

Sample List

In this case, the new sequence includes only unspiked, unknown samples (⇒*Type (Sample Type)*: **Unspiked**) and the associated **Spiked** samples. For example, the new sequence could look as follows:

No.	Name	Type	Std. Add. Group	Ref. Amount Set
1	Sample 1	Unspiked	1	
2	Spiked 1_1	Spiked	1	Spike1
3	Spiked 1_2	Spiked	1	Spike2
4	Spiked 1_3	Spiked	1	Spike3
5	Sample 2	Unspiked	2	
6	Spiked 2_1	Spiked	2	Spike1
7	Spiked 2_2	Spiked	2	Spike2
8	Spiked 2_3	Spiked	2	Spike3
9	Sample 3	Unspiked	3	
10	Spiked 3_1	Spiked	3	Spike1
11	Spiked 3_2	Spiked	3	Spike2
12	Spiked 3_3	Spiked	3	Spike3

Each unknown sample was spiked three times with the same known amounts. In this example, several unknown samples are analyzed.

Therefore, the unknown samples must be assigned to the associated spiked samples, via the ⇒*Std. Add. Group* column. In this column, the samples are assigned to a common standard addition group. For example, in this way, **Sample 1** is calibrated with the spiked samples **Spiked 1_1**, **Spiked 1_2**, and **Spiked 1_3**.

Dionex recommends spiking unknown samples always with the same amount of the same substances. In the ⇒*Ref. Amount Set* column, you can then assign the spiked samples to the same amount values in the **Amount Table** of the QNT Editor. Enter the same ID for the associated spiked samples into this column (here: **Spike1** for samples **Spiked 1_1**, **Spiked 2_1**, and **Spiked 3_1**).

QNT Method/General Tab

Select **Standard Addition** as ⇒*Calibration Mode* in the **Global Calibration Settings** section:

Re-calibration is performed automatically; it cannot be disabled.

QNT Method/Amount Table Tab

On the **Amount Table** tab page, insert standard columns for every spiked sample:

- On the context menu, select **Columns**, and then select **Edit Amount Columns**.

- From the **Assign Standards on the basis of** list, select **Ref. Amount Set** if you have used the associated column in the sample list (see above) or, select another option from the list, e.g., **Name**.

- Click **Auto-Generate** and select **Generate a separate amount column for EACH standard** from the list:
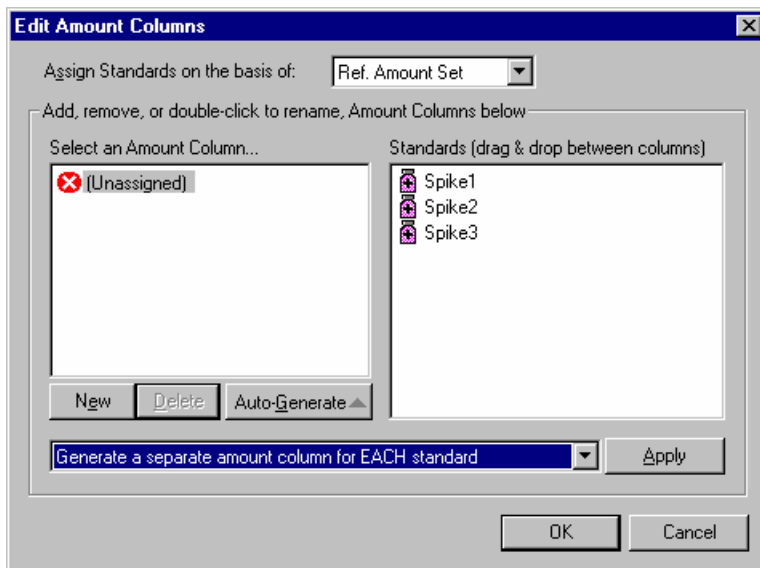
- Click **Apply** to add a column for all identically spiked samples in the Amount Table.

Concentrations

If your dimension of amount is a concentration, enter the added concentrations of the various substances in the spiked samples into the associated **Amount** columns.

or

Amounts

If your dimension of amount is an amount, enter the added amounts of the various substances into the associated **Amount** columns.

Chromeleon automatically analyzes the **Unspiked** sample(s) using the Standard Addition method.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …: Calibrating**.

▣ Calibrating Unstable Substances

If you want to calibrate unstable substances, the concentration in the samples that are analyzed later may be considerably lower than the concentration in those samples that are analyzed first although originally the concentration was the same. The instability of the substance makes calibrating more difficult. Chromeleon provides two possible solutions:

Sample List

To consider the instability of substances one or several standard samples are added to a series of unknown samples every now and then. The sequence will then appear as follows, for example:

QNT Method/General Tab

The bracketed calibration illustrated in the above figure has been achieved using the **Bracketed** ⇒*Calibration Mode* (set in the **Global Calibration Settings** section). The four less decayed standards 1-4 (from positions 1, 2, 5, and 6) are used for calibrating the less decayed unknown samples (samples 1 and 2 from positions 3 and 4). The more decayed standards 3-6 (from positions 5, 6, 9, and 10) are used to calibrate the higher decayed samples 3 and 4 from positions 7 and 8. The calibration curve shows the corresponding ➢*Calibration Points*, only.

For more information about the **Bracketed** mode see ▣ **Calibration Mode: Bracketed.**

☞ *Tip:*

Knowing the half-life of an unstable substance (this is especially true for radioactive substances) is a clever way to calculate the chromatogram, as it would be without the decay. Use a virtual channel (≻Virtual Channel Driver) to record all chromatograms in this special channel as if the substance would not decay. For a program example, refer to **Practical Tips for Device Control ⬛ Program Examples for Virtual Channels***. Having recorded these ≻Virtual Signals, perform the calibration as described in the above examples for stable substances.*

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** ⬛ **Calibrating**.

⬛ No Pure Substance Available - Known Relative Extinction Coefficient

If you wish to quantify substance A although the pure substance A is not available, calibration can be performed nevertheless if the ratio of the extinction coefficient to the extinction coefficient of a different substance B is known. First, create the corresponding sample list following the description in ⬛ **Several Standards with Several Substances Each**.

Then, create the ⇒*Amount* columns for your standards. As the pure substance A is not available and thus is not contained in the standards, the cells for substance A remain empty in the **Amount** table:

No.	Peak Name	Ret.Time	Window	Cal.Type	Resp.Fact.	Amount Sample 1	Amount Sample 2	Amount Sample 3	Amount Sample 4
1	*Substance A*	*9.144 min*	*0.065 AG*	*Lin*	*0.750000 Substance B*				
2	Substance B	9.772 min	0.078 AG	Lin	1.000000	0.983000	2.034000	4.051000	7.890000

The entry made in the ⇒*Response Factor* column is the decisive entry. This column allows you to use the calibration of substance B for substance A. Double-click the selected cell (see above) to open the **Response Factor for Substance A** dialog box and enter the factor of the extinction coefficient at the measuring wavelength between the two substances:

$$Resp.Fact. = \frac{Ext.Coeff_A}{Ext.Coeff_B}$$

Set the interpretation to **Relative to Peak** and select substance B:
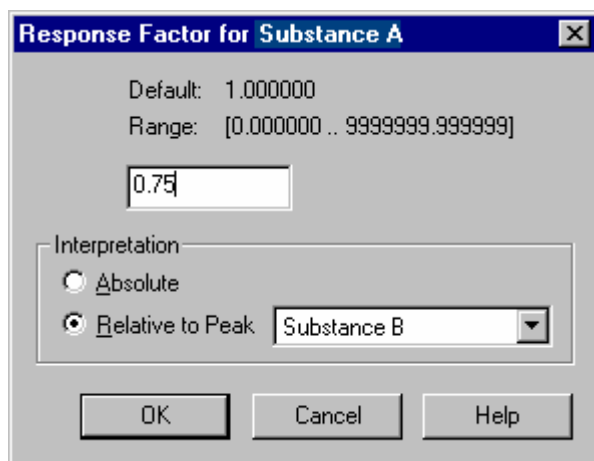
The resulting calibration curve is 75% of the ➤*Calibration Function* of substance B. No calibration points are indicated in the calibration curve because no points were acquired for substance A:
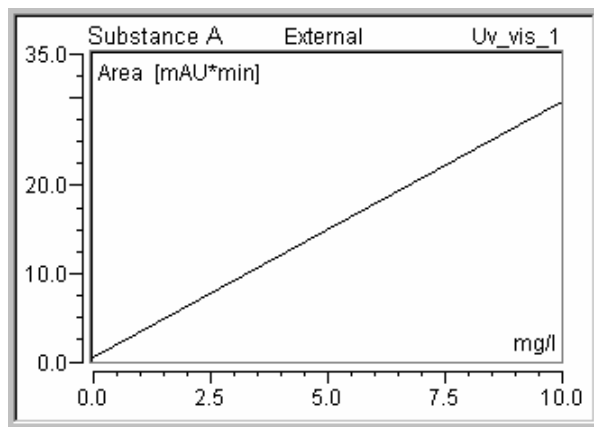
Extinction coefficients depend on the measuring wavelength. Thus, enter the corresponding response factor needs for each channel.

Create a separate Response Factor column for each channel:

Select **Columns** on the context menu of the existing **Response Factor** column. Select **Duplicate** and then select a channel by clicking **Add Channel**.

Note:

The calibration curve of the reference peak is the decisive factor. If possible, it should not have an offset. Otherwise, errors may occur when calculating the amount using the response factor. This is especially true for lower amounts.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …: I Calibrating**.

Calibrating with an Internal Standard Substance

Especially in gas chromatography but also in HPLC or IC, calibration using an internal standard substance, i.e., an ➤*Internal Standard* (= ISTD), is used to eliminate possible measuring and sample preparation errors. Chromeleon provides different calibration possibilities using one single internal standard substance:
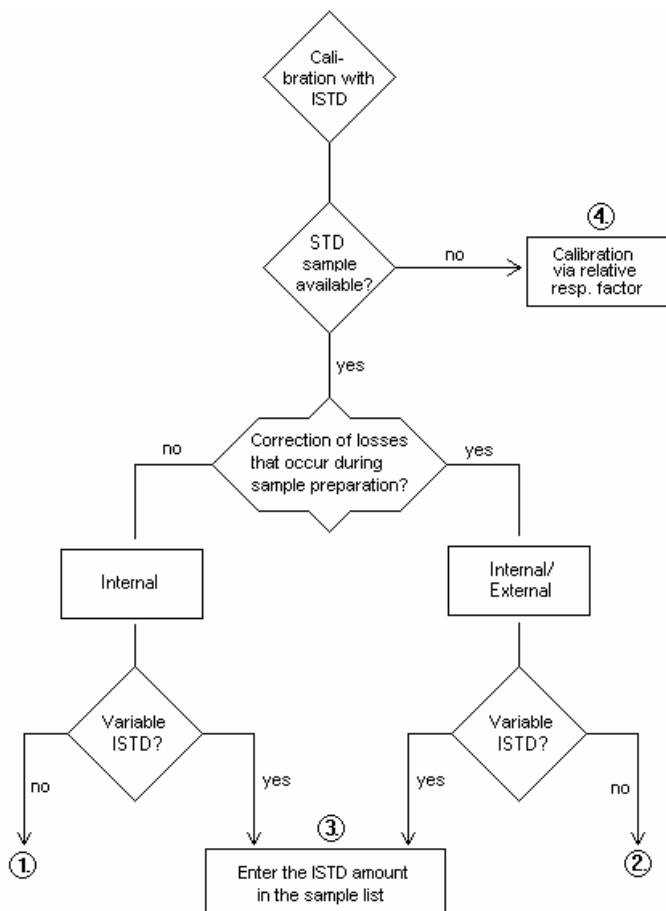
If several internal standard substances are available, the flow chart changes accordingly. For more information about the numbered options in the flow chart, refer to:

1. 🔳 Compensating Measurement Errors (Internal Calibration)

2. 🔳 Correcting Sample Preparation Errors (Internal/External Calibration)

3. 🔳 Using Different ISTD Amounts (Variable ISTD)

4. 🔳 Calibrating without Standard Samples

Calibrating Samples with Several Channels with an Internal Standard Substance

A special procedure is required for analyzing samples with several channels (e.g., SIM or UV channels). In this case, the internal standard I is detected only on the channel for which the retention time is specified in the QNT File. Therefore, a retention time need not be specified for the internal standard I on all channels. However, if no retention time is specified in the QNT Editor for the channel to be analyzed, amount calculation cannot be performed for the internal standard I. As a result, the amount of substance A, which uses the internal standard I for amount calculation, cannot be calculated either.

To calculate the amount of substance A on a channel for which no retention time is specified for the internal standard I, Chromeleon uses a channel for which a retention time is specified in the QNT Editor. Chromeleon searches all retention time columns on the **Amount Table** tab for the corresponding channels. The first channel for which a retention time is defined is used to analyze the internal standard I and thus, to calculate A.

This searching approach allows you to determine which channel is used for calculating the amount. To do so, add a retention time column for the channel:

Right-click the retention time column and select **Columns** on the context menu. Select **Duplicate Column**. Click **Add Channel** and select the channel for which you want to add a new column from the **Channel** drop-down list. Arrange the retention time columns in the order in which you want them to be searched (the first column being the retention time of the channel that is used for the analysis).

The retention times and their assigned channels are entered in the QNT Editor and will be searched in the defined order.

◪ Compensating Measurement Errors (Internal Calibration)

It is possible to compensate measuring errors, such as deviations that occur during the injection, by calibrating with an internal standard substance, i.e., the ➤*Internal Standard*. In the purely internal standard method, calibration is performed relatively to the internal standard substance, using area ratios instead of absolute areas.

Sample List

In the simplest case, only one standard sample is available. In this case, the sample list looks, for example, as follows:

No.	Name	Type	Pos	Inj. Vol.
1	Standard	Standard	R99	10.00
2	Sample 1	Unknown	RA1	10.00
3	Sample 2	Unknown	RA2	10.00

QNT Method/Amount Table Tab

Enter the amounts of the single substances contained in the standard sample in the **Amount** column of the **Amount** table of the QNT Editor:

No.	Peak Name	Ret.Time △	Resp.Fact.	Amount
1	Subst. 1	5.400 min	1.000000	1.00000
2	Subst. 2	7.800 min	1.000000	17.00000

QNT Method/Peak Table Tab

The internal standard substance is defined in the peak table. If all three samples contain two relevant substances only as in the example, make the following entries in the **Standard** column, using the F8 input box.

- For substance 1, select **Use this peak as Internal Standard** and thus define this substance as the internal standard. (The light yellow background of the line and the **ISTD: Internal** entry indicate that the assignment is correct.)

- In the F8 input box of substance 2, select **Internal**. From the **Associated ISTD Peak** field, select the standard substance serving as internal standard (here: Subst. 1).

- If necessary, repeat this operation for every additional substance that should be calibrated based on the internal method.

After completing the input, the substance 2 is labeled **Internal Subst. 1** in the **Standard** column. In addition, substance 1 is labeled as the internal standard for internal calibration:

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type
1	Subst. 1	5.400 min	0.100 AG	STD Internal	Area	Lin
2	Subst. 2	7.800 min	0.100 AG	Internal Subst. 1	Area	Lin

Amount Calculation

Chromeleon automatically calculates the amount values for the "unknown" substance. The calculation is performed according to the formula for amount calculation (rel. to ISTD) (for more information, refer to ➢*Formula for Amount Calculation (Rel. to ISTD)* in the Glossary section). Chromeleon calculates the amount for substance 2 adapted by the deviation of substance 1.

For detailed information about how to calculate the amount for unknown substances using internal calibration, refer to 🔳 **Internal Calibration: Calculation**.

For an overview of the different calibration possibilities with internal standard substances provided by Chromeleon, refer to **How to …: Calibration Examples** 🔳 **Calibrating with an Internal Standard Substance**.

🔳 Internal Calibration: Calculation

In the purely internal standard method, calibration is performed only by means of an internal standard substance, i.e., the ➢*Internal Standard*. Calculation is performed using area ratios instead of absolute areas. That is why in the ➢*Formula for Amount Calculation (Rel. to ISTD)*

$$Amount_p = f_p \left(\frac{y_{Peak}}{y_{ISTD}} \right) * Resp.Fact._p * \frac{Dil.Fact._n}{Weight_n}$$

Example

In a clinic lab, prepared urine samples of two patients are examined for the catechol level. Adrenaline and dopamine are to be determined. Two standard solutions of different concentrations are available (STD 1: 50 ng/µl each; STD 2: 100 ng/µl adrenaline/dopamine each). To correct possible inaccuracies regarding the precise dosing of the autosampler, catechol is added. The internal standard method is selected. 20 µl of each unknown sample and each standard sample is mixed with 20 µl of the catechol solution. As the concentration of the added catechol solution is 10ng per µl, each sample and standard vial (40 µl) contains 10 x 20 = 200 ng catechol. This means that exactly 200/4 = 50 ng catechol is injected with each 10 µl injection.

a) User Input

Sample List

No.	Name	Type	Pos	Inj. Vol.	Program	Method
1	🔒 STD 1	Standard	1	10.0	ADR_DOPA	QNT_DOPA
2	🔒 STD 2	Standard	2	20.0	ADR_DOPA	QNT_DOPA
3	❓ Sample I	Unknown	3	10.0	ADR_DOPA	QNT_DOPA
4	❓ Sample II	Unknown	4	10.0	ADR_DOPA	QNT_DOPA

In contrast to the examples in the external and internal/external calibration, the second calibration point is not determined by means of the modified injection volume but by means of a second standard sample with the double concentration. As a result, two different autosampler positions are used (1 and 2).

QNT Method/Peak Table Tab

No.	Peak Name	Ret.Time	Window	Standard	Cal.Type	Amount 1	Amount 2
1	Adrenaline	3.400 min	0.400 AG	Internal Catechol	Lin	50.000000	100.000000
2	Dopamine	5.500 min	0.400 AG	Internal Catechol	Lin	50.000000	100.000000
3	Catechol	9.600 min	0.400 AG	ISTD: Internal	Lin	50.000000	50.000000

As the calibration of adrenaline and dopamine is performed with two standard concentrations (STD 1, STD 2; different autosampler position in the sample list), two amount values are entered for each peak. Then, the internal standard substance is defined.

How to define the **Internal Standard** substance:

- Select the **Standard** column in the **Catechol** line and open the F8 edit box.

- Select the **Use this peak as internal Standard** option and thus define catechol as Internal Standard.

The yellow coloring of the line and the **ISTD: Internal** entry indicate the correct assignment.

- Change to the **Standard** column in the **Adrenaline** line and open the F8 edit box again.

- Select the option **Internal**, and then select the standard substance serving as Internal Standard (here: catechol) in the **Associated ISTD Peak** field.

- Perform this operation for each peak that should be calibrated with the **Internal** method (here dopamine).

After completing the input, the following occurs: In the **Standard** column, alanine, and dopamine are labeled **Internal Catechol**. In addition, catechol is marked as the internal standard substance by a darker shade of yellow.

Note:

In addition to the color changes from light yellow to dark yellow, there are two other possible colors. If the retention time is expressed depending on a selected reference peak (see ⇒Retention Time), a light blue background highlights this reference peak in the peak table. If this reference peak is also used as the internal standard peak, the corresponding line is displayed in green (blue + yellow = green).

QNT Method/General Tab

The **Total** mode is selected. This ensures that the calibration of all samples (Sample I and II) is performed based on *all* standard samples (STD 1, STD 2).

QNT Method/Calibration Tab

This page shows all *standard samples* (of a sequence) that are inserted for calibrating the current sample.

Press the F4 key or the SHIFT+F4 key combination to open the samples of a sequence one after the other. The standard samples forming the basis for calibration are shown for each sample.

Due to the selected mode, sample I and II appear as follows:

No	Enabled	Name	Pos.	Inj. Vol.	Inj. Date/Time	Calib. Comment	
1	☐	🔒 STD 1	1	10.0	10/12/00 1:16:30 PM	Disabled	
2	☑	🔒 STD 2	2	20.0	10/12/00 1:29:05 PM	Ok	

◄ ► Ⲭ Peak Tracking Ⲭ MS Tracking Ⲭ Calibr ◄

If you notice that an error occurred during the analysis of the standard sample, you can "exclude" this standard sample. Remove the standard in the **Enable** column on the **Calibration** tab page of the QNT Editor. Only the standard samples labeled **X** are considered for the calibration.

b) Analysis Structure

Injection is four times. During the first run, the first calibration point of the adrenaline and dopamine calibration curve is determined via STD 1, and during the second run the second point is determined accordingly via STD 2. Run three serves to determine the concentration of adrenaline and dopamine in sample I. In the fourth run, the concentrations of adrenaline and dopamine in sample II are determined. In addition, the area of the added catechol is determined in each run.

Chromeleon determines the following area values:

Name	Area Adrenaline	Area Dopamine	Area Catechol
STD 1	125	200	250
STD 2	250	400	250
Sample I	223	150	245
Sample II	178	380	255

The area values determined for catechol reflect the ratio of the injected volume or amounts (except for minor inaccuracies).

c) Calculation of $Amount_{p,rel}$ and $x_{p,rel}$

The ratio of Area (Peak) to Area (ISTD) results in $x_{p,rel}$:

$$x_{(prel)} = \frac{Area_p}{Area_{ISTD}}$$

Substance	Area	Area (ISTD)	x (p, rel)
Adrenaline (STD 1)	125	250	0.5
Adrenaline (STD 2)	250	250	1.0
Dopamine (STD 1)	200	50	0.8
Dopamine (STD 2)	400		1.6

The ratio of Amount (Peak) to Amount (ISTD) results in $Amount_{p,rel}$:

$$Amount_{(p,rel)} = \frac{Amount_{Peak}}{Amount_{ISTD}}$$

Substance	Amount	Amount (ISTD)	Amount (p, rel)
Adrenaline (STD 1)	50	50	1.0
Adrenaline (STD 2)	100	50	2.0
Dopamine (STD 1)	50	50	1.0
Dopamine (STD 2)	100	50	2.0

d) Calculation of the Calibration Coefficients

A linear calibration curve through the origin (calibration type: **Linear**) can already be described by one calibration coefficient (c_1). If the example is selected so that the calibration points in each calibration curve are located exactly on a straight line, that is, for example, in an exact measurement, c_1 results as the y/x-quotient of each value pair **Amount p rel** to **x p rel**:

Substance	Amount (p rel) / x (p rel)	c1
Adrenaline	1.0/0.5	2.000
Adrenaline	2.0/1.0	2.000
Dopamine	1.0/0.8	1.250
Dopamine	2.0/1.6	1.250

If the calibration points are not located exactly on one line, Chromeleon calculates an optimized c_1 approximate value for each substance.

If a different calibration type is selected, Chromeleon calculates the corresponding calibration coefficients (c_0, c_1, and c_2).

e) Amount Calculation

By means of the ➢*Formula for Amount Calculation*, the relative amount (=relative to the amount of the ISTD) of the sample content adrenaline and dopamine can be calculated from the known c_1 and from the ratio peak area (sample) to peak area (ISTD). If the ⇒*Dil. Factor* (Dilution Factor) and ⇒*Weight* (Sample Weight Factor) correction factors are assumed to be 1, the following Amount/Amount ISTD values result:

Sample I	Calculation: Amount/Amount ISTD
Adrenaline	2.000 x (223/245) = 1.820
Dopamine	1.250 x (150/245) = 0.765

Sample II	Calculation: Amount/Amount ISTD
Adrenaline	2.000 x (178/255) = 1.396
Dopamine	1.250 x (380/255) = 1.863

By multiplication with the amount values of the internal standard substance, the actual amount values for adrenaline and dopamine in the analysis samples can be calculated.

Sample I	Calculation: Amount
Adrenaline	1.820 x 50 = **91.00** [ng]
Dopamine	0.765 x 50 = **38.25** [ng]

Sample II	Calculation: Amount
Adrenaline	1.396 x 50 = **69.80** [ng]
Dopamine	1.863 x 50 = **93.15** [ng]

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …: 🔲 Calibrating**.

🔲 Correcting Sample Preparation Errors (Internal/External Calibration)

It is possible to eliminate errors occurring during sample preparation by calibrating using the internal/external method. If you use the internal/external method, an internal standard substance, i.e., the ➢ *Internal Standard* (= ISTD), is used to adapt the external calibration to the corresponding sample. Exactly that amount of the internal standard substance is added to each standard sample <u>and</u> each unknown sample that makes sure that the concentration is identical in all sample vials. The following entries are required:

Sample List

In the simplest case, only one standard sample is available. In this case, the sample list looks, for example, as follows:

No.	Name	Type	Pos	Inj. Vol.
1	⚠ Standard	Standard	R99	10.00
2	❓ Sample 1	Unknown	RA1	10.00
3	❓ Sample 2	Unknown	RA2	10.00

QNT Method/Amount Table Tab

Enter the amounts of the single substances contained in the standard sample in the **Amount** column of the **Amount** table of the QNT Editor:

No.	Peak Name	Ret.Time	Resp.Fact.	Amount
1	*Subst. 1*	*5.400 min*	*1.000000*	*12.00000*
2	Subst. 2	7.800 min	1.000000	17.00000

QNT Method/Peak Table Tab

The internal standard substance is defined in the peak table. If all three samples contain two relevant substances only as in the example, make the following entries in the Standard column, using the F8 input box.

* For substance 1, select **Use this peak as Internal Standard** and thus define this substance as the internal standard substance. (The light yellow background of the line and the **ISTD: Internal** entry indicate that the assignment is correct.)

* In the F8 input box of substance 2, select **Internal/External**. In the **Associated ISTD Peak** field, select the standard substance serving as internal/external standard (here: Subst. 1).

* If necessary, repeat this operation for every additional substance that should be calibrated based on the internal/external method.

After completing the input, the substance 2 is labeled **Int/Ext Subst. 1** in the **Standard** column. In addition, substance 1 is labeled as the internal standard for internal/external calibration. The description **ISTD: Internal** is changed to **ISTD: Int/Ext** and the yellow coloring of the Subst. 1 line is intensified:

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type
1	Peak 1	5.400 min	0.350 AG	ISTD Int/Ext	Area	Lin
2	Peak 2 Subst	7.800 min	0.400 AG	Int/Ext Peak 1	Area	Lin

Amount Calculation

Chromeleon automatically calculates the amount values for the substance 2. The calculation is performed according to the formula for amount calculation (for more information, refer to ➢*Formula for Amount Calculation* in the Glossary section). In case of internal/external calibration, the ISTD factor is used to correct the external calibration, using the ISTD amount determined for the respective sample.

The ISTD factor considers the ratio of the (nominal) amount entered in the peak table to the amount that was determined for the respective sample due to the peak area:

$$ISTD\ Factor = \frac{Amount_{ISTD(PeakTable)}}{Amount_{ISTD(Sample)}}$$

Using this formula, Chromeleon calculates the amount for substance 2 that has been adapted by the deviation of substance 1.

For detailed information about how to calculate the amount of unknown substances using internal/external calibration, refer to ◩ **Internal/External Calibration: Calculation**.

For an overview of the different calibration possibilities with internal standard substances provided by Chromeleon, refer to **How to …: Calibrating** ◩ **Calibrating with an Internal Standard Substance**.

◪ Internal/External Calibration: Calculation

In a calibration with the internal/external method, external calibration is adapted to the corresponding sample, by using an internal standard substance, i.e., an ➤*Internal Standard* (= ISTD):

Each standard sample <u>and</u> each unknown sample is added exactly that amount of internal standard substance to make sure the concentration is identical in each vial. The internal standard substance and the substance to be determined are calibrated using known standard solutions; that is, the ➤*Calibration Coefficients* are determined from the ⇒*Amount* values of the standard sample and the corresponding peak area values by means of the ➤*Calibration Function*. Thus, the amounts of all substances (including the ISTD) can be determined. As the concentration of the internal standard substance is identical in all samples, the same ISTD amount should result.

If this is not the case, an error occurred in the chromatography system (sample preparation, injection, carry-over, etc). The deviation of the actual ISTD amount from the nominal ISTD quantifies of the error. If the substances to be determined and the internal standard substance are similar, it can be assumed that the values of the remaining contents of the sample deviate in the same way; that is, they are incorrect. A correction by the deviation of the nominal and the actual internal standard substance supplies the actual values.

Example:

You want to determine the concentration of alanine and glycine in two samples. One standard sample is available. The internal standard substance norvaline is added to all three sample vials, so that the final concentration is 10 mmol/l. During the subsequent pre-column derivatization, 10 μl sample + 20 μl OPA reagent + 20 μl stop reagent are pipetted together. A constant concentration of 2 mmol/l is added from the norvaline.

The chromatographic separation follows the derivatization of the amino acids in OPA derivatives. 10 and 20 μl of the standard solution (➢*Dilution Series*) and 10 μl of each sample (autosampler position 2 and 3) are injected.

a) User Input:

Sample List

No.	Name	Type	Pos	Inj. Vol.	Program	Method
1	⚠ STD 1	Standard	1	10.0	ALA_GLY	QNT_ALA
2	⚠ STD 1	Standard	1	20.0	ALA_GLY	QNT_ALA
3	❓ Sample I	Unknown	2	10.0	ALA_GLY	QNT_ALA
4	❓ Sample II	Unknown	3	10.0	ALA_GLY	QNT_ALA

QNT Method/Peak Table Tab

No.	Peak Name	Ret.Time	Window	Standard	Cal.Type	Amount 1	Amount 2
1	Alanine	5.400 min	0.400 AG	Int/Ext Norvaline	Lin	50.000000	
2	Glycine	7.800 min	0.400 AG	Int/Ext Norvaline	Lin	50.000000	
3	Norvaline	13.600 min	0.400 AG	STD: Int/Ext	Lin	20.000000	

Only one standard concentration (STD1; same autosampler position in the sample list) is required to perform the calibration of alanine and glycine. Therefore, only one amount value is required for each peak. Afterward, the internal standard substance is defined.

How to define the **Internal Standard** substance:

- In the **Norvaline** line, select the **Standard** column and open the F8 edit box.

- Select **Use this peak as internal Standard** and thus define norvaline as the internal standard substance.

A light yellow background and the **ISTD: Internal** entry indicate that the assignment is correct.

- In the **Alanine** line, change to the **Standard** column and open the F8 edit box again.

- Select the option **Internal/External**. In the **Associated ISTD Peak** field, select the standard substance serving as **Internal/External Standard** (here: norvaline).

- Repeat this operation for each peak that should be calibrated using the Internal/External method (here: glycine).

After completing the input, the following occurs: In the **Standard** column, alanine and glycine are labeled **Int/Ext Norvaline**. In addition, norvaline is labeled as the internal standard for the Internal/External calibration. The **ISTD: Internal** description is changed to **ISTD: Int/Ext** and the yellow coloring of the norvaline line intensifies.

Note:

In addition to the color changes from light yellow to dark yellow, there are two other possible colors. If the retention time is expressed depending on a selected reference peak (see ⇒Retention Time), a light blue background highlights this reference peak in the peak table. If this reference peak is also used as the internal standard peak, the corresponding line is displayed in green (blue + yellow = green).

QNT Method/General Tab

The **Total** mode is selected. This ensures that the calibration of all samples (Samples I and II) is performed based on *all* standard samples (STD 1).

QNT Method/Calibration Tab

The page shows all *standard samples* (of a sequence) that are used for calibrating the current sample.

Press the F4 key or the SHIFT+F4 key combination to open the samples of a sequence one after the other. The standard samples forming the basis for calibration are shown for each sample.

Due to the selected mode, samples I and II appear as follows:

No	Enabled	Name	Pos.	Inj. Vol.	Inj. Date/Time	Calib. Comment	▲
1	☐	🔒 STD1	1	10.0	10/12/00 1:16:30 PM	Disabled	
2	☑	🔒 STD1	1	20.0	10/12/00 1:29:05 PM	Ok	▼

◄ ► Ɫ Peak Tracking Ɫ MS Tracking Ɫ Calibr ◄   ►

If you notice that an error occurred during the analysis of the standard sample, you can "exclude" this standard sample. Remove the standard in the **Enable** column on the **Calibration** tab page of the QNT Editor. Only the standard samples labeled **X** are considered for the calibration.

b) Analysis Structure:

Injection is four times. During the first run, the first calibration point of the calibration curve is determined, and during the second run, the second point is determined. Run three serves to determine the concentration of alanine and glycine in sample I. In the fourth run, the concentrations of alanine and glycine in sample II are determined.

Chromeleon determines the following area values:

Name	Area Alanine	Area Glycine	Area Norvaline
STD 1 (first run)	55	80	40
STD 1 (second run)	110	160	80
Sample I	45	75	39
Sample II	80	150	41

The determined area values of the internal standard substance norvaline reflect the ratio of the injected volumes (amounts), except for minor inaccuracies.

c) Calibration Points

From the known amount values and from the determined area values of the standard samples, the value pairs of the individual calibration points can be established:

Substance	Area value	Amount value [1]	Amount value [2]
Alanine	45	50	
Alanine	90		100
Glycine	80	50	
Glycine	160		100
Norvaline	40	20	
Norvaline	80		40

Chromeleon determines all calibration coefficients, depending on the selected calibration function.

d) Calculation of the Calibration Coefficients

Only one calibration coefficient (c_1) is required to describe a linear calibration curve through the origin (calibration type **Linear**). If the example is selected so that the calibration points in each calibration curve are located exactly on a straight line, that is, for example, in an exact measurement, c_1 results as the y/x-quotient of each value pair (= slope of the calibration curve).

Substance	y/x-Value pair	c1
Alanine	50/45	1.111
Alanine	100/90	1.111
Glycine	50/80	0.625
Glycine	100/160	0.625
Norvaline	20/40	0.500
Norvaline	40/80	0.500

If the calibration points are not located exactly on one line, Chromeleon calculates an optimized c1 approximate value for each substance. If a different calibration type were selected, Chromeleon would also calculate the remaining calibration coefficients (c0 and c2) according to the calibration function.

e) Amount Calculation: Internal Standard Substance in Unknown Samples

If the area values of the internal standard substances from samples I and II are known, the amount of the internal standard substance norvaline can be determined in the two samples by means of the calibration coefficient c1 (here = 0.5) established for the calibration curve of the norvaline.

Sample	Calculation	Amount (ISTD)
I	39 x 0.5	19.50
II	41 x 0.5	20.50

The ratio between the (nominal) amount value entered in the peak table and the ⇒*ISTD Amount (Amount of the Internal Standard)* of the internal standard in the corresponding sample is referred to as ISTD factor.

$$ISTD - Factor = \frac{Amount_{ISTD(Peak-Tab.)}}{Amount_{ISTD(Sample)}}$$

The following values are resulting:

Sample	Calculation	ISTD Factor
I	20 / 19.5 =	1.026
II	20 / 20.5 =	0.976

The result states that an error was made by 1.026 (sample 1) or 0.976 (sample 2). The actual amounts of alanine and glycine deviate in all probability by 2.6 or 2.4% from the "real" values. They are corrected by this amount.

f) Amount Calculation: Alanine and Glycine

The ➤*Formula for Amount Calculation* is used to calculate the amount values of glycine and alanine. In contrast to an external calibration, the **ISTD Factor** is not equal to 0; i.e., the results are corrected by the calculated ISTD factor.

Sample	Calculation (Area x c1 x ISTD Fact. =)	Amount
I	45 x 1.111 x 1.026 =	51.30 (Alanine)
I	90 x 0.625 x 1.026 =	54.90 (Glycine)
II	80 x 1.111 x 0.976 =	86.75 (Alanine)
II	160 x 0.625 x 0.976 =	97.60 (Glycine)

The alanine or glycine amount values corrected by the norvaline deviation are resulting.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** 🔲 **Calibrating**.

🔳 Using Different ISTD Amounts (Variable ISTD)

Due to the method itself or the properties of the internal standard substance, i.e., the ➤*Internal Standard*, it is sometimes impossible to add exactly the same amount of ISTD. To solve this problem, Chromeleon provides the **Use sample amount as reference (Variable Internal Standard)** option. Use this option to define the ISTD as variable internal standard substance. (The option is accessible via the F8 box of the **Standard** column in the peak table.)

In this case, you do not need to enter the amount of the internal standard substance in the **Amount** column of the peak table (this is not possible), but in the ⇒*ISTD Amount* column of the sample list. It is not important whether **Internal/External** or **Internal** is selected as calibration method. In this way, it is possible to enter the amount of an internal standard substance separately for each sample. The following entries are required:

Sample List

Enter the amount of the internal standard substance directly in the **ISTD Amount** column of the sample list:

No.	Name	Type	Pos	Inj. Vol.	ISTD Amount
1	Standard	Standard	R99	10.00	29.5670
2	Sample 1	Unknown	RA1	10.00	30.2430
3	Sample 2	Unknown	RA2	10.00	30.5840

QNT Method/Peak Table Tab

Apart from the substance serving as internal standard, enter all amount values as before. Then, select the internal standard substance as follows:

If all three samples contain two relevant substances only as in the example, make the following entries in the **Standard** column, using the F8 input box.

- For substance 1, select **Use this peak as Internal Standard** and thus define this substance as the internal standard.

- In addition, enable the **Use sample amount as reference (Variable Internal Standard)** option to define the ISTD as variable internal standard substance.

The yellow coloring of the line and the **ISTD: Var. Internal** or **ISTD: Var. Int/Ext** entry indicate that the assignment is correct. The corresponding **Amount** column cells are automatically set to 1.

Make all other QNT File entries as before. The peak table appears as follows:

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type
1	Subst. 1	5.400 min	0.100 AG	ISTD Var.Internal	Area	Lin
2	Subst. 2	7.800 min	0.100 AG	Var.Internal Subst. 1	Area	Lin

QNT Method/Amount Table Tab

Enter the amount values for the unknown substances contained in the standard sample in the **Amount** column of the **Amount** table of the QNT Editor:

No.	Peak Name	Ret.Time △	Resp.Fact.	Amount
1	Subst. 1	5.400 min	1.000000	1.000000
2	Subst. 2	7.800 min	1.000000	17.00000

Evaluation

Evaluation is similar to the corresponding evaluation with a constant amount of the internal standard substance. However, slightly different calibration and evaluation formulas are used.

For a detailed description of the differences between standard methods, refer to **Calibration 🔳 Evaluation with Various Standard Methods**.

For an overview of the different calibration possibilities with internal standard substances provided by Chromeleon, refer to **How to …: Calibrating ⬛ Calibrating with an Internal Standard Substance**.

⬛ Calibrating without Standard Sample

If you calibrate with an internal standard substance, i.e., an ➢*Internal Standard*, you may as well perform the calibration without standard samples. The prerequisite is that you know the relative ⇒*Response Factor* of the respective substance for the ISTD. The following entries are required:

Sample List

Assign all samples the Unknown sample type. Enter the amount for the ISTD in the ISTD Amount column:

No.	Name	Type	Pos	Inj. Vol.	ISTD Am
1	Sample 1	Unknown	RA1	10.00	30.0000
2	Sample 2	Unknown	RA2	10.00	30.0000
3	Sample 3	Unknown	RA3	10.00	30.0000

QNT Method/General Tab

On the General tab page, select Fixed as ⇒*Calibration Mode*.

QNT Method/Peak Table Tab

The internal standard substance is defined in the peak table. If all three samples contain two relevant substances only as in the example, make the following entries in the **Standard** column, using the F8 input box.

- For substance 1, select **Use this peak as Internal Standard** and enable **the Use sample amount as reference (Variable Internal Standard)**.

- In the F8 input box of substance 2, select **Internal**. In the **Associated ISTD Peak** field, select the standard substance serving as internal standard (here: Subst. 1).

- If necessary, repeat this operation for every additional substance that should be calibrated based on the internal method.

After completing the input, the substance 2 is labeled **Var. Internal Subst. 1** in the **Standard** column. In addition, substance 1 is labeled **ISTD Var. Internal** to indicate that substance 1 is used as internal standard for the internal calibration:

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type
1	Subst. 1	5.400 min	0.100 AG	ISTD Var.Internal	Area	Lin
2	Subst. 2	7.800 min	0.100 AG	Var.Internal Subst. 1	Area	Lin

QNT Method/Amount Table Tab

Define the corresponding calibration curve in the **Amount** table of the QNT Editor:

- Enter =1.000 as C1 value for both the ISTD and the unknown substance.

☞ *Tip:*

*Do not recalibrate afterwards by clicking **Calibrate** on the **General** tab page because this action overwrites the values for the ➢Calibration Coefficient.*

- Enter the known response factor. This factor automatically refers to the ISTD because the ISTD is used for calibration. It is not necessary to define the ISTD as reference.

No.	Peak Name	Ret.Time	Resp.Fact.	Amount	C0 Uv_vis_1	C1 Uv_vis_1	C2 Uv_vis_1
1	Subst. 1	5.400 min	1.000000	1.000000	0.000000	1.000000	0.000000
2	Subst. 2	7.800 min	2.540000	1.000000	0.000000	1.000000	0.000000

Amount Calculation

Chromeleon automatically calculates the amount values for the substance 2. The calculation is performed according to the formula for amount calculation (rel. to ISTD) (for more information, refer to ➢*Formula for Amount Calculation (Rel. to ISTD)* in the Glossary section). In case of internal calibration, the external calibration of the internal standard substance is adapted to the corresponding sample, using the area ratio of the peak to the ISTD in the corresponding sample.

In addition, in this special case, the response factor is used to take the absorption of substance 2 in relation to the ISTD (here: Subst. 1) into account.

Chromeleon calculates the amount for substance 2 adapted by the deviation of substance 1.

For detailed information about how to calculate the amount for unknown substance using internal calibration, refer to 🔲 **Internal Calibration: Calculation**.

For an overview of the different calibration possibilities with internal standard substances provided by Chromeleon, refer to **How to …: Calibrating** 🔲 **Calibrating with an Internal Standard Substance**.

🔲 Calibration Modes for External Calibration

The ⇒*Calibration Mode* determines the standard samples that are used for calibrating specific unknown samples. The following calibration modes are available:

Calibration Mode	Description
Total	All standard samples of the current sequence
Group	Grouping calibration
Additional	Additional standard samples for the samples that appear later in the sequence.
Bracketed	Unknown samples are "bracketed" by standard samples.
Fixed	Specific standard samples (also from other sequences)

For a schematic representation of the **Group**, **Additional**, and **Bracketed** calibration modes, refer to the example below:
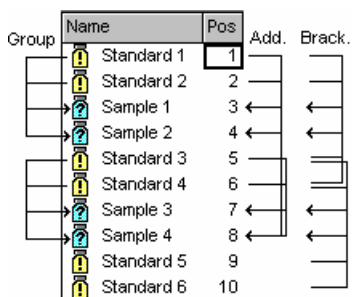
For more information about the different calibration modes including calibration curve calculation, refer to the topics below:

🔲 **Calibration Mode: Total**

🔲 **Calibration Mode: Additional**

🔲 **Calibration Mode: Group**

🔲 **Calibration Mode: Bracketed**

🔲 **Calibration Mode: Fixed**

📝 *Note:*

*Please note that the procedure is described for the **Total** mode. All other examples describe the differences between the calibration modes only.*

◾ Calibration Mode: Total

If standard samples exist, the ratio between the amount and the peak area can be used to calculate the calibration coefficients c0 (offset), c1 (slope), and c2 (curvature) by means of the selected ➢*Calibration Function*. The resulting values are entered in the ➢*Formula for Amount Calculation*, together with the area values of the substance of an unknown sample. As the result, Chromeleon provides the ⇒*Amount* of substance A in the unknown sample. You can also use the concentration instead of the amount. However, in this case, the concentration must be seen in relation to the injection volume.

Example:

You want to determine the concentration of the substances A and B in two samples (Sample 1 and Sample 2). One standard solution (Std 1) is available containing substance A in a concentration of 12 mg/l and substance B in a concentration of 17 mg/l. The calibration curve for substances A and B shall be linear and run through the origin (calibration type: linear). The curve shall show two calibration points for each substance. As only one standard solution is available, two different volumes (10 and 20 µl) must be injected (➢*Dilution Series*). Two ➢*Calibration Points* are resulting. The autosampler injects a volume of 10 µl for the two unknown samples. The standard and analysis samples occupy the autosampler positions R99, RA1, and RA2.

a) User Input

Sample List

No.	Name	Type	Pos.	Inj. Vol.	Program	Method	Status
1	Std - 1. Inj.	Standard	R99	10.00	Control 1 ▼	QNT 1	Single
2	Std - 2. Inj.	Standard	R99	20.00	Control 1	QNT 1	Single
3	Sample 1	Unknown	RA1	10.00	Control 1	QNT 1	Single
4	Sample 2	Unknown	RA2	10.00	Control 1	QNT 1	Single

For more information, refer to **How to …: Creating and Managing Files and Data 🔳 Creating a Sample List (Sequence)**.

QNT Method/Peak Table Tab

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount
1	Subst. A	1.310 min	0.100 AG	External	Area	Lin	12.00000
2	Subst. B	2.450 min	0.100 AG	External	Area	Lin	17.00000

Regarding this example, note the following:

- Select the **External** standard method in the **Standard** column using the F8 edit box.

- Only one standard concentration (Std 1) is available for calibrating the substances A and B. That is why one concentration value for each substance is entered in the **Amount** column of the peak table. The same applies if several sample list entries are generated by analyzing the same standard sample several times (twice, in this case). If the injection volume is identical, the calibration is referred to as 1-point calibration with several replicates. If the injection volume varies (10 and 20 µl) as in this case, this is referred to as multiple-point calibration, that is, 2-point calibration. This is a special case of the ➤*Dilution Series* ("concentration series").

  However, if two separate standards with different concentrations are available (two vials, different autosampler positions), two concentration values need to be entered in the **Amount** column of the peak table.

  All other entries in the peak table are based on the criteria described in **How to …: Integrating Chromatograms and Identifying Peaks 🔳 Creating a Peak Table**.

QNT Method/General Tab

To calibrate all samples (here: samples 1 and 2) with the two standard samples, select the **Total** $\Rightarrow$ *Calibration Mode*.
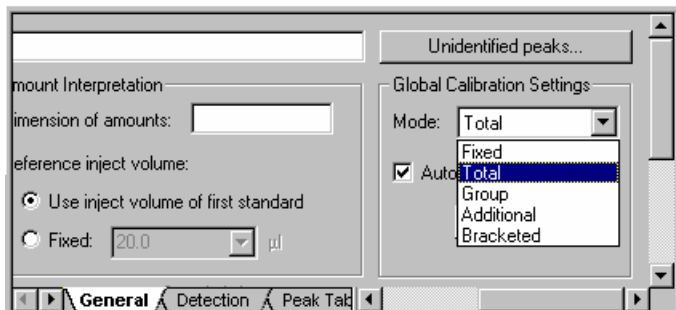
QNT Method/Calibration Tab

This page shows all standard samples (of a sequence) that are used for calibrating the current sample.

Press the F4 key or the SHIFT+F4 key combination to open all samples of a sequence one after the other. The standard samples used for calibrating the sample are shown for each sample.

If you notice that an error occurred during the analysis of the standard sample, you can "exclude" this standard. Remove the standard in the **Enable** column on the **Calibration** tab page of the QNT Editor. Only the standard samples labeled **X** are considered for the calibration.

b) Analysis Structure

Injection is four times. During the first run, the first calibration point of the calibration curves of the substances A and B is determined based on the determined area values. In the second run, the second point is determined accordingly. Run three serves to determine the area values of the substances A and B of sample 1. In the fourth run, the area values of A and B of sample 2 are determined.

The following area values are resulting:

Name	Area Subst. A	Area Subst. B
STD 1 (first run)	150	200
STD 2 (second run)	300	400
Sample I	175	150
Sample II	95	180

c) Calculation of the Calibration Coefficients

From the area values of the standard samples and the corresponding concentration values, you can determine the four value pairs. The intersection of each value pair represents a calibration point. Based on the selected calibration type (in the example: ⬛ **Calibration Type (Linear)**), Chromeleon calculates the optimum course of the calibration curve; that is, the system tempts to find a course with four calibration points on or near the curve. If the course of the curve is established, the corresponding calibration coefficients (c0, c1, c2) can be calculated.

Substance	y/x-value pair	c1
A	12/150	0.08
A	24/300	0.08
B	17/200	0.085
B	34/400	0.085

With a linear course through the origin, the calibration curve can be described by one single coefficient (c1). C1 expresses the slope of the curve. If all calibration points are located exactly on the calibration line, the resulting c1 calibration coefficient is the direct y/x-quotient of each value pair.
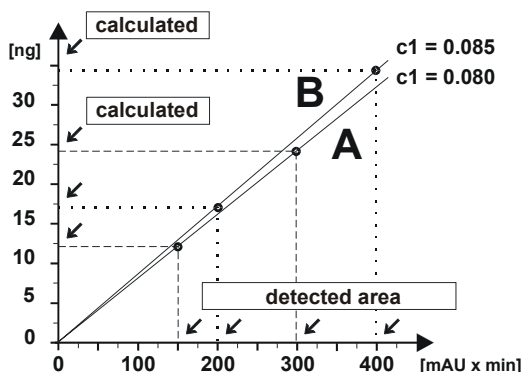
If the calibration points are not located exactly on one line, Chromeleon calculates an optimized approximate c1 value for each substance from the ➢*Calibration Function*.

If a different calibration type (**Theory of Calibration** **Calibration Type (Non-Linear)**) is used, Chromeleon also calculates the remaining calibration coefficients (c0 and c2).

d) Amount Calculation

If the calibration coefficients of a substance A are known, each area value from an unknown sample for substance A can be converted into an amount value by inserting the values in the calibration function. When inserting this value into the ➢*Formula for Amount Calculation*, the actual amount value will result.

When you perform this action for the peak areas of the substances A and B in samples I and II (the correction factors of the formula for amount calculation are assumed with 1.0), the following amount values are calculated:

Sample	Calculation	Amount. A	Amount B
I	175 x 0.08	14.00	
I	150 x 0.085		12.75
II	95 x 0.08	7.60	
II	180 x 0.085		15.30

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** **Calibrating**.

Calibration Mode: Additional

The following example illustrates the difference between the **Total** and **Additional** ⇒*Calibration Modes*.

Independently from the standard method, this mode determines which standard samples are used for evaluating a specific unknown sample. The position of the unknown sample in the sample list is decisive here.

Extending the Example: External Calibration/Mode: Total

Two standard samples are injected after two unknown autosampler have been injected. This results in an alternating list of two standard samples: two unknown samples, two standard samples, etc. All other settings are maintained; that is, two-point calibration is performed. The calibration is verified by additional replicates.

a) User Input:

Sample List

	Name	Type	Pos	Inj.Vol	Program	Method	Status
1	STD 1	Standard	1	10.0	Control 2	QNT2	Single
2	STD 1	Standard	1	20.0	Control 2	QNT2	Single
3	Sample I	Unknown	2	10.0	Control 2	QNT2	Single
4	Sample II	Unknown	3	10.0	Control 2	QNT2	Single
5	STD 1	Standard	1	10.0	Control 2	QNT2	Single
6	STD 1	Standard	1	20.0	Control 2	QNT2	Single
7	Sample III	Unknown	4	10.0	Control 2	QNT2	Single
8	Sample IV	Unknown	5	10.0	Control 2	QNT2	Single
9	STD 1	Standard	1	10.0	Control 2	QNT2	Single
10	STD 1	Standard	1	20.0	Control 2	QNT2	Single
11	Sample V	Unknown	6	10.0	Control 2	QNT2	Single
12	Sample VI	Unknown	7	10.0	Control 2	QNT2	Single

QNT Method/Peak Table Tab

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount1	Amount2
1	Subst. A	1.500 min	0.400 AG	External	Area	Lin	12.000000	
2	Subst. B	2.600 min	0.400 AG	External	Area	Lin	17.000000	

QNT Method/General Tab

If the user selects the **Additional** mode, each unknown sample is evaluated based on the standard samples analyzed so far.
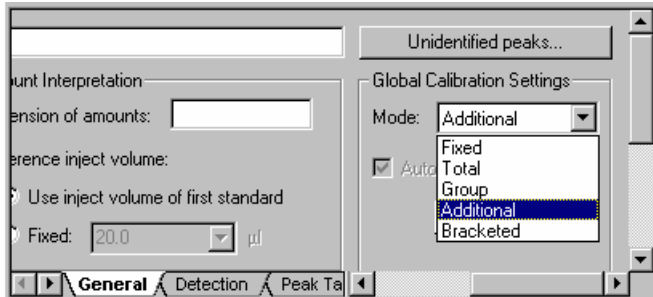
This means:

As before, samples I and II are evaluated by means of a two-point calibration. However, four analyzed standard samples are available for the samples III and IV. The result is a two-point calibration with two replicates each. Finally, the samples V and VI are evaluated by means of a two-point calibration with three replicates each.

QNT Method/Calibration Tab

The page shows all standard samples (of one sequence) that can be used for calibrating the current sample.

Press the F4 key or the SHIFT+F4 key combination to open the samples one after the other. The standard samples forming the basis for calibration are shown for each sample.

Thus, two standard samples are displayed for the samples I and II, four standard samples are displayed for the samples III and IV, and six standard samples are displayed for the samples V and VI (see below).

If you notice that an error occurred during the analysis of the standard sample, you can "exclude" this standard. Remove the standard in the **Enable** column on the **Calibration** tab page of the QNT Editor. Only the standard samples labeled **X** are considered for the calibration.

b) Evaluation:

Evaluation is similar to the example with external calibration (mode: **Total**). The only difference is:

The calibration curve is created for the different samples based on a different number of replicates.

This can affect the calculated calibration coefficients and thus the result, but does not necessarily do so. Normally this type of calibration is used to adapt to changed column conditions. Thus, it may happen that after a several samples, a specific substance cannot elute 100 percent from the column. As this will also be the case with the standard substance, the result is corrected automatically.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** **Calibrating**.

Calibration Mode: Group

The **Group** ⇒*Calibration Mode* completes the **Total** and **Additional** modes. The **Group** mode is used when there are time-dependent modifications during the analysis, for example, decomposition of the analyzed substance.

The mode is described using the example of the **Additional** mode (sample list of alternating sample pairs (2 standards, 2 samples, 2 standards, etc.).

a) User Input:

Sample List

	Name	Type	Pos	Inj.Vol	Program	Method	Status
1	STD 1	Standard	1	10.0	Control 3	QNT 3	Single
2	STD 1	Standard	1	20.0	Control 3	QNT 3	Single
3	Sample I	Unknown	2	10.0	Control 3	QNT 3	Single
4	Sample II	Unknown	3	10.0	Control 3	QNT 3	Single
5	STD 1	Standard	1	10.0	Control 3	QNT 3	Single
6	STD 1	Standard	1	20.0	Control 3	QNT 3	Single
7	Sample III	Unknown	4	10.0	Control 3	QNT 3	Single
8	Sample IV	Unknown	5	10.0	Control 3	QNT 3	Single
9	STD 1	Standard	1	10.0	Control 3	QNT 3	Single
10	STD 1	Standard	1	20.0	Control 3	QNT 3	Single
11	Sample V	Unknown	6	10.0	Control 3	QNT 3	Single
12	Sample VI	Unknown	7	10.0	Control 3	QNT 3	Single

QNT Method/Peak Table Tab

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount 1	Amount 2
1	Subst. A	1.500 min	0.400 AG	External	Area	Lin	12.000000	
2	Subst. B	2.600 min	0.400 AG	External	Area	Lin	17.000000	

QNT Method/General Tab

If the user selects the **Group** mode, each sample is evaluated based on the standard sample in the sample list that has been analyzed immediately before.

QNT Method/Calibration Tab

This page indicates all *standard samples* (of a sequence) that are used for calibrating the current sample.

Press the F4 key or the SHIFT+F4 key combination to open the samples of a sequence one after the other. The standard samples forming the basis for calibration are shown for each sample.

This means in the example that in the **Group** mode the standard samples from the lines 1 and 2 are displayed for the samples I and II. The standard samples from lines 5 and 6 are displayed for the samples III and IV, and the standard samples from lines 9 and 10 for the samples V and VI.

b) Evaluation:

Evaluation is similar to the examples with the external calibration (modes: **Total** and **Additional**). The only difference is that the calibration curve is constantly updated without considering the previously analyzed standard samples.

For the example, this means:

Samples I and II are evaluated by means of a two-point calibration of the standard samples in the lines no. 1 and 2.

In contrast to the **Additional** mode, samples III and IV are also evaluated by means of a two-point calibration. However, only the standard samples STD 1 from lines no. 5 and 6 are used.

Correspondingly, the samples V and VI are evaluated by means of the standard samples STD 1 in the lines no. 9 and no. 10.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** **Calibrating**.

Calibration Mode: Bracketed

In a bracketed calibration, a standard sample is included in a series of unknown samples so that modifications, such as column or detector drift, can also be considered in the calibration function. Amount calculation of an unknown sample is always performed using the calibration coefficients of the surrounding standard samples.

New Example

You want to determine the concentration of substance A in four samples (Samples I-IV). Two standard solutions (STD 1, STD 2) of substance A with different concentrations (50 and 100ng/µl) are available for external calibration. At the beginning, after the second and after the fourth sample, a two-point calibration is to be performed. To receive exacter results, a bracketed calibration is performed. The basis for calibrating samples I and II is provided by standards STD 1 and STD 2 from lines no. 1, 2, 5, and 6, while the samples III and IV are calibrated on the basis of the standards no. 5, 6, 9, and 10.

a) User Input:

Sample List

	Name	Type	Pos	Inj.Vol	Program	Method	Status
1	STD 1	Standard	10	20.0	Control 4	QNT 4	Single
2	STD 2	Standard	11	20.0	Control 4	QNT 4	Single
3	Sample I	Unknown	1	10.0	Control 4	QNT 4	Single
4	Sample II	Unknown	2	10.0	Control 4	QNT 4	Single
5	STD 1	Standard	10	20.0	Control 4	QNT 4	Single
6	STD 2	Standard	11	20.0	Control 4	QNT 4	Single
7	Sample III	Unknown	3	10.0	Control 4	QNT 4	Single
8	Sample IV	Unknown	4	10.0	Control 4	QNT 4	Single
9	STD 1	Standard	10	20.0	Control 4	QNT 4	Single
10	STD 2	Standard	11	20.0	Control 4	QNT 4	Single

QNT Method/Peak Table Tab

The external calibration (**Standard** = External) is performed via two standard samples (autosampler positions 10 and 11) of different concentrations. In contrast to the **Total**, **Additional**, and **Group** examples, two different ⇒*Amount* values must be entered in the corresponding amount columns [1] and [2] of the peak table.

No.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Amount 1	Amount 2
1	Subst. A	4.400 min	0.100 AG	External	Area	Lin	50.000000	100.000000

QNT Method/General Tab

To evaluate each sample based on the neighboring standard samples in the sample list, select the Bracketed ⇒*Calibration Mode*.

QNT Method/Calibration Tab

This page shows all *standard samples* (of a sequence) that are used for calibrating the current sample.

Press the F4 key or the SHIFT+F4 key combination to open the samples of a sequence one after the other. The standard samples forming the basis for calibration are shown for each sample.

For the samples I and II, the page will look is as follows:

No	Enabled	Name	Smp.No	Pos.	Inj. Date/Time	Calib. Comment	
1	☑	🔅 STD 1	1	10	12/6/00 1:20:03	Ok	
2	☐	🔅 STD 2	2	11	12/6/00 1:24:05	Disabled	
3	☑	🔅 STD 1	5	10	12/6/00 1:27:03	Ok	
4	☑	🔅 STD 2	6	11	12/6/00 1:30:14	Ok	

◀ ▶ 人 **Calibration** 人 Spectra Library Scree ◀   ▶

For the samples III and IV, the page will look as follows:

No	Enabled	Name	Smp.No	Pos.	Inj. Date/Time	Calib. Comment	
1	☑	🔅 STD 1	5	10	12/6/00 1:20:03	Ok	
2	☑	🔅 STD 2	6	11	12/6/00 1:24:05	Ok	
5	☑	🔅 STD 1	9	10	12/6/00 1:27:03	Ok	
6	☑	🔅 STD 2	10	11	12/6/00 1:30:14	Ok	

◀ ▶ 人 **Calibration** 人 Spectra Library Scree ◀   ▶

Note that the standards in line 5 and 6 are determined only once.

b) Analysis Structure:

Injection is eight times. The following area values are determined:

Name	Area substance A
STD 1 (conc.1)	218
STD 2 (conc.2)	439
Sample I	167
Sample II	152
STD 1 (conc.1)	224
STD 2 (conc.2)	442
Sample III	283
Sample IV	305
STD 1 (conc.1)	219
STD 2 (conc.2)	441

c) Calculation of the Calibration Coefficients

From the determined area values of the standard samples and the amount values from the peak table, the value pairs of the six calibration points can be listed. Note that depending on the standard (STD 1 or STD 2) the amount value is once taken from the **Amount [1]** peak table column and once from the **Amount[2]** column.

No.	Substance	y/x-value pair	y/x	c1 (K1)	c1 (K2)
1	STD 1 (conc.1)	50/218	0.2294	0.2267	
2	STD 2 (conc.2)	100/439	0.2278	0.2267	
5	STD 1 (conc.1)	50/224	0.2232	0.2267	0.2261
6	STD 2 (conc.2)	100/442	0.2262	0.2267	0.2261
9	STD 1 (conc.1)	50/219	0.2283		0.2261
10	STD 2 (conc.2)	100/441	0.2268		0.2261

If all calibration points are used simultaneously, a two-point calibration with three replicates of the same calibration level results.

As amount determination of samples I and II or III and IV is performed only according to the neighboring standard samples, there are two calibrations for substance A instead of one. Each calibration is a two-point calibration with two replicates each. That is why Chromeleon calculates two different sets of calibration coefficients.

If a linear calibration function without offset is assumed as in the previous examples, Chromeleon determines two different c1-values. One value (K1) is calculated for the numbers 1, 2, 5, and 6, and another value (K2) is calculated for the numbers 5, 6, 9, and 10.

d) Amount Calculation

If the calibration coefficients of a substance A are known, the amount of substance A contained in each sample can be calculated by inserting the peak areas determined from the unknown samples in the calibration function. Inserting this value in the ➢*Formula for Amount Calculation* returns the actual amount value.

If this action is performed for the determined peak areas of substance A in the samples I and II (taking K1 into account), the following amount values are calculated (the correction factors of the formula for amount calculation are assumed to be 1.0):

Sample	Calculation	Amount A
I	167 x 0.2267	37.86
II	152 x 0.2267	34.46

For the samples III and IV, the result is as follows (taking K2 into account):

Sample	Calculation	Amount A
III	283 x 0.2261	63.99
IV	305 x 0.2261	68.96

🖙 *Tip:*

Calculation of the amount values is continuously*; that is, after analyzing an unknown sample, the values are calculated based on the standard samples calculated so far!*

Calculation of the area values of samples I and II is performed depending on the current processing status, first with one, then with two and three, and finally with four standard samples. The same applies to the samples III and IV that are evaluated based on the standard samples in the lines 5,6,9, and 10 of the sample list.

For example, if a ➢*Report* is generated after each sample, it will only include the results from the samples analyzed so far. The result may differ from the final result.

In the **Bracketed** ⇒*Calibration Mode*, processing of all standard samples should be completed before printing the results.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** 🔳 **Calibrating**.

🔳 Calibration Mode: Fixed

To use any standard samples of an existing calibration for determining current unknown samples, perform calibration based on the **Fixed** ⇒*Calibration Mode*.

⚠️ *Caution:*

*Always perform the calibration manually (**Calibrate**). The **Auto-Recalibrate** option is not available.*

a) User Input:

Sample List

Only unknown samples are entered.

If the sample list is generated using the Sequence Wizard, decide whether to refer to an existing QNT File (which is recommended) or whether to leave out this information. When closing the Wizard, Chromeleon recognizes that the sequence does not contain any standard samples and copies the QNT File with the entire information to the sequence directory. The **Fixed** mode is selected at the same time.

If no QNT File is defined in the Sequence Wizard, enter the file name in the **Method** column of the sample list after the QNT File has been created.

QNT Method/Peak Table Tab

If an existing QNT File is copied, a peak table already exists. Change the table according to your requirements.

However, if a new (empty) QNT File is used, a new peak table must be created, also. It is not necessary to enter the ⇒*Amount* because calibration is based on an existing calibration.

QNT Method/General Tab

If an existing QNT File is copied in which the **Fixed** calibration mode is selected, this mode is automatically selected.

If a new QNT File was generated, select the **Fixed** mode. On the **Calibration** tab page, determine the standard samples to be used for the calibration.

QNT Method/Calibration Tab

If a QNT File was copied, this page lists all standard samples to which the QNT-File originally referred.

With each newly created QNT File, no standard samples are listed. Select **Insert Standard** or **Append Standard** on the context menu to insert the standard samples to be used for calibration and to evaluate the current samples according to their results.

The standard samples listed here always apply to all samples of a sequence. Press the F4 key or the SHIFT+F4 key combination to display the same maximum number of standard samples.

b) Analysis and Evaluation

Performing the analysis and the evaluation of the individual samples is similar to the previous examples.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** 🔳 **Calibrating**.

Entering the Concentration/Amount of the Validation Sample

➢ *Validation Samples* are used to check the calibration. They correspond to standard samples in as much as the amount of analyte is known. They are used to validate the calibration, but not to determine the calibration curve.

Enter the known concentration (or amounts) of the different analytes in the validation samples in the QNT Editor. The two sections below explain the ways in which the validation and standard samples can interact and the most common means to implement them. It is up to each user to decide which method is appropriate for a particular application.

1. The validation sample is equal to the standard sample

All individual concentrations/amounts correspond to the concentrations/amounts of a standard sample that has already been used for the calibration curve, especially if the validation sample is injected from a vial containing standard sample.

In this case, an ⇒*Amount* column for the corresponding standard sample is already available in the QNT Editor. The validation sample should have already been assigned to the correct vial. To verify this, proceed as follows:

- On the **Amount Table** tab page, place the cursor in the header of the **Amount** column of the corresponding standard sample. Double-click the column header to open the **Edit Amount Columns** dialog box.

- Select an **Amount** column (or **Unassigned**) in the **Amount Column** window to display the associated standards.

- Select one or more validation samples (☑) in the **Standards** window and drag them to the appropriate column in the **Amount Column** window.

- Click **OK** to close the dialog box and return to the amount table.

All values in the **Amount** column now apply to the validation sample(s), also.

2. The validation sample is not equal to the standard sample

When using a validation sample whose composition is different from all standard samples (if only in its concentration of amount of one analyte), a separate **Amount** column must be created for the validation sample. Follow the steps below:

- On the **Amount Table** tab page of the QNT Editor, double-click the header of an existing **Amount** column (or select **Columns > Edit Amount Columns** on the **Edit** or context menu) to open the **Edit Amount Columns** dialog box.

- Click **New**, type a unique column name in the edit field that appears in the **Amount Column** window, and press **Enter**.

- Select an **Amount** column (or **Unassigned**) in the **Amount Column** window to display the associated standards.

- Select one or more validation samples () in the **Standards** window and drag them to the new column in the **Amount Column** window.

- If the column created for the validation sample is valid for other validation samples or for standard samples, assign the additional standards as described in **The validation sample is equal to the standard sample**.

- Click **OK** to close the dialog box.

For information about how to use the validation sample to check the calibration curve, refer to **Validating the Calibration Curve**.

For an overview of the calibration options provided by Chromeleon, refer to **How to …:** **Calibrating**.

Displaying Calibration Curves

Chromeleon displays the ➢*Calibration Curve* calculated from the ➢*Calibration Points* in the **Calibration Curve** window. The **Calibration** tab page of the **Report** allows you to display the calibration data of the peaks of the active chromatogram.

If a single standard was excluded from the calculations (**Enabled** column) on the **Calibration** tab in the QNT Editor, the corresponding calibration point is red. Calibration points of this type will not be used for calculating the calibration curve(s) for which they were disabled (see **Disabling the Calibration Sample**). The display color of the calibration points of the current sample is purple.

In the **Calibration Curve**, select **Disable Standard** on the context menu to exclude standards, completely or in part, from the calibration. The **Disable (Enable) Standard xyz** dialog box appears for the selected calibration point. (This point refers to the selected peak in the active chromatogram. Double-click a different calibration point to select a different standard.) Define whether the corresponding standard sample shall be used for calibration purposes. You can exclude the respective standard from the calibration for all peaks and all channels (default) or for the selected peak and/or channel only.

Select **Decoration** on the context menu to perform the following actions on the different tab pages:

- Select the peak for which to show the calibration curve (**Peaks** tab page).

- Determine the axis scale (**Scale** tab page).

- Define the display and decoration of the axes, frames, and grids (**Frame & Axes** tab).

- Show validation samples (select **Draw validation sample points** on the **Options** tab). Validation samples are indicated by the following symbol: ⊕.

- Draw lines for the ➢*Confidence Interval* (**Options** tab, see 🔳 **Show Confidence Interval**)

- Change the colors of the single elements (**Colors** tab).

In addition, you can select **Next Peak** or **Prev. Peak** to display the calibration curve of the next/previous peak.

For more information, also refer to 🔳 **Displaying Values**.

🔳 Displaying Values

A purple cross marks the standard sample or the ➢*Spiked Sample* that is currently used: ⊹. (The sample has been selected on the **Calibration** tab page of the QNT Editor. For information about the editor, see **Data Representation and Reprocessing** 🔳 **The QNT Editor**.) If you disable a specific sample in the **Enabled** column on the **Calibration** tab page of the QNT Editor, e.g., because an error occurred during determination of the area value, this sample is excluded from calibration. A red asterisk marks the corresponding calibration point: ✳. This kind of calibration points is not considered when the calibration curve is determined.

To exclude standards, completely or in part, from calibration, select **Disable Standard** on the context menu of the **Calibration Curve**.

To display validation samples, Select **Decoration** on the context menu. The **Calibration Plot Decoration** dialog box is opened. On the **Options** tab page, select the **Draw validation sample points** check box. Validation samples are indicated by the following symbol: ⊕. They are not considered for calibration.
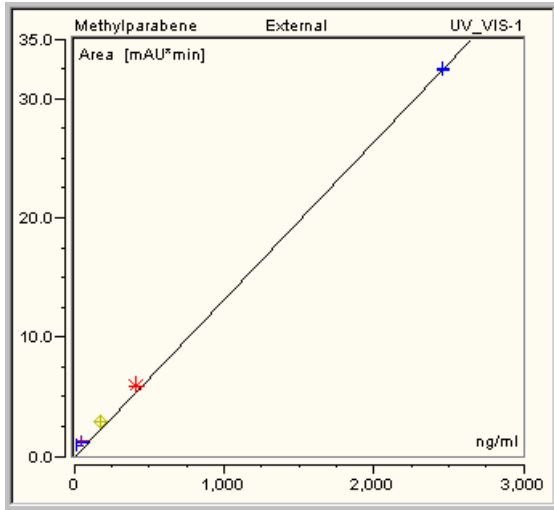
◾ Indicating the Confidence Interval

The confidence interval describes the range in which the "true" calibration curve will be found with a given probability. The range of the ➤*Confidence Interval* grows linearly with growing standard deviation. The easiest way is to indicate the confidence interval graphically.

How To

- Open the **Calibration Plot Decoration** dialog box by selecting **Decoration** on the context menu.

- Select **Draw upper & lower confidence limits** on the **Options** tab page.

- Select the probability with which the values should be within the confidence interval. The following options are available: 90%, 95%, 98%, 99%, 99.8%, 99.9%, and 99.99%.

The following example shows the confidence interval of a calibration at a probability of 99%:
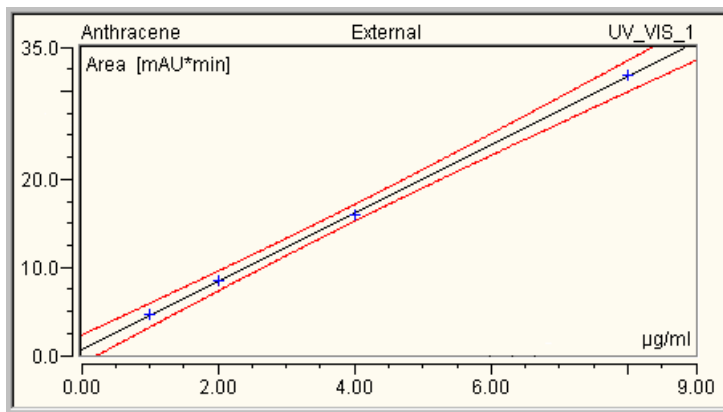
You can indicate the limits of the confidence interval in the Report using the **Upper** and/or **Lower Confidence Limit** variables of the ⇒*Peak Calibration* peak category.

For more information, refer to **How to …: Creating and Using Report Tables** 🔃 **Setting Parameters for Variables (e.g., for the confidence interval)**.

🔲 **Validating the Calibration Curve**

To check the calibration using the respective values of the ➢*Validation Samples* (perhaps of the standard sample as well) you have to compare the expected concentration or amount values to the measured/calculated values either numerically by means of the Amount Deviation report variable or visually by means of the calibration function.

1. Numerically, by means of the Amount Deviation report variable:

The determined (actual) area values are converted to concentration or amount values by means of the ➢*Calibration Function* and compared to the expected (nominal) values of the ⇒*Amount* table.

- Add a new column to a report or a ➢*Printer Layout* page or change the assignment of an existing column as described in **How to …: Creating and Using Report Tables** 🔃 **Defining the Contents of a Report**.

- Select the **Amount Deviation** variable in the ⇒*Peak Results* report category. Click **Parameter** to define whether the result of the variance comparison shall be expressed as absolute amount value or as deviation in percent (**Relative in % of the expected Amount**).

2. Visually, by means of the Calibration Curve

For checking the calibration curve visually, the validation samples can be displayed (specially marked) in the curve:

- Select **Decoration** on the context menu of the calibration curve (respectively, the **Calibration Plot Properties** command in the Printer Layout).

- Select the **Draw validation sample points** check box on the **Options** tab page.

Note:

*Even if this action is performed in the QNT Editor, these validation samples will not be considered for the calibration. Nevertheless, if you wish to use a validation sample as standard sample later and consider it for calculating the calibration curve, set the ⇒Type (Sample Type) to **Standard** in the sample list of the Browser.*

3. By Means of the Confidence Interval

a) In addition, the confidence region interval can be indicated in the calibration curve. Select the **Draw upper and lower confidence limit** check box on the **Options** tab page and define the confidence region to check whether your validation and standard samples are within the corresponding confidence interval (also, refer to **How to …: Displaying Calibration Curves** 🔼 **Indicating the Confidence Interval**).

b) For single values, you can also indicate the limits for the respective ➢*Confidence Interval* in the Report using the **Upper/Lower Confidence Limit** variables of the ⇒*Peak Calibration* report category.

For information about how to enter the concentration/amount, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔼 **Entering the Concentration/Amount of the Validation Sample**.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to …:** 🔼 **Calibrating**.

■ Inverting Dependent and Independent Variables

☞ *Tip*:

Chromeleon supports this function to improve compatibility with PeakNet5.

Normally, calibration curves are fitted using normal regression analysis, in which the known amount (or concentration) of the analyte is treated as the independent variable (x) and the corresponding response (peak area or peak height) is treated as the dependent variable (y):
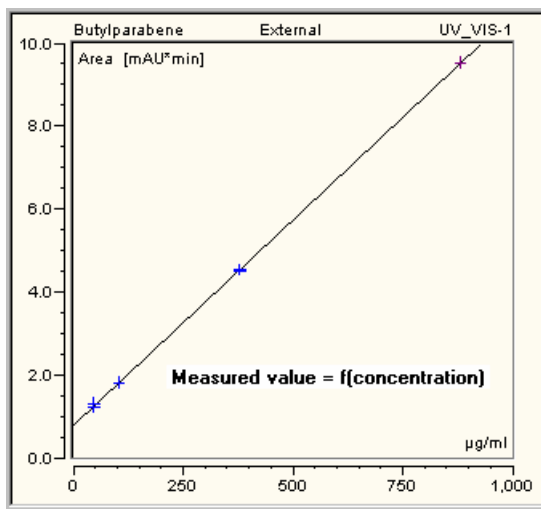
Many legacy data systems use inverted curve fitting, which treats the response as if it were the independent variable (x), and amount as if it were the dependent variable (y):
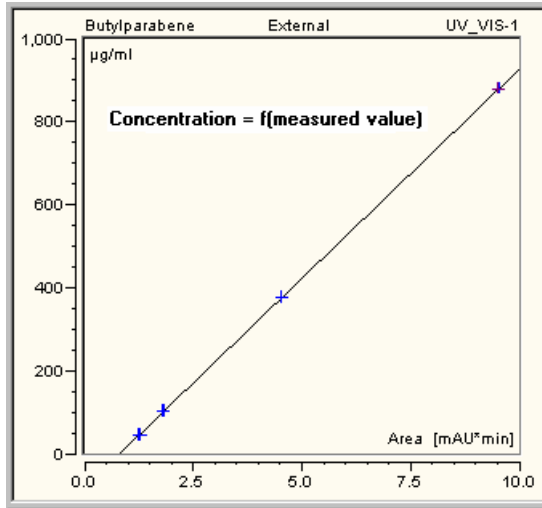
You can select the **Inverted** curve fitting option on the **General** tab page:
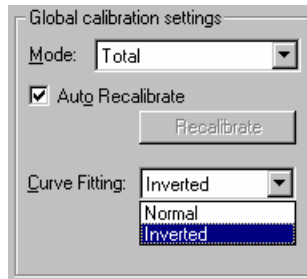
Although standard statistical theory does not support inverted curve fitting, it was often taken because it makes amounts of analytes in unknowns easier to calculate when quadratic and cubic calibration functions are used. With linear regression, the differences between normal and inverted curve fitting are usually small, but the differences can be significant with higher-order polynomials. In a few applications, most notably the determination of ammonium by suppressed conductivity detection, a better fit to the empirical data may actually be achieved using inverted curve fitting.

To provide consistency of results for customers migrating from legacy systems, and to satisfy users of special applications, Chromeleon supports inverted curve fitting via an option in the General tab page of the QNT Editor.

Methods imported from PeakNet 5 default to using inverted curve fitting, the approach used by PeakNet 5. Normal curve fitting remains the default setting for new methods created in Chromeleon.

⚠️ *Caution:*

It is the user's responsibility to decide whether inverted curve fitting is sensible for their application.

If you select **Inverted**, the dependent and independent variables are inverted. Thus, different values are usually obtained for the following variables:

⇒*Peak Results* **Category**

- ⇒*Amount* (concentration or amount)

- Amount Deviation (deviation from the expected concentration or amount)

- Concentration (concentration, if **Amount** is really an amount)

- Relative Amount (relative concentration or amount)

- Group Amount (concentration or amount of a group)

⇒*Peak Calibration* **Category**

- ➢*Offset c0* (intersection with the y-axis)

- ➢*Slope c1*

- ➢*Curve c2* (curvature)

- ➢*Cubic Coefficient c3*

- ➢*RF Value* (1/slope)

- ➢*Variance*

- ➢*Variance Coefficient*

- ➢*Standard Deviation*

- ➢*Relative Standard Deviation*

- ➤*Coefficient of Determination*

- ➤*DOF-Adjusted Coefficient of Determination*

- Calibration Point X

- Calibration Point Y

- Evaluation of Cal.Function for X ()

- Residual for Calibration Point X

- Upper Confidence Limit (upper limit for the ➤*Confidence Interval*)

- Lower Confidence Limit (lower limit for the confidence interval)

Working with Chromatograms

All operations for processing single chromatograms are performed in the **Chromatogram** window. This window displays the integrated chromatogram of the current sample. The peak areas are determined and integrated automatically, based on the ⇒*Detection Parameters*.

If the representation and/or evaluation of single samples do not correspond to the expected result, you can select another chromatogram section to specifically change the peak delimiters and the baseline manually. It is also possible to display more chromatograms for comparison. In addition, you can assign the peaks manually and define a baseline for the entire chromatogram (averaged baseline). You can also apply a data filter to smooth the chromatogram and to improve the reproducibility of the peak baselines.

For more information, refer to:

■ **Using Keyboard Shortcuts in the Chromatogram**

■ **Manual Re-Integration**

■ **Manual Peak Assignment**

■ **Comparing Chromatograms**

■ **Performing Data Smoothing**

You can also generate additional channels after data acquisition. Refer to:

■ **Copying a Channel**

■ **Combining Channels via Arithmetic Operations**

Due to the increased noise, Dionex recommends performing background subtraction for mass spectra. This is also possible for UV channels. In both cases, determine background subtraction in the chromatogram (see ■ **Subtracting Background Spectra**).

In addition, you can ■ **Display Peak Information in a Separate Area**.

To graphically move the retention window, refer to ■ **Moving a Retention Window Graphically**.

If you want to change the detection parameters, for example, because peaks that are too small or unimportant are integrated or because the baseline does not meet the requirements, you can perform these changes graphically in the chromatogram; see **Defining Detection Parameters Graphically**.

You can define the detection parameters according to your requirements. All detection parameters are available in the QNT Editor on the **Detection** tab page (see **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Detection Parameters**; for information about the editor, refer to **Data Representation and Reprocessing** **The QNT Editor**).

For more information about how to display a spectra plot, an I-t plot, or a ➢*Report*, refer to **How to …:**

 Displaying and Using UV Spectra

 Displaying and Using I-t Plots

 Creating and Using Report Tables

Using Keyboard Shortcuts in the Chromatogram

Often, it may be necessary to display specified chromatogram areas only. In this case, use the mouse to zoom to the area. In addition, keyboard keys and key combinations are supported for user-friendly operation. These keys refer to the number and navigation area on the keyboard.

Scrolling

<←> = <LEFT> scrolls all chromatograms to the right; <→> = <RIGHT> scrolls them to the left. If you press the respective key, the chromatogram is scrolled by 5% of the window width.

<↑>= <UP> (<↓> = <DOWN>) scrolls the active chromatogram plot up (down). If you press the respective key, the plot is scrolled by 10% of the plot height.

 Tip:

Scrolling beyond the chromatogram limits is supported for both cases.

<HOME> (<END>) brings you to the first (last) datapoint of the active chromatogram. The entry is ignored if the chromatogram is already displayed in full-size mode.

Zoom/UnZoom

Press <x> (MULTIPLY) to zoom in horizontally by 20%. The anchor point is the center of the time axis; for example, 0.0 to 10.0 min > 1.0 to 9.0 min.

Press <÷> (DIVIDE) to unzoom horizontally by 25%. The anchor point is the center of the time axis.

Press <CTRL><x> or <CTRL><÷> to display all chromatograms in full size. This action corresponds to an Autoscale of the time axis.

Press <+> (PLUS SIGN) to zoom in vertically by 20%. The anchor point is 10% of the signal height because the baseline region at the beginning or end of a peak is usually more interesting than the course of the peak at its maximum; for example, 0.0 - 100.0 mAU > 2.0 - 82.0 mAU.

Press the <-> (MINUS SIGN) key to vertically unzoom by 25%. The anchor point is near the baseline as described above.

☞ *Tip:*

If the unzoom operation is performed after the zoom operation, the original chromatogram view will be restored.

Press <CTRL><+> or <CTRL><-> to autoscale the active chromatogram.

Press <NUM5> to autoscale the time and signal axes in all currently open plots. This operation corresponds to the **Full Size** command on the **View** menu.

Peak Selection

Press <CTRL><←> to select the previous peak of the active chromatogram. If no peak is selected, the first peak of the chromatogram will be selected.

Press <CTRL><→> to select the next peak of the active chromatogram. If no peak is selected, the first peak of the chromatogram will be selected.

Press <CTRL><HOME> to select the first peak of the active chromatogram.

Press <CTRL><END> to select the last peak of the active chromatogram.

�’ *Tip:*

If the newly selected peak is outside the current view, the peak is automatically scrolled into the view.

Chromatogram Selection

The following keys are available for chromatogram ➢*Overlay*:

Press <PAGE UP ↑> or <CTRL><↑> to activate the next upper chromatogram; that is, the chromatogram whose caption is found above the active chromatogram.

Press <PAGE DOWN ↓> or <CTRL><↓> to activate the next lower chromatogram; that is, the chromatogram whose caption is found below the active chromatogram.

Chromeleon supports many additional " quick access" keys. For a summary of the most important keys, refer to the table in **Basic Operation** 🔳 **Quick Access Keys**.

Manual Re-Integration

On the Integration ➢*Toolbar*, Chromeleon provides different icons for manual re-integration of a chromatogram via mouse-click. However, please note that these actions affect only the active chromatogram:

🔳 **Moving Peak Delimiters**

🔳 **Modifying the Baseline Manually**

🔳 **Inserting or Deleting Peaks**

🔳 **Changing the Peak Type**

🔳 **Defining an Averaged Baseline**

Prerequisite

Manual re-integration is possible only for integrated chromatograms. They have a red baseline below the peaks and blue peak delimiters on the left and right ends of the baseline (= integration limit).

☞ *Tip:*

*To display these elements on the chromatogram, select **Decoration > Peak Decoration** on the context menu.*

For manual re-integration, Dionex recommends zooming the area in which you want to make the changes. Select **Automatic** on the context menu. The corresponding mouse pointer will then mark all places in the chromatogram that can be changed manually. The shape of the mouse pointer indicates which action can be performed.

If you have not yet saved the modifications, you can undo them by selecting **Delete Manipulations** on the **Edit** menu.

▪ Moving Peak Delimiters

Near the peak delimiters (blue color by default), the mouse pointer changes its appearance (if the **Automatic Tool** or the **Delimiter Tool** is selected). The ⬐ pointer indicates the peak start and the ⬑ pointer indicates the peak end.

Left-click and move the mouse pointer to the new peak start or peak end.

It is not possible to cross another peak delimiter. This peak delimiter is moved in the same direction, too. After positioning, Chromeleon draws a new baseline. Chromeleon immediately updates and displays the modified peak properties (Area, Width, Amount, etc.) in the integration report.

📋 *Notes:*

You can also move the peak delimiters of negative peaks.

For special cases, it is possible to move the peak delimiter beyond the peak maximum. However, this is usually not reasonable.

▪ Modifying the Baseline Manually

The usual way to determine the baseline is to use the detection parameters of the QNT Method (see **Integrating Chromatograms and Identifying Peaks/Defining Detection Parameters** ▪ ;**Modifying the Baseline**). However, in some cases it may be necessary to modify the baseline for a single chromatogram:

To integrate two peaks individually that are not completely separated in the chromatogram, drop a perpendicular line from the minimum between the two peaks to the baseline. The intersection with the baseline is referred to as "baseline node" (a).
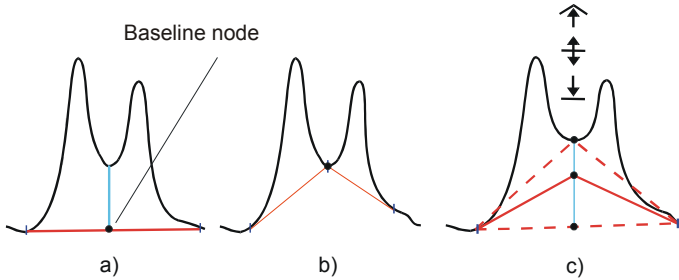
To draw the baseline from peak end to peak end, enable the ⇒*Valley to Valley* detection parameter. The baseline node automatically moves towards the signal curve until it rests on the curve in the minimum between two peaks (b).

You can move each baseline node along the perpendicular line and position it at any position ("freely floating baseline node" (c)). If you approach state (a) or (b), the mouse pointer automatically clicks into place. The shape of the mouse pointer (⬈, ↕) indicates which action is currently performed. Select the **Automatic Tool** or the **Baseline Tool**.

At the end of a baseline, you can generate freely floating baseline nodes (d, e).
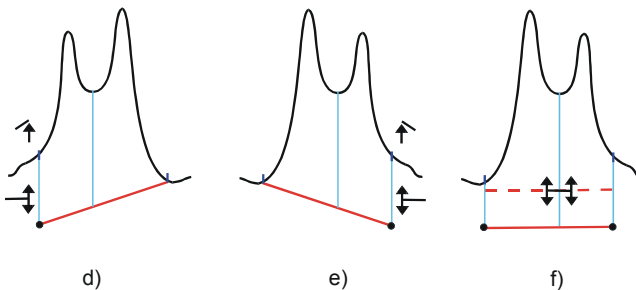
Depending on the direction in which you drag the mouse pointer, the system automatically distinguishes between moving a peak delimiter horizontally and moving a baseline node vertically.

Between two baseline nodes, you can move the entire baseline in vertical direction (f). The shape of the mouse pointer (⇕) indicates whether you can perform this action.

 Note:

For exponentially skimmed ➢Rider Peaks, you cannot move the baseline manually.

Inserting or Deleting Peaks

Inserting a Peak

You can later insert a new peak, i.e., a baseline and two peak delimiters, at any free position on the chromatogram and on the leading and trailing edges of a peak. The new peak is added to the peak table in the QNT Editor, also.

If a small peak (⋀) appears on the right side of the mouse pointer, you can insert a new at this position. If a warning sign (⊘) appears instead, you cannot insert a new peak.

- Select **Insert Peak Tool** on the context menu to have the mouse pointer indicate only those positions where you can or cannot insert a peak. If the Insert Peak Tool is active, you cannot perform any other actions (edit modes).

Deleting a Peak

- To delete a peak, point to the peak.

- Select **Delete Peak** on the context menu to remove the peak's delimiter and its baseline. This also removes the peak from the peak table in the QNT Editor.

Splitting Peaks

Sometimes only one peak is recorded when two substances elute approximately at the same time. It may happen that one peak is detected instead of two. This depends on the concentration ratio of the substances and the arrangement of the single peaks. You can then add a second peak below the existing one (also, refer to **Inserting or Deleting Peaks**). The following commands are available:

Split Peak

Move the mouse pointer to the position where you want to split the existing peak. Select **Split Peak** on the context menu to split the peak into two main peaks. The perpendicular line splits the peak exactly at the pointer position.

Note:

Splitting the peak does not change the baseline.

Shape Shoulder

Select **Shape Shoulder** on the context menu to exponentially skim a ➤*Peak Shoulder* from the existing peak. First, move the pointer to the position for the peak start of the peak shoulder and select **Shape Shoulder**. The exponential-skimming algorithm automatically calculates the peak end for the peak shoulder.

Note:

The shoulder maximum is calculated relative to the baseline. Thus, it must not correspond to the highest signal value.

Dionex recommends using the ⇒*Peak Shoulder Threshold* to define shoulder shaping for the entire quantification method.

Use the ⇒*Rider Threshold* and ⇒*Maximum Rider Ratio* detection parameters to define the peaks that will be detected as rider peaks. For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Rider Peaks**.

Changing the Peak Type

To change the peak type, point to the respective peak.

- Select **Change to Main Peak** or **Change to Rider** on the context menu.

Use this function to change a main peak into a ➤*Rider Peak* and vice versa. Single peaks are interpreted always as main peaks. That is why you cannot convert them into rider peaks. The context menu indicates: **Can't Change Peak Type**.

With automatic classification, the ⇒*Rider Threshold* and ⇒*Maximum Rider Ratio* parameters determine whether a peak is classified as a main peak or a rider.

◼ Defining an Averaged Baseline

By means of two points entered in the chromatogram, you can define a baseline for the entire chromatogram (= averaged baseline):

How To

- In the chromatogram, right-click to select an area at the beginning of the chromatogram. The first ⇒*Baseline Point* shall be in the middle of this area.

- Select **Set Averaged Baseline Start** on the context menu.
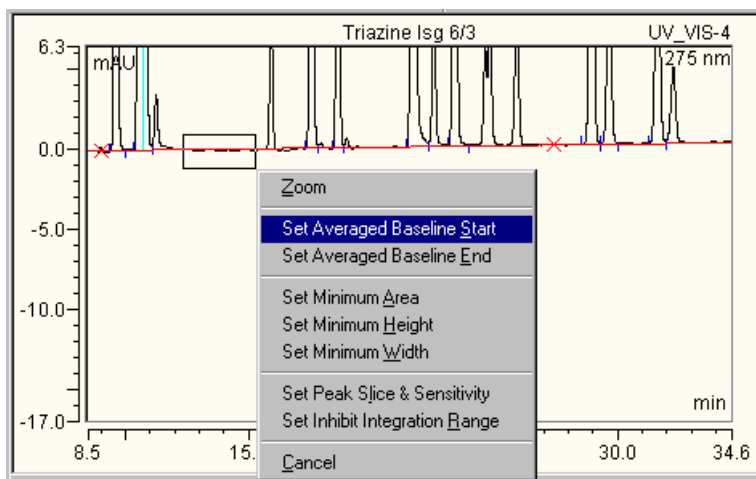
This sets the first baseline point. The x-value is the middle of the selected area while the y-value is the averaged value of the signal values weighted by the data rate. This first value is not marked, as it is not sufficient for setting the baseline. A second point is required as well.

📑 *Note:*

If you exit Chromeleon without having entered the second point, the first point is deleted.

Enter the second point as follows:

- In the chromatogram, right-click and select an area somewhere at the end of the chromatogram. The second baseline point shall be in the middle of this area.

- Select **Set Averaged Baseline End** on the context menu.

This action sets the second baseline point. The new baseline is drawn through both points, which are marked by a red cross.

You can modify the averaged baseline later in two ways:

1. Select a new baseline point, which then replaces one of the former ones.

2. Use the baseline tool on the Integration ➤*Toolbar*, see **How to …: Working with Chromatograms** 🔼 **Modifying the Baseline Manually**.

Manual Peak Assignment

(Dialog box "Properties of Peak No. x")

If you have inserted a new peak, or wish to name existing or unidentified peaks, or rename incorrectly identified peaks, you can do this in the chromatogram either via the QNT Editor (see **Data Representation and Reprocessing** 🔼 **The QNT Editor**) or in a report.

- Click the respective peak and select **Peak Properties** on the context menu to open the dialog box **Properties of Peak No. x** or double-click the peak.

- Enter the corresponding name under **Component** or select one of the names listed in the combo box. Click <Return> or the lowest symbol bottom to insert the peak into the peak table of the QNT Method using the retention time of the active chromatogram and the settings of the previous peak (as far as sensible).
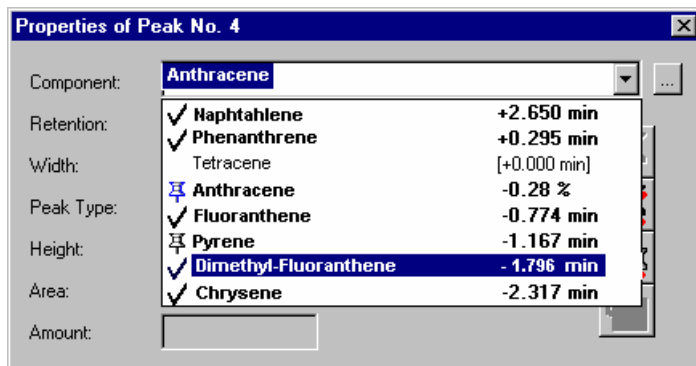
- It is also possible to rename an identified peak by manually assigning it a different name. Enter the new name or select one of the names listed in the combo box and confirm your entry by clicking the first symbol button. Via the additional symbol buttons, you can cancel this action or delete all manual peak assignments (see quick info for the buttons). Manual peak assignments of identified peaks are not transferred to the QNT Method but apply to the currently open chromatogram only.
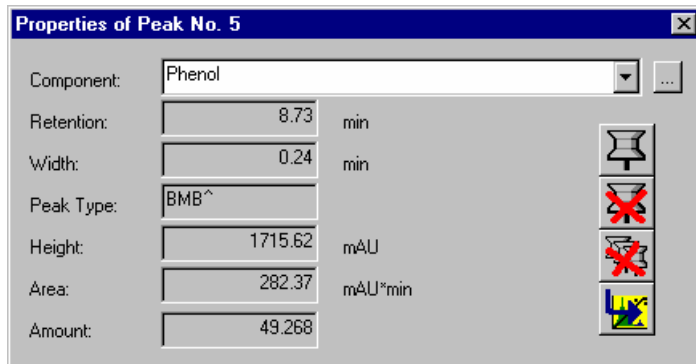
You do not have to close the dialog box to continue working in the chromatogram. <Return> accepts a new entry in the peak table of the QNT Editor (if the peak has not yet been assigned there). The dialog box then indicates the values of the next peak to be assigned. The dialog box remains open until explicitly closed or until the chromatogram is closed.

 Note:

The manual peak assignments are saved in the ➢Quantification Method. However, manual peak assignments for identified peaks are not included in the QNT Method and are valid for the currently open chromatogram only.

Comparing Chromatograms

Chromeleon allows you to compare several chromatograms by simultaneously displaying different samples or several ➢*Channels* of the same sample. Besides, you can also compare different channels of different samples. To compare chromatograms, select the samples or channels and relate them to each other.

For more information, refer to:

 Selecting the Samples and Channels

 Displaying Several Chromatograms

 Mirroring Chromatograms

 Normalizing Chromatograms

 Printing Overlaid Chromatograms

Selecting the Samples and Channels

Different alternatives are available to select samples and channels that are to be displayed as ➢*Overlays*:

- Display the chromatogram for a sample in an integration window, e.g., by double-clicking the sample name in the ➢*Browser*.

- Afterward, select one or several samples of a ➢*Sequence* in the Browser and drag the sample(s) into an open integration window while holding down the left mouse button. The action is indicated by a **+** sign on the mouse pointer. When you release the mouse button, the chromatogram of the sample(s) is displayed, showing the same channel as for the sample already displayed.

  Instead of selecting a sample in the Browser and dragging it into an open integration window, you may also select the sample in the integration view using the **Add Overlay** command on the **File** menu.

The chromatogram of the first sample will then be overlaid by the chromatogram of the selected one.

Or:

- Select several samples in the Browser and then select **Compare** on the context menu. The **Compare** submenu lists all recorded channel types. After selecting a channel, the chromatograms of all samples with raw data for this channel are compared.

Or:

- Select a sample in the Browser, and then select **Open > All Channels** on the context menu to compare all channels of a sample in a separate window. Press F10 or SHIFT+F10 to display the channels of all samples in a sequence successively.

  If several samples are selected, all channels of the first selected sample are displayed. Press F4 or SHIFT+F4 to browse through the selected samples.

Or:

- Hold down the CTRL key and click the **Next** (or **Previous**) **Chromatogram** icon (or) to insert the chromatogram of the next (previous) sample.

Or:

- Perform a ➢*Query* over several sequences or ➢*Datasources*. Select **Compare** on the context menu. The **Compare** submenu lists all recorded channel types. After selecting a channel, the chromatograms of all samples with raw data for this channel are compared.

Or:

- Hold down the CTRL key and click the **Next Channel** icon ( ) to insert the next channel of the same sample.

- Hold down the CTRL key and click the **Previous Channel** icon ( ) to insert the previous channel of the same sample.

Chromeleon supports several actions for overlaying chromatograms. For an overview, refer to **Comparing Chromatograms**.

▣ Displaying Several Chromatograms

After adding an ➢*Overlay* (= an additional chromatogram), you can define the display arrangement for the two chromatograms.

How To

- Select **Decoration** on the context menu of the chromatogram plot.

- In the **Chromatogram Decoration** dialog box, select the **Comparison** tab page and define the chromatogram **Arrangement**:

  Select **Overlay** to overlay the single chromatograms on one single plot (see the left section of the screenshot below).

  Select **Stack** to display the chromatograms one below the other in different plots (see the right section of the screenshot below).

  Select **Mixed** to combine the **Overlay** and the **Stack** option. Chromatograms of different detectors are displayed as single plots.
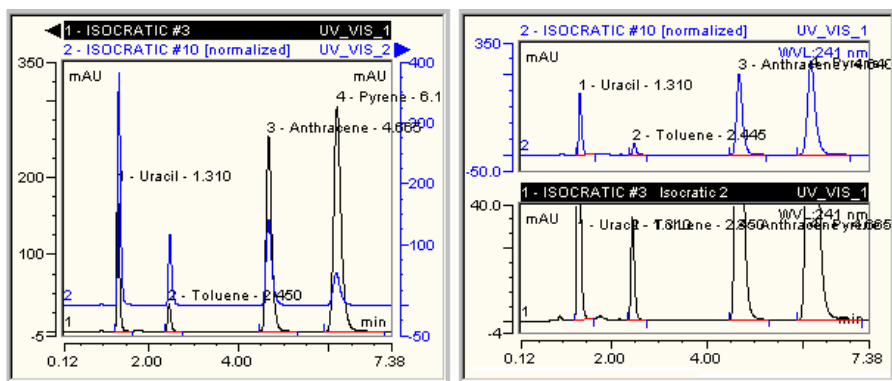
Use the **Overlay** view to add an additional signal axis (on the right). The axis refers to the overlaid chromatogram that was added last using the **Overlay with right signal axis** command. This command, too, is available on the **Comparison** tab page of the **Chromatogram Decoration** dialog box. Thus, you can select a different signal range for the chromatogram that was added last. Use the **Stack** view for individual scaling of the signal axis for each single chromatogram.

To offset the chromatograms in x- and/or y-direction in the **Overlay** view, set the signal or time offset on the **Comparison** tab page.

- Select the **Time** check box to move the active chromatogram in the x-direction.

- Select the **Signal** check box to move the active chromatogram in the y-direction.

The offset is specified in percent of the signal or time axis. A signal offset of 5% shifts each of the following chromatograms upwards by 5%. Accordingly, a time offset causes a percentage alteration in the x-direction. This results in a "pseudo-3D" presentation.

Chromeleon supports several actions for overlaying chromatograms. For an overview, refer to **Comparing Chromatograms**.

Mirroring Chromatograms

If you have inserted an ➤*Overlay* (an additional chromatogram), you can mirror the overlay.

- Select **Decoration** on the context menu on the chromatogram.

- In the **Chromatogram Decoration** dialog box, select the **Comparison** tab page. Verify that **Overlay** is selected under **Arrangement**.

- You can mirror the chromatogram that was added first by selecting the **Mirror chromatogram** check box in the **Overlay** section.
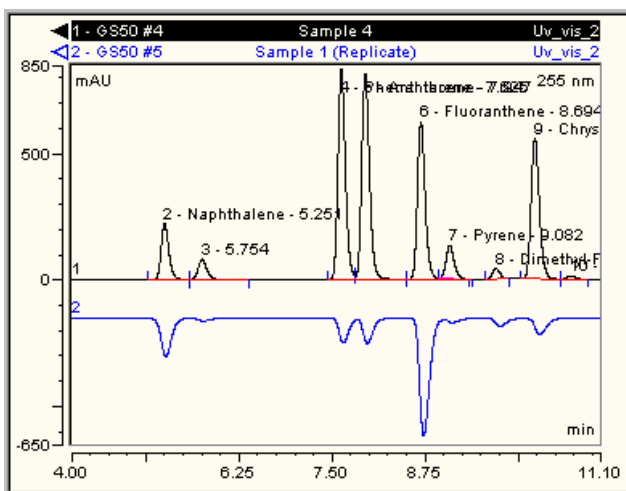
If you have selected the **Overlay with right signal axis** check box, also, this setting is ignored. In this case, the overlay is displayed with the right signal axis; it is not mirrored. However, if you add another chromatogram, it is displayed with the right signal axis and the previous chromatogram is mirrored.

Chromeleon supports several actions for overlaying chromatograms. For an overview, refer to ■ **Comparing Chromatograms**.

■ Normalizing Chromatograms

For a sensible comparison of different chromatograms, often a common reference point is chosen for the overlaid chromatograms (see ➤*Overlay*). The chromatograms are normalized. As the default, the ⇒*Retention Time* is used for the normalization, that is, all chromatograms are arranged so that their start points match (t = 0).

Alternatively, chromatograms can use a common peak as their reference point.

- Select **Decoration** on the context menu and open the **Comparison** tab page.

- Select the reference peak from the **At Peak** list box.

- Select **Shift** if this is to be performed independently from the length of the chromatogram.

- Select **Stretch** if the chromatograms are to be stretched or compressed in x-direction, so that there is a time scaling in all chromatograms. The stretch 0 to 1min (or n min) has the same length for all chromatograms.

- Enable the **Normalize Signal** check box if the height of the selected chromatogram peak should match in addition. Depending on how much the reference peak must be stretched or compressed, all other peaks in the chromatogram are also stretched or compressed. This enables amount estimation for the same peaks from different chromatograms. This option is not available in the **Stack** view.

Chromeleon supports several actions for overlaying chromatograms. For an overview, refer to ■ **Comparing Chromatograms**.

▣ Printing Overlaid Chromatograms

On single-colored printouts, it is difficult to distinguish multi-colored chromatograms. To enhance legibility, determine that dashed and dotted lines are used for the chromatograms in the ➢*Printer Layout* and printout:

On the Browser, select **Preferences** on the **File** menu to open the **Preferences** dialog box. On the **Print** tab page, select the **Use dashed and dotted lines instead of colored lines** check box. To define the line width, select an option from the **Line weight** drop-down list. Select **hair** for very thin lines. Or else, select an entry between **1 pt**, which is the default setting, and **10 pt** (for thick lines).
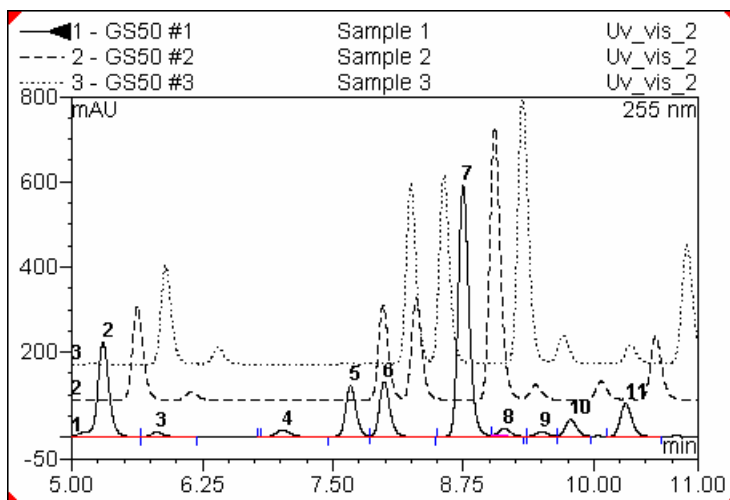
Chromeleon supports several actions for overlaying chromatograms. For an overview, refer to ▣ **Comparing Chromatograms**.

Performing Data Smoothing

Data ➢*Smoothing* can help improve the appearance of chromatograms and the reproducibility of peak baselines by reducing noise through digital filtering. Smoothing affects both the display and the integration of the chromatogram. When smoothing is completed, the new chromatogram is displayed overlaid on the original chromatogram. The original data file is **not** altered and the smoothed data is saved separately.

☞ *Tip:*

You can also define data smoothing in the ➢Post-Acquisition Steps view of the PGM Editor (see **Control** 🔳 *The PGM Editor). For instructions, see below.*
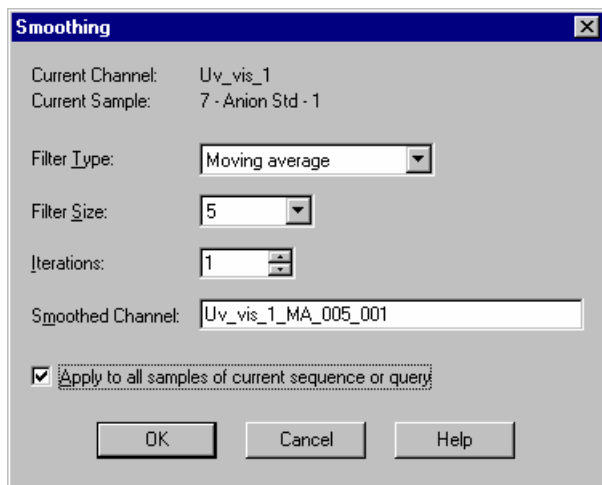
How To

1. Display the sample's chromatogram by double-clicking its name in the ➢*Browser*.

☞ *Tip:*

Open the PGM File in which you want to define data smoothing and select the **Post-acquisition steps** *view, instead.*

2. Select **Smoothing** on the context menu. The **Smoothing** dialog box appears:

☞ *Tip:*

To open the **Smoothing** *dialog box from the PGM Editor: Select* **Insert line** *on the context menu; when the* **New post-acquisition step** *dialog box appears, select* **Smooth data**.

3. Select the **Filter Type, Filter Size**, and **Iterations**. For more information, refer to 🔳 **Data Smoothing**.

4. In the **Smoothed Channel** box, enter a name for the smoothed channel. The suggested name is the current channel name, followed by the type of filter (MA for Moving Average, OL for Olympic, or SG for Savitzky-Golay), the filter size, and the number of iterations.

5. To smooth all the samples in the sequence or ➢ *Query*, select the **Apply to all samples in the current sequence or query** check box.

🖐☞ *Tip:*

*This option is not provided in the **Smoothing** dialog box in the PGM Editor.*

6. Click **OK** to start the smoothing.

For information about how to smooth MS chromatograms during mass trace extraction, refer to **How to …: Using Mass Spectrometers** 🔲 **Extracting Mass Traces Afterward**.

How to delete a smoothed channel

- Right-click the sequence name to open the context menu.

- Select **Delete** to open the **Delete Sequence "<Name>"** dialog box.

- Select **Only selected raw data**.

- Select the smoothed channel.

- Click **Yes** to delete the channel.

🖐☞ *Tip:*

This action deletes the channel from all samples in the sequence.

Copying a Channel

Chromeleon allows you to copy a channel to evaluate the same raw data in different ways; for example, to determine both the ingredients and the contaminants of a pharmaceutical product in one chromatogram. To do so, evaluate the original channel with one QNT Method, and then evaluate the copied channel with a different QNT Method.

🖘 *Tip:*

You may also define copying of channels in the ➤Post-Acquisition Steps view of the PGM Editor (see Control 🔲 The PGM Editor). The sample ➤GUID from the raw data file is copied automatically. For instructions, see below.

How To

1. Display the sample's chromatogram by double-clicking its name in the ➤*Browser*.

🖘 *Tip:*

Open the PGM File in which you want to specify that a channel is copied and select the Post-acquisition steps view, instead.

2. Select **Copy Channel** on the context menu. The **Copy Channel** dialog box appears.

🖘 *Tip:*

To open the Copy Channel dialog box from the PGM Editor: Select Insert line on the context menu; when the New post-acquisition step dialog box appears, select Copy Channel.

3. In the **New Channel** box, enter a name for the new channel. The suggested name is the name of the current channel plus _COPY.

4. To copy the channel for all samples in the sequence or ➤*Query*, select the **Apply to all samples in the current sequence or query** check box.

🖘 *Tip:*

This option is not provided in the Copy Channel dialog box in the PGM Editor.

5. Click **OK** to start the procedure.

Combining Channels via Arithmetic Operations

Chromeleon also allows you to combine two channels of different samples, using arithmetic operations. Each data point is created by combining the associated two data points from the existing channels (i.e., the data points at the corresponding time), using the selected operation.

Arithmetic combinations are not restricted to chromatograms in the strict sense. They can be used for all 2D channels, except for temporary channels.

☞ *Tip:*
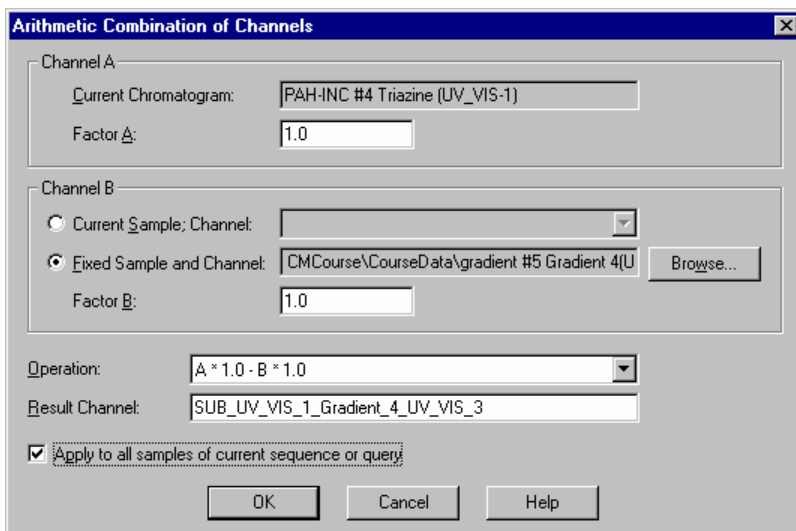
You may also define the combination of two channels in the ➢Post-Acquisition Steps view of the PGM Editor (see Control ▧ The PGM Editor). Please observe the corresponding hints below.

How To

1. Display the sample's chromatogram by double-clicking its name in the ➢*Browser*.

☞ *Tip:*

*Open the PGM File in which you want to define that two channels are combined and select the **Post-acquisition steps** view, instead.*

2. Select **Arithmetic combination of channels** on the context menu. The **Arithmetic combination of channels** dialog box appears.

Tip:

*In the PGM Editor, follow the steps below: Select **Insert line** on the context menu to add a new post-acquisition step. The **New post-acquisition step** dialog box is opened. Select **Arithmetic combination of channels** to open the **Arithmetic combination of channels** dialog box.*

3. The current channel is **Channel A**. Select the factor with which the channel shall be multiplied.
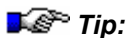
Tip:

In the PGM Editor, select the channel of the corresponding sample from the channels defined in the PGM File. Channels defined in previous post-acquisition steps are also available for selection.

4. Select a channel of the same sample or of any other sample of your choice as Channel B. Also, determine the factor with which Channel B is multiplied.

5. Select an **Operation**.

6. In the **Result Channel** box, enter the name for the new channel. The suggested name is the abbreviation for the operation plus the names of the two channels. However, you are free to enter any name of your choice as well.

7. To combine the selected channels for all samples in the sequence or ➢*Query*, select the **Apply to all samples in the current sequence or query** check box.

Tip:

*This option is not provided in the **Arithmetic combination of channels** dialog box of the PGM Editor.*

8. Click **OK** to start the procedure.

Subtracting Background Spectra

➢*Mass Spectra* usually have a higher noise level than UV spectra. Therefore, you may want to subtract the background spectra. (However, for UV channels, it is usually not necessary to change the default background subtraction.) There are two ways to subtract background spectra.

Peak Dependent Background Subtraction

Enable **Background Subtraction** on the context menu of the chromatogram. Return to the context menu and select **Peak Dependent Ranges**. Chromeleon automatically determines two ranges for calculating the background and then subtracts the entire mass or UV spectrum of these ranges. On the **MS** or **UV** tab page of the QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor**), define the number of mass spectra or UV spectra to be used for the two ranges. (For more information, refer to **How to …: Using Mass Spectrometers** 🔲 **Processing Mass Spectra** and/or **How to …: Displaying and Using UV Spectra** 🔲 **Processing UV Spectra**.)

Fixed Background Subtraction for the Entire Chromatogram

At the beginning of the chromatogram, hold down the right mouse button and select the baseline range for which the mass spectra (UV spectra) is subtracted. A context menu appears. Select **Set Background Subtraction Range 1** to use the defined range as the first range for which to subtract the mass spectra (UV spectra) from the mass spectra (UV spectra) of the single peaks and/or from the retention time spectrum.

🔲 *Note:*

*Selecting **Set Background Subtraction to Range 1** automatically enables the **Fixed Background Ranges** option of the **Background Subtraction** command on the context menu.*

In the same way, select a baseline range at the end of the chromatogram. Select **Set Background Subtraction Range 2** on the context menu to define this range as the second range for which mass spectra (UV spectra) is subtracted from the mass spectra (UV spectra) of the single peaks.

 Note:

It is useful, but not imperative, to define a second range; background subtraction can also be performed using only the mass spectra (UV spectra) of the first range.

The two defined ranges are marked by a horizontal line (in the same color as the spectrum, or in blue if no spectrum is available) and labeled "SB1" or "SB2" (SB = subtracted background). If the background subtraction range was used, the number of single mass spectra or UV spectra that were averaged is indicated in parentheses:
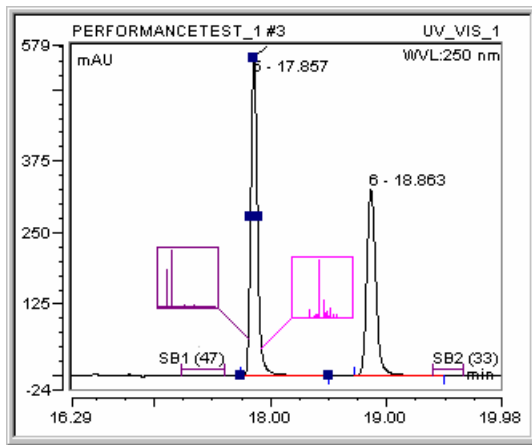
 Note:

It is possible to set the two ranges for background subtraction of mass spectra in a UV channel. However, Dionex recommends setting the two ranges in the corresponding MS channel because the respective peaks are visible only there.

Clear Background Subtraction Ranges removes the previously defined ranges.

Effects

The settings selected in the chromatogram are saved in the QNT File of the current sample. These settings overwrite the settings on the **MS** or **UV** tab page of the QNT Editor. It makes no difference whether background subtraction is defined on the **Integration** plot or in the QNT Editor. Thus, your input affects all samples that are evaluated using this QNT File.

📑☞ *Tip:*

*Make sure that no peak of another sample is within the retention time used as the **Background Subtraction Range** in **Fixed** mode.*

For a mass spectrum (UV spectrum) that was recorded between two ranges, the ranges are averaged and the result is subtracted. The mass spectra (UV spectra) of the two ranges are weighted based on the time distance from where the respective mass spectrum (UV spectrum) was recorded; that is, the range that is nearer to the respective mass spectrum (UV spectrum) is considered more.

If a mass spectrum (UV spectrum) is not located between the two ranges, only the averaged mass spectrum (UV spectrum) of the range next to the spectrum is subtracted.

Displaying Peak Information in a Separate Area

Chromeleon supports displaying the peak information in a separate area above the actual chromatogram:

- Double-click the chromatogram to open the **Chromatogram Decoration** dialog box.

- On the **Peak Calipers** tab page, select **Show peak calipers** to display the peak information.

In addition, you can also display the following information:

- The name of the current QNT method: **Show QNT info**.

- The lines indicating the width of the retention time ⇒*Window* of the associated substance: **Show all caliper drop lines**.

The chromatogram looks as follows:
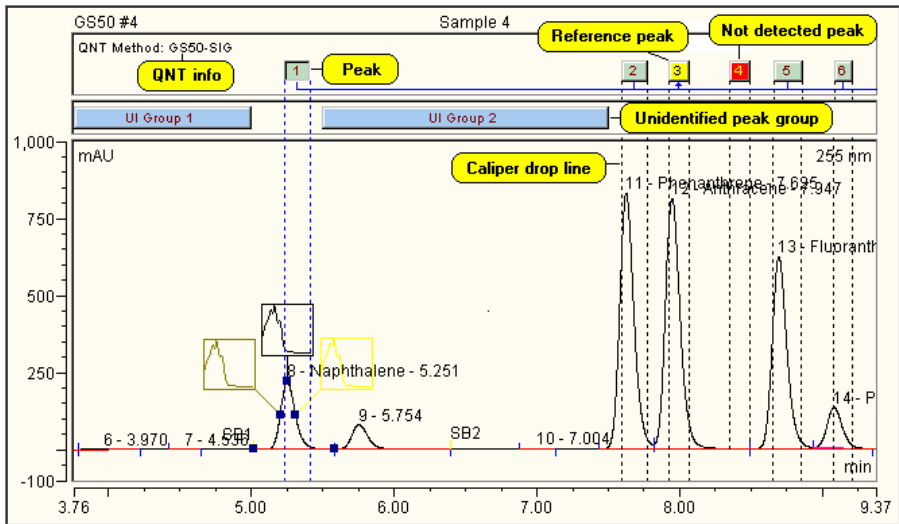
In addition, you can move the retention time window graphically. For more information, refer to 🔴 **Moving a Retention Window Graphically**.

Moving a Retention Window Graphically

Chromeleon allows you to change the retention window, i.e., the expected ⇒*Retention Time* and the time ⇒*Window*, for single peaks in the chromatogram:

- Go to the **Peak Table** tab page of the QNT Editor to check the graphical move of the retention window described below:

- Option: Add the lines that indicate the time limit of the window to the chromatogram: In the **Chromatogram Decoration** dialog box, select the **Show all caliper drop lines** check box on the **Peak Calipers** tab page. (For more information, refer to 🔴 **Showing the Peak Information in a Separate Area**.)

To graphically move the retention window, perform one of the steps below:

1. **Move the entire window**: To move the retention window of a peak, point to the center of the associated peak caliper. The pointer changes its appearance and looks as follows: ⥋.

Hold down the left mouse button and drag the caliper to the desired position, i.e., to the desired time.

The entry in the **Retention Time** column of the peak table is updated automatically.

2. **Move the start or end time**: To change the time of a specific line, point the edge of the caliper or on the associated dotted line. The pointer changes its appearance and looks as follows ⟷.

Hold down the left mouse button and drag the line to the desired position, i.e., to the desired time.

The entries in the **Window** and **Retention Time** columns of the peak table are updated automatically.

3. **Move the start or end time symmetrically**: Hold down the **SHIFT** key when you point to the edge of a caliper or on a dotted line. The pointer changes its appearance and looks as follows ⟷ˢ.

Hold down the left mouse button and drag the lines to the desired position, i.e., to the desired time.

The entry in the **Window** column of the peak table is updated automatically.

Tip:

In the same way, you can change the start and end time of an area for groups of unidentified peaks.

Defining Detection Parameters Graphically

Chromeleon supports entering detection parameters graphically: This considerably facilitates defining the time for the ⇒*Detection Parameters*.

How To

- If no parameter is displayed in the chromatogram, use the **Detection Parameter Tool**. Either select this command on the context menu or click the following icon on the integration ➤*Toolbar*: ⟷.

- In the chromatogram, move to the exact location where you want to enter a parameter.

- Select **Detection Parameters** on the context menu.

- You can then select

  Insert Inhibit Integration On or **Off** to inhibit or allow integration (see **How to …: Integrating Chromatograms and Identifying Peaks 🔳 Inhibiting Peak Integration**).

  Insert Valley to Valley On or **Off** to enable or disable integration from valley to valley.

  Insert Detect Negative Peaks On or **Off** to enable or disable integration of negative peaks.

  Insert Baseline Point to insert a new baseline point (for more information about the last three commands, refer to **How to …: Integrating Chromatograms and Identifying Peaks 🔳 Modifying the Baseline**).

  Insert to insert a new parameter.

- The **Detection Parameter Tools** moves the entered parameters to the desired location in the chromatogram.

The example below corresponds to the table input in **How to …: Integrating Chromatograms and Identifying Peaks 🔳 Defining Detection Parameters**.
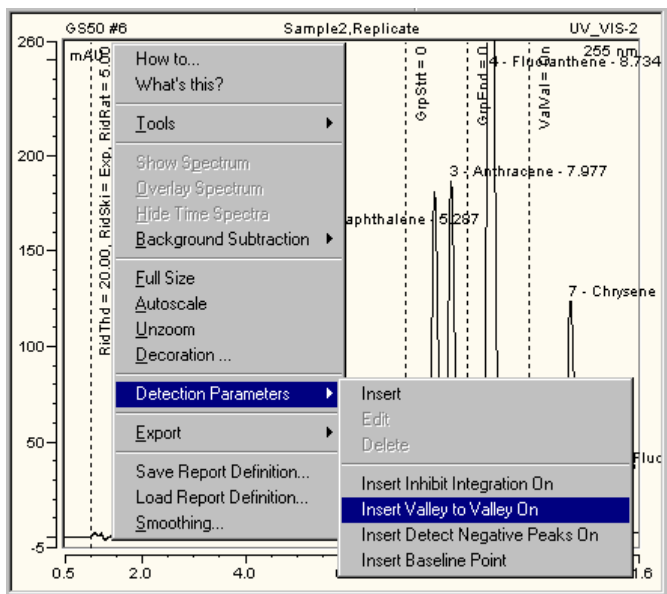
A dotted line indicates the parameters in the chromatogram. The short form for the parameters and their values are displayed along the line. The picture shows, for example, the Rider Threshold (in short: RidThd), Rider Skimming (RidSki), and Maximum Rider Ratio (RidRat) parameter values at 1.000 min.

To enter the detection parameters or other settings that influence peak calculation graphically, you also select a chromatogram area while holding the right mouse key. The context menu opens automatically. The menu contains the following commands:

- **Set Averaged Baseline Start** and **Set Averaged Baseline End** to set an averaged baseline (see **How to …: Working with Chromatograms** **Defining an Averaged Baseline**).

- **Set Minimum Area** to define the minimum peak area.

- **Set Minimum Height** to define the minimum peak height.

- **Set Minimum Width** to define the minimum peak width (for more information about the **Set Minimum** commands, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Reducing the Number of Evaluated Peaks**).

- **Set Peak Slice & Sensitivity** to define the peak recognition algorithm (see **How to …: Integrating Chromatograms and Identifying Peaks** **Modifying the Peak Recognition Algorithm**).

- **Set Inhibit Integration Range** to select a range for the peak inhibition.

- **Set Void Volume Treatment Range** to determine the range for recognition of the negative water peak. This overwrites all previous ranges for recognition of the negative water peak.

 Note:

*You can undo the graphical input of detection parameters. Click one of the QNT Editor tables, and then select **Undo** on the **Edit** menu. (In the chromatogram itself, you can only undo the modifications made for the active chromatogram.)*

When new detection parameters are entered graphically, they are automatically copied to the **Detection** tab page of the QNT Editor. (For more information about the editor, see **Data Representation and Reprocessing** **The QNT Editor**.) They will be used for all samples that are evaluated with the QNT File of the current sample. As an alternative, you can also enter the detection parameters directly on the **Detection** sheet. (For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Detection Parameters**).

Displaying and Using UV Spectra

After recording UV spectra with a ➢*Photodiode Array Detector*, Chromeleon supports all possibilities for using spectra with a modern chromatography data system.

Displaying Spectra

UV spectra are displayed in the Spectra Plot window. Select **Show Spectra** on the **View** menu or click

The spectra plot represents UV spectra and allows their identification by means of a spectra library.

For more information, refer to:

🔲 **Displaying Peak Spectra**

🔲 **Displaying and Overlaying Single Spectra**

🔲 **Processing UV Spectra**

🔲 **Match Factor, Difference Spectra, 1st /2nd Derivatives of Spectra**

In addition, you can

🔲 **Create and Use Spectra Libraries**

🔲 **Search Reference Spectra**

The **PPA** (Peak Purity Analysis) window allows you to

🔲 **Analyze the Peak Purity**

🔲 **Select the Optimum Integration Path**

and 🔲 **Extract Spectra, Chromatograms, and 3D Fields**

🔲 Displaying Peak Spectra

- In the chromatogram, select the peak of which you want to display the spectrum. If the associated raw data is available, thumbnail spectra of the peak are displayed in the chromatogram.

- Select **Show Spectra** on the **View** menu or click

- Select **Decorations** on the context menu of the Spectra window. On the **Peak Spectra** tab, determine from which peak height spectra are displayed.

Depending on the settings, up to five spectra of the same peak can be displayed in different colors. Normally, the spectra are extracted at 10 and 50% peak height from the leading and the trailing peak edges and at the run time of the peak. The representation of the spectrum is normalized. If the spectra largely match, this can be an indication for good peak purity.

Displaying and Overlaying Single Spectra

In addition to peak spectra (= spectrum in the peak maximum or spectrum at a defined peak height), Chromeleon is capable of displaying any other spectrum of a chromatogram at the time t.

To extract any spectra of a chromatogram via a mouse click, follow the steps below:

- Enable the **Spectra/I-t Plots Tool** on the context menu or click the corresponding icon on the **Integration Toolbar**

- A spectra symbol that is appended to the mouse pointer indicates that the mode was changed.

- Click anywhere in the chromatogram to display the corresponding spectrum.

- Repeat the action while pressing the SHIFT key to overlay several spectra.

Displaying Spectra of Different Samples

To compare spectra of different samples objectively, perform a chromatogram comparison:

- Compare two chromatograms by displaying an additional chromatogram in the Integration window (see **Integration** **Chromatogram Comparison**).

- Enable the **Spectra/I-t Plots Tool** and select single spectra by clicking various places in the chromatogram while pressing the SHIFT key.

 Note:

UV spectra are overlaid in one window.

Processing UV Spectra

For a better comparability of UV spectra, it is useful to subtract the background spectrum (of the solvent). Chromeleon performs this automatically, using the following algorithm:

- The background spectra are determined for both peak ends. At both peak ends, several UV spectra can be averaged over a specified range.

- The background peak spectrum (usually at the peak maximum) is determined via linear interpolation of these two background spectra and subtracted from the single spectrum (usually of the peak maximum).

 Tip:

In addition, it is possible to combine several spectra to the Apex spectrum, and then subtract the background spectrum from this averaged spectrum. However, this is usually not necessary for UV spectra as they have a relatively low noise level compared to ➢Mass Spectra.

Use the **UV** tab of the **QNT Editor** to determine background subtraction for UV spectra. (For more information about the editor, see (see **Data Representation and Reprocessing** **The QNT Editor**.)

The **Enable Background Subtraction** check box is enabled indicating that spectrum subtraction is active:
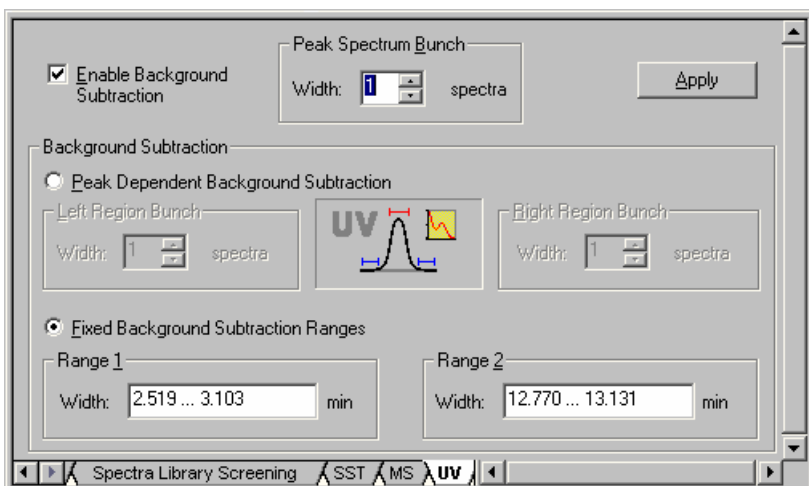
Only for UV spectra with a high noise level, it is necessary to average spectra to reduce the noise level. For example, spectra averaging may be required for very small peaks that are only slightly above the ➤*Limit of Detection*. In the combo box under **Peak Spectrum Bunch**, enter the number of single spectra to be bunched to form the spectrum at the peak maximum. A maximum of 99 single spectra can be averaged. For symmetry reasons, it is possible to enter odd numbers only. Therefore, by default, only one spectrum is selected as **Width** of the **Peak Spectrum Bunch**.

Peak Dependent Background Subtraction allows automatic background subtraction for each peak. In the edit fields under **Left Region Bunch** and **Right Region Bunch**, enter the number of spectra to be used for forming the two background spectra. You can select up to 99 single spectra. Zero and even numbers are permitted as well. These settings apply to peak spectra and time spectra below peaks. The default setting is that one spectrum each is subtracted on both sides of the peak.

Select **Fixed Background Subtraction Ranges** to define two fixed ranges for background subtraction for the entire chromatogram. It usually makes sense to set one range at the beginning and the other one at the end of the chromatogram. These settings apply to peak spectra and to all time spectra.

Click **Apply** to accept the settings and calculate the resulting UV spectrum. When the **Show Spectra** view is enabled, the newly calculated UV spectrum is displayed at once.

🗇 *Note:*

*Defining the background subtraction manually in the chromatogram affects the settings on the **UV** tab page. For example, if you select **UV Background Subtraction** on the context menu of the chromatogram, and then select **Fixed Background Ranges**, the corresponding option is automatically selected on the **UV** tab page of the QNT Editor.*

▪ Match Factor, Difference Spectra, 1st/2nd Derivatives of Spectra

When two or more spectra are represented on the spectra plot, the similarity of the UV spectra becomes an issue.

The match factor can express the similarity, by forming difference spectra or by representing the first or second derivative of a UV spectrum.

- Select **Decorations** on the **View** or context menu and select the **Show match** check box on the **Label** tab page. Chromeleon issues a value for each represented spectrum, expressing the match degree relative to the main spectrum (0 = no match; 1000 = perfect match).

- On the **Analysis** tab, define whether the difference spectrum or the first or second derivative of a spectrum is displayed in a second window in addition to the actual spectra.

In the case of the match factor and the difference spectrum, the question which UV spectrum is considered a main spectrum is especially important, as this is the basis of comparison or the basis for all calculations.

Usually, this is the peak spectrum extracted at the retention time. If there is no peak spectrum, distinguish two situations: If you select the **Spectra/I-t Plots Tool** to extract the single UV spectra from the chromatogram, the first spectrum that is extracted is the main spectrum. If spectra are extracted automatically at different peak heights, the spectrum with the "oldest" retention time is considered the main spectrum. When representing difference spectra, the **Difference to** entry indicates the basis of calculation.

Creating and Using Spectra Libraries

The **Spectra Library** pane allows you to compare ➤*Normalized* and single ➤*baseline-corrected* spectra with spectra from various libraries.

For more information, refer to:

🔳 **Creating a New Library**

🔳 **Using Spectra Libraries**

🔳 **Comparing Spectra**

🔳 Creating a New Library

First, select **New > Spectra Library** on the **File** menu to create a new and empty spectra library. Then, include the spectra of interest:

- From the **PPA** or **Spectra Plot** panes: Copy a spectrum to the Windows clipboard, using the **Copy** command or the **Extract > Lib-Spectrum to clipboard** commands on the context menu. Insert the spectrum in the open library, using the **Paste Spectra** command. (For information about PPA and/or the spectra plot, refer to **Data Representation and Reprocessing** 🔳 **PPA: Peak Purity Analysis** or **Integration** 🔳 **The Spectra Plot**.)

- Or else, generate a subset of an existing selection based on specific ➤*Hit Criteria*.

📝 *Note:*

To be able to compare UV spectra, the spectrum and the reference spectrum should be recorded under identical conditions. That is why the best search results are obtained based on spectra that were recorded and saved by the user. Dionex recommends to writing down the conditions under which the spectrum is recorded. These notes will be very useful when there are several spectra for the same sample.

Saving the library

Select **Save as** to save an open library under a different name to a directory of your choice. Libraries have the file extension **LIB**.

▣ Using Spectra Libraries

Spectra Table

The upper section lists all spectra that are contained in the active ➢*Spectra Library*, as well as their data. Double-click a column header to sort the list according to the criteria of the selected column, such as the name, ID, or number of extremes. Select the first column of the table to select the corresponding spectrum.

- When you move the mouse pointer into the left column, the cursor becomes a horizontal arrow. Left-click to select a spectrum.

- To select several spectra, press and hold the CTRL key.

Spectra Plot

The spectra plot is displayed at the bottom left, underneath the table, showing the spectra of all substances selected in the spectra table.

- Double-click the window or select **Decoration** on the context menu to change the spectra representation. For more information, refer to **Integration** 🔼 **The Spectra Plot**.

Working with the Spectra Library

The objective of spectra administration is the identification of an unknown substance based on its UV spectrum. If you have a spectrum of a previously unidentified substance, for example, in the spectra window of the PPA method, you can start searching various libraries from there.

Chromeleon compares the curve form of the two spectra (comparison), calculates a similarity value (evaluation), and displays similar spectra (hit criterion).

The distinction made by Chromeleon depends on whether a single spectrum is identified or all peaks of a sample.

Hit List

The hit list lists all library spectra that have a certain similarity to the extracted (and normalized) spectrum. For more information, refer to **How to …: Displaying and Using UV Spectra** 🔼 **Searching Single Reference Spectra**.

Peak Tracking

Assigning each peak in a chromatogram the spectrum of a library that matches the spectrum extracted at the peak maximum best is referred to as peak tracking.

🔲 Comparing Spectra

To compare two spectra with each other, the curve of the normalized spectrum can be compared to the curves of single library spectra. In some cases, better results are achieved when the first or second derivations are compared instead of the spectra themselves.

Use the comparison function (see ⇒*Check Derivative*) to determine the curve form to be used for the comparison.

In the next step, determine how the single curves are compared with each other. Select one of three mathematical methods; they are referred to as ⇒*Match Criterion*.

The combination of comparison function and standard of comparison results in a "similarity value" between 0 and 1000 that expresses the match degree between the search spectrum and various library spectra. The similarity value is known as ➢*Match Factor*. A perfect match has the value 1000.

Depending on the method (hit list or peak tracking), a similarity list is displayed or each peak is assigned the most similar spectrum. If you enter a minimum similarity value (threshold), only the hits above the threshold value are displayed.

Result Presentation in a Hit List

Using the hit list, a similarity list sorted by the match factor and a representation of the original spectrum and the library spectrum with the best match are displayed. Entering a minimum similarity value excludes dissimilar spectra.

Due to the frequently insufficient characteristics of UV spectra, it may be necessary to further limit the hit lists. ➢*Hit Criteria* are available as additional filters. Only the spectra fulfilling the selected criteria will be displayed.

For information about how to search reference spectra, refer to ⬛ **Searching Reference Spectra**.

Searching Reference Spectra

Chromeleon allows you to search for reference spectra as follows:

1. Searching single spectra: Perform the search from the Spectra windows; refer to ⬛ **Searching Single Reference Spectra**.

2. Searching spectra from any number of samples that use the same quantification method: Perform the search from the **Spectra Library Screening** tab in the QNT Editor.

For more information, refer to ⬛ **Entering Criteria for the Spectra Library Screening,** ⬛ **Starting Library Screening and Viewing Results**, and/or ⬛ **Integrating Screening Results in Reports and Peak Labels**.

◾ Searching Single Reference Spectra

To facilitate substance identification, the peak spectrum, which is displayed on the **Spectra Plots** of the **Integration** or **PPA** method, can be compared to the UV spectra of a spectra library.

Based on the available library, Chromeleon creates a spectra list sorted by the degree of similarity, the **Hit List**. The number of possible hits can be limited via comprehensive search criteria.

• If the pointer is in a spectra window, select **Library Search** on the context menu of the UV spectrum to start the spectra search.

In the edit box, specify the library to be searched. The following are minimum entries required to receive a valid search result:

• Select a spectra library (LIB file) from the **Spectra Library to be searched in** list. If no LIB file is displayed, click **Browse** to search for the file.

• In the ⇒*Match Criterion* field, determine the mathematical method based on which the original spectrum and the library spectrum are compared with each other. The best search results are generally received with **Least Squares**.

• Click **OK** to start a spectra search.

📒 *Note:*

To be able to compare UV spectra with each other, the spectrum and the reference spectrum should be recorded under identical conditions. For best results, compare spectra to your own spectra library.

Result

A list of possible candidates is displayed. The top spectrum has the highest match value and the best similarity to the original spectrum.

Detailed Search

To accelerate the search and to receive very specific results, there are numerous options:

- Normally, the **Spectrum Derivative** option is disabled, that is, spectra comparison is based on the actual curve shape. If the **1´st Derivative** option is selected, the comparison of the two spectra is performed based on the first derivative. Consequently, the curve characteristics are more significant (shoulders become real extremes), which allows a more precise comparison. The drawback of this option is the reduction of the signal-to-noise ratio, which causes sections with weaker signals to loose significance.

- Select **Hit Threshold** and enter a value between 0 and 1000, for example, 950. Only the spectra with a match above 950 is displayed. Spectra with a lower match value is not displayed. Spectra with a match value below 900 are usually spectra of other substances (if derivatives are not used for the comparison). Exceptions to this are acceptable only if, for example, the signal-to-noise ratio is low.

Select the following options to ensure precise search results:

- Enter the number of relative extremes the spectrum should have (**Number of Relative Extrema**). This option is useful to exclude spectra that are similar but have an additional side maximum.

- Select **Check Greatest Relative Maximum** to use only spectra with the greatest relative maximum at the same position. Select **Allowed Deviation** to define a tolerance range. The range should not be more than 10 nm to avoid that the criterion weakens.

- Via **Retention Time Window**, define a time window that includes the retention time of the peak. Use this option to exclude substances with very similar spectra, but which are eluted at very different retention times.

Additional criteria are only required in special cases. However, they are useful for searching large libraries containing numerous spectra of the same substance, but extracted under different conditions; for example, different solvents and detectors, different users, different date, etc.

Note:

When creating your own spectra library, please enter information in all fields, even in the fields that may seem unnecessary. In the course of time and with a growing library, this may become a major advantage.

■ Entering Criteria for the Spectra Library Screening

Enter the criteria for a spectra library search on the **Spectra Library Screening** (= **SLS**) page of the QNT Editor:
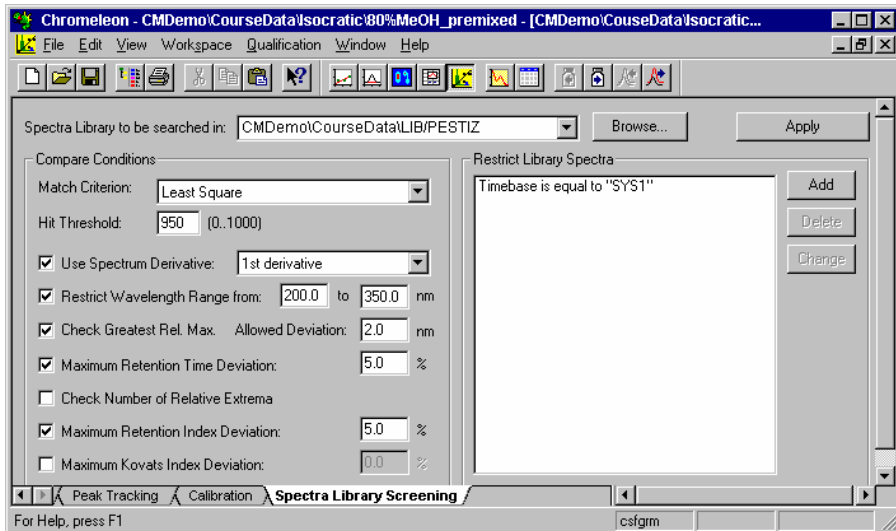
- Select a spectra library (LIB file) from the **Spectra Library to be searched in** field. If the required LIB file is not included, click the **Browse** button to search for the file.

- In the **Match Criterion** field, select the method (see ⇒*Match Criterion*) used for comparing the original spectrum and the library. The best search results are usually received via the setting **Least Square**.

- In the **Hit Threshold** field, enter a ➢*Match Factor* between 0 and 1000; for example, 950. Only spectra with a match value above 950 will be shown. Spectra with less similarity will not be included. Unless derivatives of spectra are compared with each other, reference spectra with a match value below 900 are usually spectra of other substances. Exceptions to this rule are only acceptable, if the signal-to-noise ratio is low.

📝 *Note:*

To be able to compare UV spectra, the spectrum and the reference spectrum should be recorded under identical conditions. For best results, create your own library of spectra you recorded yourself.

Additional conditions are possible to perform a more exact search:

Usually, the **Use Spectrum Derivative** option is disabled; that is, the comparison of spectra is based on their actual curve. If the **1st derivative** option is selected, the comparison of two spectra is based on the first derivative. As a result, the curve characteristics increase (shoulders become extrema), allowing a more exact comparison of extrema. However, the signal-to-noise ratio will considerably decrease; and sections with lower signals will be less significant.

Select **Restrict Wavelength Range** to limit the spectra comparison to the relevant spectral range.

Select **Check Greatest Rel. Max.** to use only spectra with the greatest relative maximum at the same position. Select **Allowed Deviation** to specify a tolerance range. The range should not exceed 10 nm to avoid that the criterion loses significance.

Click **Maximum Retention Time Deviation** to determine the maximum retention time deviation of the reference spectrum in percent. This prevents including substances with very similar spectra that are eluted at different retention times.

If you select **Check Number of Relative Extrema**, Chromeleon checks the number of relative extrema of the reference spectrum. Select this option to exclude spectra that are very similar but have an additional maximum.

Select **Maximum Retention Index Deviation:** or **Maximum Kovats Index Deviation:** to include only those spectra in the comparison for which the retention index or, respectively, the ⇒*Kovats Index* is identical with the index of the sample substance. The respective tolerances are defined in the right field.

👉 *Tip*

*With an increased noise level, noise peaks can be considered extrema. In this case, Dionex recommends disabling **Check Number of Relative Extrema**.*

On the right side of the window, you can further restrict the resulting library spectra. Open the dialog box **Restrict Library Search** with **Add**. Via **Field Name**, you can then select type of parameter to be determined from the following search criteria:

Solvent Composition	**Detector Serial Number**
Control Program	**Timebase**
Substance Name	**Sequence Name**
Unique ID	**Sample Name**
Comment	**Extract Operator**
Detector Name	

These items can be linked (partly) via the following operators (**Conditions**) with a freely selectable value:

Operator	Restricts the search to spectra with parameters that
is equal to:	fulfill the specified condition.
starts with:	start with the entered string.
does not start with:	do not start with the entered string.
ends with:	end with the entered string.
does not end with:	do not end with the entered string.
contains:	contain the entered string.
does not contain:	do not contain the entered string.

 Note:

*If you want to search all libraries in a directory, simply enter the character ***** as the file name. The wildcard characters (known from MSDOS) ***** and **?** are valid. Example: LIB::\CMDATA\LIB\D* searches all libraries starting with the letter **D** in the directory **LIB** of the datasource CMDATA.*

◼ Starting Library Screening and Viewing the Results

Click **Apply** to use the entered search parameters. This command starts the library search for each peak in the active chromatogram.

The spectra plot is displayed in addition to the chromatogram. The reference spectrum (hit) with the corresponding match criterion is shown. The displayed spectrum is the reference spectrum of the selected peak. You can display the search results for the other substances by clicking the corresponding peaks in the chromatogram.

If more than one library spectrum fulfills the entered criteria, you can display the other hits. Place the cursor on the spectrum, open the context menu, and open the **Spectra Decoration** tab page. Use the **Peak Spectra** tab page to enter the number of reference spectra to overlay:
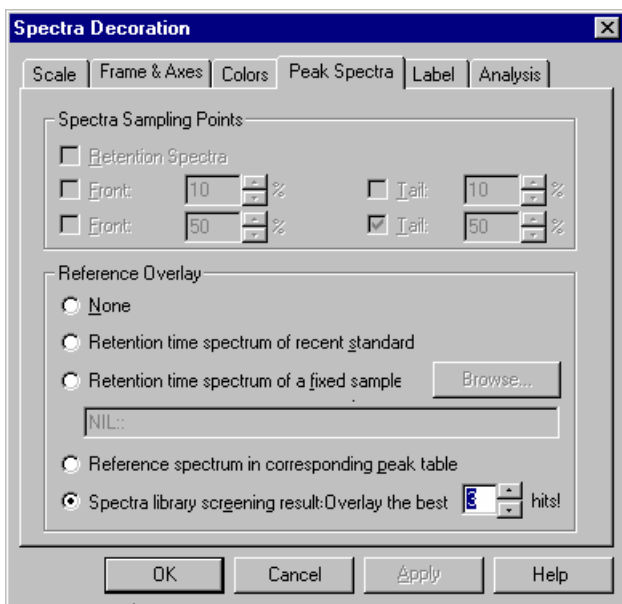
In this example, you will receive a list of three reference spectra. The first spectrum has the highest match value and thus the greatest similarity to the original spectrum.

▣ Inserting Screening Results in Reports and Peak Labels

If you save the search parameters in the QNT File, the results of the **Spectra Library Screening** can be used for the peak label or in report tables or templates (➤*Printer Layout*).

Inserting Screening Results in ➤*Report Definition Files*

To display the screening results in the report, the **Peak Purity** report category supports the following variables:

Number of SLS Hits calculates the number of library screening hits for a peak.

SLS Hit opens the ⇒*Hit Spectrum* report category that includes all variables of the corresponding library spectrum:

Designation	Description
Substance Name	
Match Factor	➤*Match Factor*
Library Name	Name of the spectra library
Library Record	Opens the ⇒*Spectra Library* category
Number of rel. Extrema	
Solvents	
Comment	➤*Comment*
Sequence Name	
Sequence Header Record	
Sample Name	
Sample Record	Branches to the ⇒*Sample* category
Acquisition Time	Acquisition date and time
Timebase	➤*Timebase*
Program	➤*PGM File*
Sample Rate	
Retention Time	⇒*Retention Time*
Lambda Min.	Minimum wavelength
Lambda Max.	Maximum wavelength
Lambda Range	Wavelength range

Designation	Description
Lambda Resolution	Spectral wavelength resolution
Detector Name	
Detector Serial Nr.	
Extract Time	Time when the sample was added to the library
Extract Operator	User who added the sample to the library
Retention Index	Linear ⇒*Retention Index*
Kovats Index	⇒*Kovats Index*

The **Formula** field shows a short version of your selection; for example, **peak.hitSpec(1).name** for the spectra name of the best hit. The digit indicates the hit: 1 is the best, 2 is the second best hit, etc.

Including Screening Results in the Peak Label

To include the screening results in the chromatogram, follow the steps below:

- Position the cursor in the chromatogram.

- Select **Chromatogram Decoration** on the context menu.

- On the **Peak Label** tab page of the **Chromatogram Decoration** dialog box, click **{...}** to open the **Insert Variable into Peak Label Formula** dialog box. Select the **Number of SLS Hits** and **SLS Hit** variables (see **Inserting Screening Results in Report Definition Files** above) from the **Peak Purity** category.

Example

To label each peak in the chromatogram with the name of the best hit and the corresponding match factor, follow the steps below:

- From the category **Peak Purity**, select the **Substance Name** under **SLS Hit**.

- In the **Formula** field, enter " - " after the formula, then click **Match Factor** under **SLS Hit**.

- Click **OK**. You receive the following **Formula** input on the **Peak Label** tab page of the **Chromatogram Decoration** dialog box:
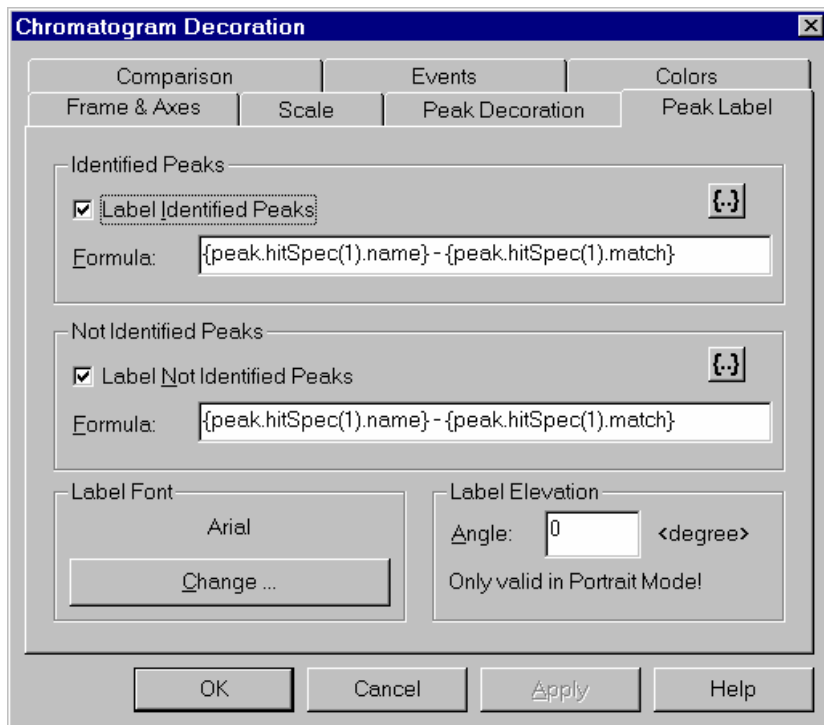
☞ *Tip:*

*If you have saved the search parameters in the QNT File, it is sufficient to enable **Spectra library screening results**; for example, on the spectra plot. Just open the context menu and select the option via **Spectra Decoration** and **Peak Spectra**. In this case, you do not need to open the **Spectra Library Screening** dialog box and click **Apply**.*

Analyzing the Peak Purity

Use the PPA window (see **Data Representation and Reprocessing** ⬛ **PPA: Peak Purity Analysis**) to check the peak purity. The PPA window displays the ➤*3D Field* of a sample if there is a corresponding raw data file. This is the case if the **3DFIELD** channel was selected for ➤*Data Acquisition*. The 3D field in the lower right-hand window section has cross-wires. With the mouse, the axes can be moved separately or together. The spectrum or chromatogram extracted along the current y and x-axis will be displayed on the left or above the 3D field.

- Move the y-axis of the cross-wires to display all spectra of the active chromatogram in succession

- Move the x-axis of the cross-wires to display the appearance of a chromatogram at different wavelengths.

- The status bar shows the retention time, wavelength value, and signal height of the current cross wire position.

To evaluate peak purity, use the following methods:

⬛ **Visual Check of Iso Line Plot**

⬛ **PPI and PPI Match Factor**

⬛ **Normalized Spectra Overlay**

⬛ **Peak Inhibition via Virtual Channels**

⬛ **Multiple Ratio**

⬛ Visual Check of Iso Line Plot

The visual check of the Iso line plot is a method that allows peak purity evaluation. High peak purity is indicated by:

- All recognizable absorption maxima are located vertically below one another in direction of the wavelength axis.

- The maxima are separated so that the surrounding iso lines do not touch (no overlapping).

- Pure peaks have (local) symmetry or an idealized ellipse shape in the maximum.
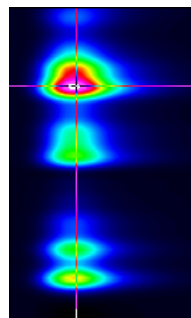
◼ PPI and PPI Match Factor

In the chromatogram window of the PPA method, curves for the ➢*PPI (Peak Purity Index)* and the ➢*Peak Purity Match Factor* can be displayed:

- Double-click within the window and select the options **Draw PPI** and **Draw Match** from the **Chromatogram Plot** tab page.

The peak purity index is represented by a purple curve, and the match factor is indicated by a green curve. A distinctive rectangular shape is one criterion (of many) for peak purity. The exact match value at the current mouse pointer position is shown on the status bar. The ideal value is 1000 and requires approximately 80% of the available window height.

PPI, match value, and the corresponding standard deviation (RSD) can be included in a report. The report variables are available in the **Peak Purity and Identification** category.

The smaller the standard deviation, the more critical the quality of the rectangular curve should be judged; that is, the better the two spectra will match in various points of a peak.

◼ Normalized Spectra Overlay

In a normalized spectra overlay, single spectra of the spectra recorded for the peak are extracted and are compared with each other. Usually, extraction is at the run time of the peak and at a specific peak height in the leading and trailing edges of the peak; for example, at 10% and 50% of the peak height. Comparing spectra that are ➢*normalized* by the same method is a means of evaluating the peak purity.

The more closely the spectra match, the higher the possibility that the peak is pure. The following are indications for the impurity of a peak:

- The number of relative maxima and minima of two spectra does not match
- The relative maxima are clearly offset against each other
- The height of the relative maxima strongly deviates

However, please note the following:

- Especially near peak limits, spectra have an increased noise level due to the normalization procedure. This applies in particular to the UV range.

- In the case of very long peaks, baseline correction cannot completely compensate for detector drift.

- The spectra shape depends on the solution; that is, the spectra of a peak can only be compared if the mixing ratio was constant during detection.

- It is possible only to compare spectra with the (complete) absorption in the dynamic validity range of the Lambert Beer Law. If the peak maximum, including the baseline spectrum, is considerably above 1 AU, caution is called for.

📖☞ *Tip:*

*The normalized spectra overlay via peak height is currently possible only on the spectra plot (also see **Integration** 🔳 **The Spectra Plot***). Within the PPA method, the "animated" extraction of spectra is possible. Pressing the CTRL key in the 3D field extracts the current spectrum. If the y-axis of the cross-wires is moved simultaneously, all spectra existing for this range are displayed in the spectra window. A different color indicates the extraction period in the chromatogram. This process can be repeated; for example, for different retention times within the peak width. If you release the CTRL key between the individual extractions, the spectra extracted per range are displayed in a separate color.*

🔲 Peak Inhibition via Virtual Channels

If two overlapping peaks have different spectra $s1(\lambda)$ and $s2(\lambda)$ the following equation is true when the area below the baseline is ignored:

$A(\lambda, t) = s1(\lambda)*c1(t) + s2(\lambda)*c2(t)$ (1).

c1 and c2 stand for the time-dependant concentrations of the corresponding components in the flow cell. Then, two wavelengths $\lambda1$ and $\lambda2$ are selected and the following signal is created:

$d(t) = A(\lambda1, t) - K*A(\lambda2, t) = c1(t)[s1(\lambda1) - K*s1(\lambda2)] + c2(t)[s2(\lambda1) - K*s2(\lambda2)]$.   (2),

The c2 term disappears provided the appropriate expression was selected for K, that is:

$$K = s2(\lambda 1) / s2(\lambda 2) \quad (3)$$

This is the channel ratio of the second peak. It can be read off the height ratio of the two peaks if they do not overlap completely. In order to be able to use this approach, the channel ratio of the first peak
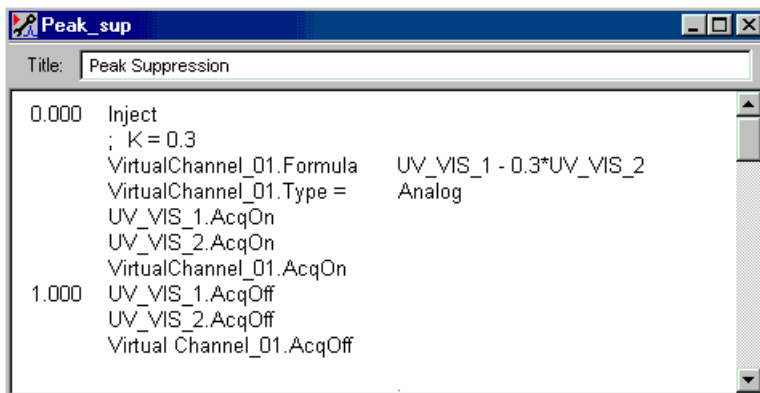
$$K' = s1(\lambda 1) / s1(\lambda 2) \quad (4)$$

must be different from K. If not, the first term in equation (2) will disappear as well. Select the $\lambda 1$ and $\lambda 2$ wavelength in such a way that the difference between K and K', that is:

$$\Delta K = |K - K'| \quad (5)$$

is the maximum difference. The best way to determine the appropriate wavelengths is to do so in the PPA Window. After this, the virtual channel d(t) can be defined. Of course, it is possible to inhibit the first peak in the same way.

If you know the wavelengths for which ΔK is the maximum before you record the chromatogram, the two channels, for example, UV_VIS_1 and UV_VIS_2 can be adjusted to those wavelengths. You can then use the following ➤*Program*:

```
Peak_sup                                              _ □ ×
Title:  Peak Suppression

0.000   Inject
        ;  K = 0.3
        VirtualChannel_01.Formula    UV_VIS_1 - 0.3*UV_VIS_2
        VirtualChannel_01.Type =     Analog
        UV_VIS_1.AcqOn
        UV_VIS_2.AcqOn
        VirtualChannel_01.AcqOn
1.000   UV_VIS_1.AcqOff
        UV_VIS_2.AcqOff
        Virtual Channel_01.AcqOff
```

As mentioned in the comment line of the program, the following equation is true for this example:

$$K = 0.3.$$

◾ Multiple Ratio

Each channel extracted from a 3D field can be used for performing the ratio test. Especially suited are chromatograms in the range of spectral minima or maxima. If the ratio condition is met, this can indicate, but does not prove peak purity. Performing the test with a larger number of channels does not change this fact.

Selecting the Optimum Integration Path

Chromeleon is capable of calculating the ➤*Optimum Integration Path* within a 3D field. The calculation is performed automatically. The result can be displayed in the 3D field window of the PPA method.

- Double-click within the window and select the **Draw Opt-Int-Path** option on the **Iso/3D Plot** tab page.

👉 *Tip:*

You may also define extraction of the optimum integration path in the ***Post-acquisition steps*** *view of the PGM Editor (see* ***Control*** ◾ ***The PGM Editor****.) Open the PGM File in which you want to define extraction of the optimum integration path, and then select the* ***Post-acquisition steps*** *view.*

A green line in the 3D field indicates the integration path. Similar to chromatograms, it can be saved as a separate channel with variable wavelength.

- Select **Extract > Opt. Int. Path to file** on the **View** or context menu and determine the name under which the path is saved as a separate channel or accept the default name (OPTINT).

👉 *Tip:*

In the PGM Editor, follow the steps below: Select Insert line on the context menu to add a new post-acquisition step. The New post-acquisition step dialog box is opened. Select Extract optimum integration path to open the Extract optimum integration path dialog box.

- Select **Extract from all samples of current sequence or query** option, if the path should be extracted for all samples of the underlying sequence or query. The shape of the path is identical for all samples!

👉 *Tip:*

This option is not provided in the dialog box of the PGM Editor.

 Notes:

*Chromeleon does not consider rider peaks (**Type (⇒Peak Type)**: Rider) when calculating the optimum integration path.*

The channel extracted in this way may have baseline jumps. For distinctly absorbing solvents, they may be due to changes in the wavelength.

If the path should serve as a basis for a ➢*Wavelength Switch* in future samples, the switch times and the selected wavelength values must be entered in a program with exact time specifications. This can be performed, also, by automatically inserting the data.

- Select **Extract > Opt.Int.Path to clipboard** on the **View** or context menu to copy the data to the clipboard.

- Select the **Name** of the channel, for which the wavelength is switched automatically in the future.

- Enter a value for the **Bandwidth** if several chromatograms are to be averaged to one. The bandwidth determines the range of the path. All sections of a chromatogram within this range are averaged to one chromatogram.

- Open a ➢*PGM File* and insert the data at the beginning of the program (**Commands** view) via the **Paste** command.

The resulting PGM File could have the following appearance:

```
0.000    UV_VIS_1.Bandwidth = 0
         UV_VIS_1.Wavelength = 210
3.320    UV_VIS_1.Wavelength = 210
4.830    UV_VIS_1.Wavelength = 206
6.100    UV_VIS_1.Wavelength = 272
8.100    UV_VIS_1.Wavelength = 262
9.660    UV_VIS_1.Wavelength = 278
10.480   UV_VIS_1.Wavelength = 250
```

- Enter more commands in the program to complete it. Sort it according to ascending retention times.

Extracting and Exporting Spectra, Chromatograms, and 3D Fields

Spectra

You can copy each spectrum extracted from a 3D field in the PPA method or on the spectra plot (see **Integration** ⬛ **The Spectra Plot**) to the Windows clipboard (**Copy** command). From the clipboard, you can then paste the spectrum in a spectra library (**Paste** command). (For information about spectra libraries, refer to **Data Representation and Reprocessing** ⬛ **Spectra Libraries**). Follow the steps below:

- Select **Extract > Spectrum to clipboard** on the **View** or context menu to copy the current spectrum from the 3D field of the method PPA to the Windows clipboard.

- Open an existing Spectra Library. Select the command by opening the corresponding LIB file from the Browser, or

- Create a new library via the **File> New > Spectra Library** commands.

- Select **Paste Spectra** to save the spectrum and the data in the library.

Chromatograms

From an open 3D field in the method PPA, you can extract a chromatogram of any wavelength:

- Select **Extract > Chromatogram to file** on the **View** or context menu to save the active chromatogram as a separate channel.

- Select the wavelength and the bandwidth at which to extract the chromatogram.

Chromeleon will suggest a name for the extracted chromatogram, considering the wavelength. However, you may also enter any other name of your choice. The chromatogram is saved in addition to the raw data of the existing 3D field. Simultaneously, the extracted chromatogram is opened via the **Integration** method to give the user an overview of the saved data.

In addition, the **Extract > Chromatogram to file** command provides a special option. Instead of saving one single chromatogram, another chromatogram of the same wavelength can be extracted and saved from all samples of the underlying sequence or query. Enable **Extract from all samples of current sequence or query**. Especially in this case, automatically naming the extracted chromatogram (see above) is very useful.

3D Field Data

Select **Export > 3DFIELD** to include and display the current 3D field raw data in other applications, such as Microsoft Excel. Data is converted into a general ASCII format.

- After you have executed the **Export 3DFIELD** command, change to the other application and insert the data, using the **Paste** command. In addition to the pure raw data, additional sample and sequence information is transferred to the application.

Displaying and Using I-t Plots

When ➢*3D_Amp* data is collected for a sample, ➢*I-t Plots* can be displayed in the Integration window.

Displaying I-t Plots

1. Open the sample in the Integration window (see 🔲 **Opening a Sample**).

2. Select **Show I-t Plots** on the **View** menu or click the following icon on the **Method** toolbar:

Two types of I-t plots can be displayed:

- Peak I-t plots are I-t plots at the peak apex or at a defined peak height. Peak I-t plots at the peak apex are also called retention time I-t plots.

- Time I-t plots are I-t plots corresponding to any selected retention time in the chromatogram. They are not defined by a peak height.

🖎 *Tips:*

*3D amperometry data collection is available only with the ED electrochemical detector running in integrated amperometry mode. The Chromeleon PC must also have the 3D Data Acquisition license. To turn on collection of 3D amperometry data, enable the 3D_Amp channel on the **ED Options** tab page of the Program Wizard or PGM Editor.*

I-t plots can also be displayed in the QNT Editor. Plot types and other options are the same as for I-t plots displayed in the Integration window.

Background subtraction is always performed on I-t plots displayed in the Integration and QNT Editor windows.

For more information, refer to:

🔲 **Displaying Peak I-t Plots**

🔲 **Displaying and Overlaying Time I-t Plots**

🔲 **Displaying Difference, 1st Derivative, or 2nd Derivative I-t Plots**

⊡ Displaying Peak I-t Plots

1. Select a peak in the chromatogram.

2. Select **Show I-t Plots** on the **View** menu or click the following icon on the **Method** toolbar:

If the associated ➢*3D_Amp* data is available, thumbnail I-t plots of the peak are displayed in the chromatogram. The I-t plots identified by the thumbnails are displayed in the I-t plot window.

Depending on the settings, up to five I-t plots from the same peak can be displayed in different colors. These include the I-t plot at the retention time of the peak, and up to four I-t plots extracted at specified peak heights along the leading and trailing edges of the peak (for example, at 10% and 50% peak heights).

• To set the peak heights from which I-t plots are extracted, select **Decoration** on the **View** or context menu and select the **Peak I-t Plot** tab.

A reference I-t plot can be overlaid on the peak I-t plot. The reference plot is an I-t plot of the peak with the same name as the displayed I-t plot. It is extracted from a recent standard, a fixed sample, or the peak table.

• To overlay a reference I-t plot, select **Decoration** on the **View** or context menu and select the **Peak I-t Plot** tab.

I-t plots can be (height) ➢*Normalized*. Normalization allows objectively comparing two I-t plots of different concentrations. I-t plots that largely match can indicate good peak purity.

• To select the type of normalization applied to the I-t plots, select **Decoration** on the **View** or context menu and select the **Frame & Axes** tab. Normalization is off by default.

Also, refer to:

⬚ Displaying and Overlaying Time I-t Plots
⬚ Displaying Difference, 1st Derivative, or 2nd Derivative I-t Plots

Displaying and Overlaying Time I-t Plots

In addition to peak I-t plots (I-t plots corresponding to the peak apex or at defined peak heights), Chromeleon can display the I-t plot corresponding to any selected time of the chromatogram.

Displaying a Time I-t Plot from a Chromatogram

1. Select **Tools>Spectra/I-t Plots Tool** on the context menu or click the following icon on the **Integration Toolbar**:

   The icon is appended to the mouse pointer, indicating that the mode was changed.

2. Click anywhere on the chromatogram to display the corresponding I-t plot.

Overlaying Several Time I-t Plots

1. Select the **Spectra/I-t Plots Tool**.

2. Press and hold the SHIFT key and click multiple places on the chromatogram.

Comparing Time I-t Plots from Different Samples

1. Overlay chromatograms of the desired samples in the Integration window (see **Comparing Chromatograms** **Selecting the Samples and Channels**).

2. Select the **Spectra/I-t Plots Tool**.

3. Press and hold the SHIFT key and click on the different chromatograms.

Also, refer to:

Displaying Peak I-t Plots

Displaying Difference, 1st Derivative, or 2nd Derivative I-t Plots

◼ Displaying Difference, 1st Derivative, or 2nd Derivative I-t Plots

When two or more I-t plots are displayed in the integration window, the similarity of the plots is often of interest. Chromeleon can analyze the I-t plots and display a difference I-t plot, or a first or second derivative I-t plot of each displayed plot.

1. Select **Decoration** on the **View** or context menu.
2. Select the **Analysis** tab and select the desired analysis option.

The **Difference** option displays a plot of the difference between the main I-t plot and the other I-t plots currently displayed. The main I-t plot is determined as follows:

*   If the peak I-t plot extracted at the retention time is displayed, it is the main I-t plot.

*   If the retention time plot is not displayed and I-t plots were selected with the **Spectra/I-t Plots** tool, the main I-t plot is the first plot selected.

*   If the retention time plot is not displayed and I-t plots were extracted automatically at different peak heights, the main plot is the I-t plot with the oldest retention time.

The **1st derivative** option displays the first derivative of each I-t plot displayed.

The **2nd derivative** option displays the second derivative of each I-t plot displayed.

Also, refer to:

Displaying Peak I-t Plots

Displaying and Overlaying Time I-t Plots

Answering Frequently Asked Questions

Question: How do I perform spectra scanning?

Answer: In the Program Wizard, select the **3DField** check box on the **UV Options** page to record the 3D channel for the corresponding samples.

Question: How do I change the ⇒*Detection Parameters*, such as ⇒*Minimum Area* or ⇒*Inhibit Integration?*

Answer: Enter the parameters on the **Detection** tab page of the QNT Editor (see **Data Representation and Reprocessing** **The QNT Editor**).

Question: How do I save the modifications to the layout of the on-screen report?

Answer: Modifications to the layout of the on-screen report are saved in the workspace. To save any modifications, select **Save Workspace** on the **Workspace** menu. The workspace contains the arrangement of the individual windows. Which information is displayed in the single windows is defined by the information saved in the corresponding file. For example, for the Printer Layout window, the information is saved in the Report Definition File (RDF).

Creating and Using Report Tables

Chromeleon provides numerous options to work with ➤*Reports*. You can add report tables to the screen report and in the ➤*Printer Layout*. The related settings are stored in the ➤*Report Definition File (RDF)*.

You can use existing report definition files or create special files to meet your individual requirements.

For more information about how to use report tables in the screen report, refer to 🔺 **Using Report Tables in the Screen Report**.

For information about how to use report tables in the Printer Layout, refer to 🔺 **Creating Report Tables for the Printout**.

🔳 Using Report Tables in the Screen Report

Before you can use a report table in the screen report, you have to display the report table (see 🔺 **Displaying a Report**.)

For more information about how to use report tables in the screen report, refer to:

🔺 **Defining the Contents of a Report**

🔺 **Defining the Appearance of a Report**

🔺 **Optimizing the Line Height**

🔺 **Saving a New Report Definition File**

🔺 **Linking Report Variables**

🔺 **Calculating the Peak Variable "Amount"**

🔺 **Adding and/or Renaming a Worksheet**

🔺 **Displaying the Peak Summary**

🔺 **Displaying an Audit Trail**

🔺 **Creating a History Report**

🔺 **Displaying MS Reports**

🔺 **Selecting Other Special Reports**

🔺 **Setting Parameters for Variables (e.g., for the confidence interval)**

▪ Displaying a Report

A numerical ➤*Report* can be included in the Integration, QNT Editor, and PPA method windows.

* Select **Show Report** on the **View** menu.

If you have not generated a report before, Chromeleon opens the default report (called **Default**). The default report contains the following worksheets: **Integration**, **Calibration**, **Peak Analysis**, **Summary**, and **Audit**. If you are working with a ➤*Photodiode Array Detector*, select the default report, **DEFLTDAD**. This report contains two additional worksheets: **Peak Purity** and **Lib Search**.

* To select a worksheet, click the corresponding tab.

Each worksheet includes several default variables that are specific to the selected report type. For example, in an integration report, there are columns for **Ret.Time**, **Area**, and ⇒*Amount*. A calibration report contains columns for **Offset (c0), Slope (c1),** and **Curve (c2)**, etc.

These predefined report sheets can be used for various purposes. Additional input is not required.

▪ Defining the Contents of a Report

To change the *contents* of a ➤*Report Definition File (RDF)*, select a command on the **Table** or context menu:

* Select **Insert Column** to insert a column in the report table on the left of the current cursor position.

* Select **Add Column** to add a column to the report table on the utmost right.

  📋 *Note:*

  *A new column created using the **Add Column** command has no format yet.*

* Select **Fix Column** to move the selected columns to the far left. Columns of this type are permanently visible, even when scrolling.

* Select **Delete Column** to delete one or several columns.

* Select **Column Properties** (or double-click the column header) to modify the column properties. You can then replace the current column contents, for example, number of theoretical plates, with a different variable.

- To change the formula of a cell, enable the **Layout Mode** on the **Table** menu. (The Layout Mode command is not available on the context menu.) An edit line appears above the table. In the table, click the cell for which you want to edit the formula. To the left of the edit line, the number of the cell is displayed. Edit the formula as desired. You can also use the ➢*Additional Functions* of the ➢*Report Publisher* module, if your Chromeleon license supports this.

🖙 *Tip:*

When Layout Mode is enabled, do not change the format.

🖙 *Tip:*

*Each column usually shows one report variable. However, it is possible to link several report variables (see **How to …: Creating and Using Report Tables** 🔳 Linking Report Variables). Mathematical and statistical functions such as SUM, AVERAGE, etc. are only available in the Report and the ➢Printer Layout together with the Report Publisher.*

- Select **Table Properties** to modify the properties of the entire report. Use this command to sort the table in groups or remove peaks below a certain area value.

🔳 Defining the Appearance of a Report

To modify the appearance of a report, select the **Table** menu. Then, select **Format >...**

- **Alignment** to position the text horizontally and vertically in a cell or a column.

- **Font** to determine the font, font style, and size.

- **Border** to determine the location, the color, and the shape of the frame.

- **Pattern** to determine the color and the pattern of the cell background.

- **Number Format** to determine the format of the represented values.

- **Autoformat** to design the entire report by selecting a ready-made default template. The **Preview** window shows the appearance of the report in the currently selected format.

🖙 *Tip:*

When Layout Mode is enabled, do not change the format.

◼ Optimizing the Line Height

Chromeleon allows you to optimize the line height. Do this especially for sample variables, for which the single values are displayed in several lines in one cell:

- Enable **Layout Mode** on the **Table** menu.

- In the left column, double-click the separation line under the line for which you want to adjust the height automatically. (In the picture below, for example, double-click the blue line that separates lines 6 and 7. (Note: The blue color is used in the picture only for illustration purposes; it does not appear in Chromeleon.) This optimizes the height of line 6 so that the entry in the **List of Aggr. Smp.** column is completely displayed.)

(In the example in the picture, the heights of lines 3 to 5 were already adjusted automatically, i.e., optimized. Thus, the list of aggregated samples is completely displayed in each line. The height of lines 6 to 9 was defined by a fixed value so that the lists were not displayed completely.)

▪ Saving a New Report Definition File

You can save all modifications made in a report to a ➢*Report Definition File (RDF)*:

- Select **Save Report Definition** and then enter the file name under which to save the RDF.

- If you change a default Report Definition File from the **Dionex Templates > Report** directory, you usually cannot save the new file to this Report folder because the Dionex Templates directory is locked. Save the modified file to a different directory; for example, create a new 'Report' directory under your local datasource.

☞ *Tip:*

If you do not save the RDF before you exit the screen report, any changes made to the report's layout are lost. There will be no hints as to the loss of these changes because a warning would then have to appear for every other change, as well, e.g., when resizing single windows.

▪ Linking Report Variables

To generate user-defined report variables, use the four basic arithmetic operations or powers to link two or more report variables. Link the single variables, using the formulas of the ⇒*Report Categories*.

How to

- Select a report column, and then select **Add Column** on the **Table** menu.

- In the **Formula** field of the edit box (**Add Report Column**), enter the formulas you wish to link by mathematical operators.

- Note that the variable names are not identical with their "formulas"; for example, **peak height** and **peak.height**). For the correct syntax, refer to ⇒*Report Categories*. You can copy the syntax by selecting the required formula and pressing the CTRL+C. Change to the edit box again and insert the formula by pressing CTRL+V.

  Example: To put the peak height in relation to the corresponding ⇒*Amount*, use the following expression:

  peak.height / peak.amount

- You can also use the following operators to link expressions: **+**, **-**, **x**, and **^** (for powers)

- Enter a column header in the **Header** field.

- Click **OK** to complete your input.

☞ *Tip:*

Mathematical and statistical functions such as SUM, AVERAGE, etc. are only available in the Report and in the ➢*Printer Layout together with the* ➢*Report Publisher!*

◢ Calculating the Peak Variable "Amount"

If you wish to check the indicated ⇒*Amount* values, have a look at the respective coefficients of the calibration function first. Then, add the columns offset (c0), slope (c1), and curve (c2), which are all part of the **Peak Calibration** category.

For the amount calculation of an unknown sample, the inverse function f(y) of the calibration function F(x) must be calculated. Calibration functions are calculated as follows:

Linear:
$$f(y) = \frac{1}{c_1} y$$

Linear with offset:
$$f(y) = -\frac{c_0}{c_1} + \frac{1}{c_1} y$$

Quadratic:
$$f(y) = \frac{1}{2 * c_2} * \left(-c_1 \pm \sqrt{c_1{}^2 + 4 * c_2 * y} \right)$$

Quadratic with offset:
$$f(y) = \frac{1}{2 * c_2} * \left(-c_1 \pm \sqrt{c_1{}^2 - 4 * c_2 * (c_0 - y)} \right)$$

Exponential:
$$f(y) = \left(\frac{1}{c_0} \right)^{\left(\frac{1}{c_1} \right)} * y^{\left(\frac{1}{c_1} \right)}$$

 Note:

For ambiguous inverse functions (two possible values for quadratic with or without offset) always use the value which comes "closest" to the X values of the respective calibration.

If it is not possible to calculate the expression (c1=0 for linear with or without offset, the radiant < 0 for quadratic with or without offset, or c0=0, or c2=0 for exponential), "n.a." is returned as amount.

If the response factor, dilution factor, weight, and a factor for the ➤*Internal Standard* are available, that is, if they do not equal 1, they have to be considered for the calculation as well (see ➤*Formula for Amount Calculation*).

▪ Adding and/or Renaming a Worksheet

You can add new worksheets to the ➤*Report Definition File (RDF)* and define their contents and appearance.

- Select **Insert Report** on the **Table** menu and determine the type of report to be created.
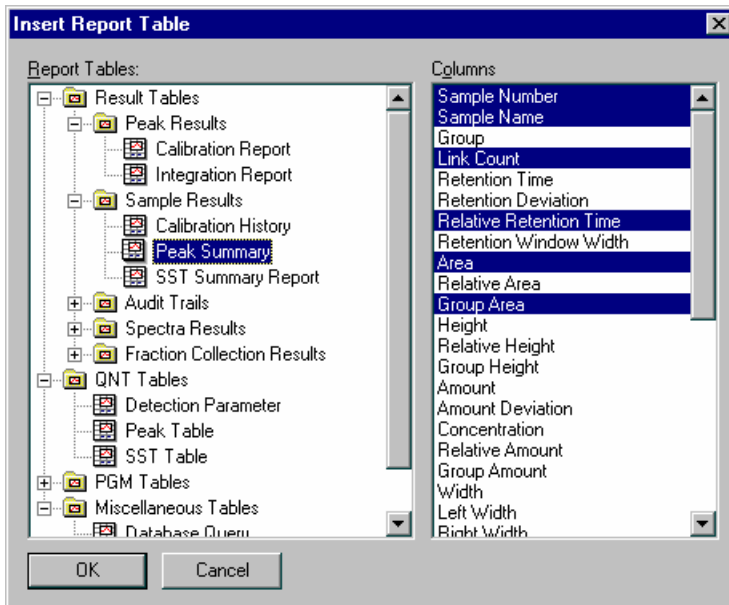
The following reports are available (the order is the same as in the dialog box):

Result Tables

Peak Results

Calibration Report	Displays all variables required for creating a calibration report on the right.
Integration Report	Displays all variables required for creating an integration report.

Sample Results

Calibration History	Displays all variables documenting the course of the calibration on the right.
Peak Summary	Displays all variables required for creating a peak summary.
SST Summary Report	Displays all variables required for documenting the results of the ➢ *System Suitability Test* for the entire sequence.

Audit Trails

Audit Trail (Commands, SST, ...)	Displays the Audit Trail of the current sample (see **Data Management** 🔳 **Audit Trails**).
MS Instrument Info Report	Displays information about the ➢ *Mass Spectrometer*.
MS Status Log Report	Displays the mass spectrometer settings.
MS Tune Data Report	Displays the tune data of the ➢ *Xcalibur* raw data file.
MS Instr. Method Report (XRaw)	Displays the MS method.

Spectra Results

MS Raw Report	Displays the raw data of the current ➢ *Mass Spectrum*.

Fraction Collection Results

Fraction Report	Displays all variables required to create a fraction report.
Tube Report	Displays the different variables describing the single fraction collection tubes. This also includes the fraction report variables. However, please note that these variables are not selected by default.

QNT Tables

Detection Parameter	Displays all ⇒*Detection Parameters* of the current sample.
Peak Table	Displays all variables that are required for displaying the peak table of the QNT Editor.
SST Table	Displays all variables that are required for the representation of the ➢*System Suitability Test*.

PGM Tables

Commands	Displays the ➢*Program* for the current sample.
Post Acq. Processing	Displays the ➢*Post-Acquisition Steps* for the current sample.
MS Method	Displays information about the current method of the ➢*Mass Spectrometer*.

Miscellaneous Tables

Database Query	Inserts any kind of database queries into the report.
History Report	Displays all variables required for creating a ➢*History* report.

- From the variables that are available on the right-hand side, click those you wish to include in the new report. Click **OK** to create the worksheet.

- In the report, double-click the newly added tab and edit the name.

Saving

To save the content and appearance of a report, select **Save Report Definition**.

☞ *Tip:*

*Please note that the appearance and the contents of the on-screen report needs not to be identical with the actually printed report, the **Report Templates**. The printout is defined in the ➢Printer Layout. Thus, it allows you to initiate printing independently of what is displayed on screen.*

▪ Displaying the Peak Summary

Use the **Summary** worksheet in the **Report** (see **Report Tables** 🔺 **The Peak Summary Report**) to show certain results from all samples.

The required steps are described in **How to …: Creating and Using Report Tables**:

🔺 **Displaying a Report** and

🔺 **Adding and/or Renaming a Worksheet**.

The **Summary** worksheet represents, e.g., data of a specific peak from *all* samples (generally from a ➢*Sequence*) while the **Integration** and **Calibration** worksheets of the ➢*Report Definition File (RDF)* list the data of the peaks from *one* sample.

To display, for example, the amount of different peaks of all samples of a sequence in one table, select **Column Properties** on the context menu. Select **Peak Results** from the **Categories** list, and then select **Amount** from the **Variables** list and have Chromeleon display the value for certain peaks (**Fixed Peak(s)**). The default setting shows the values for the currently **Selected Peak**. Determine the peak for which the data are displayed by clicking the peak in the above chromatogram.

The default **Summary** report contains several columns. In addition to the sample name and the retention time, it also includes the **Area**, **Height**, ⇒*Amount*, **Types**, and **Plates** columns. When you select a peak in the chromatogram, the values of this peak are included in the Summary report. If the selected peak is not contained in a sample, this line remains empty (n.a. = not available).

Below the sample list, there are the lines **Average** and **Relative Standard Deviation**. The average value of a column is calculated and displayed. The relative standard deviation from this value is indicated in percent.

The user can modify the default Summary report at any time. However, it is recommended to keep this report page. If another report is required, create an additional Peak Summary report page with new column assignments.

🛅 *Note:*

Instead of a single sequence, the Summary report can also be started based on a ➢Query. Then, the Summary may include different samples from different sequences.

◾ Displaying an Audit Trail

You can include the Sample Audit Trail (see **Data Management** ◾ **The Sample Audit Trail**) as a worksheet in a ➤*Report Definition File (RDF)*. The worksheet always shows the Audit Trail for the currently selected sample.

- The **Day Time** column indicates the time of a command or message.

- The **Ret.Time** column indicates the corresponding retention time.

- The **Command/Message** column shows the command itself, the text of a message, or an event.

To select additional settings, select the **Table Properties** command on the context menu. The **Audit Trail Report Properties** dialog box is opened; define the data to be displayed.

In addition, you can select one of the following **Display** options:

- **Run only** displays only the entries for the sample run (default option).

- **Preconditions only** displays only the conditions before a sample run.

- **Preconditions and Run** displays all entries.

The display filter (**Filter Level**) defines the type and extent of the Audit Trail entries for a sample run.

Normal:	Displays only the most important commands and properties.
Advanced:	Displays Normal and Advanced level commands and properties.
Expert:	Displays Normal, Advanced, and Expert level commands and properties (for experts only).

Errors and Warnings: Displays error messages and warnings, but no commands.

You can add the contents of the worksheet to other documents, using the **Cut** and **Paste** commands. You can print the entire Audit Trail in the ➤*Printer Layout*.

Using Audit Trail Variables in Other Worksheets

Certain events (such as performing a ⇒*Trigger* or changing the wavelength at a specific time) can be included in any Report worksheet.

- Open the corresponding worksheet and select **Column Properties** on the context menu.

- Select **Audit Trail** from the **Categories** list.

- In the **Variables** field, select one of the Audit Trail variables. The variables that are available for selection depend on the events listed in the Sample Audit Trail.

- Use the **Formula** field to display events that are not listed (for example, the system pressure that is recorded using a Log command). In this case, the event variable is appended to the name AUDIT; separated by a period (AUDIT.pressure).

- Click **OK** to confirm your input.

Your report now includes an additional column for an Audit Trail variable. Normally, the value for the corresponding peak at the retention time is entered in each line. If there is no value at this time, the value that was recorded last is entered. When forming a gradient, Chromeleon calculates the corresponding values (%A, %B, %C . . .).

If you want to display a specific Audit Trail event for all peaks in a report at a specific time:

- Select **Column Properties** and then select an **Audit Trail** variable.

- Click **Parameter**.

A dialog box opens, allowing you to enter a retention time. Click **OK** to confirm the entry. A report column with a fixed retention time is generated. For example, the **Wavelength** Audit Trail variable displays the wavelength at time t.

You can also search for the next associated entry in the Audit Trail. The starting point of the search is the entered retention time. To find the last entry before this retention time, select **backward** as the **Search Direction**. To find the first entry after this retention time, select **forward** as the **Search Direction**.

▣ Creating a History Report

The ➢*History* report is not part of a default report. Therefore, you must select **Insert Report** to add a history worksheet to a ➢*Report Definition File (RDF)* (see **How to …: Creating and Using Report Tables** ▣ **Adding and/or Renaming a Worksheet**).

By default, the worksheet shows the history of the current sample. Select **Table Properties** on the context menu to open the **History Report Properties** dialog box and change the settings:

- On the **History Objects** tab page, select the object for which you want to display history entries.

- On the **Time Restrictions** tab page, specify the time when the history entries to be displayed must have been made.

- On the **Operations** tab page, determine the changes to be displayed.

- On the **Users** tab page, determine the user(s) whose changes are displayed.

- On the **Sorting** tab page, define the sorting order for the history entries.

- A special layout mode is provided for the history report. Select the **Layout Mode** on the **Layout** tab page. However, this is only possible if detail columns are available. If detail columns exist, select the **Design template mode** to display a shortened history report (this simplifies the layout definition).

▣ Displaying MS Reports

Similar to the ➢*History* report, the different MS reports are not part of the default ➢*Report Definition File (RDF)*. Select **Insert > Chromeleon Report Table** to add them as separate worksheets to a report definition file (see **How to …: Creating and Using Report Tables** ▣ **Adding and/or Renaming a Worksheet**):

- Select the **MS Instrument Info Report** to display information about the ➢*Mass Spectrometer*. If there is no MS data, the following message appears in the report: *"No MS Instrument Info found".*

- The **MS Instrument Method Report** indicates the MS method. If there is no MS data, the following message appears in the report: *"No MS Instrument Method found."*

🛍 *Note:*

*The **MS Method** (under PGM tables) indicates the current method. It is possible that both MS methods are identical.*

- The **MS Raw Report** shows the raw data (mass, intensity, and relative intensity) of the ➢*Mass Spectrum* of the current sample. If no MS data is available, the report just says: *"No MS Raw Data found."*

- The **MS Status Log Report** shows the mass spectrometer settings. If there is no MS data, the report just says: *"No MS Status Log found."*

- Select the **MS Tune Data Report** to display the tune data of the ➢*Xcalibur* raw data file. If there is no MS data, the report just says: *"No Tune Data found".*

🛍 *Notes:*

If the MS Control option is disabled on your PC, the MS reports will not be displayed in the Insert Report Table dialog box.

All MS reports comprise only the default columns. It is not possible to add more columns.

▣ Selecting Other Special Reports

Similar to the ➢*History* report, the following reports are not part of the default ➢*Report Definition File (RDF)* either. Select **Insert Report** to add them as separate worksheets to a report definition file (see **How to …: Creating and Using Report Tables** 🛅 **Adding and/or Renaming a Worksheet**):

Result Tables

- The **Calibration History** documents the calibration run.

- The **SST Summary Report** shows the results of the ➢*System Suitability Test* for the entire sequence. The default columns are part of the ⇒*Sample* and ⇒*System Suitability Test* categories. It is also possible to edit the columns or to use different columns for the report.

QNT Tables

- The **Detection Parameter Report** shows the ⇒*Detection Parameters* used for the current sample.

- The **Peak Table** report shows certain entries of the peak table of the QNT Editor (such as the expected ➤*Retention Time* or the retention ⇒*Window*).

- The **SST Report** shows the parameters used and the results of the System Suitability Test. The default columns are part of the System Suitability Test category.

PGM Tables

- The **Commands** report shows the ➤*Program* of the current sample.

- The **Post-Acquisition Processing** report shows the ➤*Post-Acquisition Steps* of the current sample.

- The **MS Method** shows the current method of the ➤*Mass Spectrometer*.

Note:

*The **MS Instrument Method Report** (under Result Tables) indicates the method that is used for data acquisition. It is possible that both MS method reports are identical.*

Miscellaneous Tables

- To integrate any kind of database query in the report, select the **Database Query** report. To open the table properties and specify the datasource in which the query is performed, select **Table Properties** on the context menu. Use ➤*SQL* statements to define the properties for which the query will be performed.

Note:

*The **Detection Parameter Report** and the **Commands, Post-Acquisition Processing, MS Method,** and **Database Query** reports comprise only the default columns. It is not possible to add more columns.*

■ Setting Parameters for Variables (e.g., for the confidence interval)

You can set special parameters for many of the report variables. For an example, refer to the description below for the limits of the ➤*Confidence Interval*.

The **Parameter** button is enabled for those variables for which special parameters can be set.
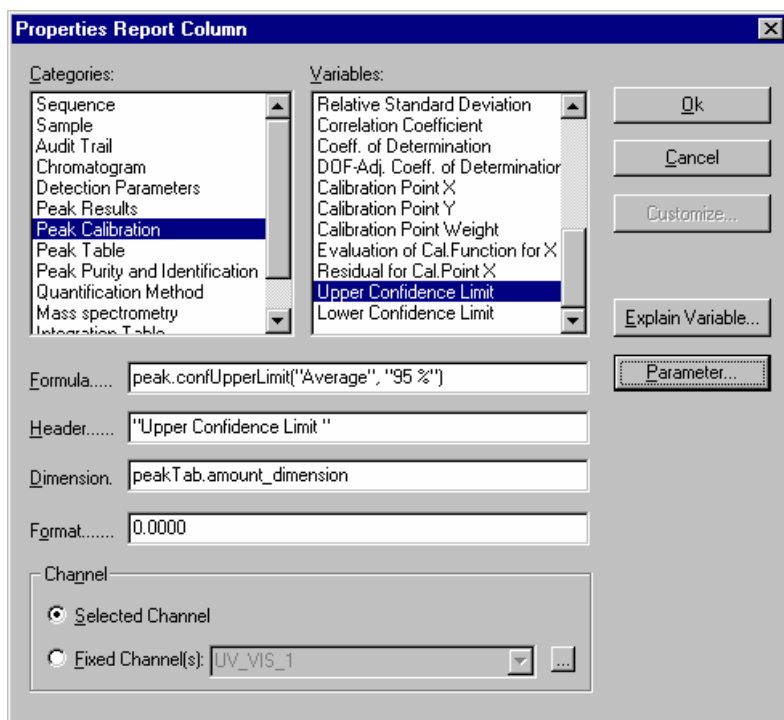
Click **Parameter** to open the **Parameter Input …** dialog box. Use this dialog box to make the settings for the displayed report variable:
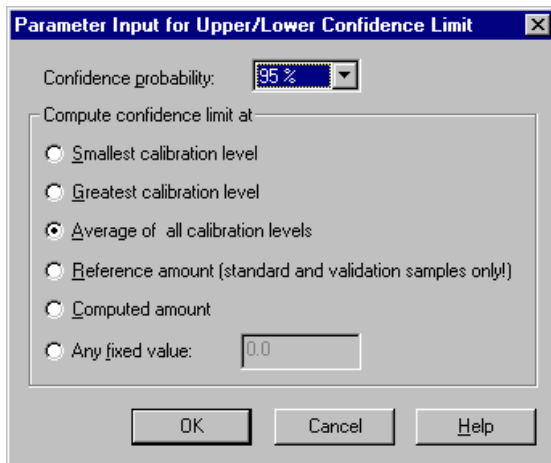
The **Parameter Input for Upper Confidence Limit** dialog box allows you, for example, to define the probability and the calibration level at which the upper limit of the confidence interval is computed:

Confidence probability Select the probability with which the indicated values shall apply via the arrow key. The following probability values are available:

90%, 95%, 98%, 99%, 99.7%, 99.8%, 99.9%, and 99.99%.

Compute confidence limit at Define the level for which to compute the respective limit of the confidence interval. Select one of the following options:

- **Lowest calibration level**

- **Highest calibration level**

- **Average of all calibration levels**

- **Reference amount (standard and validation samples only)** for reference values. The corresponding values appear for standard and validation samples only if an entry for the respective peak is available in the Amount column. If a peak has no entry or if the Amount column is not available for a standard or validation sample, **n.a.** is returned.

- **Computed amount** (for the computed amount of the respective peak in the single samples).

- **Any fixed value**. Define the Amount value in the right-hand field.

The graphical representation of the confidence interval in the calibration curve is possible as well. For more information, refer to **How to …: Displaying Calibration Curves** 🔳 **Indicating the Confidence Interval**.

The limits of the confidence interval, which are defined using the corresponding Report variables, are determined as follows: For a given amount, the system determines the intersections with the limiting curves of the confidence interval In the height of the value that is defined via the calibration curve.
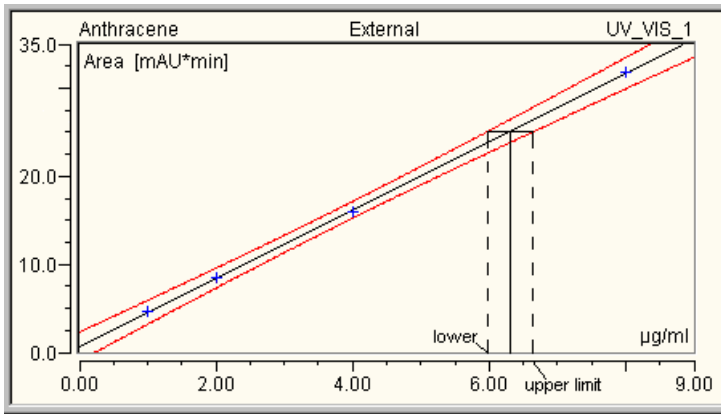
The image shows the calibration curve together with the corresponding confidence interval at a probability of 99%. In the above example, the upper and lower limits belonging to a concentration of 6.3 µg/ml are derived. Their values are 6.0 and 6.6 µg/ml, respectively. (The distance between the limiting values and the given Amount value must not necessarily be symmetrical.)

🔳 Creating Report Tables for the Printout

Similar to the screen report, you can use report tables in the Printer Layout, also. For information, refer to:

🔳 **Inserting and Editing a Table in the Printer Layout**

🔳 **Inserting a Column into an Existing Table**

Users who have the ➢*Report Publisher* add-on product can use the additional features described in 🔳 **Entering User-defined Formulas**.

Thus, they have numerous possibilities, e.g., for

🔲 **Calculating the Amount Percentage (for Identified Peaks)**

🔲 **Calculating the Concentration Percentage (in Relation to the Total Concentration)**

🔲 **Calculating the Retention Time Difference of Two Channels**

🔲 **Creating Dynamic Columns That Contain Flexible Formulas**

🔲 **Creating Dynamic Links to Lines Other than the Current Line**

◾ Inserting and Editing a Table in the Printer Layout

- Enable ➤ *Layout Mode* on the **Edit** menu.

- Select **Insert > Chromeleon Report Table** on the **Edit** or context menu. The **Insert Report Table** dialog box appears.

- In the Report Tables field, mark the report table you want to insert. If necessary, click the + character beside the report table groups to display the tables underneath. The columns available for the selected table are displayed in the **Columns** field.

- Some columns are marked by default. Select the columns that should be included in the table and deselect all others. Press and hold the **Ctrl** key to select or deselect several columns simultaneously.

- Clicking **OK** inserts the table into the worksheet.

- To indicate that the table is a Chromeleon object all four corners of the table are marked by red triangles. The last row(s) are not marked by red triangles because they are no Chromeleon objects.

⚠ *Caution:*

If the worksheet already contains a report table, insert the new table above or below the existing one. It is not possible to insert several report tables next to each other.

👉 *Tip:*

You cannot move or copy an entire table. When moving or copying a table, only the current content (= the values) is copied to the clipboard but not the underlying variables! (The red triangles are missing indicating that this table is no longer a Chromeleon object.) Besides, it is not possible to move single columns. Instead, insert a new column at the corresponding position and delete the 'old' column.

- Select a column header and then select **Report Column Properties** on the context menu. Determine the header, the dimension, and the format of the column or of the column values.

- To modify the appearance of a single cell, a table area, or the entire table (font size and style, frame, color, etc.), select the cell, the area or the entire table and then select the corresponding command on the **Format** menu.

- Select the column or the row you want to delete, and then select **Delete Column(s)** or **Delete Row(s)** on the **Edit** menu. To insert additional rows or columns in front of the selected area, select **Insert Row(s)** or **Insert Column(s)** on the **Edit** menu.

Inserting a "Total" Row

The new table does not comprise a **Total** row. Insert the Total row as follows:

- Copy any cell from another **Total** row; for example, from another ➢*Printer Layout* table or from an integration report.

- Select the cells of the new table, which should indicate the sum of all cell values of the corresponding column.

- Select **Paste**.

🖙 *Tip:*

Proceed in the same way to update any existing entries in the rows **Average** *and/or* **Rel.Std.Dev** *rows. These rows are not marked by red triangles, because they are no Chromeleon objects.*

Creating the correct cell reference

The values in the **Total** row must receive the correct cell reference. This is especially important when the new table is longer than the table from which the cell was copied.

- Enable ➢*Layout Mode* on the **Edit** menu. The edit line is displayed.

- Select the first value in the **Total** row. In the edit line, the corresponding formula appears; for example, =SUM(C10:C22).

- Select the cell range indicated in parentheses in the edit line with the mouse; for example, C10:C22.

- Then, select the actual cell range in the table with the mouse or type the cell range in the edit line via the keyboard.

- Press <Enter> to confirm your input.

The sum cell value is recalculated based on the new cell range. Then perform the individual steps for the remaining sum cell values.

For more information, see **Inserting a Column into an Existing Table**.

Inserting a Column into an Existing Table

To insert a new column into an existing table:

- Enable ➢*Layout Mode* on the **Edit** menu.

- Select the entire column, including its header and it's the cell **Total**, to the left of which you want insert the new column:

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
2	5.30	Naphthalene	86.662	10.321	2.90	1.011	BM
4	7.65	Phenanthrene	207.567	23.854	6.71	1.746	BM
5	7.97	Anthracene	139.321	15.955	4.49	1.791	MB
6	8.72	Fluoranthene	2122.275	259.255	72.90	1.017	BMB
7	9.11	Pyrene	264.949	32.018	9.00	1.244	Rd
8	9.75	Dimethyl-Fluora	10.922	1.391	0.39	1.110	BMB
9	10.29	Chrysene	76.265	9.695	2.73	1.802	BMB
Total:			2907.962	352.489	99.12	9.721	

- Select **Insert Column(s)** on the context menu. A new table column is inserted that also contains a **Total** cell.

- Double-click the header of the new column to open the **Report Column Properties** dialog box. Select a variable from the **Variables** list (Please note: The picture only shows part of the dialog box):
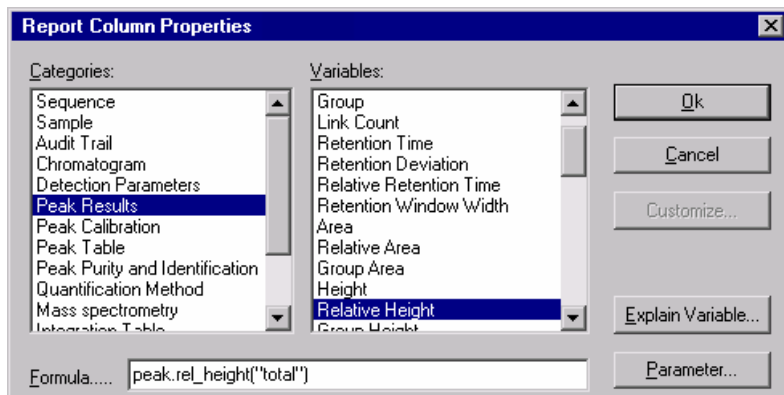
- Click **OK** to assign the selected variable to the new column.

- Fill the cell **Total**. Click a different cell in this line, which already contains a value. Copy the cell reference by pressing the Ctrl + C keys and then paste it into the new cell by the pressing Ctrl + V keys. (The format of the copied cell is automatically transferred, too. Adapt the format to the new column if necessary.)

For more information, refer to 🔲 **Inserting and Editing Tables**.

🔲 **Entering User-defined Formulas**

👉 *Tip:*

In order to use the options described below, the ⏵Report Publisher add-on product must be installed on your computer.

Creating user-defined formulas is analogous to the Microsoft Excel spreadsheet. The following description is intended for users who are not familiar with entering formulas:

Example 1:

Let's assume that a table containing two columns (A and B) and three lines (1 to 3) is extended by one column (C), for which there is *no* Chromeleon report variable. Thus, for example, the quotient of the cell contents of columns A and B can be included in column C.

To enter a formula in a cell, select the corresponding cell first. In this example, select the cell C1. Enter the equal sign. The entire input is displayed in the edit line (here indicated in blue print).

C1	=		
	A	B	C
1	12	5	
2	17	10	
3	13	20	
4			

Select the cell A1, enter a division sign (slash), select the cell B1, and complete your input by pressing <Enter>. The formula (C1=A1/B1) is displayed in the edit line; the cell C1 displays the result of the operation (2.4). The cursor moves to cell C2.

C1	=A1/B1		
	A	B	C
1	12	5	2.4
2	17	10	
3	13	20	
4			

Follow the description to output the corresponding results in the cells C2 and C3. To facilitate the procedure, click the cell C1 again, grab the selection frame on the lower right corner, and drag it to the required cells (C2, C3). For calculating the cell values, Chromeleon uses the previously entered formula and automatically creates the correct reference, line by line.

C1	=A1/B1		
	A	B	C
1	12	5	2.4
2	17	10	1.7
3	13	20	0.65
4			

Example 1 (continued)

How to sum the cells of column C in the field C4:

Select the cell C4 and enter an equal sign. Enter the **SUM** command required for adding cell values and enter an opening bracket.

C4	=SUM(		
	A	**B**	**C**
1	12	5	2.4
2	17	10	1.7
3	13	20	0.65
4			

Select all cells (C1, C2, C3) that you want to sum up. Close the bracket after the last cell. Press <Enter> to complete your input.

C4	=SUM(C1:C3)		
	A	**B**	**C**
1	12	5	2.4
2	17	10	1.7
3	13	20	0.65
4			**4.75**

In addition to the SUM command, many other functions are entered in the same way. Thus, you can form the average (see **AVERAGE**), express conditions (see **IF**, **True**, **FALSE**), create logical operations (see **AND**, **OR**), or enter time values (see **TIME**, **DATE**, **DAY**, **YEAR**).

Tip:

For an alphabetical list of the available formulas, refer to ➤*Additional Functions.*

Example 1 (continued)

Besides, it is also possible to use "fixed references." Contrary to the variable value pairs described above (A1/B1, A2/B2, A3/B3), form the quotient of a variable and a fixed value (A1/C4, A2/C4, A3/C4). In this example, the result is displayed in column D.

Follow the description for Example 1 above to create the first cell reference (D1).

D1	=A1/C4			
	A	**B**	**C**	**D**
1	12	5	2.4	2.5263
2	17	10	1.7	
3	13	20	0.65	
4			4.75	

Extend the formula by adding two **$** signs. They convert a variable reference into a fixed cell reference.

D1	=A1/$C$4			
	A	**B**	**C**	**D**
1	12	5	2.4	2.5263
2	17	10	1.7	
3	13	20	0.65	
4			4.75	

When the formula is copied, the reference to cell C4 will be retained. To calculate the fields (D2 and D3), simultaneously select the cells D1, D2, and D3.

D1	=A1/$C$4			
	A	**B**	**C**	**D**
1	12	5	2.4	2.5263
2	17	10	1.7	3.5790
3	13	20	0.65	2.3768
4			4.75	

For practical examples, refer to:

Calculating the Percentage Value for the Amount (for Identified Peaks)

Calculating the Percentage Value for the Concentration (in Relation to the Total Concentration)

Creating Dynamic Columns That Contain Flexible Formulas

Creating Dynamic Links to Lines Other than the Current Line

Calculating the Amount Percentage (for Identified Peaks)

If you use samples with ➢*Internal Standard* but wish to calculate the percentage values of the different substances that have been identified in the sample (without internal standard):

1. First, disable the display of unidentified peaks in the report table. Select **Report Table Properties** on the context menu. On the **General** tab page of the **Integration Report Properties** dialog box, clear the **Including unidentified peaks** check box.

2. Select a column that you do not need (in the example below: column D). Select **Add Column** or **Insert Column** on the context menu. From the **Categories** list, select **Peak Table**, and then select **Standard Method** from the **Variables** list.

3. a) Select a different column (here: column G) that you do not need. Double-click the column header to open the related dialog box. Delete the entry in the **Formula** field. Afterward, delete the entries in the column (except the last line, i.e., the line named **Total**).

b) In the field G29, enter the formula

=IF(OR(D29="ISTD Internal ";D29="ISTD Int/Ext ");"ISTD";F29)

(Observe the space following **Internal**!) Copy the formula to the following lines.

☞ *Tip:*

*Do not omit the space in the formula behind **Internal** and **Int/Ext**, respectively.*

4. a) Select another column which you do not need either (here: column H). Press F8 to open the dialog box. Delete the entry in the **Formula** field. Afterward, delete the entries in the column (except the last line).

b) Enter the formula **=100*G29/G$36** in the field H29 and copy it to the following lines

G29		=IF(OR(D29="ISTD Internal ";D29="ISTD Int/Ext ");"ISTD";F29)						
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
27	**No.**	**Ret.Time**	**Peak Name**	**Standard**	**Area**	**Amount**	**Amount**	**%-Amount**
28		**min**			**mAU*min**	**µg/ml**	**µg/ml**	**µg/ml**
29	2	5.30	Naphthalene	ISTD Internal	10.478	1.000	ISTD	
30	4	7.67	Phenanthrene	External	8.659	1.435	1.435	30.03
31	5	7.99	Anthracene	External	4.589	1.148	1.148	24.03
32	6	8.75	Fluoranthene	ISTD Internal	244.113	1.000	ISTD	
33	7	9.15	Pyrene	Int/Ext 'Dime	8.590	1.108	1.108	23.18
34	8	9.78	Dimethyl-Fluor	ISTD Int/Ext	1.203	0.934	ISTD	
35	9	10.31	Chrysene	External	2.659	1.088	1.088	22.77
36	**Total:**				280.292	7.71	4.779	100.00

◄ ► \ Integration ∕ Calibration(Curr.Peak) ∕ Calibration(Batch) ∕ Peak Analysis ∕ ◄ ►

Also, refer to **How to …: Creating and Using Report Tables** 🔲 **Calculating the Concentration Percentage (in Relation to the Total Concentration)**

▣ Calculating the Concentration Percentage (in Relation to the Total Concentration)

Entering and Calculating the Total Concentration

To calculate the percentage concentration in relation to the total concentration, calculate the total concentration using your exact sample weight (without any added ➤*Internal Standards*) and the liquid volume. Enter the concentration, for example, in the **Weight** column of the sample list. However, it may be better to create a ➤*User-defined Column*, name the column **concentration**, and assign **Floating Point** as the **Value Type**.

(For more information, refer to **How to …: Creating and Managing Files and Data** ▣ **Creating User-defined Columns**.) Enter the corresponding total concentrations of the single samples in this column.

Creating the Report Table

1. Double-click a variable in the page header that is not necessarily required (here: H6). For this variable, define the concentration column created before:

Properties Report Variable ☒

Categories:	Variables:	
General	Vial Number	**Ok**
Sequence	Replicate ID	
Sample	Status	**Cancel**
Audit Trail	Date & Time Sample Run	
Chromatogram	Inject Volume	
Detection Parameters	Dilution Factor	Customize...
Peak Results	Sample Weight	
Peak Calibration	ISTD Amount	
Peak Table	Program	
Peak Purity and Identification	Quantification Method	
Quantification Method	*concentration	Explain Variable...

Formula..... `smp.concentration`

Format....... `0.000`

Parameter...

2. Follow the description in the previous topic (see **How to …: Creating and Using Report Tables ⬜ Calculating the Percentage Amount (for Identified peaks)**). Instead of the formula described there in 4b, enter the following formula (here: in the field H13): **=IF(G13="ISTD";"ISTD";100*F13/$H$6)**.

■☞ *Tip:*

Unless otherwise defined, enter no spaces before and after ISTD.

The value in the **Total** line of the **%-Amount** column indicates the percentage of the substances defined by you in relation of the total concentration:

H13		`=IF(G13="ISTD";"ISTD";100*F13/$H$6)`						
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
6	Control Program:	**WELL2**				Concentration:		**5.000**
7	Quantif. Method:	**GS50-SIG**				Dilution Factor:		**1.0000**
8	Recording Time:	**4.1.1994 14:25**				Sample Weight:		**1.0000**
9	Run Time (min):	**14.10**				Sample Amount:		**1.0000**
10								
11	**No.**	**Ret.Time**	**Peak Name**	**Standard**	**Area**	**Amount**	**Amount**	**%-Amount**
12		**min**			**mAU*min**	**µg/ml**	**µg/ml**	**µg/ml**
13	2	5.30	Naphthalene	ISTD Internal	10.478	1.000	ISTD	ISTD
14	4	7.67	Phenanthrene	External	8.659	1.435	1.435	28.70
15	5	7.99	Anthracene	External	4.589	1.148	1.148	22.97
16	6	8.75	Fluoranthene	ISTD Internal	244.113	1.000	ISTD	ISTD
17	7	9.15	Pyrene	Int/Ext 'Dime	8.590	1.108	1.108	22.16
18	8	9.78	Dimethyl-Fluor	ISTD Int/Ext	1.203	0.934	ISTD	ISTD
19	9	10.31	Chrysene	External	2.659	1.088	1.088	21.76
20	**Total:**				280.292	7.71	4.779	95.58

◀ ▶ \ Integration ⋀ Calibration(Curr.Peak) ⋀ Calibration(Batch) ⋀ Peak Analysis ⋀ ⬛ ◀ ▶

smp.amount   SfGrm

▣ Calculating the Retention Time Difference of Two Channels

If data acquisition is performed with two different detectors (for example, in an ➢*APS*), the retention times may be different. In this case, the retention time deviation should be documented in a separate column.

A special formula allows you to determine for a peak how the retention time of one channel deviates from the retention time of the current channel.

🗒 *Note:*

You can create the related column in both the ➢Printer Layout and screen report. In this case, the ➢Report Publisher add-on module is not required.

Follow the steps below to subtract the retention time of a channel from the retention time of the current channel:

- Double-click in the header of an unused or newly inserted column to open the **Properties Report Column** dialog box.

- In the **Formula** field, enter the following formula (also, refer to the explanation below):
  (retention_time - smp.chm("TIC").peak("By Retention Time", peak.retention_time, 0.100, "AN").retention_time)*60.

- In the **Header** field, enter the column header, e.g., **"Ret.Diff. (UV-MS)"**.

- In the **Dimension** field, enter **"s"** (for seconds).

- Usually, two positions after the decimal point (i.e., **"0.00"**) will be sufficient.

Explanation and possibly required modifications

To use the formula efficiently, you do not need to understand the formula in detail. It will be sufficient to know the parts you may wish to modify:

- **Channel**: The formula calculates the retention time difference between the ➢*TIC* channel and the current channel for a peak. In the formula, replace TIC with a different channel if you want to subtract the retention time of this channel from the retention time of the current channel.

- **Dimension**: Usually, the retention times are in minutes. However, the retention time difference is relatively small for one peak. That is why the value is multiplied by a factor of 60 to convert it into seconds. You may delete this factor. In this case, set the entry in the **Dimension** field to **"min"**.

- **Peak detection**: The main part of the formula refers to peak detection in another channel: **peak("By Retention Time", peak.retention_time, 0.100, "AN")**. The expression in brackets finds the peak that is nearest to the expected retention time (= **N**) in a windows of **± 0.100** min (absolute = **A**) around the expected retention time (determined in the peak table of the QNT Editor). This is required to find the peak in a different channel.

▣ Creating Dynamic Columns that Contain Flexible Formulas

Follow the instructions below to create a table column that contains flexible formulas and dynamically adapts to the number of existing peaks. (This procedure is important only if the table contains columns with report variables, and if these columns are next to the newly added column.)

See the following example for information about how to create a column that lets you automatically check whether the signal noise is below 0.005 mAU:

- Add a new column to the table. In the Printer Layout, new columns always appear to the left of the selected column. Select the first field below the header in the column that will appear to the right of the new column:

	A	B	E	F
1	Sample	Sample Name	Noise	Amount
2	No.		mAU	
3				Pyrene
4			Uv_vis_1	Uv_vis_1
5	1	Sample 1	0.004	1.1078
6	2	Sample 2	0.004	1.9434
7	3	Sample 3	0.004	3.6269
8	4	Sample 4	0.004	8.4621

- On the context menu, select **Insert** and then select **Insert Chromeleon Report Column**.

- The **Insert Report Column** dialog box opens. Select a variable.

- Delete any existing entry in the **Formula** field.

- Click **OK** to exit the dialog. This action automatically selects the first field in the new column.

- Enter the formula in the layout line without changing the selection in the report table. Press **Enter** to confirm your entry.

- Do not change the selection, i.e., the first field below the header is selected in the new column. Place the cursor on the black rectangle on the bottom right corner of the selection frame. The cursor becomes a solid black cross. Left-click and draw the formula into all lines of the selected report table column.

- Double-click in any field in the new column to open the **Report Column Properties** dialog box for the new report column. Edit the **Header** and **Dimension** fields as appropriate:

◾ Creating Dynamic Links to Lines Other than the Current Line

To reference lines in a report table other than the current line, use indirect references instead of direct references such as G6. For indirect references, use the following ➢*Report Publisher* variables:

```
INDIRECT and ADDRESS
```

The following formula references the cell G6 from the cell E2:

```
=INDIRECT(ADDRESS(ROW()+4;COLUMN()+2))
```

Note:

Use the `ROW()` *and* `COLUMN()` *formulas to return the current line and column numbers.*

Preparing the Printout

Use the Printer Layout in the ➢*Report Definition File (RDF)* to prepare your data for printing. To help you prepare the first printout and create your own report definition files (also called *report templates* below), Chromeleon provides some default RDF templates. During the Chromeleon installation, the default RDFs are written to the **Dionex Templates > Reports** directory of your local datasource.

To print data that is not included in the default report definition files, Chromeleon lets you create individual worksheets via the ➢*Printer Layout* window. These worksheets can be combined and then saved as a report template. The results of sample processing can thus be printed in a number of layouts.

For more information, refer to:

🔳 **Opening and Editing the Printer Layout**

🔳 **Saving or Loading a Report Definition File**

🔳 **Specifying the Pages to Be Printed**

🔳 **Specifying the Printout**

🔳 **Printing the Results of a Single Sample**

🔳 **Printing the Results of a Sequence or Sample Batch**

🔳 **Setting Print Area and Print Title(s)**

🔳 **Automatically Repeating the Output for the Selected Objects**

🔳 **Saving the Contents of a Printer Layout Page**

In addition, you can use the Printer Layout to create charts (see 🔳 **Creating Charts**).

For help with formatting pages or with creating headers and footers, refer to:

🔳 **Changing the Page Format**

🔳 **Notes on the Page Setup**

◾ Opening and Editing the Printer Layout

To access the ➢*Printer Layout*, first open a sample and then change to the Printer Layout:

- Select and double-click the sample in the Browser. The (screen) report for the sample appears.

- Click the **Printer Layout** icon ▦ on the **Method** toolbar to change to the **Printer Layout**. The Printer Layout of the corresponding report definition file (RDF) is opened, i.e., the Preferred RDF File of the sequence to which the sample belongs.

📒 *Note:*

If you open the report definition file in the Browser, e.g. by double-clicking, the Printer Layout is opened with the data that were saved last. In this case, it is not possible to load the desired sample data.

The Printer Layout consists of several (work)sheets. To change to a different sheet, click its tab on the bottom edge. The 'default' report definition contains, for example, an Integration tab, a Calibration (Curr. Peak) tab, and a Calibration (Batch) tab. The area of the single sheets is virtually unlimited in horizontal and vertical direction. However, depending on the amount of information, it may comprise different print pages. The different sheets comprise chromatograms, tables, diagrams, calibration curves, or other elements indicating the corresponding values of the current sample.

If you need more variables, chromatograms, tables, etc. than those available in the open Report Definition File, you can insert them into the existing worksheets.

Or else, create a new worksheet:

- Enable **Layout Mode** on the **Edit** menu.

- Select **Insert Sheet** on the **Edit** menu. An additional worksheet appears before the open sheet.

For information about how to edit existing worksheets, refer to:

◾ **Inserting a Chromatogram**

◾ **Inserting a Plot**

◾ **Inserting and Deleting Individual Variables**

◾ **Inserting Text**

In addition, you can insert tables in the Printer Layout. For more information, refer to **Creating and Using Report Tables** 🔲 **Creating Report Tables for the Printout**.

🔲 Inserting a Chromatogram

- Enable ➤*Layout Mode* on the **Edit** menu.

- Select **Insert > Chromatogram** on the **Edit** menu or context menu. The mouse pointer becomes a + sign.

- Draw a rectangular frame of any size. The frame reserves this area for the chromatogram of the current sample.

- To indicate that the chromatogram is a Chromeleon object all four corners of the chromatogram inserted in this way are marked by a red triangle.

- Click the chromatogram to select it completely. You can then move it, or reduce or enlarge it in size.

- Select **Chromatogram Properties** on the **Edit** menu or context menu. Format the chromatogram as desired on the corresponding tab pages of the **Chromatogram Decoration** dialog box (caption, axes, font size, etc.).

- Press the F4 or the SHIFT+F4 to display the chromatogram of the next or previous sample and to check whether the selected settings are appropriate for all chromatograms.

🔲 Inserting a Plot

Inserting a Plot

1. Open the Printer Layout (see 🔲 **Opening and Editing the Printer Layout**).

2. Select ➤*Layout Mode* on the **Edit** menu.

3. Select the worksheet on which you want to insert the plot, or add a new worksheet (select **Insert Sheet** on the **Edit** menu).

4. On the **Edit** or context menu, select **Insert >** and select the type of plot from the list (**Calibration**, **Spectra**, **I-t**, **3D Amperometry**, etc). The mouse pointer becomes a + sign.

5. Drag the mouse to draw a rectangular frame of the approximate size that you want the plot to be. When you release the mouse, the rectangle will display the selected plot type.

   To indicate that the plot is a Chromeleon object, all four corners of the plot are marked by a red triangle.

Modifying a Plot

1. To select the complete area, click inside the frame.

2. You can now move, reduce, or enlarge the frame.

3. To define the data and format of the plot, select **x Plot Properties** on the **Edit** or context menu, where **x** is the plot type (**Calibration**, **Spectra**, **I-t**, **3D Amperometry**, etc).

4. Select the options on the corresponding tab pages of the **Decoration** dialog box.

◨ Inserting and Deleting Individual Variables

Usually, general sample data, such as the sequence name, the corresponding ➤*Datasource*, the user name, etc. appear at the beginning of a printout or in addition to a table or a chromatogram. To insert more variables:

- Enable ➤*Layout Mode* on the **Edit** menu.

- Select a single cell and then select **Insert > Chromeleon Report Variable** on the **Edit** menu.

- In the **Select Report Variable** dialog box, select the variable category from the **Categories** list and then select a variable from the **Variables** list.

- Clicking **OK** includes the variable into the selected cell.

- To indicate that the variable is a Chromeleon object, a red triangle appears in the upper right corner of the cell.

- To modify the appearance of the variable, select the respective cell and then select the corresponding command on the **Format** menu.

Deleting a Variable

To delete a variable, it is not sufficient to delete the value indicated in the edit line. Proceed as follows:

- Select the cell for which you want to delete the contents.

- Select **Clear** on the context menu or press the **Del** key on the keyboard. The **Clear** dialog box appears.

- Select the **Values** option to delete only the values. Select **All** to delete the formats as well, e.g., the frames assigned to the cell. Click **OK** to clear the cell.

- Check whether the clear action was successful. Click a different cell and then click the cell again, for which you wanted to delete the contents. The edit line is updated for this cell. If the clear action was successful, the edit line does not contain any information.

 Notes:

Cells containing an underlying variable can be recognized by the formula name that is displayed in the status line whenever the cell is selected, for example, peak.height. If the selected cell contains only text, the status line is empty.

You cannot insert new variables by entering the corresponding formula, for example, smp.name for the sample name. Chromeleon interprets formulas that are entered in this way as pure text. Thus, only the text 'smp.name' appears in the edit line and not the value of the formula, i.e., the name of the corresponding sample.

Inserting Text

You can insert text in any empty text cell of the worksheet. However, the size of the cell limits the length of the text that is displayed in the cell. Note the following:

- When you type longer texts into a cell than permitted by the cell size, the entire text is displayed using the neighboring cells to the right as long as these cells are not used. If the neighboring cells are used, the visible text is 'cut' to the cell size; the entire text is displayed only in the edit line.

- Text cells are not marked by a red triangle because they are no Chromeleon objects.

- To insert a line break, select the **Wrap Text** check box in the **Format Cells** dialog box. (To open this dialog box, select **Alignment** on the **Format** menu.)

- If graphics, such as chromatograms, are inserted, they will always hide the text.

◾ Saving or Loading a Report Definition File

The ➢*Printer Layout* of a report template (➢*Report Definition File (RDF)*) can contain one or several worksheets. If you save or load a Report Definition File, all worksheets included in the Printer Layout are saved or loaded.

- Select **Save Report Definition** on the context menu to save the Report Definition File and its worksheets under either an existing or a new name.

- Select **Load Report Definition** on the context menu to open an existing Report Definition File.

☞ *Tip:*

In addition, a Report Definition File contains various settings of the on-screen report, such as the window size, the type, and number of the columns in a report, axis captions, etc. When you save the Printer Layout, any modifications made to the on-screen report are saved as well.

◾ Specifying the Pages to Be Printed

To specify which pages of the ➢*Printer Layout* to print, enable **Layout Mode** on the **Edit** menu. Select **Batch Report Setup** on the **File** menu to open the **Batch Report Setup** dialog box:

Batch Setup dialog box:

- Tabs: Excel format (*.xls) | PDF format (*.pdf) | CM Archive (*.cmb) | External Program
- Tabs: Print Sheets | Export | AIA format (*.cdf) | ASCII format (*.txt)

Select sheets to be printed during report batch

Sheets:
- ☑ Integration
- ☐ Calibration(Curr.Peak)
- ☑ Calibration(Batch)
- ☑ Peak Analysis
- ☑ Summary
- ☐ Purity (Curr.Peak)
- ☑ Purity (Batch)
- ☑ Audit Trail

- ○ Do not print
- ○ Print for every sample
- ● Print under certain conditions
  - Conditions...

[OK] [Cancel] [Apply] [Help]
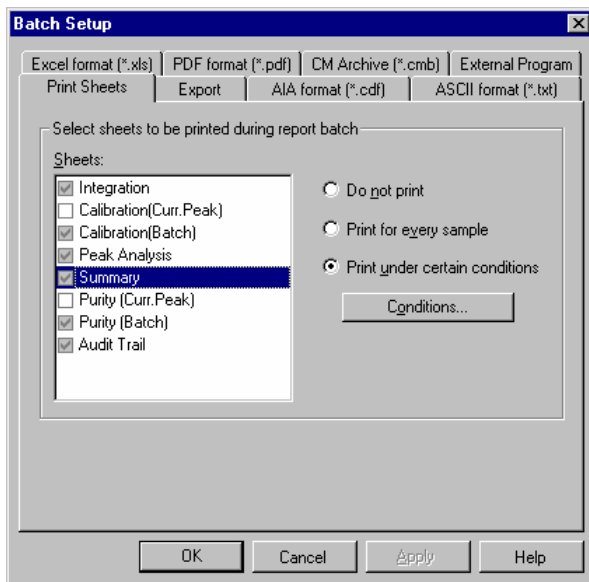
Click **Conditions** to specify the print conditions for the corresponding worksheet. For example, it would be sensible to print the Summary page only for the last sample of a sequence:

Print Conditions for Sheet 'Summary' dialog box:

Print Sheet if Sample Type is
- ☐ Unknown
- ☐ Standard
- ☐ Validate
- ☐ Blank Run
- ☐ Matrix Blank

Or Print if Sample is the
- ☐ First Sample in the Sequence
- ☑ Last Sample in the Sequence
- ☐ Last Sample in a List of Standards

Or Print if
- ☐ Sample Name   is equal   [   ]
- or ☐ Sample Comment is equal [   ]

[Ok] [Cancel]
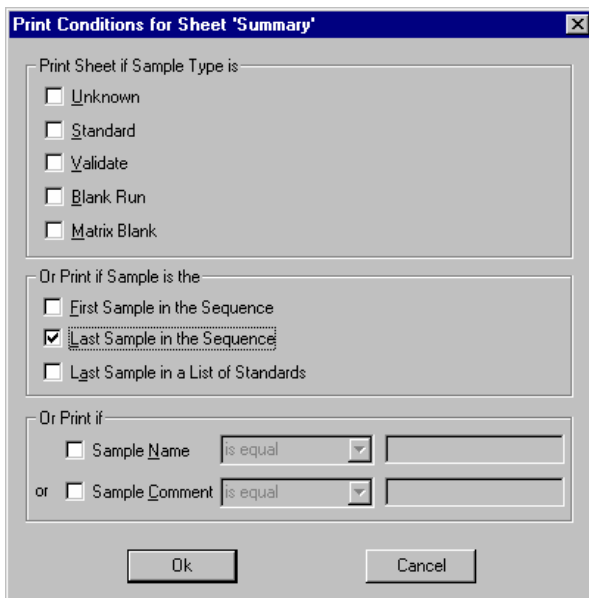
However, you could print the **Calibration (Batch)** sheet for the **Last Sample in a List of Standards**.

☞ *Tip:*

These settings are saved in the report definition file (RDF). They are used as defaults for Batch Report printing. The same defaults are used for electronically signing sequences (see ➢Electronic Signature).

▪ **Specifying the Printout**

Before you can print your data, define the printer to be used. In addition, define the headers and footers, margins, etc. in the **Print** dialog box and the **Page Setup** dialog box. Select **Print Setup** and **Page Setup** on the **File** menu.

The **Print Setup** and **Page Setup** settings are stored separately for each worksheet in a ➢*Report Definition* file (**Integration**, **Calibration**, **Peak Analysis**, **Summary**, etc.). Therefore, select the settings for each worksheet separately.

- Select **Print Setup** on the **File** menu to determine the printer, the paper size, and the format (portrait or landscape). After selecting these settings, a message box appears. Determine whether these settings will be used only for the current worksheet or for all worksheets of the Printer Layout of the current Report Definition File (RDF).

  ☞ *Tip:*

  *The **Print Setup** settings selected here apply only to the report definition file of the current sequence. They do not affect the default settings for Windows. On the other hand, changing the default printer, paper size, and format in Windows does not affect the settings specified here.*

- Select **Page Setup** on the **File** menu to determine the appearance of the headers and footers, the margins, the alignment, etc. You can also set grid lines, determine whether column and/or row headers are displayed, and whether the printout is in black and white or in color. For more information, refer to **How to …: Preparing the Printout** ▪ **Notes on the Page Setup**.

Tip:

*For technical reasons, direct help information for the **Print Setup** dialog box is not available.*

Also, refer to **How to …: Preparing the Printout:** **🔲 Saving/Loading a Report Definition File** and **🔲 Saving the Contents of a Printer Layout Page**

🔲 Printing the Results of a Single Sample

You can print the results of single samples from either the Printer Layout or the on-screen report (integration plot).

For each printout, the ➢*Printer Layout* pages saved in the ➢*Report Definition File (RDF)* are used as a template. The results of a single sample or of a sequence are output in the defined way (see **🔲 Printing the Results of a Sequence or a Sample Batch)**. Please note that the Report Definition File and the worksheets define only the appearance of the printout, but not the contents. Unless you have already specified the worksheets to be printed, specify them now:

In the Printer Layout

Select **Batch Report Setup** on the **File** menu to specify which worksheets shall be printed. The **Batch Report Setup** dialog box is opened:
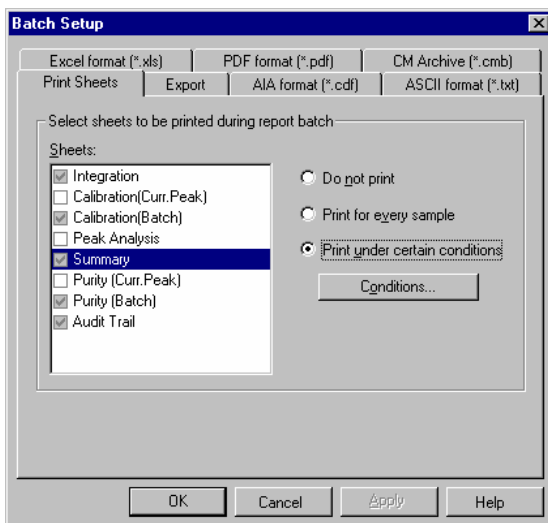
Select the corresponding settings on the **Print Sheets** tab page. The available worksheets are listed in the **Sheets** field. Define which pages shall be printed and under which conditions. Click **Conditions…** to define the print conditions. For example, you can specify for which sample type a page shall be printed. For instance, it would be sensible to print a summary report only for the last sample of a sequence.

In the Report (Screen Report)

Select **Print** on the **File** menu to print the results of the currently open sample from the on-screen report:
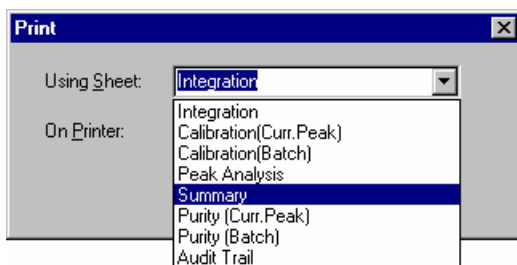
On the **Print** dialog box, select the worksheet to be printed from the **Using Sheet** drop-down list. The worksheet is always printed with the on-screen results if they are part of the defined view. For example, the current ➤*Mass Spectrum* is only printed if a mass spectrum is part of the selected worksheet.

☞ *Tip:*

In the Printer Layout, you can only select peak spectra but no retention time spectra. If you want to print a retention time spectrum, open the Report, select a spectrum on-screen, and then print a worksheet that includes the spectrum.

▣ Printing the Results of a Sequence or Sample Batch

You may also print the results of an entire sequence, a ➢*Query* or a ➢*Batch*. Unless you have already specified which of the worksheets created in the ➢*Printer Layout* should be printed, specify the worksheets now.

In the Browser

Click a ➢*Sequence* or a sample, and then select **Batch Report** on the **File** menu. The **Batch Report** dialog box appears. Select a report definition file from the **Use Report Definition** drop-down list. Or else, click the "**…**" button and navigate to the file of interest.
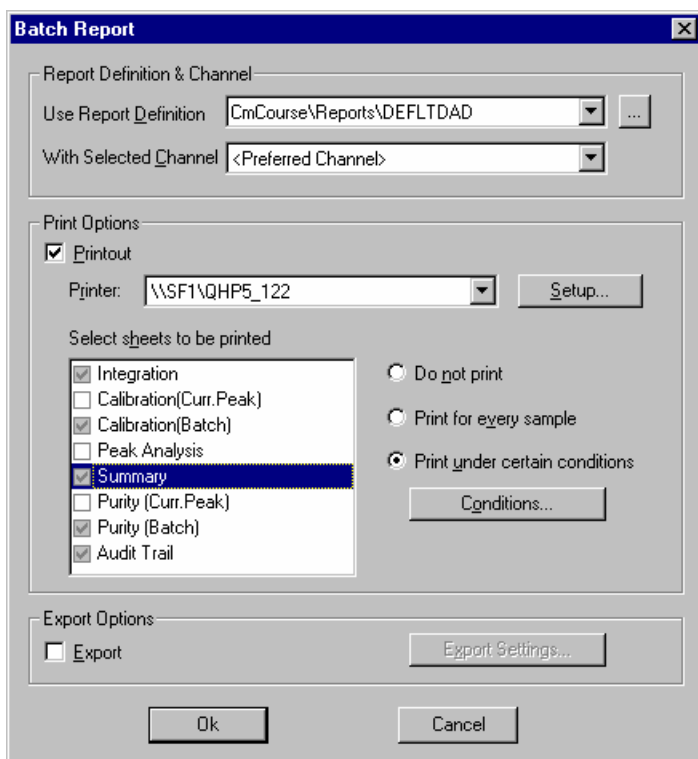
The worksheets defined in the Printer Layout of the Report Definition File are listed in the **Select sheets to be printed** field. Specify which pages shall be printed. To print the pages under certain conditions only, select the **Print under certain conditions** only option and then click **Conditions.** Define the print conditions in the **Print Conditions** dialog box.

Click **OK** to print the entire sequence or the selected samples of a query. To print the entire query, select all samples of a query by clicking the **No**. field at the top left of the sample list.

On the Control Panel: Printing the Results Directly after Data Acquisition

To print the results directly after data acquisition, select **Reporting** on the **Batch** menu. The **Reporting** tab page of the **Batch** dialog box appears:
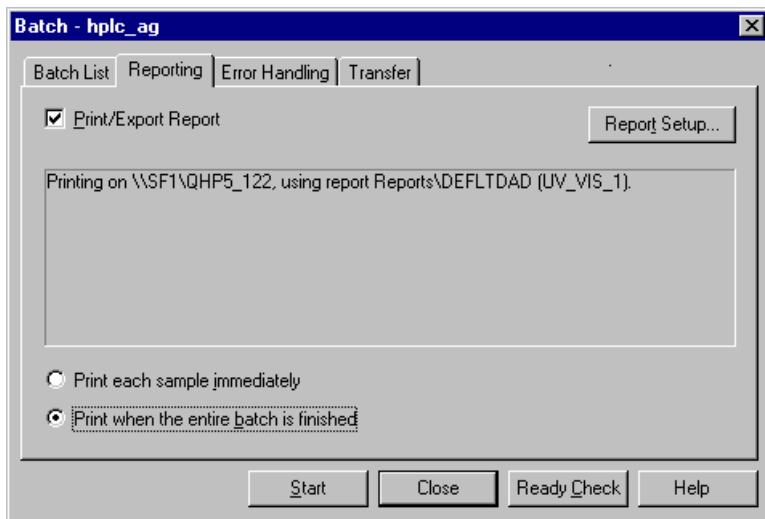
Select the **Print/Export Report** check box to reopen the **Batch Report** dialog box (see the picture in the Browser section above). Follow the description in the Browser section. The tab dialog box indicates the printer, the report definition file (RDF), and the channel selected for the printout. Click OK to return to the **Reporting** tab page. Specify whether each sample shall be printed separately and immediately after data acquisition or whether all samples shall be printed when the batch is finished.

👉 *Tip:*

The settings made in the Browser or on the control panel are saved in the report definition file of the current sequence. They apply to all sequences that use this report definition file. The same defaults are used for electronically signing the worksheets of these sequences (see ➢Electronic Signature).

For more information, refer to 🔖 **Printing the Results of a Single Sample**.

◼ Setting Print Area and Print Title(s)

To print a defined area of a ➢*Printer Layout* page:

- Press the left mouse button and drag the mouse to select an area.

- Select **Printing > Set Print Area** on the **Format** menu.

- Select **Printing > Release Print Area** on the **Format** menu to deselect the defined print area. The defined print area is indicated in parentheses behind the command.

When printing a table that exceeds one page, you can print the title on every page:

- Select the entire line(s) for the print title with the left mouse button.

- Select **Printing > Set Print Titles** on the **Format** menu.

- Select **Printing > Release Print Titles** to deselect the set print titles. The set print title area is indicated in parentheses after the command.

◼ Automatically Repeating the Output for the Selected Objects

It is often useful to repeat the output of reports for other channels, samples, and/or peaks. In Chromeleon, you can automatically repeat the output of reports for:

- Each sample of a sequence or selection

- Each peak in a peak table or the current chromatogram

- Each channel of a sample

Follow the steps below:

- Select the lines completely, i.e., in the grey column at the utmost left that indicates the line numbers.

☞ *Tip:*

Verify for graphic objects that you have selected all lines covered by the object.

- To select more than one area, hold the CTRL key down, and then select the next area by the lines as described above.

- Select **Printing > Set Autorepeat Area** on the **Format** menu to specify the selected area as the area for which the output is automatically repeated.

- The **Autorepeat Area Properties** dialog box is opened. Use this dialog box to define for which channels, samples, and/or peaks the selected area shall be output.

For an example, refer to **Automatically Repeating the Output for the Selected Objects: Example**.

It depends on the objects in the selected area whether it makes sense to repeat the output for other samples and/or peaks. Therefore, please note:

- The **Repeat Samples** tab page is disabled if at least one **Peak Summary** table and/or one **SST Summary** table is selected.

- The **Print and Repeat Peaks** tab page is disabled if at least one **Integration** table is selected.

You can repeat the output for other channels, samples, and/or peaks when you

- Print a batch

- Sign sequences

- Print interactively from the Printer Layout

 Note:

*When you repeat the output for channels and peaks, only those objects are replaced for which no fixed channel/peak is specified. A warning appears if the **Fixed Channel** or **Fixed Peak** option is selected for objects that shall be output for different channels or peaks.*

*For example, this applies to chromatograms for which the **Fixed Channel** option is selected on the **Channel** tab page. (To access the **Channel** tab page, select **Chromatogram Properties** on the chromatogram's context menu).*

◨ Automatically Repeating the Output for the Selected Objects: Example

To repeat the output for the header and the table below the chromatogram, select the following lines:

	A	B	C	D	E	F	G	H
1	7	Anion Std - 1						
2								
3	Sample Name:		Anion Std - 1			Injection Volume:		25.0
4	Vial Number:		7			Channel:		ECD_1
5	Sample Type:		standard			Wavelength:		n.a.
6	Control Program:		Test Run1			Bandwidth:		n.a.
7	Quantif. Method:		Anion Qnt			Dilution Factor:		1.0000
8	Recording Time:		13.6.2001 19:36			Sample Weight:		1.0000
9	Run Time (min):		30.00			Sample Amount:		1.0000
10								

CALIBRATION #7   Anion Std - 1   ECD_1

1 - Floride - 3.603
2 - Chloride - 6.643
3 - Nitrite - 3.630
4 - Bromide - Nitrate 14.077
7 - Sulfate - 20.603
6 - Phosphate - 17.430

No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount	Type
	min		µS	µS*min	%	µg/g	
1	3.60	Floride	14.622	1.511	15.72	4.947	BMB
2	6.64	Chloride	12.137	1.994	20.75	9.656	BMB
3	8.58	Nitrite	5.036	1.144	11.90	9.910	BMB
4	11.61	Bromide	2.800	0.826	8.60	9.954	BMB
5	14.08	Nitrate	2.911	1.063	11.05	9.958	BMB
6	17.43	Phosphate	1.840	0.983	10.23	17.384	BMB
7	20.60	Sulfate	3.545	2.091	21.75	14.925	BMB
Total:			42.890	9.612	100.00	76.734	

If your sequence contains only two standards of interest, you can specify that the repetition include all standards; refer to the image below. In addition, select the **Page break after each sample** check box to output the second sample on a separate page:
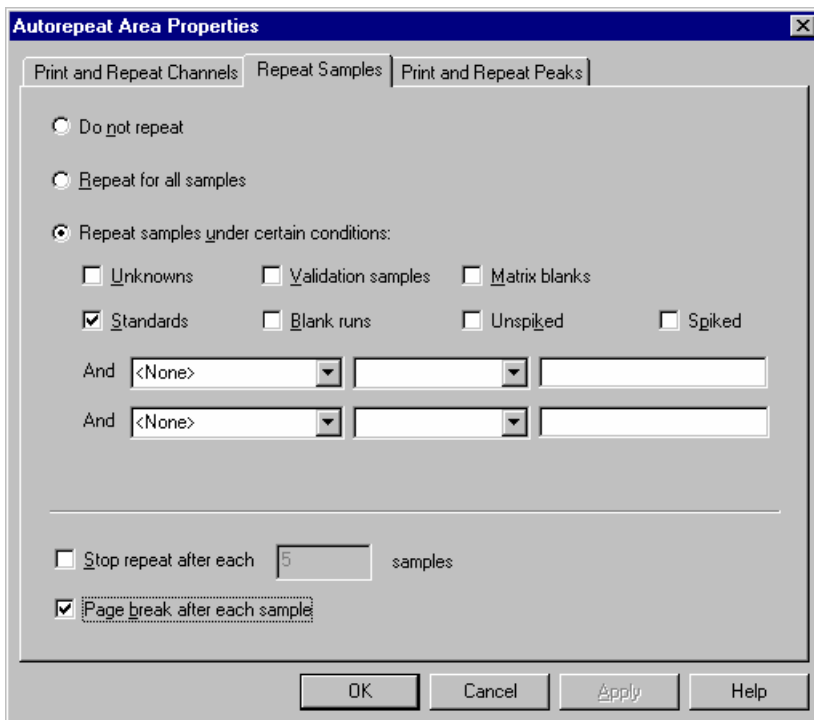
With these settings, the following two pages are output via the **Print** command (**File** menu):

7 Anion Std - 1

Sample Name:	Anion Std - 1	Injection Volume:	25.0
Vial Number:	7	Channel:	ECD_1
Sample Type:	standard	Wavelength:	n.a.
Control Program:	Test Run1	Bandwidth:	n.a.
Quantif. Method:	Anion Qnt	Dilution Factor:	1.0000
Recording Time:	13.6.2001 19:36	Sample Weight:	1.0000
Run Time (min):	30.00	Sample Amount:	1.0000

No.	Ret.Time min	Peak Name	Height µS	Area µS*min	Rel.Area %	Amount µg/g	Type
1	3.60	Fioride	14.622	1.511	15.72	4.947	BMB
2	6.64	Chloride	12.137	1.994	20.75	9.656	BMB
3	8.58	Nitrite	5.036	1.144	11.90	9.910	BMB
4	11.61	Bromide	2.800	0.826	8.60	9.954	BMB
5	14.08	Nitrate	2.911	1.053	11.05	9.958	BMB
6	17.43	Phosphate	1.840	0.983	10.23	17.384	BMB
7	20.60	Sulfate	3.545	2.091	21.75	14.925	BMB
Total:			42.890	9.612	100.00	76.734	

For general information, refer to **Automatically Repeating the Output for the Selected Objects**.

Saving the Contents of a Printer Layout Page

Chromeleon provides various formats for saving the report data of the current sample:

- To save the active page of the Printer Layout, select **Save as** on the **File** menu. The **Save As** dialog box is opened. Under **Save as type**:

- Select **Excel ... (*.xls)** to convert your data into the Excel format. Enter the file name and click **Save** to save your data as a separate file. In addition to the values of the table, any graphics and diagrams included in the table are also saved to the new file. If the ➢*Printer Layout* of the ➢*Report Definition File (RDF)* contains several worksheets, the Excel file has the same structure. Please note that different Excel file types are available, depending on the Excel version (Excel V4 or Excel V5 or V7).

- Select **HTML (*.htm)** to save your data as an HTML page. Graphics and diagrams cannot be converted and saved in HTML format.

- Select **Tabbed Text (*.txt)** to save your data as pure text. The content of the individual report columns is separated by a tab stop. Graphics and diagrams cannot be converted and saved in Tabbed Text format.

▣ Creating Charts

👉 *Tip:*

In order to perform the operations described below, verify that the ≻Report Publisher add-on product is licensed.

The Chart Wizard assists you in creating charts:

- Enable ≻*Layout Mode* on the **Edit** menu.

- Select the columns and rows that shall be represented in a chart.

- Select **Insert > Charts** on the context menu. The shape of the mouse pointer changes to a **+** character.

- Hold down the left mouse button down and draw a rectangle in the size required for the chart.

- The Wizard guides you through the chart creation process. Select the chart type and style and then enter the layout settings and axis titles.

- Click **Finish** to exit the Wizard and insert the chart.

The new chart is displayed in the previously drawn frame. If required, you can edit the chart later. For example, you can change the width, color, and pattern of lines, areas, or captions, smooth curves, or select a different chart type.

- Select a single chart element by (double-) clicking and then double-click the selected element to change it. The Chart Designer is opened. After you have made the changes and click **OK** to apply the changes to the chart.

You can also change the size and position of the entire chart:

- Click inside the chart. Hold down the left mouse button and move the chart to the desired position. Resize the chart by dragging the corresponding size markers.

◧ Changing the Page Format

In the ➢*Printer Layout*, the page format can be changed as follows:

Define the printer settings

- Select **Print Setup** on the **File** menu. The **Print Setup** dialog box appears.

- In the Paper section, specify the paper size. Click the arrow then select a size from the list. In the Orientation section, select Portrait or Landscape. The size and orientation is used for all pages of the respective printout but is not accepted as default.

☞ *Tip:*

The settings are only valid for the report definition file of the current sequence. They do not overwrite the default Windows settings. In addition, any change of the default printer, paper size, or format made under Windows does not affect the settings made here.

Directly printing in the desired format:

- Select **Print** on the **File** menu.

- Click **Properties** to open the **Properties** dialog box.

- Select a paper size and orientation.

◧ Notes on the Page Setup

Headers and footers are printed at the upper and lower page margins. Select **Page Setup** on the **File** menu. Make the settings in the **Page Setup** dialog box.

Headers and footers can contain text and specific format codes. The syntax is compatible with Microsoft Excel. Observe the order of the single entries. First, define the alignment of the single header and/or footer, using one of the following commands:

&L	Left-aligns the characters that follow
&C	Centers the characters that follow (default)
&R	Right-aligns the characters that follow

The default font is Arial 10. You can only define a different font after you have defined the header and/or footer alignment. Define the new font as follows:

&B	Bold
&I	Italic
&U	Underline
&S	Strikeout
&"fontname"	Uses the specified font
&nn	Uses the specified font size

📖 *Tip:*

If you do not enter these font definitions after the header and/or footer alignment, they will be ignored. You can change the font after each alignment code (&l/&C/&R).

Finally, enter the expression to be printed:

&A	Prints the current sheet name
&D	Prints the current date
&T	Prints the current time
&F	Prints the ➢*Report Definition File (RDF)*
&P	Prints the page number
&P+Number	Prints the following page number: current page + entered number. For example: &P+4 prints page 16 if the current page is page 12 and you entered 4.
&&	Prints an ampersand
&N	Prints the total number of pages in the document

When entering headers and footers longer than one line, note that the alignment codes (&L,&C,&R) must be column-oriented. Enter all left-aligned rows first, then enter all centered rows, and finally enter all right-aligned rows. To separate rows, press <Ctrl> <Enter>.

In addition to the pre-defined variables above, you can enter variables pertaining to Chromeleon. Enclose these variables in braces.

Example:

&LOperator: {gen.operator} , Timebase: {seq.timebase} , Sequence:{seq.name} &RPage &P of &N

&D

This example produces the following header or footer

Operator: cmadmin, Timebase: HPLC, Sequence: Calibration	**Page 1 of 12**
	4/6/2002

You can include any Chromeleon variable in the header. However, including sample and sequence variables makes the most sense. The Chromeleon formula variables are identical with the ones generated in the dialog box for entering report variables. Enter the decimal places for numerical variables after the formula. Separate them by ";" (default: 0). Example: {smp.inject_volume;2}. Time entries cannot be formatted.

Note:

All worksheets of the ➢Printer Layout that are selected for printing are considered one single print job; that is, page numbering is consecutive for all worksheets.

▪ Answering Frequently Asked Questions

Question: How can I have the results printed out automatically after the analysis?

Answer: Mark the sequence of interest in the Browser. Select **Reporting** on the **Batch** menu and select **Print/Export Report** by the box. Afterward select an option: **Print each sample immediately** or **Print when the entire batch is finished**.

Question: The view in the ➢Printer Layout window and the printed report have a different layout. Why?

Answer: This may happen. There are different ways how to print a sample report. Usually, you will print the report via the **Batch Report** dialog window. When you print the report in this way, the report definition defined in this dialog is used. When you have the results printed automatically via **Reporting** as described above, it may happen that the report definition used to display the data in the Printer Layout is different from the report definition used for the printout.

 Notes:

*When you either select **Print** on the **File** menu in the Printer Layout or click the **Print** icon on the standard toolbar, it is always the current view that is printed.*

Question: Where is the used ➢ *Report Definition File (RDF)* saved?

Answer: Some predefined Report Definition Files (RDFs) are available in the **Dionex Templates > Reports** folder. However, it is not imperative that the used RDF is stored in this folder. To find out where the used RDF is stored, proceed as follows: Mark the sequence of interest in the Browser. Right-click and select **Properties** on the context menu. In the **Preferred Report & Channel** section, the entry in the **Preferred RDF File** field indicates the path of the current report definition file. To determine a different report definition file as preferred RDF, click the Browse button ("**…**") and navigate to the report definition of interest.

The settings of this RDF are used whenever you open a sample of this sequence afterward. However, you can select a different RDF in the on-screen report or in the Printer Layout, using the **Load Report Definition** command on the **Workspace** menu.

 Notes:

If you select the RDF via Load Report Definition, this does only apply to the current view. The Preferred RDF File of the sequence is not changed.

Question: What do the terms Printer Layout, Report Template, and Report Definition File refer to?

Answer: The Printer Layout is an editor window that allows you to define the content and the layout of the pages to be printed. This information is saved to the Report Definition File (RDF) together with the layout of the on-screen report. Sometimes, the Report Definition File is also referred to as report template.

Question: How can I save the scaling?

Answer: The scaling, too, is saved to the Report Definition File. Select **Save Report Definition** on the Workspace menu. Afterward, select the previously saved RDF as Preferred RDF File in the properties of the sequence (see above). (Unlike the peak parameters, the scaling is not saved to the QNT File.)

Question: How can I save comments in the report? Where can I enter information about the column I am using?

Answer: Chromeleon provides different possibilities for doing this. For example, you can use the Title field to enter information about the column: Mark the sequence in the Browser and select **Properties** on the context menu. Afterward, add the **Title** variable of the ⇒*Sequence* category to the Printer Layout.

☞ *Tip:*

*Keep in mind to update the **Title** field entry whenever you change the column.*

For tips to solve similar questions, refer to **How to …:** 🔼 **Preparing the Printout** and the corresponding subtopics.

Analyzing 3D Amperometry Data

The ICS-3000 Electrochemical Detector (ED) can collect 3D amperometry data when it is running in integrated amperometry mode. To turn on collection of 3D amperometry data, enable the ➢*3D_Amp* channel check box in the ➢*Program* Wizard or Program Editor. This adds a **3D_Amp.AcqOn** command to the control program.

After collecting the 3D data, you can view and analyze it in the 3D amperometry window of any Chromeleon PC that has Release 6.7 or higher installed.

 Note:

The 3D Data Acquisition license is required to acquire 3D amperometry data. See *Chromeleon (Overview) Chromeleon Licenses.*

For more information, refer to:

 Displaying a Sample's 3D Amperometry Data

 Selecting the Integrated or Raw 3D Amperometry View

 Changing the 3D Amperometry Plot Scale

 Viewing Chromatograms in the 3D Amperometry Window

 Viewing I-t Plots in the 3D Amperometry Window

 Editing Integration Intervals

 Extracting a Chromatogram from 3D Amperometry Data

 Exporting a Chromatogram, an I-t Plot, or 3D Amperometry Data

See also, ➢*Baseline Correction of I-t Plots*

For an overview of 3D amperometry window features, refer to **The 3D Amperometry Window**.

◾ Displaying a Sample's 3D Amperometry Data

Use one of the following methods to display a sample's 3D amperometry data:

- Select the sample in the Browser and then select **Open>3D_Amp** from the context menu.

- If the sample is already open in another window (for example, the integration window), select **3D Amperometry** on the **View** menu or click the following icon on the Method toolbar ▣.

☞ *Tips:*

3D amperometry data collection is available only with the ED electrochemical detector running in integrated amperometry mode and on a Chromeleon PC that has the 3D Data Acquisition license. To turn on collection of 3D amperometry data, enable the ⯈3D_Amp channel on the ED Options tab page of the Program Wizard or PGM Editor.

The 3D amperometry data can also be viewed on a control panel during data acquisition.

Also, refer to:

◾ **Analyzing 3D Amperometry Data**

◾ **The 3D Amperometry Window**

◾ **The Program Wizard**

◾ **The PGM Editor: Device Views**

◾ Selecting the Integrated or Raw 3D Amperometry View

The 3D Amperometry Window has two options for viewing chromatographic ⯈3D_Amp data:

- Integrated data view
- Raw data view

To distinguish between the two views:

- In integrated data view, parallel integration interval line cursors appear on the 3D data and I-t plots. The Y-axis units on the chromatogram displayed above the 3D data plot are nC (nanoCoulombs).

- In Raw data view, a single horizontal line cursor for selecting waveform time is displayed instead of the integration cursors. The Y-axis units on the chromatogram displayed above the 3D data plot are nA (nanoAmps).

To select the view option:

1. Double-click the 3D or I-t plot or select **Decoration** on the context menu.

2. On the **Chromatogram Plot** tab page, select either the **Show Raw Data** option or the **Show Integrated Data** option.
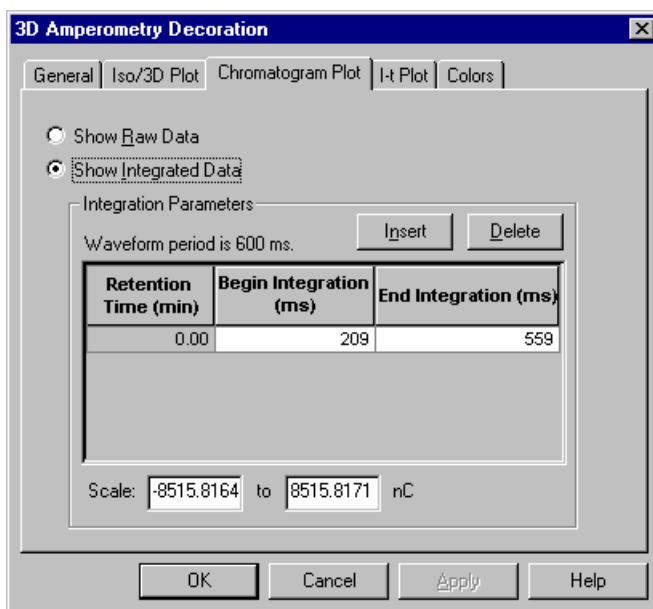
Also, refer to:

🔼 **Analyzing 3D Amperometry Data**

🔼 **The 3D Amperometry Window**

◾ Changing the 3D Amperometry Plot Scale

When you initially open a sample's 3D amperometry data, the window displays the data in full-scale view (i.e., the axes on each plot include the full range of data values from the analysis). There are several methods for adjusting the scale of a plot to zoom in or out to show the data of interest:

☞ *Tip:*

Changing the scale on one of the plots (chromatogram, raw 3D data, or I-t) also changes the scale of the other plots.

- Point to an area on a plot and then drag to define the area to zoom into.

- Select commands from the **View** or context menu (right-click to open the context menu):

Full Size	Adjusts the scale of all plot axes to include the entire 3D data set.
Autoscale	Adjusts the signal axis optimally for the selected data.
Zoom to Integration Interval	Adjusts the waveform period axis (ms) on the 3D and I-t plots to display only the integrated portion of the data. The response axis (nC) on the chromatogram and 3D plots is adjusted to the range of responses in the integrated data.
Unzoom	Reverses the previous zoom steps one by one.

- Adjust scaling parameters manually using the **Decoration** dialog box:

  1. Double-click the plot or select **Decoration** on the context menu to open the dialog box.

  2. Then, go to the **General** and/or **Chromatogram Plot** tab pages to adjust scaling parameters:

     - On the **General** tab page, clear the **Autoscale** check boxes and then enter the values for the **Time**, **Waveform**, and **Signal** axes.

       ### ☞ *Tip:*

       *When the **Autoscale** check boxes are enabled, the plot axes are adjusted to include the full range of data values from the analysis. Clearing the check boxes lets you enter specific values for each axis.*

     - On the **Chromatogram Plot** tab page, enter the **Scale** values for the response axis.

In the following examples, the first window shows 3D data in the initial full size scale.
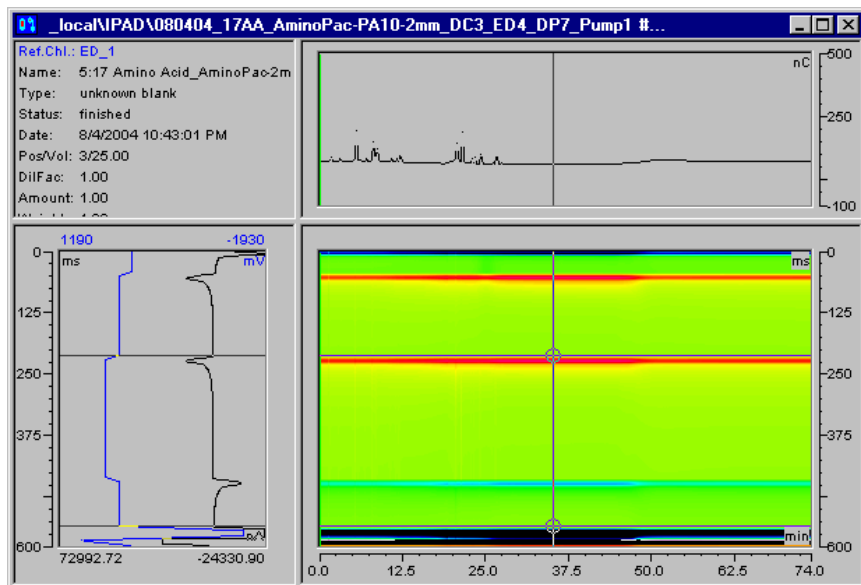
After selecting **Zoom to Integration Interval**, the window looks like this:
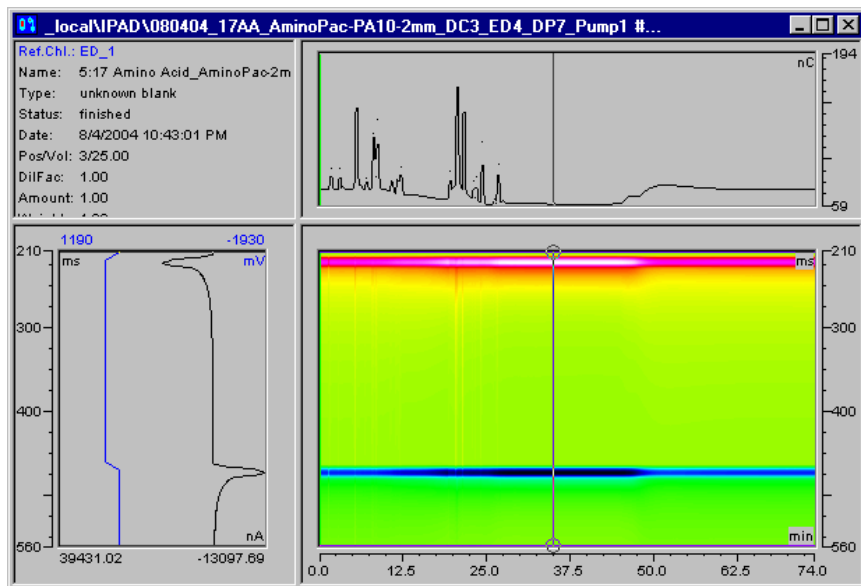
The **Scale** parameters on the **General** tab page of the **Decoration** dialog box can be used to adjust the scale further:
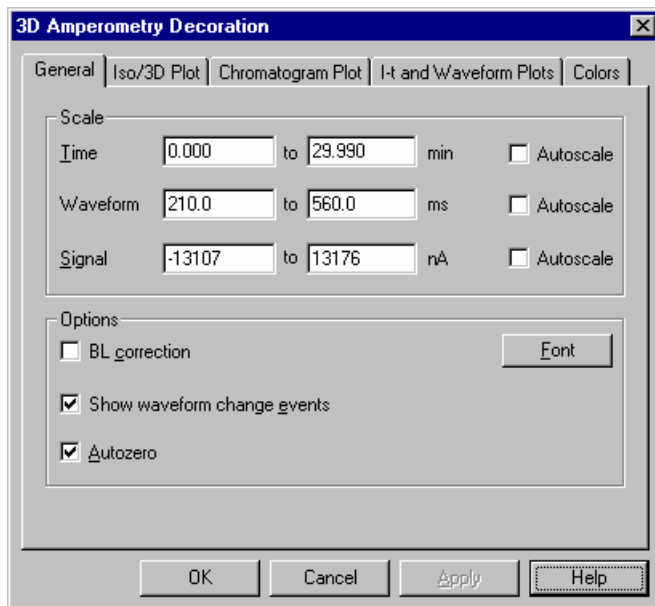
Time: Adjusted to include only the peaks of interest (no peaks of interest occurred after about 30 minutes).

Waveform: Adjusted to the length of the integration interval (done by the **Zoom to Integration Interval** command).

Signal: Adjusted to the highest response within the integration interval (done by the **Zoom to Integration Interval** command).

These settings result in the following plot display:
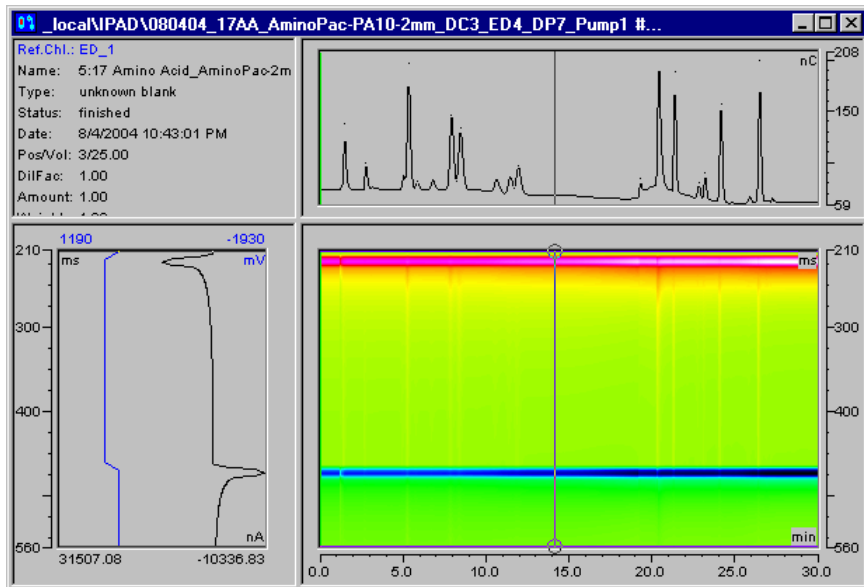
Also, refer to:

🔼 Analyzing 3D Amperometry Data

🔼 The 3D Amperometry Window

🔲 Viewing Chromatograms in the 3D Amperometry Window

The upper right pane of the 3D amperometry window displays a chromatogram of the selected 3D data. In integration view, the chromatogram is generated by adding responses measured throughout the integration interval to produce one integrated data point per waveform cycle. In raw data view, the chromatogram is generated by extracting one data point per waveform from the selected waveform period time slice.

🖎 *Tip:*

*If the chromatogram pane is not displayed, enable the **Show Chromatogram** option on the context menu or click the following icon on the Method toolbar 📈.*

To select a different chromatogram:

The following methods can be used to select data and generate a different chromatogram:

- In integration view, drag the upper integration interval line cursor on the 3D or I-t plot to move the integration interval. Drag the lower integration interval line cursor to increase or decrease the interval.

  ### ☞ *Tip:*

  If multiple integration intervals are defined, you must first drag the vertical retention time line cursor into the interval that you want to adjust and then move the integration interval line cursor.

- In raw data view, drag the single horizontal waveform time line cursor to select a different waveform period time from which to generate the chromatogram.

- In integration view, add or delete integration intervals.

- Enable or disable the Autozero.

Also, refer to:

Analyzing 3D Amperometry Data

Editing Integration Intervals

Extracting a Chromatogram

The 3D Amperometry Window

▣ **Viewing I-t Plots in the 3D Amperometry Window**

The lower left pane of the 3D amperometry window displays a plot of current (I) vs. waveform time (t). To better visualize this plot, imagine that a vertical slice of the 3D data is taken at retention time (T) and the slice is then laid flat. The left axis is the waveform period (ms) and the bottom axis is the current (nA). When the ➤*Waveform* is displayed, the top axis on the plot indicates the applied voltage (mV).

1	Current (I) axis
2	Waveform time (t) axis
3	I-t plot (detector current I at time t)
4	Integration interval line cursors
5	Applied voltage (mV) axis
6	Waveform (mV at time t)
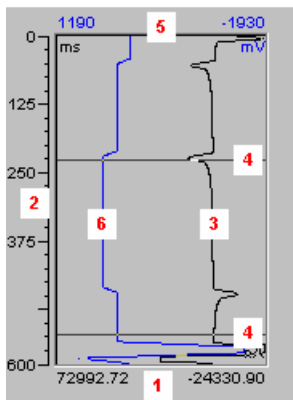
☞ *Tip:*

*If the I-t plot pane is not displayed, enable the **Show I-t Plot** option on the context menu or click the following icon on the Method toolbar* 🧩*.*

To display a different I-t plot:

On the 3D or chromatogram plot, drag the vertical retention time line cursor to a new position.

To show or hide the waveform display:

1. Double-click the 3D or I-t plot or select **Decoration** on the context menu.

2. Select the **I-t and Waveform Plots** tab.

3. Select the **Show waveform** check box to display the waveform or clear the check box to hide the waveform display.
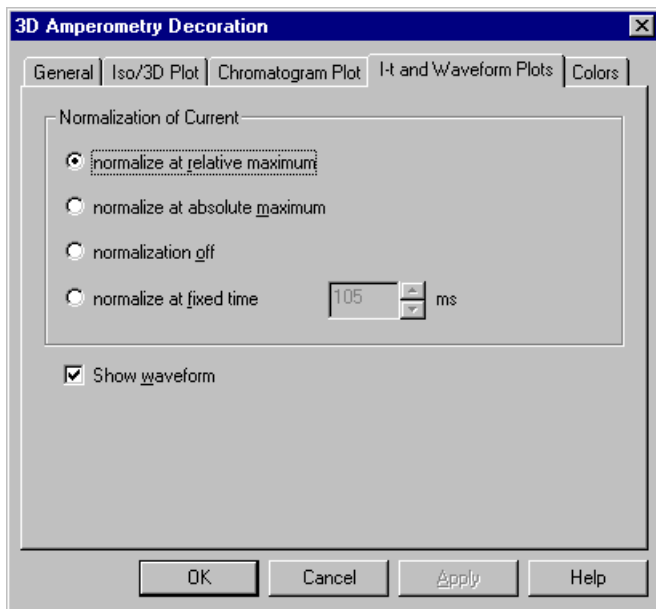
Also, refer to:

🔼 **Analyzing 3D Amperometry Data**

🔼 **The 3D Amperometry Window**

◾ Editing Integration Intervals

In addition to editing integration intervals by moving the line cursors in the 3D amperometry window (see 🔼 **Viewing Chromatograms in the 3D Amperometry Window**), you can also edit integration intervals on the **Chromatogram Plot** tab of the **Decoration** dialog box.

Use the **Chromatogram Plot** tab page to add or delete intervals and edit existing intervals.

1. Double-click the 3D or I-t plot or select **Decoration** on the context menu.

2. Select the **Chromatogram Plot** tab.

3. To add an integration interval, click **Insert** and then enter the **Retention Time (min)**, the **Begin Integration**, and the **End Integration** parameters. See the guidelines below for details.

When you add an integration interval at a particular retention time, the new interval is in effect from that time forward until the next integration interval is specified.

4. To delete an integration interval, select the interval and click **Delete**.

Note:

Integration intervals that were defined in the control ➢*Program cannot be deleted. The* **Retention Time (min)** *cell for these intervals is disabled (gray).*

Guidelines for Defining New Integration Intervals

- The retention time of the last integration interval in the table must be before the end of the run.

- The **Begin Integration** must be at least 10 ms after the start of the waveform period.

- The **End Integration** must be at least 10 ms before the end of the waveform period.

- Up to 15 intervals can be defined.

Also, refer to:

Analyzing 3D Amperometry Data

The 3D Amperometry Window

Extracting a Chromatogram from 3D Amperometry Data

You can extract a chromatogram from ➢*3D_Amp* data displayed in the 3D amperometry window.

1. In the 3D amperometry window, select **Extract>Chromatogram to file** from the **View** or context menu (right-click to open the context menu).

   A dialog box opens with settings for the chromatogram currently displayed in the 3D amperometry window.

2. Enter a **Channel name** in which to save the chromatogram, or retain the default name if you wish to overwrite the existing channel data.

3. Click **OK** to accept these settings and extract the current chromatogram to a separate channel. The chromatogram opens in the integration window.

You can also change the following settings before extracting the chromatogram:

- **Autozero**: When Autozero is enabled, the extracted 3D channel data is adjusted by the signal offset. When disabled, the total 3D channel signal is extracted.

- **Extract Raw Data**: The chromatogram is generated by extracting one data point per waveform from the selected ➤ *Waveform Period* time slice. Enter the time at which to extract the data.

- **Extract Integrated Data**: The chromatogram is generated by calculating one data point per waveform from the selected integration interval(s). Edit the beginning and ending integration times for existing integration intervals, add integration intervals, or delete intervals. See **Editing Integration Intervals** for additional information.

- **Extract from all samples of current sequence or query**: A chromatogram is extracted for all of the samples in the sequence or in a list of samples created with a query. Each chromatogram is named with the channel name entered in the **Channel name** field. After you click **OK**, Chromeleon extracts all of the chromatograms and then opens the chromatogram of the selected sample. To view an extracted chromatogram for a different sample, right-click the sample in the Browser, select **Open** from the context menu and select the channel name from the list.

Also, refer to:

Analyzing 3D Amperometry Data

The 3D Amperometry Window

◾ Exporting a Chromatogram, an I-t Plot, or 3D Amperometry Data

You can export a chromatogram, an I-t plot, or raw ➤*3D_Amp* data from the 3D amperometry window.

1. Select **Export>Chromatogram** from the **View** or context menu.

2. Select whether to copy the chromatogram to the Windows clipboard or to a file. A text file containing the data values corresponding to the chromatogram currently displayed in the 3D amperometry window is copied.

Follow the same procedure to export an I-t plot or raw 3D amperometry data.

☞ *Tip:*

*You can also export an I-t plot from the integration window. Select **Export I-t Plot (text format)** from the context menu.*

Also, refer to:

� **Analyzing 3D Amperometry Data**

🔺 **The 3D Amperometry Window**

Working with Cyclic Voltammetry Data

You can use the ICS-3000 Electrochemical Detector to perform ➤ *Cyclic Voltammetry* (CV) by issuing commands from a control panel (see **Detector Control** 🔲 **Performing Cyclic Voltammetry**). The recorded data can be viewed in a CV view window. CV data is acquired as a **3D_Amp** channel at 1 kHz and then compressed to 20 Hz before it is stored. The data from all the cycles in a run is stored in the **manual** sequence as a single sample, with a default name of **Manual Acquisition**.

🗒️ *Note:*

The 3D Data Acquisition license is required to acquire and process cyclic voltammetry data.

Also, refer to:

🔲 **Opening a Cyclic Voltammetry Data Plot**

🔲 **Viewing Cyclic Voltammetry Data**

🔲 **Copying Cyclic Voltammetry Plots**

◾ Opening a Cyclic Voltammetry Data Plot

1. To view cyclic voltammetry data plot, right-click the **Manual Acquisition** sample in the manual sequence in which the data was saved.

2. Select **Open** on the context menu and then select **3D_Amp** from the list of channels.

The data opens in a CV view window.
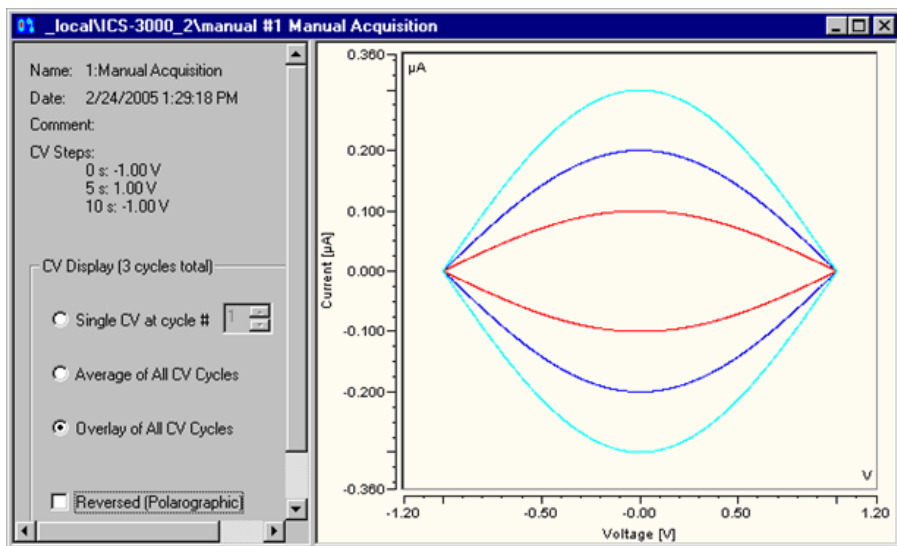
Also, refer to:

⤴ Viewing Cyclic Voltammetry Data
⤴ Copying Cyclic Voltammetry Plots

■ Viewing Cyclic Voltammetry Data

➢*Cyclic Voltammetry* data is displayed in a CV view window (see ▣ **Opening a Cyclic Voltammetry Data Plot**).

The right pane of the window displays the cyclic ➢*Voltammogram*. The x-axis is the applied voltage and the y-axis is the detected current. To change the appearance of the plot (for example, to display a grid, change the plot or background colors, change the scale, or change the font) right-click anywhere on the plot and select **Decoration** on the context menu.

The left pane of the CV view window displays basic information about when and how the data was collected.

Name	The ➢*Name* assigned to the sample.
Date	The ⇒*Injection Date/Time*.
Comment	Additional sample description (if any).
CV Steps	The steps (the three voltages and the number of seconds they were applied) that define one CV cycle. See **Detector Control:** ▣ **Performing Cyclic Voltammetry**.

The left pane also provides options for changing the display mode:

Single CV at cycle # n	Displays one cyclic voltammogram at a time. For example, if the CV run consisted of 6 cycles, you can view voltammograms 1 through 6 individually.
Average of All CV Cycles	Averages the voltammograms from each cycle in the run and displays the resulting voltammogram.
Overlay of All CV Cycles	This mode displays all of the voltammograms recorded in the run. Each one is plotted in a different color. To see (or change) the color assigned to each plot, select **Decoration** from the **View** or context menu.
Reversed (Polarographic)	Selecting this check box inverts the plot axes. The voltage will be plotted from positive to negative (left to right) and the current from positive to negative (bottom to top).

Also, refer to ▣ **Opening a Cyclic Voltammetry Data Plot** and ▣ **Copying Cyclic Voltammetry Plots**.

◾ Copying Cyclic Voltammetry Plots

You can copy a cyclic voltammetry plot to the Windows Clipboard and then paste it into another application (for example, a Word document).

1. Display the desired plot in the CV view window (see 🔺 **Viewing Cyclic Voltammetry Data**).

2. Click the right pane of the CV view window to select the plot.

3. Select **Copy** on the **Edit** menu.

Also, refer to:

🔺 **Opening a Cyclic Voltammetry Data Plot**
🔺 **Viewing Cyclic Voltammetry Data**

Using Mass Spectrometers

When used together with the Thermo Finnigan aQa or MSQ ➢*Mass Spectrometer*, Chromeleon supports MS data acquisition. However, before you can record and process mass spectra, several conditions must be fulfilled. For more information, refer to:

⬛ **Creating an MS Program and Sequence**

⬛ **Creating a PGM File for the MSQ**

⬛ **Creating MSQ Channels with the MSQ PGM File**

⬛ **Creating a PGM File for the aQa MS**

⬛ **Creating aQa MS Channels with the aQa PGM File**

⬛ **Acquiring MS Data in MCA Mode**

⬛ **Extracting Mass Traces Online**

⬛ **Extracting Mass Traces Afterward**

⬛ **Extracting a Temporary Mass Trace**

⬛ **Showing Mass Spectra**

⬛ **Minimizing the Noise of Mass Spectra**

⬛ **Processing Mass Spectra**

⬛ **Defining Further QNT Settings for MS**

⬛ Creating an MS Program and Sequence

Manual data acquisition is not supported for ➢*Mass Spectrometers*. Therefore, you must prepare a sequence before you can start data acquisition. This requires a control file (➢*PGM File*).

Program Wizard (PGM Editor: Commands)
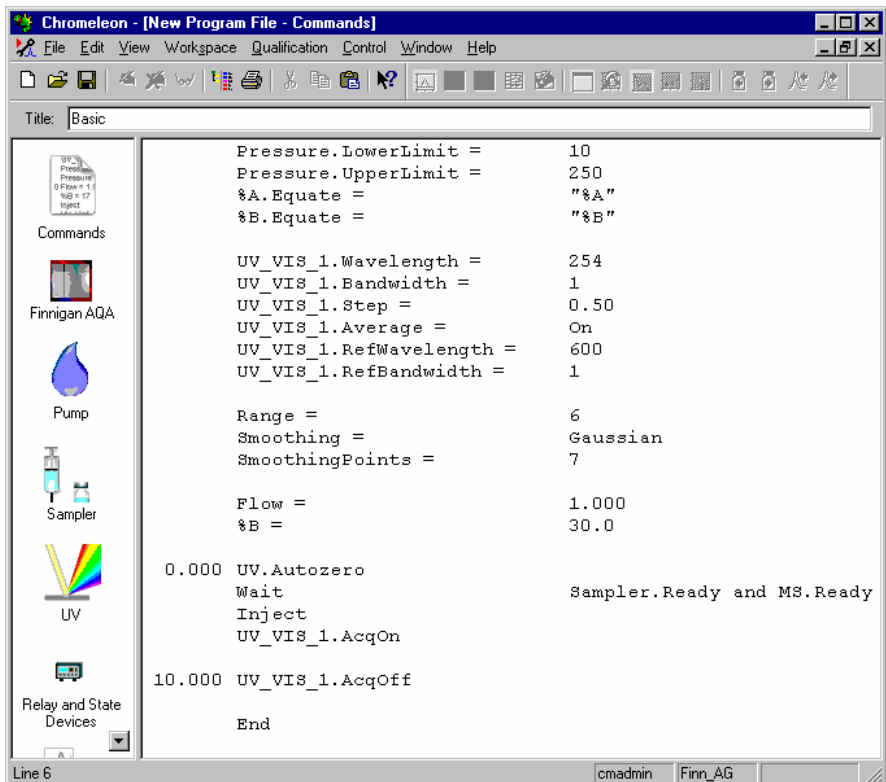
Create a new PGM File, using the Program Wizard (see **The Control Program** ⬛ **The Program Wizard**). If a mass spectrometer is installed in the current timebase, select data acquisition with the MS channel on the **Acquisition Options** tab page. This action opens the page for MS-specific parameters.

🖙 *Tip:*

Log on to the PC as a main user or as administrator if you wish to create a program for the MSQ mass spectrometer. If you do not log on this way, a ***No MS device configured*** *error message appears.*

Set the parameters required for ➢*Mass Trace* processing (= MS chromatogram). The **Range** parameter is the scaling factor for the online display of mass traces on the control panel; for example, Range = 5 allows a maximum value of 100.000 = (1 E+5) counts in the online display. However, the stored data are not affected. The ➢*Smoothing* parameter determines which type of smoothing filter is used for smoothing MS chromatograms.

In the **Points** field, define the number of data points to be used for smoothing. Select the number of data points such that the width of the smoothing filter approximately equals the peak's half width. For example, the following program will be created:

The PGM File that was generated using the **PGM Wizard** does not include ⇒*AcqOn/Off (Data Acquisition On/Off)* commands for the MS channels. The channels that are needed for mass trace acquisition depend on the contents of the MS method.

The Program Wizard automatically generates the **wait MS.ready** command before the inject command. This synchronization is required between the mass spectrometer and Chromeleon. If you want to create your own PGM Files manually, always add the **wait MS.ready** command before the inject command.

If you want to use ➢*Blank Run Samples*, verify that the inject mode is set to **Inject**. The aQa mass spectrometer will not start data acquisition unless an injection signal is received.

Example:
```
Wait MS.Ready
Inject Blank=Inject
```

On the last wizard page, select the **Review the program in a new window** option. This action automatically opens the PGM Editor as soon as the new PGM File is saved. Use the PGM Editor to define the mass spectrometer settings for the PGM File. Select the **Mass Spectrometer** view by clicking the corresponding icon in the left editor section. For information about mass spectrometer methods, refer to 🔺 **Creating a PGM File for the aQa MS** or 🔺 **Creating a PGM File for the MSQ**.

Sequence

Then, create a sequence for your HPLC or IC system.

a) Using the Sequence Wizard:

Enter the created PGM File in step 4.

b) From a previous sequence:

Copy the PGM File to a new sequence and enter the PGM File in the sample list.

🔖 *Tip:*

*Verify that the **Operation** property of the aQa mass spectrometer is in the **On** state. Otherwise, the gas flow and the probe heating are turned off and data acquisition cannot be started.*

Then, start data acquisition as usual (see **Practical Tips for Device Control** 🔺 **Starting Data Acquisition**). The current mass spectrum can be displayed on the ➢*Control Panel*.

Creating a PGM File for the MSQ

Use the **Surveyor MSQ** view of the PGM Editor (see **Control** **The PGM Editor**) to create a new instrument method for the MSQ ➢*Mass Spectrometer* as part of a PGM File. Open this view by clicking this icon on the shortcut bar in the left PGM Editor section:

 Tip:

*If the PGM File was not created for a timebase that includes a mass spectrometer, neither an MS method nor the MSQ symbol will be available. To use such a PGM File for a timebase that includes a mass spectrometer, select **Add MS Instrument Method** on the **Edit** or context menu. This adds the **Surveyor MSQ** view. From the standard MS methods (Surveyor MSQ Templates) in the dialog box, select an MS method. The appropriate MS method depends on the ionization mode, the MS mode (➢Full Scan or ➢SIM), and the polarity of the recorded ions. In addition, enter the following command in the ➢Program (**Commands** view) at the time t = 0.000 min:*

> `0.000 WaitMS.Ready`

The **Surveyor MSQ** view is part of the ➢*Xcalibur* software and allows you to specify the method used by the MSQ mass spectrometer.

The upper left section shows a runtime preview of the single channels to record. In the **Preview** section, select:

- All Scans to display all channels.

- **Full Scans** to display the ➢*Full Scan* channels (orange) only.

- **SIM** to display the ➢*SIM* channels (green) only.

For an overview of the defined channels, including their measurement parameters, select the **Parameter** option.

If a chromatogram was recorded with one MS channel only, you can also have the chromatogram displayed in the preview. Select **Show Method Options** to open the method options, and then select a sample by clicking the "**...**" button under **Display**.

On the top right, in the **Per Method Parameters** section, select ESI (**E**lectro**s**pray **I**onization) or **APCI** (**A**tmospheric **P**ressure **C**hemical **I**onization) as the ionization mode, depending on your MSQ installation. In addition, enter the nominal temperature on the ion source (range: 0 to 655°C).

In the **Full/SIM Scan Events** section, enter the MSQ-specific signal parameters for ➢ *Mass Spectra* acquisition:

In the **Mass Range** field, define the mass range (➢ *Full Scan*). In the **Mass** field, define the mass. Define the corresponding bandwidth (➢ *SIM*) in the **Span** field. In addition, define the duration of the data acquisition (**Time Range**), the data acquisition rate (**Dwell Time** (SIM) or **Scan Time** (Full Scan)), the **Polarity**, and the ionization voltage (**Cone [V]**).

📑 *Note:*

For a list of SIM masses for anions, cations, and amines in water, refer to How to …: Using Mass Spectrometers 🔊 *SIM Mass Lists for IC-MS*.

In the **Scans** section, select the **Add Full** option to add a new full-scan channel or **Add SIM** to add a new SIM channel. The settings from the preceding channel are adopted. Use the **Create Group** option to group all SIM channels that are recorded in the same retention time window. (To do so, hold down the CTRL key and click to select the SIM channels.) Select **Ungroup** to undo this action. To delete a channel, select the channel, and then click **Delete**.

📖 *Tip:*

This view of the PGM Editor is part of the Xcalibur software. The Xcalibur Help system provides detailed information about mass spectra acquisition. First, click the question mark (at the top right) and then click the option of interest to open the corresponding Help topic.

📖 *Tip:*

When saving the PGM File, only use ASCII characters to name the entire path, i.e., including the datasource and the directories. If you use other characters, too, it may be impossible to start data acquisition.

For more information, refer to 🔲 **Creating MSQ Channels with the MSQ PGM File**.

🔲 Creating MSQ Channels with the MSQ PGM File

Chromeleon creates a channel for each scan event of the MSQ instrument method. In the case of ➢*SIM* groups, this also applies to each sub scan event. The examples below describe which channels Chromeleon creates automatically for the different types of data acquisition with the MSQ.

📒 *Note:*

For a list of SIM masses for anions, cations, and amines in water, refer to How to …: Using Mass Spectrometers 🔲 SIM Mass Lists for IC-MS.

The first example creates four SIM channels plus the TIC channel:

SIM Scan Events

Name	Mass	Span	Time Range (min)
SIM 1:	80.00	1.00	0.00-1.00
SIM 2:	85.00	1.00	0.00-1.00
SIM 3:	90.00	1.00	0.00-1.00
SIM 4:	100.00	1.00	0.00-1.00

The SIM1 channel records the chromatogram at a mass of 80.00 m/z, while the other channels record the same chromatogram at a mass of 85.00 m/z (SIM2), 90.00 m/z (SIM3), and 100.00 m/z (SIM 4).

Data acquisition is performed for all four channels at the same time (0 to 1 min). That is why you can group all four channels. In this case, only SIM channels and the TIC channel would be created.

SIM Scan Events

Name	Mass	Span	Time Range (min)
GROUP 1:			0.00-1.00
SIM 1:	80.00	1.00	0.00-1.00
SIM 2:	85.00	1.00	0.00-1.00
SIM 3:	90.00	1.00	0.00-1.00
SIM 4:	100.00	1.00	0.00-1.00

The next example creates 11 SIM channels (SIM 01 to SIM 11) and the TIC channel:

SIM Scan Events

Name	Mass	Span	Time Range (min)	Dwell Time (s)	Polarity	Cone (V)
GROUP 1:			0.00-2.00			
SIM 1:	100.00	1.00	0.00-2.00	1.00	-ve	200.00
SIM 2:	200.00	1.00	0.00-2.00	1.00	+ve	100.00
SIM 3:	300.00	1.00	0.00-2.00	1.00	-ve	200.00
SIM 4:	400.00	1.00	0.00-2.00	1.00	+ve	100.00
GROUP 2:			2.00-3.00			
SIM 1:	100.00	1.00	2.00-3.00	1.00	-ve	200.00
SIM 2:	100.00	3.00	2.00-3.00	0.80	-ve	200.00
SIM 3:	100.00	5.00	2.00-3.00	2.10	-ve	75.00
SIM 4:	150.00	1.00	2.00-3.00	0.90	+ve	100.00
SIM 5:	200.00	1.00	2.00-3.00	1.20	+ve	50.00
SIM 1:	99.00	1.00	0.00-1.00	1.00	+ve	200.00
SIM 2:	111.00	1.00	0.00-1.00	1.00	+ve	200.00

Copyright © 2002, Thermo Finnigan

The channel assignment is as follows:

Xcalibur (CM program: MSQ)			Chromeleon	
Name	Mass [m/z]	Span [m/z]	Name	Mass Range [m/z]
GROUP 1: Retention time 0.00 to 2.00 min				
SIM 1	100	1	SIM_01	99.5 - 100.5
SIM 2	200	1	SIM_02	199.5 - 200.5
SIM 3	300	1	SIM_03	299.5 - 300.5
SIM 4	400	1	SIM_04	399.5 - 400.5

Xcalibur (CM program: MSQ)			Chromeleon	
Name	Mass [m/z]	Span [m/z]	Name	Mass Range [m/z]
GROUP 2: Retention time 2.00 to 3.00 min				
SIM 1	100	1	SIM_05	99.5 - 100.5
SIM 2	100	3	SIM_06	98.5 - 101.5
SIM 3	100	5	SIM_07	97.5 - 102.5
SIM 4	150	1	SIM_08	149.5 - 150.5
SIM 5	200		SIM_09	199.5 - 200.5
Single channels:				
SIM 1	99	1	SIM_10	98.5 - 99.5
SIM 2	110	1	SIM_11	109.5 - 110.5

The next examples creates 3 TICF channels and the TIC channel:

Full Scan Events

Name	Mass Range	Time Range (min)	Peak Format
FS 1:	30.00-500.00	0.00-1.00	Centroid
FS 2:	60.00-400.00	2.00-4.00	Profile
FS 3:	600.00-800.00	0.00-2.00	Centroid

The channel assignment is as follows:

TICF_1:
FS 1, Mass range 30.00 to 500.00 m/z; retention time 0.00 to 1.00 min,

TICF_2:
FS 2, Mass range 60.00 to 400.00 m/z; retention time 2.00 to 4.00 min,

TICF_3:
FS 3, Mass range 600.00 to 800.00 m/z; retention time 0.00 to 2.00 min.

☞ *Tip:*

When saving the PGM File, always use ASCII characters to name the entire path, i.e., including the datasource and the directories. If you use other characters, too, it may be impossible to start data acquisition.

If you notice after data acquisition that a channel is missing, you can extract separate ➢*Mass Traces* (or mass ranges) from the ➢*Mass Spectrum* and save them as new channels. (For more information, refer to **How to …: Using Mass Spectrometers** 🔲 **Extracting Mass Traces Afterward**.)

▣ Creating a PGM File for the aQa MS

Use the **Mass Spectrometer** view of the PGM Editor (see **Control** ▣ **The PGM Editor**) to create a new instrument method for the aQa ➢*Mass Spectrometer* as part of the PGM File. Open this view by clicking this icon on the shortcut bar in the left PGM Editor section:

👈☞ *Tip:*

*If the PGM File was not created for a timebase that includes a mass spectrometer, neither the MS method nor the Thermo Finnigan aQa symbol will be available. To use such a PGM File for a timebase that includes a mass spectrometer, select the **Add MS Instrument Method** command to the **Edit** or context menu. This creates a standard MS method and adds the **Mass Spectrometer** view. In addition, enter the following command in the ➢Program (**Commands** view) at the time t = 0.000 min:*

> *0.000 WaitMS.Ready*

The **Mass Spectrometer** view is part of the ➢*Xcalibur* software and allows you to specify the method used by the aQa mass spectrometer.

On the **Ionization Mode** tab page, select **Electrospray** (**E**lectro**s**pray **I**onization) or **APCI** (**A**tmospheric **P**ressure **C**hemical **I**onization) as the ionization mode.

On the **Analysis** tab page, set the sensitivity of the mass spectrometer via the detector voltage. Via **Advanced (from tune file)**, load a tune file that has been previously defined. To fine-tune the mass spectrometer directly, click **Tune**.

⚠ *Caution:*

*When you use the Xcalibur method editor of Chromeleon, the **Other detectors** section is irrelevant. Do **not** use this section for data acquisition with other detectors, such as UV detectors! In this case, perform data acquisition as usual.*

On the **Acquisition** tab page, enter the aQa-specific signal parameters for ➢*Mass Spectra* acquisition:

Select the data acquisition mode first: Select ➢*Full-Scan* to acquire the entire mass spectrum for each analyte or ➢*SIM* to obtain MS chromatogram at a defined mass.

Note:

For a list of SIM masses for anions, cations, and amines in water, refer to How to …: Using Mass Spectrometers 📄 *SIM Mass Lists for IC-MS.*

In Full-Scan mode, use the **Simultaneous acquisitions** tab page to set the polarity and maximum voltage on the aQa MS for four single channels (➤*TIC*F channels). In the **Acquisition rate** field, specify the rate for data acquisition; in the **Mass spectrum** field, specify the mass range for which to perform data acquisition.

☞ *Tips:*

*This view of the PGM Editor is part of the Xcalibur software. Thus, you can open the Xcalibur help either via the **Help** menu or by clicking **Help**. The Xcalibur help provides detailed information about mass spectra acquisition.*

When saving the PGM File, only use ASCII characters to name the entire path, i.e., including the datasource and the directories. If you use other characters, too, it may be impossible to start data acquisition.

For more information, refer to **Creating aQa MS Channels with the aQa PGM File**.

■ Creating aQa MS Channels with the aQa PGM File

The examples below describe which channels Chromeleon creates for the different types of data acquisition with the aQa MS.

🗐 *Note:*

*For a list of SIM masses for anions, cations, and amines in water, refer to **How to …: Using Mass Spectrometers** **SIM Mass Lists for IC-MS**.*

The first example creates two SIM channels plus the TIC channel:

The SIM_01 channel records the chromatogram at a mass of 100 amu, while the SIM_02 channel records the corresponding chromatograms at a mass of 200 amu.

The second example creates six SIM channels and the TIC channel:

The channel assignment is as follows:

SIM_01: Mass 100 amu, positive voltage, 100V,

SIM_02: Mass 100 amu, negative voltage, -80V,

SIM_03: Mass 200 amu, positive voltage, 100V,

SIM_04: Mass 200 amu, negative voltage, -100V,

SIM_05: Mass 300 amu, positive voltage, 100V,

SIM_06: Mass 300 amu, positive voltage, 80V.

The third example creates three TICF channels and the TIC channel:

The channel assignment is as follows:

TICF1: positive voltage, 100V

TICF2: positive voltage, 200V

TICF3: negative voltage, -100V.

☞ *Tip:*

When saving the PGM File, only use ASCII characters to name the entire path, i.e., including the datasource and the directories. If you use other characters, too, it may be impossible to start data acquisition.

If you notice after data acquisition that a channel is missing, you can extract separate ➢*Mass Traces* (or mass ranges) from the ➢*Mass Spectrum* and save them as new channels. (For more information, refer to **How to …: Using Mass Spectrometers** 🔲 **Extracting Mass Traces Afterward**.)

◼ SIM Mass Lists for IC-MS

➢*SIM (Selected Ion Monitoring)* is the ➢*Mass Spectrometer* method used for recording an MS chromatogram at a specific mass-to-charge ratio.

The tables below list the SIM masses for anions, cations, and amines in water. Refer to the appropriate table when entering SIM masses in aQa or MSQ ➢*PGM Files*, or to identify found masses.

Anion SIM Mass List for IC-MS (in water)

m/z	Anion	Detected As	Isotopes (decreasing frequency)
19	Fluoride	F^-	
35	Chloride	Cl^-	
45	Formate	$HCOO^-$	
46	Nitrite	NO_2^-	
58	Thiocyanate	SCN^-	
59	Acetate	CH_3COO^-	
61	Bicarbonate	HCO_3^-	
62	Nitrate	NO_3^-	
67	Chlorite	ClO_2^-	67/69
73	Glyoxylate	$CHOCOO^-$	
73	Propionate	$CH_3CH_2COO^-$	
75	Glycolate	$HOCH_2COO^-$	
79	Bromide	Br^-	79/81
83	Chlorate	ClO_3^-	83/85
87	Butyrate	$CH_3CH_2CH_2COO^-$	
87	Pyruvate	CH_3COCOO^-	
89	Lactate	$CH_3CH(OH)COO^-$	
89	Oxalate	$COOHCOO^-$	
93	Chloroacetate	$ClCH_2COO^-$	93/95

Anion SIM Mass List for IC-MS (in water)

m/z	Anion	Detected As	Isotopes (decreasing frequency)
95	Methanesulfonate	$CH_3SO_3^-$	95/97
96	Sulfamate	$NH_2SO_3^-$	96/98
97	Sulfate	HSO_4^-	97/99
97	Phosphate	$H_2PO_4^-$	
99	Perchlorate	ClO_4^-	99/101
101	Valerate	$CH_3CH_2CH_2CH_2COO^-$	
103	Hydroxybutyrate	$CH_3CHOHCH_2COO^-$	
103	Malonate	$COOHCH_2COO^-$	
113	Thiosulfate	$HS_2O_3^-$	113/115
113	Trifluoroacetate	F_3CCOO^-	
115	Maleate	$COOHCHCHCOO^-$	
115	Fumarate	$COOHCHCHCOO^-$	
117	Succinate	$COOHCH_2CH_2COO^-$	
127	Bromate	BrO_3^-	127/129
127	Dichloroacetate	Cl_2CHCOO^-	127/129
127	Iodide	I^-	
128	Selenite	SeO_3^-	128/126
131	Glutarate	$COOH(CH_2)_3COO^-$	
133	Malate	$COOHCH_2CHOHCOO^-$	
133	Tartrate	$COOH(CHOH)_2COO^-$	
137	Bromoacetate	$BrCH_2COO^-$	137/139
144	Selenate	SeO_4^-	144/142
145	Adipate	$COOH(CH_2)_4COO^-$	
173	Bromochloroacetate	$BrClCHCOO^-$	173/171/175
183	Styrenesulfonate	$CH_2CHC_6H_4SO_3^-$	

Anion SIM Mass List for IC-MS (in water)

m/z	Anion	Detected As	Isotopes (decreasing frequency)
191	Citrate	$HOOCCH_2COH(COOH)CH_2COO^-$	
191	Quinate	$C_6H_7(OH)_4COO^-$	
191	Isocitrate	$HOOCCHOHCH(COOH)CH_2COO^-$	
207	Dichlorobromoacetate	$Cl_2BrCCOO^-$	207/205/209/211
217	Dibromoacetate	Br_2CHCOO^-	297/295/293/299
251	Dibromochloroacetate	$Br_2ClCCOO^-$	251/253/249/255
297	Tribromoacetate	Br_3CCOO^-	297/295/293/299

Cation and Amine SIM Mass List for IC-MS (in water)

m/z	Cation	Detected As
18	Ammonium	NH_4^+
19	Hydronium	H_3O^+
20	Calcium	$1/2Ca_2^+$
23	Sodium	Na^+
39	Potassium	K^+
46	Ethylamine	$CH_3CH_2NH_3^+$
46	Dimethylamine	$(CH_3)_2NH_2^+$
60	Trimethylamine	$(CH_3)_3NH^+$
60	Guanidine	$(NH_2)_2CNH_2^+$
61	Ethanediamine	$NH_2CH_2CH_2NH_3^+$
62	Monoethanolamine	$OHCH_2CH_2NH_3^+$
69	Imidazole	$C_3H_4N_2H^+$
74	Diethylamine	$(CH_3CH_2)_2NH_2^+$
74	Dimethylethylamine	$(CH_3)_2(C_2H_5)NH^+$

Cation and Amine SIM Mass List for IC-MS (in water)

m/z	Cation	Detected As
74	Butylamine	$C_4H_9NH_3^+$
75	Propanediamine	$NH_2CH_2CH_2CH_2NH_3^+$
76	Methylethanolamine	$HOCH_2CH(CH_3)NH_3^+$
76	Dimethylmethanolamine	$HOC(CH_3)_2NH_3^+$
76	Propanolamine	$C_3H_6OHNH_3^+$
80	Pyridine	$C_5H_5NH^+$
85	Lysidine	$C_4H_8N_2H^+$
88	Morpholine	$C_4H_8ONH_2^+$
88	Methyldiethylamine	$CH_3(C_2H_5)_2NH^+$
89	Butanediamine (Putrescine)	$H_2NC_4H_8NH_3^+$
90	Dimethylethanolamine	$C_5H_{13}NH^+$
90	Methylpropanolamine	$C_4H_8(OH)NH_3^+$
100	Cyclohexylamine	$C_6H_{11}NH_3^+$
102	Triethylamine	$(CH_3CH_2)_3NH^+$
103	Pentanediamine (Cadaverine)	$H_2NC_5H_{10}NH_3^+$
104	Dimethylamino-2-propanol	$(C_3H_6OH)(CH_3)_2NH^+$
106	Diethanolamine	$(OHCH_2CH_2)_2NH_2^+$
118	Diethylethanolamine	$C_6H_{15}ONH^+$
120	Methyldiethanolamine	$C_5H_{13}O_2NH^+$
132	Diethylaminopropanol	$C_7H_{17}ONH^+$
146	Spermidine	$H_2N(CH_2)_3NH(CH_2)_4NH_3^+$
150	Triethanolamine	$(OHCH_2CH_2)_3NH^+$
203	Spermine	$C_{10}H_{26}N_4H^+$

▪ Acquiring MS Data in MCA Mode

Use the MCA (= Multi-Channel Analysis) mode to calibrate the aQa ➤*Mass Spectrometer* and analyze pure, low-concentration solutions of substances. Usually, the solution in question is provided to the mass spectrometer via infusion.

☞ *Tip:*

The ➤MCA mode is not available for the MSQ.

The MCA mode summarizes all ➤*Mass Spectra* of the single scans. Only the resulting averaged mass spectrum from each of the scan filters (up to four) is saved when the analysis is finished.

⚠ *Caution:*

*The MCA mode does not allow recording and showing mass spectra at a defined time of the chromatogram. Therefore, the MCA mode **is not suitable for chromatographic analyses**!*

Data acquisition in MCA mode is as follows:

- Create a PGM File, using the Program Wizard (see **The Control Program** ▪ **The Program Wizard**).

- Verify that the PGM File includes an **Inject** command even if you do not use an autosampler. Otherwise, data acquisition cannot start.

- In the PGM Editor (see **Control** ▪ **The PGM Editor**), open the **Acquisition** tab page of the Finnigan aQa window. In the **Mass spectrum** field, select **MCA** as **Peak format**.

- Save the PGM File and close the PGM Editor.

- Enter the PGM File in your sequence and start the sequence in a batch.

☞ *Tip:*

➤MCA data acquisition is not possible in demo mode!

The ➢*Xcalibur* window below opens automatically when data acquisition starts:
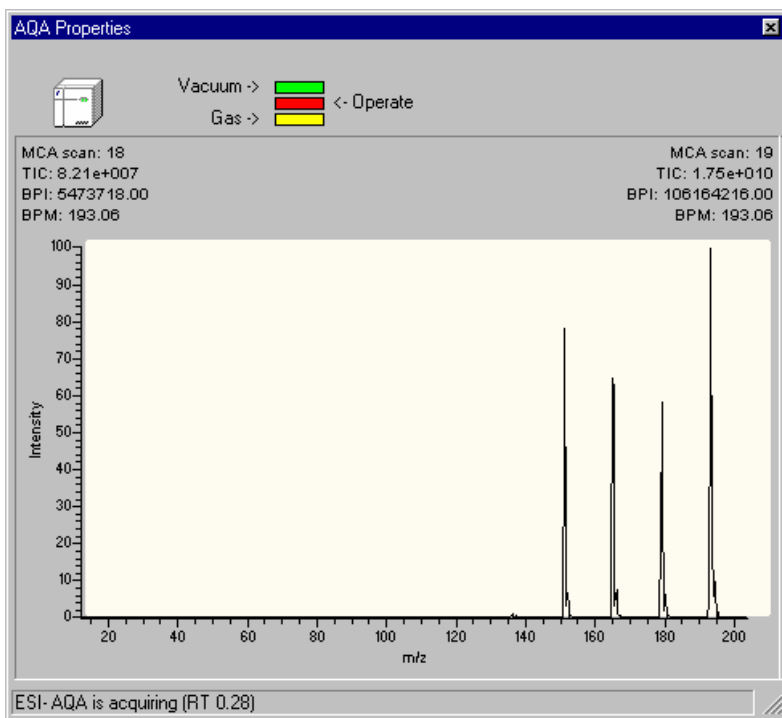
The status bar indicates the time that passed since the data acquisition was started. The results of the last two scans appear at the top left and right.

As soon as the data acquisition is finished, you can display the results in the Chromeleon report. To display the results click the line at the highest retention time on the chromatogram plot using the **Spectra/I-t Plots Tool**.

This action opens the following view:
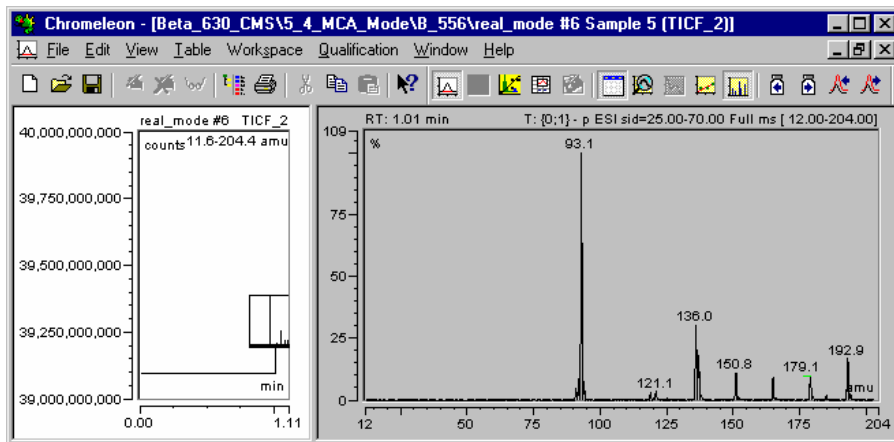
The chromatogram in the left pane contains one data point only that is at the highest retention time. To illustrate the summing up of the entire acquisition period, it shows one line in the height of the entire counts of all summed up mass spectra. In addition, only one single (entire) mass spectrum is displayed in the right window section.

☞ *Tip:*

As MCA mass spectra are not spectra from chromatographic peaks, but are formally retention time spectra, they cannot be inserted in the ➢*Printer Layout. Therefore, select* **Print** *on the* **File** *menu to print mass spectra. A dialog box appears. Select the Printer Layout page that contains the mass spectrum.*

▪ Extracting Mass Traces Online

It is possible to extract ➤*Mass Traces* (MS chromatograms) and save them as additional channels. This can be done either during data acquisition (online) or later (see 🔼 **Extracting Mass Traces Afterward**).

In the ➤*Server Configuration Program*, define the required number of Online Mass Extract Channels on the **Installed Channels** tab page. (Up to 32 channels can be defined.) These channels are automatically named MS_01 to MS_32.

Create a ➤*PGM File* for data acquisition in ➤*Full Scan* mode, using the Program Wizard (see **The Control Program** 🔼 **The Program Wizard**). Each single MS channel needs its own ⇒*AcqOn/Off* command. Define the following parameters for each single MS channel:

Parameter	Min.	Max.	Default	Usage
FilterIndex	0: NoFilter	n: TICF_0n (MSQ) or TICF_n (aQa) (n = number of the TICF channel configured in the Server Configuration)	0	Selects the filter for extraction. The filter indexes correspond to the nine MSQ filters or the four aQa filters that can be defined in the MS method. 0 = NoFilter means that the ➤*TIC* channel is used.
MinMass	0.00 m/z (MSQ) 2.0 m/z (aQa)	2000.00 m/z (MSQ) 1636.0 m/z (aQa)		Minimum mass of interval that will be extracted.
MaxMass	0.00 m/z (MSQ) 2.0 m/z (aQa)	2000.00 m/z (MSQ) 1636.0 m/z (aQa)		Maximum mass of interval that will be extracted.

These parameters cannot be set using the Program Wizard. Therefore, follow the steps below:

- Open the PGM File.

- Select **Command** on the **Control** menu to open the **Commands** dialog box.

- Select your ➤*Mass Spectrometer* (listed by the name defined in the Server Configuration).

- Open the mass channel to be extracted; for example, MS_01.

- Specify the individual parameters:
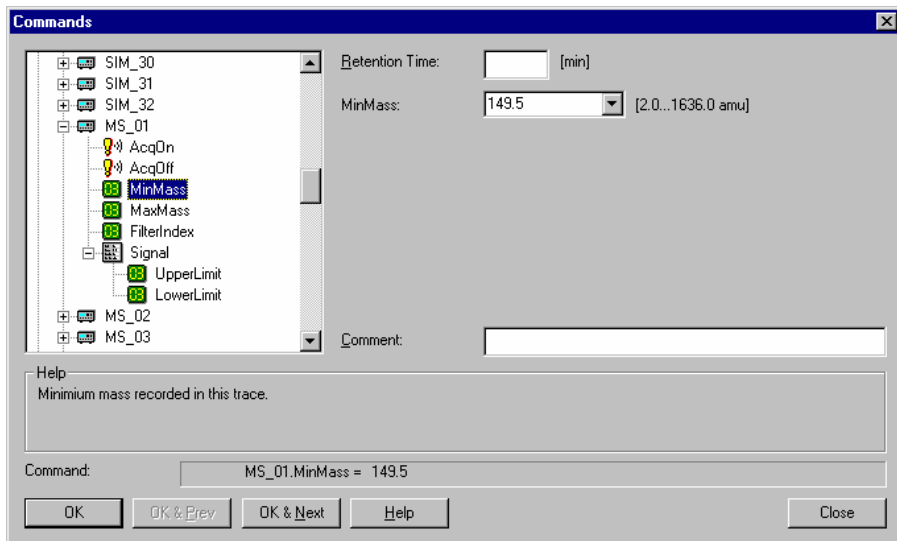
Use the **Upper/Lower Limit** parameters to specify the signal limits.

You can also extract the trace of the ➢*Base Peak*. In this case, verify that the **BasePeakMode** property is set to **Yes**.

📖 *Tip:*

Do not change the mass trace settings during a run. This might result in confusion. Therefore, Dionex recommends not entering a retention time.

The corresponding section in the ➢*Program* could look as follows:

```
MS_01.MinMass = 149.5
MS_01.MaxMass = 150.5
MS_01.FilterIndex = 1
```

📖 *Tip:*

To start data acquisition, manually enter the following command for the MS_01 channel:

```
MS_01.AcqOn
```

To stop data acquisition for the MS_01 channel, enter:

```
MS_01.AcqOff
```

You can display these channels on the ➢*Control Panel* during data acquisition.

◼ Extracting Mass Traces Afterward

If you did not extract a ➢*Mass Trace* online, during data acquisition (see ◰ **Extracting Mass Traces Online**), you can do this afterward:

☞ *Tip:*

To view a mass trace before extracting it, extract a temporary mass trace first (see ◰ Extracting a Temporary Mass Trace).

1. To extract a mass trace, open the ➢*Mass Spectrum* from the **Integration** plot or the QNT Editor. (For more information about the editor, see **Data Representation and Reprocessing ◰ The QNT Editor**.)

   ## ☞ *Tip:*

   *You may also define mass trace extraction in the **Post-acquisition steps** view of the PGM Editor (see **Control ◰ The PGM Editor**). Open the PGM File in which you want to define mass trace extraction, and then select the **Post-acquisition steps** view.*

2. Select **Extract Mass Trace…** on the context menu to open the **Extract Mass Trace** dialog box:

📌 *Tip:*

*In the PGM Editor, follow the steps below: Select **Insert line** on the context menu to add a new post-acquisition step. The **New post-acquisition step** dialog box is opened. Select **Extract MS channel** to open the **Extract Mass Trace** dialog box.*

3. Select a filter and mass range, as well as the ➢*Smoothing* type for the MS chromatograms, the number of data points to be used, and the type of the mass trace to be extracted.

4. In the **Channel Name** box, enter the name for the new channel or accept the default name. Chromeleon creates the suggested name from the **Filter Index**, the **Mass**, if indicated, the **Smoothing** information, and the **Trace Type** for the mass trace to be extracted.

📝 *Note:*

If the Trace Type is ➢TIC, Chromeleon does not consider the mass (or the mass range if < 1.00 m/z) for the channel name.

5. To extract the mass trace for all samples in the sequence or ➢*Query*, select the **Apply to all samples in the current sequence or query** check box.

📌 *Tip:*

*This option is not available in the **Extract Mass Trace** dialog box of the PGM Editor.*

6. Click **Extract** to make the new channel available for chromatographic representation.

If you know prior to data acquisition which channels you will need, you can omit this step and record the required channels right from the beginning (see **How to …: Installing and Configuring Mass Spectrometers** 🔲 **Defining the Number of MS Channels** in the **Administrator Help** section) or extract them as described above.

📝 *Note:*

*If you need help identifying found masses, refer to **How to …: Using Mass Spectrometers** 🔲 **SIM Mass Lists for IC-MS**.*

▣ **Extracting a Temporary Mass Trace**
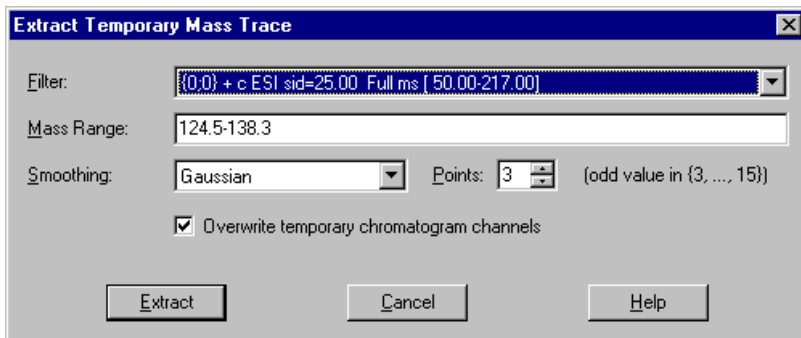
Before you extract a ➢*Mass Trace* (see 🛐 **Extracting Mass Traces Afterward**), you can create it temporarily by just clicking the mouse. The following options are available:

Using the mouse pointer

Cursor	Activated by	What it does
⿊	Placing the pointer near a mass data point or a mass needle.	A temporary MS channel is extracted for the current mass. An existing temporary MS channel will be overwritten.
⿊	Placing the pointer near a mass data point or a mass needle and simultaneously pressing the SHIFT key.	A temporary MS channel is extracted for the current mass. The new channel will overlay existing temporary MS channels.
↔	Placing the pointer between two mass range delimiters.	This action moves the mass range as desired.
↔	Placing the pointer on or near a mass range delimiter.	This action moves the left or right delimiter in the desired direction.

On the context menu

To extract a mass trace, click the corresponding mass or right-click the mass range. On the context menu, select **Extract Temporary Mass Trace** and define the corresponding parameters in the dialog box:

■ Showing Mass Spectra

The ➢*Mass Spectra* view can be added to almost all Chromeleon plots (Integration, QNT Editor (see **Data Representation and Reprocessing** 🔳 **The QNT Editor**), Printer Layout) if ➢*Xcalibur* is installed. To enable the mass spectra view, click the following icon: 📊

Or else, select **Show Mass Spectra** on the **View** menu:
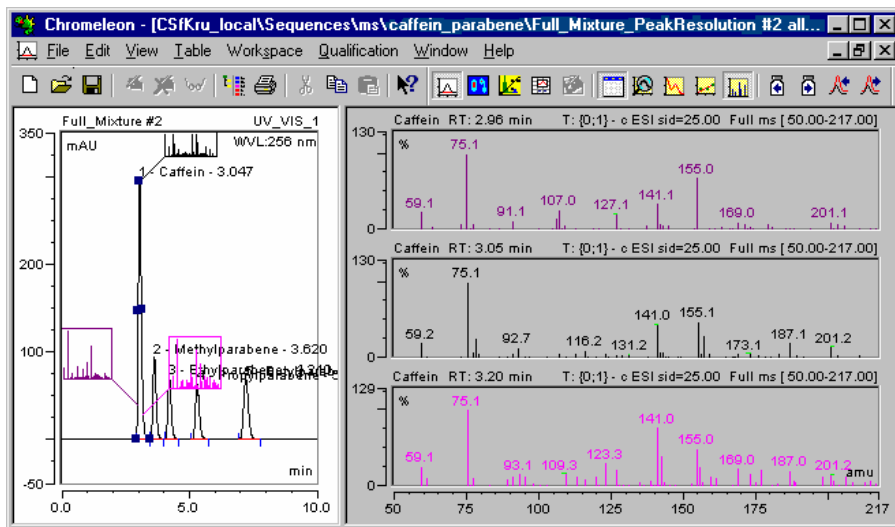
To add a mass spectrum to the ➢*Printer Layout*, enable **Layout Mode** on the **Edit** menu. Select **Insert** on the **View** or context menu, and then select **Mass Spectra Plot**.

The appearance of the displayed mass spectra may be quite different, depending on which the MS instrument method was used. For more information, refer to **How to …: Using Mass Spectrometers** 🔳 **Creating a PGM File for the aQa MS** or 🔳 **Creating a PGM File for the MSQ**. For information about which the parameters should be used in this method to receive a certain mass spectrum, refer to the Xcalibur help.

📋 *Note:*

MS spectra are displayed one below the other to enhance the clearness of representation. For a large enough representation, enlarge the upper section of the entire window, if necessary.

In the captions of the single mass spectra plots, the peak name (if the mass spectrum of a peak is given) plus the retention time of the mass spectrum appear on the left. The acquisition mode appears on the right: **Full ms** indicates ➤ *Full-Scan* mode; **SIM ms** indicates ➤ *SIM* mode.

In the caption of full-scan mass spectra, the entire mass range appears in parentheses on the right. The fragmentation voltage that appears in front of the mode is important, as well.

With mass spectra that were acquired in SIM mode, no entire mass spectra are available. These SIM mass spectra are extracted from single mass traces, so the resulting SIM mass spectrum usually shows gaps between the single traces. In the caption, the single mass ranges appear together with the corresponding fragmentation voltage behind the respective mass range (following a @ sign) on the right.

How to set the MS specific parameters of the view

Place the pointer on the mass spectrum and right-click to open the context menu. Select **Decoration**, go to the **MS Filter** tab page, and then make the desired settings:
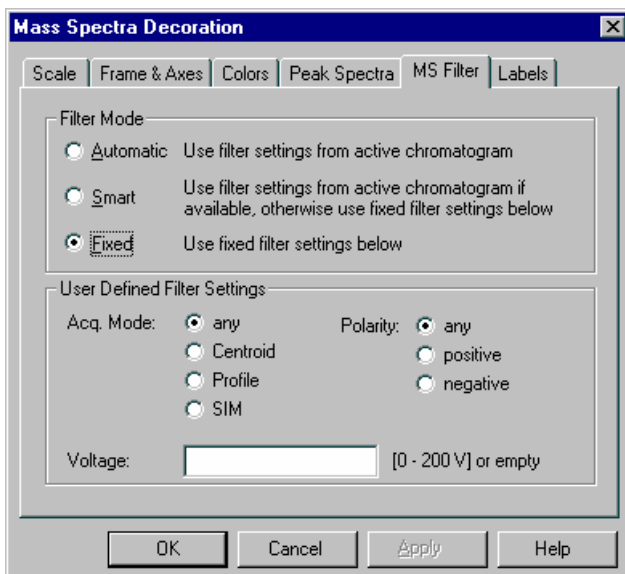
▣ Minimizing the Noise of Mass Spectra

➢*Mass Spectra* usually include more details than UV spectra. However, they often have an increased noise level. They are considerably affected by the background spectrum, especially with low signal intensity. To use the information of mass spectra in the best possible way, Dionex recommends that you reprocess them, using one of the techniques described here.

Spectra Bunching

To reduce mass spectra noise, you can bunch several single mass spectra to one entire mass spectrum. Spectra bunching can be performed for both peak spectra and retention time spectra.

Use the **MS** tab page of the QNT Editor to bunch several single spectra to the left and the right of the chromatogram peak together with the peak maximum spectrum to one entire **peak spectrum**. (For more information about the editor, see **Data Representation and Reprocessing** ▣ **The QNT Editor**.)

In the chromatogram, define the range for which to display the entire **retention time spectrum**. Use the **Spectra/I-t Plots Tool** to select a range while left-clicking. You can perform this in a UV channel, also.

Subtracting Background Mass Spectra

Background subtraction of mass spectra eliminates the influence of the background on the mass spectra. The background mass spectrum to be subtracted can be defined either for the entire chromatogram or automatically by Chromeleon for each single peak.

The corresponding setting is selected on the MS tab page of the QNT Editor or in the chromatogram. The settings made in the chromatogram are saved to the QNT File of the current sample as well. Thus, your input affects all samples that are evaluated using this QNT File.

For more information, refer to:

▣ **Processing Mass Spectra**

How to …: Working with Chromatograms ▣ **Subtracting Background Spectra**

Tracking the Effects of Background Subtraction

You can track the effects of spectra subtraction directly on the mass spectra plot:

- Select **Decoration** on the context menu to open the mass spectrum decoration and then select the **Peak Spectra** tab.

- In the **Background Subtraction Overlay** section, select the **Background Spectrum** to display the subtracted background mass spectrum in addition to the peak and retention time spectra.

- In addition, select the **Original Spectra** to display the respective mass spectrum without subtraction.

■ Processing Mass Spectra

➤*Mass Traces* have a relatively high noise level. Therefore, they must be processed before being used further. Chromeleon uses the following algorithm:

- First, the spectrum of the peak maximum is determined by averaging several spectra in the peak maximum on both ends.

- Then, the background spectra of both peak ends are determined, also by averaging several spectra.

- The background peak spectrum (usually at the peak maximum) is then determined via linear interpolation of these two background spectra and subtracted from the single spectrum (usually of the peak maximum).

■☞ *Tip:*

Only mass spectra recorded with the same filter settings are averaged and subtracted. The filter settings of the Apex mass spectrum of the current peak are used for this. For time spectra, the filter settings of the mass spectrum nearest to the selected retention time are used.

In the QNT Editor, use the **MS** tab page to specify how mass spectra are formed from individual spectra. (For more information about the editor, see **Data Representation and Reprocessing** ⬛ **The QNT Editor**.) Select the **Enable Background Subtraction** check box to enable spectrum subtraction:
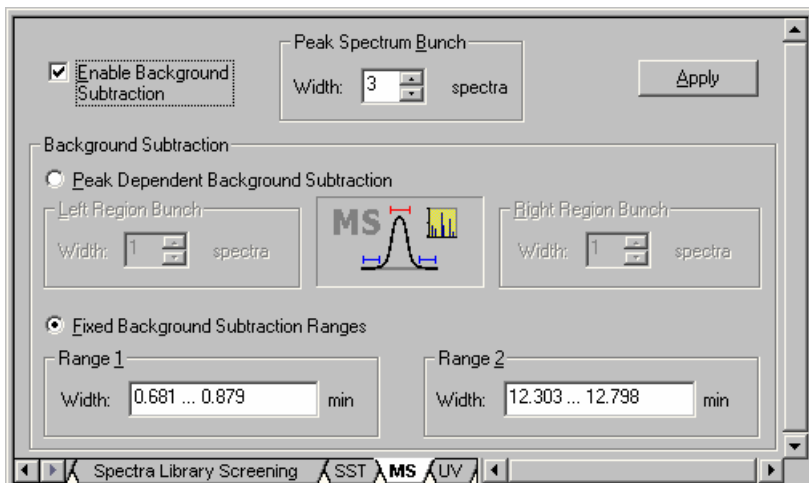
In the combo box under **Peak Spectrum Bunch**, enter the number of single spectra to be bunched to form the spectrum at the peak maximum. A maximum of 99 single spectra can be averaged. For symmetry reasons, it is possible to enter odd numbers only.

Select **Peak Dependent Background Subtraction** to allow automatic background subtraction for each peak.

In the edit fields under **Left Region Bunch** and **Right Region Bunch**, enter the number of spectra to be used to form the two background spectra. You can select up to 99 single spectra. Zero and even numbers are permitted as well. These settings apply to peak spectra and time spectra below peaks.

Select **Fixed Background Subtraction Ranges** to define two fixed ranges for background subtraction for the entire chromatogram. It usually makes sense to set one range at the beginning of the chromatogram and another range at the end. These settings apply to peak spectra and to all time spectra.

Click **Apply** to accept the settings and calculate the resulting mass spectrum. When the **Show Spectra** view is enabled, the newly calculated mass spectrum is displayed immediately.

 Note:

Defining the background subtraction manually in the chromatogram affects the settings on the **MS** *tab page. For example, if you select* **MS Background Subtraction** *on the context menu of the chromatogram, and then select* **Fixed Background Ranges***, the corresponding option is automatically selected on the* **MS** *tab page of the QNT Editor.*

▣ Defining Further QNT Settings for MS

Handling the Retention Time Delay as against a second detector

You can use the ➢*Delay Time* option on the **General** tab page to take the retention time difference into account. This is the time needed by the substances to travel from the first detector, for example, to the MSQ ➢*Mass Spectrometer*.

For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining the QNT Method for Several Detectors** in the **Creating a Peak Table** section.

Defining Peaks via Mass Spectra

For peak identification via mass spectra, use the six MS columns:

- Mass Peak 1 (as well as Mass Peak 2 and Mass Peak 3)
- MS threshold
- MS filter conditions
- Check MS retention times

For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Identifying Peaks via Their Mass Spectra (MS Tracking) Detectors**.

Collecting Fractions

➢*Fraction Collectors* are often used for fraction collection after the detector. The online interpretation of the signal can be used to automate fractionation.

Fractions can be collected for preparative use, if desired. However, in many cases, the objective is further analysis of each fraction. In either case, you can collect the fractions based on the signal. Signal-based fraction collection is quite complex, but the Fraction Collection driver provided by Chromeleon facilitates the process. In the Server Configuration program, install the Fraction Collection driver in addition to the device driver for your fraction collector.

👉 *Tips:*

*In order to perform fraction collection, a **Fraction** or **Purification** license must be installed.*

The Fraction Collection driver also allows you to collect fractions independently of the signal, with a fixed volume at predefined retention times.

For more information about fraction collection, refer to:

🔺 **Setting up Fraction Collection**

🔺 **Enabling Fraction Collection (General Options)**

🔺 **Setting the Fraction Collection Options**

🔺 **Selecting the Channel for Fraction Collection (Channel Selection Options)**

🔺 **Detecting the Peak Start, Peak Maximum, and Peak End (Peak Detection Options)**

🔺 **Entering More Peak Detection Parameters (Peak Detection Options)**

🔺 **Determining the Delay Time (Delay and Detector Offsets)**

🔺 **Editing Signal-Dependent Fraction Collection Parameters**

🔺 **Editing Signal-Independent Fraction Collection Parameters**

🔺 **Defining the Reactions to Certain Events**

🔲 **Program Example (One Detection Channel)**

🔲 **Program Example (Two Detection Channels)**

🔲 **Fraction Collection Control via an MS**

🔲 **Fraction Collection Control via an MS for Different Samples**

🔲 **Checking the Fraction Collection Status on the Control Panel**

🔲 **Tracking Fraction Collection in the Chromatogram**

🔲 **Tracking Fraction Collection in the Report**

If you select the appropriate ➢*Post-Acquisition Steps*, Chromeleon can create new samples and perform fractionation automatically. This process is also known as ➢*Autopurification*. Before beginning, verify that a Purification license is installed. For more information, refer to 🔲 **Collecting Fractions Automatically (Autopurification)** and to the autopurification manual.

📑 *Note:*

*The **Fraction Collection Automation** driver from earlier Chromeleon versions is obsolete. It is included on the Chromeleon software CD only for compatibility reasons (**Server Configuration program > Add device to timebase > Manufacturers > Obsolete**). Dionex recommends that you install the new **Fraction Collection** driver, instead (**Server Configuration program > Add device to timebase > Manufacturers > General**).*

▪ Setting up Fraction Collection

Before you can collect fractions, you have to set up fraction collection as follows:

Server Configuration

Install the **Fraction Collection** driver in the timebase. (Select **Add Device** on the context menu, select **Generic** from the left list box, and then select **Fraction Collection** from the right list box.) In addition, install the ➢*Device Driver* for the respective fraction collector.

Determine how the fractions are collected, either in the Program Wizard (see **The Control Program** 🔲 **The Program Wizard**) or on a ➢*Control Panel*. On a control panel, select **Command** on the **Control** menu and then select the required settings under **Fraction Collection**.
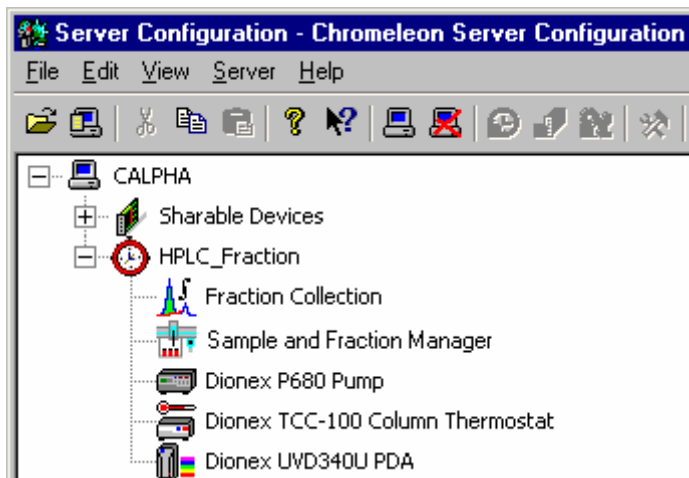
Program Wizard

The Program Wizard assists you in creating a ➢*Program* for fraction collection control:

- On the **Fraction Collection - General Options** page, determine the fraction collection period (see 🔲 **PGM Wizard: Fraction Collection - General Options**).

- On the **Fraction Collection Options** page, define the fraction collection control parameters (see 🔲 **Setting the Fraction Collection Options**).

- On the **Fraction Collection - Channel Selection Options** page, determine the channel(s) for peak recognition during fraction collection (see 🔲 **Selecting the Channel for Fraction Collection (Channel Selection Options)**).

- On the **Peak Detection Options** page, specify the peak detection algorithm for fraction collection (see 🔲 **Detecting the Peak Start, Peak Maximum, and Peak End (Peak Detection Options)**).

Standard Program Example

If you have not yet saved a FractionCollectionTemplate.pgm, the PGM Wizard creates the following standard program. (The program includes the default fraction collection commands for a single detection channel plus the standard commands.)

```
;***************************************************************
;* Definition of triggers for fraction collection starts here.
;***************************************************************
;     Definitions     copied     from     template     <Timebase>
\FractionCollectionTemplate!
Trigger            FracStart       FracStartDetected
EndTrigger
Trigger            TubeChange      FracTubeChange
EndTrigger
Trigger            FracEnd         FracEndDetected
EndTrigger
;***************************************************************
;* Definition of triggers for fraction collection ends here.
;***************************************************************
PumpDevice =                "Pump"
TubeMaxVolume =             Unlimited
FractionCollection.TotalNumberInstalled =  Unlimited
MaxTubesPerFraction =       Unlimited
TubeWrapping =              No
TubeChangeDuration          2.0
DelayTime =                 0.0
OffsetTime =                0.0
DetectionChannel1.Name =                    "UV_VIS_1"
PeakStartSlope =                            0.500
PeakStartTrueTime =                         1.00
PeakStartThreshold =                        10.00
PeakStartCurve                              Off
PeakMaxSlope =                              0.000
PeakMaxTrueTime                             1.00
PeakEndSlope =                              -1.000
PeakEndThreshold =                          10.00
PeakEndCurve                                Off
PeakEndTrueTime                             1.000
ThresholdNoPeakEnd =                        2000.000
BaselineOffset =                            0.000
BaselineDrift =                             0.000
Flow =1.00
%B = 0.0
%C = 0.0
%D = 0.0
```

```
 0.000 Autozero
       Wait Frac.Ready and Sampler.Ready
       Inject
       3DFIELD.AcqOn
       UV_VIS_1.AcqOn
       CollectFractions =      By_Peak
       CollectOutsidePeaks =   No

10.000 3DFIELD.AcqOff
    UV_VIS_1.AcqOff

10.100 End
```

🗒️ *Note:*

To make sure that the fractions are collected exactly, you have to enter and/or consider the actual delay time or the actual delay volume.

For more complex program examples, refer to 🔳 **Program Example (One Detection Channel)**, 🔳 **Program Example (Two Detection Channels) and/or** 🔳 **Fraction Collection Control via an MS**. For an overview of the fraction collection topics, refer to **How to …:** 🔳 **Collecting Fractions**.

🔳 Enabling Fraction Collection (General Options)

On the **General Options** page of the Program Wizard or PGM Editor, determine whether peaks are to be collected depending on peak detection or set the time range for fraction collection. (The **Control Program** section provides more information about the 🔳 **Program Wizard** and 🔳 **PGM Editor**.)

When you click **Next>**, more fraction collection wizard pages are displayed. The settings selected on this page determine which pages are displayed and which program commands are generated:

Off (do not collect at all)

Select this option to disable fraction collection. When this option is selected, no other fraction collection wizard pages are displayed. The program includes the following command:

```
CollectFractions = No
```

Collect by peak

Select this option to collect fractions depending on peak detector. The program includes the following fraction collection triggers:

```
;*******************************************************************
;* Definition of triggers for fraction collection starts here.
;*******************************************************************
; Definitions copied from <Timebase>\FractionCollectionTemplate!
TriggerFracStart  FracStartDetected
EndTrigger
TriggerTubeChange FracTubeChange
EndTrigger
TriggerFracEndFracEndDetected
EndTrigger
;*******************************************************************
;* Definition of triggers for fraction collection ends here.
;*******************************************************************
       (…)
       CollectFractions = By Peak
       CollectOutsidePeaks = No ;(or Yes - see Note below)
```

 Note:

*It is also possible to collect fractions outside peaks (CollectOutsidePeaks = Yes). To do so, select the **Collect outside peaks** check box.*

Collect by time

Select this option to collect fractions independent of peak detection. The program includes the following command:

```
CollectFractions = By_Time
```

With this option, use the **Collection Period** input field to specify the time in [s] after which a new tube shall be used. The default setting is 30[s]. The program includes the following command:

```
CollectPeriod =  30 [s]
```

Collect all the time

Select this option to collect fractions during the entire chromatogram.

Collect only from ... to ...

Select this option to collect fractions only during a specified period. For example, if the time ranges from 2.000 to 8.000 min and if peak-dependent fraction collection is selected, the program includes the following commands:

```
;******************************************************************
;* Definition of triggers for fraction collection starts here.
;******************************************************************
; Definitions copied from <Timebase>\FractionCollectionTemplate!
TriggerFracStart  FracStartDetected
EndTrigger
TriggerTubeChange FracTubeChange
EndTrigger
TriggerFracEndFracEndDetected
EndTrigger
;******************************************************************
;* Definition of triggers for fraction collection ends here.
;******************************************************************

2.000  CollectFractions = By_Peak
       CollectOutsidePeaks =  Yes ;(oder No - see Note)

8.000  CollectFractions = No
```

🗒️ *Note:*

*It is also possible to collect fractions outside peaks (CollectOutsidePeaks = Yes). To do so, select the **Collect outside peaks** check box.*

Restricted time range & user-defined columns

You can use ➢*User-defined Columns* in the sample list to define when fraction collection starts and stops:

- Create two user-defined columns (see **How to …: Creating and Managing Files and Data** 🔳 **Creating User-defined Columns**) in the ➢*Standard Datasource* of the server.

- Name the two columns, e.g., **FractionStart** and **FractionEnd**:
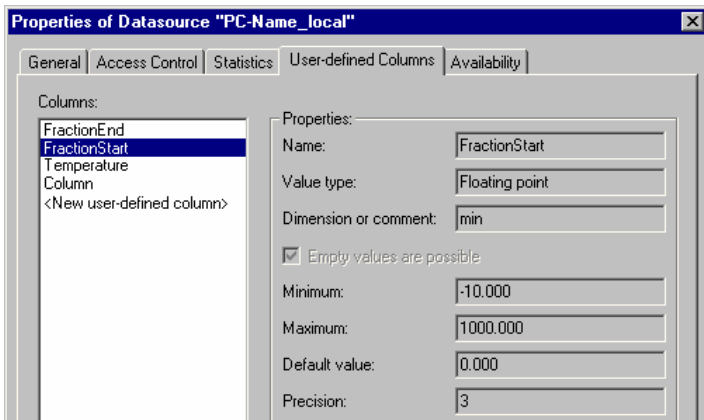
- Restart the server.
- On the **General Options** page, select **Collect only from ... to**. In the **from** field, select the user-defined column **FractionStart**; in the **to** field, select **FractionEnd**. The following triggers are generated (if peak-dependent fraction collection is enabled):

```
Trigger FractCollectionOn   System.Retention >
Sample.FractionStart,True=0.0,Hysteresis=0.0
 CollectFraction =       By Peak
 CollectOutsidePeaks =   Yes
 EndTrigger

Trigger FractCollectionOff  System.Retention >
Sample.FractionEnd,True=0.0,Hysteresis=0.0
 CollectFraction =       No
 EndTrigger
```
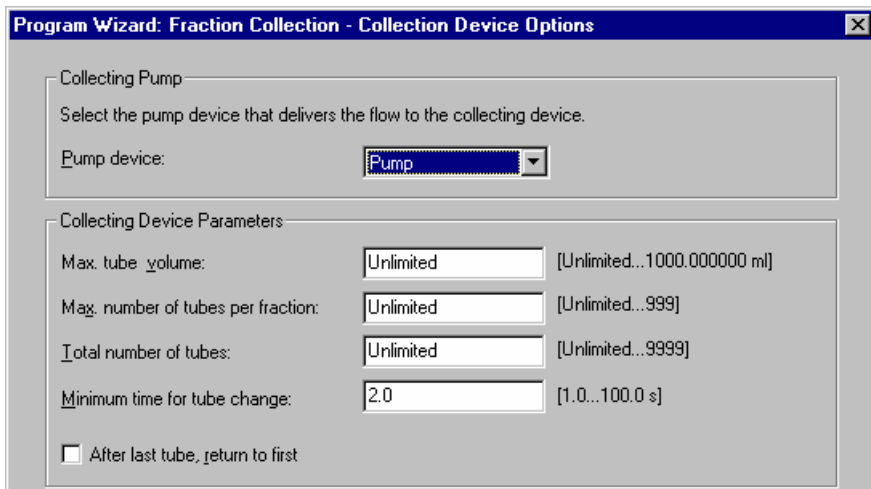
This program starts fraction collection at the retention time entered in the ***FractionStart** column for the corresponding sample. Fraction collection is stopped at the retention time from the ***FractionEnd** column.

For an overview of the fraction collection topics, refer to **How to …:** 🔳 **Collecting Fractions**.

◼ Setting the Fraction Collection Options (Collection Device Options)

On the **Collection Device Options** page of the Program Wizard or PGM Editor, determine the most important parameters for fraction collection. (The **Control Program** section provides more information about the 🔺 **Program Wizard** and 🔺 **PGM Editor**.)

Pump device:	Select the name under which the pump is identified in the installation environment (see ➤*Server Configuration* program). It is usually not necessary to change the default.

🔖 *Note:*

This entry defines the pump and thus, the pump flow for which the time required to fill the tubes is calculated.

Max. tube volume:	Enter the maximum tube volume. The allowed range is 0.000000 to 1000.000000 ml or unlimited.
Max number of tubes per fraction:	Enter the maximum number of tubes per fraction. The allowed range is 0 to 99 or unlimited.

Total number of tubes: Enter the total number of fractions. The allowed range is 0 to 9999.

Minimum time for tube change: Enter the time that the tube change must take at least. The allowed range is 1.0 to 100.0 s.

After last tube, return to first   Select this check box to return to the first tube after the last tube is filled. If this check box is cleared, the ➢*Batch* will be aborted when the last tube is reached.

                      📖☞ *Tip:*

                      If this box is checked, be sure to replace the filled tubes with empty ones before the last tube is reached. This is to prevent the next fractions from being collected in tubes already used.

Delay   Under Delay, enter the ⇒*Delay Time* or the **Delay Volume** between the detector and the ➢*Fraction Collector*. The delay time is the time that a substance needs to travel from the detector cell of the first detector to the switching valve or to the tube, depending on the device type. The delay volume is the volume between the output of the detector cell of the first detector and the switching valve or to the tube, depending on the device type. (If you have any questions, please contact the device manufacturer.)

                      📖☞ *Tip:*
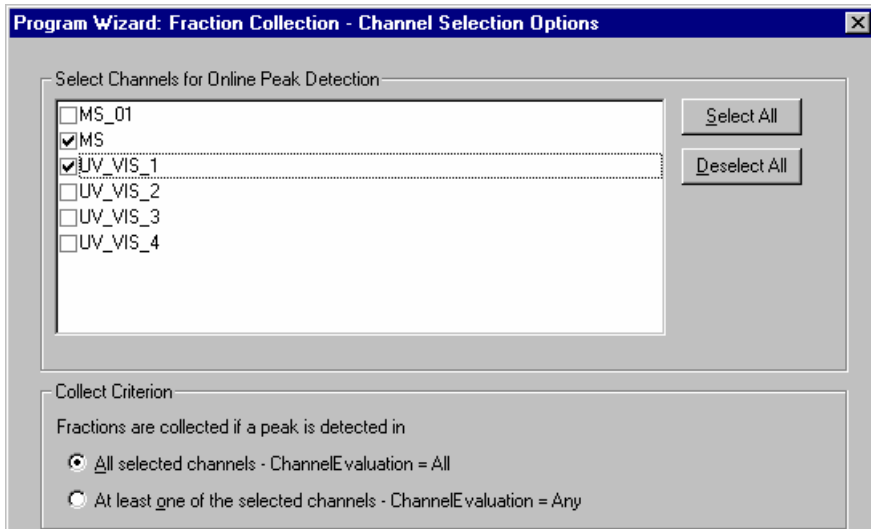
                      The prerequisite for entering the Delay Time is that the flow is constant. If the flow rate changes, the actual delay time between the detector and the fraction collector would change during the run, which is not supported by the system. If you find it easier to determine the Delay Volume rather than the Delay Time, enter the Delay Volume instead and let the system use the (constant) Flow to calculate the appropriate Delay Time.

                      📝 *Note:*

                      If you use several detectors, this delay time entry always refers to the first detector after the column.

◾ Selecting the Channel for Fraction Collection (Channel Selection Options)

On the **Channel Selection Options** page of the Program Wizard or PGM Editor, select the channel(s) for fraction collection. (The **Control Program** section provides more information about the ◾ **Program Wizard** and ◾ **PGM Editor**.)

Select one or more channels to evaluate for fraction collection. Also, select one of the following options:

Option	Description
All selected channels	(Default) The system starts fraction collection when a peak start is detected **in all** detection channels.
	The system stops fraction collection when the end of the first peak is detected.
	Note:
	The program includes the following command:
	ChannelEvaluation = All.

Option	Description
At least one of the selected channels	The system starts fraction collection when a peak start is detected **in at least one** detection channel.
	The system stops fraction collection when the end of the last peak is detected.

Note:

The program includes the following command:

```
ChannelEvaluation = Any.
```
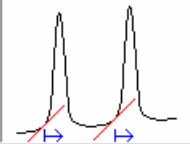
▣ Detecting the Peak Start, Peak Maximum, and Peak End (Peak Detection Options)

To ensure that the ➤*Fraction Collector* fills the tube, the peak start, peak maximum, and peak end all must be correctly detected. Select the necessary settings on the **Peak Detection Options** page of the Program Wizard or PGM Editor, select the channel(s) for fraction collection. (The **Control Program** section provides more information about the 🔳 **Program Wizard** and 🔳 **PGM Editor**.)

Program Wizard: Fraction Collection - Peak Detection Options

Info
A peak start is detected, if the signal value and the signal slope are both above their corresponding thresholds for at least the true time.

Slope that must be exceeded so that the peak start is recognized. ("Off" means "do not use parameter").

	MS	UV_VIS_1	Detection ▲
Peak Start Conditions			<not assigne
Peak Start Threshold	2000.00 counts	10.00 mAU	
Peak Start Slope	20.000 counts/s	0.500 mAU/s	
Peak Start True Time	1.00 s	1.00 s	
Peak End Conditions			
Peak End Threshold	1000.00 counts	10.00 mAU	
Peak End Slope	-10.000 counts/s	-1.000 mAU/s	
Peak End True Time	1.00 s	1.00 s	
Time Step for Slope and Curve			
Derivative Step	1.00 s	1.00 s	

Peak Start

The prerequisites for detecting the peak start (and hence, the beginning of a fraction) are as follows:

- No peak start has been detected so far.

- Fraction collection and peak detection are enabled via the **CollectFractions = ByPeak**.

- The signal of the detection channel is greater than the signal defined by **PeakStartThreshold**.

- The signal slope is greater than the slope defined by **PeakStartSlope**.

- Data acquisition on the selected channel is not yet completed.

- The run itself is not completed yet

- The conditions are fulfilled for the **PeakStartTrueTime**. (The default setting is 1.00 s. The allowed range is 0.00 to 4.00 s.)

The **PeakStartThreshold** and **PeakStartSlope** variables influence the peak detection sensitivity.

The **PeakStartSlope** variable can be changed within broad limits. The higher the value is, the later the peak start is detected. If the detector signal exceeds the **PeakStartThreshold**, the peak is detected only if the slope threshold value is also exceeded.

Chromeleon remembers a peak start so that a peak maximum can be detected next. Thus, a peak maximum can be detected only if a peak start has been detected before.

Peak Maximum

The peak maximum is detected only if

- A peak start has been detected before.

- No peak maximum has been detected yet.

- The signal slope is smaller than the signal slope defined by **PeakMaxSlope**.

- The conditions are fulfilled for the time specified by **PeakMaxTrueTime**. (The default setting is 1.00 s. The allowed range is 0.00 to 4.00 s.)

A peak maximum can be detected only if a peak start has been detected before. Thus, if no peak start has been detected, no maximum will be detected either. The **PeakMaxSlope** variable is defined as negative slope value as it applies to the tailing side of the peak. The closer the value is to zero, the closer to the peak maximum the **PeakMaxRecognition** ⇒*Trigger* is executed.

Chromeleon remembers a peak maximum so that a peak end can be detected next. Thus, a peak end can be detected only if a peak maximum has been detected before (unless one of the end-run conditions apply).

Peak End

A peak end (and hence the end of a fraction) is detected if:

- The signal slope is less than the signal slope defined by **PeakEndSlope** <u>and</u>

- The signal of the detection channel is less than the signal defined by **TresholdNoPeakEnd** <u>and</u>

- A peak maximum has been detected before.

OR:
- The signal of the detection channel is less than the signal defined by **PeakEndThreshold** <u>and</u>

- A peak maximum has been detected before.

OR:
- A peak start has been detected before <u>and</u>

- Data acquisition on the signal channel has been finished

AND:
- The conditions are fulfilled for the **PeakEndTrueTime**. (The default setting is 1.00 s. The allowed range is 0.00 to 4.00 s.)

The first group of conditions checks whether the signal is below a limit, which is defined by the **ThresholdNoPeakEnd** variable. With heavily overloaded detector signals, there is a lot of signal noise, so a peak end and/or start would be detected several times near the top of the peak. The top of the peak could also be formed like a plateau. To inhibit this, set the **ThresholdNoPeakEnd** variable to a value below this level. If the value is set to **Off**, this part of the condition will always be true and this check will be disabled. **PeakEndSlope** delays the peak end.

The second group of conditions uses the signal height criterion. If the signal value falls below **PeakEndThreshold**, the peak is completed.

The third group of conditions completes the current peak in case the data acquisition is disabled.

For more information, refer to:

 Entering More Peak Detection Parameters (Peak Detection Options)

 Defining the Reactions to Certain Events

For an overview of the fraction collection topics, refer to **Collecting Fractions**.

Entering More Peak Detection Parameters (Peak Detection Options)

Use the PGM Wizard (see **The Control Program** **The Program Wizard**) to enter the basic peak detection parameters as described in **Detecting the Peak Start, Peak Maximum, and Peak End (Peak Detection Options)**. In addition, you can enter special parameters on the **Peak Detection Options** page.

Derivative Step

Use this parameter to determine for which period the signal values are evaluated to determine the slope and curve of the signal. The slope and curve are determined at the associated retention time t for the range (t - DerivStep/2) to (t + DerivStep/2). You can have Chromeleon display the retention time on the control panel via the **RetentionTime** variable (see **Checking the Fraction Collection Status on the Control Panel**).

(Dimension: s; range: 0.02 to 60.00)

 Note:

*The higher the **DerivStep** value is, the lower the noise for the **Slope** and the **Curve**.*

PeakStartTrueTime, PeakMaxTrueTime, and PeakEndTrueTime

Use these parameters to set the time in seconds for which the different conditions must be fulfilled. If the conditions are not fulfilled within this time period, the peak start, peak maximum, and peak end cannot be detected.

(Dimension: s; range: 0.0 to 4.0)

Notes:

If you perform smoothing when using a mass spectrometer, calculating a signal value for a channel may take up to 5 seconds. If you do not allow for this time before the peaks reach the fraction collector, peaks cannot be collected. Therefore, set the respective parameter to at least 5 seconds.

*In addition, a peak can be detected only after the **DerivStep** and **PeakStartTrueTime** (or **PeakEndTrueTime**) have expired. To make sure that a peak is completely collected as a fraction on an MS detection channel, the following must apply:*

 5 + PeakStartTrueTime (or PeakEndTrueTime) + DerivStep < DelayTime.

(The ⇒Delay Time is the time between the detector (here: an MS) and the ➢Fraction Collector. If necessary, install a longer loop before the fraction collector.)

Shoulder detection

Use the **PeakStartCurve** and **PeakEndCurve** parameters to determine the curve in [Signal]/s^2 that must be exceeded so that a shoulder is detected after the peak start (PeakStartCurve). If the value is below this value, a shoulder can be detected after the peak maximum (PeakEndCurve).

(Dimension: [Signal]/s^2; range: 0 to 10^10; default setting: Off)

Tip:

Chromeleon supports these parameters only for the Purification license.

Threshold for PeakEndSlope enabling and disabling

Use the **ThresholdDoNotResolve** parameter to disable peak end detection via the **PeakEndSlope** parameter. Determine the threshold above which **PeakEndSlope** is disabled.

(Dimension: [Signal]/s; range: -10^10 to 10^10; default setting: Off)

If the minimum between these peaks is above the threshold value, a peak end is not detected before. Instead, a new peak is detected and a tube change is triggered when the signal slope is 0.0. The advantage is that the fraction for the first peak is collected until the minimum; the fraction for the second peak is then collected from the minimum.
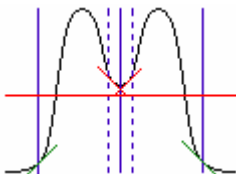
In the picture, the red horizontal line indicates the threshold value. The solid lines indicate the fractions for which the minimum between the peaks is above the threshold value.

If the minimum is below the threshold value, a peak end is detected before the minimum (via the **PeakEndSlope** parameter; red descending line). Thus, the first fraction is collected only until the first dotted line. Then, a peak start is detected (red ascending line), so that the second peak is collected only from the second dotted line.

The area between these two lines contains a compound of the two substances. This area is collected as a separate fraction only if the program contains the following line:

```
CollectOutsidePeaks =  Yes
```

Peak starts and peak ends that occur underneath the ThresholdDoNotResolveLine are detected as usual (indicated in the picture by the green diagonal lines).

Drift compensation

In the **Baseline Drift** field, enter the baseline drift that is used to correct the signal value. Enter the related baseline offset in [Signal] in the Baseline Offset field. The related thresholds (Peak Start Threshold, Peak End Threshold, and Threshold No Peak End) are compared to the signal value:
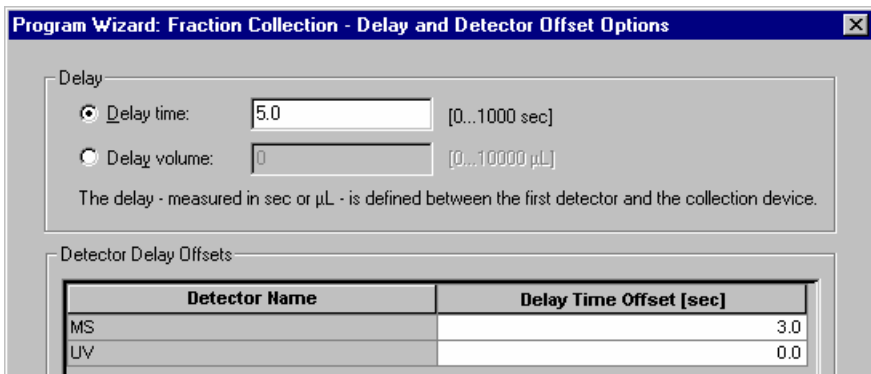
$$\text{Signal value} - (\text{BaselineOffset} + \Delta t * \text{BaselineDrift})$$

Whenever Baseline Drift changes, Δt is set to 0. In addition, Baseline Offset is set to the current value of the correction term. Thus, the BaselineDrift value always matches the actual drift.

For an overview of the fraction collection topics, refer to **Collecting Fractions**.

▣ Determining the Delay Time (Delay and Detector Offsets)

On the **Delay and Detector Offsets** page of the Program Wizard or PGM Editor, enter the delay time or delay volume between the detector and the fraction collector. (The **Control Program** section provides more information about the ▣ **Program Wizard** and ▣ **PGM Editor**.)

Delay
Enter the ⇒*Delay Time* or the **Delay Volume** between the detector and the ➤*Fraction Collector*. The delay time is the time that a substance needs to travel from the detector cell of the first detector to the switching valve or tube. The delay volume is the volume between the detector cell and the switching valve or tube, respective. (The delay time and/or volume depend on the device type. Contact the device manufacturer for more information.)

☞ *Tip:*

The prerequisite for entering the Delay Time is that the flow is constant. If the flow rate changes, the actual delay time between the detector and the fraction collector would change during the run, which is not supported by the system. If you find it easier to determine the Delay Volume rather than the Delay Time, enter the Delay Volume instead and let the system use the (constant) Flow to calculate the appropriate Delay Time.

📝 *Note:*

If you use several detectors, this entry always refers to the first detector after the column.

If the system includes several detectors (in the example above, MS and UV), the **Detector Delay Offsets** section appears. Enter the delay time between the first detector after the column and any other detector(s) in the **Delay Time Offset** column. Keep the following in mind:

 Tips:

If no channel has been selected for a certain detector in the channel selection field on the ***Channel Selection Options*** *page, you cannot enter a delay time for this detector.*

It is not possible to enter a delay time exceeding the time between the first detector and the ➤*Fraction Collector (also, see* ***Setting the Fraction Collection Options****).*

For an overview of the fraction collection topics, refer to **Collecting Fractions**.

Editing Signal-Dependent Fraction Collection Parameters

After you have created a basic program with the PGM Wizard, you can later edit this program in the PGM Editor as described in **Creating and Modifying PGM Files**.

Independently of the installed ➤*Fraction Collector*, the **Fraction Collection** driver supports several PGM Wizard parameters. These parameters, which are described in the tables below, allow you to start fraction collection in the ➤*PGM File* (or on the ➤*Control Panel*).

Press the F8 key to open the **Commands** dialog box, and then enter the parameters in the PGM Editor. You can also establish controls with the desired functionality on the control panel. (For more information, refer to **How to …: Controlling Devices from the Control Panel** **Modifying a Control Panel**.)

The tables below list the parameters in the **Commands** dialog box for the associated **DetectionChannel**. (For information about the read-only parameters, refer to **Checking the Fraction Collection Status on the Control Panel**.)

Signal-Based Parameters (Min., Max., and Default depend on the detector; here, they are shown for a UV detector)

Parameter	Min.	Max.	Description
BaselineDrift [2]	-1e10	1e10	Baseline drift in [Signal]/s used to correct the signal value. The specified thresholds (PeakStartThreshold, PeakEndThreshold, and ThresholdNoPeakEnd) are compared to: signal value - (BaselineOffset + Δt*BaselineDrift)
			Δt is set to 0 whenever BaselineDrift changes.
			Baseline Offset is then set to the current value of the correction term. Thus, the BaselineDrift value always matches the actual drift.
BaselineOffset [2]	-1e10	1e10	Baseline offset in [Signal] used to correct the signal value.
DerivStep	0.02 s	60.00 s	Time for which the signal values are evaluated to determine the signal slope and the curve.
Name	N/a	n/a	Name of the signal channel used for peak detection (this may be a ➢ *Virtual Signal*, also).
OffsetTime	0.0 s	9999.9 s	Retention time offsets between the first detector and other detectors.
			☞ *Tip:*
			Make sure the retention time setting for this command is later than the DelayTime (see below).
OffsetVolume	0.0 µl	5000.0 µl	Offset volume between the first detector and other detectors. (Based on the flow, the offset volume is converted into the corresponding OffsetTime.)
PeakEndCurve *	0	10^10	Curve in [Signal]/s^2 that must be exceeded for a shoulder to be recognized after the peak maximum.
PeakEndSlope * [2]	-1e10	0.000	The slope value in [Signal]/s must exceed the PeakEndSlope value for the peak end to be recognized.
PeakEndThreshold * [2]	-1e10	1e10	The actual signal value in [Signal] must be below the PeakEndThreshold value for the peak end to be recognized.
PeakEndTrueTime *	0.0 s	4.0 s	The time must be fulfilled for the different conditions for a peak end to be recognized.

Parameter	Min.	Max.	Description
PeakMaxSlope * [2]	-1e10	0	The actual slope value in [Singal]/s must be below the PeakMaxSlope value for the peak maximum to be recognized.
PeakMaxTrueTime *	0.0 s	4.0 s	The time must be fulfilled for the different conditions for a peak maximum to be recognized.
PeakStartCurve *	0	10^10	Curve in [Signal]/s^2 that must be exceeded for a shoulder to be recognized after the peak start.
PeakStartSlope * [2]	0.000	1e10	The actual slope value in [Signal]/s must exceed the PeakStartSlope value for the peak start to be recognized.
PeakStartThreshold * [2]	-1e10	1e10	The actual signal value in [Signal]/s must exceed the PeakStartThreshold value for the peak start to be recognized.
PeakStartTrueTime *	0.0 s	4.0 s	The time must be fulfilled for the different conditions for a peak start to be recognized.
ThresholdDoNot Resolve *	-10^10	10^10	Threshold value for the slope in [Signal]/s above which peak detection via the PeakEndSlope parameter is disabled. Instead, a new peak is detected and a tube change is triggered when the signal slope is 0.0.
ThresholdNoPeak End * [2]	-1e10	1e10	The actual signal threshold value in [Signal] must be below the ThresholdNoPeakEnd value for the peak end to be recognized. (This parameter supports the PeakEndSlope parameter for peak detection.)

👉 *Tips:*

** To ignore the parameter for peak detection, set the parameter to **Off**.*

*[2] Set these parameters on the **Peak Detection Options** page in the PGM Wizard.*

Commands

In addition, you can enter the following commands manually for a specified retention time:

Command	Description
ManualPeakEnd	Stops the current peak at the specified retention time, independently of the peak detection parameters.
ManualPeakStart	Starts the next peak at the specified retention time, independently of the peak detection parameters.
ManualTubeChange	Changes to the next tube at the specified retention time, independently of the peak detection parameters.

For the signal-independent parameters, refer to 🔳 **Editing Signal-Independent Fraction Collection Parameters**.

For an overview of the fraction collection topics, refer to 🔳 **Collecting Fractions**.

🔳 Editing Signal-Independent Fraction Collection Parameters

After you have created a basic program with the PGM Wizard, you can later edit this program in the PGM Editor as described in 🔳 **Creating and Modifying PGM Files**.

The following signal-independent parameters are listed in the **Commands** dialog box under Fraction Collection. (To open the Commands dialog box, press the **F8** key in the PGM Editor.)

Parameter	Min.	Max.	Description
ChannelEvaluation	All	Any	Determines how the results of the single detection channels are used for peak detection.
CollectFractions	No	By_Peak	Performs fraction collection based on peak detection.
			Note: Detected fractions will trigger related events after the delay time, even if CollectFractions is **No** at that time.
		By_Time	Performs fraction collection independent of peak detection.
CollectOutsidePeaks	No	Yes	Determines whether fractions are collected outside peaks, also.

Parameter	Min.	Max.	Description
➢*DelayTime*	0.0 s	9999.9 s	Delay time between the first detector and the ➢*Fraction Collector*.
DelayVolume	0.0 µl	5000.0 µl	Delay volume between the first detector and the fraction collector. (Based on the flow, the delay volume is converted into the corresponding **DelayTime**.)
MaxTubesPerFraction	0	999	Maximum number of tubes per fraction. If the specified number is reached, no additional tubes are used to collect the current peak. Set this parameter to **Unlimited** to collect all peaks completely.
PumpDevice			Pump whose flow is used to convert between time and volume.
TotalNumberInstalled	0	9999	Number of tubes installed in the rack. If this number is exceeded, the TubePosition parameter is reset to 1 if the TubeWrapping option has been selected. Otherwise, the batch is terminated.

Tip:

A warning appears in the Audit Trail when the TubePosition parameter is reset to 1. |
| **TubeChangeDuration** | 1.0 | 100.0 | Time in seconds until the next fraction can be collected.

Tip:

This property is especially useful if several channels are used for peak detection. If one peak is detected in one channel a little later than in another channel, a new fraction is usually started. Set the TubeChangeDuration parameter to prevent this. |

Parameter	Min.	Max.	Description
TubeMaxVolume	0.000000	1000.000000	Maximum liquid level in the tube in [ml]. If the specified volume is reached, the next tube is filled.
			👉 *Tip:*
			*Set the TubeMaxVolume parameter to **Unlimited** to prevent the next tube from being filled automatically.*
TubePosition	1	9999	Position of the tube in the current fraction.
TubeWrapping	No	Yes	If this option is selected, the TubePosition parameter is reset to 1 when TotalNumberInstalled is reached.

👉 *Tips:*

You can set the following properties in the PGM Wizard, also:

*Set **CollectFractions** and **CollectOutsidePeaks** on the **General Options** page.*

*Set **DelayTime** to **TubeWrapping** on the **Peak Detection Options** page.*

*Set **ChannelEvaluation** on the **Channel Selection** page.*

These properties cannot be changed during a peak. Any attempted change will produce the following warning: "Parameters cannot be changed until the current peak has ended. New value will be assigned at peak end." The change will become effective as soon as the current peak ends.

👉 *Tip:*

*When performing ➢Autopurification, you can correct the parameters set in the PGM File for all other samples in the sequence on the control panel. Use the corresponding parameters of the **Dionex_Purification_Parameters** device driver (see **Collecting Fractions Automatically (Autopurification)** 🔲 **Setting Correction Parameters**).*

For the signal-dependent parameters, refer to 🔲 **Editing Signal-dependent Fraction Collection Parameters**. In addition to these parameters, various read-only variables are available. These variables are only displayed if you open the **Commands** dialog box on a control panel. They allow you to check the peak detection status (see 🔲 **Checking the Fraction Collection Status on the Control Panel**).

For an overview of the fraction collection topics, refer to 🔲 **Collecting Fractions**.

Defining the Reactions to Certain Events

A peak start, peak maximum, and peak end are recognized if certain conditions are fulfilled (see **Recognizing Peak Start, Peak Maximum, and Peak End**).

If one of these events occurs, Chromeleon issues certain commands to the ➤*Fraction Collector*. These commands can be defined in the ➤*Program*. In the respective ⇒*Trigger* block, you can define device-specific actions for your Sample and Fraction Manager or fraction collector.

Event	Condition	Action
FracStartDetected	A peak start, which means the start of a new fraction, "arrives" at the switching valve/ tube. *Note:* *If, during a peak, a peak start is detected on another channel, a new fraction is also started for* **ChannelEvaluation = Any***.*	Switch to next fraction.* Switch to **collect**.
FracTubeChange	The tube is filled to the limit.	Switch to next tube.
FracEndDetected	A peak end, which means the end of a fraction, "arrives" at the switching valve/ tube.	Switch to **waste**. Switch to next fraction.*

* Switching to the next fraction is needed only once. It depends on the type of hardware whether this is better to switch to the first tube of the next fraction at the peak start or at the peak end.

For an overview of the fraction collection topics, refer to **Collecting Fractions**.

▣ Program Example (One Detection Channel)

A ➤*Program* for a timebase including a ➤*Fraction Collector* and one detection channel might look as follows:

```
-0.300 Flow =                                     20.000
       %B =                                       10
       UV_VIS_1.Step =                            0.50
       UV_VIS_1.Average =                         On
       UV_VIS_1.MaxAutoStep =                     1.0
       Pressure.LowerLimit =                      10.00
       Pressure.UpperLimit =                      200.00


;*********************************************************************
;*Definition of triggers for fraction collection starts here
;*********************************************************************
;        Definition         copied          from         template
<Timebase>\FractionCollectionTemplate!
;FracStart: Start of fractionation collection
       Trigger FracStart    FracStartDetected
       Collect
       Log                  SFM_A.Tray
       EndTrigger

;TubeChange: The fraction is collected to another tube when the
current tube is full
       Trigger TubeChange   FracTubeChange
       Drain
       SFM_A.TubePosition = FractionCollection.TubePosition
       Collect
       EndTrigger

;FracEnd: End of fraction collection
       Trigger FracEnd      FracEndDetected
       MovementMode =       InterruptAndEject
       Drain
       SFM_A.TubePosition = FractionCollection.TubePosition
       EndTrigger
;*********************************************************************
;*Definition of triggers for fraction collection ends here
;*********************************************************************

; Maximum liquid level in a tube in ml:
       PumpDevice =                               "Pump"
       TubeMaxVolume =                            10

; Maximum number of installed fraction tubes:
       FractionCollection.TotalNumberInstalled = 1092

; Basic fraction collection parameters:
       MaxTubesPerFraction =                      Unlimited
       TubeWrapping =                             No
```

```
; Delay time between detector output and switching valve or tube:
      DelayTime =                                 0.4
      OffsetTime =                                0.0
      TubeChangeDuration =                        2.0 [s]
      ChannelEvaluation =                         All

; Conditions for online peak recognition at program start:
      Name =                                      "UV_VIS_1"
      ;Slope at peak start:
      PeakStartSlope =                            2.000
      ;Minimum signal height at peak start:
      PeakStartThreshold =                        10.00
      ;Slope after the peak maximum:
      PeakMaxSlope =                              -4.000
      ;Slope at peak end:
      PeakEndSlope =                              -4.000
      ;Maximum signal height at peak end:
      PeakEndThreshold =                          5.00
      ;Signal must be less than this threshold value
      ;before a new peak start can be recognized:
      ThresholdNoPeakEnd =                        200
      ;Baseline drift correction:
      BaselineOffset =                            0.000
      BaselineDrift =                             0.000

; Separation start with injection:
-0.100 UV.Autozero
0.000  Wait                      Sampler.Ready
       Flow =                    20.000
       %B =                      10
       Sampler.Inject
       UV_VIS_1.AcqOn

; Basic fraction collection parameters:
      CollectFractions =      By_Peak

; Gradient program:
      Flow =           20.000
      %B =             10.0
10.000 %B =            90
13.000 %B =            90

13.000 UV_VIS_1.AcqOff
      CollectFractions =      No

; Regeneration and equilibration phase of the gradient program:
15.000 Flow =          20.000
    %B =               90

16.000 %B =            10
  End
```

Note:

The trigger block used in this program example refers to a preparative application with a Dionex Sample and Fraction Manager (SMF). Besides, the trigger block is intended for a preparative pump.

For more program examples, refer to:

Program Example (Two Detection Channels)

Fraction Collection Control via an MS

Fraction Collection Control via an MS for Different Samples

For an overview of the fraction collection topics, refer to **Collecting Fractions**.

Program Example (Two Detection Channels)

Tip:

*If the **Fraction** license is installed, Chromeleon supports two detection channels. You need the **Purification** license if you want to use more than two detection channels.*

A ➢*Program* for a timebase including a ➢*Fraction Collector* and two detection channels might look as follows:

```
-0.300  Flow =                                    20.000
        %B =                                      10
        UV_VIS_1.Step =                           0.50
        UV_VIS_1.Average =                        On
        UV_VIS_1.MaxAutoStep =                    1.0
        Pressure.LowerLimit =                     10.00
        Pressure.UpperLimit =                     200.00


;********************************************************************
;*Definition of triggers for fraction collection starts here
;********************************************************************
;Definition              copied              from              template
<Timebase>\FractionCollectionTemplate!
;FracStart: Start of fraction collection
        Trigger FracStart    FracStartDetected
        Collect
        Log                  SFM_A.Tray
        EndTrigger
```

```
;TubeChange: The  fraction  is  collected  to  another  tube  when  the
current tube is full
       Trigger TubeChange    FracTubeChange
       Drain
       SFM_A.TubePosition =  FractionCollection.TubePosition
       Collect
       EndTrigger

;FracEnd: End of fraction collection
       Trigger FracEnd       FracEndDetected
       MovementMode =        InterruptAndEject
       Drain
       SFM_A.TubePosition =  FractionCollection.TubePosition
       EndTrigger
;*********************************************************************
;*Definition of triggers for fraction collection ends here
;*********************************************************************

; Maximum liquid level in a tube in ml:
       PumpDevice =                           "Pump"
       TubeMaxVolume =                        10

; Maximum number of installed tubes:
       FractionCollection.TotalNumberInstalled =   1092

; Basic fraction collection parameters:
       MaxTubesPerFraction =                  Unlimited
       TubeWrapping =                         No
       TubeChangeDuration =                   2.0 [s]
       ChannelEvaluation =                    All

; Delay time between detector output and switching valve or tube:
       DelayTime =                            0.8
       DetectionChannel2.OffsetTime =         0.0
       DetectionChannel3.OffsetTime =         0.0

; Conditions for online peak recognition at program start:
       DetectionChannel2.Name =               "UV_VIS_1"
       ;Slope at peak start:
       DetectionChannel2.PeakStartSlope =     2.000
       ;Minimum signal height at peak start:
       DetectionChannel2.PeakStartThreshold = 10.00
       ;Slope after peak maximum:
       DetectionChannel2.PeakMaxSlope =       -4.000
       ;Slope at peak end:
       DetectionChannel2.PeakEndSlope =       -4.000
       ;Maximum signal height at peak end:
       DetectionChannel2.PeakEndThreshold =   5.00
       ;Signal must be less than this threshold value
       ;before a new peak start can be recognized
       DetectionChannel2.ThresholdNoPeakEnd = 200
       ;Baseline drift correction:
       DetectionChannel2.BaselineOffset =     0.000
       DetectionChannel2.BaselineDrift =      0.000
```

```
; Conditions  for  online  peak  recognition  for  the  2.  detection
channel:
      DetectionChannel3.Name =                  "UV_VIS_2"
      DetectionChannel3.PeakStartSlope =        2.000
      DetectionChannel3.PeakStartThreshold =    10.00
      DetectionChannel3.PeakMaxSlope =          -5.000
      DetectionChannel3.PeakEndSlope =          -4.000
      DetectionChannel3.PeakEndThreshold =      5.00
      DetectionChannel3.ThresholdNoPeakEnd =    200
      DetectionChannel3.BaselineOffset =        0.000
      DetectionChannel3.BaselineDrift =         0.000

; Separation start with injection:
-0.100 UV.Autozero
0.000  Wait       Ready
       Flow =      20.000
       %B =        10
       Sampler.Inject
       UV_VIS_1.AcqOn

; Basic fraction collection parameters:
       CollectFractions =      By_Peak

; Gradient Program:
       Flow =      20.000
       %B =        10.0
10.000 %B =        90
13.000 %B =        90

13.000 UV_VIS_1.AcqOff
       CollectFractions =      No

; Regeneration and equilibration phase of the gradient program:
15.000 Flow =      20.000
       %B =        90

16.000 %B =        10
  End
```

📝 *Notes:*

The trigger block used in this program example refers to a preparative application with a Dionex Sample and Fraction Manager (SFM). Besides, the trigger block is intended for a preparative pump.

In the example, DetectionChannel1 has not been used as the first detection channel because '1' and 'l' look almost identical when Courier is the selected font.

For more program examples, refer to:

🔼 **Program Example (One Detection Channel)**

🔼 **Fraction Collection Control via an MS**

🔼 **Fraction Collection Control via an MS for Different Samples**

For an overview of the fraction collection topics, refer to 🔼 **Collecting Fractions**.

🔳 Fraction Collection Control via an MS

➢*Mass Traces* can be used to selectively collect certain substances in fractions. This selective collection is possible since the occurrence of peaks with data acquisition via ➢*Mass Spectrometers* depends much more selectively on the corresponding masses than it depends on the wavelength with ➢*UV Detectors*.

It is possible to use certain mass traces at specific times to ➢*trigger* the fraction collection. For this purpose, online extraction of mass traces is required (see **How to …: Using Mass Spectrometers 🔼 Extracting Mass Traces Online**). The following program requires five channels for online extraction of mass traces:

```
; Definition of the minimum/maximum mass and of the filter index for
the channels MS_01 to MS_05:
     MS_01.MinMass =                    243.5
     MS_01.MaxMass =                    244.5
     MS_01.FilterIndex =                2

     MS_02.MinMass =                    145.5
     MS_02.MaxMass =                    146.5
     MS_02.FilterIndex =                2

     MS_03.MinMass =                    164.5
     MS_03.MaxMass =                    165.5
     MS_03.FilterIndex =                2

     MS_04.MinMass =                    178.5
     MS_04.MaxMass =                    179.5
     MS_04.FilterIndex =                2

     MS_05.MinMass =                    192.5
     MS_05.MaxMass =                    193.5
     MS_05.FilterIndex =                2
```

```
;**********************************************************************
;*Definition of triggers for fraction collection starts here
;**********************************************************************
;Definition             copied             from             template
<Timebase>\FractionCollectionTemplate!
;FracStart: Start of fraction collection
      Trigger FracStart    FracStartDetected
      Collect
      Log                  SFM_A.Tray
      EndTrigger

;TubeChange: The fraction is collected to another tube when the
current tube is full
      Trigger TubeChange    FracTubeChange
      Drain
      SFM_A.TubePosition =  FractionCollection.TubePosition
      Collect
      EndTrigger

;FracEnd: End of fraction collection
      Trigger FracEnd       FracEndDetected
      MovementMode =        InterruptAndEject
      Drain
      SFM_A.TubePosition =  FractionCollection.TubePosition
      EndTrigger
;**********************************************************************
;*Definition of triggers for fraction collection ends here
;**********************************************************************

; Maximum liquid level in a tube in ml:
      PumpDevice =                              "Pump"
      TubeMaxVolume =                           10

; Maximum number of installed fraction tubes:
      FractionCollection.TotalNumberInstalled =   240

; Basic fraction collection parameters:
      MaxTubesPerFraction =                     Unlimited
      TubeWrapping =                            No
      TubeChangeDuration =                      2.0 [s]
      ChannelEvaluation =                       All

; Delay time between detector output and switching valve or tube:
      DelayTime =                               0.8
      DetectionChannel2.OffsetTime =            0.0
      DetectionChannel3.OffsetTime =            0.4

; Conditions for online peak recognition at program start:
      CollectFractions =                        Yes
      DetectionChannel2.Name =                  "UV_VIS_01"
      ;Slope at peak start [mAU/s]:
      DetectionChannel2.PeakStartSlope =        2.000
      ;Minimum signal height at peak start [mAU]
      DetectionChannel2.PeakStartThreshold =    5000.00
      ;Slope after peak maximum [mAU/s]
```

```
      DetectionChannel2.PeakMaxSlope =                  -5.000
      ;Slope at peak end [mAU/s]
      DetectionChannel2.PeakEndSlope =                  -4.000
      ;Maximum signal height at peak end [mAU]
      DetectionChannel2.PeakEndThreshold =             5000.00
      ;Signal mst be less than this threshold value
      ;before a new peak start can be recognized [mAU]
      DetectionChannel2.ThresholdNoPeakEnd =           10000
      ;Baseline drift correction:
      DetectionChannel2.BaselineOffset =               0.000
      DetectionChannel2.BaselineDrift =                0.000

; Conditions  for  online  peak  recognition  for  the  MS  detection
channel:
      DetectionChannel3.Name =                         "MS_01"
      DetectionChannel3.PeakStartSlope =               2.000
      DetectionChannel3.PeakStartThreshold =           10.00
      DetectionChannel3.PeakMaxSlope =                 -5.000
      DetectionChannel3.PeakEndSlope =                 -4.000
      DetectionChannel3.PeakEndThreshold =             5.00
      DetectionChannel3.ThresholdNoPeakEnd =           200
      DetectionChannel3.BaselineOffset =               0.000
      DetectionChannel3.BaselineDrift =                0.000

; Separation start with injection and acquisition start
0.000 UV.Autozero
  Wait Sampler.Ready and MS.Ready
  Inject
  3DFIELD.AcqOn
  UV_VIS_1.AcqOn
  MS_01.AcqOn
  MS_02.AcqOn
  MS_03.AcqOn
  MS_04.AcqOn
  MS_05.AcqOn

; Basic fraction collection parameters:
      CollectFractions =      By_Peak


; Change  of  the  detection  channel  according  to  the  expected
substances
3.20    DetectionChannel3 =      "MS_02"

3.7     DetectionChannel3 =      "MS_03"

4.3     DetectionChannel3 =      "MS_04"

5.5     DetectionChannel3 =      "MS_05"

; End of data acquisition
10.000 3DFIELD.AcqOff
  UV_VIS_1.AcqOff
  MS_01.AcqOff
  MS_02.AcqOff
```

```
MS_03.AcqOff
MS_04.AcqOff
MS_05.AcqOff
      CollectFractions =      No

End
```

📋 *Notes:*

The trigger block used in this program example refers to the Dionex Sample and Fraction Manager (SFM).

In the example, DetectionChannel1 has not been used as the first detection channel because '1' and 'I' look identical when Courier is the selected font.

If the above ➤*Program* is used, fractions would be collected at the following retention times, provided that a peak occurs within the following mass traces:

Retention time-interval [min]	Channel	Mass [m/z]	Positions of the collection vials
0.000 - 3.200	MS_01	244	1
3.200 - 3.700	MS_02	146	2
3.700 - 4.200	MS_03	165	3
4.200 - 5.500	MS_04	179	4
5.500 - 10.000	MS_05	193	5

(Usually, the desired mass peak occurs only once within the corresponding time interval - this is assumed in the above table. Otherwise, the positions of the collection vials will change accordingly.)

This program example for mass-controlled fraction collection presumes that the same substances will be collected in all samples. If there are different substances in the samples, fraction collection via a mass channel needs to be adapted accordingly. For more information, refer to 🔺 **Fraction Collection Control via an MS for Different Samples**.

For more program examples, refer to:

🔺 **Program Example (One Detection Channel)**

🔺 **Program Example (Two Detection Channels)**

For an overview of the fraction collection topics, refer to 🔺 **Collecting Fractions**.

▣ Fraction Collection Control via an MS for Different Samples

If there are samples with different substances, the procedure described in ▣ **Fraction Collection Control via an MS** has to be changed accordingly.

In this case, ➢*Mass Traces* have to be extracted online, as well (see **How to …: Using Mass Spectrometers** ▣ **Extracting Mass Traces Online**).

1. Define User-defined Columns

Define the ➢*User-defined Columns* **Mass1** to **Mass5** and **FilterIndex** to **FilterIndex5** as Integer columns (see **How to …: Creating and Managing Files and Data** ▣ **Creating User-defined Columns**). The program part below checks the content of the corresponding user-defined columns during data acquisition and thus allows you to control fraction collection ➢*Triggering*.

2. Modify the Program

In the example described in **Fraction Collection via an MS** (see above), replace the corresponding paragraph in the ➢*Program* with the following program part:

```
; Definition of the minimum/maximum mass and of the filter index for
the channels MS_01 to MS_05:
  MS_01.MinMass = sample.mass1-0.5
  MS_01.MaxMass = sample.mass1+0.5
  MS_01.FilterIndex = sample.filterindex

  MS_02.MinMass = sample.mass2-0.5
  MS_02.MaxMass = sample.mass2+0.5
  MS_02.FilterIndex = sample.filterindex2

  MS_03.MinMass = sample.mass3-0.5
  MS_03.MaxMass = sample.mass3+0.5
  MS_03.FilterIndex = sample.filterindex3

  MS_04.MinMass = sample.mass4-0.5
  MS_04.MaxMass = sample.mass4+0.5
  MS_04.FilterIndex = sample.filterindex4

  MS_05.MinMass = sample.mass5-0.5
  MS_05.MaxMass = sample.mass5+0.5
  MS_05.FilterIndex = sample.filterindex5
```

3. Enter the Values in Sample List

Finally, enter the corresponding values of the single samples into each column of the sample table:

No.	Name	Pos.	*Mass1	*FilterIndex	*Mass2	*FilterIndex2	*Mass3	*FilterIndex3
1	Standard 1	R99	149	1	197	1	275	1
2	Standard 2	R99	149	1	197	1	275	1
3	Sample 1	RA1	176	0	153	0	235	0
4	Sample 2	RA1	176	0	153	0	235	0
5	Sample 3	RA2	163	0	215	0	179	0
6	Sample 4	RA2	163	0	215	0	179	0
7	Standard 3	G99	149	1	197	1	275	1
8	Standard 4	G99	149	1	197	1	275	1

In this example, the following mass traces are relevant for controlling the ➢*Fraction Collector* at the corresponding samples:

Retention time interval [min]	Channel	Standards Mass [m/z]	Sample 1 /2 Mass [m/z]	Sample3/4 Mass [m/z]
0.000 - 3.200	MS_01	149 ± 0,5	176 ± 0,5	163 ± 0,5
3.200 - 3.700	MS_02	197 ± 0,5	153 ± 0,5	215 ± 0,5
3.700 - 4.200	MS_03	275 ± 0,5	235 ± 0,5	179 ± 0,5

Note:

The retention times refer to the program example described in **Fraction Collection Control via an MS** *(see above).*

For an overview of the fraction collection topics, refer to **Collecting Fractions**.

Checking the Fraction Collection Status on the Control Panel

The **Fraction Collection** driver supports various properties that allow you to check the fraction collection status on a ➢*Control Panel*. These properties are read-only. They are available independently of the installed ➢*Fraction Collector*.

On the control panel, create display elements indicating the status of the desired parameter. You can use a Color Box, String Display, Gauge Indicator, or Lamp. (For more information, refer to **How to …: Controlling Devices from the Control Panel** **Modifying a Control Panel** and the following topics.)

The tables below list the properties in the order in which they appear in the dialog box. (For information about the parameters for active fraction collection control, refer to 🔲 **Editing Signal-Dependent Fraction Collection Parameters** and 🔲 **Editing Signal-Independent Fraction Collection Parameters**.)

Properties for the single channels

*These properties are available on the **Link** tab page of the **Properties** (context menu) for the corresponding control if the associated **DetectionChannelIN** is selected as **Object**.*

Property	Description
Curve	Indicates the last signal curve in [Signal]/s^2 evaluated by the Fraction Collection driver for peak detection.
PeakDetectCode	Use this property to have Chromeleon display the peak detection status:

Unknown (= 0)	The status is not defined.
Ready for Start (= 1)	The driver waits for the peak start, i.e., waits until the peak start conditions are fulfilled.
Start is Detected (= 2)	A peak start was detected.
Ready for Shoulder Up (= 3)	A ➤*Peak Shoulder* may have been detected at the fronting edge of the peak. However, this is not yet clearly detected. ■☞ *Tip:* *Chromeleon supports this parameter only for the **Purification** license.*
Max is Detected (= 4)	A peak maximum was detected.
Ready for Shoulder Down (= 5)	A peak shoulder may have been detected at the tailing edge of the peak. However, this is not yet clearly detected. ■☞ *Tip:* *Chromeleon supports this parameter only for the **Purification** license.*

Property	Description	
PeakDetectCode	**Waiting for Manual Peak End (= 6)**	The **Manual Peak Start** command was performed. Thus, automatic peak detection is disabled. The driver waits for the **Manual Peak End** command.
PeakEndTime **PeakMaxTime** **PeakStartTime**	Indicates the last retention times evaluated by the Fraction Collection driver for detecting a peak start, peak maximum, or peak end.	
RetentionTime	Indicates the retention time in minutes evaluated by the Fraction Collection driver for peak detection.	
Signal	Indicates the last signal value evaluated by the Fraction Collection driver for peak detection.	
Slope	Indicates the last slope in [Signal]/s evaluated by the Fraction Collection driver for peak detection.	

Properties for the Fraction Collection Driver

📖 *Tip:*

*These properties are available if the selected **Object** is **FractionCollection**.*

Property	Description
FracEndDetected	Set to **Yes** when a fraction start is detected. When the fraction end is detected, the property is reset to **No**. *(Delayed)*
FracStartDetected	Set to **Yes** when a fraction start is detected. When the fraction end is detected, the property is reset to **No**. *(Delayed)*
FractionOn	Set to **Yes** during fraction collection. *(Corresponds to PeakOn, but delayed)*
FractionTubeCount	Number of filled tubes in the current fraction. *(Delayed)*
FracTubeChange	Set to **Yes** when a filled tube is detected. When a filled tube is not detected, the property is automatically reset to **No**. *(Delayed)*
TubeFilling	Volume collected in the current tube. *(Delayed)*

■ Tracking Fraction Collection in the Chromatogram

After collecting fractions during an analysis, it is important to be able to track which substances were collected in which tube. Chromeleon lets you track this information in:

- The chromatogram

- The report (see 🔲 **Tracking Fraction Collection in the Report**)

In the chromatogram, you can view which fractions were collected and when they were collected:
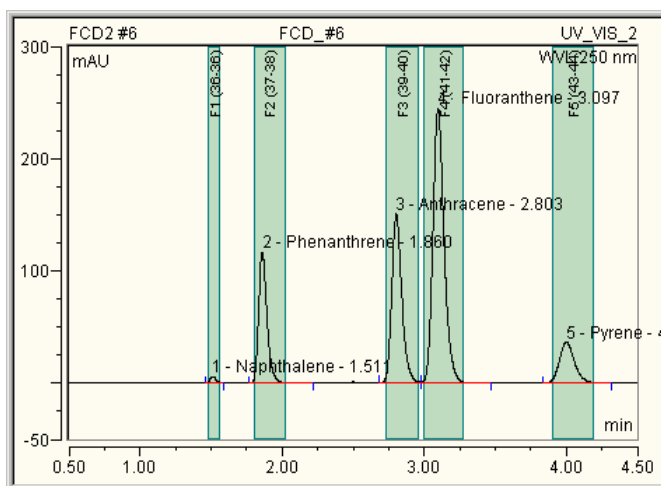
To modify the appearance of the display in the chromatogram, select **Decoration** on the context menu. The **Chromatogram Decoration** dialog box appears. On the **Fractions** tab page, select the display options:
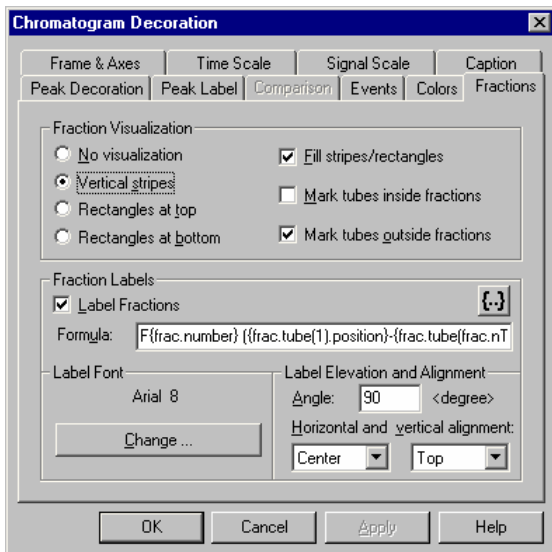
📝 *Note:*

The settings in the picture correspond to the settings used for the above chromatogram.

In the **Fraction Visualization** section, determine how the fractions are displayed. It is usually a good idea to select the **Vertical stripes** and **Fill stripes** options; selecting these options lets you display the exact time assignment of individual peaks to the corresponding fractions.

In the lower section, determine how the fractions are labeled. Select the **Label Fractions** check box first, and then determine the label in the **Formula** input field. The following formula is a good choice:

   F{frac.number} ({frac.tube(1).position}-{frac.tube(frac.nTubes).position})

The corresponding label could then read, e.g., "F2 (37-38)". This means that the second fraction was collected in tubes 37 and 38. (Note: To mark tubes in the chromatogram with dotted lines, select the **Mark tubes inside fraction** or **Mark tubes outside fraction** check box.) The '**F**' in the above formula distinguishes fraction labels from peak labels.

 *Tip***:**

You can also track fraction collection in the chromatogram during data acquisition. However, this requires several complex settings that must be tailored to the individual installation. A description of these settings goes beyond the scope of this online Help. For more information, please contact your local Dionex representative.

For an overview of the fraction collection topics, refer to **Collecting Fractions**.

Tracking Fraction Collection in the Report

You can display fraction collection information in the report at any time. The easiest way is to insert one of the two predefined report tables:

- Double-click to open a sequence, and then display the report.

- On the **Table** menu, select **Insert Report**.

- The **Insert Report Table** dialog box appears. Click the '+' characters in front of **Result Tables** and **Fraction Collection Results** to display the reports underneath.

- Select one of the two reports:
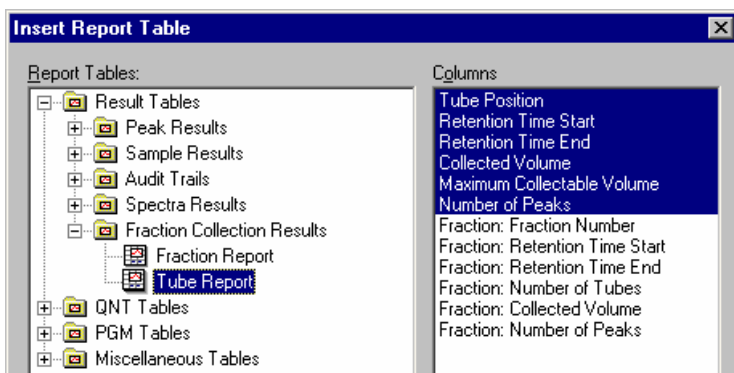
- Select the **Fraction Report** to display an overview of the fractions.

- Select **Tube Report** to add an overview of the single tubes to the report:

A	B	C	D	E	F
Pos.	Start min	End min	Volume ml	Max.Volume ml	#Peaks
4	1.485	1.568	0.083	1.0	1
5	1.801	2.035	0.234	1.0	1
6	2.735	2.968	0.350	1.0	1
7	3.001	3.285	0.426	1.0	1
8	3.901	4.202	0.301	1.0	1

◄ ► \ Integration ∧ Tube Report ∧ Audit Trail /

The picture shows a standard tube report that was created with the default settings. To insert additional columns:

- Select the column in front of which you want to insert the new column.

- Select **Insert Column** on the context menu. The **Insert Report Column** dialog box appears.

- Select one of the variables provided in the **Fraction** and **Fraction Tube** report categories. These categories provide the variables shown in the first picture plus some more.

- To access the variables of the **Fraction Detection Parameter** category, select the **Channel Parameter** variable of the **Fraction** category.

For a list of all available variables, refer to the following topics in the **Reference Information** section:

⇒ *'Fraction' Category*

⇒ *'Fraction Tube' Category*

⇒ *'Fraction Detection Parameter' Category*

For an overview of the fraction collection topics, refer to ◪ **Collecting Fractions**.

Collecting Fractions Automatically (Autopurification)

Chromeleon supports automatic fraction collection. This is also referred to as ➢*Autopurification*. Autopurification includes three steps:

- Pre-analysis of the preparative samples

- Preparative purification of the remaining samples, based on the pre-analysis results

- Post-analysis of the collected fractions for purity and yield evaluation

In order to perform autopurification, make sure that

1. A **Purification** license is installed.

2. A Dionex Sample and Fraction Manager is installed. (For installation details, refer to **Installing Dionex Devices** 🌐 **HPLC Sample and Fraction Manager (SFM)** in the **Administrator Help** section.)

For general information about fraction collection, refer to 🔙 **Collecting Fractions**.

For more information about autopurification, refer to:

🔙 **Setting up Autopurification**

🔙 **Creating PGM Files for Autopurification Samples**

🔙 **Using Triggers in Autopurification Programs**

🔙 **Performing Autopurification**

🔙 **Autopurification Samples in the Sample List**

🔙 **Autopurification Samples in the Chromatogram**

🔙 **Autopurification Samples in the Tray Views**

◼ Setting up Autopurification

Before you can collect fractions automatically, you need to take the necessary preparative steps in Chromeleon, using the corresponding files from the **Additional_Software_Autopurification** CD. First, select the appropriate directory for your installation, e.g., **APS_2222_UV_MS**.

Windows Explorer

Copy the **Dionex_Purification_Parameters.GEN** file from the **Drivers** subdirectory to the **Chromel/bin** directory.

☞ *Tip:*

Usually, a Dionex Service Representative or an authorized Dionex distributor copies the file. (Make sure that you do not move any chromatographic files in the Windows Explorer. The reason is that, except for the visible results, processes are performed below the surface.)

Server Configuration

Install the devices in the Server Configuration program. Usually, you have to install the single devices manually. However, for autopurification, Chromeleon provides several predefined device configurations on the CD that is shipped with the product. For example:

APS_2222_UV_MS.CFG (in the **cfg file** directory)

Select **Import** on the **File** menu and import the configuration.
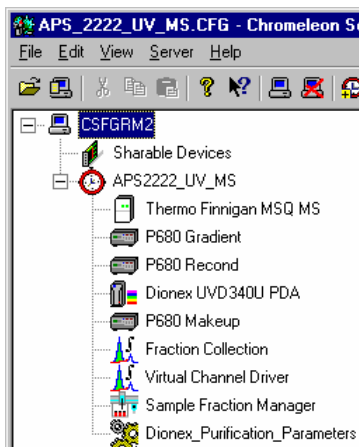
PGM Files

In the PGM File, define how the single fractions are to be collected. The Programs directory provides different programs as backup files. Select ➢*Restore* on the **File** menu and specify the location to which you want to restore the program(s).

Or else, you may create a program in the Program Wizard (see **The Control Program** **The Program Wizard**). For more information, refer to **Collecting Fractions**.

Control Panels

Install the predefined control panels from the **Panels** directory on the CD to an appropriate location, e.g., the **Dionex Templates>Panels** directory in the local datasource of the Chromeleon Browser. For example, create an **Autopurification** folder in this directory.

Reports

In the same way, you may copy the report templates from the **Reports** directory on the CD to an appropriate location, e.g., the **Dionex Templates>Reports** directory in the local datasource of the Chromeleon Browser. For example, create an **Autopurification** folder in this directory.

Demo Data

In addition, demo data backup files are provided in the **Demo_data** directory. Copy the data to an appropriate location, if required.

For an overview of how to perform ➢*Autopurification*, refer to **Collecting Fractions Automatically (Autopurification)**.

▪ Creating PGM Files for Autopurification Samples

The purification process includes three steps. Therefore, three different PGM Files are required for

1. Pre-analysis of the preparative samples

2. Fraction collection

3. Post-analysis of the collected fractions

No special PGM Files are required for steps 1 and 3. For step 2, use the PGM Wizard to create a program for fraction collection. Follow the steps in **How to …: Collecting Fractions** 🔼 **Setting up Fraction Collection**.

The Dionex_Purificiation_Parameters device driver supports additional parameters. For more information, refer to:

🔼 **Setting the General Parameters for the Dionex_Purification_Parameters Driver**

🔼 **Setting the Safety Parameters**

🔼 **Setting the Correction Parameters (Settings Panel)**

For an overview of how to perform ➤*Autopurification*, refer to 🔼 **Collecting Fractions Automatically (Autopurification)**.

▪ Setting the General Parameters for the Dionex_Purification_Parameters Driver

The Dionex_Purification_Parameters driver supports the following general parameters.

Property	Description
AnalOn	When the variable is set to **On**, the control panel shows that the analytical pump flow is directed through the analytical column.
AutoCh*	Enables or disables the ➤*ARC* logic, e.g., on the **Operation** panel. (ARC logic must be off for isomers.)
Col1On	(Tandem operation only) When the variable is set to **On**, the control panel shows that the preparative pump flow is directed through the preparative column 1.
Col2On	(Tandem operation only) When the variable is set to **On**, the control panel shows that the preparative pump flow is directed through the preparative column 2.

Property	Description
FirstRack	Indicates the rack that contains the first fraction tube of a sample (report variable).
FirstTube	Indicates the first fraction tube of a sample.
LastRack	Indicates the rack that contains the last fraction tube of a sample.
LastTube	Indicates the last fraction tube of a sample.
MSTgtPkEnd*	The variable is set to **Yes** when the system has detected a target compound (required for the ARC logic).
MSThrFact	Multiplication factor for absolute threshold values for peak start and peak end in the MS.
NextImpuls	Controls the impulse transmitter for software function monitoring of the Security and Solvent Monitoring System (SSM).
PkPrevRunEnd	Indicates whether fractions were collected during the last two runs. If no fractions were collected, you have to reset the variable manually on the **Operation** panel to allow a new sample to be injected.
TenPortState	Controls valve switching for two preparative columns in tandem operation.
ThrMSTgtPkDet*	Absolute threshold value for detecting a target mass in the MS (required for the ARC logic).
ThrUVTgtPkDet*	Absolute threshold value for detecting a target mass in the UV (required for the ARC logic).
TrigBusy	Inhibits that several triggers are performed in parallel.
UVThrFact	Multiplication factor for absolute threshold values for peak start and peak end in the UV.

☞ *Tip:*

On the control panel, you can correct the parameters marked with an asterisk (*) for all other samples of the current sequence, using the corresponding parameters that are supported by the **Dionex_Purification_Parameters** *device driver (see **Collecting Fractions Automatically (Autopurification)** 🔼 **Setting Correction Parameters**).*

For an overview of how to perform ➢*Autopurification*, refer to 🔼 **Collecting Fractions Automatically (Autopurification)**.

◾ Setting the Safety Parameters

The Dionex_Purification_Parameters driver supports several parameters that can be used to improve system safety.

Parameter	Description
Disc*	Enables or disables the **Disconnect** function. If the parameter is set to **Yes**, all modules are disconnected when the ➢*Emergency Program* is running.
LeakOn	Enables or disables leak detection. If the parameter is enabled (**Yes**), a ➢*Message* appears when the emergency program is running explaining why the system has stopped when a leak is detected, i.e., when the value is below the **PMinAlarm**.
MaxPress	Highest pressure measured in the system (used for early pressure alarm).
PMaxAlarm*	Upper pressure limit. If the pressure exceeds this value, a warning appears and no sample will be injected.
PMinAlarm*	Lower pressure limit. When the pressure is below this value, a warning appears and the system is stopped immediately.
PressAlarm	Enables or disables the pressure alarm. If parameter is set to **On**, no sample will be injected.
PressMem	At a specified time during the program run (usually, at the highest pressure), the parameter is assigned the current system pressure.
RunDone	If the parameter is set to **Yes**, fractionation is stopped at the end of the sample.

◾ *Tip:*

On the control panel, you can correct the parameters marked with an asterisk (*) for all other samples of the current sequence, using the corresponding parameters that are supported by the Dionex_Purification_Parameters device driver (see Collecting Fractions Automatically (Autopurification) ◾ Setting Correction Parameters).

For an overview of how to perform ➢*Autopurification*, refer to ◾ **Collecting Fractions Automatically (Autopurification)**.

◨ Setting the Correction Parameters (Settings Panel)

The **Dionex_Purification_Parameters** driver supports several parameters that allow you to later change the settings for single fraction collection parameters defined in the PGM File, e.g., if you come across an unfavorable setting for a special parameter when processing a long sequence.

◨☞ *Tip:*

*Unlike all other properties, the values for these parameters are **not** reset to the values in the PGM File when a new sample is started. Thus, these values are valid for all other samples.*

To change a setting, click **Settings** on the **Administration.pan** panel. The **Settings** sub panel appears.

◨☞ *Tips:*

*To use the default values for the parameters listed in the panel sections below, click the associated **SET DEFAULT VALUES** button.*

*For many parameters, a **NOW** button is provided. Click this button to apply the new value immediately. In all other cases, the new value is used after the current sample has been processed completely.*

The appearance of the panels may vary, depending on the installed version. Therefore, the parameter names listed below may be slightly different from the names on your panel version.

Pressure Monitoring

Enable use of the following pressure monitoring parameters by selecting the **Use global parameters for pressure monitoring** check box or via the **GlobParPressMem** parameter in the **Commands** dialog box:

Option	Parameter	Description
Upper pressure alarm level	PMaxAlarm2	Upper limit for the system pressure. If the pressure exceeds this value, a warning appears and no sample will be injected.
Lower pressure alarm level	PMinAlarm2	Indicates the lower limit for the system pressure during a run. A pressure below this value indicates a leak. A warning appears and the system is stopped immediately.

Safety

To enable the disconnect function, select the **Disconnect System in Case of Emergency** check box or use the **Disc** command in the **Commands** dialog box. When this function is active, Chromeleon interrupts software control for all modules while the ➢*Emergency Program* is running.

Threshold Values for Fraction Collection

Enable use of the related peak detection parameters by selecting the **Use parameters below for peak detection** check box or via the **GlobParUVDet** or **GlobParMSDet** parameter in the **Commands** dialog box:

Option	Parameter	Description
Peak Start Threshold (UV)	**UVStartThr**	The signal threshold must exceed this value for a peak start to be detected (PeakStartThreshold of DetectionChannel1).
Peak End Threshold (UV)	**UVEndThr**	The signal value must be below this value for a peak end to be detected (PeakEndThreshold of DetectionChannel1).
Threshold Do Not Resolve (UV)	**UVThrRes**	Above this threshold value, a new fraction tube is used in the minimum between two peaks if these peaks are not baseline separated (ThresholdDoNotResolve of DetectionChannel1).
Threshold No Peak End (UV)	**UVThrNoPkEnd**	The signal value must be below this value for a peak end to be detected (PeakEndThreshold of DetectionChannel1)
Peak Start Slope (UV)	**UVStartSlp**	The slope must exceed this value for a peak start to be detected (PeakStartSlope of DetectionChannel1).

Option	Parameter	Description
Peak End Slope (UV)	**UVEndSlp**	The signal slope must be below this value for a peak end to be detected (PeakEndSlope of DetectionChannel1)
Peak Start Curve (UV)	**UVStCurv**	The curvature in [Signal]/s^2 must exceed this value for a peak start to be detected (PeakStartCurve of DetectionChannel2).
Peak End Curve (UV)	**UVEndCurv**	The curvature in [Signal]/s^2 must be below this value for a peak end to be detected (PeakEndCurve of DetectionChannel2).
Peak Start True Time (UV)	**UVStTrTi**	Time in seconds for which the PeakStart conditions must be fulfilled. Only then, a peak start will be detected (PeakStartTrueTime of DetectionChannel1).
Peak End True Time (UV)	**UVEndTrTi**	Time in seconds for which the PeakEnd conditions must be fulfilled. Only then, a peak end will be detected (PeakEndTrueTime of DetectionChannel1).
Peak Start Threshold (MS)	**MSStartThr**	The signal threshold must exceed this value for a peak start to be detected (PeakStartThreshold of DetectionChannel2).
Peak End Threshold (MS)	**MSEndThr**	The signal threshold must be below this value for a peak end to be detected (PeakEndThreshold of DetectionChannel2).

Option	Parameter	Description
Threshold No Peak End (MS)	**MSThrNoPkEnd**	The signal value must be below this value for a peak end to be detected (ThresholdNoPeakEnd of DetectionChannel2).
Peak Start Slope (MS)	**MSStartSlp**	The slope must exceed this value for a peak start to be detected (PeakStartSlope of DetectionChannel2).
Peak End Slope (MS)	**MSEndSlp**	The signal slope must be below this value for a peak end to be detected (PeakEndSlope of DetectionChannel2)
Peak Start True Time (MS)	**MSStTrTi**	Time in seconds for which the PeakStart conditions must be fulfilled. Only then, a peak start will be detected (PeakStartTrueTime of DetectionChannel2).
Peak End True Time (MS)	**MSEndTrTi**	Time in seconds for which the PeakEnd conditions must be fulfilled. Only then, a peak end will be detected (PeakEndTrueTime of DetectionChannel2).

🖝 *Tip:*

The parameter names determined for DetectionChannel1 and DetectionChannel2 imply that DetectionChannel1 is a UV channel and DetectionChannel2 is an MS channel.

Fraction collection

Option	Parameter	Description
Channel Evaluation "ALL"	**ChanEval**	Determine how the results of the detection channels are to be used for peak detection:
		Selecting this check box, activates **All**, i.e., the condition must be true for all channels.
		Clearing this check box, activates **Any**, i.e., the condition must be true for only one channel.

Enable use of the following fraction collection parameters by selecting the **use parameters below for fraction collection** check box or via the **GlobParFr** parameter in the **Commands** dialog box:

Option	Parameter	Description
# of Tubes Installed	**MaxTubes**	Maximum number of tubes available for fraction collection in the system.
Tube Max Volume	**TbMaxVol**	Maximum volume per tube.
max # Tubes/Fraction	**MaxTbPerFr**	Maximum number of tubes per fraction.

High throughput

Enable or disable the procedure for increasing the sample throughput:

Option	Parameter	Description
ARC Logic	**ARCLogic**	Enables or disables ➢*ARC* logic. (ARC logic must be off for isomers.)
Injection Mode	**InjectMode**	Enables or disables overlapping sample preparation.

Threshold values ARC Logic

Enable the following fraction collection parameters by selecting the **use parameters below for ARC logic** check box or via the **GlobParEarlTerm** parameter in the **Commands** dialog box:

Option	Parameter	Description
UV	**ThrUVTgtPkDet2**	UV threshold value for a target peak
MS	**ThrMSTgtPkDet2**	MS threshold value for a target mass

Continuous Fraction Collection

To enable fraction collection outside of peaks by selecting the **Collect outside peaks** check box or via the **ColOuts** parameter in the **Commands** dialog box.

For an overview of how to perform ➢*Autopurification*, refer to 🔳 **Collecting Fractions Automatically (Autopurification)**.

🔳 Using Triggers in Autopurification Programs

Using ⇒*Triggers* allows performing one or more actions as soon as certain conditions are true. Usually, three triggers are required for fraction collection:

- To start fraction collection

- To change the tube

- To finish fraction collection

For an example, refer to **Commands for Controlling Dionex Devices** 🔳 **Sample and Fraction Manager: PGM File for Fraction Collection**.

Miscellaneous and freely definable conditions allow linking triggers to certain action, thus making them a flexible and versatile tool for controlling and monitoring various functions of the APS-2000 Series systems. For example, you can use a trigger to control and monitor the

- System pressure,

- Correct communication between the system and the software,

- ➢*ARC* logic,

- And to transfer various variables from the panel to the program.

For information about special autopurification triggers, refer to the operating instructions for the APS-2000 Series systems.

For an overview of how to perform ➢*Autopurification*, refer to 🔳 **Collecting Fractions Automatically (Autopurification)**.

🔳 Performing Autopurification

First, create a sequence that contains the samples for fractionation.

Then, have Chromeleon analyze the samples chromatographically to check whether fractionation is appropriate. From the original analytical samples (⇒*Auto Purif. Type* = **Analytic**), Chromeleon can automatically create the necessary preparative samples (**Auto Purif. Type** = **Preparation**). Determine the conditions in the **Create Purification Samples** ➢*Post-Acquisition Step*.

📒 *Notes:*

*The Create Purification Samples post-acquisition step is available only if the timebase is connected to the related server, if this server is running, and if a **Purification** license is installed.*

The preparative samples are injected from the same vials/tubes as the original analytical samples.

For more information, refer to:

🔳 **Creating Preparation-Type Samples**

🔳 **Using Optimized MS Threshold Values**

Afterward, fractionation is performed. This step, too, can be monitored chromatographically. Use the **Create Fraction Analysis Samples** post-acquisition step to create the necessary fractionated samples (**Auto Purif. Type = Fraction**). Only then will it be possible to chromatographically reanalyze the individual fractions later.

 Note:

*The Create Fraction Analysis Samples post-acquisition step is available only if the timebase is connected to the related server, if this server is running, and if a **Purification** license is installed.*

For more information, refer to **Creating Fraction-Type Samples**.

For an overview of how to perform ➤*Autopurification*, refer to **Collecting Fractions Automatically (Autopurification)**.

Creating Preparation-Type Samples

Preparative samples are required to allow fractionation of the content of a tube or vial. Create preparative samples in the **Create Purification Samples** ➤*Post-Acquisition Steps*. A wizard guides you through the creation process.

 Note:

*The Create Purification Samples post-acquisition step is available only if the timebase is connected to the related server, if this server is running, and if a **Purification** license is installed.*

The flow chart on the first wizard page illustrates how preparative samples are created.

In addition, determine for how many target compounds is searched:
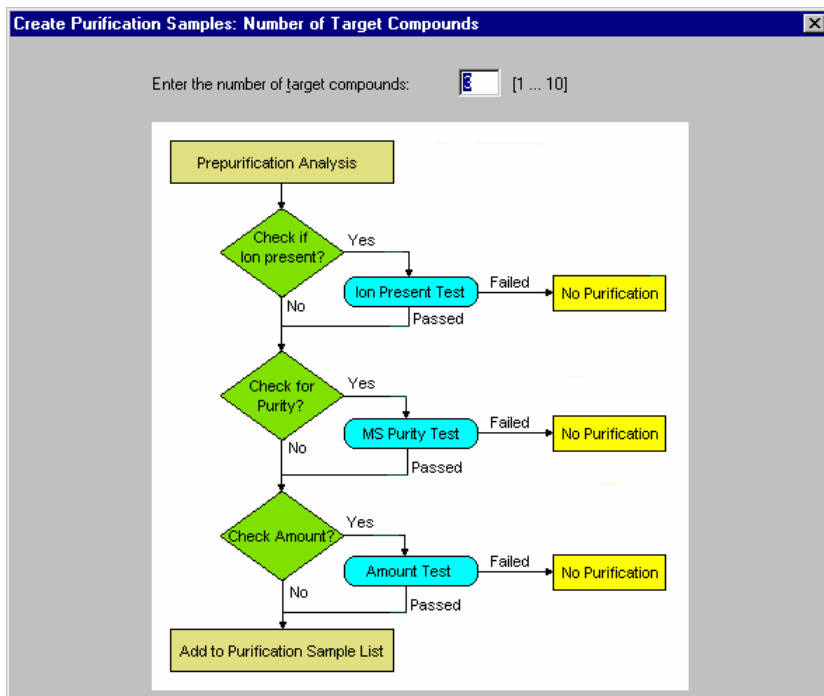
On the second wizard page, define the channel and the target compound:

Select the search criterion for the target compound from the **Select Peak…** drop-down list:

by Name:

by Retention Time:

by Greatest Height& Threshold: (= Highest peak)

by Greatest Area& Threshold: (= Peak with the largest area)

In the **Mass Spectrometry Settings** section, enter the **Target Mass** in the related input field. In addition, enter the mass range in the **Isotope bunch** field. If necessary, enter the mass of one or two adducts of the target compound, e.g., with a solvent, in the **Adduct Mass 1** and/or **Adduct Mass 2** input fields.

On the third wizard page, define the tests to be performed:

Ion present: Checks whether the target compound is present.

Mass spectrum purity: Checks whether the target compound is pure enough.

Amount check: Checks whether the amount of the target compound is sufficient.

<New customized>:   Allows you to define your own test.

For more information about this wizard page, refer to **Create Purification Samples: Target Compound X**.

Use the next wizard page to enable special options:

Use Conditional Program   Select this check box if you want to define an additional gradient program.

Optimize MS Threshold for Target Compound X   (X is a number between 1 and 10) Select this check box if you want to further analyze tailing and fronting of the target compound for a specified channel.

Selecting one of these options and clicking **Next>** takes you to the related wizard page. If you did not select an option, clicking **Next>** takes you to the three last wizard pages. Use these pages to determine the properties for the purification sequence and its samples ($\Rightarrow$*Auto Purif. Type*: **Preparation**). For more information, refer to the online Help for **Create Fraction Analysis Samples: Sequence Properties**, **Create Fraction Analysis Samples: Sample Properties**, and **Create Fraction Analysis Samples: Extended Sample Properties**. Click **Finish** to complete creation of the post-acquisition step.

For more information, refer to:

🔼 **Performing Autopurification**

🔼 **Creating Fraction-Type Samples**

For an overview of how to perform ➢*Autopurification*, refer to 🔼 **Collecting Fractions Automatically (Autopurification)**.

▣ Using Optimized MS Threshold Values

The **Purification Sample Wizard** supports the input of automatically optimizing threshold values for peak detection. For example, this may be useful in the following situation:

When the ion source of the ➢*Mass Spectrometer* is overloaded, it may appear in the MS channel as if a target compound is still eluting from the column. To avoid collecting fractions that do not contain the target compound, you can enter two automatically optimizing MS threshold values for the peak start and peak end on the **Optimized MS Threshold TCx** page. Follow the steps below:

From the **Channel for peak height** list, select the channel to be used to determine the retention time at the peak height specified in the **Peak Front Evaluation** and **Peak End Evaluation** sections. From the **Signal evaluation channel** list, select the channel whose signal height at this retention time is used. The target channel is the default setting, but you may select a different channel, instead. In addition, you may enter a **Multiplication Factor**. The signal value of this channel is multiplied with this factor.
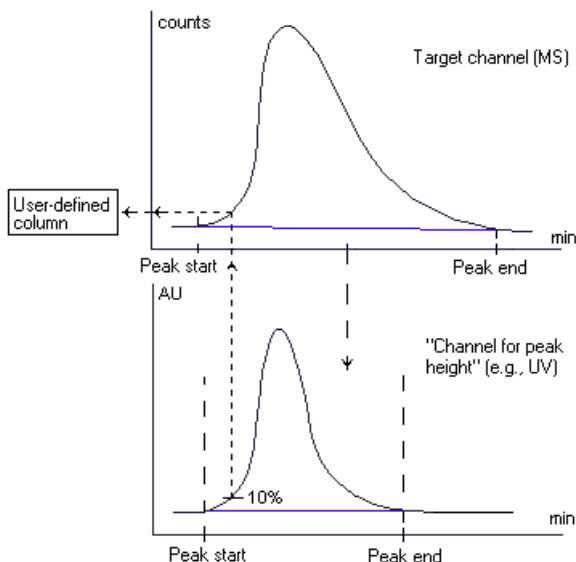
In the example, the ion source is overloaded, resulting in tailing of the peak on the MS channel (contrary to the peak in the UV channel). To check whether the peak exists on the UV channel, Chromeleon first determines the retention time that is exactly between the peak start and peak end on the MS channel. This retention time is also between the peak start and peak end on the UV channel (dashed lines), thus proving that the peak exists on the UV channel, too.

Afterward, Chromeleon determines the retention time at which the signal on the UV channel has reached (in this example) 10% of the maximum on the ascending peak edge (dotted line). Finally, Chromeleon determines the height of the signal on the **Signal evaluation channel** at this retention time. In the example, the signal evaluation channel is the target channel.

In the pre-analysis (i.e., during the analysis of the sample for which the ⇒*Auto Purif. Type* is **Analytic**), the signal value is written to the specified ➢*User-defined Column* and evaluated during fraction collection (i.e., during the analysis of the sample for which the Auto Purif. Type is **Preparation**). When the signal value is exceeded during the analysis of the preparation-type sample, a peak is detected and a fraction is collected.

To determine the peak end and thus stop fraction collection, you can enter the height for **Peak Tail Evaluation** in the same way.

👉 *Tip:*

Samples consisting of several substances with clearly different degrees of ionization are another example. When a fixed threshold value is used, it is possible that only a small portion (or no portion) of weakly ionizing substances might be collected. However, with an optimized MS threshold, the same portion of substances would be collected even if the ionization degrees were different.

▪ Creating Fraction-Type Samples

When fractionating preparative samples, the samples are chromatographically analyzed. If you want to reanalyze these fractions later again, you need one Chromeleon sample for each fraction. Chromeleon can create the samples automatically during fraction collection via the **Create Fraction Analysis Samples** ➢*Post-Acquisition Step*. A wizard guides you in creating this step.

📝 *Note:*

*The Create Fraction Analysis Samples post-acquisition step is available only if the timebase is connected to the related server, if this server is running, and if a **Purification** license is installed.*

On the first wizard page, determine which fractions are to be chromatographically reanalyzed:
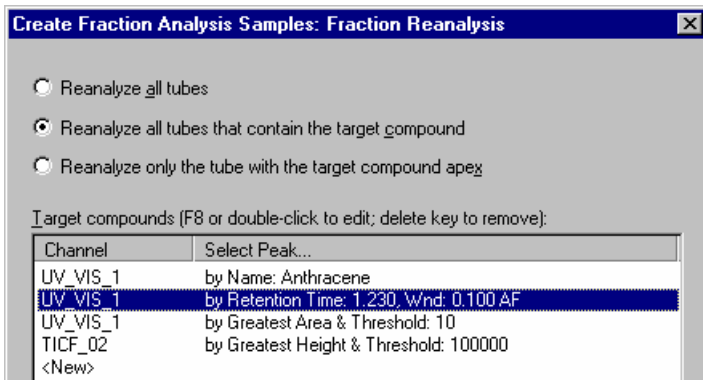
Select:

Reanalyze all tubes	To reanalyze all tubes containing fractionated samples.
Reanalyze all tubes that contain the target compound	To reanalyze only those tubes that contain the target compound.
Reanalyze only the tube with the target compound apex	To reanalyze only the tubes into which the fractions containing the peak maximum of the target compounds were collected.

The **Target Compound** table lists all defined target compounds. Click **<New>** to add a new target compound. The **Select Target Compound Context** dialog box opens:
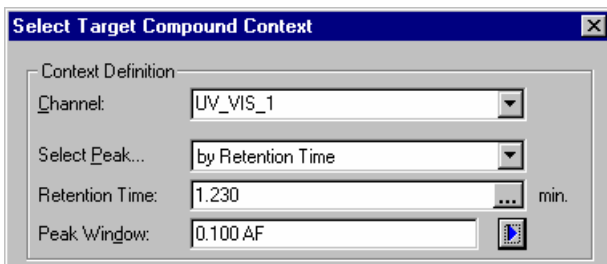
Select the search criterion for the target compound from the **Select Peak…** drop-down list. For information about the criteria, refer to ⬛ **Creating Preparation-Type Samples**.

Clicking **Next>** takes you to the next wizard page. Determine the properties for the fractionation sequence and its samples (⇒*Auto Purif. Type*: **Fraction**). For more information, refer to the online Help for **Create Fraction Analysis Samples: Sequence Properties**, **Create Fraction Analysis Samples: Sample Properties**, and **Create Fraction Analysis Samples: Extended Sample Properties**. Click **Finish** to complete creation of the post-acquisition step.

For more information, refer to:

⬛ **Performing Autopurification**

⬛ **Creating Preparation-Type Samples**

For an overview of how to perform ➤*Autopurification*, refer to ⬛ **Collecting Fractions Automatically (Autopurification)**.

⬛ Autopurification Samples in the Sample List

Click **Display Columns** on the context menu to include the following columns in the sample list:

⇒*Auto Purif. Frac (Autopurification Fraction)*

⇒*Auto Purif. Ref. (Autopurification Sample Reference)*

⇒*Auto Purif. Type (Autopurification Type)*

📝 Note:

The columns display the values assigned by Chromeleon. These values are read-only.

The **Auto Purif. Type** sample variable indicates the ➤*Autopurification* stage for the associated sample:

1. **Analytic** = original analytical sample

2. **Preparation** = preparative sample

3. **Fraction** = fractionated sample

An identical entry in the **Auto Purif. Ref.** column of the sample list indicates that the samples of these three sample types belong together. The fraction ID assigned by Chromeleon appears in the **Auto Purif. Frac.** column.

Chromeleon numbers the fractionated samples so that the order of the samples remains unchanged even if they are moved to a different sequence or folder.

The picture shows an example in which the analytical samples are stored in the sequence 1_Analytic, the preparative samples in the sequence 2_Preparation, and the fractionated samples in the 3_Fractions sequence:

For an overview of how to perform autopurification, refer to **Collecting Fractions Automatically (Autopurification)**.

Autopurification Samples in the Chromatogram

After ➤*Autopurification* has been performed, you can have Chromeleon display the results of the different samples in the associated chromatograms. The default setting is that all samples of the **Analytic**, **Preparation**, and **Fraction** ⇒*Auto Purification Types* that belong together are displayed:
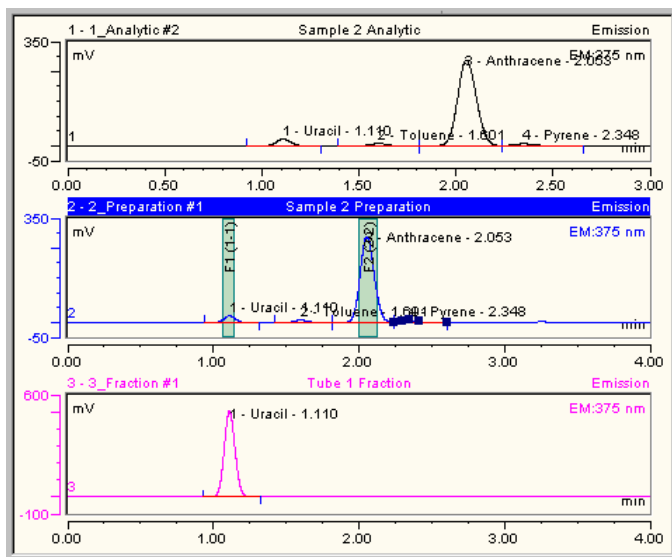
The topmost chromatogram shows the original analytical sample. If this sample fulfills the conditions determined in the **Create Purification Samples** ➢*Post-Acquisition Steps*, a preparation-type sample is created and fractionated. The chromatogram for the preparation-type sample is displayed next. The vertical stripes indicate the chromatogram ranges for which fractions were collected.

The individual fractions are chromatographically reanalyzed, based on the conditions determined in the **Create Fraction Analysis Samples** post-acquisition step. The chromatogram for the associated fractions is displayed last.

The bottommost chromatogram shows the fraction for the selected peak of the preparative sample. This is the fraction under the peak maximum. Of, if no fraction was collected at the peak maximum, this is the fraction overlapping most with the peak. If no peak was selected in the preparative chromatogram or if no fraction was collected for the selected peak, the chromatogram for the first peak is displayed.

 Note:

To display the next or previous sample or channel of the current chromatogram, click the associated icon: Click for the next chromatogram, for the previous chromatogram, for the next channels or for the previous channel. If another chromatogram is displayed, the associated chromatograms are updated simultaneously. For example, if Chromeleon displays the next analytical chromatogram, the associated preparative chromatogram and the associated chromatogram of the first fraction are displayed as well.

To change the representation of the single chromatograms, double-click the chromatogram or select **Decoration** on the context menu. The **Chromatogram Decoration** dialog box is opened. On the **Autopurification** tab page, determine which autopurification samples are displayed. On the **Fractions** tab page, determine how fraction collection is displayed in the chromatogram.

Also, refer to **Autopurification Samples in the Tray Views**.

For an overview of how to perform autopurification, refer to **Collecting Fractions Automatically (Autopurification)**.

▣ Autopurification Samples in the Tray Views

Chromeleon supports two tray views for displaying the different samples and/or fractions. Analytical and preparation-type samples are displayed in the **Inject Trays** view. To open the **Inject Trays** view, click the following icon on the **Method** toolbar: ▦. Tubes with fraction-type samples are displayed in the **Fraction Racks** view. To open the **Fraction Racks** view, click the following icon: ▦.
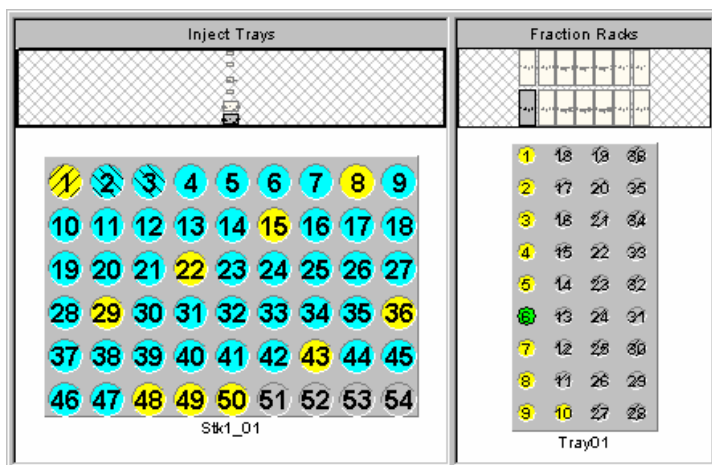
The default setting is that an overview of the single racks is displayed below the title line, also showing the racks that do not contain samples and/or fractions. The racks and their samples are then displayed below this general overview. The samples and/or fractions are color-coded, based on their ⇒*Type (Sample Type)* and ⇒*Status*.

To change the representation of the samples and/or fractions, double-click the respective tray view or select **Properties…** on the context menu. The **Inject Tray Properties** or **Fraction Tray Properties** dialog box is opened. On the **Format**, tab page, determine the formats in which the trays are displayed. On the **Tube Format** tab page, define the formats for the vials and/or tubes. Use the **Overview** tab page to display, hide, and/or format the trays.

Also, refer to ▣ **Autopurification Samples in the Chromatogram**.

For an overview of how to perform ⮞*Autopurification*, refer to ▣ **Collecting Fractions Automatically (Autopurification)**.

Simulating Chromatograms

To use ➢ *Virtual Column* to simulate retention data and chromatograms, you first select the analysis parameters (analytes of interest, methodology, column, etc.). Virtual Column then calculates retention data for the selected parameters, and displays the resulting *Resolution Response Surface* and *Virtual Chromatogram*.*

🔖 *Note:*

Results obtained with Virtual Column are intended to represent typical results for a particular column type. Because no two columns or systems are identical, the results you obtain in an actual analysis may differ somewhat from the Virtual Column predictions.

Starting Virtual Column

In the Chromeleon Browser, select **Virtual Column** on the **Tools** menu.
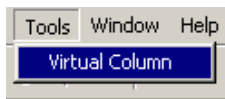
👉 *Tip*:

You can open more than one Virtual Column window.

Using Virtual Column (Overview)

1. Select an analyte category.

2. Select two or more analytes.

3. Select a column.

4. View the Resolution Response Surface (a plot of the lowest resolution values found at each eluent condition).

5. View the Virtual Chromatogram.
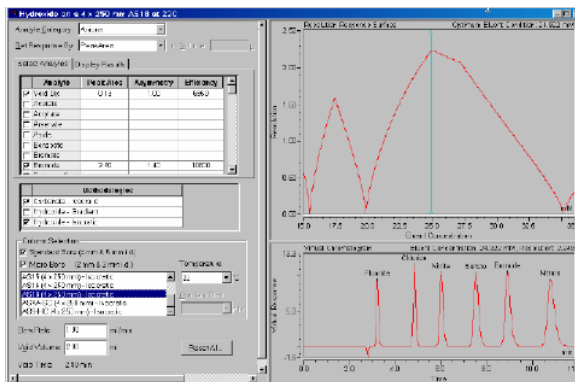
👉 *Tip*:

Right-click on the Resolution Response Surface to select a different resolution criterion.

 Note:

Parts of the Virtual Column software were developed jointly by Dionex Corporation and the Australian Centre for Research on Separation Science (ACROSS) at the University of Tasmania, Australia.

For details about how to use Virtual Column, refer to:

 Selecting the Analysis Parameters

 Viewing the Results Table

 Viewing the Resolution Response Surface

 Viewing the Virtual Chromatogram

 Selecting a Resolution Criterion

 Finding the Fastest Chromatogram

 Finding the Global Optimum

 Selecting the Column Quality Assurance Conditions

 Manually Selecting an Eluent Condition

 Saving and Reloading Virtual Column Settings

 Viewing the Eluent Composition

 Modeling Gradient Separations

Selecting the Analysis Parameters

The left side of the ➢*Virtual Column* window provides controls for selecting the analysis parameters.

Tip:

*To clear selections and return the Virtual Column window to the initial blank state, click **Reset All**.*

1. Select an Analyte Category.

To begin a Virtual Column simulation, first select the **Analyte Category**. The table on the **Select Analytes** tab lists all analytes in the selected category for which embedded data are available. The example shows the list when **Anions** is the selected category.
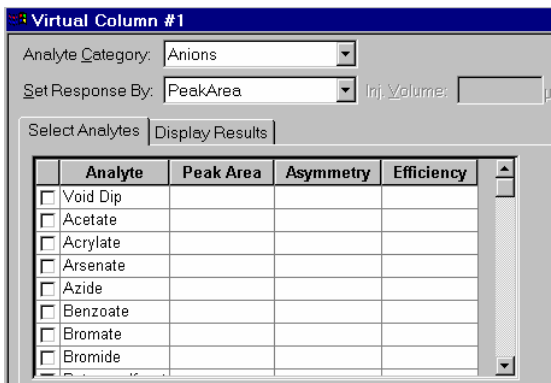
2. Select the analytes.

Select the check box for each analyte to be included in the Virtual Chromatogram.

Tip:

*If you want Virtual Column to calculate retention data for the void dip, or if you want to change its **Peak Area**, **Asymmetry**, or **Efficiency** values, select the **Void Dip** check box. If the check box is not selected, the void dip is displayed on the Virtual Chromatogram, but retention and resolution data are not calculated for it.*
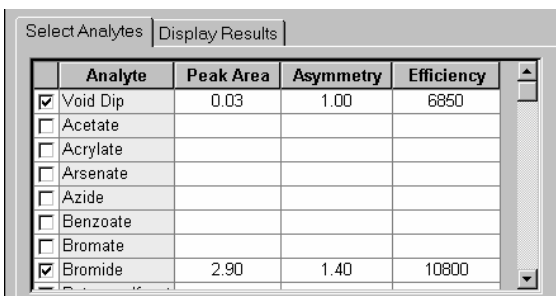
3. (Optional) Select methodologies.

After you select two or more analytes, the methodologies and columns available for the selection are displayed. If more than one methodology is shown, you can restrict the column choices by clearing the check boxes from methodologies you do not want to consider.

4. (Optional) Select a column diameter.

To restrict the column choices to a particular diameter category, clear the check box for the diameter of column (**Standard Bore** or **Micro Bore**) you do not want to consider.

5. Select a column.

Select a column from the list. Virtual Column calculates retention and resolution data for the selected parameters and displays the resulting Resolution Response Surface and Virtual Chromatogram. The display defaults to the best **Minimum Resolution** case (see **How to …:** Selecting a Resolution Criterion).

Note:

*Gradient cases are listed only if a Virtual Column Linear Gradient license is present and a gradient **Methodology** option is selected.*

The following example shows the plot and chromatogram for Fluoride, Chloride, Nitrite, Sulfate, Bromide, and Nitrate when the AS18 column is selected using isocratic conditions.
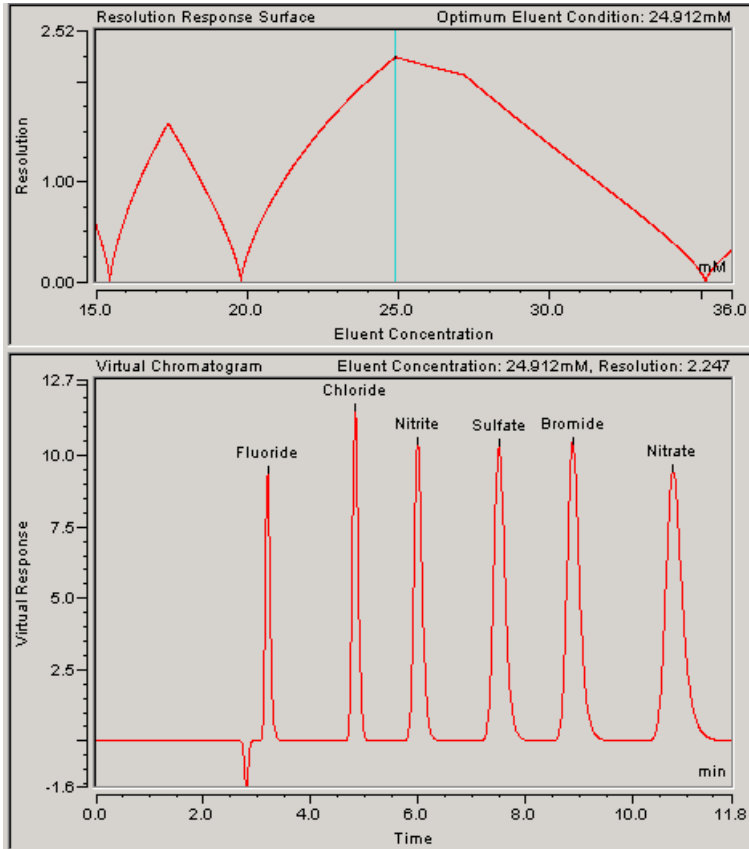
6. **(Optional) Change the Set Response By option.**

The default response factor is **Peak Area**. You can change this to **Concentration (mg/l)** or **Concentration (mM)**.

7. **(Optional) Change the Inj. Volume.**

After you select the **Concentration** option for **Set Response By**, a default injection volume is displayed. You can enter a different value in the edit box.

8. **(Optional) Change the Peak Area, Concentration, Asymmetry, and Efficiency Values.**

   After you select the analytes and choose a column, the **Select Analytes** table displays the default ➢*Peak Area* or **Concentration**, ➢*Asymmetry*, and **Efficiency** values for each selected analyte. The values displayed were obtained from the experimental data embedded in Virtual Column. If you have data specific for your system, you can enter those values in the table. Virtual Column uses the new values in retention and resolution calculations.

   After entering the values, you can save them to a file for later use (see **How to …: 🔳 Saving and Reloading Virtual Column Settings**).

9. **(Optional) Change the Temperature.**

   For some columns, you can select a different temperature to view the effect of a temperature change on the Virtual Chromatogram.

10. **(Optional) Change the Gradient Start.**

    After you select a column for a gradient simulation, a default value for the eluent concentration at the beginning of the gradient is displayed. You can select a different value from the drop-down list.

🗒️ *Note:*

See **How to …: 🔳 Modeling Gradient Separations** *for more information about gradient analyses.*

11. **Optional) Change the Flow Rate, Void Volume, and Void Time.**

    After you select the analytes and choose a column, Virtual Column displays a default flow rate, void volume (also called ➢*Dead Volume*), and void time (also called ➢*Dead Time*) for the selected column. You can change the flow rate and/or void volume values to more accurately model your specific system. Changing either value affects the void time (void time = void volume/flow rate).
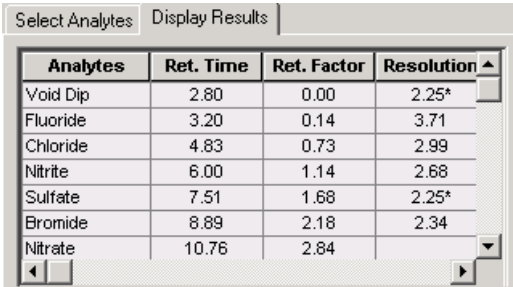
🗒️ *Note:*

If you change the flow rate in Virtual Column, the peak shapes on the virtual chromatogram are not affected. However, under actual operating conditions, peak shapes are affected by changes in the flow rate.

12. (Optional) View the retention data.

To view the calculated retention data, click the **Display Results** tab. See **How to …:** 🔼 **Viewing the Results Table** for details.

Analytes	Ret. Time	Ret. Factor	Resolution ▲
Void Dip	2.80	0.00	2.25*
Fluoride	3.20	0.14	3.71
Chloride	4.83	0.73	2.99
Nitrite	6.00	1.14	2.68
Sulfate	7.51	1.68	2.25*
Bromide	8.89	2.18	2.34
Nitrate	10.76	2.84	▼

Select Analytes / Display Results

🔳 Viewing the Results Table

After you select the analytes and choose a column, click the **Display Results** tab to view the calculated retention data. The results table lists the calculated results (**Retention Time**, **Retention Factor**, and **Resolution**) for each selected analyte. The analytes are listed in order of ascending retention time. Results are calculated based on the analyte data (peak area or concentration, asymmetry, and efficiency) and on the selected column, temperature, void time, and eluent conditions.

- ➢*Retention Time* is the time (in minutes) since injection.

- ➢*Capacity Factor* (also called retention factor) is the ratio of the net retention time to the ➢*Dead Time* (also called void time).

- ➢*Resolution* is the degree of separation between the current peak and the next peak in the chromatogram.

🔳 Viewing the Resolution Response Surface

The Resolution Response Surface is a plot of the lowest resolution values found for the Virtual Chromatogram at each possible eluent condition. Specific features of the plot vary, depending on which resolution criterion is selected (see **How to …:** 🔼 **Selecting a Resolution Criterion**) and on whether the eluent is a single- or dual-species type.

Single-Species Eluents

When a single-species eluent is used, the Resolution Response Surface is represented by a line plot. In this example, the **Minimum Resolution** criterion is selected.
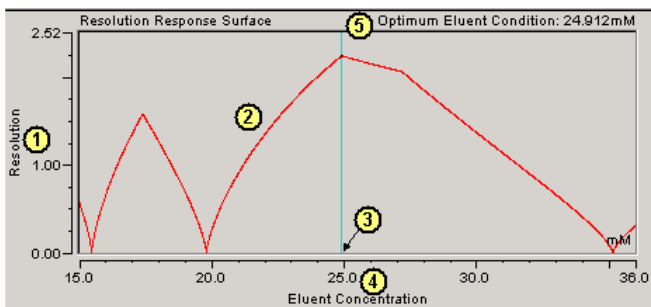
① Resolution range of the least resolved peak pairs in the Virtual Chromatogram

② Plot of the lowest resolution value found at each eluent concentration

③ Currently selected eluent concentration (the concentration at which the Virtual Chromatogram is modeled)

④ Range of eluent concentrations available in the Virtual Column database for the selected column

⑤ Optimum eluent concentration, based on the current anlayte, column, void time, temperature, and resolution criterion selections

Dual-Species Eluents

When a dual-species eluent is used, the Resolution Response Surface is represented by a contour plot. In this example, the **Minimum Resolution** criterion is selected.
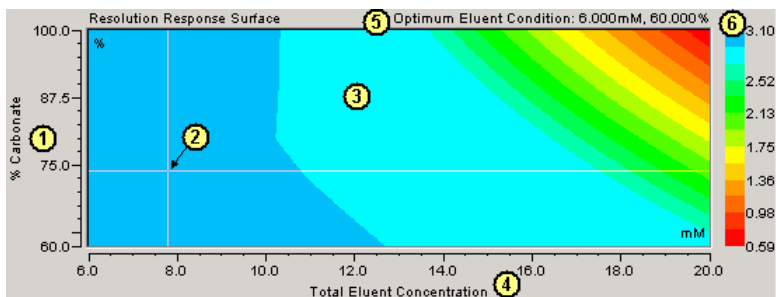
① Percentage of carbonate in the eluent

② Currently selected eluent condition (the condition at which the Virtual Chromatogram is modeled)

③ Plot of the lowest resolution value found at each eluent condition

④ Range of eluent concentrations available in the Virtual Column database for the selected column

⑤ Optimum eluent condition, based on the current analyte, column, void time, temperature, and resolution criterion selections

⑥ Range of resolutions (and the color assignment) of the least resolved peak pairs in the Virtual Chromatogram

▣ Viewing the Virtual Chromatogram

The Virtual Chromatogram simulates an actual analysis, using the currently selected analytes, column, void time, temperature, resolution criterion, and eluent condition. The Virtual Chromatogram is updated whenever the selected analytes or other parameters are changed.

The figure below describes the features of the Virtual Chromatogram. In this example, the **Minimum Resolution** criterion is selected.

Zooming/Unzooming

To zoom into an area of the chromatogram, press the left mouse button and drag to form a box around the area.
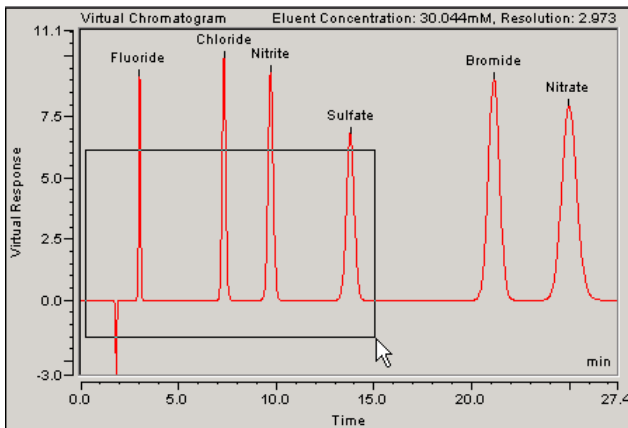
To return to the previous view, right-click and select **Unzoom**. To display the full chromatogram in the pane**,** right-click and select **Full Size**.
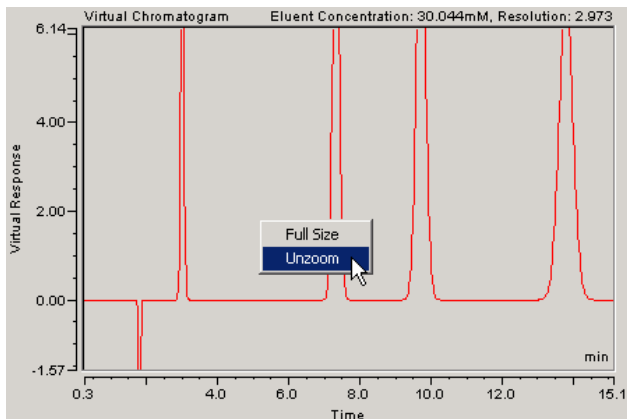
Gradient Profile

To superimpose the gradient profile (in blue) over the Virtual Chromatogram (in red), right-click and select **Gradient Profile**. This option is available only when a gradient simulation is selected.
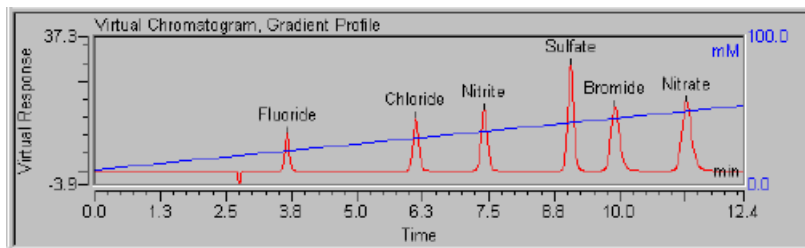
▣ Selecting a Resolution Criterion

➢*Virtual Column* provides three options for determining the optimal eluent condition for peak resolution:

- **Minimum Resolution**

- **Normalized Resolution Product**

- **Resolution Optimized for Analyte**

To select a resolution criterion, right-click on the Resolution Response Surface and select a criterion on the menu, or select an option on the **Criterion** menu on the Chromeleon menu bar.

The sections below describe each resolution criterion. Examples illustrate how each criterion affects the same Virtual Chromatogram. For the examples, the AS18 column was selected and the following list of anions was used: Bromide, Carbonate, Chloride, Fluoride, Nitrate, Nitrite, and Sulfate. **Note:** Carbonate was added to the list to better illustrate the differences among the criteria.

Minimum Resolution

When **Minimum Resolution** is selected, Virtual Column finds the least resolved peak pair for the selected eluent condition. The resolution of the entire chromatogram for that eluent condition is defined as the resolution of the least resolved peak pair. All other peak pairs of higher resolution are ignored. **Minimum Resolution** is the default criterion. This option is useful for difficult separations because it optimizes the resolution of any peak pairs that are hard to separate.

In general, a resolution value of at least 1.5 (peak areas overlap less than 0.2%) is regarded as good baseline separation. For many applications, a value of 1.2 (peak areas overlap less than 2%) is considered to be an acceptable separation. A value of 0 indicates that at least two peaks are eluting at the same retention time.
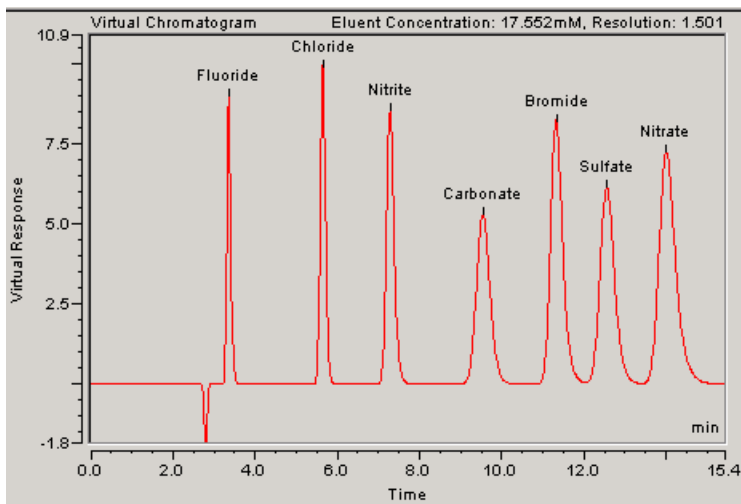
Normalized Resolution Product

When **Normalized Resolution Product** is selected, Virtual Column finds the eluent condition that provides the most evenly spaced peaks across the entire chromatogram. A normalized resolution product value of 1 indicates that all peaks are evenly resolved across the chromatogram. A value of 0 indicates that at least one peak pair is co-eluting. **Normalized Resolution Product** is useful for easy separations, as it optimizes the resolution of all peak pairs. However, for more difficult separations, Normalized Resolution Product may find a chromatogram with evenly spaced peaks, but the peaks may not all be resolved.

The normalized resolution product (r) is defined by the following equation:

$$r = \prod_{i=1}^{n-1} \left(\frac{R_{s_{i,i+1}}}{\dfrac{1}{n-1} \displaystyle\sum_{i=1}^{n-1} R_{s_{i,i+1}}} \right)$$

where n is the number of peaks and $R_{s_{i,i+1}}$ is the resolution of peaks *i* and *i*+1.

Notice that with this example, when the **Normalized Resolution Product** option is selected, the Carbonate/Nitrite peak pair is no longer resolved.
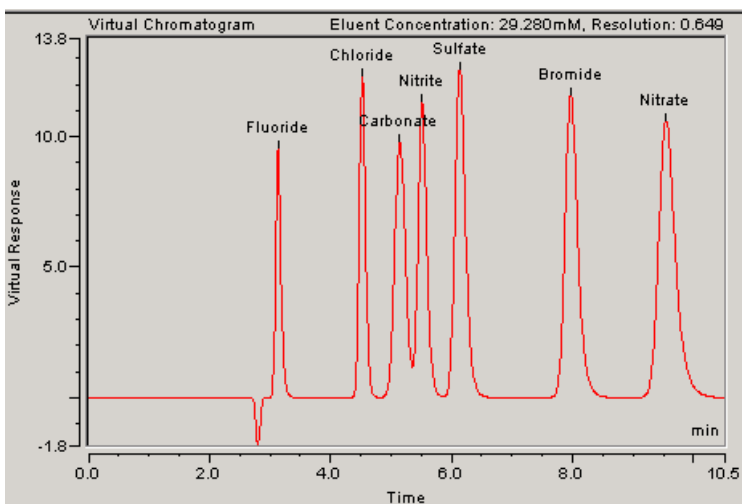
Resolution Optimized for Analyte

When **Resolution Optimized for Analyte** is selected, Virtual Column finds the eluent condition that optimizes the resolution of a selected peak. Virtual Column finds the best resolution for the selected analyte. The resolution of the other peaks is not taken into account. **Resolution Optimized for Analyte** is useful if resolving a particular analyte's peak is more critical than resolving all other peak pairs.

In this example, Chloride is the selected analyte (indicated by the asterisk next to the **Resolution** value in the **Display Results** table and the blue markers on the peak). Notice that, with this option, the Carbonate/Bromide and Nitrate/Sulfate peaks are not resolved.

Analytes	Ret. Time	Ret. Factor	Resolution
Void Dip	2.80	0.00	3.55
Fluoride	3.45	0.23	4.73
Chloride	6.12	1.18	3.63*
Nitrite	8.02	1.86	3.92
Carbonate	12.07	3.31	0.98
Bromide	12.72	3.54	2.65
Nitrate	15.88	4.67	0.57
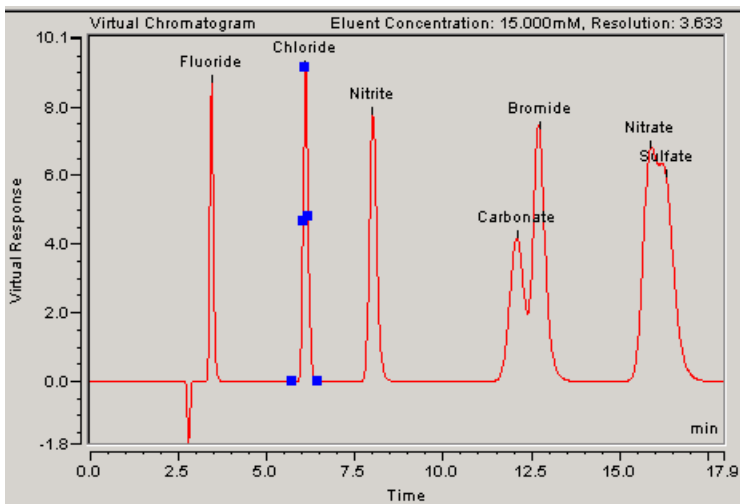
☞ *Tip*:

*When **Resolution Optimized for Analyte** is selected, you can select a different analyte to be optimized by double-clicking the analyte peak on the Virtual Chromatogram.*

◼ **Finding the Fastest Chromatogram**

➢*Virtual Column* provides an option that lets you optimize a separation for speed rather than for peak resolution.

To select this option, right-click on the Resolution Response Surface and select **Find Fastest Chromatogram** on the menu, or select the option on the **Criterion** menu on the Chromeleon menu bar. Enter the minimum acceptable resolution and click **OK**. Virtual Column finds the eluent condition that gives the fastest chromatogram that satisfies the currently selected resolution criterion.

The example below shows the results of a **Find Fastest Chromatogram** command. The minimum acceptable resolution entered was 1.5. Notice that the eluent concentration selection bar was moved from 24.912 to 29.459 mM and the chromatogram time was reduced from 11.8 to 10.5 minutes.
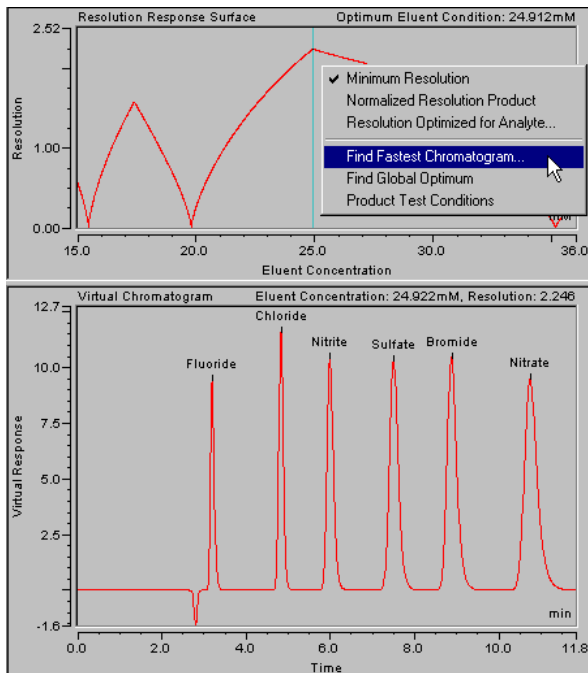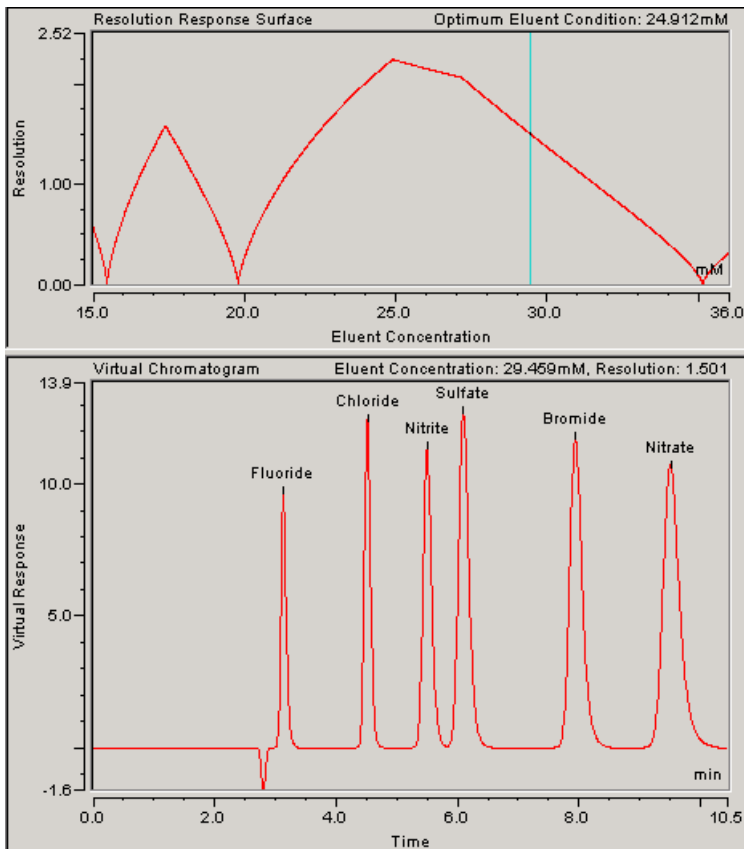
▣ Finding the Global Optimum

The ➢*Virtual Column* **Find Global Optimum** option finds the eluent composition that gives the maximum value for the selected resolution criterion (see **How to …:** ▣ **Selecting a Resolution Criterion**). To select this option, right-click on the Resolution Response Surface and select **Find Global Optimum** on the menu, or select the option on the **Criterion** menu on the Chromeleon menu bar. This option is the default when you select a column or resolution criterion.

◨ Selecting the Column Quality Assurance Conditions

The ➢*Virtual Column* **Column Quality Assurance Conditions** option sets the eluent composition to that specified in the production control test performed on every Dionex column before it is shipped. For details about the production test, refer to the column manual.

To select this option, right-click on the Resolution Response Surface and select **Column Quality Assurance Conditions** on the menu, or select the option on the **Criterion** menu on the Chromeleon menu bar. This option is available only when an isocratic modeling case is selected.

📒 *Note:*

*The **Column Quality Assurance Conditions** option changes the eluent composition, but does not affect the analyte selections. If the production test includes more analytes than those selected for display in Virtual Column, the missing analytes are not automatically added to the Virtual Chromatogram.*

◨ Manually Selecting an Eluent Condition

To determine the optimum eluent condition, you can select an option (**Find Fastest Chromatogram**, **Global Optimum**, or **Column Quality Assurance Conditions**) on the ➢*Virtual Column* **Criterion** menu or you can manually select the eluent condition on the Resolution Response Surface.

To manually select the eluent condition if you are working with a single-species eluent, drag the single vertical bar to the desired eluent concentration (see the example below), or click on the plot where you want to move the bar. The virtual chromatogram is updated to reflect the new concentration.
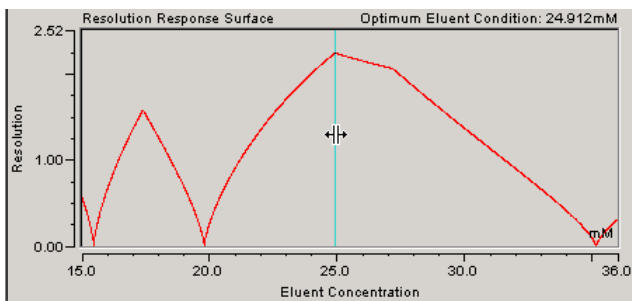
 Note:

*If a gradient **Methodology** option is selected, moving the vertical bar adjusts the gradient slope rather than the eluent concentration.*

If you are working with a dual-species eluent, two eluent selection bars are provided: one for total eluent concentration and one for ratio of the species. You can move both bars together or drag each individually. To move both bars, click on the Resolution Response Surface at the desired eluent composition, or point to the intersection of the two bars, wait for the pointer to change to a four-directional arrow and then drag the bars. See the following example.
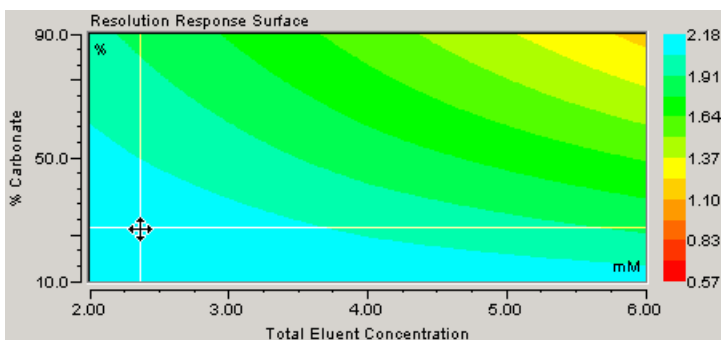
To move only one of the bars, point to the bar, wait for the pointer to change to a two-directional arrow, and drag the bar to the desired location. Drag the horizontal bar to change the ratio of the species (see the example below) or drag the vertical bar to change the total eluent concentration.
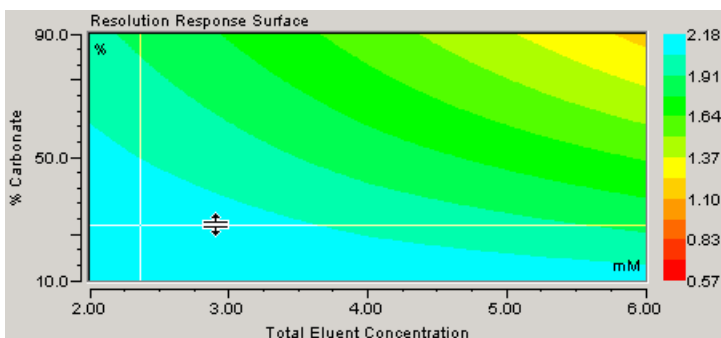
▣ Saving and Reloading Virtual Column Settings

After you have selected analytes and a column, you can save the selected settings to a file to be available for later use. The following information is saved and can be reloaded from the saved file:

- Each analyte's name, peak area or concentration, asymmetry, efficiency, and retention time information

- Resolution criterion

- Injection volume

- Methodologies

- Column name and size

- Temperature

- Eluent concentration at the gradient start

- Flow rate

- System void time and void volume

When you reload the saved file, the settings are restored and the corresponding Resolution Response Surface and Virtual Chromatogram are displayed.

To save settings:

Select **Save** on the **File** menu. The .vcol extension is assigned to the saved file.

☞ *Tip***:**

Virtual Column settings files are saved in the ..\My Documents\Virtual Column Custom Files folder.

To reload a saved file:

1. Select **Open** on the **File** menu.

2. Select a .vcol file and click **OK**.

   The **Load Selected File** dialog box opens. This dialog box displays the analyte data saved in the selected .vcol file and gives you the opportunity to choose the data from the saved file that you want loaded into the Virtual Column analyte table.

3. Clear the check box above any column (**Peak Area**, **mg/l**, **mM**, **Asymmetry**, or **Efficiency**) that you do not want loaded.

4. Clear the **Void Time** check box if you do not want the void time loaded.

5. Clear the **Inj. Volume** check box if you do not want the injection volume loaded.

 Note:

*If you clear a check box in the **Load Selected File** dialog box, Virtual Column loads the current user interface setting for the item instead of the data from the saved file. If no setting for the item exists (or if the setting is invalid), Virtual Column loads data for the item from the embedded Virtual Column database.*

6. Click **OK**.

Viewing the Eluent Composition

➢*Virtual Column* can provide detailed instructions for preparing the eluents corresponding to the currently selected conditions. This option is available only when an isocratic simulation is selected.

1. Select **Eluent Composition** on the **View** menu on the Chromeleon menu bar.

   The **Eluent Composition** dialog box opens.

2. Click the **Eluent Species** option button to display information for the eluent of interest.

3. Click **Print** to print the contents of the dialog box.

4. Click **Close** to exit.

▣ Modeling Gradient Separations

➢*Virtual Column's* gradient predictions are based on gradient data acquired under conditions that equate to zero delay time. This means that the start of the concentration ramp reaches the column at exactly the same moment as the sample, thus eliminating any effects of isocratic elution of the sample before the ramp begins.

If you try to reproduce results obtained in Virtual Column, take into consideration that your results may include a delay time, while those obtained with Virtual Column do not. This should have little effect on the results unless high starting concentrations are used in conjunction with low slopes; in that case, monovalent species with short run times may experience a shift in retention time compared to other species.

 Note:

The Chromeleon Virtual Column Linear Gradient license is required to use Virtual Column to simulate gradient separations.

Device Control

Device Control (Overview)

Chromeleon allows you to control several HPLC, IC, and GC devices from various manufacturers. To support the different device functions, you can use the corresponding commands in Chromeleon. For more information, refer to:

 General Commands for Device Control

 Commands for Controlling Dionex Devices

 Commands and Tips for Third-Party Devices

Also, refer to **Practical Tips for Device Control**

General Commands for Device Control

Certain commands are used independent of the installed devices, for example:

🔲 **System Commands**
🔲 **General Commands**
🔲 **General Device Commands**

For information about special commands for the respective devices, refer to:

🔲 **Commands for Controlling Dionex Devices**
🔲 **Commands and Tips for Third-Party Devices**

🔲 System Commands

System control comprises all commands that concern the entire chromatographic process or the entire system. The commands are available via both **Command** on the **Control** menu and the **F8** key in the **Commands** view of the PGM Editor. Some commands are available on the toolbar, also.

Command	Description
⇒*AbortSample*	Stop data acquisition and sample; continue the ➢*Batch* with the next sample.
⇒*AbortBatch*	Stop data acquisition and batch processing.
ConsumableChanged	Log in the ➢*Audit Trail* that a consumable has been changed.
⇒*Continue*	Continue all operations that are in **Hold** mode.
EluentChanged	Log in the ➢*Audit Trail* that the solvent has been changed.
⇒*Hold*	Freeze a running gradient program, stop data acquisition, and stop batch processing.
HoldMode	If **Hold** mode is enabled, the timebase waits for an event, e.g., a **Continue** command. In **Hold** mode, the retention time and the pump gradient are stopped. The pump continues to deliver the solvent; the flow rate and the solvent composition remain unchanged.
⇒*Sound*	Generate a sound of selectable frequency and duration that is heard on the PC loudspeaker.
⇒*StopFlow*	Stop pump flow, interrupt data acquisition, and stop batch processing.
StopMode	If **Stop** mode is enabled, the timebase waits for an event, e.g., a **Continue** command. In **Stop** mode, the retention time and the pump flow are stopped.

▪ General Commands

Independent from the installed instruments, Chromeleon supports the following general commands (under the device commands):

Command	Description
⇒*Branch*	Completes the active ➢*Program* and starts another one
⇒*Delay*	Especially with trigger commands, execution of the next program command is delayed for the specified time.
⇒*EndTrigger*	Continues all processes in hold mode.
⇒*End*	Starts or stops data acquisition.
⇒*If*	Control flow for conditionally executing a group of statements.
⇒*ElseIf* ⇒*Else* ⇒*EndIf*	The IF structure allows you to execute a command only if a certain condition is true. If the IF condition is true, the statements enclosed between IF and ENDIF are executed. Otherwise, they are ignored. By using ELSEIF, it is possible to combine several conditions. The statements of the final ELSE will be executed if none of the ELSEIF conditions is true either.
⇒*Log*	Logs device variable values in the Audit Trail (see **Data Management** 🔲 **Audit Trails**).
⇒*Message*	Displays a message to the user and interrupts the program until the message is clicked.
⇒*Protocol*	Logs any message in the ➢*Audit Trail*
⇒*Trigger*	Freezes a running gradient program, stops data acquisition, stops sample processing.
⇒*Wait*	Interrupts the program until a certain condition is fulfilled.

▪ General Device Commands

Most device commands are specific for a certain type of device or even for a certain device. For example, the ⇒*Flow* command is available for pumps and the ⇒*Inject* command is available for autosamplers. However, some commands are available independent of the device type:

Command	Description
⇒*Connect*	Connects the device to Chromeleon (also, see Connected, Disconnect).
Connected	Indicates whether the pump is connected to Chromeleon, that is, under computer control (also, see Connect, Disconnect)
Disconnect	Disconnects the pump from Chromeleon (also, see Connect, Connected).

The following commands and properties appear very often. However, they are not available for every device:

Command/Property	Description
FirmwareVersion	Indicates the firmware version of the device (read-only).
ModelNo	Indicates the device type (read-only).
Ready	**Ready** indicates that the device is ready for operation, i.e., no Autozero is started, the temperature has been reached, no error has occurred. **NotReady** indicates that the device is not ready for operation (read-only).
⇒*Reset*	Resets the device to its initial conditions, as attained after power-up.
SerialNo	Indicates the serial number of the device (read-only).

Commands for Controlling Dionex Devices

Chromeleon supports all functions and commands of Dionex devices. This section describes the special commands that Chromeleon supports for these devices.

For information about the general commands supported for the respective device types and often for third-party devices, too, refer to:

🔲 **Dionex Pumps**

🔲 **Dionex Sample and Fraction Manager**

🔲 **Dionex Autosamplers**

🔲 **Dionex Flow Manager and Thermostatted Column Compartments**

🔲 **Dionex Detectors**

🔲 **Dionex Ion Chromatography Systems**

🔲 **Additional Dionex Components**

Optimum support for all functions is ensured for Dionex devices. For a list of the third-party devices that are currently supported, refer to **Hardware Installation** 🔵 **Installing and Controlling Third-Party Devices** in the **Administrator Help** section.

For information about the commands for third-party devices, refer to 🔲 **Commands and Tips for Third-Party Devices**.

Dionex Pumps

In addition to the ⬛ **General Device Commands**, Chromeleon supports the following commands for the control of Dionex HPLC and IC pumps:

Command	Description
⇒*Flow*	Enter the flow rate.
⇒*Pressure.Lower/UpperLimit*	Sets the pressure limits.
⇒*WasteLevel*.Value	Enter the actual waste level (i.e., the liquid level in the waste container) before starting a sequence.
WasteLevel.UpperLimit	Enter the upper limit for the liquid level in the waste container in [l].
⇒*WasteWarningLimit*	Enter the warning limit for monitoring the liquid level in the waste container. Enter the limit as a percentage of the **WasteLevel.UpperLimit**.

The following property is related to this:

Property	Description
⇒*WasteRemainTime*	Reports the time remaining until the liquid level in the waste container reaches the upper limit (**WasteLevel.UpperLimit**).

The following additional commands are available for controlling gradient pumps (DGP-3600, DP/SP, GP40/GP50, GS50, HPG-3x00, ISO-3100, LPG-3x00, P580, and P680):

Command	Description
⇒*%B, %C, %D*	Changes the solvent composition and determines the gradient profile.
⇒*%A, %B, %C, %D_Level*	Enter the solvent volume that is available when you start the sequence.
%A-, %B-, %C-, %D_Level.LowerLimit	Enter the lower limit for the solvent volume in [l].
⇒*%A, %B, %C, %D_WarningLimit*	Enter the warning limit for monitoring the liquid level in the solvent reservoir. Enter the limit as a percentage of the **%A, %B, %C, %D_Level.LowerLimit**.

The following property is related to this:

Property	Description
⇒*%A-, %B-, %C-, %D_RemainTime*	Reports the time remaining until the liquid level in the solvent reservoir reaches the lower limit (**%A, %B, %C, %D_Level.LowerLimit**).

Commands can be selected directly (via the toolbar, menu bar, or a control) or as part of a ➢*Program* (programmable button).

They enable delivery of a specific liquid volume of defined composition, as well as starting and holding the pump. The pump is automatically placed on hold as soon as the upper or lower pressure limit is exceeded. Changing the flow rate creates a ➢*Flow Gradient*, while changing the solvent composition creates a ➢*% Gradient*. Flow and % gradients can be realized simultaneously.

🖙 *Tip:*

*Dionex GP40/GP50, GS50, IC20/IC25, IP20/IP25, and IS25 pumps do not deliver flow gradient ramps. Instead, changing the flow rate creates a step change; that is, flow rate changes are made immediately, not gradually over time. Refer to **Practical Tips for Device Control** 🔳 **Pump Commands** for additional information.*

DGP-3600, DP/SP, HPG-3x00, ISO-3100, LPG-3400, LPG-3x00, P580, and P680 Pumps

The ➢*UltiMate 3000* system pumps (the DGP-3600, HPG-3x00, ISO-3100, and LPG-3400), as well as the DP/SP, LPG-3x00, P580, and P680 pumps, all support automatic pre-compression control. The pumps are capable of adjusting to the compressibility of commonly used solvents. In addition, the **Learn** and **Freeze** commands allow the P580 pump to extend pre-compression control to unknown solvent types. Pre-compression control is fully automatic for the DGP-3600, DP/SP, HPG-3x00, LPG-3x00, and P680 pumps; therefore, the **Learn** and **Freeze** commands are not required.

Command input is identical for the ➢*Low-Pressure Gradient System* and ➢*High-Pressure Gradient System*.

⚠️ *Caution:*

Chromatography pumps are high-precision instruments! Dry operation or crystallization of buffer solutions within the fluidic system must be avoided. The most common cause of such problems is stopping pump flow without turning off the lamp in an optical detector. The flow cell heats up, and the solvent evaporates. Deposits of substances can result, for example, from the presence of salt in a buffer solution.

For information about special commands for the pumps, refer to

🔳 **Dionex DGP-3600 Pump**

🔳 **Dionex DP/SP IC Pumps**

🔳 **Dionex HPG-3x00 Pumps**

🔳 **Dionex ISO-3100 Pump**

🔳 **Dionex LPG-3400 Pump**

🔳 **Dionex LPG-3x00 Micro Pump**

🔳 **Dionex P680 HPLC Pump**

GP40/GP50, GS50, IC20/IC25, IP20/IP25, and IS25 Pumps

In addition to the general pump commands, the following commands are available for controlling the isocratic or gradient pumps listed above:

Command	Description
On	Turns the pump motor on
Off	Turns the pump motor off
Prime	Primes the pump

For pump operating specifications, refer to the individual pump operator's manuals.

Also, refer to:

🔳 **Dionex/LC Packings UltiMate Capillary/Nano HPLC Pump**

🔳 **PP-150 Preparative HPLC Pump**

🔳 **Controlling Pumps Without a Separate Device Driver**

▣ Dionex UltiMate 3000 Series - Pumps

In addition to the standard pump commands (see ▣ **Dionex Pumps**) and the P680 pump commands (see ▣ **Dionex P680 HPLC Pump**), the UltiMate 3000 pumps support the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Flow and Pressure commands and properties

Chromeleon supports the following commands and properties only if the pump is connected to a flow splitter. (In the standard configuration, Dionex recommends assigning the flow splitter to the right pump (MicroPump).)

Command/Property	Range	Description
Flow	0.000 - 2500.000	Sets the flow rate [µl/min]. This is the total flow through the column. (The valid range depends on the split ratio.)
MasterFlow	0.000 - 2500.000	Reports the flow through the master pump, i.e., the flow before the flow splitter, in [µl/min].
MasterPressure	0 - 500 bar	Reports the pressure of the master pump, i.e., the pressure before the flow splitter. (This property is read-only. The channel is recorded as MicroPump/_MasterPressure.)
*.LowerLimit *.UpperLimit		Sets the lower and upper pressure limits at the master pump. If the pressure exceeds the upper limit for 0.15 seconds or if it is below the lower limit for more than 60 seconds, the system aborts the running batch and starts error handling (see ⇒*Pressure.Lower/UpperLimit*).
MaximumFlowRampDown	infinite - 9999999 µl/min²	Sets the upper limit for the flow rate deceleration.
MaximumFlowRampUp	infinite - 9999999 µl/min².	Sets the upper limit for the flow rate acceleration.
Pressure	0 - 350 bar	Reports the current column pressure. (This property is read-only. The channel is recorded as ColumnPressure.)
*.LowerLimit *.UpperLimit		Sets the lower and upper pressure limits at the column. If the pressure exceeds the upper limit or if it is below the lower limit for more than 5 minutes, the system aborts the running batch and starts error handling.

If the pump has no flow splitter assigned, Flow and Pressure refer directly to the pump outlet. In the standard configuration, this is the LoadingPump, which is the left pump.

Command/Property	Range	Description
Flow	0.000 - 2500.000	Sets the flow rate [µl/min]. This is the total flow through the pump.
Pressure	0 - 500 bar	Reports the pressure. (This property is read-only. The channel is recorded as LoadingPump_Pressure.)
*.LowerLimit *.UpperLimit		Sets the lower and upper pressure limits at the loading pump. If the pressure exceeds the upper limit for 0.15 seconds or if it is below the lower limit for more than 60 seconds, the system aborts the running batch and starts error handling (see ⇒*Pressure.Lower/UpperLimit*).

Display commands and properties

Command/Property	Range/Values	Description
Brightness	0 - 100 %	Adapts the brightness of the pump's front panel display to your requirements.
Contrast	0 - 100 %	Adapts the screen contrast of the pump's front panel display to your requirements.
DisplayMode	Master Column	Specifies which information appears on the pump's front panel display. (This property is available only for micro pumps.)

Additional commands and properties

Command/Property	Range/Value	Description
InstalledOn		Indicates the timebase in which the pump is installed.
⇒*ParkPercentage*	0.00 - 100.00 %	Enables peak parking. The flow is reduced to the specified percentage of the current flow.
PeakParked	No, Yes	Reports the status of peak parking (read-only). Reports **Yes** while the pump delivers the reduced flow set by **ParkPercentage**.
		(This command is not available for the HPG-3x00, ISO-3100A, LPG-3400A, and LPG-3600A pumps.)
Purge	On, Off	Set to On to enable purging of the pump. Also, see **PurgeTime** and **PurgeFlow**.

Command/Property	Range/Value	Description
PurgeFlow		Enter the flow [in mL/min] to be delivered by the pump during purging. Also, see **Purge** and **PurgeTime**.
PurgeTime		Specify how long purging shall be performed [s]. Also, see **Purge** and **PurgeFlow**.
PurgeViaSampler	Yes, No	Set to **Yes** to enable purging of the pump via the autosampler. See also **Purge** and **SamplerDevice**.
		Note:
		This function is only available if your UltiMate 3000 system includes a WPS-3000(T)SL autosampler.
RearSealWashPump	Idle, Active	Indicates whether the peristaltic pump of the rear seal wash system is running or not (read-only).
RearSealWashSystem		Turns the rear seal wash system on (**Interval** or **Automatic**) or off. **Interval** activates rear-seal washing once per hour for five minutes. However, the drop sensor on the liquid reservoir is not active, i.e., piston seal monitoring is disabled. **Automatic** periodically activates rear-seal washing once per hour until the drop sensor has counted 50 drops. The drop sensor is active, i.e., piston seal monitoring is enabled. **Off** turns the rear-seal wash system off.
RearSealWashInterval		Specifies the time interval [min] when the next rear seal wash is performed.
Relay2Enabled	Yes (0), No (1)	Determines whether relay 2 can be used in Chromeleon (**Yes**) or whether it is controlled by the pump for the Left Cam Sync Out signal (**No**).
Relay3Enabled	Yes (0), No (1)	Determines whether relay 3 can be used in Chromeleon (**Yes**) or whether it is controlled by the pump for the Operable Out signal (**No**).
Relay4Enabled	Yes (0), No (1)	Determines whether relay 4 can be used in Chromeleon (**Yes**) or whether it is controlled by the pump for the Right Cam Sync Out signal (**No**).
SamplerDevice		Indicates the name of the autosampler that is used to purge the pump. See also **Purge**, **PurgeViaSampler**.

Command/Property	Range/Value	Description
SelfTest	Passed or NotPassed	Performs a self-test. 🗒️ *Note:* *You cannot start a batch when the pump failed the self-test.*
SelfTestPassed	Yes, No	Reports whether the pump has passed the self-test (read-only). **Yes** means that the pump has passed the self-test. See also **SelfTest**.
Standby	NoStandby, Standby	Sets the pump into the Standby mode or cancels this mode (NoStandby). The pump remains connected to Chromeleon. 🗒️ *Note:* *You cannot start a batch when the pump is in standby mode.*

Equilibration commands and properties

👉 *Tip:*

The Chromeleon version and pump type determine which commands and properties are available for equilibration.

Use the equilibration commands and properties only for the ➢*SmartStartup* feature.

The SmartStartup Wizard supports the following pumps of UltiMate 3000 pump series:

- ISO-3100A

- DGP-3600A, DGP-3600M

- LPG-3400A, LPG-3400M, LPG-3300M

- HPG-3200A, HPG-3400A, HPG-3200P

Command/Property	Description
Equilibration	Reports the equilibration status.
Ripple	Reports the ripple of the pump pressure.
UpperLimit	Determines the upper limit for the ripple of the pressure signal in [%]. The upper limit can be any value between 1.0% and 5.0%. The default setting is 3.0%.
Value	Reports the actual value, which is usually averaged over the last 5 minutes (read-only). At the beginning (i.e., after installation of the pump), the value field is empty, indicating that no measurement has been performed yet. Chromeleon calculates the value as soon as a 1-minute segment has been measured, and updates the value with each new segment. The value of the property does not change after the measurement and is displayed under ➢*Preconditions*.
RippleStatus	Reports the ripple equilibration status of the pump pressure (read-only).
N/A	Equilibration has not started.
Measuring	Equilibration is running, but the amount of data is not yet sufficient to report a result.
Good	Equilibration is running or has finished. The values are within the specified limits.
NotReady	Equilibration is running. The current data is outside the specified limits.
Failed	Equilibration is completed. The result is outside the specified limits.
NotTested	The limit was set to 0, thus disabling ripple checking.
StartEquilibration	Starts equilibration. For more information, refer to **How to ...:** **Equilibrating the Chromatography System**.

System Wellness and Predictive Performance commands and properties

Tip:

The Chromeleon version and pump type determine which commands and properties are available for checking the pump's system wellness and performance.

Command/Property	Description
CheckValveServiceLeft/ Right	Reports service information about the related check valve.
*.LastDate	Reports the date when the check valve was serviced last (read-only).
*.Interval	Specifies the interval (in days) when the next service for the check valve is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
CheckValveServiceDone Left/Right	After you have replaced the related check valve, perform this command to update the related service information.
OperatingHours	Counts the operating hours since initial operation (excluding the operating hours in standby mode; read-only).
OutletFritChangeLeft/ Right	Provides service information about the filter frits in the related outlet unit.
*.LastDate	Reports the date when the frit was replaced last (read-only).
*.Interval	Specifies the interval (in days) after which a service of the outlet frit is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
OutletFritChangedLeft/ Right	After you have replaced the outlet frit, perform this command to update the related service information.
PistonsChangedLeft/Right	After you have replaced a piston, perform this command to update the related service information.
PistonChangeDateLeft/ Right	Reports the date when the piston was changed last (read-only).
PistonWorkLoadLeft/Right	Provides information about the piston work load.
*.Value	Indicates the current work load [MJ] of the related piston (read-only).
*.Warning	A warning appears when the next service is due within the specified period.
*.Limit	An error message appears when the limit is reached.
PowerOnTime	Counts the time since last power-up (or last wake-up from standby) (read-only).
ProportioningValve_A/B/ C/D_CyclesLeft/Right	Counts how many times the related valve was switched (read-only).

Command/Property	Description
PumpBlockServiceDone Left/Right	Perform this command to update the related service information after you have serviced a pump block.
PumpStrokesLeft/Right	Counts the number of piston strokes in the related pump block (read-only).
Qualification	Provides information about system qualification.
*.LastDate	Reports the date when system qualification was performed (read-only).
*.LastOperator	Reports the name of the person who performed the system qualification (read-only).
*.Interval	Reports the interval (in days) when the next system qualification is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
*.GracePeriod	Indicates the period during which the device may be operated after the due date for re-qualification.
QualificationDone	Perform this command after qualification to update the related system data.
RearSealLeakage	Provides information about pump leakage.
*.Value	Reports how many drops passed the main piston seal in the past hour (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message when the specified value is reached.
RearSealWashPump Hours	Counts the operating hours of the peristaltic pump (read-only).
RearSealWashTube Changed	Perform this command to update the related service data after you have replaced the wash tubing.
RearSealWashTube ChangeDate	Reports when the wash tubing was replaced last (read-only).
RearSealWashTubeHours	Provides information about the operating time [h] of the peristaltic pump with the currently installed wash tube.
*.Value	Reports the current operating time [h]of the peristaltic pump with the currently installed wash tube (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
SealChangeDate Left/Right	Reports when the related seal was replaced last (read-only).
SealWorkLoad Left/Right	Counts the work load of the related rear seal since the seal was replaced last.
*.Value	Reports the current work load of the rear seal since the seal was replaced last (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
SealsChangedLeft/Right	After you have replaced the seals of the related pump block, perform this command to update the related service information.

Command/Property	Description
Service	Provides service information.
*.LastDate	Reports the date of the last service (read-only).
*.Interval	Specify the interval (in days) after which a service is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
ServiceDone	After the device has been serviced, perform this command to update the related information.
StandbyHours	Counts the total operating hours in standby mode since the module was operated for the first time (read-only).
WorkLoadSinceLast ServiceLeft/Right	Counts the work load [MJ] of the related pump block since the last service (read-only).
WorkLoadLeft/Right	Reports the total work load [MJ] of the related pump block since initial operation of the module (read-only).

For information about how to install the pumps, refer to **Hardware Installation** 🔵 **UltiMate 3000 Series - Pumps: Overview** and
🔵 **LPG-3x00 Micro Pump: Overview** in the **Administrator Help** section.

🔳 Dionex P680 HPLC Pump

In addition to the standard pump commands (see 🔳 **Dionex Pumps**), the P680 pumps support the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Description
AnalogOut	Determines whether the pressure of the left or right pump is monitored via the Analog Out port on the pump (available for pumps with firmware 2.50.05 or higher).
CamRevolutionsRight CamRevolutionsLeft	Indicates the number of revolutions of the camshaft of the right of left pump block. The **CamRevolutionsLeft** command is available for HPG pumps only.
Curve	Determines the curvature for flow gradients (see ➢*Gradient Curves*).
Degasser	Turns the solvent rack degasser **On** or **Off** (P680A LPG-4, SOR-100, or SRD-3x00 only). The integrated degasser should always be on.
DegasserVacuum	Reports the status of the solvent rack degasser (P680A LPG-4, SOR-100, or SRD-3x00 only): **OK** or **NotOK** (read-only; available for pumps with firmware 2.03 or higher).
HeadType	Indicates the head type of the pump: **Analytical** or **Semi-Prep** (read-only).

Command/Property	Description
Leak	Indicates the status of the central leak sensor: **OK** or **Leak** (read-only).
LeakAlarm	**On** indicates that there is a leak alarm (read-only; available for pumps with firmware 2.50.05 or higher).
LeakAlarmOff	Turns off the acoustic beep for the current alarm (available for pumps with firmware 2.50.05 or higher).
LeakDelay	Specifies how much time elapses between the detection of a leak and the pump shutdown (available for pumps with firmware 2.50.05 or higher).
LeakSensorMode	Specifies how leak detection is performed: **Enabled**–Enables leak detection; an acoustic beep sounds when the leak sensor is activated. **Silent**–Enables leak detection; no beep sounds when the leak sensor is activated. **Disabled**–Disables leak detection.
MaximumFlowRamp	Specifies the upper limit for the flow rate acceleration (0.1 - 10,000.00 ml/min²).
ModelVariant	Indicates the variant: **Isocratic**, **LPG**, or **HPG** (read-only).
Motor	Indicates whether the pump is delivering: **On** or **Off** (read-only).
Pump_Pressure	Commands and properties for the pressure signal (see below).
⇒*Purge*	Enables purging when set to **On** (available for pumps with firmware 2.50.06 or higher).
PurgeFlow	Enter the flow [in mL/min] to be delivered by the pump during purging (range: 0.000 to 10.000 ml/min or 20.000 ml/min for HPG pumps with enabled Double Flow option; available for pumps with firmware 2.50.05 or higher).
PurgeTime	Specifies for how long purging is performed (0 - 1000 s) (available for pumps with firmware 2.50.05 or higher).
RearSealLeakCounter	Indicates the number of drops that passed the piston seal in the past hour.
RearSealLeakLimit	Specifies the leak detection threshold for the rear seal wash system. If the limit is met, a message is displayed in the Audit Trail and on the pump.
RearSealWashPump	Indicates whether the peristaltic pump of the rear seal wash system is running (**Active**) or not (**Idle**) (read-only).
RearSealWashSystem	Turns the rear seal wash system on (**Interval** or **Automatic**) or **Off**.
	Interval–Activates rear-seal washing for 5 minutes every hour. However, the drop sensor on the liquid reservoir is not active, i.e., the piston seals are not monitored for tightness.
	Automatic–Periodically activates rear-seal washing once per hour until the drop sensor has counted 50 drops. The drop sensor is active, i.e., the piston seals are monitored for tightness.
	Off–Turns the rear-seal wash system off.

Command/Property	Description
SelfTest	Performs a self-test to check that all main components are functioning correctly.
SelfTestPassed	Reports the results of the self-test (read-only). If the pump fails the test, eliminate the cause of the error and/or fault. For troubleshooting help, refer to the operator's manual for the pump.
SolventRackLeak	Reports whether the leak sensor in the solvent rack detected a leak: **Leak** or **NoLeak** (read-only; available for pumps with firmware 2.03 or higher).
WorkLoadLeft	Indicates the workload of the left pump block in [MJ] (read-only). The **WorkLoadLeft** command is available for HPG pumps only.
WorkLoadRight	Indicates the workload of the right pump block in [MJ] (read-only).

Pump_Pressure

If you select the **Pressure Signal(s)** check box on the **Devices** page in the Server Configuration program, Chromeleon records the pump pressure as a separate channel (**Pump_Pressure**). The following commands and properties are available:

⇒*AcqOn/AcqOff*	AcqOn starts data acquisition. AcqOff terminates data acquisition.
⇒*Average*	Averages all measured values.
⇒*Delta*	Reports the signal's slope, i.e., the difference between the value and the value 1 second ago (read-only). This is useful for ⇒*Triggers*.
⇒*MaxAutoStep*	Sets the maximum step rate for Step = Auto.
Retention	Indicates the retention time for the signal.
⇒*Step*	Sets the step for data acquisition.

Equilibration Commands and Properties

■☞ *Tip:*

You should use the equilibration commands and properties only for the
➢*SmartStartup feature.*

Equilibration		Reports the equilibration status.
Ripple		Reports the ripple of the pump pressure.
	UpperLimit	Determines the upper limit for the ripple of the pressure signal in [%]. The upper limit can be any value between 1.0% and 5.0%. The default setting is 3.0%.
	Value	Reports the actual value, which is usually averaged over the last 5 minutes (read-only). At the beginning (i.e., after installation of the pump), the value field is empty, indicating that no measurement has been performed yet. Chromeleon calculates the value as soon as a 1-minute segment has been measured, and updates the value with each new segment. The value of the property does not change after the measurement and is displayed under ➢*Preconditions*.
RippleStatus		Reports the ripple equilibration status of the pump pressure (read-only).
	N/A	Equilibration has not started.
	Measuring	Equilibration is running, but the amount of data is not yet sufficient to report a result.
	Good	Equilibration is running or has finished. The values are within the specified limits.
	NotReady	Equilibration is running. The current data is outside the specified limits.
	Failed	Equilibration is completed. The result is outside the specified limits.
	NotTested	The limit was set to 0, thus disabling ripple checking.
StartEquilibration		Starts equilibration. For more information, refer to **How to ...:** 🔳 **Equilibrating the Chromatography System**.

For information about how to install the pump, refer to **Hardware
Installation** 🔵 **P680 HPLC Pump: Overview** in the **Administrator Help**
section.

▣ Dionex DP/SP Pumps

In addition to the standard pump commands (see ▣ **Dionex Pumps**), the DP/SP pumps support the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Description
CamRevolutionsLeft CamRevolutionsRight	Indicates the number of revolutions of the camshaft of the left or right pump block.
Degasser	Turns the integrated vacuum degassing module **on** or **off**. The degassing module normally remains on at all times.
HeadType	Indicates the head type of the pump: **Analytical** or **Semi-Prep** (read-only).
Leak	Indicates the status of the main leak sensor: **Ok** or **Leak** (read-only).
MaximumFlowRamp	Upper limit for the flow rate acceleration: (0.1 to 10,000.00 ml/min²).
ModelVariant	Indicates the variant: **Isocratic** or **Gradient** (read-only).
Motor	Indicates whether the pump is delivering: **On** or **Off** (read-only).
RearSealLeakCounter	Indicates the number of drops that passed the piston seal in the past hour.
RearSealLeakLimit	Specifies the leak detection threshold for the rear seal wash system. If the limit is reached, a message is displayed in the Audit Trail.
RearSealWashPump	Indicates whether the peristaltic pump of the rear seal wash system is running (**Active**) or not running (**Idle**) (read-only).
RearSealWashSystem	Turns the rear seal wash system on (**Interval** or **Automatic**) or **Off**. **Interval** activates rear-seal washing for 5 minutes every hour. During this time, the drop sensor on the liquid reservoir is not active, i.e., the pistons seals are not monitored for tightness. **Automatic** periodically activates rear-seal washing once per hour until the drop sensor has counted 50 drops. During this time, the drop sensor is active, i.e., the piston seals are monitored for tightness. **Off** turns off the rear-seal wash system.
WorkLoadLeft	Indicates the workload of the left pump block in MJ (read-only).
WorkLoadRight	Indicates the workload of the right pump block in MJ (read-only).

▣ Dionex/LC Packings UltiMate Capillary/Nano HPLC Pump

In addition to the standard pump commands (see ▣ **Dionex Pumps**), the pumps of the UltiMate system support the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Description
CycleTime	Indicates the time in [s] for a switching cycle of the LPG pump proportioning valves; values between 1 and 5s are possible.
CalibratorType	Indicates the calibrator type, for example, NAN-75 (read-only).
Description	Optional device description (read-only).
⇒*Flow*	Indicates the system flow, i.e., flow through the separation column: 0.000 to 200.000 µl/min
HeadType	Indicates the pump head (read-only):
	None
	Steel_10ml
	Ceramic_10ml
InstallationDate	Indicates the installation date (read-only).
LastHeadRevolutionCounter	Indicates the total number of cycles performed by the corresponding pump head (read-only).
LastService Date	Indicates the date when the last service procedure was performed (read-only).
LastServiceCode	Indicates the code number of the last service (read-only).
Motor	Turns the pump on or off.
⇒*ParkPercentage*	Enables or disables peak parking and sets the new flow to the entered percentage of the previous flow (values: 0 - 100% or Disabled).
PeakParked	State of peak parking (read-only).
PowerIndex	Pressure index in [MPah] (read-only) - a measure of pump wear: $\int$ Pressure dt
Pressure.LowerLimit Pressure.UpperLimit	If these limits are exceeded/not met for more than 60 seconds, the system aborts the running batch and starts emergency handling (see ⇒*Pressure.Lower/UpperLimit*)
TotalPumpCycles	Indicates the total number of pump cycles (read-only).
TotalVolume	Indicates the total volume in [ml] (read-only).
TotalWorkingTime	Indicates the total number of power-on hours (read-only).

The UltiMate system splits the flow before the column. Usually, only a part of the flow delivered by the pump is directed through the column. This "system flow" is determined via the **Flow** command. The flow that must be delivered by the pump to achieve the desired system flow is calculated via the CRP value. The maximum system flow is 200.000 µl/min; the maximum permissible pressure is 400 bar.

The micro pump in the UltiMate system is the "master pump." The master pump is responsible for generating the system flow. The four standard parameters for the master pump (before the flow splitter) are Flow, PressureLowerLimit, PressureUpperLimit, and PressureValue. These parameters can be checked separately, if desired, by entering the term "Master" before the command (for example, MasterPressureUpperLimit). The pressure limits for the master pump can be issued separately. The range for the pressure limits corresponds to the range for the pressure limits of the entire system. However, the flow of the master pump can be no more than 0.5 ml/min. With a Bypass Calibrator installed, up to 5 ml/min is possible.

Commands without the Master prefix refer to the values behind the flow splitter, i.e., to the column flow and the column pressure. In this connection, ColumnPressure refers to the column and TrapColumnPressure refers to the precolumn on the Switchos Switching Device.

The following commands and properties are supported for the flow sensor:

Command/Property	Description
CalibrateCRP [When={BeforeFirstSample| BeforeEachSample}]	Performs flow measurement and recalculates CRP. (The property is visible only at the Expert level.)
Ready	Indicates the state of the flow sensor: Ready or NotReady. (The property is visible only at the Advanced level and is read-only.) (NotReady is indicated during the flow recalibration procedure.)
Type	Indicates the type of the flow sensor type. (The property is visible only at the Expert level and is read-only.)

For information about how to install the UltiMate pump, refer to **Hardware Installation** ⬇ **UltiMate Capillary/Nano HPLC System: Overview** in the **Administrator Help** section.

▣ Dionex/LC Packings UltiMate Pump: Columns

In addition to the pump commands (see ▣ **Dionex/LC Packings UltiMate Pump**), the following commands and properties are available for controlling columns in the UltiMate system (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Minimum	Maximum	Description
BatchNo	64 characters		Column batch number.
CRP	0.0	2000.0	Factor for calculating the master flow [2].
Diameter	Other, 50µm, 75µm, 100µm, 180µm, 300µm, 500µm, 800µm, 1000µm		Internal diameter of the column (required for calculating the CRP value).
Length	Other, 50mm, 100mm, 150mm, 200mm, 250mm		Column length (required for calculating the CRP value).
Manufacturer	64 characters		Column manufacturer.
Material	64 characters		Column material[3].
MeasuredFlowRate	0.000 µl/min	10.000 µl/min	Measured flow rate. If set, the CRP value is calculated automatically via the measured flow rate (also, refer to [2]).
ParticleSize	0.0 µm	2000.0 µm	Particle size[3] of the column material.
PoreWidth	0.0 Å	2000.0 Å	Pore width[3] of the column material.
SerialNo	64 characters		Column serial number.
StationaryPhase	Other, C18_3µm_100A, C18_5µm_100A, C18_3µm_300A, C18_5µm_300A		Stationary phase of the column (required for calculating the CRP value).

[2] Master flow [ml/min] = CRP*system flow [µl/min]
If possible, this value is automatically calculated from the lookup table (using the settings for Length, Diameter, and Stationary Phase) whenever one of these parameters is changed. Override the value if your column is not mentioned in the table. For more information, refer to **Practical Tips for Device Control** ▣ **Determining the CRP Value**.

[3] This value is automatically set whenever the StationaryPhase parameter changes. The user can override the value.

The different parameters describing the column material (BatchNo, Manufacturer, Material, ParticleSize, PoreWidth, and SerialNo) are only available from the Advanced level on. All other parameters are available on the **Normal** ➢*Filter* level.

▪ PP-150 Preparative HPLC Pump

In addition to the standard pump commands (see **Commands for Controlling Dionex Devices** 🔳 **Dionex Pumps**), the commands and properties from the table below are available (please note that the display ➢*Filter* level determines which commands and properties are displayed).

Property	Values	Description
⇒*%B, %C, %D*	0.0 - 100.0 %	Use %B, %C, and/or %D to determine the solvent composition.
⇒*Flow*	0.000 - 9.999 ml/min	Sets the flow rate.
Motor	On/Off	(Only available for the first pump) Turns the (all) pump(s) on or off
ModelVariant	Unknown Isocratic LPG HPG	Indicates the pump's operating mode (read-only). Set the operating mode on the **Pumps** tab page in the Server Configuration program.
Pressure	0 - 432 bar	Indicates the pressure (read-only).
Pressure.LowerLimit	0 - 392 bar	Sets the lower pressure limit.
Pressure.UpperLimit	10 - 432 bar	Sets the upper pressure limit.
Solvent (Pump: PASV, PBSV, PCSV)	A, B, C, D	(For pumps with connected FCV Solvent Selector) Selects the solvent.

👉 *Tip:*

*To use the **Motor On/Off**, **CycleMode**, and **Solvent** properties, you have to enter them manually in the PGM Editor.*

Observe the following when creating a program:

- When the pressure unit is psi, always add the entry in brackets, e.g.

  `PrepPump.Pressure.LowerLimit =  400 [psi]`

- Try to avoid ⇒*Delay*. Instead, use the retention time whenever possible.

- In triggers, use ⇒*AbortSample* instead of ⇒*End*.

- Use ⇒*Protocol* instead of ⇒*Message*.

For information about how to install the pump, refer to **Hardware Installation** 🌐 **PP-150 Preparative HPLC-Pump: Overview** in the **Administrator Help** section.

◼ Controlling Pumps without a Separate Device Driver

In addition to the device drivers for 🔳 **Dionex Pumps**, Chromeleon provides many drivers for controlling third-party pumps. For an overview of the different manufacturers whose devices can be controlled by Chromeleon, refer to **Hardware Installation** 🔳 **Installing and Controlling Third-Party Devices** in the **Administrator Help** section.

Besides, pumps can be controlled for which separate device drivers or serial interfaces are not available (for example, the former Gynkotek M300 pump). Depending on the type of pump to be controlled, the following plug-in boards are available:

1. "Dionex Pump DA Converter (12 Bit)" (for *voltage-controlled* pumps) or

2. "Dionex Pump Control Card" (for *frequency-controlled* pumps).

The **Administrator Help** section provides more information; refer to **Hardware Installation:**

🔵 **Installing the Dionex 12-Bit DAC Card**

🔵 **Installing the Pump Control Card**

In addition, install the respective device driver, for example, **DAC Pumps** (for *voltage-controlled* pumps) or **Pump Control Board Pump(s)** (for *frequency-controlled* pumps). (For more information, refer to **How to …: Configuring the Chromeleon Server** 🔳 **Adding, Configuring, or Deleting Components** in the **Administrator Help** section.)

Chromeleon supports the following commands for voltage-controlled pumps (**DAC pumps**):

Command	Description
⇒*Flow*:	Change the flow rate
⇒*%B, %C, %D*:	Change the solvent composition, determines the course of the gradient

In addition, a reset can be performed for frequency-controlled pumps, which are installed via the **Pump Control Board Pump(s)** device driver.

Dionex Sample and Fraction Manager

For information about the different commands, refer to:

🔼 **Sample and Fraction Manager Commands**

🔼 **Commands for Fraction Collection**

For additional tips and program examples for the different injection modes, refer to:

🔼 **Tips for Injection**

🔼 **Overlapping Areas**

🔼 **PGM File for Fraction Collection**

For information about how to solve possible problems, refer to 🔼 **Sample and Fraction Manager: Troubleshooting**

▪ Sample and Fraction Manager Commands

Apart from 🔼 **General Device Commands**, the Sample and Fraction Manager supports the following commands and properties (please note that display ➤*Filter* level determines which commands and properties are displayed):

Status properties

Property	Value Range	Filter	Description
Busy	Busy/Idle	Expert	Indicates whether the device driver has not yet sent all commands or whether execution of a command is yet complete (*read-only*).
CPUSerialNo	-	Expert	Indicates the CPU serial number of the connected sampler (*read-only*).
DeviceState	Ready/Busy/ Init(Error)/Unknown	Normal	Indicates the current device status (*read-only*).
StatusDescription	-	Normal	Indicates the current action of the sampler. If no action is performed, **Idle** is displayed (*read-only*).
Syringe	-	Normal	Indicates the installed syringe type (*read-only*).

Inject properties

Command	Value Range	Description
AirVolume	Depends on the volume of the installed syringe; indicated in **µl**. Special value: **Default***	Specifies the air volume that is drawn for the **Inject** and/or **WashWithSample** commands (in *LC_Inject and LC_Overlap mode*).
FillSpeed	Depends on the minimum and maximum speed of the installed syringe; indicated in **µl/s**. Special value: **Default***	Fill speed for the preparing fill strokes for the **Inject** and/or **WashWithSample** commands.
FillStrokes	0-99 or **Default***	Number of preparing fill strokes for the **Inject** and/or **WashWithSample** commands (*in LC_Inject and LC_Overlap mode*).
FillVolume	Depends on the syringe volume; indicated in **µl**. Special value: **Default***	Fill volume for the single piston strokes (*for the **WashWithSample** command*).
InjectMode	LC_Inject LC_Overlap	Injection mode. In sample programs, this value must be set prior to the **Inject** command.
Injector	List of injection objects for the respective arm of the installed Sample and Fraction Manager	Injector to be used for the **Inject** and/or **WashWithSample** commands (in *LC_Inject and LC_Overlap* mode*). The setting is ignored in **Custom** injection mode.
InjectSpeed	Depends on the minimum and maximum speed of the installed syringe; Special value: **Default***	Syringe speed in µl/s for injections to the sample loop (*in LC_Inject and LC_Overlap mode*).
NoOfPlungerStrokes		Indicates the number of strokes since the last reset (**Value**) and maximum allowed number (**Max.**) as reported by the device. (The property is read-only.)
NoOfInjects		Indicates the number of injections since the last reset (**Value**) and maximum allowed number (**Max.**) as reported by the device. (The property is read-only.)
Penetration	0 - maximum value of all installed tray types	Defines how deep the needle descends into the vial (parameter for Inject, PrepareThisSample, PrepareNextSample, WashWithSolvent).

Command	Value Range	Description
Position	1 to maximum number of tubes allowed for the trays used in the current configuration.	Defines the absolute position via an index entry, which can be defined as <TrayName> <Tube>. Note: When you select a different position, the **Tube**, **Tray**, and **TrayName** properties are updated automatically.
PostInjectDelay	0 - 99000 ms or **Default***	Time between the injection and the removing of the needle from the injector (*in LC_Inject and LC_Overlap mode*).
PreInjectDelay	0 - 99000 ms or **Default***	Time between inserting the needle in the injector and injecting the liquid into the sample loop (*in LC_Inject mode*).
PullupDelay	0 - 10000 ms or **Default***	Indicates the delay time between drawing the liquid into the syringe and dispensing the volume that is not required (*for the WashWithSample command*).
Tray		Tray used (*read-only; determined via the Position property*).
TrayName	String	Indicates the name of the current tray Note: If you select a different tray, the **Tray** property is updated automatically.
Tube		Number of the current tube in the tray *(read-only; determined via the Position property)*

*⬚ Note:

*For the **Default** parameter, the default value set on the respective device is used.*

Command for the analytical SFM

Command	Description
CloseStack	Closes all stacks that might be open. This might be useful when fraction collection is finished.

Commands for the LC_Overlap mode

Command	Description
PrepareNextSample	The autosampler fills the sample loop with the *next* sample, observing the parameters defined in the PGM file for that sample (Volume, Position, Tray, AirVolume, FillSpeed, FillStrokes, and PullupDelay). The syringe then returns to the **Park** position.
	This command is ignored for InjectModes other than LC_Overlap.
	This command is available only after the Inject command in the PGM File.
PrepareThisSample	The autosampler fills the sample loop with the *current* sample, observing the parameters defined in the PGM File for the current sample (also, see PrepareNextSample). This command is available only before the Inject command in the PGM File.

For more information, refer to:

 Commands for Fraction Collection

 Tips for Injection

 Overlapping Areas

 PGM File for Fraction Collection

Sample and Fraction Manager: Commands for Fraction Collection

Chromeleon supports the following commands and properties for fraction collection with the Sample and Fraction Manager (SFM) (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Commands

Command	Description
Collect	Moves the syringe to the Collect position.
Drain	Moves the syringe to the Drain position without ejecting the contents of syringe.
Eject	Ejects the remaining contents of the syringe before the syringe is moved to the Drain position.

Properties

Property	Value Range	Description
CollectMode	Vertical, SawVertical, Horizontal, SawHorizontal	Indicates the collect mode set on the **Options** tab page in the Server Configuration program. (The property is read-only.)
FracTrayName	Available trays	Indicates the name of the tray specified via the **TubePosition** property. (The property is read-only.)
FracTrayTube	1 to maximum number of tubes per tray	Indicates the tube index in the tray, referring to the absolute **TubePosition**. (The property is read-only.)
MovementMode	StopAndEject, Stop, Collect	Specifies the behavior of the SFM if the **TubePosition** is changed during collection:
		StopAndEject: The remaining sample is ejected into the original tube, the SFM moves to the next tube position and continues collecting.
		Stop: Before moving to the next tube, the syringe moves to the Drain position without ejecting the remaining contents of the syringe.
		Collect: The syringe moves to the next tube position and continues collecting.
TotalNumberInstalled	-	Indicates the total number of tubes. This property can be used to set the respective property of the Fraction Collection driver. (The property is read-only.)
TubePosition	1 to number of tubes	Enter the absolute position of the tube to which the sample arm shall be moved. If this property is set while the SFM is collecting, the behavior depends on the MovementMode setting.

For more information, refer to:

🔧 **Sample and Fraction Manager Commands**

🔧 **Tips for Injection**

🔧 **Overlapping Areas**

🔧 **PGM File for Fraction Collection**

▣ Sample and Fraction Manager: Tips for Injection

Chromeleon supports various parameters for sample loading and injection.

Parameters supported for sample loading:

Position, **Volume**, **AirVolume**, **FillSpeed**, **FillStrokes**, and **PullupDelay**

Parameters supported for injection:

PreInjDelay, PostInjDelay, InjSpeed, and **Injector.**

When performing an inject command, the Sample and Fraction Manager performs the following steps:

1. The sample needle is moved to the specified tube.

2. The specified sample volume is drawn into the syringe.

3. *Optional:* If the **AirVolume** parameter is set to a value > 0, air is drawn from outside the tube.

📝 *Note:*

   Steps 1 through 3 are omitted if they were already performed via the PrepareThisSample or PrepareNextSample commands.

4. The sample needle is moved to the specified injector.

5. The Sample and Fraction Manager (SFM) waits for the Inject Sync signal. (The Inject Sync signal is an SFM synchronization signal.) Set this signal to **immediately** if no waiting time is required. Alternatively, you can set the signal to an input port of the SFM if the instrument is to wait for a signal from the pump. (For information about the settings, refer to **Installing Dionex Devices** 🔵 **HPLC Sample and Fraction Manager: Installation** in the **Administrator Help** section.)

6. The needle is moved into the injector.

7. The sampler pauses for the time specified by **PreInjDelay**.

8. The injection valve is moved into the Load position.

9. The contents of the syringe are dispensed into the sample loop.

10. The sampler pauses for the time specified by **PostInjDelay**.

11. The injection valve is moved into the Inject position.

12. The needle is moved out of the injector.

13. The needle returns to the Home position.

For more information, refer to:

⬛ **Sample and Fraction Manager Commands**

⬛ **Commands for Fraction Collection**

⬛ **Overlapping Areas**

⬛ **PGM File for Fraction Collection**

⬛ Sample and Fraction Manager: Overlapping Areas

The Sample and Fraction Manager has two independent arms for which the working areas partly overlap. To prevent collision of the arms in the overlapping area, only one arm is allowed in the area at a time:

For example, if arm 2 is in the overlapping area and arm 1 is to perform an operation in the overlapping area, arm 1 has to wait until arm 2 has completed its operation. Arm 2 is then moved to the Park position and arm 1 can enter and perform its operation in the overlapping area.

This is performed after each operation. To have one arm perform subsequent operations in the overlapping area without being interrupted by the other arm, Chromeleon supports the following commands:

Command	Description
AcquireOverlapArea	Provides exclusive access to the overlapping area; i.e., the other arm cannot enter the overlapping area. ⬛ *Note:* *If the second arm already has exclusive access to the overlapping area, the first arm will wait until the second arm no longer has exclusive access.*
ReleaseOverlapArea	Releases the exclusive access right for the overlapping area. The other arm can now enter and perform operations in this area. ⬛ *Note:* *At the beginning of a sample program, an implicit UnblockOverlap command is performed.*

Example:

```
2.000 SFM_A.AcquireOverlapArea
; Empty syringe of the SFM_A if necessary:
   SFM_A.Waste
; Wash syringe of the SFM_A:
   SFM_A.Wash
; Wash the SFM_A injector:
   SFM_A.WashInjector
   SFM_A.ReleaseOverlapArea
   SFM_P.PrepareNextSample
```

This program ensures that the arm of the SFM_A is not returned to the Park position between emptying the syringe and washing the injector. Before starting sample preparation, the arm of the SFM_P waits until the SFM_A has completed its operations and left the overlapping area.

Dionex recommends minimizing the blocking time of the overlapping area because the arm for which the area is blocked cannot perform any other operation until the area is no longer blocked.

 Note:

If an arm is not connected, Chromeleon does not know its position and thus, assumes that it might be in the overlapping area. That is why the other arm cannot enter the overlapping area. Therefore, always connect both arms even if you want to use only one arm.

For more information, refer to:

 Sample and Fraction Manager Commands

 Commands for Fraction Collection

 Tips for Injection

 PGM File for Fraction Collection

◼ Sample and Fraction Manager: PGM File for Fraction Collection

You can use the left arm of the Sample and Fraction Manager (SFM) for fraction collection. Chromeleon supports the following high-level commands for fractionation: Collect, Drain, Eject, and TubePosition.

A typical program for a timebase with SFM (device name: SFM_A and SFM_P), Fraction Collection driver (device name: Fraction Collection), pump (here: Dionex P680P HPG-4), and detector (here: Dionex UVD340U PDA) if only the UV_VIS_1 channel is used for peak detection could look as follows:

```
; Preparing the devices for the next sample
-1.000   PumpDevice =   "Pump"
   Flow =   50.000
   %B =  0.0
   %C =  0.0
   %D =  0.0
   Pressure.LowerLimit =   0
   Pressure.UpperLimit =   150
   %A.Equate =     "%A"
   %B.Equate =     "%B"
   %C.Equate =     "%C"
   %D.Equate =     "%D"

   CollectFractions =   Yes
   CollectOutsidePeaks =   No

; Preparing the SFM_A for fraction collection
   SFM_A.MovementMode =     Interrupt
   SFM_A.TubePosition = FractionCollection.TubePosition

   SFM_P.InjectMode =   LC_Overlap

;********************************************************************
*
;* Definition of triggers for fraction collection starts here.
;********************************************************************
*
; Note: The Fraction Collection driver increments its TubePosition
property before the TubeChange and FracEnd triggers are executed.
Thus, the SFM driver only has to synchronize its TubePosition with
the fraction collection property.
   Trigger FracStart   FracStartDetected
```

```
; When a new fraction collection starts, the sample needle should be
positioned already over the correct tube to start collecting
immediately.
   Collect
   EndTrigger

   Trigger  TubeChange  FracTubeChange
   ; Tube change necessary: synchronize SFM_A with fraction
collection position
   SFM_A.TubePosition = FractionCollection.TubePosition
   EndTrigger

   Trigger  FracEnd  FracEndDetected
; Fraction collection ends, the remaining content of the syringe is
ejected, the plunger is moved to Drain position, and the arm is moved
to next tube to be prepared for next collection
   Eject
   SFM_A.TubePosition = FractionCollection.TubePosition
   EndTrigger

;*********************************************************************
*
;* Definition of triggers for fraction collection ends here.
;*********************************************************************
*

      TubeMaxVolume =                      2.000000
      FractionCollection.TotalNumberInstalled                    =
SFM_A.TotalNumberInstalled
      MaxTubesPerFraction =                Unlimited
      TubeWrapping =                       No
      DelayTime =                          1.0
      DetectionChannel2.OffsetTime =       0.5
      DetectionChannel2.Name =             "UV_VIS_1"
      DetectionChannel2.PeakStartSlope =   0.500
      DetectionChannel2.PeakStartThreshold =   10.00
      DetectionChannel2.PeakMaxSlope =     0.000
      DetectionChannel2.PeakEndSlope =     -1.000
      DetectionChannel2.PeakEndThreshold =   10.00
      DetectionChannel2.ThresholdNoPeakEnd =   2000.00
      DetectionChannel2.BaselineOffset =   0.000
      DetectionChannel2.BaselineDrift =    0.000
      UV_VIS_1.Wavelength =                225
      UV_VIS_1.Bandwidth =                 1
      UV_VIS_1.RefWavelength =             600
```

```
        UV_VIS_1.RefBandwidth =                      1
        UV_VIS_1.Step =                              Auto
        UV_VIS_1.Average =                           On
        UV_VIS_2.Wavelength =                        250
        UV_VIS_2.Bandwidth =                         1
        UV_VIS_2.RefWavelength =                     600
        UV_VIS_2.RefBandwidth =                      1
        UV_VIS_2.Step =                              Auto
        UV_VIS_2.Average =                           On
        UV_VIS_3.Wavelength =                        275
        UV_VIS_3.Bandwidth =                         1
        UV_VIS_3.RefWavelength =                     600
        UV_VIS_3.RefBandwidth =                      1
        UV_VIS_3.Step =                              Auto
        UV_VIS_3.Average =                           On
        UV_VIS_4.Wavelength =                        300
        UV_VIS_4.Bandwidth =                         1
        UV_VIS_4.RefWavelength =                     600
        UV_VIS_4.RefBandwidth =                      1
        UV_VIS_4.Step =                              Auto
        UV_VIS_4.Average =                           On
        3DFIELD.RefWavelength =                      600.0
        3DFIELD.RefBandwidth =                       1.9
        3DFIELD.Step =                               0.5
        3DFIELD.MinWavelength =                      200.0
        3DFIELD.MaxWavelength =                      595.2
        3DFIELD.BunchWidth =                         1.9
0.000 Flow =   50.000
        %B =      0.0
        %C =      0.0
        %D =      0.0
        Autozero
        Wait    SFM_P.Ready
        SFM_P.Inject
        UV_VIS_1.AcqOn
        UV_VIS_2.AcqOn
        UV_VIS_3.AcqOn
        UV_VIS_4.AcqOn
        3DFIELD.AcqOn

; If the SFM_A does not operate in the overlapping area during
fractionation, the SFM_P can be cleaned (not shown here) and the next
sample can be prepared if necessary, e.g.:
```

```
8.000   SFM_P.PrepareNextSample

CollectFractions = No
SFM_A.Wash WashStation=Wash1, Cycles=2
        UV_VIS_1.AcqOff
        UV_VIS_2.AcqOff
        UV_VIS_3.AcqOff
        UV_VIS_4.AcqOff
        3DFIELD.AcqOff

10.20 End
```

Note:

In the example, DetectionChannel1 is not used for the first detection channel because '1' and 'l' look almost identical when Courier is the selected font.

For more information, refer to:

Sample and Fraction Manager Commands

Commands for Fraction Collection

Tips for Injection

Overlapping Areas

Sample and Fraction Manager: Troubleshooting

The following problems may occur during operation of the Sample and Fraction Manager:

Missing vial:

If no vial is present at the position from which the Sample and Fraction Manager performs injection, the batch is aborted. The Sample and Fraction Manager may then remain in an inconsistent state.

Therefore, Dionex recommends performing a **Home** command. If the tray in which the error occurred is part of a tray stack, you have to close the stack drawer manually.

Z collision error:

If the syringe holder collides with another component of the Sample and Fraction Manager while moving in z direction (e.g., because of an incorrect configuration), the instrument reports a z collision error.

First, find and eliminate the cause of the z collision error. Then, perform a **Home** command to re-calibrate the motor in the x, y, and z directions. The driver cannot perform movement commands until the motor has been recalibrated.

☞ *Tip:*

The Sample and Fraction Manager is not equipped with collision sensors for the x and y directions. Therefore, stop the batch immediately if you observe a collision in the x or y direction. Afterward, perform the same steps as described for a z collision error.

Dionex Autosamplers

Automatic sample injection with a modern ➢*Autosampler* has several advantages:

- Efficient processing of large sample batches

- Reproducible and verifiable dosing precision

- Loss-free and bubble-free injection of the sample

In spite of the vast scope of performance, only the "standard" commands (**Inject**, **Position**, and **Volume**) are usually required for automatic control of the Dionex autosamplers:

Standard Commands

In addition to the 🔲 **General Device Commands**, Chromeleon supports the following standard autosampler commands:

Command	Description
⇒*Dispense*	Dispenses the previously drawn amount of liquid.
⇒*Draw*	Draws the specified amount of liquid.
⇒*Inject*	Injects the sample.
	The **Inject** command is not used when the AS or AS50 (USB) is in Concentrate or Sequential Concentrate mode.

Command	Description
Mix	Mixes the contents of a vial. (The mixing procedure depends on the autosampler.)
⇒*Position*	Indicates the vial position.
⇒*Volume*	Sets the volume to be injected.
⇒*Wash*	Performs a wash cycle.

For information about the individual autosamplers and their specifications, refer to:

🔺 **Dionex UltiMate 3000 Series - Autosamplers**

🔺 **Dionex ASI-100 Autosampler Series**

🔺 **Dionex AS/AS50 Autosamplers**

🔺 **Dionex/LC Packings FAMOS Capillary/Nano HPLC Autosampler**

🔺 **Dionex GINA 50 Autosampler**

🔳 Dionex UltiMate 3000 Series - Autosamplers

In addition to the standard autosampler commands (see 🔺 **Dionex Autosamplers**), the UltiMate 3000 Autosampler provides additional commands for controlling autosampler functions. The following general commands are available (please note that the display ➢*Filter* level determines which commands and properties are displayed, and that, depending on the autosampler type, some commands or properties may not be available):

Command/Property	Description
AcquireExclusiveAccess	Acquires exclusive access for the current timebase.
BlueTray	Reports which sample containers are installed in the blue segment (corresponds to the settings on the **Segments** tab page in the autosampler's properties).
BootLoaderVersion	Indicates the bootloader version (read-only).
Brightness	Adapts the brightness of the autosampler's front panel display to your requirements.
BufferTubingVolume	Reports the volume of the installed buffer tubing (read-only).
Contrast	Adapts the screen contrast of the autosampler's front panel display to your requirements.
DispenseDelay	Sets the time that the needle shall remain in the sample container after dispensing.
DispSpeed	Sets the speed at which the sample is expelled for standard injections

Command/Property	Description
DrawDelay	Sets the speed at which the sample is drawn for standard injections.
DrawSpeed	Sets the speed at which the sample is drawn for standard injections.
ExclusiveAccess	Reports which timebase has exclusive access to the autosampler (read-only).
FillWashPort	Sets the wash port volume.
FirstTransportVial	Sets the position for the first transport vial. Transport vials are required only for microliter pick-up injections. (Also, see LastTransportVial.)
FlushOutFactor	Sets the flush out factor (only for split-loop injections). The flush out factor is the time after which the injection valve switches into the **Load** position. The solvent flow is directly onto the separation column, bypassing the needle and sample loop (bypass mode). This reduces the flush and gradient delay times.
FlushVolume	Sets the flush volume that shall be used for the first Full-Loop, Partial-Loop, or microliter pick-up injection.
FlushVolume2	Sets the flush volume that shall be used for additional injections from the same vial for Full-Loop and Partial-Loop injections when RinseBetweenInjections=No.
GreenTray	Reports which sample containers are installed in the blue segment (corresponds to the settings on the **Segments** tab page in the autosampler's properties).
InitiateChangeNeedle	Moves the needle arm to the left and the sample needle upwards to enable changing of the needle.
InitiateChangeSyringe	Initiates the procedure for exchanging the syringe. (Also, see TerminateChangeSyringe.)
InitiateChangeVial	Rotates the sample containers specified by the **Tray** parameter to the front, allowing you to change the vials or plates easily. Select **BlueTray**, **GreenTray**, or **RedTray** to move the corresponding plate or tray to the front). Or else, select **BlueVials**, **GreenVials**, or **RedVials** to move the corresponding segment with the transport vials to the front.
⇒*Inject*	Injects the sample. If you create a user-defined WPS-3000 program, e.g., in the 🔳 **Program Wizard**, use the **Inject** command to start the program. In this case, injection is performed after the **UdpInjectMarker** command of the user-defined program.
InjectionValvePosition	Reports the current position of the injection valve: **Inject**, **Load**, **SwitchToInject**, **SwitchToLoad**, or **Unknown**.

Command/Property	Description
InjectMode	Injection mode (for more information, refer to the operating instructions for the instrument):
	UserProg — The autosampler executes the program or sample preparation steps defined by the user.
	Partial — The loop volume is injected in part only (recommended: at most 50% of the sample loop volume).
	FullLoop — The loop volume is injected completely.
	µlPickUp — The loop volume is injected completely. However, it contains partly sample volume only. Transport liquid is drawn first, then sample, and then transport liquid again, i.e. both ends of the buffer loop, sample loop and sample needle are filled with transport liquid. That is why the TransportVial should contain solvent. The sample volume is in the middle of the sample loop.
	Normal — The autosampler performs a standard injection. (WPS-3000SL only)
InjectValveToInject	Switches the injection valve into the **Inject** position.
InjectValveToLoad	Switches the injection valve into the **Load** position.
InjectWaitTime	Reports the time that has passed between the issuing of the injection command by Chromeleon and the response from the autosampler (read-only).
InjectWash	Specifies whether the wash procedure is part of the standard inject procedure (only if InjectMode = Normal). Select an option: **NoWash** (recommended for typical applications), **BeforeInj**, **AfterInj**, or **Both**. For user-defined injection procedures, you have to set wash steps explicitly.
InstalledOn	Indicates the timebase in which the autosampler is installed.
LastTransportVial	Sets the position for the last transport vial. Transport vials are required only for microliter pick-up injections. (Also, see FirstTransportVial.)
Leak	Reports whether the leak sensor detected a leak **NoLeak** or **Leak**.
LeakAlarmOff	Turns off the beep for the current alarm. A new beep sounds when the leak sensor detects another leak.
LeakSensorMode	The following options are available: **Enabled** - Enables leak detection. If the sensor is activated, a message appears in the Chromeleon Audit Trail and an acoustic beep sounds. **Silent** - Enables leak detection. If the sensor is activated, a message appears in the Chromeleon Audit Trail, but no beep sounds. **Disabled** - Disables leak detection.

Command/Property	Description
LoopOverfill	(Used only for full-loop injections) The volume of the sample loop and the LoopOverfill factor determine the volume that is drawn in and/or transported through the sample loop before the sample is injected. Example: Loop volume: 5 µl, LoopOverfill: 2 = Volume to be drawn: 10 µl
LoopVolume	Reports the volume of the installed sample loop (read-only*).*
LoopWashFactor	Factor for the loop wash volume. The contents of the buffer loop and the LoopWashFactor determine the loop wash volume. The default factor is 1.
LowDispersionFactor	Determines the volume for ➢*Low Dispersion Injections*. The volume is calculated by multiplying the factor with the original sample volume.
LowDispersionFlow	Sets the flow rate with which the sample is flushed out of the sample loop in Low Dispersion Mode.
LowDispersionMode	Enables low dispersion mode.
MaxInjectVolume	Sets the maximum inject volume of the application. This parameter is required for calculation of the **Bypass Time** (see also **Flush Out Factor**).
ModelVariant	(read-only) Reports the autosampler variant (**DualNeedle** or **SplitLoop**).
MotorControllerVersion	Indicates the version of the motor controller (read-only).
MoveNeedleDown	Moves the needle to the position specified by **NeedleHeight**.
MoveNeedleHome	Moves the needle to the topmost position (Home position).
MoveTo	Moves the needle over the position specified with the **Position** property.
NeedleHeight	Determines to which position the needle moves with the **MoveNeedleDown** command.
NeedleVolume	Reports the volume of the installed needle (read-only).
Operable	Reports whether the autosampler is ready to operate.
Position	Sets the sample position on the carousel.
PrepareNextSample	Starts the inject procedure for the next sample (if another sample is available). The inject valve will be switched at the next **Inject** command.
PrepareThisSample	Starts the injection procedure for the current sample. However, the injection valve will be switched only at the next injection command.
PrepVial	Sets the position for the sample container used for sample preparation. This position is used for UdpDraw and UdpDispense operations if PrepVial has been selected in the **From** and/or **To** fields of the **UdpDraw** and/or **UdpDispense** commands.

Command/Property	Description
PrimeSyringe	Primes the syringe by filling and emptying it. Use **PrimeSyringeNumber** to determine how often the syringe is primed.
PrimeSyringeNumber	Specifies how often the syringe is filled and emptied during the **PrimeSyringe** operation.
PumpDevice	This property is available only if you have specified on the **Segments** tab in the Chromeleon Server Configuration in the autosampler's properties that the injection command be synchronized with the strokes of an UltiMate 3000 series pump. Usually, the setting from the **Segments** tab page is displayed by default, e.g., MicroPump or Loading Pump. If you want to change the synchronization assignment for a specific application, enter the pump in the input field. This does not overwrite the default setting on the **Segments** tab page.
PuncturerDepth	Sets how deep the puncturer descends into the sample container, measured from the top of the sample container.
PuncturerDownNeedleDown	Moves the puncturer and needle down.
PuncturerDownNeedleUp	Moves the puncturer down and the needle up.
PunctureOffset	Specifies how far the tray will be rotated to improve piercing of vial septa.
PuncturerUpNeedleDown	Moves the puncturer up and the needle down.
PuncturerUpNeedleUp	Moves the puncturer and needle up.
PurgePumpOn	Moves needle to the wash port and draws mobile phase or air from the pump to reduce the pump priming time.
PurgePumpOff	Stops the purge pump.
ReadyTempDelta *(WPS-3000T(SL) with enabled thermostatting option only")*	When the current temperature deviates from the target temperature by more than the **ReadyTempDelta**, the autosampler is not ready to operate (TemperatureReady = NotReady).
ReagentLiquidHeight	Sets the distance (in mm) between the bottom of the sample vial and the tip of the needle, defining how deep the needle descends into the container for sampling.
ReagentA/B/C/DVial	Indicates which vial (A, B, C or D) is used in **Draw/Dispense** commands, if **From/To** is set to **ReagentA/B/C/DVia**l. See also: **UdpDraw/UdpDispense**.
ReagentXVial *(where X = A to D)*	Sets the position of the sample container with the associated reagent (if the injection mode is UserProg). This position is used for **UdpDraw** and **UdpDispense** operations if the associated **ReagentxVial** has been selected in the **From** and/or **To** fields of the **Draw** and/or **Dispense** commands.
RedTray	Reports, which sample containers are installed in the blue segment (corresponds to the settings on the **Segments** tab page in the autosampler's properties).

Command/Property	Description
Relay4Enabled	Sets whether relay 4 controls the inject response. If you specify the duration, relay 4 will be active for this time upon each-inject. Select **Yes** for user-defined control, i.e., if you want to control the relay via the `Relay_4.On` and "`Relay_4.Off` commands.
ReleaseExclusiveAccess	Releases the exclusive access of the current timebase to the autosampler.
Reset	Sets the autosampler to the initial conditions when the instrument was turned on.
RinsebetweenReinjections	Sets whether a wash cycle is performed after each injection. If set to **Yes**, the system is washed after each injection. If set to **No**, the wash cycle is skipped when the next injection is performed from the same vial.
	Notes:
	This command allows you to make several injections from the same vial one after the other without loosing sample between the injections by rinsing.
	A wash cycle is always performed after the last sample in a sequence.
SampleHeight	Sets the distance (in mm) between the bottom of the sample container (well-plate, deep-well plate, or vial), as measured from the exterior, and the tip of the needle for a standard injection, defining how deep the needle descends into the container for sampling.
SeatCapillaryVolume	Reports the seat capillary volume in [µl]. Possible range: 0.000 µl to 1000.000 µl.
SetFactoryDefaults	Resets the autosampler settings to the Dionex factory default settings.
SelfTest	Performs a self-test.
	Note:
	You cannot start a batch when the sampler did not pass the self-test.
Standby	Sets the autosampler into the **Standby** mode or cancels this mode (**NoStandby**).
	Note:
	You cannot start a batch when the sampler is in standby mode.

Command/Property	Description
State	Reports whether the autosampler has injected: **On** (= injection has been performed) or **Off** (= injection has not yet been performed). *(Read-only)*
Status	Indicates which operation the autosampler is currently performing (read-only).
StopInject	Stops the running injection.
SyncWithPump	This property is available only if you have specified on the **Segments** tab in the autosampler's properties that the injection command be synchronized with the strokes of an UltiMate 3000 series pump. In this case, the setting is On. If you want to disable synchronization for a specific application, set the property to Off. (Also, see PumpDevice.) 📁 *Note:* *SyncWithPump is automatically set to **On** when you change the PumpDevice setting.*
Syringe	Reports the volume of the installed syringe (read-only).
SyringeValvePosition	Reports the current position of the syringe valve: **Needle**, **Wash**, **Waste**, or **Unknown**.
SyringeWashFactor	Factor for the syringe wash volume. The syringe volume and the SyringeWashFactor determine the syringe wash volume. The default factor is 1.
TempCtrl *(WPS-3000T(SL) with installed thermostatting option only)*	Temperature control of the sample rack. The following settings are available: **On** (enables temperature control) or **Off** (disables temperature control) (read-only).
Temperature *(WPS-3000T with installed thermostatting option only)*	Sets the target temperature for the carousel and thus, for the sample. The temperature can be between 4°C to 50°C (39.2°F to 122°F). Click the "+" character beside the name to display the items underneath: **Value** (reports the actual temperature), **Nominal** (sets the target temperature), **UpperLimit** (sets the upper temperature limit), and **LowerLimit** (sets the lower temperature limit). The system aborts the sample batch and/or starts error handling if the nominal temperature is outside the specified limits.
TemperatureReady *(WPS-3000T(SL) with installed Cooling Option only)*	Reports whether the autosampler is ready to operate. When Chromeleon reports NotReady, the current temperature deviates from the target temperature by more than the **ReadyTempDelta** (read-only).
TerminateChangeSyringe	Terminates the procedure for exchanging the syringe (Also, see InitiateChangeSyringe.)
TransLiquidHeight	Sets how deep the needle descends into the container for sampling (= distance in mm between the bottom of the transport vial, as measured from the exterior, and the tip of the needle).

Command/Property	Description
TransportVialCapacity	Sets how often transport liquid can be drawn from one transport vial. *Tip:* *Keep in mind that two draw operations are required for microliter pick-up.*
TransportVialUsecount	Reports how often transport liquid was drawn from the transport vials. *Tip:* *Reset the counter to zero after you have refilled the transport vials. If the maximum permissible number of draw operations, as defined with TransportVialCapacity, has been reached, a message ("out of transport liquid") is logged in the Chromeleon Audit Trail.*
TransVialPunctureDepth	Sets how deep the puncturer descends into the transport vial, measured from the top of the transport vial.
Volume	Sets the injection volume for standard injections.
⇒*Wash*	Performs a wash operation.
WashBufferLoop	Sets the volume for washing the buffer loop.
WashSampleLoop	Washes the sample loop.
WashSpeed	Sets the syringe speed for the wash operation.
WashVolume	Sets the volume that is drawn for the needle wash cycle after an injection.
WasteSpeed	Sets the syringe speed for expelling liquid to the waste.

System Wellness and Predictive Performance commands and properties

Tip:

Note that the installed Chromeleon version and autosampler type determine which commands and properties are available for checking the autosampler's system wellness and performance.

Command/Property	Description
CapillaryDriveMovements	Counts how many times the needle capillary moves (nano-and-cap instruments only).
CoolingWorkLoad	Reports the total cooling work load [K*min] since the module has been shipped (read-only).
DrainPumpHours	Counts the operating time [h] of the peristaltic pump (read-only).

Command/Property	Description
DrainPumpTubeChanged	After you have replaced the peristaltic pump tube, perform this command to update the related service information.
DrainPumpTubeChange Date	Reports the date when the peristaltic pump tube was changed last (read-only).
DrainPumpTubeHours	Counts the operating hours of the peristaltic pump with the currently installed tube.
*.Value	Reports the current operating hours of the peristaltic pump (read-only) with the currently installed tube.
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
HeatingWorkLoad	Reports the total heating work load [K*min] since the module has been shipped (read-only).
HorizontalDrive Movements	Counts how many times the horizontal drive moves into a new position (read-only).
InjectValveSwitches	Counts how many times the injection valve switches (read-only).
NeedleChangeDate	Reports the date when the needle was replaced last (read-only).
NeedleChanged	After you have replaced the needle, perform this command to update the related service information.
NeedleMovements	Counts how many times the needle moves.
*.Value	Reports the current number of movements (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
NeedleDriveMovements	Counts how many times the needle drive moves into a new position (read-only).
NeedleObstructions	Counts how many times the needle descends on an obstacle.
NeedleSeatChanged	After you have replaced the needle seat, perform this command to update the related service information.
NeedleSeatChangeDate	Reports when the needle seat was replaced last (read-only).
NeedleSeatCounter	Counts how many times the needle moves into the needle seat.
*.Value	Reports the current number of needle movements Into the needle seat (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
OperatingHours	Counts the operating hours since initial operation (excluding the operating hours in standby mode; read-only).
PowerOnTime	Counts the time since last power-up (or last wake-up from standby) (read-only).

Command/Property	Description
PurgePumpHours	Counts the operating hours of the purge pump (read-only).
Qualification	Provides information about system qualification.
*.LastDate	Reports the date when autosampler qualification was performed last (read-only).
*.LastOperator	Reports the name of the person who performed the autosampler qualification (read-only).
*.Interval	Reports the interval (in days) when the next system qualification is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
*.GracePeriod	Indicates the period during which the device may be operated after the due date for re-qualification.
QualificationDone	Perform this command after qualification to update the related system data.
RotorSealChanged	After you have replaced the rotor seal, perform this command to update the related service information.
RotorSealChangeDate	Reports when the rotor seal was replaced last (read-only).
RotorSealSwitches	Counts how many times the injection valve has switched since the rotor seal was replaced last.
*.Value	Reports the current number of injection valve switchings (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
Service	Provides service information.
*.LastDate	Reports the date of the last service (read-only).
*.Interval	Specify the interval (in days) after which a service is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
ServiceDone	After the device has been serviced, perform this command to update the related information.
StandbyHours	Counts the total operating hours in standby mode since the module was operated for the first time (read-only).
SyringeChanged	After you have replaced the syringe, perform this command to update the related service information.
SyringeChangeDate	Reports when the syringe was replaced last (read-only).
SyringeCounter	Reports the total number of syringe movements (read-only).
SyringeDriveMovements	Counts how many times the syringe drive moves into a new position. (read-only)

Command/Property	Description
SyringeMovements	Counts how many times the syringe moves into a new position.
*.Value	Reports the current value of syringe movements. (read-only)
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
SyringeValveCounter	Counts the valve movements.
SyringeValveSwitches	Counts how many times the syringe valve switches. (read-only)
TotalInjections	Counts how many times the injection valve switches. (read-only)
TrayDriveMovements	Counts how many times the tray moves to a new position. (read-only)

For a basic description of Dionex autosamplers, including the standard commands, refer to 🔲 **Dionex Autosamplers**.

For more information about the sample preparation commands for the WPS-3000 autosamplers, refer to 🔲 **Dionex WPS-3000 Autosampler: Commands for User-Defined Programs**

For information about how to use the commands in practical operation, refer to **Practical Tips for Device Control** 🔲 **Examples for User-Defined Programs for the WPS-3000 Autosampler**.

For information about how to install the autosampler, refer to **Hardware Installation** 🔵 **UltiMate 3000 Series - Autosamplers: Overview** in the **Administrator Help** section.

🔳 Dionex WPS-3000 Autosampler: Commands for User-Defined Programs

The Dionex WPS-3000 Autosampler provides many sample preparation commands that are used to further define the individual sample preparation steps. The sample preparation commands are accessible from the **Advanced** level only. Use the 🔲 **Program Wizard** to create a user-defined sample preparation program. This program is started by the ⇒*Inject* command.

Chromeleon supports the following sample preparation commands (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Description
UdpAirPump	Sets whether outside air shall be used to dry the needle. Select **On** or **Off**.
Udp⇒*Dispense*	Dispenses a specified volume of liquid. Make the following settings:
	To: Enter the position to which the specified volume is delivered.
	Volume: Enter the volume to be dispensed (0.0 to 25.0 µl).
	SyringeSpeed: Specify the speed with which the volume is expelled (10 to 8333 nl/s; option: GlobalSpeed here = DispSpeed).
	SampleHeight: Determine how deep the needle descends into the sample container (0 to 40 mm; option: GlobalHeight = SampleHeight).
Udp⇒*Draw*	Draws a specified volume of liquid. Make the following settings:
	From: Enter the position from which the specified volume is drawn.
	Volume: Enter the volume to be drawn (0.0 to 25.0 µl).
	SyringeSpeed: Specify the speed with which the volume is drawn (10 to 8333 nl/s; option: GlobalSpeed here = DrawSpeed).
	SampleHeight: Determine how deep the needle descends into the sample container (0 to 40 mm; option: GlobalHeight here = SampleHeight).
UdpInjectMarker	Generates an inject marker pulse. This is required in user-specific programs, i.e., when **InjectMode** = **UserProg**. The injection can be performed only after this pulse.
UdpInjectValve	Switches the injection valve to the specified position: **Inject** or **Load**.
UdpMixNeedleWash	Sets the volume that is used for washing the needle during sample preparation.
UdpMixWait	Sets the time that the system waits between the single sample preparation steps.
UdpMoveSyringe	Moves the syringe. Enter the volume to be drawn in the **Load** field; or else, enter the volume to be dispensed in the **Unload** field. Only set one of these parameters. Enter the speed for the syringe movement in the **SyringeSpeed** field.
	📖☞ *Tip:*
	Make sure that both parameters appear in the same line of the PGM File. If they appear in different lines, the PGM File does not pass the ➢Ready Check.

Command/Property	Description
UdpMoveSyringeHome	Moves the syringe it to its Home position and empties it.
UdpOutput	Sets the specified output into the defined state. Select the output (**Out1** ... **Out4**) and the state (**On** or **Off**) from the associated drop-down lists.
UdpSyringeValve	Switches the syringe valve to the specified position: **Needle**, **Wash**, or **Waste**.
UdpWaitInput	Waits until the specified input is in the selected state. Select the input (**Inp1** ... **Inp4**) and the state (**Low** or **High**) from the associated drop-down lists.
UdpWaitStrokeSync	Waits with the continuation of a program for **InjectMode** = **UserProg** until the pump has sent the synchronization signal. (Also, see PumpDevice, SyncWithPump.)

For a basic description of Dionex autosamplers, including the standard commands, refer to 🔲 **Dionex Autosamplers**.

For information about the general commands for the autosampler, refer to 🔲 **UltiMate 3000 Series - Autosampler**.

For information about how to use the commands in practical operation, refer to **Practical Tips for Device Control** 🔲 **Examples for User-Defined Programs for the WPS-3000 Autosampler**.

◾ Dionex ASI-100 Autosampler Series

In addition to the standard autosampler commands (see 🔲 **Dionex Autosamplers**), the autosamplers of the Dionex ASI-100 series provide many additional commands for controlling autosampler functions, such as the speed with which a sample is drawn (= DrawSpeed), the repeat count for mixing operations (= MixRepeat), or how deep the needle dips into the vial for the washing operations (= WashHeight). You can specify the standard commands further, for example, via the vial whose contents you wish to prepare (= PrepSubject) or via the volume to be used for the **Draw**, **Dispense**, and **Mix** (= PrepVolume) commands. For a list of the supported commands and properties, refer to the table below. (Please note that the display ➤*Filter* level determines which commands and properties are displayed.)

Special Commands and Properties

Command/Property	Description
AcquireExclusiveAccess	Assigns exclusive access to the current timebase when the autosampler is shared between two timebases. (Also, see ReleaseExclusiveAccess).
BlueSegment	Select the segment type that is used in the blue segment. The following types are available: Analytical - Semiprep - Mini - Eppendorf.
CoolingPower (only ASI-100T/ASI-100PT)	Indicates the cooling power (for diagnosis purposes only).
DispSpeed	Specify the speed at which the contents of the syringe are dispensed.
DownSpeed	Specify the speed at which the needle moves down.
DrawSpeed	Specify the speed at which the syringe is filled.
ExclusiveAccess	Reports which timebase has exclusive access to an autosampler that is shared by two timebases (read-only).
ExternalMSV	State of the external valve. The property is available only if the External MSV installed check box has been selected on the **Segments & MSV** tab page in the **Server Configuration** program.
GreenSegment	Select the segment type that is used in the green segment. Available types are: Analytical - Semiprep - Mini - Eppendorf.
HeatSinkTemperature (only ASI-100T/ASI-100PT)	Indicates the heat sink temperature (read-only).
InjectionCounter	Number of injections.
InjectMode	Inject Mode. If set to **Normal**, the autosampler draws the specified volume from the specified position and injects. If set to **Mix**, the autosampler injects whatever volume is left in the syringe after preceding draw/dispense operations.
InjectWaitTime	Period between the issuing of the command by Chromeleon and the acknowledgement from the autosampler.
InternalMSV	State of the internal motorized switching valve.
Mix	From the position that is determined via the **PrepSubject** parameter, the syringe draws and dispenses the volume that is determined via the **PrepVolume** parameter. If **PrepVial** is selected as **PrepSubject**, the **PrepVial** parameter must be specified as well. The **MixRepeat** parameter indicates the number of replicates during mixing.
MixRepeat	Repeat count for draw, dispense, and mix operations for mixing.
Msv2ToInject	Switch the external valve to **Inject**.
Msv2ToLoad	Switch the external valve to **Load**.
MsvToInject	Switch the injection valve to **Inject**.
MsvToLoad	Switch the injection valve to **Load**.
NeedleSealCounter	Needle seal wear.

Command/Property	Description
PrepareNextSample	Starts the injection procedure for the next sample, if available. However, the injection valve is only switched at the next **Inject** command (Display ➤*Filter* level: Advanced).
PrepareThisSample	Starts the injection procedure for the current sample. However, the injection valve is switched only at the next **Inject** command (Display ➤*Filter* level: Advanced).
PrepHeight	Needle height for draw, dispense and mix operations, specify how deep the needle will dip into the vial for mixing.
PrepSpeed	Syringe speed for draw and dispense operations for mixing.
PrepSubject	Specify the subject to be used for draw, dispense, and mix operations. The following subjects are only available: **PrepVial, SampleVial, WashVial, Air, ReagentAVial, ReagentBVial, ReagentCVial, ReagentDVial**. If **Air** is specified as mix subject, air is drawn in. During **Dispense,** the needle moves into the needle port and is dispensed there.
PrepVial	Specify the vial position if **PrepVial** is selected as **PrepSubject** (see **PrepSubject**).
PrepVolume	Volume to be used for the **Draw**, **Dispense**, and **Mix**.
➤*PrimeSyringe*	Primes the syringe by flushing it several times.
PumpDevice	Enter the name of the pump for which you want to synchronize the strokes with the autosampler. (This property is available only if you have selected an entry other than **<None>** from the **Sync Inject with pump** drop-down list on the **Syringe & Stroke Sync** tab page in the ➤*Server Configuration* program.)
RadialSpeed	The speed of the radial needle movement.
ReagentACapacity	Specify how often the volume can be drawn from the vial with reagent A (reagent B, C, and D, respectively).
ReagentAVial	Position of the reagent A (reagent B, C, and D, respectively) vial - is used only if **ReagentAVial** has been selected as **PrepSubject** (see **PrepSubject**).
RedSegment	Select the segment type that is used in the red segment. Available types are: Analytical - Semiprep - Mini - Eppendorf.
Relay1*, 2, 3 or 4*	The following commands are available: **State** (indicates or sets the state of the relay), **Duration** (when set, the relay's state toggles after the specified time), **On** (turns the relay on), and **Off** (turns the relay off).
Relay3Enabled	Relay 3 mode. If set to **Yes**, the relay can be controlled by Chromeleon. If set to **No**, the autosampler controls the relay and the relay indicates, whether the autosampler is operable.
Relay4Enabled	Relay 4 mode. If set to **Yes**, the relay can be controlled by Chromeleon. If set to **No**, the relay is controlled by the autosampler and indicates injection.
ReleaseExclusiveAccess	Releases exclusive access to the current timebase for an autosampler that is shared between two timebases. (Also, see ReleaseExclusiveAccess).
SampleHeight	Indicates the height at which the sample is drawn, measured from the vial bottom to the needle tip.

Command/Property	Description
State	Indicates that the autosampler has injected.
SyncWithPump	Synchronizes the injection with the cycle of a P680A LPG or P680A DGP-6 pump. (This property is available only if you have selected an entry other than **<None>** from the **Sync Inject with pump** drop-down list on the **Syringe & Stroke Sync** tab page in the ➢*Server Configuration* program.)
Syringe	Indicates the volume of the syringe that is installed in the autosampler.
SyringeCounter	Indicates the number of syringe movements.
SyringeDelay	Specify for how long the needle remains in the vial after loading.
⇒*Temperature* (only ASI-100T/ASI-100PT)	Define the set temperature of the autosampler's tray and hence of the sample. Click the + character to open the command tree; the following commands are available: **Value** (= actual temperature, read-only), **Nominal** (= set temperature), **UpperLimit**, and **Lower Limit**. With **UpperLimit** and **LowerLimit**, the system aborts the batch and starts emergency handling if the nominal temperature is outside these limits.
TempCtrl (only ASI-100T/ASI-100PT)	The following settings are available: **On** (= cooling/heating enabled) and **Off** (= cooling/heating disabled) (comp. **Nominal, Value, HeatSinkTemperature, CoolingPower**).
Test	Moves the needle to the specified vial. If no vial is specified, MixSubject is used (comp. **PrepSubject**).
TrayDetection	Turn tray detection (including manual interference monitoring) on or off. When tray detection is enabled, a home run is executed when the tray is installed. If the tray was removed while tray detection was disabled, a self-test must be performed when the tray is installed again. This is to make sure that the sample is injected from the correct position.
UpSpeed	The speed used to move the needle up.
⇒*Wash*	The internal MSV is switched to the **Load** position, if necessary. The volume specified under **WashVolume** is drawn from the position specified under **WashVial** and dispensed into the needle port/waste. The **WashSpeed** and **WashHeight** parameters are also considered.
WaitInput1, 2, 3, or 4	State of the digital input 1, 2, 3, or 4.
WashHeight	Wash height = distance between the bottom of the wash vial and the needle tip (= the depth with which the needle will dip into the vial).
WashSpeed	The speed with which the syringe draws the wash volume.
WashVial	Position of the wash vial.
WashVialVolume	Total volume of the wash vial.
WashVolume	Specify the volume to be drawn from the wash vial.

 Note:

All autosampler inputs are also available as CM input ports so that other device drivers can use them as well (for example, Remote Inject).

For a basic description of Dionex autosamplers, including the standard commands, refer to **Dionex Autosamplers**.

For information about how to use the commands in practical operation, refer to **Practical Tips for Device Control** **Autosampler Commands (ASI-100 Series)**.

For information about how to install the autosamplers of the ASI-100 series, refer to **Hardware Installation** **ASI-100 HPLC Autosampler Series: Overview** in the **Administrator Help** section.

Dionex AS/AS50 Autosamplers

In addition to the standard autosampler commands (see **Dionex Autosamplers**), the Dionex AS and AS50 Autosamplers support many additional commands for controlling autosampler functions. The following commands are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Special Commands

Command	Description
DoorSafetyTime	Displays the amount of time the autosampler door can remain open without aborting the sequence (read-only).
Flush	Flushes the inject port. During the flush cycle, the needle moves to the waste port and expels any fluid. The needle then moves to the inject port and delivers a volume of fluid (see FlushVolume below) for flushing the inject port. The needle then moves to the flush port and delivers a factory-set volume of flush fluid, which washes the outside of the needle.
FlushVolume	Sets the volume of liquid used for flushing the inject port. If the volume is greater than the sample syringe volume, the AS or AS50 performs multiple syringe strokes until the volume is reached.
Home	Moves the needle arm to home position.
➢*InjectionType*	Displays the type of injection (full-loop, partial-loop, or partial-loop limited sample) the AS or AS50 is performing (read-only).

Command	Description
LoopSize	Displays the size (volume) of loop installed (read-only).
Mode	(For AS or AS50 (USB) only) Displays the sample mode (normal, simultaneous, concentrate, sequential, or sequential concentrate) (read-only). The sample mode cannot be selected in Chromeleon. It is selected on the System Parameters screen on the AS or AS50 (USB) front panel.
Prime	Primes the liquid lines.
PrimeReservoir	Sets the reservoir to be used as a source for priming. The Flush_Reservoir is the default. If the sample preparation option is installed, Reservoir_A through D can be specified.
PrimeSyringe	Sets the syringe to be used for priming. The Sample syringe is the default. If the sample preparation option is installed, the Prep syringe can be specified.
PrimeVolume	Sets the volume of liquid used for priming the liquid lines.
SecondLoopSize	(For AS or AS50 (USB) Simultaneous mode only)
	Displays the size (volume) of loop installed on the second injection valve (read-only).
SerialNumber	Displays the autosampler serial number.
Status	Displays the current action being performed by the autosampler (read-only).
Stop	Stops the current process.
TrayType	Reports the type of sample tray (rack) installed (read-only).

Special Commands for Sampler Setup

The following commands set options that remain constant throughout the sample preparation steps and the time-dependent commands of a program.

Command	Description
ColumnTemperature	(Only for autosamplers equipped with the thermal compartment)
	Sets the temperature of the thermal compartment.
CutSegmentVolume	Sets the volume of sample discarded from each end of the aspirated sample. The middle portion of the sample is positioned in the loop and injected. The CutSegmentVolume is used only for partial-loop injections. To perform a partial-loop injection from a limited amount of sample, specify a CutSegmentVolume of 0. For full-loop injections, the CutSegmentVolume is ignored.

Command	Description
CycleTime	Sets the time between injections. To set a uniform time between injections, specify a cycle time in minutes (1 to 240). When a cycle time is specified, the autosampler delays sample injection until the specified time has elapsed since the previous injection. If the cycle time is set to 0, the time between injections is determined by the commands in the ➢ *Program* specified for each sample injection in a ➢ *Sequence*.
SetNeedleHeight	Sets the distance between the needle tip and the vial bottom. Note: This position is used for all functions, except for sample preparation functions that use a separately-specified NeedleHeight(see below).
SyringeSpeed	Sets the syringe speed. 1 is the slowest speed and 5 is the fastest. Select a slower speed for more viscous samples.
TrayTemperature	(Only for autosamplers equipped with the sample temperature control option)
	Sets the temperature of the sample tray.
WaitForTemperature	(Only for autosamplers equipped with a thermal compartment or a sample temperature control option)
	Instructs the autosampler to wait for the column or tray temperature to stabilize before proceeding to the next command.

Special Commands for Sample Preparation

The AS and AS50 provide several commands for preparing the sample before injection. The standard AS or AS50 supports eight sample preparation commands. If the autosampler is equipped with the sample preparation option, two additional commands are available.

Command	Description
DelaySP	Pauses sample preparation.
FlushSP	Flushes the inject port during sample preparation.
Mix	Mixes the vial contents.
NeedleHeight	Positions the needle above the vial bottom (used for sample preparation functions only).
Pipet	Moves sample between vials.
Concentrate	Loads and injects sample onto the concentrator column.
ReagentPrime	Primes the injector lines with reagent in preparation for a ReagentFlush.
ReagentFlush	Flushes reagent onto the concentrator column.

The following commands are available only if the AS or AS50 is equipped with the sample preparation option:

Command	Description
Dilute	Dilutes the sample with reagent.
Dispense	Dispenses reagent to a vial.

 Note:

*The **Concentrate, ReagentPrime**, and **ReagentFlush** commands require AS or AS50 (USB) Moduleware version 2.0.0 (or later). These commands are not available for the DX-LAN model of the AS50, regardless of which Moduleware version is installed in the autosampler.*

Special Commands for Exclusive Access

The exclusive access feature allows one AS or AS50 to be shared by two timebases.

Command	Description
AcquireExclusiveAccess	A timebase requests exclusive access to the autosampler as soon as the other timebase relinquishes exclusive access.
ReleaseExclusiveAccess	A timebase relinquishes exclusive access to the autosampler.

 Note:

*The DX-LAN model of the AS50 does not support the exclusive access feature. If a program created for this autosampler includes **AcquireExclusiveAccess** and **ReleaseExclusiveAccess**, the commands are ignored. However, both the AS and AS50 (USB) autosamplers can run any program created for the DX-LAN autosampler that includes these commands.*

Sample overlap is not allowed when the autosampler is shared by two timebases.

Calibration Commands

Command	Description
DownloadCalibration	Downloads the selected set of calibration values to the autosampler.
DownloadCalibrationParameter	Selects the set of calibration values (Current, Previous, or Factory) to be downloaded.
ExtLeakDet	Performs the leak detector calibration on the chromatography or thermal compartment.
InjPortVolCurVal	Specifies the current volume of the inject port. This value is determined when the inject port is manually calibrated. Calibration is not performed with Chromeleon. See the AS or AS50 operator's manual for details.
TrayLeakDet	Performs the autosampler tray leak detector calibration.

Diagnostic Commands

Command	Description
ExtLeakDetTest	Performs a test of the chromatography or thermal compartment leak detector.
ExtLeakDetTestDate	Reports the date of the leak detector test.
ExtLeakDetTestResult	Reports the result (pass or fail) of the leak detector test.
TrayLeakDetTest	Performs a test of the autosampler tray leak detector.
TrayLeakDetTestDate	Reports the date of the leak detector test.
TrayLeakDetTestResult	Reports the result (pass or fail) of the leak detector test.

For information about how to use the commands in practical operation, refer to **Practical Tips for Device Control** **Autosampler Commands (AS/AS50)**.

For a basic description of Dionex autosamplers, including the standard commands, refer to **Dionex Autosamplers**.

▪ Dionex AS-HV Autosampler

In addition to the standard autosampler commands (see ▪ **Dionex Autosamplers**), the Dionex AS-HV Autosampler supports several additional commands for controlling autosampler functions. The following commands are available (please note that the display ➢*Filter* level determines which commands and properties are displayed).

Special Commands for the AS-HV

Command	Description
ArmSpeed	Set the speed of the sampling arm. If septum-capped vials are loaded, always select **Septum** (the slowest speed).
DelayVolume	The volume of the sample loop.
GotoPosition	Moves the needle to the specified sample position.
Inject	Injects the sample.
NeedleDown	Lowers the needle.
NeedleHeight	Sets the distance between the needle and the vial bottom (0 is the lowest needle position; 160 is the highest position).
NeedleHome	Moves the needle to the home position.
PrepareNextSample	(Sample overlap programs only) Rinses the sample loop or concentrator column and, if a sample loop is installed, loads the next sample onto the loop.
	📝 *Note:*
	Chromeleon ignores the PrepareNextSample command if this is the last sample of a sequence, or if the next program is a blank run.
Rinse	Rinses the sample loop and the sampling needle.
RinseAndPrefill	Rinses the sample loop and then loads the delay volume onto the loop.
RinseSource	Reports the source of wash fluid for the rinse station (read-only).
SampleLoadingPump	Reports the sample loading pump selected in the AS-HV configuration properties (read-only).
SampleLoadingType	Reports whether sample will be loaded onto a sample loop or a concentrator column (read-only).
SamplesRackType	Identifies the type of sample rack installed (read-only).
SerialNo	Displays the serial number of the AS-HV (read-only).
StandardsRackType	Identifies the type of standards rack installed (read-only).

Command	Description
WashPumpOff	Turns off the rinse station's peristaltic pump (through Relay A).
WashPumpOn	Turns on the rinse station's peristaltic pump (through Relay A).

Special Commands for the Syringe Pump

The following commands are available when the syringe pump is selected as the AS-HV sample loading pump.

Command	Description
FlushAspirateSpeed	Specifies the speed at which the sampling needle is filled with wash fluid. For details, refer to the **Note** below.
RinseVolume	Specifies the volume of wash fluid used to rinse the sampling needle.
SampleAspirateSpeed	Specifies the speed at which the syringe is filled with sample. For details, refer to the **Note** below.
SyringeAspirate	Specifies the speed at which the syringe aspirates sample.
SyringeDispense	Specifies the speed at which the syringe dispenses sample. For details, refer to the **Note** below.
SyringeReset	Resets the syringe to its default values.
SyrPumpPosition	Reports the position of the syringe pump plunger (read-only).
SyrPumpStatus	Reports the status of the syringe pump: whether the pump is busy and whether an error condition has been detected (read-only).
SyringeSize	Reports the syringe volume (read-only).
ValvePosition	Sets the position of the three-way distribution valve in the syringe pump.
WashPortDispenseSpeed	Specifies the speed at which wash fluid is dispensed through the sampling needle. For details, refer to the **Note** below.
WasteDispenseSpeed	Specifies the speed at which liquid is directed to waste.

📝 *Note:*

There are two syringe speeds (fast and slow), and each speed has 10 levels. The actual flow rate depends on the command, the speed setting, and the syringe size. For more information, refer to the AS-HV operator's manual.

Special Commands for the AXP Pump

The following commands are available when the AXP pump is selected as the AS-HV sample loading pump.

Command	Description
AxpCurrentSetup	Reports the AXP pump configuration and the status of several operating parameters (read-only). To display details, click the plus sign beside this command.
AxpState	Turns the AXP pump on and off.
Flow	Sets the flow rate for the AXP pump.
Pressure.LowerLimit	Sets the lower pressure limit for the AXP pump. For details, refer to the **Note** below.
Pressure.UpperLimit	Sets the upper pressure limit for the AXP pump. For details, refer to the **Note** below.
Pressure.Value	Reports the current AXP pump pressure (read-only). For details, refer to the **Note** below.
RinseVolume	Specifies the volume of wash fluid used to rinse the sampling needle.

Note:

To display this command, click the plus sign beside the Pressure command.

Special Commands for the Internal Peristaltic Pump

The following commands are available when the internal peristaltic pump is selected as the AS-HV sample loading pump.

Command	Description
Flow	Sets the flow rate for the internal peristaltic pump.
InternalPumpOff	Turns off the internal peristaltic pump.
InternalPumpOn	Turns on the internal peristaltic pump.
IntPumpDirection	Specifies the direction of operation for the internal peristaltic pump.
RinseVolume	Specifies the volume of wash fluid used to rinse the sampling needle.

Special Commands for a Relay-Controlled Pump

The following commands are available when an external relay-controlled pump is selected as the AS-HV sample loading pump.

Command	Description
Flow	Sets the flow rate for the relay-controlled pump.
RinseVolume	Specifies the volume of wash fluid used to rinse the sampling needle.

For a basic description of Dionex autosamplers, including the standard commands, refer to 🔲 **Dionex Autosamplers**.

🔲 Dionex/LC Packings FAMOS Capillary/Nano HPLC Autosamplers

In addition to the standard autosampler commands (see 🔲 **Dionex Autosamplers**), the Dionex/LC Packings FAMOS Autosampler provides additional commands for controlling autosampler functions. The following general commands are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Description
AlarmBuzzer	When set to **On**, the buzzer will signal error conditions.
	🔳☞ *Tip:*
	If the buzzer signals an error condition (AlarmBuzzer=On), you have to turn the autosampler off and on again to reset the instrument and continue operation. Therefore, avoid using this command when controlling the autosampler under Chromeleon.
CoolingOnTime	Indicates cooling time (in total) of the autosampler (read-only).
⇒*Dispense*	Dispenses a specified volume. The following options are available:
	To: Sample position to which to dispense the volume.
	Volume: Volume to dispense (0.0 to 25.0 µl).
	SyringeSpeed: Speed used to dispense the volume (1 to 9 - optional setting: if the SyringeSpeed is not set, the PrepSpeed will be used instead (see below)).
	SampleHeight: Height at which the sample is dispensed (0 to 40 mm - see below).

Command/Property	Description
⇒*Draw*	Draws a specified volume. The following options are available:
	From: Sample position from which to draw the volume.
	Volume: Volume to dispense (0.0 to 25.0 µl).
	SyringeSpeed: Speed used to draw the volume (1 to 9 - optional setting: if the SyringeSpeed is not set, the PrepSpeed will be used instead (see below).)
	SampleHeight: Height at which the sample is drawn (0 to 40 mm - see below).
FirstTransportVial	Defines the vials to be used for µlPickUp injections. Used together with LastTransportVial.
FlushVolume	Flush volume used for FullLoop and PartialLoop injections (0.1 to 25.0 µl).
⇒*Inject*	Injects the sample. If you have created a user-defined FAMOS program, e.g., in the 🔳 **Program Wizard**, use the **Inject** command to start the program. In this case, injection is performed after the **InjectMarker** command of the user-defined program.
	📑 *Note:*
	*Injection can be performed only if the autosampler is in **Not running** state. This is usually ensured by the **WaitSampler.Ready** command that is generated by the PGM Wizard. If the system detects a different state, the following message appears in the Audit Trail: Abort] hh:mm:ss {Sampler} Sampler status must be "Not running" before inject."*
InjectionCounter	Total number of injections (read-only).
InjectMode	Injection mode (for more information, refer to the operating instructions for the instrument):
	UserProg The autosampler executes the program or sample preparation steps defined by the user.
	Partial The syringe volume is injected in part only (at most 50% of the sample loop volume).
	FullLoop The syringe volume is injected completely.
	µlPickUp The syringe volume is injected completely. However, it contains partly sample volume only. Transport liquid is drawn first, then sample, and then transport liquid again, i.e. the syringe, the sample needle, and both ends of the sample loop are filled with transport liquid. That is why the TransportVial should contain solvent. The sample volume is in the middle of the sample loop.
InjectValve	Injection valve can be in the following positions: **Inject** or **Load**.
ISS_A / ISS_B_Immediate	Switches the associated valve immediately.

Command/Property	Description
LastTransportVial	Defines the vials to be used for µIPickUp injections. Used together with **FirstTransportVial**.
LoopVolume	Volume of the installed loop (read-only*).*
LowDispersionFactor	(In Micro mode only) For more information, refer to the FAMOS User's Manual.
LowDispersionFlow	(In Micro mode only) For more information, refer to the FAMOS User's Manual.
LowDispersionMode	Enable low dispersion mode (in Micro mode only).
OperationalMode	Operational mode (Micro or Conventional - also, refer to **Hardware Installation** 🔵 **UltiMate Capillay/Nano HPLC System: Overview** in the **Administrator Help** section). Affects some limits, changing resets many operational parameters (read-only).
Output	Sets the corresponding output to the specified state.
PowerOnTime	Indicates the time (in total) the autosampler was turned on (read-only).
PrepSpeed	Speed used as the default syringe speed.
PrepVial	Sample preparation vial for the Draw and Dispense actions.
ReagentXVial *(where X = A to D)*	Position of the vial that contains the reagent X or the transportation fluid when **UserProg** is selected as Inject Mode.
RinsebetweenReinjections	If this parameter is set to **No**, the rinse step is omitted. 📝 *Note:* *This command allows you to make several injections from the same vial one after the other without loosing sample between the injections by rinsing.*
RunScreen	If the command is set to **On**, the action currently performed by the autosampler is displayed on the instrument.
SampleHeight	The height at which the sample is drawn or dispensed.
Status	Status (read-only).
Syringe	Volume of the installed syringe (read-only).
SyringeCounter	Total number of syringe movements (read-only).
SyringeSpeed	Syringe speed used for draw and dispense operations (**Low**, **Normal**, or **High**). The flow depends on the volume of the installed syringe. (For more information, refer to the FAMOS User's Manual.) 📝 *Note:* *The flow into or from the syringe is the product of the speed defined by **SyringeSpeed** and the **SyringeSpeedFactor**.* *Example: SyringeSpeed=Low corresponds to 31.3µl/min for the 25µl syringe), SyringeSpeedFactor=0.5. The resulting flow is 15.65µl/min.*

Command/Property	Description
SyringeSpeedFactor	Factor used for scaling the syringe speed (see SyringeSpeed).
SyringeValve	Can be in the following positions: Needle, Wash, or Waste
SyringeValveCounter	Counts the valve movements.
TempCtrl *(with installed Cooling Option only)*	Temperature control of the sample rack. The following settings are available: On (cooling/heating enabled) or Off (cooling/heating disabled).
Temperature *(with installed Cooling Option only)*	You can indicate the nominal temperature for the tray and thus for the sample. The temperature can be between 4°C to 40°C (39.2°F to 104°F). If you open the command tree, the following commands are available: **Value** (= current tray temperature, read-only), **Nominal** (= nominal tray temperature), **UpperLimit**, and **LowerLimit**. If the current temperature exceeds the limits, the system aborts the batch and starts emergency handling.
TubingVolume	Volume of the installed needle capillary (read-only).
UseAirSegment	When set to On, an additional air segment is added when loading the sample loop. This reduces sample contamination. (For more information, refer to the FAMOS User's Manual.)
UseHeadSpace	When set to On, additional pressure is applied to the vial while the sample is transported to the loop. This increases the reproducibility but requires airtight vials and/or well septa. (For more information, refer to the FAMOS User's Manual.)
⇒*Wash*	Starts the wash cycle.
WashVolume	Volume used for the wash cycle.

For a basic description of Dionex autosamplers, including the standard commands, refer to 🔼 **Dionex Autosamplers**.

For more information about the sample preparation commands for the FAMOS autosampler, refer to 🔼 **Dionex/LC Packings FAMOS Autosampler: Sample Preparation**.

For information about how to use the commands in practical operation, refer to **Practical Tips for Device Control** 🔼 **Examples for User-Defined Programs for the FAMOS Autosampler (Dionex/LC Packings)**.

For installation instructions for the FAMOS autosampler, refer to **Hardware Installation** 🔵 **FAMOS Capillary/Nano Autosampler: Overview** in the **Administrator Help** section.

▪ Dionex/LC Packings FAMOS Autosampler: Sample Preparation

The Dionex/LC Packings FAMOS Autosampler provides many sample preparation commands that are used to further define the individual sample preparation steps. The sample preparation commands are accessible from the **Advanced** level only. Use the ▪ **Program Wizard** to create a user-defined sample preparation program. This program is started by the ⇒*Inject* command.

Chromeleon supports the following sample preparation commands (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Command/Property	Description
CodeOutput	Communicates a code number (between 0 and 15) to the **P2** output (VialNo&Marker (➤*TTL*)). For more information, refer to the FAMOS User's Manual.
Compressor	Serves to build up pressure (On) or relieve pressure (Off)
⇒*Dispense*	Dispenses a specified volume. The following options are available:
	To: Sample position to which to dispense the volume.
	Volume: Volume to dispense (0.0 to 25.0 µl).
	SyringeSpeed: Speed used to dispense the volume (Either select an option (**Low**, **Normal**, or **High**) from the drop-down list or enter the any number between **1** and **9**).
	SampleHeight: Height at which the sample is dispensed (0 to 40 mm).
⇒*Draw*	Draws a specified volume. The following options are available:
	From: Sample position from which to draw the volume.
	Volume: Volume to dispense (0.0 to 25.0 µl).
	SyringeSpeed: Speed used to draw the volume (Either select an option (**Low**, **Normal**, or **High**) from the drop-down list or enter the any number between **1** and **9**).
	SampleHeight: Height at which the sample is drawn (0 to 40 mm).
InjectMarker	Required to start injection.
InjectValve	Injection valve. The valve can be either in Inject or in Load position.
LabeledVialMarker	Creates another marker pulse for different vials, e.g., standard samples.
MixWait	Specify the time the system waits between steps (0s (0:00:00h) to 35.999s (9:59:59h))
MixNeedleWash	Volume used for washing the needle (10 to 9.999µl).

Command/Property	Description
MoveSyringe	Determines the volume and the speed for loading and dispensing the syringe. For this command, the **Load** and **Unload** parameters are available. 🖙 *Tip:* *Do not use both parameters in the same line of the PGM File because in this case, the PGM File does not pass the ⇗Ready Check.*
MoveSyringeHome	Returns the syringe to its home position.
Output	Sets the corresponding output to the selected state.
SSV	Solvent Selection Valve. Specify the **Position**. The following options are available: **Solvent A-D**.
SyringeValve	Specify the Position. The following options are available: **Needle**, **Wash**, or **Waste**.
Valve_A	Specify the **Position**. The following options are available: **1_2** (concentrating the sample) or **10_1** (eluting the sample concentrated on the pre-column to the analytical column).
Valve_B	Specify the **Position**. The following options are available: **10_1** (concentrating the sample) or **1_2** (eluting the sample concentrated on the pre-column to the analytical column).
VialMarker	Generates a marker pulse for sample vials, e.g., unknown samples.
WaitInput	Defines the incoming signal for which to wait.
⇒*Wash*	Starts a wash cycle.

For a basic description of Dionex autosamplers, including the standard commands, refer to 🔲 **Dionex Autosamplers**.

For information about the general commands for the FAMOS autosampler, refer to 🔲 **Dionex/LC Packings FAMOS Autosampler**. (The general FAMOS autosampler commands partly overlap the sample preparation commands listed above.)

For information about how to use the commands in practical operation, refer to **Practical Tips for Device Control 🔲 Examples for User-Defined Programs for the FAMOS Autosampler (Dionex/LC Packings)**.

Dionex GINA 50 Autosamplers

In addition to the standard commands (see **Dionex Autosamplers**), the Dionex GINA 50 autosampler provides additional commands for controlling autosampler functions such as multiple draw operations (**Suck**) from one sample, washing the sample loop (**Wash**), moving the sample needle (**NeedleUp**), or cooling the sample vials. The following commands are available (note that it depends on the display ➤*Filter* level which commands are displayed):

Special Commands

Command	Description
⇒*Dispense*:	Dispenses a specified volume; Please note: If position = 101, the autosampler dispenses the volume into the needle port.
Load	Switches the internal switching valve without moving the needle (also, refer to NeedleUp)
⇒*NeedleUp*	Moves the needle up.
⇒*Suck*:	Draws a specified volume. Please note: If position = 100, the autosampler draws an air segment from an imaginary air vial.
TempCtrl	Enables temperature control of the Dionex GINA 50T autosampler. The Gina 50T autosampler is capable of controlling the rack temperature and, thus, the sample temperature within a 15°C range. At room temperature (20°C), maximum sample cooling is to 5°C above zero.

For a general description of the Dionex autosamplers, including the standard commands, refer to **Dionex Autosamplers**.

For information about how to use the commands in practical operation, refer to **Practical Tips for Device Control Autosampler Commands (GINA 50).**

For installation instructions for the GINA 50 autosampler, refer to **Hardware Installation GINA50 HPLC Autosampler: Overview** in the **Administrator Help** section.

Dionex Flow Manager and Thermostatted Column Compartments

In addition to the **General Device Commands**, the **Temperature** property is available for controlling the operating functions of the Dionex Thermostatted Column Compartments:

Property	Description
Temperature	Click the "+" character beside the name to display the items underneath. Depending on the column compartment or flow manager type, some commands may not be available.
	Value-reports the current temperature. **Nominal**-sets the nominal temperature. **UpperLimit**-sets the upper temperature limit **LowerLimit**-sets the lower temperature limit.
	If the nominal temperature is not in the limits, the command is not executed.

For information about the special commands and properties available for the flow manager and thermostatted column compartments, refer to:

 Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments

 Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments: Column ID Properties

 Dionex FLM-3x00 Flow Manager and Thermostatted Column Compartment

 Dionex FLM-3x00: Column ID Properties

 Dionex FLM-3x00: Splitter Commands

For an example program, refer to **Practical Tips for Device Control** **Controlling the Column Temperature**.

▪ Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments

In addition to the commands supported for the different column compartments, such as Connect, Disconnect, and Temperature (see ▪ **Dionex Flow Manager and Thermostatted Column Compartments**), Chromeleon supports the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed and that, depending on the column compartment type, some commands or properties may not be available):

Command/Property	Description
ActiveColumn	see ▪ **Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments: Column ID Properties**.
AnalyticalPump	Reports the name of the pump that is used for making the gradient.
Brightness	Adapts the brightness setting of the column compartment's front panel display to your requirements.
ClearDisplayError	Clears the error message on the column thermostat's display.
Column_A Column_B	The column names correspond to the names entered on the **Configuration** tab page in the Server Configuration program. The various column commands and properties allow you to enter and check information about the column. For more information, refer to ▪ **Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments: Column ID Properties**.
ColumnA(B/C/D)_ LeftValve ColumnA(B/C/D)_ RightValve	Sets the position of the related column switching valve to redirect the flow to column A (B, C, or D).

Command/Property	Description
ColumnOven_Temp	This channel is available only if you have selected the **TemperatureSignal** check box in the TCC-100's properties (on the **Configuration** tab page in the Server Configuration program.) If the check box is selected, Chromeleon records the temperature as a separate channel. The channels's name is the name entered on the **Configuration** tab page in the Server Configuration program.
	Click the "+ "character beside the name to display the items underneath:
	Delta–reports the signal's slope, i.e., the difference between the current value and the value one second ago. This is useful for triggers.
	Signal–has the following commands: **Value** (current signal value, read-only), **UpperLimit** and **LowerLimit**. If the current signal value is outside these limits, a warning appears in the Audit Trail.
	AcqOff–terminates data acquisition.
	AcqOn–starts data acquisition.
	Retention–reports the retention time of the signal (read-only).
	MaxAutoStep = Maximum step rate for Auto Step Mode; range: 0.1 - 5.1 s; default: 5.1 s
	Step–sets the step for data acquisition; range: 0.01 - 4.80 s; **Auto** selects the best step dynamically
	Average–averages all measured values over the step interval. Default: On. Off records only the last point of each interval.
ColumnsToUse	Sets the two columns to be used alternately for injection (see also **CurrentColumn**, **ColumnsToUse**, **NextColumn)**.
Contrast	Adapts the screen contrast of the column compartment's front panel display to your requirements.
CurrentColumn	Selects the current column (see also **ColumnsToUse**, **NextColumn**, **ColumnX_XxxValve**).
Door	Reports whether the compartment door is open or closed (**Open** or **Closed**; read-only).
EquilibrationTime	The column compartment enters the **Ready** state only if the deviation between the current temperature and the set point temperature did not exceed the maximum deviation specified under **ReadyTempDelta** for the time specified here (also, see **ReadyTempDelta** and **Ready**).
FirmwareVersion	Reports the firmware version of the thermostatted column compartments (read-only).
GasLeak	Reports whether the gas sensor has detected a leak (**NoLeak** or **Leak**; read-only).
GasLeakSensor	Sets the sensitivity with which the sensor responds to gas in the column chamber. Click the arrow in the **GasLeakSensor** combo box and select an option from the list: **Low**, **Standard**, or **High**. To turn the sensor off, select **Off**.

Command/Property	Description
HardwareVersion	Indicates the compartment's hardware version.
HumidityLeak	Indicates whether the humidity sensor has detected a leak (**NoLeak** or **Leak**; read-only).
HumidityLeakSensor	Sets the sensitivity with which the sensor responds to humidity. Click the arrow in the **HumidityLeakSensor** combo box and select an option from the list: **Low**, **Standard**, or **High**. To turn the sensor off, select **Off**.
InstalledOn	Indicates the timebase in which the autosampler is installed.
Leak	Indicates whether at least one leak sensor has detected leakage (**NoLeak** or **Leak**; read-only; also see **HumidityLeak** and **GasLeak**).
LeakAlarmOff	Deactivates the leak alarm.
LeakSensorState	Reports whether the sensors are ready for leak detection (**Ready**, **NotReady**, or **Error**; read-only).
ModelNo	Reports the column compartment's model number (read-only).
MsvPosition	If a column switching valve is installed and if the **Internal MSV** check box is selected on the **Configuration** tab page in the Server **Configuration** program, the column switching valve is switched to the desired position (A (1) or B (2)).
NotReadyCauses	Reports the column compartment's current status (read-only).
Ready	Reports whether the compartment is ready to operate (**Ready** or **NotReady**; read-only; also see **ReadyTempDelta** and **EquilibrationTime**).
ReadyTempDelta	If the current temperature deviates from the temperature set point by more than the **ReadyTempDelta**, the column compartment enters the NotReady state. If the ReadyTempDelta is set to None, Chromeleon does not check whether the temperature set point deviates from the actual temperature (also see **Ready** and **EquilibrationTime**).
ReconditionPump	Selects the pump that is used to recondition the column. ☞ *Tip:* *This command applies only to Summitx2 devices and is used by Chromeleon only for the **NextColumn** command.*
SerialNo	Serial number of the column compartment (read-only).
SetFactoryDefaults	Resets the column thermostat settings to the Dionex factory default settings.
Standby	Sets the column compartment to standby mode (**Standby**) or turns it off (**NoStandby**). In the standby mode, the connection between the column compartment and Chromeleon is not interrupted.
TCC_INPUT_1 and/or TCC_INPUT_2	Reports the state of the related digital input (**On** or **Off**).

Command/Property	Description
TCC_RELAY_1 and/or TCC_RELAY_2	Relay 1 and/or relay 2. Click the "+"character beside the relay name to display the items underneath: **State**–reports the state of the relay. **Duration**–when set, the relay toggles after the specified time. **On**–turns the relay on. **Off**–turns the relay off
Temperature	Reports the column oven's current temperature. The following commands are available if you open the command tree: **Value** (current signal value, read-only), **Nominal** (nominal temperature), **UpperLimit**,and **LowerLimit**. If the current signal value of **UpperLimit** and **LowerLimit** is outside these limits, a warning appears in the Audit Trail.
TempCtrl	Turns temperature control **on** or **off**.
TempReady	Reports whether the temperature is within the specified limits (**Ready** or **NotReady**).
ValveLeft ValveRight	Indicates and sets the position of the related valve.

	Moving	The valve switches.
	6_1/10_11	Depending on the valve type, ports 6 and 1 or 10 and 1 are fluidically connected.
	1_2	Ports 1 and 2 are fluidically connected.
	NotConnected	The control electronics is not connected. Please contact Dionex Service.
	Error	An error occurred. Please contact Dionex Service.

Command/Property	Description
Vendor	Indicates the manufacturer's name (read-only).
WorkingHours	Counts the operating hours of the column thermostat.

System Wellness and Predictive Performance commands and properties

🖙 *Tip:*

Note that the installed Chromeleon version and TCC type determine which commands and properties are available for checking the column compartment's system wellness and performance.

Command/Property	Description
CoolingWorkLoad	Reports the total cooling work load [K*min] since the column compartment was operated for the first time (read-only).
HeatingWorkLoad	Reports the total heating work load [K*min] since the column compartment was operated for the first time (read-only).

Command/Property	Description
LeftRotorSealChanged	After you have replaced the left rotor seal, perform this command to update the related service information.
LeftRotorSealChangeDate	Reports when the rotor seal was replaced last (read-only).
LeftRotorSealSwitches	Counts how many times the left valve has moved since the rotor seal was replaced last.
*.Value	Reports the current number of valve movements (read-only).
LeftValveSwitches	Counts how many times the left valve switches. (read-only)
OperatingHours	Counts the operating hours since initial operation (excluding the operating hours during standby mode; read-only).
PowerOnTime	Counts the operating hours since last power-up (or wake-up from standby) (read-only).
Qualification	Provides information about system qualification.
*.LastDate	Reports the date when detector qualification was performed last (read-only).
*.LastOperator	Reports the name of the person who performed the qualification (read-only).
*.Interval	Reports the interval (in days) when the next system qualification is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
*.GracePeriod	Indicates the period during which the device may be operated after the due date for re-qualification.
RightRotorSealChanged	After you have replaced the rotor seal, perform this command to update the related service information.
RightRotorSealChangeDate	Reports when the right rotor seal was replaced last (read-only).
RightRotorSealSwitches	Counts how many times the right valve has switched since the rotor seal was replaced last.
*.Value	Reports the current number of valve movements (read-only).
RightValveSwitches	Counts how many times the right valve switches. (read-only).
Service	Provides detector service information.
*.LastDate	Reports the date of the last service (read-only).
*.Interval	Specify the interval (in days) after which a service is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
StandbyHours	Counts the total operating hours in standby mode since the module was operated for the first time (read-only).

For information about column identification, refer to **Dionex TCC-100/TCC-3x00 Thermostatted HPLC Column Compartments: Column ID Properties**.

For an example program for the Dionex themostatted column compartments, refer to **Practical Tips for Device Control** ▣ **Controlling the Column Temperature**.

For information about how to install the column compartment, refer to **Hardware Installation** ⮑ **UltiMate 3000 Series - Thermostatted HPLC Column Compartment: Overview** and **Hardware Installation** ⮑ **TCC-100 Column Compartment: Overview** in the **Administrator Help** section.

▣ Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments: Column ID Properties

The column thermostats support the following column ID properties. The column properties appear underneath the column name specified in the Server Configuration program. (Note that the display ➤*Filter* level determines which properties are displayed and that, depending on the TCC type, some commands or properties may not be available.)

Primary column properties

These properties are used for column identification. They are entered once by either the column manufacturer or the user and cannot be changed.

Property	Description
BatchNumber	Batch or lot number of the column.
CustomId	You can enter any number you want, for example, the column's inventory number. (Note: The number cannot be changed once it is entered.)
DateOfManufacture	Manufacturing date of the column.
ProductId	Product number of the column.
SerialNo	Serial number of the column.

Secondary column properties

The user can enter and later change the following properties if necessary:

Property	Description
ActiveColumn	**TCC-100**: Determine for which column the column identification information is updated (A or B). The names correspond to the names entered on the **Configuration** tab page in the Server Configuration program.
	TCC-3x00: Specify whether the column identification information is updated for the currently used column (**Yes** or **No**); see also **NextColumn**.
CardState	Reports whether the memory card is present (read-only):
	NoChip The card is missing.
	DoorOpened The door is open. (The card is not writeable while the door is open.)
	UnknownFormat The card format is unknown.
	BusBlocked One of the cards is not inserted correctly. Thus, it may happen that another card is not readable although it is correctly.
DateOfExpiration	Sets the expiration date of the column. If a date has been entered, the current date is compared with the expiration date during injection or at the beginning of batch processing. If the current date exceeds the expiration date or if batch processing starts less than 24 h before the expiration date, a warning appears once per ➢Batch.
Description	Column description.
Diameter	Column diameter.
FormatChipCard	Select this command to format a chip card that contains invalid or corrupt data.
Injections_Limit	Maximum number of injections allowed for the column.
Length	Column length.
Manufacturer	Column manufacturer.
NextColumn	Switches the column switching valve to the next position, directing the flow through either of the alternately used columns.
PackingMaterial	Packing material of the column.
ParticleSize	Particle size
pH_LowerLimit pH_UpperLimit	Upper and lower pH limits. If both limits are set to the same value, this means that no limit exists.
	Note:
	These values are for information purposes only. The device driver cannot check the pH value.

Property	Description
Pressure_UpperLimit	If the column pressure exceeds the upper pressure limit, a warning appears. 🔲 *Note:* *Accept the default (0.0) if you do not want to have the system check the pressure.*
Temp_LowerLimit Temp_UpperLimit	If the column temperature is above the upper temperature limit or below the lower temperature limit, a warning appears. If both limits are set to the same value (default: 25°C each), this means that no limit exists. (These values also apply to the inactive column.)
VoidVolume	The column's void volume in [µl]

System properties

System properties are read-only and cannot be changed by the user. Chromeleon determines and updates the following properties during each injection:

Property	Description
DateOfFirstUse	Date of the first injection
DateOfLastUse	Date of the last injection
Injections_Counter	Number of injections
Pressure_Max	Maximum pressure measured while the column was in use
Temp_Min	Minimum temperature measured while the column was in the column compartment
Temp_Max	Maximum temperature measured while the column was in the column compartment

User properties

The user can change the following properties at any time:

Property	Description
Application	Enter any text to describe the application.
Comment	Enter any comment text.
SystemPressure	Name of the device to be used for system pressure measurement.

For information about the special commands supported by the thermostatted column compartment, refer to 🔲 **Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments**.

For information about the standard commands supported for the different column compartments, refer to 🔲 **Dionex Flow Manager and Thermostatted Column Compartments**.

Dionex FLM-3x00 Flow Manager and Thermostatted Column Compartment

For the flow manager, several subentries appear in the Commands dialog box. They provide the following commands and properties:

Command/Property	Description
Column_1 Column_2 (possibly Column_3)	The column names correspond to the names entered on the **Components** tab page in the Server Configuration program. The various commands and properties allow you to enter and check information about the column. For more information, refer to **Dionex FLM-3x00 Flow Manager and Thermostatted Column Compartment: Column ID Properties**.
ColumnOven_Temp	This channel is available only if you have selected the **TemperatureSignal** check box in the FLM's properties (on the **Configuration** tab page in the Server Configuration program.) If the check box is selected, Chromeleon records the temperature as a separate channel. The channel's name is the name entered on the **Configuration** tab page in the Server Configuration program.
	Click the "+ "character beside the name to display the items underneath:
	Delta–reports the signal's slope, i.e., the difference between the current value and the value one second ago. This is useful for triggers. **Signal**–has the following commands: **Value** (current signal value, read-only), **UpperLimit** and **LowerLimit**. If the current signal value is outside these limits, a warning appears in the Audit Trail.
	AcqOff–terminates data acquisition.
	AcqOn–starts data acquisition.
	Retention–reports the retention time of the signal (read-only).
	MaxAutoStep = Maximum step rate for Auto Step Mode; range: 0.1...5.1 s; default: 5.1 s
	Step–sets the step for data acquisition; range: 0.01...4.80 s; **Auto** selects the best step dynamically
	Average–averages all measured values over the step interval. Default: On. Off records only the last point of each interval.
FLM-3x00_INPUT_1 and/or FLM-3x00_INPUT_2	Reports the state of the related digital input (**On** or **Off**). The property is read-only.

Command/Property	Description
FLM-3x00_RELAY_1 and/or FLM-3x00_RELAY_2	Relay 1 and/or relay 2. Click the "+"character beside the relay name to display the items underneath: **State**–reports the state of the relay. **Duration**–when set, the relay toggles after the specified time. **On**–turns the relay on. **Off**–turns the relay off
FlowSplitter_1	The splitter name corresponds to the name entered on the **Configuration** tab page in the Server Configuration program. For information about the commands and properties that are available, refer to 🔳 **Dionex FLM-3x00 Flow Manager and Thermostatted Column Compartment: Flow Splitter Properties**.

In addition to the standard column compartment commands, such as Connect, Disconnect, and Temperature, (see 🔳 **Dionex Flow Manager and Thermostatted Column Compartments**), the FLM-3x00 supports the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Description	
Brightness	Adapts the brightness of the pump's front panel display to your requirements (0 - 100 %).	
Contrast	Adapts the contrast of the pump's front panel display to your requirements (0 - 100 %).	
Door	Reports whether the front panel door is open or closed (**Open** or **Closed**; read-only). 📑 *Note:* *You cannot start a batch when the front panel door is open.*	
FlowController1Error	Reports whether the flow splitter 1 is working properly (**OK** or **Error**) (read-only).	
FluidLeak	Reports whether the fluid leak sensor has detected a leak (**Ok** or **Leak**; read-only).	
FluidLeakSensor	Settings of the fluid leak sensor:	
	Enabled	Enables leak detection via the fluid leak sensor. If a leak is detected, a message appears in the Chromeleon Audit Trail and an acoustic beep sounds.
	Silent	Enables leak detection. If a leak is detected, a message appears but no beep sounds.
	Off	Disables the leak detection.

Command/Property	Description
GasLeak	Reports whether the gas sensor has detected a leak (**Ok** or **Leak**; read-only).
GasLeakSensor	Sets the sensitivity with which the sensor responds to gas in the column chamber. Click the arrow in the **GasLeakSensor** combo box and select an option from the list: **Low**, **Standard**, or **High** (if the sensor is activated, a message appears in the Chromeleon Audit Trail and an acoustic beep sounds) or **Low_Silent**, **Standard_Silent**, or **High_Silent**, respectively (a message appears but no beep sounds). To turn the sensor off, select **Off**.
HardwareVersion	Indicates the flow manager's hardware version.
HumidityLeak	Indicates whether the humidity sensor has detected a leak (**Ok** or **Leak**; read-only).
HumidityLeakSensor	Sets the sensitivity with which the sensor responds to humidity. Click the arrow in the **HumidityLeakSensor** combo box and select an option from the list: **Low**, **Standard**, or **High** (if the sensor is activated, a message appears in the Chromeleon Audit Trail and an acoustic beep sounds) or **Low_Silent**, **Standard_Silent**, or **High_Silent**, respectively (a message appears but no beep sounds). To turn the sensor off, select **Off**.
InstalledOn	Indicates the timebase in which the autosampler is installed.
Leak	Indicates whether at least one leak sensor has detected a leakage (**Ok** or **Leak**; read-only; also see **FluidLeak**, **GasLeak**, and **HumidityLeak**).
LeakAlarmOff	Turns off the beep for the current alarm. A new beep sounds when one of the leak sensors detects another leak.
LeakSensorState	Reports whether the sensors are ready for leak detection (**Ready**, **NotReady**, or **Error**; read-only).
MSVPosition	*(FLM-3300 only)* Reports and/or sets the current valve position:
	Moving — The valve is switching.
	10_1 — Indicates the fluid connections in the valve: ports 10 and 1 are connected.
	1_2 — Indicates the fluid connections in the valve: ports 1 and 2 are connected.
	NotConnected — The control electronics is not connected; contact Dionex Service.
	Error — An error occurred. Contact Dionex Service.
MSVPosition_Counter	Counts how often the left and/or right valve was switched since being installed (read-only).
Ready	Reports whether the instrument is ready to operate (**Ready** or **NotReady**; read-only; also see **ReadyTempDelta** and **EquilibrationTime**). *Note:* You cannot start a batch when the flow manager is not ready.

Command/Property	Description
ReadyTemperatureDelta	Range: 0.0 - 5.0°C. If the current temperature deviates from the temperature set point by more than the **ReadyTempDelta**, the flow manager enters the NotReady state. If the ReadyTempDelta is set to 0,Chromeleon does not check whether the temperature set point deviates from the actual temperature (also see **Ready** and **EquilibrationTime**).
SerialNo	Serial number of the manager (read-only).
SetFactoryDefaults	Resets the flow manager settings to the Dionex factory default settings.
Standby	Sets the flow manager to **Standby** mode or cancels this mode (**NoStandby**) to resume operation (values: **NoStandby** (0), **Standby** (1)). *Note:* *You cannot start a batch when the flow manager is in standby mode.*
TempCtrl	Turns temperature control **on** or **off**.
TempReady	Reports whether the temperature is within the defined limits (**Ready** or **NotReady**). (Read-only, also see Temperature.) *Note:* *You cannot start a batch when the temperature is not within the defined limits.*
ValveLeft ValveRight	*(FLM-3100 and FLM-3200 only)* Reports and/or sets the current valve position:
	Moving — The valve is switching.
	10_1 (or **6-1**) — Indicates the fluid connections in the valve: ports 10 (6) and 1 are connected.
	1_2 — Indicates the fluid connections in the valve: ports 1 and 2 are connected.
	NotConnected — The control electronics is not connected; contact Dionex Service.
	Error — An error occurred. Contact Dionex Service.
ValveLeftCounter ValveRightCounter	*(FLM-3100 and FLM-3200 only)* Counts how often the left and/or right valve was switched since being installed (read-only).
Vendor	Indicates the device manufacturer (read-only).
WorkingHours	Counts the operating hours of the column thermostat.

For information about column identification, refer to **Dionex FLM-3x00: Column ID Properties**, for information about the flow splitter commands and properties, refer to **Dionex FLM-3x00: Flow Splitter Commands**.

For information about how to install the flow manager, refer to **Hardware Installation FLM-3x00 Flow Manager and Thermostatted Column Compartment: Overview** in the **Administrator Help** section.

▣ Dionex FLM-3x00: Column ID Properties

The Dionex FLM-3x00 Flow Manager and Thermostatted Column Compartment supports the following column ID properties. The column properties appear underneath the column name specified in the Server Configuration program. (Note that the display ➤*Filter* level determines which properties are displayed.)

Primary column properties

These properties are used for column identification. They are entered once by either the column manufacturer or the user and cannot be changed.

Property	Description
BatchNumber	Batch or lot number of the column.
CustomId	You can enter any number you want, for example, the column's inventory number. (Note: The number cannot be changed once it is entered.)
DateOfManufacture	Manufacturing date of the column.
ProductId	Product number of the column.
SerialNo	Serial number of the column.

Secondary column commands and properties

The user can enter and later change the following commands and properties if necessary:

Property/Command	Description	
ActiveColumn	Set to **Yes** to have Chromeleon update the information for the associated column.	
CardState	Reports whether the memory card is present:	
	NoChip	The card is missing.
	DoorOpened	The door is open. (The card is not writeable while the door is open.)
	UnknownFormat	The card format is unknown.
	BusBlocked	One of the cards is not inserted correctly. Thus, it may happen that another card is not readable although it is inserted correctly.
DateOfExpiration	Expiration date of the column. If a date has been entered, the current date is compared with the expiration date during injection or at the beginning of batch processing. If the current date exceeds the expiration date or if batch processing starts less than 24 h before the expiration date, a warning appears once per ➤*Batch*.	
Description	Column description.	

Property/Command	Description
Diameter	Column diameter in [mm].
FormatChipCard	Select this command to format a chip card that contains invalid or corrupt data.
Injections_Limit	Maximum number of injections allowed for the column.
Length	Column length in [mm].
Manufacturer	Column manufacturer.
PackingMaterial	Packing material of the column.
ParticleSize	Particle size in [µm]
pH_LowerLimit pH_UpperLimit	Upper and lower pH limits. If both limits are set to the same value, this means that no limit exists.

🛈 *Note:*

These values are for information purposes only. The device driver cannot check the pH value.

Pressure_UpperLimit	If the column pressure exceeds the upper pressure limit, a warning appears.

🛈 *Note:*

Accept the default (0.0) if you do not want to have the system check the pressure.

Temp_LowerLimit Temp_UpperLimit	If the column temperature is above the upper temperature limit or below the lower temperature limit, a warning appears. If both limits are set to the same value (default: 25°C each), this means that no limit exists. (These values also apply to the inactive column.)
VoidVolume	The column's void volume in [µl].

System properties

System properties are read-only and cannot be changed by the user. Chromeleon determines and updates the following properties during each injection:

Property	Description
DateOfFirstUse	Date of the first injection.
DateOfLastUse	Date of the last injection.
Injections_Counter	Number of injections.
Pressure_Max	Maximum pressure measured while the column was in use.
Temp_Min	Minimum temperature measured while the column was in the column compartment.
Temp_Max	Maximum temperature measured while the column was in the column compartment.

User properties

The user can change the following properties at any time:

Property	Description
Application	Enter any text to describe the application.
Comment	Enter any comment text.
SystemPressure	Name of the device to be used for system pressure measurement. The default setting is **Pump.** If the system does not include a pump, see below.

Troubleshooting

If the system does not include a pump and if you select **Check** on the **Control** menu in the PGM Editor, the following error message appears for all columns in the system. The same message also appears when the batch is started:

[Error] {Column_1} Can't use device 'Pump' as a source of the column pressure. Please check the property "SystemPressure" for a valid device name. The device must have the 'Pressure' property available.

[Error] {Column_2} Can't use device 'Pump' as a source of the column pressure. Please check the property "SystemPressure" for a valid device name. The device must have the 'Pressure' property available.

To avoid this message, clear the values of the corresponding pump names:

```
        TempCtrl =                  On
        Temperature.Nominal =       28.0 [°C]
        ...
        Column_1.SystemPressure =   ""
        Column_2.SystemPressure =   ""
        ...
 0.000  Inject
```

For information about the special commands supported by the FLM-3x00, refer to 🔳 **Dionex FLM-3x00 Flow Manager and Thermostatted Column Compartment**, for information about the flow splitter commands and properties, refer to 🔳 **Dionex FLM-3x00: Flow Splitter Commands**.

For information about the standard commands supported for the different column compartments, refer to 🔳 **Dionex Flow Manager and Thermostatted Column Compartments**.

For information about how to install the flow manager, refer to **Hardware Installation** 🔵 **FLM-3x00 Flow Manager and Thermostatted Column Compartment: Overview** in the **Administrator Help** section.

Dionex FLM-3x00: Flow Splitter Commands

The FLM-3x00 supports the following commands and properties for flow splitter control (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Note:

The name specified on the Components page in the Server Configuration program determines the name under which the flow splitter commands and properties appear in the Commands dialog box.

Property/Command	Description
ActualSplitRatio	Indicates the actual split ratio of the flow splitter. A value of 1000 means that a master flow of 100 µL/min will result in a nano flow of 100 nL/min.
CardState	Reports whether the flow splitter chip card is present
	NoChip — The card is missing.
	DoorOpened — The front panel door is open. (The card is not writeable while the door is open.)
	UnknownFormat — The card format is unknown.
	BusBlocked — One of the cards is not inserted correctly. Thus, it may happen that another card is not readable although it is correctly installed.
Comment	Enter any user-specific comment text.
Description	Describes the flow splitter.
FSColPressDesired	Sets the desired pressure when **FSControlMode** is set to **Pressure**. For test purposes only.
FSControlMode	Sets the flow control operation mode. The standard operation mode is Auto. All other modes are for test purposes only.

Property/Command	Description
FSFlowControllerStatus	Reports the flow control status (read-only). This includes the status of the flow sensor (see **FSFlowSensorStatus**) and the status of the flow splitter valve (see **FSValveStatus**).
	OK — The flow splitter is working correctly.
	PressureLow — The necessary pressure is not built up. This can be due to:
	a) The flow delivered by the pump is too low. *--Or--*
	b) Pressure buildup is insufficient due to, e.g., leakage in the system.
	Inspect the system for signs of leakage. Verify that the configuration settings match the actual system structure.
	PressureHigh — There is too much backpressure, for example, because a capillary or the column is blocked.
	Perform a self-test for the valve. Perform a cleaning cycle for the valve if necessary. Exchange the capillaries and/or column if necessary.
	ValveFail — The flow control valve is malfunctioning. Check the valve and perform a cleaning cycle if necessary..
	ValvePositionLimit — The flow control valve does not build up enough pressure. For remedial actions, refer to **PressureLow** and **ValveFail**.
	FlowSensorFail — The flow sensor is malfunctioning. Turn the flow manager off and on.
	RegulationErrorr — The control accuracy is insufficient. In most cases, this is due to excessive pump pressure pulsation. Make sure that the pump works bubble-free. Check the valves if necessary. .
FSFlowSensorStatus	Reports the status of the flow sensor (read-only):
	OK — The flow splitter is working correctly.
	SensorNotFound TimeoutError DataTransferError DataIntervalError InvalidData DataFrameErrorr — The flow sensor is malfunctioning. (The exact wording is important only for Dionex Service.) Turn the flow manager off and on.
FSValveInit	Resets the flow control actuator; can be used to recover from malfunction (for service purposes only).

Property/Command	Description
FSValveStatus	Reports the status of the flow splitter valve (read-only):
	Init The valve is initializing.
	OK The valve is working correctly.
	OutOfRange The flow splitter valve is malfunctioning. This error can correspond to the errors described in **FSFlowControllerStatus**. Refer to the section above for information about remedial actions.
	PressureLimit This error is also reported as **PressureLow** and/or **PressureHigh** for the **FSFlowControllerStatus** property. Refer to the sections above for information about remedial actions.
	PositionLimit This error is also reported as **ValvePositionLimit** for the **FSFlowControllerStatus** property. Refer to the sections above for information about remedial actions.
InitFlowControlSelfTest	Starting from zero, the flow control actuator runs a linear ramp with the specified step (StepRate; range 10-100 ms). The resulting course of the column pressure allows you to draw conclusions when an error or fault occurs.
ProductId	Indicates the product number of the flow splitter.
SerialNo	Indicates the serial number of the flow splitter.

For information about the special commands supported by the FLM-3x00, refer to **Dionex FLM-3x00 Flow Manager and Thermostatted Column Compartment**.

For information about the column ID properties, refer to **Dionex FLM-3x00: Column ID Properties**.

For information about how to install the flow manager, refer to **Hardware Installation FLM-3x00 Flow Manager and Thermostatted Column Compartment: Overview** in the **Administrator Help** section.

Dionex Detectors

In addition to the **General Device Commands**, Chromeleon supports the following standard commands for representing the recorded data. The commands are available for all detectors, even if they supply analog signals, for example, via the ➢*UCI Universal Chromatography Interface*.

Standard Commands

Command	Description
⇒*AcqOn/Off*	Start/stop data acquisition.
⇒*Autozero*	Automatic autozero.
➢*AutoAutoscale*	Axis/scaling adjustment.
⇒*Average*	Average all measured values over the Step interval.
⇒*Delta*	Indicates the signal's slope, that is, the difference between the current value and the value one second ago; this is useful for ⇒*Triggers*.
⇒*Lamp*	Turns the lamp on or off.
⇒*MaxAutoStep*	Sets the maximum step if Step = Auto.
⇒*Step*	Step width adjustment
⇒*Wavelength*	Sets the wavelength.

For information about special commands and properties available for individual detectors, refer to:

 Dionex VWD-3x00 Detectors

 Dionex UVD 170/340 UV/PDA Detectors

 Dionex PDA-100/PDA-3000 Photodiode Array Detector

 Dionex AD20/AD25 Absorbance Detectors

 Dionex UVD-3000 UV Detector

 Dionex/LC Packings UltiMate Capillary/Nano HPLC UV Detector

 Dionex RF2000 Fluorescence Detector

 Dionex CD20/CD25/CD25A, ED40/ED50/ED50A, and IC20/IC25/IC25A Electrochemical Detectors

 Dionex CD Conductivity Detector

 Dionex ED Electrochemical Detector

Please note: The Shodex RI-101 Refractive Index Detector is available from Dionex as part of the Summit HPLC System; refer to **Commands and Tips for Third-Party Devices Shodex RI-101 Refractive Index Detector**.

▣ Dionex UltiMate 3000 Series - Variable Wavelength Detectors

In addition to the standard commands available for all detectors (see ▣ **Dionex Detectors**), the VWD-3x00 detectors provide the following control commands (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Command/Property	Description
AmbientTemp	Reports the temperature [°C] outside the optical system (read-only).
Analog1_/Mode Analog2_Mode	Sets the signal available at analog output 1 or 2.
Analog1_Offset Analog2_Offset	Sets a permanent offset voltage for analog outputs 1 or 2.
Analog1_Range Analog2_Range	Sets the voltage range of analog outputs 1 or 2. Available values are: 1AU_per_1V=0, 01AU_per_1V=1, 5AU_per_1V=2, 1AU_per_10V=3, 01AU_per_10V=4, 5AU_per_10V=5
AutoactivateUV_Lamp	**On:** The UV lamp is turned on automatically whenever the detector is powered up.
AutoactivateVisible_Lamp	**On:** The visible lamp is turned on automatically whenever the detector is powered up.
⇒*Autozero*	Performs automatic null balancing (= the current detector signal is interpreted as 0).
BootLoaderVersion	Reports the bootloader version (read-only).
Brightness	Adapts brightness of the detector's front panel display to your requirements.
ClearDisplayError	Deletes the error messages on the detector's display.
Contrast	Adapts the screen contrast of the detector's front panel display to your requirements.
Data_Collection_Rate	Enter the rate [Hz] at which Chromeleon collects digital data from the detector
DisplayMode	Specifies which type of data appears on the detector's front panel display (**Raw**, **Transmittance**, or **Absorbance**).
Door	Reports whether the compartment door is **Open** or **Closed** (read-only).
DriverID	Indicates the driver's unique name (read-only).
DriverVersion	Indicates the driver version (read-only).
ElectronicsTemp	Indicates the temperature [°C] of the electronics unit (read-only).
FlowcellType	Indicates the flow cell type (read-only).
FPGAVersion	Indicates the detector's FPGA version (read-only).
HardwareRevision	Indicates the detector's hardware reversion (read-only).

Command/Property	Description
HardwareRevision_ Optics	Indicates the optics' hardware revision (read-only).
LampCover	Indicates whether the lamp cover is installed (read-only).
LamphouseTemp	Indicates the lamp house temperature [°C] (read-only).
Leak	Indicates whether the leak sensor has detected a leak: **NoLeak** or **Leak** (read-only).
LeakDelay	Reports the delay time [s] after a leak was detected
LeakSensorMode	Specifies how leak detection is performed: **Enabled** - Enables leak detection; an acoustic beep sounds when the leak sensor is activated. **Silent** - Enables leak detection, but no beep sounds when the leak sensor is activated. **Disabled** - Disables leak detection.
Manufacturer	Indicates the manufacturer of the driver (read-only).
NotReadyCauses	Reports why the module is not yet ready for the next analysis (read-only).
OpticsTemp	Reports the temperature [°C] of the optics (read-only).
Ready	Indicates whether the detector is ready for injection (read-only).
Reset	Resets the device to its initial conditions, as attained after power-up.
Scan	Saves a scan based on the current settings to a spectra library.
ScanBaseline	Saves a baseline scan based on the current settings to a spectra library.
ScanEndWavelength	Sets the upper limit [nm] of the wavelength range for spectra scan. The allowed range is 190 to 900 nm. (Also, see **ScanBaseline** and **Scan**.)
ScanSpeed	Defines the time interval [ms] between successive measurements during a wavelength scan. (Also, see **ScanBaseline** and **Scan**.)
ScanStartWavelength	Sets the lower limit [nm] of the wavelength range for spectra scan. The allowed range is 190 to 900 nm. (Also, see **ScanBaseline** and **Scan**.)
ScanStepSize	Defines the wavelength interval [nm] between successive measurements during a wavelength scan. (Also, see **ScanBaseline** and **Scan**.)
SerialNo_Flowcell	Reports the serial number of flow cell (read-only).
SerialNo_Mainboard	Reports the serial number of the main board (read-only).
SerialNo_Optics	Reports the serial number of the optics (read-only).
SetFactoryDefaults	Restores the Dionex standard settings.
Standby	Switches the detector to standby mode (**Standby**) or cancels standby mode (**NoStandby**). During standby mode, the detector remains connected in Chromeleon.
TimeConstant	Specify the time constant for the filter.

Command/Property	Description
UV_Lamp	Reports the state of the UV lamp: **Error**, **Ignition**, **Off**, **On**.
UvLamp_Manufacturer	Indicates the manufacturer of the UV lamp (read-only).
UvTransmission_Flowcell	Indicates the factor for calculation of absorption to take the typical transmission for the flow cell into account (read-only). If the flow cell has a chip, the values are read from the chip. Otherwise, the value 100 is used.
UvLamp_SerialNo	Indicates the serial number of the UV lamp (read-only).
Visible_Lamp	Reports the visible lamp's state: **Error**, **Ignition**, **Off**, **On**.
VisLamp_Manufacturer	Indicates the manufacturer of the visible lamp (read-only).
VisLamp_SerialNo	Indicates the serial number of the visible lamp (read-only).
VisTransmission_Flowcell	Indicates the factor for calculation of absorption to take the typical transmission for the flow cell into account (read-only). If the flow cell has a chip, the values are read from the chip. Otherwise, the value 100 is used.
WavelengthCalibration	Performs wavelength calibration. In addition, wavelength calibration is performed automatically whenever the detector is powered up. (Also, see **WvlCalibStatus**)
WavelengthPeak1/2/3 Measured	Reports the measured wavelength for peaks 1, 2, or 3.
WavelengthPeak1/2/3 ResultMeasm	Reports the result (pass or fail) of the wavelength measurement for peaks 1, 2, or 3.
WavelengthPeak1/2/3 Theoretical	Reports the wavelengths expected for the wavelength verification via the holmium oxide filter for peaks 1, 2, or 3.
WavelengthVerTest	Performs wavelength verification via the ➤*Holmium Oxide Filter*.
WavelengthVerTest Result	Reports the result (pass or fail) of the wavelength verification. (Also, see **WavelengthVerTest**.)
WvlCalibStatus	Indicates the status of the wavelength calibration (read-only): **Initialized**, **Running**, **Calibrated**, **Error** (Also, see **WavelengthCalibration**)

UV_VIS channel commands

Command/Property	Description
MaxAutoStep	Sets the maximum step for the Auto-Step mode. (The available range is from 0.1 to 5.1 s, the default is 5.1 s.)
Retention	Reports the signal's retention time (read-only).
Signal	Reports the current signal value [mAU] (read-only).
Wavelength	Sets the wavelength (The available range is from 190 to 900 nm).

Equilibration commands and properties

Tips:

The Chromeleon version and detector type determine which commands and properties are available for equilibration.

Use the equilibration commands and properties only for the ➤SmartStartup feature.

Command/Property	Description
Drift	Provides information about the detector ➤*Drift*, determined by equilibration.
*.UpperLimit	Sets the upper drift limit [mAU/h]. Available values are: 0.8 and 20.0 mAU/h.
*.Value	Reports the current drift value in mAU/h. The value is calculated as soon as at least a one-minute segment was measured. It is updated with each new recorded data point. The property value does not change after measurement and can be viewed in the PreconditionLog (<u>Preconditions</u>GLOSSARY_PRECOND>glossary) of the subsequent samples.
DriftEquilibration	Reports the status/result of the drift measurement: **OK** or **NotOK** (read-only).
DriftStatus	Reports the status/result of the drift measurement (read-only).
	N/A — Equilibration has not started.
	Measuring — Equilibration is running, but the amount of data is not yet sufficient to report a result.
	NotReady — Equilibration is running. The current data is outside the specified limits.
	Good — Equilibration is running or has finished. The values are within the specified limits.
	Failed — Equilibration is completed. The result is outside the specified limits.
	NotTested — The limit was set to 0, thus disabling ripple checking.
Equilibration	Provides information about the total status of the equilibration: **OK** or **NotOK** (read-only).
Noise	Provides information on the detector ➤*Noise*.
*.UpperLimit	Sets the upper noise limit in [mAU]. The allowed range is: 0.03 to 0.50 mAU.
*.Value	Indicates the current detector noise. Usually, this is the value averaged over 5 minutes (read-only). Also, see **Drift.Value**.
NoiseEquilibration	Reports the status/result of the noise measurement: **OK** or **NotOK** (read-only).
NoiseStatus	Reports the status/result of the noise measurement (read-only). For the available values, see **DriftStatus**.
StartEquilibration	Starts equilibration (noise/drift measurement).

System Wellness and Predictive Performance commands and properties

☞ *Tip:*

The Chromeleon version and detector type determine which commands and properties are available for checking the pump's system wellness and performance.

Command/Property	Description
FilterMovements	Counts the movements of the holmium oxide filter (read-only).
GratingMovements	Counts the movements of the grating (read-only).
OperatingHours	Counts the operating hours since initial operation (excluding the operating hours in standby mode (read-only).
PowerOnTime	Counts the time since last power-up (or last wake-up from standby) (read-only).
PreviousUVLampOperation Time	Counts the previous operating hours of the UV lamp (read-only).
PreviousVISLampOperation Time	Counts the previous operating hours of the visible lamp (read-only).
Qualification	Provides information about system qualification.
*.LastDate	Reports the date when detector qualification was performed last (read-only).
*.LastOperator	Reports the name of the person who performed the detector qualification (read-only).
*.Interval	Reports the interval (in days) when the next system qualification is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
*.GracePeriod	Indicates the period during which the device may be operated after the due date for re-qualification.
Service	Provides detector service information.
*.LastDate	Reports the date of the last service (read-only).
*.Interval	Specify the interval (in days) after which a service is due.
*.WarningPeriod	Specifies the period during which a warning appears before the next service is due.
StandbyHours	Counts the total operating hours in standby mode since the module was operated for the first time (read-only).
UpdateLampIntensity	Updates the lamp intensity values.
UVLampChangeDate	Reports when the UV lamp was replaced last (read-only).
UVLampIgnitions	Counts the total number of successful UV lamp ignitions (read-only).
UVLampIgnitionCurrent Trials	Reports the number of ignition trials when the UV lamp was started last (read-only).

Command/Property	Description
UVLampIgnitionFailures	Counts how many times the UV lamp ignition fails (read-only). After a maximum of 10 retries, the counter is incremented by one.
UVLampOnTime	Counts the operating hours of the UV lamp since the lamp was ignited last (read-only).
UVLampOperationTime	Provides information on the UV lamp's operating time.
*.Value	Reports the total operating hours of the UV lamp (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
UVRelIntensity	Provides information regarding the relative intensity of the UV lamp.
*.Value	Reports the current relative intensity of the UV lamp (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
VISLampChangeDate	Reports when the visible lamp was replaced last (read-only).
VISLampIgnitionFailures	Counts how many times the visible lamp ignition fails (read-only). After a maximum of 10 retries, the counter is incremented by one.
VISLampIgnitions	Counts the total number of successful visible lamp ignitions (read-only).
VISLampOnTime	Counts the operating hours of the visible lamp since the lamp was ignited last (read-only).
VISLampOperationTime	Provides information on the visible lamp's operating time.
*.Value	Reports the total operating hours of the visible lamp (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.
VISRelIntensity	Provides information regarding the relative intensity of the visible lamp.
*.Value	Reports the current relative intensity of the visible lamp (read-only).
*.Warning	A warning appears when the specified value is reached.
*.Limit	An error message appears when the specified value is reached.

For information about how to install the detector, refer to **Hardware Installation ➡ UltiMate 3000 Series - Variable Wavelength Detector: Overview**.

▣ Dionex UVD 170/340 UV/PDA Detectors

In addition to the standard commands available for all detectors (see ▣ **Dionex Detectors**), the UVD 170 and UVD 340 detectors support additional control commands (please note that the display ➤*Filter* level determines which commands are displayed):

Special Commands

Command	Description
⇒*Bandwidth*	Sets the bandwidth of a UV-VIS channel.
⇒*BunchWidth*	For UVD 340U only: Averages photodiode signals for 3D field acquisition.
CheckWavelength	Checks wavelength calibration, using a ➤*Holmium Oxide Filter*.
⇒*LampIntensity*	States the lamp intensity at 254 nm in counts/seconds. The value can be used as a comparison value to determine how much the lamp intensity decreased. This requires that the value be first measured when the lamp is new.
⇒*RefWavelength*	Sets the reference wavelength.
⇒*RefBandwidth*	Sets the reference bandwidth.

The Dionex UVD 170 and UVD 340 detectors can be completely controlled by Chromeleon, using the above commands. In addition, the following special commands are supported:

Equilibration command and properties

☞ *Tip:*

You should use the equilibration commands and properties only for the ➤*SmartStartup feature.*

Command	Description	
Drift	Reports the signal ➤*Drift*.	
	UpperLimit	Determine the upper limit for the drift in [mAU/h]. The upper limit can be any value between 0.8 and 20.0. The default setting is 3.0.
	Value	*(Read-only)* Reports the actual value (usually, this value is averaged over the last 5 minutes.): At the beginning (i.e., after installation of the pump), the value field is empty, indicating that no measurement has been performed yet. Chromeleon calculates the value as soon as a 1-minute segment has been measured and updates the value with each new segment. The value of the property does not change after the measurement and displayed under ➤*Preconditions*.

Command	Description
DriftEquilibration	(Read-only) Reports drift whether equilibration was successful: **Ok** or **NotOK**.
DriftStatus	Reports the status of drift equilibration (read-only).
	N/A Equilibration was not yet performed.
	Measuring Equilibration is running but the amount of data is not yet sufficient to report a result.
	Good Equilibration is running or terminated. The values are within the specified limits.
	NotReady Equilibration is running. The current data is outside the specified limits.
	Failed Equilibration is completed. The result is outside the specified limits.
	NotTested The limit was set to 0, thus disabling ripple checking.
Equilibration	(Read-only) Reports the overall status of equilibration.
Noise	Reports the ➢ *Signal Noise*.
	UpperLimit Determine the upper limit for the signal noise in [mAU]. The upper limit can be any value between 0.03 and 0.50. The default setting is 0.10.
	Value (Read-only) Reports the actual signal noise (usually, this value is averaged over 5 minutes. For more information, refer to Drift.Value above.
NoiseEquilibration	(Read-only) Reports whether noise equilibration was successful: **Ok** or **NotOk**
NoiseStatus	(Read-only) Reports the status of noise equilibration. For more information, refer to DriftStatus above.

Special Commands for the Dionex UVD 170U/340U Detectors

Command	Description
LampAge	Indicates the lamp age (in hours).
LampIgnitions	Indicates the number of lamp ignitions.
MinLampIntensity	Enter the value for the minimum lamp intensity. If the lamp intensity is below this value, a warning is written to the Audit Trail.
SnapshotGetSpectrum	For UVD 340U only: Stores the current spectrum in a text file.
SnapshotResolution	For UVD 340U only: Defines the wavelength resolution for a UV spectrum that is recorded using the **SnapshotGetSpectrum** command (Range: 0.1 to 20.0 nm).
SnapshotDirPath	For UVD 340U only: Defines the destination directory for a UV spectrum that is recorded using the **SnapshotGetSpectrum** command.

Command	Description
SnapshotGetBLSpectrum	For UVD 340U only: Saves the current spectrum as baseline spectrum. The baseline spectrum is always subtracted from the spectrum recorded with the **SnapshotGetSpectrum** command.
SnapshotClearBLSpectrum	For UVD 340U only: Clears the buffer for baseline spectrum that was recorded using the **SnapshotGetBLSpectrum** command.

🖼️ *Note:*

Using a registry key, you can define a voltage offset (in millivolt) on the 16-bit DAC card for the analog output of the Dionex UVD 170/340 detector signal. The offset values for all 16-bit DAC channels of the CM server are identical.

Enter the respective offset in millivolt into the registry key:

Example for 100 millivolt (hexadecimal entry):
[HKEY_LOCAL_MACHINE\Software\Dionex\Chromeleon\drivers\UVD340]

"DAC Offset"=dword:00000064

The **Administrator Help** section provides information about how to install the UVD 170 or UVD 340 detector; refer to **Hardware Installation**

🔵 **UVD 170S and UVD 340S HPLC Detectors: Overview**

🔵 **UVD 170U and UVD 340U HPLC Detectors: Overview**

▣ Dionex PDA-100/PDA-3000 Photodiode Array Detectors

In addition to the standard commands available for all detectors (see ▣ **Dionex Detectors**), the PDA-100 and PDA-3000 detectors provide several additional control commands (please note that the display ➢*Filter* level determines which commands are displayed).

UV_VIS Channel Commands

Command	Description
⇒*Bandwidth*	Sets the bandwidth (the wavelength range at which the chromatogram is measured).
➢*Offset_Level*	Sets the offset for the analog output signal.
➢*Recorder_Range*	Sets the range of a full-scale recorder response (analog output).
⇒*RefBandwidth*	Sets the bandwidth of the reference wavelength (if one is selected). If RefWavelength is **Off**, the RefBandwidth setting has no effect.
⇒*RefWavelength*	Sets the reference wavelength.

3DFIELD Channel Commands

Command	Description
⇒*BunchWidth*	Determines how many wavelengths are averaged when collecting 3D data. Bunching (averaging) the signals of adjacent wavelengths will reduce the amount of 3D data stored.
MaxWavelength	Sets the maximum wavelength of the 3D field.
MinWavelength	Sets the minimum wavelength of the 3D field.
RefBandwidth	Sets the bandwidth of the reference wavelength (if one is selected). If RefWavelength is **Off**, the RefBandwidth setting has no effect.
RefWavelength	Sets the reference bandwidth.

Other Special Detector Commands

Command	Description
AmbientTemp	Reports the temperature outside the optical assembly.
AZ_Done	Reports the status of an autozero command (**Busy** or **Done**).
➢*Data_Collection_Rate*	Sets the rate at which Chromeleon collects digital data points from the detector.
➢*NegativeAbsorption Level*	Sets the amount of increased light transmission from autozero (negative absorption) to which the detector responds. This permits peaks to be seen even when the eluent decreases absorbance.

Command	Description
OpticalBenchTemp	Reports the temperature of the optical bench.
➢*Rise_Time*	Sets how quickly the detector responds to a change in signal.
➢*TTL_Input_Mode*	Sets the TTL input signal mode.
➢*UV_Lamp*	Turns the deuterium lamp on and off.
UVLampPowerOnSetting	Selects whether the deuterium lamp is turned on when the detector power is turned on. The default setting is On.
➢*Visible_Lamp*	Turns the tungsten lamp on and off.
VisLampPowerOnSetting	Selects whether the tungsten lamp is turned on when the detector power is turned on. The default setting is On.

UV Calibration Commands

Command	Description
DownloadCalibration	Downloads the selected set of calibration values to the detector.
DownloadCalibration Parameter	Selects the set of calibration values (Current, Previous, or Factory) to be downloaded.
FilterWheel	Calibrates the filter wheel (not applicable to the PDA-100 (USB) or the PDA-3000).
Intensity	Calibrates the intensity of the deuterium lamp.
LeakDet	Performs the leak detector calibration (not applicable to the PDA-100 (USB) or the PDA-3000).
WavelengthCal	Performs a wavelength calibration.
WavelenthCalDate	Reports the date of the wavelength calibration.
WavelenthCalResult	Reports the result (pass or fail) of the wavelength calibration.

UV Diagnostic Commands

Command	Description
LeakDetTest	Performs a test of the leak detector (not applicable to the PDA-100 (USB) or the PDA-3000).
LeakDetTestDate	Reports the date of the leak detector test.
LeakDetTestResult	Reports the result (pass or fail) of the leak detector test.
WavelengthVerTest	Performs wavelength verification via the ➢*Holmium Oxide Filter*.
WavelengthVerTestDate	Reports the date of the wavelength verification.
WavelengthVerTestResult	Reports the result (pass or fail) of the wavelength verification.

System Wellness and Predictive Performance commands and properties

🖅 *Tip:*

The Chromeleon version and the detector type determine which commands and properties are available for checking the detector wellness and performance.

Command/Property	Description
⇒*LampIntensity*	Reports the deuterium lamp's intensity at 254 nm in counts/second.
ModuleAge	Reports (or resets) the number of hours the detector has been on.
Qualification	Provides information about detector qualification.
*.LastDate	Reports when qualification was last performed (read-only).
*.LastOperator	Reports the name of the user who performed the last qualification (read-only).
*.Interval	Specifies the period of time (in days) between qualifications.
*.WarningPeriod	Specifies the number of days before qualification expiration that Chromeleon will display a reminder that requalification will be necessary.
*.GracePeriod	Indicates for how many more days the user can continue to operate the detector after failing to perform a scheduled qualification.
QualificationDone	Selecting this command after qualification sets *.LastDate to the current date and sets *.LastOperator to the name of the currently logged in user.
UVLampAge	Reports (or resets) the number of hours the deuterium lamp has been on.
VisLampAge	Reports (or resets) the number of hours the tungsten lamp has been on.
VisLampIntensity	Reports the tungsten lamp's intensity at 520 nm. Reported in counts per second.

For information about how to install the detector, refer to **Hardware Installation** 🔵 **PDA-100 (DX-LAN) Detector: Overview** or 🔵 **PDA-100 (USB)/PDA-3000 Detector: Overview** in the **Administrator Help** section.

▪ Dionex AD20/AD25 Absorbance Detectors

In addition to the standard commands available for all detectors (see
🔳 **Dionex Detectors**), Dionex absorbance detectors provide the following
commands (please note that the display ➢*Filter* level determines which
commands and properties are displayed):

Special Commands

Command	Description
➢*Data_Collection_Rate*	Sets the rate at which Chromeleon collects digital data points from the detector.
⇒*Rise_Time*	Sets how quickly the detector responds to a change in signal.
UV_Cutover	For AD20 only: Sets the wavelength above which the second order filter is inserted in the light path. Typically, the UV cutover wavelength is 380nm.
➢*UV_Lamp*	Turns the deuterium lamp to Off, Low, or High (AD20 detector), or Off or On (AD25 detector).
➢*Visible_Lamp*	Turns the tungsten lamp to Off, Low, or High (AD20 detector), or Off or On (AD25 detector).
⇒*Wavelength*	Sets the wavelength.

Analog Output Commands

Command	Description
➢*Full_Scale*	For AD20 only: Sets the full-scale analog out voltage.
➢*Mark*	Sends a positive pulse to the detector's analog output as an event marker.
➢*Offset_Level*	Sets the offset for the analog output signal.
➢*Polarity*	Switches the analog output polarity (+/-).
➢*Recorder_Calibration*	For AD25 only: Allows calibration of the recorder's response to three detector analog output settings (AU, Zero, Full_Scale)
➢*Recorder_Range*	Sets the range of a full-scale recorder response.

UV Calibration Commands (For AD25 Only)

Command	Description
DownloadCalibration	Downloads the selected set of calibration values to the detector.
DownloadCalibration Parameter	Selects the set of calibration values (Current, Previous, or Factory) to be downloaded.
LeakDet	Performs the leak detector calibration.

Command	Description
WavelengthCal	Performs a wavelength calibration.
WavelengthCalDate	Reports the date of the wavelength calibration.
WavelengthCalResult	Reports the result (pass or fail) of the wavelength calibration.

UV Diagnostic Commands (For AD25 Only)

Command	Description
LeakDetTest	Performs a test of the leak detector.
LeakDetTestDate	Reports the date of the leak detector test.
LeakDetTestResult	Reports the result (pass or fail) of the leak detector test.
WavelengthVerTest	Performs a wavelength verification via the ➤*Holmium Oxide Filter*.
WavelengthVerTestDate	Reports the date of the wavelength verification.
WavelengthVerTest Result	Reports the result (pass or fail) of the wavelength verification.

◼ Dionex UVD-3000 UV Detector

In addition to the standard commands available for all detectors (see ◼ **Dionex Detectors**), the UVD-3000 detector provides the following control commands (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Command/Property	Description
Adjust_Calibration	Performs wavelength calibration by using an internal test.
	▪☞ *Tip:*
	The command stops the data acquisition.
AnalogOffset1	Sets the offset voltage for analog output 1.
AnalogOffset2	Sets the offset voltage for analog output 2.
AnalogScale	Sets the sensitivity of the analog outputs.
⇒*Autozero*	Performs automatic null balancing.
	▪☞ *Tip:*
	*Use this command only before you start data acquisition. For null balancing whenever the wavelength is changed in the program, use **AutozeroOnWlChange = On**. Use **AutozeroOnWlChange = Off** to cancel null balancing. Use:*
	``` AutozeroOnWlChange = On UV_VIS_1 = 300 ```
	(not: AutozeroOnWlChange = Off UV_VIS_1 = 300 Autozero)

Command/Property	Description
AutozeroOnWlChange	When set to **On**, automatic null balancing is performed whenever the wavelength is changed.
➤*Data_Collection_Rate*	Enter the rate at which Chromeleon collects digital data from the detector.
DemoFileName	Name of the file that is read in the ➤*Demo Mode*.
GetDiodeReading	Determines the values for SignalDiode and ReferenceDiode.
LampType	Indicates the lamp type (read-only*)*.
ReferenceDiode	Indicates the brightness of the reference signal.
Response	Time constant. This is the time the detector requires for rising from 10% to 90% in response to a signal step. The constant is used for noise suppression (values: 0.1; 0.2; 0.5; 1; 2; 5, or 10s) 📖☞ *Tip:* *Most noise is suppressed if the Response is 10s. However, for the acquisition of narrow peaks a lower time response will be more appropriate.*
SignalDiode	Indicates the brightness of the measured signal.

📖☞ *Tip:*

If you perform the ➤*Data Collection Rate command, the default* ⇒*Step value is set to the reciprocal value. That is why you have to perform the Step command after the Data Collection Rate command if you need a different step.*

The **UV_Diagnostic** section provides information about detector operation and service. The entries are read-only:

Command/Property	Description
LampAge	Reports the total operating hours of the lamp.
LampIgnitions	Reports the number of lamp starts.
LampNumber	Indicates the lamp number. (The value is incremented when a new lamp is installed.)
LampOnTime	Reports how many hours the lamp has been burning since it was turned on.
LastServiceDate	Reports when the detector was last serviced. (The Dionex Service Engineer enters the date.)
LastServiceCode	Reports the service code, providing information about the service. (The Dionex Service Engineer sets the code.)
ManufacturingDate	Reports the detector manufacturing date.
MotorWorkTime	Reports the total operating hours of the motor.

For information about how to install the detector, refer to **Hardware Installation** 🔵 **UVD-3000 UV Detector: Overview** in the **Administrator Help** section.

◼ Dionex/LC Packings UltiMate Capillary/Nano HPLC UV Detectors

In addition to the standard commands available for all detectors (see ◼ **Dionex Detectors**), the UltiMate detector provides the following control commands (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Description
Adjust_Calibration	Performs wavelength calibration by using an internal test.
	👉 *Tip:*
	The command stops the data acquisition.
➢*Data_Collection_Rate*	Enter the rate at which Chromeleon collects digital data from the detector.
DemoFileName	Name of the file that is read in the ➢*Demo Mode*.
LampType	Indicates the lamp type: Deuterium or Tungsten (read-only).
TimeConstant	Time constant. This is the time the detector requires for rising from 10% to 90% in response to a signal step. The constant is used for noise suppression (values: 0.1; 0.2; 0.5; 1; 2; 5, or 10s)
	👉 *Tip:*
	Most noise is suppressed if the Response is 10s. However, for the acquisition of narrow peaks a lower response will be more appropriate.

👉 *Tip:*

If you perform the ➢Data Collection Rate command, the default ⇒Step value is set to the reciprocal value. That is why you have to perform the Step command after the Data Collection Rate command if you need a different step.

The UV_Diagnostic section provides information about the detector operation and service. The entries are read-only:

Command/Property	Description
LampAge	Reports the total operating hours of the lamp.
LampIgnitions	Reports the number of lamp starts.
LampNumber	Indicates the lamp number. (The value is incremented when a new lamp is installed.)
LampOnTime	Reports how many hours the lamp has been burning since it was turned on.

Command/Property	Description
LastServiceDate	Reports when the detector was last serviced. (The Dionex Service Engineer enters the date.)
LastServiceCode	Reports the service code, providing information about the service. (The Dionex Service Engineer sets the code.)
ManufacturingDate	Reports the detector manufacturing date.
MotorWorkTime	Reports the total operating hours of the motor.

For information about how to install the UltiMate UV detector, refer to **Hardware Installation** 🔵 **UltiMate Capillary/Nano HPLC System: Overview** in the **Administrator Help** section.

🔹 Dionex RF2000 Fluorescence Detector

In addition to the commands available for various detector types, such as **Step**, **Average**, **Autozero** (see 🔳 **Dionex Detectors**), controllable fluorescence detectors allow you to modify the **Emission** and **Excitation** wavelengths.

Command	Description
➢*Emission*	Modifies the measuring wavelength.
➢*Excitation*	Modifies the excitation wavelength.

In addition, the RF2000 detector supports the Scan mode for recording emission and excitation spectra. For a PGM File example, refer to **Practical Tips for Device Control** 🔳 **Determining the Optimum Emission Wavelength (RF2000)**.

For information about the following RF2000 commands, refer to **Dionex RF2000 Fluorescence Detector**:

🔳 **Excitation Wavelength**

🔳 **Emission Wavelength**

🔳 **Gain**

🔳 **Response**

🔳 **Sensitivity**

🔳 **Scanning**

🔳 **(Scan)Start and (Scan)End Wavelengths**

🔳 **ScanSpeed**

🔳 **ScanWithAnalogOutput**

For information about how to install the Dionex RF2000 Fluorescence Detector, refer to **Hardware Installation** ➡ **RF2000 Fluorescence Detector: Overview** in the **Administrator Help** section.

The **Administrator Help** section provides information about the pin assignment of the RF2000 cable; refer to ➡ **RF2000 RS Cable** in the **Appendix** of the **Hardware Installation** section.

▪ Dionex RF2000 Fluorescence Detector: Excitation Wavelength

Use the ➤*Excitation* parameter to determine the excitation wavelength of the fluorescence detector. This is the wavelength required to induce a specific substance to fluoresce.

To be able to measure the light emitted by the substance, set the fluorescence detector exactly to this wavelength, using the ➤*Emission* parameter.

▪ Dionex RF2000 Fluorescence Detector: Emission Wavelength

To be able to measure the light emitted by the substance, set the fluorescence detector exactly to the corresponding wavelength, using the ➤*Emission* parameter.

To set the wavelength required for excitation, use the ➤*Excitation* parameter.

◩ Dionex RF2000 Fluorescence Detector: Gain

Use the **Gain** signal parameter to determine the factor by which the analog output signal of the RF2000 fluorescence spectrometer is increased or decreased. Thus, the **Gain** parameter corresponds to the ➢*Range* parameter that is supported for other detector types.

📝 *Note:*

*For detectors with analog data acquisition, the ➢UCI Universal Chromatography Interface ranges from -10V to +10V and thus covers virtually all detector output signals. It is possible to record signals directly, without considering the **Gain** parameter. If size adjustment is necessary anyway, adjust the size, using the **Factor** parameter of the **signal configuration**. (Select the **Signals** tab page of the fluorescence detector properties in the ➢Server Configuration program.)*

◩ Dionex RF2000 Fluorescence Detector: Response

Response is a filter parameter.

A larger time interval improves the ➢*Signal-to-Noise Ratio*, but lowers the resolution. Use low values for narrow peaks:

Value	Minimum Peak Width (at 50% of the peak height)
1	0.2s
2	1.1s
3	3.6s
4	6.6s

◩ Dionex RF2000 Fluorescence Detector: Sensitivity

Use the **Sensitivity** parameter to determine the detector sensitivity. The detector sensitivity increases with a higher Sensitivity value. However, increasing the Sensitivity value also increases the noise; that is, the ➢*Signal-to-Noise Ratio* decreases.

The RF2000 fluorescence detector supports the following values: LOW, MED, and HIGH.

The actual sensitivity results from the product of the Sensitivity (LOW = 1, MED = 32, HIGH = 1024) and the Gain (1/4/16).

▣ Dionex RF2000 Fluorescence Detector: Scanning

Scanning indicates that the detector is currently in the Scan Mode. The Scan Mode functionality determines optimum values for the ➢*Excitation* and ➢*Emission* wavelengths. However, these values cannot be determined simultaneously. The procedure for determining the emission wavelength is described below, as an example. For a corresponding ➢*Program*, refer to **Practical Tips for Device Control** 🔳 **Determining the Optimum Emission Wavelength (RF2000).**

Scanning

- Stop the pump flow, using the **Flow = 0** command.

- Execute the **ScanEmission** command.

The duration of the scanning procedure depends on the wavelength range to be scanned (**Start and End Wavelengths**) and on the scan speed (see 🔳 **Dionex RF2000 Fluorescence Detector: ScanSpeed**). You can add an LED or a color box to the ➢*Control Panel* to visually indicate whether scanning is underway.

When the detector finishes scanning, the resulting spectrum is saved in the detector and overwritten with each new recording. To retain the data, Chromeleon saves each scan spectrum automatically to the local datasource. A ➢*Spectra Library* named RF2000 is created in the sequence directory of the associated ➢*Timebase*.

Depending on when the pump flow is stopped and when the scan procedure was started, either a background spectrum or the spectrum of a peak is saved.

- To determine an optimum excitation wavelength (excitation value), follow the steps above.

☞ *Tip:*

While the RF2000 Fluorescence Detector is scanning, it cannot receive any other commands. The scanning process cannot be interrupted.

How to form a difference spectrum

To receive a difference spectrum (the current spectrum minus the background spectrum):

- Record a background spectrum as described above.

- Use the **Save Background Spectrum** command to save this spectrum as a separate spectrum in the detector.

- Reset the pump flow to the original value, using the **Flow= ...** command. Continue until the maximum of the peak to be detected is reached.

- Stop the pump flow and record a new spectrum.

The two spectra now exist both in the RF2000 spectra library and as individual spectra that are stored in two different locations in the detector.

- Execute the GetSpectraDifference command to receive a difference spectrum.

The detector responds exactly as if a scan procedure had been started. The result of the difference formation is also saved to the RF2000 spectra library. This spectrum indicates the optimum emission or excitation value.

📖 *Tip:*

The basic requirement for forming difference spectra is that the wavelength range defined by the start wavelength and the end wavelength corresponds to the spectra type (Excitation or Emission).

▪ Dionex RF2000 Fluorescence Detector: (Scan)Start and End Wavelengths

Use the **(Scan)Start** parameter to determine the lower limit of the wavelength range to be scanned. Use the **(Scan)End** parameter to determine the upper limit of range.

To set the speed with which the range is scanned, use the **ScanSpeed** parameter (see 🔺 **Dionex RF2000 Fluorescence Detector: ScanSpeed**).

For more information about the Scan procedure, refer to 🔺 **Dionex RF2000 Fluorescence Detector: Scanning**.

◼ Dionex RF2000 Fluorescence Detector: ScanSpeed

Use the **ScanSpeed** parameter to determine the speed with which the determined wavelength range is scanned. The range is defined by the start and end wavelengths (see ◼ **Dionex RF2000 Fluorescence Detector: (Scan)Start and End Wavelengths**).

The following settings are available:

Setting	Scan Speed	Required time from 200 to 900 nm
Slow	(24 nm/min)	1900 sec.
Medium	(120 nm/min)	370 sec.
Fast (default)	(600 nm/min)	85 sec.
Super	(3000 nm/min)	25 sec.

☞ *Tip:*

While the RF2000 Fluorescence Detector is performing a scan operation, it cannot receive any other commands. It is not possible to interrupt the scanning process.

For more information about the scanning process, refer to ◼ **Dionex RF2000 Fluorescence Detector: Scanning**.

◼ Dionex RF2000 Fluorescence Detector: Scan With Analog Output

Enable **Scan With Analog Output** to display the scanned spectrum at the integrator or recorder output. On the instrument's rear panel, these outputs are labeled as follows: **INTEG.1V** and **REC. 10mV** or **REC. 1mV**.

◼ Dionex CD20/CD25/CD25A, ED40/ED50/ED50A, and IC20/IC25/IC25A Electrochemical Detectors

In addition to the standard commands available for all detectors (see ◼ **Dionex Detectors**), Dionex electrochemical detectors provide special commands (see the table below; please note that the display ➤*Filter* level determines which commands are displayed). The **Data_Collection_Rate** command and the analog output commands are available for all Dionex electrochemical detectors in any operating mode.

The **Mode** command is available only for the ED40/ED50/ED50A detectors. Other special commands depend on the selected operating mode.

Command	Description
➤*Data_Collection_Rate*	Sets the rate at which Chromeleon collects digital data points from the detector.
Mode	(Available only for the ED40/ED50/ED50A) Select the operating mode: conductivity, integrated amperometry, or DC amperometry.

Analog Output Commands (All Modes)

Command	Description
➤*Full_Scale*	Sets the full-scale analog out voltage.
➤*Mark*	Sends a positive pulse to the detector's analog output as an event marker.
➤*Offset_Level*	Sets the offset for the analog output signal.
➤*Polarity*	Switches the analog output polarity (+/-).
➤*Recorder_Range*	Sets the range of a full-scale recorder response.
➤*Rise_Time*	Sets how quickly the detector responds to a change in signal.

Special Commands (Conductivity Mode)

The following commands are available for conductivity detectors (CD20/CD25/CD25A, ED40/ED50/ED50A, or IC20/IC25/IC25A):

Command	Description
DS3_Temperature	Sets the ➤*DS3* temperature.
SRS_Current	For CD20/CD25, ED40/ED50, IC20/IC25 only: Sets the current supplied to the SRS.
Suppressor_Current	For CD25A, ED50A, and IC25A only: Sets the current supplied to the suppressor.
Suppressor_Type	For CD25A, ED50A, and IC25A only: Sets the installed ➤*Suppressor*.
➤*Temperature_Compensation*	Sets the temperature compensation factor, which is used to stabilize conductivity readings.

Special Commands (DC Amperometry Mode)

The following commands are available for the ED40/ED50/ED50A detectors in DC amperometry mode:

Command	Description
Cell	Turns the cell on and off.
➢*DC_Voltage*	Sets the voltage applied to the cell.
Electrode	Select the type of electrode installed.

Special Commands (Integrated Amperometry Mode)

The following commands are available for the ED40/ED50/ED50A detectors in integrated amperometry mode:

Command	Description
Cell	Turns the cell on and off.
Electrode	Select the reference electrode type.
➢*Waveform*	Define a waveform (a plot of potential vs. time).

Calibration Commands (For CD25A, ED50A, and IC25A Only)

Command	Description
LeakDet	Performs the leak detector calibration.
CondCell	Performs the conductivity cell calibration
DownloadCalibration	Downloads the selected set of calibration values to the detector.
DownloadCalibration Parameter	Selects the set of calibration values (Current, Previous, or Factory) to be downloaded.

Diagnostic Commands (For CD25A, ED50A, and IC25A Only)

Command	Description
LeakDetTest	Performs a test of the leak detector.
LeakDetTestDate	Reports the date of the leak detector test.
LeakDetTestResult	Reports the result (pass or fail) of the leak detector test.

Dionex CD Conductivity Detector

In addition to the standard commands available for all detectors (see **Dionex Detectors**), the CD provides special commands (see the table below; please note that the display ➢*Filter* level determines which commands are displayed).

Command	Description
➢*DataCollectionRate*	Sets the rate at which Chromeleon collects digital data points from the detector.
PressureStatus	Reports the status of the pressure sensor (over pressure or not over pressure).
➢*Rise_Time*	Sets how quickly the detector responds to a change in signal.
➢*Temperature_Compensation*	Sets the temperature compensation factor, which is used to stabilize conductivity readings.

Cell Heater Commands

Command	Description
CellHeater.Mode	Selects whether the cell heater is on or off.
CellHeater.Status	Reports whether the cell heater is under the set temperature, at the set temperature, or over the set temperature.
CellHeater.TemperatureActual	Reports the actual temperature.
CellHeater.TemperatureSet	Sets the temperature of the cell heater.
CellHeater.TemperatureState	Reports the state of the cell heater (**Ready** or **NotReady**)

Analog Output Commands (Available only if I/O option is installed)

Command	Description
➢*Full_Scale*	Sets the full-scale analog out voltage.
➢*Mark*	Sends a positive pulse to the detector's analog output as an event marker.
➢*Offset_Level*	Sets the offset for the analog output signal.
➢*Polarity*	Switches the analog output polarity (+/-).
Recorder_Calibration	Calibrates a recording device. **Zero** sets the output signal to zero volts. **Full Scale** sets the output signal to the selected full-scale voltage (10, 100, or 1000 mV). **Normal** (the default) outputs a signal corresponding to the detector output.
➢*Recorder_Range*	Sets the range of a full-scale recorder response.

Dionex ED Electrochemical Detector

In addition to the standard commands available for all detectors (see **Dionex Detectors**), the ED provides special commands (see the table below; please note that the display ➢*Filter* level determines which commands are displayed).

Command/Property	Description
CellControl	Turns the cell on and off, or turns the dummy cell on and off.
CellStatus	Reports whether the cell is connected or disconnected.
➢*DataCollectionRate*	Sets the rate at which Chromeleon collects digital data points from the detector.
➢*DC_Voltage*	Sets the voltage applied to the cell.
Electrode	Select the reference electrode type (**AgCl** or **pH**).
ElectrodeStatus	Reports whether the electrode is connected or disconnected.
pH	Reports the current pH
pHAtTime0	Reports the pH at the start of acquisition.
pH.LowerLimit	Sets the lower limit for pH. An error is reported in the Audit Trail if the pH is lower than this value at the start of acquisition.
pH.UpperLimit	Sets the upper limit for pH. An error is reported in the Audit Trail if the pH is higher than this value at the start of acquisition.
EDet1.Mode	Select the operating mode: **IntAmp** (integrated amperometry), **DCAmp** (DC amperometry), or **Cyclic** (cyclic voltammetry). **Note:** EDet1 is the default name for the first or only ED detector in a timebase. EDet2 is the default name for a second ED detector in a timebase.
➢*Rise_Time*	Sets how quickly the detector responds to a change in signal.
➢*Waveform*	Define a waveform (a plot of potential vs. time).

Commands for Cyclic Voltammetry (for the 3D_Amp Channel)

Command/Property	Description
CV_CycleTime	Sets the time in seconds for one CV cycle, which is the time it takes to go from the **CV Low Voltage** to the **CV High Voltage** and then back to the **CV Low Voltage**. This creates a triangle ➢*Waveform*.
CV_LoVoltage	Sets the lowest voltage to be applied during the CV cycle. This voltage begins and ends the cycle.
CV_HiVoltage	Sets the highest voltage to be applied during the CV cycle. This is the peak of the triangle waveform.
CV_Cycles	Sets the number of times to repeat the CV cycle.
StartCV	Starts the CV run.

Analog Output Commands (Available only if I/O option is installed)

Command/Property	Description
➢*Full_Scale*	Sets the full-scale analog out voltage.
➢*Mark*	Sends a positive pulse to the detector's analog output as an event marker.
➢*Offset_Level*	Sets the offset for the analog output signal.
➢*Polarity*	Switches the analog output polarity (+/-).
Recorder_Calibration	Calibrates a recording device. **Zero** sets the output signal to zero volts. **Full Scale** sets the output signal to the selected full-scale voltage (10, 100, or 1000 mV). **Normal** (the default) outputs a signal corresponding to the detector output.
➢*Recorder_Range*	Sets the range of a full-scale recorder response.

Dionex Ion Chromatography Systems

Refer to the following topics for information about control commands for the current Dionex ion chromatography systems:

🔼 **Dionex DX-120 Ion Chromatograph**

🔼 **Dionex ICS-90 Ion Chromatography System**

🔼 **Dionex ICS-1000/1500/2000 Ion Chromatography System**

■ Dionex DX-120 Ion Chromatograph

The following commands are available for control of DX-120 operating functions (please note that the display ➢*Filter* level determines which commands are displayed):

Command	Description
Column	If the DX-120 is in ➢*Column Mode*, the **Column** command sets the flow path to column A or column B.
Controlled AC	Turns the AC power outlet on the DX-120 rear panel on and off. This provides on off control of an external accessory connected to the outlet. Refer to the DX-120 operator's manual for cabling instructions.
➢*Data_Collection_Rate*	Sets the rate at which Chromeleon collects digital data points from the detector.
Eluent	If the DX-120 is in ➢*Eluent Mode*, the **Eluent** command selects the eluent reservoir (A or B).
Eluent Pressure	Turns the pressure to the eluent reservoir(s) on and off.

Command	Description
Pressure Unit	Select the units of pressure to use (psi or MPa).
Pump	Turns the pump on and off.
SRS	Turns the current supplied to the ➢*Self-Regenerating Suppressor* (SRS) on and off.

Dionex ICS-90 Ion Chromatography System

In addition to the **General Device Commands**, the following commands are available for control of ICS-90 operating functions (please note that the display ➢*Filter* level determines which commands are displayed):

Detector Commands

Command	Description
⇒*AcqOn/Off*	Starts/stops data acquisition.
⇒*Autozero*	Automatic autozero.

Pump Commands

Command	Description
Pump (on/off)	Turns the pump on or off.
⇒*Pressure.Lower/ UpperLimit*	Sets pressure limits.

Injection and TTL Commands

Command	Description
InjectPosition	Sets the injection valve to the inject position.
LoadPosition	Sets the injection valve to the load position.
TTL_1	The following commands are available:
	State indicates or sets the state of the ➢*TTL*
	0v sets the TTL to 0 volts
	5v sets the TTL to 5 volts
	Duration toggles the TTL's state after the specified time.
	The TTL outputs are normally at 5 volts. Setting a TTL output to 0 volts turns on the action in the connected device.
	TTL-1 on the ICS-90 is typically connected to an AS40 autosampler. Setting TTL-1 to **0v** starts the AS40 Load cycle.

In addition to the commands listed above, calibration and diagnostic commands are also available (see **How to …: Performing Validation and Qualification** **Ensuring System Wellness** for details).

Dionex ICS-1000/1500/2000 Ion Chromatography System

In addition to the **General Device Commands**, the following commands are available for control of ICS-1000/1500/2000 operating functions (please note that the display ➤*Filter* level determines which commands are displayed).

Pump Commands

In addition to the standard pump commands (see **Dionex Pumps**), the following commands are available for controlling the ICS-1000/1500/2000 pump.

Command	Description
EluentBottleLevel	Indicates the volume of liquid (in liters) in the eluent reservoir. To ensure the accuracy of this value, enter the volume of liquid in the reservoir each time you fill the reservoir.
EluentValve	Indicates the eluent valve position (**Open** or **Closed**).
On, Off, Prime	Turns the pump on or off, or starts the pump priming function.
Pump_InjectValve. InjectPosition	(If no AS50 is installed) Injects the sample *Tip:* *For the ICS ion chromatography system, injection is performed with the **Pump_InjectValve.InjectPosition** command (if no AS50 is installed). With the **Pump_InjectValve.InjectPosition** command, an injection is always performed, also for a ➤Blank Run Sample. This is contrary to the autosampler ⇒Inject command.*
PumpMode	Indicates whether the pump is on, off, or priming.

Detector Commands

In addition to the standard detector commands (see **Dionex Detectors**), the following commands are available for controlling the ICS-1000/1500/2000 detector.

Command	Description
Acquisition_Ready	Indicates when the detector is ready to start data acquisition.
CellTemperature	Sets the temperature of the DS6 conductivity cell.
➤*Data_Collection_Rate*	Set the rate at which Chromeleon collects digital data points from the detector

Command	Description
DataPolarity	Sets the signal polarity to Normal or Inverted.
MaxAutoStep	Sets the maximum step rate if ⇒*Step* is set to **Auto**.
Offset_Level	(Analog Out) Sets the offset (0 to 100%) applied to the recorder output.
Polarity	(Analog Out) Sets the recorder output polarity.
Recorder_Calibration	(Analog Out) Calibrates the recorder output (Normal, Zero or Full-Scale).
Recorder_Range	(Analog Out) Sets the full-scale output range of the recorder.
➢*Rise_Time*	Sets how quickly the detector responds to a change in signal.
TotalConductivity	Reports the measured conductivity with no offset applied.

Column Temperature and Suppressor Commands

Command	Description
ColumnTemperature	Sets the column heater temperature.
Suppressor_Current	Sets the suppressor current (in mA).
Suppressor_Mode	Turns the suppressor on or off.
Suppressor_Type	Selects the type of ➢*Suppressor* installed.

Eluent Generator Commands (ICS-2000 only)

In addition to the standard eluent generator commands (see 🔲 **Dionex Eluent Generators**), the following commands are available for controlling the ICS-2000 eluent generator.

Command	Description
CR_TC	Turns the CR-TC on or off.
EgcCurrent	Indicates the EGC current value.
EgcVoltage	Indicates the EGC voltage value.
Mode	Turns the eluent generator on or off.

State Commands

Command	Description
InjectPosition	Sets the injection valve to the inject position.
LoadPosition	Sets the injection valve to the load position.
Relay_1, Relay_2	The following commands are available: **State** (indicates or sets the state of the relay), **Closed** (closes the relay), **Open** (opens the relay), and **Duration** (toggles the relay's state after the specified time). The relay-contact closures are normally open. When the relay is closed, current flows to the connected device (turns on the action).
TTL_1, TTL_2	The following commands are available: **State** (indicates or sets the state of the ➤*TTL*), **0v** (sets the TTL to zero volts), **5v** (sets the TTL to 5 volts), and **Duration** (toggles the TTL's state after the specified time). The TTL outputs are normally at 5 volts. Setting a TTL output to 0 volts turns on the action in the connected device.

In addition to the commands listed above, calibration and diagnostic commands for the ICS-1000/1500/2000 are also available (see **How to …: Performing Validation and Qualification** 🔲 **Ensuring System Wellness** for details).

Additional Dionex Components

In addition, Chromeleon supports the Additional components. For a description of the commands and properties supported for these components, refer to:

🔲 **Dionex Eluent Generators**

🔲 **Dionex External MSVs**

🔲 **Dionex Detector/Chromatography Module**

🔲 **Dionex Active Flow Splitter (MRA**)

▪ Dionex Eluent Generators

In addition to the ▪ **General Device Commands**, the following commands are available for controlling the operating functions of Dionex eluent generators (please note that the display ➤*Filter* level determines which commands are displayed).

Commands Available for All Eluent Generators

Command	Description
CartridgeExpirationDate	Reports the expiration date of the cartridge (read-only).
CartridgeNumber	Reports the serial number of the cartridge.
CartridgeRemainingLifeTime	Reports the percentage of the original ion count remaining in the cartridge (read-only).
CartridgeType	Reports the cartridge type currently installed (read-only).
Concentration	Sets the eluent concentration to be generated.
Curve	Sets the curve number for gradients. Refer to ➤*Gradient Curves* for an explanation of curve numbers.

Commands Available for the ICS-2000 and ICS-3000 EG Only:

Command	Description
CR_TC	Turns the CR-TC (Continuously Regenerated Trap Column) on or off. The CR-TC removes anionic or cationic contaminants in the eluent or deionized water.
EgcCurrent	Indicates the current value of the cartridge (read-only).
EgcVoltage	Indicates the voltage value of the cartridge (read-only).
FlowStatus	Reports the status of the pump flow (flow detected or not flow detected) (read-only).
InstConcentration	Reports the concentration of eluent currently being generated (read-only).
Mode	Turns the eluent generator on or off.
SerialNumber	Reports the serial number of the eluent generator (read-only).
TotalConcentration	Reports the total concentration of eluent being generated by the K_2CO_3 cartridge when carbonate/bicarbonate is the eluent type. The total is equal to the set concentration of the K_2CO_3 cartridge plus the set concentration of the pH modifier (read-only).

For additional information, refer to **Practical Tips for Device Control:**

▪ **Controlling the Eluent Generator Concentration**

▪ **Monitoring the Eluent Generator Cartridge Lifetime**

Calibration Commands (Available for the EG40 and EG50 Only)

Command	Description
DownloadCalibration	Downloads the selected set of calibration values to the detector.
DownloadCalibration Parameter	Selects the set of calibration values (Current, Previous, or Factory) to be downloaded.
LeakDet	Performs the leak detector calibration.

Diagnostic Commands (Available for the EG40 and EG50 Only)

Command	Description
LeakDetTest	Performs a test of the leak detector.
LeakDetTestDate	Reports the date of the leak detector test.
LeakDetTestResult	Reports the result (pass or fail) of the leak detector test.

▪ External MSVs

In addition to the 🔼 **General Device Commands**, the following commands and properties are available for controlling the operating functions of the switching valves (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command	Min.	Max.	Default	Description
Direction	0 Shortest 1 Counterclockwise 2 Clockwise		0	Defines the direction in which the valve moves to the specified position.
Home				Moves the valve to position 1. (Note: The command ignores the Direction property.)
Position	1	Depends on the type.	Read from the device on connect.	The current position of the valve. (The valve moves to the specified position, obeying the Direction property.)
Test				Tests the valve. (Moves the valve to position 1, steps to all positions, and returns the valve to position 1) Level: Expert.

Property	Values	Default	Description
ModelNo	Text string (64 characters) describing the valve type	Read from the device on connect.	Read-only. Level: Expert.
SerialNo	Text string (64 characters) indicating the serial number.	Read from the device on connect.	Read-only. Level: Expert.
FirmwareVersion	Text string (64 characters) indicating the firmware version.	Read from the device on connect.	Read-only. Level: Expert.

◻ Dionex Detector/Chromatography Module

In addition to the ◻ **General Device Commands**, the following commands are available for control of DC operating functions (please note that the display ➢ *Filter* level determines which commands are displayed).

Property	Description
LowerDoor	Reports whether the compartment lower door is open or closed.
UpperDoor	Reports whether the compartment upper door is open or closed.
LowerLeakCondition	Reports whether the lower leak sensor is wet or dry.
UpperLeakCondition	Reports whether the upper leak sensor is wet or dry.

Suppressor Control Commands

The following commands are used for controlling a suppressor installed in a DC.

Property	Description
FlowStatus	Reports the status of the flow sensor (flow detected or flow not detected).
SuppressorName.Current	Reports the actual suppressor current.
SuppressorName.CurrentSet	Sets the current supplied to the suppressor and turns on the suppressor (**SuppressorName.Mode=On**).
SuppresorName.Mode	Turns the suppressor on and off.
SuppressorName.Type	Sets the installed ➢ *Suppressor* type.
Suppressorname.Voltage	Reports the suppressor voltage.

Notes on Suppressor Names

The following names are assigned to the suppressors by default:

Suppressor1: The first (or only) suppressor in the timebase

Suppressor2: The second suppressor in the timebase

Temperature Control Commands

The following commands are used for controlling the various DC temperature control zones (conductivity cell, compartment, column, and reaction coil heater).

Command/Property	Description
DeviceName AcquireExclusiveAccess	Gives exclusive access of the temperature zone to the current timebase. Exclusive access commands allow two timebases to share the same DC temperature zone.
DeviceName ReleaseExclusiveAccess	Takes exclusive access of the temperature zone away from the current timebase, to allow another timebase to acquire exclusive access.
DeviceName Mode	Selects whether the temperature zone is on or off.
DeviceName Status	Reports whether the temperature of the zone is under the set temperature, at the set temperature, or over the set temperature.
DeviceName TemperatureActual	Reports the actual temperature.
DeviceName TemperatureSet	Sets the temperature of the temperature zone.
DeviceName TemperatureState	Reports the state of the temperature zone (**Ready** or **NotReady**).

Notes on Temperature Control Device Names:

The following names are assigned to the temperature control devices by default:

Compartment_TC: The DC upper compartment

Column_TC: The DC lower (column) compartment

ReactionCoilHeater: The reaction coil heater

Injection Valve Control Commands

The following commands are used for controlling the high-pressure injection valves installed in the DC.

Command/Property	Description
ValveName.State	Indicates (and can set) the valve position (**Load** or **Inject**).
ValveName.LoadPosition	Switches the valve to the Load position.
ValveName.InjectPosition	Switches the valve to the Inject position.
Valve1Button	Reports whether manual control of the **VALVE 1** button is enabled or disabled.
Valve2Button	Reports whether manual control of the **VALVE 2** button is enabled or disabled.

Notes on Injection Valve Names

The following names are assigned to the injection valves by default:

InjectValve_1: The first (or only) injection valve in the timebase

InjectValve_2: The second injection valve in the timebase

🛡️ *Note:*

*When the **VALVE 1** or **VALVE 2** button is enabled in Chromeleon, pressing the button triggers the injection valve. When a **VALVE** button is enabled at the same time that a remote inject device is configured in the same timebase as the DC, pressing the button instructs the program to automatically continue collecting data after executing the inject command.*

High-Pressure Valve Control Commands

The following commands are used for controlling the high-pressure valves installed in the Automation Manager.

Command/Property	Description
ValveName.State	Indicates (and can set) the valve position (**A** or **B**).
ValveName.A	Switches the valve to the A position.
ValveName.B	Switches the valve to the B position.

Notes on Default High-Pressure Valve Names

The following names are assigned to the Automation Manager high-pressure valves by default:

AM_HP_1: The first (or only) Automation Manager high-pressure switching valve in the timebase

AM_HP_2: The second Automation Manager high-pressure switching valve in the timebase

Low-Pressure Valve Control Commands

The following commands are used for controlling the various low-pressure valves installed in the DC.

Command/Property	Description
ValveName.State	Indicates and can set the valve position (**Open** or **Closed**).
ValveName.Open	Switches the valve to the open position.
ValveName.Closed	Switches the valve to the closed position.

Notes on Default Low-Pressure Valve Names

The following names are assigned to the low-pressure valves by default:

AM_LP1: The first (or only) Automation Manager low-pressure valve in the timebase

AM_LP2: The second Automation Manager low-pressure valve in the timebase

DC_LP1: The first (or only) DC low-pressure valve in the timebase

DC_LP2: The second DC low-pressure valve in the timebase

DC_LP3...DC_LP6: The third through sixth DC low-pressure valve in the timebase

Relay and TTL Control Commands

The following commands are used for controlling the relay and TTL outputs installed in the DC.

Command/Property	Description
RelayTTLName.State	Indicates (and can set) the relay position (**Open** or **Closed**).
RelayTTLName.Open	Switches the relay to the open position.
RelayTTLName.Closed	Switches the relay to the closed position.

Notes on Default Relay and TTL Output Names

The following names are assigned to the DC relay and TTL outputs by default:

DC_Relay_1: DC relay output 1

DC_Relay_2: DC relay output 2

DC_ACRelay_1: DC AC relay output 1

DC_ACRelay_2: DC AC relay output 2

DC_TTL_1: DC TTL output 1

DC_TTL_2: DC TTL output 2

Active Flow Splitter (MRA)

In addition to the **General Device Commands**, the following commands are available for controlling the operating functions of the flow splitter (please note that the display ➤*Filter* level determines which commands are displayed):

Command	Min.	Max.	Default	Description
Flow	1.0	100.0	10.0	Sets the flow to be split in mL/min.
GrooveVolume	0	300	-	Indicates the groove volume in nL (read-only). (The property is available from the Expert level on.)

Command	Min.	Max.	Default	Description
Home				Moves the splitter to position 1.
ModuleType	Text (64 characters), describing the flow splitter type		Read from the splitter upon connect.	Indicates the module type (should be MRA100-000; read-only). (The property is available from the Expert level on.)
RunState	On/Off		Off	Sets the status of the MRA: **On** splits the flow based on the related parameters.
SplitFactor	1	59	47	Reports the split factor set on the device (read-only). (The property is available from the Expert level on.)
SplitRatio.Nominal	100	100000	20000	Sets the nominal split ratio.
SplitRatio.Value	100	100000	-	Indicates the actual split ratio (read-only). The ratio is calculated from the HPLC flow and SplitRatio.Nominal.
SwitchCounter				Counts how often the flow splitter has been switched since the counter was last reset.
UpperLimit	0	NoLimit; 10000001	NoLimit	If the total number of switches exceeds the upper limit, a warning appears. To disable the function, set the upper limit to **NoLimit**.
Value	Reset; 0	10000000	1	Reports the total number of splitter switches. Select **Reset** to reset the counter to 0.
SwitchFrequency	0.00	100.00	-	Reports the switching frequency in Hz (read-only). (The property is available from the Expert level on.)
Test				Tests the flow splitter. (Moves the splitter to position 1, steps to all positions, and returns the splitter to position 1.) (The property is available from the Expert level on.)

Commands and Tips for Third-Party Devices

Commands and Tips for Third-Party Devices

Chromeleon allows you to control both Dionex devices and numerous third-party devices. For an overview of manufacturers whose instruments can be controlled by Chromeleon, refer to **Hardware Installation ➲ Installing and Controlling Third-Party Devices** in the **Administrator Help** section.

For many third-party devices, Chromeleon provides the same commands as for Dionex devices. For more information about these standard commands, refer to **Device Control 🔲 Practical Tips for Device Control (Overview)** and 🔲 **Commands for Controlling Dionex Devices**.

In addition to the standard commands, several third-party devices support special commands. For information about special commands and useful tips for third-party instruments, refer to:

🔲 **Agilent**

🔲 **CTC Analytics PAL Samplers**

Finnigan (see 🔲 **ThermoFinnigan/TQ/TSP)**

Fisons (see 🔲 **ThermoFinnigan/TQ/TSP)**

🔲 **Gilson**

Hewlett Packard (see 🔲 **Agilent**)

Hitachi (see 🔲 **Merck Hitachi**)

🔲 **Kontron**

🔲 **Merck Hitachi**

🔲 **Perkin Elmer**

🔲 **Rheodyne Valves**

🔲 **Shodex Refractive Index Detectors (RI-101, RI-102, RI-104)**

🔲 **Shimadzu**

🔲 **ThermoFinnigan/TQ/TSP**

🔲 **Valco (= VICI) Valves**

🔲 **Varian**

🔲 **Waters**

Agilent (formerly HP): Commands and Tips

For information about the special commands that Chromeleon supports for the different Agilent devices and for tips for practical operation, refer to:

1100 HPLC System

Agilent 1050 HPLC System: UV Detector

6890/6850 GCs

Agilent HP5890 GC: Application

Agilent 7683 GC Autosampler

Agilent 1100 HPLC System: Commands and Tips

Refer to the following pages for information about the special commands supported for the Agilent 1100 HPLC System and for tips for practical operation:

General

Pump

Autosampler (G1329)

Wellplate-Sampler (G1367)

Column Compartment

UV Detectors

Fluorescence Detectors

Refractive Index Detector

Program Tips

Example for a Derivatization Program

Scan Modes for the Fluorescence Detector

Properties and Restrictions of the Refractive Index Detector

Tips for Lamp Commands

Checking the Solvent Liquid Level

Diagnostic Functions

Troubleshooting

◾ Agilent 1100 HPLC System: General

The following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Description
Firmware	Indicates the firmware version of the module (read-only)
ModelNo	Reports the module number (read-only)
ModuleType	Reports the module type, e.g., G1315A for the DAD detector (read-only)
NotReadyCauses	String to explain why the module is not yet ready for the next analysis (read-only)
SerialNo	Indicates the serial number of the module (read-only)

Command	Description
⇒*Connect*	Connects the device.
Disconnect	Disconnects the device.
Identify	Identifies the module (the module LED will flash).
⇒*Reset*	Resets the device to the default values.

Name	Possible States	Description
AnalysisState	NoAnalysis, Analysis Pending	Retention time state
AnalysisTime	0 outside of analysis, analysis time in minutes since analysis start.	Retention time
ErrorState	NoError Error	Error state
ReadyState	NotReady Ready	Ready state
RuntimeState	Prerun Run Postrun Leak Shutdown	Run time state
RunTime	0 outside of run, runtime in minutes since run start.	Run time
TestState	NoTest Test	Test state

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

⬤ **Agilent 1100 HPLC System: Overview**

⬤ **Agilent 1100 HPLC System: Installation**

▪ Agilent 1100 HPLC System: Pump

In addition to (or different from) the standard pump commands (see **Commands for Controlling Dionex Devices** ▣ **Dionex Pumps**) and the general commands for the 1100 HPLC system (see ▣ **Agilent 1100 HPLC System: General**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

	Range/Values			
Property	Min	Max	Default.	Description
⇒*%B, %C, %D*	0.0 %	100.0 %	100.0 %A	
Compressibility	autom., no compensation, 50..150	-	100	Compressibility [1e-6/bar] (Available only for the isocratic and quaternary pumps.)
Compressibility LeftPump	autom., no compensation, 50..150	-	50	Compressibility of the left pump [1e-6/bar] (Available only for the binary pump.)
Compressibility RightPump	autom., no compensation, 50..150	-	115	Compressibility of the right pump [1e-6/bar] (Available only for the binary pump.)
EmptySolventError	Off	On	Off	*
⇒*Flow*	0.000	10.000 (quaternary) 5.000 (binary)	0.000	Flow in [ml/min]
Pressure	0.0	400.0 (Flow < 5 ml/min) 200.0 bar (else)	Min.: 0 Max. 400.0	Pressure in [bar]

Property	Min	Max	Default.	Description
PrimaryChannel	autom., A, B, C, D	-	autom.	(Quaternary LPG pump only) Select the channel from which the pump shall draw liquid first. The pump starts with drawing half the volume required for the composition, then draws the required volume from the other channels, and then draws the second half of the volume required for the first channel.
				Automatic selects the channel with the maximum percentage.
SolventFillingX	0.00 l	1000.00 l	n/a	* (Where X = A1, A2, B1 or B2 for the binary pump and X = A to D for the quaternary pump.)
SolventLeftPump	A1, A2		A1	Eluent of the left pump. (Available only for the binary pump.)
SolventNotReady Limit	0.20 l	1000.00 l	Off	*
SolventRightPump	B1, B2		B1	Eluent of the right pump. (Available only for the binary pump.)
Stroke	autom., 20..100 µl		autom.	Pump stroke (Available only for the isocratic and quaternary pumps.)
StrokeLeftPump	autom., 20..100 µl		autom.	Stroke of the left pump. (Available only for the binary pump.)
StrokeRightPump	autom., 20..100 µl		autom.	Stroke of the right pump. (Available only for the binary pump.)

* For more information about these commands, refer to **Agilent 1100 HPLC System: Checking the Solvent Liquid Level**.

Rear Seal Wash Commands

The following commands are available only if the pump is equipped with the rear seal wash option and the **Rear Seal Wash** check box is selected on the **Options** tab page in the Server Configuration:

🖐☞ *Tip:*

This function is available only if the Rear Seal Wash option is installed for the pump.

Command	Description
System	Specifies a wash option: **Off**, **Single**, or **Interval**.
Duration	Specifies the duration of a *single* wash. This command is associated with the **Single** function of the **System** command.
Interval	Specifies the duration of the entire wash interval, i.e., the idle period and run time of the pump. This command is associated with the **Interval** function of the **System** command.
On Time	Specifies the actual run time of the pump during a wash period. This command is associated with the **Interval** function of the **System** command.

🖐☞ *Tip:*

*The time specified here may not exceed 70% of the duration specified under **Interval**.*

Diagnostic Commands

Command	Description
CountDown	Keeps the specified flow rate for the CountDown time. (The default setting is 0.00 min. The allowed range is 0.00 to 99,999.00 min.)
LeakTestInit	Prepares the pump for the leak test by moving pistons A1 and B1 of the quaternary pump and/or piston 2 of the binary pump to the top of its stroke.
LeakTestRun	Performs a leak test.

🖐☞ *Tip:*

Dionex recommends performing the command from the related diagnostic panel (see 🔧 Agilent 1100 HPLC System Diagnostics: Leak Test).

PressureTest	Performs a pressure test.

🖐☞ *Tip:*

Dionex recommends performing the command from the related diagnostic panel (see 🔧 Agilent 1100 HPLC System 1100 Diagnostics: Pump Pressure).

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

➡ **Agilent 1100 HPLC System: Overview**

➡ **Agilent 1100 HPLC System: Installation**

▪ Agilent 1100 HPLC System: Autosampler (G1329)

In addition to (or different from) the standard autosampler commands (see **Commands for Controlling Dionex Devices** ▪ **Dionex Autosamplers**) and the general commands for the 1100 HPLC system (see ▪ **Agilent 1100 HPLC System: General**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Default	Description
DispSpeed	10 µl/min *or* 90 µl/min	1000 µl/min	200 µl/min	Speed for dispensing the sample
DrawSpeed	10 µl/min *or* 90 µl/min	1000 µl/min	200 µl/min	Speed for drawing the sample
Position	0	(Tray dependent)	n/a	Sample vial number
SampleHeight	-2.5 mm	35 mm	0.0 mm	Needle draw/eject position offset
Syringe	100 µl	900 µl	as configured	Syringe volume [µl]
⇒*Volume*	0.0 µl	8000.0 µl	5.0 µl	Injection volume
WashVial	0	Greatest sample position (tray dependent)	0	Wash vial number 0: no wash

Command	Description
⇒*Dispense*	Injects sample into the loop.
⇒*Draw*	Draws sample from a vial into the syringe.
InitiateChangeGripper	Initiates the gripper change by moving the gripper to the front.
InitiateChangeNeedle	Initiates the needle change by removing the safety covering and moving the needle carrier to an easily accessible mounting position. In addition, the needle is raised to the uppermost position.
InitiateChangePiston	Initiates the piston change by releasing the spring on the proportioning unit.
⇒*Inject*	Performs an injection (see note below).

Command	Description
Mix	Mixes the sample using the syringe.
Repeat/EndRepeat	Repeats the enclosed sample preparation steps.
TerminateChangeGripper	Terminates the gripper change by returning the gripper to the Home position.
TerminateChangeNeedle	Terminates the needle changes by returning the needle to the Home position.
TerminateChangePiston	Terminates the piston change by tensing the spring on the proportioning unit.
Valve	Switches the injection valve.
Wait	Waits a given time during the injection program.

Parameters of the commands

Command	Values	Description
Dispense, parameter: *Vial*	SampleVial (current sample vial) SampleVialPlus (current sample vial + n) Vial (sample vial No. n) Seat (needle seat) Air	Position of the vial for dispense
Dispense, Mix parameter: *Speed*	10 (or 90 µl) to 1000 µl/min Maximum Default (uses DispSpeed)	Speed for dispense or mix
Draw, parameter: *Source*	SampleVial (current sample vial) SampleVialPlus (current sample vial + n) Vial (sample vial No. n) Seat (needle seat) Air	Position of the vial for draw
Draw, parameter: *Speed*	10 (or 90) to 1000 µl/min Maximum Default (uses DrawSpeed)	Speed for draw
Draw/Dispense, parameter: *Offset*	-2.5 to 35.0 mm SampleHeight	Needle draw/eject position offset for draw or dispense
Draw, Dispense, Mix parameter: *Volume*	0.0 to 100.0 µl (or 900.0 µl) Maximum InjectVolume	Volume for draw, dispense, or mix
Needle, parameter: *Vial*	SampleVial (current sample vial) SampleVialPlus (current sample vial + n) Vial (sample vial No. n)	Position to which the needle is moved

🖎 *Tips:*

*The **Inject** command sends a start impulse to all modules. Then the analysis will start with the following steps:*

- *The detector performs an ⇒Autozero if the program file contains the corresponding command at the time t=0.000 min.*

- *An injection/sample preparation program is executed if the program contains the corresponding commands at the t=0.000 min.*

- *The injection will take place and the run will start.*

Three different injection programs are available:

Standard (injection volume from the respective position)	WashVial=None and no sample preparation commands
Standard (with needle wash)	WashVial=None and no sample preparation commands
User-defined injection program	If sample preparation commands are issued

Autosampler/Thermostat

Property	Min	Max	Default	Description
AutoActivateSetpoint	Off	On	On	If set to **On**, temperature control is automatically enabled right after power-up.
TempCtrl	Off	On	Off	Turns temperature control on or off.
Temperature.Nominal	4°C	40°C	20°C	Temperature set point for the air stream.
Temperature.Value	4°C	40°C	n/a	Current temperature of the air stream.

Also, see 🔲 **Agilent HPLC System 1100: Wellplate Sampler (G1367)**.

Diagnostic Commands

Command	Description
GripperHome	Moves the gripper to home position.
GripperPick	Picks the vial at the current position.
GripperPut	Positions the vial at the current position.
GripperToPosition	Moves the gripper to the specified position.
NeedleIntoSeat NeedleIntoVial NeedleToPosition	(For the G1329 autosampler, the commands have no functionality.)
NeedleUp	Moves the needle to its topmost position.

Command	Description
PlungerDraw	Draws the plunger to load sample into the sample loop.
PlungerHome	Moves the plunger back to its home position.
VialToSeat	Positions the vial in the seat.
VialToTray	Positions the vial in the tray.
ValveToBypass	Switches the sample loop into the bypass position.
ValveToMainpass	Switches the sample loop into the inject position.

For more information about the diagnostic functions for the autosampler, refer to:

Agilent 1100 HPLC System Diagnostics: Injector Steps

Agilent 1100 HPLC System Diagnostics: Gripper Verification

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

Agilent 1100 HPLC System: Overview

Agilent 1100 HPLC System: Installation

Agilent 1100 HPLC System: Wellplate Sampler (G1367)

In addition to the commands supported by the autosampler of the 1100 HPLC System (see **Agilent 1100 HPLC System: Autosampler (1329)**), the wellplate sampler supports the some special commands (please note that the display ➢*Filter* level determines which commands and properties are displayed). Please note: For some of the HP 1100 autosampler commands, the ranges and parameters may be different for the wellplate sampler (refer to the table below).

Special Wellplate Sampler Parameters and Values

Command	Values	Description
Dispense, parameter: *Vial*	SampleVial (current sample vial) Location (sample position No. n) Seat (needle seat)	Position of the vial for dispense
Dispense, Mix parameter: *Speed*	10-1000 µl/min Maximum Default (uses DispSpeed)	Speed for dispense or mix
DispSpeed	10-1000 µl/min	Speed for ejecting the sample

Command	Values	Description
Draw, parameter: *Source*	SampleVial (current sample vial) Location (sample position No. n) Seat (needle seat) Air	Position of the vial for draw
Draw, parameter: *Speed*	10-1000 µl/min Maximum Default (uses DrawSpeed)	Speed for draw
Draw/Dispense, parameter: *Offset*	-10.0 to 60.0 mm SampleHeight	Needle draw/eject position offset for draw or dispense
Draw/Dispense, Mix parameter: *Volume*	0.00-100.00 µl Maximum InjectVolume	Volume for draw, dispense, or mix
DrawSpeed	10-1000 µl/min	Speed for drawing the sample
Needle, parameter: *Vial*	SampleVial (current sample vial) Location (sample position No. n)	Position to which the needle is moved
SampleHeight	-10.0 to 60.0 mm	Needle draw/eject position offset
Volume	0.0-100.0 µl	Injection volume
WashVial	Sample position No. no wash flush (see WashTime)	Wash vial number. If the setting is **no wash**, a wash cycle is not performed.

Special Wellplate Sampler Commands

Property	Min.	Max.	Default	Description
DelayVolumeReduction²	Off	On	Off (read from device on connect)	Enables or disables automatic delay volume reduction.
EquilibrationTime³	0.0 s	100.0 s	0.0 s (sent to device on connect	Time until equilibration is complete.
FlushFactor	1.0	10.0	5.0 (read from device on connect)	Indicates how often the flush port is filled for flushing the needle (exterior).
PrimePump	1	1000	5	Primes needle for the time specified (in [s]).
WashRepeat	1	5	1 (read from device on connect)	Number of washes when WashVial is set to a vial location
WashTime	0.0 s	100.0 s	1.0 s (read from device on connect)	Time to wash when WashVial is set to Flush.

📠 *Tip:*

² Switches the injection valve back to BYPASS after the sample is eluted beyond the injection valve.

³ On connect of the wellplate sampler EquilibrationTime = 0.0 s is sent to the device. If necessary, enter another value explicitly.

Command	Options			
Wash	*In*	*Time*	*Repeat*	*Offset*
(For user-defined inject programs)	*In =* AsMethod, Flush, SampleVial *or* osition of sample	*Time =* 0.0 to 100.0 s	*Repeat =* 1 to 5	*Offset =* -10.0 to 60.0 mm
Function	Defines the location of the wash solution	See WashTime required if *In* = Flush	See WashRepeat required if *In* > 0 or SampleVial	Height of the needle above the bottom of the vial

For example, the following parameters are possible for the Wash command:

1. Wash In=Flush   Time=3
(Washes for 3 seconds, using the eluent from the flush port.)

2. Wash In=SampleVial   Repeat=2
(Washes twice, using the solution from the current sample position.)

3. Wash In=15   Repeat=5
(Washes five times, using the solution from sample position 15.)

4. Wash In=AsMethod
(With this option, the parameters entered via the WashVial, WashTime, and WashRepeat commands are used.)

Also, see 🖳 **Agilent 1100 HPLC System: Autosampler (G1329)**.

Diagnostic Commands

Command	Description
GripperHome GripperPick GripperPut GripperToPosition	(For the G1367 autosampler, the commands have no functionality.)
NeedleIntoSeat	Moves the needle into the needle seat.
NeedleIntoVial	Lowers the needle into the vial.
NeedleToPosition	Moves the needle to the specified position.
NeedleUp	Moves the needle to its topmost position.
PlungerDraw	Draws the plunger to load sample into the sample loop.
PlungerHome	Moves the plunger back to its home position.
VialToSeat VialToTray	(For the G1367 autosampler, the commands have no functionality.)
ValveToBypass	Switches the sample loop into the bypass position.
ValveToMainpass	Switches the sample loop into the inject position.

For more information about the diagnostic functions for the autosampler, refer to 🔳 **Agilent 1100 HPLC System Diagnostics: Injector Steps**.

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

🔵 **Agilent 1100 HPLC System: Overview**

🔵 **Agilent 1100 HPLC System: Installation**

◾ Agilent 1100 HPLC System: Column Compartment

In addition to the standard oven commands (see **Commands for Controlling Dionex Devices** ◾ **Dionex Flow Manager and Thermostatted Column Compartments**) and the general commands for the 1100 HPLC system (see ◾ **Agilent 1100 HPLC System: General**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Range/Values Min	Max	Default	Description
AutoActivateSetpoint	Off (0) On (1)		Off	If set to **On**, temperature control is automatically enabled right after power-up.
LeftTemperature.Delta *or* RightTemperature.Delta	0°C	100°C	0.8°C	Temperature deviation permitted for the temperature set point in the left or right column compartment.
LeftTemperature.Nominal *or* RightTemperature.Nominal	-5°C	80°C	40°C	Temperature set point for the left or right column compartment.
LeftTemperature.Value *or* RightTemperature.Value	-5°C	80°C	40°C	Current temperature of the left column or right compartment.
Mode	Combined (0) Separate (1)		Combined	Enables independent control of the right column compartment. If set to **Combined**, the settings for the left column compartment apply to both compartments.
TempCtrl	Off (0) On (1)		Off	Turns temperature control on or off.
Valve	RightColumn (0) LeftColumn (1)		LeftColumn	Switches the column selector valve (option).

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

🔵 **Agilent 1100 HPLC System: Overview**

🔵 **Agilent 1100 HPLC System: Installation**

▣ Agilent 1100 HPLC System: UV Detectors

In addition to (or different from) the standard detector commands (see **Commands for Controlling Dionex Devices 🔺 Dionex Detectors**) and the general commands for the 1100 HPLC system (see 🔺 **Agilent 1100 HPLC System: General**), the following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Default	Description
AutoActivateUV_Lamp	Off	On	Off	If set to **On**, the deuterium lamp is automatically turned on upon power-up.
UV_Lamp	Off	On	Off, (On in ➢*Demo Mode*)	Reports the state of the deuterium lamp.

Property	Min	Max	Default	Description
FilterMovements	0	UINT_MAX	0 in demo mode, else read from module on connect.	Counter for the movements of the holmium oxide filter. Set the UpperLimit to 0 to disable the associated limit test. (PDA only)
ReferenceDiode Exposure	0	UINT_MAX	0 in demo mode, else read from module on connect.	Counter of the reference diode exposure. Set the UpperLimit to 0 to disable the associated limit test. (VWD only)
SampleDiode Exposure	0	UINT_MAX	0 in demo mode, else read from module on connect.	Counter of the sample diode exposure. Set the UpperLimit to 0 to disable the associated limit test. (VWD only)

Property	Min	Max	Default	Description
SlitMovements	0	UINT_MAX	0 in demo mode, else read from module on connect.	Slit movement counter. Set the UpperLimit to 0 to disable the associated limit test. (PDA only)
UvLampIgnitions	0	UINT_MAX	0 in demo mode, else read from module on connect.	UV lamp ignition counter. Set the UpperLimit to 0 to disable the associated limit test.
UvLampLifetime	0.0h	UINT_MAX /3600	0 in demo mode, else read from module on connect.	Total burn time of the UV lamp [h] Set the UpperLimit to 0 to disable the associated limit test.
UvLampOntime	0.0h	UINT_MAX /3600	0 in demo mode, else read from module on connect.	Burn time of the UV lamp since the last time it was turned on. Set the UpperLimit to 0 to disable the associated limit test.
VisLampIgnitions	0	UINT_MAX	0 in demo mode, else read from module on connect.	VIS lamp ignition counter. Set the UpperLimit to 0 to disable the associated limit test. (DAD only)
VisLampLifetime	0.0h	UINT_MAX /3600	0 in demo mode, else read from module on connect.	Total burn time of the VIS lamp [h]] Set the UpperLimit to 0 to disable the associated limit test. (DAD only)
VisLampOntime	0.0h	UINT_MAX /3600	0 in demo mode, else read from module on connect.	Burn time of the VIS lamp since the last time it was turned on. Set the UpperLimit to 0 to disable the associated limit test. (DAD only)

Command	Description
Autozero	Performs ⇒*Autozero*. The command is performed immediately, when issued manually, or at a ➢*Program* time <0.000 min. With a program time of 0.000, the command is performed before the injection program; with a program >0.000, the command is performed at this program time.

Name	Possible States	Description
DeviceState	Idle Prepare Reset Startup Whol TestWcal Scan	Detector state
LampState	Off On LampWarmup Ignition Retry	Lamp state

Diode Array Detector

	Range/Values			
Property	Min	Max	Default	Description
AutoactivateVisible_Lamp	Off	On	Off	If set to **On**, the Tungsten lamp is automatically turned on right after power-up of the detector.
⇒*Bandwidth*	2nm	400nm	Channel dependent	Signal bandwidth
⇒*BunchWidth*	0.01nm	100nm	2nm	Bandwidth of the 3D wavelength
DemoFileName	n/a	n/a	As configured	File name of the demo file. (May contain %d that will be replaced by the sample number)
⇒*MaxAutoStep*	0.1s	5.1s	0.1x	Maximum signal and spectra data rate if Step = Auto.
MaxWavelength	190nm	950nm	400	Maximum 3D wavelength
MinWavelength	190nm	950nm	190	Minimum 3D wavelength

Property	Min	Max	Default	Description
PeakWidth	0.00, 0.01, 0.02, 0.05, 0.10, 0.20, 0.20, 0.40, 0.85		Read from the detector upon connect	Expected minimum peak width in [min] (The setting affects the Step: see below).
⇒*RefBandwidth*	0;2nm	400nm	Channel dependent	Reference bandwidth (set to 0 if RefWavelength = 0)
⇒*RefWavelength*	0;190nm	950nm	Channel dependent	Reference wavelength (0 means: not used)
SlitWidth	1nm	16nm	4nm	Width of the micro slit (also MWD). A small slit allows using the fine structures of the UV spectrum. A wide slit provides higher sensitivity.
⇒*Step*	0.05s	3.2s	0.4s	Signal and spectra data rate (The setting affects the peak width: see below).
UV_LampRequired	Off, On		Off	If set to **On**: The detector is not ready for operation if the deuterium lamp is off.
Visible_Lamp	Off, On		Off, (On in demo mode)	Tungsten lamp state
Visible_LampRequired	Off, On		Off	If set to **On**: The detector is not ready for operation if the Tungsten lamp is off.
⇒*Wavelength*	190nm	950nm	Channel dependent	Signal wavelength

For the PDA/MWD, the relation between the expected peak width and the corresponding step is as follows:

Expected peak width [min]	0.00	0.01	0.02	0.05	0.10	0.20	0.40	0.85
Corresponding step [s]	0.05	0.05	0.10	0.20	0.40	0.80	1.60	3.20

For the VWD, the relation is as follows:

Expected peak width [min]	0.000	0.005	0.010	0.025	0.050	0.100	0.200	0.400
Corresponding step [s]	0.073	0.073	0.073	0.073	0.146	0.291	0.582	1.165

Name	Possible States	Description
DeviceState	Idle Prepare Reset Startup	Detector state
UVLampState	Off On Ignition	UV lamp state
VISLampState	Off On	VIS lamp state (DAD only)

Property	Min.	Max.	Default	Description
AnalogAttenuation	-10 ($\equiv 2\^-10$ AU)	1 ($\equiv 2$ AU)	0 ($\equiv 1$ AU)	Attenuation of the analog output (signal value that corresponds to the maximum output voltage) Note: logarithmic scale
AnalogOffset	1%	99%	5	Zero offset of the analog output in % of the full output range.
AnalogRange	0.1V	1.0V	1.0V	Voltage range of the analog output (may be set to 0.1 or 1.0V only).

Property	Min	Max	Default	Description
Adjust_Calibration	n/a	n/a	n/a	Adjusts the wavelength calibration using the absolute deviations determined by the test.
D2_Alpha_Deviation_ Absolute *or* D2_Beta_Deviation_ Absolute	-2.000nm	2.000nm	0 in demo mode, else read from module on connect.	Current deviation of D2 alpha or D2 beta line, ignoring current calibration settings. Valid only after the **Test_Calibration** command has been executed.
D2_Alpha_Deviation_ Correction *or* D2_Beta_Deviation_ Correction	-2.000nm	2.000nm	0 in demo mode, else read from module on connect.	Current calibration setting for the D2 alpha or D2 beta line.

Property	Min	Max	Default	Description
D2_Alpha_Deviation_ Current *or* D2_Beta_Deviation_ Current	-2.000nm	2.000nm	0 in demo mode, else read from module on connect.	Current deviation of the D2 alpha or D2 beta line, considering current calibration. Valid only after the **Test_Calibration** command has been executed.
Test_Calibration	n/a	n/a	n/a	Performs a wavelength calibration test using the D2-alpha and D2-beta lines. You need to remove the flow cell before executing this command. In addition, the UV lamp must be on.

Diagnostic Commands and Properties

Command	Description
CellTest	Performs a cell test.
CellTestEvaluation	Evaluates the cell test by averaging the spectra of all test data.
CellTestInfo	Indicates whether the cell test in the next step is performed with or without cell.
DarkCurrentTest	Performs a dark current test.
DarkCurrentTestEvaluation	Evaluates the cark current test by averaging the minimum and maximum values of all selected spectra ranges.
FilterTest	Performs a holmium filter test.
IntensityTest	Performs an intensity test.
IntensityTestEvaluation	Evaluates the intensity test by calculating the minimum and maximum values of the 1024 diodes for all test data.

For more information about the diagnostic functions for the DAD detector, refer to:

Agilent 1100 HPLC System Diagnostics: Dark Current Test

Agilent 1100 HPLC System Diagnostics: CellT est

Agilent 1100 HPLC System Diagnostics: Filter Test

Agilent 1100 HPLC System Diagnostics: Intensity Test

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

Agilent 1100 HPLC System: Overview

Agilent 1100 HPLC System: Installation

Agilent 1100 HPLC System: Fluorescence Detector

In addition to the general commands for the 1100 HPLC system (see **Agilent 1100 HPLC System: General**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Device Properties

Property	Range/Values		Description
	Min.	**Max.**	
AutoActivateLamp	Off	On	If set to **On**, the lamp is automatically turned on right after power-up.
BaselineBehavior	Append, Free, Zero		Determines how the baseline behaves after the wavelength or photomultiplier gain has changed:
			Append: Resets the baseline to the previous position.
			Free: No adjustment, shifts may occur in the baseline position.
			Zero: Sets the baseline to 0 LU (luminescence units).
Dark_Pmt²	0	999999	Last value (in counts) for the dark current of the photomultiplier (read-only). The value should not exceed 10000 counts.
Dark_Reference²	0	999999	Last value (in counts) of the dark current for the reference diode (read-only). The value should not exceed 5000 counts.
DetectionMode³	➤*Fluorescence,* ➤*Phosphorescence*		Specifies the operating mode.
			☞ *Tip:*
			The property can be set to ***Phosporescence*** *only for detectors with a firmware version A.03.6x and higher. In addition,* ***ScanMode*** *must be set to* ***Off***.
Emission_Deviation_ Absolute²	-12.000	-12.000	Correction value for the emission wavelength as established during **Test_Calibration** as the optimum value (read-only).
Emission_Deviation_ Correction²	-12.000	-12.000	Currently used correction value for the emission wavelength (read-only).

Property	Min.	Max.	Description
EmScanDuration	0	99999	Indicates the time in seconds needed to scan an emission spectrum (read-only). (This property can be used only if **ScanMode=Emission**.) *Tip:* *To perform meaningful scans, make sure that this time is less than the width of the smallest peak. A warning appears if the time exceeds one of the peak widths defined in the QNT Method.*
EmScanStepSize	1	20	Specifies the wavelength increments at which the detector scans the emission spectrum. (You can set this property only if **ScanMode = Emission**.)
EmScanWhen	None, Apex, AllInPeak, All, AllWithoutSignals		Determines when the scans of an emission spectrum are taken. (You can set this property only if **ScanMode = Emission**.) For more information, refer to **Scan Modes for the Fluorescence Detector**.
Excitation_Deviation_ Absolute²	-12.000	-12.000	Correction value for the excitation wavelength as established during **Test_Calibration** as the optimum value (read-only).
Excitation_Deviation_ Correction²	-12.000	-12.000	Currently used correction value for the excitation wavelength (read-only).
ExScanDuration	0	99999	Indicates the time in seconds needed to scan an excitation spectrum (read-only). (This property can be used only if **ScanMode=Excitation**.) *Tip:* *To perform meaningful scans, make sure that this time is less than the width of the smallest peak. A warning appears if the time exceeds one of the peak widths defined in the QNT Method.*
ExScanStepSize	1	20	Specifies the wavelength increments at which the detector scans the excitation spectrum. (You can set this property only if **ScanMode = Excitation**.).
ExScanWhen	None, Apex, AllInPeak, All, AllWithoutSignals		Determines when the scans of an emission spectrum are taken. (You can set this property only if **ScanMode = Excitation**.) For more information, refer to **Scan Modes for the Fluorescence Detector**.

Property	Min.	Max.	Description
FitSpectralRange[3]	No	Yes	When set to **Yes**, the spectral ranges have to be set in such a way that there is always a difference of at least 25 nm between the shortest emission wavelength and the longest excitation wavelength. This avoids that emission is disturbed by stray light.
			Tip:
			A warning appears if, in a program, the distance between the longest excitation wavelength and the shortest emission wavelength is less than 25 nm.
HighPowerFlashes	0	99999	Number of lamp flashes at high power (in thousands). **HighPowerFlashes.Value** indicates the current value. Set the **HighPowerFlashes.UpperLimit** to 0 to disable the associated limit test.
LampEnergyReference	Off	On	Set to **On** to compensate for variations in light intensity of the lamp, using a reference diode. This may improve the signal-to-noise ratio.
LampFlashRate	Standard, Economy		Specifies the rate at which the lamp pulses:
			Economy: 74 Hz - prolongs the lamp life.
			Standard: 296 Hz - increases the detector sensitivity.
LampOnOnlyDuringRun	No	Yes	Specifies the lamp state when no analysis is running:
			Yes: Turns the lamp off after each analysis.
			No: The lamp keeps burning after each analysis.
LampRequired	No	Yes	Specifies whether the lamp is required for the next analysis:
			Yes: If the lamp is turned off, the detector is in NotReady state.
			No: The configuration includes several detectors; the fluorescence detector is not required for the next analysis.
LowPowerFlashes	0	99999	Number of lamp flashes at low power (in thousands). **LowPowerFlashes.Value** indicates the current value. Set the **LowPowerFlashes.UpperLimit** to 0 to disable the associated limit test.

Property	Min.	Max.	Description
PeakWidth[3]	<0.005, >0.005, >0.010, >0.025, >0.050, >0.100, >0.200, >0.400		Specifies the detector's response time (in minutes).
PeakWidthPD[3]	<0.005, >0.005, >0.010, >0.025, >0.050, >0.100, >0.200, >0.400		Select from which signal width the peak shall be recognized. This setting plus the Threshold (see below) determine the internal peak recognition of the device firmware. For **EmScanWhen** or **ExScanWhen** = **Apex** or **AllnPeak**, scanning is performed only if a peak is recognized in the first channel.
			Specifies the expected peak width (in minutes) for the internal peak detector.
			👉 *Tip:*
			The property may be set only once and only at retention times <0.000 min.
PhosphorescenceDelay[3]	0.00	5000.00	Specifies the time (in µs) for which the lamp must be on before the detector starts recording data.
			👉 *Tip:*
			*This property can be set only if **DetectionMode** = **Phosphorescence**.*
PhosphorescenceGate[3]	20.00	5000.00	Specifies the time (in µs) during which data is recorded after the lamp was on.
			👉 *Tip:*
			*This property can be set only if **DetectionMode** = **Phosphorescence**.*
PMTGain[3]	0	18	Specifies the photomultiplier gain. (A typical setting is between 10 and 15.) Each step approximately doubles the gain. Use lower values for very high concentrations. Higher values may improve the signal-to-noise ratio.
Polarity	Positive, Negative		Specifies the signal polarity. Set the polarity to the expected signal polarity.
			👉 *Tip:*
			The property may be set only once and only at retention times <0.000 min.
RemainingLifetime	0	100	Remaining lifetime of the lamp (in percent). **RemainingLifetime.Value** indicates the current percentage. Set the **RemainingLifetime.UpperLimit** to 0 to disable the associated limit test.

Property	Min.	Max.	Description
ScanEndEmWavelength	281	900	Specifies the emission wavelength at which the scan should end. (This property is used only if **ScanMode** = Emission.) In addition, the following must be true: **ScanEndEmWavelength >ScanStartEmWavelength** and **>ExWavelength**
ScanEndExWavelength	201	700	Specifies the excitation wavelength at which the scan should end. (This property is used only if **ScanMode** = Excitation.) In addition, the following must be true: **ScanEndExWavelength >ScanStartExWavelength** and **<EmWavelength**
ScanMode[3]	Off, Emission Excitation		Specifies the scan mode. For more information, refer to 🔲 **Scan Modes for the Fluorescence Detector**. 🖝 *Tip:* *Changing the mode takes about 5-10 seconds. During this time, the detector is in NotReady state.*
ScanStartEmWavelength	280	899	Specifies the emission wavelength at which the scan should start. (This property is used only if **ScanMode** = Emission.) In addition, the following must be true: **ScanStartEmWavelength <ScanEndEmWavelength** and **>ExWavelength**
ScanStartExWavelength	200	699	Specifies the excitation wavelength at which the scan should start. (This property is used only if **ScanMode** = Excitation.) In addition, the following must be true: **ScanStartExWavelength <ScanEndExWavelength** and **<EmWavelength**
Threshold	0.0001	1^5	Select from which signal height in LU (Luminescence Units) the peak shall be recognized. This setting plus PeakWidthPD (see above) determine the internal peak recognition of the device firmware. For **EmScanWhen** or **ExScanWhen** = **Apex** or **AllnPeak**, scanning is performed only if a peak is recognized in the first channel. Specifies the height of the smallest expected peak in LU (Luminescence Units). The detector ignores all peaks below this threshold value.

 Tips:

The default setting is that the current values are read from the detector.

*² This property is used only if an internal calibration was successfully performed via the **Test_Calibration** command.*

³ The property may be set only once and only at retention times <0.000 min.

Device Commands

Command	Description
Abort_Calibration	Aborts the detector's internal calibration procedure. *Tip:* *This command can be performed only when the instrument is performing a calibration.*
Adjust_Calibration	After the internal calibration of the detector, you can use this command to update the internal calibration settings, based on the test. That means that the measured **Excitation_Deviation_Absolute** value is assigned to **Excitation_Deviation_Correction** and the measured **Emission_Deviation_Absolute** value to **Emission_Deviation_Correction**. *Tip:* *This command can be used only if an internal calibration was successfully performed via the **Test_Calibration** command.*
DarkCurrentTest	Performs a dark current test. The results of the last dark current test are saved as properties (**Dark_Reference** and **Dark_Pmt**, see above). *Tip:* *This command can be performed only if the detector is idle. In addition, the lamp must be on.*
PmtTest	Calculates an appropriate photomultiplier gain, based on the settings. *Tip:* *This command can be performed only if the detector is idle.*
Scan	Saves the spectrum scan. *Tip:* *This command can be performed only during data acquisition. In addition, verify that **ScanMode** is not set to **Off** and that **ScanWhen** is set to **All**.*
Test_Calibration	Starts the detector's internal calibration procedure and updates the associated properties with the results. *Tip:* *This command can be performed only if the detector is idle. In addition, the lamp must be on.*

Channel Properties

Property	Min.	Max.	Description
EmWavelength	200 to 700 nm, Off, ZeroOrder		Emission wavelength in nm
			Off: No data is recorded on this channel. This improves the signal-to-noise ratio on the other channels because more single values are averaged.
			ZeroOrder: The monochromator grating serves as mirror. The entire emission spectrum is recorded on the channel.
			🖎 *Tip:*
			*The EmWavelength must be longer than all used excitation wavelengths. In addition, it must be identical for all channels in programs with **ScanMode = Excitation**.*
ExWavelength	200 to 700 nm, Off, ZeroOrder		Excitation wavelength in nm
			Off: No data is recorded on this channel. This improves the signal-to-noise ratio on the other channels because more single values are averaged.
			ZeroOrder: The monochromator grating serves as mirror. The entire lamp spectrum is used for excitation. Overlaps with the emission wavelength result in increased stray light and thus, in increased background noise.
			🖎 *Tip:*
			*The ExWavelength must be shorter than all used emission wavelengths. In addition, it must be identical for all channels in programs with **ScanMode = Emission**.*

🖎 *Tip:*

The default setting is that the current values are read from the detector.

For more information, refer to:

Agilent 1100 HPLC System: Scan Modes for the Fluorescence Detector

Agilent 1100 HPLC System: Example Programs for the Fluorescence Detector

Agilent 1100 HPLC System: Tips for Lamp Commands

◾ Agilent 1100 HPLC System: Refractive Index Detector

In addition to the standard detector commands (see **Commands for Controlling Dionex Devices** 🔼 **Dionex Detectors**) and the general commands for the 1100 HPLC system (see 🔼 **Agilent 1100 HPLC System: General**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Command	Description
Autozero	Performs ⇒*Autozero*. When issued manually, the command is performed immediately; otherwise, it is performed at a ➤*Program* time <0.000 min. With a program time of 0.000, the command is performed before the injection program; with a program time >0.000, the command is performed at this program time.

	Range/Values			
Command/Property	Min.	Max.	Default	Description
AnalogAttenuation	488, 976, 1953, 3906, 7812, 15625, 31250, 62500, 125000, 250000, 500000, 1000000 nRIU		Read from the detector upon connect	Attenuation of the analog output (signal value that corresponds to the maximum output voltage)
AnalogOffset	1%	99%	Read from the detector upon connect	Zero offset of the analog output in % of the full output range.
AnalogRange	0.1V	1.0V	Read from the detector upon connect	Voltage range of the analog output (may be set to 0.1 or 1.0V only)
AutoActivateTempCtrl	Off (0), On (1)			Turns temperature control automatically on (or off) when the detector is powered up
AutoRecycle	Off (0), On (1)			Automatic recycling after the analysis. The property can be set once only and only at retention time <0.
DemoFileName	n/a	n/a	As configured	Name of the demo file (Use "%d" as wildcard for the sample number.)

Command/Property	Min.	Max.	Default	Description
EquilibrationTime	0.00	99,999.00	Read from the detector upon connect	Equilibration time [min] after purging The property can be set once only and only at retention time <0.
LampAge	0.0	596523.0	Read periodically from the detector	Time [h] that the lamp has been on since installation (read-only).
Optical Balance	-1.0	1.0	Read periodically from the detector	Optical balancing: (D1-D2)/(D1+D2) with D = value from the related diode (read-only)
PeakWidth	0.000, 0.005, 0.010, 0.025, 0.050, 0.100, 0.200, 0.400		Read from the detector upon connect	Expected minimum peak width in [min] (This setting affects the Step setting, see below.)
Polarity	Positive (0), Negative (1)		Read periodically from the detector	Polarity of the signal output
Purge	Off (0), On (1)		Read periodically from the detector	Purge valve switching: Off = The flow is to the sample cell. On = The flow is to the reference cell.
PurgeTime	0.00	99,999.00	Read from the detector upon connect	Purge time [min] before the analysis The property can be set once only and only at retention time <0.
PurgeValveSince	0.00	99,999.00	Read periodically from the detector	Time since the last switching of the purge valve [min]
RecycleValve	Off (0), On (1)		Read periodically from the detector	Recycle valve switching Off = Flow to waste On = Flow to recycle output
RecycleValveSince	0.00	99.999,00	Read periodically from the detector	Time since the last switching of the recycle valve [min]

Command/Property	Min.	Max.	Default	Description
RefLiquidAge	0.0	596523.0	Read periodically from the detector	Time [h] that the solvent remained in the reference cell (read-only)
RefLiquidAgeLimit	0.0	596523.0		Maximum time [h] that the solvent may remain in the reference cell This property can be set once only and only at retention time <0.
⇒*Step*	0.01s	4.8s	Auto	Rate at which data points are recorded (This setting affects the PeakWidth, see above.)
TempCtrl	Off (0), On (1)			Temperature control for the optical unit
Temperature.Nominal	5°C	55°C		Setpoint temperature for the optical unit (The detector is not able to cool. Therefore, the setpoint temperature should be at least 5°C above the ambient temperature.)
Temperature.Value	5 °C	55°C	Read from the detector upon connect	Current temperature of the optical unit (read-only)

Also, refer to 🔳 **Properties and Restrictions of the Refractive Index Detector**.

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

🔵 **Agilent 1100 HPLC System: Overview**

🔵 **Agilent 1100 HPLC System: Installation**

◾ Agilent 1100 HPLC System: Program Tips

- **Flow ramps** ($\Rightarrow$*Flow* and $\Rightarrow$*%B, %C, %D* commands) cannot be executed at negative **program times** (t < 0) or in ➢*Programs* without an **Inject** command. (The pumps of the 1100 HPLC System do not support this.)

- The $\Rightarrow$*Inject* command and all **sample preparation** commands are only accepted at the time t = 0. The ➢*Autosampler* of the 1100 HPLC System executes these commands as a block during the transition from Prerun to Run following a possibly specified **UV.Autozero** command.

- Interpretation of the **Inject** command **with explicit sample preparation**: If sample preparation commands are specified the position of the required inject command among these commands is irrelevant. The actual injection occurs either explicitly via the **Valve ValvePosition=MainpassOnStart** command or automatically at the end of the sample preparation if the command is not issued. (See the example below.)

- Changes to the **3D field parameter** may result in an automatic $\Rightarrow$*Autozero* for the PDA. During the execution of the autozero (see UV.NotReadyCauses(Prepare) property), no further command must be issued that would result in another autozero. If necessary, use the **Wait UV.Ready** command to achieve this. During the execution of the autozero, no Run must be started. Therefore, the Program Wizard adds a Wait UV.Ready command before the Inject command.

Hints for manual program creation

Programs for the 1100 HPLC System that were developed manually should include a $\Rightarrow$*Wait* command before the **Inject** command for each module:

```
0.000  UV.Autozero

    Wait UV.Ready    and    ColumnComp.Ready    and
    Sampler.Ready and Pump.Ready

  Inject

  3DFIELD.AcqOn

  UV_VIS_1.AcqOn

  UV_VIS_2.AcqOn

  UV_VIS_3.AcqOn
```

```
UV_VIS_4.AcqOn
UV_VIS_5.AcqOn
```

(These commands are automatically inserted if the Program Wizard is used.)

 Note:

Generally, a time should appear at the beginning of a program (pump and detector settings, such as at the time "-1.000") thus defining a time for each command.

Example for a user-defined wash and injection program

```
0.000   Valve ValvePosition=Bypass
        ; Washes from vial 91 to vial 92
        Draw Volume=Maximum, Source=Vial, Vial=91
        Dispense Volume=Maximum, Source=Vial, Vial=92
        ; Draws the sample
        Draw Volume=InjectVolume, Source=SampleVial
        Needle Function=Seat
        Valve ValvePosition=MainpassOnStart
        ; Injection performed
        ; Option: further steps after the injection, e.g., for
        washing:
        ; Valve ValvePosition=Bypass
        ; Washes from vial 91 to vial 92
        ; Draw Volume=Maximum, Source=Vial, Vial=91
        ; Dispense Volume=Maximum, Source=Vial, Vial=92
        ; Valve ValvePosition=Mainpass
        UV.Autozero
        Wait UV.Ready  and  ColumnComp.Ready  and  Sampler.Ready  and
        Pump.Ready
        Inject
```

Also, see **Agilent 1100 HPLC System: Program Example for Derivatization**.

Data acquisition

The detectors of the 1100 HPLC System do not always record data until the program end. Thus, it may happen that data acquisition ends approximately 0.3 min before the AcqOff command so that raw data are not available in Chromeleon until the end of the program. (Note: This corresponds to the ChemStation behavior.)

To avoid this, perform the End command 0.5 (or more) minutes after the AcqOff command:

```
0.000   Inject
        Wait UV.Ready  and  ColumnComp.Ready  and  Sampler.Ready  and
        Pump.Ready
        UV_VIS_1.AcqOn
(...)
7.500 UV_VIS_1.AcqOff
8.000 End
```

Example for a shutdown program

To shut down the 1100 HPLC System, use a shutdown program. For example:

```
0.000 Flow  = 0
       Wait   UV.Ready and ColumnComp.Ready and Sampler.Ready and
              Pump.Ready
       %B   = 100
       UV_Lamp = Off

       UV.Disconnect

       End
```

At the end of each Chromeleon sample, the program must make sure (for technical reasons) that all connected modules of the 1100 HPLC System are ready. If you turn off the detector lamp, the unit is no longer ready for operation. Therefore, the UV_Lamp.Off command can be performed only after the readiness for operation has been ensured. However, if you add a UV.Disconnect command to the program, the sample will be finished as expected.

Note:

*Select **Blank** as sample type to process the PGM File without an Inject command.*

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

Agilent 1100 HPLC System: Overview

Agilent 1100 HPLC System: Installation

■ Agilent 1100 HPLC System: Example for a Derivatization Program

The Agilent 1100 HPLC System independently processes sample preparation programs. Thus, time control by Chromeleon is not supported. To wait during sample preparation, you can only use the **Sampler.Wait Time** command.

```
0.000 UV.Autozero
   Flow =   0.450
   %B =  0.0
       Wait UV.Ready  and  ColumnComp.Ready  and  Sampler.Ready  and
       Pump.Ready

   ;Derivatization program:
   Sampler.Valve  ValvePosition=Bypass
   Draw  Volume=5.0, SyringeSpeed=200, Source=Vial, Vial=11
   Draw  Volume=1.0, SyringeSpeed=200, Source=Vial, Vial=12
   Draw  Volume=0.0, SyringeSpeed=200, Source=Vial, Vial=14
   ;Draws the sample:
   Draw  Volume=1.0, SyringeSpeed=200, Source=SampleVial
   ;Mixes 6 times:
       Mix Volume=8.0, SyringeSpeed=Maximum, Repeat=6, Source=Seat
   Draw  Volume=1.0, SyringeSpeed=200, Source=Vial, Vial=13
   Draw  Volume=0.0, SyringeSpeed=200, Source=Vial, Vial=15
   ;Mixes 3 times:
       Mix Volume=9.0, SyringeSpeed=Maximum, Repeat=3, Source=Seat
   Sampler.Wait   Time=0.5 ; if required
   Sampler.Valve  ValvePosition=MainpassOnStart ;(*)

   ;Wash program:
       ;you can enter additional steps, e.g., to wash the needle.
       These steps are performed only after the injection, i.e.,
       while the sample is running.

   Inject
       ;Executes the commands of the wash and sample preparation
       programs. Injection to the column is performed only after the
       line (*) has been reached.

   Flow =   0.450
   %B =  0
   UV_VIS_1.AcqOn
   UV_VIS_2.AcqOn
```

In a user-defined wash and/or sample preparation program, verify that you have entered all desired steps. After the ⇒*Inject* has been issued, Chromeleon starts processing the single steps to prepare injection. However, the actual injection is performed only when the **Valve ValvePosition=MainpassOnStart** command has been reached.

🖜 *Tip:*

*Keep in mind that you have to enter the **Valve ValvePosition=MainpassOnStart** command as the last step of the sample preparation program.*

Also, refer to 🔳 **Agilent 1100 HPLC System: Program Tips**.

🔳 Agilent 1100 HPLC System: Scan Modes for the Fluorescence Detector

The ➢*Fluorescence* detector supports the following operating modes:

ScanMode = Off: The monochromators are set to a fixed wavelength. In this mode, the detector can acquire the emission signal only for this wavelength. It is possible to change the wavelength setting during a sample run. However, it is not possible to acquire scans. Compared to the other operating modes, data acquisition is performed with the best signal-to-noise ratio.

ScanMode = Excitation/Emission: One monochromator is set to a fixed wavelength; the other monochromator rotates at 4000 rpm. In this way, it is possible to perform measurements at different wavelengths quasi-parallel. You can record data from up to four emission channels. In addition, you can determine whether and when you want to perform spectra scans:

- **ScanWhen = None***:* No spectra scans are recorded. Use this mode if you want to record data from several channels and no spectra.

- **ScanWhen** = **AllInPeak** or **Apex** supports data acquisition from several channels and automatically saves the scans, based on peak detection from the fluorescence detector. The **PeakWidthPD** and **Threshold** parameters determine the peak width and the peak height for this. Scanning is performed only if the device firmware recognizes a peak via these parameters.

- **ScanWhen = All**: Spectra are not saved automatically. Nevertheless, a Scan command is provided. You can use this command at any time to save the spectrum (either by using it in a program or by executing a trigger).

 Note:

*If no spectrum is recorded (**ScanWhen = None**), the signal-to-noise ratio is only slightly lower as for **ScanMode = Off**. If spectra are recorded (**ScanWhen = AllInPeak, Apex, All**), the signal-to-noise ratio is approximately half the ratio as for **ScanMode = Off**.*

Also, refer to **Agilent 1100 HPLC: Program Examples for the Fluorescence Detector**.

For information about the commands for fluorescence detector control, refer to **Agilent 1100 HPLC System: Fluorescence Detector**.

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

 Agilent 1100 HPLC System: Overview

 Agilent 1100 HPLC System: Installation

Agilent 1100 HPLC System: Program Examples for the Fluorescence Detector

1. One channel, no spectra acquisition

Set the operating mode to **ScanMode = Off**.

```
        Fluorescence.PeakWidth =    0.200
        ScanMode =                  Off
        Emission_1.ExWavelength =   335
        Emission_1.EmWavelength =   390
        Emission_1.Step =           Auto
        Emission_1.Average =        On

0.000   Wait Sampler.Ready and Fluorescence.Ready
        Inject
        Emission_1.AcqOn

2.000   Emission_1.AcqOff
        End
```

2. Several channels, no spectra acquisition

Set the operating mode to **ScanMode = Emission** or **Excitation**. In addition, set **EmScanWhen** (and/or **ExScanWhen**) to **None**.

📳 *Note:*

*Set **ExWavelength** (or **EmWavelength**, respectively) to **Off** for all channels for which data acquisition will not be performed. The other wavelength must be identical for all channels.*

```
        Fluorescence.PeakWidth =    0.200
        ScanMode =                  Emission
        EmScanWhen =                None
        PeakWidthPD =               0.200
        Threshold =                 1.000
        Emission_1.ExWavelength =   335
        Emission_1.EmWavelength =   390
        Emission_1.Step =           Auto
        Emission_1.Average =        On
        Emission_2.ExWavelength =   335
        Emission_2.EmWavelength =   450
        Emission_3.ExWavelength =   Off
        Emission_3.EmWavelength =   390
        Emission_4.ExWavelength =   Off
        Emission_4.EmWavelength =   390
0.000   Wait Sampler.Ready and Fluorescence.Ready
        Inject
        Emission_1.AcqOn
        Emission_2.AcqOn

2.000   Emission_1.AcqOff
        Emission_2.AcqOff
        End
```

3. Peak-dependent spectra acquisition

Set the operating mode to **ScanMode = Emission** (or **Excitation**). In addition, set **EmScanWhen** (or **ExScanWhen**, respectively) to **Apex** (only at the peak maximum) or to **AllInPeak** (= all spectra during the peaks).

👉 *Tip:*

*Verify that the settings for **PeakWidthPD** and **Threshold** match the peaks recorded on the first channel (Emission_1).*

```
        Fluorescence.PeakWidth =    0.200
        ScanMode =                  Emission
        EmScanWhen =                Apex
        PeakWidthPD =               0.200
        Threshold =                 1.000
        Emission_1.ExWavelength =   335
        Emission_1.EmWavelength =   390
        Emission_1.Step =           Auto
        Emission_1.Average =        On
        Emission_2.ExWavelength =   Off
        Emission_2.EmWavelength =   390
        Emission_3.ExWavelength =   Off
        Emission_3.EmWavelength =   390
        Emission_4.ExWavelength =   Off
        Emission_4.EmWavelength =   390

0.000   Wait Sampler.Ready and Fluorescence.Ready
        Inject
        Emission_1.AcqOn

2.000   Emission_1.AcqOff
        End
```

4. User-defined spectra acquisition

Set the operating mode to **ScanMode = Emission** (or **Excitation**). In addition, set **EmScanWhen** (or **ExScanWhen**, respectively) to **All**:

```
Trigger DoScan Emission_1.Signal > 10.000
   Scan
EndTrigger

        Fluorescence.PeakWidth =    0.200
        ScanMode =                  Emission
        EmScanWhen =                All
        Emission_1.ExWavelength =   335
        Emission_1.EmWavelength =   390
        Emission_1.Step =           Auto
        Emission_1.Average =        On
        Emission_2.ExWavelength =   340
        Emission_2.EmWavelength =   390
        Emission_2.Step =           Auto
        Emission_2.Average =        On
        Emission_3.ExWavelength =   Off
        Emission_3.EmWavelength =   390
        Emission_4.ExWavelength =   Off
        Emission_4.EmWavelength =   390

0.000   Wait Sampler.Ready and Fluorescence.Ready
        Inject
        Emission_1.AcqOn
        Emission_2.AcqOn
```

```
1.000   Scan

2.000   Emission_1.AcqOff
        Emission_2.AcqOff
        End
```

The DoScan ⇒*Trigger* determines that a fluorescence spectrum is recorded when the signal of the **Emission_1** channel exceeds 10.

Also, refer to 🔳 **Agilent 1100 HPLC System: Scan Modes for the Fluorescence Detector**.

For information about the commands for fluorescence detector control, refer to 🔳 **Agilent 1100 HPLC System: Fluorescence Detector**.

🔳 Agilent 1100 HPLC System Fluorescence Detector: Tips for Lamp Commands

Chromeleon supports several commands for defining the state of the Xenon lamp of the fluorescence detector that is installed in the 1100 HPLC System. Depending on the situation, use one of the first three settings in your PGM Files:

1. No data acquisition

The fluorescence detector is available but data acquisition is not performed for fluorescence data (e.g., only UV data are recorded):

```
LampRequired = No
Lamp = Off
LampOnOnlyDuringRun = No
```

🖙 *Tip:*

If the PGM File contains an Emission_n.AcqOn command, the PGM File or the batch does not pass the Ready Check.

2. The lamp is turned on only if required

The fluorescence detector is used for data acquisition. However, the lamp is turned on only if required.

```
LampRequired = Yes
Lamp = On
LampOnOnlyDuringRun = Yes
```

The lamp is turned off after each sample. However, the instrument remains ready for operation after the analysis. The lamp is automatically turned on when the next sample is started.

👉 *Tip:*

It may be necessary to turn on the lamp (i.e., to issue a Lamp=On command) manually before starting the analysis of the first sample; e.g., if the detector is in NotReady state.

3. The lamp is burning permanently

The fluorescence detector is used for data acquisition. The lamp is burning permanently.

```
LampRequired = Yes
Lamp = On
LampOnOnlyDuringRun = No
```

👉 *Tip:*

This reduces the lamp's life cycle. However, analysis is performed more quickly because the lamp is warmed up only once (at the very beginning); it does not need to warm up before each sample (see 2. above).

4. Not allowed

You should not combine the following commands:

```
LampRequired = Yes
Lamp = Off
LampOnOnlyDuringRun = No
```

With this sequence of commands, the Ready Check would fail. The detector remains in NotReady state.

For more information, refer to:

🔲 **Agilent 1100 HPLC System: Scan Modes for the Fluorescence Detector**

🔲 **Agilent 1100 HPLC System: Example Programs for the Fluorescence Detector**

For information about the commands for fluorescence detector control, refer to 🔲 **Agilent 1100 HPLC System: Fluorescence Detector**.

▣ Agilent 1100 HPLC System: Properties and Restrictions of the Refractive Index Detector

Supported properties

Chromeleon supports the following detector properties:

- Digital data acquisition

- Temperature control of the optical unit

- Flow path control

- EMF (Early Maintenance Feedback) counter providing information about how long liquid is in the reference cell

Restrictions

Chromeleon does not support the following detector features:

- Data acquisition with six additional channels, such as Diode 1 signal, Optical unit temperature, and Polarity

- Editing of the Error method

- Refractive index calibration

- External contacts

- Integrated DAC (digital/analog converter) test

- Integrated test chromatogram

For information about the commands that are available for controlling the detector, refer to ▣ **Agilent 1100 HPLC System: Refractive Index Detector**.

◪ Agilent 1100 HPLC System: Checking the Solvent Liquid Level

For the 1100 HPLC System, you can have Chromeleon check the liquid level for each solvent and determine the system's reaction if a specified minimum volume is not met. The following commands are available (the display ➢*Filter* level determines which commands are displayed):

Command	Min.	Max.	Default	Description
EmptySolventError	Off	On	Off	Determine how the system reacts when the **SolventFilling xx** reaches the value 0:
				On = The pump motor is turned off. (The red LED lights.)
				Off = Deactivates this function.
SolventFillingXX	0.20 l	1000.00 l	n/a	Enter the volume of solvent in [l] originally added to the bottle (XX = A1, A2, B1, or B2).
SolventNotReadyLimit	0.20 l	1000.00 l	Off	Determine the minimum liquid level. If the actual solvent volume falls below this value, a warning appears. The current sample is finished before Chormeleon aborts the running batch, because the pump is in the **Not Ready** state. (The yellow LED lights).
				Off = Deactivates this function.

The value entered for the **SolventNotReadyLimit** should be large enough to ensure that the last sample can be completely processed.

☞ *Tip:*

*Pump firmware versions below A.02.32 do not support this check of the solvent liquid level. For these pumps, select the **Disable Solvent Limits Features** option on the **Options** tab page in the Server Configuration program. (For more information about the program, refer to **Software Installation and Communication** 🔁 *The Server Configuration Program in the **Administrator Help** section.)*

The **Administrator Help** section provides general information and installation instructions for the 1100 HPLC system; refer to **Hardware Installation**:

🔁 **Agilent 1100 HPLC System: Overview**

🔁 **Agilent 1100 HPLC System: Installation**

◾ Agilent 1100 HPLC System: Diagnostic Functions

The HP1100.pan control panel supports several diagnostic functions for the Agilent 1100 HPLC system. Click **Diagnostic Functions** to open the diagnostic panel. To perform a test, click the related button:
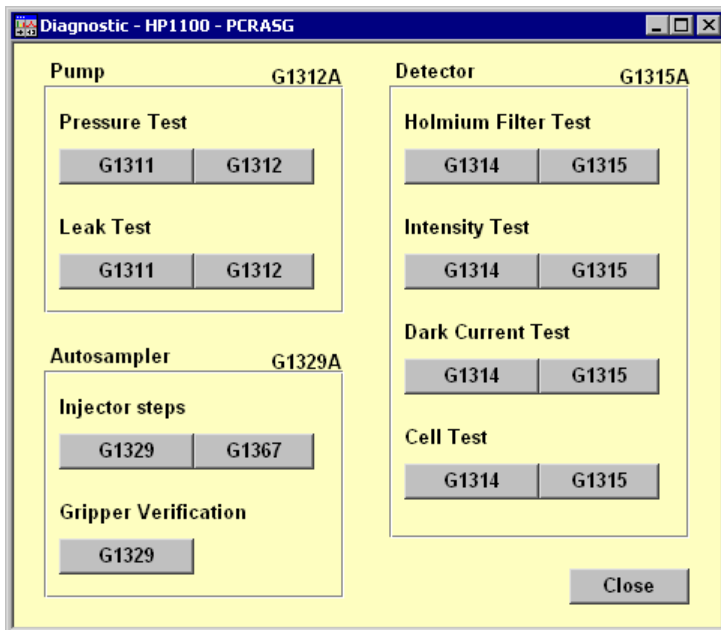
Chromeleon supports the tests listed below.

Pump:

    Pressure Test

    Leak Test

Autosampler:

    Injector Steps

    Gripper Verification

Photodiode Array Detector (DAD):

    Holmium Filter Test

    Intensity Test

    Dark Current Test

    Cell Test

◾ Agilent 1100 HPLC System Diagnostics: Pressure Test

The pressure test allows you to test the pressure resistance of the system up to 400 bar. Chromeleon supports this test for the following pumps: G1312 (binary pump) and G1311 (quaternary pump). To perform the test, click the button for the pump on the main diagnostic panel.

A subpanel is opened:
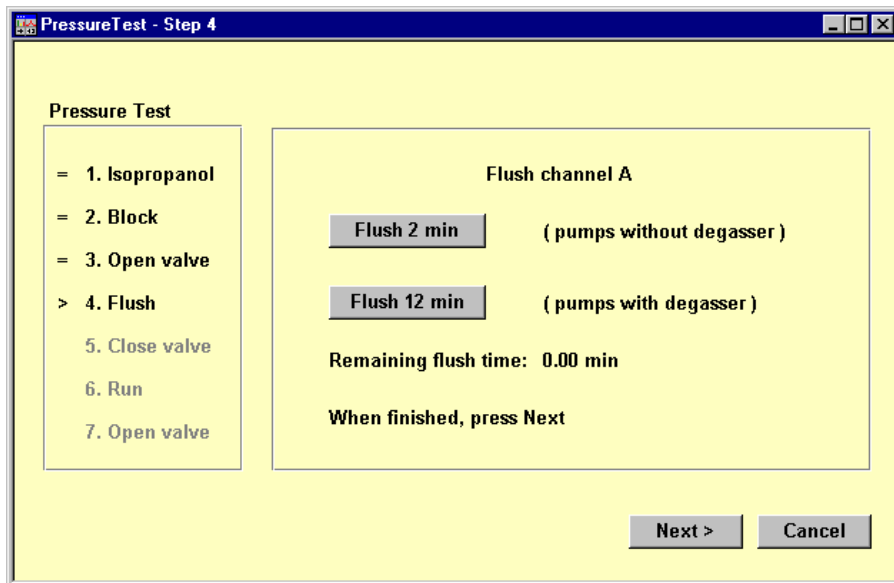
Follow the instructions on the right side of the subpanel. Clicking **Next>** takes you to the next step. For example, the instructions below apply to a G1312 pump with a solvent selector valve.

1. Isopropanol Connect a reservoir containing isopropanol solvent to channel A(2).

2. Block Seal the column compartment outlet with a blank nut.

3. Open valve Open the purge valve.

4. Flush Flush the system with isopropanol from channel A(2). (Pumps without degasser: Flush 2 minutes. Pumps with degasser: Flush 12 minutes.)

5. Close valve Close the purge valve.

6. Run This step starts the pressure test. The pump delivers at a flow rate of 0.51 ml/min until the pressure reaches 390 bar. The test results appear in the Audit Trail on the right.

7. Open valve Open the purge valve and remove the blank nut.

☞ *Tips:*
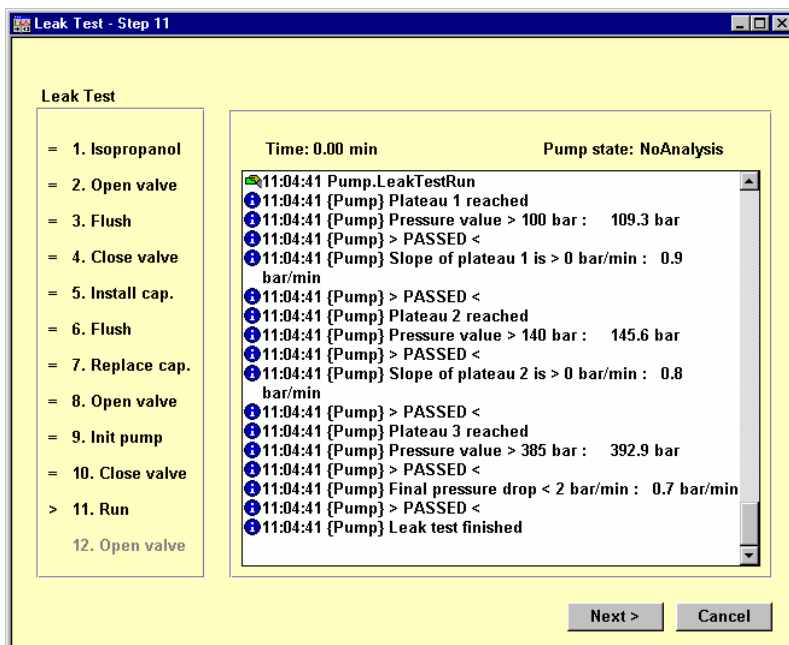
*To close the subpanel, click **Cancel** or **Finish**. This ensures that the pump is in a consistent state afterward.*

For more information, refer to the pump manual.

◾ Agilent 1100 HPLC System Diagnostics: Leak Test

The leak test allows you to check for leaks in the system. Chromeleon supports this test for the following pumps: G1312 (binary pump) and G1311 (quaternary pump). To perform the test, click the button for the pump on the main diagnostic panel.

A subpanel is opened:

Follow the instructions on the right side of the subpanel. Clicking **Next>** takes you to the next step. For example, the instructions below apply to a G1312 pump with a solvent selector valve.

1. Isopropanol   Connect a reservoir containing isopropanol solvent to channels A(2) and B(2).

2. Open valve   Open the purge valve.

3. Flush   Flush the system with isopropanol from channels A(2) (51%) and B(2) (49%). (Pumps without degasser: Flush 2x2 minutes. Pumps with degasser: Flush 2x12 minutes.)

4. Close valve   Close the purge valve.

5. Install cap.   Install the restriction capillary G1313-87305.

6. Flush   Click the button to flush the system with isopropanol from channels A(2) (50%) and B(2) (50%).

7. Replace cap.   Replace the restriction capillary with a blank nut.

8. Open valve   Open the purge valve.

9. Init pump   Initialize the pump.

10. Close valve   Close the purge valve.

11. Run   This step starts the leak test. The LeakTestRun command is slightly different for the binary pump (G1312) and the quaternary pump (G1311). The results appear in the Audit Trail on the right.

12. Open valve   Open the purge valve and remove the blank nut.

The ramps and plateaus in the leak test vary slightly, depending on whether you are testing the binary pump (G1312) or the quaternary pump (G1311). (For a detailed description, refer to the pump manual.)

For the G1311 pump, the leak test is successful if:

a) The pressure reaches a certain value for the plateaus, e.g., 100 bar for the first plateau,

b) There is no increase in pressure on the first two plateaus, and

c) The pressure increase on the third plateau does not exceed 2 bar.

☞ *Tips:*
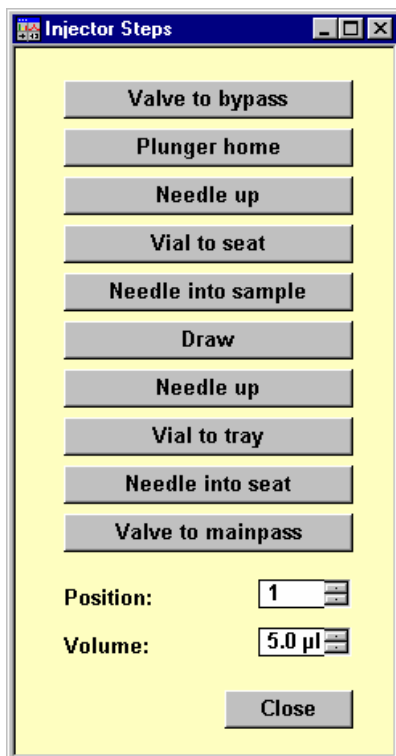
*To close the subpanel, click **Cancel** or **Finish**. This ensures that the pump is in a consistent state afterward.*

For more information, refer to the pump manual.

▣ Agilent 1100 HPLC System Diagnostics: Injector Steps

The injector steps test allows you to check several injector functions. Chromeleon supports this test for the following autosamplers: G1329 and G1367 (wellplate sampler). To perform the test, click the button for the autosampler on the main diagnostic panel.

A subpanel is opened:

First, enter the sample position and the injection volume in the related input fields.

Then, click a button to perform the following operations:

Sampler G1329

Valve to bypass	Switches the injection valve to the bypass position.
Plunger home	Moves the plunger to the home position.
Needle up	Moves the needle arm to its topmost position.
Vial to seat	Positions the specified vial in the seat.
Needle into sample	Lowers the needle into the vial.
Draw	Draws the specified injection volume.
Needle up	Moves the needle arm to its topmost position.
Vial to tray	Returns the vial to the tray.
Needle into seat	Lowers the needle into the needle seat.
Valve to mainpass	Switches the valve to the inject position.

📖 *Tip:*

*For the G1367 Wellplate Sampler, the needle is moved to the specified sample position. Therefore, the **Move to location** button is available instead of the **Vial to seat** and **Needle into sample** buttons.*

Move to location	Moves the needle to the specified position.

📖 *Tip:*

For more information, refer to the autosampler manual.

◪ Agilent 1100 HPLC System Diagnostics: Gripper Verification

The gripper verification test allows you to check the functions of the G1329 autosampler gripper. To perform the test, click the button for the autosampler on the main diagnostic panel.

A subpanel is opened:
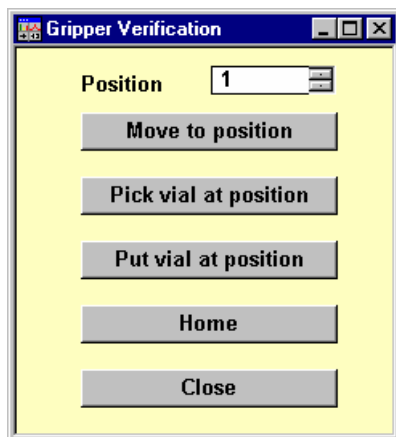
First, enter the sample position in the **Position** field. Then, click a button to perform the following operation:

Move to position	Moves the gripper to the specified position.
Pick vial at position	Picks the vial at the specified position.
Put vial at position	Moves the vial to the specified position.
Home	Returns the gripper to its home position.

☞ *Tip:*

For more information, refer to the autosampler manual.

◪ Agilent 1100 HPLC System Diagnostics: Holmium Filter Test

The filter test allows you to check the holmium oxide filter in the diode array detector (DAD: G1315) or variable wavelength detector (VWD: G1314). To perform the test, click the detector button under **Holmium Filter Test** on the main diagnostic panel.

A subpanel is opened. Click **Start Test** to start the test.

During the test, the holmium oxide filter is first installed in the light path and then removed. The test is successful if the measured intensities are within the limits. Click **Eval Test** to view the test results:
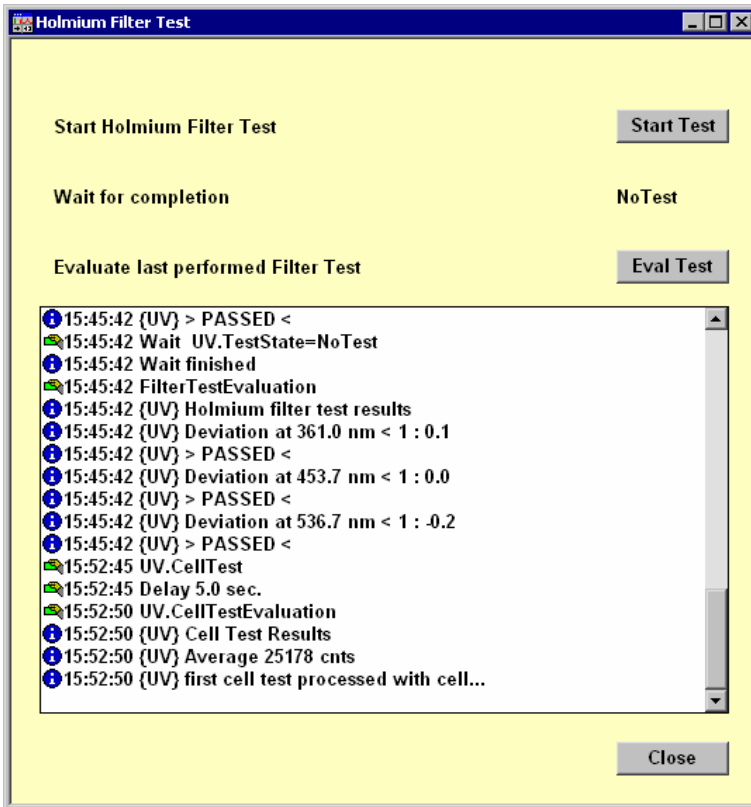
🖝☞ *Tip:*

For more information, refer to the detector manual.

◻ Agilent 1100 HPLC System Diagnostics: Intensity Test

The intensity test allows you to check the intensity of the diode array detector (DAD: G1315) or variable wavelength detector (VWD: G1314). To perform the test, click the detector button under **Intensity Test** on the main diagnostic panel.

A subpanel is opened:
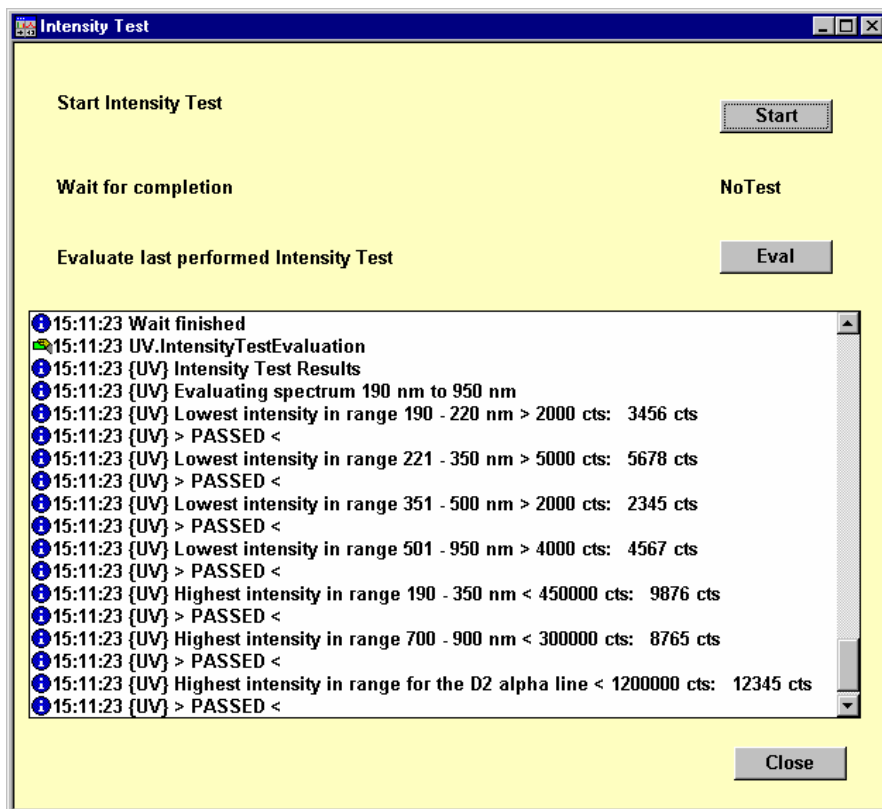
Click **Start Test** to start the test. Click **Eval Test** to view the test results. The test is successful if the measured intensities are within the limits.

☞ *Tip:*　　　　　*For more information, refer to the detector manual.*

Agilent 1100 HPLC System Diagnostics: Dark Current Test

The dark current test allows you to check the dark current of the diode array detector (DAD: G1315) or variable wavelength detector (VWD: G1314). To perform the test, click the detector button under **Dark Current Test** on the main diagnostic panel. A subpanel is opened:
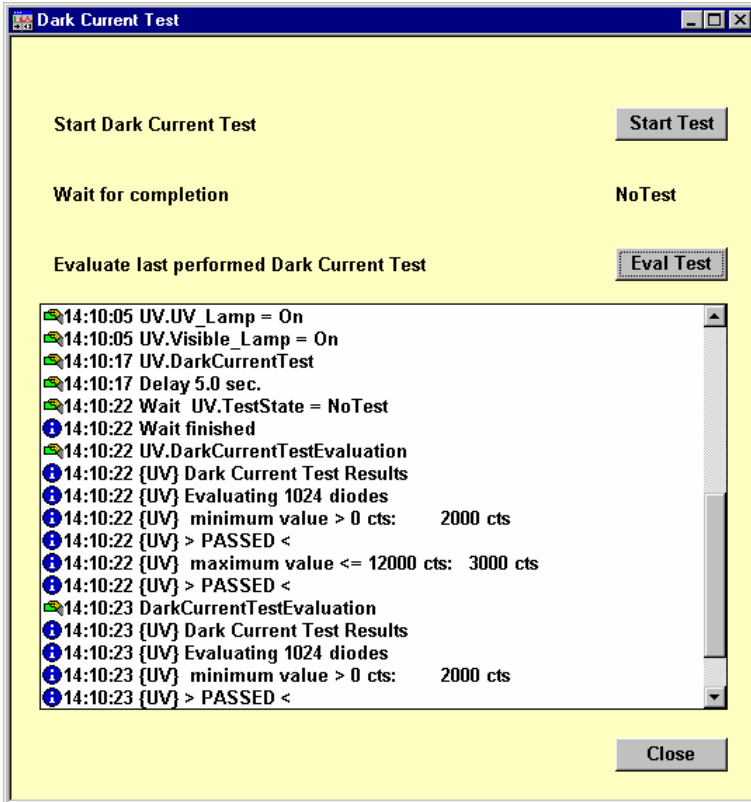
Click **Start Test** to start the test. For the DAD, click **Eval Test** to view the test results. The test is successful when the measured dark current is within the limits. Before resuming operation with the VWD, click **Lamp On** to turn on the lamp again.

Tip: *For more information, refer to the detector manual.*

◼ Agilent 1100 HPLC System Diagnostics: Cell Test

The cell test allows you to check the flow cell of the diode array detector (DAD: G1315) or variable wavelength detector (VWD: G1314) by measuring the light intensity of the deuterium and tungsten lamps over the entire wavelength range (190 - 905 nm). To perform the test, click the detector button under **Cell Test** on the main diagnostic panel.

Diode array detector (DAD)

A subpanel is opened, listing the test steps. Perform the steps in the listed order:

1. If the flow cell has not been installed, install the cell now. Fill the cell with water.

2. Click **Start test with cell** to measure the cell's light intensity.

3. Remove the cell from the light path.

4. Click **Start test without cell** to measure the light intensity without the cell.

The results appear in the Audit Trail section of the panel:
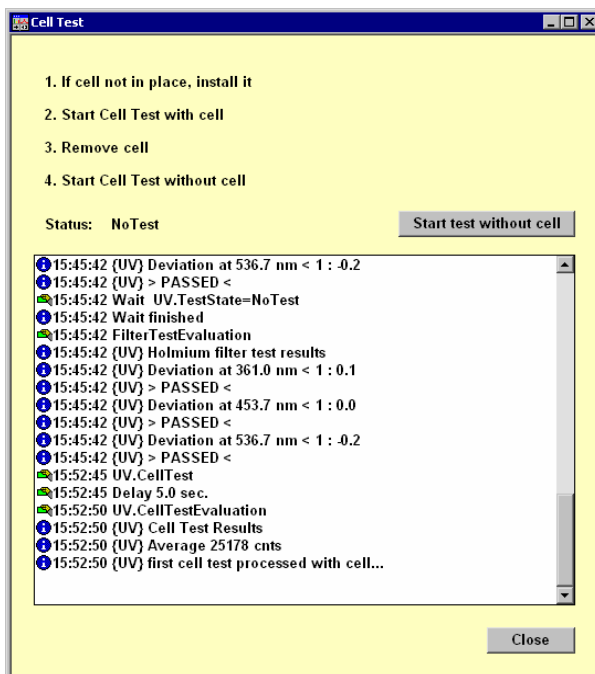
The final result is the ratio of the intensity with and without the cell (here: 25,000/38,000 = 0.66).

Variable wavelength detector (VWD)

Click **Start Test** to start the test. The test result is displayed in the Audit Trail.

📇 *Tip:*   *For more information, refer to the detector manual.*

◪ Agilent 1100 HPLC System: Troubleshooting

- After installation of a device LAN card, the device in which the card is installed may not initialize properly during setup.

  This may occur when each device in your HPLC system has a different firmware version. Perform a firmware update from the ChemStation to make sure that all devices are running the same version of the main firmware.

- The connect commands of the Chromeleon server are not completed successfully.

  See the troubleshooting section of 🔵 **Installing the BootP Server**.

- After a sample run starts, one or more devices in the HPLC system turn off.

  This may occur when you are using a refractive index detector and one or more devices in your HPLC system are running outdated firmware. Perform a firmware update from the ChemStation to make sure that all devices are running firmware version A.05.11 or higher.

- An error occurs if any of the devices in the HP 1100 HPLC system is turned off while it is still connected; for example:

  [Abort] 08:12:04 {UV} Fatal device notification (configuration change)

  Re-start the Chromeleon server.

- If the pump stops delivering and the red LED lights, although no leak or any other obvious hardware problem is apparent, this may be due to the settings selected for checking the solvent liquid level. This also applies if the pump does not return to the **Ready** state after **Motor = On** and the yellow LED remains lighted. For more information about these commands, refer to **Commands and Tips for Third-Party Devices** 🔵 **Agilent 1100 HPLC System: Checking the Solvent Liquid Level**.

- If the **Communication error: LICOP command not acknowledged. (RE0503 command unknown (outdated firmware?))** error message appears after the pump is installed, select the **Disable Sovent Limit Features** check box on the **Options** tab page.

- The following message may appear for detectors:

  [Warning] 15:10:47 1.257 {UV} Raw data delivered by detector has ended prematurely. Finishing acquisition on all channels.

  Detectors in the 1100 HPLC system sometimes do not record data until the end of the program. Data acquisition may end approximately 0.3 minutes before the **AcqOff** command; thus, raw data is not available in Chromeleon up to the end of the program. (Note: This corresponds to the ChemStation behavior.) To avoid this, do not perform the **End** command until at least 0.5 minute after the **AcqOff** command:

```
0.000 Inject
Wait  UV.Ready and ColumnComp.Ready and Sampler.Ready
      and Pump.Ready
      UV_VIS_1.AcqOn
      (…)
7.500 UV_VIS_1.AcqOff
8.000 End
```

- When connecting, the 1100 HPLC system sends different device notifications to the Audit Trail. These notifications are usually for informational purposes; however, they may facilitate troubleshooting in the event of system failure.

- The fluorescence detector may remain in the **Not Ready** state after the detector and the lamp are turned on. In this case, the yellow LED on the detector is on and Chromeleon displays the following message: **Initializing excitation monochromator, initializing emission monochromator**. Execute the **Fluorescence.Reset** command to remedy the situation.

- If your system does not reach the **Ready** state, check the **NotReadyCauses** property and take appropriate remedial action.

- Dionex recommends performing blank runs with injection. Specify the position of a vial that contains solvent, and then specify the injection command as follows:

```
Inject Blank=Inject
```

  The sampler is running also, thus improving synchronization of the entire 1100 HPLC system.

◼ Agilent 1050 HPLC System: UV Detector

The following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Device properties and commands

Property	Min.	Max.	Default	Description
Lamp	Off	On	Read from the device on connect (On in ➤*Demo Mode*).	Lamp state.
ModelNo	"HP1050VWD"			Type description (read-only)
PeakWidth	1.600 min 1.060 min 0.530 min 0.270 min 0.130 min 0.053 min 0.026 min 0.013 min		Read from the device on connect, (0.530 min in Demo mode).	Expected minimum peak width (This setting influences the **Response** property, see below.)
Response	0.25 s 1.00 s 4.00 s		Set to the corresponding value on connect, based on the PeakWidth setting (4.0 s in Demo mode).	Parameter for time-related data filtering. Use low values for narrow peaks. Use higher values to reduce the noise.

Channel properties and commands

Command/Property	Min.	Max.	Default	Description
⇒*Delta*	$-2*10^4$	$2*10^4$	Not available.	Signal slope [mAU/s].
⇒*MaxAutoStep*	0.1	5.1	0.1	Maximum data rate [s] of signals and spectra when Step = Auto
Retention	0.000	10^7	Not available.	Retention time [min].
Signal	-10^4	10^4	Read from the device during data acquisition.	Signal value [mAU].
⇒*Step*	0.05	3.2	0.4	Data rate [s] for signal and spectra.
⇒*Wavelength*	190	600	Read from the device on connect (254 in Demo mode).	Signal wavelength [nm].

The **Administrator Help** section provides general information and installation instructions for the 1050 HPLC system; refer to **Hardware Installation**:

Agilent HP 1050 HPLC System: Overview

Agilent HP 1050 HPLC System: Installation

Agilent 6890/6850 GCs: Commands and Tips

For information about the special commands supported for the Agilent 6890/6850 GCs and for tips for practical operation, refer to:

General

Injector

Inlet (General)

Inlet (Purged Packed EPC, Cool On-Column EPC, ACI, PCM, Volatiles)

Inlet (Split/Splitless EPC, Gerstel PTV, PTV, CIS3, CSI4)

Column

Detectors

ECD

FID and FPD

NPD (Nitrogen Phosphorous Detector)

TCD (Thermal Conductivity Detector)

µ-ECD

Auxiliary Devices

Application

PGM File - Entering Pressure or Flow?

▣ Agilent 6890/6850 GCs: General

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
Ambient	0°C	450°C in increments of 1.	² (Demo mode: 24°C)	Temperature in the laboratory. (The property is available only if Cryo is selected on the Options configuration tab page.)
Cryo	Off	On	² (Demo mode: Off)	If set to On, the cryogenic valve operates automatically. If set to Off, the valve is disabled. (The property is available only if Cryo is selected on the Options configuration tab page.)
CryoFault	Off	On	Off	The oven shuts down if it does not reach the set point temperature after the time specified for cryo operation. (The property is available only if Cryo is selected on the Options configuration tab page.)
CryoTimeout	0 min	999.99 min in 0.01 increments	² (Demo mode: 0.00 min)	Indicates the time that the GC waits before turning off the cooling if no sample has been injected.
CryoTimeoutOn	Off	On	² (Demo mode: On)	Turns on CryoTimeout on or off. (The property is available only if Cryo is selected on the Options configuration tab page.)

Property	Min.	Max.	Default	Description
DualOperation	FrontOnly, BackOnly Shared			Enables the dual-inject mode when set to **Shared**. In addition, **Sharable** must be set to **On** for the single samples (see **Practical Tips for Device Control/GC and Temperature** **Injecting Two GC Samples Simultaneously (6890)**).
EquilibrationTime	0 min	999.99 min in 0.01 increments	² (Demo mode: 0.5)	After reaching the target temperature, the GC waits for the equilibration time. When the equilibration time has passed, the GC is ready.
FirmwareVersion	n/a	n/a	n/a	Firmware version (20 characters; read-only).
ModelNo	n/a	n/a	n/a	Device number, i.e., 6850 or 6890 (20 characters; read-only).
Oven Type	Fast	Regular	Fast	Regular or fast oven type.
PrepRun	-	-	-	Sets the instrument into the PreRun state.
QuickCool	Off	On	² (Demo mode: Off)	If set to "On", the oven is cooled faster before the next sample is injected. (The property is available only if Cryo is selected on the Options configuration tab page.)
Ready	Not Ready	Ready	Depends on the Ready state of the instrument. Demo mode: Ready	Indicates whether the GC is ready for injection (read-only).
ReportReady	-	-	-	Status of the instrument; reports whether the instrument is not ready for operation and why.

Property	Min.	Max.	Default	Description
RunLog	-	-	-	Writes the sample protocol to the Audit Trail.
RunState	Idle, PreRun, Run, PostRun		² (In Demo mode: Idle)	GC state: Idle, PreRun, Run, or PostRun (read-only).
Sharable	Off, On		-	When set to **On**, the related sample can be processed in dual-inject mode (see **DualOperation** above and **Practical Tips for Device Control/GC and Temperature** 🔺 **Injecting Two GC Samples Simultaneously (6890)**).
SerialNo	n/a	n/a	n/a	Serial number (20 characters; read-only).
Temperature	-	-	-	Properties related to the column compartment temperature.
Temperature. Value	Depends on the system and installed cryo type; refer to the column compartment temperature section.		-	Current column compartment temperature (read-only).
Temperature. Nominal	Depends on the system and installed cryo type; refer to the column compartment temperature section. In increments of 1.		² (Demo mode: 80°C)	Determines the nominal temperature of the column compartment.
Temperature. UpperLimit	Depends on the system and installed cryo type; refer to the column compartment temperature section. In increments of 1.		² (Demo mode: highest temperature	The system aborts the sample batch and starts emergency handling when the value is outside the limits.
TempCtrl	Off	On	² (Demo mode: On)	

🗒️ *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

⮕ **Agilent 6890 GC: Overview**

⮕ **Agilent 6890 GC: Installation**

⮕ **Agilent 6850 GC: Overview**

⮕ **Agilent 6850 GC: Installation**

▪ Agilent 6890/6850 GCs: Injector

The following properties are available (please note that the display ➢*Filter* level determines which properties are displayed):

Property	Min.	Max.	Default	Description
6890 GC				
Mode	Normal	on_column	² (Demo mode: on_column).	Injection mode. Must be set to on_column for cooling on the column inlet. Otherwise, set to Normal. Usually, the correct setting is automatically selected in the Server Configuration program. It is not necessary to change this setting.
6850 and 6890 GCs				
Depth	0.0 mm	1.0 mm in increments of 1	0.0 mm	Position of the needle in the vial. The default position is about 3.6 mm from the bottom of the vial. The default position is the reference of all other positions.
Fan	Off	On	² (Demo mode: Off)	Turns the injector fan on and off.
FirmwareVersion	-	-	-	Firmware version (6 characters, read-only)
InjectWaitTime	0.0 s	1 E7 s	0.0 s	Time between the inject command and the inject response.

Property	Min.	Max.	Default	Description
ModelNo	-	-	-	Model number (8 characters, read-only)
Position	1	22 or 27 in increments of 1	1	Current sample position on the tablet.
Post-a	0	15	0	Indicates how often the syringe is rinsed with solvent from the solvent reservoir A after the injection.
Post-b	0	15	0	Indicates how often the syringe is rinsed with solvent from the solvent reservoir B after the injection.
PostDwell	0.00 min	1.00 min in 0.01 increments		Time that the needle remains in the inlet after the injection before it is withdrawn.
Pre-a	0	15	0	Indicates how often the syringe is rinsed with solvent from the solvent reservoir A prior to the injection.
Pre-b	0	15	0	Indicates how often the syringe is rinsed with solvent from the solvent reservoir B prior to the injection.
PreDwell	0.00 min	1.00 min in 0.01 increments	0.00 min	Time that the needle remains in the inlet before the piston moves to inject the sample.
Prewash	0	15	0	Indicates how often the syringe is rinsed with the sample prior to the injection.
Pumps	0	15	0	Indicates the how often the pressure plunger of the syringe is moved up and down while the needle is in the sample. This is to expel gas bubbles and to enhance the reproducibility.
Speed	Fast	Slow	0	Average piston speed[3]

Property	Min.	Max.	Default	Description
State	Off	On	Off	Indicates whether the autosampler has injected (read-only).
Syringe	5 µl, 10 µl, 25 µl, 50 µl, 100 µl.		0	Indicates the syringe type as specified in the Server Configuration program (read-only).
Viscosity	0 s	7 s in increments of 1	0 s	Number of seconds the pressure plunger of the syringe pauses after the pump and injection strokes. This pause allows viscous samples to flow into the vacuum created in the syringe.
Volume	Fixed volume which corresponds to 2%, 10%, 20%, 30%, 40%, or 50% of the syringe volume.		2% of the volume	Specifies the amount of sample to be injected (in µl).
Waste	A-only, B-only, A-and-B		² (Demo mode: Off)	Specifies which waste bottles shall be used (alternating).

Notes:

² *When connecting, Chromeleon reads the value from the instrument and accepts it as default.*

³ *For information about which plunger speed can be achieved with the* **Speed** *command for the different syringes, refer to the table below:*

Syringe (µl)	Plunger Speed (µl/min)	
	Fast	**Slow**
5	3000	150
10	6000	300
25	15000	750
50	30000	1500
100	60000	3000

In addition, the following commands are available, depending on the filter level:

Command	Description
EraseErrorLog	Deletes the sample error log.
StopInject	Stops the running injection.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

Agilent 6890 GC: Overview

Agilent 6890 GC: Installation

Agilent 6850 GC: Overview

Agilent 6850 GC: Installation

Agilent 6890/6850 GCs: Inlet (General)

The following commands and properties are available for all inlets:

Property	Min.	Max.	Default	Description
TempCtrl	Off	On	² (Demo mode: Off)	Specifies the state of the heated zone control: If set to Off, temperature control is disabled. The property is automatically set to On if an assignment is made in the Temperature field.
Temperature	-	-	-	Properties related to the temperature of the heated zone.
Temperature. Nominal	°C in increments of 1. Depends on the inlet type (see Inlets temperature limits).		² (Demo mode: 250°C)	Specifies the target operating temperature for the heated zone.
Temperature. Value	Depends on the inlet type (see Inlets temperature limits)		-	Current temperature of the heated zone (read-only).

Property	Min.	Max.	Default	Description
Type	-	-	-	Type of the installed inlet:
				6890: Purged Packed EPC, Cool On-Column EPC, Split/Splitless EPC, <Other PTV>, Purged Packed, Cool on-Column, Split/Splitless, Unknown/Other, None, ACI, Gerstel PTV, <manual ACI>, PTV, PCM, Gerstel CSI3, <JIB>, Volatiles
				6850: Purged Packed EPC, Split/Splitless EPC, Purged Packed, Split/Splitless, None.
				(33 characters, read-only)

🗔 *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

Additional commands and properties are available, depending on the inlet type:

🔲 **Inlet (Purged Packed (EPC), Cool On-Column (EPC), ACI, PCM, Volatiles)**

🔲 **Inlet (Split/Splitless (EPC), Gerstel PTV, PTV, CIS3, CSI4)**

Also, refer to:

🔲 **Agilent 6890/6850 GCs: PGM File - Entering Pressure or Flow?**

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

🔵 **Agilent 6890 GC: Overview**

🔵 **Agilent 6890 GC: Installation**

🔵 **Agilent 6850 GC: Overview**

🔵 **Agilent 6850 GC: Installation**

◲ Agilent 6890/6850 GCs: Inlet (Purged Packed (EPC), Cool On-Column (EPC), ACI, PCM, Volatiles)

In addition to the general commands described in ⬛ **Inlet (General)**, the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
Flow	-	-	-	Properties related to the gas flow.
Flow.Nominal	0.0 ml/min	1000.0 ml/min in 0.1 increments	² (Demo mode: 80 ml/min)	Specifies the target gas flow.
Flow.Value	0.0 ml/min	1000.0 ml/min	-	Measured gas flow (read-only).
FlowEquilibration Time	0.00 min	999.99 min in 0.01 increments	0.00 min	After reaching the target gas flow, the GC waits the time specified under FlowEquilibrationTime before signaling Ready.
GasType	Nitrogen, Hydrogen, Helium, Argon Methane, Unknown		² (Demo mode: Nitrogen)	Gas type.
Mode (only Cool On-Column)	RampedTemperature TrackOven		²	Operating mode³ RampedTemperature for temperature ramps or constant temperature TrackOven, i.e., the inlet temperature is automatically set to the oven temperature.
Pressure	-		-	Properties related to the gas pressure
Pressure. Nominal	0.00 bar	10.00 bar in 0.01 increments	² (Demo mode: 1.30 bar)	Specifies the target gas pressure.
Pressure.Value	0.00 bar	10.00 bar	-	Measured gas pressure (read-only).
Pressure EquilibrationTime	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 0.00 min)	After reaching the target gas pressure, the GC waits the time specified under PressureEquilibration Time before signaling Ready.

 Notes:

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

³ When a temperature has been defined, the mode is automatically set to RampedTemperature. It is not possible to set the temperature in TrackOven mode (the inlet temperature is automatically set to the oven temperature).

Also, refer to:

 Agilent 6890/6850 GCs: PGM File - Entering Pressure or Flow?

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

 Agilent 6890 GC: Overview

 Agilent 6890 GC: Installation

 Agilent 6850 GC: Overview

 Agilent 6850 GC: Installation

Agilent 6890/6850 GCs: Inlet (Split/Splitless (EPC), Gerstel PTV, PTV, CIS3, CIS4)

In addition to the general commands described in **Inlet (General)**, the following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
ColumnDefined	Off	On	² (Demo mode: Off)	Indicates whether the column is defined (read-only).
Flow	-	-	-	Properties related to the gas flow.
Flow.Nominal	0.0 ml/min	1000.0 ml/min in 0.1 increments	² (Demo mode: 80.0 ml/min)	Target gas flow.

Property	Min.	Max.	Default	Description
Flow.Value	0.0 ml/min	1000.0 ml/min	-	Measured gas flow (read-only).
FlowEquilibration Time	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 0.00 min)	After reaching the target gas flow, the GC waits the time specified under FlowEquilibrationTime before signaling Ready
GasSaver	Off	On	Off	Turns the gas saver on and off.
GasSaverFlow	0.0 ml/min in 0.1 increments	1000.0ml/min in 0.1 increments	² (Demo mode: 70.0 ml/min)	Reduced flow from the split vent when the gas saver is active. The value must be at least 15 ml/min higher than the value for the column flow.
GasSaverTime	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 2.00 min)	Retention time when the gas saver shall become active. The time setting must be after the injection and purge times.
GasType	Nitrogen, Hydrogen, Helium, Argon Methane, Unknown		² (Demo mode: Off)	Gas type.
Mode	Split, Splitless) PulsedSplitless, PulsedSplit, SolventVent/Vent		² (Demo mode: Split)	Operating mode (split or splitless, with or without pulse).
Pressure	-	-	-	Properties related to the gas pressure.
Pressure. Nominal	0.00 bar	10.00 bar in 0.01 increments	² (Demo mode: 1.30 ml/min)	Target gas pressure.
Pressure.Value	0.00 bar	10.00 bar	-	Measure gas pressure (read-only).
Pressure EquilibrationTime	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 0.00 min)	After reaching the target gas pressure, the GC waits the time specified under PressureEquilibration Time before signaling Ready.

Property	Min.	Max.	Default	Description
PulsePressure	0.00 bar	10.00 bar in 0.01 increments	² (Demo mode: 2.00 bar)	(Only in Pulsed mode) Inlet pressure during injection: The pressure rises to this value at the start of the sample (PrepRun) and remains at this value until the PulseTime is reached.
PulseTime	0.00 min	999.99 in 0.01 increments	² (Demo mode: 1.00 min)	(Only in Pulsed mode) Duration of the pressure pulse.
PurgeFlow	0.0 ml/min	1000.0 ml/min in increments of 1	² (Demo mode: 60 ml/min)	Properties related to the flow from the purge vent.
PurgeTime	0.00 min	999.99 min in increments of 1	² (Demo mode: 0.75 min).	(Only in Splitless mode) Time when the purge valve shall be opened.
SplitRatio	0.1	7500.0 in 0.1 increments	² (Demo mode: 50.0)	Ratio of the split flow to the column flow. (The column flow is set as a column property.) This property is valid in split mode only if the column is defined.
VentEndTime	0.00 min	999.99 min in 0.01 increments.	² (Demo mode: 0.00 min)	(Only valid in SolventVent mode) Time at which solvent venting ends. The time should be greater than the time needed for all injections.
VentFlow	0.0 ml/min	1000.0ml/min in 0.1 increments	² (Demo mode: 0.0 ml/min)	(Only valid in SolventVent mode) Properties related to the flow from the split vent during the vent period (before the VentEndTime). .
VentPressure	0.00 bar	10,00 bar in 0.01 increments	² (Demo mode: 0.00 bar)	Inlet pressure during the vent period. Set the property to 0 for the lowest possible pressure.

Note:

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

Also, refer to:

Agilent 6890/6850 GCs: PGM File - Entering Pressure or Flow?

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

Agilent 6890 GC: Overview

Agilent 6890 GC: Installation

Agilent 6850 GC: Overview

Agilent 6850 GC: Installation

Agilent 6890/6850 GCs: Column

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
Detector	**6890:** Front, Back, MSD, AED, Unknown		**6890:** Front for the front detector	Detector to which the column is connected.
	6850: Front, MSD, AED, Unknown		**6850:** Front	
Diameter	0.0 (Unknown) µm	1000.00 µm in 0.01 increments	250.00	Specifies the inner diameter of the column.
FilmThickness	0.0 (Unknown) µm	1000.00 µm in 0.01 increments	10.00 µm	Specifies the film thickness of the column.
Flow	-	-	-	Properties related to the flow; see **PGM File - Entering Pressure or Flow**.
Flow.Nominal	0.0 ml/min	1000.0 ml/min in increments of 1	Emulation of the ramp	Specifies the target flow value.
Flow.Value	0.0 ml/min	1000.0 ml/min	-	Current flow value (read-only).

Property	Min.	Max.	Default	Description
FlowMode	Pressure-Ctrl	FlowCtrl	PressureCtrl	Defines the pneumatics mode for the pressure or flow profile for the column.
Inlet	**6890:** Front, Back, Aux3, Aux 4, Aux 5, Unknown **6850:** Front, Aux3, Aux 4, Aux 5, Unknown		**6890:** Front for the front detector, Back for the back detector **6850:** Front	Inlet to which the detector is connected.
Length	0.0 (Unknown) mm	1000,00 mm in 0.01 increments	30.00 mm	Specifies the column length.
OutletPressure	0.0 bar	10.0 bar in increments of 1	² (Demo mode: 1.0 bar)	Specifies the pressure at the column end if the column is neither in the vacuum nor under atmospheric pressure, e.g., if the column is directly connected to an ECD.
OutletPressure Correction	Off	On	² (Demo mode: Off)	Set to On if the column end is neither in the vacuum nor under atmospheric pressure. Specify the pressure at the column end as OutletPressure.
Pressure	-	-	-	Properties related to the pressure.
Pressure. Nominal	0.0 bar	10.0 bar in increments of 1	Emulation of the ramp.	Specifies the target pressure value.
Pressure.Value	0.0 bar	10.0 bar	-	Measured pressure value (read-only).
VacuumCorrection	Off	On	² (Demo mode: Off)	Set to On if the column is connected to the vacuum, i.e., if the column is directly connected to a mass spectrometer.
Velocity	-	-	-	Properties related to the average linear gas velocity.
Velocity. Nominal	0 cm/s	1000 cm/s in increments of 1	-	Specifies the desired average target gas velocity. .
Velocity.Value	0 cm/s	1000 cm/s	-	Average linear gas velocity (read-only).

🗔 *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

🔵 **Agilent 6890 GC: Overview**

🔵 **Agilent 6890 GC: Installation**

🔵 **Agilent 6850 GC: Overview**

🔵 **Agilent 6850 GC: Installation**

🔳 **Agilent 6890/6850 GCs: Detectors**

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
Autozero	-	-	-	Chromeleon supports this command for compatibility reasons. You cannot perform the command from the command interface on the GC. Perform the command via the device keypad if necessary.
CombinedFlow)	0.0 ml/min	1000.0 ml/min	-	Combined gas flow (makeup + inlet gas). (For detectors without EPC option, this property is read-only.)
Combined Flow.Nominal	0.0 ml/min	1000.0 ml/min	-	(For detectors with EPC option) Specifies the target combined flow.
Combined Flow.Value	0.0 ml/min	1000.0 ml/min	-	(For detectors with EPC option) Indicates the current combined flow (read-only)
Data_Collection_ Rate	0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0 Hz		² (Demo mode: 1.0 Hz)	The property is available only for instruments with digital data acquisition mode.

Property	Min.	Max.	Default	Description
FastPeaks	Off	On	² (Demo mode: Off)	If set to On, the peak width is 0.004 min (8 Hz bandwidth). Otherwise, the peak width is 0.001 min (1.6 Hz bandwidth).
MakeupFlow	0.0 ml/min	100.0 ml/min	² (Demo mode: 10.0 ml/min)	Properties related to the makeup gas flow (see **Front/Back Detector** tab page in the Server Configuration program).
MakeupFlow. Nominal	0.0 ml/min	100.0 ml/min	² (Demo mode: 10.0 ml/min)	Specifies the target makeup gas flow.
MakeupFlow. Value	0.0 ml/min	100.0 ml/min	-	Current makeup gas flow (read-only).
MakeupFlowCtrl (Detectors with EPC option)	Off	On)	² (Demo mode: On)	Turns the makeup gas flowon and off.
MakeupMode	Make-up	Combined)	² (Demo mode: Makeup)	Determines which flow is kept constant: Make-up or Combined.
				(The property is available only for FID; TCD, ECD, µ-ECD, NPD, and FPD detectors.)
Range	0	13 in increments of 1	² (Demo mode: 0)	Data acquisition range. The range sets an amplication factor. The signal range and the smallest signal step depend on the range. The smaller the range, the smoother the signal.
				Therefore, if a small range is selected, small signals are more precise, however, high peaks may be cut off.
				Analog data acquisition: the range has an effect on the signal amplitude (in mV). The maximum signal value is always 10V (or 1V for some cables).
				Digital data acquisition: Chromeleon compensates the signal value. The signal amplitude (in pA) is independent of the range.

Property	Min.	Max.	Default	Description
TempCtrl	Off	On	² (Demo mode: Off)	Specifies the state of the temperature control. If set to Off, temperature control is disabled. The property is automatically set to On when a temperature value is entered in the Temperature field.
Temperature	-	-	-	Properties related to the temperature of the heated zone.
Temperature. Nominal	0	450°C in increments of 1	² (Demo mode: 300°C)	Specifies the target temperature for the heated zone.
Temperature. Value	0.0°C	450.0°C	-	Actual temperature of the heated zone (read-only).
Type	-	-	-	Installed detector type:
				6890: None, FID (flame ionization), TCD (thermal conductivity), ECD (electron capture), NPD (nitrogen phosphorous), FPD (flame photometric), Other, µ-ECD
				6850: None, FID, TCD
				(read-only)

🗒️ *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as the default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

🔵 **Agilent 6890 GC: Overview**

🔵 **Agilent 6890 GC: Installation**

🔵 **Agilent 6850 GC: Overview**

🔵 **Agilent 6850 GC: Installation**

Agilent 6890/6850 GCs: ECD

The following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
AdjustOffset	Off, On, or Abort		² (Demo mode: On)	Adjusts the reference current until the desired output is reached as defined by the TargetOutput property.
AnodeFlow (Detectors without EPC option)	0.0 ml/min	12.0 ml/min	² (Demo mode: 0.0 ml/min)	Anode gas flow. (In this form, the property is available only for detectors without EPC option, see the Detector configuration tab page. The property is read-only.)
AnodeFlow (Detectors with EPC option)	-	-	-	Properties related to the anode gas flow (The property is available for detectors with EPC option, see the Detector configuration tab page.)
AnodeFlow. Nominal	0.0 ml/min	12.0 ml/min	0.0 ml/min	Specifies the target anode gas flow.
AnodeFlow. Value	0.0 ml/min	12.0 ml/min	-	Current anode gas flow (read-only).
Anode FlowCtrl (Detectors with EPC option)	Off	On	² (Demo mode: On).	Turns the pneumatics on and off. In this form, the property is available for detectors with EPC option, see the **Front/Back Detector** tab page in the Server Configuration program.)
MakeupGasType (Detectors with EPC option)	Nitrogen, Argon Methane, Unknown		² (Demo mode: Nitrogen)	Makeup gas type. (The property is available only for detectors with EPC option, see the Detector configuration tab page.)

Property	Min.	Max.	Default	Description
Pulser	Off	On	² (Demo mode: Off)	Turns the detector electronics on and off.
RefCurrent	0.5 nA	5.0 nA in increments of 1	² (Demo mode: 1.5)	
TargetOutput	20	200 in increments of 1	² (Demo mode: 60)	Specifies the desired detector output for the AdjustOffset procedure.

📝 *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

➡ **Agilent 6890 GC: Overview**

➡ **Agilent 6890 GC: Installation**

➡ **Agilent 6850 GC: Overview**

➡ **Agilent 6850 GC: Installation**

▪ Agilent 6890/6850 GCs: FID and FPD

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
AirFlow (Detectors without EPC option)	0.0 ml/min	800.0 ml/min	² (Demo mode: 400.0 ml/min)	Air gas flow. In this form, the property is available only for detectors without EPC option, see the Detector configuration tab page. The property is read-only.)

Property	Min.	Max.	Default	Description
AirFlow (Detectors with EPC option)	-	-	-	Properties related to the air gas flow. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.
AirFlow.Value	0.0 ml/min	800.0 ml/min	-	Current air gas flow (read-only).
AirFlow. Nominal	0.0 ml/min	800.0 ml/min	0.0 ml/min	Specifies the target air gas flow.
AirFlowCtrl (Detectors with EPC option)	Off	On	² (Demo mode: Off)	Turns the pneumatics on and off. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.
Electrometer	Off	On	² (Demo mode: Off)	Turns the detector electronics on and off.
Flame	Off	On	² (Demo mode: Off)	Turns the flame on and off.
H2Flow (Detectors without EPC option)	0.0 ml/min	100.0 ml/min	² (Demo mode: 30 ml/min)	H2 gas flow. In this form, the property is available only for detectors without EPC option, see the Detector configuration tab page. The property is read-only.
H2Flow (Detectors with EPC option)	-	-	-	Properties related to the H2 gas flow. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.
H2Flow. Nominal	0,0 ml/min	100,0 ml/min in in increments of 1	² (Demo mode: 30 ml/min)	Specifies the target H2 gas flow.
H2Flow.Value	0.0 ml/min	100.0 ml/min		Current H2 gas flow.
H2FlowCtrl	Off	On	² (Demo mode: Off)	Turns the pneumatics on and off. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.)

Property	Min.	Max.	Default	Description
LitOffset	0.00 pA	99.9 pA in increments of 1	² (Demo mode: 0.0 pA)	If the detector output is below this level, the GC assumes that the flame is no longer burning and tries to reignite the flame.
MakeupGasType	Nitrogen, Hydrogen, Helium, Argon Methane, Unknown		² (Demo mode: Nitrogen)	Makeup gas type. The property is available only for FID detectors with EPC option, see the Detector configuration tab page.
OxidizerGasType	Oxygen) Air, Unknown			The property is available only for FPD detectors with EPC option, see the Detector configuration tab page.

📝 *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

🔵 **Agilent 6890 GC: Overview**

🔵 **Agilent 6890 GC: Installation**

🔵 **Agilent 6850 GC: Overview**

🔵 **Agilent 6850 GC: Installation**

◘ Agilent 6890/6850 GCs: NPD (Nitrogen Phosphorous Detector)

The following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
AdjustOffset	Off) On) Abort)		² (Demo mode: Off)	Adjusts the reference current until the desired output is reached as defined by the TargetOutput property.
AirFlow (Detectors without EPC option)	0.0 ml/min	200.0 ml/min	² (Demo mode: 60.0 ml/min)	Air gas flow. In this form, the property is available only for detectors without EPC option, see the Detector configuration tab page.)
AirFlow (Detectors with EPC option)	-	-	-	Properties related to the air gas flow. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.)
AirFlow. Nominal	0.0 ml/min	200.0 ml/min in increments of 1	² (Demo mode: 60)	Specifies the target air gas flow.
AirFlow.Value	0.0 ml/min	200.0 ml/min in increments of 1	-	Current air gas flow (read-only).
AirFlowCtrl (Detectors with EPC option)	Off	On	² (Demo mode: On)	Turns the pneumatics on and off. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.
BeadPower	Off	On	² (Demo mode: On)	Turns the bead power on and off.
BeadVoltage	0.000 pA	4.095 pA in 0.001 increments	² (Demo mode: 2.500 pA)	The bead voltage can be set either via AdjustOffset or directly.

Property	Min.	Max.	Default	Description
H2Flow (Detectors without EPC option)	0.0 ml/min	30.0 ml/min	² (Demo mode: 3.0 ml/min)	H2 gas flow. In this form, the property is available only for detectors without EPC option, see the Detector configuration tab page. The property is read-only.
H2Flow (Detectors with EPC option)	-	-	.	Properties related to the H2 gas flow. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.)
H2Flow. Nominal	0.0 ml/min	30.0 ml/min in increments of 1	² (Demo mode: 3.0 ml/min)	Specifies the target H2 gas flow.
H2Flow.Value	0.0 ml/min	30.0 ml/min	-	Current H2 gas flow (read-only).
H2FlowCtrl (Detectors with EPC option)	Off	On	² (Demo mode: On)	Turns the pneumatics on and off. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.
MakeupGasType (Detectors with EPC option)	Nitrogen) Hydrogen, Helium, Argon Methane, Unknown		² (Demo mode: Nitrogen)	Makeup gas type. The property is available only for detectors with EPC option, see the Detector configuration tab page.)
OffsetEquilibration Time	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 5.00 min)	Specifies how long the detector output must be stable before the AdjustOffset procedure is finished.
PolVoltage	Off	On	² (Demo mode: On)	Turns the detector electronics on and off.
RefCurrent	0.5 nA	50 nA in 0.1 increments	² (Demo mode: 1.5 nA)	Specifies the target detector output for the AdjustOffset procedure.
TargetOutput	20	200 in increments of 1	² (Demo mode: 50)	Specifies the target detector output for the AdjustOffset procedure.

📝 *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

🔵 **Agilent 6890 GC: Overview**

🔵 **Agilent 6890 GC: Installation**

🔵 **Agilent 6850 GC: Overview**

🔵 **Agilent 6850 GC: Installation**

▪ Agilent 6890/6850 GCs: TCD (Thermal Conductivity Detector)

The following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
Filament	Off	On	1	Turns the filament on and off.
MakeupGasType (FID Detectors with EPC option)	Nitrogen, Hydrogen, Helium, Argon Methane, Unknown)		² (Demo mode: Nitrogen)	Makeup gas type. In this form, the property is available only for FID detectors with EPC option, see the Detector configuration tab page.
Polarity	Positive	Negative	² (Demo mode: Positive)	Set to Negative to invert negative peaks. Set to Positive for normal peaks.
RefFlow (Detectors without EPC option)	0.0 ml/min	100.0 ml/min	² (Demo mode: 0.0 ml/min)	Reference gas flow. In this form, the property is available only for detectors without EPC option, see the Detector configuration tab page.
RefFlow (Detectors with EPC option)	-	-	-	Properties related to the reference gas flow.

Property	Min.	Max.	Default	Description
Ref.Flow. Nominal	0.0 ml/min	100.0 ml/min in increments of 1	0.0 ml/min	Specifies the target reference gas flow.
RefFlow.Value	0.0 ml/min	100.0 ml/min	-	Current reference gas flow (read-only).
RefFlowCtrl (Detectors with EPC option)	Off	On	² (Demo mode: On)	Turns the pneumatics on and off. In this form, the property is available only for detectors with EPC option, see the Detector configuration tab page.

Note:

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

Agilent 6890 GC: Overview

Agilent 6890 GC: Installation

Agilent 6850 GC: Overview

Agilent 6850 GC: Installation

▪ Agilent 6890/6850 GCs: µ-ECD

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
AdjustOffset	Off, On, Abort		² (Demo mode: Off)	Adjusts the reference current to the desired output as defined by the TargetOutput property.
MakeupGasType (Detectors with EPC option)	Nitrogen, ArgonMethane, Unknown		² (Demo mode: Nitrogen)	Makeup gas type. The property is available only for detectors with EPC option, see the Detector configuration tab page.
Pulser	Off	On	² (Demo mode: On)	Turns the detector electronics on and off.
RefCurrent	0.5 nA	5.0 nA in 0.1 increments	² (Demo mode: 1.5 nA)	Reference current.
TargetOutput	20	200 in increments of 1	² (Demo mode: 60)	Specifies the target detector output for the AdjustOffset procedure.

📝 *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

🔵 **Agilent 6890 GC: Overview**

🔵 **Agilent 6890 GC: Installation**

🔵 **Agilent 6850 GC: Overview**

🔵 **Agilent 6850 GC: Installation**

▣ Agilent 6890/6850 GCs: Auxiliary Devices

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Default	Description
PresCtrl	Off	On	² (Demo mode: Off)	Specifies the state of the pressure control. The property is automatically set to On if an assignment is made in the Pressure field.
Pressure	-	-	-	Properties related to the gas pressure.
Pressure.Nominal	0.00 bar	10.00 bar	Downloaded from the instrument.	Specifies the target gas pressure.
Pressure.Value	0.00 bar	10.00 bar	-	Measured gas pressure (read-only).
Temperature				Properties related to the temperature of the heated zone.
Temperature. Nominal	0.0°C	500.0°C	Downloaded from the instrument.	Target operating temperature for the heated zone.
Temperature. Value	0.0°C	500.0°C	-	Current temperature of the heated zone.
TempCtrl	Off	On	² (Demo mode: Off)	Specifies the state of the heated zone control. If set to Off, temperature control is disabled. The property is automatically set to On if an assignment is made in the Temperature field.

📝 *Note:*

² When connecting, Chromeleon reads the value from the instrument and accepts it as default.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

🔹 **Agilent 6890 GC: Overview**

🔹 **Agilent 6890 GC: Installation**

🔹 **Agilent 6850 GC: Overview**

🔹 **Agilent 6850 GC: Installation**

🔳 Agilent 6890/6850 GCs: Application

Notes on the Device Driver:

General

Experienced 6890/6850 GC users should be able to control the instrument without any problems. In Chromeleon, short descriptions of the GC commands are available in the **Command** dialog box or the **Properties/Link** box.

In Chromeleon, pressure is always stated in bar and the pressure unit cannot be changed. For more information, please contact Dionex Service.

Inlet

The inlet is usually controlled by the use of `Inlet.*` commands. However, the inlet can be connected to the column via the PGM File or via a direct command, e.g., using

```
Column.Inlet =  Front
```

The column commands also control the inlet. In this case, the last command overrides previous commands. For example, with the following command order

```
Inlet.Flow.Nominal =  20.0
Column.Pressure.Nominal =5.00
```

the inlet flow is adjusted according to a column pressure of 5.00 bar, instead of being set to 20.0 ml per minute.

Also, refer to 🔳 Agilent 6890/6850 GCs: PGM File - Entering Pressure or Flow?

Cool On-Column Inlet

It is not possible to set both the temperature and Mode = TrackOven in the same program. If you attempt to do so, the following error message appears:

```
[Error]  {FrontInlet} Can't set Temperature in TrackOven mode. Please
set either a temperature or Mode = TrackOven, but not both.
```

Setting a temperature ramp automatically selects the RampedTemperature mode.

For a **constant temperature**, set Mode = RampedTemperature and enter a temperature value.

Column

It is possible to enter column dimensions and connections, but not the calibration. If column dimensions and connections are not defined, certain commands (Velocity, MakeupMode = Combined, etc.) cannot be executed

It is not advisable to enter the column dimensions and connections in a sample program. However, you should include them in the ➢*Audit Trail*, using the ⇒*Log* command.

Valves

Chromeleon does not support certain valve types (Multiposition, Gas Sampling, etc.). For more information, please contact Dionex Service.

Aux

For the 6850 GC, one auxiliary device can be installed. For the 6890 GC, a maximum of two auxiliary devices for temperature control can be installed.

For both GC types, a maximum of three auxiliary devices for pressure control can be installed.

Sampler

For the 6890 GC, the extended capabilities of the 7683 autosampler, such as solvent prewashes, are supported. Note that these capabilities are not supported by the 7673 autosampler.

When Chromeleon connects to the 6890 GC or 6850 ALS for the first time (see ⇒*Connect*), some (or many) error messages may appear, starting with **Error log at**. These are previous errors recorded by the autosampler and can be ignored.

Barcode Reader (only for the 6890 GC): If **UseBCR = On**, the barcode of the sample is read during the Inject command and is logged in the Audit Trail.

Application

Entering a Temperature Gradient

You can enter a temperature gradient directly, either by selecting **Flow** on the **Control** menu (**GC** tab page) or by including a command in the ➢*PGM File*.

For the 6890 GC, the temperature profile can include a maximum of six ascents or descents. (The 6890 GC cannot store more steps!) The maximum temperature change (ascent) is up to 120°C per minute, depending on the oven type.

Gradients are entered in the Program Wizard or online in the typical format for GC applications. (The starting and end temperatures are entered, as well as the modification rate.) In the program, however, the "base point philosophy" is used (similar to entering a flow or percent gradient in HPLC). Each **temperature** command is a base point of the gradient program. The wizard automatically converts the entered rates into the base point representation.

Temperature gradients can only begin after the Inject command. If a program does not contain an Inject command, this does not apply.

Temperature Setting Commands

Use the following commands to enable or disable temperature control of the oven, the inlet (two inlets for the 6890 GC), and the detector (two detectors for the 6890 GC):

```
0.000     FrontInlet.TempCtrl=On     Or = Off
0.000     BackInlet.TempCtrl=On      Or = Off
0.000     GC.TempCtrl=On             Or = Off
0.000     FrontDetector.TempCtrl=On  Or = Off
0.000     BackDetector.TempCtrl=On   Or = Off
```

Use the following commands to set the temperature of each module:

```
0.000     FrontInlet.Temperature = 80
0.000     BackInlet.Temperature = 80
0.000     GC.Temperature = 80
0.000     FrontDetector.Temperature = 80
0.000     BackDetector.Temperature = 80
```

It may take some time for the instrument to reach the nominal temperature. Please note that the oven heats faster than the injector and the detector system. As soon as the nominal temperature is reached, the GC sends a **Ready** signal. An injection via the autosampler is possible only after this signal.

Example:

The following program waits until the nominal temperature of 150°C is reached before the Inject command is executed:

```
0.000   GC.Temperature = 150
  Wait  GC.Ready
  Inject
......  ..............
```

Tip:

After the instrument has received the nominal temperature value from Chromeleon, it implements the desired value as quickly as possible. When the value is "almost" reached, the instrument waits until the user-defined Equilibration Time has elapsed before sending a confirmation message to Chromeleon.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

Agilent 6890 GC: Overview

Agilent 6890 GC: Installation

Agilent 6850 GC: Overview

Agilent 6850 GC: Installation

◼ Agilent 6890/6850 GCs: PGM File - Entering Pressure or Flow?

Depending on the inlet (or auxiliary device), you have to define pressure or flow in the PGM file. In addition, this depends on the column dimensions. The GC can calculate the flow from the pressure (or vice versa) only if the column diameter, the column length, and the film thickness are known. In all other cases, you have to enter the values required by the inlet or auxiliary device.

Whether the pressure or the flow must be entered also depends on the control mode to be used by the GC for the separation.

The following control modes are available:

- Constant flow

- Constant pressure

- Flow ramp

- Pressure ramp

Known column dimensions

Inlet/AUX	Con. Pressure	Con. Flow	Press. Ramp	Flow Ramp
Purge Packed	Pressure	Flow	Pressure	Flow
Cool On - Column	Pressure	Flow	Pressure	Flow
Split/Splitless	Pressure	Flow	Pressure	Flow
PTV	Pressure	Flow	Pressure	Flow
ACI	Pressure	Flow	Pressure	Flow
Gerstel PTV	-	-	-	-
CIS3	Pressure	Flow	Pressure	Flow
JIB	Pressure	Flow	Pressure	Flow
Volatiles	Pressure	Flow	Pressure	Flow
AUX	Pressure	Flow	Pressure	Flow

Unknown column dimensions

Inlet/AUX	Con. Pressure	Con. Flow	Press. Ramp	Flow Ramp
Purge Packed (with EPC)	-	Flow	-	Flow
Cool On Column (with EPC)	Pressure	Pressure	Pressure	-
Split/Splitless (with EPC)	Pressure	Pressure	Pressure	-
PTV	Pressure	Pressure	Pressure	-

Inlet/AUX	Con. Pressure	Con. Flow	Press. Ramp	Flow Ramp
ACI	-	Flow	-	Flow
Gerstel PTV	-	-	-	-
CIS3	Pressure	Pressure	Pressure	-
JIB	-	Flow	-	-
Volatiles	Pressure	Pressure	Pressure	-
AUX	Pressure	Pressure	Pressure	-

☞ *Tips:*

If you want to work with a constant flow but if you do not know the column dimensions, you have to enter pressure instead of the flow nevertheless, e.g., for Cool-On-Column-Inlet. In this case, you have to determine the pressure by trial and error to achieve the desired flow.

Agilent does not support the applications for which the fields in the above table are empty. Agilent does not support the Purge Packed, Cool on Column and Split/Splitless inlets without ECP when the column dimensions are not known.

You can only operate the PTV inlet in the constant pressure/flow and pressure ramp modes if no column dimensions are defined.

You can only operate the Gerstel PTV without control mode.

The **Administrator Help** section provides general information and installation instructions for the 6890 and 6850 GCs; refer to **Hardware Installation**:

➡ **Agilent 6890 GC: Overview**

➡ **Agilent 6890 GC: Installation**

➡ **Agilent 6850 GC: Overview**

➡ **Agilent 6850 GC: Installation**

◼ Agilent HP 5890 GC: Application

Entering a Temperature Gradient

You can enter a temperature gradient either directly, using the **Flow** command on the **Control** menu (GC tab page) or by a PGM File.

Under Chromeleon, a temperature profile is possible with a maximum of three ascents or descents. (The HP 5890 GC cannot store more steps.) The maximum temperature change (ascent) is 70°C per minute.

When entering a temperature gradient in the ➢*Program*, the "base point philosophy" is used (similar to entering a flow or percent gradient in HPLC). Each **Temperature** command is a base point for the gradient program. Understandably, no gradient is executed before the Inject command. Temperature gradients can only begin after the Inject command. If the program does not contain an Inject command, this does not apply.

Temperature Settings

Use the following commands to enable or disable temperature control of the injector, oven, and detector:

```
0.00      InjectorA.TempCtrl = On      or = Off
0.00      InjectorB.TempCtrl = On      or = Off
0.00      GC.TempCtrl = On             or = Off
0.00      DetectorA.TempCtrl = On      or = Off
0.00      DetectorB.TempCtrl = On      or = Off
```

To set the temperature of the single modules, for example, to 80°C, you have to use the following commands:

```
0.00      InjectorA.Temperature = 80
0.00      InjectorB.Temperature = 80
0.00      GC.Temperature = 80
0.00      DetectorA.Temperature = 80
0.00      DetectorB.Temperature = 80
```

The temperature of the injector system and the detector should always be approximately 15 - 20°C (59 - 68°F) above the current oven temperature. Reaching the nominal temperature on the instrument can take some time. Please note that the oven heats faster than the injector and the detector system. As soon as the temperature is reached, the GC sends a **Ready** signal. An injection via the autosampler is possible only after this signal.

Example:

The following program waits until the nominal temperature of 150°C is reached, before the ⇒*Inject* command is executed:

```
0.000   GC.Temperature = 150
  Wait  GC.Ready
  Inject
......  ..............
```

☞ *Tip:*

*After Chromeleon has communicated the nominal temperature value to the instrument, the instrument implements the desired value as fast as possible. If the value is "almost" reached, the **Equilibration Time** passes until the instrument sends the confirmation message to Chromeleon. You can specify the duration of this time interval using the **Equilibration Time** parameter in Chromeleon.*

The **Administrator Help** section provides general information and installation instructions for the 5890 GC; refer to **Hardware Installation**:

🔵 **Agilent HP5890 GC: Overview**

🔵 **Agilent HP5890 GC: Installation**

▪ Agilent 7683 GC Autosampler

In addition to 🔲 **General Device Commands**, the following commands and properties are available for the **rear** and **front** injectors under **Sampler** (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min.	Max.	Description
Depth	-2,0 mm	30,0 mm	Position of the needle in the vial. The default position is about 3.6 mm from the bottom of the vial. The default position is the reference of all other positions.
DualInject	On	Off	Turns the dual injection on and off. When dual injection is activated, an injection will be performed on both injectors simultaneously.
Mode	normal	On_column	Indicates the injection mode as specified in the installation.

Property	Min.	Max.	Description
Post_a	0	15	Indicates how often the syringe is rinsed with solvent from the solvent reservoir A after the injection.
Post_b	0	15	Indicates how often the syringe is rinsed with solvent from the solvent reservoir B after the injection.
PostDwell	0,00 min	1,00 min	Time that the needle remains in the inlet after the injection before it is withdrawn.
Pre_a	0	15	Indicates how often the syringe is rinsed with solvent from the solvent reservoir A prior to the injection.
Pre_b	0	15	Indicates how often the syringe is rinsed with solvent from the solvent reservoir B prior to the injection.
PreDwell	0,00 min	1,00 min	Time that the needle remains in the inlet before the piston moves to inject the sample.If this value is nonzero, one injector stop of air is drawn (except at maximum injection volume, i.e., five stops).
Present	Yes	No	Indicates whether the device is connected to the data system and under computer control.
Prewash	0	15	Indicates how often the syringe is rinsed with the sample prior to the injection.
Pumps	0	15	Indicates the how often the pressure plunger of the syringe is moved up and down while the needle is in the sample. This is to expel gas bubbles and to enhance the reproducibility.
Speed	Fast	Slow	Average piston speed (only during injection). Slow: approx. 5 µl/s (using a 10µl syringe) and
			Fast: approx. 100 µl/s (using a 10µl syringe).
StopInject			Stops the running injection.
			*Note:*This command has the same effect as pressing the STOP button of the injector.
Syringe	0,5 µl	100,0 µl	Indicates the syringe type as specified in the installation.
			Note: Other values in the given range are accepted.
			Agilent provides syringes in the listed sizes.

Property	Min.	Max.	Description
Syringe *(Cont'd)*			*Tip:*
			The device does not automatically detect a change of syringe volume. If you want to change the syringe volume, enter the desired value in the corresponding field on the panel to ensure that the correct volumes are injected.
Viscosity	0 s	7 s	Number of seconds the pressure plunger of the syringe pauses after the pump and injection strokes. This pause allows viscous samples to flow into the vacuum created in the syringe.

Agilent 7683 Autosampler: Troubleshooting

- Error message:

  {Sampler} Command aborted by sampler: Rear - Turret Error.

  While the turret is still rotating, the gripper moves towards the sample. The autosampler stops and aborts the sequence. The Fault light flashes three times and after a short break flashes three times again and so on. This error may be caused by a remote start/stop cable that connects the autosampler and the GC. In this case, remove the remote cable.

  If the error occurs again, check if the turret is defective. For details, refer to the autosampler's operating instructions.

- Error message:

  {Sampler} Command aborted by sampler: No Bottle in Gripper

  The gripper grips the bottle while the turret is not rotating and places it onto the tray. However, the gripper does not "remember" this process, and thus retries to grip the bottle. After three attempts, the autosampler aborts the sequence. This error may be caused by a remote start/stop cable that connects the autosampler and the GC. In this case, remove the remote cable.

  If the error occurs again, verify that the bottles are in a correct position. For details, refer to the autosampler's operating instructions.

For commands and more information on this autosampler, refer to
 Agilent 7863 Autosampler

CTC Analytics: Commands and Tips

The first topic in this section provides information about the different ⬆ **CTC PAL Sampler Commands**.

For information about the different injection modes, refer to:

⬆ **Tips for Injection**

⬆ **PGM File for LC_Inject Mode**

⬆ **PGM File for Custom Inject Mode**

⬆ **Tips for Headspace Operation**

⬆ **PGM File for HS_Progr Inject Mode**

For information about how to solve possible problems, refer to ⬆ **CTC Analytics PAL: Troubleshooting**

▪ CTC Analytics PAL Sampler Commands

In addition to ⬆ **General Device Commands**, the CTC PAL Samplers support the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Status properties

Property	Value Range	Filter	Description
Busy	Busy/Idle	Expert	Indicates whether the device driver has sent all commands or whether execution of a command is complete (*read-only*).
CPUSerialNo	-	Expert	Indicates the CPU serial number of the connected sampler (*read-only*).
DeviceState	Ready/Busy/ Init(Error)/Unknown	Normal	Indicates the current device status (*read-only*).
StatusDescription	-	Normal	Indicates the current action of the sampler. If no action is being performed, **Idle** is displayed (*read-only*).
Syringe	-	Normal	Indicates the installed syringe type (*read-only*).

Inject properties

Command	Value Range	Filter	Description
AgitationOffTime	0-99 s or **Default***	Normal	Specifies the period during which the agitator is idle.
AgitationOnTime	0-99 s or **Default***	Normal	Specifies the period during which the agitator is active.
AgitationSpeed	Depends on the agitator; indicated in **rpm**. Special values: **Off** and **Default***	Normal	Sets the agitation speed in revolutions per minute and starts the agitator (**Off** deactivates the agitator).
AirVolume	Depends on the volume of the installed syringe; indicated in **µl**. Special value: **Default***	Normal	Specifies the air volume to be drawn for the **Inject** and/or **WashWithSample** commands (in *LC_Inject and GC_Inject* modes).
FillSpeed	Depends on the minimum and maximum speed of the installed syringe; indicated in **µl/s**. Special value: **Default***	Normal	Fill speed for the preparing fill strokes for the **Inject** and/or **WashWithSample** commands.
FillStrokes	0-99 or **Default***	Normal	Number of preparing fill strokes for the **Inject** and/or **WashWithSample** commands (*in all injection modes except **Custom***).
FillVolume	Depends on the syringe volume; indicated in **µl**. Special value: **Default***	Normal	Fill volume for the single piston strokes (*for the **Inject** command in **GC_Inject** mode and for the **WashWithSample** command*).
GCCycleTime	0 - 1440 min	Normal	Specifies the run time for the GC between two injections. In sample programs, this value must be set prior to the **Inject** command
IncubationTime	0 - 1440 min	Normal	Specifies the incubation time for the sample. In sample programs, this value must be set prior to the **Inject** command (*only in **HS_Single** injection mode*).

Command	Value Range	Filter	Description
IncubTimeOffset	0 - 1440 min	Normal	Additional incubation time with respect to the previous sample in a sequence. This value can be set only once per sequence (*only in HS_Progr injection mode*).
IncubTimeStart	0 - 1440 min	Normal	Incubation time for the first sample in a sequence. This value can only be set once per sequence (*only in HS_Progr injection mode*).
InjectMode	LC_Inject Custom (for LC), GC_Inject/HS_Single/HS_Progr/ Custom (for GC)	Advanced	Injection mode. In sample programs, this value must be set prior to the **Inject** command.
Injector	List of all injection objects for the installed PAL	Normal	Injector to be used for the **Inject** and/or **WashWithSample** commands (for all injection modes except *Custom)*. The setting is ignored in **Custom** injection mode.
InjectSpeed	Depends on the min. and max. speed of the installed syringe; Special value: **Default***	Normal	Syringe speed in µl/s for injections to the sample loop/GC inlet (*in LC_Inject, GC_Inject, and HS_Single modes*).
PostInjectDelay	0 - 99000 ms or **Default***	Normal	Time between the injection and the removal of the needle from the injector (*in LC_Inject, GC_Inject, HS_Single, and HS_Progr modes*).
PreInjectDelay	0 - 99000 ms or **Default***	Normal	Time between insertion of the needle into the injector and injection of liquid into the sample loop (GC inlet) (*in LC_Inject, GC_Inject, HS_Single, and HS_Progr modes*).

Command	Value Range	Filter	Description
PreparationTime	0 - 1440 min	Normal	Preparation time. This value is set in **HS_Single** mode at the start time of a new sample (*read-only*). Corresponds to the remaining incubation time of the running sample (marked green), i.e., the time until the sample is injected.
PullupDelay	0 - 10000 ms or **Default***	Normal	Indicates the delay time between drawing the liquid into the syringe and dispensing the volume that is not required (*in GC_Inject mode and for the* **WashWithSample** *command*).
Tray	List of all tray objects of the installed PAL	Normal	Tray to be used for **Inject** (*for all injection modes except **Custom***). Note: If you select a different tray, the **TrayName** property is updated automatically.
TrayName	String	Normal	Indicates the name of the current tray Note: If you select a different tray, the **Tray** property is updated automatically.

* Note:

*For the **Default** parameter, the default value set by CTC on the respective device is used.*

◼ CTC Analytics PAL Samplers: Tips for Injection

CTC PAL samplers support the following injection modes:

PAL Sampler for LC:

Standard HPLC injections -- **LC_Inject** injection mode (see ⬏ **PGM File for LC_Inject Mode**)

User-defined HPLC injections -- **Custom** injection mode (see ⬏ **PGM File for Custom Inject Mode**)

PAL Sampler for GC:

Standard GC injections -- **GC_Inject** injection mode

Headspace injections -- **HS_Single** and **HS_Progr** injection modes (see ⬏ **PGM File for HS_Progr Inject Mode**)

The table below indicates which parameters must be defined for each injection mode.

Step	Parameters for LC_Inject	Parameters for GC_Inject	Parameters for HS_Single and HS_Progr headspace Modes
1. Preparation			IncubationTime *or* IncubStartTime/IncubOffset, GCCycleTime
2. Load	AirVolume	AirVolume	AirVolume = 0
	FillSpeed	FillVolume	FillSpeed
	FillStrokes	FillSpeed	PullupDelay
	PullupDelay	PullupDelay	FillStrokes
		FillStrokes	
3. Injection	PreInjDelay	PreInjDelay	PreInjDelay
	PostInjDelay	PostInjDelay	PostInjDelay
	InjSpeed	InjSpeed	InjSpeed
	Injector	Injector	Injector

When performing an inject command, the LC PAL sampler performs the following steps:

1. The sample needle is moved to the specified vial (in the tray or agitator).

2. The specified sample volume is drawn into the syringe.

3. *Optional:* If the **AirVolume** parameter is set to a value > 0, air is drawn from outside the vial.

4. The sample needle is moved to the specified injector.

5. The sampler waits for the Inject Sync signal. (The Inject Sync signal is a PAL synchronization signal.) Set this signal to **immediately** if no waiting time is required. Alternatively, you can set the signal to the input port of the PAL if the sampler is to wait for a signal from the pump. (For information about the settings, refer to **Installing and Controlling Third-Party Devices** 🔵 **CTC Analytics PAL LC/GC Samplers: Installation** in the **Administrator Help** section.)

6. The needle is moved into the injector.

7. The sampler pauses for the time specified by **PreInjDelay**.

8. The injection valve is moved into the Load position (**LC_Inject** mode only).

9. The content of the syringe is dispensed into the sample loop or the GC inlet.

10. The sampler pauses for the time specified by **PostInjDelay**.

11. The injection valve is moved into the Inject position (**LC_Inject** mode only).

12. The needle is moved out of the injector.

13. The needle returns to the Home position.

Tip:

Steps 4 and 5 are omitted in the GC injection mode. Additional steps are required for headspace injections (refer to Tips for Headspace Operation).

⊡ CTC Analytics PAL Samplers: PGM File for LC_Inject Mode

Use the **LC_Inject** mode for HPLC standard injections. A typical program might look as follows:

```
; Pump/detector commands
    ...
; PAL commands
   TrayName =  Sample.PALTray
; (Prerequisite: User-defined column named PALTray)
   InjectMode =   LC_Inject
   Injector =  LC_Inj1
   InjectSpeed =  50.00
   FillSpeed = 100.00
   FillStrokes =  2
; Preparation steps of the PAL Sampler
   Wash  WashStation=Wash1, WashCycles=1
   WashWithSample    WashCycles=2
; Injection procedure
0.000 Wait  Ready
   Inject
   UV.AcqOn
; Cleansing steps of the PAL Sampler
   Wash  WashStation=Wash1, WashCycles=1
       WashInjector        WashStation=Wash2, Injector=GC_Inj1,
                           WashCycles=1
10.00 UV.AcqOff

   End
```

🖙 *Tip:*

*You can use the **TrayName** command only if the corresponding ➢User-Defined Column (here: **PALTray**) has been defined.*

For more information, refer to:

🔝 **CTC Analytics PAL Sampler Commands**

🔝 **Tips for Injection**

🔝 **PGM File for Custom Inject Mode**

For information about how to solve possible problems, refer to 🔝 **CTC Analytics PAL Samplers: Troubleshooting**.

⊞ CTC Analytics PAL Samplers: PGM File for Custom Inject Mode

The **Custom** injection mode supports special injections, e.g., from different trays with irregularly distributed sample positions and varying injection volumes.

The ⇒*Inject* command only defines the injection. You have to define all sample preparation steps and the injection procedure in the PGM File. The **Inject** command is the last command in the injection procedure.

A typical program for the **Custom** injection mode might look as follows:

```
; Pump/detector commands
   ...
; PAL commands
   InjectMode =   Custom
; Preparation steps of the PAL Sampler
   Wash  WashStation=Wash1, WashCycles=1
      WashWithSample; WashCycles=2
; Injection procedure
0.000  Wait Sampler.Ready
       GET_SAMPLE  Source=Tray01, Index=Sample.Position,
                   Sample_Volume = Sample.Volume
   SWITCH_INJ_Position = Standby
   SWITCH_INJ Injector=LCInj
   PUT_SAMPLE Destination=LCInj, Index=1
   SWITCH_INJ_Position = Active
   SWITCH_INJ Injector=LCInj
   Inject
; (The Inject command completes the injection procedure)
   UV.AcqOn
   . . .
; Cleansing steps of the PAL Sampler
   Wash  WashStation=Wash1, WashCycles=1
      WashInjector        WashStation=Wash2, Injector=GC_Inj1,
                          WashCycles=1
10.00 UV.AcqOff

   End
```

For more information, refer to ⊡ **CTC Analytics PAL Sampler Commands**, ⊡ **Tips for Injection**, and ⊡ **PGM File for LC_Inject Mode**.

For information about how to solve possible problems, refer to ⊡ **CTC Analytics PAL Samplers: Troubleshooting**

◼ CTC Analytics PAL Samplers: Tips for Headspace Operation

Chromeleon supports two headspace injection modes:

HS_Single: Standard headspace injection

HS_Progr: Progressive headspace injection

In **HS_Single** mode, the sampler performs a standard headspace injection, including sample preparation. You must specify an incubation time (**IncubationTime** parameter) for every sample in the sequence.

In **HS_Progr** mode, you do not have to explicitly set the incubation time for samples. Instead, you can increase the incubation time of the previous sample by a certain value (**IncubOffset**). The **IncubTimeStart** parameter determines the start of the incubation time of the first sample in a sequence. Thus, the incubation time for the sample number *n* is calculated as follows:

```
IncubTimeStart  + (n-1) * IncubOffset .
```

👉 *Tip:*

*You are not allowed to change the **IncubOffset** or **IncubTimeStart** parameters in a sequence, i.e., they can be set before the start of the sequence or they must have the same value in each sample program that is used in the sequence.*

In both modes, Chromeleon needs information about the minimum time difference between two sample injections. Use the **GCCycleTime** parameter to specify the time required by the GC after injection until the instrument is ready for the next injection.

👉 *Tip:*

Make sure the cycle time is long enough for completion of all actions by the GC (injection procedure, chromatographic separation, cleaning, warming up of the inlet, etc.) and the sampler (cleaning before and after injection, preparation of new samples, etc.).

The PAL sampler automatically places the vials in the agitator for sample preparation. After the injection procedure, the vials are moved back to their original positions in the tray.

- If you start a new sequence, make sure that no samples were left in the agitator. If necessary, remove all vials from the agitator manually before starting a new sequence.

⚠ *Caution:*

Always run an emergency program to cool down the agitator (or other temperature-controlled components) before removing the samples manually from the agitator.

- If you interrupt a current sequence (**immediately** or **after current sample**) or if an abort error occurs, Chromeleon attempts to remove all remaining samples from the agitator. This attempt may fail in case of a general device error. In this case, remove the vials manually.

⚠ *Caution:*

Always run an emergency program to cool down the agitator (or other temperature-controlled components) before removing the samples manually from the agitator.

- Chromeleon checks whether the specified GC cycle time of a sample exceeds the program end time minus the start time of the next sample. Nevertheless, Dionex recommends allowing for some extra time.

👉 *Tip:*

*The GC may require more time, e.g., to heat up the inlet, column, and/or detector until it has reached the new temperature. In this case, certain commands, such as **Wait** and **Inject**, delay the execution of the program, possibly even beyond the sample incubation time. Therefore, Dionex recommends setting the real time of the GC cycle to **GCCycleTime** and adding 30 to 60 seconds for safety reasons. (You might have to add more time, depending on the GC running conditions; for more information, refer to the GC instruction manual.)*

- Do not use the **TRANSP_VIAL** command to transport vials to or from the agitator while the sequence is being processed in HS_Inject mode. The mechanism for sample preparation is not connected with this command. If you use this command while a sample is being processed, Chromeleon may attempt to place a vial in a position that is already occupied or to fetch a vial from a position where no vial is present.

- In headspace injection mode, you can assign the tray to be used either manually or explicitly in the PGM File via the tray name (e.g., TrayName = Tray1 or Tray = Tray1). If you use the **Tray** and **TrayName** parameters, it is not possible to make assignments via user-defined columns or formulae.

- Actions for the current sample have priority over preparation steps for the next sample. Therefore, sample preparation steps are only performed when the sampler is idle. The injection is performed when the PAL sampler has completed sample preparation. Chromeleon does not place a new sample in the agitator if the remaining preparation time for the running sample (marked green) is less than 30 seconds.

- If you use the headspace mode for injection, all samples must use the same injection mode. It is not possible to change the headspace mode during a sequence or use non-headspace and headspace modes in one sequence.

- The following applies to blank samples (the **Blank** sample type, for which the inject command contains the **Skipped** parameter) and samples without an inject command: Sample preparation is simulated, i.e., the system waits for the incubation time but the sample is not placed in the agitator and no injection is performed.

 Note:

The Audit Trail messages for sample preparation are displayed both in the currently running sample and in the sample that is prepared.

For more information, refer to **PGM File for HS_Progr Inject Mode**.

CTC Analytics PAL Samplers: PGM File for HS_Progr Inject Mode

GC injections are similar to LC injections. If you want to perform headspace injections, you have to define the appropriate sample preparation steps via the **HS_Progr** injection mode. A typical program for headspace injections might look as follows:

```
   Agitator_Temperature.Nominal =  100.0
   Agitator_Temperature.Accuracy = 1.0
   TrayName =  "Tray1"
   Injector =  GC_Inj1
   InjectMode =   HS_Progr
   FillStrokes =  3
; Delay times in [ms]:
   PreInjectDelay =  3000
   PostInjectDelay = 5000
; Incubation time for the first sample of a sequence in [min]
   IncubTimeStart =  2.0
; Additional incubation time of the last sample
   IncubTimeOffset = 1.0
```

```
; Minimum run time between two injections(buffer: 10min + 1.5min)
   GCCycleTime =  700
; Agitation times in [s]
   AgitationOnTime = 20
   AgitationOffTime =   10
   AgitationSpeed =  250

0.000 Wait  Sampler.Ready
   Inject
   UV.AcqOn
; Cleansing steps sampler
   Wash  WashStation=Wash1, WashCycles=1
       WashInjector        WashStation=Wash2, Injector=GC_Inj1,
                           WashCycles=1
10.000   UV.AcqOff

   End
```

Also, refer to **Tips for Headspace Operation**.

CTC Analytics PAL Samplers: Troubleshooting

The following problems may occur during operation of the PAL sampler:

Missing vial:

If no vial is present at the position from which the sampler performs injections, the batch is aborted. Due to a firmware limitation, the sampler may then remain in an inconsistent state.

Therefore, Dionex recommends performing a **Home** command. If the tray in which the error occurred is part of a tray stack, close the stack drawer manually.

Z collision error:

If the syringe holder collides with another component of the sampler while moving in the z direction (e.g., because of an incorrect configuration), the sampler reports a z collision error.

First, find and eliminate the cause of the z collision error. Then, perform a **Home** command to recalibrate the motor in the x, y, and z directions. The driver cannot perform movement commands until the motor has been recalibrated.

Tip:

The sampler is not equipped with collision sensors for the x and y directions. Therefore, stop the batch immediately if you observe a collision in the x or y direction. Afterward, perform the same steps described for a z collision error.

Aborting a sequence or abort error in headspace mode:

Unless a z collision error caused the abort error, the driver attempts to remove all samples that are still in the agitator. However, samples may remain in the (heated) agitator. For security reasons, Dionex recommends generating an emergency program for cooling the agitator (or other temperature-controlled components) down to ambient temperature.

Caution:

Always run the emergency program before manually removing the samples from the agitator.

Gilson: Commands and Tips

For information about the special commands that Chromeleon supports for the different Gilson devices and for tips for practical operation, refer to:

🔼 **Gilson 321 and 322 Pumps**

🔼 **Gilson 331 to 334 Pumps**

🔼 **Gilson 202 Fraction Collector (Ext.)/204 Fraction Collector**

🔼 **Gilson: Injection Modes**

🔼 **Gilson 156 UV/VIS Detector**

■ Gilson 321 and 322 Pumps

In addition to the standard pump commands (see **Commands for Controlling Dionex Devices 🔼 Dionex Pumps**), the following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	(Min/Max) Values	Description
ModelNo	N/A	Indicates the model number of the pump (read-only)
Firmware Version	x.xx	Indicates the pump's firmware version (read-only)
ChartOut (321 only)	Possible values: Pressure, Flow, Percentage %A, %B	Determines the property to be monitored via the digital output (pin no. 9).
Pump Properties		
%A_RefillTime, %B_RefillTime	125 –1000 ms	Determines how long the piston takes to return to its initial state. Enter a higher value in case of cavitation.
%A_LiquidCompr %B_LiquidCompr	0 – 2000 1/Mbar	Determines the solvent compressibility.

Property	(Min/Max) Values	Description
	Prime Commands	
StartPriming	PumpHead parameter (possible values: RightHead, LeftHead)	Starts a priming sequence for the specified pump head. Note: After performing the **StartPriming** command, no other command or property is allowed until the **StopPriming** command was performed.
StopPriming	-	Finishes a running priming sequence.
	Maintenance Counters	
Right_PistonSeal, Left_PistonSeal	0 - 65534 h	Maintenance counter for the right or left piston seal (read-only)
Right_Piston, Left_Piston	0 - 65534 h	Maintenance counter for the right or left piston (read-only)
Right_OutletValve, Left_OutletValve	0 - 65534 h	Maintenance counter for the right or left outlet valve (read-only)
Right_InletValve, Left_InletValve	0 - 65534 h	Maintenance counter for the right or left inlet valve (read-only)
Right_Actuator, Left_Actuator	0 - 65534 h	Maintenance counter for the right or left actuator (read-only)

Further Information

☞ *Tip:*

For more information, for example, about the empirical compressibility correction values for the Gilson 321 and 322 pumps, refer to the pump's User's Guide.

For information about how to install these pumps, refer to **Hardware Installation** 🔵 **Gilson 321 and 322 Pumps**: **Installation** in the **Administrator Help** section.

■ Gilson 331 to 334 Pumps

In addition to the standard pump commands (see **Commands for Controlling Dionex Devices** ■ **Dionex Pumps**), the following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	(Min/Max) Values	Description
ModelNo	N/A	Indicates the model number and firmware version of the master pump (read-only)
ChartOut	Possible values: Pressure, Flow, Percentage %A, %B, %C	Determines the property to be monitored via the digital output (pin no. 9).
Selection of Predefined Solvents		
%A_UseSolvent *(%B_UseSolvent, %C_UseSolvent; only available if a second/third pump is installed)* *The property is visible only if the SVB is enabled for the pump.*	Possible values: Solvent1-Solvent4	Determines which solvent is used when the Solvent Valve Block (SVB) is installed for the pump.
%A_Type, %B_Type, %C_Type *(%B_… and %C_…; only available if a second/third pump is installed)* *If the SVB is enabled for a pump, e.g., A, four properties are available for this pump:* *%A1_Type … %A4_Type*	Possible values: Custom or one of the predefined solvents of the Gilson 331 or 333 pump.	Indicates the type of the used solvent. If you change this property, the ..._K0 and ..._X0 properties (see below) are also changed unless the setting is Custom.
%A_K0, %B_K0, %C_K0 *(%B_… and %C_ …; only available if a second/third pump is installed)* *If the SVB is enabled for a pump, e.g., A, four properties are available for this pump:* *%A1_K0 … %A4_K0*	0 … 2000 1/Mbar	Specifies the empirical compressibility correction K0 for the selected solvent (for the Gilson 331/332 and/or 333/334 pumps, refer to the Reference Information section of the pump's User's Guide). This value can only be changed if the corresponding **Type** property is set to **Custom**. The value is adapted again if the Type property is reset to a value other than **Custom**.

Property	(Min/Max) Values	Description
%A_X0, %B_X0, %C_X0 (%B_… and %C_….; only available if a second/third pump is installed) *If the SVB is enabled for a pump, e.g., A, four properties are available for this pump:* *%A1_X0 … %A4_X0*	0 … 2000 1/Mbar	Specifies the liquid compressibility X0. This value can only be changed if the corresponding **Type** property is set to **Custom**. The value is adapted again if the Type property is reset to a value other than **Custom**.
'Type' *sub-property of the %A (%B, %C) property*	Possible values: Custom or one of the predefined solvents of the Gilson 331 or 333 pump	This type refers to the currently selected solvent of a pump. If you change this property, e.g., for pump %A, the type of the selected solvent is changed as well. For example, if %A_UseSolvent=Solvent2 the %A2_Type is also changed (plus the compressibility, unless the **Type** setting is **Custom**.

<div align="center">

Maintenance Counters

</div>

Property	(Min/Max) Values	Description
%A<x>Count_PistonSeal, *<x>='R', 'L'*	0 - 65535 h	Maintenance counter for the right (R) or left (L) piston seal (read-only)
%A<x>Limit_PistonSeal, *<x>='R', 'L'*	0 - 65535 h	Counter limit for the right (R) or left (L) piston seal (read-only)
%A<x>Count_Piston, *<x>='R', 'L'*	0 - 65535 h	Maintenance counter for the right (R) or left (L) piston (read-only)
%A<x>Limit_Piston, *<x>='R', 'L'*	0 - 65535 h	Counter limit for the right (R) or left (L) piston seal (read-only)
%A<x>Count_OutChkValve, *<x>='R', 'L'*	0 - 65535 h	Maintenance counter for the right (R) or left (L) outlet valve (read-only)
%A<x>Limit_OutChkValve, *<x>='R', 'L'*	0 - 65535 h	Counter limit for the right (R) or left (L) outlet valve (read-only)
%A<x>Count_InlChkValve, *<x>='R', 'L'*	0 - 65535 h	Maintenance counter for the right (R) or left (L) inlet valve (read-only)
%A<x>Limit_InlChkValve, *<x>='R', 'L'*	0 - 65535 h	Counter limit for the right (R) or left (L) outlet valve (read-only)

🗒️ *Note:*

If one or two dependent 332 or 334 pumps (= slave pumps) are installed, more maintenance counters (%B…, %C…) appear.

Further Information

👉 *Tip:*
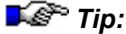
For more information, for example, about the empirical K0 compressibility correction values for these Gilson pumps, refer to the pump's User's Guide.

The **Administrator Help** section provides information about how to install these Gilson pumps. Refer to **Hardware Installation**:

➡ **Gilson 331 and 332 Pumps: Installation**

➡ **Gilson 333 and 334 Pumps**: **Installation**

◼ Gilson 202 Fraction Collector (Ext.)/204 Fraction Collector

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	(Min/Max) Values	Purpose/Remarks
ModelNo	N/A	Indicates the model number (for the Gilson 202 including the firmware version; (read-only).
FirmwareVersion	N/A	Indicates the firmware version
ValvePosition	Possible values: Drain and Collect	Switches the fractionation valve to the Drain or Collect position (The property is available only if a fractionation valve is installed.)
RackType	Possible values: All Gilson 202 rack types + Custom (user-defined setting)	Indicates the used rack type, specified in the Server Configuration program (read-only).
TubePosition	Range: 1 … n. n depends on the rack type and the number of used racks (see Server Configuration program): n = number of racks * number of tubes per line * number of lines	Moves the collect head to the specified position.
MovementMode	Possible values: normal and secure. Default: secure	In Secure mode, the fractionation valve switches to the Drain position before the collect head starts moving and switches back to the Collect position when the tube position has been reached. If the fractionation valve is already in the Drain position, the instrument does not automatically switch to the Collect position. (The property is available only if a fractionation valve is installed.)

Command	Purpose/Remark
Home	Moves the collect head to the Home position (drain outlet)
Next	Moves the collect head to the next tube. If the collect head is positioned at the last tube, it moves to the first one.
Drain	If the rack type is code 10, the collect head is positioned over the central rack hole. For all other rack types, the fractionation valve is switched to the Drain position. (The property is available only if a fractionation valve is installed or if Code 10 is selected as the rack type.)

Further Information

For information about how to install the Gilson 202 fraction collector with the Gilson 202 Fraction Collector (ext.) device driver and the Gilson 204 fraction collector with the Gilson 204 Fraction Collector device driver, refer to **nstallation Instructions** 🔵 **Gilson 202 Fraction Collector (Ext.)/204 Fraction Collector: Installation** in the **Administrator Help** section.

Gilson: Injection Modes

Chromeleon supports the following injection modes:

Mode	Description
Auto:	Default setting. Depending on the injection volume, either the entire loop (**Total Loop**) or a section of it (**Partial Loop**) is filled with the sample.
Total Loop:	The entire injection loop is filled with the sample liquid; the injection volume therefore corresponds to the injection loop.
	If the syringe volume is larger than the loop volume, it is possible to use a higher volume than the loop volume (**IntoLoopFactor** > 1). The volume exceeding the loop volume is used to pre-wash the injection loop with the sample liquid.
	To prevent dilution effects, the volume extracted from the sample reservoir can be larger than the volume injected into the loop (**Sample Factor**>1). The sample liquid remaining in the syringe after the injection (= excessive volume) is then dispensed during the final wash cycle.

$$V_S \leq F_I * V_S \leq F_S * V_S$$

V_S : Sample volume

F_I : IntoLoopFactor

F_S : Sample Factor

Mode	Description
Partial Loop:	Only a part of the injection loop is filled with the sample liquid. The injection volume is therefore smaller than the volume of the injection loop.
	To compensate for the dead volume between the injection port and the injection loop, it is possible to inject a small quantity of solvent after the sample. The volume of this injection can be specified under **Push Volume**. Please note:

$$V_S \leq V_S + V_P \leq V_I$$

V_S : Sample volume

V_P : Push volume

V_I : Injection volume

Centered Loop: (only for the 235 Autosampler)	Only a part of the injection loop is filled with the sample liquid. Solvent is drawn into the injection loop before and after the sample. Contrary to the Partial Loop injection mode, the entire content of the injection loop is injected.
Custom:	With the setting **Custom**, only the injection valve is switched to **Inject**. The user must perform any other steps.

◾ Gilson 156 UV/VIS Detector

In addition to the standard detector commands (see **Commands for Controlling Dionex Devices** 🔲 **Dionex Detectors**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min/Max	Description
ModelNo	N/A	Indicates the model number (read-only).
FirmwareVersion	N/A	Indicates the firmware version (read-only).
VisLampAge		Age of the VIS lamp
VisLampAge.Value	0 .. 9999 [h]	Current value. The allowed input is to reset the value to 0.
VisLampAge.UpperLimit	0 .. 9999 [h]	Upper limit. If the burning hours exceed the upper limit, an error message appears. Enter **0** to disable this check.
UVLampAge		Age of the UV lamp
UVLampAge.Value	0 .. 9999 [h]	Current value. The allowed input is to reset the value to 0.
UVLampAge.UpperLimit	0 .. 9999 [h]	Upper limit. If the burning hours exceed the upper limit, an error message appears. Enter **0** to disable this check.

Property	Min/Max	Description
LampSaver	On / Off	Set to **On** to have Chromeleon automatically turn off a lamp that is not used. This may increase the lamp's lifetime.
Reference	-9999999 .. 9999999	Indicates the current of the reference absorption in µFS (µ full scale; read-only). This value is for control only and not for data acquisition.
LampIntensity	-9999999 .. 9999999	Indicates the measured absorption current in µFS (µ full scale; read-only). This value is for control only and not for data acquisition.
Mode	single / dual	Toggle between single-channel and dual-channel mode.

Channel Characteristics

Property	Min/Max	Description
Wavelength	190 .. 700 [nm]	Wavelength at which the channel records the signal.
Response	**0.0** / 4.0.. 99.0 (single mode) **4.0** .. 99.0 (dual mode)	Filter parameter. Use low values for narrow peaks. Use higher values to reduce the noise.
Range	0.001 .. 2.000	Measuring range in [AU]

Kontron (Bio-Tek): Commands and Tips

For information about the special commands that Chromeleon supports for the different Kontron devices, refer to:

Kontron 465 Autosampler

Kontron SFM 25 Fluorescence Detector

Kontron 465 Autosampler

In addition to the standard autosampler commands (see **Dionex Autosamplers**), Chromeleon supports the following Kontron 465 commands for pre-column derivatization (please note that the display ➤*Filter* level determines which commands and properties are displayed). Also, refer to the information in the manual shipped with the instrument.

Command	Description
StartPosition	Returns to the start position.
GetReagent	The reagent is withdrawn from a pre-defined position.
Mix	Mixes the reagents.
AddReagentAndMix	The reagent is added and mixed with the sample.
AirSegment	Draws an air segment.

Parameter	Description
ReagentPosition	Position of the reagent.
ReagentVolume	Reagent volume to be used.
AirVolume	Air volume to be drawn up after pulling the syringe needle out of the vial.
MixingVolume	Mixing volume.

☞ *Tip:*

*The prerequisite for controlling this instrument is that the **Extended Device Control** feature must licensed on your system.*

For more information, refer to **Hardware Installation** 🌐 **Kontron Autosamplers** in the **Administrator Help** section.

▣ **Kontron SFM25 Fluorescence Detector**

Chromeleon supports the following special commands and parameters for the Kontron SFM25 Fluorescence Detector (please note that the display ➢*Filter* level determines which commands and properties are displayed). Also, refer to the information in the manual shipped with the instrument.

Command	Description
Autoblank	Sets the zero value of the amplifying factor.
Calibrate	Sets the maximum value of the amplifying factor.

Parameter	Description
HighVoltage	Enter the maximum output voltage (100...999V). Pressing **Calibrate** command automatically sets the value of the voltage.
Factor	Enter the multiplying factor (0.01...10.00). Pressing **Autoblank** automatically calculates and stores the value.

For more information, refer to **Hardware Installation** 🔵 **Kontron SFM25 Fluorescence Detector** in the **Administrator Help** section.

Merck Hitachi: Commands and Tips

For information about the special commands that Chromeleon supports for the different Merck Hitachi devices and for tips for practical operation, refer to:

🔺 **LaChrom HPLC System: Tips**

🔺 **Merck Hitachi Pumps: Program Example**

🔺 **Merck Hitachi Autosamplers**

🔺 **Merck Hitachi AS4000: Program Example**

🔲 Merck Hitachi LaChrom HPLC System: Tips

Chromatogram (Detector)

The detector allows negative signals to pass up to 250 mV only. Thus, Chromeleon displays negative signals only up to 250 mV, although the detector displays values up to 500 mV.

Rack Display (Autosampler)

If the rack you selected in the ➢*Server Configuration* is not the one used in the current sequence, the Browser displays the wrong rack.

🔲 Merck Hitachi Pumps: Program Example

To avoid overloading the Merck pumps with too fast a sequence of commands, allow for at least 0.1 min between the individual commands. For a program example for the L6200 pump, see below:

```
-0.100   Pressure.LowerLimit =   0
   Pressure.UpperLimit =   400
   %A.Equate =     "%A"
   %B.Equate =     "%B"
   %C.Equate =     "%C"
;******* Detector commands: ****************
   UV_VIS_1.Mode =   UV
   UV_VIS_1.Range = 0.200
   UV_VIS_1.Response = 1.0
```

```
   UV_VIS_1.Step =   Auto
   UV_VIS_1.Average =   On
   UV_VIS_1.Wavelength =   260


0.000   UV_VIS_1.Autozero
   Flow =   1.000
   %B =  10
   %C =  0
   Inject
   UV_VIS_1.AcqOn
   Flow =   1.000
   %B =  10
   %C =  0


;The gradient starts after 0.1 minutes to avoid overload the pump
with too fast a sequence of commands:
0.100 %B =  10

10.000   %B =  90

15.000   %B =  90

16.000   %B =  10

30.000   UV_VIS_1.AcqOff
   Flow =   1.000
   %B =  10
   %C =  0

   End
```

For an overview of the Merck Hitachi pumps (L6200/L6210 and L6250), refer to **Hardware Installation** 🌐 **Merck Hitachi L6200/L6210 and L6250 Pumps: Overview** in the **Administrator Help** section.

▣ Merck Hitachi Autosamplers

Supported Autosamplers

For ➢*Autosampler…*	…controlled via…	…use device driver
AS2000	RS-232	Merck Hitachi AS2000 Autosampler
AS4000	RS-232	Merck Hitachi AS4000 Autosampler
L7250	RS-232	Merck Hitachi L7250 Autosampler
L7250	D-Line	Merck Hitachi L7200/L7250 AS (D-Line)
L7200	D-Line	Merck Hitachi L7200/L7250 AS (D-Line)

Supported Device Properties

In addition to the standard autosampler commands (see ▣ **Dionex Autosamplers**), the Merck Hitachi autosampler support the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	AS2000	AS4000	L7250	L7200
InjectPortWashs	0-20	0-20	0-20	0-20[3]
InjectPortWashSpeed	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)3
NeedleWashs	1-20	1-20	1-20	1-20[3]
NeedleWashSpeed	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)[3]
WasteVolume	Not supported	Not supported	0-4000.0 µl	0-4000.0 µl[2]
WashVolume	10.0-100.0 µl	10.0-1000.0 µl	10.0-1000.0 µl	
LeadVolume	0-100.0 µl	0-1000.0 µl	0-1000.0 µl	0-500.0 µl[2]
RearVolume	0-100.0 µl	0-1000.0 µl	0-1000.0 µl	0-500.0 µl[2]
FeedVolume	0-100.0 µl	0-1000.0 µl	0-1000.0 µl	0-500.0 µl[2]
DeadVolume	0-100.0 µl	0-1000.0 µl	0-1000.0 µl	
Syringe	0-100.0 µl	0-1000.0 µl	0-1000.0 µl	
InjectionMethod	0 (Compatibility) 1 (Cut) 2 (All)	0 (Compatibil.) 1 (Cut) 2 (All)	0 (Compatibil.) 1 (Cut) 3 (FullLoop)	1 (Cut) ... 3 (FullLoop)[2]
SyringeSpeed	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)	1 (Low) ... 5 (High)	
NeedleDownSpeed	1 (Slow) ... 2 (Fast)	1 (Slow) ... 2 (Fast)	1 (Slow) ... 2 (Fast)	1 (Slow) ... 2 (Fast)
VolSyringe	1 (100µl) - 2 (500 µl)	1 (100 µl) - 5 (5000 µl)	1 (100 µl) - 5 (5000 µl)	Not supported
PumpPlungerWash	Not supported	Not supported	0 (No) or 1 (Yes)	0 (No) or 1 (Yes)[3]

Property	AS2000	AS4000	L7250	L7200
VolOptionalSyringe	Not supported	1 (100 µl) - 5 (5000 µl)	Not supported	Not supported
NumberOfDilutors	Not supported	1 or 2	Not supported	Not supported
Dilutor	Not supported	1 or 2	Not supported	Not supported
RackCode	1 - 16	1 - 16	1 - 16	Not supported
⇒*Volume*	1 - 500.0 µl	1 - 5000.0 µl	1 - 5000.0 µl	1 - 500.0 µl
⇒*Position*	1 - 100	1 - 200	1 - 200	1 - 200

² If one of these properties is set, all other settings that are marked with ² are set, too. Check the parameters sent to the autosampler with extra care.

³ If one of these properties is set, all other settings that are marked with ³ are set, too. Check the parameters sent to the autosampler with extra care.

Supported Device Commands

Command	AS2000	AS4000	L7250	L7200
⇒*Connect (/Disc.)*	Supported	Supported	Supported	Supported
Disconnect	Supported	Supported	Supported	Not supported
Aspirate (see ⇒*Draw*)	0 - 500.0 µl, Speed	Dilutor, 0 - 5000.0 µl, Speed	0 - 5000.0 µl, Speed	Not supported
⇒*Dispense*	0 - 500.0 µl, Speed	Dilutor, 0- 5000.0 µl, Speed	0-5000.0 µl, Speed	Not supported
GotoTube	1 - 100, RackCode	1 - 200, RackCode	1 - 200, RackCode	Not supported
GotoWashPort	Supported	Supported	Supported	Not supported
GotoInjectPort	Supported	Supported	Supported	Not supported
MoveNeedle	X:0-163.0 mm	X:0-244.0 mm	X:0-156.0 mm	Not supported
	Y:0-68.0 mm	Y:0-163.0 mm	Y:0-156.0 mm	
	Z:0-38.0 mm	Z:0-57.0 mm	Z:0-57.0 mm	
Height	Z:0-38.0 mm	Z:0-57.0 mm	Z:0-57.0 mm	Not supported
GotoSyringePos	0 - 500.0 µl, Speed	Dilutor, 0- 5000.0 µl, Speed	0 - 5000.0 µl, Speed	Not supported
GotoSValvePos	0 (Bottle) or 1 (Needle)	Dilutor, 0 (Bottle) or 1 (Needle)	0 (Bottle) or 1 (Needle)	Not supported
GotoValvePos	0 (Inject) or 1 (Load)	0 (Inject) or 1 (Load)	0 (Inject) or 1 (Load)	Not supported
Home	Supported	Supported	Supported	Not supported
Reset	Supported	Supported	Supported	Supported
Wash	Supported	Supported	Supported	Not supported

Command	AS2000	AS4000	L7250	L7200
WashPumpPlunger	Not supported	Not supported	Supported*	Not supported
Inject	Supported - see below	Supported - see below	Supported - see below	Supported
Wash_X	Supported - see below	Not supported	Supported - see below	Not supported
WashDil_X	Not supported	Supported - see below	Not supported	Not supported
GotoOpValvePos	Not supported	Not supported	0 (Inject) or 1 (Load)*	Not supported

Inject Programs

AS2000
AS4000
L7250

Depending on the current value of the **Syringe** device property, three different programs are internally executed during processing an ⇒*Inject* command. For AS2000 and L7250 the dilutor property always equals 1.

Syringe>0   GotoSyringePos   SyringePos=0

Speed=<*current value of property **Speed***>

Dilutor=<*current value of property **Dilutor***>

GotoSValvePosSValvePos=Bottle

Dilutor=<*current value of property **Dilutor***>

GotoSyringePos   SyringePos=<*current value of property **Syringe***>

Speed=<*current value of property **Speed***>

Dilutor=<*current value of property **Dilutor***>

GotoTube   TubeNumber=<*current value of property **Position***>

RackCode=<*current value of property **RackCode***>

Aspirate   AspirateVol=<*current value of property **Volume***>

Speed=<*current value of property **Speed***>

Dilutor=<*current value of property **Dilutor***>

GotoInjectPort

GotoValvePos   ValvePos=Load

GotoSyringePos   SyringePos=0

Speed=<*current value of property **Speed***>

Dilutor=<*current value of property **Dilutor***>

GotoValvePos   ValvePos=Inject

Syringe=0   GotoTube   TubeNumber=*<current value of property Position>*

RackCode=*<current value of property RackCode>*

Aspirate   AspirateVol=*<current value of property Volume>*

Speed=*<current value of property Speed>*

Dilutor=*<current value of property Dilutor>*

GotoInjectPort

GotoValvePos   ValvePos=Load

GotoSyringePos   SyringePos=0

Speed=*<current value of property Speed>*

Dilutor=*<current value of property Dilutor>*

GotoValvePos   ValvePos=Inject

Syringe<0   GotoValvePos   ValvePos=Inject

L7200   Based on the current parameter settings, the autosampler performs an injection. The **Inject** command transmits the vial number and the sample volume only.

Wash Programs

AS2000
AS4000
L7250

This is the program that is run when the extended wash command (Wash_X resp. WashDil_X) is executed. For AS2000 and L7250 the dilutor property always equals 1.

GotoWashPort

GotoSValvePosSValvePos=Bottle

Dilutor=*<current value of property Dilutor>*

GotoSyringePos   SyringePos=*<current value of property Syringe>*

Speed=*<current value of property Speed>*

Dilutor=*<current value of property Dilutor>*

GotoSValvePosSValvePos=Needle

Dilutor=*<current value of property Dilutor>*

GotoSyringePos   SyringePos=0

Speed=*<current value of property Speed>*

Dilutor=*<current value of property Dilutor>*

GotoInjectPort

GotoSValvePosSvalvePos=Bottle

Dilutor=*<current value of property Dilutor>*

GotoSyringePos   SyringePos=*<current value of property Syringe>*

Speed=*<current value of property Speed>*

Dilutor=*<current value of property Dilutor>*

GotoSValvePosSValvePos=Needle

Dilutor=*<current value of property Dilutor>*

> GotoSyringePos   SyringePos=0
>> Speed=<*current value of property **Speed***>
>> Dilutor=<*current value of property **Dilutor***>

L7200   not supported

The **Administrator Help** section provides more information about the different autosampler types; refer to **Hardware Installation**:

🔵 **Merck Hitachi AS2000 and AS4000 Autosamplers: Overview**

🔵 **Merck Hitachi L7250 Autosampler: Overview**

🔵 **Merck Hitachi L7200 Autosampler: Overview**

◾ Merck Hitachi AS4000: Program Example

To avoid stressing the Merck ➤*Autosamplers* with a fast succession of commands, allow at least 0.1 min between the individual commands. A program example for the AS4000 could look as follows:

```
;******* Pump Commands: ****************
      Pressure.LowerLimit = 2
   Pressure.UpperLimit =  200
   %A.Equate =    "Water"
   %A.Type =   Automatic

;******* Detector Commands: ****************
   UV_VIS_1.Wavelength =  254
   UV_VIS_1.Bandwidth =   1
   UV_VIS_1.Step =  0.20
   UV_VIS_1.Average =   On
   UV_VIS_1.RefWavelength =  600
   UV_VIS_1.RefBandwidth =   1

   Flow =  0.300

;******* Autosampler Commands: ****************
;(Allow for at least 0.1 min between the individual commands to avoid
stressing the autosampler with a rapid succession of commands)
-2.100   Home
   Wash

-1.600   LeadVolume = 30.0
-1.550   RearVolume = 30.0
```

```
-1.500   Height Z = 0.0, NeedleSpeed = Fast
         Aspirate  DilutorNumber = 1, SyringePos = 5.0, PlungerSpeed =
         VeryFast
;Aspirate 5 µl air

    GotoTube RackCode = 1, TubeNumber = Position
;Go to the sample vial
         Aspirate  DilutorNumber = 1, SyringePos = LeadVolume + Volume
         + RearVolume, PlungerSpeed = VeryFast
;Aspirate sample from sample vial

    Height Z = 0.0, NeedleSpeed = Fast
         Aspirate  DilutorNumber = 1, SyringePos = 5.0, PlungerSpeed =
         VeryFast
;Aspirate 5 µl air

-1.000   GotoWashPort
;Wash outside of needle

-0.500   GotoInjectPort
         Dispense  DilutorNumber = 1, SyringePos = 5.0 + LeadVolume +
         14.9, PlungerSpeed = VeryFast
;Dispense air + leadVolume + dead volume
    GotoValvePos   ValvePos = Load
         Dispense  DilutorNumber  =  1,   SyringePos  =   Volume,
         PlungerSpeed = VeryFast
;Dispense injection volume

    Syringe = -1

0.000 UV.Autozero
    Inject
    UV_VIS_1.AcqOn
;do not execute another program, Inject switches the valve only
    GotoWashPort
    Home

8.000 UV_VIS_1.AcqOff

    End
```

The **Administrator Help** section provides more information about the different autosampler types; refer to **Hardware Installation**:

🔁 **Merck Hitachi AS2000 and AS4000 Autosamplers: Overview**

🔁 **Merck Hitachi L7250 Autosampler: Overview**

🔁 **Merck Hitachi L7200 Autosampler: Overview**

Perkin Elmer: Commands and Tips

For information about the special commands that Chromeleon supports for the different Perkin Elmer devices and for tips for practical operation, refer to:

Autosystem (XL) and Clarus 500 GCs

TurboMatrix Headspace Sampler

Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Commands and Tips

Chromeleon supports special commands for the Perkin Elmer Autosystem (XL) and Clarus 500 gas chromatographs. For more information, refer to:

General

Sampler

Inlet

Column

Detectors

For application examples, refer to:

Changing the Syringe Type

PSplit Valve Control for PSS and CAP Inlets

Pressure and Flow Ramps

If problems occur, refer to **Troubleshooting**.

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

◾ Perkin Elmer Autosystem (XL) and Clarus 500 GCs: General

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Values		Description
Data_Collection_Rate	1.625Hz, 3.125Hz, 6.25Hz, 12.5Hz, 25Hz		Sets the data collection rate. (Level: Advanced or higher) 🗒 *Note:* *The property cannot be changed during data acquisition.*
EquilibrationTime	0 min	99.0 min in 0.01 increments	After reaching the desired start values (temperature and/or flow), the GC waits for the equilibration time. When the equilibration time has passed, the GC is ready.
FirmwareVersion	n/a	n/a	Indicates the firmware version. (Level: Expert or higher).
OvenReady	Not Ready	Ready	Indicates whether the oven has reached the target temperature (read-only).
RampState	Initial, Time1, Ramp1, Time2, …, Ramp3, Time4, Unknown		Indicates the current phase of the oven's temperature range (read-only; level: Advanced or higher).
Ready	Not Ready	Ready	Indicates whether the GC is ready for injection (read-only).
RunState	PowerUp, OvenOff, NotReady, Equilibration, PreRun, Ready, Running, Hold, Unknown		Indicates the GC state (read-only; level: Advanced or higher).
TempCtrl	Off	On	Enables or disables temperature control for the oven.
Temperature	-	-	Properties related to the column oven. 🗒 *Notes:* *A PGM File can include a maximum of 3 temperature ramps. The temperature can be changed between 0 and 45°C.* *If a temperature is set, TempCtrl is automatically set to On.*

Property	Range/Values	Description
Temperature. Value	-99°C - 450°C	Current temperature (read-only).
Temperature. Nominal	-99°C - UpperLimit	Sets the nominal temperature.
Temperature. UpperLimit	50 - 450°C	Sets the upper limit for the oven temperature.

In addition, Chromeleon supports the following commands and/or properties if the Autosystem GC includes a PPC:

Property	Values	Description
PPCAlarm	On or Off	Enables or disables the PPC alarm. (Level: Advanced or higher)
PPCVersion		Indicates the firmware part number and version of the PPC. (Level: Expert or higher)
PressUnit	kpa, psig, or bar	Indicates the pressure unit used for the PPC settings. (Level: Advanced or higher; read-only). Select the unit on the **Components** tab page in the Server Configuration program.

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

◪ Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Sampler

The following commands and properties are available for autosampler control (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
FillStrokes	0-15	Specifies how often the syringe is filled with sample before the syringe content is drawn for injection. (Level: Advanced or higher)
InletPosition	Injector1-Injector2 (if available)	Specifies the inlet used for the injection.
PostWash	0-15	Specifies how often the syringe is filled with solvent and dispensed to the waste after the injection. (Level: Advanced or higher)
PreWash	0-2	Specifies how often the syringe is filled with solvent and dispensed to the waste before the injection. (Level: Advanced or higher)
Ready	Ready/NotReady	Set to Ready when the internal autosampler is ready for injection, i.e., when it has finished its prewash cycle. (Level: Advanced or higher)
SampleWash	0-15	Specifies how often the syringe is filled with sample and emptied to the waste before the injection is performed. (Level: Advanced or higher)
Status	Unknown, Ready, Priority, Running, Sampling, Pause, Clean	Indicates the current status of the autosampler (read-only; level: Advanced or higher).
Syringe	0.1 - 50µl	Indicates the syringe type (read-only). Chromeleon reads the type from the GC.
SyringeSpeed	Normal, Fast, OnColumn	Sets the syringe speed for the injection: Normal: 1 s, Fast: 0.3 s, OnColumn: 7.5 s
Visc_Delay	0-15 s	Specifies the time in seconds that the autosampler waits after the piston has moved back (can be set according to the sample's viscosity).

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

🔳 Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Inlet

The following commands and properties are available for inlet control (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
FollowOven	On or Off	(Only for PPS and POC injectors) If the property is set to On, the inlet temperature follows the oven temperature (+5°C).
InletType	PKD, CAP, GSV, PSSO, PSSI, POCI, POCO	Indicates the inlet type (read-only): PKD: Packed column CAP: Capillary GSV: Gas Sampling Valve PSSO: Split/Splitless follow oven PSSI: Split/Splitless POCI: On Column POCO: On Column follow oven
Ready	Ready/NotReady	Set to Ready when the inlet heating component is ready (level: Advanced or higher). (If a PPC is installed, the SplitStatus setting must also be Ready.)
Status	None Ready NotReady FilamentOver Unknown	Indicates the current status of the associated heating component (read-only; level: Advanced or higher).
TempCtrl	On or Off	Enables or disables temperature control for the inlet.
Temperature	Without PTR: 100 - 450°C or 50 - 450°C CAP/PKD (50 - 350°C for GSV) With PTR: -99 - 500°C	Properties related to the column oven. 📒 *Note:* *If a temperature is set, TempCtrl is automatically set to On.*

Property	Range/Values	Description
Temperature. Nominal		Sets the nominal temperature. For the PSSI and POCI, this command sets a temperature ramp, instead. A maximum of two inlet temperature ramps are allowed.
Temperature. Value		Indicates the current temperature (read-only)

In addition, Chromeleon supports the following commands and/or properties if the Autosystem GC includes a PPC:

Property	Values	Description
PurgeCtrlMode	Auto, Fixed	Sets the purge flow compensation mode (level: Advanced or higher). (For more information, refer to the manual for the instrument.)
PurgeFlow	0.0 – 100.0 ml/min	Sets the split flow offset value to compensate the reduction of the split flow due to septum purge flow (level: Advanced or higher). (The property can be set only if PurgeCtrlMode = Fixed.)
SplitCtrlMode	Flow, Ratio	Sets the split control mode. Specifies whether the flow or the flow ratio is controlled.
SplitFlow	0 – 500 ml/min	Sets the target value for the total split flow. (The property can be set only if SplitCtrlMode = Flow.)
SplitRatio	1-6000	Sets the target value for the split ratio. (The property can be set only if SplitCtrlMode = Ratio.)
SplitStatus	NotUsed, Ready, NotReady, Alarm, Unknown	Indicates the current split status (read-only; level: Advanced or higher).
TotalSplitFlow	0 – 500 ml/min	Indicates the current total flow (split flow + purge flow; read-only).

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🜨 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🜨 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

▣ Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Column

The following commands and properties are available for column control (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
Flow	0 - 99.99 ml/min	(For SetPointUnit = flow)
Flow. Nominal	Observe the restrictions for ramps.	Sets the target value for the gas flow. This does not include control: If the actual flow deviates too much from nominal flow, the column enters NotReady state.
Flow Value		Indicates the current gas flow (read-only).
GasType	He, N2, H2, AR/CH4, Air	Indicates the type of the used gas (as set in the Server Configuration program; read-only).
PressureCtrl	On or Off	Turns gas pressure control on or off.
Pressure	0-6.89 bar; 0-100 psig; 0-689 kPa (The unit depends on the PPC pressure unit set in the Server Configuration program.)	(For SetPointUnit = flow) Properties related to the gas pressure.
Pressure. Nominal		Sets the nominal pressure. This does not include control: If the actual gas pressure deviates too much from the nominal pressure, the column enters NotReady state.
Pressure. Value		Indicates the current pressure (read-only).
Ready	Ready or NotReady	Indicates whether the carrier unit is ready. The carrier unit is ready when the set pressure (or flow or speed; set in the Server Configuration program) has been reached. (The property is read-only.)
SetPointUnit	None, psig, kPa, flow	Indicates the value of interest and the unit used for observing the gas flow (read-only). The property can be set on only on the device..

In addition, Chromeleon supports the following commands and/or properties if the Autosystem GC includes a PPC:

Property	Values	Description
CapCtrlMode	On/Off	(Only for PSS and CAP inlets) Enables or disables capillary column control (level: Advanced or higher).
		Note:
		If CapCtrlMode is set to Off, FlowCtrlMode is set to Flow and SplitCtrlMode is set to Split (also, refer to Inlet).
Diameter	50 - 1000 µm	Specifies the diameter of the capillary column (level: Advanced or higher).
Flow	0 - 99.99 ml/min	Properties related to the carrier flow.
	Observe the restrictions for ramps (see below ²)	
Flow.Nominal		(Only if CapCtrlMode is set to On and if FlowMode is set to Flow) Sets the nominal flow.
Flow.Value		Indicates the current flow (read-only).
FlowCtrl	On/Off	Enables or disables carrier flow.
FlowMode	for CAP, PSS : Off, PressOvenTrack, Flow, Velocity, Pressure	Sets the flow control mode (level: Advanced or higher).
	For POC, PKD: Off, Pressure, Flow	
Length	1.00 - 200.0 m	Specifies the length of the capillary column (level: Advanced or higher).
Pressure	0-6.89 bar; 0-100 psig; 0-689 kPa (The unit depends PPC pressure unit set in the Server Configuration program)	
	Observe the restrictions for ramps (see below ²)	
Pressure.Nominal		(Only if CapCtrlMode is set to On and if FlowMode is set to Pressure) Sets the nominal gas pressure.
Pressure.Value		Indicates the current gas pressure (read-only):
Ready	Ready/NotReady	Indicates Ready if the PPC is ready or if the specified carrier gas is not used (read-only).
Status	NotUsed, Ready, NotReady, Alarm	Indicates the current status of the PPC for the specified carrier gas.

Property	Values	Description
VacuumCorrection	On/Off	(Only for PPS and CAP inlets) Turns vacuum compensation on or off (level: Advanced or higher). *Note:* *This property can be set only if CapCtrlMode is set to On.*
Velocity	0 - 200.0 cm/s Observe the restrictions for ramps (see below ²)	Properties related to the carrier flow.
Velocity.Nominal		(Only if CapCtrlMode is set to On and if FlowMode is set to Velocity) Sets the nominal flow.
Velocity.Value		Indicates the current speed (read-only)

² Observe the following restrictions when you run ramps:

- The single steps must be between 0 and 999.

- A PGM File can include a maximum of 4 ramp commands, depending on the FlowMode settings: Flow, Pressure, or Velocity).

- Maximum ramp steepness:

  1. Pressure ramps: 0-6.89 bar/min or 0-99.9psig/min

  2. Flow ramps: 0-99.99 ml/min²

  3. Speed rams: 0-200.0 cm/(s·min)

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

◪ Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Detectors

The following commands and properties are available for detector control (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
Attenuation	1, 2, 4, 8, 16, 32, 64, (and 128, 256, … 65536 if Output is set to Recorder in the Server Configuration program))	Sets the detector attenuation.[2]
AZCompensation	On or Off	Enables or disables the ➢*Autozero*-option.[2]
DetectorType	Output, FID, ECD, TCD, NPD	Indicates the detector type (read-only). Select the detector type in the Server Configuration program: FID: Flame Ionization Detector ECD: Electron Capture Detector TCD: Thermal Conductivity Detector NPD: Nitrogen Phosphorous Detector
FilterTimeConstant	50 ms (not for the ECD), 200ms, 800ms	Specifies the time constant for the noise filter of the related detector.
FlameThreshold	0.00-9.99 mV	Flame threshold for evaluating whether the detector flame is lit. If the signal exceeds this value, the GC assumes that the flame is lit. Otherwise, the flame is ignited automatically. (Level: Advanced or higher)
MaxAutoStep	0.1 to 5.1 s	Specifies the maximum step for the auto step mode. The default setting is 5.1 s.
Range	1, 20 (FID, NPD) 1 (ECD) -4 - 4 (TCD)	Sets the detector output range.[2]
Ready	Ready or NotReady	Indicates whether the detector is ready (read-only; level: Advanced or higher). If a PPC is installed, the status of the gas flows is taken into account, also.
Retention	0 - 10^8 min	Indicates the retention time.
Signal	-10000 - 10000mV	Indicates the current signal value (read-only).
Status	None, Ready, NotReady, FilamentOver, Unknown	Indicates the detector status (read-only; level: Advanced or higher).
Step	0.01 to 4.80 s Auto	Sets the step. The default setting is Auto (recommended). With this setting, the optimum step is selected dynamically.

Property	Range/Values	Description
TempCtrl	On/Off	Turns the heating component of the detector on or off.
Temperature	100 to 450°C (FID, ECD, NPD) 100 to 350°C (TCD)	Properties related to the detector temperature. *Note:* *If a temperature is set, TempCtrl is automatically set to On.*
Temperature. Nominal		Sets the nominal temperature.
Temperature. Value		Indicates the current temperature (read-only)

² If the value is entered manually or at time t=0, the value used at the beginning of the run is adapted.

 Tip:

If a value is entered at another time, this value remains valid until the next setting or the beginning of the next GC run.

In addition, Chromeleon supports the following commands and/or properties if the Autosystem GC includes a PPC:

Property	Values	Description
<Gasname>	0–500 ml/min	<Gasname> is the gas specified on the **Detectors** tab page in the Server Configuration program, e.g., He, N2, H2, Ar/CH4, or Air. *Note:* *You can enter the gas name on the **Detectors** tab page in the Server Configuration program.*
<Gasname>. Nominal		Sets the nominal flow for the associated gas (level: Advanced or higher).
<Gasname>. Value		Indicates the current flow for the associated gas (read-only; level: Advanced or higher).
<GasName>_Ctrl	On/Off	
<GasName>_GasType	He, N2, H2, Ar/CH4, Air	Indicates the gas type in the PPC module (read-only; level: Advanced or higher). Note: The gas type is set in the Server Configuration program.
<GasName>_Status	NotUsed, Ready, NotReady, Alarm	Indicates the current status in the PPC module (read-only; level: Advanced or higher).

For an overview of the Autosystem GC series and Clarus 500, refer to **nstallation Instructions: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

▪ Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Changing the Syringe Type

You may change the syringe type for the internal autosampler without the need to reconfigure the device driver in the Server Configuration program:

1. Disconnect the GC in Chromeleon (Disconnect command).

2. Exchange the syringe, as described in the instrument's manual.

3. Set the new syringe type on the GC's display, as described in the instrument's manual.

4. Reconnect the GC in Chromeleon (Connect command). Verify that the Syringe property of the internal autosampler indicates the correct type.

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

◾ Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Split Valve Control for PSS and CAP Inlets

If you have a Perkin Elmer capillary split inlet installed, the GC cannot control the split valve automatically, neither with split programs nor with splitless programs. To control the split valve manually, enter the initial value and operate the valve during analysis. Please note: CAP = not programmable, PSS = programmable.

In split mode, the split valve remains open during the entire analyzing process (the usual setting in Chromeleon is **Split1** or **Split2**, for inlets 1 or 2). Therefore, it is sufficient to set the valve to On at the retention time 0.000 min.

```
   . . .
0.000 Split1.On
      Inject
      GC_1.AcqOn
      . . .
```

In **Splitless** mode, the split valve remains closed during injection and is opened afterward; e.g.:

```
-0.500  Split1.Off  ;(optional)
0.000 Split1.Off  ;(necessary)
   Inject
   GC_1.AcqOn
   . . .
1.000 Split1.On   ;(enables the split flow to purge the inlet)
   . . .
2.000 GC_1.AcqOff
```

For more information about how to control the various split modes, refer to the Perkin Elmer operating manuals for the instruments.

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** ⊕ **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** ⊕ **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

◘ Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Pressure and Flow Ramps

Programming pressure ramps is very similar to programming temperature ramps. Different modes available for controlling the pressure of the carrier gas and the inlet but not all possible combinations are allowed. Therefore, Dionex recommends specifying the various properties in each PGM file. **Ready Check** then verifies whether the various PGM settings represent a valid combination.

The examples below show common combinations for a PPC column and a capillary inlet with a pressure controlled split flow. For more information, refer to **PPC Fundamentals** in the Perkin Elmer operating manual for the instrument.

Constant Pressure/pressure ramps for PPC columns:

```
  GC_1.TempCtrl =   On
  GC_1.Temperature.Nominal =   100

  ; setting of the pressure modes
  Column_1.CapCtrlMode =  On
  Column_1.FlowMode =  Pressure
  Column_1.VacuumCorrection =   Off
  . . .
 0.000   Column_1.Pressure = 10.0
  Wait  GC.Ready
  Inject
  GC_1.AcqOn
  Column_1.Pressure = 10.0

 0.500   Column_1.Pressure = 10.0 ; pressure ramp

 1.500   Column_1.Pressure = 15.0 ; pressure ramp

 2.000   Column_1.Pressure = 15.0 ; pressure ramp
  GC_1.AcqOff
      End
```

In **PressOvenTrack** mode, you can define pressure modes that follow the oven temperature to hold the column flow constant. In this mode, you may enter only an initial pressure value:

```
  . . .
  ; setting of the pressure modes:
  Column_1.CapCtrlMode =  On
  Column_1.FlowMode =  PressOvenTrack
  Column_1.VacuumCorrection =   Off
  . . .
```

```
 0.000   Column_1.Pressure =  10.0 ; initial pressure value
   Wait  GC.Ready
   Inject
   GC_1.AcqOn
   . . .
2.000 GC_1.AcqOff
   End
```

Use the following modes to realize flow and velocity ramps:

```
Column_1.FlowMode = Flow
```

and

```
Column_1.FlowMode = Velocity.
```

Split flow control allows you to run step gradients, to specify the absolute flow, or to define the split ratio. For a split injection in a flow-controlled program (flow mode), refer to the example below. In the example, the split flow is increased after 0.5 minutes. (For more information on split and splitless injection techniques, refer to the Perkin Elmer operating manual for the instrument.)

```
       . . .
; setting of the pressure modes
   Column_1.CapCtrlMode =  On
   Column_1.FlowMode =  Pressure
   Column_1.VacuumCorrection =   Off
   Injector_1.SplitCtrlMode = Flow
       ; uses absolute split flow
   Injector_1.PurgeMode =  Fixed
       ; uses a fixed purge flow
   Injector_1.PurgeFlow =  6.0
       ; sets the purge flow to 6.0 ml/min
   . . .
; Program
 0.000   Column_1.Pressure =  10.0
   Valve1.On
       ; split valve is open
   Injector_1.SplitFlow =  50.0
       ; sets the initial split flow
   Wait  GC.Ready
   Inject
   GC_1.AcqOn
   Column_1.Pressure =  10.0
 0.500   Column_1.Pressure =  10.0; pressure ramp
   Injector_1.SplitFlow = 200.0 ; increases the split flow
 1.500   Column_1.Pressure =  15.0; pressure ramp
 2.000   Column_1.Pressure =  15.0; pressure ramp
   GC_1.AcqOff
       End
```

For an overview of the Autosystem GC series and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

🔲 Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Troubleshooting

Problem: Missing Connection

It is not possible to connect the GC with Chromeleon, e.g., with a panel.

Explanation:

Each time you connect the GC, the driver compares the actual configuration with the configuration entered in the Server Configuration program. If those configurations do not match, the connection cannot be established.

Solution:

Change the settings in the Server Configuration program according to the installed hardware.

Problem: Missing vials

It depends on the instrument whether the cause for the error is eliminated automatically (Clarus 500) or whether you have to eliminate it manually (Autosystem (XL)):

Autosystem (XL):

Chromeleon cannot remedy the situation automatically. The GC stops sample processing but remains in Ready state. Chromeleon remains in Waiting for inject response state.

Solution:

Abort and restart the batch.

Clarus 500:

Chromeleon automatically remedies the situation for the Clarus 500. An error message is logged in the Audit Trail. Chromeleon skips the sample and continues processing with the next sample. The status for the missing sample is **Interrupted**.

 Note:

If a wash vial or a waste vial is missing, Chromeleon automatically aborts the batch because the remaining samples are affected as well. Correct execution of the analysis cannot be guaranteed. Place the missing wash or waste vial on the rack and restart the batch.

For an overview of the Autosystem GC and Clarus 500, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Perkin Elmer Autosystem (XL) and Clarus 500 GCs: Installation** in the **Administrator Help** section.

Perkin Elmer TurboMatrix Headspace Sampler: Commands and Tips

Chromeleon supports special commands for the Perkin Elmer TurboMatrix Headspace sampler; refer to

 State

 Options

 Temperatures

 Time Programs

 Oven

 Pressure

For more information, practical examples, and tips, refer to:

 Single and Progressive Injection Modes

 Program Example

 MHE Mode

 Tips

For an overview of the Elmer TurboMatrix Headspace Sampler, refer to **Installing and Controlling Third-Party Devices** **Perkin Elmer TurboMatrix Headspace Sampler: Overview** in the **Administrator Help** section.

For installation details, refer to **Installing and Controlling Third-Party Devices** **Perkin Elmer TurboMatrix Headspace Sampler: Installation** in the **Administrator Help** section.

Perkin Elmer TurboMatrix Headspace Sampler: State

In addition to the **General Commands** described in the **General Commands for Device Control** section, the following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
Busy	Busy or Idle	Busy indicates that the device driver still has to send commands to the sampler or that the commands are not yet performed completely (read-only).
DeviceState	Indicates the state in which the sampler is (read-only):	
	Initializing	The sampler is initializing (start/reset).
	Standby	The sampler is idle.
	Preparation	The sampler prepares for a new run.
	Equilibration	The sampler performs equilibration.
	GCNotReady	The GC is not ready for operation.
	Thermostating	The sampler is thermostating.
	Pressurizing	Pressure is build up.
	ExtThermostat	Thermostating is exceeded.
	Injecting	The sampler is performing an injection.
	WaitingForWithdraw	Time between the end of the injection and the withdrawal of the needle.
	Venting	The sample is being vented.
	Analyzing	A sample analysis is in progress.
	RunBackflush	Backflushing is performed.
	Finishing	The sample run is finished.
	BackflushCal	Backflush calibration is performed.
	GasLeakTest	A gas leak test is performed.
	Error	A fault or error has occured.
	EconomyMode	The sampler is in the economy mode.
	CryoFocus	A cryo focus test is performed.
	ValveLeakTest1	The first valve leak test is performed.
	ValveLeak_Test2	The second valve leak test is performed.
	Unknown	Unknown.

Chromeleon does not yet support the following states

PressurizeVial	The vial is being pressurized (cryo trap).
DecayPressToTrap	The pressure buildup in the cryo trap is decayed.
TrapDesorb	Desorption of the trap.
TrapHold	The cryo trap process is hold.
TrapDryPurge	The cryo trap is being purged.
TrayGCAnalysis	A GC analysis of the tray is performed.
InsertNeedle	The needle is inserted.
RemoveNeedle	The needle is removed.
TrapLeakTest	A trap leak test is performed.

☞ *Tip:*

*Please refer to the instrument manual for more information about the **State** properties.*

For more commands and properties that Chromeleon supports for the TurboMatrix Headspace sampler, refer to:

🔲 **Options**

🔲 **Temperatures**

🔲 **Time Programs**

🔲 **Oven**

🔲 **Pressure**

🔳 Perkin Elmer TurboMatrix Headspace Sampler: Options

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Value	Description
CryoFocus	Off, On	Enables or disables cryo cooling. (This property is available only if cryo cooling is installed on the device and configured in Chromeleon.)
FlowRate	0.01 - 50 ml/min	Determines the expected flow rate. The sampler uses this setting to calculate the time required for injecting the specified volume.

Property	Range/Value	Description
InjectMode	Single, Progressive, MHE	Determines **the injection mode. For more information, refer to:** **Single and Progressive Injection Modes** **Program Example** **MHE Mode**
InjectionsPerVial	1 - 9	Specifies the number of injections to be performed from the same vial.
InjectWaitTime		Indicates the time between the Inject command in Chromeleon and the response to Chromeleon from the autosampler (read-only).
Position		Indicates the sample position (read-only). The position is set in the sequence; it depends on the type:
	1 - 16	TM16
	1 - 40	TM40
	1 - 110	TM110
Shaker	Off, On	Enables or disables the shaker. (This property is available only if the shaker is installed on the device and configured in Chromeleon.)
VentingTime	1 - 60s	Specifies how long the vial is vented.
VialVenting	Off, On	Specifies whether the vial is vented.
Volume	0 - 9.99 ml	Indicates the injection volume (read-only). The injection volume is set in the sequence.
WaterTrap	Off, On	Enables or disables the water trap. (This property is available only if cryo cooling is installed on the device and configured in Chromeleon.)

 Tip:

Please refer to the instrument manual for more information about the **Options** *properties.*

For more commands and properties that Chromeleon supports for the TurboMatrix Headspace sampler, refer to:

 State

 Temperatures

 Time Programs

 Oven

 Pressure

◘ Perkin Elmer TurboMatrix Headspace Sampler: Temperatures

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
CryoTemperature		Properties for the cryo cooling temperature. (The properties are available only if Cyro-Focus is installed on the device and configured in Chromeleon.)
CryoTemperature. Nominal	-180 to -10°C	Sets the nominal temperature for cryo cooling.
CryoTemperature. Value	-180 to -10°C	Reports the current temperature of the cryo cooling (read-only).
FollowOven	On or Off	(Name on the sampler: *Track Oven*)
		If the property is set to **Off**, you can set **NeedleTemperature**, **OvenTemperature**, and **TransferTemperature** separately.
		Setting the property to **On** defines the **OvenTemperature**. Any settings for the **NeedleTemperature** and **TransferTemperature** are ignored. The target temperature for the needle heating and the transfer temperature is automatically set to a value that is 5°C higher than the **OvenTemperature**.
NeedleTempCtrl	On or Off	Enables or disables needle heating.
NeedleTemperature		Properties related to the temperature for the needle heater (when **FollowOven** is set to **Off**).
		🗒 *Note:*
		*NeedleTempCtrl is automatically set to **On** when the corresponding temperature command is performed.*
NeedleTemperature. Nominal	35 to 210°C	Sets the target temperature for needle heating.
NeedleTemperature. Value	35 to 210°C	Indicates the actual needle temperature (read-only).
OvenTempCtrl	On or Off	Turn the incubation oven on or off.

Property	Range/Values	Description
OvenTemperature		Properties related to the temperature of the incubation oven.
		Note:
		*OvenTempCtrl is automatically set to **On** when the corresponding temperature command is performed.*
OvenTemperature. Nominal	35 to 210°C	Sets the target temperature for the incubation oven.
OvenTemperature. Value	35 to 210°C	Indicates the current temperature of the incubation oven (read-only).
TransferTempCtrl	On or Off	Enables or disables transfer line heating.
TransferTemperature		Properties related to transfer line heating (when **FollowOven** is set to **Off**).
		Note:
		*TransferTempCtrl is automatically set to **On** when the corresponding temperature command is performed.*
TransferTemperature. Nominal	35 to 210°C	Sets the target temperature for transfer line heating.
TransferTemperature. Value	35 to 210°C	Indicates the actual transfer line temperature (read-only).

For more commands and properties that Chromeleon supports for the TurboMatrix Headspace sampler, refer to:

🔲 **State**

🔲 **Options**

🔲 **Time Programs**

🔲 **Oven**

🔲 **Pressure**

▣ Perkin Elmer TurboMatrix Headspace Sampler: Time Programs

The following commands and properties are available (range: 0.1 to 999.9 min; please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Description
GCCycleTime	Sets the GC cycle time (on the device: *cycle time*). The range 0.1 to 999.99 min. The GC cycle time is the minimum time between the start of a GC run and the time when the GC is ready for the next injection.

 Note:

A error message appears when the GC cycle time is shorter than the time between two injections (PGM end time for the current sample and PGM start time for the next sample).

Property	Description
IncubationTime	Sets the incubation time (on the device: *Thermostatting Time* and *Thermo*). The range is 0.1 to 999.99 min.
InjectTime	Indicates the injection time (read-only). The injection volume and the column flow allow you to determine the injection time.
PostCryoTime	If Cryo-Focus is installed on the device and configured in Chromeleon (CryoFocus = On), the parameter sets the time between the injection and the end of cooling (range: 0.1 to 99.99 min).
PreCryoTime	If Cryo-Focus is installed on the device and configured in Chromeleon (CryoFocus = On), the parameter sets the time between the start of cooling and the injection (range: 0.1 to 99.99 min).
PreparationTime	Indicates the estimated preparation time, i.e., the time until the next injection (read-only).
PressTime	Sets the time for sample pressurizing (on the device: *Pressurization time* and *Pressurize*). The range is 0.1 to 999.99 min.
WithdrawTime	Sets the time between the end of the injection cycle and the withdrawal of the needle from the sample (= venting). The range is 0.1 to 99.99 min.

For more commands and properties that Chromeleon supports for the TurboMatrix Headspace sampler, refer to:

▣ **State**

▣ **Options**

▣ **Temperatures**

▣ **Oven**

▣ **Pressure**

◾ Perkin Elmer TurboMatrix Headspace Sampler: Oven

The oven supports 15 different positions for sample conditioning. A new sample is always placed at position 0. Injection is performed from position 2. The following properties are available on the Expert ➢*Filter* level:

Property	Range/Values	Description
_IncTime\<i\>	-1000 to 1000 min	If the oven position is occupied, the variable reports the remaining incubation time (read-only).
_Occupied\<i\>	Off, On	Set to **On** if the oven position is occupied. If the position is free, the setting is **Off**. (This property is read-only.)
_Vial\<i\>	0 to 14. 0='Empty'	If the oven position is occupied, the variable reports the number of the related sample (read-only).

Where: \<i\> = 0 to 14

For more commands and properties that Chromeleon supports for the TurboMatrix Headspace sampler, refer to:

◾ **State**

◾ **Options**

◾ **Temperatures**

◾ **Time Programs**

◾ **Pressure**

◾ Perkin Elmer TurboMatrix Headspace Sampler: Pressure

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
ColumnPressure		Properties related to the column pressure.
Column Pressure. Nominal²	0.00 - 4.00 bar, 0 - 400 kPa or 0 - 60.0 psig	Sets the target pressure in the column.
Column Pressure. Value²	0.00 - 4.00 bar 0 - 400 kPa or 0 - 60.0 psig	Indicates the current column pressure (read-only).

Property	Range/Values	Description
HighPressInject	On or Off	Enables or disables high-pressure injection mode (on the device: *Hi Psi Inject*).

 Note:

*This property is available only if you have selected the **Programmable Pneumatic (PPC)** check box on the **Options** tab page in the Server Configuration program.*

Property	Range/Values	Description
InjectPressure²	0.00 - 4.00 bar 0 - 400 kPa or 0 - 60.0 psig	Sets the pressure for high-pressure injections (HighPressInject = On) for the time between the start of the pressure period and sample venting (withdrawing the needle).

 Notes:

*This property is available only if you have selected the **Programmable Pneumatic (PPC)** check box on the **Options** tab page in the Server Configuration program.*

*When **HighPressInject** is set to **Off**, the device does not use this value.*

 Notes²:

The sampler accepts Chromeleon pressure commands only in psi. That is why Chromeleon must convert bar or kPa to psig before sending these commands to the sampler. This conversion may result in minor rounding differences, e.g., 120 kPa in Chromeleon is transferred to the sampler as 119.9 kPa.

The range of 0 - 60 psi (or 0 - 413.7 kPa) corresponds to the range that can be set on the device.

For more commands and properties that Chromeleon supports for the TurboMatrix Headspace sampler, refer to:
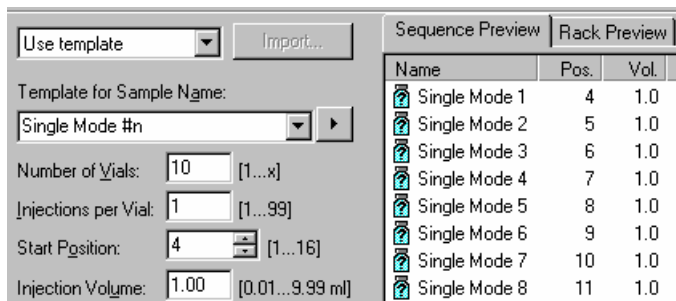
 State

 Options

 Temperatures

 Time Programs

 Oven

▪ Perkin Elmer TurboMatrix Headspace Sampler: Single and Progressive Injection Modes

The Perkin Elmer TurboMatrix Headspace Sampler supports different injection modes:

- Single mode (in routine operation: all samples are thermostatted the same time)

- Progressive mode (for method generation: increasing thermostatting time)

- MHE mode (for multiple injections from the same vial; refer to
  🔖 **MHE Mode**)

In Single and Progressive modes, the sampler assumes that the injection volume and the parameters are identical for all samples and that the vial positions are incremented. To achieve optimum sample overlapping, Dionex recommends taking this into account when creating a sequence in Chromeleon.

However, in Chromeleon, the parameters can vary for each sample. This allows you, e.g., to process the samples with different PGM Files or to vary the injection volumes. Chromeleon regards this as independent sampler runs. Sample overlapping is possible only in the single sampler runs.

This is especially important in Progressive mode. In this mode, the sample uses the **IncubationTime** parameter as the thermostatting time for the first sample in the sampler sequence. The thermostatting time for the other samples in the sampler run is prolonged, based on the sample number: Sample n is thermostatted n times as long as sample 1.

Example:

No.	Name	Type	Pos.	Inj. Vol.	Program	Method	Status
1	Test 1	Unknown	3	4.0	PETMProgressive	GC_23	Single
2	Test 2	Unknown	4	4.0	PETMProgressive	GC_23	Single
3	Test 3	Unknown	5	4.0	PETMProgressive	GC_23	Single
4	Test 4	Unknown	6	4.0	PETMProgressive	GC_23	Single
5	Test 5	Unknown	3	3.0	PETMProgressive	GC_23	Single
6	Test 6	Unknown	4	3.0	PETMProgressive	GC_23	Single
7	Test 7	Unknown	5	3.0	PETMProgressive	GC_23	Single
8	Test 8	Unknown	6	3.0	PETMProgressive	GC_23	Single

Setting the **IncubationTime** to 10 min results in the following thermostatting times for the single samples:

No.	1	2	3	4	5	6	7	8
Time [min]	10	20	30	40	10	20	30	40

The injection volume changes after the 4th sample. Thus, a new sampler run starts with the 5th sample. In this run, the Chromeleon sample no. 5 is the first sample.

For a program example for the single or progressive injection mode, refer to **Perkin Elmer TurboMatrix Headspace Sampler: Program Example**.

For tips for practical operation, refer to **Perkin Elmer TurboMatrix Headspace Sampler: Tips**.

For an overview of the Elmer TurboMatrix Headspace Sampler, refer to **Installing and Controlling Third-Party Devices** **Perkin Elmer TurboMatrix Headspace Sampler: Overview** in the **Administrator Help** section.

For installation details, refer to **Installing and Controlling Third-Party Devices** **Perkin Elmer TurboMatrix Headspace Sampler: Installation** in the **Administrator Help** section.

◾ Perkin Elmer TurboMatrix Headspace Sampler: Program Example

Below please find a typical PGM File for the ◾ **Single and Progressive Injection Modes**

```
; Specify the injection mode
        InjectMode =          Single ; or 'Progressive'
        InjectionsPerVial =   1

; Sample thermostatting
        IncubationTime =    6.5 [min]
        OvenTemperature =  59 [°C]

; GC cycle time: The time should be sufficiently longer than the PGM
; run time so that the GC has enough time to reset the temperature
and
; the flow to the initial values and to get ready for the next
; injection
        GCCycleTime =          13.5 [min]

; Remaining time program
        PressTime =           0.2 [min]
        WithdrawTime =        0.3 [min]
        VialVenting =         On
        VentingTime =         5 [s]

; Temperatur program: e.g., set all temperature separately
        FollowOven =          Off
        NeedleTemperature =   60 [°C]
        TransferTemperature = 62 [°C]

; Pressure, e.g., high-pressure injection
        HighPressInject =     On
        CarrierPressure =     100.0 [kPa]
        InjectPressure =      110.0 [kPa]

; You can then enter the GC settings
; ...

; The injection procedure starts:
0.000 Wait  Sampler.Ready AND GC.Ready
        Inject

        ; the following commands refer to GC analysis
        ; ...

12.000   End
```

After making some modification, you can use this program for the MHE mode, also. For information about the modifications, refer to 🔳 **MHE Mode**.

For tips for practical operation, refer to 🔳 **Perkin Elmer TurboMatrix Headspace Sampler: Tips**.

For an overview of the Elmer TurboMatrix Headspace Sampler, refer to **Installing and Controlling Third-Party Devices** 🟣 **Perkin Elmer TurboMatrix Headspace Sampler: Overview** in the **Administrator Help** section.

For installation details, refer to **Installing and Controlling Third-Party Devices** 🟣 **Perkin Elmer TurboMatrix Headspace Sampler: Installation** in the **Administrator Help** section.

🔳 Perkin Elmer TurboMatrix Headspace Sampler: MHE Mode

In MHE mode (= Multiple headspace extraction), multiple injections are performed from the same vial. Use the Sequence Wizard to create such a sequence (3 injections per vial), e.g.:

The PGM file for the MHE mode is similar to the PGM File for the other injection modes (see 🔳 **Program Example**). The only difference is that you have to specify in the program file how often the injection shall be performed from the same vial, in addition to the sequence. The entry must match the setting in the sequence. Otherwise, the ➤*Ready Check* fails.

```
; Specify the injection mode
   InjectMode =   MHE
   InjectionsPerVial = 3
; Sample thermostatting
   IncubationTime = 6.5
   OvenTemperature = 59 [°C]
```

🖝☞ *Tip:*

*In MHE mode, avoid explicitly setting a cycle time (**GCCycleTime**). The sampler uses the **IncubationTime** as cycle time. Thus, you only need to set the cycle time. Even if you set the GCCycleTime explicitly, the sampler uses the IncubationTime, instead. (Note: The selected GCCycleTime would be saved in the device and thus, it would then be used as preset cycle time in programs for Single and/or Progressive mode.)*

For information about the other two injection modes, refer to 🔝 **Perkin Elmer TurboMatrix Headspace Sampler: Single and Progressive Injection Modes**.

For tips for practical operation, refer to 🔝 **Perkin Elmer TurboMatrix Headspace Sampler: Tips**.

For an overview of the Elmer TurboMatrix Headspace Sampler, refer to **Installing and Controlling Third-Party Devices** 🌐 **Perkin Elmer TurboMatrix Headspace Sampler: Overview** in the **Administrator Help** section.

For installation details, refer to **Installing and Controlling Third-Party Devices** 🌐 **Perkin Elmer TurboMatrix Headspace Sampler: Installation** in the **Administrator Help** section.

🔳 Perkin Elmer TurboMatrix Headspace Sampler: Tips

Injection time

You can set an injection time on the sampler. In Chromeleon, it is not possible to explicitly set the injection time. However, the relationship between the injection time and the injection volume is as follows:

Injection time (min) = volume (ml) / column flow (ml/min)

Therefore, to determine the injection time, specify the **Volume** and the **ColumnFlow**.

📝 *Note:*

*Besides, the injection time is indicated in Chromeleon via the **InjectTime** property.*

Missing vials

When the sampler detects a missing sample vial, it does not interrupt the run. The sampler waits for the time specified for that sample and continues with the next sample afterward.

Chromeleon, too, notices that a vial is missing and

- Logs the missing sample in the Audit Trail.

- Sets the sample status to Interrupted.

- Does not start the GC run.

- Continues the run with the next sample as soon as the sampler is in Ready state.

Blank Run Samples

The TurboMatrix Headspace sampler does not know blank run samples. The sampler injects every specified sample. If a Chromeleon sequence includes a blank run sample, Chromeleon splits the sequence into several subsequences and the sampler then processes them one after the other. When the blank run sample is processed, the sampler is in Standby mode.

No.	Name	Type	Pos	Inj. Vol.
1	Sample 1	Unknown	3	2.00
2	Sample 2	Unknown	4	2.00
3	Sample 3	Blank	5	2.00
4	Sample 4	Unknown	6	2.00
5	Sample 5	Unknown	7	2.00
6	Sample 6	Unknown	8	2.00
7	Sample 7	Blank	9	2.00
8	Sample 8	Unknown	10	2.00

In the example (injection mode: Single), the sequence would be split into three subsequences:

1. Samples 1 2
2. Samples 4 through 6
3. Sample 8

When sample 3 and 7 are processed, the sample is in Standby mode.

🖝 *Tips:*

In Progressive mode, only the first sample of a Chromeleon sequence can be a blank run sample.

It is not possible in MHE mode to enter single samples as blank run sample. All samples that are injected from the same vial must be defined as blank run samples, instead.

No.	Name	Type	Pos	Inj. Vol.
1	HME 4_1	Unknown	4	2.00
2	HME 4_2	Unknown	4	2.00
3	HME 4_3	Unknown	4	2.00
4	HME 5_1	Blank	5	2.00
5	HME 5_2	Blank	5	2.00
6	HME 5_3	Blank	5	2.00
7	HME 6_1	Unknown	6	2.00
8	HME 6_2	Unknown	6	2.00
9	HME 6_3	Unknown	6	2.00

Interrupting a batch

1. When a batch was started, Chromeleon combines as many samples as possible in a sampler method and sends the associated data to the sampler so that the sampler can start a run with overlapping sample preparation (thermostatting). For all samples sent to the sampler that have not yet been injected, the sample status is **Preparing**.

When a batch is interrupted, the status for samples having the status **Prepapring** changes to **Interrupted**. It is irrelevant whether the samples are already in the oven.

2. When a batch is interrupted with the **Abort after current sample** option, Chromeleon waits until the sampler has finished the current injection cycle, i.e., until the sampler is in **GCAnalysis** or **Finishing** state.

It may happen that another sample vial is moved into the oven for preparation even if no injection can be performed. You cannot prevent this because the sampler does not allow you to stop the preparation without interrupting the entire run.

Actual incubation time

The actual incubation time may be different from the specified time. This may happen, e.g., when, after the previous run was finished, the GC does not enter the **Ready** state in time before the next injection. Chromeleon records the status of the oven and the devices to determine how long the single sample vials were actually incubated. When a sample was injected, i.e., after it was pressurized, Chromeleon logs the actual incubation time in the Audit Trail:

👉 *Tips:*

In almost all cases, the actual incubation time will be slightly different from the specified time because Chromeleon receives data from the sampler only every second. Thus, a measuring error of at least 1 second (= 0.017 min) can be expected. In addition, data processing in the sampler must be considered so that the measuring error may be some hundredth minutes.

To include the actual incubation time in the report, use the **Real Incubation Time (Sampler)** variable that is available in the **Audit Trail** category.

Inject synchronization

The injection process starts by pressurizing the sample (device state: **Pressurize**). (The sampler sends the **GC start run** signal when the device status changes from **Pressurize** to **Injecting**. Chromeleon sets the sampler to **Ready** when the pressure phase starts and sends an **Inject Response** signal when starting the injection.)

For information about the different injection modes, refer to **Perkin Elmer TurboMatrix Headspace Sampler:**

 Single and Progressive Injection Modes

 Program Example

 MHE Mode

For an overview of the Elmer TurboMatrix Headspace Sampler, refer to **Installing and Controlling Third-Party Devices** **Perkin Elmer TurboMatrix Headspace Sampler: Overview** in the **Administrator Help** section.

For installation details, refer to **Installing and Controlling Third-Party Devices** **Perkin Elmer TurboMatrix Headspace Sampler: Installation** in the **Administrator Help** section.

Rheodyne Valves: Commands and Tips

The **Rheodyne External MSV** driver supports the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Command	Min.	Max.	Default	Description
Direction	0 Shortest 1 Counterclockwise 2 Clockwise		0	Defines the direction in which the valve moves to the specified position.
Home				Moves the valve to position 1. (Note: The command ignores the Direction property.)
Position	1	Depends on the type.	Read from the device on connect.	The current position of the valve. (The valve moves to the specified position, obeying the Direction property.)
Test				Tests the valve. (Moves the valve to position 1, steps to all positions, and returns the valve to position 1) Level: Expert.

Property	Values	Default	Description
FirmwareVersion	Text string (64 characters) indicating the firmware number	Read from the device on connect.	Read-only. Level: Expert.
ModelNo	Text string (64 characters) describing the valve type.	Read from the device on connect.	Read-only. Level: Expert.
SerialNo	Text string (64 characters) indicating the serial number.	Read from the device on connect.	Read-only. Level: Expert.

Chromeleon does not read the serial number for the **Rheodyne LabPro** driver. Also, note that the following properties are available instead of the **Direction** property:

Property	Description
StepCounterClockwise	Moves the valve counterclockwise to the next position (Level: Advanced).
StepClockwise	Moves the valve clockwise to the next position (Level: Advanced).

Shimadzu: Commands and Tips

For information about the special commands that Chromeleon supports for the different Shimadzu devices and for tips for practical operation, refer to:

🔼 **Shimadzu HPLC Systems: Commands and Tips**

🔼 **Shimadzu GC-2010 GC System: Commands and Tips**

🔼 **Shimadzu PDA Detector**

🔳 Shimadzu HPLC Systems: Commands and Tips

For information about the special commands that Chromeleon supports for the Shimadzu LC-10A and LC-2010 HPLC systems and for tips for practical operation, refer to:

🔼 **System Settings**

🔼 **Pump**

🔼 **Autosampler**

🔼 **Inject Synchronization** (relates only to the Shimadzu LC-10A HPLC System)

🔼 **SIL-10AD*vp* Sample Preparation** (relates only to the Shimadzu LC-10A HPLC System)

🔼 **Column Oven**

🔼 **UV/VIS Detector**

🔼 **General Tips**

🔼 **Troubleshooting**

For program examples for sample preparation with the SIL-10ADvp autosampler, which is part of the LC-10A HPLC system, refer to:

🔼 **Program Example for the Standard Injection Mode**

🔼 **Program Example for the Reagent Injection Mode**

🔼 **Program Example for the Dilution Injection Mode**

🖱☞ *Tip:*

*You can only set the injection mode if you have selected the **Prep Loop** option on the **Sampler** tab page in the Server Configuration program.*

◼ Shimadzu LC-10A and LC-2010 HPLC Systems: System Settings

The table below lists the commands and properties that are available for the Shimadzu LC-10A and LC-2010 HPLC Systems (please note that the display ➢*Filter* level determines which commands and properties are displayed).

📋 *Note:*

The names of the Shimadzu commands and properties may be slightly different, depending on version of your system.

Chromeleon	Shimadzu	Values	Description
Activated		No, Yes	Activates or deactivates the instrument or system. Reactivate the system if it was deactivated, e.g., because the pressure was below the pressure limit.
			📋 *Note:*
			Activating the system automatically enables temperature control and turns the pump(s) on (Motor = On).
ExternalStart	EXT_S	None, AllRuns, InjOnly	Determines which program starts are signalled by the START otuput (relay 1 on the system controller).
Status	STATUS		Indicates the overall instrument or system status. The instrument or system is:
		PURGE	purging.
		PAUSE	in Pause mode.
		HOLD	in Hold mode.
		RUN	running.
		ERROR	An error has occurred.
		WAIT_OVEN	waiting for the column oven.
		OVEN_NOT_READY	The oven is not yet ready for operation.
		TRANSMIT	transmitting data.
		WAIT_DP	waiting for INJ_IN.
		PRETREAT	busy with sample prepration
		STOP	stopped.
		RINSE	rinsing.
		READY	ready for operation.

Chromeleon	Shimadzu	Values	Description
WaitForRemoteStart	INJ_IN	Run, Sync	Run: The autoinjector injects the samples immediately after drawing them.
			Sync: The injector waits for the manual injection signal before injecting the sample (read-only).

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation** 🔵 **Shimadzu LC-2010 HPLC System: Installation** and 🔵 **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

🔳 Shimadzu LC-10A and LC-2010 HPLC Systems: Pump

In addition to the standard pump commands (see **Commands for Controlling Dionex Devices** 🔳 **Dionex Pumps**), the commands and properties from the table below are available (please note that the display ➢*Filter* level determines which commands and properties are displayed).

📒 *Note:*

The names of the Shimadzu commands and properties may be slightly different, depending on version of your pump.

Property	Values	Description
⇒*%B, %C, %D*	0.0 - 100.0 %	Use %B, %C, and/or %D to determine the solvent composition.
CycleMode (Shimadzu: LPGE.M)	Auto OneCycle FourCycles	(Only available for LC-2010 and only for Pump_A in quaternary LPG pumps) Sets the cycle mode.
Degasser (Shimadzu: DEGAS)	On / Off	(Only for LC-2010) Turns the degasser on or off.
⇒*Flow*	0.000 - 9.999 ml/min	Sets the flow rate.
Motor	On/Off	(Only available for the first pump) Turns the (all) pump(s) on or off.
ModelVariant	Unknown Isocratic LPG HPG	Indicates the pump's operating mode (read-only). Set the operating mode on the **Pumps** tab page in the Server Configuration program.
Pressure	0 - 432 bar	Indicates the pressure (read-only).
Pressure.LowerLimit	LC-10A: 0 - 392 bar	Sets the lower pressure limit.
	LC-2010: 0 - 343 bar	

Property	Values	Description
Pressure.UpperLimit	LC-10A: 10 - 432 bar	Sets the upper pressure limit.
	LC-2010: 10 - 378 bar	
Solvent (Shimadzu: PASV, PBSV, PCSV)	A, B, C, D	(For pumps with connected FCV Solvent Selector) Selects the solvent.

▛☞ *Tips:*

*To use the **Motor On/Off**, **CycleMode**, and **Solvent** properties, you have to enter them manually in the PGM Editor.*

*The **CycleMode**, **Degasser**, and **Solvent** properties supported by Chromeleon correspond to the Shimadzu **LPGE.M, DEGAS,** and **PASV, PBSV,** and **PCSV** properties (see the table above).*

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation** ⟳ **Shimadzu LC-2010 HPLC System: Installation** and ⟳ **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

▣ Shimadzu LC-10A and LC-2010 HPLC Systems: Autosampler

In addition to the standard autosampler commands (see **Commands for Controlling Dionex Devices** ▣ **Dionex Autosamplers**), the commands and properties from the table below are available (please note that the display ➤*Filter* level determines which commands and properties are displayed).

▣ *Note:*

The names of the Shimadzu commands and properties may be slightly different, depending on version of your autosampler.

Command	Description
Purge	Purges the autosampler.
Wash	Washes the needle.

The table below lists the Shimadzu autosampler properties that are supported by Chromeleon.

🖎 *Tip:*

In Chromeleon, you can set the tray in the server configuration program, based on the Shimadzu MTPTRAY property. Sample processing in Chromeleon (Shimadzu MTP S.ORD property) is performed column by column.

Chromeleon	Shimadzu	Values	Description
HighPressure ValveRotations	SILNVLR	-	(Only for the LC-2010) Counts the rotations of the high-pressure valve (read-only).
InjectionCounter	INJCNT	-	Counts the injections (read-only).
LowPressure ValveRotations	SILNLVR	-	(Only for the LC-2010) Counts the rotations of the low-pressure valve (read-only)
NeedleStroke	NDLSTK	17-54 mm	Sets the distance that the needle moves down when drawing sample.
PurgeTime	P.MAXT	SIL-HT*A*/HT*C*: 0.1 - 25.0 s SIL-10ADvp: 0.1 - 10.0 s	(Only for the LC-10A) Sets the time for which the autosampler is purged.
SyringeSpeed	SMPSD	0.1-15.0 µl/s	Sets the speed with which the syringe draws the sample.
Temperature	CLTEMP	1-35 µl/s	(Only for the SIL-HTC) Sets the temperature for the cooling unit.
Volume	-	Standard syringe: 0.1-100.0 µl 2ml syringe: 0.1-2000 µl	Injection volume
WashCounter	SILNRNS	-	(Only for the LC-2010) Counts the needle wash operations (read-only).
WashDuration	DIPTIME	0-60 s	(Only for the SIL-HT*A*/HT*C*/10ADvp:) Sets the duration for the needle wash.

Chromeleon	Shimadzu	Values	Description
WashMode	RINSE.MODE	NoWash WashBeforeSample WashAfterSample Both	Determines when the needle is washed.
WashSpeed	RNSSPD	1-35 µl/s	Sets the speed with which the syringe draws the volume for washing the needle.
WashVolume	RNSVOL	1-2000 µl	Sets the volume with which the needle is washed.

☞ *Tips:*

*To use the **Purge** and **Wash** commands or the **NeedleStroke**, **WashVolume**, **WashSpeed**, **SyringeSpeed**, **PurgeTime**, **WashMode**, and **WashDuration** properties, you have to enter them manually in the PGM Editor.*

*The **NeedleStroke**, **WashVolume**, **WashSpeed**, **SyringeSpeed**, **Temperature**, **PurgeTime**, **WashMode**, and **WashDuration** properties are allowed only once in the program. Besides, they are allowed only at the time t = 0.000 min. Otherwise, an error message appears during the ➢Ready Check.*

Washing is always performed between two injections. Chromeleon waits for this either at the end of sampling or before the next injection.

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation** 🔄 **Shimadzu LC-2010 HPLC System: Installation** and 🔄 **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

▣ Shimadzu LC-10A HPLC System: Inject Synchronization

Mode	Injection	Standard Sample	Blank Run Sample
<u>Shimadzu</u> sampler installed in Chromeleon	Shimadzu Sampler (i.e., the program includes the Sampler.Inject command)	**WaitForRemoteStart** is not allowed. Chromeleon starts a 1-sample batch (i.e., a batch that contains one sample) on the system controller (SCL). The autosampler draws and injects the sample. Via the driver's internal inject port, Chromeleon generates an inject response upon the SCL start.	**WaitForRemoteStart** is not allowed. Chromeleon starts a 0-sample batch (i.e., a batch that does not contain a sample) on the system controller (SCL). Via the driver's internal inject port, Chromeleon generates an inject response upon the SCL start.
No <u>Shimadzu</u> sampler installed in Chromeleon	Manual injection via the SCL's contact input (i.e., an inject port is assigned)	**WaitForRemoteStart** must be set. Chromeleon starts a 1-sample batch on the SCL from the position "-1". The SCL waits until the contact is closed. Chromeleon changes the inject port state upon SCL start. The manual injection device detects this change and generates an inject response.	**WaitForRemoteStart** must be set. Chromeleon starts a 0-sample batch on the SCL. The SCL does not wait until the contact is closed. Chromeleon changes the inject port state upon SCL start. The manual injection device does not wait for a change of the inject port state. An inject response is generated immediately, instead.
	Inject response from another sampler (i.e., no inject port is assigned)	**WaitForRemoteStart** is not allowed. When the Sample2.Inject command is performed, the other sampler injects as usual. Upon receipt of the inject response, Chromeleon starts a 0-sample batch (i.e., a batch that does not contain a sample) on the SCL.	**WaitForRemoteStart** is not allowed. When the Sample2.Inject command is performed, the other sampler injects as usual. Upon receipt of the inject response, Chromeleon starts a 0-sample batch (i.e., a batch that does not contain a sample) on the SCL.

☞ *Tip:*

*The two cases described for **No sampler installed in Chromeleon** are true for **Sampler installed in Chromeleon**, also.*

▣ Shimadzu LC-10A HPLC System: SIL-10AD*vp* Sample Preparation

To perform sample preparation when the SIL10AD*vp* autosampler is part of the LC-10A HPLC System, you have to select the injection mode first.

 Note:

The names of the Shimadzu commands and properties may be slightly different, depending on version of your autosampler.

Chromeleon	Shimadzu	Values	Description
InjectMode	PR.MODE	Standard Advanced Dilution Reagent	Sets the mode for sample preparation. *Note:* *The Dilution and Reagent modes are not available for titer plates.*

Dilution and Reagent modes

Chromeleon supports the following properties (please note that the display ➢*Filter* level determines which properties are displayed):

Chromeleon	Shimadzu	Values	Description
Bubbling	BUBBLING	On/Off	On: Turns bubbling on, directing air through the sample.
MixRepeat	MIXCNT	1-10	Counts the mix operations after the diluent was added.
PrepTime	REACTM	0.1 - 120.0 min	Sets the time that the system waits after the mix operation has been completed.
PrepVolume	MIXVOL	1-1000 µl	Sets the volume that is drawn and dispensed for a mix operation.
SourceVial	S.VIAL	Depends on the rack type.	Specifies the sample container from which the injection is performed. (It is also possible to mix several substances in that container.)
			UpperHalf: Specifies the corresponding position in the upper half of the rack.
			R101, R102, R103: Additional reagent vials (if available on the rack)

Only Dilution mode:

In addition to the parameters listed in **Dilution** and **Reagent** modes above, Chromeleon supports the following properties (please note that the display ➤*Filter* level determines which properties are displayed):

Chromeleon	Shimadzu	Values	Description
DiluentVial	D.VIAL	Depends on the rack type.	Vial for the diluent
			Bottle: Large bottle
			R101, R102, R103: Additional reagent vials (if available on the rack)
DilutFactor	D.FACT	2-50	Ratio between the diluent and the original sample in the injected sample.
TotalVolume	T.VOL	200-1000 µl	Sets the volume that is to be present in the vial after dilution. (This volume should exceed the injection volume.)

Only Reagent mode

In addition to the parameters listed in **Dilution** and **Reagent** modes above, Chromeleon supports the following properties:

Chromeleon	Shimadzu	Values	Description
ReagentAVial	VIAL1	Depends on the rack type.	Reagent vials
ReagentBVial	VIAL2		UpperHalf: Specifies the corresponding position in the upper half of the rack.
ReagentCVial	VIAL3		R101, R102, R103: Additional reagent vials (if available on the rack)
ReagentAVolume	VOL1	1-1000 µl	Specifies the volume drawn from the reagent vial.
ReagentBVolume	VOL2		
ReagentCVolume	VOL3		
SourceVolume	S.VOL	1-1000 µl	Specifies the volume drawn from the vial.

For program examples, refer to:

🔺 **Program Example for the Standard Injection Mode**

🔺 **Program Example for the Reagent Injection Mode**

🔺 **Program Example for the Dilution Injection Mode**

☞ *Tip:*

*You can set the injection mode only if you have selected the Prep Loop option on the **Sampler**tab page in the Server Configuration program.*

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation** 🔵 **Shimadzu LC-2010 HPLC System: Installation** and 🔵 **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

🔳 Shimadzu LC-10A and LC-2010 HPLC Systems: Column Oven

In addition to the standard oven commands (see **Commands for Controlling Dionex Devices** 🔳 **Dionex Flow Manager and Thermostatted Column Compartments**), the commands and properties from the table below are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

📋 *Note:*

The names of the Shimadzu commands and properties may be slightly different, depending on version of your column oven.

Chromeleon	Shimadzu	Values	Description
AmbientTemperature	ROOM	5-85°C	(Only for the *vp* module) Indicates the ambient temperature (read-only).
TempCtrl	OVEN OVEN.T	On/Off	Enables or disables temperature control.
Temperature.Nominal	OVEN.T	CTO-10A: 4-80°C LC-2010: 4-60°C	Temperature setpoint for the column oven
Temperature.Upper Limit	T.MAX	CTO-10A: 5-85°C LC-2010: 5-65°C	Sets the upper temperature limit. Chromeleon aborts the sample batch and starts emergency handling if the current value exceeds the temperature limit.
Temperature.Value	TEMPm	CTO-10A: 4-85°C LC-2010: 4-65°C	Current temperature of the column oven

☞ *Tips:*

*You cannot set **Temperature.Nominal** and **TempCtrl** for retention times <0.000 min.*

*Each program may include only one **Temperature.UpperLimit** setting. In addition, the setting must be entered at the time t = 0.000 min. Otherwise, an error message appears during the ➢Ready Check.*

*Setting **Temperature.Nominal** automatically sets **TempCtrl** to **On**. If **TempCtrl** was set to **Off** before, **Log.TempCtrl=On** appears in the Audit Trail.*

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation ◉ Shimadzu LC-2010 HPLC System: Installation** and **◉ Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

◾ Shimadzu LC-10A and LC-2010 HPLC Systems: UV/VIS Detector

In addition to the standard detector commands (see **Commands for Controlling Dionex Devices ◾ Dionex Detectors**), the commands and properties from the table below are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

📒 *Note:*

The names of the Shimadzu commands and properties may be slightly different, depending on version of your detector.

Chromeleon	Shimadzu	Range	Description
CellTemp.Nominal	ACELL.T	Off, Low (40°C), High (50°C)	(Only for the LC2010) Sets the nominal cell temperature.
CellTemp.Value	ACELL.T		(Only for the LC2010) Indicates the current cell temperature (read-only).
ReferenceEnergy	AREFENG		Indicates the light energy in [mV] in the reference beam (read-only).
SampleEnergy	ASMPENG		Indicates the light energy in [mV] in the sample beam (read-only).
UV.Autozero	AZERO		Performs autozero.

Chromeleon	Shimadzu	Range	Description
UV.Mode²	ADUAL	Single Dual	See below
UV.Response²	ARESP	0.05; 0.1; 0.5; 1.0; 1.5; 2.0; 3.0; 6.0; 8.0; 10.0	Sets the time in [s] required by the detector signal to rise from 0% to 63% of the full-scale deflection.
UV.UV_Lamp UV.Visible_Lamp	ALAMP	Off/On	Specifies which lamp is to be turned on. Only one lamp at a time can be burning.
			Tip: *Changing from the UV lamp to the VIS lamp takes some time. Therefore, make sure that this does not interfere with sample processing.*
UV_VIS_1.Wavelength² UV_VIS_2.Wavelength²	AWAVE AWAVE2	SPD-10A*vp* and LC2010: 190-600 nm	Specifies the wavelength for the channel.
		SPD-10AV*vp* : D2: 190-370 nm W: 371-900 nm	
UVLampOnTime	AD2TIME		Counts the operating hours of the UV (D2) lamp (read-only).
VisLampOnTime	AWTIME		Counts the operating hours of the VIS (tungsten) lamp (read-only).

 Tips:

*To use the **UV_Lamp, Visible_Lamp, Mode**, and **Response** commands, you have to enter them manually in the Commands view of the PGM Editor. This also applies to any additional Autozero commands.*

*You cannot set **UV_VIS_X.Wavelength**, **UV.Lamp**, **UV.Response**, and **UV.Autozero** for retention times <0.000 min.*

Data acquisition can be started only at the retention time t = 0.000 min.

The system automatically performs an Autozero command at t = 0.1 min. Therefore, leaps may occur in the absorption at the beginning of the chromatogram.

The wavelengths must be appropriate for the lamp that is currently burning: When the UV (D2) lamp is burning, both wavelengths must be between 190 and 370 nm.

For the SPD-10AVvp with tungsten lamp, both wavelengths must be between 371 and 700 nm or between 701 and 900 nm. For the LC-2010 and the SPD-10Avp with tungsten lamp, both wavelengths must be between 371 and 600 nm. If the one or both wavelengths are not in these ranges, an Abort message appears during the ➢Ready Check and the program does not start. Manual data acquisition is limited to 100 min. Chromeleon automatically aborts manual data acquisition on all channels after that time.

² **Single Wavelength Mode and Dual Wavelength Mode**

To perform data acquisition on two channels (dual wavelength mode: Mode = Dual), two channels must be enabled on the **UV/VIS** tab page in the Server Configuration program. If only one channel is enabled, the UV.Mode property is not available. Thus, the detector runs always in single wavelength mode (Mode = Single).

If two channels were enabled, the mode depends on the number and type of the channels to be recorded. If two channels are requested for data acquisition, the detector runs in dual wavelength mode. If only the first channel is requested (UV_VIS_1), the detector runs in single wavelength mode. However, if only the second channel is requested for data acquisition (UV_VIS_2), dual wavelength mode is required to make the second channel available. If the detector is configured only for channel 2, a warning appears during the Ready Check. It is still possible to start the program.

When the detector runs in dual wavelength mode, the sampling step is 1.2 s per channel. Therefore, this mode is appropriate only if the width of the smallest peak is at least 7.2 s at 50% of the peak height.

Response settings <= 1.0s are allowed only in single wavelength mode. In all other cases, an error message appears during the Ready Check and the program cannot start.

When the mode is changed, Chromeleon automatically disconnects and reconnects the HPLC system. Thus, changing the mode is possible only if no other program is running. When the mode is changed, instrument settings may be reset to the default settings. Therefore, a warning appears for each program that changes the mode. However, the program can be started, nevertheless.

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation** 🔄 **Shimadzu LC-2010 HPLC System: Installation** and 🔵 **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

◼ Shimadzu LC-10A and LC-2010 HPLC Systems: General Tips

LC-10A and LC-2010

- When the system is operated in stand-alone mode, the system conditions, such as the oven temperature, are reset to the initial values at the end of the sample program (called Time Program by Shimadzu). **If Chromeleon controls the HPLC System, the system conditions entered last are retained.**

- Chromeleon also disconnects and automatically reconnects to the HPLC System when you change the **Mode** parameter (single or dual wavelength) for the **UV detector**. This may take some time. After reconnect, an audible beep sounds. Make sure that this process does not interfere with sample processing.

LC-10A

- Parameter settings changed on the instrument in stand-alone operation are lost when Chromeleon connects to the instrument. Chromeleon always restores the preset values when connecting to the instrument.

- Changes made in Chromeleon do not appear immediately on the ANALYSIS FILE and TIME PROGRAM menus on the System Controller. To see the changes, exit and reenter the corresponding display. The MONITOR menu is updated immediately. However, the nominal values are not displayed.

- Depending on the operation (with either the Shimadzu sampler or a manual injection valve), Chromeleon sets the **WaitForRemoteStart** property automatically to the appropriate value for the selected option.

- Chromeleon disconnects and automatically reconnects to the HPLC System when you change the setting for **ExternalStart** or when Chromeleon changes the setting for the **WaitForRemoteStart** property (see above). Make sure that this process does not interfere with sample processing.

- To change from the UV lamp to the VIS lamp takes considerable time. Make sure that this process does not interfere with sample processing.

LC-2010

- If the HPLC System detects an error or if data acquisition is active, you cannot connect to Chromeleon.

- Parameter settings changed on the instrument in stand-alone operation are lost when Chromeleon connects to the instrument. Chromeleon always restores the preset values when connecting to the instrument:

  WashDuration: 0
  WashMode: NoWash
  PurgeTime: 25.0
  WashSpeed: 35
  WashVolume: 200
  SyringeSpeed: 15.0
  NeedleStroke: 52

  SourceVial: UpperHalf
  DiluentVial: Bottle
  TotalVolume: 400
  Sampler.DilutionFactor: 2
  MixRepeat: 3
  PrepVolume: 200
  Bubbling: On
  PrepTime: 1.0

  SourceVolume: 100
  ReagentAVolume: 100
  ReagentBVolume: 100
  ReagentCVolume: 100
  ReagentAVial: None
  ReagentBVial: None
  ReagentCVial: None

- Changes made in Chromeleon do not appear immediately on the METHOD menu. To see the changes, exit and reenter the corresponding display. The MONITOR menu is updated immediately. However, the nominal values are not displayed.

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation ⮕ Shimadzu LC-2010 HPLC System: Installation** and **⮕ Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

◾ Shimadzu LC-10A HPLC System: Program Example for the Standard Injection Mode

👉 *Tip:*

The Shimadzu devices support only programs that start at the time t = 0.000 min. It is not possible to perform commands at negative retention times. If the program includes a command at a negative retention time, an abort error occurs during the ➢Ready Check.

The following program is an example for sample preparation with the Shimadzu SIL-10ADvp autosampler in Standard injection mode:

```
; Pump settings:
   Pressure.LowerLimit =   2
   Pressure.UpperLimit =   150
   %A.Equate = "Water"
   %B.Equate = "Methanol"
   Flow =   1.000
   %B =  80.0

; Detector settings:
   Mode =   Dual
   Response =  2.00
   UV_VIS_1.Wavelength =   272
   UV_VIS_1.Step =   0.20
   UV_VIS_1.Average =   On
   UV_VIS_2.Wavelength =   254
   UV_VIS_2.Step =   0.20
   UV_VIS_2.Average =   On


; The following autosampler settings are valid for each InjectMode:
   WashMode = WashAfterSample
;   Determines   when   the   needle   is   washed (Options:   NoWash,
WashBeforeSample   (before   injection),   WashAfterSample   (after
injection), Both (before and after injection)

   WashSpeed = 35
; Syringe speed when washing the needle

   WashVolume =   1
; Volume used for washing the sample loop

   SyringeSpeed = 15.0
; Speed with which the syringe draws the sample

   NeedleStroke = 52
```

```
; Indicates how deep the needle moves into the vial when drawing the
sample

   Position =  0
   Volume = 5
; You can specify the vial position in the PGM File or in the sample
list of the sequence.

   InjectMode =   Standard

; Injection and data acquisition:
 0.000   UV.Autozero
   Inject
   UV_VIS_1.AcqOn
   UV_VIS_2.AcqOn


10.000   UV_VIS_1.AcqOff
   UV_VIS_2.AcqOff
   End
```

For other example programs, refer to:

🔲 **Shimadzu LC-10A HPLC System: Program Example for the Reagent Injection Mode**

🔲 **Shimadzu LC-10A HPLC System: Program Example for the Dilution Injection Mode**

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation** 🔵 **Shimadzu LC-2010 HPLC System: Installation** and 🔵 **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

🔳 Shimadzu LC-10A HPLC System: Program Example for the Reagent Injection Mode

👉 *Tip:*

The Shimadzu devices support only programs that start at the time t = 0.000 min. If the program includes a command at a negative retention time, an abort error occurs during the ➢Ready Check.

You can only set the injection mode if you have selected the **Prep Loop** option on the **Sampler** tab page in the Server Configuration program.

The following program is an example for sample preparation with the Shimadzu SIL-10ADvp autosampler in Reagent injection mode:

```
; Pump settings:
   Pressure.LowerLimit =   2
   Pressure.UpperLimit =   150
   %A.Equate = "Water"
   %B.Equate = "Methanol"
   Flow =   1.000
   %B =  80.0

; Detector settings:
   Mode =   Dual
   Response =  2.00
   UV_VIS_1.Wavelength =   272
   UV_VIS_1.Step =   0.20
   UV_VIS_1.Average =   On
   UV_VIS_2.Wavelength =   254
   UV_VIS_2.Step =   0.20
   UV_VIS_2.Average =   On

; Settings for the Reagent InjectMode:
   InjectMode =   Reagent
   SourceVial =   51
   SourceVolume = 20
; 20 µl of air is automatically drawn first, and then the source
volume, e.g., 20 µl, is drawn from position 51. Finally, 30 µl (=
source volume + 10 µl) are delivered to the injection vial (defined
position).
; Afterward, a Wash command is executed for the sample loop and the
needle, using the settings for WashVolume and WashSpeed.

   ReagentAVial = 30
   ReagentAVolume =  5

   ReagentBVial = 31
   ReagentBVolume =  10

   ReagentCVial = 32
   ReagentCVolume =  15

; For each reagent, 20 µl of air is automatically drawn first, and
then the reagent volume, e.g. AVolume = 5 µl, is drawn from the
indicated position. The reagent volume + 10 µl (here = 15 µl) are
then delivered to the injection vial (defined position).
```

; Each time after a reagent has been delivered to the injection vial,
a Wash command is performed for the sample loop and the needle, using
the settings for WashVolume and WashSpeed. After the last reagent has
been delivered to the injection vial, the contents of the vials is
mixed; no additional wash cycle is performed. For information about
the general commands, refer to the previous topic: 🔲 **Program Example
for the Standard Injection Mode.**

```
   Position =  0
   Volume = 5
```

; The position of the injection vial is the position at which the
sample and the reagent are mixed and from which injection is
performed. You can specify the position in the PGM File or in the
sample list of the sequence.

; Settings for the mixing cycle
```
   MixRepeat = 3
   PrepVolume =   25
```
; Number of mixing cylces that are performed after the SourceVolume
and the Reagent have been delivered to the injection vial. The
PrepVolume is drawn and expelled for each mixing cycle.

```
   Bubbling =  Off
```
; If Bubbling is set to On, the PrepVolume is used as volume.
; After all mixing cycles have been performed, a Wash command is
executed for the sample loop and the needle, using the settings for
WashVolume and WashSpeed.

```
   PrepTime =  1.0
```
; Indicates the time the system waits after the mixing cycles have
been completed (here: 1.0 min).

; Injection and data acquisition:
```
 0.000   UV.Autozero
   Inject
   UV_VIS_1.AcqOn
   UV_VIS_2.AcqOn

10.000   UV_VIS_1.AcqOff
   UV_VIS_2.AcqOff
   End
```

For another example program, refer to 🔲 **Shimadzu LC-10A HPLC
System: Program Example for the Dilution Injection Mode**.

For information about how to install the Shimadzu HPLC Systems, refer to
Hardware Installation 🔵 Shimadzu LC-2010 HPLC System: Installation
and **🔵 Shimadzu LC-10A HPLC System: Installation** in the
Administrator Help section.

▣ Shimadzu LC-10A HPLC System: Program Example for the Dilution Injection Mode

▣🖙 *Tip:*

The Shimadzu devices support only programs that start at the time t = 0.000 min. If the program includes a command at a negative retention time, an abort error occurs during the ➢Ready Check.

You can only set the injection mode if you have selected the **Prep Loop** option on the **Sampler** tab page in the Server Configuration program.

The following program is an example for sample preparation with the Shimadzu SIL-10ADvp autosampler in Dilution injection mode:

```
; Pump settings:
   Pressure.LowerLimit =   2
   Pressure.UpperLimit =   150
   %A.Equate = "Water"
   Flow =   1.000
   %B =  0.0

; Detector settings:
   Mode =   Dual
   Response =  2.00
   UV_VIS_1.Wavelength =   272
   UV_VIS_1.Step =   0.20
   UV_VIS_1.Average =   On
   UV_VIS_2.Wavelength =   254
   UV_VIS_2.Step =   0.20
   UV_VIS_2.Average =   On

; Settings for the Dilution InjectMode
; The commands below are necessary to allow the autosampler execute
the desired actions:
   InjectMode =   Dilution
   SourceVial =   30
; 20 µl of air is automatically drawn first, and then the calculate
source volume, e.g., 200 µl, is drawn from position 30. Finally, 210
µl (= source volume + 10 µl) are delivered to the injection vial
(defined position).
; Afterward, a Wash command is executed for the sample loop and the
needle, using the settings for WashVolume and WashSpeed. For
information about the general commands, refer to the previous topic:
```

➢ ▣ **Program Example for the Standard Injection Mode.**

```
   DiluentVial =  51
; 20 µl of air is automatically drawn first, and then the calculated
DilutVolume, e.g. 200 µl, is drawn from the position 51. Afterward,
410 µl (= DilutVolume + 10 µl) are then delivered to the injection
vial (defined position).

   TotalVolume =  600
; Volume that shall be delivered to the injection vial after dilution
(should exceed the injection volume).

   DilutFactor =  3
; Ratio between the diluent/sample ratio in the resulting mixture. If
the total volume is 600 µl and if the dilution factor is 3, 200 µl
are drawn from the SourceVial and 400 µl are drawn from the diluent
vial and delivered to the injection vial.

   Position =  0

   Volume = 5

; The position of the injection vial is the position at which the
sample and the reagent are mixed and from which injection is
performed. You can specify the position in the PGM File or in the
sample list of the sequence.

   MixRepeat = 3
   PrepVolume =  300
   Bubbling =  Off
   PrepTime =  1.0

; Injection and data acquition:
 0.000   UV.Autozero
   Inject
   UV_VIS_1.AcqOn
   UV_VIS_2.AcqOn

 3.000   UV_VIS_1.AcqOff
   UV_VIS_2.AcqOff
   End
```

For another example program, refer to **Shimadzu LC-10A HPLC System: Program Example for the Reagent Injection Mode**.

For information about how to install the Shimadzu HPLC Systems, refer to **Hardware Installation** **Shimadzu LC-2010 HPLC System: Installation** and **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

▣ Shimadzu LC-10A and LC-2010 HPLC Systems: Troubleshooting

LC-10A and LC2010

- If communication to the Chromeleon **cannot** be **re-established** after a **fatal communication error**, the Chromeleon sever might not run stable afterward (due to the Shimadzu software). Restart the server to solve the problem.

- When **communication** to Chromeleon is **interrupted**, the instrument may be in KEYLOCK state afterward. In this case, it is not possible to control the instrument by Chromeleon. Cancel KEYLOCK state by pressing the F5 key from the System Controller's Main menu and enter the system password.

- Chromeleon uses the **ANALYSIS FILE #0** file and the associated **TIME PROGRAM**. Thus, changes made while the instrument is operated in stand-alone mode are lost when you reconnect to Chromeleon later.

LC-10A

- *Error:* "[Abort] {HPLC_System} Fatal Instrument Error (NOT LINKED, 0)."

- *Cause:* This error occurs if the server configuration includes an instrument that is not connected to the LC-10A system

- *Remedial action:* Adapt the server configuration to the actually installed instruments.

- *Error:* "[Error] 09:00:58 {HPLC_System} Error in task Remote: Configuration settings mismatch with the actual connection."

- *Cause:* This error may occur if the operating mode set in the server configuration on the **Pumps** tab page does not match the setting on the instrument.

- *Remedial action:* Check and change the operating mode on the device if necessary:

  1. Press the key 1 to open the ANALYSIS FILE menu.

  2. Press the F3 key (T.PROG) to open the TIME PROGRAM menu.

  3. Press F1 (P.CTRL), and then use the F1 - F4 key to select the operating mode (ISO, B.GE, T.GE, LPGE).

LC-2010

- When configuring the LC_2010 HPLC System for the first time in the Chromeleon Server Configuration program, the following error may occur:

- "[Error] 10:50:51 {HPLC_System} Error in task PollCfg: Configuration settings mismatch with the actual HPLC system."This error may indicate that you have installed an LC-10A HPLC System in Server Configuration program. Uninstall the LC-10A HPLC System and install the LC-2010 HPLC System instead.

- "[Error] 10:51:27 {HPLC_System} Error in task PollCfg: Invalid Parameter."This error may indicate that self-test of the LC-2010 HPLC system failed. Check the system and press **CE** or restart the system if necessary.

- "[Error] 17:51:25 {HPLC_System} Error in task Remote: Invalid function."This error may indicate that the LC-2010 HPLC System was not in Ready state when the Connect command was issued. Check the system; stop data acquisition, or perform a SIL rinse operation if necessary.

- In very rare cases, it may happen that the LC-2010 displays "Calculating…" and seems to be frozen. You may have to wait some time until the system responds again. If you do not want to wait, you may turn the system off and on again and perform the following steps:

☞ *Tip:*

If you perform these steps, ALL settings, including the communication, start, and shutdown settings, are reset to the factory defaults. This means that you have to reenter all settings on the system (for example, the settings described in the Device Settings section in the ***Administrator Help*** *section:* ***Hardware Installation*** 🔄 ***Shimadzu LC-2010 HPLC System: Installation***).

1. Press the [VP] key.

2. Press the [F4](CALIB) key.

3. Enter the login ID "00000" on the system.

4. Press the [F2](OK) key.

5. Select "7: Initialize Parameters."

6. Press the [Enter] key.

7. Press the [F2](OK) key.

8. Restart the system.

- *Problem***:** Only 100 µl are injected although the injection volume is larger than 100 µl and the 2000 µl syringe was configured in the Server Configuration program.

  *Reason***:** The 100 µl injection was set on the device. Set the 2000 µl syringe on the device, following the steps in the **Device Settings** section in the **Shimadzu LC-2010 HPLC System: Installation** topic in the **Administrator Help** section.

For a list of error messages that might appear in Chromeleon refer to the Error Levels tab page in the Server Configuration program.

For information about how to install the Shimadzu LC-10A HPLC System, refer to **Hardware Installation** 🔵 **Shimadzu LC-10A HPLC System: Installation** in the **Administrator Help** section.

🔲 Shimadzu GC-2010 GC System: Commands and Tips

For information about the special commands that Chromeleon supports for the Shimadzu GC-2010 GC system and for tips for practical operation, refer to:

🔲 **General**

🔲 **Autosampler**

🔲 **Inlet (General)**

🔲 **Inlet (Split/Splitless SPL-2010)**

🔲 **Inlet (On Column OCI/PTV-2010)**

🔲 **Detectors**

▣ Shimadzu GC-2010 GC System: General

Please note that the display ➤*Filter* level determines which commands and properties are displayed:

Command/Property	Range/Values	Description
AmbientTemperature	-5°C to 80°C	Reports the ambient temperature (read-only).
AtmosphericPressure	47 to 107 kPa	Reports the atmospheric pressure (read-only).
Connect		Connects the device to the data system and enables control from the PC. The connect command fails if a method is started or another software controls the GC.
Connected	Connected/Disconnected	Indicates whether the device is connected to Chromeleon, i.e., whether it is controlled from the PC (see Connect, Disconnect).
Disconnect		Disconnects the device from the data system and enables manual control from the device's control panel.
EquilibrationTime	0 min to 9999.99 min	Indicates the time that the GC waits after reaching the target temperature.
ErrorCode	0 to 1000	Indicates the error code if an error occurred (read-only).
FirmwareVersion		Indicates the firmware version of the device (read-only).
GC-System	On/Off	Activates the GC according to the parameters on the system's main screen.
OverTemp	OK, Fault	Indicates the temperature status of the column oven (read-only).
Ready	Ready/NotReady	Indicates whether the GC is ready for injection (read-only). Enter a **Wait GC Ready** command directly before the injection. This ensures that the temperature gradient is performed in due time.
Reset		Restores the default settings.
RunState	Ready, NotReady, Diagnosis, Baking, Run, Pre-Run, Post-Run	Reports the GC status (read-only).
SerialNo		Reports the serial number of the GC (read-only).

Command/Property	Range/Values	Description
TempCtrl	On/Off	Turns column oven's temperature control on or off.
Temperature		Sets the oven temperature [°C].
Temperature.Nominal	0°C to 450°C	Sets the target operating temperature for the column oven. The temperature ramp can be programmed with a maximum rate of up to 250°C/min and up to 20 steps (not counting the first one).
Temperature. UpperLimit	50°C to 450°C	Determines the maximum allowed oven temperature. The GC interrupts the power supply for the oven if the temperature exceeds the upper limit.
Temperature.Value	0°C to 450°C	Reports the actual oven temperature (read-only).

For an overview of the system, refer to **Hardware Installation** **Shimadzu GC-2010 GC System: Overview** in the **Administrator Help** section.

For information about how to install the system, refer to **Hardware Installation** **Shimadzu GC-2010 GC System: Installation**.

Shimadzu GC-2010 GC System: Autosampler

Command/Property	Range/Values	Description
AirGap	Yes, No	Allows using an air gap of 1 µl after the sample was drawn into the syringe.
DwellTime	0.0 s to 99.9 s	Indicates the dwell time after the injection and before the needle is removed from the inlet.
ErrorCode	0 to 33	Indicates the error code of the sampler or injector if an error occurred.
Inject	Blank Position Volume	Performs an injection using the specified parameters.
InjectMode	SampleAir SolventAirSampleAir SolventSampleAir SolventAirStdAirSampleAir SolventStdSampleAir	Sets the inject mode.

Command/Property	Range/Values	Description
InjectWaitTime	0.0 s to 10,000,000 s.	Specifies the time between the inject command and the response of the autosampler.
VialType	1.5 ml, 4 ml	Indicates the vial type.
LoadsPerInject	1 to 99	Sets the number of injections per sample.
DispenseSpeed	Slow, Middle, Fast	Sets the injection speed.
DrawSpeed	Slow, Middle, Fast	Sets the drawing speed.
Position		Sets the position of the sample in the rack.
PreSolventWash	0 to 99	Sets the number of pre-injection cleaning cycles during which the needle is washed with a solvent. Applies only when the selected injection mode is 0 (SampleAir).
Pumps	0 to 99	Determines how often the syringe plunger moves up and down before the sample is actually drawn. This eliminates gas bubbles, and thus enhances reproducibility.
RackType	Short Rack (1-6) Long Rack (1-12)	Displays the rack type.
SampleWash	0 to 99	Sets the number of cleaning cycles with sample before injection.
SolventSelect	All A only B only C only	Select the solvent.
SolventWash	0 to 99	Sets the number of post-injection cleaning cycles during which the needle is washed with a solvent.
SpeedOfPlunger	Slow, Middle, Fast	Sets the plunger speed for pumping or cleaning the syringe with sample liquid.
State	On, Off	Indicates the injection state (read-only): On = The injection has been performed Off = The injection has not yet been performed
Status		Indicates the autosampler's status (read-only).
SyringeSpeed	Slow, Fast	Sets the syringe speed with which the syringe descends into the vial.

Command/Property	Range/Values	Description
SyringeHeightDown	0 to 2 mm (for 1.5 ml vials) 0 to 10 mm (for 4 ml vials) default: 0 mm	Sets how deep the needle descends into the vial. The **Down** parameter defines a position *below* the default Shimadzu position.
SyringeHeightInj	0 to 22 mm default: 0 mm	Defines how deep the needle descends into the injector during sample injection.
SyringeHeightUp	0 to 20 mm default: 0 mm	Sets how deep the needle descends into the vial. The **Up** parameter defines a position *above* the default Shimadzu position.
Syringe	10 µl, 50 µl, 250 µl	Indicates the syringe type (read-only).
Viscosity	0.0 s to 99.9 s	Sets the number of seconds that the pressure plunger of the syringe pauses after the pump and injection strokes. (Adapt this value to the viscosity of the sample.) While drawing sample before the injection, the system pauses for the defined time or a minimum of 4 seconds. The waiting time during the solvent wash is always 0.2 seconds.
Volume	0.1 µl to 8.0 µl	Specifies the injection volume of the sample.

For an overview of the system, refer to **Hardware Installation** **Shimadzu GC-2010 GC System: Overview** in the **Administrator Help** section.

For information about how to install the system, refer to **Hardware Installation** **Shimadzu GC-2010 GC System: Installation**.

■ Shimadzu GC-2010 GC System: Inlet (General)

The following commands and properties are available for all inlets:

Command/Property	Range/Values	Description
ColumnFlow		Indicates the carrier gas flow properties [ml/min].
*.Nominal	0 to 1200 ml/min	Sets the nominal flow for the carrier gas at the output of the capillary column. The temperature ramp can be programmed with a maximum rate of up to 400 ml/min and up to seven steps (not counting the first one).
*.Value		Indicates the current carrier gas flow measured at the output of the capillary column.
Diameter	0.01 mm to 6.00 mm	Indicates the internal diameter of the column (read-only).
FilmThickness	0.0 μm to 300.0 μm	Indicates the film thickness of the column.
FlowCtrl	On, Off	Turns the column flow on or off.
FlowMode	Pressure; Velocity; Flow	Determines which parameter is controlled for the column.
GasSaver	On, Off	Turns the gas saver on or off.
GasSaverSplitRatio	0.0 to 9999.9	Sets the split ratio to reduce the the split flow and thus carrier gas consumption.
GasSaverTime	0 to 9999.9 min	Sets the retention time when the gas saver shall become active. The time setting must be after the injection and purge times.
GasType	He, N2, H2, Ar	Indicates the type of carrier gas.
Length	0.1 m to 250.0 m	Indicates the column length.
Mode	Split, Splitless, Direct	Indicates the split mode.
PulseEnable	Off, On / Auto	Turns the pressure pulse on or off.
PulsePressure	0 to 970 kPa	Sets the input pressure for high-pressure injection.
PulseTime	0 to 9999,9 min	Sets the duration of the pressure pulse.
Pressure		Indicates the properties of the inlet's column pressure [kPa].
*.Nominal	0.0 to 979.0 kPa	Sets the nominal pressure of the column (read-only).
*.Value		Indicates the current pressure in the column head.

Command/Property	Range/Values	Description
PurgeCtrl	On, Off	Turns the purge option on or off.
PurgeFlow		Provides information on the purge flow [ml/min].
*.Nominal	0.0 to 1200.0 ml/min	Sets the nominal purge flow. The flow ramp can be programmed with a maximum rate of up to 400 ml/min and up to seven steps (not counting the first one).
*.Value		Indicates the current purge flow.
SplitRatio		Specifies the split ratio. Programmable with up to seven steps.
*.Nominal	-1.0 to 9999.9	Sets the nominal value for the split ratio.
*.Value		Indicates the current split ratio (read-only).
Temperature		Sets the temperature properties [°C].
*.Value	0°C to 450°C	Sets the current inlet temperature (read-only).
*.Nominal	0°C to 450°C	Sets the target operating temperature for the inlet. When the PTV type is used, use this parameter to program a temperature ramp with a rate of up to 250°C/min and up to seven steps (not counting the first one).
*.UpperLimit	10°C to 470°C	Sets the maximum inlet operating temperature.
TotalFlow		Provides information on the total flow [ml/min].
*.Nominal	0.0 to 1200 ml/min	Determines the total flow calculated from the sum of the column flow rate, split flow rate and the flow in the column's purge head septum.
*.Value		Indicates the current total flow.
Type	Unknown PTV-2010 SPL-2010	Indicates the selected inlet type (read-only).
Velocity		Indicates the linear speed in the column [cm/s].
*.Nominal	From 0 to a value where the calculated column input pressure is 970 kPa.	Sets the average linear speed of the carrier gas in the column.
*.Value		Indicates the current linear speed in the column (read-only).

For more information on additional commands and properties that are available for certain inlet types, refer to:

🔳 **Shimadzu GC-2010 GC System: Inlet (Split/Splitless SPL-2010)**

🔳 **Shimadzu GC-2010 GC System: Inlet (On Column OCI/PTV-2010)**

For an overview of the system, refer to **Hardware Installation** 🔵 **Shimadzu GC-2010 GC System: Overview** in the **Administrator Help** section.

For information about how to install the system, refer to **Hardware Installation** 🔵 **Shimadzu GC-2010 GC System: Installation**.

🔲 Shimadzu GC-2010 GC System: Inlet (Split/Splitless SPL-2010)

In addition to the standard inlet commands (see 🔳 **Inlet (General)**), the following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Command/Property	Range/Values	Description
PurgeTime	0.00 to 9999.99 min	In splitless mode, this parameter sets the period of time during which the flow is not split. After this period, the flow corresponds to the flow set in the split mode.

Also, refer to 🔳 **Shimadzu GC-2010 GC System: Inlet (On Column OCI/PTV-2010)**.

For an overview of the system, refer to **Hardware Installation** 🔵 **Shimadzu GC-2010 GC System: Overview** in the **Administrator Help** section.

For information about how to install the system, refer to **Hardware Installation** 🔵 **Shimadzu GC-2010 GC System: Installation**.

■ Shimadzu GC-2010 GC System: Inlet (On Colum OCI/PTV-2010)

In addition to the standard inlet commands (see ■ **Inlet (General)**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Command/Property	Range/Values	Description
FanTemperature	0°C to 100°C	Sets the cooling temperature of the air fan.

Also, refer to ■ **Shimadzu GC-2010 GC System: Inlet (Split/Splitless SPL-2010)**.

For an overview of the system, refer to **Hardware Installation** 🌐 **Shimadzu GC-2010 GC System: Overview** in the **Administrator Help** section.

For information about how to install the system, refer to **Hardware Installation** 🌐 **Shimadzu GC-2010 GC System: Installation**.

■ Shimadzu GC-2010 GC System: Detectors

In addition to the standard detector commands (see ■ **Dionex Detectors**), the following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Command/Property	Range/Values	Description
AirCtrl	On, Off	Turns the air flow control on or off.
AirFlow	0.0 to 1200.0 ml/min	Sets the air flow.
AutoAutoZero	On, Off	If activated, autozero is performed automatically.
AutoIgnite	On, Off	Turns automatic ignition on or off.
AutoReIgnite	On, Off	Turns automatic re-ignition on or off.
AutoZero		Performs an autozero, i.e., sets the signal to 0.
DetectorCtrl	On, Off	Turns the detector on or off.
DetectorType	FID, ECD	Indicates the selected detector type (read-only).
Extinguish		Extinguishes the flame.

Command/Property	Range/Values	Description
FilterTimeConst	4 ms, 5 ms, 10 ms, 20 ms, 50 ms, 100 ms, 200 ms, 500 ms, 1 s, 2 s	Sets the time constant for the noise filter of the detector.
Flame	On, Off	Turns the flame on or off.
H2Ctrl	On, Off	Turns the H2 gas flow control on or off.
H2Flow	0.0 to 1200.0 ml/min	Sets the H2 gas flow.
Ignite		Ignites the flame.
MakeUpCtrl	On, Off	Turns the makeup gas flow control on or off.
MakeUpFlow	0.0 to 1200.0 ml/min	Sets the makeup gas flow.
MakeUpGasType	He, N2, H2, Ar	Enter the current gas type of the makeup gas.
Temperature		Sets the detector oven temperature.
*.Nominal	0.0 to 450.0°C	Sets the nominal detector oven temperature.
*.UpperLimit	10°C to 470°C	Sets the maximum detector oven temperature.
*.Value	0.0 to 450.0°C	Indicates the actual detector oven temperature.

For an overview of the system, refer to **Hardware Installation** 🔵 **Shimadzu GC-2010 GC System: Overview** in the **Administrator Help** section.

For information about how to install the system, refer to **Hardware Installation** 🔵 **Shimadzu GC-2010 GC System: Installation**.

▪ Shimadzu SPD-M10Avp/M20A PDA Detector

In addition to the ▪ **General Properties** and the standard detector commands (see **Commands for Controlling Dionex Devices** ▪ **Dionex Detectors**), the commands and properties from the table below are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Detector Commands and Properties

SPD-M10Avp and SPD-M20A:

Command/Property	Range/Values	Description
Data_Collection_Rate	0.50000 to 20.00000 Hz	Sets the data collection rate in [Hz].
		▪ *Tip:*
		Changes to this parameter affect the 3D signal and the channel signals.
OperatingHours	0.0 to 1000000.0 hours	Displays the total duration of exposure of the photo diode array (read-only).
LampMode		Specifies the lamp mode.
	Off	No lamp
	UV	Only UV lamp
	VIS	Only VIS lamp
	Both	UV and VIS lamp
ModelVariant		Reports the detector ID (read-only).
Response	0 ms (no averaging), 25 ms (SPD-M20A only), 80 ms, 160 ms, 240 ms, 320 ms, 640 ms, 1280 ms, 2000 ms	Sets the time filter of the measuring data.
		▪ *Tip:*
		Changes to this parameter affect the 3D signal and the channel signals.
SamplingTime	25 ms (SPD-M20A only), 80 ms, 160 ms, 240 ms 320 ms, 640 ms, 1280 ms, 2000 ms.	Displays the time between two scans (read only). Corresponds to the reciprocal Chromeleon value of the data collection rate. Exception: For 20 Hz (SPD-M20A), the sampling time is 25 ms.
Status		Displays the statuses of the data collection and the detector (read-only).
UVExposureTime	0.0 to 1000000.0 ms	Reports the exposure time of the photo diode for the current slit width when a UV (D2) lamp or a UV and VIS (tungsten) lamp are used simultaneously (read-only).

Command/Property	Range/Values	Description
UVLampOnTime	0.0 to 000000.0 hrs	Counts the operating hours of the UV lamp since the lamp was replaced last (read-only).
ValidationDate		Reports the date when the device was validated last (read-only).
VISExposureTime	0.0 to 1000000.0 ms	Reports the exposure time of the photo diode when a VIS (tungsten) lamp is used (read-only).
VisLampOnTime	0.0 to 1000000.0 hrs	Counts the operating hours of the VIS lamp since the lamp was replaced last (read-only).
VISVoltage	0.0 to 1000000.0 V	Reports the operating voltage of the VIS lamp (read-only).

SPD-M20A only:

Command/Property	Range/Values	Description
SlitWidth	1.2 nm or 8 nm	Sets the width of the micro slit. A small slit increases spectral resolution. A wide slit improves the signal-to-noise ratio.
TempCtrl	On or Off	Turns temperature control of the flow cell on or off.
Temperature		Indicates the flow cell temperature.
Temperature. Nominal	9°C to 50°C, default: 40°C, Off = 0	Sets the nominal flow cell temperature.
Temperature. Value		Reports the actual flow cell temperature.

UV-VIS Channel Properties and Commands

The following properties refer to digital single-channel data only:

Command/Property	Range/Values	Description
Average	ON OFF	Averages two data points. ON: The complete step interval is used for averaging (recommended). OFF: Only the last sample of a step is used.
Bandwidth	1 nm to 64 nm	Sets the bandwidth. The higher the bandwidth is, the better is the signal-to-noise ratio.
		☞ *Tip:*
		Changes to this parameter affect the digital channel data and the analog signal of the optional D/A board.

Command/Property	Range/Values	Description
Polarity	Positive Negative	Sets the polarity of the analog output signal of the corresponding channel. 🖙 *Tip:* *This property is available only for the SPD-M20A or when the D/A converter board is selected in the Chromeleon Server Configuration program. The setting does not affect the shape of the digital signal.*
Range	500, 1000, 1250, 2000, 2500, and 4000 mAU/V.	Sets the output range of the analog output of the corresponding channel. 🖙 *Tip:* *This property is available only for the SPD-M20A or when the D/A converter board is selected in the Chromeleon Server Configuration program. The setting does not affect the shape of the digital signal.*
Retention	0.000 min to 10000000.000 min	Specifies the signal retention time.
Signal		Indicates the signal properties.
Signal.Value	-2147483.750 to 0.000 mAu	Reports the current signal value.
Signal.UpperLimit **Signal.LowerLimit**	-2147483.750 to 0.000 mAu	Sets the upper or lower limit for the signal. 🖙 *Tip:* *If the actual value is above the upper limit or below the lower limit, Chromeleon aborts the batch and starts emergency handling.*
Step	0.001 to 4.80 s Auto	Sets the step. The default setting is Auto. Dionex recommends using this setting because the optimum step is selected dynamically.
Wavelength	190 nm to 800 nm	Sets the wavelength. 🖙 *Tip:* *Changes to this parameter affect the digital channel data and the analog signal of the optional D/A board.*

Commands and Properties of the 3D field:

Command/Property	Range/Values	Description
BunchWidth	1 nm to 32 nm	Determines how many wavelengths are averaged when collecting photo diode signals.
MaxWavelength	190 nm to 800 nm	Sets the maximum 3D wavelength.
MinWavelength	190 nm to 800 nm	Sets the minimum 3D wavelength.
RefBandwidth	1 nm to 64 nm	Sets the reference bandwidth.
RefWavelength	190 nm to 800 nm Off	Sets the reference wavelength.
Retention	0.000 min to 10000000.000 min	Indicates the signal retention time.

Note:

You can change the digital data. also, using the parameters specified for the main device, e.g., Data_Collection_Rate or SlitWidth.

In addition, if the PDA detector is fitted with a D/A board and if this option is selected in the Server Configuration program, the analog output of the related D/A channel can be controlled with the channel device.

For an overview of the detector, refer to **Installing and Controlling Third Party Devices** Shimadzu SPD-M10Avp/M20A PDA Detector: Overview** in the **Administrator Help** section.

For a program example, refer to **Commands and Tips for Third-Party Devices** Shimadzu SPD-M10Avp/M20A PDA Detector: Program Example**.

▣ Shimadzu SPD-M10Avp/M20A PDA Detector: Program Example

The following program section shows examples for sampler and pump settings as well as the relevent settings for any important PDA parameters:

```
; ================================================
;  Sampler settings (Example, may vary depending  on
sampler type)
; ================================================
         DispSpeed =          10.00 [µl/s]
         DrawSpeed =          10.00 [µl/s]
…


; ================================================
;  Pump settings (Example, may vary depending  on  pump
type)
; ================================================
         Flow =               0.000 [ml/min]
         %B =                 0.0 [%]
         %C =                 0.0 [%]
         %D =                 0.0 [%]
…


; ================================================
; Detector settings
; ================================================

; Temperature (if available, SPD-M20A only)
         TempCtrl =               On
         Temperature.Nominal =    40 [°C]

; General device and signal settings
         Data_Collection_Rate =   6.25000 [Hz]
         Response =               160 [ms]
; Slit width (available for SPD-M20A only)
         SlitWidth =              1.2 [nm]

; 3D field
         3DFIELD.MaxWavelength =   600 [nm]
         3DFIELD.MinWavelength =   200 [nm]
         3DFIELD.BunchWidth =      4    [nm]
         3DFIELD.RefWavelength =   Off
         3DFIELD.RefBandwidth =    1    [nm]
```

```
; Single channel – Signal properties
        UV_VIS_1.Wavelength = 254 [nm]
        UV_VIS_1.Bandwidth =  4   [nm]
        UV_VIS_1.Step =       Auto
        UV_VIS_1.Average =    On

; Single channel – D/A converter board settings (if
available))
        UV_VIS_1.Range =      4000 [mAU/V]
        UV_VIS_1.Polarity =   Positive

; Add parameters for any further channels here
; if required
        …

 0.000     3DFIELD.Autozero
           UV_VIS_1.Autozero
           Wait              UV.Ready and Sampler.Ready
           Inject
           3DFIELD.AcqOn
           UV_VIS_1.AcqOn
; Autozero commands can be added during a run
; if required
 5.00      3DFIELD.Autozero

 10.000    3DFIELD.AcqOff
           UV_VIS_1.AcqOff

           End
```

For an overview of the detectors, refer to **Installing and Controlling Third Party Devices** 🔄 **Shimadzu SPD-M10Avp/M20A PDA Detector: Overview** in the **Administrator Help** section.

For information about the special commands that Chromeleon supports for controlling the detectors, refer to **Commands and Tips for Third-Party Devices** 🔖 **Shimadzu SPD-M10Avp/M20A PDA Detector.**

Shodex Refractive Index Detectors (RI-101, RI-102, RI-104): Commands and Tips

☞ *Tips:*

The Shodex RI-101 Refractive Index Detector is available from Dionex as part of the Summit HPLC System whereas the RI-102 and RI-104 detectors are not. However, the commands listed below are also supported for the Shodex RI-102 and RI-104 detectors.

In addition to the commands available for various detector types, such as **Step**, **Average**, and **Autozero** (see ⬛ **Dionex Detectors**), the Shodex RI-101 Refractive Index Detector supports the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Minimum	Maximum	Description
Baseline_Shift	0 mV	500 mV	Baseline offset (10 mV increments only). (Display level: Advanced)
DemoFileName			Name of the standard demo file (Display level: Expert. The property is read-only.)
Drift	0 nRIU/h	9990 nRIU/h	Drift value (Display level: Advanced. The property is read-only.)
FirmwareVersion	"1.06" (Note: This is the version number displayed on the startup screen. Other information is not retrievable.)		Standard firmware version.
Integrator_Range	125 µRIU/V or 500 µRIU/V		Voltage scale for analog integrator output (display level: Advanced)
LampAge	0 h	99999 h	Lamp age (The value should not exceed 2000h. Display level: Advanced. The property is read-only.)
LampVoltage	0.0V	9.9V	Lamp voltage (The value should not exceed 4.5V. Display level: Advanced. The property is read-only.)
Noise	0 nRIU	9990 nRIU	Noise value (Display level: Advanced. The property is read-only.)
NotReadyCauses	Text string (128 characters)		Explanation of why the device is not ready (read-only).
Polarity	Plus	Minus	Output polarity (Display level: Advanced)

Property	Minimum	Maximum	Description
Purge	Off	On	Controls the purge valve.
Recorder_Range	0.25 µRIU	512.00 µRIU	Voltage scale for full-scale analog recorder output. Only powers of 2 are allowed. (Display level: Advanced)
Rise_Time	0.10, 0.25, 0.50,1.00, 1.50, 2.00, 3.00, 6.00 s		Rise time constant.
Span	0 µRIU	999 µRIU	Validation span (The value should be between 487 and 537 µRIU. Display level: Advanced. The property is read-only.)
TemperatureDelta	-99°C	99°C	Temperature delta (also see: Temperature.Nominal and Temperature.Value. Display level: Advanced. The property is read-only.)
Temperature.Nominal	30°C Off	50°C	Temperature set point.
Temperature.Value	0°C	99°C	Measured temperature.

For information about how to install the Shodex RI-detectors, refer to **Hardware Installation** 🔁 **Shodex HPLC Refractive Index Detectors (RI-101, RI-102, RI-104): Overview** in the **Administrator Help** section.

Thermo Finnigan/TQ/TSP: Commands and Tips

For information about the special commands that Chromeleon supports for the different Thermo Finnigan/TQ/TSP devices and for tips for practical operation, refer to:

 Surveyor MSQ Mass Spectrometer

 aQa Mass Spectrometer

 Trace and Focus GCs

 AS2000 Autosamplers (corresponds to the Fisons AS800 Autosampler)

 AS3000 and AI3000 GC Autosamplers

 HS850 and HS2000 Headspace Samplers

 AS3500/AS3000 Autosamplers: Sample Preparation

 UV1000 Detector

 UV2000 Detector

 UV3000 Detector

 UV6000 PDA Detector

 FL2000 and FL3000 Fluorescence Detectors

◪ Thermo Finnigan/TQ/TSP Surveyor MSQ Mass Spectrometer

TIC, SIM, and MS Channel Properties and Commands

These channels are standard data channels with the following properties (please note that the display ➢*Filter* level determines which properties are displayed):

Property	Min	Max		Description
⇒*AcqOn/Off*	-	-	MS_n only	Starts and stops data acquisition.
MinMass	0.00 m/z	2000.00	Read-only	Minimum mass recorded in this trace. (SIM channels only)
MaxMass	0.00 m/z	2000.00	Read-only	Maximum mass recorded in this trace. (SIM channels only)
Polarity	Negative	Positive	Read-only	Polarity
SourceVoltage	0 V	200 V	Read-only	Maximum voltage.
FilterIndex	0: NoFilter	n (dep. on installation): 9 (TIC/MS) 41 (SIM)	Read-only (for TICF and SIM channels)	Extraction filter. The filter corresponds to the filters defined in the MS method. For 0 = NoFilter, the TIC channel is used.
➢*Filter*	n/a	n/a	Read-only	Filter string as used in Thermo Finnigan MSQ method.
BasePeakMode	No	Yes	MS only	Extracts ➢*Base Peak* chromatograms during data acquisition
⇒*Delta*	-	-	Read-only	Signal change (difference between the current value and the value one second before).

☞ *Tip:*

*Support for data compression is not available. Thus, the **Step**, **MaxAutoStop**, and **Average** commands cannot be set. Manual data acquisition is not possible, either. In addition, acquisition is started at the time 0.000 and stopped at the end of the program for all channels that deliver data according to an MSQ method file. These channels do not appear in the Program Wizard or on the control panel.*

Device Properties and Commands

Property	Min	Max	Default	Description
Ready	Ready, Busy		Read-only	Indicates whether the spectrometer is busy or idle.
Operation	On, Off, Standby		Off	Indicates whether the spectrometer is busy or idle.
Status	n/a		Read-only	Indicates the spectrometer's operational status.
Range	1 (for exp(1) Counts)	10 (for exp(10) Counts)		Scaling range used for online signal plot during data acquisition. (Can be set only before data acquisition starts.)
➢*Smoothing*	None, Gaussian, Boxcar	None		Smoothing algorithm. **Note**: This parameter is used by Xcalibur raw data extraction during online and post-run channel extraction.
SmoothingPoints	3, 5, 7, 9, 11, 13, 15	3		Number of data points used for smoothing. **Note**: This parameter is used by Xcalibur raw data extraction during online and post-run channel extraction.
HardwareVersion	n/a		Read-only	The mass spectrometer's hardware version. **Note**: This value is available only after the first raw data acquisition.
FirmwareVersion	n/a		Read-only	The spectrometer's firmware version. **Note**: This value is available only after the first raw data acquisition.

Command	Description
⇒*Connect*	Connects the device
Disconnect	Disconnects the device
⇒*Reset*	Resets the device to the default values

For troubleshooting information, refer to **Thermo Finnigan/TQ/TSP Surveyor MSQ: Troubleshooting**.

◾ Thermo Finnigan/TQ/TSP Surveyor MSQ: Troubleshooting

Problem: Interruption of the Xcalibur server

Grounding of the USB cable and connector on the MSQ is intermittent and can interrupt the ➤*Xcalibur* server. Interruption of the Xcalibur server results in the following symptoms:

- The Xcalibur Manual Tune software spinning "tune note" stops spinning. This indicates that communication is interrupted between the MSQ and the PC; the MSQ can neither be controlled nor transmit values to the Chromeleon.

  ☞ *Tip:*

  The MSQ communicates with the PC via the USB port. Communications function is monitored with the Xcalibur Manual Tune software with a spinning "tune note" in the upper right corner of the screen. Spinning of the "tune note" is the primary indication that the Xcalibur server is operational.

- The Xcalibur Manual Tune spectra section "freezes". This, too, indicates that communication between the MSQ and the PC is interrupted.

Solution:

1. Restart the Xcalibur server, following the steps below:

- Exit Xcalibur Manual Tune.

- Reset the MSQ by depressing the hardware reset button on the MSQ's rear panel.

- Restart Xcalibur Manual Tune and observe the active Xcalibur server: The MSQ makes an audible beep, the spinning "tune note" in the upper right corner of Manual Tune will be spinning, and the MSQ can be controlled and transmits values to Chromeleon.

2. Ensure that the length of the USB cable between the MSQ and PC is of minimum length (preferably 2 meters or less).

3. Ensure that the MSQ USB connector is grounded. Temporarily insert a conductive material, such as a paper clip or similar, between the MSQ USB connector and the sheet metal panel from which it protrudes. If the Xcalibur server interrupt problem does not re-occur, a more permanent solution would be to utilize a powered USB hub. (A suitable product is available, for example, from Belkin.) Install the hub between the MSQ and PC, using USB cables of minimum length.

Problem: Leakage on the return-valve

If the LC pump continues running after shutting down the MSQ, the non-return valve can leak inside the instrument. This is problem especially for high flow applications.

The Turbo pump controller is located directly below the non-return valve. When the non-return valve leaks, it does so onto the controller and pools on the main chassis bottom. In extreme cases, such a leak can cause the Turbo pump controller to fail. In minor cases, the leak causes the inside of the MSQ to be unsightly.

Solution:

A non-return valve removal kit and instruction sheet is available from Dionex. Contact the Callcenter at Callcenter@Dionex.com or 1-800-346-6390. Removal of the valve takes 30 to 60 minutes and can be performed during any other scheduled service.

 Note:

The removal kit includes an apparatus to provide a continuous nitrogen leak into the MSQ source, when the instrument is not in operation. This configuration consumes more nitrogen and customers may need to take measures to conserve nitrogen when supply is limited. In these cases, Dionex recommends turning off nitrogen at the source when the instrument is not in use. Closely monitor the level of the waste in the source waste bottle and/or leave the source door open slightly when the MSQ is not in use. This will prevent waste from being drawn back into the source when the instrument is turned off.

Problem: No data acquisition for Blank Run Samples

The Inject command of the PGM File is not performed for Blank Run samples (sample type: **Blank**) because it is not required. However, the MSQ needs this signal from the sample to start data acquisition.

Solution:

There are two possible solutions:

1. For Blank Run Samples (sample type: Blank), inject the solvent that is in the sample loop. Specify the Inject command as follows:

```
Inject  Blank=Inject
```

2. To analyze blank run samples without an injection, connect a relay, e.g., from the pump or detector, to the IN1 digital input on the Surveyor MSQ User Interface, using the black and the red wire of the three-core cable from the MSQ's accessory kit. In addition, add the following line to the PGM File directly after the Inject command, e.g.:

```
Pump_Relay_1.Open  Duration=1.00
```

🗒️ *Note:*

This example refers to Relay_1 on the pump.

🔲 Thermo Finnigan/TQ/TSP aQa Mass Spectrometer

TIC and SIM Channel Properties and Commands

These channels are standard data channels with the following properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max		Description
MinMass	2.00	1636.00	Read-only	Minimum mass recorded in this trace. (SIM channels only)
MaxMass	2.00	1636.00	Read-only	Maximum mass recorded in this trace. (SIM channels only)
Polarity	Negative	Positive	Read-only	Polarity
SourceVoltage	0 V	200 V	Read-only	aQa max voltage. (SIM channels only)
SourceVoltage	0 V	200 V	Read-only	aQa max voltage range. (TIC/TICF channels only)
BasePeakMode	No	Yes	MS only	Extracts ➢*Base Peak* chromatograms during data acquisition
Filter	n/a	n/a	Read-only	Filter string as used in Thermo Finnigan aQa method.

🖘 *Tip:*

*Support for data compression is not available. Thus, the **Step**, **MaxAutoStop**, and **Average** commands cannot be set. Manual data acquisition is not possible, either. In addition, acquisition is started at the time 0.000 and stopped at the end of the program for all channels that deliver data according to an aQa method file. These channels do not appear in the Program Wizard or on the control panel.*

Device Properties and Commands

Property	Min	Max	Default	Description
Ready	Ready, Busy		Read-only	Indicates whether the spectrometer is busy or idle.
Operation	On, Off, Standby		Off	Indicates whether the spectrometer is busy or idle.
Status	n/a		Read-only	Indicates the spectrometer's operational status.
Range	1 (for exp(1) Counts)	10 (for exp(10) Counts)		Scaling range used for online signal plot during data acquisition. (Can be set only before data acquisition starts.)
Smoothing	None, Gaussian, Boxcar	None		Smoothing algorithm.
				Note: This parameter is used by Xcalibur raw data extraction during online and post-run channel extraction.
SmoothingPoints	3, 5, 7, 9, 11, 13, 15	3		Number of data points used for smoothing.
				Note: This parameter is used by Xcalibur raw data extraction during online and post-run channel extraction.
HardwareVersion	n/a		Read-only	The mass spectrometer's hardware version.
				Note: This value is available only after the first raw data acquisition.
FirmwareVersion	n/a		Read-only	The spectrometer's firmware version.
				Note: This value is available only after the first raw data acquisition.

Command	Description
⇒*Connect*	Connects the device
Disconnect	Disconnects the device
⇒*Reset*	Resets the device to the default values.

◼ Thermo Finnigan/TQ/TSP Trace and Focus GC Commands

Lots of commands are available for the individual options of the Trace and Focus GCs. Only the most important of them are described below (please note that the display ➤*Filter* level determines which commands and properties are displayed). They are common to the respective device types (for example, the different detectors). For a short description of all GC commands, refer to the corresponding **Command** dialog box (press the F8 key in the program) or the Properties/Link box.

GC

Command	Min	Max	Default	Description
Cryo *(Trace GC only)*	Off	On	Off	ON: The cryo valve will operate when needed. OFF: The cryo valve is disabled. (The property is visible only if the cryo option is enabled.)
Range	0	3	1	Sets the output range of the detector signal. Note: Logarithmic scale with 10^n nA.
SignalSource				Sets the signal source of the relevant GC channel. The following settings are available:
				None (no assignment) DetA (Detector A) DetB (Detector B) DetC (Detector C) OvenSetpoint (nominal oven temperature) OvenTemp (current oven temperature) AmbientTemp (ambient temperature) PercentPower (relative power input)
				☞ *Tip:*
				If all three available detectors are installed, assign the relevant signal sources as follows: "Right" detector (= GC1) -> DetA"„Left" detector (= GC2) -> DetB "Aux" detector (= GC3) -> DetC. If no right detector is installed, the left detector is assigned to the DetA signal source instead of DetB.

Command	Min	Max	Default	Description
TempCtrl	OFF	ON	ON	Turns the oven's temperature control on or off.
Temperature				The allowed maximum temperature change is 120°C/min (1200°C/min for the Trace UltraFast). A maximum of 6 ramps per program are possible.
.Nominal	30 Note 1	Note 2	50	Nominal oven temperature in [°C].
.Value	30 Note 1	Note 2	N/A	Actual oven temperature in [°C] (read-only).
.UpperLimit	70	Note 2	350 (Focus) 450 (Trace)	Upper limit for the oven temperature in [°C].
.LowerLimit	30 Note 1	50°C	30	Lower limit for the oven temperature in [°C].

Note 1: Trace GC: Depending on the cryo settings, the minimum temperature can be 30°C, -55°C, or -99°C.

Note 2: Trace GC: Depending on the cryo settings, the maximum temperature can be 450°C or 500°C.
Focus GC: Depending on the cryo settings, the maximum temperature can be 350°C.

Inlet

Command	Min	Max	Default	Description
TempCtrl	OFF	ON	ON	Turns temperature control on or off.
Temperature				
.Nominal	30 (Trace) 50 (Focus)	450 (Trace) 375 (Focus)	50 (Trace) 150 (Focus)	Nominal inlet temperature in [°C]
.Value	30 (Trace) 50 (Focus)	450 (Trace) 375 (Focus)	N/A	Actual inlet temperature in [°C] (read-only).
Mode	N/A	N/A	Split	Inlet mode: Split or Splitless

Detector(s)

Command	Min	Max	Default	Description
⇒*Autozero*				Sets the signal output to 0.
TempCtrl	OFF	ON	ON	Turns temperature control on or off.
Temp				
.Nominal	30 (Trace) 100 (Focus)	450 (Trace) 375 (Focus)	150	Nominal detector temperature in [°C]
.Value	30 (Trace) 100 (Focus)	450 (Trace) 375 (Focus)	N/A	Actual detector temperature in [°C] (read-only).

Column(s)

Command	Min	Max	Default	Description
PressureCtrl	OFF	ON	ON	Turns the control of the column gas pressure on or off.
Pressure				
.Nominal	0.03	10.00	N/A	Nominal pressure in the column [bar].
.Value	0.03r	10.00	N/A	Actual pressure in the column [bar] (read-only).
FlowCtrl	OFF	ON	ON	Turns the column flow control on or off.
Flow				
.Nominal	0.0	50.0 (Trace) 100.0 (Focus)	N/A	Nominal flow in the column [ml/min].
.Value	0.0	50.0 (Trace) 100.0 (Focus)	N/A	Actual flow in the column [ml/min].

Further Information

The **Administrator** Help section provides more information about the Trace and Focus GCs. Refer to **Hardware Installation**:

🔵 **Thermo Finnigan/TQ/TSP Trace GC: Overview** and 🔵 **Thermo Finnigan/TQ/TSP Trace GC: Installation**.

🔵 **Thermo Finnigan/TQ/TSP Focus GC: Overview** and 🔵 **Thermo Finnigan/TQ/TSP Focus GC: Installation**.

▣ Thermo Finnigan/TQ/TSP Trace GC: Application

General

The Thermo Finnigan Trace GC uses a packet-oriented, binary data format to communicate with the PC via the RS-232 interface. When the GC is connected in Chromeleon, Chromeleon enables remote mode for the GC and locks the GC keypad. When Chromeleon terminates the connection to the GC, the GC switches back to local mode after 30 seconds.

The GC will not start communication by itself; it will only respond to commands and requests sent by Chromeleon.

Sampler

Using the AS2000 autosampler with the GC: If a sample vial is missing at the position specified in the sequence, the autosampler treats this as a ➢*Blank Run Sample* (refer to the AS2000 manual, page 118). However, the sequence is not stopped.

If the GC is waiting for an inject response from the autosampler and suddenly becomes **Not Ready** due to gas supply or flow problems, an error message appears and the sequence is stopped.

▣ Thermo Finnigan/TQ/TSP Trace GC: Setting the Temperature

Entering a Temperature Gradient

To enter a temperature gradient, use a ➢*Program*.

For the Thermo Finnigan Trace GC, a temperature profile can be entered with a maximum of six ascents or descents. The maximum temperature change (ascent) is up to 120°C per minute (1200°C/min for the Trace UltraFast), depending on the oven type.

Gradients are entered in the Program Wizard in the typical format for GC applications. (The starting and end temperatures are entered, as well as the modification rate.) In the program, however, the "base point philosophy" is used (similar to entering a flow or percent gradient in HPLC). Each **temperature** command is the base point of the gradient program. The wizard automatically converts the entered rates into the base point representation.

An Inject command is indispensable. No gradient is executed before the Inject command. Thus, temperature gradients can only begin after the Inject command

Temperature Setting

Reaching the nominal temperature on the instrument can take some time. Please note that the oven heats faster than the injector and the detector system. As soon as all temperatures and the specified pressure and flow parameter are reached, the GC sends a **Ready** signal. An injection from the autosampler is possible only after this signal.

Example:

The following program waits until the nominal temperature (150°C) is reached before the Inject command is executed:

```
0.000   GC.Temperature = 150
  Wait  GC.Ready
  Inject
...... ..............
```

Tip:

*After the instrument has received the method parameters from Chromeleon, it implements the desired value as quickly as possible. When the value is "almost" reached, the **Equilibration Time** passes until the instrument sends the confirmation message to Chromeleon. You can specify the duration of this time interval, using the **Equilibration Time** parameter in Chromeleon.*

ThermoQuest AS2000/Fisons AS800 Autosamplers

Chromeleon supports the following special commands and parameters for these autosamplers, which are identical in construction (please note that the display ➢*Filter* level determines which commands and properties are displayed). Also, refer to the information in the manual shipped with the instrument.

Command	Description
Clean	Cleans the syringe using the previously defined parameters (see below).
Inject	Injects the sample using the previously defined parameters (see below).
StopInject	Aborts a running injection.
	Tip:
	Use with Care! Might cause incorrect behavior!

Use the following parameters to specify the **Inject** command in detail. Define all injection parameters before initiating the **Inject** command. Otherwise, they will not take effect.

Parameter	Description
AirVolume	Select this parameter to specify the volume of air (0.0 - 500.0 µl) drawn after pulling the syringe needle out of the vial. This reduces evaporation from the syringe needle. The total of **AirVolume** plus **FillingVolume** must not exceed the syringe volume.
CleanCycles	Select this parameter to determine the number of cleaning cycles before injection (0...15). Specify the volume with the **FillingVolume** parameter.
Cleaning Cycles before Injection (Pre Inj Clean Cyc) [Solvent A/B/C/D]	Select this parameter to set the number of pre-injection cleaning cycles with solvent A/B/C/D. Please note: Only the solvent that has been specified last is used.
Cleaning Cycles after Injection (Post Inj Clean Cyc) [Solvent A/B/C/D]	Select this parameter to set the number of post-injection cleaning cycles with solvent A/B/C/D. Please note: Only the solvent that has been specified last is used.
Delay (PullUpDelayTime)	Select this parameter to determine the delay following the up and down movements of the syringe plunger (0.0 - 15.0 s).
Delay after (Post Inj Delay Time)	Select this parameter to determine for how long the syringe remains in the injector after the injection (0 - 63 s).
Delay before (Pre Inj Delay Time)	Select this parameter to determine the delay the syringe remains in the injector before the injection (0 - 63 s).
FillingVolume	Select this parameter to set the sample volume to be used for cleaning and eliminating gas bubbles (see **CleanCycles** and **PullupCount**). If the **FillingVolume** (0.0 - 500.0 µl) is less than the sample volume (**Volume**), the sample volume will be used instead.
InjectionSpeed	Select this parameter to determine how fast the sample is injected (1 - 100 µl/s).
Post Inj Clean Cyc A/B/C/D	Select this parameter to set post-injection cleaning cycles with solvent A/B/C/D. Please note: Only the solvent that has been specified last is used
PullupSpeed (AspirationSpeed)	Select this parameter to determine how fast the sample is drawn (1 - 100 µl/s).
Pumps (PullupCount)	Select this parameter to determine how often the syringe plunger is moved up and down before the sample is actually drawn up (0...15). This eliminates bubbles and thus, enhances reproducibility.
Volume (Pre Inj Clean Vol)	Select this parameter to set the pre-injection cleaning volume (0,0 - 500,0 µl).
With Sample (Position)	Select this parameter to specify the sample that shall be used for cleaning (positions 0...15).

The following parameters appear only for the **AS2000**. Use these parameters to specify the **Inject** command in detail:

Parameter	Description
AirAspirationMode	If the selected **InjectMethod** is **IntStd**, set this parameter to specify the sequence used to draw air, sample, and standard into the syringe (possible values: **Post**=0 (PST*); **Double**=1(DBL*)).
InjectMethod	Set this parameter to specify the injection method. **Normal**=0 (Method A*), **IntStd**=1 (Method B*).
	For more information about the parameters for the Internal Standard injection method, refer to the AS2000 manual.
Injector	Set this parameter to specify the GC inlet into which the standard sample is injected. (possible values: Right=0, Left=1) Note: The AS 2000 **Both** mode is currently not supported.
InternalStdVol	If the selected **InjectMethod** is **IntStd**, set this parameter to specify the volume of the internal standard sample (0-500µl).
IntStdVial	If the selected **InjectMethod** is **IntStd**, set this parameter to specify the vial containing the internal standard sample.
RelAirVol	If the selected **InjectMethod** is **IntStd**, set this parameter to specify the air volume that is drawn between the internal standard and the sample (0-500µl).

*ThermoQuest nomenclature; refer to the AS2000 manual for details.

For more information, refer to:

ThermoQuest AS2000/Fisons AS800 Autosamplers: Program Example

ThermoQuest AS2000/Fisons AS800 Autosamplers: Troubleshooting

The **Administrator Help** section provides installation details; refer to **Hardware Installation**:

Fisons AS800 GC Autosampler

Thermo Finnigan/TQ/TSP AS2000 GC Autosamplers

ThermoQuest AS2000/Fisons AS800: Program Example

After the ⇒*Connect* command, the parameter values are initially unknown to Chromeleon (blank fields in the AS800 control panel). You should enter meaningful values before injection. Use one of the **Method A/B/C/D** buttons on the control panel or specify them in the sample program. (For more information, refer to **How to …: Creating and Modifying Programs** **Creating a Program**.) This might be a good starting point:

```
; Standard 10 µl syringe.
; Injection Volume: 1.0 µL.
PreInjCleanVol = 10.0
PreInjCleanCycA = 3
CleanCycles = 1
PostInjCleanCycC = 0
; PostInjCleanVol = 10.0
PullUpCount = 6
PullUpDelayTime = 2.0
AirVolume = 3.0
FillingVolume = 5.0
AspirationSpeed = 100
InjectionSpeed = 100
PreInjDelayTime = 1
PostInjDelayTime = 2
```

For the commands supported for these instruments and for additional tips, refer to:

ThermoQuest AS2000/Fisons AS800 Autosamplers

ThermoQuest AS2000/Fisons AS800: Troubleshooting

The **Administrator Help** section provides installation details; refer to **Hardware Installation**:

Fisons AS800 GC Autosampler

Thermo Finnigan/TQ/TSP AS2000 GC Autosampler

ThermoQuest AS2000/Fisons AS800: Troubleshooting

Note:

*If an error occurs during operation, for example, excessive sample volume, the error is displayed on the instrument only. To continue the analysis, confirm the error message directly on the autosampler by pressing the <Enter>. This is possible after the autosampler keycard has been unlocked in Chromeleon. Use either the **Disconnect** command or the **KeycardUnlocked** command. Better yet: **Disconnect** the autosampler, turn it off and on again, wait until the autosampler is ready, and then reconnect the unit (see ⇒Connect).*

Error	Description	Remedial Action

Missing vial	The following messages appears on the instrument's display:
	`End Sample`
	`Total Injections 0`
	In addition, Chromeleon waits forever for an inject response.
		Abort the batch.

Bad vial	After some retries, a message like this appears on the instrument's display:
	`PLG NDL INJ TUR CNTR`
	`--- BSY --- ---  *`
	This happens quite easily if the vials are not perfectly crimped.
		Disconnect the autosampler and press the EXIT key (on the autosampler's keycard). When prompted whether to pause, continue, or abort the sample, select Abort. Abort the batch and reconnect.

Illegal parameter (e.g., Volume/ Position > Max or Air + Sample Volume > Max)	An error message appears on the autosampler's display.	Disconnect the autosampler. Press the autosampler key, as necessary.
Continuous beep and display cleared	Severe damage may occur (destroyed syringe, vials etc.)!	**Turn off the autosampler immediately!**
Strange behavior of any other kind		Disconnect the autosampler, turn it off and on again, wait until it is ready, and reconnect.

For the commands supported for these instruments and for additional tips, refer to:

ThermoQuest AS2000/Fisons AS800 Autosamplers

ThermoQuest AS2000/Fisons AS800: Program Example

▣ Thermo Finnigan/TQ/TSP: AS3000 and AI3000 Autosampler Commands

Chromeleon supports the following special commands and parameters for these autosamplers (please note that the display ➢*Filter* level determines which commands and properties are displayed). Also, refer to the information in the manual supplied with the instrument.

Command	Description
Clean	Cleans the syringe using the previously defined parameters (see below).
Inject	Injects the sample using the previously defined parameters (see below).
Recalibrate	Performs the start procedure again, allowing the autosampler to recalibrate the internal positions, such as injector, wash vials, tray. This command is required when a new tray was installed.
StopInject	Aborts a running injection.

Use the following parameters to specify the **Inject** command in detail. Define all injection parameters before initiating the **Inject** command. Otherwise, they will not take effect!

Parameter	Description
AirVolume	Select this parameter to specify the volume of air (0.0 - 3.0 µl) drawn after pulling the syringe needle out of the vial. This reduces evaporation from the syringe needle. The total of **AirVolume** plus **Volume** must not exceed the syringe volume.
DrawSpeed	Select this parameter to determine how fast the sample is drawn (slow or fast).
FillStrokes	Select this parameter to determine how often the syringe plunger is moved up and down before the sample is actually drawn up (0...15). This eliminates bubbles and thus, enhances reproducibility.
InjectWaitTime	Set the time between the injection command and the response from the autosampler (0 - 10.000.000 s)
PostInjDelayTime	Select this parameter to determine for how long the syringe remains in the injector after the injection (0 - 63 s).
PostWash	Select this parameter to set the number of post-injection cleaning cycles (0 - 15).
PostWashVial	Specify which vial(s) is(are) used for washing the syringe after the injection with the solvent(s): A, B, C, D, A+B, C+D.
PreInjDelayTime	Select this parameter to determine the delay the syringe remains in the injector before the injection (0 - 63 s).
PreWash	Select this parameter to set the number of pre-injection cleaning cycles (0 - 15)
PreWashVial	Specify which vial(s) is(are) used for washing the syringe before the injection with the solvents: A, B, C, D, A+B, C+D.

Parameter	Description
SampleDepth	Specify how deep the needle descends into the vial when drawing the sample for injection (bottom or half).
SampleWash	Select this parameter to determine the number of cleaning cycles with sample before injection (0...15).
State	Indicates the status of the autosampler (read-only): On = the injection has been performed Off = the injection has not yet been performed

For more information, refer to **Hardware Installation** 🔵 **Thermo Finnigan/TQ/TSP AS3000 and AI3000 GC Autosamplers** in the **Administrator Help** section.

🔳 Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers

For information about the headspace samplers, refer to:

🔳 **Commands**

🔳 **Specifying the Sample Volume**

🔳 **Tips for Sequences**

🔳 **Creating a Program**

🔳 **Status of Sample Preparation**

🔳 **Miscellaneous**

For more information about how to install the samplers, refer to **Hardware Installation** 🔵 **Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers** in the **Administrator Help** section.

▣ Thermo Finnigan/TQ/TSP HS850/HS2000 Headspace Sampler Commands

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Value Range	Description
_IncTime<X>	-1499.0 to 1499.0 min	Reports the remaining incubation time for a sampler at oven position X (where X = A through F; read-only).
_Occupied<X>	Off (0), On (1)	Reports whether oven position X is occupied (where X = A through F; read-only).
_Vial<X>	1 - 32, Empty (0)	Reports the number of the vial that is prepared for the injection at oven position X (where X = A through F; read-only).
Busy	Busy Idle	Reports whether the sampler is busy or idle (read-only).
DrawSpeed	1 - 99.9 ml/min	Sets the speed with which the sampler draws the sample volume and the preparing fill strokes.
FillStrokes	0 - 15	Sets the number of preparing fill strokes that the sampler draws before drawing the sample volume.
FillVolume	0 - 4.50 ml	Sets the volume that the sampler draws before drawing the sample volume.
FirmwareVersion	-	Indicates the sampler's firmware version (read-only).
FlushMode	AfterInject (0) , Always (1)	Sets the mode for flushing the needle (FLSH on the sampler display): Always = FLSH=ON AfterInject = FLSH=Off (For details, refer to the headspace sampler manual.)
FlushPressure	0 – 200 kPa or 0 – 29 psi or 0 – 2.00 bar (depending on the unit selected in the Server Configuration program)	Sets the nominal pressure for flushing the syringe.This command is available only if the sampler has an EPC (Electronic Pressure Control) option installed.

Property	Value Range	Description
GCCycleTime	0.1 - 1499 min	Sets the expected GC cycle time, i.e., the time between two injections. Enter the maximum time that the GC requires after an injection to be ready for the next sample. In sample programs, the cycle time must be set before the Inject command. In addition, the following must be true: GCCycleTime > PGM run time The following is recommended: GCCycleTime > PGM run time + equilibration time (of the GC)
GCStartMode	Normal (0), Anticipated (2), TrapNormal (4), TrapAnticipated (6)	Reports the start mode of the GC. The start mode determines when the sampler sends the start signal to the GC (read-only).
HardwareVersion	-	Indicates the sampler's hardware version (read-only).
IncubationTime	0.1 - 1499 min	Sets the incubation time for the sample (in **Single (constant)** mode).
IncubTimeOffset	0.1 - 599 min	Sets the incubation time offset for the previous sample in the sequence. The property can be set only once per sequence (in **Progressive** mode).
IncubTimeStart	0.1 - 1499 min	Sets the incubation time for the first sample in the sequence (in **Progessive** mode).
InjectionVolume	0 - 4.5 ml	*(Read-only)* Reports the sample volume for the injection of one sample during an enrichment cycle. If sample enrichment is disabled (LoadsPerInject = 1), this value matches the total injection volume ($\Rightarrow$*Inj. Vol.*).
InjectMode	Single (0); Progressive (1)	Sets the inject mode. (Note: The **Single** mode corresponds to the **Constant** Thermo mode.)
InjectSpeed	1 - 99.9 ml/min	Sets the injection speed.
LoadsPerInject	1 - 9	Sets the number of sample enrichments during an injection cycle.
LoadRepitition Time	0 - 63 min	Only for LoadsPerInject > 1: Sets the delay time between two sample injections during an enrichment cycle.
ModelNo	THERMO_2000; THERMO_850	Indicates the sampler's model number (read-only). The model number is used in the PGM Editor.

Property	Value Range	Description
NeedleTempCtrl	Off (0), On (1)	Turns on the needle heater while a sample is running. 🔖 *Note:* *NeedleTempCtrl is automatically set to On if a NeedleTemperature (see below) is set.*
Needle Temperature	40 - 150°C	Sets the temperature setpoint for the needle heater during a sample run.
OvenTempCtrl	Off (0), On (1)	Turns on the incubation oven while a sample is running. 🔖 *Note:* *OvenTempCtrl is automatically set to On if an OvenTemperatue (see below) is set.*
OvenTemperature	40 - 150°C	Sets the temperature setpoint for the incubation oven heater during a sample run.
PostInjectDelay	0 - 63 s	Sets the delay time after the injection, i.e., the time between the end of the sample injection and the removal of the needle from the GC inlet.
PreFill	Off (0), On (1)	Set to **On** to enable pressurization before sampling the headspace vapor. Enable only when using sample enrichment. (For details, refer to the headspace sampler manual.)
PreInjectDelay	0 - 63 s	Sets the delay time before the injection, i.e., the time between the insertion of the needle into the GC inlet and the start of the injection.
PreparationTime	0.1 - 1499 min	Reports the remaining preparation time for the sample to be injected next.
PullupDelay	0 - 63 s	Sets the delay time between drawing and dispensing the preparing fill strokes.
Ready	Ready NotReady	Reports whether the sample is ready for the next injection (read-only).
RunState	Unknown Standby CheckOven LoadVial PrimaryConditioning EnrichmentConditioning WaitForGCReady WaitForNextLoad WaitOvenTempReady InjectSample CleanHeadspace Error or Error_<I> (where I = 1 through 9)	Reports the device state and/or the action that is currently being performed (read-only).

Property	Value Range	Description
RunStateVial	1 through 32	Reports the vial number for the device states (see RunState) that include the vial number as additional information (read-only).
Shaker	Off (0), On (1)	Turns on the shaker. 🗎 *Note:* *If you did not specify a positive ShakerOnTime before, Shaker = On has no effect.*
ShakerOnTime²	0 - 594 s	Specifies for how long the shaker is on during a shaker cycle. 🗎 *Note:* *ShakerOnTime = 0 automatically turns off the shaker. ShakerOnTime > 0 automatically turns on the shaker.*
ShakerOffTime²	0 - 594 s	Specifies for how long the shaker is off during a shaker cycle. 🗎 *Note:* *If ShakerOffTime = 0, the shaker runs continuously.*
StandbyNeedle TempCtrl	Off (0), On (1)	Turns on the needle heater while the sampler is on standby. 🗎 *Note:* *StandbyNeedleTempCtrl is automatically set to On if a StandbyNeedleTemperature is set.*
StandbyNeedle Temperature	40 – 150°C	Sets the setpoint temperature for the needle heater while the sampler is on standby.
StandbyOven TempCtrl	Off (0), On (1)	Turns on the incubation oven while the sampler is on standby. 🗎 *Note:* *StandbyOvenTempCtrl is automatically set to On if a StandbyOvenTemperature is set.*
StandbyOven Temperature	40 – 150°C	Specifies the setpoint for the incubation oven temperature while the sampler is on standby.
Syringe	5.0 ml (0), 2.5 ml (1), 1.0 ml (2), Unknown (3)	Reports which syringe type is installed (read-only).
VialType	10 ml (0), 20 ml (1), Unknown (2)	Reports which vial type is installed (read-only).

² The ShakerOnTime and ShakerOffTime properties determine the shaker behavior:
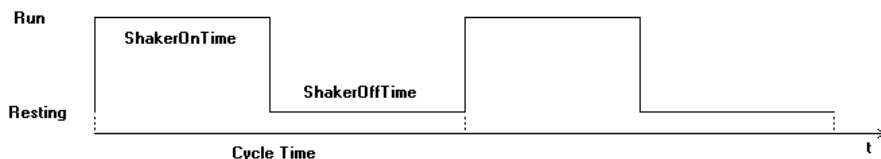
For more information, refer to **Hardware Installation ⬥ Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers** in the **Administrator Help** section.

▪ Thermo Finnigan/TQ/TSP HS850/HS2000 Headspace Samplers: Specifying the Sample Volume

Changing the syringe type

Chromeleon supports three syringe types for the HS850 and HS2000 headspace samplers: 5.0 ml, 2.5 ml, and 1.0 ml. The installed type is read from the device upon connect, so it is not necessary to update the configuration after changing the syringe type. To change the syringe type:

1. Disconnect the headspace sampler in Chromeleon.

2. Replace the syringe as described in the headspace sampler manual.

3. If the new syringe has a different volume, adapt the device configuration as necessary from the control module display. Refer to the headspace sampler manual for details.

4. Reconnect the headspace sampler in Chromeleon.

5. Verify that the device driver correctly recognized the syringe type. To do so, use the Syringe property. Have Chromeleon display the Log.Syringe value.

Using sample enrichment

Chromeleon supports sample enrichment by the headspace sampler. Using the GC's cold trap, large sample volumes (i.e., injection volume > max. syringe volume) are injected in several load cycles.

In Chromeleon, specify the sample volume (Volume). The sample volume is considered the total injection volume (V). In addition, specify the number of enrichments (n) via LoadsPerInject. The load cycle volume (V_L) is calculated as follows:

$$V = V_L * n$$

☞ *Tip:*

To inhibit enrichment, set LoadsPerInject to 1.

The load cycle volume must be a multiple of 10 µl; if it is not, a warning appears. For example, if Volume = 1 ml and LoadPerInject = 3, the warning reads as follows:

```
[Warning]  {Sampler} The sampler accepts only values for the volume
per load that are multiples of 10µl. Instead of the specified volume
(1.000 ml), 0.990 ml would be injected.
```

If you decide to inject anyway, Chromeleon will modify the total injection volume accordingly and an error message will be logged in the Audit Trail.

For more information, refer to **Hardware Installation** 🔄 **Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers** in the **Administrator Help** section.

🔲 Thermo Finnigan/TQ/TSP HS850/HS2000 Headspace Samplers: Tips for Sequences

Restrictions for headspace sequences

HS850 and HS2000 headspace sampler runs must meet the following conditions:

- Each run can contain only one program. If you want to use a different program, you must set up a new run.

- The sample position of adjacent samples is incremented by one.

- The injection volume must be the same for all samples.

- The run must not contain blank run samples with Inject = None. The headspace sampler injects every sample.

- The GCCycleTime should exceed the total of the GC's program run time and equilibration time.

For optimal use of the sampler's overlapping sample preparation, Chromeleon sequences should meet these conditions, also. The Chromeleon sequence is split into several headspace runs if:

- The Chromeleon sequence contains more than one program.

- The sample positions are not numbered consecutively.

- The sample volumes are different.

For example, the following sequence is split into two headspace runs because of the sample positions:

No.	Name	Type	Pos	Inj. Vol.	Program	Method
1	Sample1	Unknown	20	1.00	ATP_Progressive	default
2	Sample2	Unknown	21	1.00	ATP_Progressive	default
3	Sample3	Unknown	22	1.00	ATP_Progressive	default
4	Sample4	Unknown	23	1.00	ATP_Progressive	default
5	Sample5	Unknown	10	1.00	ATP_Progressive	default
6	Sample6	Unknown	11	1.00	ATP_Progressive	default
7	Sample7	Unknown	12	1.00	ATP_Progressive	default
8	Sample8	Unknown	13	1.00	ATP_Progressive	default

The first headspace run includes Sample1 through Sample4 (positions 20 through 23); the second run includes Sample5 through Sample8 (positions 10 through 13). If the incubation start time (*IncubTimeStart)* is 10 min and if the additional incubation time (*IncubTimeOffset*) is 5 min, the incubation times for the single samples are as follows:

Sample no.:	1	2	3	4	5	6	7	8
Incubation time [min]:	10	15	20	25	10	15	20	25

Appending samples to a running sequence

Chromeleon supports appending samples to a running sequence, but the HS580 and HS2000 headspace samplers do not. Therefore, Chromeleon downloads any appended sample(s) to an additional headspace sampler run that is processed after the current one has finished. This means that:

- In the **Single** mode, the appended samples are prepared only after the previous run has finished.

- In **Progressive** mode, this may lead to unexpected results because the headspace sampler considers the appended sample(s) a new sequence.

For example, if the original sequence includes 10 samples and one sample is appended during the run, the incubation time for sample 11 is *IncubTimeStart* (not *IncubTimeStart+10*IncubTimeOffset*, which would be the incubation time if sample 11 had been included in the original sequence).

For more information, refer to **Hardware Installation** 🔵 **Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers** in the **Administrator Help** section.

🔳 Thermo Finnigan/TQ/TSP HS850/HS2000 Headspace Samplers: Creating a Program

Follow the steps below to create a program for the headspace sampler:

- Use the PGM Wizard to create a program for the timebase.

- Add all relevant settings for the headspace sampler in the PGM Editor. The settings must appear before the first time step, i.e., before t = 0.000 min.

- Add a **Wait Sampler.Ready AND GC.Ready** statement directly before the Inject command.

👉 *Tip:*

The GCCycleTime should exceed the total of the GC's program run time and equilibration time.

Example program for Single mode

The following program is a typical example of a sequence for a headspace sampler (here, the Focus GC) in Single mode:

```
; ==============================================================
; Sampler settings
; ==============================================================
          ; Inject mode
          InjectMode =                    Single

          ; Time program
          IncubationTime =                5 [min]
          GCCycleTime =                   12 [min]

          ; Enrichment settings: split volume in two loads
          ; without delay between the loads
          LoadsPerInject =        2
```

```
          LoadRepetitionTime =            0 [min]

          ; Shaker
          Shaker =                        On
          ShakerOnTime =                  10 [s]
          ShakerOffTime =                 30 [s]

          ; Temperatures
          NeedleTemperature =             65 [°C]
          OvenTemperature =               60 [°C]
          StandbyNeedleTemperature =      45 [°C]
          StandbyOvenTemperature =        40 [°C]

          ; Sample flushing
          FlushMode =                     Always
          FlushPressure =                 2 [kPa]

          ; Inject options
          PreFill=                        Off
          FillStrokes =                   2
          DrawSpeed =                     50.00 [ml/min]
          FillVolume =                    1.50 [ml]
          PullupDelay =                   2 [s]
          PreInjectDelay =                5 [s]
          PostInjectDelay =               5 [s]
          InjectSpeed =                   20.00 [ml/min]

; ============================================================
; GC settings (Thermo Focus GC)
; ============================================================
          Inlet.TempCtrl =                On
          Inlet.Temperature.Nominal =     100 [°C]
          GC_1.Temp =                     110 [°C]
          GC_1.Step =                     0.10 [s]
          GC_1.Average =                  Off
          GC_1.Range = 0
          GC_1.Data_Collection_Rate =     10 [Hz]
          GasSaverFlow = 10.0
          . . .

; ================================================================
; Injection
; ================================================================
0.000     GC_1.Autozero
          GC.Temperature =                100 [°C]
          Wait    Sampler.Ready and GC.Ready
          Inject
          Off
          GC_1.AcqOn

 10.000   GC.Temperature =                100 [°C]
          GC_1.AcqOff
          End
```

Example program for Progressive mode

The program for a headspace sampler in Progressive mode is similar to the above program except for the injection mode and time program settings. The following program lines show the relevant settings for a PGM file in which the first sample of the sequence incubated for 4 minutes, the second sample for 6 minutes, the third for 8 minutes, etc.:

```
; Inject mode
InjectMode =                     Progressive

; Time program
IncubTimeStart =                 4 [min]
IncubTimeOffset =                2 [min]
GCCycleTime =                    12 [min]
```

For more information aboput the headspace samplers, refer to **Hardware Installation** 🌐 **Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers** in the **Administrator Help** section.

🔲 Thermo Finnigan/TQ/TSP HS850/HS2000 Headspace Samplers: Status of Sample Preparation

Chromeleon allows you to monitor the sample preparation process:

In the Browser in a Sequence

The ⇒*Status* column indicates the status of the samples:

No.	Name	Type	Pos	Inj. Vol.	Program	Method	Status
▷	🔞 Sample1	Unknown	20	1.00	ATP_Single	default	Running
2	🔞 Sample2	Unknown	21	1.00	ATP_Single	default	Preparing
3	🔞 Sample3	Unknown	22	1.00	ATP_Single	default	Preparing
4	🔞 Sample4	Unknown	23	1.00	ATP_Single	default	Preparing
5	🔞 Sample5	Unknown	24	1.00	ATP_Single	default	Preparing
6	🔞 Sample6	Unknown	25	1.00	ATP_Single	default	Preparing
7	🔞 Sample7	Unknown	26	1.00	ATP_Single	default	Preparing
8	🔞 Sample8	Unknown	27	1.00	ATP_Single	default	Preparing

👉 *Tip:*

*Apart from the current sample, the ⇒Status of all samples sent to the sampler is **Preparing**. This is independent of whether the sample is already in the oven. Check the Audit Trail to see whether a sample has already been moved to the oven.*

On the Thermo_HS850_2000.pan control panel

Open the **Thermo_HS850_2000.pan** control panel from the **GC** folder of the **Dionex Templates**. Click **Oven / Tray…** for a graphical representation of the sample status:
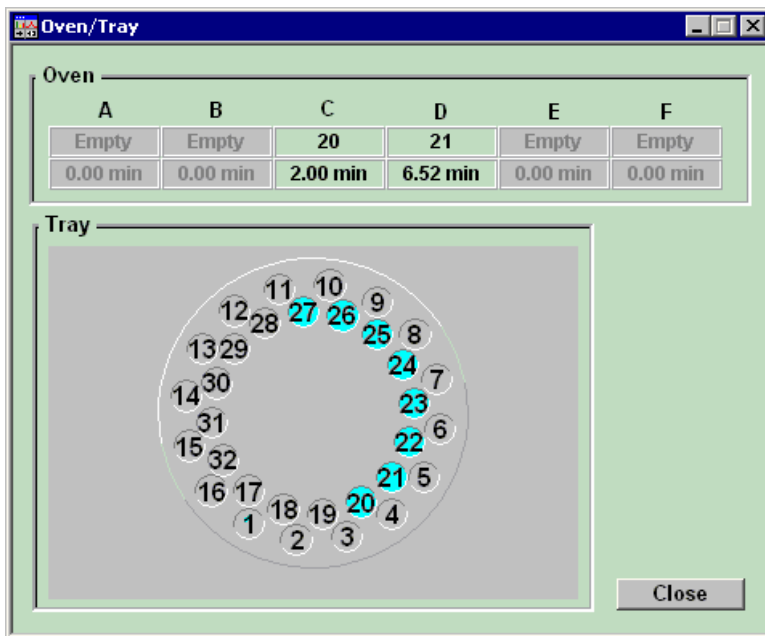
In the Audit Trail (sample preparation and real incubation time)

In addition, the Audit Trail (see **Overview, Operation, and Data Management** ⬛ **Audit Trails**) provides information about the progress of sample preparation.

The real incubation time may differ from the specified time (e.g, if the GC does not reach the Ready state in time for the next injection). Therefore, Chromeleon logs the real incubation time (**RealIncubationTime**) in the Audit Trail:

Lines relating to the Audit Trail of the current sample and at least one sample in prepration have a yellow background.

If the real incubation time is different from the specified time, a warning appears in the Audit Trail:

In the Report (real incubation time)

You can include the real incubation time in the report, using the **Real Incubation Time (Sampler)** report variable of the **Audit Trail** report category.

For more information about the samplers, refer to **Hardware Installation** 🔵 **Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers** in the **Administrator Help** section.

▣ Thermo Finnigan/TQ/TSP HS850/HS2000 Headspace Samplers: Miscellaneous

Blank run samples

The headspace sampler does not recognize ➢*Blank Run Samples*. If a Chromeleon sequence contains a blank run sample, the sequence is split into several headspace sampler runs. While the blank run sample is running, the sampler is set to standby.

For example, the sequence below is split into three sampler runs with the following sub-sequences: Sample1+2, Sample4-6, and Sample8-10:

No.	Name	Type	Pos	Inj. Vol.	Program	Method	Status
▷	Sample1	Unknown	20	1.00	ATP_Single	default	Running
2	Sample2	Unknown	21	1.00	ATP_Single	default	Preparing
3	Sample3	Blank	22	1.00	ATP_Single	default	Single
4	Sample4	Unknown	23	1.00	ATP_Single	default	Single
5	Sample5	Unknown	24	1.00	ATP_Single	default	Single
6	Sample6	Blank	25	1.00	ATP_Single	default	Single
7	Sample7	Blank	26	1.00	ATP_Single	default	Single
8	Sample8	Unknown	27	1.00	ATP_Single	default	Single
9	Sample8	Unknown	28	1.00	ATP_Single	default	Single
10	Sample8	Unknown	29	1.00	ATP_Single	default	Single

During the blank run samples (Sample3, 6, and 7), the sampler is set to standby.

☞ *Tip:*

*In **Progessive** mode, only the first sample is allowed to be a blank run sample. Otherwise, the sequence will not start.*

Missing vials

When detecting a missing vial, the sampler does not stop the sequence. It sends a warning and continues the sequence as if the vial were present. The sampler handles the error as follows:

- A warning is logged in the Audit Trail.

- The current sample (of the missing vial) is set to **Interrupted**.

- The GC run for the missing sample is cancelled.

- Chromeleon continues with the next sample.

Demo mode

In ➢*Demo Mode*, Chromeleon does not simulate the following features of the headspace sampler:

- During a run, the sampler ignores sample enrichments.

- The incubation time should not be shorter than the GC cycle time.

- Only one sample is prepared, even if the incubation time is significantly larger than the GC cycle time.

Aborting a batch

When a batch is aborted:

- If you select **Immediately**, all samples with the ⇒*Status* **Preparing** are set to **Interrupted**. This is independent of whether the sample is already in the oven when the batch is aborted. Check the Audit Trail to see whether the sample has already been moved to the oven.

- If you select **Abort after current sample**, the sampler stops loading samples. At the end of the current sample, Chromeleon stops the batch.

- If you select **Abort after current sequence**, sequence refers to the Chromeleon sequence and not to the sampler sub-sequence(s). This means that all sampler runs of the current sequence are finished regularly.

For more information about the samplers, refer to **Hardware Installation** 🌐 **Thermo Finnigan/TQ/TSP HS850 and HS2000 Headspace Samplers** in the **Administrator Help** section.

🔲 Thermo Finnigan/TQ/TSP AS3500/AS3000 Autosamplers: Sample Preparation

Follow these guidelines to ensure successful sample preparation with an AS3500 or AS3000 Autosampler:

- Place extra vials in the three positions after the sample vial position (FirstVial+1, FirstVial+2, and FirstVial+3).

- When you create the ➢*Program*, make sure the sample prep steps are in the correct order and that all required steps are present. For guidance, refer to the AS3500/AS3000 Basic Template example program shown below.

- Make sure that each dilution cycle includes all of the required steps.

- Specify the desired final volume in the Add step.

- To skip any step (except the Add step), set its volume to 0.

- To perform an operation on the target vial (but not deliver sample to it), set the Add step's volume to 0. This is useful, for example, when you want to mix or heat the contents of a vial.

- Make sure the volume specified in the Add step in a given dilution cycle is less than or equal to the sum of the PickUpLg and the Load volumes.

- Remember that the autosampler always injects from the FirstVial, which is specified in the Pos. column of the sequence.

Example AS3500/AS3000 Sample Preparation Programs

1. AS3500/AS3000 Basic Template

```
; Basic Template for sample preparation
; Injection will be made from FirstVial
      ; Optionally specify Reagent positions
      ReagentA =    A35
      ReagentB =    B35
      ReagentC =    C35

      ; Required to tell autosampler prep is starting.   Must be
      first command!
      PrepStart      ; required

      ; Prep steps defined.   Up to 4 sets.   Must be done in this
      exact order!
      PrepLoad       Volume=0.0, Reservoir=Solvent_1 ; optional
      PrepPickUp1    Volume=0.0, Reservoir=FirstVial+1 ; optional
      PrepPickUp2    Volume=0.0, Reservoir=FirstVial+2 ; optional
      PrepPickUpLg   Volume=0.0, Reservoir=FirstVial+3 ; optional
      PrepAdd        Volume=0.0, Reservoir=FirstVial ; required
      PrepMix        PreHeatMix=0.00, Heat=0.00, PostHeatMix=0.00
                     ; optional
      PrepWait       Time = 0.00 ; optional
      PrepRemove     Volume=0.0, SampleHeight=2.0 ; optional
      PrepRetvial    ; required
      PrepMovearm    ; required
      PrepFlush      ; required
      PrepFlushBtl   ; required
      ;Repeat previous commands for additional 3 cycles
```

```
      ;...

      ; required end of prep steps.  Must be last commands!
      PrepFlushP     Volume=0.0, Solvent=Solvent_1 ; optional
      PrepEnd        ; required

0.000  Inject ; Required for sample prep to execute.

      End
```

2. AS3500/AS3000 Dilution Template

```
; Template is a dilution program that you can use to perform a 1:5
dilution
; Injection will be made from FirstVial

      ; Required to tell sampler prep is starting  Must be first
      command!
      PrepStart      ; required

      ; Prep steps defined.  Up to 4 sets.  Must be done in this
      exact order!
      PrepLoad       Volume=500.0, Reservoir=Solvent_1 ; optional
      PrepPickUp1    Volume=100.0, Reservoir=FirstVial+1 ; optional
      PrepPickUp2    Volume=0.0, Reservoir=FirstVial+1 ; optional
      PrepPickUpLg   Volume=0.0, Reservoir=FirstVial+1 ; optional
      PrepAdd        Volume=500, Reservoir=FirstVial ; required
      PrepMix        PreHeatMix=0.50, Heat=0.00, PostHeatMix=0.00
                     ; optional
      PrepWait       Time = 0.00 ; optional
      PrepRemove     Volume=0.0, SampleHeight=0.0 ; optional
      PrepRetvial    ; required
      PrepMovearm    ; required
      PrepFlush      ; required
      PrepFlushBtl   ; required

      ; required end of prep steps.  Must be last commands!
      PrepFlushP     Volume=0.0, Solvent=Solvent_1 ; optional
      PrepEnd        ; required

0.000  Inject ; Required for sample prep to execute.

      End
```

3. AS3500/AS3000 DABS-CL Template

```
; Template heats your samples to a given temperature
; Injection will be made from FirstVial

        ; Turn on heater and set to desired temperature for mix/heat
        step
        Heater =        On
        HeaterTemperature = 65

        ; Optionally specify Reagent positions
        ReagentA =     A35
        ReagentB =     B35
        ReagentC =     C35

        ; Required to tell autosampler prep is starting.   Must be
        first command!
        PrepStart      ; required

        ; Prep steps defined.   Up to 4 sets. Must be done in this
        exact order!
        ; Cycle 1
        PrepLoad       Volume=200.0, Reservoir=Solvent_1 ; optional
        PrepPickUp1    Volume=100.0, Reservoir=ReagentA ; optional
        PrepPickUp2    Volume=40.0, Reservoir=ReagentB ; optional
        PrepPickUpLg   Volume=0.0, Reservoir=ReagentC ; optional
        PrepAdd        Volume=140, Reservoir=FirstVial ; required
        PrepMix        PreHeatMix=0.30, Heat=4.50, PostHeatMix=0.30
                       ;optional
        PrepWait       Time = 0.00 ; optional
        PrepRemove     Volume=0.0, SampleHeight=0.0 ; optional
        PrepRetvial    ; required
        PrepMovearm    ; required
        PrepFlush      ; required
        PrepFlushBtl   ; required
        ; Cycle 2
        PrepLoad       Volume=1000.0, Reservoir=Solvent_1 ; optional
        PrepPickUp1    Volume=0.0, Reservoir=ReagentA ; optional
        PrepPickUp2    Volume=0.0, Reservoir=ReagentB ; optional
        PrepPickUpLg   Volume=0.0, Reservoir=ReagentC ; optional
        PrepAdd        Volume=860, Reservoir=FirstVial+1 ; required
        PrepMix        PreHeatMix=0.30, Heat=0.00, PostHeatMix=0.00
                       ; optional
        PrepWait       Time = 0.00 ;optional
        PrepRemove     Volume=0.0, SampleHeight=0.0 ; optional
        PrepRetvial    ; required
        PrepMovearm    ; required
```

```
      PrepFlush       ; required
      PrepFlushBtl    ; required

      ; required end of prep steps.  Must be last commands!
      PrepFlushP      Volume=0.0, Solvent=Solvent_1 ; optional
      PrepEnd         ; required

0.000  Inject ; Required for sample prep to execute.

      End
```

4. AS3500/AS3000 Rainbow Template

```
; Prepares four samples
; Injection will be made from FirstVial
      ; Setup Reagent positions
      ReagentA =    A35
      ReagentB =    B35

      ; Required to tell autosampler prep is starting.  Must be
      first prep command!
      PrepStart       ; required

      ; Prep steps defined.  Up to 4 sets. Must be done in this
      exact order!
      ; Cycle 1
      PrepLoad        Volume=1000.0, Reservoir=Solvent_1 ; optional
      PrepPickUp1     Volume=30.0, Reservoir=Reagent_B ; optional
      PrepPickUp2     Volume=0.0, Reservoir=Reagent_A ; optional
      PrepPickUpLg    Volume=0.0, Reservoir=FirstVial ; optional
      PrepAdd         Volume=1000.0, Reservoir=FirstVial ; required
      PrepMix         PreHeatMix=0.50, Heat=0.00, PostHeatMix=0.00
                      ; optional
      PrepWait        0 ; optional
      PrepRemove      Volume=0.0, SampleHeight=2.0 ; optional
      PrepRetvial     ; required
      PrepMovearm     ; required
      PrepFlush       ; required
      PrepFlushBtl    ; required
      ; Cycle 2
      PrepLoad        Volume=1000.0, Reservoir=Solvent_1 ; optional
      PrepPickUp1     Volume=20.0, Reservoir=Reagent_B ; optional
      PrepPickUp2     Volume=10.0, Reservoir=Reagent_A ; optional
      PrepPickUpLg    Volume=0.0, Reservoir=FirstVial ; optional
      PrepAdd         Volume=1000.0, Reservoir=FirstVial+1
                      ; required
```

```
        PrepMix         PreHeatMix=0.50, Heat=0.00, PostHeatMix=0.00
                        ;optional
        PrepWait        0 ;optional
        PrepRemove      Volume=0.0, SampleHeight=2.0 ; optional
        PrepRetvial     ; required
        PrepMovearm     ; required
        PrepFlush       ; required
        PrepFlushBtl    ; required
        ; Cycle 3
        PrepLoad        Volume=1000.0, Reservoir=Solvent_1 ; optional
        PrepPickUp1     Volume=10.0, Reservoir=Reagent_B ; optional
        PrepPickUp2     Volume=20.0, Reservoir=Reagent_A ; optional
        PrepPickUpLg    Volume=0.0, Reservoir=FirstVial ; optional
        PrepAdd         Volume=1000.0, Reservoir=FirstVial+2
                        ; required
        PrepMix         PreHeatMix=0.50, Heat=0.00, PostHeatMix=0.00
                        ;optional
        PrepWait        0 ;optional
        PrepRemove      Volume=0.0, SampleHeight=2.0 ;o ptional
        PrepRetvial     ; required
        PrepMovearm     ; required
        PrepFlush       ; required
        PrepFlushBtl    ; required
        ; Cycle 4
        PrepLoad        Volume=1000.0, Reservoir=Solvent_1 ;optional
        PrepPickUp1     Volume=0.0, Reservoir=Reagent_B ; optional
        PrepPickUp2     Volume=30.0, Reservoir=Reagent_A ; optional
        PrepPickUpLg    Volume=0.0, Reservoir=FirstVial ; optional
        PrepAdd         Volume=1000.0, Reservoir=FirstVial+3
                        ; required
        PrepMix         PreHeatMix=0.50, Heat=0.00, PostHeatMix=0.00
                        ; optional
        PrepWait        0 ;optional
        PrepRemove      Volume=0.0, SampleHeight=2.0 ; optional
        PrepRetvial     ; required
        PrepMovearm     ; required
        PrepFlush       ; required
        PrepFlushBtl    ; required

        ; required end of prep steps.  Must be last of the prep
        commands!
        PrepFlushP      Volume=0.0, Solvent=Solvent_1 ; optional
        PrepEnd         ; required
0.000   Inject ; Required for sample prep to execute.

        End
```

5. AS3500/AS3000 Linear Template

```
; Template allows you to do linear dilutions
; Injection will be made from FirstVial

        ; Required to tell autosampler prep is starting.  Must be
        first prep command!
        PrepStart       ; required

        ; Prep steps defined.  Up to 4 sets. Must be done in this
        exact order!
        ; Cycle 1
        PrepLoad        Volume=1000.0, Reservoir=Solvent_1 ; optional
        PrepPickUp1     Volume=100.0, Reservoir=FirstVial+3 ; optional
        PrepPickUp2     Volume=0.0, Reservoir=FirstVial ; optional
        PrepPickUpLg    Volume=0.0, Reservoir=FirstVial ; optional
        PrepAdd         Volume=1000.0, Reservoir=FirstVial+2 ; required
        PrepMix         PreHeatMix=0.30, Heat=0.00, PostHeatMix=0.00
                        ;optional
        PrepWait        0 ; optional
        PrepRemove      Volume=0.0, SampleHeight=2.0 ; optional
        PrepRetvial     ; required
        PrepMovearm     ; required
        PrepFlush       ; required
        PrepFlushBtl    ; required
        ; Cycle 2
        PrepLoad        Volume=1000.0, Reservoir=Solvent_1 ; optional
        PrepPickUp1     Volume=50.0, Reservoir=FirstVial+3 ; optional
        PrepPickUp2     Volume=0.0, Reservoir=FirstVial ; optional
        PrepPickUpLg    Volume=0.0, Reservoir=FirstVial ; optional
        PrepAdd         Volume=1000.0, Reservoir=FirstVial+1 ; required
        PrepMix         PreHeatMix=0.30, Heat=0.00, PostHeatMix=0.00
                        ;optional
        PrepWait        0 ; optional
        PrepRemove      Volume=0.0, SampleHeight=2.0 ; optional
        PrepRetvial     ; required
        PrepMovearm     ; required
        PrepFlush       ; required
        PrepFlushBtl    ; required
        ; Cycle 3
        PrepLoad        Volume=1000.0, Reservoir=Solvent_1 ; optional
        PrepPickUp1     Volume=5.0, Reservoir=FirstVial+3 ; optional
        PrepPickUp2     Volume=0.0, Reservoir=FirstVial ; optional
        PrepPickUpLg    Volume=0.0, Reservoir=FirstVial ; optional
        PrepAdd         Volume=1000.0, Reservoir=FirstVial ; required
```

```
        PrepMix         PreHeatMix=0.30, Heat=0.00, PostHeatMix=0.00
                        ;optional
        PrepWait        0 ;optional
        PrepRemove      Volume=0.0, SampleHeight=2.0 ; optional
        PrepRetvial     ; required
        PrepMovearm     ; required
        PrepFlush       ; required
        PrepFlushBtl    ; required


        ; required end of prep steps.  Must be last of the prep
        commands!
        PrepFlushP      Volume=0.0, Solvent=Solvent_1 ; optional
        PrepEnd         ; required

0.000   Inject ; Required for sample prep to execute.

        End
```

For more information, refer to **Hardware Installation** 🔵 **Thermo Finnigan/TQ/TSP AS3500 and AS3000 Autosamplers** in the **Administrator Help** section.

🔲 Thermo Finnigan/TQ/TSP UV1000 Detector

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Default	Description
AvailableLamps	None	UV & VIS lamps	UV lamp only	Indicates which lamps are available (read-only).
Range	0.0005, 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0		1.0000	Amplifying factor in [AUFS] for the output signal.
Response	0.0	5.0	1.0	Time constant. Indicates the time needed by the detector to reach 63% of the full-scale deflection.
Wavelength	190 nm	800 nm	254 nm	Wavelength at which the detector measures the signal: UV lamp: 190 - 380 nm VIS lamp: 366 - 800 nm Both lamps: 190 - 800 nm
ZeroOnLambda Change	Off	On	Off	Determines whether the detector performs an ⇒*Autozero* every time the wavelength is changed.

Command	Description
UV_LampOn	Turns on the deuterium lamp.
UV_LampOff	Turns off the deuterium lamp.
Visible_LampOn	Turns on the tungsten lamp.
Visible_LampOff	Turns off the tungsten lamp.

☞ *Tip:*

After a ⇒Reset command, it takes 90 seconds until the detector is ready to execute further commands.

▪ Thermo Finnigan/TQ/TSP UV2000 Detector

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Default	Description
DetectionMode	-	-	-	Sets the wavelength mode:
				Single Mode: 190 - 800 nm (only one detector channel is used)
				Dual1 Mode: 190 ... 450 nm
				Dual2 Mode: 366 ... 800 nm
Response	0.0	5.0	1.0	Time constant. Indicates the time needed by the detector to reach 63% of the full-scale deflection.

Property	Description
UV_LampOn	Turns on the deuterium lamp.
UV_LampOff	Turns off the deuterium lamp.
Visible_LampOn	Turns on the tungsten lamp.
Visible_LampOff	Turns off the tungsten lamp.

Channel-Specific Commands and Properties

Property	Min	Max	Default	Description
Range	0.0005 AUFS	3.0000 AUFS	1.0000 AUFS	Amplifier gain for the output signal
Wavelength	190 nm	800 nm	254 nm	Wavelength at which the detector measures the signal. Legal range: see **DetectionMode**.

▣ Thermo Finnigan/TQ/TSP UV3000 Detector

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Default	Description
AcquisitionMode	Single Wavelength	Multi Wavelength	N/A	Changes the data acquisition mode.
Connected	Discon-nected	Connec-ted	N/A	Indicates whether the detector is connected.
UV_Lamp	Off	On	N/A	Turns the deuterium lamp on or off (λ = 190 - 365 nm)
UV_Lamp_Intensity	Intensity_low	Intensity_OK	N/A	Indicates whether the deuterium lamp intensity is too low or OK (read-only)
Visible_Lamp	Off	On	N/A	Turns the tungsten lamp on or off (λ = 366 - 800 nm)
Visible_Lamp_Intensity	Intensity_low	Intensity_OK	N/A	Indicates whether the tungsten lamp intensity is too low or OK (read-only)

Channel-specific Commands and Properties

Property	Min	Max	Default	Description
Response	0.0	9.9	1.0	Time constant. Indicates the time needed by the detector to reach 63% of the full-scale deflection.
⇒*Wavelength*	190 nm	800 nm	254 nm	Wavelength at which the detector measures the signal.

All other channel properties and commands correspond to the standard detector commands (refer to **Practical Tips for Device Control** ▣ **Detector Commands**).

 Notes:

*The **Range** property provided by the TSP UV3000 (analog data acquisition) driver is not required for digital data acquisition; thus, it is not available with the TSP UV3000 (digital data acquisition) driver.*

Due to extensive communication during the ⇒Connect procedure, it may take up to 1 min until the instrument is finally connected. The corresponding message is logged in the Audit Trail.

 Tip:

The device driver does not support the Scan mode of the detector. Therefore, it is not possible to acquire single spectra.

◼ Thermo Finnigan/TQ/TSP: Troubleshooting

Why can't you start the data acquisition?

Is an AS3500 or an AS3000 autosampler installed for which **Inj Hold Active** has been set to **HI**? Set this option to **LO**; otherwise, data acquisition cannot start.

Why does the batch abort?

a) The batch aborts due to a communication timeout.

b) A TSP device is not ready for communication.

In both cases, the reason may be that TSP devices communicate via the Start and/or Stop input. Make sure that no cables are connected to these inputs or remove them if necessary.

AS3500 and AS300:
Why can it take some time until the program is started?

Chromeleon needs to **Wait for the Equilibration Time** or **Wait for Temperature** that is set on the instrument until the autosampler can inject. Reduce the Equilibration Time if your system needs less time to equilibrate or disable the **Wait for Temperature** option. For more information, refer to the device manual.

Only UV3000:
Why doesn't the UV detector respond?

Due to the extensive communication during connecting the device (following the **Connect** command), it may take up to 1 min until the device finally responds. The respective message is logged in the Audit Trail.

UV2000, UV1000, P2000, P4000, AS3500, and AS3000:
How to cure a communication error?

The **Communication error while sending** may occur periodically in the Audit Trail. In this case, reduce the input buffer of the COM port to **Low**: Under Windows 2000 (or correspondingly under another Windows system), open the Device Manager via Settings>Control Panel>System>Hardware. Double-click the respective COM port and open the **Advanced Settings** dialog box by clicking **Advanced** on the **Settings** tab page. Reduce the input buffer as far as possible and confirm the input by clicking **OK**. Disconnect and reconnect the device in Chromeleon.

Only UV1000:
Why is a command not executed?

Have 90 s passed since the last reset? Otherwise, the command cannot be executed because the detector is not yet ready.

◾ Thermo Finnigan/TQ/TSP UV6000 PDA Detector

The following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Default	Description
Data_Collection_Rate (For more information, refer to ➢*Data Collection Rate* in the **Glossary** section.)	0.5 Hz	20.0 Hz	1.0 Hz Read from the device on connect	Indicates the data collection rate for the channels.
FirmwareVersion	Text string (max.32 characters) Read from the device on connect - typically: ROM 0.2 RAM 1.4			Indicates the module's firmware version (read-only)
Rise_Time	0.0 s	10.0 s	2.0 s Read from the device on connect	Indicates the response time after a signal has been changed.

Property	Min	Max	Default	Description
XY_Lamp	Off	On	N/A	Turns the tungsten/deuterium lamp on and off.
XY_LampAge	0 h	9999 h	Periodically read from the device.	Indicates the time the tungsten/deuterium lamp has been on since its installation.
XY_LampReplacedAt	Date string (periodically read from the device)			Indicates the time when the tungsten/deuterium lamp has been installed.

In the tables above and below, XY can be either **UV** (deuterium lamp λ = 190 - 340 nm) or **Visible** (tungsten lamp: (λ = 340 - 800 nm).

☞ *Tip:*

*When the **Data Collection Rate** command is executed, the default ⇒Step value is set to the reciprocal value. Thus, you must issue the **Step** command after the **Data Collection Rate** command, if you need a different step.*

Command	Description
⇒*Autozero*	Sets the detector signal to 0.
⇒*Connect*	Connects the PDA detector to Chromeleon.
Disconnect	Disconnects the PDA detector from Chromeleon.
⇒*Reset*	Resets the PDA detector to the default values.
XY_LampReset	Resets the **XY_LampReplacedAt** counter to **now**

Commands and Properties of UV Channels

Property	Min	Max	Default	Description
⇒*Average*	Off	On	Off	Allows signal averaging.
⇒*Bandwidth*	1 nm	121 nm	1 nm	(Odd values only)
			Read from the device on connect.	
⇒*MaxAutoStep*	0.01 s	5.10 s	5.10 s	Indicates the maximum step for Step = Auto
⇒*Step*	Auto, 0.01 s	5.10 s	Auto	Indicates the time interval between data points.
⇒*Wavelength*	190 nm	800 nm	254 nm	Indicates the wavelength at which the detector measures the signal.

🖐 *Tip:*

*After a **Reset** command, it takes 90 s until the detector is ready to execute another command.*

Commands and Properties of the 3D Field

Property	Min	Max	Default	Description
⇒*Bandwidth*	1	121	1 (Read from the device on connect.)	Indicates the bandwidth in [nm] for all wavelengths in the spectrum² (odd values only)
⇒*BunchWidth*		1 2 4 5 10	1 (Read from the device on connect.)	Indicates the distance in [nm] between the wavelengths in the spectrum².
MaxWavelength	190	800	800	Indicates the upper limit of the measuring range in [nm].
MinWavelength	190	800	190	Indicates the lower limit of the measuring range in [nm].
⇒*Step*		0.05 0.10 0.20 0.25 0.50 1.00 2.00	1.00 (Read from the device on connect.)	Indicates the time interval between recorded spectra in [s].

🖐 *Tip:*

² For example, to scan the wavelengths between 400 nm and 500 nm with a BunchWidth of 10 mm, you can select the following bandwidths, e.g., 11 nm or 5 nm.

a) 11 nm

Select a bandwidth of 11 nm to scan the following wavelength ranges:

395-405, 405-415, 415-425, 425-435, 435-445, 445-455, 455-465, 465-475, 475-485, 485-495, and 495-505 nm.

b) 5 nm

Select a bandwidth of 5 nm to scan the following wavelength ranges:

398-402, 408-412, 418-422, 428-432, 438-442, 448-452, 458-462, 468-472, 478-482, 488-492, and 498-502 nm.

In this case, the wavelength ranges 403-407, 413-417, 423-427 nm etc. are not scanned.

Thermo Finnigan/TQ/TSP UV6000 Detector: Troubleshooting

How can you turn on the lamps?

The PDA detector does not automatically turn on the lamps at power-up. To turn on the lamps, connect the PDA detector to Chromeleon, and issue the corresponding commands.

Why can't you reconnect the PDA detector?

It is not always possible to reconnect the PDA detector after a breakdown in communication. In this case, turn the detector off and on before reconnecting.

Why can't you start the data acquisition?

Is an AS3500 or an AS3000 autosampler installed for which **Inj Hold Active** has been set to **HI**? Set this option to **LO**; otherwise, data acquisition cannot start.

Why does the batch abort?

a) The batch aborts due to a communication timeout.

b) A TSP device is not ready for communication.

In both cases, the reason may be that TSP devices communicate via the Start and/or Stop input. Make sure that no cables are connected to these inputs or remove them if necessary.

◾ Thermo Finnigan/TQ/TSP: FL2000 and FL3000 Fluorescence Detectors

The following commands and properties are available (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Description
AnOut_1_Offset, AnOut_2_Offset	-50; -20; -10; -5; -2; -1; 0; 1; 2; 5; 10; 20; 50 %FS		Offset on the analog output. (This value is added to the associated measured value.)
AnOut_1_Range, AnOut_2_Range	500; 200; 100; 50; 20; 10; 5; 2; 1; 0,5; 0,2; 0,1; 0,05; 0,02; 0,01 FUFS		Output range for the analog output.
DetectionMode	➤*Fluorescence,* ➤*Phosphorescence*		Operating mode of the detector.
Lamp	Off	On	Turns the lamp on or off. (Note: It is not possible to turn the lamp off during data acquisition. Also, it is not possible to start data acquisition if the lamp is off.)
LampFlashRate	20 Hz	100 Hz	Pulse rate of the xenon lamp (20 Hz prolongs the lamp's life cycle; 100 Hz increases the sensitivity for the measurements).
NumberOfScans²	2 - 32 (in 2 nm increments)		This property is available only for the **FL3000**.
			It specifies how often the monochromator repeats each scan for averaging.
PmtVoltage	0, 500, 600, 700, 800, 900, 1000 V		Specifies the voltage applied to the photomultiplier tube (PMT). (The PMT's sensitivity increases proportional to the applied voltage. However, higher voltages shorten the PMT's life cycle.)
ScanEndEm Wavelength²	200 - 650 nm (or 800 nm; in 2 nm increments)		This property is available only for the **FL3000**.
			Emission wavelenth at the end of the scan. (Ignored for ScanMode = Excitation)
			The difference between **ScanStartEmWavelength** and **ScanEndEmWavelength** must be a multiple of 10.

Property	Min	Max	Description
ScanEndEx Wavelength²	200 - 650 nm (in 2 nm increments)		This property is available only for the **FL3000**.
			Excitation wavelength at the end of the scan (Ignored for ScanMode = Emission)
			The difference between **ScanStartEmWavelength** and **ScanEndEmWavelength** must be a multiple of 10.
			If **ScanMode**=*Delta*, the differences between the emission and the excitation scan ranges must be identical: **(ScanEndEmWavelength - ScanStartEmWavelength)** equals **(ScanEndExWavelength - ScanStartExWavelength)**.
ScanMode	Excit.	Emission	This property is available only for the **FL3000**.
			Scan mode:
			• *Excitation*: The emission wavelength is constant; the excitation monochromator scans the wavelengths.
			• *Emission*: The excitation wavelength is constant; the emission monochromator scans the wavelengths.
			• *Delta*: Both monochromators perform the scans simultaneously; the difference between the wavelengths is constant.
ScanStartEm Wavelength²	200 - 650 nm (or 800 nm; in 2 nm increments)		This property is available only for the **FL3000**.
			Emission wavelength at the beginning of the scan (ignored for ScanMode = Excitation)
ScanStartEx Wavelength²	200 - 650 nm (in 2 nm increments)		This property is available only for the **FL3000**.
			Excitation wavelength at the beginning of the scan (ignored for ScanMode = Emission)
ScanStepSize	2, 4, 8, 16 nm		This property is available only for the **FL3000**.
			Specifies the step for the wavelength scan.

☞ *Tip:*

² If the value for NumberOfScans and/or for the area to be scanned, specified by either ScanStartEmWavelength and ScanEndEmWavelength or ScanStartExWavelength and ScanEndExWavelength, is too large, the firmware may fail to respond. If this occurs, the sample is interrupted and the batch stops. To resume operation, turn the instrument off and on again.

Command	Description
Scan	This property, which is available only for the **FL3000**, starts the scan for the emission and/or excitation wavelength.
	Scanning is possible only while a PGM File is running.
	During the run of a PGM File, manual scanning is possible only if the detector provides enough storage capacity.
	A scan can start only after the previous baseline scan is finished (see ScanAutozero).
	👉 *Tip:*
	The chromatogram is disturbed during the scan.
ScanAutozero	This property, which is available only for the **FL3000**, starts the baseline scan for the emission and/or excitation wavelength.
	A baseline scan can start only after the previous baseline scan is finished.

Data acquisition channel

Property	Min	Max	Description
AutozeroOnWlChange	Off, On		If the setting is *On*, autozeroing is performed each time the wavelength (see EmWavelength and ExWavelength) is entered.
			👉 *Tip:*
			Autoszeroing is performed each time the wavelength is changed, even if the new value is the same as the previous one.
EmWavelength	200 - 650 (or 800; in increments of 2)		Emission wavelength in [nm].
ExWavelength	200 - 650 (in increments of 2)		Excitation wavelength in [nm].
Response	0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0		Specifies the response time in [s] for the detector (see ➢*Response*).
Signal.UpperLimit Signal.LowerLimit	-1000	1000 FU	Upper and lower signal limit.
			👉 *Tip:*
			If the actual value is above the upper limit or below the lower limit, Chromeleon aborts the batch and starts emergency handling.
Signal.Value	-1000	1000 FU	Indicates the current signal value.

 Tip:

*After a **Reset** command, it takes 90 s until the detector is ready to execute another command.*

Also, refer to **Thermo Finnigan/TQ/TSP: FL2000 and FL3000 Fluorescence Detectors: Different Names in Firmware and Chromeleon**

Thermo Finnigan/TQ/TSP: FL2000 and FL3000 Fluorescence Detectors: Different Names in Firmware and Chromeleon

For consistency reasons, the names of some Chromeleon properties and commands are different from the names used in the instrument's firmware:

Instrument	Chromeleon	Description
Lamp Status: *On*, *Off*, *Run*, *Off@End*	**Lamp**: *On*, *Off*	The instrument provides two additional lamp modes:
		Run (The lamp is turned on at the beginning of the sample run; it is turned off at the end of the run.) *Off@End* (The lamp is turned off at the end of the sequence.)
		The device driver does not support these modes because you can control the lamp by the corresponding commands in the PGM File.)
Scan	**Scan**	This command corresponds to 'Scan' entry on the instrument's **COMMANDS** menu (manual scanning).
Scan Zero	**ScanAutozero**	The Chromeleon command corresponds to the 'Scan Zero' command on the instrument's COMMANDS menu (manual baseline scanning).
		The first **ScanAutozero** command that is included in the PGM File at a non-negative time is not executed immediately. The time is downloaded to the instrument as **Autozero Time** parameter. The instrument performs the **Autozero** command at this time.
Autozero Time	Supported via the **ScanAutozero** command (see above)	
Auto Spectra: *On*, *Off*		Chromeleon sets this parameter always to Off because Chromeleon can perform automatic scanning more reliably by using ➢ *Trigger Commands*.

Instrument	Chromeleon	Description
Zero on λ Change	**AutozeroOnWlChange**	
Detection Type:	**DetectionMode**:	In addition, it is possible to measure the ➤*Chemiluminescence*. Use one of the two modes and turn off the lamp.
Fluor	➤*Fluorescence*	
Phos	➤*Phosphorescence*	

🖰☞ *Tip:*

*After a **Reset** command, it takes 90 s until the detector is ready to execute another command.*

Also, refer to 🔲 **Thermo Finnigan/TQ/TSP: FL2000 and FL3000 Fluorescence Detectors**

🔲 Thermo Finnigan/TQ/TSP: FL2000 and FL3000 Detectors: Troubleshooting

Why does the batch and the sample abort and the detector does not respond?

If the value for NumberOfScans and/or for the area to be scanned, specified by either ScanStartEmWavelength and ScanEndEmWavelength or ScanStartExWavelength and ScanEndExWavelength, is too large, it may happen that the sample is interrupted and the batch is stopped. (The reason is that the firmware does not respond.) Turn the instrument off and on again.

Why does the batch abort?

a) The batch aborts due to a communication timeout.

b) A TSP device is not ready for communication.

In both cases, the reason may be that TSP devices communicate via the Start and/or Stop input. Make sure that no cables are connected to these inputs or remove them if necessary.

Why doesn't the fluorescence detector respond?

Due to the extensive communication during connecting the device (following the **Connect** command), it may take up to 1 min until the device finally responds. The respective message is logged in the Audit Trail.

Valco Valves: Commands and Tips

The Two-Position Valve has two valve positions (A and B). The Multi-Position Valve has up to 16 valve positions (1, 2, 3, ...16).

The valve is switched to position A (Two-Position Valve) or position 1 (Multi-Position Valve) when connecting to Chromeleon.

Valve Commands and Properties

In addition to the standard commands, the following commands and properties are available (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Value	Description
Direction (Available only for the Multi-Position Valve.)	Clockwise or Counterclockwise	Direction in which the valve is switched.
FirmwareDate		Firmware date (read-only).
FirmwareVersion		Firmware version (read-only).
FlipDelay (Available only for the Two-Position Valve.)	0 to 65.000 ms	Delay time for the **Flip** command.
MaxPosition (Available only for the Multi-Position Valve.)	4, 6, 8, 10, 12, 16	Maximum number of positions (read-only). The property is set on the **General** tab page in the Server Configuration program.
Position	A or B 1 through MaxPosition, respectively.	Current valve position.

Command	Description
Flip (Available only for the Two-Position Valve.)	Switches the valve to the opposite position, waits for the time specified by the **FilpDelay** property, and then returns to the original position.
Home	Switches the Two-Position Valve to position A. Switches the Multi-Position Valve to position 1.
Reset	Performs a Disconnect command, and then a ⇒*Connect* command.
Toggle (Available only for the Two-Position Valve.)	Switches the valve to the other position.

For an overview of the Valco valves, refer to **Hardware Installation** 🌐 **Valco Valves** in the **Administrator Help** section.

Varian: Commands and Tips

For information about the special commands that Chromeleon supports for the different Varian devices and for tips for practical operation, refer to:

Varian 3800 GC:

 General

 Sampler

 Injector

 Column

 Detectors

 Troubleshooting

Varian 3400 and 3600 GCs:

 Varian 3400 and Varian 3600 GCs

 Different Names in Firmware and Chromeleon

 Detectors

 Different Names in Firmware and Chromeleon

 Tips for Operation

 Troubleshooting

▪ Varian 3800 GC: General

In addition to the 🔳 **General Device Commands**, the Varian 3800 GC supports the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Range/Value	Description
AtmosphericPressure²	0.000 - 2.067 bar	Indicates the atmospheric pressure
Cryo	On/Off	(If cryo cooling is installed) Enables or disables cyro cooling.
CryoHasTimeout²	Yes, No	(If cryo cooling is installed) = Yes if the temperature zone did not reach the target temperature, despite Cryo.
CryoTimeout	0.01 - 999.99 min	(If cryo cooling is installed) Time that the GC waits before turning off the cooling if no sample has been injected.
CryoThreshold	30-450°C	(If cryo cooling is installed) Temperature above which the GC shall use cryo for cooling.
EndRunTime²	0.01 - 999.99 min	Actual run time of the current program.
Fault²	Fault, OK	Indicates whether a fault occurred in the GC*.
OvenFault²	Fault, OK	Indicates whether a fault occurred in the column oven.
OvenPower²	On, Off	Indicates whether the column oven is on.
OvenReady²	Ready, NotReady	Indicates whether the column oven has reached the target temperature and is ready.
ProtocolVersion	n/a n/a	Protocol version of the instrument (must be '7').
RunState	NotReady, Equilibrating, Stabilizing, Monitoring, Ready, Running	Indicates the status of the GC.
RunTime	0.01 - 999.99 min	Indicates the actual retention time during sample processing.
StabilizationTime²	0.00 - 10.00 min	Time that the GC waits after reaching the target temperature.
Temperature		Properties related to the column oven temperature.
Temperature. Value²	-99°C - 450°C	Current temperature.
Temperature. Nominal	-99°C - UpperLimit	Sets the nominal temperature.
Temperature. UpperLimit	50 - 450°C	The system aborts the sample batch and starts emergency handling if the temperature exceeds the upper limit.

 Note:

² These properties are read-only.

For an overview of the Varian 3800 GC, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Varian 3800 GC: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** **Varian 3800 GC: Installation** in the **Administrator Help** section.

Varian 3800 GC: Sampler

In addition to the **General Device Commands** and the standard autosampler commands (see **Dionex Autosamplers**), the Varian 3800 GC supports the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property/Command	Range/Values	Description
AbortWashVial	I, II, III	(Only 8400) Wash vial that is used when the injection was aborted.
AdvanceTray	Yes, No	Sets whether both injections are performed from the same tray (No) or whether the second injection is performed from the next tray (Yes).²
AirPlugAfterSample	0.0 - 5.0 µl (5µl syringe) 0.0 - 10.0 µl (10µl syringe) 0.0 - 100 µl (100µl syringe)	Volume of the air gap after the sample.³
Buzzer	On, Off	(Only 8400) Turns the buzzer on or off at the beginning of the injection cycle.
CleanBetweenInjections	Yes, No	Sets whether a wash cycle is performed between injections (Yes).
Depth_1 (*or* Depth_2)	0 - 100 %	(Only 8400 if the injection port 1 (or 2) is enabled in the configuration) Sets how deep the syringe needle descends into the injector.
FillStrokes	0 - 99	Number of piston strokes for filling the syringe.

Property/Command	Range/Values	Description
FillVolume	0.0 - 5.0 µl (5µl syringe)	Volume with which the syringe is to be filled.
	0.0 - 10.0 µl (10µl syringe)	
	0.0 - 100 µl (100µl syringe)	
FirstInjectorUsed	Position_1, Position_2	Sets which injector is used first for the injection. (If only one injector is installed, the property is read-only.)
Home		(Only 8400) Recalibrates the sampler's Home position.
		👉 *Tip:*
		Select this command when a mechanical error occurred after the sampler accessed a position.
InjectionDelay	0.0 - 10.0 min	Determines how long the sampler waits between injections.
InjectionMode	Split_Splitless, On_ Column, Neat, Viscous, Volatile, User_Defined,	Only if the injection mode is set to User Defined, all sample parameter can be controlled in Chromeleon.
InjectorPosition	Position_1, Position_2, Both	Sets which injector is used first for the injection. (If only one injector is installed, the property is read-only.)
InjectWaitTime	0.0 - 10000000.0	Indicates the time between the Inject command in Chromeleon and the response to Chromeleon from the autosampler.
IntStd_AirGap	On, Off	(Only 8400 for the **Viscous**, **Volatile**, and **User_Defined** injection modes) Enables usage of an air gap for the internal standards.
IntStd_Delay	0.0 - 9.9 s	(Only 8400 for the **Viscous**, **Volatile**, and **User_Defined** injection modes) Delay time for the internal standards.
IntStd_Enable	On, Off	(Only 8400 for the **Viscous**, **Volatile**, and **User_Defined** injection modes) Enables usage of an internal standard.
IntStd_Position	I, II, III	Position of the internal standard.
IntStd_Speed	0.1 - 25.0 µl/s (5µl syringe)	(Only 8400 for the **Viscous**, **Volatile**, and **User_Defined** injection modes) Speed with which an internal standard is drawn.
	0.1 - 50.0 µl/s (10µl syringe)	
	1 - 100 µl/s (100µl syringe)	

Property/Command	Range/Values	Description
IntStd_Volume	0.0 - 4.9 µl (5µl syringe) 0.0 - 9.9 µl (10µl syringe) 0.0 - 99 µl (100µl syringe)	(Only 8400 for the **Viscous**, **Volatile**, and **User_Defined** injection modes) Volume of the internal standards.
PlungerStrokes.Upperlimit	1 - 10000	(Only 8400) Sets the upper limit for the number of plunger strokes.
PlungerStrokes.Value	0 - 10000	(Only 8400) Indicates the number of plunger strokes. The property can be reset to 0 after a piston has been exchanged.
PostWash	0 - 99	(Only 8400) Sets how often the syringe is washed with solvent after the injection.
PreWash	0 - 99	(Only 8400) Sets how often the syringe is washed with solvent before the injection.
SampleAirGap	On, Off	Enables usage of an air gap for samples.
SampleDepth	0 - 100 %	(Only 8400) Sets how deep the syringe needle descends into the sample vial.
SampleWash	0 - 99	(Only 8400) Sets how often the syringe is filled with sample before the injection.
SecondInjectionVolume	0.1 - 5.0 µl (5µl syringe) 0.1 - 10.0 µl (10µl syringe) 0.1 - 100 µl (100µl syringe)	(Only 8400 if both injection ports are enabled in the configuration) Sets the volume for the second injection.
SolventDepth	0 - 100 %	(Only 8400) Sets how deep the syringe needle descends into the solvent vial.
SolventPlug_AirGap	On, Off	Enables usage of an air gap for solvent plugs[3].
SolventPlug_Delay	0.0 - 9.9 s	Delay time for solvent plugs[3].
SolventPlug_Position	I, II, III	Position of the solvent plug[3].
SolventPlug_Speed	0.1 - 25.0 µl/s (5µl syringe) 0.1 - 50.0 µl/s (10µl syringe) 1 - 100 µl/s (100µl syringe)	Speed with which the solvent plugs are drawn[3].

Property/Command	Range/Values	Description
SolventPlug_Volume	0.0 - 5 µl (5µl syringe) 0.0 - 10 µl (10µl syringe) 0.0 - 100 µl (100µl syringe)	Solvent plug volume[3].
Speed_1 (*or* Speed_2)	1 - 30 %	(Only 8400 if the injection port 1 (or 2) is enabled in the configuration) Sets how fast the syringe needle descends into the injector.
State	On, Off	(Only 8400) Indicates whether the sampler has injected.
Status	Ready, Running, Suspended, Resetting	(Only 8400) Indicates the autosampler status*.
SyringeVolume	5µl, 10µl, 100µl	Indicates the syringe volume*.
VialDetect	On, Off	(Only 8400) If **VialDetect = On** (default setting), the sample checks whether a sample vial is available at the next position. If the vial is missing, the injection is aborted. If **VialDetect = Off**, no error message is logged if the vial is missing.
Visc_Delay	0.0 - 9.9 s	Delivery time for viscous samples[3].
Visc_DispenseSpeed	0.1 - 25.0 µl/s (5µl syringe) 0.1 - 50.0 µl/s (10µl syringe) 1 - 100 µl/s (100µl syringe)	Speed with which viscous samples are dispensed[3].
Visc_DrawSpeed	0.1 - 25.0 µl/s (5µl syringe) 0.1 - 50.0 µl/s (10µl syringe) 1 - 100 µl/s (100µl syringe)	Speed with which viscous samples are drawn[3].
Visc_PostInjectionDelay	0.0 - 99.9 s	Time that the needle remains in the injector after a viscous sample was injected[3].
Visc_PreInjectionDelay	0.0 - 99.9 s	Time that the needle remains in the injector before a viscous sample is injected[3].

Property/Command	Range/Values	Description
⇒*Volume*	0.1 - 5.0 µl (5µl syringe)	Injection volume.
	0.1 - 10.0 µl (10µl syringe)	An error message appears if the total injection volume (Volume + IntStd_Volume + SolventPlug_Volume + AirPlugAfterSample) exceeds the syringe volume.
	0.1 - 100 µl (100µl syringe)	
	For the 8100/8200: Max. ¾ of the syringe volume	
WashCycles	0 - 10	(Only 8400) Sets how often the syringe is washed after an abort.
WashSpeed	0.1 - 25.0 µl/s (5µl syringe)	(Only 8400) Sets the speed with which the wash volume is drawn.
	0.1 - 50.0 µl/s (10µl syringe)	
	1 - 100 µl/s (100µl syringe)	
WashVials	I, II, III, I_II, I_III, I_II_III	(Only 8400) Sets from which wash vial(s) the wash solvent is drawn.
WashVolume	0.1 - 5.0 µl (5µl syringe)	Wash volume.
	0.1 - 10.0 µl (10µl syringe)	
	0.1 - 100 µl (100µl syringe)	
	For 8100/8200: maximum ¾ of the syringe volume	

📝 *Notes:*

* These properties are read-only.

*² Chromeleon supports these properties only if the injection port setting on the **Sampler** tab page in the Server Configuration is **Injection ports = Position 1 and 2***.

*³ Chromeleon supports these properties only for the 8400 in the **User_Defined** injection mode. An error message is displayed if these properties are set in an injection mode other than User_Defined:*

For more information about the Varian 3800 GC, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Varian 3800 GC: Installation** in the **Administrator Help** section.

◾ Varian 3800 GC: Injector

In addition to the ◾ **General Device Commands**, the Varian 3800 GC supports the following injector commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
ConstantFlow	0.0 - 50.0 ml/min	(Only if a type 1 electronic flow control (EFC) is installed) Nominal value for a constant flow rate.
ConstantFlowMode	On, Off	(Only if an EFC type 1 is installed) Enables or disables **ConstantFlow**.
		If ConstantFlowMode is disabled and Pressure.Nominal is not in the allowed range, the nominal pressure is set to the minimum pressure of 0.007 bar.
Cryo	On, Off	(Only if a cooling element is installed) Turns the cooling element on or off.
CryoTimeout	Off, 0.01 - 999.99 min	(Only if a cooling element is installed) Time that the GC waits before turning off the cooling if no sample was injected.
CryoThreshold	30 - 450°C	(Only if a cooling element is installed) Temperature above which the GC shall use cryo for cooling.
Description	On-Column Injector, Universal Capillary Injector, Split/Splitless Capillary Injector, EFC only	Indicates the inlet type*. EFC_only means that only EFC is installed (without injector).
EFCFault	Fault, OK	(Only if electronic flow control (EFC) is installed) Indicates an error in the injector EFC.
EFCReady	Ready, NotReady	(Only if EFC is installed) Indicates whether the injector EFC has reached the target temperature and/or flow and is ready,
EFCType	None, 1, 3, 4, Generic (Reserved)	Indicates the type of injector electronic flow control (EFC)

Property	Range/Values	Description
Flow		Properties related to the total flow
Flow. Value	1.0 - 1000.0 ml/min	(Only if EFC type 1 and 3 is installed) Indicates the current flow*.
Flow.Nominal	0.1 - 100.0 ml/min	(Only if EFC type 3 is installed) Sets the target flow.
		The pressure range can be programmed; the allowed range is 0.007 to 27.576 bar/min in 8 increments (including the first value).
GasSaverFlow	1 - 100 ml/min	(Only if EFC type 1 is installed) Sets the minimum gas flow to which the flow is reduced.
GasSaverTimeout	0.00 .. 999.99 min	(Only if EFC type 1 is installed) Time after which the gas flow is reduced to the minimum gas flow (see GasSaverFlow).
Mode	Split, Splitless	(Only for 1079 and 1177 with EFC) Indicates split or splitless mode.
OvenFault²	Fault, OK	**Fault** indicates a fault in the injector oven.
OvenReady²	Ready, NotReady	**Ready** indicates whether the injector oven reached the target temperature and is ready.
Pressure		Properties related to the column pressure.
Pressure.Value	0.007 - 6.894 bar	(Only if EFC type 1 and 3 is installed) Indicates the current pressure in the column head*.
Pressure.Nominal	0.007 - 6.894 bar	(Only if EFC type 1 is installed) Sets the injector target pressure.
		The pressure range can be programmed; the allowed range is 0.007 to 27.576 bar/min in 8 increments (including the first value).
PulseEnable	On, Off	(Only if EFC type 1 is installed) Enables or disables the pressure pulse.
PulsePressure	0.007 - 6.894 bar	(Only if EFC type 1 is installed) Sets the target value for the pressure pulse.
PulseTime	0.01 - 5.00 min	(Only if EFC type 1 is installed) Sets the duration of the pressure pulse.
SplitRatio	1 - 10000	(Only for the 1079 and 1177 with EFC). Sets the split ratio.
		The ratio can be programmed; a maximum of six increments (including the first value) is allowed. An error message appears if the SplitRatio is set for **SplitMode = Splitless**.
SplitlessVentFlow	0 - 500 ml/min	(Only if EFC type 1 is installed) System flow when the split valve is closed.

Property	Range/Values	Description
Temperature²		Properties related to the injector temperature
Temperature. Value²	-99°C - 450°C	Indicates the current temperature*.
Temperature. Nominal²	-99°C - UpperLimit	Sets the target temperature.
Temperature. UpperLimit²	50 - 450°C	If the temperature exceeds this value, Chromeleon aborts the batch and starts emergency handling.
Type	1041, 1079, 1177, EFC only	Indicates the inlet type*. EFC_only means that only EFC is installed (without injector).

📝 *Notes:*

* These properties are read-only.

² These properties are available only if a heating zone was specified for the associated injector in the Server Configuration program.

For an overview of the Varian 3800 GC, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Varian 3800 GC: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Varian 3800 GC: Installation** in the **Administrator Help** section.

🔲 **Varian 3800 GC: Column**

In addition to the 🔲 **General Device Commands**, the Varian 3800 GC supports the following column commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
Diameter	0 - 999 µm	Inner column diameter (read-only; for packed columns, the entry is 0 µm).
Flow	0.0 - 999.99 min	Indicates the flow rate in the column (read-only).
GasType	He, H2, N2	Type of the carrier gas (read-only).
Length	0.00 - 250 m	Column length (read-only; for packed columns, the entry is 0.00 m)

Property	Range/Values	Description
Mode	Standard, Rapid_MS	Column mode.
SeptumPurgePressure	0.007 .. 6.894 bar	Pressure at the column's purge head septum (read-only).
SeptumPurgeFlow	0.1 ..100.0 ml/min	Flow rate at the column's purge head septum (read-only).
Velocity	0.0 - 999.99 cm/s	Linear velocity in the column (read-only).

For an overview of the Varian 3800 GC, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔄 **Varian 3800 GC: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔄 **Varian 3800 GC: Installation** in the **Administrator Help** section.

▪ Varian 3800 GC: Detectors

In addition to the 🔳 **General Device Commands** and the standard detector commands (see 🔳 **Dionex Detectors**), the Varian 3800 GC supports the following detector commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Range/Values	Description
AirFlow		Properties related to the air flow.
AirFlow. Value	0 - 500 ml/min	(Only for type 11 or type 12 electronic flow control (EFC)) Current air flow (read-only).
AirFlow.Nominal	0 - 500 ml/min	(Only for type 11 or type 12 EFC) Sets the target air flow.
BalancePercentage	-100 - 100 %	Balance percentage for the bridge (read-only). The property is set during Autozero in such a way that the signal value is 0.
Baseline	-10^8 - 10^8 mV	Current baseline value (read-only).
BeadCurrent	2.4 .. 3.8 A	(Only TSD) Sets the current at the bead.
BeadPower	On, Off	(Only TSD) Turns the bead power on or off (read-only).
CellContactPotential	-800 - 800V	(Only ECD) Cell contact voltage.
CellCurrentSelection	N2_High, N2_Standard, CAP, AR_CH4, ZERO	(Only ECD) Selection of the cell current, based on the gas type.
Channel1_GasType	Helium Makeup, Nitrogen Makeup, Argon Makeup	(Only for type 11, 12, or 14 EFC) Type of the carrier gas in channel 1 (read-only)

Property	Range/Values	Description
Channel2_GasType (Type 11 or 12 EFC)	Hydrogen	(Only for type 11 or type 12 EFC) Type of the carrier gas in channel 2 (read-only)
Channel2_GasType (Type 14 EFC)	Helium Makeup, Nitrogen Makeup, Argon Makeup, Helium Reference, Nitrogen Reference, Argon Reference	(Only for type 14 EFC) Type of the carrier gas in channel 2 (read-only)
Channel3_GasType	Air	(Only for type 11 or type 12 EFC) Type of the carrier gas in channel 3 (read-only)
CheckFlameOut	On, Off	(Only FID) Enables the check whether the flame is burning.
Description	Flame Ionization Detector, Thermal Conductivity Detector, Thermionic Specific Detector, Electron Capture Detector	Detector type (read-only)
DetFault	Fault, OK	**Fault** indicates a detector error.
DetReady	Ready/NotReady	Indicates whether the detector has reached the initial condition and is ready.
EFCFault	Fault, OK	(Only if EFC is installed) Indicates an error in the detector EFC (read-only).
EFCReady	Ready, NotReady	(Only if EFC is installed) Indicates whether the detector EFC has reached the target flow and is ready (read-only).
EFCType	None, 11, 12, 15, 16	Indicates the type of detector electronic flow control (read-only; electronic flow control = EFC)
Electronics	On, Off	(FID, TCD, and ECD) Turns the electronic elements of the detector on or off.

Note:

If the electronic elements are off, the GC can be Ready even if, e.g., the flame of the FID is not burning.

Property	Range/Values	Description
FilamentCurrent	0 .. 500 mA	(Only TCD) Indicates the current of the filament (read-only).
FilamentTemerature. Nominal	0 - 490°C	(Only TCD) Sets the target temperature for the filament.
FilamentTemerature. UpperLimit	390°C or 490°C	(Only TCD) Upper limit for the filament temperature (read-only)

Property	Range/Values	Description
FilamentTemerature. Value	0 - 490°C	(Only TCD) Current temperature of the filament (read-only).
GasType	He, N2_Ar	Type of the carrier gas.
HydrogenFlow		Properties related to the hydrogen flow
HydrogenFlow. Value	0 - 500 ml/min	(Only for type 11 or type 12 EFC) Current flow (read-only)
HydrogenFlow. Nominal	0 - 500 ml/min	(Only for type 11 or type 12 EFC) Sets the target hydrogen flow.
MakeupFlow		Properties related to the carrier gas flow.
MakeupFlow. Value	0 - 50 ml/min (Type 11, 12, or 14 EFC)	(Only for type 11, 12, or 14 EFC) Current carrier gas flow (read-only)
MakeupFlow. Nominal	0 - 50 ml/min (Type 11, 12, or 14 EFC)	(Only for type 11, 12, or 14 EFC) Sets the carrier gas target flow.
OvenFault	Fault, OK	**Fault** indicates a detector oven error.
OvenReady	Ready/NotReady	Indicates whether the detector oven has reached the target tempeature and is ready.
Polarity	Negative, Positive	Sets the detector polarity. The polarity can be programmed in up to 7 increments (including Range and Autozero).
Range	9, 10, 11, 12 (FID, TSD) 0.05, 0.5, 5.0 (TCD) 1, 10 (ECD)	Sets the output range of the detector. The range can be programmed in up to 7 increments (including Polarity and Autozero).

🗒️ *Note:*

This range also refers to digital signals.

Property	Range/Values	Description
ReferenceFlow		Properties related to the reference gas flow
ReferenceFlow. Value	0 - 100 ml/min	(Only for type 14 EFC) Current reference gas flow (read-only)
ReferenceFlow. Nominal	0 - 100 ml/min	(Only for type 14 EFC) Sets the reference gas target flow.
Signal	Signal.LowerLimit - Signal.UpperLimit	Current detector signal (read-only)
Signal.LowerLimit	-10^8 mV bis 10^8 mV	Lower limit for the detector signal (read-only)
Signal.UpperLimit	-10^8 mV bis 10^8 mV	Upper limit for the detector signal (read-only)
TempCtrl	On / Off	Turns the detector oven on or off.
Temperature. Nominal	-99 - Upperlimit	Sets the target temperature for the detector.
Temperature. Upperlimit	50 - 450°C	Upper limit for the detector temperature (read-only)

Property	Range/Values	Description
Temperature. Value	-99 - 450°C	Current detector temperature (read-only)
TimeConstant	Slow, Fast	Sets the time constant for the noise filter of the detector.
Type	FID, ECD, TCD, TSD	Indicates the detector type (read-only).

For an overview of the Varian 3800 GC, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Varian 3800 GC: Overview** in the **Administrator Help** section.

For installation details, refer to **Hardware Installation: Installing and Controlling Third-Party Devices** 🔵 **Varian 3800 GC: Installation** in the **Administrator Help** section.

▪ Varian 3800 GC: Troubleshooting

Problem:

The gas chromatograph does not start after reconfiguration has been performed in the Ethernet setup. This may be due to errors in the Ethernet configuration.

Solution:

Follow the steps below:

1. Uninstall the network card. Please note that the network card is located behind the EPROM card. Therefore, uninstall the EPROM card first.

2. Reinstall the EPROM card.

3. Start the gas chromatograph.

4. Reconfigure the gas chromatograph as follows:

   - Set BOOTP to No.

   - Enter the associated network address in the Network Address field. Verify that no other PC or device is already using this address.

   - In addition, make sure that the subnet mask is identical to the subnet mask of the PC and that the gateway addresses and the IP addresses of the gas chromatograph and the PC are identical as well.

5. Restart the instrument via Save+Exit (without the network card) to check whether the instrument accepted the changes.

6. Turn the instrument off.

7. Reinstall the network card.

8. Reconnect and restart the instrument.

Problem:

It looks as if changes to different parameters, such as ConstFlowMode, ConstFlow, and Pulse are not transmitted to the instrument.

Solution:

The method that is displayed on the instrument is not the method that is actually used. Set **Active Method** on the instrument to display the currently used method.

☞ *Tip:*

*In addition, set **Edit Method** to **Method 8**. This ensures that **Method 8**, which is used by Chromeleon, is displayed in any case.*

◼ **Varian 3400 and Varian 3600 GCs**

The Varian 3400 GC and Varian 3600 GC device drivers support the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Columns and Main Device

Property	Min	Max	Default	Description
CausesForNotReady	N/a	N/a	N/a	Indicates why the driver is not in Ready state (read-only).
Connected	Off	On	Off	Indicates whether the GC is connected to Chromeleon.
CryoHasTimeout	Off	On	Off	Indicates whether a cryogenic coolant time out occurs (read-only).
CryoTimeout	2.00	650.00	650.00	If no run starts after the specified time (in [min]), cooling is stopped to save coolant. To disable CryoTimeout, set the property to **Infinite**.

Property	Min	Max	Default	Description
CryoToColumn	Off	On	Off	Turns cryogenic cooling for the column thermostat on and off.
CryoToInjector	Off	On	Off	Turns cryogenic cooling for the injector on and off. (For the Varian 3400 GC, this is an Injector property.)
ModelNo	N/a	N/a	N/a	Indicates the model number (read-only). This property is available only for the Varian 3600 GC.
Ready	N/a	N/a	N/a	Indicates whether the GC is ready for injection (read-only).
StabilizationTime	0.00	650.00	2.00	After reaching the desired temperature, the GC waits for the specified time (in [min]) before indicating Ready.
StabilizationTimeRemainder	N/a	N/a	N/a	Indicates how much time of the StabilizationTime is left (read-only). This property is available only for the Varian 3600 GC.
SystemState	N/a	N/a	N/a	Indicates the current GC status: Run, Equilibrate, Stabilize, Wait, Sample, Test, RunEnd, or ERROR (read-only).
TempCtrl	Off	On	On	Turns the oven on or off.
Temperature.UpperLimit	²	²	²	Sets the maximum temperature for the oven.
Temperature.Nominal	²	²	²	Sets the nominal temperature for the oven
Temperature.Value	-99°C	420°C	50°C	Indicates the actual temperature of the oven (read-only).

² The values depend on the device settings.

Command	Description
⇒*Connect*	Connects the GC to Chromeleon.
Disconnect	Disconnects the GC from Chromeleon.
⇒*Reset*	Resets the GC.

Auxiliary Heater

Property	Min	Max	Default	Description
TempCtrl	Off	On	²	Turns the auxiliary heater on and off.
Temperature.Nominal	²	²	²	Sets the nominal temperature of the auxiliary heater.
Temperature.UpperLimit	²	²	²	Sets the maximum temperature of the auxiliary heater.
Temperature.Value	-99°C	420°C	50°C	Indicates the actual temperature of the auxiliary heater (read-only)

² The values depend on the device settings.

Autosampler

Property	Min	Max	Default	Description
AirDry	Off	On	On	Enables or disables air drying after the injection.
Depth	0%	100%	90%	Sets how deep the needle moves into the vial: 0%: septum height; 100%: to the bottom of the vial.
InjectWaitTime	0.0	10000000.0	N/a	Indicates the time delay [sec] between the injection command and the inject response.
LowerAirGap	Off	On	On	Sets whether an air gap is used below the sample.
Position	101	412	101	Indicates the position of the vial used for injection. Range: 101 ... 112; 201 ... 212; 301 ... 312; 401 ... 412
PostDwell	0.00	10.00	0.10	Sets the time [min] that the needle remains in the injection port after the injection.
PreDwell	0.00	1.00	0.00	Sets how long ([min]) the needle stays in the injection port before the injection.
Purge	N/a	N/a	A_only	Sets which solvent bottles are used for the washing process: A_only, B_only, A_then_B, or No_wash (For the Varian 3400 GC, this is an Injector property.)
SamplerStatus	N/a	N/a	N/a	Indicates the current state of the autosampler: ERROR, Searching, Washing, Sampling, Injecting, Waiting, Stop_Pin_Found, No_Vial, or Not_in_method (read-only).

Property	Min	Max	Default	Description
SolventPlug	0.0 µl	3.0 µl	0 µl	Sets the solvent volume for the injection.
Speed	0.2	10.0	5.0	Sets the average injection speed [µl/sec]
State	Off	On	N/a	Indicates whether the autosampler has injected the sample.
UpperAirGap	Off	On	On	Sets whether an air gap is used above the sample.
Volume	²	²	²	Sets the injections volume.²

² The values depend on the syringe volume. The Varian 3600 GC supports a variable syringe volume.

Command	Description
⇒*Inject*	Performs an injection.

Injector

Property	Min	Max	Default	Description
Flow.Nominal³	0	100	40	Sets the nominal carrier gas flow [ml/min]
Flow.Value³	0	100	N/a	Indicates the actual carrier gas flow [ml/min] (read-only).
Pressure.Nominal³	0	60	15	Sets the nominal carrier gas pressure [psi].
Pressure.Value³	0	60	N/a	Indicates the actual carrier gas pressure [psi] (read-only).
TempCtrl	Off	On	On	Turns the injector heater on or off.
Temperature.Nominal	²	²	²	Indicates the nominal temperature of the injector heater.
Temperature.UpperLimit	²	²	²	Sets the maximum temperature of the injector heater.
Temperature.Value	-99°C	420°C	50°C	Indicates the actual temperature of the injector heater (read-only).

² The values depend on the device settings.

³ These properties are available only if you have selected the **Injector Flow Control** or **Injector Pressure Control** option on the **Components** tab page in the Server Configuration program.

Valve (available only for the Varian 3600 GC)

Property	Min	Max	Default	Description
Duration	0.00	59999.90	N/a	When set, the relay switches after the indicated time [sec].
State	Off	On	Off	Indicates and sets the valve's state.

Command	Description
Off	Turns the valve off.
On	Turns the valve on.

Also, refer to **Varian 3400 3600 GCs: Different Names in Firmware and Chromeleon**.

For an overview of the Varian 3400 GC, refer to **Hardware Installation** **Varian 3400 GC** in the **Administrator Help** section.

For an overview of the Varian 3600 GC, refer to **Hardware Installation** **Varian 3600 GC** in the **Administrator Help** section.

Varian 3400 and 3600 GCs: Different Names in Firmware and Chromeleon

 Tip:

Dionex recommends avoiding manual input on the device. Chromeleon cannot read all settings from the device. Therefore, manual settings on the device may lead to errors in the program.

For consistency reasons, the names of some Chromeleon properties and commands are different from the names used in the instrument's firmware:

Columns and Main Device

Instrument	Chromeleon
Coolant To Injector	CryoToInjector
Coolant To Column	CryoToColumn
Fault 39	CryoHasTimeout
Coolant Time Out²	CryoTimeout
div. fault codes	CausesForNotReady
Thermal Stabilize Time²	StabilizationTime
Column Oven On/Off	TempCtrl
Column Temp Limit³	Temperature.UpperLimit
Initial Column Temp	Temperature.Nominal

■☞ *Tip:*

*² Chromeleon cannot read **Coolant Time Out** and **Thermal Stabilize Time** from the instrument. Therefore, these setting are overwritten when Chromeleon connects to the instrument and when the sample is started. Please do not set these parameters on the instrument.*

Auxiliary oven

Instrument	Chromeleon
Auxiliary Oven On	TempCtrl
Auxiliary Temp Limit	Temperature.UpperLimit
Initial Aux Temp	Temperature.Nominal

Autosampler

Instrument	Chromeleon
A/S Solvent Plug Size	SolventPlug
A/S Lower Air Gap	LowerAirGap
A/S Upper Air Gap	UpperAirGap
A/S Air Dry After Wash	AirDry
A/S Solvent Select	Purge
Vial Needle Depth	Depth
A/S Residence Time	PostDwell
A/S Hot Needle Time	PreDwell
A/S Uptake Speed	Speed

Injector

Instrument	Chromeleon
Pressure	Pressure.Nominal
Flow	Flow.Nominal
Injector Oven On/Off	TempCtrl
Injector Temp Limit	Temperature.UpperLimit
(Initial) Injector Temp	Temperature.Nominal

Valve (available only for the Varian 3600 GC)

Instrument	Chromeleon
Initial Relays	State

🖏 *Tip:*

³ Chromeleon cannot read the temperature limits from the instrument. Therefore, these setting are overwritten when Chromeleon connects to the instrument and when the sample is started. Please do not change the temperature limits on the instrument.

For detailed information about the different commands and the allowed minimum and maximum values, refer to 🔺 **Varian 3400 and 3600 GCs**.

🔲 Varian 3400 and Varian 3600 GCs: Detectors

The Varian 3400 and 3600 GC device drivers support the following commands and properties for the different detectors (please note that the display ➤*Filter* level determines which commands and properties are displayed):

General Detector Properties

Property	Min	Max	Default	Description
Attenuation	1	8	1	Sets the attenuation of the detector signal
Baseline	-1315.80	1315.80	N/a	Indicates the actual baseline value.
TempCtrl	Off	On	On	Turns the detector heater on or off.
Temperature.Nominal	²	²	²	Sets the nominal temperature for the detector heater.
Temperature.UpperLimit	²	²	²	Sets the maximum temperature for the detector heater.
Temperature.Value	-99°C	420°C	50°C	Indicates the actual temperature of the detector heater (read-only).
Type	TCD, FID, TSD, or ECD		N/a	Indicates which type of detector is installed.

² The values depend on the device settings.

Command	Description
⇒*Autozero*	Sets the current detector signal to 0.

Thermal Conductivity Detector (TCD)

Property	Min	Max	Default	Description
FilamentTemp	50°C	390°C	50°C	Sets the temperature of the TCD filament.
Polarity	Negative	Positive	Positive	Sets the polarity of the detector signal.
Range	5	500	500	Range for the output signal: 5, 50, 500.

Flame Ionization Detector (FID)

Property	Min	Max	Default	Description
Range	10^8	10^{12}	10^8	Range for the output signal.

Thermionic Specific Detector (TSD)

Property	Min	Max	Default	Description
BeadCurrent	2400	3800	2400	Indicates the current bead power [mA].
BeadPower (TSD_BeadPower for the Varian 3400 GC)	Off	On	On	Turns the TSD bead power on or off.
Range	10^8	10^{12}	10^8	Range for the output signal.

Electron Capture Detector (ECD)

Property	Min	Max	Default	Description
Range	1	10	1	Range for the output signal (allowed values: 1 and 10).

Also, refer to **Varian 3400 3600 GC Detectors: Different Names in Firmware and Chromeleon**.

For an overview of the Varian 3400 GC, refer to **Hardware Installation Varian 3400 GC** in the **Administrator Help** section.

For an overview of the Varian 3600 GC, refer to **Hardware Installation Varian 3600 GC** in the **Administrator Help** section.

🔲 Varian 3400 and 3600 GC Detectors: Different Names in Firmware and Chromeleon

For consistency reasons, the names of some Chromeleon properties and commands are different from the names used in the instrument's firmware:

General Detector Properties

Instrument	Chromeleon
Detector A/B Oven On	TempCtrl
Detector Temp Limit²	Temperature.UpperLimit
Detector Temp	Temperature.Nominal
Initial Atten	Attenuation

👉 *Tip:*

² Chromeleon cannot read the temperature limits from the instrument. Therefore, these setting are overwritten when Chromeleon connects to the instrument and when the sample is started. Please do not change the temperature limits on the instrument.

Thermal Conductivity Detector (TCD)

Instrument	Chromeleon
TCD Filament Temp Off	FilamentTemp
Initial Range	Range
Polarity Positive	Polarity

Flame Ionization Detector (FID) and Electron Capture Detector (ECD)

Instrument	Chromeleon
Initial Range	Range

Thermionic Specific Detector (TSD)

Instrument	Chromeleon
Bead Power On	BeadPower
Bead Current In Amps	BeadCurrent
Initial Range	Range

For detailed information about the different commands and the allowed minimum and maximum values, refer to 🔲 **Varian 3400 and 3600 GCs: Detectors**.

◱ Varian 3400 and 3600 GCs: Tips for Operation

Method

For the 3600 GC:
The driver always uses Method 1. The method is automatically activated and overwritten with the settings in Chromeleon.

For the 3400 GC:
The driver for the Varian 3400 GC cannot identify whether a reset was performed on the instrument or a different method was selected. Therefore, Dionex recommends that you always activate Method 1 before Chromeleon connects to the instrument and that you do not change this setting while Chromeleon controls the instrument.

Setting Properties

You can set properties only if the GC is not in **Sample**, **Run**, or **RunEnd** mode. If you set a property for the 3400 GC although the GC is in one of the above modes, execution is postponed until the GC is no longer in this mode. A message is written to the Audit Trail. If you set a property for the 3600 GC although the GC is in one of the above modes, a message is written to the Audit Trail but the command will not be executed.

The only commands and properties that can be used after an **Inject** command are: temperature gradients for the column oven, **Acquisition On/Off**, **Delta**, **Average**, and **Step**. Therefore, define all properties that are required for the method before the **Inject** command. A **Reset** command can always be sent.

If you want to include the temperature for an isocratic column oven in a program, define the temperature before and after the **Inject** command, and at the final program time. Otherwise, runtime calculation will not be correct.

Example:

0.000	GC.Temperature =	100
	Wait	GC.Ready
	Inject	
	GC.Temperature =	100
30.000	GC.Temperature =	100
	End	

Entering a Temperature Gradient

Temperature gradients can be defined only for the column heater.

Enter the temperature gradient either directly, by selecting the **Flow** command on the **Control** menu, or via a ➤*Program*.

Chromeleon supports a temperature profile with up to four ascents. The maximum change in temperature is 50°C per minute. Descending temperature profiles are not supported. The GC returns to the initial temperature when the gradient is completed.

3600 GC: **Flow / Pressure**

The gas chromatograph maintains a constant pressure or flow for column A or column B, depending on the value that was entered last. This is how the column is selected.

Example:

If the following command was entered last:

```
Injector_B.Flow = 1
```

the flow for column B is kept constant at 1 ml/min.

For an overview of the Varian 3400 GC, refer to **Hardware Installation** 🔹 **Varian 3400 GC** in the **Administrator Help** section.

For an overview of the Varian 3600 GC, refer to **Hardware Installation** 🔹 **Varian 3600 GC** in the **Administrator Help** section.

■ Varian 3400 and 3600 GCs: Troubleshooting

Problem: The GC does not reach the Ready state

In addition, the following message appears in Chromeleon: GC.CausesForNotReady=Remote_In.

Solution:

Disable the Remote input on the device. Select **Set Checks** on the Configuration menu and set **Wait for ext. device ready?** to **No**.

Error messages in Chromeleon:

(GC) Communication Error: Bad Response to "0" command 0

Problem: The instrument uses a method from the GC's method table.

> The error message is repeated until Chromeleon disconnects from the instrument.

Solution: On the instrument, delete all references to Method 1:

Select Method 1.

> Press Delete/Section Table and select Section or Table, and then select Method 1. Select the section you want to delete (here: Column). The following message should appear on the instrument: Section Cleared.

> Repeat these steps for the Injection Port, Autosampler, and Detector sections of the instrument.

> If you want to use a Varian GC method (instrument) in Chromeleon, you have to add an autosampler. Select Build/Modify, and then select Method 1. Enable the autosampler in the method by selecting the autosampler and adding it to Method 1.

(GC) Communication Error: Bad Response to XB?22@DS Command: XB?22@DS COMMAND (or … **to XC?22@DX Command: XC?22@DS COMMAND**)

Problem: No autosampler has been defined in the Varian GC method (on the instrument).

Solution: Add the autosampler to the Varian GC method in Single mode. Select the autosampler on the instrument's panel via **Build/Modify > Method 1 > Autosampler > Add Autosampler Section > Yes**.

(GC) Communication Error: Bad Response to "Read Flag Not Ready" Command: ;B?22@DS COMMAND (or **C?22@DS COMMAND**)

Problem: Communication error due to incompatible EPROM or Varian GC CPU board.

Solution: Replace the four outdated EPROMs with EPROMs version 03-910617-00 Rev. 1 or higher.

Autosampler not present:

Problem: Communication error with the autosampler, mainly due to a bad connection.

Solution: Disconnect the autosampler from the power source and connect the brown autosampler cable to the CPU board.

Waters: Commands and Tips

For information about the special commands that Chromeleon supports for the different Waters devices and for tips for practical operation, refer to:

 Alliance 2690/2695 HPLC Modules

 Alliance 2790/2795 HPLC Modules

 717plus Autosampler

 996 and 2996 PDA Detectors

 2487 UV Detector

For troubleshooting information, refer to:

 Waters Instruments: Troubleshooting

 GPIB-Connected Devices: Troubleshooting

■ Waters Alliance 2690/2695 HPLC Modules: Commands and Tips

In addition to the standard commands (refer to **Practical Tips for Device Control** 🔂 **Pump and Flow Control**), the Waters 2690/2695 Separation Module device driver supports the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Pump Commands and Properties

Property	Min	Max	Description
BubbleDetection²	Off	On	Checks the System for gas bubbles in the flow path, can return an error message and perform a bubble reduction procedure.
			The following settings are required on the instrument: In the **Default** method, set the **Alarms/Bubble detect** field to **Alert User**, **Stop Funct**, or **StopFlow** as described in the instrument's manual on page 1 (Mobile Phase).
Curve	convex	concave	➢*Gradient Curve* (Default: linear)
			📒 *Note:*
			*If the gradient defined by Curve is steeper than the one defined by the **MaxAcceleration** parameter, the actually performed gradient can be different from the gradient defined here. In this case, a warning appears. This is always the case for step gradients.*
MaxAcceleration²	0.01 min	30.00 min	Sets the time needed by the pump to reach the maximum flow rate of 10 ml/min. The property serves to protect the column from major pressure changes.
			It is connected with the **MaximumFlowRamp** property:
			MaxAcceleration = 10 / MaximumFlowRamp
			(Default: 0.01 min. The property is visible only on the Advanced level.)

Property	Min	Max	Description
MaximumFlowRamp	0.34	1000.00	Sets the maximum flow rate increase in [ml/min²]. The property serves to protect the column from major pressure changes. It is connected with the **MaxAcceleration** property: **MaximumFlowRamp =** 10 / **MaxAcceleration** (Default: 1000.00 ml/min². The property is visible only on the Advanced level.)
⇒*Pressure.LowerLimit* *Pressure.UpperLimit²*	0.0 bar	344.70 bar (= 5000 psi)	Upper and lower pressure limits. For the pressure limits to take effect, the following setting is required on the instrument: In the **Default** method, set the **Alarms/Min.** and **Alarms/Max** fields to **Alert User**, **Stop Funct**, or **StopFlow** as described in the instrument's manual on page 1 (Mobile Phase). The limits entered last in Chromeleon are valid (not the values set on the instrument). 🔸 *Note:* *The instrument checks the lower pressure limit only during injection.*
PrimaryPressure	-3.45 bar	380 bar	Sets the pressure after the first piston chamber. (The property is read-only and visible only on the Expert level.)
StrokeVolume²	Auto; 25 µl; 50 µl; 100 µl		Sets the volume delivered with each piston stroke. (Default: Auto. The property is visible only on the Advanced level.)
⇒*StopFlow*, ⇒*Continue*			When using these commands for the pump of the Alliance system, please note: When the Stop command is executed while a gradient program is running, the Continue command does not restart the gradient. Instead, the pump is started with constant solvent composition and constant flow rate.

🔹 *Tip:*

² It is not possible to change the property during an injection, during gradient runs, or at negative retention times.

🔸 *Note:*

*As described in the instrument's manual, negative pressure readings are possible for the pressure behind the first piston chamber (**PrimaryPressure** property).*

Degasser Commands and Properties

The standard degasser of the Waters Alliance 2690/2695 HPLC module is a Helium degasser. Select the following property to specify which solvent reservoirs shall be helium sparged and set the sparge rate for each reservoir:

Property	Min	Max	Default	Description
HeliumSparge_A to HeliumSparge_D²	0	100	0	If the sparge rate is set to 100% for a specific reservoir, the solvent in this reservoir is helium sparged all the time. If the sparge rate is less than 100%, the corresponding valve turns on and off according to the specified percentage.

The standard degasser of the Waters Alliance 2690/2695 XE HPLC modules is a vacuum degasser (In-Line Degasser). Specify the degassing mode:

Property	Value	Default	Description
Degasser Mode²	Normal Continuous Off	Off	Normal: The degasser turns on and off, depending on the pressure. Continuous: The degasser is always on. Off: The degasser is always off.

🖢 *Tip:*

² It is not possible to change the setting during an injection, during gradient runs, or at negative retention times.

The **Pressure** property indicates the current pressure:

Property	Min	Max	Description
Degasser Pressure	-1.0	1.0	Indicates the pressure (in bar) measured by the degasser. (The property is read-only.)

Injector Commands and Properties

For information about the standard autosampler commands, refer to **Control 🔳 Dionex Autosampler**.

In addition, the Waters 2690/2695 Separation Module device driver supports the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Description
InjectWaitTime	0	1E+7	Indicates the time in seconds between the inject command from Chromeleon and the inject response from the autosampler to Chromeleon (read-only)
LoopPressure	-3.45	380	Indicates the pressure (in bar) in the sample loop (The property is read-only and visible only on the Expert level.)
⇒*Ready*	NotReady	Ready	Indicates the state of the autosampler. (It depends on the state of the sample temperature control if WaitForTemperature = Yes).
SampleHeight²	0.0 mm	20.0 mm	Indicates the distance of the needle tip from the bottom of the sample vial (default: 0.0 mm).
SyringeSpeed²'	Fast (5.0 ml/min) Normal (2.5 ml/min) Slow (1.0 ml/min)		Defines the drawing speed of the syringe. The speed depends on the installed syringe type. The values correspond to a standard 250 µl syringe.
WaitForTemperature²	No	Yes	Specifies whether the autosampler waits for the sample temperature control.

Tip:

² *It is not possible to change the setting during an injection, during gradient runs, or at negative retention times.*

Note:

As described in the instrument's manual, negative pressure readings are possible for the sample loop pressure (LoopPressure property).

Column Heater and Sample Temperature Control Commands and Properties

For information about the standard commands for temperature control, refer to **Practical Tips for Device Control** **Temperature Control (On/Off)** and **Controlling the Temperature**.

In addition, the following properties allow you to determine, e.g., the maximum allowed deviation of the actual temperature from the expected temperature:

Property	Min	Max	Default	Description
MaxTempDeviation[2]	0°C	10°C	5°C	If the actual column temperature deviates from the expected temperature by more than the set value, an error message appears.
				The following setting is required on the instrument: Follow the description in the instrument's manual to set the **Column Temperature/On error** field to **Alert User**, **Stop Funct**, or **StopFlow**. For the column oven, this setting is made on page 4 (**Column**); for sample temperature control, it is made on page 2 (**Sample**).
⇒*Ready*[2]	NotReady	Ready	Ready	Indicates the state of the column heater. The heater is Ready: • when temperature control (TempCtrl) is Off *or* • when the difference between the actual temperature and the target temperature is equal or less than 2°C for at least 30 minutes.
TempCtrl[2]	Off	On	Off	Sets the state of the temperature control. (Temperature control is active only if TempCtrl = On.)
Temperature.Nominal[2]	20°C (4°C)	60°C (40°C)	20°C	Sets the target operating temperature (sample temperature control). The temperature must be at least 5°C above ambient.
Temperature.Value[2]	0°C	80°C		Indicates the actual temperature. (The property is read-only.)

 Note:

For better understanding, [°C] is used as unit, even though [K] would be correct to indicate temperature differences.

 Tip:

² The temperature control commands of the column oven can be used only if an injection is made. In all other cases, e.g. for ➢Blank Run Samples, an error message appears.

*It is not possible to change the **MaxTempDeviation** setting during an injection, during gradient runs, or at negative retention times.*

The standard relay commands are available to control the relays of the Waters Alliance 2690 and 2695 HPLC modules (refer to **Practical Tips for Device Control** **Relay, TTL, and Remote Input Commands**).

For information about how to install the Waters Alliance 2690 and 2695 HPLC modules, refer to **Hardware Installation** **Waters Alliance 2690/2695 HPLC Modules**: **Installation** in the **Administrator Help** section.

For an overview of the Waters Alliance 2690 and 2695 HPLC modules, refer to **Hardware Installation** **Waters Alliance 2690/2695 HPLC Modules: Overview** in the **Administrator Help** section.

▪ Waters Alliance 2790/2795 HPLC Modules: Commands and Tips

The Alliance 2790/2795 Separation Module device driver supports several modules. For information about the corresponding commands and properties, refer to:

🔼 **HPLC System**

🔼 **Pump and Degasser**

🔼 **Injector**

🔼 **Column Heater and Sample Temperature Control**

🔼 **Overlapping Sample Preparation**

▪ Waters Alliance 2790/2795: HPLC System

In addition to the standard commands (refer to **Practical Tips for Device Control** 🔼 **General Device Commands**), the Waters 2790/2795 Separation Module device driver supports the following commands and properties for the HPLC System (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Values	Description
ChartOut²	³ GradA, GradB, GradC, GradD, FlowRate, SampleLoopPressure, SystemPressure, PrimaryHeadPressure, VacuumLevel, SampleTemperature, ColumnTemperature	Sets the type of the analog signal that is transmitted from the Chart Out output. The available options depend on the system configuration. The following options are available only if the corresponding module is installed:
		Option *Module*
		VacuumLevel Degasser
		SampleTemperature Sample Heater
		ColumnTemperature Column Heater
LogStatus²	On, Off	If the property is set to On, each change of the instrument's status is logged in the Audit Trail. (The default setting is Off. The property is visible only on the Advanced level.)

Property	Values	Description
PrecolumnVolume²	0.0 to 10,000.0	Specifies the solvent volume in [µl] that is pumped through the column before injection is started. (The default setting is 0.0 µl. The property is visible only on the Advanced level.)
Status	*(80 characters)*	Indicates the status of the HPLC system.
Vendor		Indicates the manufacturer (here: Waters).

Tips:

² It is not possible to change the property during injection or during a gradient run.

³ The signal condition depends on the selected ChartOut option:

Signal	Parameter at 0 mV (min)	Parameter at 2000 mV (max)
ColumnTemperature	20°C	60°C
FlowRate	0.000 ml/min	10.000 ml/min
GradA - GradD	0.0%	100.0%
PrimaryHeadPressure	-3.45 bar (-50 psi)	380 bar (5500 psi)
SampleLoopPressure	-3.45 bar (-50 psi)	380 bar (5500 psi)
SampleTemperature	4°C	40°C
SystemPressure	-3.45 bar (-50 psi)	380 bar (5500 psi)
VacuumLevel	-0.0 bar	0.34 bar (4.91 psi)

Commands

Command	Description
StopOperation	Stops a previously started operation (e.g., by the **SysPrep** or **WetPrime** commands (see below) or by the **PrimeSyringe** command, see **Waters Alliance 2790/2795 HPLC Modules: Injector**). (The command is visible only on the Advanced level.)
SysPrep	Performs system preparation including priming the fluid components of the systems, rinsing the syringe, and washing the needle. (The command is visible only on the Advanced level.)
WetPrime	Primes the fluid components of the system with the currently selected flow rate and for 100.0 minutes. Use the **StopOperation** command to stop priming. (The command is visible only on the Advanced level.)

For information about how to install the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation** 🌐 **Waters Alliance 2790/2795 HPLC Modules**: **Installation** in the **Administrator Help** section.

For an overview of the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation** 🌐 **Waters Alliance 2790/2795 HPLC Modules: Overview** in the **Administrator Help** section.

▪ Waters Alliance 2790/2795: Pump and Degasser

In addition to the standard commands (refer to **Practical Tips for Device Control** 🔳 **Pump and Flow Control**), the Waters 2790/2795 Separation Module device driver supports the following pump commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Pump Commands and Properties

Property	Min	Max	Description
BubbleDetection[2,]	Off	On	Checks the system for gas bubbles in the flow path, can return an error message and perform a bubble reduction procedure.
			The following settings are required on the instrument: In the **Default** method, set the **Alarms/Bubble detect** field to **Alert User**, **Stop Funct**, or **StopFlow** as described in the instrument's manual on page 1 (Mobile Phase).
Continue			see StopFlow
Curve	convex	concave	➤*Gradient Curve* (Default: linear)
			📒 *Note:*
			If the gradient defined by Curve is steeper than the one defined by the MaxAcceleration parameter, the actually performed gradient can be different from the gradient defined here. In this case, a warning appears. This is always the case for step gradients.

Property	Min	Max	Description
MaxAcceleration²	0.01 min	30.00 min	Sets the time needed by the pump to reach the maximum flow rate of 10 ml/min. The property serves to protect the column from major pressure changes.
			It is connected with the **MaximumFlowRamp** property:
			MaxAcceleration = 10 / MaximumFlowRamp
			(Default: 0.01 min. The property is visible only on the Advanced level.)
MaximumFlowRamp	0.34	1000.00	Maximum flow rate increase in [ml/min²]. This property serves to protect the column from major pressure changes.
			This property is connected with the **MaxAcceleration** property as follows:
			MaximumFlowRamp = 10 / MaxAcceleration
			(Default: 1000.00 ml/min². The property is visible only on the Advanced level.)
⇒*Pressure.LowerLimit* *Pressure.UpperLimit²*	0.0 bar	344.70 bar (= 5000 psi)	Upper and lower pressure limits.
			For the pressure limits to take effect, the following setting is required on the instrument: In the **Default** method, set the **Alarms/Min.** and **Alarms/Max** fields to **Alert User**, **Stop Funct**, or **StopFlow** as described in the instrument's manual on page 1 (Mobile Phase). The limits entered last in Chromeleon are valid (not the values set on the instrument).
			Note:
			The instrument checks the lower pressure limit only during injection.
Pressure.Value	0.0 bar	380 bar (= 5500 psi)	Indicates the current pump pressure.
PrimaryPressure	-3.45 bar	380 bar	Pressure after the first piston chamber (The property is read-only and visible only on the Expert level.)
StrokeVolume²	Auto; 25 µl; 50 µl; 100 µl		Volume delivered with each piston stroke (Default: Auto. The property is visible only on the Advanced level.)

Property	Min	Max	Description
⇒*StopFlow*, ⇒*Continue*			When using these commands for the pump of the Alliance system, please note: When the Stop command is executed while a gradient program is running, the Continue command does not restart the gradient. Instead, the pump is started with constant solvent composition and constant flow rate.

🖝 *Tip:*

² It is not possible to change the property during an injection, during gradient runs, or at negative retention times.

📝 *Note:*

*As described in the instrument's manual, negative pressure readings are possible for the pressure behind the first piston chamber (**PrimaryPressure** property).*

Degasser Commands and Properties

The standard degasser of the Waters Alliance 2790/2795 HPLC modules is a Helium degasser. Select the following property to specify which solvent reservoirs shall be helium sparged and set the sparge rate for each reservoir:

Property	Min	Max	Default	Description
HeliumSparge_A to HeliumSparge_D²	0	100	0	If the sparge rate is set to 100% for a specific reservoir, the solvent in this reservoir is helium sparged all the time. If the sparge rate is less than 100%, the corresponding valve turns on and off according to the specified percentage.

The standard degasser of the Waters Alliance 2790/2795 XE HPLC modules is a vacuum degasser (In-Line Degasser). Specify the degassing mode:

Property	Value	Default	Description
Degasser Mode²	Normal Continuous Off	Off	Normal: The degasser turns on and off, depending on the pressure. Continuous: The degasser is always on. Off: The degasser is always off.

🖐☞ *Tip:*

² It is not possible to change the setting during an injection, during gradient runs, or at negative retention times.

The **Pressure** property indicates the current pressure:

Property	Min	Max	Description
Degasser Pressure	-1.0	1.0	Indicates the pressure (in bar) measured by the degasser. (The property is read-only.)

For information about how to install the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation** 🔵 **Waters Alliance 2790/2795 HPLC Modules**: **Installation** in the **Administrator Help** section.

For an overview of the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation** 🔵 **Waters Alliance 2790/2795 HPLC Modules: Overview** in the **Administrator Help** section.

🔹 Waters Alliance 2790/2795: Injector

For information about the standard autosampler commands, refer to **Control** 🔳 **Dionex Autosampler**.

In addition, the Waters 2790/2795 Separation Module device driver supports the following commands and properties (please note that the display ➢*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Description
CheckPlateHeight²	Off	On	When set to On, the needle position sensor determines the plate height before the first injection is performed from this plate. The value is checked against the range of values allowed for this plate type. (The default setting is Off. The property is visible only on the Advanced level.)
FullLoopOverfill²	1.0%	99.9%	Only in InjectMode = FullLoop (see below). Specifies the excess volume drawn to rinse the loop when the loop is filled with the sample. (The default setting is 4 %. The property is visible only on the Advanced level.)

Property	Min	Max	Description
InjectWaitTime	0	1E+7	Indicates the time in seconds between the inject command from Chromeleon and the inject response from the autosampler to Chromeleon. (The property is read-only.)
InjPortWashTime²	0, 1, 2, ... 9999		Specifies the time in seconds for inject port washing. (The default setting is 6. The property is visible only on the Advanced level.)
InjectMode²	PartialLoop; FullLoop		Determines the injection mode. (The default setting is FullLoop.)
			FullLoop — The entire loop volume is delivered to the column.
			PartialLoop — Only part of the loop volume is delivered to the column.
LoopPressure	-3.45	380	Indicates the pressure (in bar) in the sample loop (The property is read-only and visible only on the Expert level.)
NeedleWashTime²	0, 1, 2, ... 9999		Specifies in seconds how long the exterior of the needle is washed. (The default setting is 15. The property is visible only on the Advanced level.)
PrepDelay³	0.0	*Loop volume*	Determines in seconds how long it may take until the loop is completely filled. (The default setting is 0.0. The property is visible only on the Advanced level.)
PrepSubject³	*Vial positions* Sample_Vial, Air, Sample_Vial ± *number (relative addressing)*		Determines which vial is used for Draw, Dispense, and Mix operations. If set to **Air**, the needle first draws air, and then dispenses the air into the needle port. (The default setting is 15. The property is visible only on the Advanced level.)
PrepVolume³	0, 1, 2, ... 9999		Determines the volume [µl] to be used for the Draw, Dispense, and Mix commands. (The default setting is 10.0. The property is visible only on the Advanced level.)
➢*PrimeSyringe*			Primes the syringe by flushing it.
			🔳☞ *Tip:*
			*After priming the syringe, always perform a **Wait Sampler.Ready** command.*
PurgeVolume³	0, 1, 2, ... 9999		Determines the volume [µl] to be used for purging. (The default setting is 100. The property is visible only on the Advanced level.)
⇒*Ready*	NotReady	Ready	State of the autosampler (depends on the state of the sample temperature control if WaitForTemperature = Yes).

Property	Min	Max	Description
SampleHeight²	0.0 mm	20.0 mm	Indicates the distance of the needle tip from the bottom of the sample vial (default: 0.0 mm).
SampleOverlapped²	Off	On	When set to On, the current sample will be prepared while the previous sample is still analysed. (The default setting is Off. The property is visible only on the Advanced level.)
SeekWellBottom²	Off	On	Can be enabled only if SampleHeight = 0.0 (see above).
			When set to On, the needle detects the well bottom for a specified plate before the first injection is performed. This value is stored for the subsequent injections from this plate. When a different plate is used, the measurement is performed again. (The default setting is Off. The property is visible only on the Advanced level.)
State	Off	On	Indicates On when the autosampler has injected a sample. (The property is read-only.)
SyringeSpeed²'	Fast (5.0 ml/min) Normal (2.5 ml/min) Slow (1.0 ml/min)		Defines the drawing speed of the syringe. The speed depends on the installed syringe type. The values correspond to a standard 250 µl syringe.
WaitForTemperature²	No	Yes	Specifies whether the autosampler waits for the sample temperature control.
⇒*Wash*			Flushes the autosampler.
			📌 *Tip:*
			*After priming the syringe, always perform a **Wait Sampler.Ready** command.*
WashCycle²	0, 1, 2, ... 9		Sets the number of wash cycles to be performed. (The default setting is 1. The property is visible only on the Advanced level.)
WashFrequency²	Never, EveryInject, EveryWell		Determines when a wash cycle shall be performed. The following options are available: *Never, EveryInject, EveryWell.*
			(The default setting is EveryInject. The property is visible only on the Advanced level.)
WashSequence²	PurgeWash, PurgeWashPump		Determines the wash sequence for the needle. (The default setting is PurgeWash. The property is visible only on the Advanced level.)

� ☞ *Tips:*

² It is not possible to change the setting during an injection, during gradient runs, or at negative retention times.

³ It is not possible to change the setting during sample analysis.

📳 *Note:*

As described in the instrument's manual, negative pressure readings are possible for the sample loop pressure (LoopPressure property).

Injector Commands

Command	Description
Draw³	Draws the PrepVolume from the PrepSubject (see above). (The command is available only on Advanced level.)

� ☞ *Tips:*

Up to 16 Draw commands are allowed per injection.

*Always use a **Mix** command after a sequence of **Draw** commands.*

LoopOffline³	Switches the sample injection valve after the injection. Thus, the loop is no longer part of the flow path. Preparation of the next sample can be started. (The property is visible only on the Advanced level.)

� ☞ *Tip:*

Make sure to issue the command only after the previous sample has completely left the loop.

Mix³	Starts sample preparation. (The property is visible only on the Advanced level.)

�I ☞ *Tips:*

*No further **Draw** commands are allowed after a **Mix** command.*

*Always use a **Mix** command after a sequence of **Draw** commands.*

PrimeSyringe	Fills the syringe with the purge solvent. (The property is visible only on the Advanced level.)

�I ☞ *Tip:*

*The command uses the values set for **PurgeVolume** and **WashCycle**.*

�I ☞ *Tip:*

³ It is not possible to change the setting during sample analysis.

For information about how to install the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation** 🔵 **Waters Alliance 2790/2795 HPLC Modules**: **Installation** in the **Administrator Help** section.

For an overview of the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation** 🔵 **Waters Alliance 2790/2795 HPLC Modules: Overview** in the **Administrator Help** section.

▪ Waters Alliance 2790/2795: Column Heater and Sample Temperature Control

For information about the standard commands for temperature control, refer to **Practical Tips for Device Control** 🔲 **Temperature Control (On/Off)** and 🔲 **Controlling the Temperature**.

In addition, the following properties allow you to determine, e.g., the maximum allowed deviation of the actual temperature from the expected temperature:

Property	Min	Max	Default	Description
MaxTempDeviation²	0°C	10°C	5°C	If the actual column temperature deviates from the expected temperature by more than the set value, an error message appears.
				The following setting is required on the instrument: Follow the description in the instrument's manual to set the **Column Temperature/On error** field to **Alert User**, **Stop Funct**, or **StopFlow**. For the column oven, this setting is made on page 4 (**Column**); for sample temperature control, it is made on page 2 (**Sample**).

Property	Min	Max	Default	Description
⇒*Ready*²	NotReady	Ready	Ready	Indicates the state of the column heater. The column heater is Ready • when temperature control (TempCtrl) is Off *or* • when the difference between the actual temperature and the target temperature is equal or less than 2°C for at least 30 minutes.
Temperature.Nominal²	20°C (4°C)	60°C (40°C)	20°C	Sets the target operating temperature (sample temperature control). The temperature must be at least 5°C above ambient.
Temperature.Value²	0°C	80°C		Indicates the actual temperature. (The property is read--only.)
TempCtrl²	Off	On	Off	Sets the state of the temperature control. (Temperature control is active only if TempCtrl = On.)

Note:

For better understanding, [°C] is used as unit, even though [K] would be correct to indicate temperature differences.

Tip:

² The temperature control commands of the column oven can be used only if an injection is made. In all other cases, e.g. for ⇒Blank Run Samples, an error message appears.

*It is not possible to change the **MaxTempDeviation** setting during an injection, during gradient runs, or at negative retention times.*

The standard relay commands are available to control the relays of the Waters Alliance 2790 and 2795 HPLC modules (refer to **Practical Tips for Device Control Relay, TTL, and Remote Input Commands**).

For information about how to install the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation Waters Alliance 2790/2795 HPLC Modules**: **Installation** in the **Administrator Help** section.

For an overview of the Waters Alliance 2790 and 2795 HPLC modules, refer to **Hardware Installation Waters Alliance 2790/2795 HPLC Modules: Overview** in the **Administrator Help** section.

⬛ Waters Alliance 2790/2795: Overlapping Sample Preparation

The following program is an example for overlapping sample preparation with the Alliance 2790/2795 HPLC Modules and a Waters 996 or 2996 PDA Detector:

```
SampleHeater.TempCtrl =   Off
ColumnOven.TempCtrl =   Off
Pressure.LowerLimit =   1.0
Pressure.UpperLimit =   345.0
%A.Equate =    "%A"
%B.Equate =    "%B"
%C.Equate =    "%C"
%D.Equate =    "%D"
3DFIELD.MaxWavelength =   799.6
3DFIELD.MinWavelength =   190.0
3DFIELD.BunchWidth =   1.2
3DFIELD.Step =   1.0
UV_VIS_1.Wavelength =   272
UV_VIS_1.Bandwidth =   1.2
UV_VIS_1.Step =   0.50
UV_VIS_1.Average =   Off
ExposureTime =   Auto
Interpolate656nm =   On
Flow =   1
%B =  0.0
%C =  0.0
%D =  0.0
Curve =  5

; Optional for overlapping sample prepration:
; SampleOverlapped =   On

  InjectMode =   PartialLoop

; The following commands are used to fill the syringe as follows:
10.0 µl air, 5.0 µl sample, 10 µl reagent, and again 10.0 µl air:
  PrepSubject = Air
  PrepVolume =   10.0
  Draw

  PrepSubject = Sample_Vial
  PrepVolume =   5.0
  Draw
  PrepSubject = 2_F8
; For this example: The reagent is in vial 2_F8 of a 48-vial rack
```

```
   PrepVolume =   10.0
   Draw

   PrepSubject = Air
   PrepVolume =   10.0
   Draw

   Mix
; The Mix command is required to complete the sample preparation
commands:

0.000 Autozero
   Wait  UV.Ready and Sampler.Ready and ColumnOven.Ready   and
HPLC_System.Ready
   Inject
   3DFIELD.AcqOn
   UV_VIS_1.AcqOn

5.000 3DFIELD.AcqOff
   UV_VIS_1.AcqOff

   End
```

☞ *Tips:*

*The **SampleOverlapped = Off** command is required to disable overlapping sample preparation.*

It is not possible to prepare the first sample after a blank sample or after a run without injection while the previous sample is being analyzed.

The PrepSubject = Air command can be specified only as the first and/or the last PrepSubject command.

For more information, also refer to **Practical Tips for Device Control ⬚ Overlapping Samples**.

▪ Waters 717plus Autosampler

For information about the standard autosampler commands, refer to **Device Control ▪ Dionex Autosamplers**.

In addition, Chromeleon supports the following properties:

Property	Min	Max	Description
SyringeVol	25, 250, 2500		Indicates the syringe volume in µl (read-only).
InjectWaitTime	0	1E+7	Sets the time between the inject command and the inject response in seconds. The default setting is 0 s.
LoopPressure	-3,45	380	Indicates the pressure (in bar) in the sample loop (read-only). This property is available only on Expert level.

📝 *Note:*

As described in the instrument's manual, negative pressure readings are possible for the sample loop pressure (LoopPressure property).

For an overview of the Waters autosampler, refer to **Hardware Installation 🔵 Waters 717plus Autosampler: Overview** in the **Administrator Help** section.

▪ Waters 996 and 2996 PDA Detectors

👉 *Tip:*

*Before data acquisition can start, the detector needs some time to load the parameter settings. That is why a **Wait** command is required before data acquisition is started.*

In addition to the standard commands, the Waters 996 PDA detector supports the following commands and properties (please note that the display ➤*Filter* level determines which commands and properties are displayed):

Property	Min	Max	Description
ExposureTime	Auto; 11 ms	500 ms	Time during which a single diode is exposed to light. (The value cannot exceed the Step value - see below.)
			Tip:
			Do not change the default ('Auto') unless absolutely required.
Interpolate656nm	Off	On	On: To protect the lamp the signal at 656.1 nm (Balmer line for deuterium) is ignored (visible only on the Expert level).
Lamp	Off	On	Turns the lamp on or off.

UV Channel Commands

Property	Min	Max	Default	Description
⇒*Average*	Off	On	On	Allows signal averaging.
⇒*Bandwidth*	1.2 nm	30 nm	1.2 nm	
⇒*Step*	Auto, 0.01 s	4.80 s	Auto	Indicates the interval between two data points. (The value must exceed the ExposureTime value.)
⇒*Wavelength*	190 nm	800 nm	240.0 nm	Indicates the wavelength at which the detector measures the signal.

 Tip:

*After a **Reset** command, it takes 90 s until the detector is ready to execute further commands.*

Commands and Properties of the 3D Field

Property	Min	Max	Default	Description
⇒*BunchWidth*	1.2 nm	60 nm	1.2 nm	Describes the bandwidth of a bunch of diodes.
MaxWavelength	MinWavelength	799.6 nm	799.6 nm	Indicates the upper limit of the measuring range. (The setting cannot be changed during data acquisition.)

Property	Min	Max	Default	Description
MinWavelength	190.0 nm	MaxWavelength	190.0 nm	Indicates the lower limit of the measuring range. (The setting cannot be changed during data acquisition.)
⇒*Step*		0.1 s 0.2 s 0.5 s 1.0 s 2.0 s 3.0 s 4.0 s	0.1 s	Indicates the interval between two data points. (The value must exceed the ExposureTime value.)

 Note:

Due to the minimum Bunch Width of 1.2 nm, the minimum and/or maximum wavelength can only have the following value: 190.0 nm + n*1.2 nm (where n = integer). If you enter a different value, the next lower value is used for the minimum wavelength and the next higher value is used for the maximum wavelength. This is logged in the Audit Trail.

The standard relay commands are available to control the relays of the Waters 996 and 2996 PDA detectors (refer to **Practical Tips for Device Control** **Relay, TTL, and Remote Input Commands**).

For an overview of the Waters 996 and 2996 PDA detectors, refer to **Hardware Installation** **Waters 996 and 2996 PDA Detectors: Overview** in the **Administrator Help** section.

For information about how to install the Waters 996 and 2996 PDA detectors, refer to **Hardware Installation** **Waters 996 and 2996 PDA Detectors: Installation** in the **Administrator Help** section.

▣ Waters 2487 UV Detector

☞ *Tip:*

*Before data acquisition can start, the detector needs some time to load the parameter settings. That is why a **Wait** command is required before data acquisition is started.*

In addition to the standard commands, the Waters 2487 UV Detector provides the following commands (please note that the display ➢*Filter* level determines which commands are displayed):

Command	Min	Max	Default	Description
Data_Collection_Rate (For more information, refer to ➢*Data Collection Rate* in the **Glossary**.)	1 Hz	10 Hz	1 Hz	Indicates the data collection rate for the channels.
Lamp	Off	On		Turns the lamp on or off.

Commands and Properties of UV Channels

Property	Min	Max	Default	Description
AutozeroOn EventIn²	On	Off	Off	If the property is enabled, autozeroing is performed whenever this is required by an event.
AutozeroOn WlChange²	On	Off	On	If the property is enabled, autozeroing is performed whenever the wavelength changes.
⇒*Average*	Off	On	On	Allows signal averaging.
FilterTime Constant³	0.0 s	99.0 s	1.0 s	Time constant for the selected noise filter.
FilterType	None	RC	Hamming	Filter type used to reduce analog and digital signal noise.
Signal. Upper/ LowerLimit	-10^10 mAU	10^10 mAU	0	Indicates the upper and lower limit for the signal.
⇒*Step*	Auto, 0.01 s	4.80 s	Auto	Interval between two data points.
⇒*Wavelength*	190 nm	700 nm	UV_VIS_1: 254.0 nm UV_VIS_2: 225.0 nm	Wavelength at which the detector measures the signal (see the **Troubleshooting** section).

☞ *Tips:*

² *It is only possible to change the **Filtertype**, **AutozeroOnEventIn**, and **AutozeroOnWIChange** properties once in the PGM File during negative retention times.*

³ *It is not possible to change the time constant for the selected filter during data acquisition.*

For an overview of the Waters 2487 UV detector, refer to **Hardware Installation** ⊙ **Waters 2487 UV Detector: Overview** in the **Administrator Help** section.

For information about how to install the Waters 2487 UV detector, refer to **Hardware Installation** ⊙ **Waters 2487 UV Detector: Installation** in the **Administrator Help** section.

◾ Waters Instruments: Troubleshooting

Waters 2690/2695 or 2790/2795 Separation Modules:

a) A Separation Module (Alliance 2690/2695 or 2790/2795 modules) and the Chromeleon Audit Trail report a communication error with another Waters instrument, e.g., The instrument stops due to error circumstances or user interaction: Absorbance detector fault.

Cause: The reason is that both Chromeleon and the Separation Module attempt to control this instrument.

Remedial action: Disconnect the other Waters instrument from the Separation Module, and then perform the following steps on the Separation Module (the example refers to a detector):

1. In the main window, press the **Develop Method** key to open the **Method** window.

2. Use the arrow key to select the **<Default>** method. Confirm your selection by pressing <Enter>.

3. Press the **Next** key until the **Detectors** window is opened:
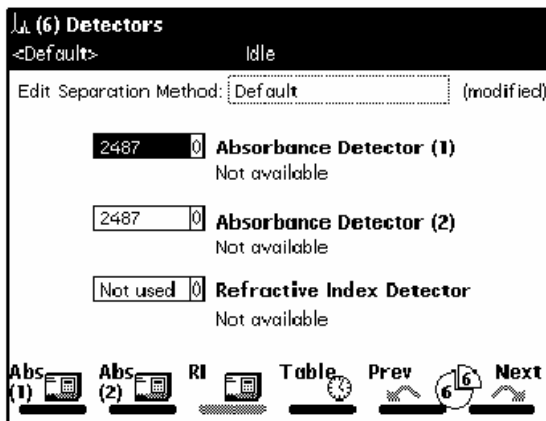
4. Use the arrow keys to select the first detector and press <Enter>.

5. Use the arrow keys to select the <Not used> option and press <Enter>.

6. If necessary, repeat steps 4 and 4 for all other detectors.

7. Press the **Exit** key to open the **Save Method** dialog box.

8. Select **Yes** to save the method.

9. In the main window, select **Default Method**.

10. Make sure that the other parameters in the method, e.g., extreme flow rates, selected solvents, etc., do not cause any damage.

11. Start the method.

12. Immediately stop the method.

b) No injection is performed although the Inject command was executed. **Conditioning Column** and a countdown are displayed on the instrument's display. The injection is not performed either after the countdown time has expired.

Remedial action: On the **Condition Column** menu on the instrument, set the conditioning time to 0:

1. Press **Menu/Status** to open the **Status** dialog.

2. From the **Method** field, select the column conditioning method.

3. Press the **Direct Function** key.

4. Select **Condition Column** and confirm by pressing **OK**. The **Condition Column** dialog box is opened.

5. (Option) Specify the column that is currently conditioned if applicable.

6. Set the time to **0** and confirm with OK.

Waters 2487 UV Detector:

When you enter a wavelength, the following warning can appear: "Wavelengths will span 370 nm during acquisition. The order filter will not be used. Do you really want to execute this command?"

Reason: The detector automatically uses a filter for wavelengths above 370 nm. In this way, unwanted UV light is filtered out. For measurements at or below 370 nm, the filter is automatically removed. The warning appears when the measuring wavelength of one channel is above 370 nm while the measuring wavelength of the other channel is below or equal to 370 nm.

Remedial Action: Click **Yes** to confirm the warning. Data acquisition is performed at the selected wavelengths **without** a filter. However, in this case, it may happen that errors occur in the data recorded at the higher wavelength, due to unwanted UV light. Or else, click **No** to use two wavelengths that are both either above or equal to 370 nm or below 370 nm.

Also, refer to **GPIB-Connected Devices: Troubleshooting**.

For an overview of the different Waters instruments for which device drivers are available in Chromeleon, refer to **Hardware Installation** **Waters** in the **Administrator Help** section.

GPIB-Connected Devices: Troubleshooting

Communication Error

A communication error may occur when you install a driver in the Server Configuration program. Possible reasons are:

- The instrument is either turned off or disconnected from the mains.

- An invalid address has been assigned to the instrument.

- Only for the Waters Separation Modules (2690/2695 or 2790/2795): The **Controlled by Millennium 32** option has not been enabled on the module. (The **Administrator Help** section provides more information; refer to **Hardware Installation** ➡ **Waters Alliance 2690/2965 HPLC Modules**: **Installation**, **Device Settings** section.)

- The instrument is not yet completely initialized.

- There are problems with the cable.

- The instruments are not connected in series. One or more instruments are connected in a star structure.

- One or more of the instruments are turned off.

- There are more than 20 devices connected.

- The cable length between two instruments exceeds 4 meters.

- The average cable length between the separate instruments connected to a bus exceeds 2 meters.

Remedy the situation, and then reinstall the driver in the Chromeleon Server Configuration.

Problems with Cables

If problems occur with a cable, follow the steps below:

1. Turn the instruments off.

2. Perform the following steps in succession for the individual instruments:

   - Connect only one instrument to the PC.

   - Make sure that the GPIB address for the connected instrument is correct.

- Wait until the instrument has completely initialized.

- Try to connect the instrument from a Chromeleon panel.

3. If you cannot connect to the instrument from Chromeleon, the reason is mainly that the cable is defective. Turn off the instrument and replace the cable.

Also, refer to **Waters Instruments: Troubleshooting.**

Practical Tips for Device Control

Practical Tips for Device Control (Overview)

In addition to the standard commands of the ➢*Program*, which are easily created with the Program Wizard (see **The Control Program** 🔲 **The Program Wizard**), Chromeleon supports various additional ⇒*Control Commands*. For more information about these commands and the command syntax, refer to:

🔲 **Pump and Flow Control**

🔲 **Autosampler Control**

🔲 **Detector Control**

🔲 **IC Control**

🔲 **GC and Temperature Control**

🔲 **Component Controller Control**

🔲 **Special Commands, Relay Control, and Miscellaneous**

- Transfer the structure and syntax of the commands that you want to use to your program file.

- Use **Cut & Paste** to install the command directly from online Help at the appropriate position in the standard program.

    Specify the time when to execute each command.

    Save the result of your input as a PGM File.

Pump and Flow Control

The following commands are available for pump and/or flow control. For the "simple" commands, refer to 🔲 **Pump Commands**.

For more information, refer to:

🔲 **Setting the Flow Rate**

🔲 **Determining the Solvent Composition**

🔲 **Determining a Gradient**

🔲 **Determining Pressure and Pressure Limits**

🔲 **Starting/Stopping the Pump Flow**

🔲 **Holding the Pump Flow**

In addition, refer to the following information about

🔲 **Recording the Pump Pressure**

🔲 **Setting Automatic Pre-Compression Control (P580)**

🔲 **Viewing Leak Sensor and Workload Status**.

🔲 **Parking Peaks**

You can use an **Active Flow Splitter (MRA)** to split the flow. For a typical program example, refer to 🔲 **Splitting the Flow (Program Example)**.

▪ Pump Commands

The following commands are supported for pump and/or flow control:

Hold/Continue Gradient

```
0.000   HoldMode              = On / Off
```

Alternatively, the following short command is valid:

```
0.000   ⇒Hold                   or
0.000   ⇒Continue
```

Stop Pump Flow/Gradient

```
0.000   StopMode              = On  or
0.000   ⇒StopFlow
```

Determining Pressure Limits (see ⇒*Pressure.Lower/UpperLimit*)

```
0.000   Pressure.LowerLimit   = Value[bar, MPa, psi]
0.000   Pressure.UpperLimit   = Value[bar, MPa, psi]
```

📋 *Note:*

The pressure unit (bar, MPa, or psi) depends on the pump type.

Generating a Flow or % Gradient

The flow or solvent value determined for the time t is continually adjusted to the following flow or solvent command. If the two values coincide, the flow or solvent value is kept at a specific level (see ramp profile 0 to 1), if they differ from each other, the value is modified. The difference between the two time values corresponds to the length of the ramp (see ramp profile 1 to 2min).

```
0.000   %B.Value    = 20
1.000   %B.Value    = 20
2.000   %B.Value    = 50
3.000   %B.Value    = 50
```

Ramp Profile
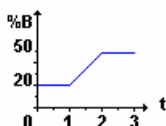
🖐☞ *Tip:*

Dionex GP40/GP50, IP20/IP25, IC20/IC25/IC25A, GS50, and IS25 pumps do not deliver flow gradient ramps. Instead, changing the flow rate between one time value and the next results in an immediate change in the flow rate (step change).

To determine a sharp increase, two different values must be defined at the same time. The start and end values must be entered at the exact times (see rectangle profile at the time t=1min).

```
0.000   %B.Value   = 20
1.000   %B.Value   = 20
1.000   %B.Value   = 40
2.000   %B.Value   = 40
2.000   %B.Value   = 20
3.000   %B.Value   = 20
```


Rectangle Profile
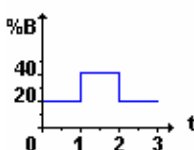
By combining the just mentioned possibilities, any multi-step gradient profile can be realized. For example:

```
0.000   %B.Value   = 20
0.500   %B.Value   = 20
1.500   %B.Value   = 40
2.000   %B.Value   = 40
2.000   %B.Value   = 30
2.500   %B.Value   = 30
2.500   %B.Value   = 20
```


Multi-Step Profile
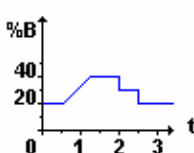
Instead of the arbitrarily selected quantity **%B** used in these examples, any other solvent (see ⇒*%B, %C, %D*) or the flow rate can be changed.

Creating a Non-Linear Gradient Ramp (Dionex GP40/GP50/GS50 pumps only)

A Curve command (also, refer to ➢*Gradient Curves*) instructs the pump to apply the selected curve number when adjusting the solvent composition between two retention times. In the example below, the Curve = 8 command at 2 min creates a concave ramp between 1 and 2 min.

Consecutive commands with identical solvent compositions generate an isocratic segment, regardless of the curve number selected (see 0 to 1 min and 2 to 3 min below).

```
0.000   %B.Value    = 20
1.000   %B.Value    = 20
2.000   %B.Value    = 50
        Curve       = 8
3.000   %B.Value    = 50
```

Non-Linear Ramp Profile
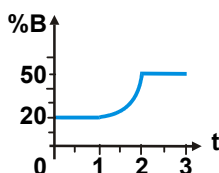
▪ Setting the Flow Rate

The ⇒*Flow* rate is set via the corresponding controls on the control panel (slider, edit field, or button). For more information about the control panel, see **Control 🔳 The Control Panel**.

- To set a higher or lower flow rate, use the mouse to move the gauge slider in the desired direction.

- Enter the flow rate in the edit field via the keyboard. Confirm your input by pressing <Enter> (Return).

- Click a button to execute the assigned function.

- Alternatively, select **Flow** on the **Control** menu.

It is also possible to include the **Flow** command in the ➢*Program* (see **How to: Creating and Modifying Programs 🔳 Creating a Program**). Example:

```
0.000   Pump.Flow        = Value[ml/min%]   ;HPLC/IC pump
0.000   InjectorB.flow   = Value[ml/min%]   ;GC HP5890
```

Several **Flow** commands result in a ➢*Flow Gradient*. For a description on how to enter gradients, refer to 🔳 **Determining a Gradient**.

▪ Determining the Solvent Composition

Manually

For non-controlled pumps, solvent composition (see ⇒*%B, %C, %D*) is set directly on the instrument (also, refer to the *Operating Instructions* of the instrument).

For controlled pumps, Chromeleon features the appropriate controls (sliders, edit fields, switches, etc.). Depending on the control panel layout, you can set the values for %B, %C, and %D via either a slider or an edit field.

- Use the mouse to move the slider of a gauge in the desired direction.

- Type a value in an edit field and press <Enter>.

- To determine a gradient, select ⇒*Flow* on the Control menu (also, see ▪ Determining a Gradient).

Programmed

To change the solvent composition via the program, enter the following commands in the ➢*Program*:

```
t=....   %B.Value    = Value[%]
t=....   %C.Value    = Value[%]
t=....   %D.Value    = Value[%]
```

If you enter the command several times for at least one component, this input results in a ➢*% Gradient*.

For more information, refer to **How to: Creating and Modifying Programs ▪ Creating a Program**.

◼ Determining a Gradient

There are two ways how to determine a gradient. You can do this either manually on a control panel or automatically via a program:

Manual Input

Different dialog boxes are provided for each device for which a gradient can be determined. Use these dialog boxes to enter the device-specific settings. You can determine:

- <u>Flow</u>REFERENCE_FLOW_RATE>referenc   and <u>%</u>GLOSSARY_PROCENT_GRADIENT>glossary gradients for HPLC and IC pumps.

- Temperature, pressure, and flow gradients for gas chromatographs.

A gradient can be run either as a ramp or as a ➤*Step Gradients* (in IC, also as a ➤*Gradient Curve*).

- Select **Flow** on the **Control** menu or press <**CTRL> + <F**>. Make the necessary settings in the dialog box.

- For HPLC/IC, determine the desired gradient on the **Gradient** tab page. For gas chromatographs, select a gradient on the **GC** and **Column1** and **Column2** tab pages.

Programmed Input

The easiest way to create a new program is to use the Program Wizard (see **The Control Program** 🔲 **The Program Wizard**). To open the wizard, select **New** on the **File** menu, and then select **Program File**.

HPLC/IC   On the **Pump Options** page, select **Multi-Step Gradient**. The **Flow Gradient Options** dialog box appears. This dialog box corresponds to the **Gradient** page during manual input.

GC   On the **GC Options** page (or the **Front/Back Inlet Options** tabs), click the arrow next to the **Type** field, and then select **Ramped Temperature** from the list. This setting allows you to program a temperature gradient. In the same way, you can program flow and pressure gradients. Select **Ramped Flow** or **Ramped Pressure** on the **Column1 Options** and **Column2 Options** pages.

Entering "Basic points"

HPLC/IC

- In the **Retention** column, enter the time (relative to the injection time) of the desired modification.

- In the **Flow** column, enter the new value for the flow rate.

- In the **%B**, **%C**, and/or **%D** columns, enter the new value for the delivered solvent composition. %A is the difference between the total of the entered values and 100%.

- For Dionex GP40, GP50, and GS50 pumps only: Enter a value in the **Curve** column to determine whether the pump delivers a linear or ➢*Curved Gradient*. Curve 5 (the default) is linear; curves 1 - 4 are convex upward and curves 6 - 9 are concave upward.

- Click **Insert Line** to append another line to the table. After appending the first new line, further lines are appended automatically, as necessary. Enable or disable this option by selecting or deselecting **Autom. Append New Lines** on the context menu.

- Click **Delete Line** to remove unnecessary lines from the table.

- If an entry is invalid, the input in the corresponding cell is displayed in red color. Invalid entries must be corrected immediately.

- Click **Clean Up** to delete superfluous information from a previously selected area.

- To calculate missing values between two or more time values can, click **Interpolate**. The values are automatically added to the table.

- Select **Fill Column** on the context menu to fill the cells in the column with the currently selected value. Only the cells below the selected value are filled.

In a graphical representation, each flow and solvent value of the value table is represented as a point. By interpolation between the basic points of a column, an area gradient profile is created. The solvent areas are represented in the color of the corresponding caption; the flow rate is displayed as a thin line.

Direct Input into a Program

If you know the ➢*Program* syntax, you can directly determine the gradient in the program. Entering the ⇒*%B, %C, %D,* ⇒*Flow*, and **Temp**. commands in the program allows you to change the solvent composition, flow rate, and temperature at a precise time. The gradient profile results from the change in value of a certain quantity at the time t.

🖙 *Tip:*

The Dionex GP40/GP50, IP20/IP25, IC20/IC25/IC25A, GS50, and IS25 pumps do not deliver flow *gradient ramps. Instead, changing the flow rate between one time value and the next, results in an immediate change in the flow rate (step change).*

Example: The three different profiles are realized via the commands listed below the profiles.
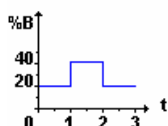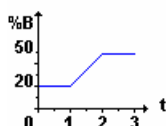
Rectangle Profile   **Ramp Profile**   **Multi-Step Profile**
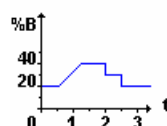

```
0.000 %B.Value = 20      0.000 %B.Value = 20      0.000 %B.Value = 20
1.000 %B.Value = 20      1.000 %B.Value = 20      0.500 %B.Value = 20
1.000 %B.Value = 40      2.000 %B.Value = 50      1.500 %B.Value = 40
2.000 %B.Value = 40                               2.000 %B.Value = 40
2.000 %B.Value = 20                               2.000 %B.Value = 30
                                                  2.500 %B.Value = 30
                                                  2.500 %B.Value = 20
```

For a rectangle profile, indicate exactly how long the specific solvent composition is valid. If the composition is changed at the same time as another percent command is defined, the change in solvent composition is executed immediately. The solvent percentage achieved is then maintained until replaced by another command.

To realize an increase over a longer period, the start value and end value must be specified with the precise time. The difference between the two time values corresponds to the duration of the increase (see ramp profile).

You can realize any multi-step gradient profile by combining the above-mentioned possibilities.

For more information about how to enter basic points in GC, refer to 🔼 **Determining a Gradient (GC)**.

◼ Determining Pressure and Pressure Limits

Pressure

The operating pressure can only be determined for gas chromatographs. It depends on the layout of the control panel which type of control is used to set the pressure.

- Determine the pressure via the corresponding slider or type the value in the input field.

For other devices, such as Dionex IC and HPLC pumps, the current operating pressure can be displayed on a control panel.

Pressure Limits

Some device drivers provide the possibility to determine the upper and lower pressure limits.

- Type the corresponding value in the input field.

Or

- Position the slider for the upper and lower pressure limits with the mouse pointer. Assign different colors to the controlling sliders so that they indicate whether a pressure limit is exceeded or whether it is within the selected limits (see **How to: Controlling Devices from the Control Panel** 🔳 **Modifying a Control**).

If defined ⇒*Pressure Limits*, for example, of an HPLC pump, are exceeded, Chromeleon automatically turns off the flow, issues an error message, and stops the sample batch, as necessary. In addition, all operations are logged in the ➢*Audit Trail*.

It is also possible to determine the pressure limits by entering the following commands in the ➢*Program*:

```
0.000    X.Pressure.LowerLimit     =Value[bar, MPa, psi]
0.000    X.Pressure.UpperLimit     =Value[bar, MPa, psi]
```

where X refers to the name of the instrument defined in the **Server Configuration**. The pressure unit depends on the pump type.

▪ Starting and Stopping the Pump Flow

- Select ⇒*StopFlow* to stop the pump flow and the ➢*Gradient* formation. During a running ➢*Batch*, batch processing is stopped.

- Select ⇒*Continue* to resume the pump flow, a running gradient program, or batch processing.

🖝 *Tip:*

The ⇒Hold command interrupts gradient formation and automatic batch processing, but not the pump flow.

▪ Holding the Pump Flow

- Select ⇒*Hold* to interrupt ➢*Gradient* formation or a running batch. During a running ➢*Batch*, sample processing is stopped.

In **Hold** mode, the pump delivers a constant flow rate and solvent composition until a stop or a ⇒*Continue* command is entered.

▪ Recording the Pump Pressure

To determine whether signal variations are related to pressure variations, that is, whether the pump causes those variations, Dionex recommends that you record the pump pressure as an additional signal.

Hardware Configuration

To display the pump pressure, an analog pressure output must be available on your pump. In addition, a ➢*UCI-100 Universal Chromatography Interface* is required. Connect the pressure output of your pump to one of the free channels of the UCI-100.

🖝 *Tip:*

If you do not have a UCI-100 installed, a virtual channel can be used to record the pump pressure. If you use a virtual channel, note the greater distance between the measured values (⇒Step). For an example, refer to 🔼 ***Program Examples for Virtual Channels***.

Server Configuration

P680:

On the **General** tab page, select the **Pressure Signal** check box to record the pump pressure. Chromeleon generates the Pump_Pressure channel for data acquisition.

Other Pumps:

Add an ➢*Integrator Driver* to the devices of the corresponding timebase. Name the unassigned signal (for example, **Pump_Pressure**) and click **Change**.
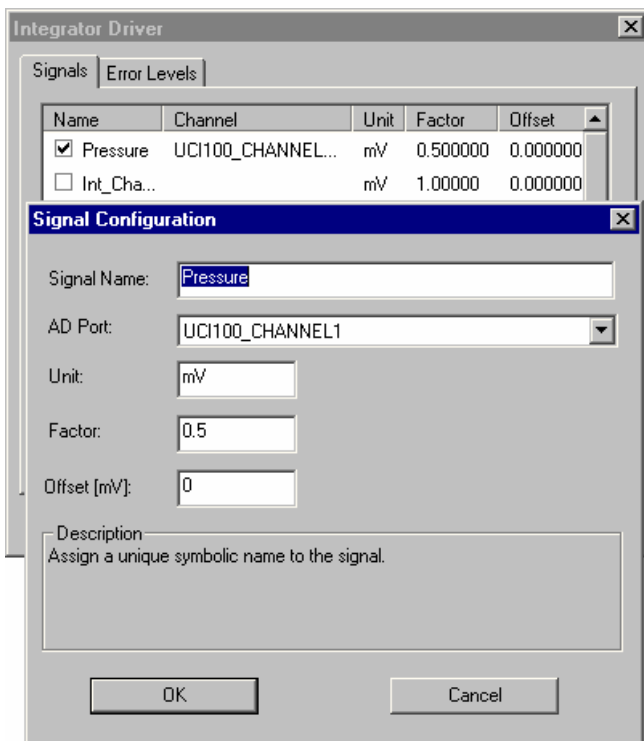
Select the channel to which the pressure output of your pump is connected as **AD Port**. Enter under **Factor** how many bar correspond to an output voltage of 1 mV. For example, enter 0.5 bar/mV (0.5 bar = 50 kPa = 7.25 psi) for the Dionex P580 pump or 5 bar/mV (5 bar = 500 kPa = 72.5 psi) for the Dionex M480 pump.

You can now record data for the **Pump_Pressure** channel. Data acquisition can also be defined in the ➢*PGM File* or by selecting ⇒*AcqOn* on the **Control** menu on the ➢*Control Panel*.

Panel

- When you are on the signal plot of your panel, select **Signals** on the context menu.

- Select a signal in the **Available Signals** field (for the above example, select **Pressure**).

- When starting the data acquisition, select the Pump_Pressure channel and define the ⇒*Step* (see 🔳 Starting Data Acquisition).

🔳 Setting Automatic Pre-Compression Control (P580)

The better the pump is set to the varying compressibility of the different components of the solvents, the lower the pump's pulsation. Automatic pre-compression control of the Dionex P580 pump considers the varying compressibility of different solvents. Automatic pre-compression control can also be used for unknown solvents.

With low-pressure gradients and isocratic pumps, pre-compression control is fully automatic. On the ➢*Control Panel*, select **Commands** on the **Control** menu. Select the pump and the solvent components one after the other and assign the component type **Automatic**.

The high-pressure gradient pumps must "learn" the automatic pre-compression control. Select **Commands** on the **Control** menu. Select the pump and the solvent component. Assign the solvent type **Custom**. Deliver 100% of this solvent at 1 ml/min and a backpressure of approximately 100 bar (= 10 MPa = 1450 psi). Select **Commands** on the **Control** menu and issue the ⇒*Learn* command. Observe the pressure signal for at least 10 minutes. Issue the corresponding pump ⇒*Freeze* command to save the optimum pre-compression setting when the pressure fluctuations from the pump are minimal.

☞ *Tip:*

*During the **Learn** phase, the backpressure should correspond to the maximum pressure in normal operation. If the backpressure is less than 25°bar (= 2.5 MPa = 362.5 psi) during this phase, pre-compression control cannot be set correctly! Increase the backpressure, for example, by installing a second column or a longer capillary before the detector. Again, wait for approximately 10 minutes before saving the pre-compression value using the Freeze command.*

Change the flow to 100% of the next solvent and set the pre-compression control as described above.

For a detailed description, refer to the P580 Operating Instructions.

▪ Viewing Leak Sensor and Workload Status

The Dionex P580 pump allows you to display of the status of the leak sensor. In addition, you can display the ➤*Cumulated Workload*.

To create the corresponding controls, follow the description in **How to: Controlling Devices from the Control Panel:**

🔲 **Modifying a Control Panel**

🔲 **Modifying a Control**

🔲 **Linking a Control to a Device**

Select **Commands** on the **Control** menu. Under **Pump**, select **Leak** to display the status of the leak sensor. Click **Cumulated WorkLoad** to display the total workload [in mega joule, MJ] of the pump.

▪ Peak Parking

The UltiMate system pump allows peak parking, for example, to increase the ➤*Mass Spectrometer* acquisition time while the peak elutes. The ⇒*ParkPercentage* enables peak parking. Select the **PeakParked** property to display the peak parking state.

Peak parking is similar to the behavior of the ⇒*StopFlow* command:

- The ➤*Gradient* program is interrupted.

- Usually, the pump flow is reduced (but not turned off).

However, unlike the behavior of the **StopFlow** command:

- Data Acquisition (see ➤*Acquisition On/Off*) is not interrupted.

- A running ➤*Batch* is not stopped.

Select **ParkPercentage** to enable and disable peak parking. The following value disables peak parking:

```
0.000   ParkPercentage          = Disabled
```

ParkPercentage > 0.00 sets the flow to the following relative value during PeakParked state:

```
        current flow * ParkPercentage.
```

The PeakParked state is entered whenever the signal at the pump's START IN input changes from Open to Closed (edge trigger) and continues until the signal changes from Open to Closed again:
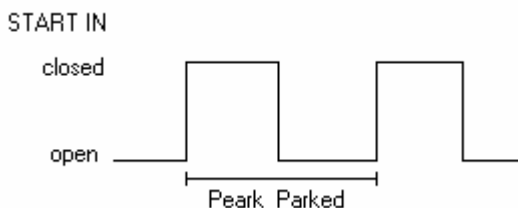
At each sample start, the PeakParked state is reset.

☞ *Tip:*

Peak parking freezes the gradient with its current composition and reduced flow while data acquisition continues. Make sure that data acquisition is long enough (that is, the expected running time of the chromatogram + expected time for peak parking). Therefore, with peak parking the retention times do not correspond to the expected times. In addition, ➤Audit Trail entries and gradient plots are no longer synchronized.

▪ Splitting the Flow (Program Example)

The Dionex Active Flow Splitter (MRA), which is usually part of an APS system for ➢*Autopurification*, splits the HPLC flow and directs the smaller portion to a ➢*Mass Spectrometer*. The following program is a typical example of how to set up flow splitting in a PGM File:

```
        . . .
        3DFIELD.Step =  0.5 [s]
        Pump.Flow =  50.000 [ml/min]

;       MRA settings: Communicate the used flow rate and the target
;       value for the split ratio

;       Caution: You might find it useful to add an implicit
;       assignment, such as MRA.Flow = Pump.Flow. However, this is
;       not possible as most pumps do not deliver the target value
;       immediately. The Flow property always sets the actual flow.
;       Therefore, you have to set the MRA flow explicitly:

        MRA.Flow = 50.000 [ml/min]
        MRA.SplitRatio = 20000
 0.000   Autozero
   Wait  Ready
   Inject
   UV_VIS_1.AcqOn

; To minimize wear, start the MRA after the injection if possible:

   MRA.RunState = On
   . . .

; You can change the split ratio during a run.

 1.300   MRA.SplitRatio =  100000
   . . .
 2.600   MRA.SplitRatio =  20000
   . . .
 10.000  UV_VIS_1.AcqOff

; To minimize wear, stop the MRA after a run if possible

   MRA.RunState = Off

   End
```

Autosampler Control

Depending on the ➢*Autosampler* type, different commands are available. For an overview of the individual commands that are available for the different Dionex autosamplers, refer to:

🔺 **Autosampler Commands (GINA 50)**

🔺 **Autosampler Commands (ASI-100 Series)**

🔺 **Autosampler Commands (AS/AS50)**

🔺 **Examples for User-Defined Programs for the WPS-3000 Autosampler**

🔺 **Examples for User-Defined Programs for the FAMOS Autosampler (LC Packings)**

The following pages provide detailed information about:

🔺 **Injecting a Sample**

🔺 **Setting Up Remote Injection**

🔺 **Priming the Syringe (ASI-100 Series)**

🔺 **Defining Sample Preparation Steps (AS/AS50)**

🔺 **Overlapping Samples**

🔺 **Injecting Two Samples Simultaneously**

🔺 **Opening the AS/AS50 Door during Operation**

🔺 **Sharing an Autosampler (AS or AS50 (USB))**

🔺 **Monitoring the Status of the AS/AS50**

◾ Autosampler Commands (GINA 50)

Suck (see ⇒*Draw*)/⇒*Dispense* Sample

```
0.000    Suck        Position = Value, Volume = Value [µl],
                     Duration = Value [s]
0.000    Dispense    Position = Value, Volume = Value [µl],
                     Duration = Value [s]
```

📳 *Note:*

*In the case of the Dionex Autosampler GINA 50, the corresponding operations can be synchronized with Chromeleon via a remote input and the **Suck** and **Dispense** commands. The device automatically reports the completion of each operation to Chromeleon. In the ⋗Program, the wait command **Sucked** must be inserted. All following commands are executed only after the autosampler confirmed the execution of the **Suck** command, that is, after the sample has been drawn. The program performs this independently of the time required by the autosampler for the suck and dispenses processes. This is shown in the following example:*

```
0.000    Suck        Position = 20, Volume = 30, Duration = 0
0.000    Wait        Sucked
0.000    Suck        Position = 21, Volume = 30, Duration = 0
0.000    Wait        Sucked
0.000    Dispense    Position = , Volume = 60, Duration = 0
0.000    Wait        Sucked
0.000    ⇒Inject
```

Description of the program part: First, the Autosampler GINA 50 draws 30 µl solution from ⇒*Position* 20 and afterward draws 30 µl solution from position 21. The entire drawn volume is dispensed in the current vial (current = last position if no other position is specified). Then, the injection volume, which has been specified in the sample list, is drawn and injected from there.

Short command syntax:

```
0.000    Suck        20, 30, 0
0.000    Wait        Sucked
0.000    Suck        21, 30, 0
0.000    Wait        Sucked
0.000    Dispense    Position = , Volume = 60, Duration = 0
0.000    Wait        Sucked
0.000    Inject
```

Relative Sample Location

The Dionex Autosampler GINA 50 is capable of executing the suck and the dispense commands relative to a certain sample position. The **Position+Location** is entered in the field **Position** of the **Suck** or **Dispense** commands. In the program this is expressed as follows:

```
0.000   Suck       Position = 20, Volume = 30, Duration = 0
0.000   Wait       Sucked
0.000   Suck       Position = Position+10, Volume = 30, Duration = 0
0.000   Wait       Sucked
0.000   Dispense   Position = Position+20, Volume = 60, Duration = 0
0.000   Wait       Sucked
```

Description of the program part: The autosampler takes 30 µl of solution from position 20, moves 10 positions from the current position in the sample list, and takes 30 µl of solution from there. Then, the 60-µl solution is dispensed into a vial that is located 20 positions from the current sample vial.

Air Segment

For the Dionex GINA 50 Autosampler, the input position = 100 is an imaginary **Air Vial** from which a certain air volume can be drawn as a separating segment; for example:

```
0.000   Suck       Position = 20, Volume = 30, Duration = 0
0.000   Wait       Sucked
0.000   Suck       Position = 100, Volume = 30, Duration = 0
0.000   Wait       Sucked
0.000   Suck       Position = 21, Volume = 30, Duration = 0
0.000   Wait       Sucked
```

Description of the program part: After 30 µl of solution is sucked from position 20, 30 µl of air is sucked before another 30 µl of solution is sucked from position 21. Thus, the two solutions do not encounter each other.

🗐 *Note:*

*The **Segment** command must always be used in combination with the **Dispense** command; otherwise, the sucked air volume will be injected, also.*

Dispense in Needle Seat

Entering position 101 in a **Dispense** command enables dispensing the volume contained in the needle into the needle seat of the Autosampler and thus into the waste container.

```
0.000   Dispense    Position = 101, Volume = 60, Duration = 0
```

Wash Injection Loop

Select the ⇒*Wash* command to rinse the injection loop of the Gina 50 autosampler with solvent. This corresponds to the **normal** solvent flow during the **Inject** command.

```
0.000   DEVICENAME.Wash    Or
0.000   Wash
```

Lift/Lower Sample Needle

Select the ⇒*NeedleUp* command to raise the sample needle. When raising the needle, an active Wash process is automatically interrupted, i.e., solvent flow is not through the injection loop any longer, but directly from the pump to the column.

```
0.000   NeedleUp
```

Execute the **Wash** command to lower the needle again and direct solvent flow through the sample loop again (for more information, see ➢*Autosampler*).

```
0.000   Wash
```

The combination of the two commands prevents crystallization of substances in the sample loop.

For an overview of the individual commands for the GINA 50, refer to **Commands for Controlling Dionex Devices** 🔲 **Dionex GINA 50 Autosamplers**.

Also, refer to 🔲 **Autosampler Commands (ASI-100 Series)**.

▪ Autosampler Commands (ASI-100 Series)

Many commands for the Dionex ASI-100 ⮞*Autosamplers* (ASI-100 T and ASI-100 PT= with temperature control) are similar to those of the GINA 50 (see ▪ **Autosampler Commands (GINA 50)**). However, there are some important differences, which are described below:

Sample Positions

The sample vials are situated in three different segments, which are distinguished by color. Within the segments, the vials are situated in different rows. Thus, the sample ⇒*Positions* are indicated as follows:

Letters according to their color describe the individual segments: R, G, or B (indicating the red, green, and blue segments, respectively). The different rows are described from the outer to the inner row: A, B, C, or D. The individual positions within the respective rows are numbered counterclockwise. For example, the RA1 position is located in the outer row of the red segment (also, refer to the Operating Instructions for the ASI-100 Series).

Autosampler Configuration

Specify the device configuration before starting the program. It is important to define the sample positions for the reagents and the wash liquid. These positions are valid throughout the entire running time of the program:

```
0.000    Sampler.ReagentAVial    BB1
0.000    Sampler.ReagentBVial    BC1
0.000    Sampler.ReagentCVial    BA1
0.000    Sampler.WashVial        G99
0.000    Sampler.PrepVial        R99
```

The following commands define the number of draw and dispense actions (for the **Mix** command):

```
0.000    Sampler.MixRepeat       3
```

⇒*Draw,* ⇒*Dispense* Sample, ⇒*Mix*

Before issuing the **Draw**, **Dispense**, or **Mix** command, specify the vial (depending on the selected option - see below) from which to draw and/or dispense and the volume to draw and/or dispense (the latter for the **Mix** command):

```
0.000    PrepSubject            Option (see below)
0.000    PrepVolume             Value [µl]
0.000    Draw

0.000    PrepSubject            Option (see below)
0.000    PrepVolume             Value [µl]
0.000    Dispense
```

The following options are available for the **PrepSubject** command:

Option	Description
PrepVial	Actual mixing vial position (current PrepVial value)
Sample_Vial	Vial for the actual sample in the sample list (current Sampler.Position value)
WashVial	Vial containing the wash liquid (current Sampler.WashVial value)
Air	Air (with the Draw command) and needle port (with the Dispense command), respectively
ReagentA (B, C or D)	Reagents A (B, C, or D) (actual Sampler.ReagentAVial value)

Some commands need to be synchronized with Chromeleon; that is, the autosampler automatically reports completion of the respective operation to Chromeleon. In addition, the **Sampler.Ready** wait condition must be part of the ➢*Program*. The following commands are executed only after the autosampler has confirmed that the command has been executed. This applies to the **Draw**, **Dispense**, **Mix**, **Test**, and ⇒*Wash* commands and is illustrated below:

```
0.000    PrepSubject            Sample_Vial
0.000    PrepVolume             30
0.000    Draw
0.000    Wait                   Sampler.Ready
0.000    PrepSubject            ReagentA
0.000    PrepVolume             30
0.000    Draw
0.000    Wait                   Sampler.Ready
0.000    PrepSubject            MixVial
0.000    PrepVolume             60
0.000    Dispense
0.000    Wait                   Sampler.Ready
0.000    ⇒Inject                Position = PrepVial
```

Description of the program part: First, the autosampler draws 30 µl of solution from the current sample vial, and, upon completion, 30 µl of solution from the reagent A vial. The entire volume drawn is dispensed into the MixVial. The injection volume, which has been specified in the sample list, is then drawn and injected from this position.

Air Segment

To draw an air segment, specify that the PrepSubject be Air. For example, use the following program part to draw two samples separated by an air segment. The air segment prevents early mixing in the needle and makes sure that an exactly defined reaction time is met.

```
0.000    PrepSubject          ReagentA
0.000    PrepVolume           100
0.000    Draw
0.000    Wait                 Sampler.Ready
0.000    PrepSubject          Air
0.000    PrepVolume           5
0.000    Draw
0.000    Wait                 Sampler.Ready
0.000    PrepSubject          Sample_Vial
0.000    PrepVolume           100
0.000    Draw
0.000    Wait                 Sampler.Ready
0.000    PrepSubject          MixVial
0.000    PrepVolume           205
0.000    Dispense
0.000    Wait                 Sampler.Ready
```

Description of the program part: Having drawn 100 µl of reagent A, 5 µl of air is drawn first before another 100 µl of sample is drawn from the vial. Thus, the two solutions encounter each other in the mixing vial only.

Relative Sample Location

1. Different rings: The autosampler can execute commands relative to a given sample position. Type the description "Position + relative entry" in the **Position** field of the **Draw** or **Dispense** commands. In the program, this is expressed as follows (if the current position is in the RA ring):

```
0.000    PrepSubject          Sample_Vial
0.000    PrepVolume           30
0.000    Draw
0.000    Wait                 Sampler.Ready
0.000    Position             Position + RB1 - RA1
0.000    PrepSubject          Sample_Vial
0.000    PrepVolume           30
0.000    Draw
0.000    Wait                 Sampler.Ready
0.000    Position             Position + RC1 - RB1
0.000    PrepSubject          Sample_Vial
0.000    PrepVolume           60
0.000    Dispense
0.000    Wait                 Sampler.Ready
0.000    Inject               Volume=30
```

Description of the program part: The autosampler draws 30 µl of solution from the actual sample vial, moves to the respective position in the RB ring, and draws 30 µl of solution from this position as well. Then, the 60 µl solution is dispensed into the vial at the respective position in the RC ring. For example, if the current sample is situated at position RA3, another 30 µl will be drawn from position RB3, and the entire volume of 60 µl will be dispensed at position RC3. After that, 30 µl are injected from position RC3.

Note:

*To complete the program, the **PrepSubject** command is repeated with the **Sample_Vial** argument. However, this command is not required, as Chromeleon keeps the latest setting. The second command (= **PrepVolume**) could also be omitted, as it only repeats the argument "30."*

2. Different segments: You can also place the samples to derivate into the red segment, for example, and execute the mixing process with a reagent in the corresponding sample vial in the green segment using the following commands:

```
0.000    Sampler.PrepVial       Position + GA1 - RA1
0.000    PrepSubject            PrepVial
0.000    Mix
```

The first line specifies that the corresponding vial in the green segment be used as the mixing vial for the sample in the red segment. For example, if you wish to process the sample at position RB5, mixing takes place at position GB5.

3. Incrementing the positions: Another way is to increment the positions. Use the following example to define a position 5 after the current position for MixSubject:

```
0.000    Sampler.PrepVial       Position + 5
0.000    PrepSubject            PrepVial
0.000    Mix
```

For example, if you wish to process the sample from position RB3, the vial at position RB8 is the mixing vial.

Wash Needle

Use the **Wash** command to rinse the autosampler's needle with the wash liquid. The wash volume is drawn and dispensed into the needle seat.

```
0.000   WashVolume        Value [µl]
0.000   Wash
```

For an overview of the individual commands for the ASI-100 Autosamplers, refer to **Commands for Controlling Dionex Devices** 🔲 **Dionex ASI-100 Autosampler Series**.

For an overview of the commands for the AS and AS50 autosamplers, refer to 🔲 **Autosampler Commands (AS/AS50)**.

🔳 Autosampler Commands (AS/AS50)

Sample Loading and Injection

With the AS and AS50 autosamplers, sample loading and injection are two distinct events. The **Load** command switches the injection valve to the load position and moves the sample from the AS or AS50 inject port into the sample loop. The **Inject** command switches the injection valve to the inject position, which directs the pump flow through the loop. The sample is then transported from the loop to the column.

👉 *Tip:*

*Clicking the **Inject** button on the control panel sends both the **Load** and the **Inject** commands to the autosampler.*

📒 *Note:*

*If the AS or AS50 is in Simultaneous mode, separate **Valve.LoadPosition** and **Valve.InjectPosition** commands are required (in addition to the **Load** and **Inject** commands). See the description of Simultaneous mode below for details.*

Flush the Inject Port

The **Flush** and **Wait** commands must be in the order shown. We recommend that these commands occur either *before* any other ➤*Autosampler* commands or *after* all other autosampler commands.

```
        Flush       Volume = Value [µl]
        Wait        FlushState
```

Autosampler Options

All AS and AS50 autosampler option commands must be grouped together in the ➤*Program*. Place them at the beginning of the program, before t = 0.000. You do not need to specify event times for the option commands.

```
Devicename.NeedleHeight          = Value [mm]
Devicename.CutSegmentVolume      = Value [µl]
Devicename.SyringeSpeed          = Value
Devicename.ColumnTemperature     = Off / Value [°C]
Devicename.TrayTemperature       = Off / Value [°C]
Devicename.Cycle                 = Value
```

Sample Preparation

All AS and AS50 sample prep commands (**Pipet**, **Mix**, **FlushSP**, **DelaySP**, **SetNeedleHeight**, **Dilute**, **Dispense**, **Concentrate**, **ReagentPrime**, and **ReagentFlush**) must be grouped together in the program. Place them at the beginning of the program, after the AS and AS50 option commands and before t=0.000. You do not need to specify event times for the sample prep commands. Include a **Wait SampleReady** command after the sample prep commands to allow them to be completed before injection.

🗔 *Note:*

*The **Dilute** and **Dispense** commands are available only if the AS or AS50 is equipped with the sample preparation option. The **Concentrate**, **ReagentPrime**, and **ReagentFlush** commands require AS or AS50 (USB) Moduleware version 2.0.0 (or later). These commands are not available for the DX-LAN model of the AS50, regardless of which Moduleware version is installed in the autosampler.*

In the example below, 20 µl are pipetted from vial 1 and delivered to vial 10. 200 µl are dispensed from reservoir A into vial 10. The contents of vial 10 is mixed by drawing in and expelling 100 µl of the vial contents. The mixing cycle is repeated 5 times.

Note:

Due to limitations here, commands are shown on two lines. In an actual program, one command must be entered on one line.

```
Sampler.Pipet     Volume   =   20.0,   SourceVial   =   1,
                  DestinationVial = 10
Sampler.Dispense  Volume   =   200.0,   SourceReservoir   =
                  Reservoir_A, DestinationVial = 10
Sampler.Mix       SourceVial  =  10,  NumberOfTimes  =  5,
                  Volume = 100.0
Wait              SampleReady
```

Also refer to **Defining Sample Preparation Steps**.

ReagentFlush (Concentrate) Applications

The AS or AS50 (USB) runs in Concentrate or Sequential Concentrate mode when the mode is selected on the autosampler front panel and a concentrator column is installed. The default ReagentFlush program for these modes contains the following parameters:

- The **Concentrate** command is automatically listed on the first line of the **Sample Prep Options** page. You may add sample prep steps before or after this line.

- The program does not include the **Load**, **Inject**, or **Wait InjectState** commands.

- The **Sampler_InjectValve.InjectPosition** command is located after the **Wait CycleTimeState** command at time 0.00.

- The **BeginOverlap** command is inserted at 5.00 minutes. Sample overlap normally begins after the **Wait InjectState** command; however, since ReagentFlush programs do not include the **Inject** and **Wait InjectState** commands, sample overlap will start at the time specified for **BeginOverlap**.

You may add sample prep commands before or after the **Concentrate** command. You may add or delete the **Concentrate**, **ReagentPrime**, **ReagentFlush**, and **BeginOverlap** commands as needed.

A program may include multiple **Concentrate**, **ReagentPrime**, and **ReagentFlush** commands. However, if the program includes multiple **BeginOverlap** commands, all but the first **BeginOverlap** command is ignored.

Make sure the multiple **Concentrate** or **ReagentFlush** commands do not exceed the volume of sample or reagent in the vial specified in the sequence.

In the following example program for the Concentrate mode, commands for the ReagentFlush function are in bold. If the Sequential Concentrate mode is selected, the program must also include exclusive access commands that allow the autosampler to be shared by two timebases.

Also, refer to 🔟 **Sharing an Autosampler (AS or AS50 (USB))**.

📝 *Note:*

Due to limitations here, commands are shown on two lines. In an actual program, one command must be entered on one line.

```
        Flush                                    Volume = 100
        Wait                                     FlushState
        NeedleHeight =                           2
        CutSegmentVolume =                       0
        SyringeSpeed =                           3
        ColumnTemperature =                      15
        CycleTime =                              0
        Pipet     Volume  =  20.0,  SourceVial  =
        CurrentVial, DestinationVial = CurrentVial
        Mix        SourceVial    =    CurrentVial,
        NumberOfTimes = 5, Volume = 250.0
        FlushSP  Volume = 250.0
        DelaySP  Time = 0.0
        Dispense   Volume = 1000.0, SourceReservoir
        =    Reservoir_A,    DestinationVial    =
        CurrentVial
        WaitForTemperature                       False
        Concentrate ValvePosition = LoadPosition
        ReagentPrime Volume = 10000.0, SourceVial =
        CurrentVial, ValvePosition = NoChange
        ReagentFlush Volume = 5000.0, SourceVial =
        CurrentVial, ValvePosition = NoChange
        Wait                                     SampleReady
0.000   Wait                                     CycleTimeState
        Sampler_InjectValve.InjectPosition
0.100   Home
5.000   BeginOverlap
30.000  End
```

Relative Vial Location

In the sample preparation commands, a vial location can be specified either as an absolute position or as a relative position. In relative positioning, the CurrentVial is the current sample vial position, specified in the sample list in the Sequence. In the example below, 20 µl of liquid are pipetted from the current sample vial and then expelled into the vial, 1 position past the current vial.

```
Sampler.Pipet     Volume = 20.0, SourceVial = CurrentVial,
                  DestinationVial = CurrentVial+1
```

Sample Overlap

If the AS or AS50 **Sample Overlap** option is enabled in the Server Configuration, the autosampler performs the following commands for the next sample in a sequence, while data acquisition is occurring for the currently running sample in the sequence:

- **Flush**

- **Autosampler Option Commands** (ColumnTemperature, TrayTemperature, WaitForTemperature, SyringeSpeed, CycleTime, SetNeedleHeight, CutSegmentVolume)

- **Sample Prep Commands** (Pipet, Mix, DelaySP, FlushSP, NeedleHeight, Dilute, Dispense, Concentrate, ReagentPrime, ReagentFlush)

When the autosampler is preparing a sample, the **Status** field in the sequence displays **Preparing** and the sample line is in yellow. A message is also logged in the Audit Trail.

Sample overlap is not allowed when the autosampler is running in the Sequential or Sequential Concentrate mode.

Also, refer to **Overlapping Samples**.

Cycle Time

Cycle time controls the time between injections. When a cycle time is specified, the autosampler delays sample injection until the specified time has elapsed since the previous injection. This is accomplished with the **Wait CycleTimeState** command. When running a batch, the **Wait CycleTimeState** command in the first PGM File in the batch is ignored.

The following example sets a cycle time of 30 minutes.

```
      Cycle              =30
0.000  Sampler.Load
      Wait               CycleTimeState
      Sampler.Inject
```

Priming the Liquid Lines

The **Prime** command is used to prime the flush reservoir line to the sampling valve and the sample transfer line. In addition, if the sample prep option is installed, the **Prime** command is used to prime the lines from each installed reagent reservoir. The following example uses the prep syringe to prime the line to reservoir A with 2000 µl.

```
0.000  Sampler.Prime    Volume=2000,   PrimeReservoir=Reservoir_A,
                        PrimeSyringe=Prep
```

Simultaneous Mode

If the Simultaneous mode is selected on the AS or AS50 front panel, the program includes additional commands for controlling two injection valves. The additional commands are used to switch the position of each valve during sample injection.

In the following example, an AS50 is connected to two ICS-2000 systems. Each ICS-2000 is equipped with an injection valve; no injection valves are installed in the AS50.

```
0.000  Pump_InjectValve.LoadPosition
      Pump_InjectValve_2.LoadPosition
      Load
      Wait   CycleTimeState
      Pump_InjectValve.InjectPosition
      Pump_InjectValve_2.InjectPosition
      Pump_ECD.Autozero
      Pump_ECD_2.Autozero
      Inject
      Wait   InjectState
```

Note:

For an overview of the individual commands for the autosamplers, refer to Dionex AS/AS50 Autosamplers.

▣ Examples for User-Defined Programs for the WPS-3000 Autosampler

The user-defined program is loaded and executed at the autosampler's start time (**Sampler.Inject** command) even if the program steps appear prior to that command in the program file. Make sure that the user-defined program includes an **UdpInjectMarker** command at the appropriate position so that the system recognizes that an injection has been made:

The following programs are typical application examples for the WPS-3000 ➢*Autosampler*:

0. User-defined standard program

```
        ;User defined program (UDP) template for WPS-3000 sampler
        ;******    PLEASE DO NOT EDIT  ******
0.000   InjectMode =            UserProg
        Sampler.ReagentAVial=   R1
        Sampler.ReagentBVial=   R2
        Sampler.ReagentCVial=   R3
        Sampler.ReagentDVial=   R4
        Sampler.PrepVial=       RA1
        Sampler.UdpDraw         From=SampleVial,            Volume=5,
                                SyringeSpeed=50, SampleHeight=2
        Sampler.UdpDispense     To=PrepVial,                Volume=5,
                                SyringeSpeed=100, SampleHeight=2
        Sampler.UdpDraw         From=ReagentAVial,         Volume=10,
                                SyringeSpeed=50, SampleHeight=2
        Sampler.UdpDispense     To=PrepVial,               Volume=10,
                                SyringeSpeed=2000,   SampleHeight=2
        Sampler.UdpMixWait      Duration=10
        Sampler.UdpDraw         From=PrepVial,              Volume=5,
                                SyringeSpeed=50, SampleHeight=2
        Sampler.UdpInjectValve  Position=Inject
        Sampler.UdpInjectMarker

        Inject
        ;starts the above program and waits for the inject response.

        Acquisition On
      ...
4.000   Acquisition Off
        End
```

1. Drawing a sample volume of 1 µl

```
        ;User defined program (UDP) template for WPS-3000 sampler
        ;******    PLEASE DO NOT EDIT  ******
0.000   InjectMode =            UserProg
        UdpInjectValve      Position=Load
        UdpSyringeValve     Position=Needle
        UdpDraw             From=SampleVial,            Volume=1.0,
```

```
                         SyringeSpeed=50, SampleHeight=2
        UdpMixWait          Duration=5
        UdpDraw             From=SampleVial,            Volume=0.0,
                            SyringeSpeed=50, SampleHeight=2
        UdpDraw             From=ReagentAVial,          Volume=25,
                            SyringeSpeed=50, SampleHeight=2
        UdpMixWait          Duration=5
        UdpInjectValve      Position=Inject
        UdpInjectMarker
        ;generates an Inject Response during the program run

        UdpMixWait            Duration=5
        UdpDraw             From=ReagentAVial,          Volume=0.0,
                            SyringeSpeed=50, SampleHeight=2
        UdpSyringeValve       Position=Waste
        UdpMoveSyringeHome    SyringeSpeed=2000
        UdpSyringeValve       Position=Needle
        UdpMixNeedleWash      Volume=50

     Inject
     ;starts the above program and waits for the inject response.

      Acquisition On
     ...
4.000   Acquisition Off
        End
```

2. Sample preparation with 2 reagents

```
        ;User defined program (UDP) template for WPS-3000 sampler
        ;******   PLEASE DO NOT EDIT  ******
0.000   InjectMode =        UserProg
        UdpDraw             From=ReagentAVial,        Volume=20.0,
                            SyringeSpeed=50, SampleHeight=2
        UdpMixWait          Duration=5
        UdpDraw             From=ReagentAVial,         Volume=0.0,
                            SyringeSpeed=50, SampleHeight=2
        UdpMixWait          Duration=5
        UdpDispense         To=SampleVial,            Volume=20.0,
                            SyringeSpeed=2000, SampleHeight=2
        UdpMixWait          Duration=10
        UdpDraw             From=ReagentBVial,        Volume=10.0,
                            SyringeSpeed=50, SampleHeight=2
        UdpMixWait          Duration=5
        UdpDraw             From=ReagentBVial,         Volume=0.0,
                            SyringeSpeed=50, SampleHeight=2
        UdpMixWait          Duration=5
        UdpDispense         To=SampleVial,            Volume=10.0,
                            SyringeSpeed=2000, SampleHeight=2
        UdpMixWait          Duration=10

        UdpInjectValve      Position=Inject
        UdpSyringeValve     Position=Needle
        UdpDraw             From=SampleVial,           Volume=3.0,
                            SyringeSpeed=50, SampleHeight=2
```

```
        UdpMixWait              Duration=5
        UdpInjectValve          Position=Load
        UdpDraw                 From=SampleVial,            Volume=5.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpMixWait              Duration=5
        UdpInjectValve          Position=Inject
        UdpInjectMarker
        ;generates am inject response during the program run

        UdpMixWait                Duration=5
        UdpDraw                 From=SampleVial,            Volume=0.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpSyringeValve           Position=Waste
        UdpMoveSyringeHome        SyringeSpeed=2000
        UdpSyringeValve           Position=Needle
        UdpMixNeedleWash          Volume=50

        Inject
        ;starts the above program and waits for the inject response.

        Acquisition On
      ...
4.000   Acquisition Off
        End
```

3. Gel extraction

```
        ;User defined program (UDP) template for WPS-3000 sampler
        ;******    PLEASE DO NOT EDIT  ******
0.000   InjectMode =            UserProg
        UdpDraw                 From=SampleVial,              Volume=5.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpDispense             To=PrepVial,                  Volume=5.0,
                                SyringeSpeed=2000, SampleHeight=2
        UdpDraw                 From=ReagentAVial,            Volume=5.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpDispense             To=SampleVial,                Volume=5.0,
                                SyringeSpeed=2000, SampleHeight=2
        UdpMixWait              Duration=10

        UdpDraw                 From=SampleVial,              Volume=5.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpDispense             To=PrepVial,                  Volume=5.0,
                                SyringeSpeed=2000, SampleHeight=2
        UdpDraw                 From=ReagentBVial,            Volume=5.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpDispense             To=PrepVial,                  Volume=5.0,
                                SyringeSpeed=2000, SampleHeight=2
        UdpMixWait              Duration=10

        UdpDraw                 From=SampleVial,              Volume=5.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpDispense             To=PrepVial,                  Volume=5.0,
                                SyringeSpeed=2000, SampleHeight=2
        UdpMixWait              Duration=5
```

```
        UdpInjectValve        Position=Inject
        UdpSyringeValve       Position=Needle
        UdpDraw               From=SampleVial,              Volume=1.0,
                              SyringeSpeed=50, SampleHeight=2
        UdpMixWait            Duration=5
        UdpInjectValve        Position=Load
        UdpDraw               From=PrepVial,               Volume=5.0,
                              SyringeSpeed=50, SampleHeight=2
        UdpMixWait            Duration=5
        UdpInjectValve        Position=Inject
        UdpInjectMarker

      ;generates an inject response during the program run

        UdpMixWait              Duration=5
        UdpDraw                 From=SampleVial,              Volume=0.0,
                                SyringeSpeed=50, SampleHeight=2
        UdpSyringeValve         Position=Waste
        UdpMoveSyringeHome      SyringeSpeed=2000
        UdpSyringeValve         Position=Needle
        UdpMixNeedleWash        Volume=50

        Inject
      ;starts the above program and waits for the inject response

        Acquisition On
        ...
4.000   Acquisition Off

        End
```

🗟 *Notes:*

Use the PGM Wizard to create a user-defined program. You can either create a new user-defined program or use an existing program as a template for the new one.

*Each user-defined program must include an **UdpInjectMarker** command. If this command is not included, a message appears in the PGM Wizard.*

For an overview of the individual commands that are available for the autosampler, refer to **Commands for Controlling Dionex Devices** 🗟 **UltiMate 3000 Series - Autosampler**.

For information about the sample preparation commands supported by the autosampler, refer to **Commands for Controlling Dionex Devices** 🗟 **Dionex WPS-3000 Autosampler: Commands for User-Defined Programs**.

◾ Examples for User-Defined Programs for the FAMOS Autosampler (LC Packings)

The user-defined program is loaded and executed at the autosampler's start time (**Sampler.Inject** command) even if the program steps appear prior to that command in the program file. Make sure that the user-defined program includes an **InjectMarker** command at the appropriate position so that the system recognizes that an injection has been made:

The following programs are typical application examples for the FAMOS HPLC ➤*Autosampler*:

0. User-Defined Standard Program

```
;User-defined program (UDP) template for FAMOS autosampler
;******    PLEASE DO NOT EDIT  ******
Sampler.ReagentAVial= 1
Sampler.ReagentBVial= 2
Sampler.ReagentCVial= 3
Sampler.ReagentDVial= 4
Sampler.PrepVial=     A1
Sampler.Draw      From=SampleVial,                Volume=5,
SyringeSpeed=Low,    SampleHeight=0
Sampler.Dispense  To=PrepVial,  Volume=5,  SyringeSpeed=Low,
                  SampleHeight=0
Sampler.Draw      From=ReagentAVial, Volume=10,
                  SyringeSpeed=Low, SampleHeight=0
Sampler.Dispense  To=PrepVial,  Volume=10,  SyringeSpeed=Low,
                  SampleHeight=0
Sampler.MixWait   Duration=10
Sampler.Draw      From=PrepVial, Volume=5, SyringeSpeed=Low,
                  SampleHeight=0
Sampler.InjectValve  Position=Inject
InjectMarker
```

1. Drawing a Sample Volume of 1 µl

```
      ;User-defined program (UDP) template for FAMOS autosampler
      ;******    PLEASE DO NOT EDIT  ******
0.000 InjectMode =    UserProg
      InjectValve     Position=Load
      SyringeValve    Position=Needle
      Draw            From=SampleVial, Volume=1.0
      MixWait         Duration=5
      Draw            From=SampleVial, Volume=0.0
      Draw            From=ReagentAVial, Volume=25
      MixWait         Duration=5
      InjectValve     Position=Inject
      InjectMarker
      ; generates an inject response during the program run
      MixWait         Duration=5
      Draw            From=ReagentAVial, Volume=0.0
```

```
        SyringeValve      Position=Waste
        MoveSyringeHome
        SyringeValve      Position=Needle
        MixNeedleWash     Volume=50

        Inject
        ; starts the above program and waits for the inject response

    Acquisition On
        ...
4.000 Acquisition Off
    End
```

2. Sample Preparation with 2 Reagents

```
        ;User-defined program (UDP) template for FAMOS autosampler
        ;******    PLEASE DO NOT EDIT  ******
0.000   InjectMode =      UserProg
        Draw              From=ReagentAVial, Volume=20.0
        MixWait           Duration=5
        Draw              From=ReagentAVial, Volume=0.0
        MixWait           Duration=5
        Dispense          To=SampleVial, Volume=20.0
        MixWait           Duration=10
        Draw              From=ReagentBVial, Volume=10.0
        MixWait           Duration=5
        Draw              From=ReagentBVial, Volume=0.0
        MixWait           Duration=5
        Dispense          To=SampleVial, Volume=10.0
        MixWait           Duration=10

        InjectValve       Position=Inject
        SyringeValve      Position=Needle
        Draw              From=SampleVial, Volume=3.0
        MixWait           Duration=5
        InjectValve       Position=Load
        Draw              From=SampleVial, Volume=5.0
        MixWait           Duration=5
        InjectValve       Position=Inject
        InjectMarker
        ; generates an inject response during the program run

        MixWait           Duration=5
        Draw              From=SampleVial, Volume=0.0
        SyringeValve      Position=Waste
        MoveSyringeHome
        SyringeValve      Position=Needle
        MixNeedleWash     Volume=50

        Inject
        ; starts the above program and waits for the inject response

        Acquisition On
        ...
4.000   Acquisition Off
        End
```

3. Gel Extraction

```
       ;User-defined program (UDP) template for FAMOS autosampler
       ;******   PLEASE DO NOT EDIT  ******
0.000  InjectMode =    UserProg
       Draw            From=SampleVial, Volume=5.0
       Dispense        To=PrepVial, Volume=5.0
       Draw            From=ReagentAVial, Volume=5.0
       Dispense        To=SampleVial, Volume=5.0
       MixWait         Duration=10

       Draw            From=SampleVial, Volume=5.0
       Dispense        To=PrepVial, Volume=5.0
       Draw            From=ReagentBVial, Volume=5.0
       Dispense        To=PrepVial, Volume=5.0
       MixWait         Duration=10

       Draw            From=SampleVial, Volume=5.0
       Dispense        To=PrepVial, Volume=5.0
       MixWait         Duration=5
       InjectValve     Position=Inject
       SyringeValve    Position=Needle
       Draw            From=SampleVial, Volume=1.0
       MixWait         Duration=5
       InjectValve     Position=Load
       Draw            From=PrepVial, Volume=5.0
       MixWait         Duration=5
       InjectValve     Position=Inject
       InjectMarker
       ; generates an inject response during the program run
       MixWait         Duration=5

       Draw            From=SampleVial, Volume=0.0
       SyringeValve    Position=Waste
       MoveSyringeHome
       SyringeValve    Position=Needle
       MixNeedleWash   Volume=50

       Inject
       ; starts the above program and waits for the inject response

   Acquisition On
       ...
4.000 Acquisition Off

   End
```

🖝 *Tips:*

*If the previous action was Draw or Dispense, the needle slowly returns to its start position while the **MixWait** command is executed. For the needle to remain at its current position, enter another Draw or Dispense command with "Volume=0.0" as shown in the above program examples.*

🛈 *Notes:*

Use the PGM Wizard to create a user-defined program. You can either create a new user-defined program or use an existing program as a template for the new one.

*Each user-defined program must include an **InjectMarker** command. If this command is not included, a message appears in the PGM Wizard.*

For an overview of the individual commands that are available for the FAMOS autosampler, refer to **Commands for Controlling Dionex Devices** 🔳 **Dionex/LC Packings FAMOS Autosampler**.

For information about the sample preparation commands supported by the FAMOS autosampler, refer to **Commands for Controlling Dionex Devices** 🔳 **Dionex/LC Packings FAMOS Autosampler: Sample Preparation**.

🔳 Injecting a Sample

There are two methods for injecting a sample using a Chromeleon controlled ➤*Autosampler*:

- Enter the ⇒*Inject* command from the **Control** menu or control panel

- Enter the **Inject** command in a ➤*Program*

In either case, the autosampler performs the injection and then reports to Chromeleon when injection is complete. When Chromeleon receives this message, the retention time clock is started.

Controlling Injection from the Control Menu or Control Panel

- Select the **Inject** command on the **Control** menu. The **Inject** dialog box opens. Enter the ⇒*Volume* and the ➤*Autosampler* position for the injection and click **OK**.

- Alternatively, if the control panel provides the corresponding controls, enter the **Volume** and **Position** parameters directly in the corresponding edit boxes and then click the **Inject** button.

The time required by the autosampler to inject the sample can be visually indicated on the control panel. Link a color area or a lamp with the **Inject Wait** property. For more information, refer to **How to ...: Actions on the Control Panel** **Linking a Control to a Device**.

Controlling Injection in a Program

- Enter the **Inject** command at the time t = 0.000 in the program. The **Volume** and **Position** parameters are entered in the ➢*Sequence*.

 Tip:

*With the AS and AS50 autosamplers, sample loading and injection are two distinct events. Include a **Load** command in the program at t = 0.000 and then the **Inject** command. See* *Autosampler Commands (AS/AS50) for details.*

Injecting Sample with an Uncontrolled Autosampler or a Syringe

You can also use an autosampler that is not controlled by Chromeleon for sample injection, or use a syringe to manually inject sample. When injection is via an uncontrolled autosampler or a syringe, Chromeleon also waits for a signal before it starts the retention time clock. For example, with a hand-operated valve, the signal is returned after the valve has switched from **Load** to **Inject**. Thus, program execution is delayed until the injection is actually performed. This type of automatic injection requires connection of a ➢*Remote Input* or remote start device via TTL or relay inputs and configuration of a ➢*Remote Inject* device in the timebase. See **How to ...: Device Control** **Setting Up Remote Injection**.

▣ Setting Up Remote Injection

If Chromeleon does not directly control the injection valve, a ➢*Remote Input* (remote start) device can be set up that communicates to Chromeleon when an injection was performed. The setup procedure includes the following main steps:

- Connect the remote input device via TTL or relay

- Install and configure a remote inject device in the ➢*Timebase*

- Add an ⇒*Inject* command to the ➢*Program*.

👉 *Tip:*

*For the UI20, remote injection can be triggered by the remote input or by pressing the **Run** button on the UI20 front panel. For the DX-120, remote injection can only be triggered by the remote input. The **Load/Inject** button on the DX-120 front panel is disabled during remote control.*

Connect the Remote Input Device (TTL or Relay Connections)

The remote input of the remote input (remote start) device must be connected via TTL or a relay to the injection valve or to another device, e.g., an ➢*Autosampler*. When injection is performed, the injection valve or autosampler sends a signal to the remote input device.The remote input device then communicates this information to Chromeleon.

Example Connections: AS40 and DX-120

👉 *Tip:*

For detailed TTL and relay connection instructions, refer to the operator's manual for each device.

The following connections allow completely automated control of the AS40 and DX-120.

1. Connect the **Relay Control Ready Out** pin on the AS40 rear panel to the **Inject TTL Input** pin on the DX-120 rear panel.

2. Connect the **Ready Out Ground** pin on the AS40 to the **TTL Inputs Ground** pin on the DX-120.

3. Connect the **Load** pin on the AS40 to the **TTL 1 Out** pin on the DX-120.

4. Connect the **Load Ground** pin on the AS40 to the **TTL Outputs Ground** pin on the DX-120.
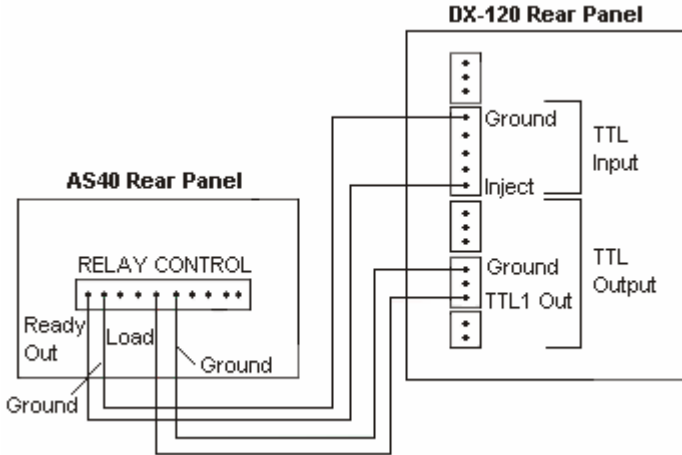
Configure a Remote Inject Device

1. Open the Server Configuration.

2. Select the timebase in which the remote input device is configured.

3. Select **Add Device** on the **Edit** or context menu. The **Add device to timebase** dialog box appears.

4. Select **General** from the **Manufacturers** list box, and then select ➢*Remote Inject* from the **Devices** list. Click **OK**.

   The Properties dialog box for the Remote Inject device appears.

5. The default device name is InjectValve. You can accept the default name or enter a different one.

6. From the **Inject Port** drop-down list, select the remote input device that was connected through TTL or relay. Click **OK**.

Add an Inject Command to the Program

Add the following command to the PGM File. (If you use the Program Wizard (see **Control** 🔳 **The Program Wizard**), the command is added automatically).

```
0.000    Inject
```

When Chromeleon executes the program, it runs the commands that occur before the Inject command and then waits for the signal from the remote input device. A message in the Audit Trail is displayed: "Wait for inject response on remote start." When the inject signal occurs, program execution resumes.

Example PGM File: AS40 and DX-120

```
        Data_Collection_Rate  5.00
        Pump =                On
        SRS =                 On
        EluentPressure =      On
        Column =              A
        ECD_TTL_1.State =     5v          Note 1
        Wait                  RinseComplete  Note 2
-0.100  ECD_TTL_1.State =     0v          Note 3

0.000   ECD.Autozero                      Note 4
        Inject                            Note 5
        ECD_1.AcqOn

10.000  ECD_1.AcqOff

        End
```

Notes:

1. TTL Out 1 turned off.

2. The TTL_1 5v and TTL_1 0 v commands must be separated by one or more commands or they must occur at different times in the program.

3. TTL Out 1 turned on. AS40 Load Cycle starts.

4. Program execution waits until inject signal is received.

5. AS40 Ready Out signals the DX-120 Inject TTL Input. Injection occurs. Program execution resumes.

◾ Priming the Syringe (ASI-100 Series)

The **PrimeSyringe** command allows removing air from the syringe without dismantling the syringe from the instrument. The steps of the PrimeSyringe procedure (see below) are automatically performed 5 times with the wash liquid. Then, repeat the procedure with the eluent.

Before performing the **PrimeSyringe** command:

- Make sure that **the pump flow is off**. Otherwise, the pump will deliver eluent to the wash vial when the injection valve switches to the Inject position (see step 3).

- Set the **WashSpeed** and the **DispSpeed** to values that correspond to the installed syringe. (Note: When a wash vial is used, the **WashSpeed** command is required instead of the **DrawSpeed** command.)

The recommended settings and the required time are listed in the table below:

Syringe	100 µL	250 µL	1000 µL*	2500 µL*
WashSpeed [µL/s]	5	10	25	25
DispSpeed [µL/s]	5	10	50	50
SyringeDelay [s]	5	5	5	5
Duration of the prime syringe cycle [min]	Approx. 5	Approx. 6	Approx. 7 (5 x 1000µl)	Approx. 9 (5 x 2500µl)

* A PrimeSyringe command is not usually required to remove gas bubbles from syringes with a volume of 1000 or 2500 µL. It is usually sufficient to inject the entire syringe volume of eluent.

- Fill the wash vial with the wash liquid. Enter the vial position in Chromeleon (**WashVial**).

Dionex recommends using isopropanol (2-propanol) for the PrimeSyringe cycle and repeating the cycle with the starting eluent of the next sample.

The ASI-100 automatically performs the following steps:

1. The injection valve is switched to the Load position. The eluent flow is from the pump through the valve directly to the column; the eluent does not come into contact with the needle or the syringe. In addition, the needle descends into the wash vial:
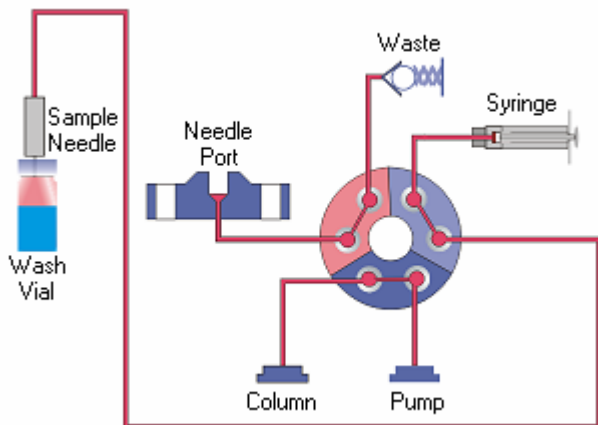
2. The syringe is completely filled:
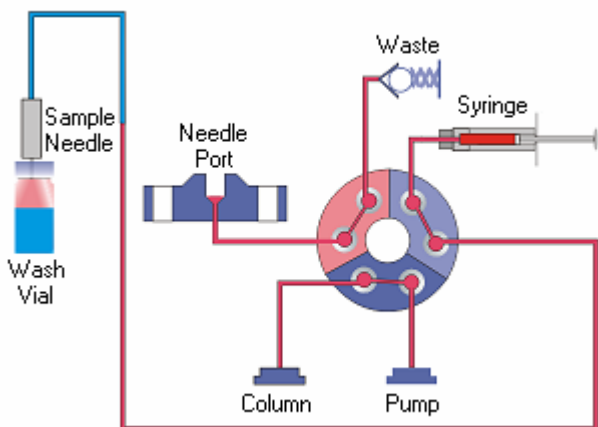
3. The injection valve is switched to the Inject position; the needle remains in the wash vial and the contents of the syringe are directed to waste:
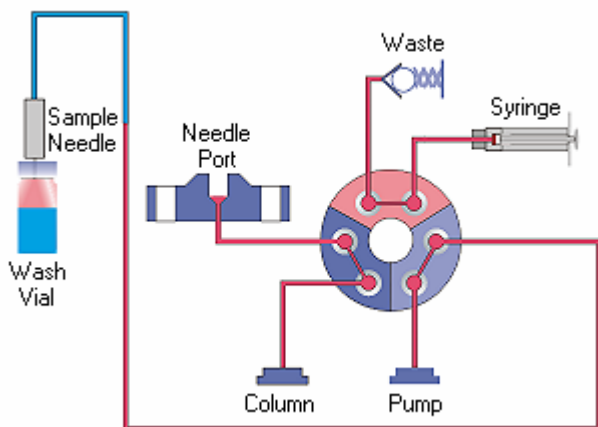
4. The injection valve is switched to the Load position

The above steps are performed five times. The wash liquid reaches the syringe, removes existing gas bubbles, and exchanges the syringe content:
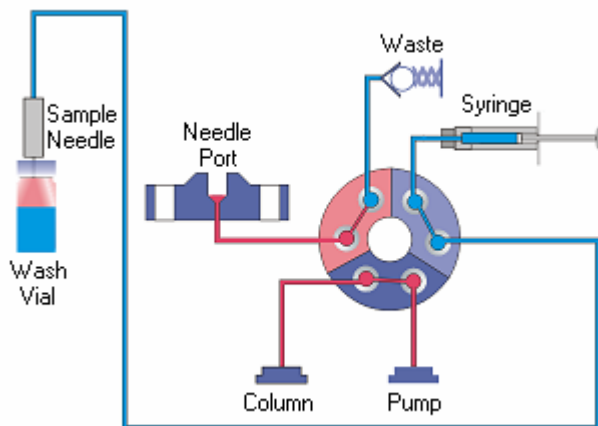
☞ *Tip:*

The PrimeSyringe command exchanges the liquid in the autosampler completely. Therefore, you may also use the PrimeSyringe procedure when switching between incompatible applications, such as between reversed-phase and normal-phase applications.

Afterward, the needle returns to the needle port and the injection valve is switched into the Inject position. Although most of the wash liquid is exchanged with the current eluent and the needle and the needle port are filled again with eluent, the segment between the syringe and the waste remains filled with the wash liquid:
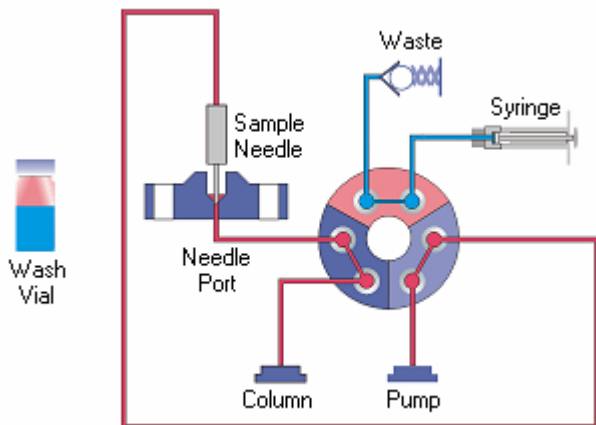
Therefore, repeat the procedure using the eluent to precondition the valve and syringe with the eluent, too. (Note: To avoid deposits, use the starting eluent without buffer additives.)
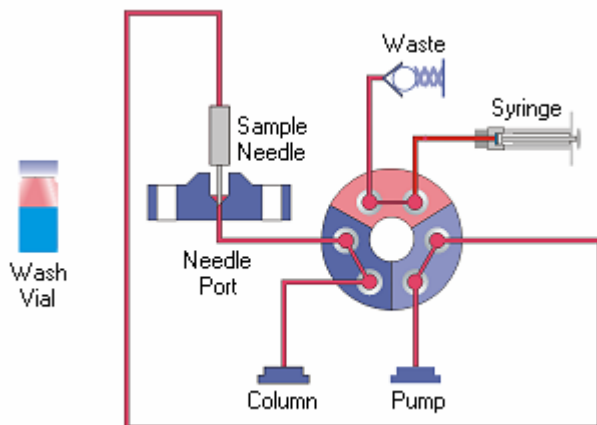
▣ Defining Sample Preparation Steps (AS/AS50)

Sample preparation steps for the AS and AS50 ➤*Autosamplers* are part of a ➤*PGM File* and can be defined using the Program Wizard (see **Control ▣ The Program Wizard**).

Perform the following functions from the **Sampler Options** dialog box in the Program Wizard.

Defining a New Sample Preparation Step

1. Select a **Function** from the list and then select the parameter values for the function (see the table below).

2. Click **Insert** to add the step to the list.

   The new step is added above the currently selected step (if any).

Changing an Existing Step

1. Select the step in the list.

2. Make the required changes, for example, select a new function and enter the function's parameters, or change the parameters for the current function.

3. Click **Enter**.

▣☞ *Tip:*

*To delete a step, select it, and click **Delete**.*

Sample Prep Functions	Description	Parameters
Pipet	Move sample between vials	**Source:** the vial from which to pick up the sample volume
		Volume: the amount (µl) of sample to be pipetted **Destination:** the vial in which to add the sample volume
Mix	Mix the contents of a vial by repeatedly drawing and expelling a volume of the vial contents	**Vial:** the vial to be mixed
		Volume: the amount (µl) of the vial contents to draw and expel
		Cycles: the number of times to draw and expel the specified volume
Flush	Flush inject port during sample preparation	**Volume:** the amount (µl) to flush through the inject port
Delay	Pause sample preparation	**Delay Time:** the number of minutes to pause

Sample Prep Functions	Description	Parameters
Needle	Position needle above vial bottom	**Height:** the distance (mm) from the vial bottom
Dilute	(Available only with the AS or AS50 sample preparation option) Dilute sample with reagent	**Concentrate Source:** the vial that contains the concentrated sample
		Concentrate Volume: the amount of concentrated sample to be diluted
		Diluent Source: the reservoir that contains the diluent
		Diluent Volume: the amount of diluent to be used
		Destination: the vial into which the concentrated sample and diluent are added
Dispense	(Available only with the AS or AS50 sample preparation option) Dispense reagent to a vial	**Source:** the reservoir from which to dispense reagent
		Volume: the amount of reagent to dispense
		Destination: the vial into which reagent is dispensed
Concentrate	(Available only when the AS or AS50 is in Concentrate mode) Load and inject sample onto the concentrator column	**Valve Position:** the position of the injection valve
ReagentPrime	(Available only when the AS or AS50 is in Concentrate mode) Prime the injection valve lines with reagent	**Source:** the reservoir from which to pick up reagent
		Volume: the amount of reagent used for priming
		Valve Position: the position of the injection valve
ReagentFlush	(Available only when the AS or AS50 is in Concentrate mode) Flush reagent onto the concentrator column	**Source:** the reservoir from which to pick up reagent
		Volume: the amount of reagent to flush through the concentrator
		Valve Position: the position of the injection valve

📒 *Note:*

*The **Concentrate**, **ReagentPrime**, and **ReagentFlush** commands require AS or AS50 (USB) Moduleware version 2.0.0 (or later). These commands are not available for the DX-LAN model of the AS50, regardless of which Moduleware version is installed in the autosampler.*

Also, refer to 🔲 **Entering Sample Preparation Vial Positions** and 🔲 **Overlapping Samples**.

◾ Entering Sample Preparation Vial Positions

In sample preparation operations for the AS and AS50 autosamplers, a vial position can be specified either as an absolute position or as a relative position.

To specify an absolute position, enter the number of the vial's position in the tray. Valid numbers depend on the tray type in use: 1 to 100 for the 2 ml tray; 1 to 49 for the 10 ml plastic tray.

To specify a relative position, select **CurrentVial** for the current sample vial, **CurrentVial+1** for one vial past the current vial, **CurrentVial+2** for two vials past the current vial, and so on, up to **CurrentVial+9**.

Some operations let you specify **FlushPort** instead of a vial.

◾ Overlapping Samples

To reduce the time between injections, some devices (for example, the AS50 and the CC80 Component Controller) support overlapping sample preparation. This means that the sample preparation steps are performed for the next sample while the current sample is still being analyzed.

When the device finishes the overlapped functions, the program pauses, if necessary, to finish the currently running sample. Then, the remaining commands in the overlapped sample's program are executed.

The sequence (displayed in the Browser) indicates that sample overlap is being performed. The sample currently being analyzed has a green background and the ⇒*Status* **Running**. The sample being simultaneously prepared has a yellow background and the status **Preparing**. This sample has not yet been injected. Therefore, the ⇒*Inj. Date/Time* column is empty:

No.	Name	Type	Pos	Inj. Vol.	Status	Inj. Date/Time
1	Standard1	Standard	91	5.0	Finished	25.11.2003 11:42:07
2	Sample1	Unknown	1	5.0	Running	25.11.2003 11:45:18
3	Sample2	Unknown	2	5.0	Preparing	
4	Sample3	Unknown	3	5.0	Single	
5	Sample4	Unknown	4	5.0	Single	

In this example, the first unknown sample ('Sample 1') is being analyzed while the next sample ('Sample 2') is being prepared.

 Note:

Sample overlap is disabled if the AS or AS50 is shared by two timebases or if the autosampler is running in Sequential or Sequential Concentrate mode.

Details about how sample overlapping is performed and which commands are required depend on the device. For more information, refer to:

 Autosampler Commands (AS/AS50)

 Defining Sample Preparation Steps (AS/AS50)

 Component Controller Control

Also, refer to **Commands and Tips for Third-Party Devices** **Waters Alliance 2790/2795: Overlapping Sample Preparation**.

Injecting Two Samples Simultaneously

Some autosamplers (for example, an AS or AS50 (USB) in the Simultaneous mode) can deliver sample to two independent ion chromatography (IC) systems. The two IC systems and the autosampler are configured into a single timebase and each system is assigned a unique device name and channel. This lets you monitor and control both systems from one ➤*Control Panel* and run all samples in one sequence.

For plumbing connections, refer to the autosampler operator's manual or the simultaneous injection kit instructions.

To set up simultaneous injections in Chromeleon, follow these basic steps:

1. In the ➤*Server Configuration* program, create a timebase that includes both systems and the AS or AS50 (if used).

2. Assign a unique name to each device in the timebase. For example, if the timebase includes two ICS-2000 systems, name the second system, **Pump_ECD_2**. In addition to renaming the device, also rename any signals and other devices associated with system #2 (for example, the detector signals, TTLs, relays, and eluent generator).

🖐️☞ *Tip:*

Although you can assign any unique device and signal names to system #2, adding _2 to the default names will allow the timebase to be connected to the template control panels (described below) without errors.

3. In the Browser, open a simultaneous injection control panel and then connect the timebase to the panel. For example, to open a simultaneous injection control panel for a timebase with two ICS-2000 systems and an AS50 autosampler, go to the **Dionex Templates\Panels\Dionex_IC\ICS-2000 System Panels** folder and double-click the **ICS-2000_System_Simultaneous_AS50.pan** file.

4. Create sequences, PGM Files, and QNT Files for simultaneous injections. Follow these guidelines:

   • Create one sequence that includes calibration standards for both systems and the unknown samples.

   • Create a PGM File for the unknown samples that includes commands for controlling both systems.

   • Calibrate each system separately by creating separate calibration PGM Files.

   • Create a single QNT File that includes the components from both systems. Use the Duplicate Column command on the Peak Table, Amount Table, and Peak Tracking pages of the method to create two extra Ret. Time columns; associate each column with a different channel.

Also, refer to:

Autosampler Control 🔼 **Autosampler Commands (AS/AS50)**
How To: Integrating Chromatograms and Identifying Peaks
🔼 **Defining the QNT Method for Several Detectors**

◧ Sharing an Autosampler (AS or AS50 (USB))

Two timebases can share a single AS or AS50 (USB) when:

- The Sequential or Sequential Concentrate mode is selected on the autosampler front panel.

- The diverter valve is installed in the autosampler.

- The autosampler is configured as a shared device in the Server Configuration program.

- The program includes commands that specify when each timebase has exclusive access to the autosampler.

🗒️ *Note:*

The DX-LAN model of the AS50 does not support the exclusive access feature. If a program created for this autosampler includes ***AcquireExclusiveAccess*** *or* ***ReleaseExclusiveAccess****, the command is ignored. However, both the AS and AS50 (USB) autosamplers can run any program created for the DX-LAN autosampler that includes these commands.*

To ensure that the autosampler commands for a timebase are exercised before it yields access to the next timebase, insert the **AcquireExclusiveAccess** command immediately before the first autosampler command (**Flush** or **Prime**) and the **ReleaseExclusiveAccess** command immediately after the last autosampler command or property (e.g., TTL or Relay). Thus, a timebase does not have to finish data acquisition before the next timebase can acquire access to the autosampler. If you want one timebase to control the autosampler for an entire sequence, you may omit the **ReleaseExclusiveAccess** command from all but the last program in the sequence.

When the autosampler is in Sequential or Sequential Concentrate mode, a **DiverterValve.Position** command is automatically added immediately after the **AcquireExclusiveAccess** command.

In the following example program, commands required for exclusive access
are in bold.

```
        Pressure.LowerLimit =              0
        Pressure.UpperLimit =              5000
        %A.Equate =                        "%A"
        %B.Equate =                        "%B"
        %C.Equate =                        "%C"
        %D.Equate =                        "%D"
        AcquireExclusiveAccess
        DiverterValve.Position_1
        Flush                              Volume = 100
        Wait                               FlushState
        NeedleHeight =                     2
        CutSegmentVolume =                 0
        SyringeSpeed =                     3
        TrayTemperature =                  10
        CycleTime =                        0
        WaitForTemperature =               False
        Data_Collection_Rate =            5.0
        Temperature_Compensation =        1.7
        DS3_Temperature =                  35
        SRS_Current =                      100
        Wait                               SampleReady
        Flow =                             1.00
        %B =                               0.0
        %C =                               0.0
        %D =                               0.0
        Curve =                            5
0.000   Load
        Wait                               CycleTimeState
        Autozero
        Inject
        Wait                               InjectState
        ECD_1.AcqOn
1.000   Sampler_TTL_1.0v
        ReleaseExclusiveAccess
30.000  ECD_1.AcqOff
        End
```

For an overview of the individual commands for the autosamplers, refer to
🔼 **Dionex AS/AS50 Autosamplers**.

▣ Opening the AS/AS50 Door during Operation

Normally, the ➢*Autosampler* door must remain closed while the AS or AS50 is running a batch. If the door is opened inadvertently, the sampling arm stops immediately and the batch is aborted. There are certain times during operation, however, when the autosampler is not busy, and it is safe to open the door. Chromeleon monitors operation and informs you when you can open the door without aborting the batch.

First, press the **Door** button on the AS or AS50 front panel. If the autosampler is busy, a message in the ➢*Audit Trail* window informs you that it is currently not safe to open the door.

When the autosampler is no longer busy, a message informs you that it is now safe to open the door. A timer then begins counting down the time remaining in which it is safe to have the door open. It continues counting down after the door is opened. The door must be closed again before the timer reaches zero, or the batch will be aborted.

If, after pressing the **Door** button, you decide you do not want to open the door, you can either allow the timer to expire *or* press any button on the autosampler's front panel to reset the timer to zero.

🗒️ *Note:*

*To display the timer on the control panel, place a string display, gauge slider, or gauge indicator ➢Control on the control panel (see ➢Layout Toolbar). Then, link the control to the object property, **DoorSafetyTime**. For more information, refer to **How to: Controlling Devices from the Control Panel** 🔲 Linking a Control to a Device.*

▣ Monitoring the Status of the AS/AS50

The ➢*Autosampler* Status control on a ➢*Control Panel* displays the action currently being performed by the AS or AS50 autosampler: Idle, Preparing to run, Injecting, and so on.

To add the autosampler status to a control panel, place a string display ➢*Control* on the panel (see ➢*Layout Toolbar*). Then, link the control via the **Link** tab page to the Sampler object property, **Status**.

Detector Control

The detector type determines which commands are available. For an overview of the individual commands available for Dionex UV and PDA detectors, refer to **Detector Commands**.

For more information, refer to:

 Starting Data Acquisition

 Defining Signals, Signal Parameters, Axis Decoration, etc.

 Modifying Signal Parameters (Overview)

 Determining Wavelength Switching

 Determining the Optimum Emission Wavelength (RF2000)

 Recording Fluorescence Spectra (RF2000)

 Defining a Waveform (Overview)

 Controlling a Suppressor

 Performing Cyclic Voltammetry

In addition, you can **View or Reset the Lamp Age**.

■ Detector Commands

Modifying Signal Parameters

```
0.000        Channelname.⇒Average        =On / Off
0.000        Channelname.⇒Bandwidth      =Value
0.000        Channelname.⇒Wavelength     =Value
0.000        Channelname.⇒Step           =Value [s] / Auto
```

☞ *Tip:*

Select the Step in such a way that 20 to 50 data points are placed on the narrowest relevant peak in your chromatogram. If you acquire more data points than necessary, this will consume more storage space and slow down data processing and the integration might become incorrect, especially when baseline noise is high. This may also happen when using the Step =Auto option.

📋 *Note:*

To implement ➢Wavelength Switching, define a new wavelength for a channel at the switch time.

Switching Detector ⇒*Lamp*

```
0.000   Devicename.Lamp          =On / Off
```

Switching Detector Lamps On/Off

AD20 Detector

```
0.000   Devicename.UV_Lamp       =Low / High / Off
0.000   Devicename.Visible_Lamp  =Low / High / Off
```

AD25, PDA-100 and PDA-3000 Detector

```
0.000   Devicename.UV_Lamp       =On / Off
0.000   Devicename.Visible_Lamp  =On / Off
```

Selecting the Detection Mode

ED40, ED50, and ED50A, Detectors

```
0.000   Devicename.Mode          =Conductivity / IntAmp / DCAmp
```

ICS-3000 ED Detectors

```
0.000   Devicename.Mode          = IntAmp / DCAmp / Cyclic
```

Turning the Amperometry Cell On/Off

ED40, ED50, ED50A Detector in Amperometry Mode

```
0.000   Devicename.Cell        =On / Off
```

ICS-3000 ED Detector

```
0.000   Devicename.CellControl  =On / Off
```

Selecting the Reference Electrode (ED and ED40/ED50/ED50A Detector in Amperometry Mode)

```
0.000   Devicename.Electrode    =pH / AgCL
```

◾ Starting Data Acquisition

- Click the ⇒*AcqOn/Off* button on the toolbar and determine the channels to record via the automatically displayed dialog box.

- Click the button again to stop data acquisition.

Alternatively, the command can be performed via the **Control** menu or the corresponding button on the Desktop. Each signal (channel) selected during the start of data acquisition is stored in a separate raw data file.

- Open the context menu from the signal plot to influence the representation of the signals (Autoscale, Replot from Beginning, Chromatogram Overlay) or the type of the recorded data (signal parameters) via the context menu.

◾ Defining Signals, Signal Parameters, Axis Decoration, etc.

Right-click a signal plot to open the context menu. Select:

- **Signals** to determine the signals to be displayed.

- **Axis/Decoration** to display axis decoration, units, timebase names, signal parameters, a grid, the signal value, or the retention time.

- **Chrom. Overlay** to display a comparable chromatogram.

Press the F1 key for context-sensitive help.

▪ Modifying Signal Parameters (Overview)

Manually or directly:

- Move the mouse pointer to the signal plot on a control panel.

- Right-click a signal plot and select **Signal Parameters**.

- Select the tab page of the signal or channel for which you want to change the parameters. Signals of different types have different ➢*Signal Parameters*.

- MS parameters are not available in this dialog box. Define them in a separate METH file.

Programmed or indirectly:

In controlled systems, the parameters can be enabled/disabled during chromatogram run time at exact times.

- Select **Command** on the **Control** menu, select the signal to modify, and then define its signal parameters - *or else*

- Enter the corresponding command in a ➢*Program* that is executed in automatic ➢*Batch* processing.

For more information, also refer to:

🔺 **Modifying the Signal Parameters of a UV Channel**

🔺 **Modifying the Signal Parameters of a 3D Field**

🔺 **Displaying the Signal Parameters of a Mass Channel**

For an example for modifying signal parameters, refer to 🔺 **Determining Wavelength Switching**.

◧ Modifying the Signal Parameters of a UV Channel

Modify one or several signal parameters to improve the signals provided by the ➢*UV Detector*:

Signal Parameter	Description	Comment
⇒*Average*	Averaging.	Use this parameter to improve the signal-to-noise ratio.
⇒*Bandwidth*	Changes the bandwidth with which the signal is recorded.	If necessary, increase the bandwidth to improve the ➢*Signal to Noise Ratio*.
Delta	Indicates the difference between the current signal value and the signal value one second before.	The value cannot be changed.
MaxAutoStep	Sets the maximum step width for the **auto** setting.	The highest possible value is the default: 5.1 s.
➢*Polarity*	Changes the polarity of the output signal.	For some detectors, you can use this parameter to change the polarity of the output signal.
Retention	Indicates the current retention time	The value cannot be changed.
Signal	**Value** indicates the current value of a ➢*Signal* Use **UpperLimit** and **LowerLimit** to set the signal acquisition limits.	If signal acquisition limits are not met, the running batch is stopped and the ➢*Emergency Program* is started.
⇒*Step*	Changes the step.	Select the step such that the narrowest peak contains approximately 20 datapoints
⇒*RefBandwidth (Reference Bandwidth)*	Changes the reference bandwidth.	If necessary, increase the bandwidth to improve the signal-to-noise ratio.
⇒*Wavelength*	Changes the recorded wavelength.	Note the possibilities for wavelength switching (see ◧ **Determining Wavelength Switching**).

▣ Modifying the Signal Parameters of a 3D Field

Modify one or several signal parameters of a ➢*Photodiode Array Detector* to improve the size and the quality of a ➢*3D Field*:

Signal Parameter	Description	Comment
⇒*BunchWidth*	Shows the current value of the bunch width.	Increasing the value improves the signal-to-noise ratio. However, the resolution decreases.
MaxWavelength MinWavelength	Determines the upper/lower limit of the 3D field.	Use these parameters to limit the size of the 3D field to the essential section.
⇒*RefWavelength (Reference Wavelength)*	Changes the reference wavelength.	If necessary, increase the bandwidth to improve the ➢*Signal-to-Noise Ratio*.
⇒*RefBandwidth (Reference Bandwidth)*	Changes the reference bandwidth.	If necessary, increase the bandwidth to improve the signal-to-noise ratio.
Retention	Indicates the current retention time.	The value cannot be modified.
⇒*Step*	Changes the step.	Select the step such that the narrowest peak contains approximately 20 datapoints

▣ Determining Wavelength Switching

➢*Wavelength Switching* is realized by time-precise modification of the ⇒*Wavelength* (UV detector) or ➢*Emission*, and ➢*Excitation* (fluorescence detector) signal parameters.

For each wavelength switch, enter the time, the channel, and the new parameter value in the ➢*Program*, for example, for the channel of a fluorescence detector:

```
10.000   UV_VIS1.Wavelength      = 280 nm
10.000   UV_VIS2.Wavelength      = 320 nm
```

or

```
10.000   Emission.ExWavelength   = 240 nm
10.000   Emission.EmWavelength   = 295 nm
```

In addition to changing the wavelength once, you may as well record spectra, for example, using the RF2000 fluorescence detector. For an example PGM File for the scan procedure with the RF2000 detector, refer to ▣ **Determining the Optimum Emission Wavelength (RF2000).**

▪ Determining the Optimum Emission Wavelength (RF2000)

Fluorescence detectors not only allow you to automatically change the wavelength from time to time (see ⬛ **Determining Wavelength Switching**), but also to scan the entire range of the spectrum. Thus, spectra acquisition is possible for determining the optimum emission and excitation wavelengths. For more information, refer to ⬛ **Recording Fluorescence Spectra (RF2000)**.

The PGM File below assists you in finding the optimum values for the emission wavelength (that is, the ➢*Emission* parameter). Start the chromatographic analysis using the same conditions as before. Decrease the pump flow to 0.000 ml/min as soon as you reach the peak for which to optimize the emission wavelength (in the example below: 5.370 min) and then start scanning.

```
-0.1     Pressure.LowerLimit =        0
         Pressure.UpperLimit =        400
         %A.Equate =                  "%A"
         %A.Type =                    Automatic
         %B.Equate =                  "%B"
         %B.Type =                    Automatic
         %C.Equate =                  "H2O/MeOH 80/20"
         %C.Type =                    Automatic
         %D.Equate =                  "%D"
         %D.Type =                    Automatic
         Flow =                       1.000
         %B =                         0.0
         %C =                         100.0
         %D =                         0.0

         Emission.ExWavelength =      275
         Emission.EmWavelength =      350
         Emission.Gain =              1.0
         Emission.Response =          0.5
         Emission.Sensitivity =       Low
         Emission.Step =              Auto
         Emission.Average =           On

 0.000   Emission.Autozero
         Inject
         Emission.AcqOn
```

```
 5.370    ; stop flow
          Flow =                        1.000
          Flow =                        0.000
          ; start scanning
          Emission.ScanEmission
          ; wait for start of scan
          Wait                          Emission.Scanning
          ; wait for end of scan
          Wait                          Not emission.Scanning
          ; continue flow
          Flow =                        0.000
          Flow =                        1.000

10.000    Emission.AcqOff
          End
```

You receive the usual chromatogram until 5.370 min. From 5.370 min on, the emission spectrum of the substance currently being in the flow cell is recorded. The excitation wavelength of 275 nm is kept.

Stop the pump flow at the corresponding retention time to optimize the emission wavelength for all other peaks as well. Determine the optimum ➢*Excitation* wavelength in the same way.

▣ Recording Fluorescence Spectra (RF2000)

It is **not** possible to record spectra simultaneously with the ➢*Emission* signal. Single spectra scans are performed, instead. To guarantee a stable status in the flow cell stop the pump flow while the scans are performed.

The duration of the scans depends on the scan speed and the scan range. A scan from 200 to 900 nm with the scan speed **Fast** takes approximately 85 seconds. The result of a scan is either an emission spectrum or an excitation spectrum. These spectra are automatically saved in a ➢*Spectra Library*. A library called **RF2000.LIB** is created in the timebase directory of the local datasource. If a scan is performed during sample processing, a library with the same name is saved in the associated sequence.

Emission Spectrum

To scan an emission spectrum:

- Stop the pump flow via the **Flow=0** command.

- Execute the **ScanEmission** command. Depending on when the pump flow was stopped and when the scan procedure was started, either a background spectrum or a peak spectrum is saved.

- Perform the analog procedure to determine an optimum ➤*Excitation* value.

Difference Spectrum

To record a difference spectrum:

- Record a background spectrum as described above.

- Select **Save Background Spectrum** to save this spectrum separately in the detector.

- Reset the pump flow to the original value via the **Flow= ...** command. Continue until the maximum of the peak to detect is reached.

- Stop the pump flow and record a new spectrum.

The two spectra now exist in the RF2000 spectra library and as individual spectra in two different storage locations within the detector.

- Execute the **GetSpectraDifference** command to receive a difference spectrum.

The detector behaves as if a **Scan** procedure was started. The result of the difference formation is also saved to the RF2000 spectra library. This spectrum indicates the optimum emission or excitation value.

🖙 *Tip:*

The basic requirement for forming difference spectra is the correspondence between the wavelength range defined by the start wavelength and the end wavelength, as well as the spectra type (Excitation or Emission).

For an example program, refer to 🔝 **Determining the Optimum Emission Wavelength (RF2000)**.

■ Displaying the Signal Parameters of a Mass Channel

Different channel types are available for ➤*Mass Spectrometers*:

TICF channels (TICF 01 to TICF 09 for the MSQ or TICF_1 to TICF_4 for the aQa: data acquisition in ➤*Full-Scan* mode)

➤*SIM* channels (SIM_01 to SIM_32: data acquisition in SIM mode)

➤*TIC* channel (total of all TICF or SIM channels)

MS_01 to MS_32 channels (data acquisition in full-scan mode)

Almost no parameters for mass spectrometer channels can be changed from the ➤*Control Panel*. Select **Axis/Decoration** on the context menu of the signal plot to display the following parameters for the channels of a mass spectrometer:

Parameter	Description	Comment
Filter	Filter of the MS method (METH file).	No input possible, not even in the F8 dialog box.
FilterIndex	Number of the ➤*Filter* of the MS method.	Only for the MS_01 to MS_32 channels (press F8): Select the number of the desired filter (1-9 for the MSQ or 1-4 for the aQa):

	Polarity	AQAmax (V)
1	+ve	100
2	-ve	100
3		0
4		0

Parameter	Description	Comment
Min/MaxMass	Limits of the mass range that is acquired in this channel.	Only for MS channels MS_01 to MS_32 (press the F8 key): Enter the lower and/or upper mass range limit.
Polarity	Ionization polarity of the mass spectrometer.	No input possible, not even in the F8 dialog box.
Source Voltage	Voltage of the ion source of the mass spectrometer.	No input possible, not even in the F8 dialog box.

Press the F8 key or select **Commands** on the **Control** menu to display the following parameters:

Parameter	Description	Comment
Delta	Difference between the current signal value and the signal value one second before.	The value cannot be changed.
Retention	Indicates the current retention time.	The value cannot be changed.
Signal	**Value** indicates the current value of a ➢*Signal*.	If signal acquisition limits are not met, the running batch is stopped and the emergency program is started.
	UpperLimit and **LowerLimit** define the signal acquisition limits.	

▣ Defining a Waveform (Overview)

A ➢*Waveform* must be defined when a Dionex electrochemical detector is used in ➢*Integrated Amperometry Mode*. Waveform definitions are part of a ➢*Program* and can be defined using the Program Wizard. Waveforms can also be entered manually into the program. The steps for defining a waveform using the Program Wizard vary, depending on the model of electrochemical detector being used.

For details, see:

🔼 **Defining a Waveform for an ED40, ED50, or ED50A Electrochemical Detector**

🔼 **Defining a Waveform for an ICS-3000 Electrochemical Detector (ED)**

Also, refer to:

The Control Program 🔼 **The Program Wizard**

How to: Creating and Modifying Programs 🔼 **Manually Editing a PGM File in the Commands View**

▣ Defining a Waveform for an ED40, ED50, or ED50A Electrochemical Detector

You can select a preprogrammed ➢*Waveform* from the Program Wizard, or define a custom waveform.

1   In the **Select Waveform Options** dialog box of the Program Wizard, select **Custom** to create a new waveform, or select a preprogrammed waveform from the list.

🗒 *Note:*

The selected waveform must match the electrode. For guidelines, see 🔼 *Selecting a Default Waveform for Integrated Amperometry.*

2.   Click **Next>**.

The **Waveform Options** dialog box appears. If you selected a preprogrammed waveform, the selected waveform's time, potential, and integration settings are displayed in the waveform table. If necessary, the settings can be modified now (see below). The waveform can also be modified in the resulting program after completing the Program Wizard.

3.   For each step in the waveform, enter or edit the parameters listed below, and then click **Enter**.

Time (Sec)	The time at which to apply the selected potential.

👉 *Tip:*

The maximum waveform period (the length of time from the first step in the waveform to the last) is 2 seconds. However, because only 1 data point can be collected per waveform period, the actual maximum length of a waveform period depends on the ➢Data Collection Rate. The relationship is as follows:

data collection rate x waveform period ≤ 1

For example, if the data collection rate is 5 Hz (5 points per second), the longest waveform period allowed is 0.2 seconds. To create a longer waveform, reduce the data collection rate.

Potential (V)	The potential to apply between the reference and working electrode.
Integration	The ➢*Integration Interval* option:
	No-Change keeps the setting selected in the previous step
	Begin starts integration at this point
	End stops integration

👉 *Tip:*

Each waveform must have one integration interval. Integration cannot begin at the first step or end at the last step in the waveform.

4. Select the **Reference Electrode Mode**.

5. Enter the **pH Lower Limit** and **pH Upper Limit.**

    The pH is read at the start of each injection.

🗒️ *Note:*

For guidelines when setting the reference electrode mode and pH limits, see 🔲 *Selecting the Reference Electrode Mode with Alkaline Eluents.*

👉 *Tips:*

*To delete a step, select it in the waveform table and click **Delete**.*

To edit an existing step, select it in the waveform table; change the time, potential, and/or integration settings; and press <Enter>.

Also, refer to:

The Control Program 🔲 **The Program Wizard**

How to: Creating and Modifying Programs 🔲 **Manually Editing a PGM File in the Commands View**

🔳 Defining a Waveform for an ICS-3000 Electrochemical Detector (ED)

You can select a preprogrammed ➤*Waveform* from the Program Wizard or PGM Editor, or define a custom waveform.

1. Go to the **ED Options** dialog box of the Program Wizard or PGM Editor.

2. In the **Waveform Selector** table, select the waveform to be edited or select **<New Waveform>**.

👉 *Tip:*

In the PGM Editor, an asterisk (*) before a waveform name indicates that Chromeleon is reading the waveform definition directly from the program (i.e., there is no associated waveform file for this waveform).

3. Click **Edit**.

   The **Waveform Editor** appears. If you selected an existing waveform, a plot of the waveform is displayed, with the waveform's times, potentials, and other settings displayed in the table below. If you did not select a waveform, you can select one now from the Waveform list or you can begin creating a new waveform.

4. Follow the guidelines below to edit an existing waveform or create a new one:

   - To add a new line to the end of the list, click **Append**.

   - To add a new line above an existing line, select the existing line and click **Insert**.

   - To delete a line, select the line and click **Delete**.

   - To edit parameters for each line, select the parameter and type or select the new value. Then, press <Enter> or <Tab>.

   - To update the plot with the new value(s), click **Apply**.

5. For each step in the waveform, enter the following parameters:

   Time (ms)   The time at which to apply the selected voltage.

   👉 *Tip:*

   The absolute maximum waveform period is 2 sec. However, for 2D data, because only 1 data point is generated per waveform period, the effective maximum length of a waveform period depends on the ➢Data Collection Rate. The relationship is as follows:

   data collection rate x waveform period ≤ 1

   For example, if the data collection rate is 5 Hz (5 points per second), the longest waveform period allowed is 0.2 s (200 ms). To create a longer waveform, reduce the data collection rate.

   Voltage (mV)   The voltage to apply between the reference and working electrode.

   Gain Region   Select whether to turn the ➢Gain Region on or off.

   Ramp   Select either a **Ramp** or a **Step** change:

   Ramp is a gradual change in potential from this step in the waveform to the next step.

   Step is an immediate change in potential at this step.
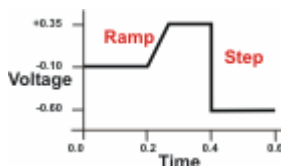
For example, enter the following information to define the waveform steps shown in the above example:

Time	Voltage	Ramp/Step
0.00	-0.10	Step
0.20	-0.10	Ramp
0.25	0.35	Step
0.40	0.60	Step

Integration   The ➢*Integration Interval* option:

On starts or continues integration

Off stops integration or keeps integration off (if it was turned off in a previous step)

🖝☞ *Tip:*

Each waveform must have one integration interval. Integration cannot begin at the first step or end at the last step in the waveform.

6. After all of the steps have been entered, save the waveform. If you are creating a new waveform or editing one of the default waveforms, enter a new name before saving. The default waveforms provided with Chromeleon are read-only.

Also, refer to:

The Control Program 🔼 **The Program Wizard**

Control 🔼 **The PGM Editor**

How to: Creating and Modifying Programs 🔼 **Manually Editing a PGM File in the Commands View**

🔼 **Selecting a Default Waveform for Integrated Amperometry**

◼ Using the Reference Electrode with Alkaline Eluents

The reference electrode inside the ED and ED40/ED50/ED50A cell is a combination pH/Ag/AgCl electrode. Selection of the **Reference Electrode Mode** is one of the steps in 🔳 **Defining a Waveform**. If you select the Ag mode, only the Ag/AgCl part of the electrode is used. If you select the pH mode, the entire pH/Ag/AgCl electrode is used.

There are several important considerations:

- The same pH/Ag/AgCl combination electrode is used in both reference modes (Ag and pH).

- The Ag/AgCl half-cell is more commonly used as the cell reference electrode. However, if the pH changes during a separation, then use the pH reference mode instead of the Ag mode.

- When selecting the pH mode, always correct the waveform for pH dependence (0.059 V/pH unit). See 🔳 **Correcting a Waveform for pH Dependence** for details.

- The pH readout matters, even if the reference mode is Ag.

When used with alkaline eluents, the reference potential undergoes a gradual change. As the silver chloride layer on the silver wire is converted to a mixture of silver oxide and hydroxide, the value of the reference potential gradually increases. This gradual change is indicated by the changes in pH readout when pumping a known composition of mobile phase.

For example, the pH readout for 60 mM sodium hydroxide should be approximately 12.8. It thus follows, from the Nernst equation, that a change in pH from 12.8 to 13.3 corresponds to approximately 30 mV of change in the reference potential. By calibrating the pH reference electrode only once, during initial installation, and by monitoring the pH readout, you can determine the status of the reference electrode. On the other hand, repeating the calibration will cause an electronic adjustment of the constant and slope of the Nernst equation in the detector electronics. Hence, the pH readout will be corrected with each recalibration, but the reference potential drift will not be apparent.

Also refer to **How to: Performing Validation and Qualification** 🔳 **Calibrating the pH Reference Electrode**.

🔖 *Note:*

The reference electrode is a consumable item and should be replaced whenever the potential has changed by more than 30 mV (typically, after 3 months of use). Continued use of a defective reference electrode will cause a passivation (loss of detection response) of the working electrode, and may result in delays and unnecessary service calls.

Chromeleon compares the pH value at the beginning of each chromatographic run with the upper and lower pH limits you selected when defining the waveform. If the pH reading falls outside this range, a warning message appears in the ➢*Audit Trail*. You may want to set the pH limits to define a narrow range and rely on the "out of range" warning to detect any unacceptable shift in the reference potential. To avoid receiving this warning, set the pH limits to a very broad range; for example, 7 to 14.

Recommendations for Specific Operating Conditions

Operating Conditions	Comments
The mobile phase composition is identical at the beginning of each run and pH <12.8	Calibrate the reference electrode only after installation of a new electrode.
	Set the pH upper and lower limits to values 0.5 higher and lower, respectively, than the known and constant initial pH. Replace the reference electrode when the "out of range" warning appears in the Audit Trail.
	This mode of operation can be used for all CarboPac PA10 and AminoPac PA10 applications; it can also be used with the CarboPac PA1 when running isocratic monosaccharide separations.
The initial mobile phase composition changes from run to run and/or the initial pH >12.8	Calibrate the reference electrode only after installation of a new electrode.
	Test the reference electrode at least twice a week, as follows:
	1. Replace the column with a length of narrow diameter PEEK tubing. The tubing must generate at least 500 psi at the flow rate for your application.
	🔖 *Note:*
	Testing the pH with PEEK tubing (instead of a column) eliminates re-equilibration periods between the high pH of actual eluents and lower pH during the reference electrode testing.
	2. Set the pH limits to 11.5 and 12.5.

3. Pump an eluent composition having pH 12.0 (for example, 90% water and 10% of 100 mM NaOH).

4. When testing is completed, do the following:

- Reset the eluent composition.

- Specify a pH range that is wide enough, for example, 7 to 14, to avoid receiving warning messages.

- Reconnect the column.

This electrode test with PEEK tubing can be used for all CarboPac MA1 applications; it can also be used for some separations, such as, sialic acids, with the CarboPac PA1.

Also, refer to 🔲 **Selecting a Default Waveform for Integrated Amperometry**

🔲 Selecting a Default Waveform for Integrated Amperometry

The following waveforms are provided with Chromeleon. You can use these waveforms as is, or modify them for your own applications. If you modify one of the default waveforms, you must save it with a different name. The default waveforms are read-only.

Waveform	Working Electrode*	Reference Mode**	Comments
Carbohydrates (std. quad. potential)	Au disposable (P/N 060139; pkg. of 6) Au (P/N 044112)	Ag/AgCl	Can be used with disposable gold electrodes.
Alcohols, Formic Acid, Glycols	Pt (P/N 044113)	Ag/AgCl	Use with platinum electrodes only.
Amino Acids (pH reference)	Au disposable (P/N 060082; pkg. of 6)	pH/Ag/AgCl	For research tasks (e.g., waveform development, separation optimization), use non-disposable gold electrode Au (P/N 055832)
Amino Acids (Ag reference)	Au disposable (P/N 060082; package of 6)	Ag/AgCl	For research tasks (e.g., waveform development, separation optimization), use non-disposable gold electrode Au (P/N 055832).

Waveform	Working Electrode*	Reference Mode**	Comments
Carbohydrates (triple potential)	Au (P/N 044112)	Ag/AgCl	Discontinued waveform. **DO NOT** use with disposable gold electrodes; dissolves gold and can cause reproducibility problems.

* Working electrode only (does not include complete electrode cell).

** The same combination electrode (P/N 044198) is used for the pH/Ag/AgCl and Ag/AgCl reference modes.

Correcting a Waveform for pH Dependence

At a mobile phase pH of 7, the reference potential of the entire electrode is the same as that of the Ag/AgCl half-cell. As the mobile phase pH is increased, the pH half-cell potential decreases approximately 0.059 V per pH unit. For example, at a mobile phase pH of 12, the reference potential of the pH half-cell would be -0.295 V relative to the Ag/AgCl half-cell. Therefore, at pH 12, the potentials applied to the working electrode must be raised approximately 0.3 V when switching from the "Ag" reference to the "pH" reference.

For example, two default waveforms for amino acids are provided with Chromeleon: one for use with the Ag reference electrode mode and one for the pH mode. The waveforms are identical except that in the pH mode waveform, the potentials are raised by 0.33 V, to compensate for an eluent with a pH of 12.7.
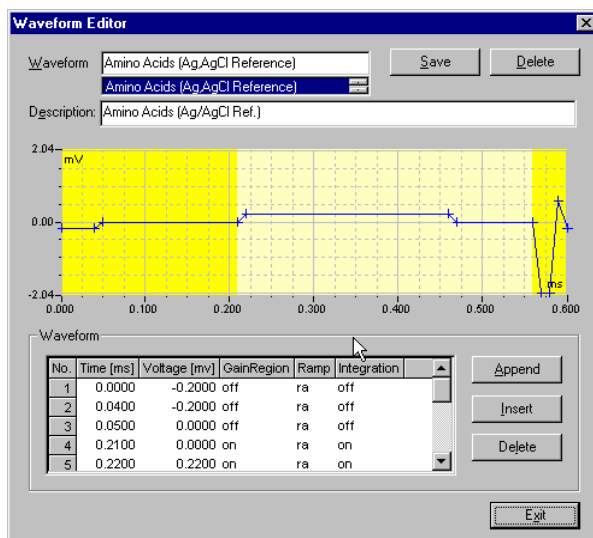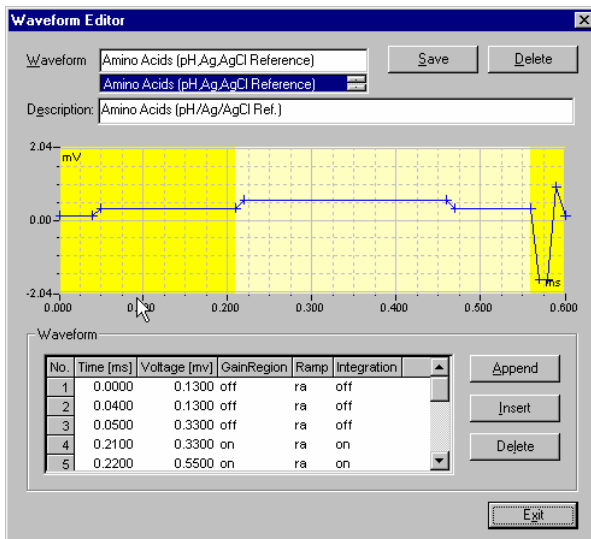
Also, refer to:

🔼 **Defining a Waveform**

🔼 **Selecting a Default Waveform for Integrated Amperometry**

▪ **Performing Cyclic Voltammetry**

The ICS-3000 ED can be used for ➤*Cyclic Voltammetry* (CV) detection. This mode is available only by issuing commands from a ➤*Control Panel*. Cyclic voltammetry commands cannot be programmed into a ➤*PGM File*.

📖 *Note:*

A 3D data license is required to acquire and process cyclic voltammetry data.

Before you begin:

Data from a CV run is stored in the **manual** sequence in the timebase. In order to ensure all data is saved and data from one cyclic voltammetry run does not overwrite the data from a previous run, make sure the datasource does not have multiple sample status enabled.

To do this:

1. On the Browser, right-click the datasource name and select **Properties** from the context menu.

2. On the **General** tab page, clear the **Enable Sample Status Multiple** check box, if it is selected.

3. In the **manual** sequence, select **Single** for the ➤*Sample Status* of the manual sample. Press **Enter**.

4. Select **Save** on the **File** menu to save the sequence change.

Setting up and starting the CV run:

1. On the panel tabset, click the **EC Detector** tab, or on the Browser, open the ED control panel and connect to the ➤*Timebase* in which the ED is configured; see **How to: Controlling Devices from the Control Panel** 🔳 **Opening a Control Panel**.

2. Enter the following CV parameters:

CV Cycle Time	Enter the time in seconds for one CV cycle, which is the time it takes to go from the **CV Low Voltage** to the **CV High Voltage** and then back to the **CV Low Voltage**. This creates a triangle ➤*Waveform*.
CV Low Voltage	Enter the lowest voltage to be applied during the CV cycle. This voltage begins and ends the cycle.
CV High Voltage	Enter the highest voltage to be applied during the CV cycle. This is the peak of the triangle waveform.
CV Cycles	Enter the number of times (2 or more) to repeat the CV cycle
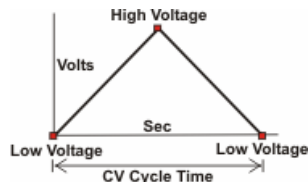
3. To start the CV run, click **Start CV**. The **Mode** automatically switches to CV.

4. The run continues until all of the specified **CV Cycles** are completed. At the end of the run, the mode, waveform, and voltage settings that were in effect before the start of the CV run are restored.

5. After the run is complete, view the data in the CV view window (see 🔳 **Viewing Cyclic Voltammetry Data**).

◼ Defining Step and Average

The time interval between two recorded data points is referred to as ⇒*Step*.

The smaller the selected step, the more data points will be recorded, thus (generally) ensuring a more precise result. As this increases required storage capacity, Dionex recommends a good compromise between the required information and the capacity.

For intelligent minimization of the required storage capacity, the individual instruments, for example, Dionex detectors, support the setting Step = Auto. However, Dionex recommends that you select a fixed step because an automatic step may result in a faulty integration.

In combination with the ⇒*Average* parameter, the step can optimize the signal-to-noise ratio. See the following example:

Example: A detector delivers 100 values per channel (A and B) and per second. For channel A, the setting Step = 0.1; Average = ON is selected, and for channel B Step = Auto; Average = ON.

Channel A:

A step of 0.1 corresponds to a sampling rate of 10 values/s. This means that out of the 100 values that are available, only every tenth value is stored. If the **Average** signal parameter is **ON**, the values recorded per step are averaged. From 10 values, one average value is formed and is stored. For channel A, one signal value per second is stored.

Channel B:

Step = Auto means that the Chromeleon decides depending on events, how many values should be recorded per second. As a different number of values is recorded at different times, a varying number of values is averaged in the final value.

☞ *Tip:*

Use Step = Auto for fast sample chromatograms for which you do not know the peak width to be expected. For a precise and reproducible analysis, a fixed step should be used.

▣ Viewing or Resetting the Lamp Age

The PDA-100 and PDA-3000 detectors allow you to display the number of hours the UV and visible lamps have been on. In addition, the lamp age can be reset to 0, if new lamps are installed.

The lamp age commands are available from the **Command** dialog box. Select **Command** on the **Control** menu. Under **UV,** select **UVLampAge** or **VisibleLampAge**. The total number of hours the lamp has been on is displayed. The number of hours can also be changed from this dialog box.

The UV and Visible lamp ages can also be displayed on the ➢*Control Panel*. To do this, place a string display ➢*Control* on the control panel (see ➢*Layout Toolbar*). Then, on the **Link** tab page, link the control to the UV object property: **UVLampAge** for the UV lamp or **VisibleLampAge** for the visible lamp.

For more information, refer to:

▣ **Modifying a Control Panel**

▣ **Modifying a Control**

▣ **Linking a Control to a Device**

▣ Controlling a Suppressor

The commands used for controlling a ➢*Suppressor* differ, depending on the model of conductivity detector used and the type of suppressor installed:

Detector Model	Supported Suppressor(s)
CD20, CD25, ED40,ED50, IC20, and IC25	SRS
CD25A, ED50A, IC25A, ICS-1000/1500/2000	AES, SRS
ICS-3000 CD	AES, SRS

CD20, CD25, ED40, ED50, IC20, and IC25 Command

```
SRS_Current            =Off, 50, 100, 300, 500 (mA)
```

CD25A, ED50A, IC25A, ICS-1000/1500/2000 Commands

```
Suppressor_Type        =AAES, CAES, ASRS_4mm, ASRS_2mm,
                        ASRS MS, CSRS_4mm, CSRS_2mm, SRS-
                        MPIC
Suppressor_Current     =mA
```

Example

Suppressor_Type	=AAES
Suppressor_Current	=20 [mA]

ICS-3000 CD Commands

SuppressorName.Type	=AAES, CAES, ASRS_4mm, ASRS_2mm, ASRS MS, CSRS_4mm, CSRS_2mm, SRS-MPIC
SuppressorName.CurrentSet	=mA

Notes on Suppressor Names for ICS-3000 CD Detectors

The following names are assigned to the suppressors by default:

Suppressor1: The first or only suppressor in the timebase

Suppressor2: The second suppressor in the timebase

Example

Suppressor1.Type	=AAES
Suppressor1.CurrentSet	=20 [mA]

Suppressor Operating Current Ranges

Suppressor Type	Current Range
AAES	=0.0...150 (mA)
CAES	=0.0...150 (mA)
ASRS_4mm	=0.0...500 (mA)
ASRS_2mm	=0.0...150 (mA)
ASRS MS	=0.0...150 (mA)
CSRS_4mm	=0.0...300 (mA)
CSRS_2mm	=0.0...110 (mA)

📝 *Note:*

The Program Wizard guides you in setting the recommended current for the suppressors. The Wizard supplies a recommended current based on the eluent concentration and flow rate settings. Chromeleon automatically enters the recommended current into the ➢Program and applies that current to the suppressor. To ensure the optimal performance of the suppressor, always accept the recommended current setting.

For more information, refer to:

🔲 **Setting Atlas Suppressor Currents**

🔲 **Setting SRS Suppressor Currents**

🔲 **Setting SRS-MPIC Suppressor Currents**

▣ Setting Atlas Suppressor Currents

For optimal suppressor performance, it is important to use the recommended suppressor currents for the target applications. The Chromeleon Program Wizard (see **The Control Program 🔄 The Program Wizard**) guides you in setting the recommended current for the Atlas Electrolytic Suppressor. The wizard supplies a recommended current based on the eluent concentration and flow rate settings (see the table below). Chromeleon automatically enters the recommended current into the ➢*Program* and applies that current to the Atlas Electrolytic ➢*Suppressor*.

⚠️ *Caution:*

It is possible to override the recommended current settings in the wizard by entering an alternate current value in the program file. However, to ensure the optimal performance of the suppressor, avoid applying excess current to it. Excess operating current leads to excess heat generation, which can reduce the suppressor lifetime and cause higher chromatographic baseline noise.

Atlas Electrolytic Suppressors have a maximum suppression capacity of 25 mN eluent at 1.0 ml/min, which is equivalent to 12.5 mM sodium carbonate for anion separations or 25 mM methanesulfonic acid (MSA) for cation separations at 1.0 ml/min.

👉 *Tip:*

For gradient separations, use the highest eluent concentration in the gradient when you enter the eluent concentration in the wizard.

Examples of Recommended Operating Currents for Atlas Electrolytic Suppressors:

Column	Eluent	Flow Rate (ml/min)	Current (mA)
4-mm AS4A-SC	1.8 mM Na2CO3/1.7 mM NaHCO3	2.00	36
2-mm AS4A-SC	1.8 mM Na2CO3/1.7 mM NaHCO3	0.50	9
4-mm AS9-HC	9.0 mM Na2CO3	1.00	60
2-mm AS9-HC	9.0 mM Na2CO3	0.25	15
4-mm AS14	3.5 mM Na2CO3/1.0 mM NaHCO3	1.20	32
2-mm AS14	3.5 mM Na2CO3/1.0 mM NaHCO3	0.30	8
3-mm AS14A	8.0 mM Na2CO3/1.0 mM NaHCO3	0.5	28
4-mm CS12A	22 mM MSA	1.00	72
3-mm CS12A-5 μm	20 mM MSA	0.50	32
2-mm CS12A	22 mM MSA	0.25	18

For other column and eluent conditions, the recommended current for the Atlas electrolytic suppressors can be calculated using the following equation:

$$Current\,(mA) = 2 \times 1.67\,(mA*\min/mN*ml) \times [eluent\,(mN)] \times flow\,rate\,(ml/\min)$$

For anion separations:

$$[eluent\,(mN)] = 2 \times [CO_3^{2-}\,(mM)] + [HCO_3^-\,(mM)] + [OH^-\,(mM)]$$

For cation separations:

$$[eluent\,(mN)] = 2 \times [H_2SO_4\,(mM)] + [MSA\,(mM)]$$

(Where: [ ] enclosing an ion or eluent indicates concentration.)

The following table lists the **maximum** operating currents for the Atlas Electrolytic Suppressors under various column diameter and flow rate conditions.

Maximum Operating Currents for the Atlas Electrolytic Suppressors:

Separation Column	Flow Rate (ml/min)	Maximum Current (mA)
2-mm column	0.25 to 0.50	30
3-mm column	0.50 to 1.00	50
4-mm column	1.00 to 2.00	100

Once the recommended suppressor current is determined, turn on the pump flow and apply the current to the suppressor.

Setting SRS Suppressor Currents

For optimal suppressor performance, it is important to use the recommended suppressor currents for the target applications. The Chromeleon Program Wizard (see **The Control Program** **The Program Wizard**) guides you in setting the recommended current for the Self-Regenerating ➢*Suppressors* (SRS). The wizard supplies a recommended current based on the eluent concentration and flow rate settings (see the table below). Chromeleon automatically enters the recommended current into the ➢*Program* and applies that current to the SRS.

⚠️ *Caution:*

It is possible to override the recommended current settings in the wizard by entering an alternate current value in the program file. However, to ensure the optimal performance of the suppressor, avoid applying excess current to it. Excess operating current leads to excess heat generation, which can reduce the suppressor lifetime and cause higher chromatographic baseline noise.

The maximum suppression capacity of the Self-Regenerating Suppressors varies, depending on the suppressor type and the flow rate. The table below indicates the absolute maximum suppression capacity for each SRS, regardless of flow rate. The required capacity for an application is calculated as [mN eluent][ml/min flow rate] and cannot exceed the absolute maximum dynamic capacity of the specific suppressor. For example, the 4-mm ASRS cannot suppress 200 mM at 2.0 ml/min.

Suppressor	Absolute Maximum Suppression Capacity
4-mm ASRS	200 μeqv/min at 1.0 ml/min
2-mm ASRS	50 μeqv/min at 0.25 ml/min
4-mm CSRS	110 μeqv/min at 1.0 ml/min
2-mm CSRS	37.5 μeqv/min at 0.25 ml/min

👉 *Tip:*

For gradient applications, select the highest eluent concentration in the gradient when you enter the eluent concentration in the wizard.

Examples of Recommended Operating Currents for Self-Regenerating Suppressors:

Column	Eluent	Flow Rate (ml/min)	Current (mA)
4-mm AS4A-SC	1.8 mM Na2CO3/1.7 mM NaHCO3	2.00	26
2-mm AS4A-SC	1.8 mM Na2CO3/1.7 mM NaHCO3	0.50	7
4-mm AS9-HC	9.0 mM Na2CO3	1.00	44
2-mm AS9-HC	9.0 mM Na2CO3	0.25	11
4-mm AS14	3.5 mM Na2CO3/1.0 mM NaHCO3	1.20	24
2-mm AS14	3.5 mM Na2CO3/1.0 mM NaHCO3	0.30	6
3-mm AS14A	8.0 mM Na2CO3/1.0 mM NaHCO3	0.5	21
4-mm CS12A	22 mM MSA	1.00	65
3-mm CS12A-5 μm	20 mM MSA	0.50	59
2-mm CS12A	22 mM MSA	0.25	16
4-mm AS11	12 mM NaOH or KOH	1.00	30

Column	Eluent	Flow Rate (ml/min)	Current (mA)
2-mm AS11	12 mM NaOH or KOH	0.25	7
4-mm AS11-HC	30 mM NaOH or KOH	1.50	111
2-mm AS11-HC	30 mM NaOH or KOH	0.38	28
4-mm AS15	38 mM NaOH or KOH	1.20	113
3-mm AS15	40 mM NaOH or KOH	0.50	49
2-mm AS15	38 mM NaOH or KOH	0.30	28
4-mm AS16	35 mM NaOH or KOH	1.00	86
2-mm AS16	35 mM NaOH or KOH	0.25	22
4-mm AS17	15 mM NaOH or KOH	1.00	37
2-mm AS17	15 mM NaOH or KOH	0.25	9
4-mm CS14	10 mM MSA	1.00	29
2-mm CS14	10 mM MSA	0.25	7
4-mm CS15	10 mM H2SO4/9% Acetonitrile	1.25	74
2-mm CS15	10 mM H2SO4/9% Acetonitrile	0.30	18
4-mm CS16	30 mM MSA	1.00	88

For other conditions, the recommended current for the Self-Regenerating Suppressors can be calculated using the following equations.

For anion separations:

$$Current\,(mA) = 2.47\,(mA*\min/mN*ml) \times [eluent\,(mN)] \times flow\,rate\,(ml/\min)$$

$$[eluent\,(mN)] = 2 \times [CO_3^{2-}\,(mM)] + [HCO_3^{-}\,(mM)] + [OH^{-}\,(mM)]$$

For cation separations:

$$Current\,(mA) = 2.94\,(mA*\min/mN*ml) \times [eluent\,(mN)] \times flow\,rate\,(ml/\min)$$

$$[eluent\,(mN)] = 2 \times [H_2SO_4\,(mM)] + [MSA\,(mM)]$$

(Where: [ ] enclosing an ion or eluent indicates concentration.)

The following table lists the **maximum** operating currents for the suppressors under various column diameter and flow rate conditions.

Maximum Operating Currents for the Self-Regenerating Suppressors:

Suppressor	Maximum Current (mA)
4-mm ASRS	500
2-mm ASRS	150
4-mm CSRS	300
2-mm CSRS	110

Once the recommended suppressor current is determined, turn on the pump flow and apply the current to the suppressor.

Setting SRS-MPIC Suppressor Currents

The Chromeleon Program Wizard (see **The Control Program** **The Program Wizard**) displays the valid current settings for Self-Regenerating ➢*Suppressors* (SRS) operating in the MPIC (mobile phase ion chromatography) Suppression Mode. For optimal suppressor performance, it is important to use the recommended suppressor currents for the target applications. The sections below indicate the recommended current for anion and cation applications, based on the eluent concentration and flow rate.

⚠ *Caution:*

It is possible to override the recommended current settings in the wizard by entering an alternate current value in the program file. However, to ensure the optimal performance of the suppressor, avoid applying excess current to it. Excess operating current leads to excess heat generation, which can reduce the suppressor lifetime and cause higher chromatographic baseline noise.

ASRS-MPIC Suppressor

The ASRS-ULTRA can be used for suppression of MPIC (ion-pairing) eluents when operating in the MPIC Suppression Mode. The MPIC Suppression Mode is a combination of the AutoSuppression External Water Mode augmented with a chemical regenerant, such as, sulfuric acid.

During operation in the MPIC Suppression Mode, the ASRS-ULTRA uses an applied current and a constant source of dilute sulfuric acid solution from a pressurized bottle delivery system. The table below lists the concentrations and flow rates of standard eluents for anion MPIC separations and the current levels and regenerant flow rates required to suppress them.

4-mm ASRS-ULTRA in the MPIC Suppression Mode:
Matching the Current Setting and Regenerant Flow Rate

Eluent	Eluent Flow Rate (ml/min)	Current (mA)	Regenerant Flow Rate (ml/min)*	Sulfuric Acid Regenerant Concentration (mN)
0.1-2.0 mM Tetrabutylammonium Hydroxide	0.5-2.0	50/100	3-5	5-10
2.0-5.0 mM Tetrabutylammonium Hydroxide	0.5-1.0	100/300	5-8	10
	1.1-1.5	300/500	5-8	10

* The flow rate is measured volumetrically with a graduated cylinder (with the power on).

☞ *Tip:*

For the lower eluent concentration in a given range, choose a correspondingly lower current setting; for a higher eluent concentration, choose a higher current setting. Higher current settings require the application of higher pressure to the pressurized regenerant delivery bottle to maintain adequate regenerant flow.

For complete operating instructions, refer to the manual for the 4-mm ASRS-ULTRA.

CSRS-MPIC Suppressor

The CSRS-ULTRA can be used for suppression of MPIC (ion-pairing) eluents when operating in either the AutoSuppression External Water Mode or the MPIC Suppression Mode; the appropriate mode depends on the specific MPIC application. The MPIC Suppression Mode is a combination of the AutoSuppression External Water Mode augmented with a chemical regenerant, if necessary (for example, boric acid). During operation in the MPIC Suppression Mode, the CSRS-ULTRA uses an applied current and a constant source of regenerant from a pressurized bottle delivery system.

The separation of alkanolamines by ion-pairing using the CSRS-ULTRA requires the addition of 10 mM boric acid to the regenerant. This increases the degree of ionization of the alkanolamines, thereby increasing their conductivity. Do not use boric acid regenerant for the separations of the fully-ionized alkali and alkaline earth metal salts; the borate ion will displace the hydroxide counter ion and lower the conductivity response. The table below lists the concentrations and flow rates of standard eluents for cation MPIC separations and the current levels and regenerant flow rates required to suppress them.

4-mm CSRS-ULTRA in the MPIC Suppression Mode:
Matching the Current Setting and Regenerant Flow Rate

Eluent	Eluent Flow Rate (ml/min)	Current (mA)*	Regenerant** Flow Rate (ml/min)***
0.1-2.0 mM Hexanesulfonic Acid	0.5-1.0	50/100	3-5
2.0-5.0 mM Hexanesulfonic Acid	0.5-1.0	100/300	5-8
1.1-2.0 mM Octanesulfonic Acid	0.5-1.0	50/100	3-5
2.0-5.0 mM Octanesulfonic Acid	0.5-1.0	100/300	3-5
0.1-2.0 mM Nonafluoropentanoic Acid	0.5-1.0	50/100	3-5
2.0-5.0 mM Nonafluoropentanoic Acid	0.5-1.0	100/300	3-5

* For optimal results, choose a current setting of 300 mA or less. Operating the 4-mm CSRS-ULTRA at current settings over 300 mA can shorten the life of the suppressor.

** The regenerant is deionized water or boric acid.

*** The flow rate is measured volumetrically with a graduated cylinder (with the power on).

👉 *Tip:*

For the lower eluent concentration in a given range, choose a correspondingly lower current setting; for a higher eluent concentration, choose a higher current setting. Higher current settings require the application of higher pressure to the pressurized regenerant delivery bottle to maintain adequate regenerant flow.

For complete operating instructions, refer to the manual for the 4-mm CSRS-ULTRA.

▪ Controlling an MMS Suppressor

Refer to the MicroMembrane Suppressor manual for operating conditions for the MMS, including the Displacement Chemical Regeneration mode.

IC Control

For the control commands for the DX-120, ICS-90, or ICS-1000/1500/2000, refer to:

🔼 **Controlling a DX-120 Ion Chromatograph**

🔼 **Controlling an ICS-1000/1500/2000 Ion Chromatography System**

🔼 **Controlling an ICS-90 Ion Chromatography System**

The following pages provide details on:

🔼 **Controlling the Eluent Generator Concentration**

🔼 **Monitoring the Eluent Generator Cartridge Lifetime**

🔼 **Monitoring the DX-120 Operating Status**

▪ Controlling a DX-120 Ion Chromatograph

The following commands are available for controlling DX-120 operating functions:

Controlled AC	Turn the AC power outlet on the DX-120 rear panel on and off. This provides on off control of an external accessory connected to the outlet. See the DX-120 operator's manual for cabling instructions.
Column/Eluent	If the DX-120 is in ➢*Column Mode*, the **Column** command sets the flow path to column A or column B. If the DX-120 is in ➢*Eluent Mode*, the **Eluent** command selects the eluent reservoir (A or B).

📋 *Note:*

These commands are active only if the DX-120 is equipped with the dual-column option.

Data Collection Rate	Sets the ➢*Data Collection Rate* of the detector.
Eluent Pressure	Turns the pressure to the eluent reservoir(s) on and off.
Pressure Unit	Selects the units of pressure to use (psi or MPa).
Pump	Turns the pump on and off.
SRS	Turns the current supplied to the ➢*Self-Regenerating Suppressor (SRS)* on and off.

These commands can be included in a ➢*Program*, executed directly from the **Commands** dialog box (to do so, select **Command…** on the **Control** menu), or linked to a ➢*Control* on the control panel.

To link a command to a control, place an edit box or switch control on the control panel (see ➤*Layout Toolbar*). Then, link the control to an object property. For example, to turn the DX-120 pump on and off from the control panel, link the **Pump** object property to a switch control. For more information, refer to **How to: Controlling Devices from the Control Panel 🔃 Linking a Control to a Device**.

The corresponding commands in the program are as follows:

```
0.000    ControlledAC            =On/Off
0.000    Column                  =A/B
;        Only for a DX-120 equipped with the dual-column option.
0.000    Data_Collection_Rate    =Value [Hz]
0.000    Eluent                  =A/B
;        Only for a DX-120 equipped with the dual-column option.
0.000    EluentPressure          =On/Off
0.000    PressureUnit            =psi/MPa
0.000    Pump                    =On/Off
0.000    SRS                     =On/Off
```

🔲 Controlling an ICS-1000/1500/2000 Ion Chromatography System

Commands for controlling the ICS-1000, ICS-1500, or ICS-2000 can be included in a ➤*Program* (see the examples below), or executed directly from either the ➤*Control Panel* or the **Commands** dialog box (select **Command…** on the **Control** menu). For a list and description of the commands used most often, refer to **Commands for Controlling Dionex Devices 🔃 ICS-1000/1500/2000 Ion Chromatography System**. Also see the **Commands** dialog box for a complete list of commands.

The following programs are created with the Program Wizard (see **The Control Program 🔃 The Program Wizard**).

Program for an ICS-1000:

```
        Pump_ECD.Pressure.LowerLimit =        200
        Pump_ECD.Pressure.UpperLimit =        3000
        Pump_ECD.%A.Equate =                  "%A"
        Pump_InjectValve.LoadPosition
        Pump_ECD.Data_Collection_Rate =       5.0
        Pump_ECD.CellTemperature =            35.0
        Pump_ECD.ColumnTemperature =          30.0
```

```
           Suppressor_Type =                        AAES
           ; Carbonate =                            3.5
           ; Bicarbonate =                          1.0
           ; Hydroxide =                            0.0
           ; Recommended Current =                  33
           Suppressor_Current =                     33
           Pump_ECD.Flow =                          1.20
-2.300     Pump_ECD_Relay_1.Closed                  Duration=138.00
           ; For AS40 injection. Replace this line
           with the following for manual injection
           ;-0.100 Message "Load  the  sample  into
           the   injection   loop,   press   OK   to
           continue"
 0.000     Pump_ECD.Autozero
           ECD_1.AcqOn
           Pump_InjectValve.InjectPosition          Duration=30.00
15.000     ECD_1.AcqOff
           End
```

📘 *Note:*

The program above assumes that the ICS-1000's TTL output is connected to the AS40's Load Relay. Refer to the ICS-1000 and AS40 operator's manuals for connection details.

Program for an ICS-2000:

```
           Pump_ECD.Pressure.LowerLimit =           200
           Pump_ECD.Pressure.UpperLimit =           3000
           Pump_ECD.%A.Equate =                     "%A"
           Pump_InjectValve.LoadPosition
           Pump_ECD.Data_Collection_Rate =          5.0
           Pump_ECD.CellTemperature =               35.0
           Pump_ECD.ColumnTemperature =             30.0
           Suppressor_Type =                        ASRS_4mm
           ; Carbonate =                            0.0
           ; Bicarbonate =                          0.0
           ; Hydroxide =                            12.0
           ; Tetraborate =                          0.0
           ; Other eluent =                         0.0
           ; Recommended Current =                  30
           Suppressor_Current =                     30
           EluentGenerator.Concentration =          12.0
           Pump_ECD.Flow =                          1.00
```

```
-2.300     Pump_ECD_Relay_1.Closed                Duration=138.00
           ; For AS40 injection. Replace this line
           with the following for manual injection
           ;-0.100 Message "Load the sample into
           the injection loop, press OK to
           continue"
0.000      Pump_ECD.Autozero
           ECD_1.AcqOn
           Pump_InjectValve.InjectPosition        Duration=60.00
15.000     ECD_1.AcqOff
           End
```

🖫 *Note:*

The program above assumes that the ICS-2000's TTL output is connected to the AS40's Load Relay. Refer to the ICS-2000 and AS40 operator's manuals for connection details.

🔲 Controlling an ICS-90 Ion Chromatography System

Commands for controlling the ICS-90 can be included in a ➢*Program* (see the examples below), or executed directly from either the ➢*Control Panel* or the Commands dialog box (select **Command…** on the **Control** menu). For a list and description of the commands used most often, refer to **Commands for Controlling Dionex Devices** 🔳 **ICS-90 Chromatography System**. Also see the **Commands** dialog box for a complete list of commands.

The following programs are created with the Program Wizard (see **The Control Program** 🔳 **The Program Wizard**). They are identical, except for the method used to load the sample loop.

Program for an ICS-90 connected to an AS40 autosampler:

```
           Pump =   On
           MeasuredFlowRate     = 1
           ;Record the flow rate set from the hardware
           Pressure.LowerLimit  = 0
           Pressure.UpperLimit  = 3000
-2.000     Pump_ECD_TTL_1. 0v   Duration=
                                120.00
           ;Turn on TTL 1 for 120 seconds,
           ;AS40 load cycle started
```

```
0.000     Pump_ECD.Autozero
          InjectPosition       Duration=
                               60.00
          ;Set the injection valve to the inject position
          ;for 60 seconds
          ECD_1.AcqOn
30.000    ECD_1.AcqOff
          End
```

📒 *Note:*

The program above assumes that the ICS-90's TTL output is connected to the AS40's Load Relay. Refer to the ICS-90 and AS40 operator's manuals for connection details.

Program for manual injections:

```
          Pump =    On
          MeasuredFlowRate     = 1
          Pressure.LowerLimit  = 0
          Pressure.UpperLimit  = 3000
0.000     Pump_ECD.Autozero
          Message  "Manually  inject  the ;Pause  the  program  and
          sample and click OK to continue. display the message
          If you are using an autosampler
          remove  this  command  from  the
          Program file."
          InjectPosition       Duration=
                               60.00
          ECD_1.AcqOn
30.000    ECD_1.AcqOff
          End
```

◾ Controlling the Eluent Generator Concentration

The eluent concentration generated by the ➤*Eluent Generator* can be set manually or controlled via a ➤*Program*. The allowable eluent concentration depends on several factors, including the flow rate, suppressor type, eluent generator cartridge type, and cartridge configuration (in the ICS-2000 and ICS-3000 EG only). See **How to: IC Control** 🔲 **Maximum Concentration Ranges for Eluent Generators** for details.

To determine the eluent concentration required for your application, refer to the column manual. Dionex column manuals are included on the Dionex Reference Library CD-ROM.

Manually Setting the Eluent Generator Concentration

1. Open the control panel for the ➤*Timebase*; see **How to: Controlling Devices from the Control Panel** 🔲 **Opening a Control Panel**.

2. Enter the value into the **Concentration** edit field. **Note:** On some control panels, the field is labeled **Target Concentration**.

3. Alternatively, select **Command** on the **Control** menu to open the **Commands** dialog box. Under **EluentGenerator**, select the **Concentration** command, and enter the value.

The **Applied Concentration** field on the control panel displays the concentration of eluent currently being generated. When the eluent generator is on, the **Applied Concentration** and the **Target Concentration** are typically equal, except in these situations:

- During a gradient run, the **Applied Concentration** indicates the currently generated (instantaneous) concentration, which changes as the ramp progresses. The **Target Concentration** is the concentration to be achieved at the end of the ramp.

- For carbonate/bicarbonate eluent, the **Applied Concentration** for the K_2CO_3 cartridge is equal to the total of the target concentrations from both cartridges.

  For carbonate/bicarbonate eluent:
  Applied Concentration K_2CO_3 = Target Concentration K_2CO_3 + Target Concentration Electrolytic pH Modifier

Programming the Eluent Generator Concentration

Commands for setting the eluent concentration can be added to a program (see **Control** 🔳 **The Control Program**).

You can use the 🔳 **Program Wizard** to enter the concentration command(s). With the wizard, you can enter a single concentration value for the entire analysis or program a ramp or multi-step gradient in which the generated concentration of eluent changes over time. When completing the wizard, click the **Help** button on the **EluentGenerator Options** tab page to display details about the eluent generator and gradient options.

🗒️ *Note:*

The ICS-2000 Gradient Generator license is required in order to create eluent gradients with the ICS-2000.

🔳 Maximum Concentration Ranges for Eluent Generators

Refer to the appropriate section below for the allowable concentration ranges for each eluent generator type and cartridge configuration.

EG40 or EG50 Eluent Generator

The EG40 or EG50 can generate eluent concentrations from 0.1 to 100 mM. The maximum concentration (X) depends on the pump flow rate and is calculated as:

0.1 to 100 mM at 0.1 to 1.0 ml/min
0.1 to X mM at 1.0 to 3.0 ml/min
where X mM = 100/Flow Rate in ml/min

ICS-2000: Single-Cartridge Configuration
ICS-3000 EG: Single-Cartridge Configuration or Independent Dual-Cartridge Configuration

In the single-cartridge configuration, the ICS-2000 or the ICS-3000 EG contains one eluent generator cartridge. In the independent dual-cartridge configuration, the ICS-3000 EG contains two cartridges that are being operated independently on separate systems (each cartridge is linked to a different pump). The following table indicates the allowable concentration range for each cartridge in single-cartridge or independent dual-cartridge configuration.

Cartridge Type	Eluent Concentration Range
K_2CO_3	0.1 to 15 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 2.0 ml/min where X = 15/Flow in ml/min
KOH	0.1 to 100 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 3.0 ml/min where X = 100/Flow in ml/min
LiOH	0.1 to 80 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 3.0 ml/min where X = 80/Flow in ml/min
MSA	0.1 to 100 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 3.0 ml/min where X = 100/Flow in ml/min
NaOH	0.1 to 100 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 3.0 ml/min where X = 100/Flow in ml/min
Electrolytic pH Modifier	Not applicable; the Electrolytic pH Modifier is available for selection only for the linked dual-cartridge configuration.

ICS-2000 or ICS-3000 EG: Linked Dual-Cartridge Configuration

In the linked dual-cartridge configuration, the ICS-2000 or ICS-3000 EG contains two eluent generator cartridges that work together. To set up the ICS-3000 EG for this configuration, link both cartridges to a single pump. The allowable concentration for each linked cartridge is less than when the cartridge is defined as independent. The following table indicates the allowable total concentration for each cartridge in this configuration.

Cartridge Types	Eluent Concentration Range	Comment
K_2CO_3/Electrolytic pH Modifier	0.1 to 15 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 2.0 ml/min where X = 15/flow in ml/min	For a carbonate/bicarbonate mixture, set the K_2CO_3 cartridge to the desired carbonate concentration and set the pH Modifier to the desired bicarbonate concentration. The combined eluent concentrations for both cartridges must not exceed the specified range. In addition, the Electrolytic pH Modifier's eluent concentration must not exceed 10 mM.

Cartridge Types	Eluent Concentration Range	Comment
K_2CO_3/Electrolytic pH Modifier		The K_2CO_3 cartridge generates potassium carbonate eluent equal to the combined concentrations specified for carbonate and bicarbonate. The potassium carbonate eluent is then modified to achieve the desired carbonate/bicarbonate mixture.
KOH/KOH KOH/MSA KOH/NaOH MSA/MSA MSA/NaOH NaOH/NaOH	0.1 to 50 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 3.0 ml/min where X = 50/flow in ml/min	The eluent concentration range for each cartridge is cut by 50%.
LiOH/LiOH	0.1 to 40 mM at 0.1 to 1.0 ml/min 0.1 to X mM at 1.0 to 3.0 ml/min where X = 40/flow in ml/min	The eluent concentration range for each cartridge is cut by 50%.

▪ Monitoring the Eluent Generator Cartridge Lifetime

Chromeleon monitors the lifetime status of the EluGen cartridge currently being used by the eluent generator and displays the information on the ➢*Control Panel* and in the Chromeleon ➢*Server Configuration* program.

The **Remaining Lifetime (Ion Count)** or **Remaining Life (%)** represents the percentage of the original ion capacity remaining in the cartridge. The ion percentage is counted down in 1% increments. At 10%, Chromeleon logs a warning each time the cartridge is used. At 0%, Chromeleon displays a message that the ion count is depleted; the cartridge must then be replaced before operation can be continued.

Note:

*If a pH modifier is installed, the **Remaining Life** field indicates how much the pH modifier has been used. The value starts at 0 and counts up with use. A count of 1 means the usage is approximately equal to the lifetime of one K_2CO_3 cartridge.*

The **Expiration Date** is 2 years from the date of manufacture. One month before expiration, Chromeleon logs a warning each time the cartridge is used. Although you can continue to operate with the cartridge after the expiration date, performance may be impaired until a new cartridge is installed.

To view the cartridge lifetime status information from the Server Configuration program:

1. In the **Server Configuration** program, select the EG40 or EG50 Eluent Generator, the ICS-2000 that contains the eluent generator, or the ICS-3000 EG.

2. Select **Properties** on the **Edit** menu.

3. For an EG40 or EG50, select the **Pump Link & Cartridge** tab.

4. For an ICS-2000, select the **Eluent Generator** tab.

5. For an ICS-3000 EG, select the **Cartridges** tab.

▪ Monitoring the DX-120 Operating Status

You can monitor the status of many DX-120 operating functions on the control panel. For example, the control panel can include ➢*Controls* displaying the current DS4 temperature set point and status, the current flow rate, and the ➢*SRS* status.

To add the corresponding controls to the control panel and link the desired status functions, follow the description in **How to: Controlling Devices from the Control Panel**:

⤴ Modifying a Control Panel

⤴ Modifying a Control

⤴ Linking a Control to a Device

The following DX-120 functions can be monitored on the control panel.

DX-120 Function	Description
Column Configuration	Indicates whether the DX-120 is configured with a single- or dual-column set.
DS4 Status	Indicates whether the current DS4 temperature is under, at (ready), or over the temperature set point.
DS4 Temperature	Displays the current DS4 temperature set point.
Flow Rate	Displays the pump flow rate.
Full Scale	Indicates the microSiemens output (100 or 1000) of a full-scale detector response.
Pressure	Displays the current pump pressure transducer reading.
SRS Status	Indicates the status of the ➢*SRS*, not installed, off, or the selected operating current (if on).
System Mode	Indicates whether the DX-120 is in ➢*Column Mode* or ➢*Eluent Mode*.

�’ *Tips:*

*The Chromeleon reports the status of the above DX-120 functions only. Except for the system mode, values for these functions are selected with DIP-switches on the DX-120. Refer to the DX-120 operator's manual for more information. The system mode is selected in the DX-120 Properties dialog box (Mode tab page) in the **Server Configuration**.*

For functions that can be controlled by Chromeleon, refer to 🔲 ***Controlling a DX-120 Ion Chromatograph***.

🔲 Monitoring the ICS-1000/1500/2000 Operating Status

You can monitor the status of many ICS-1000/1500/2000 operating functions on the control panel. For example, the control panel can include ➤*Controls* displaying the current pump flow rate, DS6 conductivity cell temperature, eluent generator status, and amount of eluent in the reservoir.

To add the corresponding controls to the control panel and link the desired status functions, follow the description in **How to: Controlling Devices from the Control Panel**:

🔲 **Modifying a Control Panel**

🔲 **Modifying a Control**

🔲 **Linking a Control to a Device**

The following ICS-1000/1500/2000 functions can be monitored on the control panel.

Function	Description
%A_Value	Indicates the partial flow of a component, expressed as a percentage of the total flow.
Acquisition_Ready	Indicates whether the ICS-1000/1500/2000 is ready to begin data acquisition.
CellTemperature	Indicates the DS6 conductivity cell temperature.
ColumnTemperature	Indicates the column temperature; available only if the column heater option is installed.
DegasCycleOff, DegasCycleOn	Indicates whether the degas cycle is off or on; available only if the vacuum degas option is installed.
DegasMode	Indicates the degas mode setting (Always Off, Always On, Cycle, or Monitor); available only if the vacuum degas option is installed.

Function	Description
DegasPressure	Indicates the degas pressure (in psi); available only if the vacuum degas option is installed.
EGC1_Connected, EGC2_Connected	Indicates whether the corresponding eluent generator cartridge is connected or disconnected (ICS-2000 only).
EgcCurrent	Indicates the operating current of the eluent generator cartridge (in mA) (ICS-2000 only).
EgcVoltage	Indicates the eluent generator cartridge voltage (ICS-2000 only).
EluentBottleLevel	Indicates the volume of liquid (in liters) in the eluent reservoir.
EluentValve	Indicates whether the eluent valve is open or closed.
Pressure	Indicates the current pump pressure transducer reading.
Rise_Time	Indicates the filter rise time to apply to the recorder output.
SuppressorStatus	Indicates whether the suppressor is on, off, or not installed. If the suppressor is on, the selected operating current is displayed.
SuppressorVoltage	Indicates the suppressor voltage.
TotalConductivity	Indicates the measured conductivity (with no offset applied).
TTL_Input_Function_1, TTL_Input_Function_2, TTL_Input_Function_3, TTL_Input_Function_4	Indicates the function assigned to the corresponding TTL input.
TTL_Input_Mode	Indicates the TTL input mode setting.

Note:

Chromeleon reports the status of the above ICS-1000/1500/2000 functions. For a list of functions that can be controlled by Chromeleon, refer to Controlling an ICS-1000/1500/2000 Ion Chromatography System.

GC and Temperature Control

Flow and pressure control is an important aspect of gas chromatographs. For more information, refer to

🔲 **Flow/Pressure Control for Gas Chromatographs**

🔲 **Determining a Gradient (GC)**.

The Agilent 6890 GC and the 7673 and 7683 GC autosamplers support simultaneous injection of two samples. For more information, refer to:

🔲 **Commands and Properties for Dual Operation (6890)**

🔲 **Injecting Two GC Samples Simultaneously (6890)**

🔲 **Injecting Two GC Samples Simultaneously (7673 and 7683 Autosamplers)**.

Some instruments support temperature control. For general information, refer to:

🔲 **Temperature Control (On/Off)**

🔲 **Controlling the Temperature**

For information about how to control a column thermostat, refer to

🔲 **Controlling the Column Temperature**

🔲 Flow/Pressure Control for Gas Chromatographs

Gas chromatographs can operate at a constant gas flow or at a constant pressure. It is possible to switch from one mode to the other.

Two mutual switches are assigned the property **FlowMode**. Setting the flow and the pressure is in the corresponding mode via a separate control (gauge or edit field).

◾ Determining a Gradient (GC)

For basic information about how to determine a gradient, refer to **◾ Determining a Gradient**.

How to enter basic points for GC:

- Select the initial temperature in the **Init** line.

- In the **Iso Time** column, enter for how long this temperature shall be maintained.

- In the next line, select the ramp (**Rate** in °C/min) to be used to change the temperature. In the **Temperature** column, select the temperature that the GC should reach with this first ramp. In the **ISO Time** column, reenter for how long this temperature shall be maintained.

You can program up to six temperature ramps.

In the same way, you can use the **FrontInlet** and **BackInlet** pages to determine a temperature gradient for an inlet. A temperature gradient can be determined for **PTV** and **Cool on Column** inlets.

For example, for the HP 6890, you can use the **Column1** and **Column2** pages to define flow and pressure gradients. Select **Type: Ramped Flow** to program a low gradient. Or else, select **Type: Ramped Pressure** to program a pressure gradient. The gradient will then be displayed in the color indicated at the top right.

☞ *Tip:*

After the program run, each sample is automatically reset to the original state, i.e., for example, the initial temperature is restored. Restoring the initial temperature in this way is usually the quicker way, compared to setting the corresponding cool-down command in the program.

▣ Commands and Properties for Dual Operation (6890)

In dual operation mode, the 6890 GC allows you to simultaneously inject two samples from different positions, and thus doubling the sample throughput. To work in dual operation mode, you have to install and configure the 6890 GC as described in **Installation Instruction 🌐 Agilent 6890 GC: Dual Operation** in the **Administrator Help** section.

After you have installed the GC as described, the following commands and devices will be available in the PGM Editor, depending on the timebase:
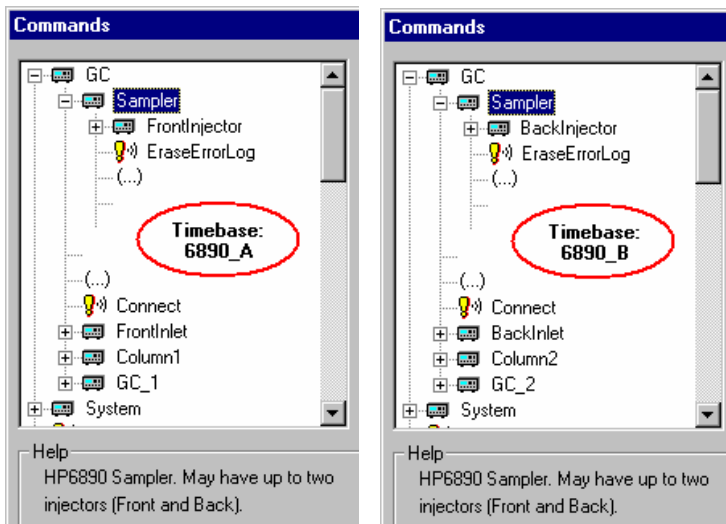
The commands and properties for the GC and the sampler are listed under both timebases. For the sampler, this is above all **UseTray**. For the GC, these are temperature commands and properties. (Note: The single commands and properties have been removed from the above picture; they are usually listed in the **Commands** dialog box between the **EraseErrorLog** and **Connect** commands.) The commands that are available for both timebases must be identical for simultaneously running samples, i.e., they must be identical in the related PGM Files.

In addition, only commands and properties are listed for the devices that are actually installed in the timebase.

For example, the commands and properties for the following devices appear under the first timebase (here: 6890_A):

- Front injector (default name: FrontInjector)

- Front inlet (FrontInlet)

- First column (Column1)

- Front detector (GC_1)

Under the second timebase (here: 6890_B), the commands and properties appear for the following devices:

- Back injector (BackInjector)

- Back inlet (BackInlet)

- Second column (Column2)

- Back detector (GC_2).

For information about how to perform dual injection, refer to ⬛ **Injecting Two GC Samples Simultaneously (6890)**.

⬛ Injecting Two GC Samples Simultaneously (6890)

To double the sample throughput, the 6890 GC supports the dual-operation mode, which lets you simultaneously inject two samples from different positions. The following conditions must be met:

- The GC must be fitted with the required hardware components.

- The GC must be installed in two timebases (refer to **Hardware Installation: Third-Party Devices** 🔵 **Agilent 6890 GC: Dual Operation** in the **Administrator Help** section).

- The PGM Files for the two timebases must match.

- A batch must be available for each timebase.

After installing the GC, follow the steps below to set up the dual-operation mode.

Creating two PGM Files

Create a PGM File for each timebase, observing the following:

- Enable dual operation with the following command (this can be done only from the control panel):

```
DualOperation      = Shared
```

- Enable dual operation for the related sample(s) (this can be done from the control panel, also):

```
DualOperation      = Shared
```

 Tip:

When a batch is running, you cannot perform manual commands in the ***Shared*** *mode (exceptions: RunLog, ReportReady, and PrepRun). To control the GC via manual commands while a batch is running, assign the GC to only one timebase:*

```
DualOperation      = FrontOnly or BackOnly
```

- Make sure that all commands and properties shared by the timebases (see **Commands and Properties for Dual Operation (6890)**) have the same values at the same retention times. This is especially important for the temperature. If the values and/or retention times are different, the samples will be processed one after the other.

 Note:

- *The ReadyCheck cannot always establish exactly in which order the samples are processed. Therefore, more or less warnings than appropriate may appear during the ReadyCheck.*

- In general, commands and properties that are assigned to only one timebase can be set independently of the setting in the PGM File for the other timebase. The **Inject** command is an exception: It must be available in both programs at the same time. (In special cases, it may be missing in **both** programs; it must not be missing in only one program.)

Creating a sequence and a batch for each timebase

It is not possible to simultaneously inject two samples from the same vial (except if `UseTray = No`).

The batches do not have to include the same number of samples. If all samples of one batch are processed, sample processing will continue for the remaining samples of the other batch.

🖙 *Tip:*

You can add new samples or sequences to the finished batch and restart the batch. Sample processing is resumed when the next sample of the running batch is started.

Starting batches

In dual operation mode, a batch must be started on both timebases simultaneously:

- Create the two batches and perform the Ready Check on both timebases. Afterward, start the two batches.

- A message may appear in the Audit Trail telling you to start a batch on the other timebase, too.

- Start the batch on the other timebase.

- The two batches run in synchronization. The Ready Check checks that the shared properties, temperature gradient, and run time for the sample pairs match and that the injections are performed from different positions.

📒 *Note:*

- *Observe the following restrictions:*
- *The Ready Check can check only the active sequences.*
- *The Ready Check can detect changes to the running sequence on the other timebase only when you stop and restart the batch on the other timebase. If the Ready Check reports an error on the other timebase, follow the steps below.*
  - *In the timebase for which the error was reported:*
    - *Stop the batch.*
    - *Eliminate the error.*
    - *For the first sample, change the status from Interrupted to Single, if necessary.*
    - *Restart the batch.*
  - *Return to the original timebase.*
  - *Run the Ready Check again.*

Examples

a) To analyze a pair of sequences (e.g., Front1 and Back1) simultaneously on two timebases (e.g., Front and Back) and then run another sequence (Front2) only on the Front timebase:

- For the pair of sequences, set `Sharable = On`.

- In the PGM File for the Front2 sequence, set `Sharable = Off`.

b) Use the following settings if you want to process samples in dual operation mode whenever possible but also analyze them one after the other if required:

- Accept the default setting: `DualOperation = Shared,` however

- Do **not** enter the `Sharable` command in the PGM File.

c) If you want to run a batch only on one timebase (e.g., on the Front timebase), select one of the following alternatives:

- In the PGM File, set `Sharable = Off` and start the batch on the Front timebase.

--Or--

- On the control panel, set `DualOperation = FrontOnly` and start the batch on the Front timebase.

In both cases, the batch is started immediately and does not wait for a batch on the Back timebase.

For information about how to install the 6890, refer to **Hardware Installation** 🔵 **Agilent 6890 GC: Overview** in the **Administrator Help** section.

◪ Injecting Two GC Samples Simultaneously (7673 and 7683 Autosamplers)

To double the sample throughput, the Agilent 7673 and 7683 GC autosamplers support dual injection, allowing you to simultaneously inject two samples from different sample positions.

🗒️ *Note:*

*The Dionex AS and AS50 autosamplers also support simultaneous injection of samples. Refer to **Practical Tips for Device Control: Autosampler Control** 🔼 **Autosampler Commands (AS/AS50)**.*

Use the following command to enable or disable dual injection:

```
DualInject        = On/Off
```

If DualInject is set to On, both injectors inject simultaneously. The master injector is always the injector mentioned in the Inject command. For example, the front injector is the master injector if the command is:

```
 0.000    Front.Inject
```

The master injector injects from the sample position defined in the sequence. The sample position for the other injector (the slave injector) needs to be defined separately, via either the Fixed Offset or the UDC method.

Fixed Offset Method

The Fixed Offset method defines the sample position from which to inject, relative to the position from which the Master Injector injects:

```
        DualInject        = On
        Rear.Position     = FrontPosition + 25
 0.000    Front.Inject
```

Dionex recommends reserving the first and third quadrants for the Front Injector and the second and fourth quadrants for the Rear Injector.

To run a chromatogram with one sample only, use a different PGM File including "DualInject = Off" for the corresponding sample.

UDC Method

For the UDC method, a ➤*User-Defined Column* is required for the datasource named; for example, RearPosition, Value type "Integer", Minimum "1", Maximum "100." The "empty values are possible" option should be enabled. In this column, enter the sample position from which the slave injector (in this case, the Rear Injector) will inject.

The corresponding program lines read as follows:

```
        DualInject          = On/Off
        Rear.Position        = Sample.RearPosition
0.000   Front.Inject
```

Use the UDC method to define a separate sample position for each injection. If the Rear Injector will not be performing an injection, leave the corresponding cell empty. (This is only possible if the "empty values are possible" option is enabled for the column.)

For information about how to install the Dual Inject option for the 7673 and 7683 autosamplers, refer to **Installation Instructions 🔵 Agilent 7673 and 7683 Autosamplers: Dual Inject** in the **Administrator Help** section.

For information about how to install the autosampler, refer to 🔵 **Agilent GC Autosamplers: Flow Chart** in the **Administrator Help** section.

▪ Temperature Control (On/Off)

Assign **TempCtrl** property to a ➤*Control* on a control panel, e.g., to a switch, to turn temperature control for the corresponding instrument on and off (see **How to: Controlling Devices from the Control Panel 🔲 Linking a Control to a Device**). The actual temperature is set by an additional control (see **Practical Tips for Device Control: GC and Temperature Control 🔲 Controlling the Temperature**).

Instruments providing temperature control include column ovens, autosamplers (Dionex ASI-100 T and ASI-100 PT), specific detectors, and gas chromatographs (HP 5890).

You can also enable the temperature control via a ➤*Program*. The corresponding command syntax is:

```
0.000    X.TempCtrl=On          or
0.000    X.TempCtrl=Off
```

Where X is the name under which the corresponding instrument is listed in the ➤*Server Configuration* program; for example:

```
0.000    GC.TempCtrl=On         or
0.000    Sampler.TempCtrl=On
```

◼ Controlling the Temperature

Instruments providing temperature control, such as column ovens, autosamplers (Dionex ASI-100T and ASI-100 PT), or gas chromatographs (HP 5890), can be operated directly by Chromeleon if the corresponding device driver supports this function. The prerequisite is that temperature control of the instrument has been enabled (see **Practical Tips for Device Control: GC and Temperature Control** 🔼 **Temperature Control (On/Off)**).

You can then control the temperature of the instrument by using the corresponding ➤*Control* (gauge or edit field) on the ➤*Control Panel*.

📝 *Note:*

To do so, assign the **Temperature** *property to the control. For more information, refer to* **How to: Controlling Devices from the Control Panel** 🔼 *Linking a Control to a Device*

Manual Cooling

- Move the slider with the mouse to the desired temperature value.

- Enter the nominal temperature in an edit field.

- A slider can have an additional gauge for the upper and lower temperature limits. Chromeleon monitors the set temperature range.

- Point to the gauge to display the exact value of the set temperature.

If an instrument reports that the set temperature value was actually reached (**Ready**), this can be made visible via an LED on the control panel (see **Practical Tips for Device Control: Special Commands, Relay Control, and Miscellaneous** 🔼 **The Ready Signal**).

Programmed Cooling

In the ➤*Program*, the temperature of an instrument can be adjusted as follows:

```
0.000   TempCtrl                        = On/Off
;       This command enables and disables
        the temperature control.
0.000   X.Temperature                   = Value[°C]
;       This command sets the nominal temperature.
;       X corresponds to name of the instrument as defined
;       in the "Server Configuration" program.
0.000   X.Temperature.UpperLimit        = Value[°C]
0.000   X.Temperature.LowerLimit        = Value[°C]
;       These commands define the upper and lower limit.
;       The limits are monitored by Chromeleon.
0.000   Wait                            SampleTempOK (or
                                        similar)
;       The Wait command delays all further operations on
;       the instrument until the system receives the feedback
;       that the nominal temperature has been reached.
```

■ Controlling the Column Temperature

The following Dionex instruments provide temperature control of the column:

Instrument	Temperature Control Range
TCC-100 Thermostatted Column Compartment (see the Administrator Help section for details)	5°C to 80°C
UltiMate 3000 Series - TCC-3x00 Thermostatted Column Compartments (see the Administrator Help section for details)	5°C to 85°C
STH585 Column Oven (see the Administrator Help section for details)	5°C to 85°C
ICS-1000/1500/2000 Ion Chromatography Systems	30°C to 60°C. (The column heater is an option for the ICS-1000.)
AS50 Thermal Compartment	10°C to 40°C for PEEK systems
	10°C to 80°C for stainless steel systems

Instrument	Temperature Control Range
ICS-3000 Detector/Chromatography Module	Units without separate column temperature control: 10°C to 40°C
	Units with separate column temperature control:
	10°C to 40°C (upper compartment)
	10°C to 70°C (column compartment)
🔵 **FLM-3100 Flow Manager and Thermostatted Column Compartment** (see the Administrator Help section for details)	5°C to 70°C

Depending on the type of ➤*Control* (see ➤*Layout Toolbar*), you can have the current temperature displayed (alphanumeric display), set a new temperature value (edit field), or determine the upper and/or lower temperature limit (gauge slider). For more information about changing controls, refer to **How to:** 🔲 **Controlling Devices from the Control Panel**.

It is also possible to integrate column temperature control into a ➤*Program*. See the following descriptions.

TCC-100/TCC-3x00 and FLM-3100 program commands:

```
0.000   ColumnOven.Temperature            = Value[°C]
;       This command determines the nominal temperature.
0.000   ColumnOven.Temperature.UpperLimit = Value[°C]
0.000   ColumnOven.Temperature.LowerLimit = Value[°C]
;       These commands determine the upper and lower limits.
;       The limits are monitored by Chromeleon.
```

ICS-1000/1500/2000 and AS50 thermal compartment program commands:

```
0.000   Column.Temperature             = Value[°C]
;       This command determines the nominal temperature.
0.000   Column.Temperature.UpperLimit  = Value[°C]
0.000   Column.Temperature.LowerLimit  = Value[°C]
;       These commands are available for the ICS-1000/1500/2000
;       only and determine the upper and lower limits.
        The limits are monitored by Chromeleon.
```

DC program commands:

Include the following commands before data acquisition begins:

```
        Column_TC.AcquireExclusiveAccess
;       This command gives exclusive control of column temperature
        to the current timebase.
        Column_TC.Mode              = On/Off
        Column_TC.TemperatureSet    = Value[°C]
;       These commands turn on or off column temperature control
;       and determine the temperature setpoint. Column temperature
        control is an option in the DC.
        Compartment_TC.AcquireExclusiveAccess
;       This  command  gives  exclusive  control  of  compartment
        temperature to the current timebase.
        Compartment_TC.Mode         = On/Off
        Compartment_TC.TemperatureSet   = Value[°C]
;       These commands turn on or off compartment temperature
;       control and determine the temperature setpoint.
```

Include the following commands after data acquisition ends:

```
        Column_TC.RelaseExclusiveAccess
        Compartment_TC.ReleaseExclusiveAccess
;       These  commands  release  control  of  the  column  and
;       compartment  temperatures  to  allow  another  timebase  to
        acquire exclusive access.
```

For the commands that Chromeleon supports for the Flow Managers and Thermostatted Column Compartments, refer to **Device Control: Commands for Controlling Dionex Devices** 🔖 **Dionex Flow Manager and Thermostatted Column Compartments**.

Component Controller Control

As with other Chromeleon controlled devices, to control a CC80 Component Controller using programmed control, commands are included in a ➢*PGM File*. However, the CC80 differs from other controlled devices in that ➢*Sequences* are not created in Chromeleon. Instead, sequences are created in the Analyzer program, which then oversees control of the CC80 and the other analyzer devices. Refer to the Analyzer program Help for how to create and run analyzer sequences.

The simplest way to create a program for the CC80 is to use the Program Wizard (see **Control** 🔖 **The Program Wizard**).

Using the wizard ensures that commands and parameters are entered with the correct syntax and in the correct order. This is especially helpful for entering the SP80 valve commands for sample preparation.

 Note:

*Direct control of a CC80 using a ➢Control Panel is used only when the CC80 is offline (not controlled via the Analyzer program). When the CC80 is online, the control panel is set to **Monitor Only**.*

Sample Preparation

CC80 sample preparation commands that will be used to prepare the sample or standard for analysis are grouped together in the program and executed before the timed commands. The beginning of the group is defined with the **BeginSamplePrep** command**,** and the end of the group is defined with the **EndSamplePrep** command. Because these grouped commands are not assigned retention times, the **Delay SP** command is used to specify a time to wait before continuing to the next command.

Sample Preparation Overlap

To reduce the time between injections, the CC80 can perform the sample preparation functions for the next sample while the current sample is still running. The overlapped functions are run while data acquisition or other program commands that do not require the CC80 are being executed. When the CC80 finishes the overlapped functions, the program pauses, if necessary, to allow the currently running sample to finish. Then, the remaining commands in the overlapped sample's program are executed.

To ensure sample overlap works correctly, if you are editing a PGM manually, be sure that the following three commands are present in the given order:

```
BeginSamplePrep
[list of CC80 sample prep commands]
EndSamplePrep
Wait SamplePrepComplete
```

The **BeginSamplePrep** and the **EndSamplePrep** commands enclose the commands that are to be overlapped. The **Wait SamplePrepComplete** command determines whether the CC80 can begin running the overlapped commands for the next sample. Chromeleon automatically sets the **SamplePrepComplete** status to true or false at the appropriate times when running the program.

CC80 sample prep commands can also be assigned retention times. When a sample prep command is assigned a time, it is not included in the overlapped commands. To ensure that timed CC80 commands do not interfere with overlapped sample preparation, commands that require a length of time to complete cannot be assigned retention times. These commands are: **DelaySP**, **Dilution Pump**, and **Loading Pump**.

 Note:

*The **Sample Preparation Overlap** option can be disabled in the CC80 server configuration properties on the **Options** tab page.*

Also, refer to **Overlapping Samples**.

Program Examples

Chromeleon-PA provides example program files, which you can use as starting points for your programs. Because every program is created for a specific system and application, the examples will not work "as is" with your analyzer, but the principles behind the types of commands included and the order in which they are listed can be applied to many systems. Refer to *Setting Up Chromeleon-PA* (Document No. 031970) for detailed descriptions of the example programs.

The example programs are included in the **CM-PA Example** datasource on the Chromeleon-PA installation CD. To connect to the datasource and view the examples, insert the CD in the drive, go to the Chromeleon Browser, and select **File>Mount Datasource>Browse**.

Also, refer to **How to:**

 Valve Control for CC81: Concentration

 Valve Control for CC82: Dilution

 Valve Control for CC83: Dilution with Reagent

 Valve Control for CC84: Concentration with Reagent

◼ Valve Control for CC81: Concentration
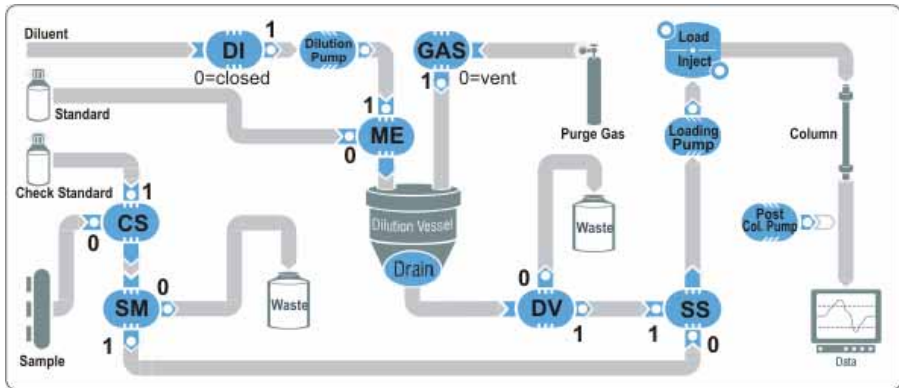
Metering Valve Detail
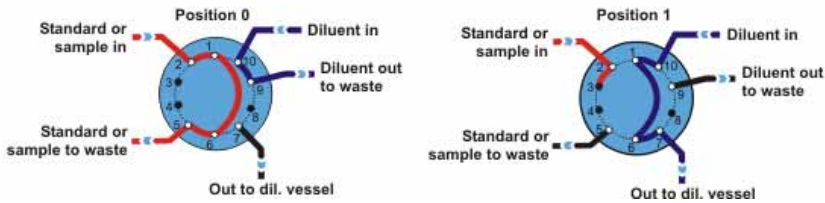
Sample (SM)	**waste (0)**—directs output from the Chk Std (CS) valve to waste
	SS valve (1)—directs output from the CS valve to the SS valve
Diluent (DI)	**closed (0)**—stops flow of diluent (typically DI water) to the dilution pump
	open (1)—allows flow of diluent to the dilution pump
Dil Vessel (DV)	**purge (0)**—directs output from the dilution vessel to waste
	SS valve (1)—directs output from the dilution vessel to the SS valve
Samp/Std (SS)	**sample (0)**—directs output from the CS valve (sample or check standard) to the Load/Inject valve
	standard (1)—directs output from the DV valve (the diluted standard) to the Load/Inject valve
Gas (GAS)	**vent (0)**—no gas pressure on the dilution vessel
	pressurize (1)—pressurizes the dilution vessel
Metering (ME)	**standard (0)**—allows the stock standard to flush and fill the loop
	diluent (1)—directs the diluent through the loop for delivery to the dilution vessel
Chk Std (CS)	**sample (0)**—directs the sample to the SM valve
	ChkStd (1)—directs the check standard to the SM valve

Valve Control for CC82: Dilution
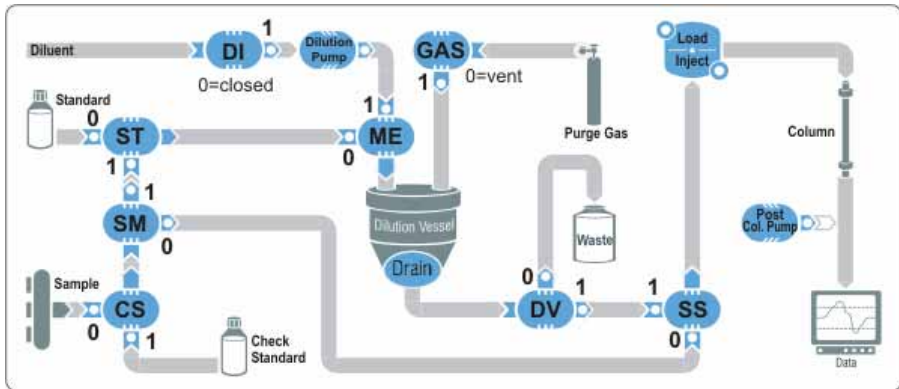
Metering Valve Detail
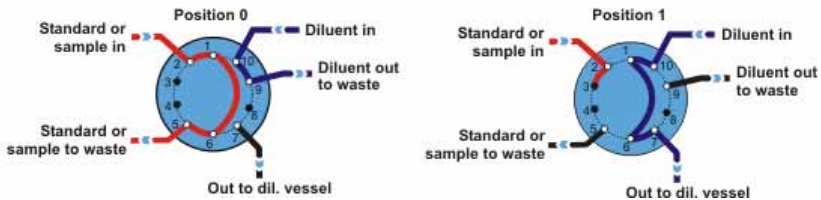
Sample (SM)	**SS valve (0)**—directs output from the SM valve (sample or check standard) to the SS valve
	ST valve (1)—directs output from the SM valve to the ST valve
Diluent (DI)	**closed (0)**—stops flow of diluent (typically DI water) to the dilution pump
	open (1)—allows flow of diluent to the dilution pump
Dil Vessel (DV)	**purge (0)**—directs output from the dilution vessel to waste
	SS valve (1)—directs output from the dilution vessel to the SS valve
Samp/Std (SS)	**sample (0)**—directs the (undiluted) sample or check standard to the Load/Inject valve
	standard (1)—directs output from the dilution vessel to the Load/Inject valve
Gas (GAS)	**vent (0)**—no gas pressure on the dilution vessel
	pressurize (1)—pressurizes the dilution vessel
Metering (ME)	**ST valve (0)**—allows the output from the ST valve (stock standard, sample, or check standard) to flush and fill the loop
	diluent (1)—directs the diluent through the loop for delivery to the dilution vessel

Chk Std (CS)	**sample (0)**—directs the sample to the SM valve
	ChkStd (1)—directs the check standard to the SM valve
Standard (ST)	**standard (0)**—directs stock standard to the ME valve
	sample (1)—directs output from the SM valve (sample or check standard) to the ME valve

◨ Valve Control for CC83: Dilution with Reagent
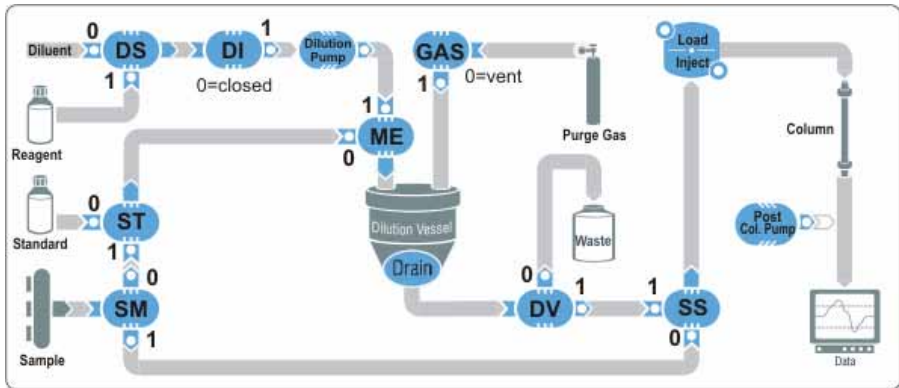
Metering Valve Detail
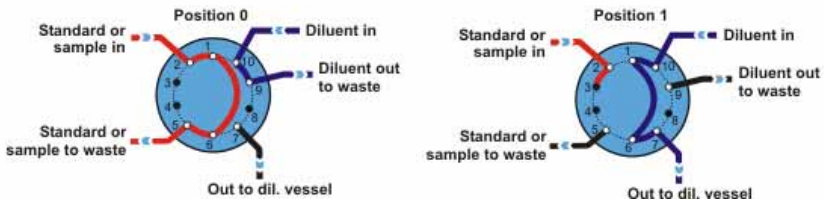
Sample (SM)	**ST valve (0)**—directs sample to the ST valve
	SS valve (1)—directs sample to the SS valve
Diluent (DI)	**closed (0)**—stops flow from the DS valve to the dilution pump
	open (1)—allows flow from the DS valve to the dilution pump
Dil Vessel (DV)	**purge (0)**—directs output from the dilution vessel to waste
	SS valve (1)—directs output from the dilution vessel to the SS valve
Samp/Std (SS)	**sample (0)**—directs the (undiluted) sample to the Load/Inject valve
	diluent (1)—directs output from the dilution vessel to the Load/Inject valve

Gas (GAS)	**vent (0)**—no gas pressure on the dilution vessel
	pressurize (1)—pressurizes the dilution vessel
Metering (ME)	**ST valve (0)**—allows the output from the ST valve (sample or standard) to flush and fill the loop
	diluent (1)—allows output from the DS valve (diluent or reagent) to flow through the loop and into the dilution vessel
Dil Select (DS)	**diluent (0)**—directs diluent to the ME valve (through the dilution pump)
	reagent (1)—directs reagent to the ME valve (through the dilution pump)
Standard (ST)	**sample (0)**—directs sample to the ME valve
	standard (1)—directs stock standard to the ME valve

◱ Valve Control for CC84: Concentration with Reagent
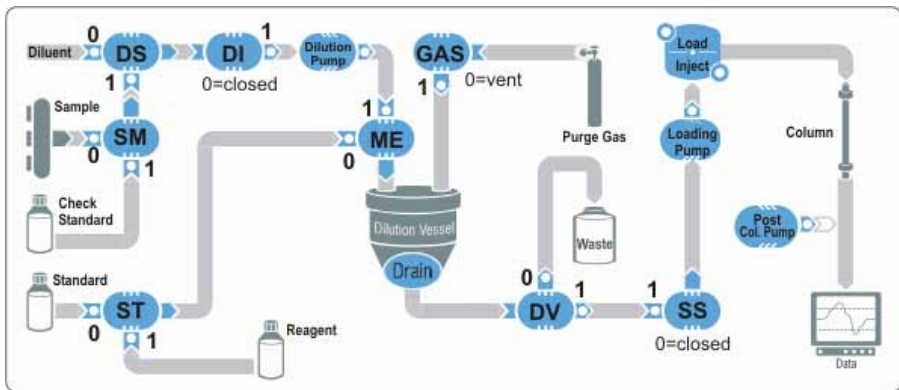
Metering Valve Detail
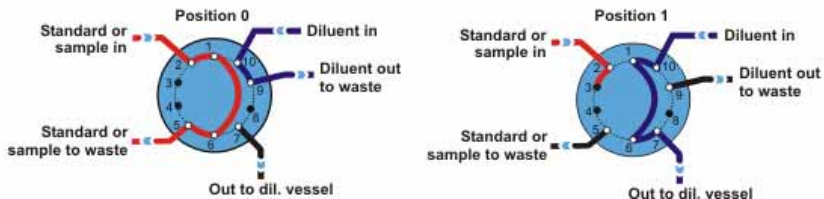
Sample (SM)	**sample (0)**—directs sample to the DS valve
	ChkStd (1)—directs check standard to the DS valve
Diluent (DI)	**closed (0)**— stops flow of diluent (typically DI water) to the dilution pump
	open (1)— allows flow of diluent to the dilution pump
Dil Vessel (DV)	**purge (0)**—directs output from the dilution vessel to waste
	SS valve (1)—directs output from the dilution vessel to the SS valve

Samp/Std (SS)	**closed (0)**—does not allow flow through the valve
	DV valve (1)—directs output from the DV valve to the loading pump
Gas (GAS)	**vent (0)**—no gas pressure on the dilution vessel
	pressurize (1)—pressurizes the dilution vessel
Metering (ME)	**ST valve (0)**—allows output from the ST valve (stock standard or reagent) to flush and fill the loop
	DS valve (1)—allows output from the DS valve to flow through the loop and into the dilution vessel
Dil Select (DS)	**diluent (0)**—directs diluent to the ME valve
	SM valve (1)—directs output from the SM valve to the ME valve
Standard (ST)	**standard (0)**—directs standard to the ME valve
	reagent (1)—directs reagent to the ME valve

Special Commands, Relay Control, and Miscellaneous

Chromeleon also supports various special commands:

 Virtual Channel Commands

 Program Examples for Virtual Channels

 Trigger Commands

 Mixed Commands

For information about relays, refer to:

 Relay, TTL, and Remote Input Commands

 Switching a Relay

For general information about instrument control, refer to:

 Device Successfully Connected

 The Ready Signal

▪ Virtual Channel Commands

Changing the Channel Type

To optimize signal recording, assign each virtual channel a type. Three types are currently defined (see **Hardware Installation 🌐 Channel Types of the Virtual Channel Driver** in the **Administrator Help** section:

```
0.000    VirtualChannel_01.Type   = Analog, Digital or Fixed
```

Analog	Suitable for expressions that	Examples: `formula =`
	a) change quickly and/or	a) `pressure.value`
	b) are composed of other analog signals.	b) `UV_VIS_1/UV_VIS_2`
Digital	Suitable for digital expressions.	`P580Relay_1.State AND P580Relay_2.State`
Fixed	Suitable for expressions that change slowly.	`Pump.%A`

👉 *Tip:*

If step gradients occur for virtual signals consisting of other signals even if the channel type is set to Analog, the resolution in y direction is insufficient. In this case, set FormulaMin/FomulaMax accordingl

The selected type affects the ⇒*Step*, ⇒*MaxAutoStep*, and ⇒*Average* settings of the corresponding signal and the selection of appropriate raw data compression. For the standard compressor, these three parameters have the same significance as with normal signal channels. For the step compressor, the values have no significance because this compressor always uses a fixed step of 0.01s. The default values are as follows:

	Step	MaxAutoStep	Average	Compression
Analog	Auto	5.1s	On	Standard compressor
Digital	Fixed, 0.01s			Special compressor for step signals
Fixed	Fixed, 0.01s	5.1s	Off	Standard compressor

For analog or fixed channels, you can change **Step**, **MaxAutoStep**, and **Average** values after you have selected the type. If these properties are set for digital channels, a warning appears.

👉 *Tip:*

Step = Auto and Average = On is not considered for fixed channels.

Defining the signal to record

Select **Formula** to specify the expression to be recorded. You can use the same expressions as for ⇒*Trigger* conditions, i.e., arithmetic and logical links. The expression must be numeric. During program execution, the **FormulaCur** property indicates the value calculated. **FormulaMin** and **FormulaMax** define the minimum and maximum signal values. If the measured signal exceeds the FormulaMax value, only the FormulaMax value is used for the data point. (This also applies to FormulaMin.)

🗊 *Note:*

The more the measuring range is limited by FormulaMin and FormulaMax, the better is the signal resolution.

As for other signal channels, you can use the **Value** property to display the current (or interpolated) signal value (changes every 0.01s).

The **Equate** property allows you to enter a user-defined name for the virtual channel during the analysis. Use the **Log** command to include the name in the Audit Trail.

It is not possible to change the **Step**, **MaxAutoStep**, **Average**, **Formula**, and **Type** properties during data acquisition. The **FormulaCur** property indicates the status.

For program examples for virtual channels, refer to 🔲 **Program Examples for Virtual Channels**.

🔲 Program Examples for Virtual Channels

Recording the Pump Pressure

You can use the ➢*Virtual Channel Driver* to record the pump pressure. This steady signal is likely to change frequently. That is why Dionex recommends setting the type of the virtual channel to **Analog**. You can use the default settings for recording this channel:

```
0.000 Pressure.LowerLimit =   0
   Pressure.UpperLimit =   400
   Inject
   VirtualChannel_01.Formula  pump.pressure
   VirtualChannel_01.Type =   Analog
   VirtualChannel_01.AcqOn
2.000 VirtualChannel_01.AcqOff
```

The signals are recorded with the ⇒*Step* determined by the corresponding pump. This step might be too large to return the pump pressure precisely. In this case, use the ➤*Integrator Driver* to receive the precise pump pressure signal. In this case, you will need an A/D converter such as the ➤*UCI-100 Universal Chromatography Interface* (also, refer to 🖻 **Recording the Pump Pressure**).

Sum of two channels

Much as the pump pressure, you can record the sum of two UV channels as a virtual channel. This steady signal is also likely to change frequently. That is why Dionex recommends setting the type of the virtual channel to **Analog**. Using the default settings, this channel is recorded as precisely as the two UV channels:

```
0.000     Pressure.LowerLimit =        0
          Pressure.UpperLimit =        400
          Inject
          VirtualChannel_01.Formula    UV_VIS_1+UV_VIS_2
          VirtualChannel_01.Type =     Analog
          UV_VIS_1.AcqOn
          UV_VIS_2.AcqOn
          VirtualChannel_01.AcqOn
2.000     UV_VIS_1.AcqOff
          UV_VIS_2.AcqOff
          VirtualChannel_01.AcqOff
```

👉 *Tip:*

If step gradients occur for virtual signals consisting of other signals even if the channel type is set to Analog, the resolution in y direction is insufficient. In this case, set FormulaMin/FomulaMax accordingly.

Relay Status

The status of a relay should be recorded as a virtual channel. For this rectangle signal, Dionex recommends setting the type of virtual channel to **Digital**:

```
0.000     Pressure.LowerLimit =        0
          Pressure.UpperLimit =        400
          Inject
          VirtualChannel_01.Formula    P580_Relay1.State
          VirtualChannel_01.Type =     Digital
          VirtualChannel_01.AcqOn
```

```
0.000       P580_Relay1.On
0.100       P580_Relay1.Off
0.200       P580_Relay1.On
0.300       P580_Relay1.Off
0.400       P580_Relay1.On
0.500       P580_Relay1.Off
0.600       P580_Relay1.On
0.700       P580_Relay1.Off
0.800       P580_Relay1.On
0.900       P580_Relay1.Off
1.000       P580_Relay1.Off
1.000       VirtualChannel_01.AcqOff
```

Recording a Gradient

The currently set gradient should be recorded as a virtual channel. This steady signal is unlikely to change frequently. That is why Dionex recommends setting the type of the virtual channel to **Fixed**. It is sufficient to record the current value once per second:

```
0.000       Pressure.LowerLimit =        0
            Pressure.UpperLimit =        400
            Inject
            Flow = 10
            %B = 100
            VirtualChannel_01.Formula    Pump.%B
            VirtualChannel_01.Type =     Fixed
            VirtualChannel_01.Step =     1
            VirtualChannel_01.AcqOn
0.500       %B = 100
1.000       %B = 50
1.500       %B = 50
2.000       %B = 0
2.000       VirtualChannel_01.AcqOff
```

Correcting a signal from a radioactive substance:

To determine the concentration of a radioactive substance, you must take the decay of the substance into account and correct the signal accordingly. Use the virtual channel driver to accomplish this task.

In the example below

- The half-life is **t½** min

- The run time of the chromatogram is **tl** min

- The uncorrected signal (UV_VIS_1) is between - 10.000 and 10.000 mAU,

```
0.000   Pressure.LowerLimit = 0
        Pressure.UpperLimit = 400
        Inject

        VirtualChannel_01.Formula   UV_VIS_1 / 2.718**
        (-0.69314718* System.Retention /t½)
        VirtualChannel_01.FormulaMin = -10000 / 2.718**
            (-0.69314718* tl /t½)
         VirtualChannel_01.FormulaMax = 10000 / 2.718**
            (-0.69314718* tl /t½)
        VirtualChannel_01.Type = Analog

        UV_VIS_1.AcqOn
        VirtualChannel_01.AcqOn

tl      UV_VIS_1.AcqOff
        VirtualChannel_01.AcqOff
```

In the program, replace the **t½** entry with the actual half-life. In addition, enter the actual run time of your program with three decimal places for **tl**.

When the program is modified accordingly, the virtual channel records the signal that would be measured by the detector if the radioactive decay were stopped with the injection into the chromatography system. The decay that occurred before the injection will not be corrected.

 Note:

If you know the signal range to be expected for your chromatogram (in the example for the channel UV_VIS_1) you may enter this value instead of -10,000 and 10,000 for calculating FormulaMin/Max to map your chromatogram more precisely.

▣ Trigger Commands

🔲 *Note:*

The trigger name must be unique; that is, no triggers by the same name can be used, nor device, relay, or signal names that were already assigned. The same trigger can initiate any number of reactions. The reactions of the trigger list are not supplied with time information. The trigger must always be completed by an EndTrigger command; for example:

```
0.000    Trigger <NAME>  Condition=      [Value](Operator)[Value]
                                         ,OperatorValue,True=Value[s]
                                         ,Delay=Value[s],Limit=Number
         Reaction 1      =Value/Status
         ...                             ...
         Reaction n      =Value/Status
         EndTrigger
```

If a trigger condition consists of several values, operators can combine them. Available operators are **+**, **-**, *****, **/**, **AND**, **OR**, **NOT**. The Trigger condition is fulfilled if the corresponding value is reached, not reached, or exceeded. This is indicated by the operators <, >, <=, >=, = and <>. Any dependence on various values can be realized via parentheses.

If the value is preceded by the name ⇒*Delta*, the slope (i.e., the first derivation) is processed as the trigger condition. Remote input signals or relay states can also be processed.

🔲 *Note:*

Due to lack of space, line breaks are inserted here. Normally, one command must be entered in one line. For a detailed description of the **Trigger** *command, refer to ⇒Trigger in the* **Reference Information** *section.*

Example 1: Peak Recognition via Absolute Value

```
0.000    Trigger EXAMPLE1        UV_VIS_1>100,True=1,Delay=5,Limit=4
         Sound                   File="TEST.WAV"
         ...                     ...
         RELAYNAME               =On
         EndTrigger
```

Description: The trigger with the Trigger Name **EXAMPLE1** monitors the channel UV_VIS_1. If the signal exceeds the value 100 for more than 1 second ((Trigger-) True), the Audio file TEST.WAV is played after a (Trigger-) Delay of 5 seconds. (If no sound card is installed in the PC, there is a short **Beep** instead.) At the same time, the RELAYNAME relay is enabled.

The entire process, i.e., exceeding the signal value 100 of channel UV_VIS_1 with a subsequent reaction, is limited to 4 times (**Limit=4**). If the condition (Trigger-) Limit is not entered, the process is always repeated if....

Example 2: Peak Recognition via Signal Slope

```
0.000    Trigger EXAMPLE2        UV_VIS_1.DELTA>1
         Sound                   File="TEST.WAV"
         EndTrigger
```

Description: The trigger **EXAMPLE2** monitors the channel UV_VIS_1. At a slope value (DELTA) above 1, the Audio file TEST.WAV is played. If no sound card is installed in the PC, there is a short beep instead.

Example 3: AND, OR, NOT

```
0.000    Trigger EXAMPLE3        ((UV_VIS_1>100) AND (UV_VIS_2>100) OR
                                 (UV_VIS_3>200)) AND NOT REMOTE1
         Sound                   File="TEST.WAV"
         EndTrigger
```

Description: The **EXAMPLE3** trigger monitors the UV_VIS_1, UV_VIS_2, and UV_VIS_3 channels as well as the REMOTE1 remote input. If the channels 1 and 2 simultaneously (**AND**) exceed the value 100, or (**OR**) if the channel UV_VIS_3 exceeds the value 200 and if the remote input REMOTE1 delivers no signal (**AND NOT**) at the same time, the TEST.WAV Audio file is played (without a sound card, there is a short beep instead).

Example 4: Further parameters that can be triggered

```
0.000    Trigger EXAMPLE4        Pressure.LowerLimit<20 OR
                                 Pressure.UpperLimit>300 OR
                                 Temperature>60

         AbortBatch
         EndTrigger
```

Description: The trigger **EXAMPLE4** monitors the pump pressure and the temperature of the column oven. If the pressure falls below 20 bar (= 2 MPa = 290 psi) or exceeds 300 bar (= 30 MPa = 4350 psi), or if temperature exceeds 60°C, the running sample batch is aborted (**Abort Batch**).

Example 5: Fraction Collector Foxy Jr.

```
         FoxiJr.Valve = Off
         ; switches the ➢Fraction Collector valve to the "Waste" position
0.000  Trigger STARTofPEAK          FoxiJr.Valve = 0 AND
                                     UV_VIS_1.Delta >1, True = 2
         FoxiJr.Valve = On
         ; switches the valve to the "Collect" position
         EndTrigger


         Trigger TUBEFULL            FoxiJr.Valve = 1,True = 60
         Protocol "Tube full"
         FoxiJr.Valve = Off
         EndTrigger


         Trigger ENDofPEAK           FoxiJr.Valve = 1 AND
                                     UV_VIS_1.Delta > -1,
                                     True = 2, Delay = 5
         FoxiJr.Valve = Off
         ; switches the valve to the "waste" position
         FoxiJr.Tube = FoxiJr.Tube +1
         EndTrigger
```

Description: The **PEAKSTART** trigger causes the valve to switch to the **Collect** position if the valve is previously in the **Waste** position and if the slope of the UV_VIS_1 channel exceeds the value 1 for more than 2 seconds (True). After 60 seconds, the TUBEFULL trigger ensures that the collection container does not overflow. The valve is switched again (back to the **Waste** position).

For larger collection containers, the time can be adjusted as needed. If the signal is below the slope value -1 for more than 2 seconds, and if the valve is still in the **Collect** position, this is interpreted as the end of the peak. After a delay of 5 seconds, the valve is switched to the **Waste** position. Simultaneously, the x/y-arm of the fraction collector is moved by one position (FoxiJr.Tube = FoxiJr.Tube +1).

Also, refer to **How to: Collecting Fractions**:

📕 **Setting Up Fraction Collection**

📕 **Program Example (One Detection Channel)**

📕 **Program Example (Two Detection Channels)**

📕 **Fraction Collection Control via an MS**

▪ Mixed Commands

Produce Sound

```
0.000   Sound             File="TEST.WAV"
```

For more information, refer to ⇒*Sound* command.

Temperature Regulation for Column Oven

```
0.000   Temperature.Nominal =Value [°C] (nominal value)
```

In combination with a ⇒*Trigger* condition, the actual value can also be inquired:

```
0.000   Trigger OVENTEST   Condition=Temperature>50[°C]
        Sound              File="TEST.WAV"
        EndTrigger
```

Description: The OVENTEST trigger monitors the current oven temperature. Each time the temperature exceeds 50°C, the TEST.WAV Audio file is played. If the PC has no sound card, there is a short beep.

Comments, Protocol and Message Texts

```
;       Comment Text...
0.000   Protocol           "Text ..."
0.000   Message            "Text ..."
```

 Note:

Comments can be added to any program instruction via " ; ". They can easily be recognized by their green color. Protocol and message texts must be indicated by quotation marks ("Text...")! Both are included in the Audit Trail. While the ⇒Protocol command is for documentation purposes only, the ⇒Message text appears on the screen. Continuation of the program is only possible after confirming with the Enter key.

Example 6: Delayed Execution of the Inject Command ("Wait")

```
0.000    Acquisition          =On
0.000    Wait                 UV_VIS_1 < 10
0.000    Inject
```

Description: Program execution is delayed until the absorption on channel UV_VIS_1 is below 10 mAU. As soon as this is the case, injection is started.

The **Wait** command is also required for Suck and Dispense operations of the Dionex GINA 50 and GINA 160 autosamplers (see **Autosampler Control**).

Relay, TTL, and Remote Input Commands

Enable/Disable Relays

```
0.000    RELAYNAME.State      =On / Off
```

Alternatively, the following short command is valid:

```
0.000    RELAYNAME.On
0.000    RELAYNAME.Off
```

Enable/Disable Relay for a specific duration

```
0.000    RELAYNAME.On         =Value[s]
0.000    RELAYNAME.Duration   =Value[s]
```

Alternatively, use the following short command:

```
0.000    RELAYNAME.On         Duration= Value[s]  or
0.000    RELAYNAME.Off        Duration= Value[s]
```

Enable/Disable TTLs

```
0.000    TTL_NAME.State          =0v / 5v
```

Alternatively, use the following short command:

```
0.000    TTL_NAME.0v
0.000    TTL_NAME.5v
```

Enable/Disable TTLs for a specific duration

```
0.000    TTL_NAME.0v             Duration=Value[s]
0.000    TTL_NAME.5v             Duration=Value[s]
```

■ Switching a Relay

Every ➢*Relay* can be integrated as a simple switch on the control panel interface. The two switch settings correspond to the relay states **On** and **Off**. In addition, ➢*Controls* on the ➢*Control Panel* are linked to **State**. Each relay can be executed with a time relay, using the **Duration** parameter.

If a 3-way valve is integrated in the fluidics on the detector output, two switch states can be controlled via an internal device relay; for example, the Dionex M480 pump.

- In the **Server Configuration**, double-click the instrument to open its **Property** dialog box, and select **Relays**.

- Enable a currently free device relay and click OK.

- Save the new ➢*Server Configuration*.

📒 *Note:*

*All settings you select on the **Relays** tab page will be automatically copied to the **State Devices** page of the Program Wizard (see **Control** 🔲 **The Program Wizard***).

- On the control panel, any switch can be linked to the new relay function (**Object=Relayname**, **Object Property=State**). See **How to: Controlling Devices from the Control Panel** 🔲 **Linking a Control to a Device**.

- If necessary, create an additional control, for example, an edit field (see ➢*Layout Toolbar*), to set the on/off duration of the valve.

In the ➢*Program,* the switch procedure of a relay can be realized via the following commands:

```
0.000    RELAYNAME.State          =On/Off
0.000    RELAYNAME.Duration       =Value[s]
```

▪ Device Successfully Connected

Many devices automatically communicate their status to Chromeleon; that is, they communicate whether they were successfully integrated into a system or not.

This signal can be displayed on the ➢*Control Panel*, for example, via an LED or color area integrated into the control panel (see ➢*Layout Toolbar*).

- Link the corresponding ➢*Control* on the **Link** tab page to the **Connected** object property. (For more information, refer to **How to: Controlling Devices from the Control Panel** ▪ **Linking a Control to a Device**.)

If the LED or the color area is active, the device was successfully integrated into the system. In this state, Chromeleon can control the device.

▪ The Ready Signal

If an instrument supplies a feedback (**Ready** signal) when reaching a nominal (set) value, this can be indicated via the corresponding ➢*Control* on the control panel (LED or color box).

Column ovens, autosamplers with temperature control, electrochemical detectors (Antec Decade), or gas chromatographs (HP 5890; Fisons 8000) are sending **Ready** signals.

The Dionex GINA 50T autosampler with temperature control sends the **SampleTempOK** signal instead of the **Ready** signal.

◩ Determining the CRP Value

With the UltiMate capillary/nano HPLC system, the pump flow is split before the column. Enter the system flow, that is, the flow through the column, in Chromeleon. Chromeleon uses the following formula to determine the flow to be delivered by the pump (= master flow):

Master Flow [ml/min] = CRP*System Flow [µl/min].

The CRP value is a conversion factor that depends on the properties of the installed column and the used calibrator. Chromeleon determines the appropriate CRP value based on the following lookup table that indicates the column length, internal diameter of the column, and the stationary phase:

Internal Diameter of the Column [µm]	Column Length [cm]				
	5	**10**	**15**	**20**	**25**
50	A: 1100	A: 1150	A: 1250	A: 1275	A: 1300
	B: 1000	B: 1100	B: 1170	B: 1235	B: 1300
	C: 1100	C: 1150	C: 1250	C: 1275	C: 1300
	D: 800	D: 850	D: 890	D: 950	D: 1000
75	A: 1100	A: 1150	A: 1250	A: 1275	A: 1300
	B: 1000	B: 1100	B: 1170	B: 1235	B: 1300
	C: 1100	C: 1150	C: 1250	C: 1275	C: 1300
	D: 800	D: 850	D: 890	D: 950	D: 1000
100	A: 440	A: 460	A: 500	A: 510	A: 520
	B: 440	B: 460	B: 500	B: 510	B: 520
	C: 440	C: 460	C: 500	C: 510	C: 520
	D: 440	D: 460	D: 500	D: 510	D: 520
180	A: 90.5	A: 105.5	A: 124.9	A: 143.1	A: 161.2
	B: 69.2	B: 79.9	B: 91.0	B: 95.5	B: 100.0
	C: 90.5	C: 105.5	C: 124.9	C: 143.1	C: 161.2
	D: 68.5	D: 74.9	D: 81.3	D: 89.5	D: 97.5
300	A: 36	A: 43	A: 50	A: 58	A: 65
	B: 28	B: 32	B: 47	B: 39	B: 40
	C: 36	C: 43	C: 50	C: 58	C: 65
	D: 33	D: 30	D: 33	D: 36	D: 40
500	20	20	20	20	20

Internal Diameter of the Column [μm]	Column Length [cm]				
	5	**10**	**15**	**20**	**25**
800	A: 6.67	A: 8.07	A: 9.46	A: 10.44	A: 11.39
	B: 5.16	B: 5.66	B: 6.17	B: 6.84	B: 7.49
	C: 6.67	C: 8.07	C: 9.46	C: 10.44	C: 11.39
	D: 4.93	D: 5.43	D: 5.94	D: 6.58	D: 7.19
1000	A: 4	A: 5	A: 6	A: 6	A: 7
	B: 3	B: 3	B: 4	B: 4	B: 5
	C: 4	C: 5	C: 6	C: 6	C: 7
	D: 3	D: 3	D: 4	D: 4	D: 4

The capital letters A-D in the table describe the column material. The meaning is as follows:

Column	Chemical Structure	Particle Size [μm]	Pore Width [Å]
A	C18	3	100
B	C18	5	100
C	C18	3	300
D	C18	5	300

This table is part of the **UltiMate.lup** text file in the **../Chromel/bin** directory. You can extend the file, i.e., the comments in the file, according to you rrequirements.

⚠️ *Caution:*

If the appropriate CRP value is missing, Chromeleon cannot determine the pump's flow rate. Thus, the pump cannot deliver the correct flow. Therefore, make sure to enter the length, internal diameter, and stationary phase of the installed column!

If your column does not correspond to an entry in the table, Chromeleon cannot determine the CRP value. In this case, enter the appropriate CRP value manually.

Determine the CRP value as follows:

- Enter a CRP value that is close to your column (according to the table above).

- Enter a high nominal flow rate. Based on the entered CRP value, Chromeleon calculates the master flow. Usually, the actual system flow deviates from the nominal flow rate as it may happen that the entered CRP value is correct only by chance.

- Determine the current system flow by measuring the volume transported through the column within a specified time.

- Enter the measured system flow as **MeasuredFlowRate**. The CRP value will then be determined automatically according to the formula.

$$CRP = \frac{CRP_{entered} * Nom.Flow_{entered}}{SystemFlow_{measured}}$$

CRP and MasterFlow will then be corrected appropriately so that the resulting system flow will match the specified system flow.

Reference Information

Sample Variables

The variables in the sample list (see **Samples and Sequences** 🔳 **The Sample List (Sequence)**) default columns are referred to as sample variables. They characterize a sample and are stored in a database as part of the ➢ *Sample Data*. For an explanation of the columns, refer to:

⇒ *Auto Purif. Frac. (Autopurification Fraction)*

⇒ *Auto Purif. Ref. (Autopurification Sample Reference)*

⇒ *Auto Purif. Type (Autopurification Sample Type)*

⇒ *Comment* (Sample Comment)

⇒ *Dil. Factor* (Dilution Factor)

⇒ *GUID* (Globally Unique Identifier)

⇒ *Inj. Date/Time*

⇒ *Inj. Vol.* (Injection Volume)

⇒ *ISTD Amount* (Amount of the ➢ *Internal Standard*)

⇒ *Method* (QNT Method)

⇒ *Name* (Sample Name)

⇒ *No.* (Sample Number)

⇒ *Pos.* (Sample Position)

⇒ *Program* (also, refer to ➢ *PGM File*)

⇒ *Ref. Amount Set* (Reference Amount ID)

⇒ *Replicate ID*

⇒ *Sample ID*

⇒ *Std. Add. Group* (Standard Addition Group)

⇒ *Status* (Sample Status)

⇒ *Type* (Sample Type)

⇒ *Weight* (Sample Weight Factor)

In addition to these default columns, Chromeleon supports ➢ *User-defined Columns* and ➢ *Sequence Report Columns*.

Auto Purif. Frac. (*Autopurification Fraction*)

Type:	**Text**
Dimension:	**---**
Value Range:	**Digits**
Default:	**---**
Related Parameters:	⇒*Auto Purif. Ref*
	⇒*Auto Purif. Type*
	⇒*Replicate ID*
	⇒*Sample ID*
	⇒*Name*
	⇒*No.*
Description:	The **Auto Purif. Frac.** column indicates the ID for the associated fraction. Chromeleon determines the ID; it is read-only.
Function:	Chromeleon uses this column to number the fractionated samples. This allows Chromeleon to keep the order of the samples if they are moved to a different sequence or to a different folder.

Auto Purif. Ref. (*Autopurification Sample Reference*)

Type:	**Text**
Dimension:	---
Value Range:	**All printable characters**
Default:	---

Related Parameters: ⇒*Auto Purif. Frac*

⇒*Auto Purif. Type*

⇒*Replicate ID*

⇒*Sample ID*

⇒*Name*

⇒*No.*

Description: The **Auto Purif. Ref.** column indicates the autopurification ID. Chromeleon determines the ID; it is read-only.

Function: Chromeleon uses this column to associate samples of the different autopurification sample types to each other. Preparative samples (sample type: Preparation) and fractionated samples (sample type: Fraction) have the same autopurification reference ID as the analytical sample from which they originate.

Auto Purif. Type (*Autopurification Sample Type*)

Type:	**Text**
Dimension:	**---**
Value Range:	**Analytic, Preparation, Fraction**
Related Parameters:	⇒*Type*
	⇒*Auto Purif. Frac.*
	⇒*Auto Purif. Ref*
Default:	**---**
Description:	The **Auto Purif. Type** column and/or ⇒*Sample Variable* reports the type of the autopurification sample:

- **Analytic** for the original analytical sample.
- **Preparation** for a preparative sample.
- **Fraction** for injections from fraction or tubes generated from a preparative sample.

🖑 *Note:*

These values are automatically set during sample creation in the related post-acquisition step. They are read-only and cannot be changed.

Function:

Analytic   Analytic indicates an **analytical sample**. The sample is to be analyzed and its content of one or several substances is to be determined. Based on the analysis and on ➢*Post-Acquisition Steps*, Chromeleon automatically determines whether the sample shall be processed further.

Preparation   **Preparative samples** are used for fraction collection.

Fraction   **Fractionated samples** are used to check the pureness of the fractions.

👉 *Tip:*

To analyze the fractioned samples, Dionex recommends using chromatographic conditions other than those used for fraction collection.

Comment *(Sample Comment)*

Type:	**Text**
Dimension:	**---**
Value Range:	**All printable characters**
Default:	**---**
Related Parameter:	⇒*Name* (sample name)
Description:	In addition to the sample name, further comments can be entered. Press the F8 key to open an enlarged edit field.

Dil. Factor *(Dilution Factor)*

Type:	**Floating point value**
Dimension:	**---**
Value Range:	**0.0001 ... 999999.9999**
Default:	**1.0000**
Related Parameters:	⇒*Inj. Vol.* (injection volume)
	⇒*Weight* (Sample Weight Factor)
Description:	The ⇒*Sample Variable* **Dilution Factor** is a correction factor for amount calculation formulas. As is the injection volume, it can also be used for multi-point calibrations to define the dilution of subsequent calibration samples. For integration samples, it serves to account for any dilution that was made before the injection.
Function:	The calculated values for all peaks of a sample are corrected by the appropriate dilution factor.
Note:	*The effect is reciprocal to the injection volume; that is, a larger dilution factor indicates that less of the component is present.*

GUID *(Globally Unique Identifier)*

Type:	128-bit character string
Dimension:	---
Value Range:	*(Consists of hexadecimal characters.)*
Default:	---
Related Parameters:	⇒*Name*
	⇒*No.*
	⇒*Replicate ID*
	⇒*Sample ID*
Description:	Chromeleon creates a globally unique 128-bit ➢*GUID* in the GUID column when the analysis is started.
Function:	The GUID allows unique identification of each sample.

Also, refer to **Creating and Managing Files/Creating a Sample List (Sequence) 🔼 Using Globally Unique Sample Identifiers**.

Inj. Date/Time *(Time of Injection)*

Type:	**Character String**
Dimension:	**Month/Day/Year Hours:Minutes:Seconds**
Value Range:	**---**
Default:	**---**
Related Parameter:	**Time**
Description:	This column is read-only. Chromeleon enters the injection time and date of the sample in the corresponding column of the sample list. For samples with the ⇒*Status (Sample Status)* **M** (multiple), the time of the last injection is entered.
	Chromeleon stores the time stamps as universal time (Greenwich time). However, the date notation is displayed according to regional settings made in the operating system.
Function:	The kind of entry (empty or time value) indicates if the sample was processed and when the sample was processed.
Note:	*The time difference between successive samples is generally the analysis time plus the time required for injecting the following sample because the report is generated in parallel to the online batch. As the injection time is generally minimal, the time value provides a reliable indication as to whether the sample batch was processed smoothly, i.e., without an incident, such as power failure or third-party interference.*

Inj. Vol. *(Injection Volume)*

Type:	**Floating point value**
Dimension:	**µl**
Value Range:	**system-dependent**
Default:	**20.0**
Related Parameters:	⇒*Dil. Factor* (dilution factor)
	⇒*Weight* (Sample Weight Factor)
Description:	The ⇒*Sample Variable* **Injection Volume** defines the injection volume in micro liters (µl). In automatic operation, the installed ➢*Driver* converts this value into a volume readable by the autosampler, then the value is sent to the ➢*Autosampler*.
	You can enter different injection volumes to create a ➢*Dilution Series* for multiple-point calibration (➢*Single-Point and Multiple-Point Calibration*).
Function:	In multiple-point calibration with differing injection volumes, the concentration of the second calibration sample is calculated from the injection volumes of the first and the second sample. The same principle (doubling injection volume equals doubling the amounts of each component) is applied to all subsequent calibration samples.
	In a multi-point calibration with a "concentration series" (varying injection volume) which was created with an automatic autosampler it is therefore only necessary to enter the component amounts for the first standard sample into the peak table. Chromeleon calculates all additional calibration values, that is, the corresponding amounts of the subsequent standard samples (Type: **Standard**).
Note:	*In order to minimize carry-over effects in such a multiple-point calibration, standard order should <u>always</u> be from lowest to highest concentration.*

ISTD Amount *(Amount of the internal standard)*

Type:	**Floating point value**
Dimension:	**---**
Value Range:	**0.0001 ... 999999.9999**
Default:	**1.0000**
Related Parameters:	⇒*Dil. Factor* (dilution factor)
	⇒*Weight (Sample Weight Factor)*
	⇒*Inj. Vol.* (injection volume)
Description:	The ⇒*Sample Variable* **ISTD Amount** is only required for a calibration based on a variable ➢*Internal Standard* (internal or internal/external).
	In this case, the column serves in the sample list for entering the amount values of the internal standards used for the different samples. Input is directly in the sample list (via the keyboard or the F8 edit dialog box). Editing the column in the QNT Editor is not possible. Entering the amount values in the peak table is omitted.
Function:	The **ISTD Amount** parameter is implemented as a multiplication factor in the ➢*Amount Calculation Formula* and is dimensionless.

Method *(QNT Method)*

Type:	**File name**
Dimension:	**---**
Value Range:	---
Default:	**Name of QNT File**
Related Parameters:	⇒*Program* (PGM File)
Description:	The **Method** column (in the Browser) contains the name of the quantification method (see **The QNT Editor** 🔴 **The Quantification Method (QNT Editor)**).
	The quantification method includes all parameters that are used for evaluating a peak or the entire chromatogram.
Function:	The QNT Method serves as the basis for calculation for sample evaluation. It includes:
	⇒*QNT Parameters* All parameters required for (qualitative) peak identification and for converting the determined peak areas into amount or concentration values (quantitatively)
	⇒*Detection Parameters* All parameters regarding, for example, peak recognition and peak area evaluation
	➤*Calibration Variables* All parameters regarding the type and performance of a calibration
	➤*Blank Run Subtraction* Information about baseline subtraction
	Peak Tracking Parameters for comparing spectra to library or sequence spectra

Function: (Cont'd)	Spectra Library Screening Parameters for spectra library screening
	➢*System Suitability Test (SST)* Parameters for performing the System Suitability Test
	MS Parameters for ➢*Mass Spectra* evaluation

☞ ***Tip:*** *Normally it is not necessary to include the QNT Method in the sample list before the analysis starts. However, if you want to perform a System Suitability Test, make sure to enter the QNT File into the sample list before starting the analysis. Otherwise, the batch cannot be aborted in case of **Fail Action** - **Abort Batch** because the SST will not yet be performed during the batch run!*

Name *(Sample Name)*

Type:	**Character String**
Dimension:	**---**
Value Range:	**All printable characters**
Default:	**[Sample] [No.]**
Related Parameters:	⇒*No.* (sample number)
	⇒*GUID*
	⇒*Sample ID*
	⇒*Replicate ID*
Description:	The ⇒*Sample Variable* **Name** serves to identify a sample and to label graphics and reports.

 Note:

If the sequence contains ⇒*Sequence Report Columns, statistical values can be displayed in the last three lines of the sample list. In this case, the names of the statistical values appear in the Name column: Sum, Average Value, and/or Rel. Std. Dev. (relative standard deviation).*

Function:	The **Fill Column** function (F9) allows automatic sample name generation. For this purpose, the currently selected name can be copied or a character string with wild cards as template can be entered. These are names that might include, for example, the sample number (#n, refer to ⇒*No.*), the position (#p, refer to ⇒*Pos.*), the replicate number (#r), and the injection volume (#i, s. ⇒*Inj. Vol.*). Thus, telling names can be easily generated using these wild cards! The replicate number is calculated from the repetition of a sample number. Example: If two injections are made from the first two vials the template „Analysis-#p_Repl.#r" yields the following names:

Function:	Analysis-1_Repl.1
(Cont'd)	Analysis-1_Repl.2
	Analysis-2_Repl.1
	Analysis-2_Repl.2

When creating a sample list using the Sequence Wizard (see **Samples and Sequences** **The Sequence Wizard**), automatic sample name generation is possible as well.

 Note: *Each line represents an individual analysis. As multiple injections of the same sample also represent multiple analyses, a sample line must be reserved for each injection.*

No. *(Sample Number)*

Type:	**Nonnegative integer**
Dimension:	**---**
Value Range:	**1 ... infinite**
Default:	**Ascending number**
Related Parameters:	⇒*Pos.* (sample position)
	⇒*Name* (sample name)
	⇒*GUID*
	⇒*Sample ID*
	⇒*Replicate ID*
Description:	You cannot edit the sample number. Each new line in the sample list, that is, each analysis, is assigned its own number.

For a sample that is currently being processed (⇒*Status* = **Running**), the sample number is replaced with a green arrow:

📒 *Note:*

If the sequence contains ➢*Sequence Report Columns, statistical values can be displayed in the last three lines of the sample list. In this case, the symbols for the statistical values appear in the No. column.*

■☞ *Tip:*   *Each injection (or replicate) occupies its own line in the sample list and is, therefore, an individual analysis for which an individual raw data file is created.*

Pos. *(Sample Position - Sample List)*

Type:	**Nonnegative integer and/or letters**
Dimension:	**---**
Value Range:	**Depending on the autosampler**
Default:	
Related Parameter:	⇒*No.*
Description:	The ⇒*Sample Variable* **Pos**. determines the position of the sample in the ➢*Autosampler*.
	For externally controlled autosamplers, this value is automatically transmitted to the autosampler for sample processing. The autosampler approaches the corresponding sample for injection. For non-controllable autosamplers, this value is for documentation purposes only. If the sample position parameter is not entered, the previous (current) value is used.
Function:	Depending on the installed segment type, the ASI-100 Autosampler supports 63 position for semiprep vials, 66 positions for Eppendorf vials, 117 positions for analytical vials, or 192 positions for mini vials.
	Letters according to their color describe the individual segments: R, G, or B (indicating the red, green, and blue segment, respectively). The different rows are described from the outer to the inner row: A, B, C, or D. The individual positions within the respective rows are number counterclockwise. The position RA1, for example, is in the outer row of the red segment (also, refer to the Operating Instructions for the ASI-100 Series).
	Some autosamplers and fraction collectors, e.g., the Dionex SFM (Sample and Fraction Manager) have several trays installed. In this case, you have to enter the **Sample tray** and **Vial** to make sure that the position is defined unambiguously.

Program *(PGM File)*

Type:	**File name**
Dimension:	**---**
Value Range:	---
Default:	**Name of PGM File**
Related Parameter:	⇒*Method* (QNT Method)
Description:	The column serves to name the control program. (For information about control program, see **Control 🔳 The Control Program**.)
	The file contains all information for chromatographic sample processing. These are commands for chromatography devices (flow, %B, etc.), analysis time, acquisition time, etc.
	For more information, refer to ➢*PGM File*.
	Input for a sequence is by directly editing the column in the Browser. All program files that are part of the sequence are listed.
Function:	The sample is processed according to the conditions contained in the PGM File.

Ref. Amount Set *(Reference Amount ID)*

Type:	**Text**
Dimension:	---
Value Range:	**All printable characters**
Default:	---
Related Parameter:	⇒*Std. Add. Group*
Description:	The **Ref. Amount Set** ⇒*Sample Variable* allows you to assign standards, ➢*Validation Samples*, and/or ➢*Spiked Samples* to the ⇒*Amount* columns of the QNT Editor.

Enter the same name for standard and validation samples for which the concentration (amount) is identical. Proceed in the same way for spiked samples.

👉 *Tip:*

If you analyze several samples using the ➢Standard Addition method, Dionex recommends adding identical amounts of the same substances to the same sample volume.

Function:	To assign the samples to the **Amount** columns, select **Columns > Edit Amount Columns** on the context or **Edit** menu in the QNT Editor.

In the **Edit Amount Columns** dialog box, select **Ref. Amount Set** from the **Assign Standards on the basis of:** drop-down list. The entries of the **Ref. Amount Set** sample column are then used to assign amount columns.

To assign one **Amount** column to each level of standard, validation, and/or spiked samples, click **Auto-Generate**, and then select **Generate a separate amount column for EACH standard** from the list.

For an application example, refer to **How to …: Calibrating 🔳 Standard Addition**.

Replicate ID

Type:	**Text**
Dimension:	**---**
Value Range:	**All printable characters**
Default:	**---**
Related Parameters:	⇒*Name* (sample name)
	⇒*No.*
	⇒*GUID*
	⇒*Sample ID*
Description:	The **Replicate ID** column serves to mark samples as ➤*Replicates*.
Function:	This column is a mere text column and not used for evaluation purposes. If a sequence is generated using the Sequence Wizard (see **Samples and Sequences** 🔼 **The Sequence Wizard**), the sample position is entered automatically as replicate ID.
	To group samples with identical replicate IDs in the **Summary** report, select the **Table Properties** command on the context menu to open the **Peak Summary Property** dialog box. Select **Sort the report table according to the evaluation of this formula 'smp.replicate'**. It is also possible to access the replicate ID for a query (see **How to …: Creating and Managing Files and Data** 🔼 **Performing a Query**).

Sample ID

Type:	**Text**
Dimension:	**---**
Value Range:	**All printable characters**
Default:	**---**
Related Parameters:	⇒*Name* (sample name)
	⇒*No.*
	⇒*GUID*
	⇒*Replicate ID*
Description:	The user or a help program enters a sample ID in the Sample ID column.
Function:	This column is a mere text column and not used for evaluation purposes. The sample ID is intended for LIMS connections that usually know sample IDs.
	To group samples with identical sample IDs in the **Summary** report, select the **Table Properties** command on the context menu to open the **Peak Summary Property** dialog box. Select **Sort the report table according to the evaluation of this formula 'smp.ident'**. It is also possible to access the replicate ID for a query (see **How to …: Creating and Managing Files and Data Performing a Query**).

Std. Add. Group *(Standard Addition Group)*

Type:	**Text**
Dimension:	**---**
Value Range:	**All printable characters**
Default:	**---**
Related Parameter:	⇒*Ref. Amount Set*
Description:	The **Std. Add. Group** ⇒*Sample Variable* allows you to assign ➢*Spiked Samples* to unspiked unknown samples.
	Enter a group name into this column. Make sure that the group name is identical for the unspiked unknown samples and the associated spiked samples.
Function:	The **Std. Add. Group** variable is required to analyze several unspiked samples using the ➢*Standard Addition* method. For more information, refer to **How to …: Calibrating 🔼 Standard Addition**.

Status *(Sample Status)*

Type:	**Text**
Dimension:	**---**
Value Range:	**Single/Multiple/Finished/Interrupted/Running/ Preparing**
Default:	**Single**
Related Parameter:	⇒*Inj. Date/Time (Time of Injection)*

Description: The ⇒*Sample Variable* **Status** determines the current sample processing status. A sample is unprocessed (Single), due for multiple processing (Multiple), processed (Finished), or currently being processed (Running).

Chromeleon also maintains a log of the sample status, that is, a **Single** sample is automatically assigned the status **Finished** as soon as processing is complete. A sample may also be excluded from processing by assigning the status Finished manually.

The sample status has a special significance for the built-in ➢*Power Failure Protection*. Upon recovery from a power failure or starting the sample batch after a manual interruption, Chromeleon continues processing the sample batch according to the selected power failure handling option.

Functions:

    Single The sample is processed only once, after which it receives the status Finished. When loading a sample list for processing (online), only samples with the status Single and Multiple are processed.

If a Single sample contains an entry in the time column, this indicates that the sample was injected but was interrupted before completion.

Multiple
(Not available for Chromeleon XPress)
The sample can be repeatedly acquired. The system just enters the ⇒*Inj. Date/Time (Time of Injection)* in the **Time** column. Existing ➢*Raw Data* are overwritten with each new data acquisition. New samples can have the status Multiple assigned only if the **Enable Sample Status Multiple** option of the corresponding datasource is enabled. The option is available in the (datasource) **Properties** (via the Properties command on the context menu) on the **General** tab page. Samples that have the status Multiple already assigned keep their status even with disabled **Enable Sample Status Multiple** option.

Finished
All original Single samples are automatically assigned the status Finished after successful processing. A sample with the status Finished will be omitted in the renewed processing (also following a ➢*Power Failure*). In ➢*GLP* ➢*Datasources*, it is not possible to reset the sample type Finished to Single.

Interrupted
If the user stops a running batch via **Immediate** while a sample is being processed of if an error occurs during the sample run resulting in an abort, the corresponding sample is labeled **Interrupted**.

After a power failure or a server breakdown the sample is started again if you selected the **Continue with interrupted sample option** on the **Error Handling** tab page. (Access the **Error Handling** tab page by selecting the **Error Handling** command on the **Batch** menu.)

Running
A running sample is marked green. When the sample has been processed, it gets the status **Finished** or **Multiple** assigned (without background color).

Tip:

*You can also open samples during the analysis, while their status is **Running** (see **Integration Opening a Sample**).*

Preparing	The autosampler prepares the next sample while the current sample is Running. The sample being prepared is marked yellow.

 Note:

*This status occurs, for example, when an AS or AS50 autosampler that has the **Sample Overlap** option enabled is preparing a sample. The Sample Overlap option is enabled in the AS or AS50 Server Configuration Properties on the Options tab page.*

Also, refer to **Practical Tips for Device Control** **Overlapping Samples**

Tip:	*Multiple status samples will be acquired each time. The raw data, therefore, are not protected from accidental overwriting. The **Time** column indicates, however, whether and when the sample was last injected. If this column is empty, the sample has not yet been acquired at all.*

Type *(Sample Type)*

Type:	**Text**
Dimension:	**---**
Value Range:	**Blank, Unknown, Standard, Validate, Matrix, Spiked, Unspiked**
Related Parameters:	**Amount, Baseline Subtraction**
Default:	**Unknown**
Description:	The **Type** ⇒*Sample Variable* indicates the sample type:

- Select **Unknown** for an unknown analysis sample: .

- Select **Standard** for a standard sample with known concentration: .

- Select **Validate** for a ➤*Validation Sample*: .

- Select **Blank** for a ➤*Blank Run Sample*: . If a sample is corrected by ➤*Raw Data* of a blank run sample, this is referred to as ➤*Blank Run Subtraction*.

- Select **Matrix** for a ➤*Matrix Blank Sample*:

- Select **Spiked** for a ➤*Spiked Sample*: .

- Select **Unspiked** for an unspiked unknown sample to be analyzed with the Standard Addition method: .

Functions:	
Blank	The sample injected with the ⇒*Inject* command is no "real" sample; it is a **Blank Run**. In this case, the baseline is stored for subsequent subtraction from a later sample. Thus, if baseline subtraction is enabled for a subsequent sample, the **baseline chromatogram** may be subtracted before integration.

Blank
(Cont'd)

🖙 *Tip:*

If an ICS 1000/1500/2000 Ion Chromatography System does not have an AS or AS50 installed, injection is performed with the **Pump_InjectValve.InjectPosition** command. Unlike the autosampler ⇒Inject command, the **Pump_InjectValve.InjectPosition** command always performs an injection, even for *a* ➢Blank Run Sample.

Unknown The (unknown) sample is analyzed to determine whether it contains one or more substances.

Standard The sample is a **calibration standard**. The corresponding ⇒*Amount* values of the single peaks are listed in the corresponding **Amount Table** column.

Validate **Validation samples** are treated as ordinary analytes. However, the type is written to the result file. The validation sample references the **Amount** column in the subordinated peak table. The **Amount** column referenced by such a validation sample contains the required amount or concentration for each peak of the sample. In the report, this amount or concentration can be compared with the calculated actual amount or concentration. The **Amnt. Diff.** (amount difference) and **Rel. Amnt. Diff.** (relative amount difference) peak variables are available for this.

Matrix Contrary to **Blank Run** samples, **Matrix Blank Samples** are "real" samples that are injected. Chromeleon automatically subtracts the peak areas or peak heights of the matrix blank sample from the corresponding peak areas or peak heights of all samples in the sequence. The resulting areas (heights) are then used for all other calculations, such as calibration. However, the respective peaks must have been identified for both the unknown sample (or standard sample) and the **Matrix Blank Sample**.

Matrix
(Cont'd)

 Note:

Thus, matrix blank samples are treated differently from "normal" ➢Blank Run Samples. For normal blank run samples, the chromatogram is subtracted point by point from the chromatogram of the current sample.

 Tip:

Matrix blank samples are subtracted only if they are evaluated in the same QNT Method. Otherwise, they are not considered.

Spiked
A known amount is added to an unspiked sample (see **Unspiked**). The spiked sample is used to analyze the original unspiked sample, using the ➢*Standard Addition* method.

Unspiked
Known amounts of the analytes to be determined are added to unspiked unknown samples. Afterward, the original (**Unspiked**) and the **Spiked** samples are analyzed. The quantitative analysis of the results is performed, using the Standard Addition ⇒*Calibration Mode*, i.e., the ➢*Standard Addition* method.

 Tip:

*If you use the **Spiked** and/or **Unspiked** sample ⇒Types, please keep in mind that:*

- *An ➢Electronic Signature created with Chromeleon 6.50 or earlier is invalid in Chromeleon 6.60 or higher.*

- *An electronic signature created with Chromeleon 6.60 or later is invalid in Chromeleon 6.50 or earlier.*

Weight *(Sample Weight Factor)*

Type:	**Floating point value**
Dimension:	**None**
Value Range:	**0.0001 ... 999999.9999**
Default:	**1.0000**

Related Parameters: ⇒*Dil. Factor* (dilution factor)

⇒*Inj. Vol.* (injection volume)

Description: The ⇒*Sample Variable* **Weight** has two functions. It serves to enter the sample weight but it can also be used as a weight correction factor.

1. **Sample Weight**: Enter the sample weight to calculate the content - normalized to the basic unit - of a substance in a sample

   For example, if the calculated concentrations should always be valid for 1 mg of a sample, enter the actual weight; for example, 124.08. Chromeleon then divides all calculated concentrations by 124.08.

2. **Weight Correction Factor** (for Standard and Validation samples)**:** If you wish to relate the concentrations to be calculated to a specific concentration, enter the corresponding correction factor as **Weight**.

   If, for example, 4.16 mg were weighed instead of 4.0 mg, you must enter 1.04 (not 4.16!) here. This procedure is called **approximate exact weighting**.

Function: The **Weight** parameter is implemented as a dimensionless multiplication factor or divisor:

1. It is used as factor for calculating the calibration curve (for the formulas, see **Theory of Calibration** **Evaluation with Various Standard Methods**).

2. It is used as divisor for amount calculation (see ➤*Formula for Amount Calculation*).

Control Commands

On the following pages, the most important control commands and device properties are described, together with their corresponding parameters. The commands and parameters that are actually available depend on the individual installation. For example, if a simple UV detector is installed, it is not possible to record a 3D field.

📖☞ *Tip:*

An extensive explanation of all existing commands for all devices that can be controlled is far beyond the scope of this reference manual. About 100 commands are available only for the 6890 GC!

For examples on the commands for various devices, refer to:

⇒*General Commands*

⇒*System Commands*

⇒*Pump Commands*

⇒*Autosampler Commands*

⇒*Detector Commands*

General Commands

The following general commands are available regardless of the installed devices:

⇒*Branch*
⇒*Delay*
⇒*Else*
⇒*ElseIf*
⇒*End*
⇒*EndIf*
⇒*EndTrigger*
⇒*If*
⇒*Log*
⇒*Message*
⇒*Protocol*
⇒*Trigger*
⇒*Wait*

System Commands

System control comprises all commands that concern the entire chromatographic process or the entire system. Some system commands are available on the **Online** toolbar and on the context menu. The most important system commands are:

⇒*Abort Batch*
⇒*AbortSample*
⇒*Acquisition On/Off*
⇒*Continue*
⇒*Hold*
⇒*Sound*
⇒*StopFlow*

Also, refer to **Control 🔼 System Commands**.

Pump Commands

For more information about the most important pump commands, refer to:

⇒*%B, %C, %D*
⇒*%A, %B, %C, %D_Level*
⇒*%A-, %B-, %C-, %D_RemainTime*
⇒*%A, %B, %C, %D_WarningLimit*
⇒*Connect/Disconnect*
⇒*Delta*
⇒*Flow*
⇒*Freeze*
⇒*Learn*
⇒*ParkPercentage*
⇒*Pressure.Lower/UpperLimit*
⇒*Purge*
⇒*WasteLevel*
⇒*WasteRemainTime*
⇒*WasteWarningLimit*

Autosampler Commands

For information about the most important autosampler commands, refer to:

⇒ *Connect/Disconnect*

⇒ *Dispense*

⇒ *Draw*

⇒ *Inject*

⇒ *Mix*

⇒ *NeedleUp*

⇒ *Position*

⇒ *Ready*

⇒ *Reset*

⇒ *Relay On/Off*

⇒ *Temperature*

⇒ *Volume*

⇒ *Wash*

Detector Commands

For more information about the most important detector commands and properties, refer to:

⇒ *AcqOn/Off*

⇒ *Autozero*

⇒ *Average*

⇒ *Bandwidth*

⇒ *Bunch Width*

⇒ *Connect/Disconnect*

⇒ *Delta*

⇒ *Lamp*

⇒ *LampAge*

⇒ *LampIgnitions*

⇒ *LampIntensity*

⇒ *MaxAutoStep*

⇒ *MinLampIntensity*

⇒ *RefBandwidth*

⇒ *RefWavelength*

⇒ *Retention*

⇒ *Signal.Value*

⇒ *Step*

⇒ *Wavelength*

%B, %C, %D *(Solvent Components)*

Instrument Type:	**Pump(s)**
Related Commands:	⇒*Flow*
	⇒*%A, %B, %C, %D_Level*
Description:	The solvent and thus the flow usually consist of different individual components. The respective amounts of the partial flow are indicated in percent of the flow. The total sum of all partial flows (solvent components) is 100% (%A+%B+%C+%D=100%), where %A is calculated from the remaining partial flows (%A=100%-(%B+%C+%D)). It is therefore sufficient to determine the values for %B, %C, and %D.
	Changing the solvent composition during the analysis is referred to as gradient (or more exactly as ➢*%-Gradient*).
	%A, %B, %C, and %D define the **partial flow rates** of the individual pumps in a high pressure mixing system (as %-values of flow), or the partial flow rates on the suction side of a low pressure mixing system. The latter generally refers to a single pump or a separate mixing vessel with controlled proportioning valves. The formation of %-gradients is by the same principle as for Flow.
	For each ➢*Timebase*, one or more fluidic systems can be installed. They can be controlled independently, either manually or via a ➢*Program*.
	In case a second fluidic system is installed, the corresponding device name has to be specified in the program. Use the F8 key or the Program Wizard to insert the device name automatically.
☞ *Tip:*	*The ⇒Hold, ⇒Continue, and ⇒StopFlow commands are always effective for all fluidic systems.*

Function:

With ➢*Ramps*, the partial flow rates of A, B, C, and D change linearly with time between two %A, %B, %C, or %D commands. By entering two percent commands at the same time, you can program ➢*Step Gradients*. When sorting, the editor does not change the order of commands having the same program time! If the partial flow rates are to remain constant during analysis, that is, isocratic, the appropriate %A, %B, %C, and/or %D commands need only be entered at the beginning of the file.

```
-2.000 Flow =           1.000
         %B =           20.0
... ...
 0.000 Inject
 0.500 %B =             20.0
        %B =            40.0
... ...
 8.500 %B.Value =       80.0
 8.500 End
```

In this example, the total flow rate remains constant at 1.0 ml/min. %B also remains constant, at 20% from -2.0 to 0.5 min, then increasing abruptly to 40%, then linearly to 80% with a gradient of 5%/min.

Parameters:

Value	Partial flow of the solvent component [%]
Equate	Name of the solvent component
Type (P580 only)	Solvent type of the respective component

🖙 *Tip:*

Dionex recommends the following setting:
Isocratic and LPG pumps: Automatic
HPG pumps: Custom

With HPG pumps, set the pre-compression control via the ⇒Learn and ⇒Freeze commands.

Tips: *Chromeleon is able to run flow and %-gradients simultaneously. However, in high pressure mixing systems, this results in non-linear partial flow rate changes for each individual pump.*

Note that due to compressibility and dead volume considerations, high pressure and low-pressure applications are not always interchangeable!

The %-values remain constant from the last %-command to the end of the program. Thus, for isocratic operation, a single %-entry at the beginning of the file is sufficient.

%A, %B, %C, %D_Level *(Solvent Volume)*

Instrument Type:	**Pump(s)**
Related Commands:	⇒*%A, %B, %C, %D_WarningLimit*
	⇒*%B, %C, %D*
	⇒*Flow*
	⇒*WasteLevel*
Description:	This command monitors solvent consumption.
Function:	Before starting a sequence, enter the existing volume of each solvent component. Based on the flow rate and the solvent composition, Chromeleon calculates whether the amount of available solvent is sufficient for the sequence.
Parameters:	

Value   Available volume of the corresponding component in [l].

LowerLimit   Lower limit for the corresponding component in [l]. If this value is reached, the batch is aborted and the following message appears:

[Abort] xx:xx:xx {Pump} The %A level is below the limit (x l)! Please refill %A.

🖘 *Tip:*

In the emergency program, reduce the flow rate to 0 ml/min. This ensures that the pump and the detector cell never run dry.

🖘 *Tip:*   *Chromeleon checks the expected demand for solvent during the ➢Ready Check. If not enough solvent is available, a warning is displayed.*

📄 *Note:*

This command is not available for all ion chromatography pumps.

%A, %B, %C, %D_RemainTime

Instrument Type:	**Pump(s)**
Related Commands:	⇒*WasteRemainTime*
	⇒*%A, %B, %C, %D_Level*
	⇒*%A, %B, %C, %D_WarningLimit*
Description:	Reports approximately how much time remains until the associated solvent reservoir reaches the lower limit (**%X_Level.LowerLimit**).
Function:	Chromeleon calculates the time remaining based on the current flow rate and the specified liquid level in the solvent reservoir.

🖝 *Tip:*

If you change the flow rate, Chromeleon will automatically recalculate the time remaining. Therefore, check this value whenever you change the flow rate.

📝 *Note:*

This command is available for all pumps except for ion chromatography pumps.

%A, %B, %C, %D_WarningLimit

Instrument Type:	**Pump(s)**
Related Commands:	⇒*%A,%B,%C,%D_Level*
	⇒*WasteWarningLimit*
Description:	This command allows you to enter a warning limit for monitoring the liquid level in the associated solvent reservoir. The input is entered as a percentage and refers to the lower liquid limit (**%X_Level.LowerLimit**).
Function:	If the liquid level in the solvent reservoir is below the lower limit plus the warning limit (**%X_Level.LowerLimit** + **%X_WarningLimit**), the following warning appears:

[Warning] xx:xx:xx {Pump} The %A level (x l) will reach the lower limit in about x.x hours! Please refill %A.

☞ *Tip:*

If the liquid level reaches the %X_Level.LowerLimit, the batch is aborted. In the emergency program, reduce the flow to 0 ml/min. This ensures that the pump and the flow cell never run dry.

📙 *Note:*

This command is not available for all ion chromatography pumps.

AbortBatch

Instrument Type:	**System Command**
Related Commands:	⇒*AbortSample*
Description:	The **AbortBatch** command aborts a running sample batch. In combination with the ⇒*Trigger* command, this command allows you to react, for instance, to external errors. Example:

0.000 Trigger   Cond=RemErr

      AbortBatch

      EndTrigger

Function:	**AbortBatch** terminates data acquisition, deletes all triggers, and aborts the current sample batch. If you have specified that Chromeleon should run an emergency program after **AbortBatch**, Chromeleon starts the program automatically (see **How to … Creating and Modifying Programs** 🔳 **Specifying an Emergency Program**).

AbortSample

Instrument Type:	**System Command**
Related Commands:	⇒*AbortBatch*
Description:	The **AbortSample** command interrupts data acquisition and aborts a running sample.
Function:	The batch continues with the next sample.
	Use this command, for example, if, by mistake, no injection was made for a sample.

AcqOn/Off *(Data Acquisition On/Off)*

Instrument Type:	**System Command, all detectors**
Related Commands:	⇒*Inject*
Description:	The **AcqOn/Off** command enables or disables data recording (➢*Raw Data*) for the selected ➢*Signal* (= channel) of a timebase. Each signal (or channel) is stored in a separate file. Signal parameters define the type of data from each signal.
Function:	To reduce raw data storage requirements, issue the **AcqOn** command some time after the **Inject** command. However, be sure to issue the command at least 30 seconds before the elution of the first peak. The reason is that Chromeleon requires a baseline segment of several seconds in order to perform a noise analysis for the auto-optimized step control functions (⇒*Step*, ➢*Sampling Rate*, or ➢*Data Collection Rate*).
	You can issue the command either manually using the **AcqOn/Off** command or automatically via the ➢*Program*.
	⇒*Trigger* commands whose conditions depend on signal values or their slope are effective during data acquisition only.
📖☞ *Tip:*	*You can change the parameters that are defined in the program, such as Step and ⇒Average at any time. For clarity, however, Dionex recommends observing the following command sequence:*

```
-2.000 Flow = 1.000

 ...   ...

 0.000 Inject

 0.500 UV_VIS_1.AcqOn

 ...   ...
```

```
 3.270 UV_VIS_1.Wavelength = 275

 ...   ...
12.000 UV_VIS_1.AcqOff

13.500 End
```

Do not change the 3DFIELD signal parameters between AcqOn and AcqOff.

*Use the ⇒Hold and ⇒StopFlow commands to interrupt data acquisition. Use the ⇒Continue command to resume acquisition. To complete raw data acquisition, select the **AcqOff** command.*

Autozero

Instrument Type:	**All detectors**
Related Commands:	**---**
Description:	The **Autozero** command resets physical or ➢*Virtual Signals* to zero.
Function:	All data recorded after an autozero is interpreted and displayed in relation to the new zero point. A sharp increase of the absorption value indicates this in the chromatogram.
	The Autozero command applies to all signals delivered by a single detector. Thus, autozeroing a **diode array detector**, such as the Dionex **UVD 340** detector, causes the entire **3DFIELD** to be zeroed. Autozero applies to *one* detector only! If several detectors are present in one system, for example, connected in series, they must be autozeroed individually.
☞ *Tip:*	*Usually, a jump is observed in the baseline after an autozero. That is why Dionex recommends performing autozero before data acquisition, unless this is specifically required during the analysis; for example, after wavelength switching.*
	If a wavelength switch is triggered, a ⇒triggered autozero command should follow! Example:

```
0.000 Inject
0.100 Trigger SwitchWave  UV_VIS_1 >
20
      UV_VIS_1.Wavelength = 280
      Autozero
      EndTrigger
0.500 UV_VIS_1.AcqOn
```

Average

Instrument Type:	**Detector**
Default:	**ON**
Related Commands:	$\Rightarrow$*Step*

Description:	The **Average** parameter allows averaging signals. Signal averaging is possible for both, digital signals sent to the server PC from the detector, such as the Dionex UVD 170U and UVD 340U detectors, and analog signals recorded via the A/D converter.
	The Dionex A/D converter records each analog signal with a frequency of 100 Hz. This corresponds to a $\Rightarrow$*Step* of 0.01 seconds or a $\triangleright$*Sampling Rate* of 100 data points per second. When increasing the step or decreasing the sampling rate, less data points are stored than theoretically possible.
Function:	When activating the **Average** signal parameter, the data points between the stored values are considered as well. Chromeleon averages all measured values that are within a step interval. The calculated average is stored in the $\triangleright$*Raw Data* file. This generally improves the $\triangleright$*Signal-to-Noise Ratio*. (Select the **Average** parameter to smooth chromatograms with an increased noise level.) This parameter does not influence the precision of integration.
Tip:	*Averaging is always performed in a 3D field.*
	For almost all detectors, concentration is proportional to peak-area; that is, it is integral of the signal over time. That is why local (time) signal averaging does not influence quantitative determination.

Bandwidth

Instrument Type:	**Detector**
Type:	**Integer**
Value Range:	**Detector-dependent**
Default:	**0 [nm]**
Related Commands:	⇒*Wavelength*
	⇒*RefBandwidth (Reference Bandwidth)*
	⇒*BunchWidth*
Description:	The **Bandwidth** command specifies the optical bandwidth [in nm] with which a chromatogram (UV VIS channel) is recorded. In general, this corresponds to the ➢*Optical Resolution* of a detector.
Function:	You can increase the bandwidth by averaging several single photodiode signals. This process is known as **Photodiode Bunching**. Averaging is performed symmetrically to the selected wavelength. Thus, at a bandwidth of 30 nm and a wavelength of 255 nm, the signals of all photodiodes between 240 and 270 nm are averaged.
☛ *Tip:*	*Changing the bandwidth can often increase the sensitivity. Quadrupling the bandwidth almost halves the noise. However, in this case, linearity usually decreases.*

Branch

Instrument Type:	**Processed in PC**
Related Commands:	---
Description:	The **Branch** command allows you to start a different program either from the active ➢*Program* or on a ➢*Control Panel*. In order to start the program on a control panel, the **Branch** command needs to be assigned to a **Script Button**. For example, you can combine the **Branch** command with the ⇒*Trigger Commands* to define dynamic program runs.
Function:	If a **Branch** command is issued while a program is running, the execution of the active control file is stopped. The newly selected control file is used instead. The times in the new control file are automatically corrected by the program time that already passed.

Example1 (within a program):

```
0.000 Trigger        Pressure > 300

      Branch         "Overpressure"

      EndTrigger
```

If the pressure exceeds 300 bar (30 MPa = 4350 psi), the **Overpressure** program is started. You can use the Overpressure program, for example, to slow down the flow, turn off the lamp, and deliver the ⇒*Message* that the pressure within the system was too high and thus, the flow has been slowed down.

☞ *Tip:*

*In example 1, make sure that the program to be started via the **Branch** command is stored in the sequence to be processed. If the program is not stored in the sequence, specify the location in the program. As with the Script Button, use slashes to separate the different levels.*

Function: *(Cont'd)*	**Example 2** (as script button on a control panel):
	Add a **Script Button** to the Control Panel (see **How to …: Controlling Devices from the Control Panel** **Creating a Script Button**). Branch to the selected program by assigning the Branch command to the button.

```
Branch
"CM_Seminar/Programs/Equilibration"
```

Click the button to start the column equilibration program that is stored in the **CM_Seminar** ➢*Datasource* in the **Programs** directory.

Parameter:	
Program	Name of the program file used for further processing
Tip:	*When making a backup of a sequence, all PGM Files that are included in the sequence are also saved automatically. Therefore, to ensure that PGM Files branched to via a Branch command are automatically added to your sequence backups, verify that they are saved in the corresponding sequence.*

BunchWidth *(Distance between Wavelengths)*

Instrument Type:	**Detector**
Type:	**Fixed point value**
Value Range:	**1.9 ... 197.6 [nm]**
Default:	**1.9 [nm]**
Related Commands:	⇒*Bandwidth*
Description:	The distance between the wavelengths of a 3D field is referred to as BunchWidth. To enhance the ➢*Signal-to-Noise Ratio* of a ➢*Photodiode Array Detector*, the signals of several photodiodes can be averaged (or bunched).
Function:	The **BunchWidth** is closely linked to the **Bandwidth**. For Dionex detectors, the **Bandwidth** is calculated automatically from the **BunchWidth**. Thus, you do not need to determine the **Bandwidth** for the 3D field of Dionex detectors.
	If a detector, for example, has 80 photodiodes distributed on a wavelength range of 160nm, the (theoretical) optical resolution (= Bandwidth) is 2 nm. If the BunchWidth is set to 8 nm, 8 : 2 = 4 photodiodes are averaged to one signal.
📖☞ *Tips:*	*Averaging the signals improves the signal-to-noise ratio. However, at the same time, ➢Optical Resolution decreases.*
	For certain instrument, the settings cannot be changed during the sample run. Thus, it may happen that commands included in the branched program are not performed.

Connect/Disconnect

Instrument Type: **All**

Related Commands: **---**

Description: Perform the **Connect Device** command to connect a device with the server to enable remote control.

Execute the **Disconnect** command to separate a user PC (client) from a timebase or to operate an instrument locally.

Functions:

Connect   The command checks whether the specified device is actually connected, and then turns on the instrument. For all installed instruments, the **Connect** command is executed automatically when the Chromeleon server is started. Thus, if working in remote operating mode only, you do not need to explicitly issue this command.

When the **Connect** command is enabled, the corresponding instrument may now be remotely operated from the PC. On most instruments, including all Dionex devices, the instrument keyboard is now locked for safety (and ➢*GLP*) reasons. The instrument can be operated remotely only. This is to ensure that the selected settings are retained. Input on the instrument itself is possible again after the **Disconnect** command.

Disconnect   The corresponding instrument reverts to local operating mode and the keyboard is unlocked again. The instrument is no longer monitored by Chromeleon, nor can it be operated via the data system.

📧☞ *Tip:*   *Connect and Disconnect should only be used in interactive (online) mode and not within a ➢Program. Otherwise, the ➢Ready Check may not be valid.*

Continue

Instrument Type:	**System Command**
Related Commands:	⇒*StopFlow*
	⇒*Hold*
Description:	The **Continue** command cancels the **Hold** and **StopFlow** commands.
Function:	An interrupted sample ➤*Batch* is continued in the same way as is an interrupted pump flow.

Delay

Instrument Type:	**System Command**
Related Commands:	⇒*Trigger*
Description:	Delays the execution of the following commands for the given time.
🖙 *Tip:*	*Delay is usually used for Trigger commands, only. Example:*

```
Trigger PEAK  UV_VIS_1 > 20
Delay  5.0
Sound  Frequency = 440, Duration = 1
EndTrigger
```

With this program, an acoustic beep sounds 5 seconds after the signal has exceeded 20 mAU.

Delta

Instrument Type:	**Detector**
Related Command:	⇒*Trigger*
Description:	Delta is a signal property that is accessible (read-only) via the ⇒*Log* command. Delta indicates the difference between the current signal value and the value measured one second ago.
	Delta is especially useful when creating complex trigger conditions. A peak cannot only be recognized by the height of its absorption signal within a chromatogram, but also, for example, by a sharp increase of the signal.

 Note:

The delta is calculated once per second independent of the ➢Sampling Rate or the ⇒Step used for data acquisition.

Function:	Delta is used especially for trigger conditions. The syntax is as follows:

<Signal.Delta><comparison operator><value>

Example:

```
Trigger PEAK   UV_VIS_1.Delta > 0.1
Sound Frequency=440, Duration=1
EndTrigger
```

With this program, an acoustic beep sounds after a peak has been detected (exactly: if the UV_VIS_1 signal ascends by more than 0.1 mAU in one second).

 Note:

Select the reference value individually depending on the peak height and the baseline noise.

Tip:	*For another example of a trigger condition, refer to* ***How to …: Practical Tips for Device Control*** *Trigger Commands.*

Dispense

Instrument Type:	➤*Autosampler*
Related Commands:	⇒*Inject*
	⇒*Draw*

Description:

The **Dispense** command causes the autosampler to dispense a specific quantity (volume) from the sample loop into a certain sample vial (⇒*Position*). The amount of time the autosampler may take for this operation is determined via the ➤*Duration* parameter.

When the operation is completed, the autosampler communicates the **Sampler.Ready** signal (for the ASI-100 Autosampler; ➤*Sucked* for the GINA 50/GINA 160 Autosamplers) to Chromeleon. The time interval between the **Dispense** command and the **Sampler.Ready** response signal can vary, depending on the instrument (ASI-100/GINA 50).

Parameters (GINA50 only):

Position	Sample position
Volume	Sample volume
Duration	This parameter indicates the minimum time required by the autosampler for the respective operation.

☞ *Tips:*

For highly viscous liquids, more time must be allowed for the autosampler to dispense the exact volume.

In manual operation, the commands are selected via the control pull-down of the unit window. When entering the commands to a ➤Program, the three parameters need not be specified. During execution of a PGM File, the missing parameters are replaced by the current sample position, the current inject volume, and by the value 0 (if there is no duration), respectively.

Draw

Instrument Type:	➢*Autosampler*
Related Commands:	⇒*Inject*
	⇒*Dispense*
Description:	The **Draw** command (**Draw** for the ASI-100, **Suck** for the GINA 50/GINA 160) induces the autosampler to draw a specific ⇒*Inj. Vol.* (injection volume) from a certain sample vial (⇒*Pos.* (Sample position)). The amount of time this operation is allowed to take is determined for the GINA50 autosampler via the ➢*Duration* parameter.
Function:	When the operation is completed, the autosampler communicates the **Sampler.Ready** signal (for the ASI-100; **Sucked** for the GINA 50/GINA 160) back to Chromeleon. The time interval between the **Draw** (or **Suck**) command and the **Sampler.Ready** (or **Sucked**) response signal can vary, depending on the instrument type.

Examples:

ASI-100 Autosamplers: The following command sequence delivers 10 µl of the current sample to the RA1 vial. The **Dispense** command is not executed until the Draw process has been completed.

```
-1.000  PrepSubject    Sample_Vial
        PrepVolume     10
        Draw
        Wait           Sampler.Ready

-1.000  PrepVial       RA1
        PrepSubject    PrepVial
        PrepVolume     10
        Dispense
```

Function: (Cont'd)	GINA 50 autosampler: The following command sequence is required to deliver 10 µl of the current sample to the vial 1. Again, the Dispense command will not be executed until the Draw process is completed.

```
-1.000   Draw    Volume=10
   Wait    Sampler.Ready
   Dispense Pos=1, Volume=10
```

Parameters (GINA 50 only):

Position	Sample position
Volume	Sample volume
Duration	This parameter indicates the minimum time required by the autosampler for the respective operation.
☞ *Tips:*	*For highly viscous or low-boiling liquids, more time must be allowed for the autosampler to draw the exact volume <u>without bubbles</u>.*
	In manner operation, the commands are selected via the control pull-down of the unit window. When entering the commands in a ➢Program, you do not have to specify the three parameters. During execution of a program, the missing parameters are replaced by the current sample position, the current inject volume, and by the value 0 (if there is no ➢Duration), respectively.

For more information, refer to **Practical Tips for Device Control:**

🔺 **Autosampler Commands (GINA 50)**

🔺 **Autosampler Commands (ASI-100 Series)**

End

Instrument Type:	**System Command**
Related Command:	$\Rightarrow$*EndTrigger*
Description:	Marks the end of a program.

EndTrigger

Instrument Type:	**System Command**
Related Command:	$\Rightarrow$*Trigger*
Description:	The **EndTrigg**er command marks the end of a block of triggered commands.
Function:	All commands between **Trigger** and **EndTrigger** are executed when the trigger condition becomes true.
☞ *Tip:*	*Define the Trigger identified by its name with the new condition „0" and mark the end of this trigger block with the EndTrigger command to finish an active Trigger.*

```
Time Trigger Name  0

  EndTrigger
```

This is necessary for each trigger separately. It is not possible to finish all triggers together.

Equate

Instrument Type: **Processed in PC**

Related Command: $\Rightarrow$*%B, %C, %D*

Description: The **Equate** command is used to assign different designations to the three partial flows of the fluidics, %A, %B, %C, and %D. Example:

```
0.000 %A.Equate =    "Water"

0.000 %B.Equate =    "MeOH"

0.000 %C.Equate =    "ACN"
```

Function: In the unit windows, you can assign the actual eluent names to the partial flows. The **Equate** commands are logged in the Audit Trail. This to keep track of the chromatographic conditions at any time. Consider that a PGM File which contains **Equate** commands may itself serve as a protocol.

☞ *Tip:*   *It is up to the user to check the consistency of %A, %B, %C, and %D with the eluents actually used!*

Flow *(Flow Rate)*

Instrument Type:	**Pump**
Related Commands:	⇒*%B, %C, %D*
	⇒*%A, %B, %C, %D_Level*
	⇒*Purge*
	⇒*WasteLevel*
Description:	The **Flow** command defines the total flow rate (in [ml/min]) through the column, i.e., the sum of all ⇒*Partial Flows* (%A+%B+%C+%D=100%). The value entered represents the **current value**. For isocratic systems, this value remains constant during the program. In **flow-gradient** systems, this value represents an interpolation point of a polygonal line to which the previous and following flow values are adjoined by straight lines.
Function:	With flow ⍍*Ramps*, the total flow rate alters linearly with time, between two flow commands. By entering two flow commands at the same time, you can program ⍍*Step Gradients*. When sorting, the editor does not change the order of commands having the same program time! If the flow is to remain constant for the entire analysis, a single entry at the beginning of the ⍍*Program* is sufficient.

```
-2.000 Flow=   0.500
 ... ...
 0.000 Inject
 0.500 Flow=   0.500
 0.500 Flow=   1.000
 ... ...
 8.500 Flow=   5.000
 8.500 End
```

In this example, the flow rate remains constant at 0.5 ml/min between -2.0 and 0.5 min. Then it increases abruptly to 1.0 ml/min and then linearly to 5 ml/min with a gradient of 0.5 ml/min.

If a second fluidic system is installed in the same ⍍*Timebase*, the corresponding device name has to be specified in the program. By using the F8 key for generating command lines, the device name is inserted automatically.

☞ *Tip:*

The ⇒Hold, ⇒Continue, and ⇒StopFlow commands are always effective for both fluidic systems!

☞ *Tips:*   *Chromeleon is able to run flow and %-gradients* simultaneously*. However, in high pressure mixing systems this results in non-linear partial flow rate changes for each individual pump.*

As there are pressures up to 400 bars in the chromatography column and as solvent mixtures such as methanol/water are subject to volume compression, the volume delivered in ➢High-Pressure Gradient Systems does not correspond to the volume transported via the column. However, the number of delivered and transported solvent particles is not changed by this fact.

The flow rate remains constant from the last flow command to the end of the program. Thus, for isocratic operation, a single flow entry at the beginning of the file is sufficient.

The first flow command must be at the beginning *of the PGM File!*

Normal flow rates are in the range 0.5 to 10 ml/min. Flow rates deviating from this range are achieved by using special micro pumps (0.1 - 0.5 ml/min) or preparative pumps (as from 10 ml/min).

Stopping the flow or decreasing the flow to 0.00 ml may result in deposits in the detector flow cell. To avoid this, turn off the lamp in UV and fluorescence detectors when the flow is stopped (⇒Lamp = Off).

For Dionex detectors, a warning appears if the lamp is not turned off. However, make sure that you have selected the installed pump from **Link to Pump** *list on the* **General** *tab page for the detector in the* **Server Configuration** *program.*

Freeze

Instrument Type:	**Pump (P580 only)**
Related Commands:	⇒*Learn*
Description:	Compressibility always varies with different solvents. The better the pump is set to the compressibility of the respective solvent the lower is the pump's pulsation. Chromeleon allows you to determine optimum pre-compression control for the Dionex P580 pumps, using the **Learn** and **Freeze** commands.
	The **Freeze** command completes the learn mode for determining the compressibility of the respective solvent component. (For more information, refer to **Practical Tips for Device Control ![icon] Setting Automatic Pre-Compression Control (P580)**)
Function:	If the **Freeze** command is issued, the measured compressibility is saved and used for pre-compression, according to the portion of the respective component in the total flow.

Hold

Instrument Type:	**System**
Related Command:	⇒*StopFlow*
	⇒*Continue*
Description:	The **Hold** command

- Stops ➢*Data Acquisition*
- Interrupts a running ➢*Gradient* program
- Stops automatic processing of the ➢*Batch*

Function:	In **Hold** mode, no data is acquired, the pump continues delivery with the current solvent composition, and evaluation of the batch samples is stopped.
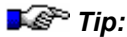
☞ *Tip:*   *To abort all processes, select the **StopFlow** command. To continue the processes, select **Continue**.*

If/ElseIf/Else/EndIf

Instrument Type: **System command**

Related Commands: ⇒*Trigger*, ⇒*Wait*

Description: The **IF** control command allows you to execute a command only if a certain condition is fulfilled. If the **IF** condition is true, the statements enclosed between **IF** and **ENDIF** are executed. Otherwise, they are ignored.

Function: When the **IF** condition is true, the associated commands are executed. Otherwise, each **ELSEIF** condition is evaluated in turn until a condition is found to be true and the associated command(s) are executed. The statements of the final **ELSE** (if available) will be executed if none of the **IF** or ELSEIF conditions are true. The IF structure always ends with **ENDIF**.

Parameters:

     Condition   The system waits until the **IF** or **ELSEIF** condition specified here is realized (see ⇒*Trigger*)

☞ *Tips:*   *Usually, while acquisition is disabled or interrupted, signal values cannot be evaluated.*

                     *Conditional execution of the **Flow**, **%B**, **%C**, and **%D** commands is restricted to isocratic separations.*

                     For certain instrument, the settings cannot be changed during the sample run. Thus, it may happen that IF commands are not performed.

Inject

Instrument Type: ➢*Autosampler*

Related Commands: ⇒*AcqOn*

    ⇒*Draw*

    ⇒*Dispense*

Description: The **Inject** command defines the beginning of a chromatogram, that is, it determines the time at which the sample enters the high-pressure system. Thus, the time of the first inject command is 0 by definition. The advantage is that the ⇒*Retention Time*, as appearing in chromatograms and reports, coincides with program execution times. All commands before inject, are assigned **negative execution times**.

    Between the Inject command and the actual injection process (Inject response by the autosampler or the hand-operated valve), a system-dependent interval is required for reaching the rack and to draw the sample. During this time, the pump remains in ⇒*Hold* state; that is, a gradient that might be running is stopped. The **Inject Wait** state is canceled only by an **Inject Response** signal. The response signal is delivered either automatically (via an interface) or via a remote input signal. Only from this point, time keeping is started.

Function: The function depends on the individual installation. For manual injection, Chromeleon simply holds the analysis time and waits for inject response. For a controlled autosampler, the inject command (at least) is relayed to this. For autosamplers with **variable injection volumes** and **random sample access**, such as the **ASI-100** autosampler, the inject volume and vial position are relayed as well. Such autosamplers are ideal for automated **multipoint calibration.**

Function: *(Cont'd)*	For **interactive operation** (online) of the inject command, the **Sample Position** and **Inject Volume** parameters must be entered as well. During **automated batch operation**, these values are read from the sequence. Thus, parameters do not have to be set individually in the ➤*Program*.
Parameters:	(When a hand-operated valve is used for injection, this information can be used for documentation purposes.)
Position	Vial ⇒*Position* in the autosampler
Inject Volume	Injection ⇒*Volume* in µl
Blank	Specifies whether ➤*Blank Run Samples* are actually injected:
	Skip: no injection
	Inject: Injection is performed.
	When you use the Famos autosampler, make sure to allow a short time before blank run samples are injected. (For more information on the autosampler, refer to **Hardware Installation** 🌐 **FAMOS Capillary/Nano HPLC Autosampler** in the **Administrator Help** section.)

```
-0.100  ; 6 seconds to process program
0.000 Inject
```

🖎 *Tips:*	*Data acquisition (⇒AcqOn/Off) should be started after the Inject signal, at least 30 seconds before the first peak. Also, there should be ➤Isocratic conditions during the injection process.*
	A program should only contain one *inject command.*
	For the ICS ion chromatography system, injection is performed with the ***Pump_InjectValve.InjectPosition*** *command (if no AS50 is installed). With the* ***Pump_InjectValve.InjectPosition*** *command, an injection is always performed, also for a ➤Blank Run Sample. This is contrary to the autosampler ⇒Inject command.*

Lamp

Instrument Type:	**All optical detectors**
Related Commands:	**---**
Description:	The **Lamp On/Off** command turns the lamp of an optical detector on and off. UV/VIS detectors often have a separate lamp (deuterium/tungsten) for each wavelength range. In this case, the commands are as follows: UV_Lamp = On/off Visible_Lamp = On/Off.
Function:	The lamp is turned on or off.

☛ *Tips:*   *Almost all optical detectors require a considerable warm-up period for high sensitivity, drift-free operation. The inject command should, therefore, be placed several minutes (minimum) after this command. Note also that lamp(s) should never be turned off during a sample batch. Many laboratories operate detectors 24 hours a day with the lamp turned on.*

Detector lamps are subject to aging. Check the ⇒LampIntensity property, using the ⇒Log command; for example, to receive the value of the current lamp intensity at 254 nm.

Stopping the flow or decreasing the flow to 0.00 ml may result in deposits in the detector flow cell. To avoid this, turn off the lamp in UV and fluorescence detectors when the flow is stopped (⇒Lamp = Off).

*For Dionex detectors, a warning appears if the lamp is not turned off. However, make sure that you have selected the installed pump from **Link to Pump** list on the **General** tab page for the detector in the **Server Configuration** program.*

LampAge

Instrument Type:	**All optical detectors**
Related Commands:	⇒*LampIgnitions*
	⇒*LampIntensity*
Description:	Detector lamps are subject to aging. After installing a new lamp, select the **LampAge** command to reset the lamp age to 0.

The **LampAge** command allows you to check the lamp age via the ⇒*Log* command. The value indicates the quality of the lamp.

☞ *Tip:*
The maximum number of operating hours for a UV lamp is usually approximately 2000 hours.

Function:	The **Log LampAge** command logs the age of the optical detector lamp in the ➢*Audit Trail*, indicating the values in operating hours. The lamp age is checked at the retention time at which the command was performed.

LampIgnitions

Instrument Type:	**All optical detectors**
Related Commands:	⇒*LampAge*
	⇒*LampIntensity*
Description:	Detector lamps are subject to aging. After installing a new lamp, select the **LampIgnitions** command to reset the number of ignitions to 0.

The **LampIgnitions** command allows you to check the number of ignitions via the ⇒*Log* command. The value indicates the quality of the lamp.

Function:	The **Log LampIngitions** command logs the number of ignitions performed by the optical detector lamp. The number of lamp ignitions is checked at the retention time at which the command was performed.

LampIntensity

Instrument Type:	**All optical detectors**
Related Commands:	⇒*LampAge*
	⇒*LampIgnitions*
	⇒*MinLampIntensity*
Description:	Detector lamps are subject to aging. The **LampIntensity** command allows you to check the intensity of the lamp via the ⇒*Log* command. The value indicates the quality of the lamp. After you have installed a new lamp, periodically check the lamp intensity via the **Log** command, and then compare the two values.
Function:	The **Log LampIntensity** command logs the lamp intensity for an optical detector in the ➤*Audit Trail*, indicating the value in counts/second. The intensity of the lamp is checked at 254 nm at the retention time when the command is performed.

📑 *Tip:*

Determine the lamp intensity at a time when it is not affected by substances in the flow cell. Therefore, it makes sense to check the value at the beginning of data acquisition.

📑 *Tips:*

*The lamp intensity can be included in the report. Select the **LampIntensity** variable from the **Audit Trail** category.*

If you check the lamp intensity while recording the chromatogram, you have to a) search for the retention time at which the intensity was read (done automatically) or b) indicate it manually.

a) Type the end retention time of the chromatogram in the Formula field. For example, for an overall run time of 15 minutes, type

AUDIT.LampIntensity(15.0)

☞ *Tips:*
(Cont'd)

Chromeleon will automatically search for the retention time (backwards from the end retention time on) at which the lamp intensity was checked, and then indicate the lamp intensity that was measured at that time.

Or, you may search for the retention time at which the lamp intensity was checked from the data acquisition start:

> *AUDIT.LampIntensity(0.0,"forward").*

b) If you know the retention time at which the intensity was checked, enter the exact time. For example, if the lamp intensity was read at 0.5 min, type

> *AUDIT.LampIntensity(0.5)*

Learn

Instrument Type: **Pump (P580 only)**

Related Command: ⇒*Freeze*

Description: Compressibility always varies with different solvents. The better the pump is set to the compressibility of the respective solvent the lower is the pump's pulsation. Chromeleon allows you to determine the optimum pre-compression control for the Dionex P580 pumps, using the **Learn** and **Freeze** commands.

The **Learn** command starts the learn mode for determining the compressibility of the respective solvent component. However, verify that the solvent type has been set to **Custom**. (For more information, refer to **Practical Tips for Device Control** 🔳 **Setting Automatic Pre-Compression Control**.)

Function: After you have issued the **Learn** command, the pump automatically adapts to the compressibility of the respective solvent.

Log

Instrument Type:	**Processed in PC**
Related Commands:	⇒*Message*
	⇒*Protocol*
Description:	The **Log** command allows you to document the values of variables in the ➢*Audit Trail* at any time.
Function:	All device settings are listed as ➢*Preconditions* in the Audit Trails. (For more information about the Audit Trails, refer to Data Management 🔳 **Audit Trail**.). In addition, you can log them at a specified time, using the **Log** command.

If, for example, the pressure at the time t = 5.000 min during sample processing should be logged, include the following line in the control program (see **Control 🔳 The Control Program**):

5.000 Log   Pressure.Value

In the example mentioned above, the current pressure will be logged. In exceptions, the **Log** command is sent directly by the device driver; for example, for ➢*Fraction Collectors*. For another example, refer to ⇒*LampIntensity*.

Using the Log command, the different values from the sample table of the Browser may be written to the Audit Trail. This is possible for:

PrevStandard: the most recent standard sample

🔖 *Note:*

This standard may not be required for the current sample.

PrevSample: the most recent sample

Sample: the currently running sample

NextSample: the next sample of the current sequence

Function:
(Cont'd)
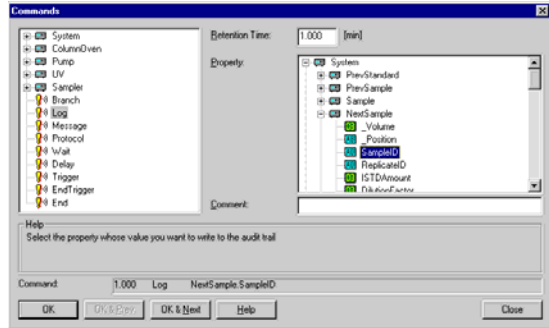
It is possible for various standard columns and for ➢*User-defined Columns* to output the corresponding values.

MaxAutoStep

Instrument Type	**Detector**
Type:	**Fixed point value**
Value Range:	**0.1 ... 5.1 [s] (not with 3D fields)**
Default:	*5.1 s*
Related Parameters:	⇒*Step*
	➢*Sampling Rate*
	➢*Data Collection Rate*
Description:	The **MaxAutostep** variable determines the maximum step if Step = Auto.

Message

Instrument Type:	**Processed in PC**
Related Commands:	⇒*Wait*
	⇒*Protocol*
	⇒*Log*
Description:	When a program is executed, an on-screen message pops up. Click **OK** to confirm the message. Only then, the program will be continued.
	The Message command allows the user to enter reminders for things to do or to consider while executing a ➢*Program*.
Function:	When executing the program, a window containing the respective message text is displayed. The program is interrupted until the user confirms the message. Besides, the command and the message are written to the Audit Trail.
	For example, it a user should be reminded to check and, if necessary, refill eluents prior to the analysis, add the following command to the program:

```
-1.000 Message "Check eluent containers!"
```

When executing the program, the text in quotation marks is displayed on screen at the specified time (in the above example one minute prior to injection). Simultaneously, the command and the message are logged in the Audit Trail. The server is then in **Hold** mode. During this time, the monitor icon indicates this status by its yellow/red coloring.

If Chromeleon is operated on a network, the message appears on the client having control privileges.

The program continues operating as soon as the message has been confirmed.

Parameter:

> **Text**   The message to be displayed.

☞ *Tip:*   *As long as the message dialog box is displayed on the screen, the time is interrupted and the flow conditions are in Hold mode.*

MinLampIntensity

Instrument Type:	**All optical detectors**
Related Commands:	⇒*LampIntensity*
Description:	Select the **MinLampIntensity** command to set the lower limit for the lamp intensity.
Function:	When the measured lamp intensity is below the **MinLampIntensity**, a warning appears when data acquisition is started.

Mix

Instrument Type:	➢*Autosampler*
Related Commands:	⇒*Inject*
	⇒*Dispense*
Description:	The **Mix** command causes the autosampler to mix defined injection volumes (see ⇒*Volume*) from several sample vials (see ⇒*Position*) in a different sample vial.
Function:	When the action is completed, the autosampler returns the **Sampler.Ready** signal.

Example (ASI-100 Series):

```
0.000  PrepVial          BA1
0.000  PrepVolume        10
0.000  Sampler.PrepVial  Position + GA1 - RA1
0.000  PrepSubject       PrepVial
0.000  Mix
```

The above command sequence mixes 10 µl from a vial in the green segment with 10 µl of a vial in the red segment in the corresponding vial of the blue segment.

Example (AS/AS50):

```
Sampler.Pipet  Volume = 20.0, SourceVial = 1,
DestinationVial = 10
Sampler.Mix SourceVial = 10, NumberOfTimes =
5, Volume = 30.0
```

In the above command sequence, 20 µl is pipetted from vial 1 and delivered to vial 10. The contents of vial 10 is mixed by drawing in and expelling 30 µl of the vial contents. The mixing cycle is repeated 5 times.

You can also use the PrepSpeed (ASI-100) or SyringeSpeed (AS/AS50) command to determine the draw speed of the syringe.

Tip: *When using highly viscous liquids or liquids with a low boiling temperature, allow the autosampler more time to draw the exact volume exactly without bubbles.*

For more information, refer to **Practical Tips for Device Control:**

Autosampler Commands (ASI-100 Series)

Autosampler Commands (AS/AS50)

NeedleUp

Instrument Type:	➢*Autosampler*
Related Commands:	⇒*Wash*
Value Range:	**Depends on device and installed syringe**
Description:	The **NeedleUp** command enables lifting the sample needle.
Function:	Then the internal sample valve is switched (see the example for a load/inject process in the Autosampler).
	When lifting the needle, a running ⇒*Wash* process is automatically interrupted, i.e., the solvent flow is not through the sample loop but directly from the pump onto the column. Use the **Load** command to perform the same operation without moving the needle up.
Tip:	*Use the **Wash** and **NeedleUp** commands to prevent crystallization of substances, such as buffers, by washing the sample loop.*

ParkPercentage

Instrument Type:	**UltiMate and LPG-3x00 pumps only**
Related Commands:	⇒*Hold*
	⇒*Continue*
	⇒*StopFlow*

Description: The **ParkPercentage** command enables the peak parking functionality. A peak can be parked only from the system if the ParkPercentage is > 0.00. For the UltiMate pump, an external signal must be sent to the pump's START IN input.

For the LPG-3x00, the ParkPercentage can be controlled via the corresponding commands, e.g., via script buttons on a panel. For example, one button sets `ParkPercentage = 5` and the other `ParkPercentage = 100`.

If peak parking is signaled, e.g., via a digital impuls from a ➢*Mass Spectrometer*, you can use triggers for one of the pump's inputs:

```
Trigger ParkPeakStart LPG3x00_Input1.State = 1
      MicroPump.ParkPercentage = 5
EndTrigger

Trigger ParkPeakEnd LPG3x00_Input1.State = 0
      MicroPump.ParkPercentage = 100
EndTrigger
```

Function: Peak parking is similar to the behavior of the ⇒*StopFlow* command:

- The ➢*Gradient* program is interrupted.

- Usually, the flow is reduced (but not turned off).

However, unlike the behavior of the **StopFlow** command:

- Data Acquisition (see ➢*Acquisition On/Off*) is not interrupted.

- A running ➢*Batch* is not stopped.

Function:
(Cont'd)

The **PeakParked** variable displays the peak parking state. Chromeleon cannot control the state.

By parking a peak, the peak is slowly pumped through the detector. Thus, you can operate, for example, a ➤*Mass Spectrometer* in a more sensitive range.

☞ *Tips:*

Peak parking freezes the gradient with its current composition and reduced flow while data acquisition continues. Please keep the following in mind::

- *The retention times do not correspond to the expected times.*

- *➤Audit Trail entries and gradient plots are no longer synchronized.*

- *➤Triggers may no longer work as programmed.*

- *Detection parameters need to be corrected.*

- *The ⇒Delay Time between two detectors is incorrect.*

The changed flow is not displayed on the pump display or in Chromeleon.

Position

Instrument Type:	➢*Autosampler*
Related Commands:	⇒*Inject*
	⇒*Volume*
Value Range:	**Depends on device and installed carrier segment**
Description:	Specifies the position of the vial from which a sample shall be injected.
Function:	If you use a controllable autosampler, the entered position is transmitted automatically to the autosampler. The autosampler approaches the corresponding sample for injection.
	If you use either a non-controlled autosampler or a hand-operated valve, this column is for documentation purposes only. If the sample position parameter is not entered, the previous (current) value is used.

Pressure.Lower/UpperLimit

Instrument Type:	**Pump**
Related Commands:	$\Rightarrow$*Flow*
Description:	Define the pressure limits within which the pump(s) is permitted to operate.
Function:	If one of the limits is exceeded, Chromeleon **turns off the flow**, displays an error message, and terminates the current sample batch.
	If the upper pressure limit is exceeded, the flow is immediately set to 0 ml/min. The pressure reading must remain below the lower pressure limit for some time (typically approximately 60 s - the time depends on the pump type), before Chromeleon turns off the flow.
Parameters:	(The values that can be entered and the pressure unit depend on the pump type.)
Pressure.LowerLimit	Lower pressure limit in **Bar, MPa**, or **psi**.
Pressure.UpperLimit	Upper pressure limit in **Bar, MPa**, or **psi**.
☞ *Tip:*	*Pressure limits apply to* all *pumps that are connected on the high-pressure side.*
	Exceeding the upper pressure limit can be due to a blocked column or capillary or to a defective injection valve.
	If the pressure is below the lower limit, this is usually due to a leak in the fluidic system.

Protocol

Instrument Type:	**Processed in PC**
Related Commands:	⇒*Message*
	⇒*Log*

Description:

Writes a comment to the Audit Trail (without screen message). Thus, program steps can be additionally commented or chromatographic conditions can be written to the Audit Trail. (PGM comments are not written to the Audit Trail.)

```
-2.000 Protocol "Separation according to DFG-
method, residue analysis"
```

```
-2.000 Protocol "0.05 M
Natriumdihydrogenephosphate buffer"
```

```
-2.000 Protocol "Column: LiChrospher RP-18"
```

Function:

Unlike simple green comments starting with a semicolon, which are only part of the program, the **Protocol** text is included in the Audit Trail and thus, it is directly linked with the corresponding sample. Therefore, the **Protocol** text can be used for commenting individual samples. The **Protocol** command does not affect the program run.

Parameter:

Text The text that is logged in the Audit Trail.

📠 *Tip:*

Event-controlled execution of the command is also possible. Example:

```
0.000  Trigger Protocol  UV_VIS_1 > 1000

       Protocol           "Valid absorption
                          range exceeded"

       EndTrigger
```

Purge

Type:	**Pump**
Related Commands:	⇒*Flow*
Description	Usually, the Purge command is used to rinse the system for a short time at a considerably higher flow rate.
Function:	The command sets the flow rate to the rate specified by PurgeFlow. The PurgeFlow is valid for the time specified by PurgeTime. Afterward, the flow rate is reset to the value specified by Flow.

Ready

Type:	⊳*Autosampler*
Related Commands:	⇒*Inject*
Value Range:	**NotReady ... Ready**
Description:	The **Ready** property indicates the state of the autosampler.
Function:	The autosampler can only execute a command, for instance, the **Inject** command, when it is in **Ready** state.

RefBandwidth (Reference Bandwidth)

Type:	**Integer**
Value Range:	**Detector-dependent**
Default:	**0 [nm]**
Related Commands:	⇒*RefWavelength (Reference Wavelength)*
	⇒*Bandwidth*
Description:	The reference bandwidth can be selected separately for each channel. In addition, the 3D field of a ➤*Photodiode Array Detector* has its own reference bandwidth.
	Analogous to the conventional bandwidth of a channel, the reference bandwidth serves to average several photodiode signals of the Reference Wavelength.
Function:	For example, if the reference wavelength is 350 nm and the reference bandwidth is 5 nm, wavelengths in the range of 348 - 352 nm are averaged and used as the reference.
👉 *Tips:*	*For the **UVD 170/340** detectors, change the reference bandwidth only if the reference wavelength cannot be set to 600 nm; that is, for substances clearly absorbing at 600 nm, especially blue substances. This is the only case where using a higher bandwidth can improve the signal-to-noise ratio.*
	*For the **PDA-100/PDA-3000** detectors and other two-lamp PDA detectors, select a reference bandwidth that includes a majority of light from the same lamp as the sample wavelength. For the PDA-100 and PDA-3000, the deuterium lamp provides wavelengths of 190 nm to 380 nm and the tungsten lamp provides wavelengths of 380 nm to 800 nm. Select a reference bandwidth that is narrow enough not to interfere with nearby compounds and select an area of the spectrum where the sample does not absorb.*

RefWavelength (Reference Wavelength)

Instrument Type	**Detector**
Type:	**Integer**
Value Range:	**Detector-dependent**
Default:	**Off**
Related Commands:	⇒*RefBandwidth (Reference Bandwidth)*
	⇒*Wavelength*
Description:	With Dionex Photodiode Array Detectors, the reference wavelength is used to correct the absorption values of the wavelength that has been selected for the analysis.
Function:	If the absorption of the reference wavelength changes during the analysis, absorption values of the analysis wavelengths are adjusted up or down accordingly. The selected reference wavelength should be in a quiet area of the spectrum where little absorption occurs. Each change in the absorption then indicates substantially changed conditions; for example, a reduction of the lamp energy (lamp drift). Each change can be used to correct the absorption in the remaining wavelength range even during the analysis (the recorded signal is reduced or amplified accordingly, as necessary).
	Reference wavelengths are especially useful for gradient analyses because, as the light intensity changes over time (due to the gradient), absorption values are adjusted. This minimizes baseline drift.
	Peaks are not detected in the reference wavelength range, where the system is "blind" by definition. Should peaks be detected, nevertheless, select a different reference wavelength. You can deliberately do this to inhibit peaks.

Function: *(Cont'd)*	If the reference wavelength is chosen in a light-deficient range, Dionex recommends that you average the signals of several photodiodes by increasing the Reference Bandwidth, thus improving the signal-to-noise ratio.

For Dionex Photodiode Array Detectors, you may change the reference wavelength after data acquisition. To do so, the PPA window has two options:

1. Extracting a channel at a wavelength other than the wavelength used for data acquisition: On the context menu, select **Extract** and then **Chromatogram to file**. Then, select a reference condition for the new channel.

2. Displaying the 3D field at a different reference wavelength: On the context menu, select **Decoration** to open the **Peak Purity Decoration**. Then, select the reference conditions on the **General** tab page.

Tip:	*Do not confuse the reference wavelength with the ➢Reference Channel used for ➢Baseline Correction of Spectra.*
Settings:	Details about reference wavelengths vary, depending on the type of detector:

Dionex UVD 170/340 Detectors

For the Dionex UVD 170/340 UV/PDA detectors, select a reference wavelength of 600 nm because relatively low absorption values are observed at this wavelength. In addition, the Dionex detectors have a special noise optimum at this value. However, when the reference wavelength is changed, the special noise optimum no longer applies.

Settings:
(Cont'd)

☞ Tip:

Use a ➢Holmium Oxide Filter (spectra calibration) to reference the measured wavelength range; that is, to verify whether the actual nanometer value corresponds to the nominal value.

Dionex PDA-100 and PDA-3000 Detectors

By default, the reference wavelength is disabled for the PDA-100 and PDA-3000. With UV channels ($\neq$ 3D Field), the absorption at the first scan after the autozero is used as a reference. This results in lower noise, but less drift compensation. The **Reference_Wavelength=Off** command in a program or on the control panel turns off the reference wavelength.

During collection of ➢*3D Field* data, the absorption after autozero is always used as a reference (as described above). After data collection, a reference wavelength can be applied to the data. In this case, the reference wavelength defaults to the highest wavelength in the analysis range. For example, if the analysis wavelength range is 190 - 350 nm, the default reference wavelength is 350 nm. If you select a different reference wavelength, the selected reference must be within the stored wavelength range.

Relay On/Off

Instrument Type:	**All relays**
Related Commands:	---
Description:	**Relay-Name.On (Off)** closes (opens) a relay output (closure contact) for a specific instructed time. Switching valves are treated as relays, also.
Function:	The specified relay is opened or closed for a specified time in seconds. **Relay-Name.On** opens the relay upon completing a certain period (➢*Duration*), **Relay-Name.Off** closes the relay after completing the duration. If no duration is specified for **Relay-Name.On (Off),** the relay remains closed (open) until the next relay command is performed.
Parameter:	
Duration	Closure or opening time in seconds [s] (optional).
🖐☞ *Tip:*	*Relay On/Off duration for the same relay may not overlap.*

Reset

Instrument Type:	**All**
Related Commands:	⇒*Autozero*
Description:	Resets an instrument to its initial conditions, as attained after power-up; i.e., to **standby mode**.
Function:	Enables (in general) a warm start, that is, the instrument regains its initial state as immediately after power-up. The reset command is particularly useful when an instrument has been operated locally, or if it has an undefined status (to Chromeleon); for example, after an instrument fault.
☞ *Tip:*	*Activating the reset of an autosampler may require a considerable time to completion, as generally this involves a mechanical recalibration and a complete wash cycle.*

Retention

Instrument Type:	**Detector**
Related Property:	⇒*Signal.Value*
Description:	Retention reports the system retention time (*System.Retention*) and signal retention time (*<Signal name>.Retention*).
	The system retention time indicates how much time has elapsed since sample injection. The signal retention time indicates when the last data transfer occurred. Not all devices transfer data continuously to the PC; some devices, e.g., detectors, transfer data packets only at certain times. The signal retention time is displayed in the online plot on the ➢ *Control Panel*.
Function:	The Retention signal property is accessible (read-only) via the ⇒*Log* command.

Signal.Value

Instrument Type:	**Instruments from which signals are recorded**
Related Property:	⇒*Delta*
Description:	Signal.Value indicates the signal value at the related retention time.
Function:	The Signal.Value signal property is accessible (read-only) via the ⇒*Log* command.
	Use the Signal.Value property in ⇒*Triggers* if you want to collect fractions based only on the signal value.

Sound

Instrument Type:	**Processed in PC**
Related Commands:	**---**
Description:	The **Sound** command generates an acoustic beep of selectable frequency and duration that is heard on the internal loudspeaker of the PC. You can use a beep to acoustically monitor the progress of a program, by setting **Sound** commands at key positions in the file. Combining the **Sound** command with a ⇒*Trigger* command allows you, e.g., to "hear" a peak elute. Select different frequencies for leading and trailing peak edges to distinguish them acoustically.

Example:

```
-2.000 Flow =        1.000

-2.000 %B =          50

 0.000 Inject

 0.000 Trigger Up    UV-VIS-1 > 20

       Sound     Frequency=440, Duration=1

       EndTrigger

       Trigger Down  UV-VIS-1 < 40

       Sound     Frequency=880, Duration=1

       EndTrigger

 0.500 Acquisition On

 ...  ...

 8.000 Acquisition Off

 9.000 Program End
```

Function: If the PC is not equipped with a sound card, the loudspeaker may not be able to process the beep generated by the **Sound** command. In this case, a default beep is generated, instead.

Also, refer to **Practical Tips for Device Control** **Trigger Commands**.

Parameters:

    File   Sound File (normally WAV file)

    Frequency   In Hertz [Hz] (concert pitch a = 440 Hz

    Duration   In seconds [s].

Step

Instrument Type:	**Detector**
Type:	**Fixed point value**
Value Range:	**Depends on the channel; for example, for a UV channel: 0.01 ... 4.8 [s] or Auto**
Default:	Depends on the detector and the channel; for example:
	0.5 s (3D field of the 340U PDA)
	Auto (UV-VIS channel of the 340U PDA)
	0.05 s (GC)

Note:

For non-controlled GCs: 0.25 s.

Related Commands:	⇒*MaxAutoStep*
	➢*Sampling Rate*
	➢*Data Collection Rate*
Description:	The Step determines the time interval between two consecutive data points within the signal's raw data file (see **Data Management** 🔳 **Raw Data**). The step is variable; you can specify a fixed step or have it selected automatically. A fixed sampling rate is especially useful if you want to export the recorded raw data to external software applications and if the destination program can only process **equidistant** data points.
Function:	**Fixed Step [0.01 .... 4.8** s**]**
	Every *step* seconds, a data point is stored in the **Raw Data** file. For example, selecting step = 0.5 means acquiring and storing 2 data points per second. The smaller the step, the more data points will be recorded per time unit (regardless of whether there is a baseline segment or a peak).

Function: *(Cont'd)*	For the 3D field, **step** (here, 0.1 - 4.0 s) defines the data rate at which the connected ➢*Photodiode Array Detector* collects spectra.

The distance between individual data is as follows:

- for the Dionex UV Detector = 0.01 s

- for the ➢*UCI Universal Chromatography Interface* = 0.01 s

- for the ➢*3D Field* = 0.1 s

Automatic Step [auto]

At a variable sampling rate, the last 10 seconds of a chromatogram are temporarily stored in the system memory with the highest sampling rate. For each new data point (every 0.01 s), the oldest data point can be removed.

A complex algorithm allows determining and storing only those data points in the raw data file that are actually required. All "unnecessary" data points are filtered out and the chromatogram is stored almost without a loss in representation.

Depending on whether the peaks are narrow or wide or a baseline segment, 0.2 to 100 data points are stored per second as the result. Thus, the step automatically varies between 0.01 and 5 seconds.

Saving raw data with automatic step reduces the storage requirement up to 75% and thus increases data processing.

Select the **Decoration** command on the context menu and then the **Raw Data Point** option on the **Peak Decoration** tab page to display the chosen raw data points in the Report.

Select the ⇒*MaxAutoStep* command to determine the maximum step for Step = Auto.

Tips: *Select the step in such a way that 20 data points are placed on the narrowest relevant peak of your chromatogram. If you acquire more data points, this will use unnecessary disk capacity and the integration might become incorrect, especially with increased baseline noise.*

*Use the **Auto** step for fast peak chromatograms for which you do not know the width of the expected peak. For a precise and reproducible analysis, always use a fixed step (see above). Especially with increased baseline noise, using **Auto** may result in incorrect integration.*

If you issue the ➢Data Collection Rate command, the default Step value is set to the reciprocal value. That is why you have to issue the Step command after the Data Collection Rate command if you need a different step.

For an overview of data acquisition, refer to **Data Management 🔳 Data Acquisition***.*

StopFlow

Instrument Type:	**System Command**
Related Commands:	⇒*Hold*
	⇒*Flow*
	⇒*Continue*
Function:	The **Stop Flow** command turns off the pump flow; data acquisition is interrupted. A running ➢*Batch* is stopped, as in the hold mode.
	Select the **Continue** command to undo this command.

Temperature

Instrument Type:	**Column Oven/Autosampler/GC**
Related Commands:	**---**
Description:	The **Temperature** command adjusts the required temperature for a column thermostat, autosampler, or GC.
Function:	If the program file contains more than one Temperature command, you will get a step profile instead of linear interpolation between two commands, which is happening with all temperature commands. In other words: Every new temperature command drives the thermostat to the new temperature as fast as possible (device-dependent). With GCs, the temperature is changed according to the selected temperature program.

Parameters:

Value	Actual column oven temperature
Nominal	Required temperature for the column oven.
Upper/LowerLimit	Upper/lower limit for the temperature of the column oven.

Note: *As with all device parameters, Chromeleon tries to display the <u>status</u> on the screen! Whether this is possible depends on the column oven connected.*

Trigger

Instrument Type:	**System Command**
Related Commands:	⇒*EndTrigger*
Description:	Executes any commands that are immediately following if previously defined conditions are met; for example, if a defined signal voltage threshold and/or your time gradients exceeds a specified threshold. Define the condition, using the **Condition** parameter.

In the program, the entire Trigger block is indicated in blue color.

Examples
- A typical use for this function is the control of a ➤*Fraction Collector*. If the absorption signal of a given channel exceeds a threshold value, a relay is enabled, switching to the next collection vial. Further important applications are:

- Signal-dependent ➤*Wavelength Switchings*; for example, immediately after completion of a peak. Thus, retention time variations can be neglected.

- Programs can be ⇒*Branched* off according to external conditions, such as a remote input or the value of an analog signal. Thus, you are flexible in determining the chromatographic reaction to external conditions (pressure, conductivity, etc.).

- Acoustic signals can be generated when certain conditions become true (⇒*Sound*). Thus, your peaks can be made audible.

Function: The trigger condition is active from the time of the trigger command and has to be specifically disabled. However, signal values are received only after the ⇒*AcqOn* command of the program. The following command is executed every time the specified conditions are med (**edge triggering**), i.e., from each transition from false to true.

Function:
(Cont'd)

Thus, in the previous example, the trigger condition must be entered only once in the program; the fraction collector will then be switched for each new peak.

Trigger commands can only be activated from programs or programmable buttons but not in the online control. The following syntax is valid:

```
Time Trigger NAME Condition, True,
                  Delay, Limit, Hysteresis

     Triggered command 1

     Triggered command 2

     Triggered command 3

     ...

     EndTrigger
```

As a response to a ➢*Trigger* condition becoming true, virtually any instruction can be performed. Examples:

- AbortBatch
- Acquisition.on/off
- BranchTo
- ⇒*Message*
- ⇒*Protocol*
- Relayname.on/off
- Signalname.Parameter
- Sound

Triggering the flow rate and the solvent composition is also possible. However, a gradient will be interrupted, and the analysis will be continued under isocratic conditions. Yet, you can use the **Branch** command to start a new gradient program.

The inject command cannot be triggered as it is linked to t = 0.000 and can be issued dependently from the time.

Note that trigger commands can be triggered, also.

Parameters:

Name   The name of the trigger command is obligatory. It serves for its identification. That is why the name must be unique, that is, the trigger cannot have the same name as a device or another command. Then, several triggers with different names can be activated simultaneously.

Note:

To be able to find the trigger in an Audit Trail or a Report, it is recommended to include the trigger name in capital letters in the program.

Condition   Defines the **trigger condition** and can have two forms:

```
<Parameter><Comparison Operator><Value> or
<Signal.Delta><Comparison Operator><Value>
```

or

```
<Remote-Input-Name>
```

<Parameter>
All installed channels, but also parameters such as **Temperature** (column temperature), **Pressure** (system pressure), **Flow** (flow rate), and **%A, %B, %C**, and **%D** can be used.

<**Signal.Delta**>
For signals, the first derivative of the parameter can be evaluated. This is achieved by adding the signal property ➤*DELTA*.

<**Comparison Operator**>
<, >, =, <=, >= and <>

<**Value**>
Fixed-point number. The dimension of the <Value> corresponds to the signal dimension. Parameters that are combined with DELTA have the dimension [signal dimension / s].

<**Remote-Input-Name**>
is reported from the instrument to Chromeleon, for example, **Sample.Ready** or **Leak**.

Condition *(Cont'd)*	If several parameters are to be linked, this can be realized via mathematical (+, -, *, / , **) or logical (AND, OR, NOT, XOR) operators. Parentheses show combinations and hierarchy, as for instance:

- (UV_VIS_1>100) AND (UV_VIS_2>100)

- (UV_VIS_1+UV_VIS_2)>200

Logical links of entire conditions are also possible.

- (UV_VIS_1>100) AND NOT (UV_VIS_2>200)

True	**Validity** in seconds. The trigger condition must be true for this time, to activate the trigger. Example:

```
Trigger DIGIN1 Remote1, True=2.0
```

The input signal must be active for at least 2 seconds to trigger DIGIN1.

The **True** parameter is also used as a criterion for the truth of signals; for example, to differentiate between **true** signals and spikes.

Delay	Indicates the delay time (in seconds) between fulfillment of the conditions and execution of the command; for example:

```
1.220 Trigger SIGNAL  UV_VIS_1>20, Delay=5.0

1.220 Relay On FracCol
```

The FraCol relay is switched 5 seconds after trigger activation only.

The **Delay** parameter is usually used for switching a fraction collector with a time delay. Thus, the dead volume between the detector and the fraction collector is taken into account.

If the **Delay** parameter is used in combination with the **True** parameter, the command is executed only <TRUE> + <DELAY> seconds after the condition is true.

Limit The upper limit for executing the trigger command. Without the limit parameter, a trigger command is carried out any number of times until the trigger off command is executed. The limit parameter automatically deletes the trigger after a corresponding number of activations. Example:

```
1.220 Trigger BRANCH RemoteIn, Limit=1

      Branch NEWPGM
```

The trigger command is only carried out once, and then the trigger is deleted.

Hysteresis To prevent multiple trigger execution with very noisy signals, each trigger receives the **Hysteresis** parameter in addition to the **True** parameter.

In contrast to the **True** parameter, **Hysteresis** is not time but signal-dependent. The parameter can vary between 0 and 100%. The default value is 5%.

A 5 % hysteresis causes, for example, the condition

```
Cond = UV_VIS_1 > 20,0
```

To change from FALSE to TRUE when reaching the value 20.0, but only changing from TRUE to FALSE at 19.0. Thus, a difference is made between signal increases and decreases. This corresponds to the different paths of a hysteresis loop.

If the operator < is used instead of >, the calculation is reversed! For the above example this means that the condition

```
Cond = UV_VIS_1 < 20,0
```

Becomes TRUE immediately when the value falls below 20, but is reset from TRUE to FALSE only at 20 + 5% = 21.

🖰☞ *Tips:*   *While acquisition is disabled or interrupted, signal values cannot be evaluated.*

*If the **True** and **Delay** parameters are used, the command is switched only <TRUE> + <DELAY> seconds after the condition becomes true.*

End program automatically disables all trigger conditions.

*Triggering the **Flow**, **%B**, **%C**, and **%D** commands is restricted to isocratic separations.*

For certain instrument, the settings cannot be changed during the sample run. Thus, it may happen that trigger commands are not performed.

Example:

When the trigger condition becomes true, the following program switches the flow to 0.1 ml/min and sets %B to 0.

```
-1.000  Trigger ELUENT     UV_VIS_1 > 20
        Flow =             0.100
        %B.Value =         0.0
        EndTrigger
 0.000  Inject
 0.000  UV_VIS_1.AcqOn
 ....   ........
15.000  UV_VIS_1.AcqOff
15.000  End
```

Volume

Instrument Type:	➢*Autosampler*
Related Commands:	⇒*Inject*
	⇒*Position*
Value Range:	**Depends on device and installed syringe**
Description:	Specifies the injection volume in microliters (μl).
Function:	In automatic operation, the installed ➢*Driver* converts this value into a volume readable by the autosampler; the value is then sent to the autosampler.
	You can enter different injection volumes to create a ➢*Dilution Series* for multiple-point calibration (➢*Single-Point and Multiple-Point Calibration*).
☞ *Tip:*	*In order to minimize carryover effects in multiple-point calibrations, you should <u>always</u> start with the sample with the highest dilution or the smallest injection volume.*

Wait

Instrument Type:	➢*Remote Input*
Related Commands:	⇒*Message*
	⇒*Inject*
	⇒*AcqOn/Off*
Description:	The **Wait** command interrupts program execution until the specified remote input signal arrives.
Function:	**Wait** stops the program execution. In the **Hold** mode, it also stops the time and data acquisition; the pump(s) are on **Hold**. In the **Continue** mode, the time, data acquisitions and pump flow continue.
	For examples of how to use the **Wait** command, refer to **Practical Tips for Device Control**:
	🔳 **Autosampler Control**
	Special Commands, Relay Control, and Miscellaneous 🔳 **Mixed Commands**
Parameter:	
Condition	The system waits until the condition specified here is realized (see ⇒*Trigger*)
Run	Switches from *Hold* to *Continue*.
Timeout	Sets the maximum period of time during which the system waits for the condition to become true. If this time is exceeded, the system aborts the running batch.
🖎 *Tip:*	*It is mandatory to specify a timeout if working in Continue mode.*

Wash

Instrument Type:	➤*Autosampler*
Related Commands:	⇒*Inject*
Value Range:	**Depends on device and installed syringe**
Description:	The **Wash** command rinses the needle and/or sample loop according to the relevant instrument procedures.
Function:	**Wash** allows washing the needle and/or sample loop with solvent in the **Inject** position. This corresponds to the normal solvent flow following an **Inject** command.
☞ *Tip:*	*Select the **Wash** command to wash the sample loop and thus prevent crystallization of substances in the sample loop.*

WasteLevel

Instrument Type:	**Pump(s)**
Related Commands:	⇒*WasteWarningLimit*
	⇒*%A, %B, %C, %D_Level*
	⇒*%B, %C, %D*
	⇒*Flow*
Description:	This command indicates the level of the solvent waste.
Function:	Before starting a sequence, enter the actual liquid level in the waste container. Chromeleon checks whether the waste container has sufficient capacity.
Parameters:	
Value	Actual waste level in [l].
UpperLimit	Upper limit for the waste level in [l]. If the level in the waste container reaches this value, the batch is aborted and the following message appears:

[Abort] xx:xx:xx {Pump} The waste level is above the limit (x.xxx l)! Please empty the waste container.

Tip:

In the emergency program, reduce the flow rate to 0 ml/min. This ensures that the waste container does not overflow.

Tip: *Chromeleon checks the calculated level of solvent waste during the ⇒Ready Check. The corresponding warning appears if necessary.*

Note:

This command is available for all pumps except for ion chromatography pumps.

WasteRemainTime

Instrument Type:	**Pump(s)**
Related Commands:	⇒*%A-, %B-, %C-, %D_RemainTime*
	⇒*WasteLevel*
	⇒*WasteWarningLimit*
Description:	Reports the approximate time until the associated waste container reaches the upper limit (**WasteLevel.UpperLimit**).
Function:	Chromeleon calculates the waste remain time based on the current flow rate and the current liquid level in the waste container.

Tip:
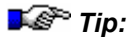
If you change the flow rate later, Chromeleon will recalculate the waste remain time. Therefore, check this value whenever you change the flow rate.

Note:

This command is available for all pumps except for ion chromatography pumps.

WasteWarningLimit

Instrument Type:	**Pump(s)**
Related Commands:	⇒*WasteLevel*
	⇒*%A-, %B-, %C-, %D_WarningLimit*
Description:	This command allows you to enter a warning limit for monitoring the liquid level in the waste container. Input is in percent and refers to the upper limit (**WasteLevel.UpperLimit**).
Function:	If the liquid level in the waste container exceeds the upper limit x the warning limit/100 (**WasteLevel.UpperLimit** x **WasteWarningLimit**/100), the following warning appears:

[Warning] xx:xx:xx {Pump} The waste level (actual level in [l]) will reach the upper limit (upper limit in [l]) in about 0.1 hours! Please empty the waste container.

☞ *Tip:*

When the liquid level reaches the upper limit, the batch is aborted. In the emergency program, reduce the flow rate to 0 ml/min. This ensures that the waste container does not overflow.

📑 *Note:*

This command is available for all pumps except for ion chromatography pumps.

Wavelength

Instrument Type:	**Detector**
Type:	**Integer**
Value Range:	**Detector-dependent**
Default:	**0 [nm]**
Related Commands:	⇒*Bandwidth*
	⇒*RefWavelength (Reference Wavelength)*
Description:	The **Wavelength** command specifies the wavelength at which the chromatogram is recorded.
Function:	For controlled detectors, such as the Dionex detectors, this value is automatically transferred to the detector (requires **control option**). The wavelength can also be entered manually during the analysis or in the program.
	In Chromeleon configurations without control option, the selected wavelength remains fixed during the analysis. In controlled versions, the wavelength can be altered during analysis.

QNT Parameters

The QNT Editor allows you to define general, peak table, and detection parameters. For information about the general parameters and peak table parameters, refer to:

⇒*Amount*

⇒*Calibration Mode*

⇒*Calibration Type*

⇒*Check Derivative*

⇒*Check Extrema*

⇒*Comment (Peak)*

⇒*Dead Time*

⇒*Delay Time*

⇒*Dimension of Amounts*

⇒*Group* (*Peak Group)*

⇒*Integration Type*

⇒*Kovats Index*

⇒*Left/Right Limit*

⇒*Match Criterion*

⇒*Maximum/Minimum Wavelength*

⇒*Name* (*Peak Name*)

⇒*Reference Spectrum*

⇒*Relative Maximum Deviation*

⇒*Response Factor*

⇒*Retention Index*

⇒*Retention Time*

⇒*Standard*

⇒*Threshold*

⇒*Type* (*Peak Type*)

⇒*Use Recently Detected Retention Time*

⇒*Window*

Amount

Location:	**Peak Table** tab page Report Variable **(Peak Table/Peak Results)**
Type:	**Floating point number**
Dimension:	Determined by the **Dimension of Amounts** parameter on the **General** tab page
Value Range:	**0.000001 ... 9999999.999999**
Default:	**1.000000**
Related Parameter:	⇒*Dimension of Amounts*
Description:	The **Amount** parameter determines the content (amount, concentration) of a standard sample or a ➤*Validation Sample* of a particular mixture component. For multi-point calibration with various standards (i.e., the injection volume does <u>not</u> vary), several values must be entered for each peak. Thus, a matrix of standard contents is created, whose lines correlate to the peaks and the columns to the various standards.
Function:	Enter the amount as concentration value (e.g., µg/µl) or as absolute amount (e.g., µg). In the report, the result is displayed accordingly, either as concentration value or as an absolute amount. The respective inverse function is used for calculation (see **How to …: Creating and Using Report Tables** 🔲 **Calculating the Peak Variable "Amount"**). The ➤*Formula for Calculating the Amount* allows you to consider the response factor, dilution factor, weight, and a factor for the ➤*Internal Standard*.
	In absolute amounts, the result always relates to the injected volume; for example, 17.6 µg per 20 µl injection volume. Select the ➤*Concentration* peak result variable to normalize the value to 1 µl by dividing the absolute value by the injection volume; for example, 17.6 / 20 = 0.88 µg/µl.

Function:
(Cont'd)

☞ *Tip:*

*If a concentration unit has been chosen as **Dimension of Amount**, the **Concentration** peak variable has lost its sense.*

In a single-point calibration, only one standard is used. This means that there is only one value per component (peak).

In a "real" multiple-point calibration (➢*Single-Point and Multiple-Point Calibration* - no variation of the injection volume), the number of amount values for each component must correspond exactly to the number of standards.

☞ *Tips:*

*In a multiple-point calibration, the values of the **Amount** columns are corrected by the ratio of the injection volumes (for which the reference volume defined on the **General** tab page of the QNT Editor is used) and the dilution factors in comparison to the <u>first</u> standard. You can thus combine the amount values with the ➢Dilution Series!*

Even for multi-point calibration, normally only <u>one</u> peak table is required!

Calibration Mode

Location:	**General** tab page Report Variable **(Peak Calibration)**
Type:	**Character**
Dimension:	**No dimension**
Value Range:	**Fixed/Total/Group/Additional/Bracketed/Standard Addition**
Default:	**Total**
Related Parameter:	$\Rightarrow$*Calibration Type*
Description:	The **Calibration Mode** parameter determines the standards that are used for calibrating a specific sample of a sequence. Chromeleon provides six different calibration modes.
	Select the mode on the **General** tab page in the QNT Editor (see **The QNT Editor** 🔳 **The General Tab Page**).
Function:	
Fixed	This setting enables the calibration using various standards (also from other sequences).
	The desired standards are entered via the **Insert Standard** or **Append Standard** commands on the **Calibration** tab page of the QNT Editor. The sequence the standards are taken from is included in the **Sequence** column.
	If desired, you can enter individual calibration coefficients via the c0, c1, c2, and c3 columns in the amount table.

📖 *Tip:*

*Always perform the calibration manually (**Calibrate**). The **Auto-Recalibrate** option is not available.*

Total Calibration is performed using all valid standard samples of a sequence. The standard samples disabled in the **Enabled** column of the **Calibration** tab page (QNT Editor) are not included.

Group The calibration of a sample series from the sample list (for example, samples 3 to 50) is performed based on the standard samples listed directly before this series; for example, no. 1 and 2. If this sample list includes additional standards (for instance, no. 51 and 52), the samples that follow (for instance, 53 to 100), will be evaluated based on these. The standard samples in lines 1 and 2 will no longer be considered. (For an example, see the picture below.)

Additional For calibrating a sample, all standard samples that are listed *before* this sample in the sample list are used. The further down the sample is listed in the table, the more standard samples will be considered. For an example, see the picture below.

Bracketed Samples are evaluated based on all standard samples immediately surrounding. In case of a list containing two samples, two standards, two samples, two standards, and so on, samples 1 and 2, located at positions 3 and 4, are evaluated with standards 1, 2, 3, and 4, located at positions 1, 2, 5, and 6. Samples 3 and 4 are evaluated using standards 3, 4, 5, and 6, located at positions 5, 6, 9, and 10. For an example, see the picture below.

Standard Addition Use this mode to analyze unspiked samples using the ➤*Standard Addition* method. In this case, only the sample types **Unspiked** and **Spiked** are considered for the analysis. (For more information about these sample types, refer to ➤*Spiked Sample*).

If several unspiked samples (sample type: Unspiked) are available, only the associated spiked samples are analyzed. Make sure that they are assigned to the same ⇒*Std. Add Group (Standard Addition Group)*, i.e., verify that the names indicated in the **Standard Addition Group** column in the sample list are identical.

Calibration Type

Location:	**Peak Table** tab page Report Variable **(Peak Calibration)**
Type:	**Code**
Dimension:	**---**
Value Range:	**Linear (Lin** and/or **LOff)** **Quadratic (Quad** and/or **QOff)** **Cubic (Cubic** and/or **COff)** **Exponential (Exp)** **Point-to-Point (P-P)**
Combine with:	**No Weights, 1/Amount (X), 1/Amount² (XX), 1/Response (Y), 1/Response² (YY)** (with **Average all response values...** additionally **1/Rel.Std.Dev., 1/Rel.Std.Dev.²**)
Default:	**Linear**
Related Parameters:	⇒*Standard* ⇒*Calibration Mode*
Description:	The **Calibration Type** parameter determines the ➤*Calibration Function* and the ➤*Weights*. In calibration, up to three calibration constants per peak are entered into the calibration file, defining the peak area/amount ratio. The minimum number of required samples depends on the type of calibration curve chosen. A maximum of 20 samples from different sample files may be marked and used as calibration samples. The calibration points can also be weighted with 1/Amount, 1/Amount^2, 1/Response, and 1/Response^2. The type of the weight function is entered together with the curve type. Dionex recommends using the F8 box for selecting the desired specifications; for example, the field value *XXQOff* stands for quadratic with offset and weight function 1/Amount^2.

Function: A series of methods, differing with respect to their model function and the number of coefficients, is available. The number of calibration samples needs to be at least equal to the number of coefficients to be determined.

Calibration functions:

Linear   Minimum number of standards or ➢*Spiked*
(Lin)   *Samples*: 1

                               The model function is a straight line leading through the origin. (In ➢*Standard Addition* calibration mode, the line leads through the average of all unspiked samples.) For more than one standard, the regression line is calculated. The calibration curve is based on the following equation:

                               Model $f(A) = c1\, x\, A$
                               function:

                               Where A is the peak area and f(A) the calculated amount.

                               c1 (linear coefficient, slope) is calculated and stored.

Linear with offset   Minimum number of standards or spiked
(LOff)   samples: 2

                               The calibration curve is a straight line, crossing the ordinate at a finite value.

                               Model $f(A) = c0 + c1\, x\, A$
                               function:

                               c0 and c1 are calculated and stored.

Quadratic (Quad)	Minimum number of standards or spiked samples: 2
	The calibration curve is a parabola leading through the origin. (In **Standard Addition** calibration mode, the curve leads through the average of all unspiked samples.) For more than two calibration samples, the regression parabola is calculated.
	Model function: $f(A) = c1 \, x \, A + c2 \, x \, A^2$
	c1 and c2 are calculated and stored.
Quadratic with offset (QOff)	Minimum number of standards or spiked samples: 3
	The calibration curve is a parabola crossing the ordinate at a finite value. For more than three calibration samples, the regression parabola is calculated.
	Model function: $f(A) = c0 + c1 \, x \, A + c2 \, x \, A^2$
	c0, c1, and c2 are calculated and stored.
Cubic	Minimum number of standards or spiked samples: 3
	The calibration curve leads through the origin. (In **Standard Addition** calibration mode, the curve leads through the average of all unspiked samples.) For more than three calibration samples, the regression curve is calculated.
	Model function: $f(A) = c1 \, x \, A + c2 \, x \, A^2 + c3 x A^3$
	c1, c2, and c3 are calculated and stored.

Cubic with offset (COff)

Minimum number of standards or spiked samples: 4

The calibration curve is a curve crossing the ordinate at a finite value. For more than four calibration samples, the regression curve is calculated.

Model function:

$$f(A) = c0 + c1\, x\, A + c2\, x\, A^2 + c3xA^3$$

c0, c1, c2, and c3 are calculated and stored.

Exponential (Exp)

Minimum number of standards or spiked samples: 2

The calibration curve presents an exponential function.

Model function:

$$f(A) = c0\, x\, A^{c1}$$

c0 and c1 are calculated and stored.

The following applies to the Standard Addition calibration mode:

Model function:

$$f(A) = \widetilde{c} + c0\, x\, A^{c1}$$

with:

$\widetilde{c}$ = Expected offset value of all calibration points with positive amount values.

Point-to-Point

Minimum number of standards or spiked samples: 1

The calibration curve is polygon, that is, a linear interpolation between two calibration points. If there are several replicates of a calibration level, they are averaged before interpolation.

⚠️ *Caution:*   *The decision whether it is physically sensible to use a calibration type other than the **linear** default is the responsibility of the user. (With a calibration type that is physically not sensible, no y values may appear for the actually measured values. For example, if, for the Quadratic calibration type, the calibration function is a bottom-down parabola-shaped curve, the values measured above the summit cannot be evaluated. In this case, Chromeleon reports "n.a.")*

Weighting:

No Weights   Default: higher weighting of higher amounts or signal values.

1/Amount (X)   Nearly cancels out the weighting of higher amounts.

1/Amount^2 (XX)   Causes over-proportional weighting of smaller amounts.

1/Response (Y)   Nearly cancels out the weighting of higher signal values. In this case, the Y-values (dependent signal values) of the calibration points are used as weight factors instead of the X-values (nominal amounts).

1/Response^2 (YY)   Causes over-proportional weighting of smaller signal values. In this case, the Y-values (dependent signal values) of the calibration points are used as weight factors instead of the X-values (nominal amounts).

1/Rel.Std.Dev. (S)   Weights signal values with small relative standard deviations more than those with large relative standard deviations.

1/Rel.Std.Dev. ² (SS)   Weights signal values with small relative standard deviations clearly more than those with large relative standard deviation.

Options: **Average all response values of each calibration level before curve fitting** averages the calibration level points before calculating the calibration curve from the average values.

Include point (0,0) for curve fitting includes the origin into the calibration curve calculation for calibration functions with offset without forcing the calibration curve through the origin.

📑 *Tip:* *All procedures are possible both, with the area method and the height method. Note that integration and calibration should fit together, that is, either area method in both cases, or height method. Only the area method can be substantiated by physics.*

For more information about the available calibration types, refer to **Theory of Calibration**:

🔺 **Calibration Types (Linear)**

🔺 **Calibration Types (Non-Linear)**

Check Derivative

Location:	**Peak Tracking** and **Spectra Library Screening** tab pages/Report Variable **(Peak Table)**
Type:	**Selection**
Dimension:	**-**
Value Range:	**Off; 1st derivative; 2nd derivative**
Default:	**Off**

Related Parameters: Additional parameters for comparing spectra:

⇒ *Check Extrema*
⇒ *Match Criterion*
⇒ *Maximum/Minimum Wavelength*
⇒ *Relative Maximum Deviation*
⇒ *Threshold*

Description: Comparison of two or more spectra with each other can be performed in three different ways (comparison functions):

- Based on the curve shape of the spectrum itself

- Based on the curve shape of the first derivative of the spectrum

- Based on the curve shape of the second derivative of the spectrum

Function: Comparing the curve shape of the first or second derivative of the spectrum is more characteristic (shoulders become extremes). However, the ➤ *Signal-to-Noise Ratio* decreases considerably. Depending on the comparison function different ⇒ *Threshold* values are useful.

Note: *Differentiating once or twice can lead to well matching hits, although the absorption heights of the original spectra differ significantly.*

Check Extrema

Location:	**Peak Tracking** and **Spectra Library Screening** tab pages/Report Variable **(Peak Table)**
Type:	**Selection**
Dimension:	**-**
Value Range:	**Off; On**
Default:	**Off**
Related Parameters:	Additional parameters for comparing spectra:

⇒ *Check Derivative*
⇒ *Match Criterion*
⇒ *Maximum/Minimum Wavelength*
⇒ *Relative Maximum Deviation*
⇒ *Threshold*

Description: The parameter indicates whether the spectra search compares the number of extrema in the reference spectra with the ones in the peak spectrum.

Note: *If there is a high noise level (see ➢Signal-to-Noise Ratio), it is possible that noise peaks are considered extrema. In this case, Dionex recommends disabling the **Check Extrema** option.*

Comment (Peak)

Location:	**Peak Table** tab page Report Variable **(Peak Table)**
Type:	**Text**
Dimension:	---
Value Range:	**All printable characters**
Default:	---
Related Parameters:	⇒*Name* (*Peak Name*)
Description:	In addition to the peak name, further comments can be entered. Double-click or press the F8 key to open an enlarged entry field.

Note:

*If you have generated the peak table in the QNT Editor via **Autogenerate Peak Table**, this will be automatically indicated in the peak table's **Comment** column:*

- *If **Enumerate peaks of current chromatogram** is enabled, the comment is **Autogenerated**.*

- *If **Use spectra library screening results** is enabled, the comment is **Autogenerated, Spectrum: Name of Reference Spectrum, Match:** ➢Match Factor **number**.*

Dead Time

Location:	**General** tab page Report Variable **(Quantification Method)**
Type:	**Floating point value**
Dimension:	**Minutes**
Value Range:	**0.000 ... 9999.999**
Default:	**Not chosen**
Related Parameters:	⇒*Retention Time* ⇒*Delay Time* ⇒*Window*
Description:	The dead time is defined as the time required for the peak maximum of an unretained substance to reach the detector from the point of injection. Thus, the dead time corresponds to the residual time (of all substances) in the mobile phase. Enter the dead time on the **General** tab page in the **QNT Editor**.
Function:	The adjusted retention time of a substance is defined as the time this substance remains in the stationary phase. The sum of the residual times in the mobile and stationary phases is the ⇒*Retention Time*:

$$t_S = t'_S + t_0$$

Where:

t_S = Retention time of a substance

t'_S = Adjusted retention time of a substance

t_0 = Dead time

The dead time is required to calculate the ⇒*Capacity Factors* and the ⇒*Kovats Indexes*.

Delay Time

Location: **General** tab page
Report Variable **(Quantification Method)**

Type: **Floating point value**

Dimension: **Minutes**

Value Range: **-9999.999 ... 9999.999**

Default: **Not selected**

Related Parameters: ⇒*Dead Time*

⇒*Retention Time*

Description: The time required for a substance to travel from the detector cell of one detector to the detector cell of a second detector is referred to as **Delay Time**.

Function: Usually, a constant ⇒*Flow* is used. With constant flow, the delay time between two detectors is constant as well. In this case, it can be measured and entered on the **General** tab page in the QNT Editor (see **The QNT Editor 🔲 The General Tab Page**). Verify that a second detector (or channel) is specified in the **Detector name** field. Otherwise, you cannot enter a delay time.

The entered delay times can be positive or negative, depending on whether the detector directly following the column or any other detector is defined as **2nd detector**.

🗒️ *Note:* *Entering a delay time for ➢Flow Gradients does not make sense because the delay is not constant in this case. Follow the description in **How to …: Integrating Chromatograms and Identifying Peaks 🔲 Defining the QNT Method for Several Detectors**.*

Dimension of Amounts

Location:	**General** tab page
	Report Variable **(Peak Table)**
Type:	**String**
Dimension:	---
Value Range:	**All printable characters**
Default:	---
Related Parameters:	⇒*Amount*
Description:	The **Dimension of Amounts** parameter defines the physical dimension of the amount values. This is usually an amount dimension, but you may select a concentration dimension, instead.
Function:	The chosen dimension is entered in the column header of the amount column of the report table. It is only relevant to the documentation of results and has no influence on the calculation algorithms.
☞ *Tips:*	*Consistency of dimensions is entirely up to the user. If weight was read as g, do not enter mg here. There will be no automatic conversion!*
	*For the calculation of the concentration in the report or the printer layout, Chromeleon presumes that a dimension of amount was chosen. If a dimension of concentration was entered as **Dimension of Amounts**, this calculation does not make sense.*

Group (Peak Group)

Location:	**Peak Table** tab page
	Report Variable **(Peak Table)**
Type:	**String**
Dimension:	**---**
Value Range:	**All printable characters**
Default:	**---**
Related Parameters:	⇒*Peak Group Start/End*
	⇒*Integration Type*
	⇒*Calibration Type*

Description: Peaks may be grouped and then be calibrated and integrated together. You may assign the group any name, whilst it is recommended to use the name of the group's most "important" member. The members must not necessarily succeed one another.

(If you wish to group peaks that lie close together, use the **Peak Group Start** and **Peak Group End** detection parameters (on the **Detection** tab page of the QNT Editor).

Function: During calibration (not during peak detection), the peak group is treated as one non-resolved peak: The calibration constants of the amount value and the area sum of all peaks being part of the group are calculated. The calibration curve, therefore, is identical for all peaks.

During integration, the amount/concentration values for each peak are calculated individually for each peak from its area and calibration constants.

📑 *Tip:* *The **Integration** and **Calibration** types must be identical for all peaks within a peak group.*

Also, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Grouping Peaks**.

Integration Type

Location:	**Peak Table** tab page
	Report Variable **(Peak Table)**
Type:	**Selection**
Dimension:	**No dimension**
Value Range:	**Area/Height/Relative Area/Relative Height/CE Area**
Can be combined with:	**All peaks, identified Peaks only/Exclude ISTD peaks** (only with **Relative Area/Relative Height**)
Default:	**Area**
Related Parameters:	⇒*Calibration Type*
	⇒*Group*
Description:	Indicates the peak property to be used for the calculation of quantitative results. Different Area and height methods are the alternatives.
Functions:	

Area Peak area integration.

All amount calculations refer to the peak area which itself is calculated numerically according to the trapezoidal method. Area delimiters in this case, consist of chromatogram, baseline and, if necessary, perpendiculars in order to exclude adjacent non-resolved peaks. In case of ➢*Riders*, an appropriate skimming tangent substitutes the baseline.

Height Peak height integration.

All amounts refer to the peak height; that is, to the height of the peak maximum as related to the baseline.

Relative Area   Relative peak area integration. The area can be chosen relative to the area of

- **All peaks** or

- **Identified peaks only**

In both cases, the internal standard(s) may be excluded (**Exclude ISTD peaks**).

Relative Height   Relative peak height integration. Reference corresponding to the relative peak area integration is possible.

CE Area   Integration type for capillary electrophoresis

CE-Area = Area / Ret. Time in [mAU])

☞ *Tips:*   *Integration and calibration always require the same method!*

*All peaks of a **Group** require the same method.*

Only the area methods can be substantiated by physics.

Kovats Index

Location:	**Peak Table** tab page
	Report Variable **(Peak Table)**
Type:	**Integer**
Dimension:	**No dimension**
Value Range:	**0 ... 999.999**
Default:	**-**
Related Parameters:	$\Rightarrow$*Retention Time*
	$\Rightarrow$*Dead Time*

Description: Data exchange between laboratories for substance identification based on relative retention times is not exact. Therefore, especially in gas chromatography, retention indexes are used. They use a series of chemically similar standard substances to achieve uniform scaling of the **Retention Time**.

Advantage is taken of the fact that within a homologous series of compounds the logarithms of the adjusted retention times are proportional to the number of carbon atoms (adiabatic and isothermal conditions presumed). The most frequently used **Kovats Index** is defined as follows:

$$KI = 100 * n + 100 \frac{[\log t'_S - \log t'_n]}{[\log t'_{n+1} - \log t'_n]}$$

Where:

KI = Kovats index

t'_S = Adjusted retention time with $\quad t_S = t'_S + t_0$

t_S = Retention time

t_0 = Dead time

n = Number of carbon atoms (normally of n-alcane)

S = Substance of interest

Description:
(Cont'd)

The following must be true:

$$t_n < t_S < t_{n+1}$$

Function:

As the Kovats indexes for the markers are freely selectable the following formula is used for the calculation:

$$KI = KI(z) + (KI(z+1) - KI(z)) \frac{[\log t'_S - \log t'_z]}{[\log t'_{z+1} - \log t'_z]}$$

With KI = Kovats index (usually KI = 100 * n is entered) and

z = number of the marker peak.

If no marker peaks or no dead time is determined, the Kovats index is not defined (n.a.). In addition, the Kovats index is not defined for any peaks that occur before the first and behind the last marker peaks. For more information about how to enter marker peaks and dead time, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining the Retention Index and the Kovats Index**.

 Note:

In Chromeleon, the substances of interest must be between two marker peaks.

Left/Right Limit

Location:	**Peak Table** tab page Report Variable **(Peak Table)**
Type:	**Fixed point number**
Dimension:	**Minutes**
Value Range:	**0 ... 999.999 [min]**
Default:	**0 (no function)**
Related Parameters:	⇒*Retention Time*
Description:	Normally, peak integration is performed automatically. It is possible to limit or extend integration on the left, right, or on both sides with the appropriate peak table parameters. This may be done in the peak table via a left and/or a right limit.
Function:	The peak is integrated between left and right limit, that is, between:

t - LL   ...   t + RL

Where:

t = Retention Time

LL = Left Limit

RL = Right Limit.

The value 0 disables the limitation.

Example 1:

For the right and left integration limits, the value 0.5 min is entered. The peak maximum is at the retention time of 8 min. The peak is integrated from 7 min 30 s to 8 min 30 s.

Note: *The LL and RL limits can also be enabled/disabled independently.*

Example 2:

For the left integration limit, the value 0.5 min is entered. For the right integration limit, the value 0 is entered. As before, the peak maximum is at the retention time of 8 min. The peak is integrated from 7 min 30 s. The end of integration is automatically determined then.

Match Criterion

Location:	**Peak Table** and **Spectra Library Screening** tab pages/ Report Variable **(Peak Table)**
Type:	**Selection**
Dimension:	**No dimension**
Value Range:	**Least Square, Weighted Least Square, or Correlation**
Default:	**Least Square**
Related Parameters:	Additional parameters for comparing spectra:

⇒*Check Derivative*
⇒*Check Extrema*
⇒*Maximum/Minimum Wavelength*
⇒*Relative Maximum Deviation*
⇒*Threshold*

Description: The match criterion defines the mathematical method with which two standardized spectra are compared with each other.

Function: The signal deviation, that is, the difference between the UV spectrum and a reference spectrum at defined wavelength, is checked (λ1, λ 2, ...). Since the square of the deviations is calculated as well, the direction of the subtraction does not matter.
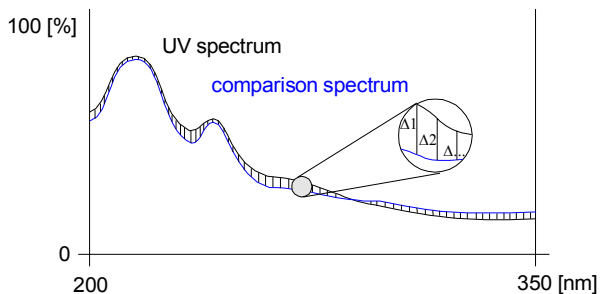
The method provides a value of similarity (match) between completely different (= 0) and identical (= 1000).

Function: *(Cont'd)*	The following mathematical methods are available in Chromeleon:
Least Square:	Forms the sum of the squared signal deviations at each wavelength and determines the average square deviation between two spectra (standard method)
Weighted Least Square:	Analogue to Least Square; the squared deviations are weighted by the signal height (for spectra extracted close to the detection limit).
Correlation:	Usual match criterion in statistics if a linear correlation between two curves is presumed (similar to Least Square; usually provides the same result).

Maximum/Minimum Wavelength

Location:	**Peak Tracking** and **Spectra Library Screening** tab pages/Report Variable **(Peak Table)**
Type:	**Floating point number**
Dimension:	**nm**
Value Range:	**Auto; 190.0 - 900.0 nm**
Default:	**Auto**
Related Parameters:	Additional parameters for comparing spectra: ⇒*Check Derivative* ⇒*Check Extrema* ⇒*Match Criterion* ⇒*Relative Maximum Deviation* ⇒*Threshold*
Description:	The **Maximum Wavelength** (**Max. WL**) and **Minimum Wavelength** (**Min. WL**) columns allow you to enter the upper and lower wavelength limits for spectra screening. In case of **Auto**, the respective limits will not be considered.
Note:	*The value entered here has no significance for any currently displayed peak spectrum.*

Name (Peak Name)

Location:	**Peak Table** tab page Report Variable **(Peak Table)**
Type:	**String**
Dimension:	**no dimension**
Value Range:	**All printable characters**
Default:	With automatic table generation: **<Name of the QNT Method>x**, where **x** is the current peak number. When appending a new cell: **Peak-x**
Description:	In this column, you may enter the chemical name of the substance that created the peak at the time t. (The default is used with auto-generated peaks, only.)
Function:	The substance name is taken for the report and the ➢*Printer Layout*.
Note:	*Only use letters, numbers, and punctuation marks, since the complete MS-DOS character set is not available on all printers.*

Reference Spectrum

Location:	**Peak Tracking** tab page
	Report Variable **(Peak Table)**
Type:	**Selection**
Dimension:	**No dimension**
Value Range:	**---**
Default:	**Empty**
Related Parameters:	$\Rightarrow$*Window*
Description:	Reference spectra are used for substance identification. A reference spectrum can be chosen for each peak. Such spectra are acquired with the Chromeleon DAD license.
	Identifying peaks by means of peak tracking can be enabled via input in the **Window** column. In the corresponding F8 dialog box (press the F8 key or double-click the **Window** column), select the **Spectrum** or **Spectrum and time** option of the **Peak Match** group.
Function:	Peak tracking is performed (limited to the specified time window in the case of **Spectrum and time**) using the reference spectra in the corresponding column. (For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔼 **Peak Tracking**.)
👉 *Tip:*	*Spectra acquisition is possible with the DAD license only.*

Relative Maximum Deviation

Location:	**Peak Tracking** and **Spectra Library Screening** tab pages/Report Variable **(Peak Table)**
Type:	**Floating point number**
Dimension:	**nm**
Value Range:	**Off; 0.0-100.0 nm**
Default:	**Off**
Related Parameters:	Additional parameters for comparing spectra:
	⇒*Check Derivative*
	⇒*Check Extrema*
	⇒*Match Criterion*
	⇒*Maximum/Minimum Wavelength*
	⇒*Threshold*
Description:	The **Relative Maximum Deviation** column allows you to enter the maximum permissible deviation of the wavelength maxima of the reference spectra from the maximum of the current peak spectrum.
Function:	If the setting is **Off**, deviation is not checked.

Response Factor

Location:	**Peak Table** tab page Report Variable **(Peak Table)**
Type:	**Floating point number**
Dimension:	**---**
Value Range:	**0.000000 ... 9999999.999999**
Default:	**1.000000**
Related Parameters:	⇒ *Weight* (Sample Weight Factor) ⇒ *Dil. Factor* (dilution factor)
Description:	The response factor is a peak-specific dimensionless, multiplicative factor, used to compensate for absorption differences in the case of differing wavelengths.
Function:	

Absolute Select **Absolute** to correct the amount value received from the peak area via the ➤ *Formula for Amount Calculation* by the entered factor. In this way, for example, absorption differences at different wavelengths can be compensated. Besides, losses of readily volatile samples can thus be considered in the calculation (especially common in gas chromatography).

Relative to Peak Select **Relative to Peak** to calculate the peak in relation to a reference peak. Any identified peak with absolute response factor can be used as the reference peak. The basis of the evaluation is the calibration curve of the selected reference peak. The amount value calculated in this way is multiplied by the reciprocal value of the relative response factor and thus corrected.

Relative to Peak
(Cont'd)   **Example: Relative to Peak**

Under certain conditions, an active agent in a tablet decomposes into various products. As standards for these products are difficult to produce or very expensive, the active agent and the decomposition products are calibrated infrequently; for example, once a year.

If the active agent and the decomposition products are calibrated via the **linear without offset** function, there is a direct correlation between the slope of the active agent calibration curve and the calibration curves of the individual decomposition products. This proportionality between the calibration coefficient c1 of the active agent and that of the decomposition product is expressed as the Response Factor and must be determined by the user.

Should the active agent and/or the decomposition product be determined in an analysis, the existing calibration and the Response Factor determined for each decomposition product is used. The factors are entered as *relative* values (**Response Factor** column) in the peak table; that is, the active agent in the tablet is used as the reference peak.

Calculating the amount values of all decomposition products is according to the calibration coefficients of the active agent, corrected by the Response Factor.

*Note***:**   *The response factor is* <u>*peak-specific*</u>*.*

In contrast to this, the Sample Weight and the Dilution Factor of the sequence are sample-specific; that is, they apply to all peaks.

Retention Index

Location:	**Peak Table** tab page
	Report Variable **(Peak Table)**
Type:	**Integer**
Dimension:	**No dimension**
Value Range:	**0 - 999.999**
Default:	**-**
Related Parameters:	⇒*Retention Time*
	⇒*Dead Time*
	⇒*Kovats Index*
Description:	Retention indexes are used to facilitate positive assignment of substances in inter-laboratory data exchange. For programmed GC as well as for HPLC linear retention indexes are defined.
Function:	Chromeleon calculates the following linear retention index:

$$RI_S = RI_Z + \frac{[RI_{Z+1} - RI_Z]*[t_S - t_Z]}{t_{Z+1} - t_Z}$$

Wherein:

RI: Retention index (usually RI = 100, 200, 300 is entered)

S: Substance

Z: number of the marker

The following is true:

$$t_Z < t_S < t_{Z+1}$$

If the peak occurs before the first marker peak, the following formula is used:

$$RI (x) = RI(1) * [t(x) / t(1)]$$

Function:
(Cont'd)

If the peak occurs behind the last marker peak, the following formula is used:

$$RI (x) = RI(last) * [t(x) / t(last)]$$

For information how to determine marker peaks, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining the Retention Index and the Kovats Index**.

Retention Time

Location:	**Peak Table** tab page Report Variable **(Peak Table)**
Type:	**Floating point value**
Dimension:	**Minutes**
Value Range:	**0.000 ... 9999.999**
Default:	**0.000** With **Autogenerate Peak Table**, the retention time of the active chromatogram is used.
Can be combined with:	**Absolute time; Time distance/ratio to reference peak; Reference peak**
Related Parameters:	⇒*Window* ⇒*Use Recently Detected Retention Times* ⇒*Dead Time*
Description:	The retention time is used for peak identification. The **Retention Time** variable refers to the time (in minutes) that passed since the injection (also, refer to ⇒*Dead Time*). By definition, the injection time always equals zero. Therefore, control commands issued before this time will have negative times. The advantage is that the times stated in the ➢*PGM File* (file type .PGM) are compatible with the retention times, rather than being delayed by the injection time. Peak retention times are generally interpreted as time between injection and peak maximum. However, indication of the distance to a reference peak is possible as well. As the reference peak does not need to be at the beginning of the chromatogram, such difference retention times can also have negative values. Relative retention times are time quotients in percent as to a reference peak.

Function: On the page **Peak Table** in the QNT Editor, the retention time can be entered either manually by the user or automatically by the system. The latter is possible via the **Autogenerate Peak Table** command on the **Edit** or context menus.

The user enters the expected retention time, while the system enters the actually detected retention time of the actually analyzed sample.

Interpretation Input in the **Retention Time for Peak X** dialog box (to be opened via **F8** or by double-clicking in the **Ret. Time** column)

Absolute time Select **Absolute time [min]** to display the retention times as usual (time period between injection and peak maximum).

Time distance to reference peak [min] Select **Time distance to reference peak [min]** to express the time difference to the reference peak in minutes. All peak lines apart from the reference peak line display time differences in the time column.

Time ratio to reference peak [%] Select **Time ratio to reference peak [%]** to express the retention time relatively to the reference peak in % values. The retention times are indicated as time ratio (percent) to the reference peak. All peak lines apart from the reference peak line display the time ratios to the reference peak in percent.

Reference peak Select a peak from the peaks listed in the selection box. The reference peak line in the peak table is in light blue. It **always** displays the **absolute retention time**!

Note: *If the* **Use Recently Detected Retention Time** *option (retention time correction) is enabled in the peak table, the retention times entered manually on the page* **Peak Table** *are no longer used as nominal retention times for all identified peaks. Instead, the values detected for the previously analyzed sample (its nominal times) are used. Distinguish between:*

- *The originally expected retention time that was entered on the* **Peak Table** *tab page.*

- *The nominal retention time which, in this case, corresponds to the actual retention time of the previously analyzed sample.*

- *The actual retention time of the actually analyzed sample.*

⇒*Retention Windows are also interpreted as percentage value of the absolute retention time for both, difference, and relative retention times! Only the retention times of identified peaks are corrected!*

In the Peak Table, you can toggle the three options. The retention time column is automatically re-calculated.

Standard

Location:	**Peak Table** tab page
	Report Variable **(Peak Table)**
Type:	**String**
Dimension:	**---**
Value Range:	**External; Internal; Internal/External; Use this peak as internal Standard**
Default:	**External**
Can be combined with:	**Use sample amount as reference** (only with **Use this peak as internal Standard**)
Related Parameter:	$\Rightarrow$*Amount*
Description:	The **Standard** parameter indicates the standard method to be used for calibration. For a detailed description including numerical examples, refer to **How to …:** 🔲 **Calibrating**.
Function:	

External
The calibration curve determines the area/amount ratio. During calibration, the calibration coefficients are calculated from mixtures of known components (see ▷*Calibration Function* and **Theory of Calibration** 🔲 **Standard Methods**).

Internal
Calibration corresponds to the External Standard method. In addition, an ▷*Internal Standard* is added. The internal standard itself is calibrated as all other peaks. When performing the integration, the calculated amounts of the remaining peaks are corrected by the nominal/actual ratio of the internal standard.

Chromeleon recognizes any number of internal standards. Theoretically, each peak may have its own internal standard! Furthermore, the standard method may be selected for each peak individually.

Internal
(Cont'd)

During calibration, the amount ratio (instead of amount against area) is displayed in the diagram with regard to the respective peak. From these data points, the calibration function is calculated.

During integration also, the area ratio is inserted into the stored calibration function and the amount ratio is calculated.

👉 *Tip:*

*Before you can use this option, at least one internal standard must be defined (see **Use this peak as internal Standard**). Select an ISTD peak in the **Associated ISTD Peak** check box.*

Entering Several Internal Standards

It is also possible to define several internal standards. Via the F8 box, define the respective peaks of the peak table in the Standard column of the peak table as ISTD peaks. The corresponding line of peak table has a yellow background.For the remaining peaks, enter the standard method to be used (external, internal/external, internal). If this should be performed in relation to an ISTD peak, it is possible to choose between the previously defined ISTD peaks.

Internal/External

When calibrating with an internal standard, calculation is with area and amount ratios instead of absolute areas and amounts. During integration, the area ratio is inserted into the saved calibration function to calculate the amount ratio. The result is the amount in relation to its reference peak. Each peak can have its own internal standard.

Calibration does not vary from the external standard method, except for a defined peak (that is, internal standard) being added to the analyses. The internal standard is calibrated as all other peaks. During integration, the calculated amounts of the other peaks are corrected according to the relation of the internal standard to the actual peak.

Interna/External (Cont'd)	🖙 *Tip:*
	*Before you can use this option, at least one internal standard must be defined as well (see **Use this peak as internal Standard**). Select an ISTD peak in the **Associated ISTD Peak** check box.*
Use this peak as Internal Standard	Select this option to define the current peak as ISTD peak. Select the **Use sample amount as reference** check box to use the amount of the internal standard from the ISTD amount column of the sample list instead of the **Amount** columns of the QNT peak table. In this way, you can enter a different amount of internal standard for each sample.
🖙 *Tips:*	*For internal standard as well as internal/external standard methods, a substance whose concentration is known, must be added to the standards and to the analyses. This can be conveniently carried out by means of an appropriate ➤Autosampler. Quantitative accuracy reached with this method, exceeds that of the external standard method by one dimension.*
	For these two options, the name of the internal standard must be indicated.
	Internal standard methods compensate even small injections deviations, since possible loss or excess apply to the internal standard as well.

Threshold

Location:	**Peak Tracking** and **Spectra Library Screening** tab pages/Report Variable **(Peak Table)**
Type:	**Integer**
Dimension:	**No dimension**
Value Range:	**0 - 1000**
Default:	**950**
Related Parameters:	Additional parameters for comparing spectra:

⇒*Check Derivative*
⇒*Check Extrema*
⇒*Match Criterion*
⇒*Maximum/Minimum Wavelength*
⇒*Relative Maximum Deviation*
⇒*Threshold*

Description: This parameter defines the threshold of the ➢*Match Factor* above which two spectra may be accepted as matching.

Function: Depending on the comparison function the following settings can usually be recommended:

Spectrum (Off): 950

1st derivative: 900

2nd derivative: 800

Type (Peak Type)

Location:	**Peak Table** tab page **(Peak Table)** Report Variable
Type:	**Alternatives**
Dimension:	**No dimension**
Value Range:	**Autodetect (Auto)/Rider (R)/Main (M)/Baseline-Main (B-M)/Main-Baseline (M-B)/Baseline-Main-Baseline (B-M-B)**
Default:	**Autodetect**
Related Parameters:	⇒*Rider Threshold* ⇒*Maximum Rider Ratio* ⇒*Valley to Valley* ⇒*Lock Baseline*
Description:	The **Type** parameter determines the type of baseline contact and the separation of other, non-resolved peaks. An integration program algorithm usually defines the peak type. You can also change the peak type as desired, using the detection parameters (see **How to …: Integrating Chromatograms and Identifying Peaks** 🔼 **Modifying Detection Parameters**). To determine the peak type regardless of other factors, this should be done in the peak table. Type specifications of the peak table have **priority**.
Functions:	
Autodetect (Auto)	The peak type is determined via an algorithm, or via detection parameters.
Determine at Calibration	Not yet implemented

Rider (R)/Main (M)	The peak is integrated as rider or main peak <u>without</u> baseline contact. This, of course, applies to non-resolved peaks, only.
	In the case of rider peaks, an additional distinction is made between peaks on the leading edge (**u** = up) and peaks on the trailing edge (**d** = down). Of course, this does not affect the type of baseline contact of the reference peak.

 Note:

Chromeleon does not consider rider peaks when calculating the ➢*Optimum Integration Path.*

Baseline-Main(B-M)/ Main-Baseline(M-B)/ Baseline-Main-Baseline (M-B-M)	The peak is integrated as main peak with left, right, or bilateral baseline contact.
	A further distinction between **b** and **B** is made in the report: If a main peak has direct baseline contact on the left and/or right side, this is, indicated by a capital **B**. If the baseline below non-resolved peaks is drawn from minimum to minimum (⇒*Valley to Valley*), that is, from peak end to peak end, this type of baseline contact is characterized by a small **b**.
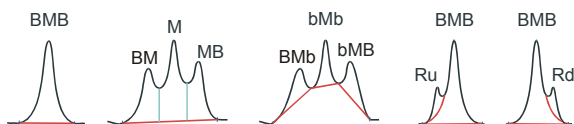
 Note:

The peak type determined here can only be realized with non-resolved peaks. For resolved peaks, peak type is always Baseline-Main-Baseline.

*The distinction between **b** and **B** is made only in the report output. The baseline is checked automatically. Then, the baseline contact is determined. In the peak table, the classification is always **B**.*

*The peak type that is defined in the peak table has priority over the type assignments of the **Rider Threshold** and **Maximum Rider Ratio** detection parameters.*

Use Recently Detected Retention Time

Location:	**General** tab page **Use Prev. Retention Time (Peak Table)** Report Variable
Type:	**Check-box**
Dimension:	**---**
Value Ranges:	**Marked/not marked** **Use recently detected ret. times of last sample/standard** **Set reference time to Last value/Average of last n values/Global average**
Defaults:	**Not marked** **Last sample** **Last value**
Related Parameters:	⇒*Retention Time* ⇒*Window*
Description:	This QNT File parameter (**General** tab page) defines whether the retention time stored in the peak table (nominal time) is used by default to identify a peak or whether the actual retention time of a peak in the last sample is used. Thus, many types of drift phenomena can be compensated; for example, evaporation of volatile components in pre-mixed solvents or column age. If the peak is not found in this sample, the chronologically previous sample in the sequence is searched, etc. (The chronological order is important and not the order in the sample list.) If you have chosen this option, you may select whether you want to use the retention times of the last unknown sample (**Sample**) or of the last **Standard**.

Description: *(Cont'd)*	Click **Options** to define whether you want to use the last retention time (**Last value**), the average of the last x retention times (**Average of last x values**), or the average of all retention times (**Global average**).
Function:	If the **Use Recently Detected Ret.Time** check box is selected, Chromeleon always stores the last actual retention time of the peak and positions the **Window** specified in the peak table around this actual value, not around the nominal value stated in the peak table.
	This allows positive identification of peaks even if the retention time changes due to column trends, and thus, if the peak leaves the defined retention time window.
Example:	The first sample of a sequence containing 10 samples is processed in an automatic batch. Based on the retention times stated in the peak table and the selected window values; for example, 0.2G, four peaks are identified in the first sample (A, B, C, and D). Each one of these peaks may have an actual retention time that deviates from the nominal retention time. Chromeleon stores these actual values and uses them as the basis for positioning the time window in the second sample. If single peaks are then identified in the second sample, the "stored" actual values are replaced by the current actual values. The time window is repositioned accordingly. If, for example, the retention time of peak A from the first sample (1 min) is delayed by 10 s in each sample, the peak will be appear at 1 min + 9 x 10 s = 150 s or 2.5 min in the tenth sample. As the time window is also moved by 10 s with each sample, peak A can be clearly identified in the tenth sample, although the peak is no longer within the originally specified time window (48 s to 1 min 12 s).
Note:	*Only retention times of identified peaks are corrected!*

Window *(Retention Window)*

Location:	**Peak Table** tab page Report Variable **(Peak Table)**
Type:	**Positive floating point number or string**
Dimension:	**Minutes or %**
Value Range:	**0.000 ... 999.999 or 0 - 100%**
	Absolute/Relative and **First/Greatest/Nearest/Spectrum/Spectrum and time**
Default:	**Autogenerate Peak Table**: A third of the distance to the preceding or the succeeding peak depending on which distance is smaller
	Else: 0.100 min or %
	Absolute Greatest
Can be combined with:	**Check the best hits only** (with the peak match options **Spectrum** and **Spectrum and Time** only)
Related Parameters:	$\Rightarrow$*Retention Time*
Description:	The **Window** parameter defines a tolerance interval within which the peak is expected. The start and end times of the retention window result from adding the window value to and subtracting it from the retention time (retention time +/- window value); that is, the window width is always twice as wide as your input. A peak outside this window is not identified. The window value defined here is either interpreted as absolute (minutes) or relative (percentage) value by the integration programs. For each peak, the interpretation can be individually specified, by adding a suffix to the window time.

Function: The dimension W of the time windows is calculated according to the following formula:

Absolute time window: W = TW

Relative time window: W = T x TW / 100

Where T is the retention time of the table and TW the entered **window** value. All peaks whose maximum falls into the time interval T+W belong to the time window.

Function of the suffixes:

- Select **Absolute** or add a capital **A** to the window value to indicate the window width in minutes.

- Select **Relative** or add a capital **R** to the window value to indicate the window width relative to the retention time.

If a peak is registered within this window, it is assigned the peak name entered in the peak table. If several peaks are located in the same window, the window parameter can be extended to determine which peak is identified:

- Select **First** to identify the first peak within the window (F).

- Select **Greatest** to identify the largest peak within the window (G).

- Select **Nearest** to identify the peak nearest to the specified retention time (N).

- Select **Spectrum** to identify a peak by a spectrum. The reference spectrum is specified in the **Spectrum** column of the peak table (S).

- Select **Spectrum and time** to identify a peak within a specific window width by a spectrum. The reference spectrum is specified in the **Reference Spectrum** column of the peak table (ST).

Via the **Check the best hits only** option, **Spectrum** and **Spectrum and time** allow you to check the hits with the largest ➢*Match Factors* for the respective peaks only (= check box selected, default). The abbreviation for this is **B** (**SB** or **STB**).

If you have MS options installed, you can also enable the **Check mass ratios** option to check whether the masses indicated on the **Mass Tracking** tab page are found in the defined ratio range.

Examples:

Window Values: 0.2 AF / 0.2 AG / = 0.2 AN

If the expected retention time of the peak is Ret.Time = 5.0min and the window value is 0.2 A, the time window is 5.0min +/- 0.2min = 4min 48sec to 5min 12sec. By adding the suffix F, the first peak in the time window, by adding the suffix G, the greatest peak in the window, and by adding the suffix N, the peak nearest to the expected retention time is assigned.

Window Values: 10 RF / 10 RG

If the expected retention time of the peak is Ret.Time = 5.0min and the window value is 10 R, the time window has the range 5.0 min ± 10% = 5 min ± 0.5 min = 4 min 30 s to 5min 30 s. By adding the suffix F, the first peak in the time window, by adding the suffix G, the largest peak in the window is assigned.

Note:

The absolute time interval of the window is twice as wide as the window value entered, since the interval reaches from T-W to T+W!

Detection Parameters

Chromeleon supports the following detection parameters:

⇒*Baseline Point*

⇒*Detect Negative Peaks*

⇒*Front Riders to Main Peaks*

⇒*Inhibit Integration*

⇒*Lock Baseline*

⇒*Maximum Area Reject*

⇒*Maximum Height Reject*

⇒*Maximum Peak Height*

⇒*Maximum Rider Ratio*

⇒*Maximum Width*

⇒*Minimum Area*

⇒*Minimum Height*

⇒*Minimum Width*

⇒*Peak Group Start/End*

⇒*Peak Purity Start/End Wavelength* (*PWlStart/PPWlEnd*)

⇒*Peak Purity Threshold (PPTrshold)*

⇒*Peak Shoulder Threshold*

⇒*Peak Slice*

⇒*Rider Skimming*

⇒*Rider Threshold*

⇒*Sensitivity*

⇒*Tailing/Fronting Sensitivity Factor*

⇒*Valley to Valley*

⇒*Void Volume Treatment*

For more information about how to define detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Detection Parameters**.

Baseline Point

Location:	**Detection** tab page
	Report Variable **(Detection Parameters)**
Type:	**Command**
Dimension:	---
Value Range:	---
Default:	---
Related Parameters:	⇒*Lock Baseline*
	⇒*Valley to Valley*
	⇒*Inhibit Integration*
	⇒*Type (Peak Type)*
Description:	The **Baseline Point** detection parameter defines a baseline point at the indicated time.
🖝 *Tips:*	*When setting a baseline point, keep in mind that this point will be valid for all chromatograms that are evaluated with the respective QNT Method. If in one of these chromatograms a peak maximum occurs by coincidence at the time of your hard entered baseline point, the peak maximum will be defined as base point and the peak will not be detected.*
	This command has priority over ⇒Lock Baseline and ⇒Valley to Valley, but not over ⇒Type (Peak Type) from the peak table.

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔺 **Defining Detection Parameters**.

Detect Negative Peaks

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Switch**
Dimension:	---
Value Range:	**On; Off**
Default:	**Off**; that is, negative peaks are not detected.
Description:	The **Detect Negative Peaks** parameter enables and disables detection of negative peaks. When the parameter is enabled, negative as well as positive peaks are detected.
Function:	Enabling detection of negative peaks automatically enables the ⇒*Lock Baseline* parameter. Disabling negative peak detection locks the baseline to the default again. In the result report, the area of negative peaks is indicated as a positive value.
	To correct the baseline without labeling the peaks or including peaks in the peak list, select the **Don't label** option.
☞ *Tip:*	*In order to be effective, this switch must be activated before the peak start.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks 🔲 Defining Detection Parameters**.

Front Riders to Main Peaks

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Selection**
Dimension:	**-**
Value Range:	**Off, On**
Default:	**Off**
Related Parameters:	⇒*Rider Threshold* ⇒*Maximum Rider Ratio* ⇒*Type (Peak Type)*
Description:	Select the **Front Riders to Main Peaks** detection parameter to change rider peaks on the leading edge of a peak into main peaks.
Function:	Set the parameter value to **On** to change upward riders to main peaks even if riders are enforced; for example, ⇒*Rider Threshold* = 0.00%, ⇒*Maximum Rider Ratio* = 100.0%).
Tip:	*This parameter has priority over the **Rider** peak type in the peak table (see ⊁Rider).*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Inhibit Integration

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Switch**
Dimension:	---
Value Range:	**On; Off**
Default:	**Off**
Description:	The **Inhibit Integration** detection parameter serves to fade out certain chromatogram areas. When set, peak detection is disabled.
Function:	If the value is set to **On** before the first peak to inhibit, peak detection will not take place until the parameter is disabled (**Off**); that is, no peaks are recognized in this area. The chromatogram is displayed on screen, but it is not integrated. Inhibit Integration can be used to inhibit the injection peak by activating the criterion at the start time of the chromatogram and by deactivating it after the dead time.
☞ *Tip:*	*To have any effect, set this parameter before the first peak to be inhibited starts.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔝 **Defining Detection Parameters**.

Lock Baseline

Location:	**Detection** tab page
	Report Variable **(Detection Parameters)**
Type:	**Alternatives**
Dimension:	**---**
Value Range:	**Off; On; At current level /**
	At global minimum
Default:	**Off**
Related Parameters:	$\Rightarrow$*Valley to Valley*
	$\Rightarrow$*Inhibit Integration*
	$\Rightarrow$*Baseline Point*
	$\Rightarrow$*Type (Peak Type)*
Description:	If the **Lock Baseline** parameter is set to Off, the detection program calculates the baseline below non-resolved peaks according to a complex pattern recognition process. The other values are used to lock the baseline on different levels.
Function:	

Off Under non-resolved peaks, the baseline is determined automatically. The **Valley-to-Valley** parameter is effective in this mode, only. With all other lock-values, lock baseline has priority, while valley-to-valley is ignored.

On In case of non-resolved peaks, the baseline is **not** pulled up to the relative minima (valleys). The baseline connects the start of the first with the end of the last non-resolved peak.

If one of the valleys in between is located below the baseline, the baseline is connected with this minimum to avoid cutting off a peak foot. The **Valley-to-Valley** parameter is not effective.

At current level The baseline is fixed at the current signal level and is extrapolated horizontally to the right. The **Valley-to-Valley** parameter will not take effect.

At global minimum Chromeleon searches to the right for the next absolute minimum. The search is performed either to the end of the chromatogram or to the next **Lock Baseline**. Within this interval, the baseline is locked on the found minimum and is horizontal. The **Valley-to-Valley** parameter is not effective.

Tips: *The end of the last non-resolved peak in a series of peaks is always a baseline point, since the chromatogram itself forms the baseline outside the peak!*

*Lock baseline needs to be disabled, before switching from **At global minimum** to another lock baseline value.*

The classification criterion ⇒Type (Peak Type) in the peak table has priority. The criterion specified here is effective only for Peak Type = AUTO!

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Maximum Area Reject

Location:	**Detection** tab page
	Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Signal dimension ∗ Minute**
Value Range:	**0.000 ... 1,000,000.000**
Default:	The default setting is **Off**; i.e., all peaks are integrated.
Related Parameters:	⇒*Minimum Area*
	⇒*Maximum Height Reject*
Description:	Select the **Maximum Area Reject** parameter to limit the number of integrated peaks. This parameter is very similar to ⇒*Minimum Area*.
Function:	The parameter defines the maximum peak area up to which a peak is rejected. No peaks with a peak area below the defined value are integrated.
☞ *Tip:*	*Contrary to the **Minimum Area** the **Maximum Area Reject** parameter does not affect the baseline course.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Maximum Height Reject

Location:	**Detection** tab page
	Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Signal dimension, such as mAU**
Value Range:	**0.000 ... 1,000,000.000**
Default:	The default setting is **Off**; i.e., all peaks are integrated.
Related Parameters:	⇒*Minimum Height*
	⇒*Maximum Peak Height*
	⇒*Maximum Area Reject*
Description:	Select the **Maximum Height Reject** parameter to limit the number of integrated peaks. This parameter is very similar to ⇒*Minimum Height*.
Function:	The parameter defines the maximum peak height up to which a peak is rejected. Not all peaks with a peak height below the defined value are integrated. (Determination of the height is always relatively to the baseline.)
📌 *Tip:*	*Contrary to **Minimum Height**, the **Maximum Height Reject** parameter does not affect the baseline course.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔺 **Defining Detection Parameters**.

Maximum Peak Height *(MaxHght)*

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Signal dimension, such as mAU**
Value Range:	**Off; 0.000 ... 1,000,000.000**
Default:	The default setting is **Off**; that is, all peaks are integrated.
Related Parameters:	⇒*Minimum Height* ⇒*Maximum Area Reject*
Description:	Positive peak identification is performed using the ⇒*Minimum Height* parameter. *All* peaks above this height value are identified.
	The **Max. Peak Height** detection parameter is used as a maximum criterion determining the height threshold above which peaks are not identified.
Function:	All peaks above the specified peak height are classified as **Unknown**; that is, despite positive identification, they are not assigned a name from the peak table. (The height is always determined relative to the baseline.)
	This option is very useful when working with two detectors switched in series. Peaks that are clearly identified in the channel of the first detector, such as a UV detector, can cause problems in the second detector, for example, a fluorescence detector, due to an excessive concentration. These peaks are outside the linear range. Due to the positive identification, they would be considered in the calibration and they would falsify the result. If they are classified as **Unknown**, they are not considered.

Maximum Rider Ratio

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Percent [%]**
Value Range:	**0 ... 99.99**
Default:	**20**
Related Parameters:	⇒*Rider Threshold* ⇒*Type*
Description:	If one or several peaks (h1 to h4) are above the **Rider Threshold** in a series of non-resolved peaks, Chromeleon determines via the **Maximum Rider Ratio** detection parameter, whether a peak is classified as main peak or ➢*Rider*.
Function:	For this, the height of the peak to classify is put in relation to the height of the greatest peak in the series (reference peak).
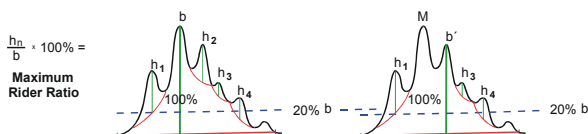
As can be seen in the figure, not the "real" peak height of the peak to be classified is evaluated but the distance between the peak maximum and the skimming tangent.

If the ratio h/b multiplied by 100 percent produces a value *larger* than the one defined as maximum rider ratio, the peak is a main peak.

If the ratio h/b multiplied by 100 percent produces a value *smaller* than the one defined as maximum rider ratio, the peak is a rider peak.

Function: *(Cont'd)*	Starting with the largest peak in the series (reference peak), all adjacent peaks are then classified. As soon as another main peak is recognized, this peak automatically becomes the new reference peak (b´). The maximum rider ratio is recalculated considering b´. The remaining peaks are classified again. The process is continued until all peaks of the series are classified.
	Riders can be recognized as such by the skimming tangent drawn on the chromatogram plot and by a Ru (Rider up) or Rd (Rider down) entry in the **Type** column of the report (**Integration** tab page).
☞ *Tips:*	*In order to be effective, the criterion must be enabled before the peak start!*
	The ⇒Type (Peak Type) criterion in the peak table has priority! The criterion specified here, is effective only in combination with PEAK TYPE = AUTO!

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔳 **Defining Detection Parameters**.

Maximum Width

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**min**
Value Range:	**0.01 ... 999.99**
Default:	**The presetting is 999.99 min so that all peaks are considered.**
Related Parameters:	⇒*Minimum Width* ⇒*Maximum Peak Height*
Description:	The **Maximum Width** detection parameter defines the maximum width above which peaks are ignored during peak detection. The peak width is measured on the baseline. For peaks that do not reach the baseline, the width is extrapolated.

🖝 *Tip:* *The criterion must be enabled before the peak end. The maximum width influences peak recognition and thus the baseline! In order to avoid this and to inhibit small peaks in the report only, select **Properties** on the **Table** menu, and then select **Reject peaks with smaller relative area than ...%** check box.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Minimum Area

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Signal dimension ∗ Minute**
Value Range:	**0.000 ... 1,000,000.000**
Default:	The default setting is **0.001**; that is, all peaks with an area > 0.001 [*Sig.dimension x minutes*] are integrated.
Related Parameters:	⇒*Maximum Area Reject* ⇒*Minimum Width* ⇒*Minimum Height*
Description:	The **Minimum Area** detection parameter is used as a minimum criterion determining the area threshold below which peaks are ignored during peak detection or integration.
Function:	Should only this inhibition criterion be applied, peak numbers in the integration report table may not be consecutive, because two detection phases are carried out for a chromatogram; that is, for peak detection and area calculation. The minimum area parameter might be effective in the second run only; that is, a peak may be sorted out only then, the result being a gap. The **No**. column of the result file thus indicates which criterion was responsible for inhibiting a peak.
☞ *Tip:*	*This parameter must be enabled at a retention time before the start of a peak. It influences peak recognition and, therefore, also the baseline! In order to avoid this and to inhibit small peaks in the report only, select **Properties** on the **Table** menu, and then select the **Reject peaks with smaller relative area than ...%** check box.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔖 **Defining Detection Parameters**.

Minimum Height

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Signal dimension**
Value Range:	**0.000 ... 1,000,000.000**
Default:	The default setting is zero; that is, all peaks with the height > 0 [*Sig.dimension*] are integrated.
Related Parameters:	⇒*Maximum Height Reject* ⇒*Minimum Area* ⇒*Minimum Width* ⇒*Maximum Peak Height*
Description:	The **Minimum Height** detection parameter is used as a minimum criterion determining the height threshold below which peaks are ignored.
Function:	The peak height is measured relative to the baseline (a). For ➤*Rider Peaks*, the height measurement is relative to the profile of the carrier peak (b). The baseline can strongly influence these criteria (c,d).
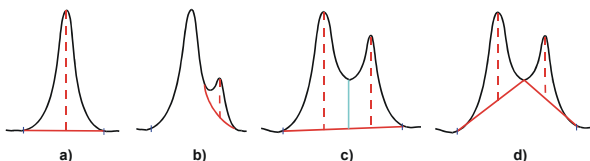
a)   b)   c)   d)

☞ *Tip:*   *Enable this parameter if the retention time is before the start of the peak. The parameter influences peak recognition and, therefore, also the baseline! In order to avoid this and to inhibit small peaks in the report only, select **Properties** on the **Table** menu, and then select the **Reject peaks with smaller relative area than ...%** check box.*

Minimum Width

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Minute**
Value Range:	**0.000 ... 1,000,000.000**
Default:	The default setting is zero; that is, all peaks with the width > 0 [*minutes*] are integrated.
Related Parameters:	⇒*Minimum Area* ⇒*Minimum Height*
Description:	The **Minimum Width** detection parameter is used as a minimum criterion defining the minimum width below which peaks are ignored during peak detection.
Function:	The peak width is measured on the baseline. In the case of peaks not reaching the baseline, the width is extrapolated.
🖝 *Tip:*	*Enable this parameter if the retention time is before the start of the peak. The parameter influences peak recognition and, therefore, also the baseline! In order to avoid this and to inhibit small peaks in the report only, select **Properties** on the **Table** menu, and then select the **Reject peaks with smaller relative area than ...%** check box.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔼 **Defining Detection Parameters**.

Peak Group Start/Peak Group End

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Selection**
Dimension:	**---**
Value range:	**Auto, Fixed**
Default:	**Auto**
Related Parameters:	⇒*Baseline Point* ⇒*Lock Baseline* ⇒*Inhibit Integration*
Description:	Select the **Peak Group Start** and **Peak Group End** detection parameters to identify several successive peaks as one peak group. A peak group that has been defined in such a way is treated as one single peak. The peak maximum of the largest peak becomes the peak maximum of the entire group. For the group, only one name and number is entered in the chromatogram. Parameters such as area value, peak height, etc. are determined and displayed only once.
Function:	The **Peak Group Start** parameter (**Peak Group End** parameter, respectively) defines the start (end) of such a group. On the **Detection** tab page of the QNT Editor, set the start of the peak group automatically (**Auto**). If you select the **Fixed** mode, the baseline will start and end exactly at the mentioned retention times. The setting made for the **Peak Group Start** parameter is also adopted for **Peak Group End**. The system toggles between **Peak Group Start** and **Peak Group End** when appending a line.

Function: *(Cont'd)*	Within one group, the entire peak detection algorithm is disabled; that is, the **Rider Threshold** and **Maximum Rider Ratio** detection parameters are not considered. The area defined by **Peak Group Start** and **Peak Group End** only indicates the limits in which the algorithm is not active. Peak start and end as well as the baseline are determined as usual.
Note:	*The time interval defined by peak group start and peak group end defines only the limits within which the algorithm is disabled. Peak start, peak end, and baseline course are determined as usual!*
	Instead of setting the peak group start and peak group end, you can group individual peaks that are not necessarily consecutive. To do so, use the ⇒Group column of the peak table in the QNT Editor. Contrary to a peak group that has been defined via peak group start and peak group end the individual peaks of this group are still indicated.

Also, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Grouping Peaks**.

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Detection Parameters**.

Peak Purity Start/End Wavelength (PPWlStart/*PPWlEnd*)

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Floating point number**
Dimension:	**nm**
Value range:	**190.0 - 900.0**
Default:	**Start: 190.0 nm; End: 900.0 nm**
Related Parameters:	⇒*Peak Purity Threshold*
Description:	The **Peak Purity Start Wavelength** and **Peak Purity End Wavelength** detection parameters limit the wavelength range for peak purity calculations.
Function:	For calculating the ➢*Peak Purity Index (PPI)* and the ➢*Peak Purity Match Factor*, only the wavelength range within the values limited by start and end is used.
Note:	*The values 190 nm (Start) and 900 nm (End) are used as defaults. The actually recorded wavelength range is used for the calculation if the recorded range of the 3D field is smaller.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Peak Purity Threshold *(PPTrshold)*

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Command**
Dimension:	**---**
Value range:	**0.00 ... 100.00**
Default:	**10.00**
Related Parameters:	⇒*Peak Purity Start/End Wavelength*
Description:	The **Peak Purity Threshold** detection parameter determines the threshold for the signal height above which spectra comparison is performed for the UV and ➤*Mass Spectra*. The parameter is important for the Peak Purity Analysis (PPA) and the Peak Ratio.

Peak Purity Analysis (PPA)

When determining the ➤*Peak Purity Index (PPI)* or the ➤*Peak Purity Match Factor*, the parameter defines the signal height above the baseline, from which the spectra are extracted for calculation.

Peak Ratio

For the Peak Ratio, the **PPTrshold** parameter defines the signal height above which the ratio is formed.

Function:	**Peak Purity Analysis (PPA)**

The value is stated in percent of the peak height, that is at the value 20%, only the spectra are used to determine the match factor for which the signal height of the peak is at least 20%.

Function:
(Cont'd)

The smaller the selected value, the wider the match factor curves. The calculation may include spectra that no longer have an optimum ➢*Signal-to-Noise Ratio*. The match factor curve then has "fringes" at the margins (peak start and peak end). This can be avoided by a smaller threshold value.

Limiting the wavelength range that is used for calculating the center wavelength of the PPI is possible via the ⇒*Peak Purity Start/End Wavelength* detection parameters. A corresponding limitation to a specified mass range is not possible for the MS Peak Purity Match.

Peak Ratio

A value of 10% means that the signal ratio between two channels is only formed where the signal intensity exceeds 10% in two channels.

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Defining Detection Parameters**.

Peak Shoulder Threshold

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**-**
Value Range:	**Off; 0.01 ... 1000.00**
Default:	**Off**; that is, shoulders without relative maximum are not recognized as peaks. Only if value ≠ Off, shoulders are detected.
Related Parameters:	⇒*Rider Threshold* ⇒*Type (Peak Type)*
Description:	The **Peak Shoulder Threshold** parameter defines a threshold value for peak shoulder recognition.
Function:	First, the average baseline curvature is determined for the entire chromatogram. The curvature threshold value is the product of the average baseline curvature and the threshold value.
	Detected peak shoulders with a maximum curvature above this threshold value are not considered. Select the ⇒*Sensitivity* detection parameter to influence the determination of the average baseline curvature. Higher sensitivity results in a smaller average baseline curvature and thus effects peak shoulder recognition.
🖾 *Note:*	*Peak shoulders are treated like "normal" peaks. All other detection parameters (such as rider criteria, minimum criteria as well as peak recognition via peak table) except the peak group parameters (⇒Peak Group Start/Peak Group End) and the ⇒Valley to Valley parameter are used for peak shoulders as well.*

Peak Slice

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Floating point number**
Dimension:	**Seconds [s]**
Value Range:	**0.01 ... 100,000.00**
Default:	**0.05**
Related Parameters:	This parameter should always be considered in combination with the ⇒*Sensitivity*!
Description:	The **Peak Slice** detection parameter determines the width (= time span) from which several successive data points are interpreted as peak or as noise. The presetting is 5/100 seconds.
Function:	**General Rule** If a specific peak should "just" be interpreted as a peak <u>and</u> if this was not reached via the Auto setting, a manual peak slice value can be specified by selecting the peak width.

Note: *This parameter only refers directly to peak detection and indirectly to integration. For area calculation, the original signal is used. (The parameter influences peak start and peak end. In this way, the peak area is influenced, also.)*

To change the variables, Sensitivity *and* Peak Slice*, is only necessary in case of chromatograms comprising very wide (many minutes) or very narrow (< 0.1 **s**) peaks. Under normal conditions, the default values should not be changed.*

*Graphical definition is recommended above all for **Sensitivity** and **Peak Slice**. For more information see. **How to …: Integrating Chromatograms and Identifying Peaks** Modifying the Peak Recognition Algorithm.*

Rider Skimming

Location:	**Detection** tab page
	Report Variable **(Detection Parameters)**
Type:	**Selection**
Dimension:	**-**
Value Range:	**Tangential at lower peak end, Tangential at both peak ends** or **Exponential**
Default:	**Tangential at lower peak end**
Related Parameters:	⇒*Rider Threshold*
	⇒*Maximum Rider Ratio*
	⇒*Type (Peak Type)*
Description:	This parameter indicates how ➢*Rider Peaks* are skimmed.
Function:	There are three options:

- **Tangential at lower peak end** (default and the common skimming method): For ascending rider peaks, the peak start, and for descending rider peaks, the peak end is defined in such a way that rider skimming is tangential to the chromatogram.

- **Tangential at both peak ends**: Peak start and peak end are determined so that rider skimming is tangential at both chromatogram ends.

- **Exponential**: The chromatogram is approximated by an exponential function, so that the slope of the chromatogram and the exponential function correspond at the peak start and the peak end of the rider peak. This option clearly distinguishes from the two others. In most cases, **Exponential** maps the actual course of the curve very accurate. With this option, the rider peak will usually receive a more realistic larger area. You can use this option only if a sufficient number of data points is available.

Tip: *You can move the baseline in the chromatogram with the mouse for tangentially skimmed riders, but you cannot for exponentially skimmed riders.*

Note: *When reprocessing a chromatogram, the skimming function can be set manually for each rider peak. The required commands are available on the context menu of the selected peak.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Rider Threshold

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Fixed point number**
Dimension:	**Percent [%]**
Value Range:	**0.00 ... 100.00**
Default:	**10.00**
Related Parameters:	⇒*Maximum Rider Ratio* ⇒*Type (Peak Type)* ⇒*Peak Shoulder Threshold*
Description:	The **Rider Threshold** and **Maximum Rider Ratio** variables determine whether a single peak in a series of non-resolved peaks is classified as a ➤*Rider Peak* (skimming peak) or as main peak.
Function:	The ratio between the ➤*Peak Height* of the single peaks (here: h1 to h5) and the height of the largest peak (here: b) determines whether a peak is classified as rider peak or main peak.

$$\frac{h_n}{b} \times 100\% =$$
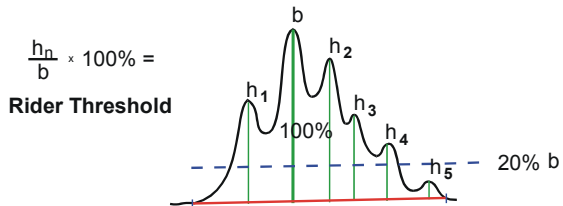
Rider Threshold

If the height ratio h/b multiplied by 100 percent produces a value below the defined rider threshold, the corresponding peak is a main peak by definition. In this example, this is only the case for the h5 peak.

If the height ratio h/b multiplied by 100 percent produces a value above the defined rider threshold, the **Rider Threshold** criterion is not sufficient for a clear classification.

Function: *(Cont'd)*	In this case, the $\Rightarrow$*Maximum Rider Ratio* is established. The resulting value allows you to classify the remaining peaks as rider peaks or main peaks.
	Riders can be recognized as such by the skimming tangent drawn on the chromatogram plot and by a Ru (Rider up) or Rd (Rider down) entry in the **Type** column of the report (**Integration** tab page).
Notes:	*In order to be effective, the criterion must be enabled before the peak start!*
	The $\Rightarrow$Type (Peak Type) classification criterion in the peak table has priority! The criterion specified here, is effective only together with PEAK TYPE = AUTO!

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Sensitivity

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Floating point number**
Dimension:	**Signal dimension**
Value Range:	**0.0001 ... 10,000,000.0**
Default:	Dynamical (= ⮞*Noise* value of the related chromatogram)
Related Parameters:	Always consider this parameter in combination with the ⮞*Peak Slice*
Description:	Together with the **Peak Slice** parameter, the **Sensitivity** parameter determines the **granularity** of the peak detection algorithm; i.e., when using small values, even the smallest signals are interpreted as peaks; for example, pump pulsation, whereas such signals are ignored when using higher values.
Function:	Peak recognition is performed using a rectangle formed by the granularity of the y-axis (**Sensitivity**) and the granularity of the x-axis (**Peak Slice**) (see figure a):
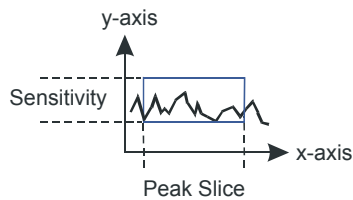
a)

As shown in the picture, this rectangle is placed with its lower left corner on the first data point, and is duplicated by mirroring in the direction of negative y-values. As long as none of the subsequent data points is above or below the mirrored rectangle, the last data point will be used for positioning further rectangles (b). The measured signal is interpreted as noise.

Function:
(Cont'd)

b)

c)
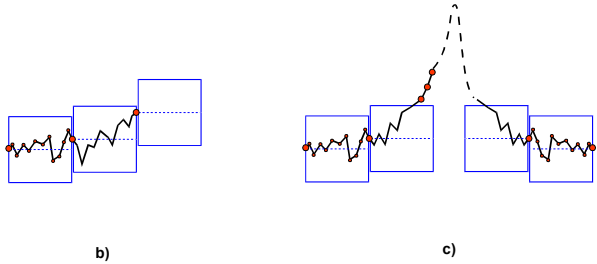
If three successive data points are located above or below the rectangle (simplified!), the peak recognition algorithm will recognize a peak. When the signal difference of one data point to the next one becomes so small that they are within the bandwidth again, the peak end is indicated (c).

Manually Setting the Parameters Sensitivity and Peak Slice

You can manually modify the granularity of the peak recognition; that is, you can determine the size of the rectangle or the bandwidth. The larger the area, the lower the sensitivity of the system. Smaller peaks are likely to be interpreted as noise.

General Rule

If a specific peak shall "just" be interpreted as a peak and if this was not achieved via the Auto setting, specify a manual Sensitivity value of a third of the signal height of the peak.

Notes: *This parameter refers directly to peak detection only and indirectly to integration. For area calculation, the original signal is used. (The parameter influences peak start and peak end. In this way, the peak area is influenced, also.)*

Notes:
(Cont'd)

*Using the **Sensitivity** and **Peak Slice** variables is only necessary in case of chromatograms comprising very wide (many minutes) or very narrow (< 0.1 s) peaks. Under normal conditions, the default values should not be changed.*

Sensitivity is always interpreted in the dimension installed; for example, mAU.

*Graphical definition is recommended above all for **Sensitivity** and **Peak Slice**. For more information, refer to **How to …: Integrating Chromatograms and Identifying Peaks** Modifying the Peak Recognition Algorithm.*

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Detection Parameters**.

Tailing/Fronting Sensitivity Factor

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Floating point number**
Dimension:	**---**
Value range:	**Off; 1.0 ... 100.0**
Default:	**Off**
Related Parameters:	**Baseline Point, Lock baseline, Inhibit Integration**
Description:	This detection parameter is an implicit threshold for setting the peak end. In the case of the **Fronting sensitivity factor**, the options refer to the peak start.
Function:	The following paragraphs describe the **Tailing sensitivity factor** options. The **Fronting sensitivity factor** behaves in the same way.
Off (=0):	The peak end is moved on the time axis until the slopes of the chromatogram and of the baseline correspond in the peak end.
Factor 1 - 100:	First, determine the time for the peak end as described above for Off (=0) and then determine the right baseline width, using the **Baseline Level** parameter of the ➢*Right Width* peak variable or **peak.right_width(0)**). Multiply the value, which is the upper limit for the distance between the peak retention time and the peak end, by the **Tailing sensitivity factor**. If this limit is exceeded, the peak end is corrected and **reset** accordingly.

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Defining Detection Parameters**.

Valley to Valley

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Switch**
Dimension:	**---**
Value Range:	**On; Off**
Default:	**Off**
Description:	Usually, an algorithm determines the baseline automatically. The **Valley to Valley** parameter enables baseline treatment from valley-to-valley, that is, from peak minimum to peak minimum, in a series of non-resolved peaks.
Function:	When the parameter is enabled, the baseline below non-resolved peaks leads from peak end to peak end.

Default ⟶ **Valley to Valley**
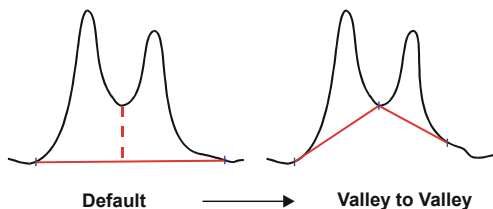
Notes: *The end of the last non-resolved peak in a series of peaks is always a baseline point since the chromatogram itself forms the baseline outside the peak.*

*The **Valley to Valley** parameter is invalidated by ⇒Lock Baseline.*

The ⇒Type (Peak Type) criterion of the peak table has absolute priority.

For information about how to use individual detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Void Volume Treatment

Location:	**Detection** tab page Report Variable **(Detection Parameters)**
Type:	**Switch**
Dimension:	**---**
Value Range	**On; Off**
Default:	**Off**
Related Parameters:	⇒*Inhibit Integration*
Description:	Use this parameter to hide a negative peak at the chromatogram start. This is especially useful for the negative water peak in ion chromatography. If Void Volume Treatment is set to On, peak detection is disabled for the range of the negative water peak.
Function:	The system searches for the negative water peak in the retention time range between Void Volume Treatment = On and Void Volume Treatment = Off. The negative water peak is the peak with the lowest signal at the retention time. Peak suppression is enabled for the time between Void Volume Treatment = On and the end of the negative water peak. The chromatogram is recorded, but it is not integrated. (This corresponds to Inhibit Integration = On.) Peak detection is enabled again only after the peak end of the negative water peak has been detected. (This corresponds to Inhibit Integration = Off.)
☞ *Tip:*	The **Void Volume Treatment** parameter becomes active only when its time is set before the start of the negative water peak.

Note: *For each chromatogram, there can only be one range in which the negative water peak is detected. If you have defined several Void Volume Treatment = On and Void Volume Treatment = Off commands, the negative water peak is searched in the first defined range.*

                    If you have selected a range in the chromatogram using the right mouse button and if you have set Void Volume Treatment to On via the Set Void Volume Treatment command on the context menu, this overwrites all previously defined ranges.

                    *If **Void Volume Treatment** is not set to Off, Chromeleon uses the end of the chromatogram as the end of the range.*

For information about how to use individual detection parameters, refer to **How to …: Working with Chromatograms** Defining Detection **Parameters Graphically**.

Report Categories

The report variables are grouped in the following categories:

Category	Formula	Variables
⇒*General*	gen.*	General variables (in the ➢*Printer Layout* only)
⇒*Sequence*	seq.*	➢*Sequence* variables
⇒*Sample*	smp.*	Sample variables
⇒*Audit Trail*	AUDIT.*	Audit trail variables (protocol variables) (see **Data Management** 📊 **Audit Trails**)
⇒*Preconditions*	precond.*	Variables at the conditions before a sample run.
⇒*Chromatogram*	chm.*	Chromatogram variables
⇒*Detection Parameters*	det.*	⇒*Detection Parameters* variables
⇒*Peak Results*	peak.*	Peak result variables
⇒*Peak Calibration*	peak.*	Calibration variables
⇒*Peak Table*	peakTab.*	Peak table variables
⇒*Mass Spectrometry*	ms.*	Mass spectrometry variables
⇒*Peak Purity and Identification*	peak.*	Peak purity and identification variables
⇒*Quantification Method*	qnt.*	Quantification method variables (in the Printer Layout only)
⇒*System Suitability Test*	sst.	➢*System Suitability Test (SST)* variables (in the Printer Layout only)
⇒*History*	history.*	➢*History* variables (in the history report only)
⇒*Integration/Summary Table*	table.group*	Variables for statistics concerning sample groups and/or replicates (named **Summary Table** from the **Summary** tab page - or else: **Integration Table**)
⇒*Program*	pgm.*	➢*Program* variables
⇒*Report Definition (RDF)*	rdf.*	➢*Report Definition File (RDF)* variables
⇒*Device Wellness*	wns.*	➢*Device Wellness* variables
⇒*Fraction*	frac.*	Fraction collection variables
⇒*Fraction Tube*	tube.*	Tube variable for fraction collection
⇒*Fraction Detection Parameter*	frac.channel.*	Peak detection variables for fraction collection

Category	Formula	Variables
⇒*Spectra Library Screening*	sls.*	Variables for the associated settings in the QNT File (accessible via the **Spectra Library Scr. Param.** variable for the **Quantification Method** category)
⇒*Mass Spectrum*	msspec.*	➢*Mass Spectra* variables (accessible via the **Select Spectrum** variable of the **Mass Spectrometry** category)
⇒*Spectrum Data*	peak.spectrum.*	Peak spectra variables (accessible via the **Reference Spectrum** variable of the **Peak Table** category)
⇒*Hit Spectrum*	hitSpec.*	Variables concerning spectra of spectra libraries (accessible via the **SLS Hit** variable of the **Peak Purity** category)
⇒*Spectra Library*	specLib.*	Spectra library variables (accessible via the **Library Record** variable of the **Hit Spectrum** category)
⇒*User Information*	~.*	Variables concerning the corresponding user (accessible via all **Operator** variables of the different categories. ~: Formula depends on the way you opened the category.)

The following pages provide a brief description of the respective variables of the corresponding categories.

☞ *Tip:*

*Follow the description in **How to …: Creating and Using Report Tables** 🔲 **Linking Report Variables** to link report variables listed on the following pages via the four basic arithmetic operations, and thus create user-defined report variables.*

"General" Category

The **General** category is only available in the ➢*Printer Layout*. It includes the following variables:

Variable	Formula	Description
Version Number	gen.version	Version number
Serial Number	gen.serialNo	Serial number
Key Code	gen.keyCode	➢*Key Code*
Computer Name	gen.computerName	Computer name
Operator Name	gen.operator	User name - opens the ⇒*User Information* category
Report Batch ID	gen.batchID	Number of the Report Definition File (RDF)
Report Batch Status	gen.batchStatus	Status of the batch printout at the end of the batch
Total Number of Sheets	gen.nSheets	Total number of worksheets to be printed
Current Sheet Number	gen.SheetNr	Number of the current worksheet
Current Time	gen.Time	Current date and time

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Sequence" Category

The ➤*Sequence* category includes the following variables:

Variable	Formula	Description
Name	seq.name	Sequence name
Directory	seq.path	Directory in which the sequence is stored
Datasource	seq.dsn	Name of the corresponding ➤*Datasource*
Timebase	seq.timebase	➤*Timebase* of last run
Title	seq.title	Sequence header
Number of Samples	seq.nSamples	Number of samples in the sequence
Creation Date & Time	seq.creation_time	See ➤*Time*
Creation Operator	seq.creation_operator	Opens the ⇒*User Information* category
Last Update Date & Time	seq.update_time	Indicates the date and time when the last update was performed.
Last Update Operator	seq.update_operator	Opens the **User Information** category
Preferred RDF File	seq.prefRdf	Preferred ➤*Report Definition File (RDF)*
Preferred Channel	seq.prefChl	Preferred ➤*Channel*
Sign Status	seq.signStatus	Status of the➤ *Electronic Signature*
Submit Date & Time	seq.submitTime	Date and time when submitting the electronic signature
Submit Operator	seq.submitOperator…	User who submitted the electronic signature - opens the **User Information** category
Submit Comment	seq.submitComment	Comment when submitting the electronic signature
Review Date & Time	seq.reviewTime	Date and time when checking the electronic signature
Review Operator	seq.reviewOperator…	User who checked the electronic signature - opens the **User Information** category

Variable	Formula	Description
Review Comment	seq.reviewComment	Comment when checking the electronic signature
Approve Date & Time	seq.approveTime	Date and time when the approving the electronic signature
Approve Operator	seq.approveOperator…	User who approved the electronic signature - opens the **User Information** category
Approve Comment	seq.approveTime	Comment when approving the electronic signature

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Sample" Category

The ⇒*Sample* category includes the following variables:

Variable	Formula	Description
Sample Number	smp.number	⇒*No.*
Sample Name	smp.name	⇒*Name*
Raw Data ID	smp.id	Show the raw data ID
Sample ID	smp.ident	⇒*Sample ID*
Comment	smp.comment	⇒*Comment*
Sample Type	smp.type	⇒*Type* (Sample type)
Sample Position	smp.position	See ⇒*Pos.* (sample position)
Replicate ID	smp.replicate	⇒*Replicate ID*
Status	smp.status	⇒*Status* (Sample status): Single/Multiple/ Finished/Interrupted/Running
Date & Time Sample Run	smp.time	See ⇒*Inj. Date/Time*
Inject Volume	smp.inject_volume	⇒*Inj. Vol.* (injection volume)
Dilution Factor	smp.dilution_factor	See ⇒*Dil. Factor*
Sample Weight	smp.weight	⇒*Weight* (Sample Weight Factor)
ISTD Amount	smp.amount	See ⇒*ISTD Amount* (Amount of the ➢*Internal Standard (ISTD)*)
Program	smp.program	➢*PGM File*
Quantification Method	smp.method	➢*Quantification Method* (QNT Method)
Standard Addition Group	smp.spike_group	➢*Standard Addition* group
Reference Amount Set	smp.refamount_set	ID for ⇒*Amount* column in the QNT Editor
Auto Purification Type	smp.autopurification_sample _type	Type of the associated autopurification sample
Auto Purification Sample Reference	smp.autopurification_reference	ID for the associated autopurification sample
Auto Purification Fraction	smp.autopurification_fraction	Fraction of the associated autopurification sample
Select Chromatogram	smp.chm.x	Selects the chromatogram for the associated sample - opens the ⇒*Chromatogram* category.
Globally Unique Sample ID	smp.guid	Creates a ⇒*Globally Unique Sample ID*
*x	smp.x	➢*User-defined Column* (x = column name)

"Audit Trail" Category

Depending on the connected instrumentation, hundreds of variables are available in the Audit Trail. (For more information about Audit Trails, refer to **Data Management** ⬛ **Audit Trails**.)

The list below contains some of the variables that are typically available during data acquisition with a P680 pump or a UVD 340 detector.

P680

Variable	Formula	Description
%B, %C, %D	AUDIT.%B (C or D)	Percentage of the respective component in the eluent (partial flow) (see ⇒*%B, %C, %D*
Average (Pump_Pressure)	AUDIT.Pump_Pressure. Average	Status of averaging (also, see ⇒*Average*)
Equate (%A)	AUDIT.%A.Equate	Name of eluent component A (components B, C, and D accordingly)
Flow	AUDIT.Flow	⇒*Flow* rate
Lower Limit (Pressure)	AUDIT.Pressure.Lower Limit	Lower pressure limit (see ⇒*Pressure.Lower/UpperLimit*)
Step (Pump_Pressure)	AUDIT.Pump_Pressure. Step	Step for each channel (also, see ⇒*Step*)
Upper Limit (Pressure)	AUDIT.Pressure.Upper Limit	Upper pressure limit (see ⇒*Pressure.Lower/UpperLimit*)

UVD340

Variable	Formula	Description
Average	AUDIT.Average	Status of averaging: enabled or disabled (see⇒ *Average*)
Bandwidth	AUDIT.BandWidth	⇒*Bandwidth* (for each UV channel)
Bunch Width	AUDIT.3DFIELD.Bunch Width	⇒*Bunch Width* of the diode bundle (3D-field only)
LampIntensity	AUDIT.LampIntensity	⇒*LampIntensity* [counts/s]
MaxAutoStep	AUDIT.MaxAutoStep	Maximum value for Step = Auto (see ⇒*MaxAutoStep*)
Max. Wavelength (3DFIELD)	AUDIT.3DFIELD.Max Wavelength	Maximum wavelength of the ➢*3D Field*
Min. Wavelength (3DFIELD)	AUDIT.3DFIELD.Min Wavelength	Minimum wavelength of the 3D field

Variable	Formula	Description
Ref Wavelength	AUDIT.RefWavelength	⇒*RefWavelength (Reference Wavelength)* (for each UV channel)
Ref Bandwidth	AUDIT.RefBandwidth	⇒*RefBandwidth (Reference Bandwidth)* (for each UV channel)
Step	AUDIT.Step	⇒*Step*
Wavelength	AUDIT.Wavelength	⇒*Wavelength* (for each UV channel)

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Preconditions" Category

Depending on the connected instrumentation, thousands of ➢*Preconditions* variables exist. They allow you to display the device settings before a sample run in the Audit Trails. (For more information about Audit Trails, refer to **Data Management** 🖼 **Audit Trails**.) The Preconditions are displayed if you selected **Preconditions only** or **Preconditions and Run** on the **View** or context menu.

The list below contains some of the variables that are typically available during data acquisition with a P680 pump or a UVD 340 detector.

P680

Variable	Formula	Description
Average (Pump_Pressure)	precond.Pump_Pressure. Average	Status of averaging (also, see ⇒*Average*)
Equate (%A)	precond.Pump.%A.Equate	Name of eluent component A (components B, C and D accordingly)
Flow	precond.Pump.Flow	⇒*Flow* rate
Lower Limit (Pressure)	precond.Pump.Pressure. LowerLimit	Lower pressure limit (see ⇒*Pressure.Lower/UpperLimit*)
Step (Pump_Pressure)	precond.Pump_Pressure. Step	Step for each channel (also, see ⇒*Step*)
Upper Limit (Pressure)	precond.Pump.Pressure. UpperLimit	Upper pressure limit (see ⇒*Pressure.Lower/UpperLimit*)
Value (Pump.%A)	precond.Pump.%A.Value	Percentage of the component in the solvent (partial flow): ⇒*%B, %C, %D* (in the same way for components B, C, and D)
Value (Pump.%A_Level)	precond.Pump.%A_Level	Value for the ⇒*%A, %B, %C, %D_Levels* (in the same way for the components B, C, and D)
Value (PumpWasteLevel)	precond.Pump.WasteLevel	Value for the ⇒*WasteLevel* (in the same way for the components B, C, and D)

UVD340

Variable	Formula	Description
Average	precond.Average	Status of averaging: enabled or disabled (see⇒ *Average*)
Bandwidth	precond.BandWidth	⇒*Bandwidth* (for each UV channel)
Bunch Width	precond.3DFIELD.Bunch Width	⇒*Bunch Width* of the diode bundle (3D-field only)
LampIntensity	precond.LampIntensity	⇒*LampIntensity* [counts/s]
MaxAutoStep	AUDIT.MaxAutoStep	Maximum value for Step = Auto (see ⇒*MaxAutoStep*)
Max. Wavelength (3DFIELD)	precond.3DFIELD.Max Wavelength	Maximum wavelength of the ➢*3D Field*
Min. Wavelength (3DFIELD)	precond.3DFIELD.Min Wavelength	Min. wavelength of the 3D field
Ref Wavelength	precond.RefWavelength	⇒*RefWavelength (Reference Wavelength)* (for each UV channel)
Ref Bandwidth	precond.RefBandwidth	⇒*RefBandwidth (Reference Bandwidth)* (for each UV channel)
Step	precond.Step	⇒*Step*
Wavelength	precond.Wavelength	⇒*Wavelength* (for each UV channel)

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Chromatogram" Category

It depends on the detector(s) that is(are) connected which chromatogram variables are available during data acquisition. The table below lists the variables that are available when either a UVD 340 detector or an aQa ➤*Mass Spectrometer* is connected.

Variable	Formula	Description
Channel Name	chm.channel	Shows the channel name.
Raw Data File Path Name	chm.rawpath	See ➤*Raw Data*
Number of Peaks	chm.nPeak	Shows the number of peaks.
Select Peak	chm.peak(*OPT*)	⇒*Select Peak* selects a peak in the chromatogram according to input; opens the ⇒*Peak Results* category
Count Peaks if ...	chm.countIF	Counts the peaks which ...
Sum Peak Results if ...	chm.sumIF	Adds up the characteristics of peaks that ....
Start Time (relative to Inject Time)	chm.start_time	Chromatogram start time [min]
End Time (relative to Inject Time)	chm.end_time	Chromatogram end time [min]
Signal Value	chm.sig_value	Signal value [for example, in mAU]
Signal Dimension	chm.sig_dim	Shows the signal dimention
Signal Noise	chm.noise	Signal noise [for example, in mAU]
Sample Rate	chm.sig_step	Shows the sample rate
Modified?	chm.manipulated	(Yes/no)
Modification Time	chm.manip_time	Corresponding date/time
Modification Operator	chm.manip_operator	Corresponding operator - opens the ⇒*User Information* category
XXXXX Start Value	chm.XXXXX	Start value of the signal parameter XXXXX when acquiring data.
Delay Time Value	chm.delayTimeValue	⇒*Delay Time* between two detectors
Delay Time Detector	chm.delayTimeDetector	Name of the second detector
Average	chm.Average	⇒*Average* (for UV channels only)
Step	chm.Step	⇒*Step* (for UV channels only)
Wavelength	chm.Wavelength	⇒*Wavelength* (for UV channels only)

Variable	Formula	Description
Bandwidth	chm.Bandwidth	⇒*Bandwidth* (for UV channels only)
RefWavelength	chm.RefWavelength	See ⇒*RefWavelength (Reference Wavelength)*) (for UV channels only)
RefBandwidth	chm.RefBandwidth	See ⇒*RefBandwidth (Reference Bandwidth)*) (for UV channels only)
MassRange	chm.MassRange	Mass range of the ➢*Mass Spectrometer* channel (for MS channels only)
Polarity	chm.Polarity	Polarity (for MS channels only)
Source Voltage	chm.Voltage	Voltage (for MS channels only)
FilterIndex	chm.FilterIndex	Number of the ➢*Filter* (MS channels only).
Smoothing	chm.Smoothing	Smoothing algorithm (in parenthesis: number of smoothing points) (for MS channels only or if explicitly performed)

For an overview of the different report variable categories, including links to the lists for these categories, refer to ⇒*Report Categories*.

"Detection Parameters" Category

The **Detection Parameters** category includes the following variables:

Variable	Formula	Description
Minimum Area	det.minArea	⇒*Minimum Peak Area*
Maximum Area Reject	det.maxAreaRj	⇒*Maximum Area Reject*
Minimum Height	det.minHeight	⇒*Minimum Height*
Maximum Height Reject	det.maxHeightRj	⇒*Maximum Height Reject*
Maximum Peak Height	det.maxHeight	⇒*Maximum Peak Height*
Minimum Width	det.minWidth	⇒*Minimum Width*
Maximum Width	det.maxWidth	⇒*Maximum Width*
Rider Threshold	det.riderMin	⇒*Rider Threshold*
Maximum Rider Ratio	det.riderRatio	⇒*Maximum Rider Ratio*
Rider Skimming	det.riderSkim	⮞*Rider*⇒ *Skimming*
Peak Shoulder Threshold	det.shoulderThrshld	⇒*Peak Shoulder Threshold*
Front Riders to Main Peaks	det.frontRiderToMain	⇒*Front Riders to Main Peaks*
Detect Negative Peaks	det.negDetect	⇒*Detect Negative Peaks*
Lock Baseline	det.lockBl	⇒*Lock Baseline*
Valley to Valley	det.valval	Baseline from ⇒*Valley to Valley*
Inhibit Integration	det.noInteg	⇒*Inhibit Integration*
Sensitivity	det.noise	⇒*Sensitivity*
Peak Slice	det.filter	⇒*Peak Slice*
Fronting Sensitivity Factor	det.frontFac	⇒*Fronting Sensitivity Factor*
Tailing Sensitivity Factor	det.tailFac	⇒*Tailing Sensitivity Factor*
Part of a Peak Group?	det.isInGroup	See ⇒*Group*
Peak Purity Threshold	det.ppThrshld	⇒*Peak Purity Threshold*
Peak Purity Start Wavelength	det.ppStartWl	⇒*Peak Purity Start Wavelength*
Peak Purity End Wavelength	det.ppEndWl	⇒*Peak Purity End Wavelength*
Void Volume Treatment	det.voidVolume Treatment	⇒*Void Volume Treatment*

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Peak Results" Category

The **Peak Results** category includes the following variables:

Variable	Formula	Description
Number	peak.number	Shows the peak number
Name	peak.name	⇒*Name* (Peak Name)
Group	peak.group	⇒*Group* (Peak Group)
Link Count	peak.linkCount	Number of substances assigned in one peak
Retention Time	peak.retention_time	⇒*Retention Time* in [min]
Retention Deviation	peak.retention_deviation	Deviation in [min] or [%], depending on parameter (**abs** or **rel**)
Relative Retention Time	peak.rel_retention_time	Shows the relative retention time
Retention Window Width	peak retention windows	Width of the Retention⇒ *Window*
Area	peak.area	Peak area for UV detectors in [min * mAU]
Relative Area	peak.rel_area	Parameters: **total**, **identified**, **group**, **istd**, **rel**
Group Area	peak.groupArea	Total of the peak ⇒*Group* areas
Height	peak.height	Peak height, for UV detectors in [mAU]
Relative Height	peak.rel_height	Parameters **total**, **identified**, **group**, **istd**, **rel**
Group Height	peak.groupHeight	Total of the heights of the peak group
Amount	peak.amount	⇒*Amount* or concentration
Amount Deviation	peak.amount_deviation	Deviation analogous to retention_deviation
Concentration	peak.concentration	Concentration (if **Amount** is really an amount): amount/injection volume
Relative Amount	peak.rel_amount	Parameters **total**, **group**, **istd**, **rel**
Group Amount	peak.groupAmount	Peak ⇒*Group* amount
Width	peak.width	Peak width (Parameters 0, 5, 10, 50 in % of signal height above baseline - 0 base width with tangent method)
Left Width	peak.left_width	For the parameters, refer to Width

Variable	Formula	Description
Right Width	peak.right_width	For the parameters, refer to Width
Peak Start Time	peak.start_time	Shows the peak start time in [min]
Peak Stop Time	peak.stop_time	Shows the peak stop time in [min]
Signal Value at Peak Start/ Signal Value at Peak End	peak.start_value peak.stop_value	➢*Signal Value at Peak Start*, for UV detectors in [mAU]
Signal Value at Peak Retention	peak.sig_value	➢*Signal Value at Peak Retention*, for UV detectors in [mAU]
Baseline Value at Peak Start/ Baseline Value at Peak End	peak.start_value_baseline peak.stop_value_baseline	➢*Baseline Value at Peak Start*, for UV detectors in [mAU]
Baseline Value at Peak Retention	peak.sig_value_baseline	➢*Baseline Value at Peak Retention*, for UV detectors in [mAU]
Detection Code at Peak Start/ Detection Code at Peak End	peak.start_detection_code peak.stop_detection_code	➢*Detection. Code at Peak Start/End*
Type	peak.type	Peak⇒ *Type*
Modified	peak.modified	Modified peak?
Manually Assigned	peak.assigned	Manually assigned peak?
Resolution	peak.resolution	➢*Resolution* (parameters **EP** and **USP**)
Asymmetry	peak.asymmetry	Peak ➢*Asymmetry*, parameters **EP** and **AIA**
Theoretical Plates	peak.theoretical_plates	➢*Theoretical Plates* (parameters **EP** and **USP**)
Skewness	peak.skewness	➢*Skewness*
Signal-to-Noise Ratio	peak.sn("Current")	➢*Signal-to-Noise-Ratio*
➢*Statistical Moments*	peak.moment("Mx")	With x =
		0 Zero moment = peak area
		1 First moment = averaged retention time
		2 Second moment = retention time variance
K'	peak.kValue	➢*Capacity Factor*
Retention Index	peak.ri	Linear ⇒*Retention Index*
Kovats Index	peak.ki	⇒*Kovats Index*

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Peak Calibration" Category

The **Peak Calibration** category includes the following variables:

Variable	Formula	Description
Calibration Mode	peak.calMode	⇒*Calibration Mode*
Auto Recalibrate	peak.autoRelCal	Auto calibration On/Off
Reference Inject Volume	peak.reference_inject_volume	Reference Injection Volume
Calibration Type	peak.calibration_type	⇒*Calibration Type*
Weights	peak.calibration_weight	➢*Weights*
Offset (c0)	peak.offset	➢*Offset c0*
Slope (c1)	peak.slope	➢*Slope c1*
Curve (c2)	peak.curve	➢*Curve c2*
Cubic Coefficient (c3)	peak.cubic	➢*Cubic Coefficient c3* of the ➢*Calibration Function*
RF-Value (Amount/Area)	peak.rf_value	(1/c1) (see ➢*RF-Value (Amount/Area)*)
Number of Calibration Points	peak.nCalpoints	➢*Number of Calibration Points*
Number of Disabled Calibr. Points	peak.nCalDisabled	➢*Number of Disabled Calibration Points*
Calibration Point Status	peak.calPointStatus	Shows the status of the calibration point
Variance	peak.variance	➢*Variance*
Variance Coefficient	peak.variance_coefficient	➢*Variance Coefficient*
Standard Deviation	peak.standard_deviation	➢*Standard Deviation*
Relative Standard Deviation	peak.rel_standard_deviation	➢*Relative Standard Deviation*
Correlation Coefficient	peak.correlation_coefficient	➢*Correlation Coefficient*
Coefficient of Determination	peak.rQuadrat	➢*Coefficient of Determination*
DOF-Adj. Coefficient of Determination	peak.rQuadratAdj	➢*DOF-Adjusted Coefficient of Determination*
Calibration Point x	peak.calPointX	Shows the calibration point x
Calibration Point y	peak.calPointY	Shows the calibration point y
Calibration Point Weight	peak.calPointWeight	➢*Calibration Point* weighting
Evaluation of Calibration Function	peak.calPointFX	Evaluates the calibration function
Residual for Cal. Point x	peak.calPointDist	Shows the residula for the calibration point
Upper Confidence Limit	peak.confUpperLimit	Upper limit of the ➢*Confidence Interval*
Lower Confidence Limit	peakconfLowerLimit	Lower limit of the confidence limit

"Peak Table" Category

The **Peak Table** category includes the following variables:

Variable	Formula	Description
Number	peakTab.number	Peak number
Peak Name	peakTab.name	Peak ⇒*Name*
Retention Time	peakTab.retention_time	⇒*Retention Time* in [min]
Retention Time Interpretation	peakTab.retention_type	Specification of retention times
Use Prev. Retention Time	peakTab.use_previous_rettime	Status of corresponding variable in QNT File (see ⇒*Use Recent Retention Time*)
Window	peakTab.window	Time ⇒*Window* width
Standard Method	peakTab.standard_method	See ⇒*Standard*
Integration Type	peakTab.integration_type	⇒*Integration Type*
Calibration Type	peakTab.calibration_type	⇒*Calibration Type*
Peak Type	peakTab.type	See ⇒*Type*
Left Limit	peakTab.left_limit	⇒*Left Limit*)
Right Limit	peakTab.right_limit	See ⇒*Right Limit*)
Peak Group	peakTab.group	See ⇒*Group*
Response Factor	peakTab.response_factor	⇒*Response Factor*
Amount	peakTab.amount	⇒*Amount*
Amount Dimension	peakTab.amount_dimension	
C0 Value	peakTab.C0	Y ⮞*Offset* of the calibration curve
C1 Value	peakTab.C1	⮞*Slope* of the calibration curve
C2 Value	peakTab.C2	Curvature of the calibration⮞ *Curve*
C3 Value	peakTab.C3	⮞*Cubic Coefficient c3* of the ⮞*Calibration Function*
Reference Spectrum	peakTab.reference_spectrum.**x**	⇒*Reference Spectrum*, opens the category ⇒*Spectrum Data*
Match Criterion	peakTab.spec_compare_method	⇒*Match Criterion*
Check Derivative	peakTab.spec_derivative	⇒*Check Derivative*
Minimum Wavelength	peakTab.spec_min_wavelength	Lower limit of the compared wavelength range (see ⇒*Minimum Wavelength*)

Variable	Formula	Description
Maximum Wavelength	peakTab.spec_max_wavelength	Upper limit of the compared wavelength range (see ⇒*Maximum Wavelength*)
Threshold	peakTab.spec_threshold	⇒*Threshold* value for spectra search
Relative Maximum Deviation	peakTab.spec_relmaxdev	⇒*Relative Maximum Deviation* during spectra search
Check Number of rel. Extrema	peakTab.spec_check_extrema	Checking the number of extrema during spectra search (see ⇒*Check Extrema*)
Comment	peakTab.comment	⇒*Comment*
Retention Index	peakTab.ri	Linear ⇒*Retention Index*
Kovats Index	peakTab.ki	⇒*Kovats Index*
Mass Peak x	peakTab.ms_peakx	Mass of Peak x
MS Threshold	peakTab.ms_threshold	Threshold for Mass
MS Filter	peakTab.ms_filter	Mass Filter
Check of mass ratios	peakTab.ms_enable_rejection	Checks the mass ratios
Check of MS retention times	peakTab.ms_check_traces	Checks the MS retention times
*x	peakTab.user_x	➢*User-defined Column* (x = column name)

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Mass Spectrometry" Category

The **Mass Spectrometry** category includes the following variables:

Variable	Formula	Description
Background Subtraction Type	ms.spec_enhanced	Indicates the type of ➢*Mass Spectra* background subtraction
Peak Spectrum Bunch	ms.nSpec_peak_top	Number of averaged single spectra at peak maximum
Left Background Subtraction Bunch	ms.nSpec_left_BG	Number of single spectra that are averaged for the left background spectrum
Right Background Subtraction Bunch	ms.nSpec_right_BG	Number of single spectra that are averaged for the right background spectrum
Left Background Subtraction Range	ms.fix_left_BG	Number of single spectra averaged for the left background spectrum at the peak maximum
Right Background Subtraction Range	ms.fix_right_BG	Number of single spectra averaged for the right background spectrum at the peak maximum
Select Spectrum	ms.spectrum.x	Opens the ⇒*Mass Spectrum* category
Instrument Information	ms.instrument	Information about the mass spectrometer
Instrument Method	ms.method	Mass spectrometry method
Tune Data	ms.tune	Tune data of the ➢*Xcalibur* raw data file
Status Log	ms.statusLog	Information about the status of the MS system at a specified time
Spectra Count	ms.spec_count	Number of mass spectra

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Peak Purity and Identification" Category

The **Peak Purity and Identification** category includes the following variables:

Variable	Formula	Description
Peak Purity Index	peak.ppi	Average ➢ *PPI (Peak Purity Index)* in nm over the entire peak (from a specific height)
RSD Peak Purity Index	peak.rsd_ppi	Correspond. Rel.Std.Dev.
Peak Purity Match	peak.match	Average ➢ *Peak Purity Match Factor* (0..1000) over the entire peak (from a specific height detection parameter)
RSD Peak Purity Match	peak.rsd_match	Corresponding ➢ *Relative Standard Deviation*
Peak Ratio Mean Value	peak.ratio	Average ratio value (BL-corrected) with the "next" channel.
RSD Peak Ratio	peak.rsd_ratio	Corresponding Rel.Std.Dev. value
Peak Spectrum	peak.spectrum.**x**	Opens the ⇒ *Spectrum Data* category
Reference Spectrum Match	peak.refMatch	(See ➢ *Match Factor*)
Number of SLS Hits	peak.nSlsHits	No. of reference spectra
SLS Hit	hitSpec.x	Opens the ⇒ *Hit Spectrum* category
Mass of n-th MS peak	peak.ms_peak_mass(n)	Shows the mass of the n-th mass peak.
Intensity of n-th MS peak	peak.ms_peak_intens(n,"peak1")	Shows the intensity of the n-th mass peak
Position of mass peak maximum	peak.ms_peak_maximum(n."retTime")	Shows the position of the mass peak maximum
MS Peak Purity Match	peak.ms_match(x)	Averaged MS ➢ *Peak Purity Match Factor* (0..1000) over the entire peak (from peak height **x**%)
RSD MS Peak Purity Match	peak.ms_rsd_match(x)	Corresponding relative standard deviation (Rel.Std.Dev.)
Peak Mass Spectrum	peak.msspectrum	Opens the ⇒ *Mass Spectrum* category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒ *Report Categories*.

"Quantification Method" Category

The **Quantification Method** category is only available in Summary tables and in individual ➢*Printer Layout* cells. It includes the following variables:

Variable	Formula	Description
Name	qnt.name	QNT File name
Directory	qnt.path	Directory where the QNT File is saved
Datasource	qnt.dsn	Name of corresponding datasource
Title	qnt.title	QNT File description
Creation Date & Time	qnt.creation_time	Date and ➢*Time* when the QNT File was created.
Creation Operator	qnt.creation_operator.x	User who created the QNT File; opens the ⇒*User Information* category
Last Update Date & Time	qnt.update_time	Date and time when the last update was performed.
Last Update Operator	qnt.update_operator	User who performed the last update; opens the **User Information** category
Last Calibration Date & Time	qnt.lastCal_time	Date and time when calibration was performed last.
Last Calibration Operator	qnt. lastCal_operator.x	User who performed the last calibration; opens the **User Information** category.
Curve Fitting Model	qnt.calFuncType	Model used for curve fitting.
Dual-Column Separate Calibration	qnt.separateCal	Separate calibration for each column
Retention Time Determination	qnt.retTimeMode	Interpretation of the retention time.
Parent Sequence Name	qnt.seq_name	Directory in which QNT File is saved.
Sequence Header Record	qnt.sequence.x	Opens the ⇒*Sequence* category
Dead Time	qnt.deadTime	⇒*Dead Time*
Delay Time Value	qnt.delayTimeValue	Also, refer to ⇒*Delay Time*
Delay Time Detector	qnt.delayTimeDetector	Corresponding detector
Blank Run Subtraction	qnt.blankRun Mode	Status of ➢*Blank Run Subtraction*
Blank Run Sample Record	qnt.blankRunSample.x	Opens the ⇒*Sample* category

Variable	Formula	Description
Matrix Blank Subtraction	qnt.matrixBlank	Subtraction status of a ➢*Matrix Blank Sample*
Number of Detection Parameters	qnt.nDet	Shows the number of detection parameters
Number of Peaks in the Peak Table	qnt.nPeaks	Shows the number of peaks in the peak table
Select Peak in the Peak Table	qnt.peakTabLine("**OPT**")	⇒*Select Peak in the Peak Table* selects peak in chromatogram according to the included option - opens the ⇒*Peak Table* category
Number of Amount Columns	qnt.nAmount	Shows the number of amount columns in the peak table.
Amount Column Name	qnt.amountColName	Shows the name of an amount column.
SST Result	qnt.sst-result	Total result of all single ➢*System Suitability Tests (SST)*
SST Rows	qnt.sst_rows	Number of lines (= number of single System Suitability Tests) on the SST page in the QNT Editor
Select SST	qnt.sst(n.)x	Opens the category: ⇒*SST*. You can then select a variable for the chosen single System Suitability Test with the number n.
Spectra Library Scr. Param.	qnt.sls.x	Parameter for Spectra Library Screening - opens the ⇒*Spectra Library Screening* category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"System Suitability Test" Category

Usually, the ➤*System Suitability Test* category is only available in the ➤*Printer Layout* or in the SST Report. In addition, you can also open a system suitability test by selecting the **Select SST** variable of the ⇒*Quantification Method*. In this case, the formulas start with **qnt.sst(n.)x** instead of **sst.x** and the variables refer to the SST referenced by the number n in brackets. In all other cases, the variables refer to the current SST.

The System Suitability Test category includes the following variables:

Variable	Formula	Description
Number	sst.number	Number of the SST
Name	sst.name	Name of the SST
Sample Condition	sst.sample_condition	Sample condition for the SST
Test Condition	sst.test_condition	Test condition
Aggregate Condition	sst.aggregate	Aggregate function
Min. Aggregation Sample Count	sst.agg_min_smps	Minimum number of samples to be aggregated
Max. Aggregation Sample Count	sst.agg_max_smps	Maximum number of samples to be aggregated
Operator	sst.operator	Indicates the operator who performed the test
Round Digits	sst.round_digits	Rounding function
Value	sst.value	Test value
Channel	sst.channel	Channel on which the test is performed.
Peak Condition	sst.peak	Peak condition for the test
N.A.	sst.n_a	User defined test result if the condition cannot be evaluated.
Fail Action	sst.fail_action	Action be taken if the test fails
Sample Condition Result	sst.sample_cond_result	Results of the sample conditions
Sample Result	sst.smp_result	Result of the text condition for the current peak and the current sample
Results of Test Condition or Aggregate	sst.test_cond_result	Shows the results of the test condition or aggregate
Result of Compare Value	sst.value_result	Shows the results of the compare value
Peak Valuation	sst.peak_valuation	Test result of the current peak
Test Results	sst.results	Result of the system suitability test

Variable	Formula	Description
Aggregated Sample Count	sst.agg_list	Number of aggregated samples
Aggregation: Average	sst.agg_average	Average of the aggregated samples
Aggregation: Standard Deviation	sst.agg_std	Standard deviation of the aggregated samples
Aggregation: Rel. Standard Deviation	sst.agg_rsd	Relative standard deviation of the aggregated samples in percent
Aggregation: Range	sst.agg_range	Result range of the aggregated samples
Aggregation: Rel. Range	sst.agg_rel_range	Relative result range of the aggregated samples in percent

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"History" Category

The ➢*History* category is only available in the History Report. It includes the following variables:

Variable	Formula	Description
Object Name	history.name	Indicates the object name.
Object Path	history.path	Data path, object path and name.
Object Version	history.version	Version of the modified object.
Date/Time	history.time	Date and Time (see ➢*Time*) of the modification.
Operator	history.operator	User - opens the ⇒*User Information* category.
Operation	history.operation	Short description of the modification.
Comment	history.comment	Short descriptive text
Detail Number	history.detail_number	
Detail Object	history.detail_object	
Detail Column	history.detail_column	
Detail Old Value	history.detail_old_value	Old value of the modified field.
Detail New Value	history.detail_new_value	New value of the modified field.
Detail Comment	history.detail_comment	

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Integration Table" and "Summary Table" Categories

Use the variables of the **Integration/Summary Table** category to calculate various statistical values of samples and replicates. It only makes sense to apply these variables if the table has been sorted before.

The category includes the following variables:

Variable	Formula	Description
Group Sum	table.groupSum	Total number of report lines with identical group criterion
Group Count	table.groupCount	Number of report lines with identical group criterion
Group Average	table.groupAverage	Average of report lines with identical group criterion
Group Standard Deviation	table.groupStdev	Standard deviation of report lines with identical group criterion
Group Relative Standard Deviation	table.groupRelStdev	Relative standard deviation of report lines with identical group criterion

 Note:

*On the **Summary** tab page, the category is named **Summary Table**. Elsewhere, it is called **Integration Table**.*

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Program" Category

The **Program** category includes the following variables:

Variable	Formula	Description
Name	pgm.name	Program name
Directory	pgm.path	Directory where the ➢*Program* is saved.
Title	pgm.title	Program title
Timebase	pgm.timebase	Name of the ➢*Timebase* to which the program belongs.
Creation Time	pgm.creation_time	Creation date/time (see➢ *Time*)
Creation Operator	pgm.creation_operator	Opens the ⇒*User Information*
Last Update Time	pgm.update_time	Date and time of the last update
Last Update Operator	pgm.update_operator	Opens the **User Information** category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Report Definition" Category

The Report Definition category (see ➢*Report Definition File (RDF)*) is available for some variables only in the ➢*Printer Layout*; it is not available in tables. It includes the following variables:

Variable	Formula	Description
Name	rdf.name	Name of the report definition file
Path	rdf.path	Path where the report definition file (RDF) is saved.
Datasource	rdf.dsn	Datasource where the report definition file (RDF) is saved.
Sheets	rdf.Sheets	Number of the sheets of the report definition file (RDF)
Current Sheet Number	rdf.CurSheetNo	The current sheet number.
Current Sheet Name	rdf. CurSheetName	The current sheet name.

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Device Wellness" Category

The Device Wellness category includes the following variables:

Variable	Formula	Description
Background	wns.Background	The background signal at the time specified in the device configuration properties.
Pressure	wns.Pump.Pressure	The pressure at the time specified in the device configuration properties.

"Fraction" Category

🗒 *Note:*

In the Printer Layout, the Fraction category is available only for report tables.

The Fraction category includes the following variables:

Variable	Formula	Description
Fraction Number	frac.number	Indicates the number of the fraction.
Retention Time Start	frac.start_time	Retention time at fraction start.
Retention Time End	frac.end_time	Retention time at fraction end.
Number of Tubes	frac.nTubes	Indicates the number of tubes in the fraction.
Select Tube	frac.tube("OPT").x	Displays the selected fraction tube - opens the ⇒*Fraction Tube* category
Tube List	frac.tubeList("OPT", "<Sep.> ")	Lists the tubes in the fraction (OPT = option and <Sep.> = used separator).
Collected Volume	frac.volume	Volume collected in the fraction
Channel Parameter	frac.channel.x	Displays the channel parameter selected for the fraction - opens the ⇒*Fraction Detection Parameter* category.
Number of Peaks	frac.nPeaks("OPT")	Number of all peaks within the fraction
⇒*Select Peak*	frac.peak("OPT").x	Selects the peak from the fraction based on the selected parameter - opens the ⇒*Peak Results* category

For an overview of the different report variable categories, including links to lists of variables for each category, refer to ⇒*Report Categories*.

"Fraction Tube" Category

The Fraction Tube category provides different variables indicating how the single tubes have been filled during fraction collection. You can also open the Fraction Tube category via the Select Tube variable of the ⇒*Fraction* category. In this case, the formulas start with **frac.tube.x** instead of **tube.x**. The variables always refer to the tube indicated in parenthesis. This can be either First, Last, or the actual number of the tube. In all other cases, the variables return the value of the present tube.

 Note:

In the Printer Layout, the Fraction Tube category is available only for report tables.

The Fraction Tube category includes the following variables:

Variable	Formula	Description
Tube Position	tube.position	Tube position (number)
Retention Time Start	tube.start_time	Retention time when the system starts to fill the tube.
Retention Time End	tube.end_time	Retention time when the system stops to fill the tube.
Collected Volume	tube.volume	Volume collected in the tube.
Maximum Collectable Volume	tube.maxVolume	Maximum tube capacity
Number of Peaks	tube.nPeaks("x")	Number of 'peaks' collected in the tube
⇒*Select Peak*	tube.peak("OPT").x	Selects the peak from the tube based on the selected parameter - opens the ⇒*Peak Results* category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Fraction Detection Parameters" Category

The Fraction Detection Parameter category includes different variables that indicate the peak detection setting for the present fraction. This category can be opened only via the ⇒*Fraction* category.

🗒 *Note:*

In the Printer Layout, the Fraction Detection Parameter category is available only for report tables.

The Fraction Detection Parameter category includes the following variables:

Variable	Formula	Description
Detection Channel	frac.channel.channel	Channel for peak detection
Channel Name	frac.channel.name	Name of the channel used for peak detection.
Peak Start Slope	frac.channel.start_slope	The slope must exceed the Peak Start Slope value for the peak start to be recognized.
Peak Start Curve	frac.channel.start_curve	Curve in [Signal]/s^2 that must be exceeded for a shoulder to be recognized after the peak start.
Peak Start True Time	frac.channel.start_truetime	The time must be fulfilled for the different conditions for a peak start to be recognized.
Peak Start Threshold	frac.channel.start_threshold	The signal threshold value must exceed the Peak Start Threshold value for the peak start to be recognized.
Peak Max Slope	frac.channel.max_slope	The slope value must be below the Peak Max Slope value for the peak maximum to be recognized.
Peak Max True Time	frac.channel.max_truetime	The time must be fulfilled for the different conditions for a peak maximum to be recognized.

Variable	Formula	Description
Peak End Slope	frac.channel.end_slope	The slope value must be below the Peak End Slope value for the peak end to be recognized.
Peak End Threshold	frac.channel.end_threshold	The signal threshold value must be below the Peak End Threshold value for the peak end to be recognized.
Peak End Curve	frac.channel.end_curve	Curve in [Signal]/s^2 that must be exceeded for a shoulder to be recognized after the peak maximum.
Peak End True Time	frac.channel.end_truetime	The time must be fulfilled for the different conditions for a peak end to be recognized.
Threshold No Peak End	frac.channel.threshold_no_peak_end	The signal threshold value must be below the Threshold No Peak End value for the peak end to be recognized.
Baseline Drift	frac.channel.baseline_drift	Start value for the baseline drift. This value is used to correct the signal value.
Baseline Offset	frac.channel.baseline_offset	Start value for the baseline offset. This value is used to correct the signal value.
Threshold Do Not Resolve	frac.channel.thrs_no_resolve	Threshold value for the slope in [Signal]/s above which peak detection via the **PeakEndSlope** parameter was disabled.
Deriv. Step	frac.channel.deriv_step	Time for which the signal values were evaluated to determine the signal slope and the curve.

For an overview of the different report variable categories, including links to a list of variables in each category, refer to ⇒*Report Categories*.

"Spectra Library Screening" Category

The **Spectra Library Screening** category includes the variables listed below. This category can be opened only via the ⇒*Quantification Method* category.

Variable	Formula	Description
Spectra Library	sls.library	⮚*Spectra Library*
Match Criterion	sls.match	⇒*Match Criterion*
Hit Threshold	sls.threshold	See ⇒*Threshold*
Spectrum Derivative	sls.derivative	See ⇒*Check Derivative*
Wavelength Range Minimum	sls.wavelength_min	See ⇒*Maximum/Minimum Wavelength*
Wavelength Range Maximum	sls.wavelength_max	Shows the maximum of the wavelength range
Max. Allowed Deviation	sls.max_deviation	⇒*Relative Maximum Deviation*
Max. Retention Time Deviation	sls.max_ret_deviation	See ⇒*Retention Time*
Check Relative Extrema	sls.rel_extrema	See ⇒*Check Extrema*
Max. Retention Index Deviation	sls.max_ret_idx_dev	See ⇒*Retention Index*
Max. Kovats Index Deviation	sls.max_kov_idx_dev	See ⇒*Kovats Index*
Spectra Library Restriction	sls.restriction	Restrictions in the spectra library
Spectra Library Restriction Count	sls.restriction_count	Counts the restrictions of the spectra library

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Spectrum Data" Category

Open the **Spectrum Data** category by selecting the **Reference Spectrum** variable of the ⇒*Peak Table* category. The Spectrum Data category includes the following variables:

Variable	Formula	Description
Lambda Min	peak.spectrum.wlmin	Lower limit of wavelength range
Lambda Max	peak.spectrum.wlmax	Upper limit of wavelength range
Lambda Resolution	peak.spectrum.wlResolution	Wavelength resolution
Sample Rate	peak.spectrum.acqStep	Data acquisition rate
Absorbance Value	peak.spectrum.sig_value("max")	
Absorbance Extremum at [nm]	peak.spectrum.extremum("relMax")	

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Mass Spectrum" Category

Open the **Mass Spectrum** category by selecting the **Select Spectrum** variable of the ⇒*Mass Spectrometry* category. The Mass Spectrum category includes the following variables:

Variable	Formula	Description
Number of Data Points	mspec.mass_count	Number of mass data points
Mass	mspec.mass	Mass [m/z]
Intensity	mspec.intensity	Intensity [counts]
Relative Intensity	mspec.rel_intensity	Relative Intensity (%)
Minimal Mass	mspec.mass_min	Minimal mass [m/z]
Maximal Mass	mspec.mass_max	Maximal mass [m/z]
Mass Range	mspec.mass_range	Mass range [m/z]
Spectrum Type	mspec.type	Spectra type (centroid/profile)
Resolution	mspec.resolution	Resolution of the ➢*Mass Spectrum*
Total Ion Current	mspec.TIC	Number of ➢*TIC* channels

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Hit Spectrum" Category

Open the **Hit Spectrum** category by selecting the **SLS Hit** variable of the ⇒*Peak Purity and Identification* category. The Hit Spectrum category includes the following variables for the matching reference spectrum:

Variable	Formula	Description
Substance Name	hitSpec.name	
Match Factor	hitSpec.match	➢*Match Factor*
Library Name	hitSpec.lib_name	Name of the ➢*Spectra Library*
Library Record	hitSpec.specLib.**x**	Opens the ⇒*Spectra Library* category
Number of rel Extrema	hitSpec.nExtrema	Number of relative extrema
Solvents	hitSpec.solvent	
Comment	hitSpec.comment	➢*Comment*
Sequence Name	hitSpec.seq_name	
Sequence Header Record	hitSpec. sequence.**x**	Opens the ⇒*Sample* category
Sample Name	hitSpec.smp_name	
Sample Record	hitSpec.sample.**x**	Opens the ⇒*Sample* category
Acquisition Time	hitSpec.acqTime	
Timebase	hitSpec.timebase	➢*Timebase*
Program	hitSpec.program	➢*PGM File* used for recording the library spectrum.
Sample Rate	hitSpec.acqStep	Data acquisition rate
Retention Time	hitSpec.retention_time	⇒*Retention Time*
Lambda Min	hitSpec.wlMin	Lower limit of wavelength range
Lambda Max	hitSpec.wlMax	Upper limit of wavelength range
Lambda Range	hitSpec.wlRange	Wavelength range
Lambda Resolution	hitSpec.wlResolution	Wavelength resolution
Detector Name	hitSpec.detectorName	Name of the detector
Detector Serial No.	hitSpec.detectorSerNo	Serial number of the detector
Extract Time	hitSpec.extrTime	Date and time of the spectra search
Extract Operator	hitSpec.operator	Opens the ⇒*User Information* category
Retention Index (lin)	hitSpec.ri	Linear ⇒*Retention Index*
Kovats Index	hitSpec.ri	⇒*Kovats Index*

"Spectra Library" Category

Open the **Spectra Library** category by selecting the **Library Record** variable of the ⇒*Hit Spectrum* category. The Spectra Library category includes the following variables:

Variable	Formula	Description
Name	specLib.name	Spectra library name
Directory	specLib.path	Directory in which the spectra library is saved
Datasource	specLib.dsn	Name of corresponding datasource
Timebase	specLib.timebase	Timebase where the reference spectrum was recorded
Title	specLib.title	Spectra library description
Number of Spectra	specLib.nSpectra	Number of spectra in the library
Creation Date & Time	specLib.creation_time	See ⇒*Time*
Creation Operator	specLib.creation_operator	Opens the ⇒*User Information* category
Creation Software Version	specLib.creation_version	Related software version
Last Update Date & Time	specLib.update_time	Date and time when the last update was performed
Last Update Operator	specLib.update_operator	Opens the **User Information** category
Last Update Software Version	specLib.update_version	Date of the last software update
Retention Index (lin)	specLib.ri	Linear ⇒*Retention Index*
Kovats Index (log)	specLib.ki	⇒*Kovats Index*

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"User Information" Category

You can open the **User Information** category via all **Operator** variables of the different categories except the **Operator** variable of the **System Suitability Test** category. For example, select the Creation Operator variable of the ⇒*Sequence* category. The User Information category includes the following variables (formulas according to the above mentioned example):

Variable	Formula	Description
User ID	seq.creation_operator.userID	ID of the user
User Name	seq.creation_operator.userName	Name of the user
Job Title	seq.creation_operator.jobTitle	Job title of the user

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒*Report Categories*.

"Select Peak" Options

Use the **Select Peak** variable of the ⇒*Chromatogram* category to select a peak in the chromatogram. The Select Peak variable opens the ⇒*Peak Results* category. Enter an option in the **Formula** field.

📝 *Note:*

*Instead of using the **Formula** field, you may as well click **Parameter** and select an option from the **Select Peak** drop-down list.*

The meaning of the options is as follows:

"Formula": chm.peak(OPT, [Name, Number])

OPT = "By Number" (default)**:**	Searches the chromatogram for the peak with the indicated number. Example: **chm.peak("By Number," 1).area** determines the area of the first peak in the chromatogram.
OPT = "By Name":	Searches the chromatogram for the peak with the indicated name. Example: **chm.peak("By Name," "Benzene".)amount** determines the amount of benzene in the sample.
OPT = "By Tab.Number":	Searches the chromatogram for the peak that was identified by the corresponding line in the peak table. Example: **chm.peak("By Tab.Number," 2).height** searches the chromatogram for the peak that was identified by the second line in the peak table, and then it determines its height.
OPT = "By Group":	Searches the chromatogram for the first peak in a specific peak group. Example: **chm.peak("By Group," "CKW").amount** searches the chromatogram for the first peak in the ⇒*Group (Peak Group)* called CKW and then determines its ⇒*Amount*.
OPT = "By SLS-Hit":	Searches the chromatogram for a peak for which at least one SLS hit is available. Example: **chm.peak("By SLS-Hit," 2).hitSpec(1).name** selects the second peak in the chromatogram for which at least one SLS hit is available. In addition, the name of the respective library spectrum with the best match is indicated.
OPT = "ISTD":	Searches the chromatogram for the ➤*Internal Standard* peak. If there are several ISTD peaks, the number of the ISTD peak can be indicated by a second parameter. Example: **chm.peak("ISTD").area** searches the chromatogram for the first ISTD peak and calculates its area. **chm.peak("ISTD," 2).area** calculates the area of the second ISTD peak.
OPT = "Ref":	Searches the chromatogram for the reference peak (Retention Time Interpretation as **Time Distance to ...** or **Time Ratio to...)** For example: **chm.peak("Ref").retention_time** searches for the reference peak and then calculates the corresponding retention time.
OPT = "Condition":	Searches the chromatogram for the first peak for which the conditions becomes true. For example: **chm.peak("By Condition","peak.area>10.000").retention_time** searches for the first peak with an area larger than 10.000 and returns its retention time.

"Select Peak in the Peak Table" Options

Use the **Select Peak in the Peak Table** variable of the ⇒*Quantification Method* category to select a peak in the peak table of the QNT Editor. This variable opens the ⇒*Peak Table* category. Enter an option in the **Formula** field.

🗒️ *Note:*

*Instead of using the **Formula** field, you may as well click **Parameter** and select an option from the **Select Peak** drop-down list.*

The meaning of the options is as follows:

"Formula": qnt.peakTabLine(OPT, [name, number])

OPT = "By Name":	Searches the peak table for the first line with the corresponding name. Example: **qnt.peakTabLine("By Name,"** "**Benzene").amount(1)** returns the value in the first amount column for the peak 'Benzene'.
OPT = "By Number" (default):	Searches the peak table for the line with the corresponding number. Example: **qnt.peakTabLine("By Number,"** **1).name** returns the name of the first peak in the peak table.
OPT = "By Group":	Searches the peak table for the first line with the specified group entry. Example: **qnt.peakTabLine("By Group,"** **"CKW").amount(1)** returns the value in the first amount column for the first peak in the group called CKW.
OPT = "ISTD":	Searches the peak table for the line with the ISTD peak. If there are several ISTD peaks, the corresponding number can be specified via the second parameter. If no number is specified, the first ISTD peak will be used. Example: **qnt.peakTabLine("ISTD,"** **2).name** returns the name of the second ISTD peak.
OPT = "Ref":	Searches the peak table for the line with the ➢*Reference Peak* (➢*Retention Time Interpretation* as **Time Distance to ...** or **Time Ratio to...)** Example: **qnt.peakTabLine ("Ref").name** returns the name of the reference peak.

Glossary

%B, %C, %D (Solvent Components)

The solvent and thus, the ⇒*Flow* usually consist of different components. The amounts of the single partial flows (solvent components) are indicated in percent of the flow. The total of all partial flows is 100% (%A + %B + %C + %D = 100%), where %A is calculated from the remaining partial flows (%A = 100% - (%B + %C + %D)). Therefore, it is sufficient to determine the values for %B, %C, and %D.

Changing the solvent composition during the analysis is referred to as ➢*%-Gradient*.

Also, refer to ⇒*%B, %C, %D (Solvent Components)*.

%-Gradient

See ➢*(%-)Gradient*.

3D_Amp

The **3D_Amp** signal is available for an ICS-3000 ED electrochemical detector in integrated amperometry mode. The signal records the detector response (current), at time t (in milliseconds) in the ➢*Waveform* period and time T (in minutes) of the run (retention time). As a result, the two-dimensional view of an integrated amperometry chromatogram (retention time and integrated current) is extended by a third dimension (waveform time).

You can display and evaluate this three-dimensional data set in the 3D Amperometry window. Each detector current value is entered as a data point in a waveform time by retention time grid, producing 3D data that stretches in the x, y, and z directions.

Also, refer to 🔳 **The 3D Amperometry Window**.

3D Field

A ➢*Photodiode Array Detector* (PDA) simultaneously measures the different absorptions in a wavelength range at the time t. The two-dimensional view of a chromatogram (retention time and absorption height) is extended by another dimension (wavelength). Each recorded data point contains information about the detected wavelength, in addition to the retention time and the absorption value.

The resulting 3D field stretches in x, y, and z-direction.

Dionex Photodiode Array Detectors record data points via the **3DFIELD** signal. You may use ➢*Signal Parameters* to influence signal recording in the same way as for any other signal.

Recording a 3D field can produce an enormous amount of data that depends on the selected ➢*Sampling Rate*, the detector's ➢*Optical Resolution*, and the selected wavelength range. This not only requires special data compression procedures (see **Data Management** 🔳 **Raw Data Compression**), but also makes it impossible to control photodiode array detectors via conventional serial interfaces. That is why powerful special connectors and separate interface cards are used for the PC connection of the Dionex PDAs, such as the UVD 340U detector. This connection even allows online representation of the 3D field on a ➢*Control Panel*.

You can display and evaluate existing 3D fields in different modes (see: ➢*3D Field: Presentation Modes*). The ➢*PPA (Peak Purity Analysis)* pane is available for this.

3D Field: Presentation Modes

The ➢*3D Field* is the relation between the retention time, wavelength, and absorption. Chromeleon supports two modes for presenting three-dimensional figures: ISO plot and 3D plot presentation. Use the **Decoration** dialog box of the ➢*PPA (Peak-Purity Analysis)* pane to toggle between the two modes.

Presentation Mode	Description
ISO Plot	In the area formed by the retention time and the wavelength, lines with equivalent absorption are displayed in the same color. The resolution increases with the number of drawn lines (levels).
	Online representation of the ISO plot, i.e., its real-time representation, is possible as well.
3D Plot	The 3D plot is a combination of perspective projection and iso-line plot. The iso-lines are moved to the third dimension, depending on the absorption degree. A grid containing all retention time and wavelength values is placed over the created profile.

 Note:

The 3D plot presentation mode requires more computing resources than the ISO plot presentation. Therefore, on-screen representation requires more time. Copying a 3D plot to the clipboard or printing is more time-consuming, as well.

3D Plot

See ➢*3D Field: Presentation Modes*

A Groups

See ➢*Access Groups*

Access Groups (A Groups)

An Access Group includes various Chromeleon users. Membership in a specific Access Group determines which objects (server, ➢*Timebases*, ➢*Datasources*, and directories) the user can access. Each user can belong to several Access Groups.

For example, the **All** Access Group can include users A, B, C, X, Y, and Z, while the **Specials** group can include only users A, B, and C.

The more different Access Groups that you create, the more precisely you can define access rights in Chromeleon. (For more information, refer to **Software Installation and Communication** **Access Control** in the **Administrator Help** section.)

After creating an Access Group in the ➢*User Manager* (CmUser program), the system administrator determines the objects the user can access:

a) In the Chromeleon ➢*Client*, the system administrator specifies the access rights to datasources, directories, and sequences.

b) In the ➢*Server Configuration* program, the system administrator specifies the rights to servers and timebases.

To assign access rights, select the object, and then select **Properties** on the context or **Edit** menu. In the **Properties** dialog box, specify which user groups are granted access to the selected object.

If a user belongs to an Access Group, certain privileges are automatically assigned to the user. These ➢*Privileges* are defined in Privilege Groups.

 Note:

If the ➢User Mode is disabled, the user has all rights but (s)he cannot access any section that is protected by an Access Group.

Account

Each user has a separate account. When the ➢*User Mode* is enabled, the account is checked each time the user logs on. To prevent penetration from the outside, the system administrator can determine in the ➢*User Manager* (CmUser program) whether to lock a user account after a specified number of failed ➢*Logons*. In addition, a user account can be locked completely (Account locked). In both cases, the user can restart the Chromeleon client only when the system administrator grants access to the account again.

Acquisition On/Off

The **Acquisition on** command enables data recording (➢*Raw Data*) from all selected ➢*Signals* or channels of a ➢*Timebase*. The signal parameters determine the type of data for signal recording.

Also, refer to ⇒*AcqOn/Off*.

Additional Functions

The ➢*Report Publisher* is an add-on module to the Chromeleon software and provides 124 additional functions for creating user-defined formulas in the ➢*Printer Layout*. For more information about the formulas, select **Additional Functions** in the index of the Chromeleon online Help.

For an application example, refer to **How to …: Creating and Using Report Tables** **Entering User-defined Formulas**.

For an overview of the available functions, refer to the following lists:

Date and Time

DATE	HOUR	SECOND	WEEKDAY
DATEVALUE	MINUTE	TIME	YEAR
DAY	MONTH	TIMEVALUE	
DAYS360	NOW	TODAY	

Mathematics and Trigonometry

ABS	COS	LOG10	SIN
ACOS	COSH	MOD	SINH
ACOSH	EVEN	ODD	SQRT
ASIN	EXP	PI	SIGN
ASINH	FACT	PRODUCT	SUM
ATAN	FLOOR	RAND	SUMIF
ATAN2	INT	ROUND	SUMSQ
ATANH	LN	ROUNDDOWN	TAN
CEILING	LOG	ROUNDUP	TANH

Statistics

AVERAGE	COUNTIF	STDEV	VARP
COUNT	MAX	STDEVP	
COUNTA	MIN	VAR	

Addressing

ADDRESS	HLOOKUP	LOOKUP	ROW
COLUMN	INDEX	MATCH	ROWS
COLUMNS	INDIRECT	OFFSET	VLOOKUP

Text

ASC	FIXED	PROPER	SUBSTITUTE
CHAR	LEFT	REPLACE	T
CLEAN	LEFTB	REPLACEB	TEXT
CODE	LEN	REPT	TRIM
CONCATENATE	LENB	RIGHT	TRUNC
DBCS	LOWER	RIGHTB	UPPER
EXACT	MID	SEARCH	VALUE
FIND	MIDB	SEARCHB	
FINDB			

Logic

AND	IF	OR	TRUE
FALSE	NOT		

Information

CHOOSE	ISERROR	ISNUMBER	N
ERROR.TYPE	ISLOGICAL	ISREF	NA
ISBLANK	ISNA	ISTEXT	TYPE
ISERR	ISNONTEXT		

AIA (ALSSA)

AIA (today called ALSSA) is the abbreviation for **Analytical Instrument Association** (ALSSA = **Analytical and Life Science Systems Association**). The AIA is an association of manufacturing and sales companies for chemical analysis instruments and software. Their aim is to develop guidelines for a standardized chromatography file format and thus, facilitate exchanging data between laboratory systems produced by different manufacturers.

This especially refers to the independence from operating systems and the selected transfer type, but standardization efforts also involve operation, documentation, maintenance, and ⯈*GLP* requirements.

The generally valid AIA (ALSSA) export format for chromatography data is known as **ANDI** (**AN**alytical **D**ata **I**nterchange/Chromatography).

Since 1992, there are five data format categories (Category 1 through 5). However, only the categories 1 and 2 are widely accepted so far.

Data formats of *category 1* comply with standard raw data, unit, and scaling guidelines, and include information about where original data and corresponding methods are stored. This allows you to exactly restore a chromatogram from its raw data.

In addition to amount values and names for substance identification, data formats of *category 2* also transmit all calculated results; for example, based on a chromatogram. Thus, the data can be managed in a database or ⯈*LIMS*.

Using the AIA format (ANDI) in Chromeleon

To export data from Chromeleon, you can use the ANDI format. Select **Export/Backup** on the **File** menu of the Browser. Select **ANDI/Chromatography (AIA)** to open the **Export ANDI/Chromatography (AIA)** dialog box.

It is also possible to export and print data simultaneously. On the **File** menu in the Browser, select **Batch Report**. Under **Export Options**, select the **Export** check box. The **Export Wizard: Common Options** dialog box appears. Select the **ANDI/Chromatography - AIA (*.cdf)** check box, and then click **Next>**. In the **Export Wizard: Options AIA Format (*.cdf)** dialog box, define the AIA format. The **Export Wizard** corresponds to the **Batch Setup** command in the Printer Layout. Enable **Layout Mode** on the **Edit** menu, and then select **Batch Report Setup** on the **File** menu. On the **AIA format (*.cdf)** tab page, define the AIA format.

You can also import AIA files into Chromeleon. On the **File** menu of the Browser, select **Import/Restore**, and then select the **ANDI/Chromatography (AIA)**. In the dialog box, select the file to be imported.

AIA Peak Type

See ➤ *Detection Code at Peak Start or Peak End*

Align

Select the **Align** function to align two or more selected ➤ *Controls* in the ➤ *Layout Mode* (**left**, **right**, **top**, **bottom**) or to assign them identical dimensions (**width**, **height**, **size**).

The ➤ *Control Frame* of the control that is selected first is the reference for the control(s) to be aligned.

Amount

The **Amount** peak table parameter defines the content of a component in a standard sample. For each component used for calibration, exactly one amount value is entered in the **Amount** column of the peak table in the QNT Editor. (For information about the editor, see **Data Representation and Reprocessing** 🔲 **The QNT Editor**.)

Also, refer to ⇒ *Amount*

Amount Deviation

The **Amount Deviation** peak table variable specifies the difference between the nominal ⇒*Amount* included in the **Amount** column of the peak table and the value actually determined by area calculation (actual amount):

Amount Deviation = Actual Amount - Nominal Amount

By default, the difference is expressed in absolute amount units (= **Absolute** parameter). Alternatively, input is possible in percent of the expected amount value (= **Relative** parameter).

Use this variable to check the quality of a calibration. The smaller the determined deviation, the closer is the corresponding calibration point to the calibration curve.

APS

The Dionex ➢*Autopurification* system series is called APS. The series includes the APS-2000 (semipreparative) and APS-3000 (preparative) systems and comprises the following devices:

Device/Accessory	Name	Description
Pumps	**P680**	HPLC pump
	PP-150	preparative HPLC pump (APS-3000 only)
Sample and Fraction Manager	**SFM**	Autosampler and fraction collector
UV detectors	**UVD 170 UV-VIS**	➢*UV Detector*
	UVD 340 PDA	➢*Photodiode Array Detector*
Mass Spectrometer	**MSQ**	Thermo Finnigan ➢*Mass Spectrometer*

In addition, the APS comprises the following devices:

- **SSM** - Safety and Solvent Monitoring System,

- **System Safety Platform**

- **Accurate Flow Splitter** or **Active Splitter**

- The **Chromeleon** software is another main part of the APS.

Also, refer to **Chromatography Components: Hardware and Software** **Chromatography Instruments**.

ARC

ARC, which is the abbreviation for Automated Run Completion, is an ➤*Autopurification* feature in Chromeleon. After the target compound has been found, Chromeleon automatically terminates the run. This saves time and increases the efficiency of sample processing and fraction collection.

Area

The **Area** peak result variable specifies the area between the signal curve, the baseline, and the perpendicular delimiters, if required. The (resolved or non-resolved) baseline considerably influences the size of the peak area.
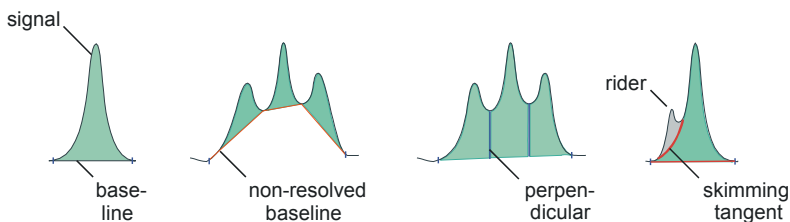
Rider peaks have a separate area that is limited by a skimming tangent and the signal curve.

The peak area is computed from the summation of trapezoids. The trapezoidal area between the two data points **(T1,A1)** and **(T2,A2)** is

$$\text{Area} = \text{(T2-T1)*((A1+A2)/2)}.$$

After the corresponding trapezoids of all data points have been summed up, the baseline is subtracted. For the **(B_T1,B_A1)** and **(B_T2,B_A2)** baseline points at peak start and at peak end, the baseline area is computed as

$$\text{Area} = \text{(B\_T2-B\_T1)*((B\_A1+B\_A2)/2)}.$$

The areas of the rider peaks are computed accordingly and subtracted from the corresponding main peaks.
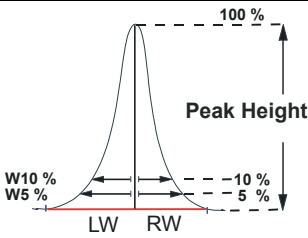
The dimension of the area depends on the used detector type. For UV detectors, the dimension is usually specified in mAU * min (milliabsorbance minutes).

Asymmetry

The **Asymmetry** peak result variable is a measure of peak fronting or tailing. The peak asymmetry helps to evaluate column quality, as long as the analysis conditions (solvent, column type, etc.) are identical.

Theoretically, peaks correspond to a symmetrical Gauss distribution. Any insufficient separation deviates from this ideal. That is why you should analyze and eliminate any distinct asymmetry.

The parameter has no dimension. The definition varies, depending on which standard (USP, EP, or AIA) Chromeleon uses to compute asymmetry:

Asymmetry	Calculation	
USP/EP standard	$A = \dfrac{RW_{5\%} + LW_{5\%}}{2 * LW_{5\%}}$	
AIA standard	$A = \dfrac{RW_{10\%}}{LW_{10\%}}$	
With: RW5% LW5% RW10/%, LW10%	➢*Right* and ➢*Left* peak width in 5% and 10% of the peak height.	

For ideal peaks, the asymmetry (A) = 1. However, for tailing peaks, A is between 1.2 and 5 (USP/EP standard). Values higher than 5 usually produce imprecise quantitative results.

If the asymmetry is calculated at 10% of the peak height, this value is referred to as ➢*Skewness*.

In addition, it is possible to determine the peak asymmetry via statistical moments, using the following formula:

$$A = \frac{\left(\mu_1 - t_R\right)}{\sqrt{\mu_2}}$$

With:

t_R	= Retention time of the current peak
μ_1	= First statistical moment (retention time average). For details, refer to ➢ *Statistical Moments*.
μ_2	= Second statistical moment (retention time variance)

Contrary to the values calculated according to the USP/EP or AIA standard, asymmetry determined via statistical moments scatters around zero. For tailing peaks, asymmetry is positive. For fronting peaks, it is negative.

1. Select the column in the report, and then select **Column Properties** on the context menu.

   The **Properties Report Column** dialog box appears.

2. Select **Peak Results** from the **Categories** list and **Asymmetry** from the **Variables** list, and then click **Parameter**.

   The **Parameter Input for 'Peak Asymmetry'** dialog box opens.

3. Specify whether the method used to compute the peak asymmetry is based on the EP formula, the AIA formula, or statistical moments.

4. Click **OK**.

 Note:

EP = European Pharmacopeia, USP = United States Pharmacopeia

Atlas Electrolytic Suppressor (AES)

See ➢ *Suppressor*

Audit Trail

The Audit Trail is a daily event log that is maintained independently for each system. It facilitates compliance with ➢ *GLP* (Good Laboratory Practice) by recording all events related to instrument operation, such as system events, pre-run device settings, executed commands, and error messages. In addition, you can include the current Audit Trail on the ➢ *Control Panel*.

For more information, refer to **Data Management** 🔳 **Audit Trails**.

Auto Autoscale

Select **Auto Autoscale** to automate ➢*Autoscaling*.

During data acquisition, **Auto Autoscale** adjusts the scaling of the signal axis exactly to the size of the open chromatogram whenever the signal leaves the signal plot in y direction.

Auto Plot Speed

Select the **Auto Plot Speed** option to automatically increase the time axis on a signal plot when the end of the signal plot is reached. The time is increased by the period defined on the **Axis/Decoration** tab page.

Autopurification

Chromeleon supports automatic fraction collection, also referred to as autopurification. In the first step, individual samples are analyzed to determine whether fractionation is appropriate. This can depend on the following criteria:

- Existence of a target compound

- Purity of the target compound

- Amount of the target compound

- Additional user-defined conditions

It is possible to define a maximum of ten target compounds. If all conditions are fulfilled, the sample is fractionated. This step, too, is chromatographically monitored. Finally, additional chromatographic analyses can be performed for the individual fractions.

This process can be automated via ➢*Post-Acquisition Steps*:

Select **Create Purification Samples** to create the samples for purification.

Select **Create Fraction Analysis Samples** to create fractionated samples.

The ⇒*Auto Purif. Type* sample variable identifies the autopurification step.

1. **Analytic** = original, analytical sample

2. **Preparation** = preparative sample

3. **Fraction** = fraction or tubes generated from the preparative sample

If samples of these three sample types belong together, their $\Rightarrow$*Auto Purif. Ref* entries in the sample list are identical.

📠 *Tip:*

In order to perform autopurification, a Purification license must be installed on your PC.

For information about how to perform autopurification, refer to **How to …:** 🔲 **Collecting Fractions Automatically (Autopurification)** and to the operating instructions for the Autopurification Systems series.

AutoQ Equipment Qualification

Dionex AutoQ combines different tools for the automatic qualification of both Chromeleon software and a wide range of chromatography instruments. Thus, AutoQ provides comprehensive functions for quick and easy compliance with the required qualification regulations. A key feature of Dionex AutoQ is that the tests are available not only for Dionex instruments, but for instruments from other manufacturers, also.

Dionex AutoQ is available for the following systems:

- **Chromeleon** software

- **Dionex** IC modules and **Summit** HPLC modules

- **Agilent 1100 HPLC System** modules

- **Shimadzu LC10** and **LC2010** HPLC instruments

- **Shodex RI-101** HPLC Refractive Index Detector

- **TSP** HPLC modules

- **Waters** HPLC devices (including the **Alliance** 2690/2695 Systems and the 996/2996 PDA's)

For more information, refer to **Validation, AutoQ, and System Wellness** 🔲 **AutoQ Equipment Qualification**.

Autosampler

Autosamplers allow routine analyses to be automated. Depending on the autosampler type and model, a number of samples can be prepared and then processed (injected) in a ➤*Batch* (automatic batch processing).

When operating an autosampler, it is important to understand the difference between sample loading and sample injection.

Loading the Sample

The fluid input and output are directly connected. The sample is transported from the pump, through the autosampler, and on to the column. In a second cycle, the volume to be injected is drawn from a sample vial into the sample loop, using a syringe.

Injecting the Sample

To inject the sample, the needle descends into the needle port (GINA 50 and ASI-100) or the inject port (AS and AS50). The injection valve is switched, directing the solvent flow through the sample loop and the needle port or inject port to the column. Any sample remaining in the syringe is directed to waste.

Example for a load/inject process (here: the Dionex ASI-100 autosampler):
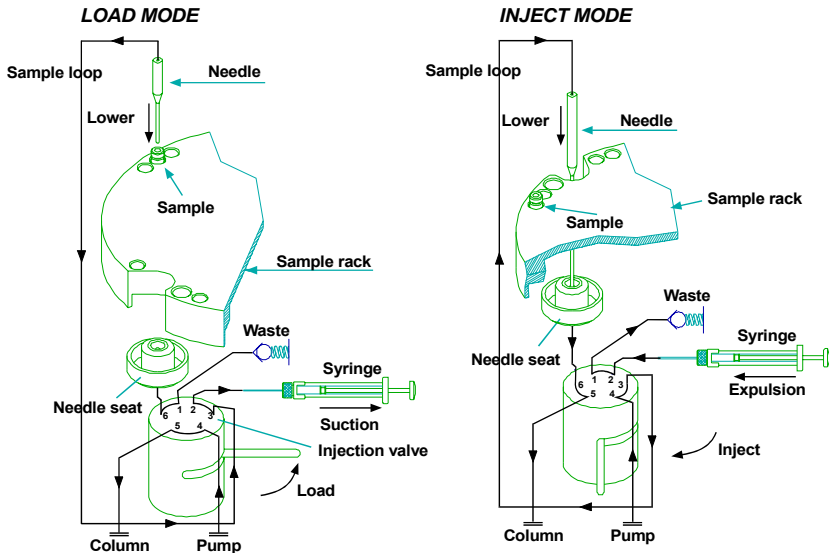
The Dionex Gina 50, ASI-100, AS, and AS50 autosamplers function on this basis. Apart from the standard models, versions providing temperature control (Gina 50T and ASI-100T) and preparative versions (ASI-100P, ASI-100PT with temperature control) are available. In addition, the standard version of ASI-100 autosamplers supports derivatization and method programming.

For more information, refer to **Control** 🔳 **Dionex Autosamplers**.

Autoscale

Each time the **Autoscale** command is selected, the scaling of the signal plot is adjusted exactly to the displayed window section; for example, the height of a peak. The adjustment depends on the method.

For instance, the difference between the maximum and minimum value of the largest peak fills 80% of the signal axis in online mode and 100% in the PPA representation.

Select ➢*Auto Autoscale* to perform autoscaling automatically.

Autozero

The **Autozero** command resets physical or ➢*Virtual Signals* to zero. All values that are subsequently measured are interpreted and displayed in relation to the new zero point. This is usually indicated in the chromatogram by a sharp increase in the absorption value.

🔖☞ *Tip:*

Enter the command separately for each detector if several detectors are switched in series.

Also, refer to ⇒*Autozero*

Average

The **Average** parameter allows you to average signals. Signal averaging is possible for digital signals that are sent to the server PC by the detector, such as the Dionex UVD 170U and UVD 340U detectors, and for analog signals recorded via the ➢*UCI Universal Chromatography Interface*.

The Dionex UCI records each analog signal with a frequency of 100 Hz. This corresponds to a ⇒*Step* of 0.01 second or a ➢*Sampling Rate* of 100 data points per second. When you increase the step or decrease the sampling rate, fewer data points are stored than theoretically possible.

Also, refer to ⇒*Average*

Averaged Baseline

The **Averaged Baseline** is a baseline that is valid throughout the entire chromatogram. The baseline can be defined for single chromatograms.

For more information, refer to **How to …: Working with Chromatograms** 🔲 **Defining an Averaged Baseline**.

Backup

Select **Backup** to store data for data security. For information about how to back up data, refer to **How to …: Creating and Managing Files and Data** 🔲 **Creating Backup Files**.

Chromeleon stores data in databases or other files organized in a hierarchical directory structure. There are many cross-links among the included objects.

For problem-oriented data archiving, it is necessary to know how the individual objects are linked with each other. As conventional backup and archiving programs do not support this, Chromeleon has its own backup program. The Chromeleon backup program supports the following functions:

- Archiving of all objects displayed in the Browser (samples, sequences, files, entire directory trees, etc.), together with all linked files (➢*Raw Data*, Audit Trails (see **Data Management** 🔲 **Audit Trails**), history, preferred ➢*Report Definition File* (RDF), etc.).

- Selective restoration of objects stored using the **Backup** command

- Compressing the stored data

- Dividing the backup files on several media; for example, disks or ZIP media

- Optionally deleting original files after the backup has been performed successfully

- Generating the corresponding entries in the ➢*History*

Backup files are located outside the ➢*Datasource*. That is why they are not stored within the directory structure of the datasources. They have no file history and the user management does not protect them.

Select ➢*Restore* to restore backup files that were created using the **Backup** command. (For more information, refer to **How to …: Creating and Managing Files and Data** 🔲 **Restoring Backup Files**.)

🔳👉 *Tip*

If you have created a backup file that contains a new feature, please keep in mind that you cannot read this file with a Chromeleon version that does not support this feature. For example, this refers to:

- *➢Sequence Report Columns (available since Chromeleon 6.50)*

- *⇒Std. Add. Group and ⇒Ref. Amount Set sample columns (available since Chromeleon 6.60)*

- *The ➢Trend Plot (available since Chromeleon 6.50)*

Bandwidth

The bandwidth is the nm range at which the chromatogram is recorded. The bandwidth usually corresponds to the ➢*Optical Resolution*.

The bandwidth can be selected by averaging several single photodiode signals. This process is known as **Diode or Wavelength Bunching**. Averaging is performed symmetrically to the selected wavelength. Thus, at a bandwidth of 31 nm and a wavelength of 255 nm, for example, the signals of all photodiodes in the range of 240 to 270 nm are averaged.

Also, refer to ⇒*Bandwidth*

Baseline

The line drawn from peak start to peak end for calculating the peak area is called baseline.

Note:

In colloquial language, the chromatogram section between two peaks is also referred to as baseline.

Baseline Correction of Spectra

Automatic correction of a spectrum by a calculated baseline spectrum allows you to compare single spectra, even under varied conditions. Especially peak spectra on a gradient or of ➢*Rider Peaks* can be compared more precisely after a large "underground portion" (due to the gradient portion or the larger main peak) is now removed.

When the spectra plot (**Show Spectra**) is displayed, it is always corrected by the baseline spectrum. If you enable **Baseline correction** in the ➢*PPA (Peak Purity Analysis)* view, the baseline spectrum is also subtracted for this view.

Establishing a Baseline Spectrum

Based on the peak recognition algorithm, Chromeleon establishes the start and end of all peaks for the ➢*Reference Channel* and marks these points by positioning peak delimiters (ta and te). The ➢*Baseline* (**I**) is drawn between the two delimiters.
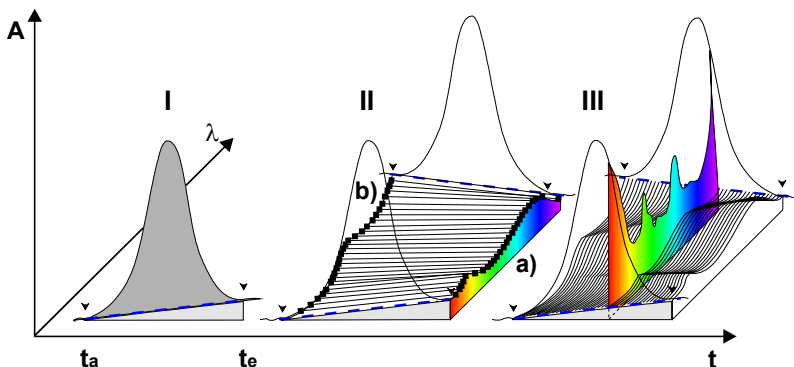
This is performed for all detected wavelengths. The absorbance values at all wavelengths measured at the time ta and te result in the baseline spectrum of the peak start and peak end (**II**, **a** and **b**). Chromeleon interpolates the two spectra and calculates a separate baseline spectrum for each data point of a peak. By definition, the 3D field is zero outside the peak delimiters if baseline correction is enabled.

Each spectrum measured at a given time t can now be corrected by a calculated baseline spectrum (**III**).

Baseline Correction with Manual Re-Integration

Manually shifting the peak delimiters necessarily influences the baseline spectrum. After each manipulation, the new baseline spectra are automatically re-interpolated and re-calculated.

Depending on the changes to the peak delimiters, this will also change the spectrum, for example, the spectra plot.

👉 *Tips:*

Baseline correction can be performed only for the spectra of those peaks that are part of the integrated reference channel.

Baseline correction near the detection limits often considerably smoothes the ➢PPI and ➢Match Curve because it eliminates the spectral baseline portion.

The PPA report is always baseline corrected.

If you add a spectrum from the spectra plot to the spectra library using the Windows clipboard, the spectrum will always be baseline-corrected.

If you add a spectrum from the PPA method to the spectra library using the Windows clipboard, the spectrum will always be baseline-corrected, independent of the settings on the General tab. (To access the General tab, open the PPA view and select Decoration on the View menu.) Baseline Correction influences only the representation of the PPA view.

Baseline Correction of I-t Plots

➢*I-t plots* can be corrected by subtracting a calculated baseline I-t plot from the ➢*3D_Amp* data. This lets you compare single I-t plots, even under varied conditions. For example, I-t plots of peaks on a gradient or of ➢*Rider Peaks* can be represented more precisely because a large "background portion" (due to the gradient portion or the larger main peak) is now removed.

🔲☞ *Tip:*

*To apply baseline correction to 3D amperometry data, select **Decoration** on the context menu and select **Baseline correction** on the **General** tab page.*

Establishing a Baseline I-t Plot

Based on the peak recognition algorithm, Chromeleon establishes the start and end of all peaks for the channel and marks these points by positioning peak delimiters (Ts and Te). The ➢*Baseline* (**I**) is drawn between the two delimiters.
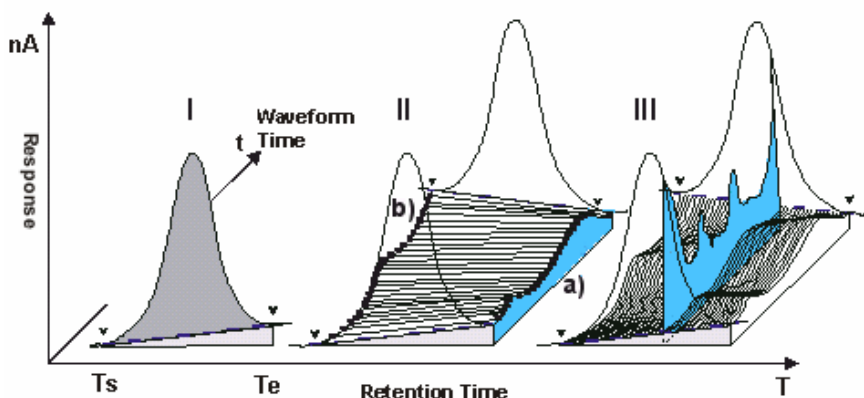
This is performed for all waveform times (t). The response (nA) at all waveform times measured at the time Ts and Te result in the baseline I-t plot of the peak start and peak end (**II**, **a** and **b**). Chromeleon interpolates the two I-t plots and calculates a separate baseline I-t plot for each data point of a peak. Each I-t plot measured at a given retention time (T) can now be corrected by a calculated baseline I-t plot (**III**).

By definition, if baseline correction is enabled, 3D amp data is zero outside the peak delimiters.

The plot below is an example of 3D amperometry data before baseline correction is enabled.
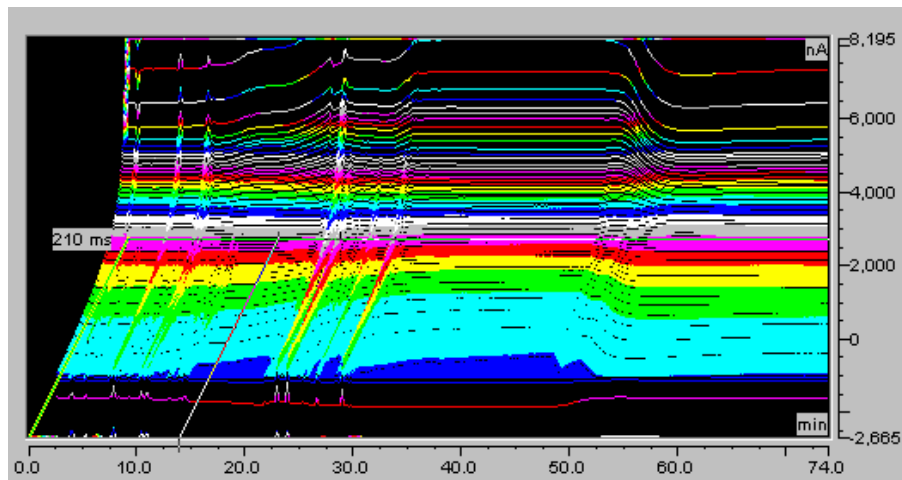
The plot below shows the same data after enabling baseline correction.
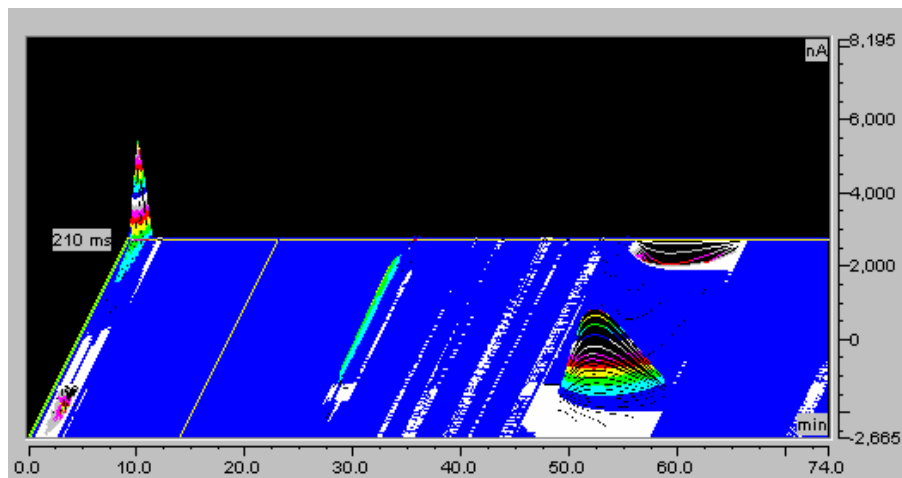
Baseline Correction with Manual Re-Integration

Manually shifting the peak delimiters necessarily influences the baseline I-t plot. After each manipulation, the new baseline I-t plots are automatically re-interpolated and re-calculated.

Also, refer to ⬛ **The 3D Amperometry Window**.

Baseline Point

The **Baseline Point** detection parameter defines a baseline point at the indicated time.

Also, refer to ⇒*Baseline Point*

For information about how to apply detection parameters refer to **How to …: Integrating Chromatograms and Identifying Peaks** ⬛ **Modifying Detection Parameters**.

Baseline Value at Peak Retention

In contrast to the ➢*Signal Value at Peak Retention* variable, which indicates the actual signal value of the signal curve, the **Baseline Value at Peak Retention** peak variable indicates the *baseline* signal value at the peak's retention time.

Baseline Value at Peak Start/End

In contrast to the ➢*Signal Value at Peak Start/End* variable, which indicates the actual signal value of the signal curve, the **Baseline Value at Peak Start/End** peak variable indicates the *baseline* signal value at the peak start or the peak end.

Base Peak

The mass peak with the highest intensity in a ➢*Mass Spectrum* is called the base peak. It serves as a reference point for representing relative intensities (base peak = 100 %).

Batch

Repeating a processing step several times is referred to as batch processing (also called **batch** or **online batch**).

Processing a batch in chromatographic applications means that several samples are automatically processed or analyzed in a defined order. Distinguish between:

- Online batch = batch during the chromatographic analysis

- Offline batch = batch after the analysis

After the chromatographic analysis of a batch, you may print or export the results or process them using an external program. To perform these actions, select **Batch Report** on the **File** menu in the ➢*Browser*. The Batch Report uses the settings selected for the **Batch Report Setup** of the ➢*Printer Layout*. A sample batch is also processed during the batch report. In this case, the batch is referred to as offline batch.

For more information, refer to **Samples and Sequences** 🔳 **Automatic Batch Processing**.

🔲 *Note:*

Each modification of a quantification method results in an immediate recalculation of all involved variables. It is not necessary to recalculate data, as the displayed data is <u>always</u> up-to-date. This is to avoid inconsistencies between the QNT Method and the results.

Binary-Coded Decimal Code ("BCD")

➢*Autosamplers* that can be controlled via binary code (BCD-enabled autosamplers) can communicate the autosampler position from which the injection was performed to Chromeleon. This requires that the autosampler outputs be connected to the BCD inputs on, for example, the ➢*UCI Universal Chromatography Interface*.

For more information about how to proceed, refer to the configuration of the **Remote Inject** ➢*Device Driver* in the ➢*Server Configuration* program (F1 key).

Blank Run Sample

A **blank run** is a chromatogram for which no sample is injected and only the solvent absorption (baseline) is recorded.

In the sample list, blank samples are labeled **Blank** and marked by the following symbol 🗋 . The ⇒*Inject* command's optional **Blank=Inject** parameter forces an injection for blank run samples. However, usually no injection is performed for these samples (**Blank=Skip**). Apart from this, blank samples are treated in the ➢*Program* like normal integration samples.

🖙 *Tips:*

*If an ICS 1000/1500/2000 Ion Chromatography System does not have an AS or AS50 installed, injection is performed with the **Pump_InjectValve.InjectPosition** command. Unlike the autosampler ⇒Inject command, the **Pump_InjectValve.InjectPosition** command always performs an injection, even for blank run samples.*

Blank samples are stored with the sequence, allowing a ➢Blank Run Subtraction to be performed. Because both the normal and the blank run samples are saved, you can undo the subtraction at any time.

Also, refer to ➢*Matrix Blank Sample*

Blank Run Subtraction

A **blank run subtraction** corrects a signal's (channel's) ➢*Raw Data* by the raw data of a comparable ➢*Blank Run* sample. The chromatogram of the blank sample is subtracted point by point from the open chromatogram. However, with a ➢*Matrix Blank Sample*, the peak areas are determined before they are subtracted from the corresponding peak areas of all samples in the sequence.

🖙 *Tips:*

Perform blank run subtraction if a reproducible shift in baseline is interfering with peak detection; for example, with gradient elution.

Because both the normal and the blank run sample are saved, you can undo or restore the subtraction at any time.

A special interpolation procedure is used to subtract two 3D fields even if the recording conditions are not identical; for example, different wavelength range or optical resolution. However, this procedure should not be used except in special situations.

Branch

Select **Branch** to branch to a different ➢*Program* from the active program or to start a program from the ➢*Control Panel* by clicking a script button on the panel.

Also, refer to ⇒*Branch*

Browser

In the Chromeleon Browser, set up, delete, and move ➢*Datasources*, subordinate directories, and files.

The Browser displays samples, methods, and programs as well as further details of a ➢*Sequence*. Double-click to open the selected file.

⚠ *Caution:*

Browser functions and structure are similar to the Windows Explorer. However, do not confuse the Browser with the Windows Explorer. ***Do not use the Windows Explorer for operations within Chromeleon datasources.*** *Administrators can prevent these operations by selecting the* **Protect Datasource Directory** *option on the* **General** *tab page (via* **Properties** *on the datasource context menu).*

For more information, refer to **Data Management 🔳 The Browser**.

Bunch Width

The signals of several photodiodes can be averaged or bunched to enhance the ➢*Signal-to-Noise Ratio* of a ➢*Photodiode Array Detector*. The bunch width describes the sample bandwidth of a bunch of photodiodes.

Also, refer to ⇒*Bunch Width*.

Bypass Mode

The Dionex autosamplers with split-loop injection principle (for example, ASI-100 or WPS-3000SL) feature a Bypass Mode that reduces the gradient delay time and minimizes the flush time. For this purpose, the injection valve is switched into the **Load** position after a specified time and the autosampler allows bypassing the needle and sample loop in the chromatographic flow. The solvent flows directly onto the separation column. In addition, the Bypass Mode allows you to increase the sample throughput because you can start preparing the next sample even before the analysis of the current sample is finished.

For more information, refer to **How to …: Creating and Modifying Programs 🔺 Setting the Bypass Mode Options**.

c0

See ➢ *Offset (c0)*

c1

See ➢ *Slope (c1)*

c2

See ➢ *Curve (c2)*

c3

See ➢ *Cubic Coefficient (c3)*

Calibration Functions, Calibration Coefficients

In each calibration, a mathematical relationship is established between the ⇒*Amount* of a standard sample f(A) and the corresponding area value (A). Depending on the location of the ➢*Calibration Points*, the relationship can be linear, a parabola, or exponential. Select the ⇒*Calibration Type* and by that determine the shape of the calibration points. The corresponding mathematical functions are called calibration functions.

Linear (Lin)
$$f(A) = c1 \, x \, A$$
min. 1 calib. point

Linear with offset (LOff)
$$f(A) = c0 + c1 \, x \, A$$
min. 2 calib. points

Quadratic (Quad)
$$f(A) = c1 \, x \, A + c2 \, x \, A^2$$
min. 2 calib. points

Quadratic with offset (QOff)
$$f(A) = c0 + c1 \, x \, A + c2 \, x \, A^2$$
min. 3 calib. points

Cubic:
$$f(A) = c_1 A + c_2 A^2 + c_3 A^3$$
min. 3 calib. points

Cubic with offset (COff):
$$f(A) = c_0 + c_1 A + c_2 A^2 + c_3 A^3$$
min. 4 calib. points

Exponential (Exp)
$$f(A) = c0 \, x \, A^{c1}$$
min. 2 calib. points

The value of the corresponding calibration coefficient (c0, c1, c2, or c3) describes the slope of the straight line (linear course) or the shape of the curve (non-linear course). The coefficients are calculated by simply replacing known amount and area values in the corresponding calibration function (standard sample). If c0, c1, c2, and c3 are known, it is also possible to calculate the amounts for an unknown sample.

For more information, refer to **Theory of Calibration** 🔺 **Calibration (Overview)** and 🔺 **Evaluation with Various Standard Methods**.

Calibration Mode

The calibration mode determines how a specific sample of a sequence is calibrated and which standards are used. Chromeleon provides six calibration modes.

Select the calibration mode on the **General** tab page of the QNT Editor (see **The QNT Editor** 🔺 **The General Tab Page**). (For information about the editor, see **Data Representation and Reprocessing** 🔺 **The QNT Editor**.)

Also, refer to ⇒*Calibration Mode*

Calibration Point, Calibration Level, Replicates

If the peak area is determined for the known amount of a standard sample, the point resulting from both values in an x, y-diagram is referred to as the calibration point.

If the corresponding peak area is determined several times for the same ⇒*Amount*, different area values are received, depending on the precision. Thus, a larger number of calibration points is obtained. Calibration points based on the same concentration are referred to as replicates of the same concentration level.

Calibration points of different levels are obtained when area values are determined for various amounts of a standard. This can be achieved by a ➤*Dilution Series* or by varying the injection volume.

Based on the calibration points, the data system calculates the calibration coefficients of the ➤*Calibration Function* selected by the user.

For more information, refer to:

Theory of Calibration 🔺 **Calibration (Overview)** and 🔺 **Calibration Principle**

How to …: Calibrating 🔺 **Weighting and Averaging Calibration Points**

Calibration Type

The **Calibration Type** peak table parameter describes the mathematical model function (➤*Calibration Function*) based on which Chromeleon calculates the calibration curve by inserting the c_0, c_1, c_2, and c_3 calibration coefficients.

Also, refer to ⇒*Calibration Type*

For more information about calibration types, refer to **Theory of Calibration**:

🔺 **Calibration Types (Linear)**

🔺 **Calibration Types (Non-linear)**

Also, refer to **How to …: Calibrating** 🔺 **Weighting and Averaging of Calibration Points**.

Calibration Variables

Numerical calibration values are referred to as **calibration variables**. They can be included as a separate column in any table created by Chromeleon. The following variables are calculated:

⇒*Calibration Type*

➤*Coefficient of Determination*

➤*Correlation Coefficient*

➤*DOF-Adjusted Coefficient of Determination*

➤*Curve c2*

➤*Number of Calibration Points*

➤*Number of Disabled Calibration Points*

➤*Offset c0*

➤*Relative Standard Deviation*

➤*RF Value*

➤*Slope c1*

➤*Standard Deviation*

➤*Variance Coefficient*

➤*Variance*

➤*Weights*

Capacity Factor (k´)

The **k´** peak result variable refers to the capacity factor (also called retention factor (Rf)), that is, to the ratio of the net retention time to the ⇒*Dead Time*:

$$k' = \frac{t_R - t_0}{t_0}$$

Where

k'   = Capacity factor

t_R = Retention time

t_0 = Dead time

To achieve a reasonable compromise between the retention time and the required analysis time, k´ should be a value between 1 and 5.

The calculation can be performed only if you have entered the dead time of the system on the **General** tab page in the QNT Editor (see **The QNT Editor** 🔼 **The General Tab Page**. For information about the editor, see **Data Representation and Reprocessing** 🔼 **The QNT Editor**.)

t_0 may either be measured at the inert peak or calculated as the quotient of the dead volume and the flow.

If $t_0 = 0,$ is not calculated.

Select the column in the report. Select **Column Properties** on the context menu, and then select the variable from the **Variables** list box. Click **Parameter** to define the dead time required for the calculation.

Cascade

Select **Cascade** on the **Window** menu to arrange the open windows one behind the other. This arrangement provides an overview of the currently open windows and allows you to change quickly to the window of interest.

Channel

If a detector delivers more than one ➤*Signal*, for example, a multi-channel UV detector, the single signals are referred to as channels.

Check Commands

Chromeleon allows you to perform various checks:

Syntax Check

A syntax check is automatically performed for each ➤*Program*. The syntax check verifies that a program complies with the program syntax. For example, the syntax check may detect the following errors:

- Exceeded value ranges
- Incorrect punctuation
- Misspellings

Errors that are detected by the syntax check are written in red letters.

Semantics Check

The semantics check verifies the (chromatographic) meaning of a program. This includes, for example, the following checks:

- Is the total of all partial flows 100%?

- Does the program contain an ⇒*Inject* command at the time t = 0.000?

- Are the **Inject/**⇒*AcqOn/Off* commands listed in the correct order?

- Is the minimum difference between the emission and the excitation value observed?

- Is the last command an ⇒*End* command? (Absolutely necessary)

To check the semantics of a PGM File, select **Check** on the context menu in the PGM Editor. To check the semantics of a script button, right-click on the button, select **Properties** on its context menu, and then click **Check** on the **Command** tab page. Detected errors are indicated in a dialog box.

Ready Check

The ➢*Ready Check* checks whether the connected instruments are ready to operate; for example, to process a sample batch. The check includes:

- Is the instrument turned on?

- Are all instruments connected correctly?

- Is the lamp turned on?

- Are all required files available?

- Is there sufficient storage capacity?

In the **Batch** dialog box, click **Ready Check** to perform the ready check. In addition, a ready check is performed automatically for each online command. If a problem occurs, an error message or warning appears.

The semantics check usually includes the syntax check. The ready check usually includes the semantics check.

Check Derivative

See ⇒*Check Derivative*

Chemiluminesence

Emission of light caused by chemical reaction is referred to as chemiluminescence. Compared to ➢*Fluorescence* and ➢*Phosphoresence*, excitation by a light source is not required.

Some reactions that cause increased chemiluminescence occur in solutions; they are used for chemical analysis. To generate chemiluminescence, commercialized GC detectors use, for example, ozone, molecular fluorine, and sodium vapor. In HPLC applications, the reactions of luminol, lucigenin, and oxalate esters are often used.

Chemiluminescence detection is more sensitive than fluorescence detection as there is no disturbance by the diffused light of an excitation light source. It is often possible to use a fluorescence detector for chemiluminescence detection by turning off the lamp.

Chromatogram Overlay

See ➢*Overlay*

Chromatographic Methods

All instructions and parameters regarding the chromatographic treatment, that is, the analysis and processing of a sample, are referred to as chromatographic methods. Depending on the included information, the following distinction is made:

Chromatographic Method	Description
➢*Program* (part of the PGM File)	Commands and instructions enabling automation of the analysis.
Quantification methods (QNT)	All parameters required for qualitative peak identification and for quantitative area determination.

The data is either stored in the ➢*Sequence Directory* or copied to this directory by Chromeleon.

Chromatography Server

See ➢ *Server*

Chromeleon Xpress

The Chromeleon Xpress program provides real-time control and monitoring of Dionex chromatography instruments. It is installed on a Tablet PC and functions as the front panel user interface for the instruments connected to one local Chromatography ➢ *Server*.

Refer to 🔳 **Welcome to Chromeleon Xpress** in the Chromeleon Xpress User Help section for details.

Client

Workstations that can start Chromeleon are called clients. The client provides a user interface for accessing chromatography data and ➢ *Datasources* as well as appropriately representing their contents.

To control or monitor a chromatography system from a client PC, the client must be connected to the Chromatography ➢ *Server*. This connection is usually established when you open a ➢ *Control Panel* and connect to the desired ➢ *Timebase*. Once the connection is established, you can access the devices installed on the server.

To manage database access, Chromeleon uses the capability of a modern operating system to share resources (see **Chromatography Systems: Hardware and Software 🔳 Operating Systems**). A client can access all files or datasources for which sharing was issued on a different workstation.

In addition, monitoring and controlling a system is only possible via the client. To do this, the client must connect to a (chromatography) ➢ *Server*.

In combination with a network (see **Chromatography Systems: Hardware and Software 🔳 Network**), this allows you both to operate a system and to represent the system status and the course of the open chromatogram from any client! Due to access conflicts, it is not possible to synchronize system control over several clients.

CmUser and CmSecure Programs

See ➢ *User Manager and Security Activation Tool*

Coefficient of Determination

Use this variable to display the **Coefficient of Determination**, which reflects the deviation of the measured data points from the calibration curve. The coefficient of determination can have any value between 0 and 1 (0 to 100%). A coefficient of 1 indicates that all calibration points are exactly on the calibration curve. Use the following formula to calculate the coefficient of determination:

$$R^2 = 1 - \frac{\sum_{i=1}^{N} W_i * (Y_i - F(X_i))^2}{\sum_{i=1}^{N} W_i * (Y_i - \overline{Y})^2}$$

With: N Number of standard samples participating in the calibration

i Index for standard samples

W_i Weight factor of the standard sample no. i

$F(x)$ Model function of the calibration

X_i X-value of the standard sample no. i

Y_i Y-value of the standard sample no. i

$\overline{Y}$ Average of all Y-values.

👉 *Tip:*

For a linear calibration plot that is not forced through the origin, the coefficient of determination is identical to the squared ➢Correlation Coefficient. In all other cases, this does not apply.

Also, refer to ➢ *DOF-Adjusted Coefficient of Determination*

Column Mode (DX-120)

Column mode enables switching of flow from one column set to another in a DX-120 Ion Chromatograph equipped with the dual-column option. When the column is switched, the eluent flow is also switched. For example, when column A is selected, flow is from eluent reservoir A through column A. If a command switches the flow to column B, flow is then from eluent reservoir B through column B. Also, refer to ➢ *Eluent Mode*

The ColumnAB command is used to switch the flow from one column set to the other. The command can be included in a ➢*Program*, executed directly from the **Commands** dialog box (select **Command…** on the **Control** menu), or linked to a ➢*Control* on the control panel. To link the command to a control, place an edit box or switch control on the control panel (see ➢*Layout Toolbar*). Then, link the control to the **ColumnAB** object property. For more information, refer to **How to …: Controlling Devices from the Control Panel** 🔲 **Linking a Control to a Device**.

To enable **Column Mode**:

1. Open the **Server Configuration**.

2. Select the DX-120 in the timebase.

3. Double-click to open the Properties dialog box (or right-click and select **Properties**).

4. Select the **Mode** tab page, and then select the **Column** option.

Column Temperature

The **Column Temperature** command determines the nominal temperature of the column thermostat (**column oven**).

After defining the nominal temperature, the thermostat is set as quickly as possible to the new temperature (device-dependent). A linear interpolation between the actual value and the nominal value is performed.

It is also possible to enter several temperature commands (➢*PGM File*). In this case, the result is a ➢*Step Profile* instead of a linear interpolation between two values.

🔖 *Tip:*

As with all instrument parameters, Chromeleon attempts to represent the actual temperature on the screen; whether this is possible will depend on the column thermostat that is connected.

Comment

Chromeleon allows you to enter a comment at different places.

- In the ⇒*Comment* column of the sample list, enter any sample description in addition to the sample name.

- In the **Comment** column of the peak table of the QNT Editor (see **Data Representation and Reprocessing** 🔳 **The QNT Editor**), enter any comments regarding the sample.

- Comments are often needed in a ➢*Program*. To enter a comment in the PGM File, enter a semicolon (;) in front of the comment text. The text is then written in green letters.

- In the **Comment** column of a spectra library, enter any comment on the individual spectra.

- On the **General** tab page of the ➢*Datasource* **Properties** (select **Properties** on the context menu), specify that all modifications be logged in the ➢*History*. If the **Comment required** option is enabled, a dialog box opens whenever you save a modification and prompts you to comment the respective change.

If required, Chromeleon automatically enters a comment at two places in the QNT Editor:

- If you have the system create the peak table automatically (select **Autogenerate Peaktable** on the **Edit** menu), the **Comment** column of the peak table shows the following entry:
  - **Autogenerated** (if **Enumerate peaks of current chromatogram** is selected) or
  - **Autogenerated. Spectrum:** *Reference spectrum name*, **Match:** *Match Factor* (if **Use spectra library screening results** has been selected).

- In the **Calibration Comment** column on the **Calibration** tab page, Chromeleon comments the calibration. Usually, this comment will read **OK**. However, it may also indicate a calibration error or inconsistencies in calibration.

You may output the respective comments using the corresponding variables of the single ⇒*Report Categories* in the Report and in the ➢*Printer Layout*.

Concentration

The **Concentration** peak result variable describes the quotient of the calculated ⇒*Amount* and the injection ⇒*Volume*. The value thus specifies the amount of a substance contained in a micro liter (μl) of the injected volume.

☞ *Tips:*

This variable is especially meaningful if you enter an absolute amount value, such as 15.00 μg, in the amount column of the peak table instead of a concentration value; for example, 1.0 μg/μl.

In this case, the value is divided by the injected volume, for example, 20 μl, which results in comparable absolute amount values that are always normalized to 1 μl; for instance, 15 μg/20 μl = 0.75 μg per μl.

Confidence Interval/Confidence Range

General Definition

The confidence interval describes the range in which the true value is found, with a given probability. According to the respective probability, you can define the corresponding confidence interval of the concentration or amount value for each measured value.

Calibration Curve

If the single points of the confidence intervals are connected in a calibration curve, they indicate the confidence range of the curve:
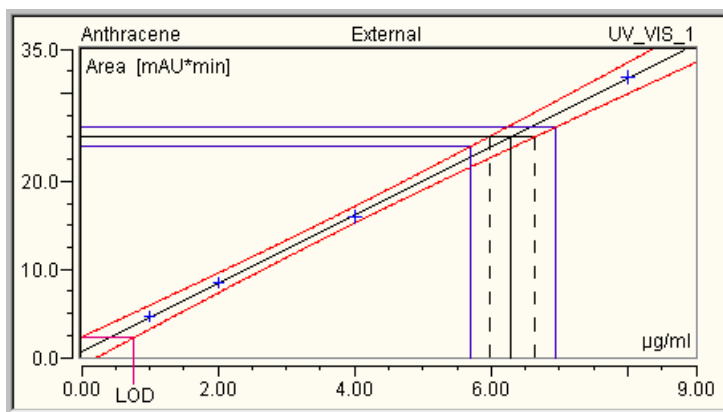
In the above example, the confidence interval appears at a probability of 99%: In addition, a measured value of 25 mAU*min is indicated. The concentration determined by the measured value and the calibration curve is 6.3 µg/ml (black, uninterrupted line). The two intersections with the limiting curves of the confidence interval indicate that the true concentration value will be between 6.0 and 6.6 µg/ml (probability = 99%). In addition, if the **measuring uncertainty** of the measured value is ±1 mAU*min, that is, between 24 and 26 mAU*min, the concentration will be between 5.7 and 6.9 µg/ml (probability = 99%; see the blue auxiliary lines).

Moreover, you can derive the ➢*Limit of Detection*   (= LOD) from the diagram. The LOD is the concentration of the lower limiting curve of the confidence interval, which corresponds to the intercept of the y-axis of the upper limiting curve. In the above example, the LOD would be at 0.75 µg/ml at a probability of 99%.

Confidence Interval in the Calibration Curve

It is also possible to indicate the confidence interval graphically in the calibration curve. For more information, refer to **How to …: Displaying Calibration Curves** **Indicating the Confidence Interval**.

Confidence Interval in the Report

In addition, you can indicate the limits of single confidence intervals in the Report using the **Upper** and/or **Lower Confidence Limit** variables of the ⇒*Peak Calibration* report category. You can choose between different parameters, which allow you defining the values for which to indicate the confidence intervals.

For more information, refer to **How to …: Creating and Using Report Tables** **Setting Parameters for Variables (e.g., for the confidence interval)**.

Connect

Select **Connect to Timebase** on the **Control** menu of a control panel to connect a user PC (➢*Client*) to a ➢*Timebase* (➢*Chromatography Server*). In a 🔳 **Network Installation**, use this command to connect the current client to any server on the network.

Select **Connect Device** to connect a device to the server and thus, to control the device by Chromeleon (remote control). The command first checks whether the specified device is actually connected, and then it activates the instrument. The **Connect** command is executed automatically for all installed instruments whenever the program is started.

📒 *Note:*

For safety and ➢GLP reasons, the instrument keyboard is locked on most instruments that have been connected using the **Connect** *command. In* **Connect** *status, the instrument is exclusively under remote control. Input on the instrument is possible again only after you have executed the* **Disconnect** *command.*

Also, refer to ⇒*Connect/Disconnect*

Contents (Button)

The **Contents** tab page in the online Help displays the table of contents. Click a **book** icon to display the topics in a chapter. Click a **document** icon to open a specific Help topic.

Continue

The **Continue** command cancels the ⇒*Hold* and ⇒*StopFlow* commands and continues an interrupted sample batch or an interrupted pump flow.

Also, refer to ⇒*Continue*

Control

Elements that can be surrounded by a ➢*Control Frame* by left-clicking in ➢*Layout Mode* are referred to as **Controls**.

On the ➢*Layout Toolbar*, Chromeleon provides various standard controls. Authorized persons can use the controls in Layout Mode to create a new ➢*Control Panel*.

If a control is later placed on another control, it is subordinate to the first one. That is why certain modifications to the reference control, such as the font or the **Autosize** command, will also affect the new control.

Press the <Shift> key to select several controls.

Control Frame

The control frame marks the outline of a ➢*Control*. Draw the control frame to move, enlarge, or reduce the control in size.

Control Panel

In online control, the window in which control and display elements are combined is called the **Control Panel**. The control and display elements allow you to control and/or monitor a ➢*Timebase*. The timebase to be controlled is typically assigned when the control panel is created. Select **Connect to Timebase** on the **Control** menu to change the assignment. To save the new assignment, select **Save As** from the **File** menu.

Use the design tools provided on the ➢*Layout Toolbar* to adapt the control panel to your requirements. If you have the corresponding rights, you can also create a completely new control panel. To create or modify a control panel, enable the ➢*Layout Mode*.

Chromeleon also provides easy-to-use default panels for new users. All control panels are saved as PAN files (*.pan).

Control panels are also included in ➢*Panel Tabsets*.

Also, refer to:

How to …: 🔲 Controlling Devices from the Control Panel

How to …: 🔲 Controlling Devices from the Panel Tabset

Correlation Coefficient (Linear)

The linear **Correlation Coefficient** indicates the "linear dependence" between two variables (for example, the peak area and the amount (concentration) of an analyte). It can range from –1 to +1 (-100% to +100%).

If all data points are located on a straight line, the correlation coefficient is exactly +1 or -1 (or ±100%). If the data points are scattered very much, the coefficient approximates 0.

Contrary to the ➢ *Coefficient of Determination*, the correlation coefficient only indicates the linear dependence between two variables. This means that, for example, with a quadratic calibration function, the correlation coefficient may be very low due to the curve shape, although all data points are located on or close to the calculated curve. In this case, the coefficient of determination will be near 1 or 100%, whereas the correlation coefficient will be near zero.

The mathematical description of the correlation coefficient is as follows:

$$CorrCoeff = \frac{\sum_{i=1}^{N} W_i * (X_i - \overline{X}) * (Y_i - \overline{Y})}{\sqrt{\left(\sum_{i=1}^{N} W_i * (X_i - \overline{X})^2\right) * \left(\sum_{i=1}^{N} W_i * (Y_i - \overline{Y})^2\right)}}$$

With:
N	Number of standard samples involved in the calibration,
i	Index for standard samples,
W_i	Weight factor of the standard sample no. i
X_i	X-value of the standard sample no. i
$\overline{X}$	Average value of all x-values
Y_i	Y-value of the standard sample no. i
$\overline{Y}$	Average value of all y-values

👉 *Tip:*

For a linear calibration plot that is not forced through the origin, the correlation coefficient corresponds to the square root of the coefficient of determination. In all other cases, this does not apply.

Use the coefficient of determination if you are using either non-linear calibration curves or a linear calibration curve forced through the origin.

CR-TC

The CR-TC (Continuously Regenerated Trap Column) removes anionic or cationic contaminants in the eluent or deionized water. The CR-TC is used with an ➤*Eluent Generator*.

Cubic Coefficient (c3)

The **Cubic Coefficient (c3)** calibration variable indicates the cubic coefficient of the ➤*Calibration Function* that has been used.

The cubic coefficient is not equal to 0 only for the following calibration functions: **Cubic** and **COff**.

Cumulated Workload

The **Cumulated Workload** variable indicates the wear and tear of a pump. For the Dionex **P 580** pump, you can display the cumulated workload in megajoule (MJ).

For more information, refer to **Practical Tips for Device Control** 🔳 **Viewing Leak Sensor and Workload Status**.

Curve (c2)

The **Curve (c2)** calibration variable indicates the curve value of the ➤*Calibration Function* that has been used.

The curve value is not equal to 0 only for the following calibration functions: **Quadratic** (i.e., **Quad** and **QOff**), **Cubic**, and **COff**.

Cyclic Voltammetry

Waveforms for integrated amperometry are usually developed using information gathered with an electrochemical technique called cyclic voltammetry, in which the current that results from oxidation or reduction reactions is measured against the voltage applied to the system. The applied voltage is changed (scanned) within preset limits.

In cyclic voltammetry, the voltage is first scanned in one direction and then the scan direction is reversed so that the voltage at the end of the scan is the same as at the beginning. This results in a triangular ➢*Waveform* as shown in the example below.
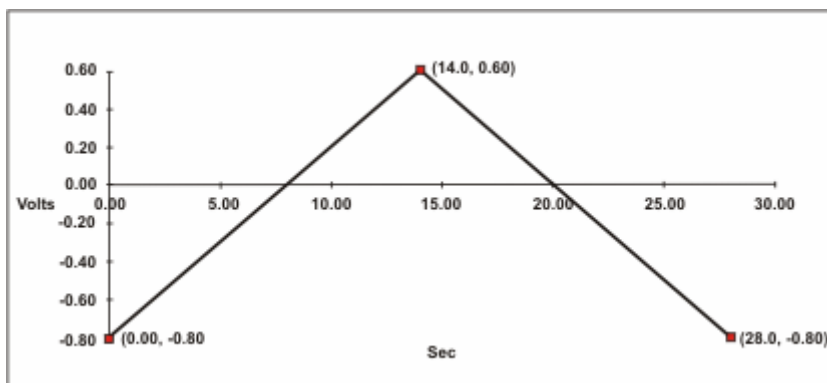
DAC Pump(s)

The **DAC Pump(s)** driver allows you to control voltage-controlled low-pressure pumps for which a separate device driver is not available. A Dionex 12-Bit DAC card is required for this. (For installation instructions, refer to **Hardware Installation** 🌐 **Installing the 12-Bit DAC Card (***"Pump DA Converter"***)** in the **Administrator Help** section.)

The solvent composition is defined in the ➢*PGM File*, as a percentage at the respective retention time. On the DAC FlowA tab page in the Server Configuration program, define the voltages that correspond to the minimum and maximum flow of the corresponding component.

Data Collection Rate

The rate at which Chromeleon collects digital data points from the detector, expressed as points per second or Hertz (Hz), is referred to as the data collection rate. (Refer to **Data Management** 🔲 **Data Acquisition** for more information.) The data collection rate of detectors corresponds to the ➢*Sampling Rate* of A/D converters.

- In general, each peak should be defined by at least 20 data points. For chromatograms with co-eluting peaks or low peak-to-noise ratios, 40 points per peak are better.

- If you expect all of the peaks to be relatively wide, use a slow data collection rate.

- If any peaks of interest are less than a few seconds wide, set a fast data collection rate.

- If the data collection rate is too slow, the peak start and end will not be precisely determined. If the data collection rate is too fast, data files will occupy more disk space and take longer than necessary to process.

Step vs. Data Collection Rate

In addition to the data collection rate, a step value is set. The step value is the reciprocal value of the selected data collection rate. For example, if you select a data collection rate of 5 Hz, the step value is automatically set to 0.2 second. In general, Dionex recommends using the automatically selected step value (see ⇒*Step* for exceptions). It is possible to specify a step value that is independent of the data collection rate; however, only advanced users should do this. Refer to the detector operator's manual for guidelines.

🖝 *Tip:*

*When you issue the **Data Collection Rate** command, the **Step** value is automatically set to the reciprocal value. Therefore, if you want to use a different step value, issue the **Step** command after the **Data Collection Rate** command.*

Setting the Data Collection Rate for Integrated Amperometry Data

For 2D integrated amperometry data, the data collection rate is governed by the ➢*Waveform* period. Chromeleon generates one integrated data point per waveform period. The data collection rate determines the rate at which this data is stored. The data collection rate is usually equal to the reciprocal of the waveform period. Thus, every integrated data point is stored. It is also possible to set the data collection rate to less than the reciprocal of the waveform:

data collection rate $\leq$ *1/waveform period*

 Note:

The data collection rate does not apply to 3D amperometry data. 3D data is collected at a fixed rate of 1 Khz.

Datasource

A database that is mounted to the Chromeleon ➢*Client* is referred to as a datasource. To mount a datasource, select **Mount Datasource** on the **File** menu in the ➢*Browser* and then specify the format and the location of the database. For more information, refer to **How to …: Working with Files, Databases, and Networks** **Setting up a Datasource** in the **Administrator Help** section.

During the initial installation of Chromeleon, a local ➢*Standard Datasource* is created on each client PC. Chromeleon data is usually stored in an Access database, that is, in an mdb (**Microsoft Data Base**) container. The default datasource is based on an Access database, too. The ➢*ODBC Capability* of Chromeleon allows you to use various other formats (dBASE, SQL, Oracle, etc.), as well. Both ➢*Sample Data* and ➢*Sequence Data* are saved in a datasource, independently of the chosen format.

You can save a datasource on a local hard disk or any other mass storage device.

Select **New Directory** on the **File** menu in the Browser to create individual subdirectories under a datasource. You can then use these directories to save ➢*Sequences* and the corresponding data and programs.

Network Datasource

If the datasource is located on a centralized network PC, all clients with the appropriate access rights, can access the database. (The system administrator assigns the access rights.) If the datasource is stored on a local hard disk, the corresponding client grants database access via the Windows **File Sharing** option.

The **Administrator Help** section provides more information; refer to **How to …: Working with Files, Databases, and Networks:**

 Setting up a Network Datasource

 Saving Chromatography Data on the Network

Chromeleon allows you to lock datasources, directories, or sequences, too. For more information, refer to **Data Management** **The Datasource**.

Non-Availability of the Network Datasource

In many companies and organizations, the database server shuts down during the daily backup. Similar to a network breakdown (see ➢*Network Failure Protection*), the network datasource will not be available then. However, in case of the daily backup the time is known during which the datasource will not be available. To ensure data integrity, disconnect Chromeleon before the shutdown. When the database is available again, Chromeleon automatically connects to the database and writes back any data that were acquired during the breakdown.

Also, refer to **How to …: Working with Files, Databases, and Networks** **Network Failure/Non-Availability** in the **Administrator Help** section.

 Note:

*"Old" GynkoSoft data has the required database structure. That is why Chromeleon is capable of handling this data as if they were "real" Chromeleon data. Simply connect to the data stored under **Drive....***

Data Smoothing

See ➢ *Smoothing*

DC Amperometry Mode

DC (direct current) amperometry mode is a basic operating mode of an electrochemical detector. In DC mode a constant potential is applied between the reference and the working electrode.

Electrochemical detection measures current resulting from the application of potential (voltage) across electrodes in flow-through cells. The applied voltage causes oxidation or reduction of analyte molecules in the sample. The current produced is dependent on many factors; the most important of these is the analyte concentration. When you apply a constant potential between reference and working electrode, you can observe a proportional current, depending on the concentration of an oxidable or reducible substance.

Also, refer to ➢ *Integrated Amperometry Mode*

DC Voltage

The **DC Voltage** command for the ED, ED40, ED50, and ED50A electrochemical detectors sets the fixed potential applied to the working electrode, in the range of -2.04V to +2.04V.

Dead Time

The dead time is defined as the time required by the peak maximum of an unretained substance to reach the detector from the point of injection. The relation between the ➢ *Dead Volume* and the ➢ *Flow Rate* is as follows:

$$t_0 = V_m / f_v$$

Where:

t_0 = Dead time

V_m = Dead volume

f_v = Flow rate

Also, refer to ⇒ *Dead Time*

Dead Volume

The volume of the mobile phase between the injector and the detection cell is referred to as dead volume. The dead volume is calculated from the ⇒*Dead Time* and the ➢*Flow Rate* based on the following formula:

$$V_m = t_0 * f_v$$

Where:

V_m = Dead volume

t_0 = Dead time

f_v = Flow rate

(Also, see ➢*Bypass Mode*)

Decimal Minute

A decimal minute (also referred to as industry minute) has 100 units instead of 60 seconds.

The value "2.500" (industry minutes) means "2 minutes, 30 seconds," "2.100" means "2 minutes, 6 seconds," etc.

Delay

The general ⇒*Delay* command specifies the time to pause before executing the next command(s). The **Delay** command is often used for ⇒*Trigger* commands.

The AS or AS50 **Delay** sample prep command specifies a number of minutes to pause before proceeding to the next step in a sample preparation sequence.

Delay Time

The time required for a substance to travel from one location in the chromatography system to another is referred to as the delay time.

In a gradient system, the delay time is the time required for a change in the gradient composition to migrate from where the gradient is formed to the head of the column. The gradient delay time is proportional to the volume between the point at which the gradient is formed and the head of the column, and is inversely proportional to the flow rate. As an example, the delay time in a Dionex RFIC (Reagent-Free Ion Chromatography) system using a gradient pump is typically about 0.8 minute.

Sometimes, the delay time is used to refer to the time related to the delay volume between the following system components:

- The separator column and the detector cell

- The first detector cell and a second detector cell

- The detector cell and the switching valve (or the tube, depending on the device type)

On the **General** tab page of the QNT Editor (see **The QNT Editor** **The General Tab Page**), enter the ⇒*Delay Time* needed by a substance to travel from the detector cell of the first detector to the detector cell of a second detector. (For information about the editor, see **Data Representation and Reprocessing** **The QNT Editor**.)

On the **Fraction Collection Options** page of the **Program Wizard** (see **Control** **The Program Wizard**), enter the delay time between the detector and the ⊳*Fraction Collector*. The wizard displays this page only if you installed the Fraction Collection driver in the ⊳*Server Configuration* program. If you use several additional detectors, enter the delay time(s) between the detectors on the **Fraction Collection - Channel Selection Options** page.

 Tip:

Enter a fixed delay time only if the flow is constant. Changing the flow rate also changes the actual delay time between two detectors or between the detector and the fraction collector.

Delta (Signal Property)

The ⇒*Delta* signal property indicates the signal slope in a second.

This property is especially useful when creating ⇒*Trigger* conditions. A peak cannot only be recognized by the height of its absorption signal within a chromatogram, but also, for example, by a sharp increase of the signal.

For information about how to express this type of trigger condition, refer to **Practical Tips for Device Control** 🔲 **Trigger Commands**.

Demo (or Virtual) Mode

Use the Demo (or Virtual) Mode to simulate device control and data acquisition without the instrument being installed. To operate an instrument in this mode, enable demo or virtual mode in the ➢*Server Configuration* program. With most ➢*Device Drivers* you can enable demo or virtual mode by selecting the corresponding option on the **General** tab page. For some detectors, for example the UVD340U PDA, select the **Read** option to enable demo mode.

Simulate data acquisition by loading a pre-recorded demo file and then displaying the data on the signal plot of the ➢*Control Panel*. The demo file is "read back" as though the data were being acquired in real time. Some detectors also allow recording your own demo data.

For more information about how to enable demo or virtual mode, simulate data acquisition, and record demo data, refer to **How to …: Controlling Devices from the Control Panel** 🔲 **Using/Recording Demo Data**.

Contrary to the usual procedure, the demo mode of the ➢*UCI Universal Chromatography Interface* does not use previously recorded signal courses. The UCI drivers include a Software Peak Generator, instead. Specify the signal properties of the chromatograms to be generated on the **Demo** tab page.

☞ *Tip:*

It is not possible to output MS ➢MCA data in demo mode!

📑 *Note:*

Do not confuse Demo or Virtual Mode with the Chromeleon ➢Evaluation Mode.

Detect Negative Peaks

The **Detect Negative Peaks** detection parameter determines whether negative peaks are recognized apart from positive peaks.

Also, refer to ⇒*Detect Negative Peaks*

For information about how to apply detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Modifying Detection Parameters.**

Detection Code at Peak Start or Peak End (AIA Peak Type)

These peak result variables indicate how the peak start and the peak end is classified. In principle, this corresponds to the ⇒*Peak Type* classification of Chromeleon. The classification described below complies with the ➢*AIA* convention.

The following AIA peak types are evaluated:

AIA Peak Type	Description	Chromeleon Peak Type
B (baseline peak)	The peak starts on the baseline.	**BM**, **bM**
VD (vertical drop)	The peak starts with a perpendicular line dropped to the baseline.	**MB**, **M**
PT (pretangent skim)	The peak is interpreted as a rider.	**Ru** or **Rd**

Device Driver

A device driver is required to control a device by Chromeleon. Each controllable instrument or integrated system has its own device driver that is activated when Chromeleon is started. The device driver translates the instructions (commands) into device-specific digital commands that are then converted into "real control commands" after the data has been transferred to the instrument. In the opposite direction, the signals of all instruments (readings, status information, etc.) are converted so that Chromeleon can read them and display them on the screen.

Device drivers are also capable of monitoring regularly requested or automatically given device parameters in a target/actual value comparison. Unexpected events can be corrected or displayed in an error message.

We can only guarantee for Dionex devices that the device driver fully supports all instrument functions. Support of third-party devices may be limited. If you can change the settings to modify the functionality of the driver (for example, if you can specify the shared A/D channels, relays, or remote inputs), this action is performed in the **Server Configuration Program**

The names of the device drivers are derived from the corresponding instruments.

If a separate device driver is not available for your instrument, special drivers often allow at least partial control of a device. (For more information, refer to **How to …: Configuring the Chromeleon Server** **Controlling Devices without a Separate Device Driver** in the **Administrator Help** section.) In special cases, you can use the generic device driver to create a user-defined device driver. (For more information, refer to **Hardware Installation** **The Generic Device Driver** in the **Administrator Help** section.)

Digital Input

See ➢ *Remote Input*

Dilution Factor

The **Dilution Factor** ⇒ *Sample Variable* is a correction factor for diluting consecutive calibration samples.

Also, refer to ⇒ *Dil. Factor* (dilution factor)

Dilution Series

A dilution series consists of several samples with different concentrations ("concentration series"). The samples were generated from a common original sample. You may as well generate a dilution series by varying the injection volume.

A dilution series is especially useful for multiple-point calibration. Instead of injecting standard samples of different concentrations, different volumes are injected from a standard sample. (The principle is: Twice the injection volume contains exactly twice the amount of each component).

The corresponding amount is calculated as follows:

$$Amount = Am._{(Peak-Tab.)} * \frac{Inj.Vol._{unknownAmount}}{Inj.Vol._{known Amount}} * \frac{Smp.Wght}{Dil.Factor}$$

☞ *Tip:*

Note that the calculated amount values refer to the same standard sample. That is why, in the peak table, you only have to enter one ⇒Amount value (column 1) for each substance contained in the standard.

Dimension of Amounts

This peak table parameter defines the physical dimension of the ⇒*Amount* values, i.e., either amount or concentration.

Also, refer to ⇒*Dimension of Amounts*

Diode Array Detector

See ➢*Photodiode Array Detector*

Disconnect

Execute the **Disconnect** command to separate a user PC (client) from a ➢*Timebase* or to operate an instrument locally. Chromeleon no longer controls or operates the specified instrument. The instrument keyboard lock of the **Connect** command is no longer valid.

📒 *Note:*

*Select **Connect** to undo the operation.*

Also, refer to ⇒*Connect/Disconnect*

Dispense

The **Dispense** command causes the ASI-100 ➤*Autosampler* to dispense a specific quantity (volume) from the sample loop into a certain sample vial (⇒*Position*). The ➤*Duration* parameter determines how long the autosampler takes to perform this operation. After the operation has been completed, the ASI-100 returns the **Sampler.Ready** signal to Chromeleon.

The **Dispense** command causes the AS and AS50 autosamplers to dispense reagent from a reservoir into a specified vial. The **Dispense** command is available only if the autosampler is equipped with the sample preparation option. See **Practical Tips for Device Control: Autosampler Control 🖼 Defining Sample Preparation Steps** for details.

Also, refer to ⇒*Dispense*

DOF-Adjusted Coefficient of Determination

The **DOF-Adjusted Coefficient of Determination** calibration variable returns the coefficient of determination corrected by the degree of freedom. The calculation is as follows:

$$r_{DOF-adj}{}^2 = 1 - \frac{(N-1) * \sum_{i=1}^{N} W_i * (Y_i - F(X_i))^2}{(N-m-1) * \sum_{i=1}^{N} W_i * (Y_i - \overline{Y})^2}$$

With:
	N	Number of standard samples involved in the calibration
	m	Number of degrees of freedom (= coefficients to be determined according to the ⇒*Calibration Type*: LIN: m = 1; LOFF: m = 2; QUAD: m = 2; QUOFF: m = 3 and EXP: m = 2),
	i	Index for standard samples
	W_i	Weight factor of the standard sample no. i
	$F(x)$	Model function of the calibration
	X_i	X-value of the standard sample no. i
	Y_i	Y-value of the standard sample no. i and
	$\overline{Y}$	Average of all Y-values.

Also, refer to ➤*Coefficient of Determination*

Dongle

A dongle is a software license device that is attached to the USB or parallel port (= LPT) of the PC. The free end of the dongle that can be attached to the LPT port is designed as a parallel interface connector.

The dongle stores the serial number of the Chromeleon workstation, much as the ➢*PAL Plug-In Board* does. Each workstation has a unique serial number.

The serial number of the dongle and the ➢*Key Code* stored in Chromeleon must match. If they do not match, Chromeleon cannot operate correctly.

Unless there is a PAL, a dongle, or a ➢*License Server*, and unless the key code is correct, Chromeleon can run in ➢*Evaluation Mode* only.

🖉 *Notes:*

*If a WIBU-KEY dongle is installed, WIBU-KEY software is automatically installed when Chromeleon is installed. Use the WIBU-KEY software to correct individual settings; for example, printing errors when your printer is connected to the dongle. For more information, refer to the WIBU-KEY Help system. To run WIBU-KEY software, click **Start > Settings > Control Panel**:*

The Aladdin Hardlock software does not provide a user interface. As it is not possible for you to select any settings, information about this software is not provided.

The **Administrator Help** section provides more information; refer to:

Software Installation and Communication 🔼 **The Software License**

Hardware Installation 🔵 **Entering the Software License** and 🔵 **Useful Tips for Installing Dongles**.

Draw

The **Draw** command (for the ASI-100) or **Suck** command (for the GINA 50/GINA 160) induces the ➤*Autosampler* to draw a specific injection ⇒*Volume* from a certain sample vial (⇒*Sample Position*). The amount of time this operation is allowed to take is determined by the ➤*Duration* parameter.

After the operation has been completed, the autosampler returns the **Sampler.➤Ready** signal (for an ASI-100) or **Sucked** (for a GINA 50/GINA 160) to Chromeleon. The time between the **Draw** (or **Suck**) command and the **Sampler.Ready** (or **Sucked**) response signal can vary, depending on the instrument type.

Also, refer to ⇒*Draw*.

Drift

A signal change over time, e.g., caused by a temperature change in the laboratory, gradient elution, or detector drift, is referred to as drift.

To show the drift in Chromeleon: Add a new report variable in the report or in the ➤*Printer Layout*. Select **Insert Chromeleon Report Variable** or **Insert Column** on the context menu to open the appropriate dialog box. In the dialog box, select ⇒*Chromatogram* from the **Categories** list and **Signal Value** from the **Variables** list.

Click **Parameter** to open the **Parameter Input for 'Signal Value'** dialog box and select **Drift**. Select the **Restrict Range from** check box and determine the range for which the drift is calculated.

To compute the drift, a regression line is drawn through all data points. The slope of the regression line is the calculated drift. Therefore, to compute the drift, always select a baseline range in which no peaks occur.

Note:

Unless the step is equidistant, all data points are weighted with their corresponding steps to determine the regression line.

The drift is always indicated in [Signal]/min.

DS3 Detection Stabilizer

The DS3 Detection Stabilizer is a temperature-controlled chamber that houses a conductivity cell and an eluent heat exchanger.

The DS3 Temperature command can be used to set the temperature of the DS3. The command can be executed directly from the ➢*Control Panel* or linked to a ➢*Control*. To link the command to a control, place a string display, gauge slider, or edit box control on the control panel (see ➢*Layout Toolbar*). Then, link the control to the object property, **DS3_Temperature.** For more information, refer to **How to …: Controlling Devices from the Control Panel 🔳 Linking a Control to a Device**.

Tip:

The DS4, which is installed in the DX-120 Ion Chromatograph, is identical in function to the DS3. The only difference is the connections required for installation in the DX-120.

Duration

Relay On/Off

Relay On/off indicates the closing or opening duration in seconds [s]. Specifying the duration is optional. The relay on and relay off times must not overlap for the same relay! Some ➢*Device Drivers* support trigger contacts that are treated as two (dependent) relays, R1 and R2: Activating R1 deactivates R2 and vice versa. Thus, you can control a trigger contact using only one **Relay On** command. However, you can also ignore the second relay, R2, and control the trigger contact via the ⇒*Relay On/Off* commands of the R1 relay.

Autosampler

The **Duration** parameter indicates the minimum time that the ➢*Autosampler* may take to perform ⇒*Draw* or ⇒*Dispense* operations. To prevent evaporation of the sample load, do not draw or dispense more than 10 µl per second. Otherwise, if the **Draw** time is too short, gas bubbles may result and considerably impair measuring precision.

Sound

The **Duration** parameter defines for how long (in seconds) the tone is produced for the ⇒*Sound* command.

DX-LAN

The DX-LAN is the local-area network (LAN) that connects Dionex modules to the PC on which Chromeleon is installed. (The **Administrator Help** section provides more information; refer to **Software Installation and Communication** 🔲 **The DX-LAN**.)

Electronic Signature

According to the "21 CFR Part 11" rules and regulations published by the FDA in 1997 electronic signature means the "a computer data compilation of any symbol or series of symbols executed, adopted, or authorized by an individual to be the legally binding equivalent of the individual's handwritten signature".

Use the Electronic Signature feature in Chromeleon to sign and thus, to protect the results generated from your ➢*Raw Data*. This is an important aspect for quality assurance and ➢*GLP*. When ➢*User Mode* is enabled, you can sign and protect ➢*Sequence* reports that have been accepted as correct. In this way, it is possible to review and reproduce the results at any time later.

Electronic signature includes three steps:

* Submit

* Review

* Approve

Typically, the user who created the report signs and **submits** it. Afterward, for example, the laboratory manager reviews the report and signs it as well. Finally, the quality assurance manager **approves** the results.

☞ *Tips:*

Enable User Mode. If User Mode is disabled, electronic signature will not be possible. Besides, electronic signature is available only for user databases that have been created with a ≻User Manager (CmUser program) version 6.10 or higher. Update your database if an error message notifies you that electronic signature will not be possible.

If you use the sample ⇒Types **Spiked** *and/or* **Unspiked***, please keep in mind that:*

- *An ≻Electronic Signature created with Chromeleon 6.50 or earlier is invalid in Chromeleon 6.60 or higher.*

- *An electronic signature created with Chromeleon 6.60 or later is invalid in Chromeleon 6.50 or earlier.*

For information about how to sign reports electronically, refer to **How to …: Creating and Managing Files and Data** 🔲 **Signing Sequences Electronically**.

Eluent Generator

Eluent generators can generate high-purity acid or base eluents online at the point of use, using only deionized water as the carrier. An eluent generator consists of a high-precision programmable current source (power supply), a ≻DX-LAN or USB automation interface, a high-pressure gas removal device, and a disposable eluent generator cartridge (EluGen or pH modifier).

Dionex offers the following eluent generators:

- The EG40 and EG50 eluent generators are configured as separate devices in a ≻*Timebase.*

- The eluent generator installed in the ICS-2000 Ion Chromatography System is configured in a timebase as part of the ICS-2000. The ICS-2000 can have one or two eluent generators configured in the timebase.

- Each eluent generator cartridge installed in the ICS-3000 EG is configured as a separate device in the timebase(s). If two cartridges are installed, they can be configured into the same timebase or into two timebases.

Chromeleon provides the following eluent generator control functions:

- Control of the generated eluent concentration

- Monitoring of the ions remaining in the EluGen cartridge

- Monitoring of the EluGen cartridge expiration date

Also, refer to **Practical Tips for Device Control**:

⬆ Controlling the Eluent Generator Concentration

⬆ Monitoring the Eluent Generator Cartridge Lifetime

Eluent Mode (DX-120)

Eluent mode enables switching the flow from one eluent to another in a DX-120 Ion Chromatograph equipped with the dual-column option. In this mode, the column-switching valve is disabled. The selected eluent always flows to the column that was selected when Eluent mode was enabled. Also, refer to ➢*Column Mode*.

The EluentAB command is used to switch the flow from one eluent reservoir to the other. The command can be included in a ➢*Program*, executed directly from the **Commands** dialog box (select **Command…** on the Control menu), or linked to a ➢*Control* on the control panel. To link the command to a control, place an edit box or switch control on the ➢*Control Panel*) (see ➢*Layout Toolbar*). Then, link the control to the **EluentAB** object property. For more information, refer to **How to …: Controlling Devices from the Control Panel ⬆ Linking a Control to a Device**.

To enable **Eluent Mode**:

1. Open the ➢*Server Configuration* program.

2. Select the DX-120 in the ➢*Timebase*.

3. Double-click to open the **Properties** dialog box (or right-click and select **Properties**).

4. Select the **Mode** tab page and select the **Eluent** option.

Emergency Program

Chromeleon can distinguish four types of errors:

- Fatal Error (**Abort** - The batch is aborted.)

- Serious Error (**Error** - The error is corrected to the extent possible and the batch continues running.)

- Error (**Warning** – A warning appears and the batch continues running.)

- Minor Error (**Ignore** - The error is logged in the ➢*Audit Trail* and the batch continues running.)

If desired, you can define an emergency program for a fatal error. When you do, the emergency program will automatically start when the fatal error occurs and the batch will not be aborted.

The retention time continues while the emergency program is active; the times are interpreted as offset. Data acquisition and Audit Trail reporting will continue, also. After the emergency program finishes running, an **End of Sample** entry is recorded in the Audit Trail.

For emergency program examples, refer to **How to …: Creating and Modifying Programs 🔲 Specifying an Emergency Program**.

Emission

If a substance is fluorescent, it emits light at a specific wavelength range. You can measure the ➢*Fluorescence* with a fluorescence detector that is set to the respective emission wavelength maximum. Select the **EmWavelength** parameter to enter the emission wavelength.

🗒️ *Note:*

*Select the **ExWavelength** parameter to enter the excitation wavelength of the fluorescence detector (also, see ➢Excitation).*

Evaluation Mode

Select the Evaluation Mode to test Chromeleon in the actual working environment. You may temporarily operate Chromeleon in evaluation mode if a software license is not available. Evaluation Mode supports the following licenses:

- Two class 3 ➤*Timebases*

- Server Control

- Two Concurrent Clients

- GLP Features License

- PDA License

- MS Control

For more information, refer to **Chromatography Components: Hardware and Software** 🔺 **Chromeleon Licenses**.

The Chromeleon server remains in evaluation mode for one hour and will shut down automatically after that period.

☞ *Tip:*

Data acquisition is not supported in Evaluation Mode.

The **Administrator Help** section provides more information; refer to:

Software Installation and Communication 🔺 **The Software License**

Hardware Installation 🔵 **Entering the Software License**.

📋 *Note:*

Do not confuse the Evaluation Mode with the ➤Demo Mode of the respective instruments!

Equilibration

Also, refer to ➢SmartStartup.

Excitation

➢*Fluorescence* can be generated only with light that is of higher energy than the emitted light. That is why the selected excitation wavelength must be lower than the emission wavelength. As the excitation wavelength, select the highest absorption maximum for which the wavelength is below the emission wavelength. This allows the substance that will be analyzed to absorb as much irradiated energy as possible. Select the **ExWavelength** parameter to enter this wavelength.

 Note:

*Select the **EmWavelength** parameter to enter the emission wavelength of the fluorescence detector (also, see ➢Emission).*

F1 Key

Press the F1 key to open context-sensitive online Help. The help text refers to the current cursor position.

Filter

Chromeleon distinguishes three types of filters:

Data Acquisition Filters

You can set these filters on the ➢*Control Panel* if the ➢*Device Driver* of the detector supports this option.

Data Reprocessing Filters

➢*Smoothing* filters are often used to improve the ➢*Signal-to-Noise Ratio*. They allow smoothing chromatograms that have already been recorded. The following smoothing filters are available:

- Moving Average (Boxcar)
- Olympic
- Savitzky-Golay
- Gaussian

Data Display Filters

1. **Audit Trail Filters:** You may specify the filter level for displaying messages on the control panel, in the Report, and in the ➢ *Printer Layout*. This is to avoid that the Audit Trail includes messages that are not relevant to you and thus becomes unnecessarily long. (Refer to **Basic Operation** 🔳 **Audit Trails** for more information) Errors, warnings, and system messages are always indicated. However, commands and properties are displayed only from the specified level on. The color codes are as follows:

   Errors and Warnings: Only error messages and warnings but no commands are displayed.

   Normal: 🟩 green (only the most important commands are displayed)

   Advanced: 🟨 yellow (Normal and Advanced level commands and properties are displayed.)

   Expert: 🟥 red (Normal, Advanced, and Expert level commands and properties are displayed.)

2. **Commands Filters:** Similar to the Audit Trail filters, you may specify the filter level for displaying commands in the **Commands** dialog box. Select **Normal**, **Advanced**, or **Expert** on the Context menu.

3. **MS Filter:** In case of ➢ *Mass Spectra*, you can define the ionization polarity and the ionization voltage for the mass spectrum plot.

   If several ionization settings are used in ➢ *Full-Scan* mode, you may enter an MS filter index on the mass spectra plot of the control panel. This is to display those mass spectra of the ➢ *TIC* signal that correspond to a specified group of settings. For example, index 1 refers to the first line in the **Simultaneous acquisitions** table (= TICF_1 - in the following example: polarity "+ve", maximum voltage 100 V):
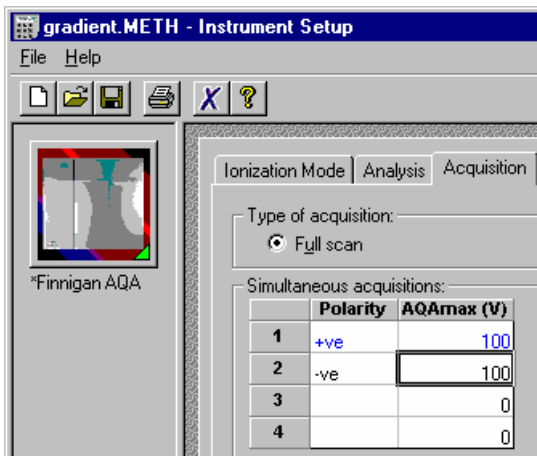
Find (Button)

The **Find** tab page in the online Help topics window allows you to find specific words, terms, or text included in the online Help. All Chromeleon help texts are scanned.

Flow Gradient

Changes to the ⇒*Flow* rate in a chromatogram are referred to as a flow gradient. This type of ➢*Gradient* is quite unusual in liquid chromatography. However, compared to the ordinary ➢*%-Gradient*, the flow gradient has the advantage that the solvent composition does not change in the column. Thus, you do not need to condition the column before starting the next analysis.

The gradient can be generated manually or program-controlled. You can change the flow either continually (➢*Ramps*) or immediately (➢*Step Gradient*).

📖 *Note:*

Pumps that are serially controlled deliver a specific flow rate by commands. It is not possible to enter an unlimited number of these commands in very short intervals. Therefore, a flow gradient generated in this way has very small steps, which, however, are almost immeasurable. Pumps with voltage, frequency, or pulse width control can continually change the delivery rate so that there are virtually no steps.

Flow Rate

The **Flow Rate** usually indicates the total volume (in [ml/min]) delivered by the chromatography pump. It is the sum of all partial flows (%A+%B+%C+%D=100%). (For more information, refer to ⇒*%B, %C, %D (Solvent Components)*.)

Flow rates are usually in the range 0.5 to 10 ml/min. Use micro pumps (0.1 - 0.5 ml/min) or especially equipped pumps (as from 10 ml/min) for flow rates below or above these values.

For gas chromatographs, the amount of mobile gas phase is also called flow rate.

☞ *Tip:*

The pressure in the chromatography column is up to 400 bar (40 MPa, 5800 psi). Solvent mixtures, for example, of methanol and water, are subject to volume compression. That is why the volume delivered by ➢*High-Pressure Gradient Systems does not correspond to the volume transported over the column. Of course, this does not change the number of the delivered and transported solvent particles.*

Also, refer to ⇒*Flow*

Fluorescence

The immediate emission of light after excitation with light energy is referred to as fluorescence. Fluorescence is often used as the detection principle in HPLC applications. The wavelength of the emitted light is longer than the excitation wavelength. In solutions, the fluorescence spectrum is independent of the excitation wavelength.

📒 *Note:*

You can also use a fluorescence detector to measure the ➢*Phosphorescence and* ➢*Chemiluminescence.*

The Molecular Orbital (MO) theory provides a more exact explanation:

Molecules are excited by the absorption of light. Absorption arises from the ground state, the lowest vibrational level of the lowest singlet state (S0) and terminates in different excited vibrational levels of the next higher singlet state (S1).

$$S_0 + h\nu_A \rightarrow S_1$$
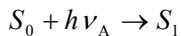
where:

h = Plank's constant

$$\nu_A = \text{light frequence at absorption} = \frac{c}{\lambda_A}$$

where:

c = speed of light

λ = wavelength

Higher vibrational levels of S1 undergo radiationless transitions to the lowest vibrational level of S1. Finally, fluorescence originates in the lowest vibrational level of S1 and terminates in different excited vibrational levels of S0:

$$S_1 \rightarrow S_0 + h\nu_F$$
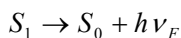
Also, refer to the image below for clarification:
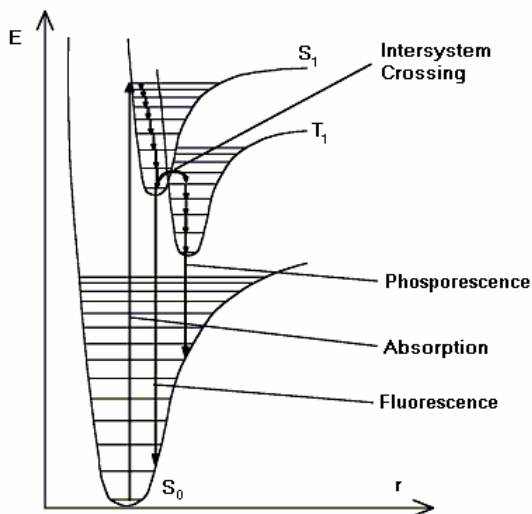
� **Note:**

Due to the fast relaxation in solution, the emission spectra of electronically excited molecules in solution is independent of the excitation wavelength (also for excitation of, e.g., S0→S2), according to the Kasha rule.

Formula for Amount Calculation

This formula calculates the amount in an unknown sample. The prerequisite is that all necessary ➤*Calibration Coefficients* and all peak areas of the sample to be analyzed have been determined.

$$Amount_p = f_p(y_p) * Resp.Fact._p * ISTD\ Fact * \frac{Dil.Factor_n}{Weight_n}$$

The ISTD factor is the ratio of the added amount of ➤*Internal Standard* to the determined amount of internal standard:

$$ISTD\ Factor = \frac{Amount_{ISTD(Peak\ Table)}}{Amount_{ISTD(Sample)}}$$

p	Indicates the peak number
f_p	Indicates the calibration function for peak p calculated during the calibration. For more information, refer to **How to …: Creating and Using Report Tables** **Calculating the Peak Variable "Amount."**
y_p	Indicates the determined area of peak p (for integration type = Area)
Resp.Fact.p	Indicates the scaling factor. The scaling factor can be specific for each peak; for example, to compensate the differing absorption behavior.
ISTD Fact.	Indicates the correction factor for the internal/external standard method. For the external standard, this factor equals 1.
Dil.Fact.	Corresponds to the Dil.Fact. parameter from the sample list.
⇒*Weight*	Is a sample-specific factor that always refers to all peaks.
n	Indicates the sample number

The calculation of the calibration curve is the precondition for amount calculation. For information about how the calibration curve is calculated, refer to **Theory of Calibration** **Evaluation with Various Standard Methods**.

Formula for Amount Calculation (Rel. to ISTD)

This function calculates the amount in an unknown sample by means of the ratio of the area to the ➢*Internal Standard*. The prerequisite is that all peak areas of the sample have been determined including the area of the internal standard.

$$Amount_p = f_p\left(100 * \frac{y_{Peak}}{y_{ISTD}}\right) * Resp.Fact._p * \frac{Dil.Fact._n}{Weight_n}$$

p	Indicates the peak number.
f_p	Indicates the calibration function for the peak p that is calculated during the calibration. For more information, refer to **How to …: Creating and Using Report Tables** 🔼 **Calculating the Peak Variable "Amount"**.
y_p	Indicates the determined area of peak p (for integration type = Area)
Resp.Fact.p	Indicates the scaling factor: The scaling factor can be specific for each peak; for example, to compensate the differing absorption behavior.
ISTD	Indicates the internal standard.
Dil.Fact.	Corresponds to the Dil.Fact. parameter from the sample list.
⇒*Weight (Sample Weight Factor)*	Is a sample-specific factor that always refers to all peaks.
n	Indicates the sample number

The calculation of the calibration curve is the precondition for amount calculation. For information about how the calibration curve is calculated, refer to **Theory of Calibration** 🔼 **Evaluation with Various Standard Methods**.

Fraction Collector

At the detector output, fractions can be collected for preparative chromatography as well as for the exact determination of the separated substances in subsequent analytical procedures. A fraction collector is imperative if this is routine operation.

The fraction collector must collect the fractions depending on the incoming signal; that is, fraction collector control is performed using extensive ➢*Programs*. If the absorption signal exceeds a specified threshold value, ⇒*Trigger* commands are used to close a relay and move to the next collecting tube.

Install the **Fraction Collection** driver in the ➤*Server Configuration* program. Using this driver considerably shortens the respective program and thus facilitates fraction collector control. In addition, install the ➤*Device Driver* for the respective fraction collector in the server configuration program. The Fraction Collection driver is comparable to the ➤*Integrator Driver* that is required, in addition to the respective pump driver, for recording the pump pressure.

For information about how to control fraction collectors, refer to **How to …:** **Collecting Fractions**.

Front Riders to Main Peaks

The **Front Riders to Main Peaks** detection parameter changes rider peaks on the leading edge of a peak into main peaks.

 Tip:

This parameter has priority over the ➤Rider peak type indicated in the peak table.

Also, refer to ⇒*Front Riders to Main Peaks*

For more information about how to use detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Defining Detection Parameters**.

Fronting Sensitivity Factor

See ➤*Tailing Sensitivity Factor*.

Full-Loop Injections (AS/AS50 Sample Prep Command)

When performing a full-loop injection, the AS or AS50 ➤*Autosampler* draws four times the loop volume from the sample vial and delivers it to the injection valve. The middle portion of the sample is positioned in the loop and injected.

☞ *Tips:*

For very small loop sizes (less than 17 μl), 2.5 times the loop volume plus 25 μl is delivered to the valve.

If an installed sample loop is too large, sample can contaminate the flush bottle because the sample volume drawn exceeds the sampling needle assembly volume. The maximum loop size for full-loop injections is 300 μl if the 1.2 ml sampling needle assembly is installed and 1100 μl if the 8.2 ml assembly is installed.

Full-Loop Injection Sequence

1. Draw air

2. Draw sample (at least 4X loop volume)

3. Draw air again to isolate the sample

4. Prepare to load sample (excess sample used)

5. Deliver sample to the loop (excess sample sent to waste)

6. Inject sample onto column. Empty syringe (remaining sample + air bubble)

KEY
— Flush
— Air
— Eluent
— Sample
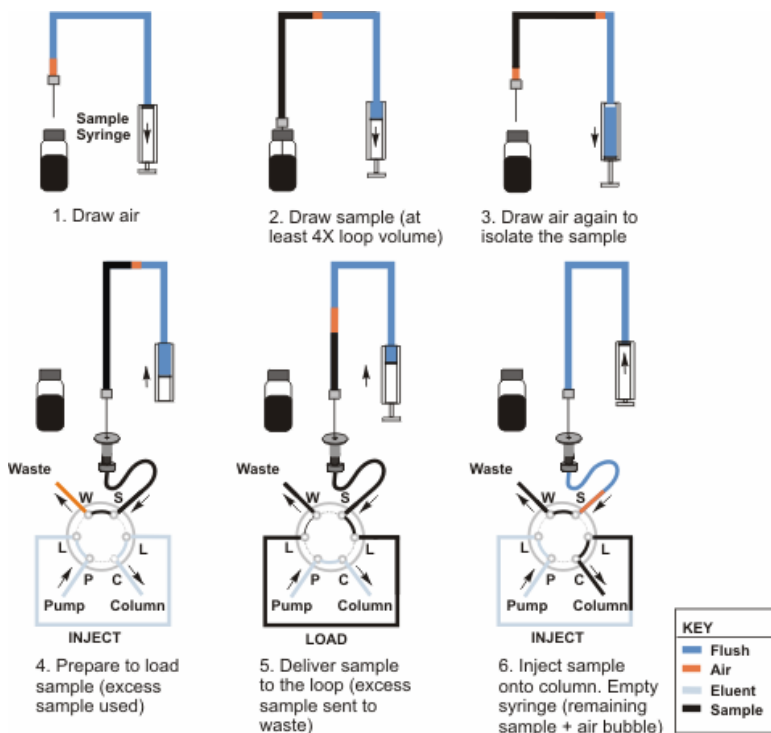
Full-Scale

The **Full-Scale** command sets the voltage for a full-scale analog output. For example, if 0.1V is selected and a recorder is connected to the detector's analog output, the recorder's full-scale response will be at 0.1V. For the AD20 detector, the full-scale voltage is fixed at 1V.

The voltage output of a full-scale detector response to use depends on the recording device to which the analog output is connected. For example, if the analog output is connected to a device that accepts input voltages up to 1 V, select a voltage output of 1000 mV.

Full-Scan

Full-Scan is the ➢*Mass Spectrometer* method for recording ➢*TIC* chromatograms. Contrary to ➢*SIM* (Selected Ion Monitoring), this method records the entire ➢*Mass Spectrum* of each single analyte.

In full-scan mode, you can already extract ➢*Mass Traces* during data acquisition. (For more information, refer to **How to …: Using Mass Spectrometers** **Extracting Mass Traces Afterward**.)

Also, refer to **How to …: Installing and Configuring Mass Spectrometers** **Defining the Number of MS Channels** in the **Administrator Help** section.

Full Size

The **Full Size** command undoes all previous **Zoom** operations. To undo only the last operation, select **Unzoom** on the context menu.

On the signal plot, double-click the ➢*Overview Window* to perform full sizing.

Gain

The Gain signal parameter determines the factor by which the analog output signal of the RF2000 fluorescence spectrometer is increased or reduced. Thus, the **Gain** parameter corresponds to the ➤*Range* parameter that is supported for other detectors.

Note:

*The ➤UCI Universal Chromatography Interface ranges from -10V to +10V and thus covers virtually all detector output signals. It is possible to record signals directly, without considering the **Gain** parameter. If size adjustment is necessary anyway, adjust the size, using the **Factor** parameter of the signal configuration. (In the ➤Server Configuration, right-click the fluorescence detector, and then go to the **Signals** tab page.)*

Gain Region

The Gain Region in an integrated amperometry waveform is a period in which the detector electronics adjust the gain (the ratio of the signal output from the cell to the input of the analog-to-digital converter). If the signal from the cell falls outside the calculated upper or lower limit of the analog-to-digital conversion value, the gain is adjusted to bring the signal back into range.

Keep the following points in mind when selecting a gain region:

- If you will be acquiring 2D data only, select a gain region that matches the ➤*Integration Interval*.

- Because 3D data can be reintegrated after acquisition, the actual integration interval is not known before the run and the gain region cannot be set to the integration interval. Instead, select a longer gain region (or more than one), making sure the region encompasses any period that will be used for integration.

- Do not include the cleaning portion of a waveform in the gain region. The signal input in this region is very high and the analog-to-digital converter may become saturated, causing noisy data.

Globally Unique Identifier

See ➢*GUID*

GLP ("Good Laboratory Practice")

Good Laboratory Practice is the adherence to regulations on the organization and conditions of planning, performing, and monitoring laboratory work.

A special focus is the complete and clear listing of the complete data in an analysis process. In addition, regular checks of the instruments and the software are required.

GLP contributes to enhancing the quality of test data. In Germany, the GLP principles were introduced in 1983. In 1990, they became part of the chemical law (ChemG).

Gradient

Usually, gradients are used in HPLC/IC and GC to accelerate the separation of substance mixtures. Due to the different separation mechanisms in HPLC/IC on the one hand and GC on the other, different gradient types can be used.

HPLC/IC: Two different gradient types

A change in the **composition** of the mobile phase during the analysis is referred to as a gradient (more exactly as ➢*%-Gradient*). A change in the flow rate is referred to as a ➢*Flow Gradient*. However, this gradient type is quite unusual. Chromeleon lets you generate flow and %-gradients. Both gradient types can be performed at the same time (!).

➤➣ *Tips:*

Performing both gradients at the same time results in non-linear partial flow alterations in the total flow. For ➢High-Pressure Gradient Systems with more than one pump, it will result in non-linear partial flow alterations for each pump. Some chromatography pumps do not support this option.

The chromatographic conditions for high-pressure and low-pressure gradients are not transferable due to the different properties in solvent compressibility and dead volume!

GC: Three different gradient types

Usually, **temperature** gradients are used in GC. In addition, some GCs can run **flow** or **pressure** gradients. With GC gradients, the flow (or the temperature, or the pressure) usually increases during the chromatogram. Changing one of the three variables automatically changes the remaining two according to the equitation of state for ideal gases and the Hagen-Poiseuille law. Nevertheless, it is also possible in GC to run a temperature and a flow or pressure gradient simultaneously. In this case, the flow or pressure change that results from the temperature increase is considered for the flow or pressure settings.

Independently of the gradient types described above, the gradient forms can be different. Distinguish ➤*Ramps* and ➤*Step Gradients*. For example, if you run a %-gradient as a ramp, the solvent composition continually changes until the desired composition is finally reached at the desired end. The composition for a step gradient is set immediately. (Nevertheless, it may take some time until the desired composition reaches the column, depending on the column, the solvent change, and the flow)

%-Gradient

A change in the composition of the delivered solvent mixture during the analysis in liquid chromatography is referred to as a gradient. Usually, the eluting power of the solvent is increased to accelerate the elution of the later eluting substances.

To distinguish this gradient type from the ➤*Flow Gradient*, which is quite unusual in liquid chromatography, it is also referred to as a %-gradient. The composition can be modified immediately (➤*Step Gradient*) or over a certain period (➤*Ramp*).

📑 *Tips:*

Depending on the dead volume of the pump, the time required for changing the mixing ratio varies. The greater the dead volume is, the later the new mixing ratio will be realized on the pump output and the later the solvent mixture will reach the column. Therefore, make sure to perform gradient courses in sensible intervals.

Usually, the solvent composition at the beginning of the analysis widely differs from the composition at the end. Therefore, before you start the next analysis, make sure that the column has been sufficiently conditioned, that is, that the solvent composition in the column corresponds again to the original composition at the beginning of the analysis.

Gradient Curves

The Dionex P680, GP40, GP50, and GS50 pumps let you specify linear or non-linear ➢*%-Gradient* profiles (gradient curves). ➢*Eluent Generators* can also deliver non-linear %-gradient profiles. For the mathematical description of the curve types, refer to ➢*Gradient Curves Equations*.

Curve 5 (the default) is linear. Changes in composition of the delivered solvent over time are constant.

Curves 1 - 4 are convex upward. Convex curves cause rapid changes in solvent composition at the beginning of the gradient and slower changes at the end. Slope changes over time become extreme as curves go from 4 (least convex) to 1 (most convex).

Curves 6 - 9 are concave upward. Concave curves cause slower changes in solvent composition at the beginning of the gradient and rapid changes at the end. Slope changes over time become extreme as curves go from 6 (least concave) to 9 (most concave).

The figure below shows the solvent composition profiles of curves 1 through 9, for a change from 0 - 100% B over 10 minutes.
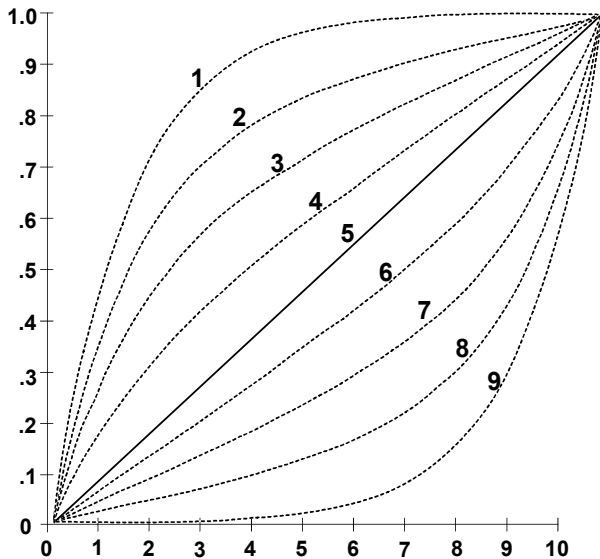
📋 *Note:*

The statements regarding curves 1 to 9 also apply to some third-party devices. However, the curvature may deviate.

Gradient Curves Equations

The following equations describe the ➢*Gradient Curve* profiles generated by the Dionex GP40, GP50, and GS50 gradient pumps, and by the ➢*Eluent Generator*.

For convex gradients:

$$Ve = Vf + (1-k)(Vt - Vf)\left(1 - 2^{\frac{-10(Te-Tf)}{(Tt-Tf)}}\right) + \frac{k(Vt - Vf)(Te - Tf)}{(Tt - Tf)}$$

where: k = 0, ¼, ½, ¾, 1 for curve numbers 1, 2, 3, 4, 5 respectively

For concave gradients:

$$Ve = Vf + (1-k)(Vt - Vf)\left(2^{\frac{-10(Tt-Te)}{(Tt-Tf)}}\right) + \frac{k(Vt - Vf)(Te - Tf)}{(Tt - Tf)}$$

Where: k = ¾, ½, ¼, 0 for curve numbers 6, 7, 8, 9 respectively

Ve	Current eluent value
Vf	Eluent value at the previous step time
Vt	Eluent value at the next step time
Te	Current elapsed time
Tf	Time at the beginning of the gradient step
Tt	Time at the ending of the gradient step
k	Parameter that is changed to produce different curve number results

Group (Peak Group)

Chromeleon lets you to group single peaks in a chromatogram and then calibrate and integrate them as a group. Assign these peaks a common name in the **Group** peak table column in the QNT Editor (see **Data Representation and Reprocessing** 🔖 **The QNT Editor**). The peaks do not have to succeed each other directly but can be scattered at will.

If a peak belongs to a user-defined group, the **Group** report peak variable indicates the group's name. In addition to the user-defined peak groups, two pre-defined groups are available:

1. The group of all unidentified peaks without explicit group entry (empty group)

2. The group of all identified peaks without explicit group entry (empty group)

Define all other groups of identified peaks on the **Peak Table** tab page in the **QNT Editor**. For unidentified peaks, the group defined in the **Unidentified Peaks** dialog box on the **General** tab page in the QNT Editor is indicated if the peak appears in the corresponding time interval.

Also, refer to ⇒*Group*

GUID

Chromeleon supports sample identification by means of a unique character string referred to as GUID (Globally Unique IDentifier). Each character string is globally unique; that means that it is not used a second time, neither on the same datasource or Chromeleon server nor on any other datasource or server.

Chromeleon uses the common 128-bit-GUID, e.g.:

<p style="text-align:center">{c7b2aa95-66a3-48a5-b77d-aee437656b2a}.</p>

The GUID is consists of hexadecimal digits (0, 1, 2, … d, e, f). Hyphens are used to enhance readability. The first four blocks have a timestamp. The last block is the network address of the server PC.

Also, refer to **Creating and Managing Files/Creating a Sample List (Sequence)** 🔖 **Using Globally Unique Sample Identifiers**.

Height (Peak Height)

This peak result variable refers to the peak height in the peak maximum, that is, at the retention time, relative to the baseline.

The height dimension depends on the detector type. For UV detectors, the area is specified usually in mAU (milliabsorbance units).

Also, refer to ➢*Relative Height*

Help

See ➢*Online Help*

High-Pressure Gradient System

In a high-pressure gradient system, each partial flow is delivered via a separate pump into the mixing chamber against the pressure of the column. Differences in volume contraction and compressibility, as observed in most solvent mixtures, are not important during delivery but when mixing the solvents on the high-pressure side of the pump.

Unlike ➢*Low-Pressure Gradient Systems*, the delivered volume of each partial flow can be determined precisely in high-pressure gradient systems. The gradient profile is more precise and can be achieved with relatively simple pumps. However, the costs for the additional pump are disadvantageous.

☞ *Tip:*

Due to solvent compressibility and differing dead volumes, the chromatographic conditions of high-pressure and low-pressure gradient systems are not interchangeable.

History

The History mode (or more exactly the File History Mode or Modification History Mode) completely documents important changes and operations performed in specific objects and files. Objects include samples, ➤*Sequences* or ➤*Datasources*, ➤*Control Panels*, ➤*Report Definition Files* (RDFs), ➤*PGM Files*, and/or ➤*QNT Files*, and of course modified chromatograms.

🔖 *Note:*

Changes in report definition files (RDFs) that are not important for GLP are not logged in the history; such as:

- *Changes of the view (Integration View)*

- *Changes of embedded graphic objects that are not part of Chromeleon, e.g., charts or bitmaps*

- *Enabling and/or disabling Layout Mode*

- *Format changes, e.g., changes of the cell frame, cell background, or alignment.*

The History mode can be enabled (or disabled) individually for each datasource. **Enable Modification history** in the **Properties** of a datasource). If the **Comment required** option is enabled, each user is prompted to enter a comment before (s)he can save the changed object.

Select a datasource, a directory, or a single object, and then select **Show History** on the context menu to display the changes that are already documented.

☞ *Tip:*

It can take some minutes to create and display the history.

To be able to enter and edit comments or to enable and disable the History Mode, the user must have the corresponding ➤*Privileges*.

For more information, refer to **How to …: Working with Files, Databases, and Networks** 🔲 **Tracking File Modifications (History)** in the **Administrator Help** section.

Hit Criteria

If there are too many hits in a spectra comparison (see **How to …: Displaying and Using UV Spectra** 🔹 **Searching Single Reference Spectra**) due to the selected comparison function (see ⇒*Check Derivative*) or ⇒*Match Criterion*, this list can be limited by adding the following selection criteria:

Comparison Conditions	Additional Limitations
Standard of comparison	Number of extrema
Minimum match degree	Retention time deviation
Comparison function	Solvent composition (*)
Wavelength range	➢*PGM File* name (*)
Comparison of the largest relative maximum	

Only spectra fulfilling the corresponding criterion will be admitted into the selection.

All fields accepting alphanumeric input (*), can be searched with a regular expression, which may contain the common wildcards '?' and '*'.

Example: If ***y?ene** is entered as the name filter, the hydrocarbons pyrene, chrysene and perylene would be hits, but not anthracene. The hit criteria can be used to comfortably generate sub-libraries. If using these filters, without comparing spectra, the hit list contains the spectra fulfilling the hit criteria. The partial quantity can be stored as a separate library.

📑 *Tip:*

Please note that a distinction is made between upper and lower case letters in the case of alphanumeric input. When using a capital letter in the filter expression, hits must also contain a capital letter at exactly the same position. When using lower case letters, the corresponding hit may contain lowercase and uppercase letters.

Hold

In the Hold mode, no more data is acquired, the pump continues delivery with the current solvent composition, and processing of the batch samples is stopped before the next sample.

Also, refer to ⇒*Hold*

Holmium Oxide Filter

Newer Dionex UV/photodiode array detectors (including the UVD 170U four-channel detector and the UVD 340U, PDA-100, and PDA-3000 ➢*Photodiode Array Detectors*) feature holmium oxide filters.

For the UVD 170U and UVD 340U detectors, the holmium oxide filter is used to calibrate UV/VIS spectra recorded with the detector due to the typical absorption spectrum of holmium oxide.

For the PDA-100 and PDA-3000 detectors, the holmium oxide filter is used to verify wavelength accuracy.

Note:

The UVD 170S/340S detectors can be fitted with holmium oxide filters. Please contact Dionex Service for more information.

If/ElseIf/Else/EndIf

The IF construct allows you to execute a command if a certain condition is true. If the **IF** condition is true, the statements enclosed between **IF** and **ENDIF** are executed. Otherwise, they are ignored. By using **ELSEIF**, it is possible to combine several conditions. The statements of the final **ELSE** will be executed if none of the ELSEIF conditions is true either.

Also, refer to ⇒*If/ElseIf/Else/EndIf*

Index (Button)

The **Index** tab page provides an alphabetical index list of terms that are explained in the Chromeleon online Help. Enter the first letter of the term of interest and click **Display** to view information about this topic.

Inhibit Integration

The **Inhibit Integration** detection parameter can be used to shut off certain chromatogram areas.

Also, refer to ⇒*Inhibit Integration*.

For information about how to apply detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔖 **Defining Detection Parameters**.

Inject Command

The **Inject** command defines the start of a chromatogram and thus the time when the sample is brought into the high-pressure cycle of the system. By definition, the time of the first **Inject** command is set to zero.

In a dialog box, define the sample vial position in the ➤*Autosampler* (⇒*Position*) and the quantity of the substance (⇒*Volume*) to be injected. When a hand-operated valve is used for injection, this information is used for documentation purposes.

Also, refer to ⇒*Inject Command*

Injection Time

The **Inj. Time** column in the sample list shows the day, month, year, hour, minute, and second when the sample was injected (Also, refer to ➤*Time*.) This column cannot be edited. Chromeleon enters the injection time of the sample in the corresponding column of the sample list. For samples with the ⇒*Status* (sample status) "M" the time of the last injection is entered.

The kind of entry (empty or time value) indicates whether and when the sample was processed.

Also, refer to ⇒*Inj. Date/Time* (Time of Injection)

Injection Types (AS/AS50 Autosampler)

Three types of sample injections are possible with an AS or AS50 ➢*Autosampler*:

➢*Full-Loop* **-** the full loop volume is injected

➢*Partial-Loop* **-** a part of the loop volume is injected

➢*Partial-Loop, Limited-Sample* **-** a partial volume of the loop is injected, and no extra sample is aspirated from the vial.

The injection type is selected automatically, depending on the settings of other variables:

• A full-loop injection is performed when the ⇒*Inj. Vol.* (injection volume) is greater than the ➢*Loop Volume*.

• A partial-loop injection is performed when the injection volume is less than the loop volume and the *cut volume* (a volume of sample discarded from each end of the aspirated sample) is greater than 0.

• A partial-loop, limited-sample injection is performed when the injection volume is less than the loop volume and the cut volume is 0.

Injection Type	Injection Volume*	Cut Volume**	Sample Volume Used	Volume Injected
Full-loop	≥ Loop volume	Ignored	4 X Loop volume or 2.5 X Loop volume + 25 µl (whichever is greater)	Loop volume
Partial-loop	< Loop volume	1-30 µl	Injection volume + 2 X cut volume	Injection volume
Partial-loop, limited sample	< Loop volume	0	Injection volume	Injection volume

* Injection volume is entered in the ➢*Sequence*.

**Cut volume is entered in the ➢*Program*.

☞ *Tips:*

If the injection valve is installed externally or if an AS or an AS50 (USB) is in Simultaneous mode, the autosampler can perform full-loop injections only.

For the best accuracy when performing partial-loop injections, specify an injection volume equal to half the loop volume or less. For example, if the loop is 100 μl, use an injection volume of 50 μl or less.

For the best accuracy when performing partial-loop, limited sample injections, specify an injection volume no greater than the loop volume minus the air bubble volume (8 μl). For example, if the loop is 25 μl, use an injection volume of 17 μl or less. Note: An air bubble is drawn before and after the sample when it is loaded into the loop.

Injection Volume *(Volume)*

The $\Rightarrow$*Sample Variable* **Inj. Vol.** is in micro liters (μl). In automatic operation, the installed $\triangleright$*Device Driver* converts this value into a volume readable by the $\triangleright$*Autosampler*, and then the value is sent to the autosampler.

By entering different injection volumes, a $\triangleright$*Dilution Series* can be created in case of a multiple-point calibration (see $\triangleright$*Single-Point and Multiple-Point Calibration*).

Also, refer to $\Rightarrow$*Inj. Vol.* (injection volume) and $\Rightarrow$*Volume* (command).

Installation Qualification (IQ)

As defined in cooperation with EURACHEM, Installation Qualification (IQ) is "the process of installing an instrument up to and including its response to the initial start of operation" [P. Bedson and M. Sargent, Accred. Qual. Assur. (1996) 1, 265-274].

An important task of Installation Qualification is to perform all formal checks necessary to ensure that the instrument and its individual components are supplied as ordered; i.e., according to the specification agreed on by the customer and the manufacturer. In addition, IQ checks that the instrument is correctly installed in the selected environment. Finally, IQ must be formally documented.

Installation Qualification is performed in accordance with this definition and the Dionex IQ manual.

To start Installation Qualification for your chromatography system, select **Instruments IQ** on the **Qualification** menu.

To start Installation Qualification for Chromeleon, select **Chromeleon IQ** on the **Qualification** menu, and then click **Check Installation**. Chromeleon IQ checks the system information and Chromeleon status, as well as all installed files. Examine the report for possible installation errors.

🔖 *Note:*

Warnings may appear stating that higher versions of the system files and the ODBC files already existed before Chromeleon was installed.

Select **Save**, **Save as**, or **Print** on the **File** menu to formally document Chromeleon IQ from the indicated report (**IQReport.log - Editor** dialog box).

For more information about Installation Qualification, refer to **Validation and Qualification** 🔲 **Chromeleon Installation Qualification** in the **Administrator Help** section.

☞ *Tip:*

Dionex ➤*AutoQ includes three qualification procedures:* ➤*Operational Qualification,* ➤*Performance Qualification, and Installation Qualification.*

Institutes and Institutions for Industry Standards

Among others, the following institutes and institutions set and control industry standards:

AOAC INTERNATIONAL. The Association of Official Agricultural Chemists is committed to be a proactive, worldwide provider and facilitator in the development, use, and harmonization of validated methods and laboratory quality assurance programs and services.

ASTM. The American Society for Testing and Materials sets technical standards and rules for the industry worldwide.

CEN. The Commité Européen de Normalisation improves the technical harmonization in Europe by setting standards and technical specifications.

DIN. The Deutsche Institut für Normierung e.V. is internationally recognized as normative body.

EMEA. The European Agency for the Evaluation of Medicinal Products is the European counterpart of the American FDA. Especially in Europe, the EMEA enforces standards for the pharmaceutical industry.

EURACHEM. Eurachem has been founded as a network of European organizations. Their aim is to create a system for the international examination of chemical measurements and to promote GLP.

FDA. The Food and Drug Administration is an agency within the U.S. Department of Health and Human Services. The FDA enforces regulations and product standards in the US and worldwide to ensure higher safety of the products.

ISO. The International Organization for Standardization contains 130 national standardization institutes. Their task is to develop standards for the safer and more efficient development, manufacturing, and sales of products and services.

NIST. The National Institute of Standards and Technology is an administrative body of the U.S. Department of Commerce. Together with the industry, the NIST develops new technologies and sets technological standards in the U.S.

Integrated Amperometry Mode

In addition to the ➢*DC Amperometry Mode*, Dionex electrochemical detectors can be operated in integrated amperometry mode. Integrated amperometry is similar to DC amperometry in that electroactive analytes are oxidized or reduced at the surface of an electrode. However, instead of a constant potential being applied, a series of potential changes is repeated over time. By repeatedly pulsing between high positive and negative potentials, the electrode surface is continually cleaned and regenerated. Current is measured by integration during a portion of the repeating potential vs. time ➢*Waveform*.

In integrated amperometric detection (also known as IA or IPAD), current is integrated at two or more potentials. Pulsed amperometric detection (also known as PAD) is similar to integrated amperometry. However, in PAD, current is integrated at a single potential.

When the Dionex ED and ED40/50/50A electrochemical detectors are operated in integrated amperometry mode, they can perform either IPAD or PAD. The applied waveform determines which technique is used.

Example IPAD Waveform

In the example integrated amperometry waveform shown below, the current is integrated at two potentials: first, while the potential is swept across the metal oxide formation wave, and then again during the reverse sweep across the oxide reduction wave. Without the presence of analyte molecules, the net charge is around zero. Positive and negative cleaning pulses are added to the waveform following the ➤*Integration Interval*.
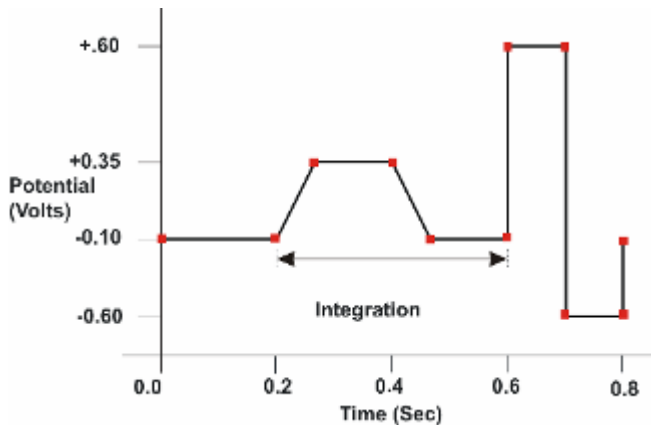
Example PAD Waveform

During a PAD waveform, the current is integrated at only one potential as shown in the example waveform below. The potentials, labeled E1, E2, and E3, are applied for durations t1, t2, and t3, respectively. At t1, the E1 potential is applied. After a delay, the signal is measured by integrating the current for a fixed time. At t2 and t3, positive and negative cleaning pulses are added to the waveform.

E1. Analytical Potential
E2. Cleaning Potential
E3. Reconditioning Potential
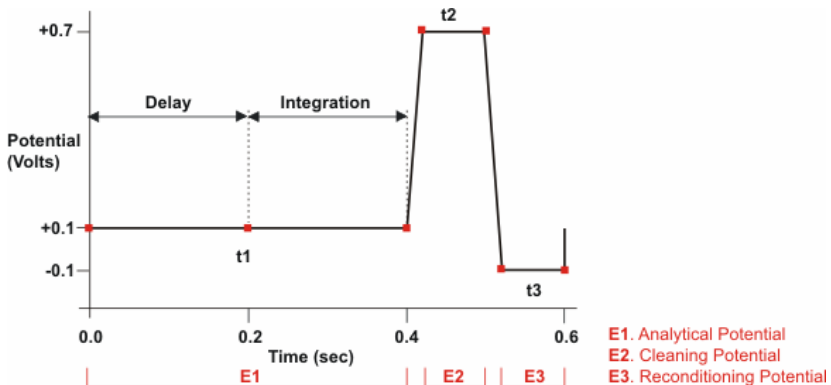
Integration Parameters

In Chromeleon, integration parameters are referred to as **Detection Parameters**. For an overview, refer to ⇒*Detection Parameters*.

Integration Interval

In Integrated Amperometry detection, the integration interval is the time during the ➢*Waveform* in which an integrated data point is recorded. The integration interval is also referred to as sampling time.

Integration Type

The **Integration Type** peak table parameter indicates which peak property is to be used for the calculation of quantitative results.

Also, refer to ⇒*Integration Type*.

Integrator Driver

Chromeleon does not directly process analog signals that are received via a Sharable Device, such as the ➢*UCI Universal Chromatography Interface*. The Integrator Driver must be installed to allow recording these analog signals, for example, from gas chromatographs or pumps, via the channels of, for example, the UCI that assigns the individual channels of the sharable device. (Also, refer to ➢*Device Driver*.)

The advantage is that you do not have to rewrite all ➢*PGM Files* concerned if you plan to connect a device to a different channel. Instead, only change the A/D port assignment in the integrator driver. This also allows you to distribute the channels of a ➢*Sharable Device* to several ➢*Timebases*, thus, eliminating the need to have, for example, one individual UCI for each timebase.

The integrator driver allows you, for example, to record the pump pressure in order to check whether signal variations in the chromatogram are caused by pump pressure variations. Also, refer to **Practical Tips for Device Control** 🔳 **Recording the Pump Pressure**.

Intercept

See ➢*Offset (c0)*

Internal Standard ("ISTD")

An internal standard is a substance of which a known amount is added both to standard and unknown samples. The internal standard should have a similar retention time behavior as the analyzed substances, but it should be easily separable from them. Internal standards are used especially in gas chromatography but also in HPLC and IC to eliminate possible sample preparation errors.

As a rule, the amount of injected internal standard is constant; that is, all samples receive precisely the required amount of internal standard so that identical amounts reach the column when injecting the same volumes. In the case of a ➢*Dilution Series*, the internal standard must be pipetted <u>after</u> the dilution. Therefore, the dilution cannot be simulated by variation of the injection volume but must be actually performed.

 Note:

*With an appropriate ➢Autosampler, such as the Dionex ASI-100, pipetting can be performed automatically. The quantitative accuracy thus reached is one level above the **External** standard method. The methods with internal standard also compensate minor injection inaccuracies, as the internal standard is equally affected by a possible loss or excess.*

Substances serving as an internal standard are labeled as such in the **Standard** column of the peak table. Depending on the type of internal calibration, they are labeled **ISTD Int/Ext** or **ISTD Internal**.

Internal/External Principle

All substances contained in a sample are calibrated as usual with known standard substances (including the internal standard). As the same amount of ISTD is contained in all samples of a sequence, a uniform value for the ISTD should result. If this is not the case, this is an indication for an error during sample processing (if the same amount of the internal standard is actually contained in all samples). The results of the remaining substances of this sample can be relativized afterward.

Internal Principle

Instead of absolute areas and amounts, calculation is based on area and amount ratios. Calculation of all substance amounts is relative to the internal standard. By forming the ratio, inaccuracies in sample processing (from adding the ISTD) can be eliminated (**const. Internal standard**).

Due to the high experimental expenses, this type of calibration is rarely used (the internal standard must be added with a pipette to each sample, in the case of a dilution series this must be after the dilution).

Exceptions (= Variable Internal Standard)

The method itself or the properties of the internal standard make it impossible to add the same amount of ISTD to all samples. To solve this problem, Chromeleon supports the **Use sample amount as reference (Variable)** option (see F8 box of the **Standard** column in the peak table).

In this case (irrelevant whether **Internal/External** or **Internal**), the amount of the internal standard is entered in the $\Rightarrow$*ISTD Amount* column of the sample list, not in the $\Rightarrow$*Amount* column of the peak table. In this way, the amount of internal standard can be entered separately for each sample.

Also, refer to $\Rightarrow$*Standard*

IQ

See $\succ$*Installation Qualification (IQ)*

ISTD

See $\succ$*Internal Standard ("ISTD")*

Isocratic

A solvent flow of constant composition is called isocratic. It is irrelevant whether it is a pure solvent or a solvent mixture of constant composition.

ISO Plot

See $\succ$*3D Field: Presentation Modes*

I-t Plot

A plot of current (I) vs. waveform time (t) for ➤*3D_Amp* data. To better visualize this plot, imagine that a vertical slice of the 3D data set is taken at retention time (T) and the slice is then laid flat. I-t plots can be viewed in the 3D amperometry, integration, and QNT Editor windows. They can also be included in the printer layout.

Also, refer to:

⬛ **Viewing I-t Plots in the 3D Amperometry Window**

⬛ **Displaying and Using I-t Plots in the Integration Window**

⬛ **The QNT Editor**

⬛ **Preparing the Printout: Inserting a Plot**

Jump to Another Help Topic

As soon as the mouse pointer is pointing to a link term, it becomes a pointing hand. All link terms are displayed in green.

Terms underlined by a dotted line, for example, ➤*F1 Key*, are links to additional information in a pop-up; terms underlined by a single line are links to a new help topic.

Key Code

The range of functions available for the Chromeleon stations is determined during installation by a key code.

The key code is a 12-digit or 24-digit number that makes specific functions and options available. Each Chromeleon station has a ➤*Dongle* or a ➤*PAL Plug-In Board* or receives its license from a ➤*License Server*. A Chromeleon station can be operated only if the serial number coded there matches the key code.

If a user expands the scope of Chromeleon functions by acquiring the corresponding licenses, a new key code is assigned to the user. The extended scope of functions is made available as soon as the new code is entered.

Enter the key code as entered in the **About** box on the Help menu or in the ➤*Server Configuration* program. Valid characters are numbers, letters, spaces, and hyphens. Input is not case-sensitive.

Kovats Index

The Kovats Index serves for achieving a uniform scaling of the ⇒*Retention Time*. Define the Kovats Index on the **Peak Table** tab page of the QNT Editor (see **Data Representation and Reprocessing** 🔊 **The QNT Editor**).

Also, refer to ⇒*Kovats Index*

Lamp

This command turns the lamp of an optical detector on and off. UV/VIS detectors often have a separate lamp for each wavelength range (deuterium/tungsten). To activate both lamps, the command usually has to be entered and performed separately for each lamp.

However, it is also possible to turn on both lamps simultaneously or to turn on the second lamp automatically when the first lamp reaches a certain value.

Also, refer to ⇒*Lamp*

LampAge

After you have installed a new lamp, select the **LampAge** command to reset the lamp age to 0.

Periodically checking the lamp age allows you to determine the quality of the lamp and decide whether to install a new lamp.

Also, refer to ⇒*LampAge*

LampIgnitions

After you have installed a new lamp, select the **LampIgnitions** command to reset the number of ignitions to 0.

Periodically checking the number of lamp ignitions allows you to determine the quality of the lamp and decide whether to install a new lamp.

Also, refer to ⇒*LampIgnitions*

LampIntensity

Select the **LampIntensity** property to check the lamp intensity via the ⇒*Log* command. Periodically checking the value allows you to check the quality of the lamp and decide whether to install a lamp.

Also, refer to ⇒*LampIntensity*.

Layout Mode

For some Chromeleon elements, a special Layout Mode is available. Use the Layout Mode to design these elements according to your requirements. The Layout Mode is available for:

- ➤*Control Panels*

- ➤*Printer Layouts* (printouts)

- Reports (screen tables)

- ➤*History* reports

Control Panel

Select **Layout Mode** on the **Edit** context menu to change from the online mode to the layout mode.

The Layout Mode lets you create new control panels, and modify the appearance and functionality of existing control panels. While the Layout Mode is enabled, active online processes are continued but it is not possible to control the system.

To add a new control to the control panel, click an icon on the ➤*Layout Toolbar*. The layout toolbar allows you to select ➤*Control* (display and control elements). A ➤*Control Frame* surrounds selected controls; they can be modified in size, shape, and function.

For more information about how to modify a control panel, refer to **How to …: Controlling Devices from the Control Panel** 🔺 **Modifying a Control Panel**.

📒 *Note:*

You can only create a new control panel or modify an existing one if you have the corresponding access rights.

Printer Layout

To enable or disable Layout Mode in the Printer Layout, select or clear **Layout Mode** on the **Edit** menu. You can only insert, modify, and/or copy the different Printer Layout elements if the Layout Mode is enabled. If the Layout Mode is enabled, an additional text input field appears in the upper report section. Input is similar to MS Excel.

Report

To enable or disable Layout Mode in the Report, select or clear **Layout Mode** on the **Table** menu. If Layout Mode is enabled, an additional text input appears in the upper report section. Use this field to enter the desired values.

History Report

A special Layout Mode is available in the ➢ *History* report. If **Detail** columns are available, you can enable the Layout Mode on the **Layout** tab page of the **History Report Properties** dialog box. A concise report is displayed in which you can easily define the layout.

Layout Toolbar

The layout toolbar contains icons for all default ➢ *Controls* and display elements of the ➢ *Control Panel* that are available in the ➢ *Layout Mode*. The layout toolbar is only accessible in the Layout Mode.

Click an icon and then left-click on the control panel to place the selected default control on the active control panel. Enable and disable the display of the layout toolbar by the **Toolbars > Layout** on the **View** menu.

The following default controls are available:

▶	Pointer (selecting, moving, etc.)	▦	Gradient Control
⊠	Check Box	🟥	➢*3D Field* Plot
🟥	Color Box	🟥	➢*3D Amp* Plot
123	String Display	⊕	Rack Control (see ➢*Rack*)
▮	Gauge Indicator	ⅲ	➢*Mass Spectrum*
▭	Script Button	🔦	Device Command Button
ⅲ	Online Signal Plot	ⅲ	➢*Trend Plot*
▦	Group Box	🗒	Timebase Command Button
🖳	Slider	▦	Sample List
🔴	Lamp	▼	Drop Down List
⇕	Switch	🖳	Wellness Gauge
A±	Entry Field (Edit Field)	ⅲ	Spectra Plot
▦	Audit Trail		

Left Limit

Peak integration is usually performed automatically. However, it is possible to limit or extend integration on the left, on the right or on both sides. To do so, enter a left and/or a right integration limit in the peak table.

Also, refer to ⟹*Left/Right Limit*

Left Width

If a perpendicular line is dropped to the baseline from the peak maximum, the ➢*Width* of the peak is divided in a left and a right section. The two sections are referred to as **left width** and **right width** and can be expressed as separate peak result variables.

Chromeleon also determines the left and right peak width at 5, 10, and 50% of the peak height. As described in the ➢*Width* topic in the glossary section, the height is an important factor for calculating the peak width. This also applies to the calculation of the left and right peak widths.

The abbreviations for the left and right peak widths are **LW** and **RW**.

How to display the left peak width in the report

- Select the column in the report.

- Select **Column Properties** on the context menu

- Select the **Left Width** variable in the selection box.

- Click **Parameter** to determine the peak height at which to determine the peak width.

License Server

If installations include a large number of computers, a License Server is available to manage the Chromeleon licenses. With this type of installation, it would not make sense to equip all ➢*Client* PCs with ➢*Dongles* or ➢*PAL Plug-In Boards* and to enter the corresponding ➢*Key Codes*.

If no PAL, dongle, or License Server is specified, Chromeleon can be operated in ➢*Evaluation Mode*, only.

For information about how to install the License Server, refer to **Hardware Installation** ⬥ **Installing the License Server** in the **Administrator Help** section.

The **Administrator Help** section provides more information; refer to:

Software Installation and Communication ⬛ **The Software License**

Hardware Installation ⬥ **Entering the Software License**

Limit of Detection

The limit of detection (LOD) is the lowest concentration that is just distinguishable from zero (➤*Blank Run Sample*). One way to calculate this is when the signal-to-noise ratio is 3; that is, the signal height of a peak is 3 times the signal ➤*Noise*. The lower the noise is during analysis, the lower are the detection limits. Unlike the sensitivity, the detection limit depends on the instruments used.

There are three different limits: Limit of Detection (LOD), Limit of Determination (LODn), and Limit of Quantification (LOQ). The LODn is reached when the signal height is 6 times the signal noise. The LOQ is reached when the signal height is 10 times the signal noise.

With a blank reading b <> 0, the three limits are determined as follows considering the standard deviation of the blank value (s):

Limit of Detection (LOD)   = b + 3s

Limit of Determination (LODn)   = b + 6s

Limit of Quantification (LOQ)= b + 10s

It is also possible to determine the limit of detection by means of the confidence range of the calibration curve (also, refer to ➤*Confidence Interval/Confidence Range*).

LIMS

A **Laboratory Information Management System**, in short **LIMS**, receives tasks in electronic from, makes sure that they are processed, and makes the results available in a defined format.

In the chromatography system, this can mean, for example, that the samples to be analyzed are read via bar code, grouped in a sequence, and then processed one after another. The results are saved in a default format, such as ➤*AIA*. Chromeleon generates the sequence if the required information is supplied in a defined format.

Chromeleon reads this data via the defined format of a ➤*Worklist*. However, you can also use the ➤*SDK (Software Development Kit)* to connect Chromeleon with the LIMS.

Linked Objects

Objects are often linked to a file, rather than saved with the file. In Chromeleon, linked objects appear:

In Sequences

You can link a sequence to a ➢ *Report Definition File (RDF)* by specifying a Preferred Report. Select **Properties**… on the context menu of the sequence. Determine the Preferred Report on the **General** tab page in the **Properties of Sequence…** dialog box. The preferred report to which the sequence is linked is used whenever the sequence is opened.

In QNT Files

QNT Files can be linked to the following:

- **Spectra Libraries**: Use the **Spectra Library Screening** tab page in the QNT Editor to specify the spectra library to be used for peak identification.

- **Samples for Blank Run Subtraction**: Select a sample for blank run subtraction on the **General** tab page of the QNT Editor to determine which sample(s) are used for blank run subtraction.

- **Standard calibration samples** from a different sequence: On the **General** tab page in the QNT Editor, select **Fixed** as the ⇒ *Calibration Mode*. To enter the standard (samples), go to the **Calibration** tab page and select **Append Standard** on the context menu.

Backup

When performing a ➢ *Backup*, you can specify whether linked objects are saved by selecting or clearing the **Include linked objects** check box. Dionex recommends saving the linked objects. This ensures that data is available in the target laboratory if data is exchanged between laboratories.

Restore

During a ➤*Restore*, you can select the linked objects to be restored:
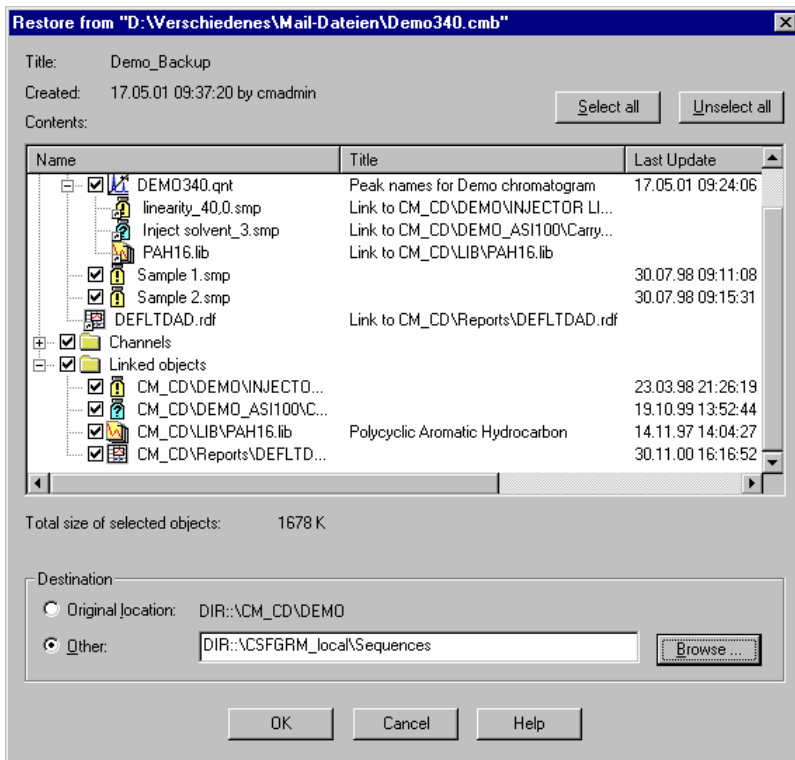
The **Restore** dialog box lists the linked objects under the file to which they are linked. Select the linked objects to be restored.

Lock Baseline

By activating this parameter, it is possible to keep the baseline on the prescribed level.

Also, refer to ⇒*Lock Baseline*

For information about how to apply detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Modifying Detection Parameters**.

Log

Use the **Log** command to document the values of variables in the Audit Trail at any time.

The command is also available directly in the **Commands** dialog box or can be included in the PGM File. To open this dialog box, select **Command…** on the **Control** menu.

Also, refer to ⇒*Log*

Logon

The **Logon** dialog ensures access to the respective program. Access is permitted for authorized users only who are identified via a ➢*Password* in the logon dialog.

In Chromeleon, access to the ➢*User Manager* (CmUser program) is always via the logon dialog. To open a Chromeleon client, you have to logon with a password only if the ➢*User Mode* is enabled.

In the CmUser program, you can enable LDAP logon, also (LDAP = Lightweight Directory Access Protocol). In this case, password authentication is delegated to Windows.

Loop Volume

Loop Volume is the sample loop volume obtained from the value entered on the Plumbing Configuration screen on the AS or AS50 ➢*Autosampler* front panel.

Low Dispersion Injection

Dispersion is one of the reasons for peak tailing. You can reduce dispersion considerably during the injection.

In the middle of the loop, the liquid flow is faster than close to the capillary walls. Thus, the following injection profile appears at the loop outlet:
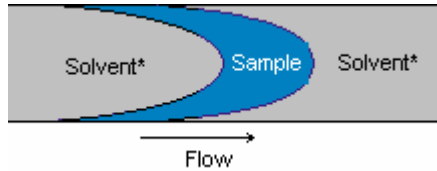
(* Solvent in the example - can be transport liquid or any a similar liquid, instead.)

You can either cut off or inject the tailing part completely, depending on when the injection valve is switched back to the Load position.

For the Dionex FAMOS and WPS-3000 micro autosamplers, Chromeleon supports the **Low Dispersion Factor** (**LD Factor**). This factor allows you to determine when the injection valve is switched back. Thus, the factor determines the amount of the original sample volume that is injected. A value of 1.0 means that the switching valve is switched back when the entire original sample volume was injected. This means that, in the middle of the capillary, the entire sample liquid is injected and that, on the capillary walls, sample liquid is cut-off:
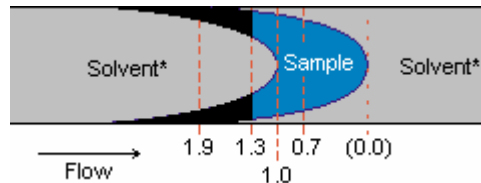
(* Solvent in the example - can be transport liquid or any a similar liquid, instead.)

If the value is greater than 1.0, the injection valve is switched back after the injected volume is greater than the original sample volume. Thus, in the middle of the capillary, not only sample is injected. Simultaneously, the amount of sample that is cut off on the capillary walls is minimized.

If the value is less than 1.0, sample liquid is cut off in the middle of the capillary, too. In this way, a vertical injection profile appears at the end of the sample plug, i.e., the tailing part is completely cut off during the injection. However, this process can avoid neither tailing caused by dead volumes or overloading of the separation column nor chemical tailing during the separation. Nevertheless, with the same conditions, peak tailing is reduced by a factor of 1.2 to 1.4, compared to common injections.

The formula for calculating the time after which the injection valve is switched back to the Inject position is as follows:

$$t_I = 60 * f * V_P / F_V$$

Where:

t_I = time [s] that the valve is in Inject position

f = low dispersion factor

V_P = original sample volume in the loop [µl]

F_V = flow rate [µl/min]

The time that the valve is in Inject position must be at least 10 s. If the calculated switching time is less than this limit, it is reset to 10 s.

Low dispersion injections are possible for full-loop and partial-loop injections as well as in user-defined programs. In Chromeleon, the low dispersion factor can be any value between 0.01 and 100. For example, if the value is 1.3, the valve is switched back when 130 % of the value of the original sample plug has been injected. Meaningful values are usually between 0.7 and 2.0, i.e., between 70 % and 200 % of the original sample volume.

Low-Pressure Gradient System

In low-pressure gradient systems, the same pump delivers all partial flows.

Simple systems generate the required solvent mixing ratio in the mixing chamber. Then, the mixture is sucked from the chamber and carried to the column. The solvent composition is controlled via different valve closing times in the feed lines of the individual components. As the mixing chamber volume represents a dead volume, producing the correct mixing ratio on the column is delayed.

Systems that are more complex mix the individual partial flow during the suction period in the piston. This requires the calculation of how much volume can be sucked. (The value is not constant as delivery is not always against the same external pressure; that is, small volumes are pumped if the external pressure is high, larger volumes are delivered if the external pressure is low. In addition, it is necessary to calculate the time of how long each valve must be opened for the individual partial flows to reach the exact mixing ratio.

Despite these mechanical and electronic procedures, the gradient profile produced by low-pressure gradient systems is less precise than ➢ *High-Pressure Gradient Systems*, however, it is the less expensive alternative.

☞ *Tip:*

Due to solvent compressibility and differing dead volumes, the chromatographic conditions of high-pressure and low-pressure gradient systems are not interchangeable.

Manipulations

Minor changes during the integration of single chromatograms are called **Manipulations**. For information about how to perform manipulations, refer to: **How to …: Working with Chromatograms** 🔳 **Manual Re-Integration**. Select **Save Manipulations** or **Delete Manipulations** on the **Edit** menu to accept or reject these manipulations.

Mark

The **Mark** command sends a positive pulse to the detector's analog output as an event marker. The pulse is 10% of the full-scale analog output. A mark is typically used to indicate a sample injection.

Mass Defect

The mass defect is the difference between the calculated mass and the nominal mass (integer). Combining the masses of all the components of the compound uses calculated mass. The masses of the elements come from the periodic table, with the mass of ^{12}C being exactly 12.0000000 amu. Most of the other elements have masses close to their nominal elemental mass:

^{1}H	1.007825 amu
^{12}C	12.00000 amu
^{13}C	13.00335 amu
^{14}N	14.00307 amu
^{16}O	15.99940 amu
^{19}F	18.99840 amu

You can calculate the mass of a compound as follows:

Formula	Nominal Mass [amu]	Actual Mass [amu]	Defect [amu]
$C_5H_{10}O_5$	5(12) + 10(1) + 5(16) = 150	5(12.00) + 10 (1.008) + 5(15.999) = 150.075	0.075
$C_{50}H_{102}$	50(12) + 102(1) = 702	50(12.00) + 102(1.008) = 702.8	0.8

Therefore, as you get to larger compounds containing more atoms, you will add all the "defects" of the individual atoms together and the difference may be substantial, especially if you display the mass as an integer. In the examples above, the first would be displayed as an integer (150 amu) either way. In the second example, the actual mass in integer format would be 703 amu; that is, a whole mass unit from the nominal mass of 702 amu.

If you have organic compounds containing nitrogens and hydrogens and the only other element is carbon, the result is a positive mass defect. Since many hydrocarbon compounds have a lot of hydrogen, the defect can be very large.

If there is also a substantial amount of oxygen, its negative mass defect can offset the nitrogen and hydrogen and reduce the mass defect. That is why the user sets the type and magnitude of the mass defect depending upon what kind or class of compounds he is working with. The correction for mass defect is generally set as x mmu per 100 amu where mmu is **m**illi**m**ass **u**nit or one thousandth of a mass unit.

If the correction is set to **100 mmu/100 amu** (it is usually set from 30 to 120 mmu/100 amu), the correction for the examples above would be:

Correction Factor [amu]	Corrected Mass [amu]	In Integer Format
150 (0,100/100) = 0.15	150.075 - 0.15 = 149.925	150 amu
702 (0,100/100) = 0.7	702.8 - 0.7 = 702.1	702 amu

Thus, the correction factor "modifies" the data so that people used to working with integer masses for their elements in a compound obtain the expected results.

 Note:

In Chromeleon, atomic masses are always indicated in [m/z] (= mass/charge number).

Mass Spectrometer

Mass spectrometers were originally developed as stand-alone instrumentation to analyze the structure of unknown, mostly organic compounds. When mass spectrometers are used together with chromatographic separation procedures, they allow the qualitative and quantitative analysis of complex mixtures of substances. The combination of GC and MS has long been part of the standard equipment in analytical laboratories. However, the combination of HPLC and MS is not yet common because it is more difficult to realize. Nevertheless, acceptance is steadily growing because

- Decisive enhancements have been achieved during the past years.

- In addition to the ➢*Photodiode Array Detector*, the MS detector, too, allows qualitative statements on individual substances of a chromatogram in HPLC.

Mass spectrometry includes four steps:

- Sample loading (neutral molecules)

- Ionization of individual molecules

- Ion separation according to their masses

- Ion registration in form of a ➢*Mass Spectrum*

Different processes can be used for ionizing and separating ions with different masses. The following image illustrates ionization by means of an electron impact and the subsequent mass separation by means of a vertical magnetic field:
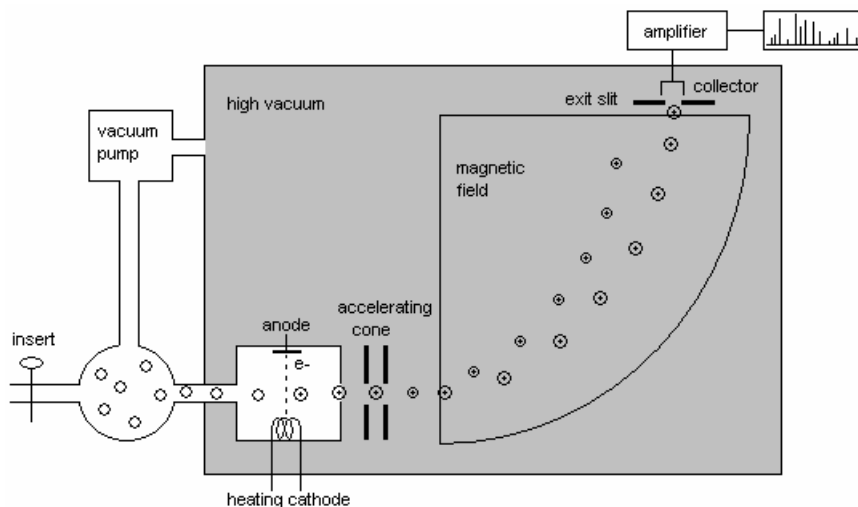
The ions that are created via the electron impact are first accelerated by means of the cones and then separated according to their mass in the magnetic field. Due to the varying intensity of the magnetic field (or the accelerating voltage), ions of different masses are directed through the exit slit into the collector and registered. In this way, mass spectrometers perform a mass scan similar to the wavelength scanning of many photodiode array detectors.

For information about how to record and process MS Data in Chromeleon, refer to **How to …:** **Using Mass Spectrometers**.

Mass Spectrum

Use an MS detector (➢*Mass Spectrometer*) to record a mass spectrum at any time *t* of a chromatogram. This extends the two-dimensional view of a chromatogram (retention time and intensity) by a third dimension (mass). In addition to the information about retention time and intensity, each of the recorded data points includes information about the masses detected in the mass spectrometer.

Mass spectra usually include a great number of narrow peaks or needles. To distinguish them from the comparatively wide chromatogram peaks, they are called mass peaks. A mass spectra representation with many needles is called **Centroid**. A mass spectra representation that combines the single needles into wide peaks is called a **Profile**.

In the report, you can display the mass spectrum of a peak or the mass spectrum at any time of the chromatogram. You can also display the current mass spectrum on the control panel during data acquisition.

In addition, you may aggregate single mass spectra to enhance their ➢*Signal-to-Noise Ratio* (see **How to …: Using Mass Spectrometers** ▣ **Processing Mass Spectra**).

To display recorded mass spectra on the mass spectra plot, click the following icon on the **Method** toolbar:

Mass spectra are generally represented via their relative intensities [%] against the mass. The unit of the mass is m/z (mass/charge number). The base peak, that is, the mass peak with the highest intensity, is the reference point for relative intensities. Thus, its relative intensity is always 100 %.

Erythromycine RT: 0.29-0.36 min
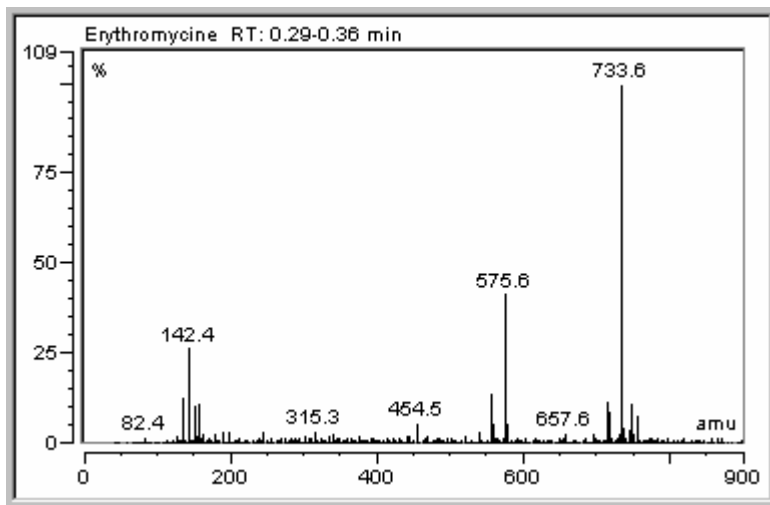
In the above example, the base peak is the mass peak at 733.6 m/z. The time when the mass spectrum was recorded is indicated, also. The mass spectra in the example were aggregated to the displayed total mass spectrum between 0.29 and 0.35 min.

For information about how to record and process MS data, refer to **How to …:** **Using Mass Spectrometers**.

Mass Trace

Contrary to original ➢*TIC* or ➢*SIM* chromatograms that are recorded during data acquisition, a mass trace is an MS chromatogram for a specific mass area. Mass traces can be extracted from a ➢*Mass Spectrum* and saved as a new channel. This is possible online during data acquisition but can be done later as well.

For more information, refer to **How to …: Using Mass Spectrometers**:

 Extracting Mass Traces Online

 Extracting Mass Traces Afterward

The **Administrator Help** section provides additional information; refer to **How to …: Installing and Configuring Mass Spectrometers** **Defining the Number of MS Channels**.

Match Criterion

The match criterion defines the mathematical method of comparison for the curve shape (= standardized spectra).

Also, refer to ⇒*Match Criterion*

Match Factor

The match factor is an indicator of the similarity between two curves. It varies from 0 (=no match) to 1000 (=perfect match).

The match factor is calculated as soon as two spectra are compared with each other in a spectra search (**Library Search** command or on the **Spectra Library Screening** tab page of the QNT Editor (see **Data Representation and Reprocessing** 🔳 **The QNT Editor**).) This command is possible in windows or panes capable of displaying a spectrum. (For more information, refer to **Data Representation and Reprocessing** 🔳 **Spectra Libraries** and 🔳 **PPA: Peak Purity Analysis**.) The displayed spectrum is used as the basis. It is calculated by averaging all spectra recorded for one peak.

The result of the spectra search is the **Hit List**. It lists all spectra that have a certain similarity with the original spectrum. The match value in the **Match** column indicates how much they correspond.

📙 *Note:*

In the method PPA, the match can be indicated as a curve. Please note that instead of determining the similarity of two independent spectra, the spectra of the same peak (at different wavelengths) are compared with each other here (see ➤Peak Purity Match Factor)!

Matrix Blank Sample

The sample matrix can considerably influence sample analysis. To consider this, the standard samples should have the same matrix or at least a similar one. If this is not the case, you can use the ➤*Standard Addition* calibration mode to analyze samples with an interfering matrix. In addition, you can use matrix blank samples to eliminate the influence of the sample matrix in the individual samples.

Select **Matrix** as sample type for the respective blank sample. Matrix blank samples are marked as follows: . For this sample, too, the different peak areas (or peak heights, if these were used for quantification) are determined. Chromeleon automatically subtracts the peak areas (or peak heights) of the matrix blank sample from the corresponding peak areas (peak heights) of all sequence samples. The resulting areas (heights) are then used for all further calculations; for example, for calibration. However, the respective peaks must have been identified for both the unknown sample (or standard sample) and the matrix blank sample.

 Note:

Thus, matrix blank values are treated differently from "normal" ➢Blank Run Samples. For normal blank run samples, the chromatogram is subtracted point by point from the chromatogram of the current sample.

If more than one matrix blank sample was recorded in the sequence, the respective peak areas (peak heights) are averaged and the resulting value is subtracted from the corresponding peak areas (peak heights) of the other samples.

 Tip:

Matrix blank samples are subtracted only if they are evaluated in the same QNT Method. Otherwise, they will not be considered.

Maximum Area Reject

The **Maximum Area Reject** parameter defines the maximum peak area up to which a peak is rejected. Peaks with a peak area below the defined value are not identified.

Also, refer to ⇒*Maximum Area Reject*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Maximum Height Reject

The **Maximum Height Reject** parameter defines the maximum peak height up to which a peak is rejected. Peaks with a peak height below the defined value are not identified. (Determination of the height is always relatively to the baseline.)

Also, refer to ⇒*Maximum Height Reject*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Maximum Peak Height *(MaxHght)*

Positive peak identification is via the ⇒*Minimum Height* parameter. *All* peaks above this height value are identified.

Also, refer to ⇒*Maximum Height Reject*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Maximum Rider Ratio

If one or several peaks (h1 to h4) is above the ➢*Rider Threshold* in a series of non-resolved peaks, Chromeleon determines via the **Maximum Rider Ratio** detection parameter whether it is classified as a peak or a ➢*Rider*.

Tip:

The ⇒Peak Type peak table classification criterion has priority! The criterion indicated here is valid for Peak Type = AUTO only!

Also, refer to ⇒*Maximum Rider Ratio*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Maximum Width

The **Maximum Width** detection parameter defines the maximum width above which peaks are ignored during peak detection.

Also, refer to ⇒*Maximum Width*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** ⬛ **Defining Detection Parameters**.

MCA

If you use an aQa ➢*Mass Spectrometer* for data acquisition, you may acquire the data in MCA (Multi Channel Analysis) mode. This mode mainly serves for calibrating the mass spectrometer. It may also be used for analyzing pure solutions of substances. Usually the solution in question is made available to the mass spectrometer via infusion.

☞ *Tip:*

The ➢MCA mode is not available for the MSQ.

In MCA mode, all ➢*Mass Spectra* of the single scans are added up. Only the resulting total mass spectra (up to four) are saved when the analysis is finished. The advantage is that the files require less storage capacity due to the reduced signal-to-noise ratio.

⚠ *Caution:*

Signal dependence according to time is not recorded in MCA mode. Therefore, a "traditional" chromatogram is not available. Thus, the MCA mode is **not suitable for chromatographic analysis***.*

In MCA mode, the "chromatogram window" looks as follows:
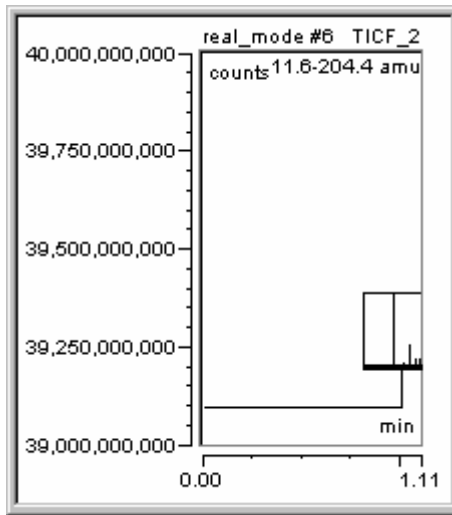
The data acquisition time is represented by a line at the height of the sum of all counts of the single mass spectra that have been added up. If, in addition, the mass spectrum is displayed in the respective window, it can also be displayed as thumbnail in the chromatogram at the end retention time, depending on the respective setting.

☞ *Tip*

MCA data acquisition is not possible in ➢Demo Mode!

For information about using the MCA mode, refer to **How to …: Using Mass Spectrometers** 🔲 **Acquiring MS Data in MCA Mode**.

Message

Use the **Message** command to enter reminders for things to do or to consider when executing a ➢*Program*. The message is then displayed on the screen. The user must confirm the message by clicking **OK** before the program is continued.

Also, refer to ⇒*Message*

Minimum Area

The **Minimum Area** detection parameter is used as a minimum criterion determining the area threshold, below which peaks are ignored during peak detection or integration.

Also, refer to ⟹*Minimum Area*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Modifying Detection Parameters**.

Minimum Height

The **Minimum Height** detection parameter is used as a height threshold, below which peaks are ignored. The peak height is measured relative to the baseline (a).

Also, refer to ⟹*Minimum Height*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Modifying Detection Parameters**.

Minimum Width

The **Minimum Width** detection parameter is used as a minimum criterion, defining the minimum width, below which peaks are ignored during peak detection.

Also, refer to ⟹*Minimum Width*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Modifying Detection Parameters**.

MinLampIntensity

Select the **MinLampIntensity** command to set the lower limit for the lamp intensity.

Also, refer to ⟹*MinLampIntensity*

Modem Cable (1:1 Cable)

Modem cables are frequently used standard cables for connecting instruments to a PC. The cable assignment is as follows (Also, refer to ➢*Pin Assignment*):

Instrument			PC	
9-pin male connector	25-pin male connector		25-pin female connector	9-pin female connector
1	8	------------	8	1
2	3	------------	3	2
3	2	------------	2	3
4	20	------------	20	4
5	7	------------	7	5
6	6	------------	6	6
7	4	------------	4	7
8	5	------------	5	8
9	22	------------	22	9

🗒 *Notes:*

The pin mapping for connectors with identical pin number is 1:1. That is why modem cables are also called 1:1 RS cables.

On the cable with two 25-pin connectors (part no. 8914.0128, see below), the remaining 16 pins are also mapped 1:1.

Dionex part numbers

8914.0143: 9-pin (male) - 9-pin (female) cable (LC Packings part no. **160070**; previous numbers: **ULT-S-COM** and **SW-S-COM**)

8914.0144A: 25-pin (male) - 9-pin (female) cable (5 m long)

8914.0128: 25-pin (male) - 25-pin (male) cable (5 m long)

☞ *Tip:*

Do not confuse non-crossover modem cables (= 1:1 RS cables) with crossover ➢Null Modem Cables!

For information about additional or other connections, refer to the installation instructions for the device. (Also, refer to **Hardware Installation** 🌐 **Installing and Controlling Third-Party Devices** in the **Administrator Help** section.)

Moduleware

Instrument control firmware installed on Dionex modules. Moduleware sends status information and data to Chromeleon and receives control parameters from Chromeleon.

"Monitor Only" Mode

Two users cannot control the same ➢*Timebase* simultaneously. A user who connects to the local server from a control panel can control the instruments connected to a timebase (control mode). However, a second user who accesses the same timebase will automatically be assigned the **monitor only** status. This status means that the second user is allowed to view all current values and states on the control panel, but cannot make any changes (e.g., change the flow rate). A user who accesses a timebase from the ⬛ **Network** is assigned the **monitor only** status, also.

Changing from Monitor Mode to Control Mode

To change between the two modes, select or clear **Monitor Only** on the **Control** menu. If user A takes over control of the timebase from user B, user B is automatically assigned the **monitor only** status.

Organizing Access Options

If ➢*User Mode* is enabled, the assigned privileges determine whether a user can control a timebase or simply monitor the timebase, and whether the user is allowed to change between monitor mode and control mode.

If access control is disabled, each user has all privileges at all times. Objects that are members of an ➢*Access Group* will not be visible.

Independently of this, access can usually be limited directly from the server or the timebase. Specified users can monitor and/or control the server or timebase from the network only if remote monitoring and/or control of a server or a timebase is explicitly allowed in the ➢*Server Configuration* program (on the **Access Control** tab page of the Properties dialog box). This does not apply to users who access their local servers.

Move to Background

Use **Move to Background** to move a selected object to the background.

Move to Front

Use **Move to Front** to move a selected object to the foreground.

Multi-Tasking

Multitasking refers to simultaneous operations of several programs on a PC. Unlike earlier Windows versions (3.1; 3.11), allocation of processing time under Windows 2000/XP is no longer between programs (cooperative), but is monitored and controlled externally (preemptive). This guarantees that each application (process) is automatically continued after a specific time. A single process cannot claim the entire processing time at the expense of other processes.

Multi-Threading

Multi-threading allows simultaneous execution of two or more application parts as separate processes.

For example, if many control commands are communicated simultaneously to a chromatography system, communication requires a certain amount of time. In the past, it was not possible to continue working during this time, regardless of whether the operation took a split second or longer.

Multi-threading allows simultaneous execution of this type of operation (**Threads**). This is possible because each operation is internally separated into many small steps that are executed with a time lag, without being noticed by the user.

Thus, the user can perform other tasks; for example, (s)he can manually re-integrate a chromatogram despite the comprehensive communication of control commands.

Name

The user enters the **Name** for the substance in the peak table. The name is available as peak result variable.

NeedleUp

Use the **NeedleUp** command to lift the sample needle. The internal sample valve is switched. (Also, refer to the example for a load/inject process in the ➢*Autosampler* section.)

Also, refer to ⇒*NeedleUp*

Negative Absorption

Negative absorbance can be the result of a decreasing absorption of the eluent after ➢*Autozero* (which usually occurs at the beginning of a run). At the default setting, the detector will become saturated when the actual transmission is 20% above the autozero level. The extent of negative absorbance is wavelength-dependent. It depends on:

- The spectrum of the lamps (deuterium and tungsten),

- The spectrum and concentration of the different eluent components (present in the cell), and

- The spectrum and concentration of other substances (present in the cell).

Usually, the PDA-100 and PDA-3000 can detect signal values down to about -0.1 AU (default). Use the **NegativeAbsorptionLevel** command to change the default and to enter the level up to which negative values can be detected. If the baseline becomes flat and truncated and shows no noise, increase the **NegativeAbsorptionLevel** setting. (Note that baseline noise may increase as the negative absorbance level increases.)

This increases the available signal range to lower values. This is especially recommended when running a gradient application in which the absorbance of the eluent decreases by several hundred mAU.

Network Failure Protection (NFP)

Often it is useful to store important data on central data server PCs. In case the network connection is interrupted or the central data server PC crashes, data acquisition should continue, of course. For this purpose, all data that are relevant for the Chromeleon server are stored locally on the server's hard disk before the sequence is started. If the network connection is interrupted, the ➢*Server* will nevertheless continue to process these sequences.

Interruption of the network connection is logged in the Audit Trail (see **Data Management** 🔲 **Audit Trails**). The server continues to process the ➢*Batch* using the last conditions. All data are first saved locally.

In some cases, network recovery might fail due to problems of the ODBC or network driver. Restart the Chromeleon server after batch processing is complete. In order to ensure that Windows and all other applications work properly again Dionex recommends rebooting your PC first.

In addition to the network-failure protection, ➢*Power Failure Protection* is available.

🔲☞ *Tip:*

If the network fails while Chromeleon accesses an Access databases, it may happen that the Chromeleon datasource (mdb file) is damaged. Therefore, Dionex recommends using an MS SQL or Oracle server as central database to protect your data even if the network fails.

Also, refer to **How to …: Working with Files, Databases, and Networks** 🔲 **Network Failure/Non-Availability** in the **Administrator Help** section.

Nice Size

Select **Nice Size** to optimize the size of one or more controls. The ➢*Control Frame* is adjusted so that the ➢*Control* including the caption is just visible.

Noise

See ➢*Signal Noise*

Normalization (Overview)

In the linear range, the response (either absorbance or current) recorded at a particular retention time is always proportional to the concentration of the substance currently in the flow cell. An objective comparison of two responses is therefore only possible if the concentration is identical.

As this condition is rarely achieved, and as it is not possible to convert a response recorded at a specific concentration to another concentration, the height of one response must be scaled to the height of the other. This is called normalization.

The following factors must be considered:

- The base area portion must be considered - especially in trace analysis (see ➤*Baseline Correction of Spectra*).

- In UV detection, the noise portion depends on the wavelength and the solvent. Thus, at 200nm, not only is the lamp energy of a deuterium lamp considerably lower than at 250nm, but the absorption of common solvents is also especially high.

- In amperometric detection, the noise intensity depends on the value of the applied potential. The higher the applied potential the higher the noise tends to be.

- Normalization should be performed where little influence on the above factors is expected and where a relative response maximum of the substances to be compared is observed.

Therefore, normalization can be performed by the ➤*Absolute Maximum*, by the ➤*Relative Maximum*, by a fixed ➤*Wavelength* (for UV spectra), or by a ➤*Fixed Time* (for I-t plots).

 Note:

Chromatograms and peaks can also be compared. They are referred to as normalized chromatograms, areas, or amounts.

Normalization (Absolute Maximum)

The absolute maximum of the response (for example, the maximum height of a spectrum in UV detection or the maximum current in an I-t curve) is determined. The response maximum is chosen as a reference point and it receives the absolute height **1** (=100%).

Also, refer to ➤*Normalization (Overview)*

Normalization (Relative Maximum)

The relative maximum of the response is determined. This will be the greatest λ-peak of the spectrum or the greatest current-peak of the I-t plot. After normalization, this peak has the height **0.5** (50%). This prevents normalization with incomplete maxima on spectrum or I-t plot margins.
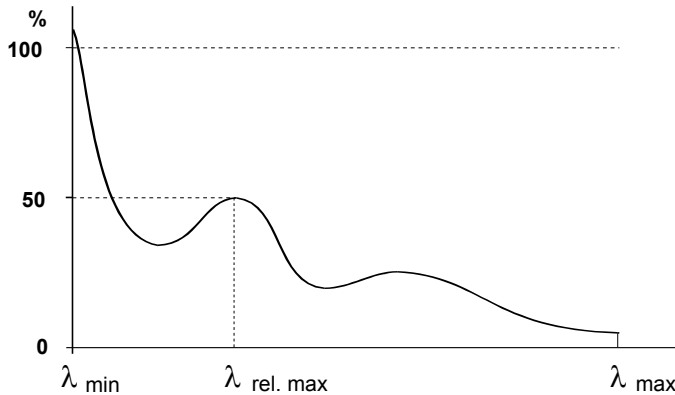
☞ *Tip:*

Note that response maxima can exceed the 100% line on the margin of the plot, due to the definition!

Also, refer to ➤*Normalization (Overview)*

Normalization (Fixed Wavelength)

Normalization with a fixed wavelength is analogous to normalization at a relative maximum.

Instead of the relative maximum, the user can choose the wavelength of the absorption value, which seems suited best for normalization.

☞ *Tips:*

Note that spectrum maxima on the margin of the spectrum can exceed 100%, due to the definition!

If you use the wavelength of the absolute maximum for normalization, it is possible that this wavelength is on the margin of the spectrum. Often, this will be on an edge. On edges, wavelength fluctuations will have stronger effects. Thus, the results may be less precise. This is especially true for detectors that have an optical bandwidth higher than 5nm.

Also, refer to:

➤*Normalization (Overview)*

➤*Normalization (Relative Maximum)*

Normalization (Fixed Time)

Normalization at a fixed time is analogous to normalization at a relative maximum.

Instead of the relative maximum, you can choose the time (in the ➤*Waveform* period) of the response value that seems best suited for normalization.

Also, refer to:

➤*Normalization (Overview)*

➤*Normalization (Relative Maximum)*

Null Modem Cable

Null modem cables are frequently used (crossover) standard cables for connecting instruments to the PC. However, the term "null modem cable" is not defined clearly and precisely, and thus it is used for a number of different cables. The cable assignment of the Dionex null modem cables is as follows: (Also, refer to ➤*Pin Assignment*):

Instrument			PC	
9-pin female connector	25-pin female connector		25-pin female connector	9-pin female connector
2	3	------------	2	3
3	2	------------	3	2
5	7	------------	7	5
			4-5	7-8

Instead of the pseudo handshakes (7-8 and 4-5), the following assignments are realized for the completely connected null modem cable:

1	------------		4
4	------------		1
7	------------		8
8	------------		7
9	------------		9
S	------------		S

■☞ *Tip:*

Do not confuse crossover null modem cables with non-crossover ➤Modem Cables (1:1 RS cables)!

To order null modem cables from Dionex, use the following part numbers:

The **completely connected** null modem cable is available as a 9-pin to 9-pin cable:

- **1310.2263** null modem cable

The following null modem cables with **pseudo handshakes** are available: **9-pin to 9-pin:**

- **8914.0129** for RS cable RU, 2.5m

- **8914.0130** for RS cable RU, 5m

- **8914.0131** for RS cable RU, 10m

25-pin - 9-pin:

- **8914.0103A** for RS cable RU, 2.5m

- **8914.0120** for RS cable RU, 5m

- **8914.0121** for RS cable RU, 10m

If necessary, use an adapter to connect the cable to a 25-pin socket. These null modem cables have been slightly modified to ensure data transmission even with enabled hardware handshake. However, the above hardware handshake is not realized.

For information about additional or other connections, refer to the installation instructions for the device. (Also, refer to **Hardware Installation** **Installing and Controlling Third-Party Devices** in the **Administrator Help** section.)

Number (No.)

The **Number** variable is the successive number assigned to a peak during peak detection. The peak number can be output as peak result variable.

If the values in this column are not successive but contain gaps, the missing peaks fell victim to one of the inhibition criteria; for example, **Relative Area Threshold** or **Report Unidentified Peaks = OFF**.

Number of Calibration Points

The **Number of Calibration Points** calibration variable gives the number of values that are used for the calibration.

The ➤*Number of Disabled Calibration Points* can be displayed separately.

Number of Disabled Calibration Points

The **Number of disabled calibration points** calibration variable indicates the number of values that were not considered in the calibration.

The ➤*Number of Calibration Points* can be displayed separately.

ODBC (Open DataBase Connectivity)

ODBC is the name of a standard database interface that allows access to various databases, such as SQL server, dBASE, or Oracle, using an ODBC driver.

Offset

Signals or chromatograms with an assigned offset value (in %) are offset vertically by this value in percent of the window size. Valid entries are from 0 to 50.

Offset (c0)

The **Offset (c0)** calibration variable returns the offset value of the used ➤*Calibration Function*.

As a y intercept is only available for the **Linear with Offset** (= LOff), **Quadratic with Offset** (= QOff), and **Exponential** (= Exp) functions, no output is possible for the **Linear** (= Lin) and **Quadratic** (= Quad) functions.

Offset Level

The **Offset Level** command (also called zero position) offsets the detector's analog output signal as a percentage of the full-scale voltage. Offsetting the output allows an attached recording device to plot the signal if it becomes negative.

Offset Volume

The ➢*Eluent Generator* **Offset Volume** option specifies the delay volume between the pump outlet and the injection valve. Because the EG40/EG50 is located between the pump and the injection valve, the eluent concentration gradient produced by the EG40/EG50 may reach the column more quickly than the ➢*%-Gradient* produced by the pump. Chromeleon uses the offset volume to calculate a time delay in starting the EG40/EG50. The delay synchronizes EG40/EG50 and gradient pump operation.

The default offset volume of 0 µL is used for most systems. The actual offset volume may be different, however, depending on the system configuration. To change the value, start the ➢*Server Configuration* program and double-click the EG40/EG50 entry in the list of devices for the ➢*Timebase*. Select the **Pump Link & Cartridge** tab page.

The offset volume has no effect during isocratic operation.

Note:

*The **Offset Volume** option is not available for the ICS-2000 or ICS-3000 eluent generator.*

Online Help

The Chromeleon **Online Help** provides on-screen help information in any situation.

- Click the **Help** cursor icon on the toolbar to display **help information about a specific item on the screen**.

- Press the F1 key to display **context-sensitive help information about a specific situation**. A description of the selected menu bar, (tab) dialog box, or the enabled control will appear.

- Select **Index** on the **Help** menu to open the Chromeleon online Help. The Chromeleon online Help provides three tabs: Contents, Index, and Find. The Contents tab page displays the table of contents, listing the different sections and topics. The Index tab allows you to search the alphabetical list of index entries for a specific term of interest. The Find tab allows you search the entire online Help text for specific words and/or phrases.

- Select **Using Help** to open the Windows online Help. The Windows Help provides information about how to use an online Help system.

On-Line SPE-LC (On-Line Solid Phase Extraction Combined with Liquid Chromatography)

On-line **S**olid **P**hase **E**xtraction combined with liquid chromatography (LC) is an automated extraction and analysis process for fluid samples. The analyte is directly enriched and analyzed in the chromatographical system. Whereas conventional HPLC methods require several manual preparatory steps to eliminate the sample matrix, on-line SPE-LC enhances the sample throughput while improving the overall analytical quality.

One of the possible setups for an analytical system includes a small dedicated SPE column and an analytical column, coupled via a 2-position switching valve (either 2-position 6-port or 2-position 10-port valve). The target analytes are selectively extracted from the matrix (integrated sample cleanup) and pre-concentrated. The trapped analytes are transferred in backflush mode to a conventional analytical HPLC column for analyte separation and detection. During this step, the SPE column can be reconditioned
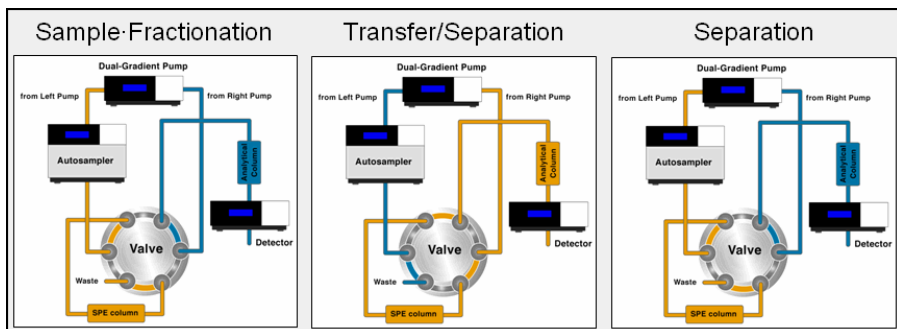
The figure shows a typical setup of an On-line SPE-LC system with a 2-position 6-port switching valve. In the sample fractionation step, the analytes are trapped on an SPE column while the matrix is flushed to waste. In the second step the analytes are transferred to the analytical column. An UltiMate 3000 x2 Dual-Gradient system integrates two pumps in one enclosure and provides a preinstalled thermostatted switching valve.

The application of column-switching for extractive sample clean-up and analysis involves four steps:

1. Matrix depletion and analyte extraction

The sample is loaded with a mobile phase delivered by one of the two pumps via an autosampler onto the SPE column . While the sample matrix is flushed to waste, the analytes are retained on the SPE column.

2. Analyte transfer from the SPE column to the analytical column

After the sample matrix has been eluted from the SPE-column, the valve is switched into a position where the flow direction changes (*Backflush Mode*) and the SPE column is coupled with the analytical LC column. The composition of the mobile phase delivered by the second HPLC pump is chosen in such a way that it desorbs the analyte(s) from the SPE column.

3. Analyte separation

After the transfer of the analyte(s) onto the analytical LC column, the valve is switched back into its original position. Separation of the analyte(s) from each other is achieved on the analytical column in the conventional manner (isocratic or gradient elution mode).

4. Reconditioning of the SPE column and re-equilibration of the analytical column

During the analytical separation, the SPE column can be washed and re-equilibrated with mobile phase delivered by the pump connected to the autosampler. This procedure eliminates carryover and at the same time retains full capacity of the SPE column. The analytical column is re-equilibrated during the analytical run.

Online Transfer Agent (OTA)

The Online Transfer Agent allows the server to transfer sequences after they have been processed. When the Online Transfer Agent is installed on your computer, you can transfer a ➢*Sequence* or a ➢*Batch* to the desired location directly after data acquisition. To do so, select **Transfer** on the **Batch** menu (either on a control panel or in the Browser). On the **Transfer** tab page, you can select the option to delete the sequence or batch at the original position after the transfer.

Tips:

During batch transfer, sequences in the destination directory may be overwritten if they have the same name as the sequences to be transferred. However, it is not possible to overwrite locked data, signed data, or sequences containing raw data. (Example: If you appended samples to a sequence that was already transferred, you have to delete the existing sequence in the destination directory manually before you can transfer the extended sequence.)

Sequences not containing raw data are overwritten; neither a warning appears before this operation nor are the ➢Privileges (e.g., DeleteSEQ) checked.

For installation details, refer to **How to …: Starting and Monitoring the Server, Setting Up the Server for Network Access 🔲 Setting Up the Online Transfer Agent for Network Access** in the **Administrator Help** section.

👉 *Tip:*

*In order to use the Online Transfer Agent, the **Multiple Network Control** license must be available on your PC (see **Chromatography Components: Hardware and Software 🔲 Chromeleon Licenses**).*

📋 *Note:*

If a transfer operation fails, the system attempts the transfer nine times in the following intervals: 10 min, 30 min, 1:10 h, 2:30 h, 5:10 h, and then four times every 6:00 hours.

Operational Qualification (OQ)

According to the definition formulated together with EURACHEM, Operational Qualification is "the process of demonstrating that an instrument will function according to its operational specification in the selected environment." [P. Bedson and M. Sargent, Accred. Qual. Assur. (1996) 1, 265-274]

The purpose of OQ is to prove and document that an analytical system functions according to its operating specification while your specific environmental conditions are taken into account. Operational Qualification is usually performed directly after a new device was installed.

On the **Qualification** menu, Chromeleon provides the **Instruments OQ** and **OQ Setup** commands. Select **Instruments OQ** to perform operational qualification. Select **OQ Setup** to generate the templates required for performing operational qualification. Usually, this is only necessary after initial installation or when the configuration has been modified.

The individual report pages of the **Instrument OQ** correspond to the ➢*Performance Qualification* pages for instruments. However, OQ limits are stricter than PQ limits.

It is also possible to perform Chromeleon Operational Qualification. To do so, select **Chromeleon OQ** on the **Qualification** menu. (For more information, refer to **Validation and Qualification** 🔳 **Chromeleon Operational Qualification (OQ)** in the **Administrator Help** section.)

☞ *Tips:*

*For more information about Instrument Operational Qualification, refer to the **Operational Qualification/Performance Qualification** Operating Instructions that you can obtain from Dionex Service.*

For more information about Chromeleon Operation Qualification, refer to the Chromeleon Operational Qualification Operating Instructions.

Dionex ➤AutoQ includes the three qualification procedures: ➤Installation Qualification, ➤Performance Qualification, and Operational Qualification.

Optical Resolution (Spectral Resolution)

The difference between two detectable wavelengths [in nm] that can only just be separated is referred to as the optical resolution of a photodiode array detector. It is determined by two factors:

- ➤*Photodiode Resolution*

- Quality of grating required for spectral dispersion (**optical bandwidth**)

The values for the optical resolution of conventional detectors are approximately 2 - 6 nm (Dionex UVD 340U Detector: 1.9 nm (UV range), Dionex PDA-100/PDA-3000 Detector: 1.0 nm).

☞ *Tip:*

The optical resolution is only one criterion for the quality of a detector. For a meaningful comparison of different detectors, combine the optical resolution and signal-to-noise ratio. In this connection, it is also important that the ratio between the individual factors is balanced. This correlation becomes clear when you loot at benzene in the 230 - 265 nm range. A fine structure ("benzene finger") is only recognizable if the number of photodiodes, the imaging optics, and the ➤Signal-to-Noise Ratio are in a sensible performance relation to each other.

Optimum Integration Path

A chromatogram that has been recorded at a fixed wavelength usually does not have an optimum ➢*Signal-to-Noise Ratio* for all included peaks. The selected wavelength is often a compromise that allows you to "see" all substances.

It would be ideal to detect, e.g., peak 1 at 230 nm, peak 2 at 254 nm, peak 3 at 320 nm, etc.

This is possible when you perform ➢*Wavelength Switching* (if the wavelength switch points are known) or when you record a ➢*3D Field* using the ➢*Photodiode Array Technology* and subsequently determine the optimum integration path.

Definition

The optimum integration path is characterized by

- An optimized signal-to-noise ratio

- Minimum overlay of neighboring peaks and/or

- Optimum linearity

This means that the integration path must pass through the absorption maximum of a peak, without being influenced by another peak. The recording precision in relative maxima and minima is higher than it is in steep spectra edges (this is especially true for large optical bandwidths of a detector), Dionex recommends selecting the largest *relative absorption maximum* instead of the absolute maximum. In addition, the absolute maximum of UV spectra is frequently located in the low UV range, i.e., near 200 nm. In this range, the detector sensitivity usually decreases, while interferences, such as solvent absorption or humidity, increase.

When dealing with large substance quantities, as in preparative chemistry, total absorption should be below 2 AU. For optimum precision, select a *relative spectra extremum* for recording.

If the complete chromatographic separation of two peaks is not possible, it makes sense to record each channel at a point where the other peak has its absorption minimum, or ideally, is inhibited entirely.

Implementation

Chromeleon automatically calculates the course of the optimum integration path and displays the path in the ➢*3D Field*. The transition between two wavelengths is exactly between the peak end of one peak and the peak start of the other peak. That is why the peak end, peak start, and switch time coincide for neighboring peaks.

You can include the result, i.e. the switch times at which the **Wavelength** signal parameter changes, in a ➢*Program*, using the **Extract: Opt.Int.Path to clipboard** command.

👉 *Tip:*

*Chromeleon ignores rider peaks (⇒**Type (Peak Type)**: Rider) when calculating the optimum integration path.*

For more information, refer to **How to …: Analyzing the Peak Purity** ⬚ **Selecting the Optimum Integration Path**.

OQ

See ➢*Operational Qualification (OQ)*

Origin

See **How to …: Integrating Chromatograms and Identifying Peaks** ⬚ **Selecting the Calibration Function**.

Original Size

See ➢*Full Size*

Overlay

An **Overlay** is a chromatogram that is displayed in addition to the open chromatogram, i.e., overlaying the open chromatogram. Overlays are often used to compare chromatograms. To do so, several chromatograms can be overlaid at several positions in Chromeleon:

Control Panel

You can overlay chromatograms during data acquisition. Open a ➤*Control Panel* and select **Chromatogram Overlay** on the context menu of the online signal plot. In addition to the currently recorded channels, a previously recorded chromatogram is displayed.

QNT Editor and Report

Select **Add Overlay** on the **File** menu in either the **Report** or the QNT Editor (see **Data Representation and Reprocessing** 🔳 **The QNT Editor**) to overlay chromatograms after data acquisition.

Printer Layout

To plot overlaid chromatograms, select **Chromatogram Properties** on the context menu of a chromatogram (in the ➤*Printer Layout*) and make the corresponding settings on the **Overlay** tab page.

In each plot, you can use a processed sample of the current sequence or of any other sequence as the reference chromatogram.

Also, refer to **How to …: Working with Chromatograms** 🔳 **Comparing Chromatograms**.

Overview Window

When zooming, an overview window is displayed in the upper right corner of the signal plot. The overview window displays the entire chromatogram and indicates the position of the enlarged section.

P Groups

See ➢*Privilege*

PAL Plug-In Card

A PAL is a PC plug-in card that is either attached to another card, such as the A/D converter card, or inserted in the PC as a separate card.

The ➢*Dongle* stores the serial number of the Chromeleon workstation, much as the PAL Plug-In card does. Each workstation has a unique serial number.

The serial number of the PAL plug-in card (or dongle) and the ➢*Key Code* stored in Chromeleon must match. If they do not match, Chromeleon cannot operate correctly.

Unless there is a PAL, a dongle, or a ➢*License Server*, and unless the key code is correct, Chromeleon can run in ➢*Evaluation Mode* only.

The **Administrator Help** section provides more information; refer to:

Software Installation and Communication 🔲 **The Software License**

Hardware Installation 🔵 **Entering the Software License**

Panel

See ➢*Control Panel*

Panel Tabset

A panel tabset provides (on one window) a set of Chromeleon ➢*Control Panels* for controlling the individual instruments in a ➢*Timebase* and for performing system-wide functions (for example, creating and running sequences). A panel tabset is designed to function as the main or only interface required for controlling and monitoring a timebase.

Also, refer to **Control** 🔲 **The Panel Tabset**

Partial Flows

See ➢*%B, %C, %D*

Partial-Loop Injections (AS/AS50 Autosampler)

When performing a partial-loop injection, the AS or AS50 autosampler draws the volume to be injected, plus two times the *cut volume* (a volume of sample discarded from each end of the aspirated sample), from the sample vial.

Partial-loop injections are possible under the following conditions:

- The valves are installed in the autosampler or, if external, are installed in one of the following modules: ICS-1000/1500/2000 or ICS-3000 DC.

- The AS or AS50 (USB) is not running in Simultaneous or Sequential mode.

If the above conditions are not met, the autosampler can perform ➢*Full-Loop Injections* only.

Partial-Loop Injection Sequence

1. Draw air

2. Draw sample (injection volume + 2X cut volume)

3. Draw air again to isolate the sample.

4. Draw flush fluid to position sample in loop.

Waste — INJECT
5. Prepare to load sample (flush fluid + air bubble + cut volume #1 is used)

Waste — LOAD
6. Deliver sample (inject volume) to the loop

Waste — INJECT
7. Inject sample onto column. Empty sample syringe (cut volume #2 + air bubble)

KEY
- Flush
- Air
- Eluent
- Sample
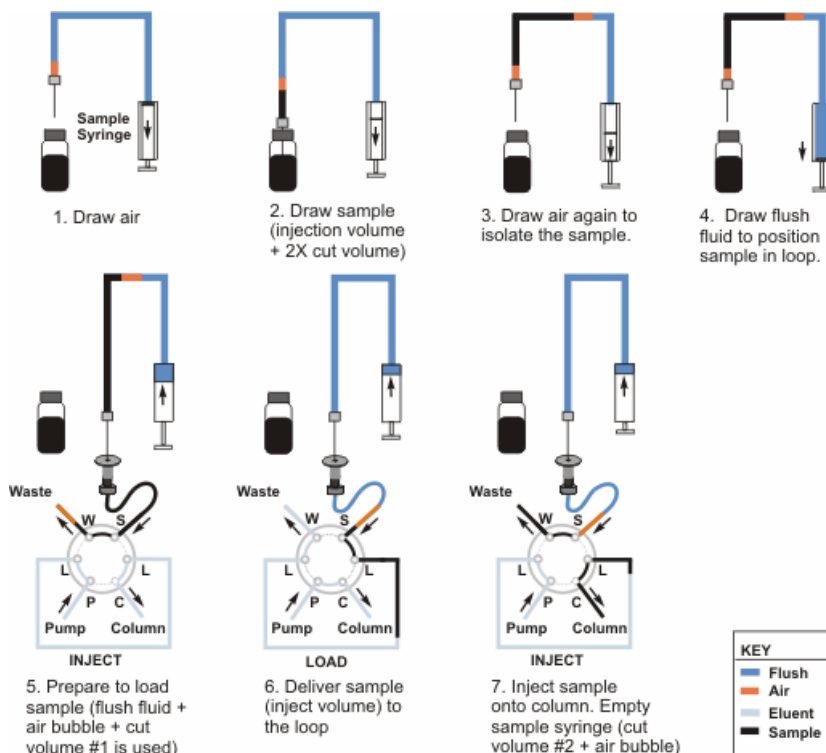
Partial-Loop, Limited-Sample Injections (AS/AS50 Autosampler)

When performing a partial-loop, limited-sample injection, the AS or AS50 autosampler draws only the volume to be injected from the sample vial.

Partial-loop injections are possible under the following conditions:

- The valves are installed in the autosampler or, if external, are installed in one of the following modules: ICS-1000/1500/2000 or ICS-3000 DC.

- The AS or AS50 (USB) is not running in Simultaneous or Sequential mode.

If the above conditions are not met, the autosampler can perform ➢*Full-Loop Injections* only.

Partial-Loop, Limited-Sample Injection Sequence

1. Draw air

2. Draw sample (injection volume)

3. Draw air again to isolate the sample.

4. Draw flush fluid for cleaning inject port.

5. Prepare to load sample (flush fluid + air bubble is used)

6. Deliver sample (inject volume) to the loop

7. Inject sample onto column. Empty sample syringe (air bubble + any excess fluid)

KEY
- Flush
- Air
- Eluent
- Sample
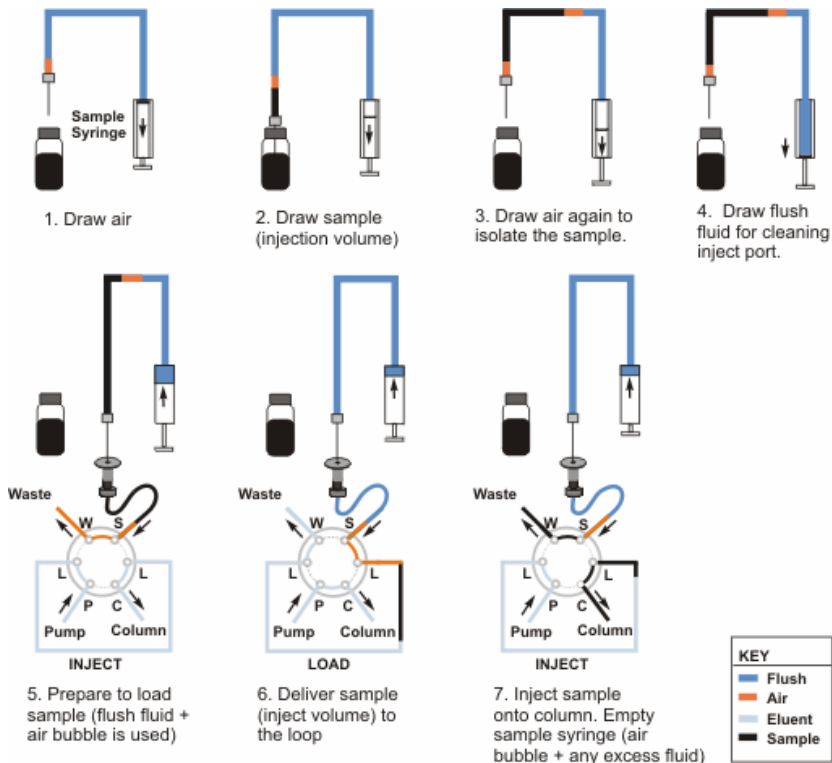
Password

Passwords protect access to specific software or areas thereof. Chromeleon requires passwords in different situations:

A password is required to open the ➢*User Manager* (CmUser program). The administrator assigns the password when s(he) creates the user database.

When the ➢*User Mode* is enabled, a user can open the Chromeleon client only after entering the correct password. The administrator associates each user with an individual password in the User Manager. However, after the user correctly enters the assigned password, s(he) can change the password at any time.

A password is also required to sign sequences electronically (see: ➢*Electronic Signature*). Each user has a password that allows him(her) to submit, review, and approve the signature according to his(her) privileges. This signature password, too, is assigned by the administrator in the User Manager program and can later be changed by the user. Electronic signature is possible only when User Mode is enabled.

Peak Group Start/Peak Group End

Select **Peak Group Start** and **Peak Group End** detection parameters to identify several successive peaks as a peak group. A peak group that has been defined in such a way is treated as one single peak.

Also, refer to ⇒*Peak Group Start/End*

For more information about the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Defining Detection Parameters**.

Peak Name

Enter the name of the substance detected at the time t in the **Peak Name** column of the peak table in the QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor**). The peak name can be indicated as a separate peak variable in the report.

Also, refer to ⇒*Name (Peak Name)*

Peak Number (No.)

See ➢*Number (No.)*

Peak Purity

See the following topics:

➢*PPA (Peak Purity Analysis)*

➢*PPI (Peak Purity Index)*

➢*PPA Report*

Peak Purity End Wavelength (PPWlEnd)

In combination with the **Peak Purity Start Wavelength** detection parameter, **Peak Purity End Wavelength** limits the wavelength range for peak purity calculations.

Also, refer to ⇒*Peak Purity Start/End Wavelength*.

For information about how to apply detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Defining Detection Parameters**.

Peak Purity Match Factor

The Match Factor expresses the similarity of two curves. A peak purity match factor can be expressed for both UV spectra and mass spectra.

The match factor for UV spectra (= UV match factor) refers to the correlation between the spectrum in the peak maximum and the spectra on the leading and trailing edges. Ideally, none of the spectra between peak start and peak end deviates from the spectrum in the peak maximum. They correspond to 100%; that is, the match value is 1000. For ➢*Mass Spectra*, the distance between the single masses and the relative height of the different mass peaks is taken into account. As with UV spectra, a match factor of 1000 indicates a perfect match. However, the MS match factor is far more selective than the UV match factor.

🔖 *Tip:*

The calculation of the match factor becomes more inaccurate on the peak margins. This is due to the poorer ➤Signal-to-Noise Ratio. Select the ⇒Peak Purity Threshold detection parameter to limit the selection of the UV and mass spectra in the QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor***) to a sensible peak height.*

If the match factor of a peak is included in a report column, the value will be averaged from all match values of the peak that have been determined so far. The UV match factor is always baseline-corrected (see ➤*Baseline Correction of Spectra*), as this portion usually cannot be neglected; it would falsify the result.

With mass spectra, define in the QNT Editor whether background mass spectra shall be subtracted. If you enable background subtraction, define which spectra shall be subtracted. This also defines whether the match factor shall be output baseline-corrected. For more information, refer to **How to …: Working with Chromatograms** 🔲 **Subtracting MS Background Spectra**.

In addition, you can include the deviation of the averaged single match values in the ➤*PPA Report* as the ➤*Peak Purity: Relative Standard Deviation of the Match Factor (RSD Match)*.

Peak Purity: Relative Standard Deviation of the Match Factor (RSD Match)

The relative standard deviation of the ➤*Peak Purity Match Factor* indicates its homogeneity over the entire course of the peak.

Calculation is analogous to the ➤*Relative Standard Deviation of the PPI (RSD PPI)*. Instead of the PPI values, the corresponding match values are used. Divide the standard deviation by the average of all match values (Match Ø) and multiply the result by 100%. The result is the relative standard deviation of the match factor (RSD Match) in percent.

$$RSD\,(Match) = \frac{SD}{Match\,\varnothing} * 100\%$$

Peak Purity Start Wavelength (PPWlStart)

In combination with **Peak Purity End Wavelength** detection parameter, **Peak Purity Start Wavelength** limits the wavelength range for peak purity calculations.

Also, refer to ⇒*Peak Purity Start/End Wavelength*.

For information about how to apply detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** ▣ **Defining Detection Parameters**.

Peak Purity Threshold (PPTrshold)

The **Peak Purity Threshold** detection parameter determines the threshold for the signal height above which spectra comparison is performed. The parameter is important for the Peak Purity Analysis (PPA) and the peak ratio.

Peak Purity Analysis (PPA)

For the peak purity analysis, the parameter defines the signal height above the baseline from which UV or ➢*Mass Spectra* shall be extracted for calculating the ➢*PPI (Peak Purity Index)* or the ➢*Peak Purity Match Factor*.

Peak Ratio

For the peak ratio, the parameter defines the signal height above which the ratio of two UV or MS channels shall be calculated.

Also, refer to ⇒*Peak Purity Threshold*

For information about how to apply detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** ▣ **Defining Detection Parameters**.

Peak Shoulder

A peak shoulder (often simply referred to as a shoulder) occurs if a second substance with a (usually) considerably lower concentration is found below a large main peak. Peak shoulders normally do not have a distinct separate relative signal maximum of their own. Unlike peak shoulders, ➢*Rider Peaks* usually have a distinct relative maximum of their own.

Chromeleon distinguishes between peak shoulders and rider peaks when defining retention time. As with main peaks, the peak retention time for rider peaks is defined as the time of the signal maximum. In contrast to this, the peak retention time for peak shoulders is the time with the maximum signal height over the baseline. Below the shoulder, the baseline is often inclined. That is why this time does not necessarily correspond to the time of the signal maximum.

Chromeleon usually skims peak shoulders in an exponential way:
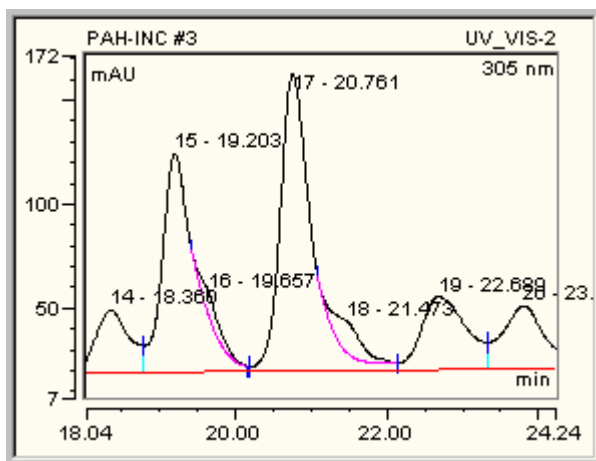
Peak Shoulder Threshold

The **Peak Shoulder Threshold** parameter defines a threshold value for peak shoulder recognition.

Also, refer to ⇒*Peak Shoulder Threshold*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks Defining Detection Parameters**.

Peak Slice

The **Peak Slice** detection parameter determines the width (= space of time) from which several successive data points are interpreted as peak or as noise.

🔲 *Note:*

Always consider this parameter in combination with the ⇒Sensitivity.

Also, refer to ⇒*Peak Slice*

For more information about how to use the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Modifying Detection Parameters**.

Peak Start Time

The **Peak Start Time** peak result variable refers to the peak start in minutes. The left peak delimiter determines the peak start.

Peak Stop Time

The **Peak Stop Time** peak result variable refers to the peak end in minutes. The right peak delimiter determines the peak end.

Peak Summary

A peak summary tabulates the peak and sample variables of any sample(s). The summary usually includes the samples of a sequence. However, you may also create a summary based on a ➢*Query*.

For more information, see **Report Tables** 🔲 **The Peak Summary Report**.

Peak Table

The peak table is part of the QNT Editor (see **Data Representation and Reprocessing** 🔲 **The QNT Editor**). The QNT Editor allows you to determine the parameters for peak identification and peak calibration.

For more information, refer to **The QNT Editor** 🔲 **Peak Table, Amount Table, and Peak Tracking**.

Peak Type

See ➤ *Type*

Peak Width

See ➤ *Width*

Performance Qualification (PQ)

Instrument and software validation is becoming increasingly important to analytical laboratories. Performance Qualification (PQ) is an important aspect of validation. PQ is defined as "the process of demonstrating that an instrument consistently performs according to a specification appropriate for its routine use" [P. Bedson and M. Sargent, Accred. Qual. Assur. (1996) 1, 265-274.]

The task of PQ is both to ensure and to document that your system functions as required by your particular (routine) operation. The frequency and scope of Performance Qualification are determined by the requirements in your laboratory. They can be specified in internal instructions or in an SOP. The parameters should be similar to those for routine operation. In addition to Performance Qualification, ➤ *Operational Qualification* and ➤ *Installation Qualification* are important factors for validating instruments and software. The term ➤ *AutoQ* includes the three qualification procedures.

To perform Performance Qualification for your instruments, select **Instrument PQ** on the **Qualification** menu in the Chromeleon Browser. For more information, refer to **Validation, AutoQ, and System Wellness** 🔲 **Instruments Operational and Performance Qualification**.

PGM File

The PGM File (also called control file) can have several sections. That is why the PGM Editor (see **Chromeleon (Overview)** 🔲 **The PGM Editor**) provides different views:

- The **Commands** view, displaying the actual ➤*Program*

- The **Post-acquisition steps** view, providing extraction and data smoothing steps that are performed after data acquisition

The PGM File and the program can be created easily, using the Program Wizard. (For information about the wizard, refer to **Control** 🔲 **The Program Wizard**.) The user input is automatically converted into uniform command syntax readable by Chromeleon. In this way, even new users can quickly create operable programs.

After creating the program, you may have to enter the ➤*Mass Spectrometer* method and the **Post acquisition steps** on the corresponding pages of the PGM Editor. To edit the commands for device control, use the Device view.

Phosphorescence

The temporally delayed emission of light after excitation with light energy is referred to as phosphorescence. The time delay observed in solutions is between 0.001 and 10 seconds. It is often possible to use a fluorescence detector to measure the phosphorescence (as well as the ➤*Chemiluminescence*). In this case, the emitting light is measured several milliseconds after the excitation flash.

📋 *Note:*

The wavelength of the emitted light is always higher than the excitation wavelength. In HPLC applications, the phosphorescence spectrum is independent of the excitation wavelength.

The Molecular Orbital (MO) theory provides a more exact explanation:

Similar to ➤*Fluorescence*, molecules are excited by the absorption of light. Absorption arises from the ground state, the lowest vibrational level of the lowest singlet state (S0) and terminates in different excited vibrational levels of the next higher singlet state (S1).

$$S_0 + h\nu_A \rightarrow S_1$$

where:

h = Plank's constant

$$\nu_A = \text{light frequence at absorption} = \frac{c}{\lambda_A}$$

where:

c = speed of light

λ = wavelength

The excited vibrational states of S1 transit via a radiationless intersystem crossing into the lowest triplet state (T1). The transition from the triplet state into the ground state is spin prohibited. Therefore, it is temporally delayed and phosphorescent light is emitted:

$$T_1 \rightarrow S_0 + h\nu_P$$
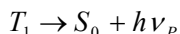
Notes:

Compared to fluorescence spectra, phosphorescence spectra are shifted to the red because the electronic T1 states are energetically below the S1 states.

Due to the fast relaxation in solution the emission spectra of electronically excited molecules in solution is independent of the excitation wavelength (also for excitation of, e.g., S0→S2), according to the Kasha rule.

Photodiode Array Detector

A photodiode array detector is a special multi-channel ➢*UV Detector*. For more information, refer to

➢*Photodiode Array Detectors (Main Features)*

➢*Photodiode Array Detectors (Functionality)*

Highest optical resolution and at the same time high sensitivity are decisive factors for the quality of a photodiode array detector. For more information, refer to ➢*Optical Resolution (Spectral Resolution)*.

◪ Main Features

➢*Photodiode Array Detectors* (PDAs) feature high sensitivity and a large linearity range, they are relatively independent from temperature fluctuations, and can be used for gradient elution (%-gradient). The simultaneous detection of all wavelengths at the time t is made possible by:

- Peak Purity Analysis

- Peak identification via the ➢*Spectra Library*

- Determination of optimum integration path for later wavelength alterations

- Normalization of spectra

- Quantification of non-resolved peaks

The measuring range of the PDA-100 and PDA-3000 detectors is from 190 to 800 nm. The measuring range of the UVD 340S detector is from 200 to 355 nm in the UV range and from 357 to 600 nm in the visible range.

◪ Functionality

Unlike conventional (1 or 2-channel) ➢*UV Detectors*, multi-channel detectors (PDAs) send the entire spectrum of the light source (a), in focused form (b), through the flow cell (c). Thus, absorption not only causes the intensity of a single wavelength to change, but that of all wavelength portions of the entire spectrum. After that, the white light is dispersed spectrally using a grating (d). The resulting spectrum is measured by adjoining photodiodes (e) almost synchronously.
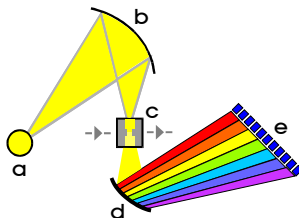
Dionex detectors store this information analog to a UV channel via the signal ➢*3D Field*. Apart from the quality of the optics and the grating, the number and size of the photodiodes determine the ➢*Optical Resolution* of the detector.

☞ *Tip:*

The optical resolution cannot be considered by itself, but must be seen in connection with the achieved signal-to-noise ratio!

Photodiode (Pixel) Resolution

The bandwidth of wavelengths on an individual photodiode is referred to as photodiode or pixel resolution.

If 150 photodiodes are available in the UV range (200 - 350 nm), the photodiode resolution is 150 nm / 150 photodiodes = 1 nm per photodiode.

The ➢*Optical Resolution* of the UV detector is determined by photodiode resolution and the optics (mirror, slit, grating, optical bandwidth).

Pin Assignment (RS-232)

Most instrument connections are made with 9-pin or 25-pin ➢*Modem Cables* or ➢*Null Modem Cables*. The pin assignments are as follows (only the most important pins are mentioned):

25-pin	9-pin	Abbrev.	Description
2	3	TxD	Transmitted data
3	2	RxD	Received data
4	7	RTS	Request to send
5	8	CTS	Clear to send
6	6	DSR	Data set ready
7	5	GND	Signal ground/common return
8	1	DCD	Received line signal detector
20	4	DTR	Data terminal ready

The pin numbering (order for plug) is as follows:
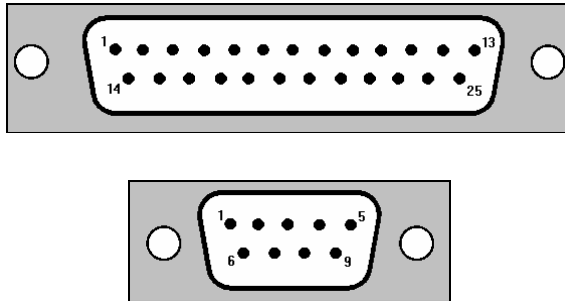
If other connectors are required, refer to **Hardware Installation** 🌐 **Installing and Controlling Third-Party Devices** in the **Administrator Help** section.

Polarity (Analog Output)

The **Polarity** command allows you to change the polarity (+/-) of the analog output signal on a Dionex detector. For example, if components are appearing as negative peaks instead of as positive peaks, use this command to change the analog output polarity from **+** to **-**.

Post-Acquisition Steps

Post-acquisition steps are data reprocessing steps for extracting and smoothing data. They can be defined in the PGM Editor. (For information about the PGM Editor, refer to **Control** 🔳 **The PGM Editor**.) They can be performed online, after data acquisition, or offline of the chromatogram, UV spectrum, or ➢*Mass Spectrum*. The following steps are available for data reprocessing:

- **Arithmetic combination of channels** (arithmetic combination of 2D channels - see 🔳 **Combining Channels via Arithmetic Operations**)

- **Copy Channel** (see 🔳 **Copying a Channel**)

- **Create Fraction Analysis Samples** (see **How to …: Collecting Fractions Automatically (Autopurification)** 🔳 **Creating Fraction-Type Samples**)

- **Create Purification Samples** (see **How to …: Collecting Fractions Automatically (Autopurification)** 🔼 **Creating Preparation-Type Samples**)

  👉 *Tip:*

  The **Create Fraction Analysis Samples** *and* **Create Purification Samples** *options are part of* ➢*Autopurification. They are visible only if the* **Purification** *license is installed and if a connection to the server exists. A connection exists if a PGM File is associated with a timebase, the server on which this timebase resides is running, and the client is connected to this server.*

- **Extract ED channel** (for extracting an ED channel - only possible if ➢*3D_Amp* data is available)

- **Extract MS channel** (for extracting a ➢*Mass Trace* - only possible if MS data is available; see **How to …: Using Mass Spectrometers** 🔼 **Extracting Mass Traces Afterward**)

- **Extract optimum integration path** (for extracting the ➢*Optimum Integration Path* - only possible if a 3D field is available; see **How to …: Creating and Using Spectra Libraries** 🔼 **Selecting the Optimum Integration Path**)

- **Extract UV channel** (for extracting a UV channel - only possible if a 3D field is available; the procedure is similar to selecting the optimum integration path)

- **Smooth data** (for chromatogram ➢*Smoothing* - see **How to …: Working with Chromatograms** 🔼 **Performing Data Smoothing**)

For more information, refer to **How to …: Creating and Modifying Programs** 🔼 **Adding Post-Acquisition Steps**.

Power Failure Protection

When Chromeleon is started, the system recognizes whether online ➤*Batch* processing was interrupted by a power failure.

This is possible because the processing date and time of each sample is entered in the sequence editor. Chromeleon checks whether all "started" samples (labeled with the date and time) have been completely processed, i.e., whether they have reached the status **F** (finished). If the samples are not labeled Finished, the **Power Failure Handling** function allows you to define how to continue processing. Select **Error Handling** on the **Batch** menu and select an option:

- Continue with the interrupted sample

- Continue with the next sample

- Reprocess the entire online batch

- Abort the batch

- Prompt the user what to do

You can also select a ➤*PGM File* that shall be started after booting the server (see **How to …: Creating and Modifying Programs** 🔲 **Creating a Power Failure Program**).

In addition to power failure protection, Chromeleon also provides ➤*Network Failure Protection*.

PPA (Peak Purity Analysis)

Peak purity analysis (PPA) is a pane of the offline module. It consists of a graphical section and the ➤*PPA Report*.

For more information, see **Data Representation and Reprocessing** 🔲 **PPA: Peak Purity Analysis**.

PPA Report

The PPA (Peak Purity Analysis) report represents the numerical addition to the PPA pane. In a table, this report combines variables characteristic for the peak purity analysis and variables freely selectable by the user.

Especially meaningful variables are:

* ➢*PPI (Peak Purity Index)*,

* ➢*Relative Standard Deviation (RSD PPI)*,

* ➢*Peak Purity Match Factor*, and

* ➢*Relative Standard Deviation of the Match Factor (RSD Match)*.

PPI (Peak Purity Index)

The peak purity index (PPI) represents the central wavelength of a spectrum. In simplified terms, this is the wavelength where the areas of the spectrum to the left and right are identical. The PPI is thus independent of the concentration.
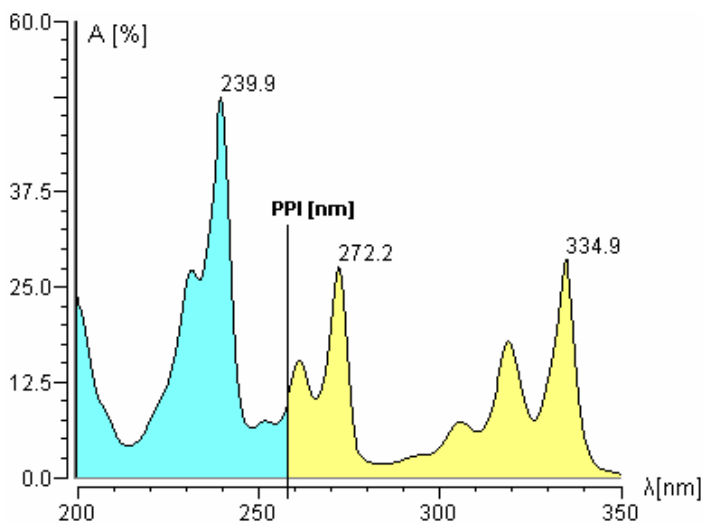
A pure single peak should supply the same PPI value from peak start to peak end for each spectrum recorded in this range.

The following conditions must be fulfilled:

- The baseline portion must be insignificant. If this is not the case, a ➢*Blank Run Subtraction* must be performed.

- During analysis, the solvent composition and thus the UV absorption of the solvent may not change significantly (special attention is required for gradient applications).

- Compared to the signal height, the drift must be very small.

- The Lambert-Beer Law must be valid for the entire range.

In the ideal case of a pure peak, entering individual PPI values results in a rectangle curve. The height of each single rectangle corresponds to the value of the central wavelength. The deviation from the rectangle shape can be expressed mathematically by the relative standard deviation of the PPI value.
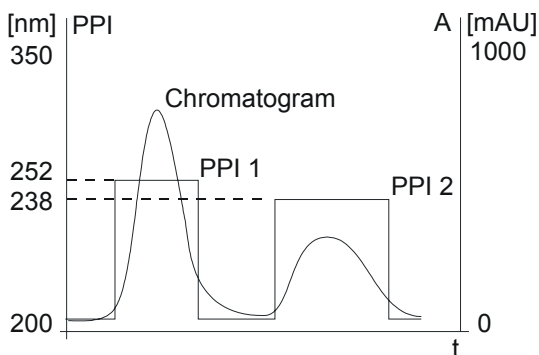
Significant deviations from the rectangle shape indicate peak impurity. However, it is not necessarily correct to deduce from the rectangular shape that the peak is pure.

☞ *Tip:*

*The calculation of the PPI value becomes inaccurate near peak limits due to the poorer ➢Signal-to-Noise Ratio. Using the ⇒Peak Purity Threshold detection parameter, the selection of spectra can be limited to a sensible peak height in the QNT Editor. (For information about the editor, see **Data Representation and Reprocessing** 🔼 **The QNT Editor**).*

If the Peak Purity Index (PPI) is entered in a report column, this value is averaged from all determined PPI values of a peak.

The deviation of single PPI values from each other can be expressed as the ➢*Relative Standard Deviation (RSD PPI)* in the ➢*PPA Report*.

PPI: Relative Standard Deviation of the PPI (RSD PPI)

The relative standard deviation of the PPI expresses the degree of deviation of the computed individual PPI values from each other.

To determine this value, all PPI values of a peak are averaged (PPIØ) and are then squared (Q-Sum).

$$Q - Sum = \sum_{i=1}^{n}(PPI - PPI\varnothing)^2$$

The standard deviation (SD) is defined as the root that is extracted from the Q-sum divided by (n-1), with n being the number of data points.

$$SD = \sqrt{\frac{Q - Sum}{(n-1)}}$$

The relative standard deviation of the PPI (RSD (PPI)) in percent is obtained by dividing the standard deviation by the average of all PPI values (PPIØ), and by multiplying the result by 100%.

$$RSD(PPI) = \frac{SD}{PPI\varnothing} * 100\%$$

PQ

See ➢*Performance Qualification (PQ)*

Preconditions

The device settings before a sample run are referred to as **Preconditions**. The **Preconditions** are saved in the Chromeleon Audit Trail files.

To display them, select **Preconditions only** or **Preconditions and Run** (to display all entries) on the context menu. The selected information will appear in the Daily and Sample Audit Trails after the following icon: 📷. (For more information about Audit Trails, refer to **Data Management 🔖 Audit Trails**.)

The device settings, which are displayed against a light blue background, are listed in the Daily Audit Trail directly after the first message (**Daily audit trail of timebase X**):
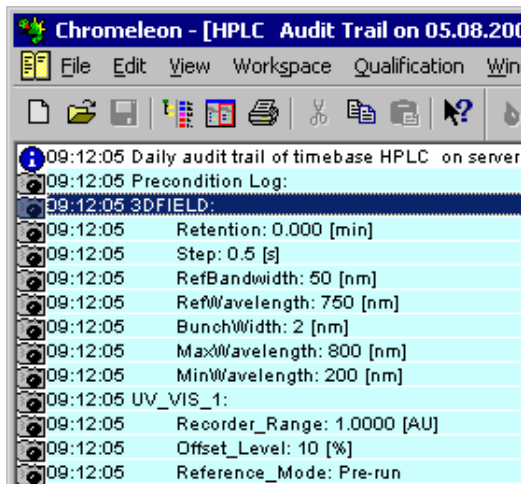
Predictive Performance

The term *Predictive Performance* refers to various functions for estimating the lifetime of replaceable parts, and for monitoring and recording service and qualification information. It helps users to define intervals for regular service and qualification dates and replace parts on a timely basis, as well as to document these measures. Colored counters and gauges on special panels are used as visual indicators of qualification and service periods, making it easy for users to get a quick overview and monitor the device status. Predictive Performance is particularly important in regulated environments, where instruments are subject to special test and qualification requirements.

The following UltiMate 3000 modules support Predictive Performance:

- DGP-3600, ISO-3100A, HPG-3x00, and LPG-3x00 HPLC pumps

- WPS-3000 (T)SL HPLC autosamplers

- TCC-3x00 HPLC thermostatted column compartments

- VWD-3x00 HPLC detectors

- PDA-3000 Photodiode Array Detector

The following Dionex module also supports Predictive Performance:

- PDA-100 (USB) Photodiode Array Detector

Various specific predictive performance commands are available on the panels or in the **Commands** dialog box. For a list of available commands, refer to:

Dionex UltiMate 3000 Series - Pumps

Dionex UltiMate 3000 Series - Autosamplers

Dionex TCC-100/TCC-3x00 Thermostatted Column Compartments

Dionex UltiMate 3000 Series - Variable Wavelength Detectors

Dionex PDA-100/PDA-3000 Photodiode Array Detectors

For more information, also refer to **System Wellness and Predictive Performance for UltiMate 3000 Devices (Overview)**

For details about how to use Predictive Performance features, refer to: How to …: **Validation and Qualification Predictive Performance**

Pressure Limits

Depending on solvent composition and the selected ⇒*Flow*, pressures of up to 450 bar (= 45 MPa = 6525 psi) occur on the high-pressure side of a chromatography system. Chromeleon is capable of monitoring the pressure value provided by the pump. The user can define upper and lower pressure limits. If one of the limits is exceeded, Chromeleon stops the flow, displays an error message, and stops the sample batch, as necessary.

Also, refer to ⇒*Pressure.Lower/Upper.Limits*

PrimeSyringe (ASI-100 Autosampler)

The **PrimeSyringe** command is supported for the ASI-100 autosampler. The command allows removing gas bubbles from the syringe without dismantling the syringe from the instrument. First, the syringe is filled 5 times with washing liquid, usually isopropanol. Then, the procedure is repeated, using the solvent.

For more information, refer to **Practical Tips for Device Control** **Priming the Syringe (ASI-100 Series)**.

Printer Layout

Use the Printer Layout to prepare your data for the printout. Enable ➢*Layout Mode* on the **Edit** menu. When Layout Mode is enabled, you can add the following elements to the Printer Layout:

- Sheets (worksheets that can also include the following elements)
- Report Tables
- Rows
- Columns
- Variables (report variables of the different ⇒*Report Categories*)
- Chromatograms
- Calibration plots (calibration curves)
- Spectra plots (UV spectra)
- Mass spectra plots (➢*Mass Spectra*)
- 3DFIELD plots (➢*3D Fields*)
- 3D amperometry plots
- Cyclic voltammetry plots
- ➢*Trend Plots*
- Charts (graphical display of the values of the individual cells/columns)

The Printer Layout is stored in the ➢*Report Definition File (RDF)* together with the (on-screen) report. The Printer Layout section of the RDF is also referred to as print template. Click the Printer Layout icon ( ) on the Method toolbar to open the Printer Layout of the selected report definition file. The report definition file contains sample data.

For information about how to design a print template, refer to **How to …: 🔧 Preparing the Printout**.

For information about how to print your results, refer to **How to …: Preparing the Printout 🔧 Specifying the Printout**.

Privileges (User Rights)

When the ➢*User Mode* is enabled, the system administrator can assign user rights that determine which actions a certain user group (= Privilege Group) is allowed to perform. The system administrator assigns the access rights in the ➢*User Manager* (CmUser program).

When the User Mode is enabled, the user can execute most actions in the Chromeleon only if (s)he has the corresponding rights (privileges). Chromeleon provides almost 100 different user privileges, such as ReadSequence, CopySample, DeleteSequence, etc. Instead of assigning each privilege separately to each user, privileges are combined in **Privilege Groups** (P Groups). Thus, the user's Privilege Group membership defines his(her) privileges.

Each Privilege Group defines a specific range of operations. These are divided in the specific areas: **CM-Server Control**, **Datasource**, **Sequence**, **Data Reduction**, **Reporting**, and **Miscellaneous**. The scope of functions available for one Privilege Group increases with the number of privileges enabled in the individual areas. (For more information about the user privileges, refer to **Chromeleon User Management ➡ Privileges (Categories)** in the **Administrator Help** section.

Members of a specific Privilege Group can use all privileges assigned to this group. Each user can be a member of one or several Privilege Group(s). The users belonging to a Privilege Group are listed by name.

The more Privilege Groups you create, the more precisely you can define the assignment of privileges in Chromeleon. The privileges of all Privilege Groups to which the user belongs define the privileges of the individual user.

Privileges, such as DeleteSequence, are assigned globally; that is, a specific privilege is granted for all datasources the user can access. To restrict access, the system administrator can establish ➢*Access Groups* in the User Manager in addition to the Privilege Groups.

Program

The control program (or program, for short) is part of the ➢*PGM File* and is displayed in the **Commands** view of the PGM Editor (see **Control** **The PGM Editor**). It can be considered a "schedule" for the execution of different ⟹*Control Commands*. In this file, the user writes all commands to be executed before, during, and after analyzing a sample plus the corresponding execution times. In this way, it is possible to automate the analysis completely, including any routine procedures that are performed before and after the analysis, for instance, column conditioning by rinsing the column with various solvents.

It is also possible to use a program to monitor specific parameters or limits and to trigger specific reactions when these limits are not met.

Follow the instructions in the ➢*Wizard* (also see **Control** **The Program Wizard**) to create the basic structure of the program. Chromeleon automatically converts the user input into uniform command syntax that is readable by the program. In this way, even new users can quickly create new programs.

For more information, refer to:

Control **The Control Program**

 The Program Syntax

Protocol

The **Protocol** command allows you to log specific events in the ➢*Audit Trail* while a ➢*PGM File* is executed.

Unlike simple comments that are only part of the ➢*Program*, the **Protocol** text is included in the Audit Trail, and thus is directly linked to the corresponding sample. Use **Protocol** text to include comments about individual samples.

It is also possible to execute the **Protocol** command event-controlled.

Also, refer to ⟹*Protocol*

Pulse Mode

In addition to the ➢*DC Mode*, some electrochemical detectors can be operated in the **Pulse Mode**. This method is also called PAD (pulsed amperometric detection) and is characterized by a series of potential changes. This method allows a continuous regeneration of the electrode surface (platinum or gold electrode).

📋 *Note:*

Instead of Pulse Mode, Dionex electrochemical detectors are operated in Integrated Amperometry Mode. In this mode, Dionex electrochemical detectors can perform PAD and IPAD (integrated amperometric detection). Refer to ➢Integrated Amperometry Mode for details.

In PAD, a distinction is made between three different potentials (E1, E2, E3) and the period (T1, T2, T3) for which a potential is connected to the electrode. T1 + T2 + T3 form a cycle that is constantly repeated.

T1 = T1´ + Ts
Ts = Measurement time
T1` = Stabilisation (Delay) time
T2 = Cleaning
T3 = Conditioning
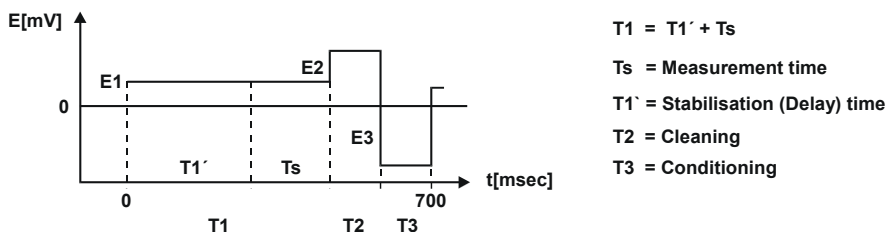
Data is recorded only during the second half of T1 (= Ts). The duration of Ts is either set directly or is determined by defining a delay T1´ (stabilizing phase).

The material of the working electrode largely determines the period and the height of the various potentials.

PWA

PWA, which is the abbreviation for Purification Workflow Automation, is the key feature of ➢*Autopurification*.

The workflow is automatically adapted to your requirements; for example:

- Fractionation is performed only for those samples that contain a sufficient amount of the specified target compound(s).

- Purification is performed only for the compounds of interest.

- Only specified fractions are reanalyzed, using different chromatographic conditions.

Qualification

The purpose of instrument and data system qualification is to check the functionality of the instrument and Chromeleon. Before that, it is the task of the manufacturer to validate the instruments and Chromeleon (see ➢*Validation*). There are several qualification steps:

- ➢*Installation Qualification* (IQ)

- ➢*Operational Qualification* (OQ; qualification in the operating environment)

- ➢*Performance Qualification* (PQ; qualification in routine operation)

Chromeleon allows you to perform the single qualification steps automatically with the ➢*AutoQ* qualification tools.

For more information, refer to **Validation, AutoQ, and System Wellness**:

Validation and Qualification
AutoQ Equipment Qualification

Quantification Method (QNT Method)

A quantification method includes all parameters that are used for the evaluation of a peak or the entire chromatogram. The QNT Method serves as the basis of calculation for evaluating a sample and contains:

- All data for the assignment according to the order of elution within defined limits (time windows) (**identification**) and

- The parameters (calibration constants) that are part of the area/concentration (amount) conversion formulas as well as the calibration points required for the calculation.

Also, refer to **Data Representation and Reprocessing** 🔼 **The QNT Editor**. The **Method** column of the sample list in the Browser contains the name of a quantification method (QNT Method). (For more information, refer to **Data Management** 🔼 **The Browser** and **The QNT Editor** 🔼 **The Quantification Method**.)

Query

Chromeleon allows you to efficiently search, e.g., directories, sequences, samples, or comment lines, for specific names or character strings:

- In the ➤*Browser*, select **Query** on the context menu or select **New > Query (using Wizard)** on the **File** menu. A wizard appears guiding you through entering the desired conditions.

- On the first page, define whether to perform the query in the currently open ➤*Datasource* or in a different one. In addition, define whether to find certain sequence properties and/or sample properties, and/or other result properties.

- After clicking **Finish**, you can enter additional conditions to limit the query.

From the entered information, Chromeleon generates an ➤*SQL* statement that can start a query within all ➤*ODBC*-capable databases.

For more information about performing a query, refer to **How to …: Creating and Managing Files and Data** 🔼 **Performing a Query**.

You can save the result of a query as a new sequence, using the **Save as** command. QNT Files and ➤*PGM Files* are copied; raw data is copied optionally. File names occurring more than once receive a consecutive number. The following symbol indicates the query sequence: 🔲

QNT Editor

The QNT Editor allows you to create and modify methods for evaluating chromatographic results. The ➤*Quantification Methods (QNT Methods)* created in the QNT Editor are used for sample evaluation. The QNT Editor usually provides:

• The chromatogram of the current sample

• The calibration curve of the current peak

• A set of tabs for parameter input

Note:

*Some tabs are displayed by default, others are hidden, e.g., the **UV** tab. To show a hidden tab, choose **Select Sheets** on the **View** menu. In the **Select Sheets** dialog box, select the check box of the tab to show and click **OK**.*

For more information about the QNT Editor, refer to **Data Representation and Reprocessing** **The QNT Editor**. Also, refer to **How to …:** **Integrating Chromatograms and Identifying Peaks**.

QNT Method

See ➤*Quantification Method (QNT Method)*

Rack

Many ➤*Autosamplers* and ➤*Fraction Collectors* provide a choice of racks to meet individual capacity and vial number requirements. For optimum operation, you have to specify the rack installed in the autosampler in the **Server Configuration** program.

If you have a Dionex autosampler, there are two ways to display the rack for the selected sequence and check the ➤*Status* of samples:

From an existing sequence

• Connect the sequence to the corresponding timebase.

• Select **Rack** on the **View** menu.

When creating a sequence

- Select **New** on the **File** menu, and then select **Sequence (using Wizard)** from the list.

- After you have entered the samples in the wizard, click the **Rack Preview** tab.

The Dionex ASI-100 Autosampler rack consists of three color-coded segments. The following segment types are available:

Rack Types	Description
Analytical	Select this option if you use the default segment. This segment can hold up to 39 1.1-ml or 1.8-ml vials plus one 4-ml vial.
Semiprep	Select this option if you use the semipreparative segment. This segment can hold up to 21 4-ml vials.
Mini	Select this option if you wish to analyze a large number of samples, or if all sample volumes are small. This segment can hold up to 64 1.2-ml vials plus one 4-ml vial.
Eppendorf	Select this option if you are using the rack for Eppendorf vials. This segment can hold up to 22 1.5-ml or 2-ml vials plus one 4-ml vial.

If your system includes an autosampler with rack cooling, the temperature can be set to between 4 and 45°C (39.2 and 113°F). For example, the ASI-100T Autosampler allows you to:

- Cool the rack to 18 C below ambient.

- Heat the samples to 35 C above ambient.

Fraction Collector

➤*Trigger Commands* are used during the analysis to define when the fraction collector starts collecting the next fraction. Fraction collection control depends on many factors, and is very complex. For more information, refer to **How to …:** Collecting Fractions.

Ramp

Continually changing the composition of the delivered solvent mixture over the desired time (for ➢*%-Gradients*) or the flow (for ➢*Flow Gradients*) is referred to as a ramp (or more precisely as a ramp gradient). Contrary to this, immediate changes of the solvent composition or flow are referred to as ➢*Step Gradient*.

To realize a ramp gradient, define one value for the beginning and another value for the end. Between these two values, Chromeleon automatically calculates the solvent composition (or flow) at the respective time (two ramps in the example below: 0 - 2 min and 3 - 5 min):

	Retention	Flow	%B	%C	%D
1	0.000	1.000	20.0	0.0	0.0
2	2.000	1.000	40.0	0.0	0.0
3	3.000	1.000	40.0	0.0	0.0
4	5.000	1.000	80.0	0.0	0.0
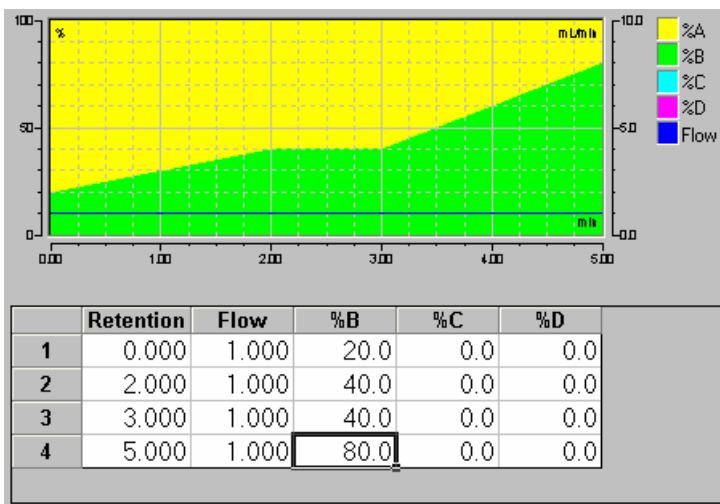
Range

The **Range** parameter determines the factor by which the analog output signal of controllable detectors is amplified or reduced.

📋 *Notes:*

*The **Range** parameter has a different meaning for the RF2000 fluorescence detector. The ➢Gain parameter is used, instead.*

For ➢*Mass Spectrometers*, the range indicates the scaling for the online plot of mass traces. You can enter any value between 1 and 10. The value describes the tenth power of the maximum value that can be displayed. (For example, if Range = 6, the maximum value that can be displayed is 1.000.000 = (1 E+6).)

Raw Data

Data generated by the system are referred to as raw data, whereas data entered by the user are referred to as user data. Raw data, such as:

- Sample data acquired on different channels (see ➢*Signal*)

- 🔲 **Audit Trails**

- Injection time (see ⇒*Inj. Date/Time*)

- ➢*History*

cannot be changed by the user.

In a narrower sense, all analog or digital values measured by a detector and stored digitally on the PC are referred to as raw data. Raw data only exists for those signals or channels that were selected by the user before data acquisition.

The scope and precision of the stored raw data depends on the selected ➢*Sampling Rate* or ⇒*Step*.

For more information, refer to **Data** 🔲 **Raw Data Storage** and 🔲 **Raw Data Compression**.

Ready (Signal)

After each autosampler operation, for example, **Draw** for the ASI-100 autosamplers or **Suck** for the GINA 60/GINA 160 Autosamplers, a ready signal command (**Sampler.Ready** for the ASI-100 Autosamplers or **Sucked** for the GINA 50/GINA 160 Autosamplers) is sent to Chromeleon as confirmation. Other commands, such as **Dispense**, can only be executed after receipt of this signal.

The time interval between the autosampler operation and the response **Sampler.Ready** (or **Sucked**) is the minimum value for the **Duration** command.

To execute two commands successively, ⇒*Wait* is entered after the first command in the ➢*Program*. The next command will then be executed immediately after the arrival of the **Sampler.Ready** (or **Sucked**) signals.

Ready Check

The Ready ➤*Check* command verifies whether it is actually possible to perform automatic batch processing and/or to execute a PGM File. Before a batch is started, the Ready Check automatically checks whether:

- The instruments in the chromatography system are ready to operate, i.e., they are turned on and connected, the detector lamp is burning, etc.

- The amount of solvent is sufficient for the batch.

- All required files exist.

- The storage capacity is sufficient.

If you perform the Ready Check by clicking the **Ready Check** button, information is also provided about, e.g., how long the system can run with the remaining solvent supply at the specified flow rate after the end of the batch. (The Ready Check supports this information only for the Dionex P680 pumps.)

If the Ready Check detects an error (Error or Abort Error), it is not possible to start the batch. If the Ready Check reports a warning, the batch can be started nevertheless. However, Dionex recommends that you take appropriate action to remedy the situation.

As the batch list can be extended by further sequences during operation, the **Ready Check** command can also be performed during operation.

👉 *Tips:*

For program reasons, the system always prompts you before the Ready Check to confirm whether you want to accept previously made changes. You are even prompted for example, when these changes refer to a sequence that is not part of the batch to be started or checked. Decide whether to accept or reject the changes. Cancel the batch start or the Ready Check if you wish to check the changes first.

Reconnect

Use the **Reconnect** command to check the connection between the user PC and the timebase or the instrument and Chromeleon. If there is no connection, the ⇒*Connect* command is performed automatically.

Recorder Calibration

The **Recorder Calibration** command allows calibration of the recorder's response to three detector analog output settings:

AU	Sets the analog output to AU, scaled from 0 to 1 V, based on the AU full-scale response selected by the ➢ *Recorder Range* command.
Zero	Sets the analog output to 0 volts.
Full Scale	Sets the analog output to the full-scale setting (1V).

Recorder Range

The **Recorder Range** command sets the range of a full-scale recorder response in the signal units appropriate for the detector. For example, for conductivity detectors, the Recorder Range is in microSiemens (μS). This command can be used to adjust the recorder output to accommodate larger or smaller peak heights.

The range to use depends on the detector readings expected for the application. For example, selecting a range of 20 microSiemens (μS) will allow you to view conductivity readings of 20 μS or less.

Reference Bandwidth

The reference bandwidth can be selected separately for each channel. The 3D field of a ➢ *Photodiode Array Detector* also has its own reference bandwidth.

Analogous to the conventional ⇒ *Bandwidth* of a channel, the reference bandwidth serves to average several photodiode signals of the ⇒ *RefWavelength (Reference Wavelength)*.

Also, refer to ⇒ *RefBandwidth (Reference Bandwidth)*

Reference Channel

= Reference Channel for Baseline Correction

To perform automatic ➢*Baseline Correction of Spectra* a reference channel is required.

Any channel, either a physical channel or a channel that was extracted from the 3D field and stored afterward can be used. The wavelength of the channel is variable; i.e., a channel recorded using ➢*Wavelength Switching* is also valid.

It depends on how the PPA window has been opened which channel is actually used as reference channel in the PPA method:

* If you open the PPA window from the Browser by selecting the **Open ... 3DFIELD** command, the channel specified in the **File** or context menu under **Properties** is used as reference channel.

* If you first open the sample, and then access the PPA window from a different method, the displayed channel is used as reference channel.

To include the current reference channel in the PPA chromatogram window, select the **Draw Reference Channel** check box, which is provided on the **Chromatogram Plot** tab page of the **Decoration** dialog box.

The reference channel serves as a basis for integration and peak detection.

☞ *Tip:*

Do not confuse the reference channel with the ⇒*RefWavelength (Reference Wavelength) of the signals from a photodiode array detector.*

Reference Peak

Reference peaks are references for calculating relative retention times for different substance peaks. The relative retention time can be indicated as an absolute time difference (in minutes) or as a percentage (retention time substance peak/retention time reference peak). In addition, reference peaks can be used for calculating the ➢*Relative Peak Area*, the ➢*Relative Peak Height*, and the ➢*Resolution*.

On the **Peak Table** tab page in the QNT Editor, you can specify a reference peak for each expected chromatogram peak. Double-click the **Ret. Time** column to open the **Retention Time for** dialog box (see ➢*Retention Time Interpretation*) or the corresponding peaks.

Select either **Time distance to reference peak** or **Time ratio to reference peak** to select a **reference peak** for each substance peak.

The retention time is re-calculated automatically when a different reference peak is selected. Only peaks with absolute retention times can be selected as reference peaks. Reference peaks can be deleted only from the **Retention Time for** dialog box

In addition, the retention time can be entered directly in the entry field in the following format:

<div align="center">[<Reference Peak>] <Ret. Time> [<Unit>]</div>

You are free to choose the order in which the fields are used. If no name is entered for the reference peak, the time is interpreted as an absolute time.

The unit is either **min** (the default) or **%**. The unit determines whether the time given is the difference (**min**) or the ratio (**%**) to the time of the reference peak. For absolute times, only **min** is permitted.

🗒 *Note:*

*Even if the retention time is then specified in **Absolute times**, Chromeleon saves the selected reference peaks.*

Reference Wavelength

For Dionex photodiode array detectors, the reference wavelength is used to correct absorption values of the wavelength(s) selected for analysis. If the absorption of the reference wavelength changes during the analysis, absorption values of the analysis wavelengths are adjusted up or down accordingly. The selected reference wavelength should be in a quiet area of the spectrum where little absorption occurs. Each change in the absorption then indicates substantially changed conditions, for example, a reduction of the lamp energy (lamp drift). Each change can be used to correct the absorption in the remaining wavelength range even during the analysis (the recorded signal is reduced or amplified accordingly, as necessary).

Reference wavelengths are especially useful for gradient analyses, because as the light intensity changes over time (due to the gradient), absorption values are adjusted, thus minimizing baseline drift.

The reference wavelength is freely selectable, not only for each channel of the detector, but also for a 3D field. Because the absorption of the reference wavelength is stored, it is possible to undo a correction later.

The reference wavelength can be changed later by extracting the absorption values of the "new" reference wavelength from the 3D field and using them for correction.

Also, refer to ⇒*RefWavelength (Reference Wavelength)*

Relative Amount

This peak result variable refers to the amount portion of a peak relative to others. The peaks used as a reference are determined via the following options:

Option	Description
All Peaks:	Amount portion relative to the sum of all amount portions of all peaks (default setting).
All Peaks of the same Group	Amount portion relative to the sum of all amount portions in one group.
The corresponding ISTD Peak	Amount portion relative to the amount of the peak of the ➢*Internal Standard*.
The corresponding Reference Peak	Amount portion relative to the amount of a ➢*Reference Peak*.

🗗 *Note:*

The values of options 2 to 4 can considerably exceed 100 %.

Select the respective column in the report, select **Column Properties** on the context menu, and select the variable in the selection box. Click **Parameter** to determine the peak to be used as reference.

Relative Area

The **Relative Area** peak variable is the portion of the peak area in the sum of all peak areas (total area).

Which peak areas form the total area, is determined by selecting one of the following options (The values of all **relative peak areas** always result in 100% for the options 1 to 3.)

Option	Description
All detected Peaks	The areas of all detected peaks are used for the calculation (default setting).
All identified Peaks	Only the areas of identified peaks are used for the calculation.
All Peaks of the same Group	Only the peaks of one group are used for the calculation.
The corresponding ISTD Peak	The area of a peak is put in relation to the area of the ➢*Internal Standard* peak
The corresponding Reference Peak	The area of a peak is put in relation to a ➢*Reference Peak*.

Select a column in the report, select **Column Properties** on the context menu, and select the variable in the selection box. Click **Parameter** to select another option.

Relative Height

This peak variable refers to the peak height relative to the average height of a certain peak group or a specific peak. As with the ➢*Height* variable, the maximum, that is, at the retention time, is measured, relative to the baseline. The dimension does not depend on the detector type.

The selection of the peak heights is possible via the following options:

Option	Description
All detected peaks	The relative peak height is indicated in relation to the average height of all detected peaks (default setting).
All identified peaks	The relative peak height is indicated in relation to the average height of all identified peaks.
All Peaks of the same group	The relative peak height is indicated in relation to the average height of all peaks in one group.
The corresponding ISTD peak	The relative peak height is indicated in relation to the height of the ➢*Internal Standard Peak*.
The corresponding reference peak	The relative peak height is indicated in relation to the height of a selected ➢*Reference Peak*.

Select the column in the report, select **Column Properties** on the context menu, and then select the variable in the selection box. Click **Parameter** to select another option.

Relative Retention Time (Rel. Ret. Time)

The **Relative Retention Time** peak variable of the **Peak Results** category is the quotient of the peak $\Rightarrow$*Retention Time* and the retention time of the corresponding reference peak expressed as a percentage:

Relative retention time = $100 * \dfrac{t_R}{t_{R, ref}}$

Where:

t_R = Retention time (of the current peak)

$t_{R, ref}$ = Retention time of the corresponding reference peak

The $\succ$*Reference Peak* is specified in the peak table of the QNT Editor (see **Data Representation and Reprocessing** 🖪 **The QNT Editor**).

Clicking the **Parameter** button opens the **Parameter input for 'Relative Retention Time'** dialog box. Select an option:

Reference Peak: The relative retention time refers to the reference peak (default)

Fixed Peak: The relative retention time refers to a user-defined peak. In this case, enter the name of the reference peak in the input field. Click the arrow, and then select a peak from the list of identified peaks.

Relative Standard Deviation

The **Relative Standard Deviation** is the standard deviation in relation to the size of the measured values (average); that is, the standard deviation is normalized. In contrast to absolute standard deviations, relative standard deviations can be compared (for values around 1000 a standard deviation of 1 is minor, for values around 10, this is a major deviation). In Chromeleon, the relative standard deviation is expressed in percent.

The relative standard deviation (relStdDev) is calculated as follows from the absolute standard deviation (StdDev):

$$relStdDev = \frac{StdDev}{\overline{Y}}$$

With: $\overline{Y}$: Average value of all Y-values.

Note:

*The standard deviation is calculated differently for calibration curves and for system suitability test and reports. For more information, refer to ➢Standard Deviation (for calibration curves) and **STDEV** (for system suitability tests and reports).*

Relay

Relays are closing contacts that can be switched by Chromeleon. Apart from the physical ("real") relays on the rear panels of various Dionex instruments, there are also virtual relays that can be used only in Chromeleon. Virtual relays are primarily used for defined functions, such as moving a needle. Virtual relays are available if the corresponding instruments or functions are installed.

Before you can use a "real" instrument relay, you must install the relay in the **Server Configuration** program; that is, the relay must be identified. This ID consists of the relay name and the relay number.

Note:

Some device drivers support trigger contacts, which function as two dependent relays. When the first relay is switched on, the second is switched off and vice versa.

Relay-controlled Gradient

Use the **Relay-controlled gradient** to control relay-controlled low-pressure pumps for which a separate device is not available. This requires the relays of a relay card or a ➢UCI Universal Chromatography Interface.

As usual, the solvent composition is defined as a percentage in the ➢PGM File. Each solvent component is controlled by a separate relay. The length of time for which the corresponding relay is open determines how the solvent is mixed.

Use the **Cycle time** parameter on the **Solvents** tab page in the Server Configuration program to specify the cycle duration; that is, the time period from when delivery of the first solvent starts until the delivery of the last solvent is complete. The shorter the cycle is, the more homogeneous the solvent. The longer the cycle is, the more precise the solvent composition.

Relay On/Off

The **Relay On (Off)** command closes (opens) a relay output (contact closure relay) for a defined time. Switching valves are also treated as contact closure relays.

The specified relay is opened or closed for a specified time in seconds. **Relay On** opens the relay upon completing a certain period (➢*Duration*), **Relay Off** closes the relay after completing the duration. If no duration is specified for **Relay On (Off),** the relay remains closed (open) until the next relay command is performed.

Also, refer to ⇒*Relay On/Off*

Remote Inject

The **Remote Inject** driver is required for non-controlled chromatography systems. Typically, non-controlled systems are GC systems or third-party HPLC systems for which a separate driver is not available.

For controlled chromatography systems, the ➢*Autosampler* directly communicates the time of injection to Chromeleon via an RS-232 cable. Since direct communication is not possible for non-controlled systems, the **Remote Inject** driver is used to communicate the time of injection from the autosampler to Chromeleon. The autosampler communicates the inject signal to Chromeleon via a remote input, i.e., typically via the detector. When the signal has been received, for example, data acquisition can be started (⇒*AcqOn/Off* command). For GC systems, the time of injection is communicated in the same way.

For example, select the following setting if the inject output of the autosampler is connected to the input of the UVD:
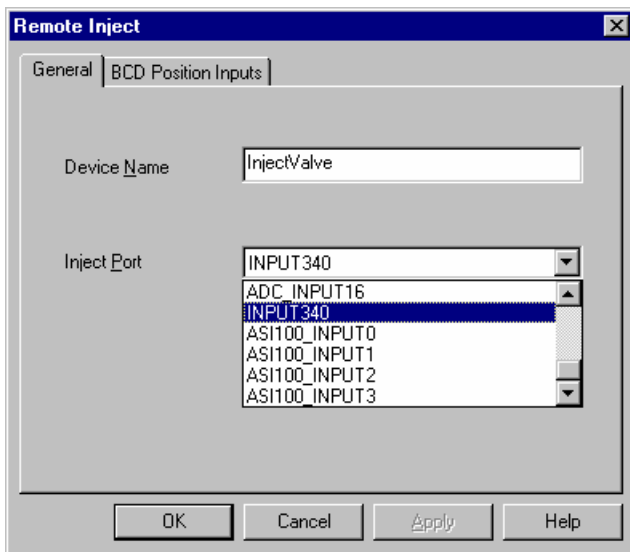
Remote Input

Several Dionex instruments and additional cards provide remote inputs that enable a reaction to external events, such as an injection. The remote inputs are suitable for input voltages between 0V and +5V (TTL level).

As the remote input level is kept at +5V by internal card resistors (pull-up resistance), a simple contact closure relay can be used as an external signal source. This contact must be switched between GND and the respective remote input. If the contact is closed, the digital voltage is grounded. The relay itself is under a low current of approximately 1mA. Opening the contact restores the initial state.

If the signal source itself is active (TTL), the polarization of the remote input must be correct. Ground must be connected to ground, and the digital output must be connected to the remote input.

Installation is for the instrument that supplies the remote inputs. Determine clear installation names and numbers for the required remote inputs.

⚠️ *Caution:*

Do not supply higher voltages (>5V) to the remote inputs, as this may result in a malfunction of the microprocessor system on the A/D card, in the destruction of the input modules, or in further damage.

For an example, refer to ➢*Remote Inject*.

Replicate

In the case of multiple injections from a standard or sample vial, all samples following the first injection are called replicates. The name **Replicate** is used independently of the injected volume (see ⇒*Inj. Vol.* (injection volume).)

Each replicate must be added to the sample list of the sequence as an independent sample! Document the injection from a single vial (identical sample position) in the ⇒*Replicate ID* column by entering the same number and/or the same text. When a new sequence is generated using the Sequence Wizard, the ⇒*Pos.* (sample position) is automatically entered as replicate ID.

Replicates of a standard sample with the same injection volume will produce ➢*Calibration Points* on the same level. Each alteration of the injection volume will generate calibration points of a new level.

Replot from Beginning

Enable **Replot from beginning** to replot automatically when the signal leaves the right-hand border of the signal plot. The active chromatogram is then replotted from the beginning. One unit scales the time axis to fit the chromatogram into the window.

For example, if the first time axis was 2min, after 2min the window is enlarged by 2min to show 4min, after 4min it is enlarged by another 2min to show 6min, etc.

Report Column

See ➢*Sequence Report Column*

Report Definition File (RDF)

Report Definition Files (**RDFs**) have two functions: They display and reprocess your results on the screen (**Report**) and prepare the printout (see ➤*Printer Layout*).

All settings regarding user-specific representation of chromatographic data in different windows are stored in the Report Definition File. Use the report template plus the ➤*Workspace* to arrange the screen according to your requirements.

If no workspace is loaded, each new window is opened based on the most recently used report definition file. If you have not yet stored a report definition file, the default Chromeleon RDF is used. The default report definition file (default.rdf) is located in the **Reports** directory.

Each RDF contains specific information about the window sections and views, window representations and captions, color and size of individual elements, and the presentation of the sample results on-screen and in the printout. The information refers to the following questions:

- Is the window content, e.g., a chromatogram, displayed in zoomed view or in full-scale view?

- Which type of scaling is used for the axes?

- Which additional objects are displayed, e.g., grid, reference chromatogram, units, etc.?

- Which colors are used for the reference chromatograms, captions, etc.?

- Are other window sections displayed, e.g., a spectra plot, calibration curve, report table, etc.?

- Which worksheets are included in the report? How is the report formatted?

- Which columns does the worksheet include?

For more information, see **Data Representation and Reprocessing** **Report Tables (Overview)**.

Report Publisher

The **Report Publisher** is an add-on product to Chromeleon. With its 141 ➢*Additional Functions*, it provides considerable enhancements to the report templates in the ➢*Printer Layout*.

Select **About Chromeleon** on the **Help** menu. The **About Chromeleon** dialog box allows you to check whether this add-on is available on your system.

Report Publisher = On

This setting indicates that the Report Publisher is available on your system. In the **Printer Layout**, select **Layout Mode** on the **Edit** menu to display the edit line. Click the cell and enter the 'equals' sign ('**=**') followed by the required formula. The **Insert/Chart** command is available on the context menu, also. Select this command to insert a graphical representation of specific cell values.

For more information about the available options, refer to **How to …:** 🖻 **Preparing the Printout**.

Report Publisher = Off

This setting indicates that the Report Publisher is not installed on your system. Please contact Dionex Service if you are interested in purchasing the add-on product.

Reserved Relay, Remote Input, and Signal Names

The following names are reserved for special functions:

	Description
3DFIELD	The **3DFIELD** signal is composed of the absorbance values recorded at the time t at different wavelengths using a photodiode array detector. Each absorbance value is entered as a data point in a wavelength by time grid, producing a three-dimensional data field. The number of data points is determined by the optical resolution and the selected bandwidth (limits) of the 3D field.
3D_Amp	The **3D_Amp** signal is reserved for an electrochemical detector in integrated amperometry mode. The signal is composed of the detector response value (current) recorded at time (t) in the ➢*Waveform* period and at time (T) in the run (retention time). Each detector current value is entered as a data point in a waveform time by retention time grid, producing a three-dimensional data field.
INJECT	Refers to the signal that is recognized as the INJECT signal by the system and that defines the start of the chromatogram.

Resolution

The **Resolution** peak result variable is a measure of the separation between two peaks. This resolution helps to evaluate the separation capability of the column. The resolution is determined via the following formulas:

USP standard:

$$R = 2 * \left| \frac{t_{\text{Ref Peak}} - t_R}{BW_{\text{Ref Peak}} + BW_R} \right|$$

EP standard:

$$R = 1.18 * \left| \frac{t_{\text{Ref Peak}} - t_R}{W_{50\%,\text{Ref Peak}} + W_{50\%,R}} \right|$$

➤*Statistical Moments*:

$$R = \left| \frac{t_{\text{Ref Peak}} - t_R}{2 * \left(\sqrt{\mu_{2,\text{Ref Peak}}} + \sqrt{\mu_2} \right)} \right|$$

With:

t_R	= Retention time of the current peak
$t_{\text{Ref Peak}}$	= Retention time of the reference peak for the resolution (By default, the reference peak is the peak after the current peak. However, you can select either the previous peak or a fixed peak as the reference peak.)
$BW_R, BW_{\text{Ref Peak}}$	= ➤*Peak Widths* of the two peaks
$W_{50\%,R}, W_{50\%,\text{Ref Peak}}$	= Widths of the two peaks at 50% of the peak height
	= Second statistical moment of both peaks (variance). For details, refer to **Statistical Moments**.

The distance between the peak maxima is divided by the arithmetic average of the peak widths.

To select the **Resolution** variable:

1. Select the column in the report, and then select **Column Properties** on the context menu.

   This opens the **Properties Report Column** dialog box.

2. Select **Peak Results** from the **Categories** list and **Resolution** from the **Variables** list.

3. Click **Parameter**.

   This opens the **Parameter Input for 'Peak Resolution'** dialog box.

4. Specify the formula Chromeleon will use to compute the resolution: the EP (EU standard) formula, the USP (US standard) formula, or statistical moments.

5. Specify which peak Chromeleon will use as the second peak for the calculation.

6. Click **OK**.

Note:

EP = European Pharmacopeia

USP = United States Pharmacopeia

Response

The **Response** parameter determines the time-related data filtering behavior. It smoothes the signal and improves the ➤*Signal-to-Noise Ratio*. Note that the definition of response varies, depending on the type of detector.

Usually, response is a measure of how long a detector signal requires to rise from a certain value (expressed as a percentage) to a higher value. It is assumed that, in theory, the parameter being measured (e.g., for a UV detector, the absorption) rises immediately from 0% to 100%.
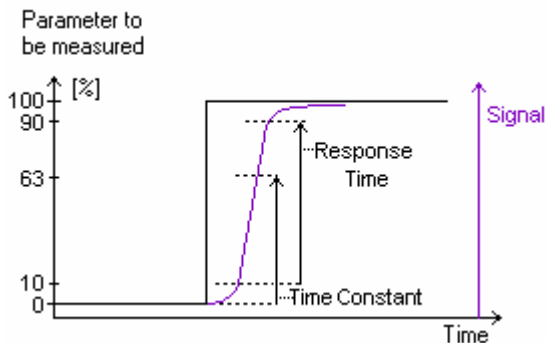
However, for many detectors, the response is a measure of how long the detector signal requires to rise from 0% to 63% of the parameter being measured. This is also referred to as the time constant.

For other detectors, including the UltiMate and UVD-3000 UV detectors, the response is the time that the detector signal requires to rise from 10% to 90% of the parameter being measured. This is also referred to as the response time.

With a longer time interval, the signal-to-noise ratio improves, but the resolution is reduced.

Response Factor

The **Response Factor** peak table parameter is a peak-specific, multiplicative factor without dimension. Its default value is 1.000 and is changed for special applications. The response factor is entered in the corresponding column of the **Amount Table** in the QNT Editor (see **Data Representation and Reprocessing** 🔟 **The QNT Editor**).

Also, refer to ⇒*Response Factor*.

Restore (File)

The **Restore** command allows you to restore files that have previously been stored using the ⮞*Backup* command. For more information, refer to **How to …: Creating and Managing Files and Data** 🔟 **Restoring Backup Files**.

To restore data, select **Import/Restore** on the **File** menu in the Browser.

🔖 *Tip:*

Archive files have the extension *.cmb. To open, double-click the cmb file in the Windows Explorer.

If you have created a backup file for a sequence that contains one or several ⮞Sequence Report Columns, this file cannot be restored with Chromeleon 6.40 or earlier.

Retention Index

The retention index allows you to save uniform scaling of the ⇒*Retention Time*

Also, refer to ⇒*Response Factor*

Retention Time

The retention time is used for peak identification. The **Retention Time** peak variable refers to the elapsed time (in minutes) since the injection (also, refer to ⇒*Dead Time*). The retention time of a peak is defined by the time of the data point with the highest response value (the peak maximum). (This does not have to be the data point with the largest distance to the baseline.)

In Chromeleon, the retention time is especially important at two points:

- In the QNT Editor (see **Data Representation and Reprocessing** 🔳 **The QNT Editor**) as part of the peak table.

- In the Report and ➢*Printer Layout* on the **Integration**, **Peak Analysis**, and **Summary** sheets.

QNT Editor

On the **Peak Table** page in the **QNT Editor**, the retention time can be entered either manually by the user or automatically by the system.

Also, refer to ⇒*Response Factor*

Report

In contrast to the QNT Editor, the actually measured retention times of the corresponding samples are stated in the report. This is the default setting for the **Retention Time** variable of the **Peak Results** category. With this variable, it is also possible to indicate the actual nominal retention time. Then, the settings of ⇒*Use Recently Detected Retention Times* are taken into account.

However, it is also possible to include the expected retention times (from the peak table of the QNT Editor) using the **Peak Table** category. Select the **Retention Time** variable from the **Peak Table** report category. It is not always the nominal retention time that is displayed.

Retention Time Correction

See ⇒*Use Recently Detected Retention Time*

Retention Time Deviation (Ret. Deviation)

The **Retention Time Deviation** peak result variable indicates the deviation of the actual retention time from the nominal ⇒*Retention Time* according to the peak table (in [minutes]).

Retention time deviation = $\qquad t_{R(Soll)} - t_{R(Ist)}$

The retention time deviation can be set via two different methods. The setting can be either

- Absolute, i.e., in minutes, *or*

- Relative, i.e., relative to the retention time.

Select the column in the report, select **Column Properties** on the context menu, and then select the variable in the selection box. Click **Parameter** to determine whether the deviation is expressed as a relative or absolute value.

Retention Time Interpretation

To determine how the corresponding retention time is interpreted, double-click the ⇒*Retention Time* in the peak table of the QNT Editor. (For information about the editor, see **Data Representation and Reprocessing** 🔲 **The QNT Editor**). The **Retention Time for X** dialog box is opened.

- Select **Absolute Times [min]** (default) to interpret the retention time as usual (time interval between injection and peak maximum).

Instead of this, relative time intervals to any peak in the peak table (reference peak) can be used:

- Select **Time distance to Reference peak [min]** to indicate the difference to the ➤*Reference Peak* in minutes.

- Select **Time ratio to Reference peak [%]** to indicate the difference to the reference peak in % values.

- The corresponding reference peak is displayed in the **Reference peak** field. Use the arrow to select a different reference peak.

The reference peak is displayed in the peak table with a light blue background. When the reference peak is in the center of the chromatogram, the difference expressed in minutes or percent can assume negative or positive values, or values below or above 100%.

🖐☞ *Tip:*

For difference and relative retention times also, relative (time) ⇒Windows are interpreted as percentage values of the <u>absolute</u> retention time! Only the retention times of identified peaks are corrected! In the peak table, you can choose between the three options, as required. The retention time column is re-calculated automatically.

Retention Time Spectrum

The retention time of a peak is determined at the peak maximum. The spectrum the photodiode array detector records at this time is referred to as retention spectrum.

Retention Time Window

See ➢*Window*

Retention Window Width

The **Retention Window Width** peak variable provides the width of the ⇒*Retention Window* (in minutes) that was used to detect the peak. This variable is also available for not identified peaks.

RF Value (Amount/Area)

The ascending slope of the calibration curve, specified as amount/area value, is referred to as **RF Value**. This is the reciprocal value of the ➢*Slope* c1.

📝 *Note:*

The RF value is sometimes called the Response Factor. Do not confuse this term with the ➢Response Factor used in the peak table.

Rider Peak

In a series of non-resolved peaks, all peaks - with the exception of the largest peak (reference peak) - are referred to as **rider peaks**. Alternatively, they are called **skimming peaks**.

Depending on the position of the rider peak (on the leading or trailing edge of the reference peak), a distinction is made between rider up and rider down.

Use the ⇒*Rider Threshold* and ⇒*Maximum Rider Ratio* detection parameters to determine the peaks that are classified as rider peaks within a series of non-resolved peaks.

A skimming tangent on the chromatogram plot indicates the peaks that are classified as rider peaks. In the result report, they are labeled **Ru** (**Rider up**) or **Rd** (**Rider down**).

Rider peaks can be skimmed in different ways (see ⇒*Rider Skimming*).

🖐☞ *Tips:*

You can move the baseline in the chromatogram with the mouse for tangentially skimmed riders, but you cannot for exponentially skimmed riders.

***Split Peak** on the context menu allows splitting one rider peak into two peaks.*

Rider peaks are different from ➢*Peak Shoulders* but the transitions can be smooth. Peak shoulders normally do not have a distinct separate relative signal maximum of their own. Unlike peak shoulders, ➢*Rider Peaks* usually do have a distinct relative maximum of their own.

For more information about rider peaks, refer to **How to …: Integrating Chromatograms and Identifying Peaks/Defining Detection Parameters** 🔳 **Defining Rider Peaks**.

Rider Skimming

This parameter indicates how ➢*Rider Peaks* are skimmed.

Also, refer to ⇒*Rider Skimming*

For information about how to apply the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔳 **Modifying Detection Parameters**.

Rider Threshold

The **Rider Threshold** detection parameter determines whether individual peaks in a series of non-resolved peaks are classified as ➤*Rider* or as main peak.

Also, refer to ⇒*Rider Threshold*

For information about how to apply the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** 🔲 **Modifying Detection Parameters**.

Right Mouse Button

The most common commands and functions are available on the context menu. The choice is context-sensitive, i.e., the commands most likely to be used in the current situation are available on the menu. Right-click to open the context menu.

Right Limit

Normally, peak integration is performed automatically. It is possible to limit or extend integration on the left, right or on both sides with the appropriate peak table parameters.

Each (minute) limit is relative to the retention time, that is, the peak is integrated between the retention time - left integration limit .... and .... retention time + right integration limit. The value 0 deactivates the limit.

Example: For the right and left integration limits, the value 0.5min is entered. The peak maximum is at the retention time of 8 minutes. The peak is integrated from 7 minutes 30 seconds to 8 minutes 30 seconds.

Example: For the left integration limit, the value 0.5min is entered. For the right integration limit, the value 0 is entered. As before, the peak maximum is at the retention time of 8 minutes. The peak is integrated from 7 minutes 30 seconds. The end of the integration is then automatically determined.

Right Width

If a perpendicular line is dropped to the baseline from the peak maximum, the ➤*Peak Width* is divided in a left and right section. The two sections are referred to as **left width** and **right width** and can be expressed as separate peak result variables.

Chromeleon also determines the left and right peak width at 5, 10, and 50% of the peak height. As described in **Peak Width** in the Glossary, the selected height is very important for the calculation of the peak width. This also applies to the calculation of the left and right peak widths.

The abbreviations for the left and right peak widths are **LW** and **RW**.

Select the column in the report, select **Column Properties** on the context menu, and then select the variable in the selection box. Click **Parameter** to determine at which peak height to determine the peak width.

Rise Time

The rise time is a measure of how quickly the detector responds to a change in signal. The rise time is defined as the time it takes the output signal to rise from 10% of its final value to 90% of its final value. Choosing an appropriate rise time value can optimize performance by keeping the ➤*Signal-to-Noise Ratio* at a minimum level.

A longer rise time allows you to average the noise frequencies. Subsequently, the baseline will contain considerably less short-term noise. However, longer rise times may have the following effects on peaks:

- The peak shape will become asymmetric.

- The peak maximum is shifted.

- The peak height is reduced.

🗒 *Note:*

The rise time should be approximately 25% of the peak width at half height of the narrowest peak of interest.

For example, for a peak width of 5 seconds, calculate the rise time as: 1/4 (5 s) = 1.25 s. As it is not possible to set the rise time to 1.25 seconds, select the next fastest rise time. In this case, select 1 second.

Tip:

In the AD2,5 PDA-100, and PDA-3000 detectors, the rise time applies to both digital and analog output.

RPC (Remote Procedure Calls)

RPC technology allows you to run separate processes of a program on different computers.

Compared to true full-control programs, such as PC-DUO or PC-ANYWHERE, the amount of transferred data is considerably lower. Processes started from a remote computer run almost without any time delay. It is not possible for the user to determine whether the process is performed on the local PC or on a remote PC.

RPC requires a network connection between the involved computers. It is not import whether the computers are part of the same **Network** or whether they are linked via a modem connection. Communication also works between different networks (Novell, Windows) if the networks use a common protocol for communication.

Chromeleon generally communicates via the IPX/SPX, TCP/IP, or NetBEUI network protocols. The **Named Pipes** protocol can be used, as well.

Sample

In Chromeleon, a single injection, i.e., the volume that is injected, is referred to as sample. Thus, different samples can be injected from the same vial.

Note:

In laboratories, the content of a vial is usually referred to as sample.

The order in which the samples are processed is determined in the sample list. The order is also called ➢*Sequence*.

Sample Data

Data that contributes to characterize a single ➢*Sample* is stored in a database by Chromeleon. This includes not only the entered data (name, volume, etc.), but all results calculated from the ➢*Raw Data* and the chromatographic conditions under which the sample was analyzed (column, temperature, solvent, instruments, etc.).

Sample Name

The **Name** ⇒*Sample Variable* serves to identify a ➢*Sample* and also to label graphics and result reports. The user generally enters the sample name.

When you create a sample list using the Sequence Wizard, it is possible to generate the sample names automatically. (For more information about the wizard, refer to **Samples and Sequences** 🔺 **The Sequence Wizard**.)

Also, refer to ⇒*Name* (sample name)

Sample-Oriented Operation

Due to the object-oriented concept, emphasis is on samples and ➢*Sequences*, not on methods or activities.

The user selects the sample or sequence to process and automatically receives the corresponding view; that is, the calibration methods are displayed on the calibration plot, integration samples on the integration plot, and sequences in the sample editor, etc. For each sample or sequence, only the selectable or expected views, methods, or functions are available to the user. This saves time and reduces the number of simultaneously active windows.

This context-sensitive procedure is also valid for options selectable on the context menu. The selection depends on the position and the time of the mouse click.

Sample Position

The **Pos**. ⇒*Sample Variable* determines the position of the ➢*Sample* in the ➢*Autosampler*.

If you use a controllable autosampler, the entered position is transmitted automatically to the autosampler. The autosampler approaches the corresponding sample for injection. If using a non-controlled autosampler or a hand-operated valve, this column only serves for documentation purposes. If the sample position parameter is not entered, the previous (current) value is used.

Also, refer to ⇒*Pos.* (sample position) and ⇒*Position* (autosampler command).

Sample Status

The **Status** ⇒*Sample Variable* determines the current sample processing status. A ➢*Sample* is either unprocessed (Single), due for multiple processing (Multiple), processed (Finished), Interrupted, currently being processed (Running), or being prepared (Preparing).

Chromeleon also maintains a log of the sample status, that is, a Single sample is automatically assigned the status Finished as soon as processing is complete. A sample may also be excluded from processing by assigning the status Finished.

The sample status has a special significance for the built-in ➢*Power Failure Protection*. Upon recovery from a power failure or starting the sample batch after a manual interruption, Chromeleon begins processing the sample batch according to the selected power failure handling option.

Also, refer to ⇒*Status* (sample status).

Sample Type

The ⇒*Sample Variable* **Type** specifies which type of ➢*Sample* is used.

Select **Unknown** if the sample is an unknown analysis sample. This sample type is indicated by the symbol: .

Select **Standard** if the sample is a standard sample with known concentration. This sample type is indicated by the symbol: .

Select **Validate** if the sample is a ➢*Validation Sample*. This sample type is indicated by the symbol: .

Select **Blank** if the sample is a ➢*Blank Run Sample*. This sample type is indicated by the symbol: . If a sample is corrected by the ➢*Raw Data*, for example, of a blank run sample, this is referred to as ➢*Blank Run Subtraction*.

Select **Matrix** for a ➢*Matrix Blank Sample*. This sample type is indicated by the symbol:

Select **Spiked** for a ➢*Spiked Sample* that shall be used in ➢*Standard Addition* calibration. This sample type is indicated by the symbol: .

Select **Unspiked** for an unspiked unknown sample to be analyzed with the Standard Addition method. This sample type is indicated by the symbol: .

Also, refer to ⇒*Type (Sample Type)*.

Sample Weight Factor (Weight)

The ⇒*Sample Variable* **Weight** has two functions. It serves to enter the sample weight, but it can be used as a weight correction factor, also.

Sample Weight is implemented as a multiplication factor in the ➢*Formula for Amount Calculation* of not explicitly entered amount values (dilution series). It is without dimension.

Also, refer to ⇒*Weight* (Sample Weight Factor)

Sampler.Ready

See ➢*Ready*

Sampling Rate

The number of stored signal values per second is referred to as the sampling rate (or ➢*Data Collection Rate*). The maximum of the stored values corresponds to the number of values generated per second and depends upon the device; for example, Dionex UVD 170S/340S Detectors = 100, ➢*UCI Universal Chromatography Interface* = 100, 3D field = 10, and Dionex AD25, PDA-100, and PDA-3000 Detectors = 10.

The reciprocal value (the time interval between data points) is referred to as the ⇒*Step* (Dionex UV detectors = 0.01, UCI = 0.01, 3D field = 0.1, and Dionex AD25, PDA-100, and PDA-3000 detectors = 0.1).

▮☞ *Tip:*

*For Dionex detectors that are installed via the DX-LAN, the Data Collection Rate command determines the data collection (sampling) rate and the step value is automatically set to the reciprocal value of the selected data collection rate. For other Dionex devices, only the step value is set and a separate Data Collection Rate command is not used. For more information, refer to **Practical Tips for Device Control** ▥ **Defining Step and Average***.

SDK (Software Development Kit)

A Software Development Kit (SDK) is available for Chromeleon. The kit is a separate product and not included in the Chromeleon distribution. The SDK allows you to use Chromeleon for tasks beyond its standard functionality. In addition, it allows programmers to access Chromeleon from their own software. The SDK supports almost all Chromeleon functions and some of its masks in the individual programs.

For example, you can use the SDK for the following applications:

- Open Access solutions that allow even untrained users to create ➤*Sequences* and start ➤*Batches*.

- Special applications for which the extensive functions of Chromeleon are not sufficient.

- Connecting Chromeleon to almost all ➤*LIMS* or Management Software solutions.

You can use the SDK together with all programming languages supporting the Microsoft Component Object Model, such as:

- Visual Basic

- MS Excel (with VBA Basic)

- MS Access (with VBA Basic)

- Visual C++

- Delphi

In order to successfully use the SDK, the programmer must be familiar with the corresponding programming language and functions and with the Chromeleon concept.

If the SDK is too extensive a tool and you do not need all its functions, the command line allows you to use simple Chromeleon applications in external programs. For more information, refer to **How to …: Working with Files, Databases, and Networks** **Using Chromeleon Data in an External Program** in the **Administrator Help** section.

Security Activation Tool

See ➤*User Manager and Security Activation Tool*

Self-Regenerating Suppressor (SRS)

See ➤*Suppressor*

Sensitivity

The sensitivity of a measuring method describes the concentration dependence of the quantity being measured that results from the method itself, that is, from the slope of the calibration line (or more generally: the calibration curve). Unlike the ➤*Limit of Detection*, the sensitivity does not depend on the instruments used.

Distinguish between the sensitivity of the measuring method and the sensitivity of the peak recognition algorithm (also, refer to ⇒*Sensitivity*).

Sensitivity (Detection Parameter)

The **Sensitivity** detection parameter determines the signal height from which the detected data points are interpreted as peak or as noise. It is interpreted in the selected dimension; for example, in mAU.

 Note:

Always consider this parameter in combination with the ⇒Peak Slice detection parameter!

Also, refer to ⇒*Sensitivity*

Sensitivity (Signal Parameter)

The **Sensitivity** parameter adjusts Chromeleon to the ➢*Signal-to-Noise Ratio* of the detector. The RF 2000 fluorescence detector supports the values LOW, MED (default), and HIGH.

Sequence

A sequence combines ➢*Samples* that belong together due to their origin or processing. The names of all samples that belong to one sequence are entered in the sample list.

When the chromatography analysis has been started, the samples that shall be analyzed are processed from the top to the bottom of the sample list. Thus, the sample list also determines the order (= sequence) in which the analysis is performed.

The sample list can contain different columns:

- Standard columns with different ⇒*Sample Variables*, e.g. Name, Inj. Date/Time or Type, etc.)

- ➢*Sequence Report Columns* to display sample results

- ➢*User-defined Columns* that allow you to enter special values that are then available as additional variables in the report.

To create a sequence, use the Sequence Wizard (see **Samples and Sequences** 🔳 **The Sequence Wizard**). For more information, refer to **How to …: Creating and Managing Files and Data** 🔳 **Creating a Sample List (Sequence)**.

Also, refer to **Samples and Sequences** 🔳 **The Sample List (Sequence)**

Sequence Data

Data that characterizes a ➢*Sequence* instead of a single sample (see ➢*Sample Data*) is referred to as sequence data.

This includes, for example, the date of the last change, the corresponding timebase or the name of the user who created the sequence. As the sample data, this information is stored in a database.

Sequence Report Column

Sequence report columns serve to display sample results in the sample list of a ➢*Sequence*. In the Browser, these report columns already provide an overview of the most important sample results. For example, you can display the number of peaks detected for each sample and the amount and retention time of the main reaction product (Pyrene in the example below):

No.	Name	#Peaks	Ret.Time (det) [min] Pyrene Uv_vis_1	Area_P [mAU*min] Pyrene Uv_vis_1	Amount_P [µg] Pyrene Uv_vis_1
1	Sample 1	9	9.146	8.590	1.035
2	Sample 2	7	9.144	16.542	2.014
3	Sample 3	9	9.112	32.018	3.918
4	Sample 4	9	9.081	65.447	8.033
5	Sample 1 (Replicate)	7	9.121	8.128	0.978
6	Sample 2 (Replicate)	8	9.126	16.059	1.954
7	Sample 3 (Replicate)	8	9.114	31.928	3.907
8	Sample 4 (Replicate)	10	9.095	63.018	7.734
9	Sample 1 (Replicate 2)	7	9.087	8.672	1.045
10	Sample 2 (Replicate 2)	8	9.113	16.260	1.979
11	Sample 3 (Replicate 2)	8	9.095	31.411	3.844
12	Sample 4 (Replicate 2)	10	9.080	63.154	7.751
Σ	Sum				44.192
X̄	Average Value		9.109		
σ	Rel. Std. Dev.		0.247 %		

In addition, you can include different statistical values in the sample list and thus, display the sum, the average value, and the relative standard deviation of the corresponding results in the sequence report columns.

☞ *Tip:*

*Sequence report columns will be lost if you save (***Save*** and ***Save As*** commands), copy, move, or ➢Backup a sequence with Chromeleon 6.40 or earlier.*

For more information, refer to **How to …: Creating and Managing Files and Data** 📄 **Creating a Sequence Report Column**.

(Chromatography) Server

PCs connected to the components of a chromatography system can be used as chromatography servers.

A chromatography server is automatically installed on the PC during the Chromeleon installation. The chromatography server controls the data exchange between the system and the PC. Upon starting, each server is capable of serving up to six controlled systems (➢*Timebases*), which together may control a maximum of two ➢*Photodiode Array Detectors*.

The server receives the commands that have been entered on the ➢*Control Panel* of a ➢*Client* PC and executes them at the specified time; for example, by communicating them to the corresponding device driver of the chromatography system. The server also assumes this buffer function in the opposite direction. Thus, the raw data of each system is stored at the location specified by the client. In addition, the entire system-relevant data is forwarded to the client.

You can start (load) the server manually or automatically. Select the required options in the ➢*Server Monitor Program*. However, to ensure optimum functionality, you must configure the server. You can do this in the Chromeleon ➢*Server Configuration* program on any client PC. (Also, refer to **Software Installation and Communication** 🔳 **Chromeleon and Windows Operating Systems** in the **Administrator Help** section.)

If the Chromeleon server software and the Chromeleon client software are installed on the same PC, it is a local installation. If they are installed on different PC's, it is a network installation (see **Chromatography Components: Hardware and Software** 🔳 **The Network**).

🔳👉 *Tips:*

In case of manual data acquisition, the ➢Raw Data is always stored in the PCNAME_LOCAL server directory (if not otherwise defined). In case of ➢Batch processing, each user can decide where the data is stored.

To minimize the risk of losing data, always store raw data temporarily on the server. You may later transfer them to a different computer for archiving purposes.

➢Chromeleon Xpress does not include data storage. To store data, you must have Chromeleon Xpress/Server installed, or the analog output on the detector must be connected to a third-party chromatography data system.

Also, refer to **How to …: Starting and Monitoring the Server, Setting up the Server for Network Access** 🔳 **Setting up the Chromeleon Server for Network Access** in the **Administrator Help** section.

Server Configuration

The server configuration provides information about the chromatography devices that are installed in the ➤*Timebases*, about the server that is connected to the timebase, and about how the single devices are configured. The server configuration is defined in the **Server Configuration** program. (For more information, refer to **Software Installation and Communication** 🔲 **The Server Configuration Program** in the **Administrator Help** section.)

The entire information is saved in an installation file (*.CFG file). The current configuration is always saved in the CMSERVER.CFG file on the chromatography server.

The user can create different installation files in the server configuration program, transfer them to any server via the **Import** function, and use them accordingly.

This configuration can then be saved as the new Cmserver.cfg on this server by selecting **Save Installation**.

For more information, refer to **How to …:** 🔲 **Configuring the Chromeleon Server** in the **Administrator Help** section.

Server Monitor Program

The program required for configuring, starting, and monitoring the ➤*Chromatography Server* is called the Server Monitor program. When you install Chromeleon, you can include the Server Monitor program in the Autostart group of the operating system.

When the Server Monitor program is started, an additional icon appears on the Windows status bar, indicating the server status.

For more information, refer to **Software Installation and Communication** 🔲 **The Server Monitor Program** in the **Administrator Help** section.

Sharable Devices

Chromeleon records signals from several devices and controls different instruments. The signals are sent to the ➤*Server* PC via PC plug-in cards or the ➤*UCI* Universal Chromatography Interface. To avoid requiring an individual card or interface be available for each device from which signals are recorded and to which signals are sent, the cards or the interface provide several channels.

The different devices need not be part of the same ➢*Timebase*. For example, to enable signal recording by detectors from different timebases, the PC plug-in cards or the interface are not assigned to one specific timebase. Instead, the card or interface is shared between multiple timebases. Therefore, the PC plug-in cards and the interfaces are called **Sharable Devices**.

Each slot of a PC plug-in card or interface corresponds to a channel. In the ➢*Server Configuration* program, assign a signal to each used channel: Install the corresponding device driver and assign the signals on the **Signals** page. The ➢*Remote Inject* and the ➢*Integrator Driver* are available for non-controlled systems (usually GC, but also non-controlled chromatography systems). The Integrator Driver can also be used to record the pump pressure.

The advantage of this conception is that you do not have to rewrite all ➢*Programs* concerned if you plan to connect a device to a different channel. Instead, just change the A/D port assignment in the respective driver.

📖 *Tip:*

Some chromatography instruments are also "sharable." For example, the AS Autosampler can be shared between two timebases, as can the ICS-3000 Detector/Chromatography (DC). Sharing of these devices is enabled in the Server Configuration program.

Shared Relays and Inputs

Relays and remote inputs of the PC plug-in cards and the ➢*UCI Universal Chromatography Interface* are installed in the **Server Configuration** program as ➢*Sharable Devices*. They are independent from the timebase because one PC plug-in board or one UCI is possibly shared among several timebases. Thus, they can be used and addressed by different ➢*Timebases*.

To make these relays and remote inputs visible as controls on a ➢*Control Panel* and to allow you to activate a relay, the corresponding relay or the used remote input must be installed in the corresponding timebase.

You have to install a ➢*Device Driver* as you would for any other device. Select **Add Device** on the **Edit** or context menu. For relays and remote inputs, select **General** from the **Manufacturers** list box, and then select **Shared Relays and Inputs** from the **Devices** list box and click **OK**.

After installing the device driver, double-click the driver name to enable configuration. Configure the relays and remote inputs on the tab pages that appear, i.e., the **Relays** tab page and the **Inputs** tab page.

Each of the two tab pages indicates the current assignment of the relays and/or remote inputs that are currently used. If there is no entry on the list, either no **Sharable Device** component has been installed or the relays and remote inputs have not been assigned. Click **ADD** to install a new relay or remote input.

Shoulder

See ➢ *Peak Shoulder*

Signal

The data transferred directly from an instrument (ideally a detector) to Chromeleon, either analog via ➢ *UCI Universal Chromatography Interface* or digitally via a USB or LAN connection or via the RS-232 or another interface, is referred to as *physical* signals. If an instrument delivers several signals, these are also called channels; for example, two different wavelengths of a UV detector.

Calculated data, such as the arithmetic average of several channels, can represent a signal. This type of signal is called ➢ *Virtual Signal*.

Each signal has its own signal name that is assigned during instrument installation. The symbolic name of each signal must be clear. Some special signals have pre-defined signal names assigned to them.

The properties of a signal are determined via separate ➢ *Signal Parameters*. They can be modified manually or program-controlled (➢ *PGM File*) at any time.

The measuring data of the different physical and virtual signals is stored in uniform raw data files.

Signal Name

You are free to select any signal name. However, make sure that the name is unique. All signals used in a timebase must have different names. ➢*Virtual Signals* also have a signal name. This name is defined by Chromeleon.

☛ *Tip:*

The signal name is also stored within PGM Files, QNT Files, and sequences. Do not change the names. Otherwise, you cannot read and/or use older data later.

Signal Noise

The Signal Noise parameter calculates the signal noise within a given time range of the chromatogram. The parameter is used for two main purposes:

- The default calculation returns a value that is used internally as the ➢*Sensitivity* during integration (peak detection).

- The signal noise can also be determined for a user-defined time range of the chromatogram, typically for calculating the ➢*Signal-to-Noise Ratio*.

Signal noise calculation in Chromeleon is based on the ASTM method: After fitting a regression line (using the method of least squares) to all the data points in the time range, Chromeleon determines the maximum distance of the data points above and below the line. (When calculating the regression line, all data points are weighted with their corresponding step unless the step is equidistant.) The signal noise equals the sum of the maximum distances above and below the line.

Determining the Signal Noise for Internal Use (Default)

When no parameters are provided, Chromeleon calculates the signal noise in the first and last 10 seconds of the chromatogram. These values are compared to an internal value. The lowest value is returned as signal noise. If the sensitivity is not explicitly set on the **Detection** tab of the QNT Editor, this value is then used as the sensitivity for integration (peak detection).

⚠️ *Caution:*

To calculate quantification and detection limits via the signal-to-noise ratio, always use the signal noise with a user-defined time range (see below). If you use the default calculation, the quantification and detection limits might not be calculated correctly.

Signal Noise in User-defined Time Range (Parameter Input)

You can also determine the signal noise for a specific time range of the chromatogram. For example, in the **Integration** view, specify this time range in the **Report Column Properties** dialog box (after double-clicking the column to edit) or in the **Add Report Column** dialog box by selecting **Add Column** on the **Table** menu. Select **Chromatogram** in the **Categories** list, and then select **Signal Noise** in the **Variables** list. Click **Parameter** to open the **Parameter Input for 'Signal Noise'** dialog box. Select the **Specific Range** check box and enter a range.

Signal Parameters

Each signal delivered by a detector has detector-specific parameters. You can modify these signal parameters either on the instrument or in Chromeleon, if the system is controlled by the data system. Thus, it is possible to change the signal parameters while the chromatogram is running, either manually or program-controlled by a ➢*PGM File*.

Chromeleon usually supports the following parameters for Dionex instruments and third-party devices that are controlled by Chromeleon device drivers:

Signal type	Parameter	Signal type	Parameter
UV channel	⇒*Average*	**Fluorescence channel**	Average
	⇒*Bandwidth*		EmWavelength (see➢ *Emission*)
	⇒*RefBandwidth (Reference Bandwidth)*		ExWavelength (see➢ *Excitation*)
	⇒*RefWavelength (Reference Wavelength)*		➢*Gain*
	⇒*Step*		➢*Response*
	⇒*Wavelength*		➢*Sensitivity*
			Step

Signal type	Parameter	Signal type	Parameter
3D field	⇒*BunchWidth*	**Electrochemical channel**	Average
	➢*Min/Max Wavelength*		➢*Data_Collection_Rate*
	RefBandwidth		
	RefWavelength		
	Step	**Pressure channel**	Average
			Step
MS Channel	FilterIndex (see➢ *Filter*)		
	Min/Max Mass	**3D_Amp**	Step
	➢*Range*		
	Reset	**Virtual channel**	Average
	➢*Smoothing*	(see ➢*Virtual Signals*)	Formula
	SmoothingPoints		Step
			Type

(Also refer to: **How to …: Using Mass Spectrometers** 🖼 **Creating an MS Program and Sequence**)

Signal-to-Noise Ratio

The signal-to-noise ratio serves to characterize electronic components such as the ➢*UCI Universal Chromatography Interface* or a UV or photodiode array detector. The lower the ➢*Noise* is, the smaller the possible signal variations that can be recorded. As a general rule, the average of a signal should be at least twice (or, if possible, three times) the standard deviation of the signal value.

The signal-to-noise ratio of the Dionex UVD 340U ➢*Photodiode Array Detector* is 1500 mAU / 0.005 mAU (254 nm, 8 nm, 1 s). Measurement of this technical specification is within a clearly defined scope. This includes the wavelength information (254 nm), the bandwidth, and the ⇒*Step*. The values can be realized only with an empty flow cell and a new lamp that has been adequately burned in (approximately 40 h). The maximum signal variation (in AU) is measured at maximum light radiation through the flow cell.

If establishing comparable specifications is not relevant, the signal-to-noise ratio can be improved by the following operations:

- Selecting a low ➢*Sampling Rate*
- Using photodiode bunching (➢*Bandwidth*)
- Selecting the ➢*Optimum Integration Path*

Signal-to-Noise Ratio *(Peak Variable)*

The peak variable *Signal-to-Noise Ratio* indicates the quantification accuracy of components and is calculated as follows:

$$S/N = 2 * \frac{PeakHeight\ (H)}{Noise\ (h)}$$

The value for *PeakHeight* always corresponds to the related value of the peak variable *Height* ('peak.height').

The following figure shows the relation of peak height and signal noise:
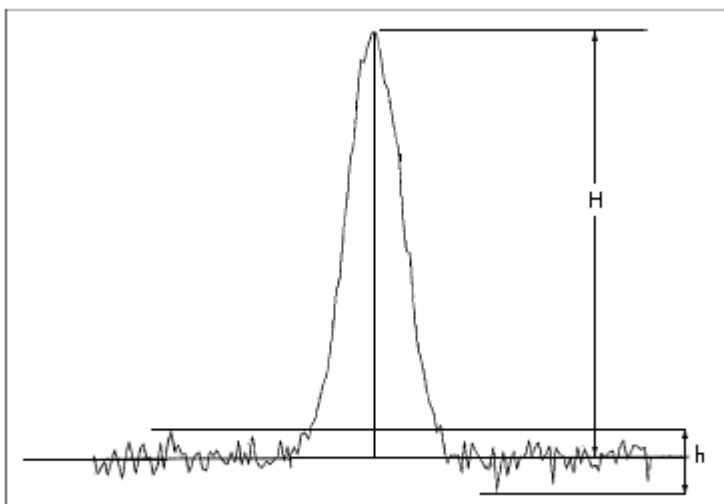
Signal Value at Peak Retention

The **Signal Value at Peak Retention** peak result variable indicates the signal value at the peak's retention time.

Signal Value at Peak Start/End

The **Signal Value at Peak Start/End** peak result variable indicates the signal value at the time of the peak start/end. The left peak delimiter defines the peak start. The right peak delimiter defines the peak end.

Also, refer to **How to …: Working with Chromatograms** 🔹 **Moving Peak Delimiters**.

Signature

See ➤*Electronic Signature*

SIM (Selected Ion Monitoring)

SIM is the ➤*Mass Spectrometer* method used for recording an MS chromatogram at a specific mass. As only single ions are recorded with specific masses, the SIM mode is more sensitive than the ➤*Full-Scan* mode. You can use up to 32 channels for data acquisition. These channels are called SIM_1 through SIM_32. In addition, one TIC channel is always recorded. The TIC channel summarizes the results of the single SIM channels.

With SIM chromatograms, the counts of specific masses (or more precisely, those with a particular mass-to-charge ratio) are added up. Thus, contrary to ➤*TIC* chromatograms, SIM chromatograms contain data of a specific mass.

SIM chromatograms are always recorded during data acquisition. This is contrary to ➤*Mass Traces* that are later extracted from ➤*Mass Spectra* (see **How to …: Using Mass Spectrometers 🔺 Extracting Mass Traces Afterward**). If you record data in full-scan mode, you can later extract the SIM channel(s) as mass trace(s).

👉 *Tip:*

The device methods of the aQa mass spectrometer (1.2) and the MSQ (1.3) are different. Therefore, please note the following difference: During data acquisition with the aQa mass spectrometer, data with the same mass range, polarity, and ionization voltage are always recorded in one channel. The Surveyor MSQ records them in different channels if the scan events are different for this data, i.e., if they are defined with different retention times. For example (same mass range, polarity, and ionization voltage):

Retention Time	aQa Channel	MSQ Channel
0-2 min	SIM_01	SIM_01
3-5 min	SIM_01	SIM_02

Also, refer to **How to …: Installing and Configuring Mass Spectrometers 🔺 Defining the Number of MS Channels** in the **Administrator Help** section.

Single-Point and Multiple-Point Calibration

If the position of the calibration curve is determined only by the ➤*Calibration Points* of one ➤*Calibration Level*, this is called single-point calibration. In this case, the ⇒*Calibration Type* **linear** must be chosen.

If calibration points on different levels determine the position of the calibration curve, this is referred to as multiple-point calibration; for example, two-point, three-point, etc. calibration. The number of calibration levels determines possible calibration types.

In both cases, the number of replicates is irrelevant for the number of calibration levels. Replicates only serve to secure the measuring results statistically.

Skewness

As is the **Asymmetry** peak result variable, the **Skewness** peak result variable, too, is a measure for the column quality.

The definition of the two variables is similar, too. The **Skewness** peak result variable is frequently mentioned in chromatography literature.

Skewness	Calculation	
S	$$S = \frac{RW_{10\%} + LW_{10\%}}{2 * LW_{10\%}}$$	
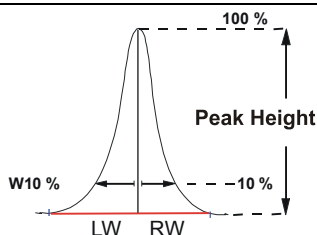
Where:

$LW_{10\%}$	Left peak width in 10% of the peak height.
$RW_{10\%}$	Right peak width in 10% of the peak height.

Also, refer to ➤*Asymmetry*.

Slope c1

The **Slope c1** calibration variable indicates the c1-value of the currently used ➢*Calibration Function*.

SmartStartup

Before you can start analyzing your samples, you have to equilibrate the chromatography system (in particular when the system was not used for a longer period of time or when the analysis conditions have changed). The SmartStartup Wizard supports the following features:

- If a device was turned off using the standby button, the SmartStartup Wizard includes a wake-up function from standby mode.

- The entire system, especially the column, is washed with the starting solvent until the system is free of any other liquid composition.

- The column thermostat and the thermostatted autosampler (if installed) are warmed or cooled to the starting temperature.

- In addition, Chromeleon checks the signal stability to avoid increased ➢*Drift* or ➢*Signal Noise*.

If you combine the SmartStartup functionalities with the ➢*SmartShutdown* Wizard, you can optimize the use of your chromatography system.

🖝☞ *Tip:*

SmartStartup is also supported when a device is shared by two timebases.

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to **How to ...:** 🔳 **Shutting down the Chromatography System (SmartShutdown)**.

The SmartStartup Wizard assists you in defining the equilibration conditions. (For more information about the wizard, refer to **Control** 🔳 **The SmartStartup Wizard**.)

For an overview of how to equilibrate the HPLC system with Chromeleon, refer to **How to …:** 🔳 **Equilibrating the Chromatography System**.

SmartShutdown

For UltiMate 3000 (excluding nano cap modules) and Summit HPLC systems, Chromeleon provides a special shutdown and standby process to automatically shut down the chromatography system or set it to standby mode. The SmartShutdown process comprises the following steps:

- The flow is reduced (standby) or stopped (shutdown) automatically at the end of the program. The SmartShutdown wizard ensures that the flow is not interrupted at high column temperatures to avoid damage to the columns.

- The detector lamp is turned off (optional for standby programs).

- The column thermostat temperature is cooled down (standby) or the temperature control is turned off (shutdown).

If you combine the SmartShutdown functionalities with the ➢ *SmartStartup* Wizard, you can optimize the use of your chromatography system.

▪☞ *Tip:*

SmartShutdown is not supported when the autosampler is shared by two timebases. In this case, an error message appears on the first wizard page.

For an overview of how to create a shutdown or standby program using the SmartShutdown Wizard, refer to **How to ...:** 🔳 **Shutting down the Chromatography System (SmartShutdown)**.

For more information about the SmartShutdown Wizard, also refer to **Control** 🔳 **The SmartShutdown Wizard**.

For an overview of how to equilibrate the HPLC system with SmartStartup, refer to **How to ...:** 🔳 **Equilibrating the Chromatography System (SmartStartup)**.

Smoothing

Data smoothing serves to reduce signal noise and help improve chromatogram appearance and reproducibility of peak baselines applying different digital filters without altering the raw data. Chromeleon provides the following filter types on the context menu of the Chromatogram window:

Moving Average (Boxcar)

The **Moving Average** (or **Boxcar**) filter equally weights each point. Thus, its ability to discriminate between noise and signal is limited (also, refer to ➢*Signal-to-Noise Ratio*).

Olympic

Compared to the **Moving Average** filter, the **Olympic** filter provides better rejection of impulse noise (spikes).

Savitzky-Golay

Savitzky-Golay smoothing is useful for reducing high-frequency noise of a data set that is continuous (such as a chromatogram) without significantly degrading the underlying signal.

For more information, refer to **How to …: Working with Chromatograms** 🔲 **Performing Data Smoothing**

In addition, Chromeleon allows you to smooth MS chromatograms during acquisition or mass trace extraction by applying the Gaussian or Boxcar (= Moving Average, refer to above) filter. The ➢*Xcalibur* software provides both filters.

Gaussian

The Gaussian filter applies the Gaussian distribution for chromatogram smoothing.

For more information, see **How to …: Using Mass Spectrometers** 🔲 **Extracting a Mass Trace**.

For more information about the different filter types and their parameters, refer to **Integration** 🔲 **Data Smoothing**.

Snap to Grid

Snap to Grid allows you to reduce, enlarge, or move the control frame.

- Select **Preferences** on the **File** menu.

- On the **Grid** tab page, to determine the mesh size of the grid.

SOR File (Signed Off Results)

SOR files (Signed Off Results) are electronically signed sequences (see: ➤*Electronic Signature*). They are indicated as follows:

Signed Results.sor
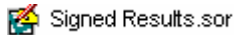
SOR files merely contain the tabs with the corresponding data for the sequence that have been selected in the respective ➤*Report Definition File (RDF)*. The data cannot be changed unless the signature is undone. However, to do so, the user must have the corresponding ➤*Privilege*: **UndoSubmit/Review/ApproveResults**. He(she) has to re-enter his(her) User ID and the respective signature ➤*Password* if this has been defined accordingly in the ➤*User Manager* (CmUser program) via the **File** menu > **Database Properties** > **Electronic Signature** tab page.

Sound

The **Sound** command allows you to play WAV files. The PC must be fitted with a sound card to support this command. WAV files are part of the Windows installation and contain various acoustic signals. Numerous example files are supplied, for example, in the WINNT/MEDIA directory (full Windows installation).

The Chromeleon command syntax for playing a WAV file is:

```
0.000  Sound    File= "Example.wav"
```

If the command cannot be performed due to a missing sound card, a default sound is generated at the PC speaker.

Also, refer to ⇒*Sound*

Spectra Library

UV spectra libraries can be helpful in identifying unknown substances. UV spectra can be saved in spectra libraries and later compared to the spectrum of the unknown substance. Make sure that the different spectra are recorded using the same solvent.

Apart from absorption values, each spectrum in a library contains various ID data fields that you can edit to facilitate spectra searches. The following fields are available:

Column	Description
Name	This column shows the name under which the substance is included in the spectra library. Select a column entry to assign a new name to the corresponding substance.
ID	This column shows the short ID that is assigned to the spectrum. Use this column to save several spectra under the same substance name in a spectra library. The Ids, such as consecutive numbers, then distinguish the spectra ID.
#Extrema	This column shows the number of extreme values in the spectrum determined by the system. Select a column entry to change the number.
Solvents:	This column shows the solvent in which the spectrum was recorded. Select a column entry to edit.
Comment:	Use this column to document details on the saved spectrum.
WL-Range:	This column indicates the wavelength range of the recorded spectrum.
WL-Resolution:	This column shows the ⇒*Bandwidth* with which the spectrum was recorded.
Ret.Time:	This column indicates the retention time at which the spectrum was extracted from a peak.
Ret. Index	If available, the column indicates the ➢*Retention Index* of the substance.
Kovats Index	If available, the column indicates the ➢*Kovats Index* of the substance.
Acq.Step:	This column indicates the Step with which the underlying ➢*3D Field* was recorded.
Detector:	This column indicates the detector with which the spectrum was recorded. The name is taken from the **Server Configuration**.

Column	Description
Detector Serial No.:	This column indicates the serial number of the detector from the **Server Configuration**.
Timebase:	This column indicates the name of the timebase with which the spectrum was recorded.
Sequence:	This column indicates the name and the patch of the sequence from which the extracted spectrum is taken.
Sample Name:	This column shows the name of the sample from which the extracted spectrum is taken.
Acq. Date/Time:	This column shows the date and time at which the spectrum or the underlying 3D field was originally acquired.
Program:	This column shows the name of the ➢*PGM File* that was used to generate the underlying chromatogram.
Extract Date/Time:	This column shows the data and time at which the spectrum was extracted and saved.
Extract Operator:	This column shows the name of the user who extracted the spectrum and saved it to the spectra library.

Spiked Sample

The sample matrix can considerably influence sample analysis, particularly in certain ion and gas chromatography applications. To account for this, a known amount of one or more substances is added to unknown samples. In this way, the concentration of these substances is increased by an exact known value. After spiking, the original (unknown) and the spiked sample are analyzed using the ➢*Standard Addition* calibration mode. The unknown and the spiked samples must be separated and analyzed with the same chromatographic method.

Spiked samples in Chromeleon have the ⇒*Type (Sample Type)* **Spiked**, indicated by the symbol .

Unspiked samples have the type **Unspiked**, indicated by the symbol .

When analyzing several unknown samples, assign the unknown samples to the associated spiked samples in the ⇒*Std. Add. Group* column. In this column, the samples are assigned to a common standard addition group.

Dionex recommends always spiking unknown samples with the same amount of the same substances. In the ⇒*Ref. Amount Set* column, you can then assign the spiked samples to the same amount values in the **Amount Table** of the QNT Editor.

SQL (Structured Query Language)

SQL is a computer language developed especially for queries in databases. Chromeleon uses an adapted SQL for ➢*Queries* in the Browser (queries for specific samples/sequences), as well as for "Database Queries" in the report and in the ➢*Printer Layout*.

Standard

The **Standard** column input in the peak table defines the reference peak to be used for calibration. The term **Standard** can also be used for a calibration sample or a standard sample. Calibration can be performed using an external and/or internal standard.

Also, refer to ⇒*Standard*

Standard Addition

Standard Addition is used as a calibration method, mainly in ion and gas chromatography. Standard Addition considers matrix effects during the analysis. Before the analysis, a known amount of one or more substances is added to a known volume of an unknown sample. In this way, the concentrations of these substances are increased by values that are exactly known. Afterward, the original and the spiked sample are analyzed. For reliable results that are more reliable, you can spike the unknown sample either several times or with various known quantities.

For analysis, the area is plotted against the concentration. (In gas chromatography, the area is plotted against the amount.)
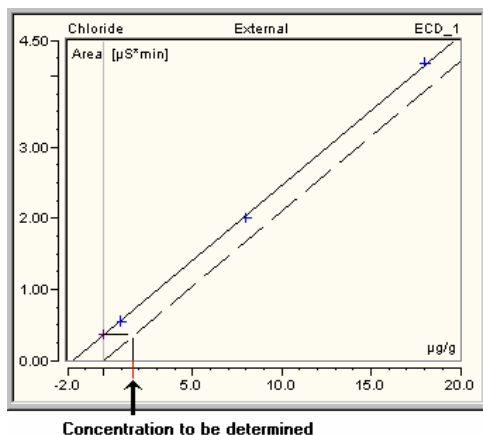
The unknown concentration (amount) of the originally unspiked sample (sample type = **Unspiked**) is set to zero, i.e., the y-axis is exactly on the concentration of the unspiked sample For evaluation purposes, the calibration line (calibration curve) is shifted parallel by setting the offset to zero. The concentration of the unspiked sample is determined as shown in the picture.

⚠️ *Caution:*

Only if the calibration curve is forced through the origin (i.e., in this case, through the calibration point of the original, unspiked sample), the amount of this sample corresponds to the negative intercept on the x-axis, i.e., to the average of all values based on this sample. For all other calibration types, the calculated amount of the original, unspiked sample may deviate from the negative intercept on the x-axis of the calibration curve.

The picture below illustrates this for the case in which the measured value of the original sample (evaluation: solid line) above the curve, i.e., the corresponding amount, is larger than the negative intercept on the x-axis (broken line):
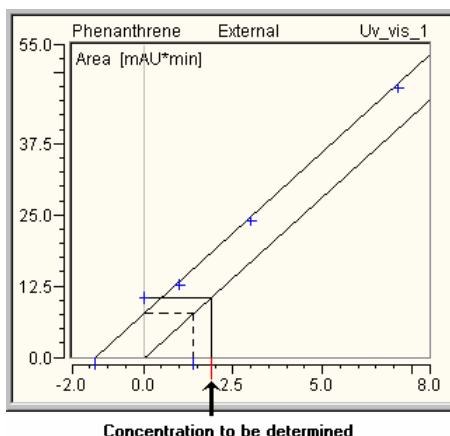
For more information, refer to **How to …: Calibrating** 🔳 **Standard Addition**.

Standard Datasource

The standard ➢*Datasource* is activated when the chromatography ➢*Server* is booted. The datasource is used for:

1. Saving the Daily ➢*Audit Trail*

2. Saving manual ➢*Sequences*

3. Saving ➢*OQ* and➢ *PQ* templates (This is the default setting, but you may as well define a different datasource for saving the templates.)

4. Reading ➢*User-defined Columns* during the program run.

During initial installation of Chromeleon, a local standard datasource is created on each client PC. In network operation, the datasource name is composed of the computer name and the suffix local (<PC Name_local>). For a local station, i.e., for a station that is not part of a 🔲 **Network**, the computer name entered in the operating system under **Start > Settings > Control Panel > System Network Identification** is used. If no identification is entered, the datasource is named **Default_local**.

In the ➢*Server Configuration* program, you may select a different datasource as standard datasource (see **How to …: Configuring the Chromeleon Server** 🔲 **Adding, Configuring, or Deleting Components** in the **Administrator Help** section).

To save chromatography data on the network, you can select a network datasource as the standard datasource. The **Administrator Help** section provides more information; refer to **How to …: Working with Files, Databases, and Networks**:

🔲 **Saving Chromatography Data on the Network**

🔲 **Defining a Network Datasource as Standard Datasource**

👉 *Tips:*

To save the Daily Audit Trails on a central PC, define different paths for different servers:

Network Datasource:Daily Audit Trail \Server1

Network Datasource:Daily Audit Trail \Server2

Standard Deviation

The standard deviation calibration variable is the square root of the ➢*Variance* calibration variable.

Statistical Moments

Statistical moments often describe the chromatographic separation much better than the values for the ➢*Theoretical Plates* and ➢*Resolution*. This is because statistical moments are not based upon an ideal Gaussian distribution, but on the actual statistical distribution of data points.

The ⇒*Peak Results* report category contains the **Statistical Moments** variable. The following options are available:

Zero Moment

The zero moment is the peak area:

$$\mu_0 = \int f(t)\, dt$$

With:

μ_0:   Zero moment

t: Time in minutes

f(t):   Baseline-corrected signal over time

The unit of the zero moment is the unit of measured value multiplied by the time in minutes, e.g., mAU*min.

First Moment

The first moment is the averaged retention time:

$$\mu_1 = \frac{\int t * f(t)\, dt}{\mu_0}$$

The unit of the first moment is minutes.

Second Moment

The second moment is the retention time variance (the narrower the peak, the smaller the variance):

$$\mu_2 = \frac{\int \left(t - \mu_1 \right)^2 * f(t)\, dt}{\mu_0}$$

The unit of the second moment is minutes squared (min²).

Status Bar

The status bar is located at the bottom of the window. Messages about the current system status appear on the left. The name of the current timebase and the command that is being performed appear on the right.

To hide the status bar, clear **Toolbars > Status Bar** on the **View** menu.

Step

The time interval between two successively recorded data points is referred to as the step; the sum of all recorded data points is called raw data. The reciprocal value of the step value is the ➢*Sampling Rate* (if the data is supplied by an A/D converter) or, by default, the ➢*Data Collection Rate* (if the data is supplied by a detector).

Also, refer to ⇒*Step* and 🔳 **Data Collection Rate**.

Step Gradient

Immediate changes of the solvent composition (for ➢*%-Gradients*) or the flow (for ➢*Flow Gradients*) are referred to as step gradients. Contrary to this continual changes of the solvent composition or flow are referred to as ➢*Ramp* (or more precisely as ramp gradient). In case of step gradients, the elution conditions change rapidly. The realization of the gradient on the column depends among others on the size of the dead volume between the pump and the column end.

To realize a step gradient, enter two percentage values (also, refer to ⇒*%B, %C, %D)*) or two flow values for the same time:

- Enter the current value (to specify that the composition or the flow of the solvent mixture does not change until this time).

- Enter the new value to which to set the solvent (or the flow) immediately:

	Retention	Flow	%B	%C	%D	
2	1.700	1.000	20.0	0.0	0.0	
3	1.700	1.000	40.0	0.0	0.0	
4	2.800	1.000	40.0	0.0	0.0	
5	2.800	1.000	80.0	0.0	0.0	
6	4.000	1.000	80.0	0.0	0.0	
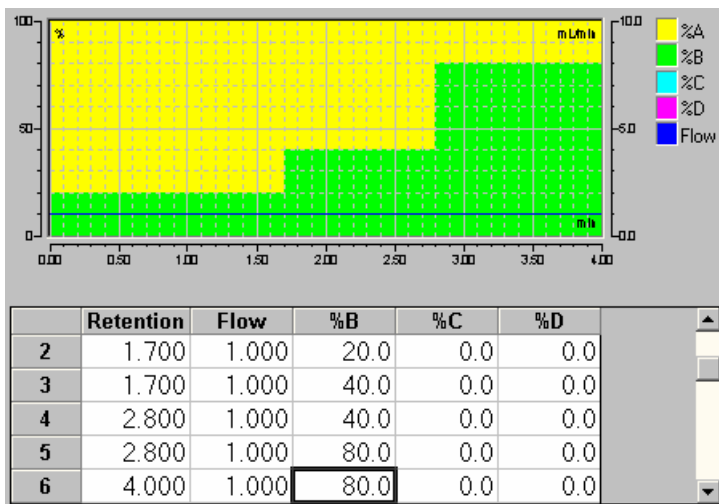
Stop Flow

Use the **Stop Flow** command to turn off the pump flow and to interrupt data acquisition. A running batch is stopped, as in the Hold mode.

Also, refer to ⇒*StopFlow*

Suck

See ➢*Draw*

Sucked

See ➢*Ready*

Summit HPLC System

The Summit HPLC System is the Dionex HPLC product line. The system comprises the following devices:

Device	Name	Description
Pump	P680 P580	HPLC gradient pumps
Autosampler	ASI-100	Temperature control option for sample thermostatting available (ASI-100T)
Column Thermostats	TCC-100 STH-585	Temperature range: 5-85°C (41°-185°F)
Detectors	UVD 170 UV-VIS UVD 340 PDA PDA-100 RF2000	➢*UV Detector* ➢*Photodiode Array Detector* Photodiode array detector Fluorescence detector
	(Shodex RI-101)	(Refractive index detector)
	(aQa MS, MSQ)	(➢*Mass Spectrometers*)

☞ *Tip:*

For automatic sample preparation, 2D chromatography, and/or optimization of sample throughput, the ➢Summit x2 Dual-Gradient HPLC System is available from Dionex.

Also, refer to **Chromatography Components: Hardware and Software** 🔳 **Chromatography Instruments**.

Summit x2 Dual-Gradient HPLC System

The Summit x2 Dual-Gradient HPLC System (in short: Summit x2 HPLC System) provides the maximum flexibility for standard HPLC and LC/MS methods and for advanced chromatographic techniques requiring two gradient pumps. The system is suitable for both standard HPLC applications and advanced chromatographic techniques, such as ➢*Tandem Operation*, online sample preparation, and 2D chromatography.

Running the Summit x2 HPLC System in tandem operation mode allows increasing the productivity, typically by 50-100%, without the need to modify existing chromatographic methods.

In addition, the Summit x2 system supports online sample preparation, e.g., for analyte enrichment or sample matrix elimination, and 2D chromatography for optimizing the chromatographic separation.

The Summit x2 HPLC System comprises the following modules:

- Two P680 HPLC gradient pumps (e.g., a P680 DGP dual-gradient pump with two ternary gradient pumps in one enclosure or two separate P680 gradient pumps with identical flow rate.)

- SOR-100 solvent rack with integrated degasser for degassing all channels of the pumps

- ASI-100 autosampler (that supports overlapping injections)

- TCC-100 thermostatted column compartment with column switching valve

- A detector of the ➢*Summit HPLC System* series, including the MSQ ➢*Mass Spectrometer*

Also, refer to **Chromatography Components: Hardware and Software** **Chromatography Instruments**.

Suppressor

A suppressor is a device used in ion chromatography to reduce the background signal from the eluent while increasing the analyte response when using a conductivity detector. Ion chromatography requires eluents that contain electrolytes, which can interfere with the detection of the ionic analytes of interest. The suppressor uses a combination of ion exchange and chemical or electrolytic regeneration to neutralize or suppress the electrolytes in the eluent. The analyte, on the other hand, generally is converted to a more conductive acid or base form.

Dionex offers suppressor products based on several patented suppression technologies. These products include the Atlas Electrolytic Suppressor, the Self-Regenerating Suppressor, and the MicroMembrane Suppressor. For more information, refer to the appropriate product manual.

For more information, refer to **Practical Tips for Device Control**:

 Controlling a Suppressor

 Setting Atlas Suppressor Currents

 Setting SRS Suppressor Currents

 Setting SRS-MPIC Suppressor Currents

 Controlling an MMS Suppressor

Syringe Type

The volume that can be injected with an autosampler depends on the installed syringe type. The default syringe types are 25, 50, 100, 250, 500, 1000, and 4000 µl. Many autosamplers support syringe in a range of different volumes. When installing a new autosampler, you must define the syringe type to be used by configuring the corresponding device driver. If the syringe type is ever changed, remember to specify the new type in the Chromeleon **Server Configuration** program.

☞ *Tip:*

Syringe types with volumes of 250 µl and 1000 µl are supported for use with the Dionex GINA 50 Autosampler. If different syringe types are substituted, the reproducibility stated in the autosampler specifications cannot be guaranteed.

System Suitability Test (SST)

As defined in cooperation with EURACHEM, System Suitability Checking (SSC) or a System Suitability Test (SST) is "a series of tests to check the performance of a measuring process " [P. Bedson and M. Sargent, Accred. Qual. Assur. (1996) 1, 265-274]. The objective of System Suitability Testing is to ensure the performance of the operating system and the system.

SST or SSC can be applied to individual measuring processes, and thus may be part of the validation process. For example, the System Suitability Test can be run to establish that the operational conditions required for a specific measurement process are being achieved.

☞ *Tip:*

*If you want to perform a System Suitability Test, be sure to enter the QNT File into the sample list before starting the analysis. Otherwise, the batch cannot be aborted in case of **Fail Action** - **Abort Batch** because the SST will not yet be performed during the batch run!*

In addition to the System Suitability Test, ➢*Operational Qualification*, and ➢*Performance Qualification* are also important for validating instruments and software (see **Validation, AutoQ, and System Wellness** ⬛ **Validation and Qualification**).

For more information about how to perform a system suitability test in Chromeleon, refer to **How to …: Performing Validation and Qualification Defining System Suitability Tests**.

System Wellness

System Wellness monitors the overall "health" of a chromatographic system. The different devices and Chromeleon provide several diagnostic and calibration features that help prevent unscheduled system shutdowns and assure reliable operation of system devices. System Wellness features are available for devices in the UltiMate 3000 and Summit HPLC product lines and for several IC devices.

Calibration and diagnostic commands for IC devices are available from Chromeleon Wellness control panels and Help topics provide instructions for performing the various tasks.

The devices in the UltiMate 3000 and Summit HPLC product lines are calibrated at the factory, e.g., the pump flow or calibration is performed automatically, e.g., for the UV detector via a ➢*Holmium Oxide Filter*. Thus, the user does not need to calibrate these devices.

The UltiMate 3000 devices additionally support various ➢*Predictive Performance* functions for estimating the lifetime of replaceable parts, and for monitoring and recording service and qualification information.

For more information about System Wellness, refer to **Validation, AutoQ, and System Wellness**:

System Wellness for IC Devices (Overview)

System Wellness for Summit HPLC Devices (Overview)

System Wellness and Predictive Performance for UltiMate 3000 Devices (Overview)

For more information about how to perform System Wellness procedures for IC devices, refer to **How to …: Performing Validation and Qualification Ensuring System Wellness**.

Tailing/Fronting Sensitivity Factor

This detection parameter is an implicit threshold for setting the peak end. The **Fronting sensitivity factor** refers to the peak start, respectively.

Also, refer to ⇒*Tailing/Fronting Sensitivity Factor*

For information about how to apply the detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Modifying Detection Parameters**.

Tandem Operation

Short analysis times in the laboratory save valuable time. Running the Dionex ⮞*UltiMate 3000 x2 Dual-Gradient HPLC System* or ⮞*Summit x2 Dual-Gradient HPLC System*, which comprise either two gradient pumps with identical flow rate or one dual-gradient pump, two columns, and a 10-port valve, in tandem operation mode allows increasing the sample throughput considerably, typically by 50 to 100 %.

For a gradient separation, it is usually necessary to wash and reequilibrate the column for the next run when the different analytes of a sample have been separated. During this time, the autosampler, detector, and fraction collector (if installed) are running idle. Analyzing the next sample during the wash and reequilibration steps can considerably enhance the productivity of these devices. Equilibration is performed offline.
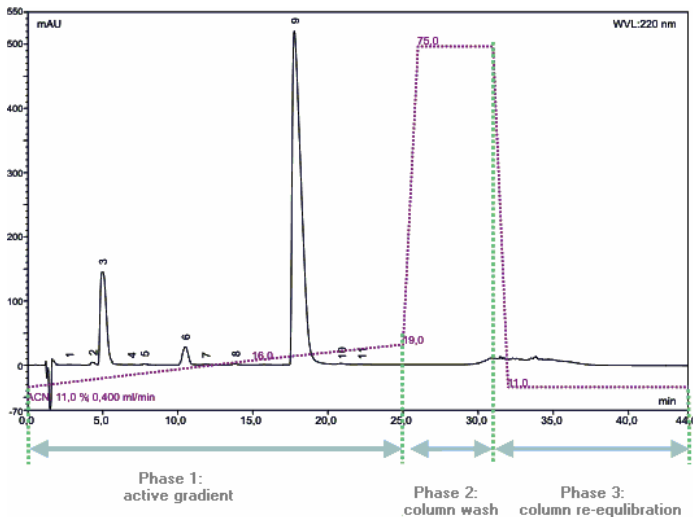
The example illustrates that phases 2 and 3 could be performed for one sample. In parallel, phase 1 could be performed for the next sample, on the second pump and second column. This requires the following system configuration:

Position A
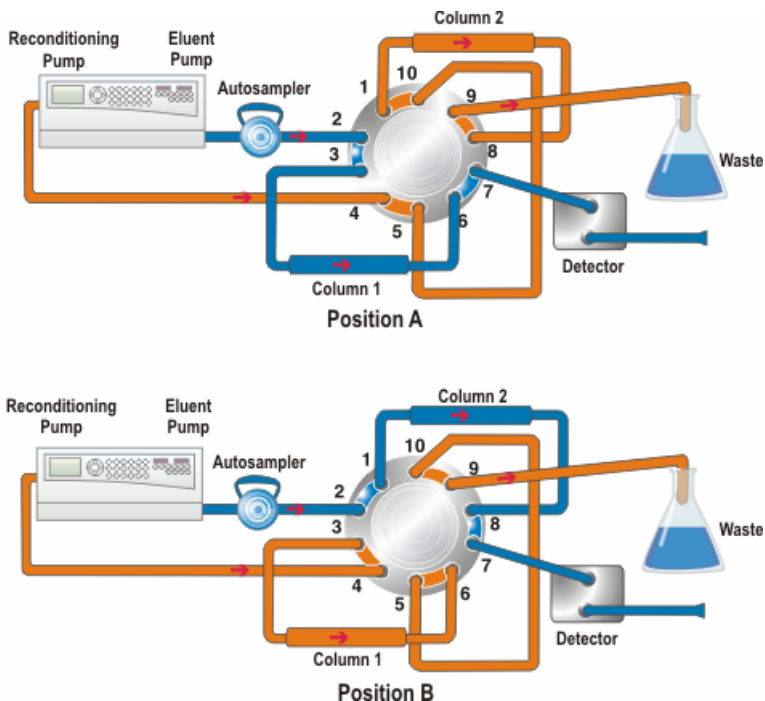
Position B

In position A, the chromatographic separation is performed on column 1. During this time, the wash and reequilibration steps are performed for column 2. When the valve is in position B, the separation is performed on column 2 while the washing and reequilibration steps are performed for column 1.

Using this configuration, you can considerably shorten the analysis time for the above example when you

- Stop the active gradient after 25 minutes.

- Set the solvent back to the starting composition (%B = 11%).

- Wash the remaining eluent out of the capillaries between the pump and the column switching valve, using this composition for 1 minute.

- After this minute, switch the valve to direct the flow to the second column and inject the next sample.
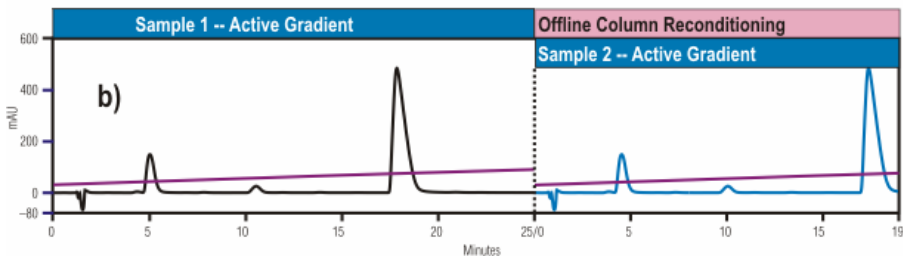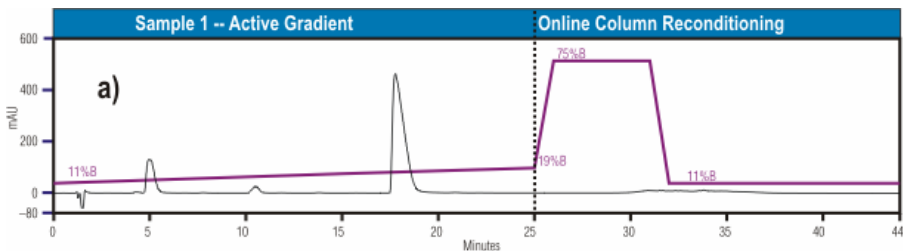
This considerably reduces the run cycle time and increases the system throughput, typically by 50% to 100%.

For more information, refer to **How to …: Creating and Modifying Programs 🔲 Defining Tandem Gradients (UltiMate 3000 x2/Summit x2)**.

Temperature Compensation Factor

A temperature coefficient used in conductivity detection to stabilize conductivity readings. The temperature compensation factor corrects for changes in ambient temperatures that occur during a run and normalizes conductivity measurements to 25°C.

If the cell is installed in a ➢*DS3 Detection Stabilizer* or a chromatography oven, the default factor of 1.7% is appropriate. When operating without a DS3 or oven, the compensation factor can be optimized to help minimize the baseline drift caused by fluctuations in ambient temperature. See the detector operator's manual for more information.

Template

Templates facilitate data input. Therefore, Chromeleon provides various pre-defined templates, such as report templates (DEFAULT.RDF and DEFLTDAD.RDF).

Theoretical Plates

The **Theoretical Plates** peak result variable is a measure of the separation capability of the column. With the standard formulas, theoretical plates are calculated from the peak width and the corresponding retention time. As with asymmetry, the USP and EP standards are different:

Parameter	Calculation	Comment
EP Formula	$$TP = 5.54 * \left(\frac{t_R}{W_{50\%}} \right)^2$$	European Pharmacopeia
USP Formula	$$TP = 16 * \left(\frac{t_R}{BW} \right)^2$$	United States Pharmacopeia
JP Formula	$$TP = 5.55 * \left(\frac{t_R}{W_{50\%}} \right)^2$$	Japanese Pharmacopeia

With:

TP	= Number of theoretical plates
t_R	= Retention time
$W_{50\%}$	= Peak width at 50% height (half-width)
BW	= ➢*Peak Width* on the baseline.

In addition, it is possible to determine the number of theoretical plates via the second statistical moment, i.e., the retention time variance of a peak, using the following formula:

Parameter	Calculation
Formula using Statistical Moments	$$TP = \frac{t_R^2}{\mu_2}$$

With:

μ_2	= Second statistical moment (variance). For details, refer to ➢*Statistical Moments*.

TP does not have a dimension. If they are placed in relation to the currently used column (L), the result is the theoretical plate height (H):

$$H = \frac{L}{TP}$$

However, the reciprocal value reveals more about the quality of the currently used column. It indicates the number of theoretical plates per column meter.

$$\frac{1}{H} = \frac{TP}{L}$$

To select the **Theoretical Plates** variable:

1. Select the column in the report, and then select **Column Properties** on the context menu.

   This opens the **Properties Report Column** dialog box.

2. Select **Peak Results** from the **Categories** list and **Theoretical Plates** from the **Variables** list.

3. Click **Parameter**.

   This opens the **Parameter Input for 'Theoretical Plates'** dialog box.

4. Specify whether Chromeleon will compute the theoretical plates based on the EP, USP, or JP formula, or using statistical moments. Click **OK**.

TIC (Total Ion Current)

TIC chromatograms summarize all counts at the corresponding retention time. Unlike ➤*SIM* chromatograms, TIC chromatograms include the data of the entire mass range.

The **TIC** channel is always recorded; it is independent of the mode used in the MS method (Full-Scan or SIM). In ➤*Full-Scan* mode, up to four additional **TICF**_n channels can be recorded for the aQa MS (nine TICF_n channels for the MSQ). The data acquisition conditions (for example, the acceleration voltage in the ➤*Mass Spectrometer*) may vary. The channels are called TICF_1 through TICF_4 for the aQa mass spectrometer and TICF_1 through TICF_9 for the MSQ spectrometer. The **TIC** channel summarizes the results of these single channels. One TIC channel is also recorded in SIM mode. In this case, it corresponds to the sum of the results of the single SIM channels.

From the ➢*Mass Spectrum*, you can extract separate ➢*Mass Traces* (or mass ranges) and save them as new channels (see **How to …: Using Mass Spectrometers** 🔲 **Extracting a Mass Trace**). In this way, you can limit the size of the mass range of TIC channels according to your requirements.

Also, refer to **How to …: Installing and Configuring Mass Spectrometers** 🔲 **Defining the Number of MS Channels** in the **Administrator Help** section.

🗐 *Note:*

TIC chromatograms represent the totals of intensity from the spectra headers. These headers are created at run time during data acquisition. Thus, the chromatograms that are later extracted using the entire mass range show a slightly different intensity profile.

Time

Chromeleon stores the time stamps as universal time (Greenwich time). However, the date notation is displayed according to the local settings chosen in the operating system (also, refer to ⇒*Inj. Date/Time* (Time of Injection)).

Timebase

All components that are combined in a chromatography system to enable chromatographic separations and are related in a time context with each other are assigned to the same timebase. A timebase can be a very complex system. It can consist, for example, of two pumps, one ➢*Autosampler*, one column oven, two detectors that are switched in series, and one ➢*Fraction Collector*. However, an isolated integrator or a gas chromatograph can also represent a timebase.

Any other system that is completely independent from the first one represents a new timebase. Administration of different timebases is on one or several ➢*(Chromatography) Servers*. Each server can serve up to six timebases; a maximum of four of these timebases can be fully-controlled Dionex LC or IC systems. However, only two Timebase Class 2 or Timebase Class 3 licenses are allowed. A timebase can have only one ➢*Photodiode Array Detector* installed. A maximum of two photodiode array detectors per server are permitted.

Specify the name of a timebase and the assignment of the devices in the ➢*Server Configuration* program.

Toolbars

Frequently used commands and features are available as icons; related functions are combined on toolbars directly below the menu bar. When starting Chromeleon, the standard toolbar is displayed. Select **Toolbars** on the **View** menu to enable or disable the display of individual toolbars. The following toolbars are available:

- Standard (standard functions)

- Online (commands for the ➢ *Control Panels* and ➢ *PGM-Files*)

- Layout (commands that can be used in ➢ *Layout Mode* are available on the ➢ *Layout Toolbar*)

- Method (for changing to other method sections, samples, or channels)

- Integration (commands for chromatogram processing)

- ➢ *Status Bar* (shows or hides the Status Bar)

Point to an icon to display the name in the **quick info** box.

Transaction Agent

The Transaction Agent serves to protect the Chromeleon datasource files against access from the outside. Under no circumstances should you edit, rename, or move Chromeleon data in the Windows Explorer! To avoid unintentional operations on these files, use the Transaction Agent to protect the Chromeleon datasource structure against these operations in the Windows Explorer. Please note that the Transaction Agent is available only on computers running under Windows 2000 or Windows XP.

A Chromeleon datasource consists of a database part and a file part. When the Transaction Agent is enabled, the standard user cannot access the file part. This prevents unauthorized access to the Chromeleon file part in the Windows Explorer or via the command prompt of the operating system. The user of the Chromeleon client can nevertheless access this part via a virtual user (TargetUser).

First, set up the Transaction Agent in the ➢ *User Manager*, and then enable this function in the **Security Activation Tool** (CmSecure program). For more information, refer to **Chromeleon User Management** ➡ **Setting up the Transaction Agent** in the **Administrator Help** section.

Transmission

When light passes through the UV detector flow cell without restraint, transmission is 100%. Transmission decreases with increasing absorption. For the UVD 340S detector, the maximum transmission value is determined via zero order at a reference wavelength of 630nm.

👉 *Tip:*

The substance mixture passing through the flow cell must not absorb in the reference wavelength range. If this is the case, either change the reference wavelength or select transmission without reference.

Trend Plot

Chromeleon's trending feature provides a way for specific data to be graphically displayed from sample to sample. In many cases, you can more quickly view progressive changes, compare runs, or identify items of interest by looking at a graphical representation of different samples, rather than by examining specific quantitative values of these samples in a report.

For example, if you view graphically the background signal in a conductivity detector for a series of runs and the trend shows a progressive increase over time, it might suggest a problem with an instrument. Or, if the same internal standard for the same column is used for a series of experiments, you can view a graphic trend of the theoretical plate calculations. A general decrease in the plate values might suggest column degradation.

In addition to depicting data graphically through a trend, Chromeleon can quickly calculate and display statistical parameters (mean, target, 1s standard deviation, 2s standard deviation, 3s standard deviation).

When a trend is shown with the target and standard deviations, it enables you to view the data within a broader context. Note that Chromeleon can depict the statistical parameters relative to ALL the samples selected, to only the samples in a particular view, or to user-entered target values. Moreover, the trend plot can be included in both ➢*Control Panels* and reports.

On the screen report, select **Show Trend** from the **View** menu to display the trend plot. For information about how to add a trend plot to a control panel, refer to **How to …: 🔳 Modifying a Control Panel**. For information about how to add a trend plot to a report template (printer layout), see **How to …: 🔳 Inserting a Trend or 3D Amperometry Plot**.

There are two types of trend variables: module-specific variables, such as pump pressure and background signal, and result variables, including retention time, area, peak height, etc. Depending on the data selected for trending, you can elect to show trends in real time, as an injection occurs, or post-run, after the run has been collected and processed. Here is an example of a trend plot:
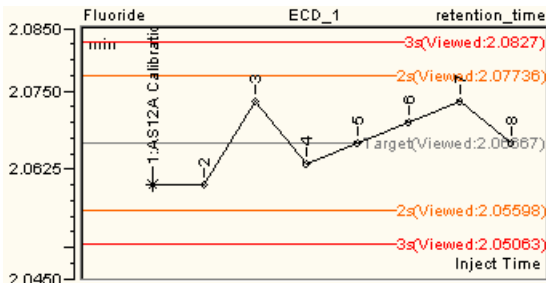
Trigger Commands

A trigger refers to the automatic execution of a command as soon as a condition becomes true.

The **Trigger** command can be included in a ➢*Program* or a programmable button, but not in the online control. The following syntax is valid:

```
Time    Trigger TRIGGERNAME    Condition,True,Delay,Limit,Hysteresis
        Reaction 1
        Reaction 2
        Reaction ...
Time    EndTrigger
```

Also, refer to ⇒*Trigger Commands*

TTL

TTL (Transistor-Transistor Logic) inputs and outputs are electronic switching devices for controlling instruments.

TTL Input Mode

The TTL inputs respond to four types of device output signals. The default TTL signal mode, normal edge, is compatible with the output signals provided by Dionex modules. If the controlling device outputs a different signal type, select a different signal mode

The four input signal modes are:

Normal Edge: In normal edge operation, the negative (trailing) edge of a signal turns on the function and the positive (leading) edge turns off the function.

Inverted Edge: The inverted edge mode works identically to the normal edge mode, except that the positive and negative edges are reversed in function.

Normal Pulse: In normal pulse operation, the negative (trailing) edge of the TTL signal is the active edge and the positive (leading) edge is ignored.

The minimum pulse width guaranteed to be detected is 50 ms. The maximum pulse width guaranteed to be ignored as noise or invalid is 4 ms. The action is undefined for pulses less than 50 ms or greater than 4 ms.

Inverted Pulse: The inverted pulse mode operates identically to the normal pulse mode, except that the positive and negative edges are reversed in function.
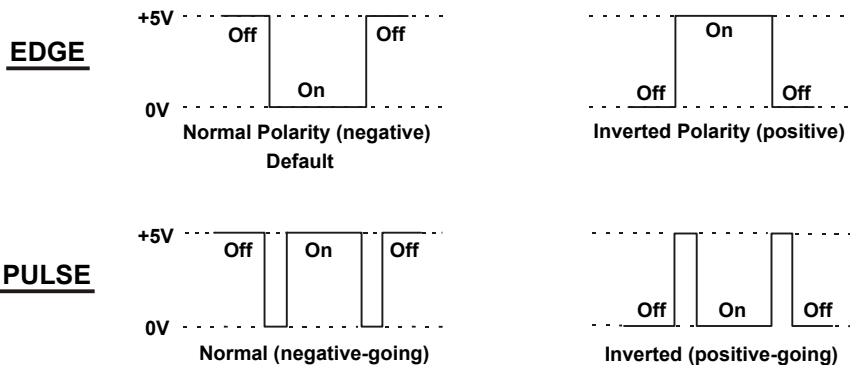
Type (Peak Type)

The **Type** peak result variable classifies a peak as a main peak (M=Main) or a ➢*Rider* (R=Rider). This variable also indicates the type of baseline contact (B=Baseline) and whether the peak data has been manually modified (*=modified).

Also, refer to ⇒*Type (Peak Type)*

UCI Universal Chromatography Interface

The UCI Universal Chromatography Interface serves as a data acquisition and device control module. It provides several different interfaces and makes them available to the chromatography server:

- Eight analog inputs (UCI-100) or two analog inputs (UCI-50)

- Four serial RS-232 ports

- Eight remote inputs

- Eight remote outputs

- One BCD input

For communication between the different devices and the server's chromatography data system, either a USB or an Ethernet connection can be used.

👉 *Tips:*

*Use the RS-232 interfaces (COM ports) via USB only (**no** TCP/IP)! If LAN connections are required, additional interface cards must be used for instrument control via RS-232. Dionex recommends using an instrument LAN for analog data acquisition (= installation of a second network interface card (= NIC; in the operating system, the card is often referred to as adapter) in the server PC). (Also, refer to **Hardware Installation** 🔵 **Connecting Dionex Devices via TCP/IP** in the **Administrator Help** section.)*

Always connect instruments requiring a GSIOC cable, such as GILSON devices and VARIAN pumps, to the multi-serial 8-fold PCI interface card (8-RS-232 Multi-COM card, Dionex part no. 5906.2095). (For more information about these devices, refer to **Hardware Installation** **GILSON** *and the* **VARIAN** *in the* **Administrator Help** *section.) Dionex no longer recommends connecting these instruments with other RS-232 ports; for example, with the COM ports of the PC or the Dionex Universal Chromatography Interface (UCI) as communication problems might occur. The power supply of the GSIOC adapter (type 605) is directly via the RS-232 interface. The RS-232 ports of the UCI Universal Chromatography Interface, however, are not designed for this.*

Also, refer to **Software Installation and Communication** **The Dionex Universal Chromatography Interface (UCI)** in the **Administrator Help** section.

UI20 Universal Interface

The UI20 Universal Interface functions as a communications and control link between the PC and instruments that are not directly connected to the ➤*DX-LAN*. This enables Chromeleon to collect data from any chromatographic detector with an analog output. The UI20 collects up to two analog detector voltage signals and converts them to digital data with 20-bit resolution.

UltiMate 3000

UltiMate 3000 is a Dionex product line for HPLC applications. The system comprises the following devices:

Device	Name	Description
Pumps	ISO-3100A	Isocratic pump (analytical)
	LPG-3400	Low-pressure gradient pump (available as micro, analytical, or biocompatible pump)
	DGP-3600	Dual low-pressure gradient pump (available as micro, analytical, or biocompatible pump)
	HPG-3x00	High-pressure gradient pump (available as micro, analytical, or semipreparative pump)
Autosampler	WPS-3000	Autosampler (available as micro, analytical, or semipreparative autosampler; temperature control option available)
Flow Manager and Thermostatted Column Compartments	TCC-3x00	Temperature range: 5-85°C
		1 or 2 thermostatted switching valves (10 or 6 ports)
	FLM-3x00	Temperature range: 4-45°C
Detectors	VWD-3x00	Variable Wavelength Detector with deuterium lamp (optional tungsten lamp available) and 4 selectable wavelengths.
	PDA-3000	➢*Photodiode Array Detector*
	UVD-3000	➢*UV Detector*

Also, refer to **Chromatography Components: Hardware and Software** 🔳 **Chromatography Instruments**.

UltiMate 3000 x2 Dual-Gradient HPLC System

The UltiMate 3000 x2 Dual-Gradient HPLC System (in short: UltiMate 3000 x2 HPLC System) provides the maximum flexibility for standard HPLC and LC/MS methods and for advanced chromatographic techniques requiring two gradient pumps. The system is suitable for both standard HPLC applications and advanced chromatographic techniques, such as ➢*Tandem Operation*, on-line sample preparation, parallel chromatography, and 2D chromatography.

Running the UltiMate 3000 x2 HPLC System in tandem operation mode allows increasing the productivity, typically by 50-100%, without the need to modify existing chromatographic methods.

The UltiMate 3000 x2 system supports on-line sample preparation, e.g., for analyte enrichment or sample matrix elimination, which automates otherwise time-consuming manual preparation steps.

The parallel setup allows doubling the throughput by sharing the autosampler and column compartment between two timebases.

The 2D chromatography setup improves selectivity and peak capacity (e.g., peptide analysis).

The UltiMate 3000 x2 HPLC System comprises the following modules:

- Two HPLC gradient pumps (e.g., DGP-3600 dual-gradient pump with two ternary gradient pumps in one enclosure or two separate LPG-3x00A or HPG-3x00A/P gradient pumps with identical flow rate)

- SRD-3200, SRD-3400, or SRD-3600 solvent rack with integrated degasser for degassing all channels of the pumps

- WPS-3000(T)SL HPLC autosampler (that supports overlapping injections)

- TCC-3x00 HPLC thermostatted column compartment with column switching valve, with a 10-port valve selected on the **Components** tab page of the Server Configuration, *and*

- Detectors of the ➢ *UltiMate 3000* series.

Also, refer to **Chromatography Components: Hardware and Software** **Chromatography Instruments**.

Unspiked

Refer to ➢ *Spiked Sample*

Unzoom

The **Unzoom** command undoes the last zoom operation.

To unzoom on the signal plot, double-click inside the ➢ *Overview Window*.

Use Recently Detected Retention Time

This QNT Editor parameter (**General** sheet) defines whether the retention time stored in the peak table (nominal time) is used by default to identify a peak or whether the actual retention time of a peak in the last sample is used. (For information about the editor, see **Data Representation and Reprocessing** 🔳 **The QNT Editor**.)

The parameter serves to compensate many types of drift appearances; for example, evaporation of volatile components in pre-mixed solvents or column aging.

Also, refer to ⇒*Use Recently Detected Retention Time*.

User Database

The user database defines the user rights (= ➢*Privileges*) and the membership of the individual users in ➢*Access Groups*. The user database is created in the ➢*User Manager* program and activated in the **Security Activation Tool** (CmSecure program). If the database is activated, ➢*User Mode* is enabled.

🔳☞ *Tip:*

Although the two terms are similar, do not confuse a user database with a Chromeleon ➢Datasource (database). Although both may be, for example, Access databases (= mdb container), they are two completely different databases.

User-defined Columns (UDC)

Various tables with different meanings appear throughout Chromeleon. For example:

- The different tables in the **Report** and in the ➢*Printer Layout*

- The sample list in the Browser (For more information, refer to **Data Management** 🔳 **The Browser**.)

- The peak table in the QNT Editor (see **Data Representation and Reprocessing** 🔳 **The QNT Editor**).

While the appearance of the sample list and the peak table is mainly default, you can define the Report and the Printer Layout according to your own requirements. You can also define your own columns in all tables mentioned above.

In the Report and the Printer Layout, use the ➢*Report Publisher* to create new columns and thus, to display special aspects of your data. For more information, refer to **How to …: Creating and Using Report Tables** 🔲 **Entering User-defined Formulas**.

In the sample list and in the peak table, user-defined columns

- Allow input of user-specific data, which can then be used as additional variables for report generation. In addition, they allow data to be imported via ➢*SDK (Software Development Kit)* or ➢*Worklist* (WLE file).

- Provide additional key words for a ➢*Query*.

- Allow you to define the fraction collection period in the PGM Wizard. (This is only possible if the Fraction Collection driver has been installed in the Server Configuration Program. This only refers to user-defined columns in the peak table.)

On the **Samples** page of the Query Wizard, select the user-defined columns for the **Sample** field type. Click the arrow next to the **Data Field** input box to display the selection list. An asterisk ('*') in front of the column name marks the user-defined columns created in the sample list of the Browser.

For the **Results** field type, it is not possible to directly access the user-defined columns by clicking the '**...**' button. Thus, to use a user-defined column that has been created in the peak table of the QNT Editor, type the formula directly in the **Formula** field. The syntax is as follows:

peak_tab.user_x

where x is the name of the user-defined column.

For more information, refer to **How to …: Creating and Managing Files and Data** 🔲 **Creating User-defined Columns**.

☞ *Tips:*

Be careful when creating user-defined columns in the sample list of the Browser. For columns having identical names in different ➤Datasources or on different computers that may communicate with each other, make sure that the column definition is identical, too. If the column definitions are different, problems may occur when you copy sequences or ➤Restore backup files. To make sure that the columns are identical in both datasources, select **Import Columns** *(select the datasource, click* **Properties** *on the context menu and then click the* **User-defined Columns** *tab). This command imports all existing user-defined columns from another datasource to the current datasource.*

To use the new and/or changed columns in the ➤PGM File, shut down and restart the ➤Server after you have entered or changed the columns in the sample list. Only then can the server access the user-defined columns.

However, access is restricted to the user-defined columns of the ➤*Standard Datasource*. It is not possible to access user-defined columns for which the **Value type** is **Time** or **Date and time**.

For more information about user-defined columns, refer to **How to …: Creating and Modifying Programs** 🔲 **Using User-defined Columns in a Program**.

User Management: User Manager and Security Activation Tool

Chromeleon provides two different administrator programs for user management: Use the **User Manager** (CmUser program) to create the ➤*User Database* and to manage the users. Use the **Security Activation Tool** (CmSecure program) to enable or disable User Mode. The setup for both programs is located in the CmUser directory on the Chromeleon software CD. Please note that the programs are **not** automatically copied to the Chromeleon program directory (e.g., c:\chromel\bin).

The User Manager is password-protected. Only the system administrator or somebody with similar authority can open the program.

☞ *Tip:*

Dionex recommends installing the Chromeleon User Management applications (CmUser directory) to a suitable network location, CD-ROM, or USB stick to allow modifications via the network. Make sure that only

Chromeleon administrators with administrator privileges for the CmUser database can access this network location. The Administrator Online Help also contains a section describing all steps and options for user management.

The User Manager, which is intended for *User*, *Privilege,* and *Access Group* management, allows you to perform the following actions:

- Create and edit ➢*Access Groups*

- Create and edit ➢*Privileges* (Privilege Groups or P Groups)

- Create and edit users in Chromeleon. The system administrator can define the user's name and job title, the ➢*Logon* and signature ➢*Passwords*, and the behavior if the logon failed.

Use the **Security Activation Tool** (CmSecure program) to enable and disable User Mode on the Chromeleon client. Besides, you can use the CmSecure program to select the user database. The user database contains the status and the rights of each user. When ➢*User Mode* is enabled, the user has to enter a password to start Chromeleon. It is only possible to sign sequences electronically when the User Mode is enabled (see ➢*Electronic Signature*).

⚠️ *Caution:*

*In the User Manager, select **User Database Properties** on the **File** menu to enable **Compatibility Mode**. This mode allows ➢Clients of previous Chromeleon versions (Chromeleon 6.01 or earlier) to log on to the system.*

User Mode

The **Security Activation Tool** (CmSecure program) allows you to enable and disable User Mode (= Access Control). When the User Mode is enabled, Chromeleon provides numerous options to limit user access to ➢*Servers*, ➢*Timebases*, ➢*Clients*, and ➢*Datasources*.

System access is password-protected. Access is only granted to users who are identified by the system after password input.

Each user identified by the system is granted personal privileges. The system administrator assigns these privileges. Via the ➢*Access Group* and ➢*Privilege* group membership of a user, the administrator defines the operations the user can perform within the system.

If access control is disabled, each user receives all rights. Datasources or directories that have access groups assigned cannot be accessed when the User Mode is disabled. With enabled User Mode, the user can access an object if he/she is a member of a group granting access to the respective object. If no access group is assigned to an object, each user can access the object independently of his/her access group membership.

If the User Mode is enabled, the user can access any object that is part of the respective access group. The sum of all privileges granted to the user (depending on his Privilege Group membership) determines what the user is allowed to do with the object. Signing sequences electronically (see ➢*Electronic Signature*) is possible with enabled User Mode only.

For more information, refer to **Software Installation and Communication** 🔲 **Access Control** in the **Administrator Help** section.

UV Cutover

The **UV Cutover** command for the AD20 Absorbance Detector sets the wavelength above which the second order filter is inserted in the light path. Typically, the UV cutover wavelength is 380nm.

UV Detector

UV detectors (strictly speaking, UV/VIS detectors) quantify absorption of UV/VIS-active substances in the range from 190 to 380nm (UV range) and 380 to 900nm (VIS range). UV detectors provide high sensitivity and a large linearity range. UV detectors are relatively independent from temperature fluctuations; they can be used for gradient elution (%-gradient).

Functionality

A grating (b) disperses the light that is emitted from a light source (a) in a discontinued spectrum. Simultaneously, the grating serves as a filter and directs only the wavelengths required for detection to a beam splitter (s). A portion of the beam is directed through the flow cell (d) onto a photodiode (e), the other part is used as a reference (f).
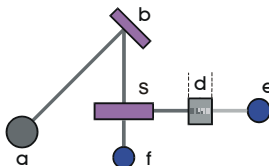
Theory

Depending on the absorptivity (ε) of the substance, the light beam is more or less attenuated when traversing the flow cell. For the absorption (E), the following is true:

$$E = \varepsilon\, c\, d$$

As the length of the cell (d) is constant and the absorptivity (ε) depends only on the substance itself or the absorbed wavelength, there is a direct connection between the substance concentration (c) and the absorption (E). The absorption measured in AU ("absorbance unit") is thus proportional to the number of particles in the beam path ("Lambert-Beer absorption law").

Wavelength Calibration

The wavelength is calibrated automatically after each $\Rightarrow$*Lamp* = On or $\Rightarrow$*Connect* command (**detector calibration**). The **CheckWavelength** command checks the currently valid wavelength calibration. The maximum deviation of this calibration compared to the instrument status is logged in the Audit Trail.

Calibration is possible only when certain conditions are met:

1. During calibration, the baseline must be sufficiently stable. This may not be the case, for example, if the solvent composition has been modified or if there are gas bubbles in the solvent.

2. The solvent in the cell must not be fully absorbing in the wavelength range that is calibrated. This will be the case, for example, if the cell is filled with 96% hexane / 4 % ethyl acetate.

3. Before calibration, make sure that the deuterium lamp is already warm because its spectrum changes a lot during the first minutes after turning on the lamp.

If these conditions are not met, the process will be interrupted with the corresponding error message. When the problem is solved, repeat the calibration by selecting **Disconnect** and **Connect**.

Detector Calibration

The detector is calibrated via a holmium oxide filter that is installed in the beam path of the deuterium lamp. The maxima are determined from the resulting transmission spectrum and compared to the holmium oxide values stated in the literature. If a difference is detected for this maximum between the measured and the known value, this maximum and the two adjacent maxima are interpolated to correct the wavelength allocation of the affected photodiodes. Wavelength calibration can take up to two minutes. During this time, data acquisition will not be possible.

Also, see ➢*Photodiode Array Detector*.

UV Lamp

This command turns the UV lamp on Dionex absorbance and PDA detectors on and off. For the AD20 Absorbance Detector (see ➢*UV Detector*), the command options are Low, High, and Off.

👈☞ *Tips:*

If you switch the AD20 lamp from Off to Low or High, there is a 22-second pause while the UV lamp warms up. During this time, the status of the lamp on the ➢Control Panel remains at Off. Once the warm-up period is complete, the status changes to the selected setting (Low or High). The Audit Trail window logs the UV_Lamp command and the warm-up time.

Detector lamps require a considerable warm-up time for high-sensitivity and drift-free operation. Therefore, after turning on the lamp, wait at least 10 minutes before you perform the ⇒Inject command. Also, avoid turning off the lamp(s) during a sample batch.

Validation

The process of ensuring that a system or analysis procedure supplies reproducible and reliable results is referred to as validation. For the user, this includes above all procedures regarding the planning, implementation, and documentation of an analytical method. Thus, validation is an integral part of ➢*GLP ("Good Laboratory Practice")*. The manufacturer of the instrument or data system performs validation. The user then performs several qualification steps (see ➢*Qualification*) to check the functionality of the instruments and Chromeleon.

For more information, refer to **Validation, AutoQ, and System, Wellness** 🔲 **Validation and Qualification**.

Validation Sample

Samples of known concentration that serve to verify a calibration are referred to as validation samples. In the sample list, they are labeled with the sample ⇒*Type* **Validate** and have the following symbol: .

Verification is by regular insertion of the validation samples in the normal analysis. The resulting (actual) area values are converted into amounts via the ➢*Calibration Function* and parameters and are then compared with the expected (nominal) values in the ⇒*Amount* table. The result of the nominal/actual comparison can be displayed as direct amount (**Amount Deviation** result variable) or as deviation in percent (**Rel. Amount Deviation**).

Checking can also be performed visually if you display the validation samples (marked by colors) in the calibration curve. They are not considered for calibration, though.

Validation samples can be injected also from vials that are normally used for injection of standard (calibration) samples. Validation samples are not relevant for the calculation of the calibration function!

For more information about validation samples, refer to **How to …: Integrating Chromatograms and Identifying Peaks**:

 Entering the Concentration/Amount of the Validation Sample

 Validating the Calibration Curve

Valley to Valley

If the **Valley to Valley** detection parameter is enabled, the baseline is drawn from peak minimum to peak minimum (i.e., from peak end to peak end), below non-resolved peaks.

Also, refer to ⇒*Valley to Valley*

For information about how to apply detection parameters, refer to **How to …: Integrating Chromatograms and Identifying Peaks** **Modifying Detection Parameters**.

Variance

The **Variance** calibration variable is the average deviation of all area values F from the corresponding ideal area value in a calibration. The ideal area value is the value at the point of intersection between the calculated calibration curve and the corresponding amount value.
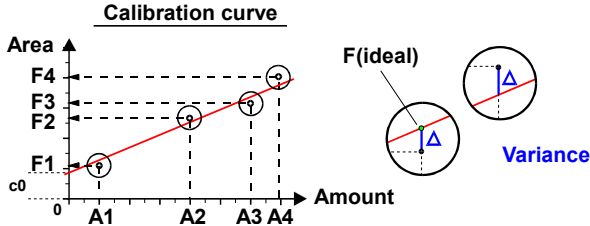
Variance is therefore a criterion for the measuring accuracy in the calibration. With an increasing value, calibration points are increasingly scattered.

The mathematical description of the variance is as follows:

$$Variance = \frac{1}{N-m}\sum_{i=1}^{N} W_i * (Y_i - F(X_i))^2$$

N: Number of standard samples involved in the calibration,

m: Number of coefficients to determine (depending on the $\Rightarrow$ *Calibration Type*: LIN: $m = 1$; LOFF: $m = 2$; QUAD: $m = 2$; QUOFF: $m = 3$ and EXP: $m = 2$),

i: Index for standard samples,

F(x):   Model function of the calibration,

Xi: X-value of the standard sample no. i,

Yi: Y-value of the standard sample no. i,

Wi: Weight factor of the standard sample no. i, and

$\varDelta i$: $Y_i - F(X_i)$

Extracting the square root of the variance value results in the $\succ$ *Standard Deviation* calibration variable; that is, the square standard deviation is the variance.

Variance Coefficient

The **Variance Coefficient** calibration variable can be considered a type of **normalized** ➤ *Variance* value. In Chromeleon, the variance coefficient is expressed in percent. This Chromeleon variable is especially useful when comparing variances of different peaks with different concentrations. A variance coefficient near zero means that the calibration curve well approximates the calibration points.

Thus, the variance coefficient indicates how well the data points correspond to the theoretically assumed course of the curve. Similar to the ➤ *Coefficient of Determination* and in contrast to the ➤ *Correlation Coefficient* it depends on the calibration type. The mathematical description of the variance coefficient is as follows:

$$VarCoeff = \sqrt{\frac{\sum_{i=1}^{N} W_i * (Y_i - F(X_i))^2}{\sum_{i=1}^{N} W_i * Y_i^2}}$$

N: Number of standard samples involved in the calibration,

i: Index for standard samples,

F(x): Model function of the calibration,

Xi: X-value of the standard sample no. i,

Yi: Y-value of the standard sample no. i,

Wi: Weight factor of the standard sample no. i.

Virtual Channel Driver (VCD)

The Virtual Channel Driver is used to record, display, save, and export the system status (for example, the relay status, gradients, or pump pressure) or ➤ *Virtual Signals* (for example, UV_VIS_1/UV_VIS_2) as signals. The virtual channel driver allows you to calculate any arithmetic expression during data acquisition, and then display the result as a signal channel and save it. (For more information about the virtual channel driver, refer to **Chromatography Components: Hardware and Software** 🔳 **Virtual Channel Driver**.) Any combination of numeric expressions can be used as terms in the formula that is used for calculating the virtual signal.

For information about how to install the Virtual Channel Driver, refer to **Hardware Installation** 🔵 **Installing the Virtual Channel Driver (VCD)** in the **Administrator Help** section.

A special program is required to record virtual channels. It is not possible to create these channels manually. For information about how to enter the corresponding commands into the program, refer to **Practical Tips for Device Control** 🔺 **Virtual Channel Commands** and 🔺 **Program Examples for Virtual Channels**.

Virtual Column

Virtual Column is a simulation tool that uses known ion chromatographic retention data to predict new retention data and chromatograms. The known retention data was acquired by Dionex using an appropriate experimental design and then embedded into Virtual Column.

📁 *Note:*

Results obtained with Virtual Column are intended to represent typical results for a particular column type. Because no two columns or systems are identical, the results you obtain in an actual analysis may differ somewhat from the Virtual Column predictions.

To use Virtual Column, you first select the application parameters (analytes of interest, methodology, column, etc.) to be simulated. Virtual Column then uses the embedded data in retention models and resolution algorithms to determine the retention data, resolution response, and Virtual Chromatogram for the selected parameters.

Virtual Column helps you to answer the following questions:

- What is the best column to use for a particular analysis?

- What eluent should I use for optimum separation of analytes?

- What eluent should I use for the fastest separation of analytes?

- How will changing the temperature of an analysis affect the separation?

Evaluation Mode

Virtual Column is an optional Chromeleon component. There are two license options: Isocratic and Linear Gradient. The Isocratic license enables modeling of isocratic separations only; the Linear Gradient license enables modeling of isocratic and linear gradient separations. (The Linear Gradient license is sold only in a Virtual Column Complete package that also includes the Isocratic license.)

If you have not purchased a Virtual Column license, you can use Virtual Column in ➢*Evaluation Mode*. Most functions are available in Evaluation Mode, with the following exceptions:

- In Evaluation Mode, the selection options for analytes are limited to the following predefined lists of analytes:

  Anions: Bromide, Chloride, Fluoride, Nitrate, Nitrite, Phosphate, and Sulfate

  Cations: Ammonium, Calcium, Lithium, Magnesium, Potassium, Sodium

  Carbohydrates: Fucose, Galactosamine, Galactose, Glucosamine, Glucose, Mannose

- In Evaluation Mode, the **Save**, **Open**, and **Reset All** commands are not available.

For details about how to use Virtual Column, refer to **How to …: Simulating Chromatograms**.

Virtual Signals

Signals that are composed of or calculated from several readings at the time t are referred to as virtual signals; for example, the arithmetic average of several channels. Virtual signals often include ➢*Reserved Signal Names*, such as 3DFIELD. Virtual signals can be generated with the ➢*Virtual Channel Driver*. They cannot be acquired manually; data acquisition is possible only in the context of a sample program.

For information about how to enter commands, refer to **Practical Tips for Device Control Virtual Channel Commands**.

Visible Lamp

This command switches the visible lamp of Dionex absorbance (see: ➢*UV Detectors*) and ➢*Photodiode Array Detectors* on and off. For the AD20 Absorbance Detector, the command options are Low, High, and Off.

Tip:

Detector lamps require a considerable warm-up time for high-sensitivity and drift-free operation. Therefore, wait at least 10 minutes after turning on the lamp before performing the ⇒Inject command. Also, avoid turning off the lamp(s) during a sample batch.

Void Time

See ➤*Dead Time*

Void Volume

See ➤*Dead Volume*

Voltammogram

A plot of current measured vs. voltage applied during a voltammetric run.

Also, refer to ➤*Cyclic Voltammetry*

Volume

See ➤*Injection Volume*

Wait

The **Wait** command interrupts program execution until the specified remote input signal arrives. During this time, program time and data acquisition are stopped. Controlled pumps are kept in the **Hold** mode.

For examples for using the **Wait** command, refer to **Practical Tips for Device Control**:

🔲 **Autosampler Control**

🔲 **Special Commands, Relay Control, and Miscellaneous**

Also, refer to ⇒*Wait*

Wash

The **Wash** command causes the autosampler to rinse the needle and/or sample loop with solvent in the **Inject** position. This corresponds to the normal solvent flow following an ⇒*Inject* command.

☞ *Tip:*

*Select the **Wash** command to wash the sample loop and thus prevent crystallization of substances in the sample loop.*

Also, refer to ⇒*Wash*.

Waveform

A waveform is a series of steps, defined as points on a plot of potential vs. time. The waveform is repeated continually throughout the run. A waveform must be defined when using the Dionex electrochemical detector in ➢*Integrated Amperometry Mode*.

The waveform period is the elapsed time from the first step in the waveform to the last.

In the example waveform below, the potentials (labeled E1, E2, and E3) are applied for durations t1, t2, and t3, respectively. The signal is measured at E1 by integrating the current for a fixed time (ts).
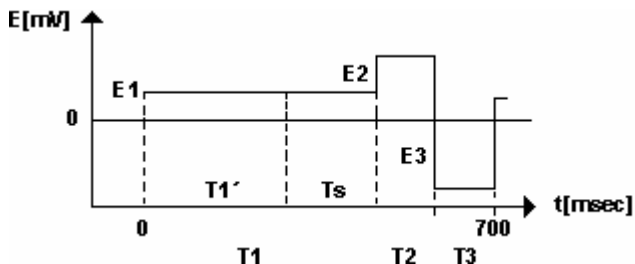
Wavelength

➢*UV Detectors* and ➢*Photodiode Array Detectors* are the most commonly used detector types in HPLC. Simple UV detectors record chromatograms at a defined wavelength (λ), while PDA detectors record them in a defined wavelength range. The wavelength and the wavelength range, respectively, depend on the detector and can vary from 190 to 900 nm.

This value indicates the wavelength at which a chromatogram is measured.

On Dionex photodiode array detectors, the wavelength is not set directly on the instrument, but via the PC. It is also possible to enter the wavelength manually, either during the analysis or in the ➢*PGM File*.

For the UV-VIS, 3DFIELD, and SPECTRA signals, wavelength means the central wavelength; that is, the wavelength around which a field with a symmetrical ⇒*Bandwidth* forms.

Also, refer to ⇒*Wavelength*

Wavelength Calibration

Dionex UV detectors fitted with a ➢*Holmium Oxide Filter* perform a wavelength calibration after each Lamp = on command (see ⇒*Lamp*) or ⇒*Connect* command.

Dionex AD2,5 PDA-100, and PDA-3000 detectors perform a wavelength calibration at power-up. New wavelengths are assigned to each pixel, based on the emission spectrum from the deuterium lamp.

With defined absorption maxima, wavelength calibration ensures that the data delivered by the detector corresponds to the expected wavelength values.

Wavelength Switching

Usually, samples contain different substances with different UV spectra and different absorption maxima. Wavelength switching allows the detection of individual peaks at their optimum wavelength. Wavelength switching changes the wavelength of the respective measurement when the solvent composition changes, resulting in a composite chromatogram of the largest absorbance for each substance. The switching should occur so long before the retention time of the respective peak that the entire peak is detected at one wavelength.

Wavelength switching also allows the simultaneous analysis of substances with strongly differing absorption maxima, and little or no absorption at the wavelength of the other maximum.

Wavelength switching is program-controlled; that is, the switch time at which the wavelength of a specific channel is changed is entered in a control ➢*Program*. The switch time and wavelength can be generated automatically by extracting the ➢*Optimum Integration Path* from an opened ➢*3D Field* (**Extract: Opt.Int.Path to Clipboard**).

■☞ *Tip:*

Wavelength switching is also possible with fluorescence detectors. Usually, the excitation wavelength should be changed at exactly the same time as the detection wavelength.

For information about how to perform wavelength switching, refer to **Practical Tips for Device Control** 🔲 **Determining Wavelength Switching**.

Weight

Also, see ➢*Sample Weight Factor (Weight)* and ⇒*Weight* (Sample Weight Factor).

Weights

The **Weights** calibration variable indicates the weighting (see **How to …: Calibrating** 🔲 **Weighting and Averaging Calibration Points** in the **Creating a Peak Table** section) assigned to the individual ➢*Calibration Points* when creating the calibration curve.

Define the weighting in the ⇒*Calibration Type* column of the QNT Editor. (For information about the editor, see **Data Representation and Reprocessing** 🔲 **The QNT Editor**.) The following options are available:

No weight	Default: Higher weighting of higher amounts and/or signal values.
1/Amount (X)	Nearly cancels out the weighting of higher amounts/signal values.
1/Amount² (XX)	Causes over-proportional weighting of smaller amounts.

1/Response (Y)	Nearly cancels out the weighting of higher signal values. In this case, the Y-values (dependent signal values) of the ➤Calibration Points are used as weight factors instead of the X-values (nominal amounts).
1/Response² (YY)	Causes over-proportional weighting of smaller signal values. In this case, the Y-values (dependent signal values) of the calibration points are used as weight factors instead of the X-values (nominal amounts).

If you select **Average all response values of each calibration level before Curve Fitting (A)**, two more options are available:

1/Rel.Std.Dev (S)	Each average value is reciprocally weighted with the related relative (and squared) standard deviation. This means that calibration levels with increased scattering are weighted less than calibration levels with less scattering.
1/Rel.Std.Dev.² (SS)	Causes over-proportional weighting of calibration levels with less scattering.

Width

This peak result variable refers to the peak width *extrapolated* on the baseline. Peak tangents are drawn from the turning points of the leading and trailing edges. Then the points of intersection with the baseline are calculated. The time distance between the two points of intersection is defined as the base width. If the base width is used for calculating other parameters, the abbreviation is **BW**.
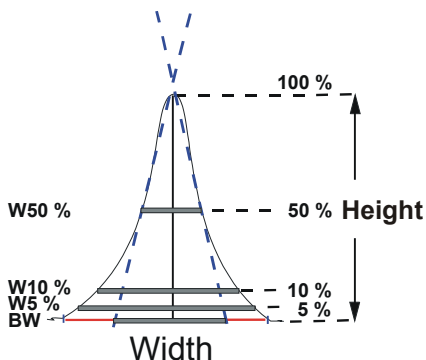
Width

Chromeleon also determines the peak width at 5%, 10%, and 50% of peak height (abbreviations: W5%, W10%, and W50%).

☞ *Tip:*

*In contrast to the base width **BW**, the peak widths at 5%, 10%, and 50% of peak height (W5%, W10%, and W50%) are not only measured up to the point of intersection with the two tangents, but up to the signal curve!*

The peak width cannot be determined unless the peak is resolved to at least one-half the peak height.

How to display the peak width in the report

- Select the column in the report.

- Select **Column Properties** on the context menu.

- Select the **Width** variable in the selection box.

- Click **Parameter** to determine the peak height at which to determine the peak width.

Window

The **Window** peak table parameter defines the tolerance interval within which the peak is expected. Adding and subtracting the window value to the retention time (retention time +/- window value) determines the window size; that is, the window always has twice the width of your input. The value can be interpreted as an absolute or relative value.

Also, refer to ⇒*Window*

Wizard

Chromeleon includes several wizards designed to facilitate procedures such as creating a ➤*Sequence* or a ➤*Program*, or defining ➤*Query* conditions.

Wizards help users avoid unnecessary typing, syntax errors in command entries, and omission of important information and parameters. Each wizard prompts the user to define conditions and to enter the required information. The wizard then adds the default elements and completes a basic structure for the procedure. Users can extend or modify this structure as required.

Worklist

The format for reading data (sequences) of a ➤*LIMS* Chromeleon is referred to as a Worklist (WLE file). The worklist describes the contents and structure of a sequence (also, refer to ➤*Worklist Format*).

To import an existing worklist, select **File > New > Sequence (From LIMS Worklist)**.

If importing is possible, the imported sequence is listed in the Browser. To prevent importing the existing worklist again, it can be deleted automatically by setting **DeleteWorklist** to **Yes** in the **[options]** section.

If an error occurs during the import, Chromeleon reports the error description and location in the worklist. The error message is also saved in a log file. This log file carries the worklist name and the extension **log**. To automatically change the extension of an incorrect worklist file to ***.err**, set **Rename On Error** to **Yes** in the **[options]** section.

For more information, refer to ➤*Worklist Format*.

Worklist Format

The file extension for ➤*Worklists* is **.wle** (Work List Export). The worklist structure and syntax are similar to those of the Windows **INI** files. A worklist consists of various sections. Each section starts with a new line. The name of the section is written in brackets. One or more entries appear below the section name, with the syntax *name* = *value*. Comments start with a semicolon and finish at the end of the line.

📖☞ *Tip:*

If information, such as the injection volume, is missing in the worklist, you can enter it in Chromeleon as usual after the worklist has been imported. However, please note that the QNT and PGM Files must already be specified in the worklist.

Example of a valid worklist plus comments (to use the worklist in Chromeleon, first delete the comments):

; Worklist generated by MegaLIMS 3.11.5622.00a
; Wed 05/27/98, 16:25:02

[options]
Application = Chromeleon
Delete Worklist = No
Rename On Error = No

[file names]
; datasource, path, and name for the generated sequence
Sequence = \labor2_local\sys58\sequences\lims4711
; default for the program column
pgm = pgmWash
pgm = Stop
; default for the QNT Method column.
qnt = qnt0815
qnt = noint
; copy ➢*PGM Files* from here (datasource and path)
pgm templates = \server1\templates\hplc

; copy ➢*Quantification Methods (QNT Methods)* as stated here (datasource, path, and name)

[qnt files]
qnt0815 = \server1\templates\hplc\pah\qnt0815
noint = \server1\templates\common\noint

[defaults]
; default for injection volume
Injection Volume = 10.0

[1]
Name=Wash
Type=Blank Run
Pos=1
; use the "Wash" program instead of the default ("pgm0815")
PGM=Wash
QNT=qnt0815

; default for the user-defined column **Water Contents** - the marking asterisk * is omitted with
➤*User-defined Columns*

Water contents = 72.3

[2]
Name=Std
Type=Standard
Pos=99

[3]
Name=Sample1
Pos=2

[4]
Name=Sample2
Pos=3

[5]
Name=Sample3
Pos=4

[6]
Name=Sample4
Pos=5

[7]
Name=Stop
Type=Blank Run
Pos=1
PGM=Stop
QNT=Nnoint

With this worklist (without the comments), a sequence named **lims4711** is created in the datasource **labor2_local** in the **sys58\sequences** directory. The sequence contains a rinse sample, a standard, four unknown samples, and a stop program.

All control programs are copied from the subdirectory **\templates\hplc** of the datasource **Server1** (central file server). The source must contain the **pgm0815**, **wash**, and **stop** programs.

The evaluation method for the rinse and stop samples is copied from **\templates\common\noint.qnt**, the method for the analysis samples is copied from **\templates\hplc\pah\qnt0815.qnt** (both in the datasource **server1**).

Path Information

The entire path information of the worklist is related to the Chromeleon data structure. Therefore, the path name starts with the ➢*Datasource*, not the hard disk name. Relative paths are not accepted. Path names can be expressed either in the internal Chromeleon syntax (**Moniker**), for example, SEQ::\labor2_local\sys58\sequences\lims4711, or in a simplified syntax (as above), which omits the type abbreviation and the double colon. In this case, a slash or a colon can be used instead of the backslash.

```
Sequence = SEQ::\labor2_local\sys58\sequences\lims4711
Sequence = \labor2_local\sys58\sequences\lims4711
Sequence = Labor2_local:sys58/sequences/lims4711
```

All of the above paths refer to the sequence lims4711 in the directory sys58/sequences of the datasource Labor2_local.

Do not include type extensions (**.PGM**, **.QNT**, **.SEQ**) in the path information.

Handling of PGM Files and QNT Files

The program and the method columns of the sequence only include the file name without the path information, as the files are always located in the sequence. The same applies to the corresponding options (PGM= and QNT=) in the WLE file (see below).

The LIMS import module creates these files by copying them to the sequence. There are two possibilities to specify where to find these templates to copy:

- If all used files of a type are located in the same directory (the template directory), its path can be determined in the section [file names] via

  PGM Templates = *path to template directory*   or. **QNT Templates**= for QNT Files

  Chromeleon searches the corresponding template directory for a file with the specified name and copies this file to the sequence.

- If the copy templates are located in different directories, the path of each section has to be included in the '[PGM File]' or '[QNT Files]' section. On the left side of the equal sign, the file name is located, and on its right side, the complete path of the template is indicated.

  Technically, it may be possible that the file name of the template differs from the name of the copied file. However, this will not be accepted.

Both methods can be mixed. In case of doubt, the second method has priority. If there is an entry in the section [PGM Files], the PGM template file is not searched.

If all samples use the same PGM and QNT Files, it does not matter which method is used.

Section [OPTIONS]

The options listed in this section will influence the import function. Usually, no entries are required here. When testing a worklist, it may be useful to disable the **Delete Worklist** and **Rename On Error** commands. Thus, the worklist is retained instead of being deleted after importing.

Application = Chromeleon indicates that this worklist is intended for Chromeleon. If there is a different entry here, the worklist will not be accepted by Chromeleon. In this case, no log file is created, and the worklist is not deleted or renamed.

Computer Name = <Name of PC> indicates where to copy the worklist. If this entry does not correspond to the local computer, the worklist is not accepted. In this case, no log file is created, and the worklist is not deleted or renamed.

Log Error = Yes creates a log file containing the error message in the event of an error.

Log Success = Yes creates a log file documenting the successful import.

Delete Worklist = Yes deletes the worklist when importing was successful.

Rename On Error = Yes renames the worklist ***.err** in the event of an error.

Character Set = Windows indicates the character set of the worklist. Any generated log file is written with this character set, also. Change this option if some special characters, such as umlauts, are not transferred correctly. Valid values: Windows or ANSI (system character set, that is, no conversion) DOS or OEM (PC character set, conversion by Windows function OEMToAnsi).

Section [FILE NAMES]

This section determines various file names. **Sequence =** indicates the path and the name of the sequence to generate. This parameter must always be specified. **PGM =** or **QNT =** determines the default value for the PGM column or the QNT column. (The default value can be overwritten for each sample.)

PGM Templates = and **QNT Templates =** determine the corresponding template directory (see above).

Sections [PGM Files], [QNT Files]

The sections define from where to copy the PGM and the QNT Files. Entries in this section take priority over a template directory. Entries have the following syntax:

name = path

On the left side of the equals sign, the file name is located, and on its right side the complete path of the template.

Section [DEFAULTS]

This section defines default values for various sample variables. **Name=**, **Comment=**, **Pos=**, **Type=Unknown**, **Status=Single**, **Sample Weight=1.0**, **Dilution Factor=1.0**, **ISTD Amount=**, **Std. Add. Group=**, **Ref. Amount Set=**, and **Injection Volume=20.0** have the same meaning as in the Browser. The corresponding value range is also identical.

Note: The default values for the PGM and the QNT Files are defined in the section [FILE NAMES].

Section [SEQUENCE]

This section defines the properties of the sequence.

Title = Created from worklist <Name of Worklist> determines the name (description) of the sequence.

Timebase = determines the timebase on which the sequence is executed.

Report = determines the default value for the report template (appears in the print dialog)

Channel = determines the default value for the channel when printing (appears in the print dialog)

Section [n] (Sample Data Records)

Each sample is described by one section. The section must have the name `[n]`, where *n* is the sample number (without leading zero). The samples must be numbered consecutively. With a new sequence, sample numbering must start with 1. Samples can be appended to existing sequences. In this case, sample numbering must start with the next free sample number in the sequence (as number of samples in the sequence plus one). A sample section can have the entries **PGM=**, **QNT=**, **Name=**, **Comment=**, **Sample ID=**, **Replicate ID=**, **Pos=**, **Type=Unknown/Blank/Validation/Standard/Matrix/Spiked/Unspiked**, **Status=Single/Multiple/Finished/Interrupted**, **Sample Weight=1.0**, **Dilution Factor=1.0** and **Injection Volume=20.0**. Missing entries are completed by the defaults in the sections [DEFAULTS] and [FILE NAMES].

☞ *Tip:*

In the worklist, omit the asterisk (*) that marks ➢User-defined Columns in the Browser.

Workspace

The area between the menu bar or ➢*Toolbar* and the ➢*Status Bar* is called the **Workspace**. The **Workspace** displays the open windows.

The **Workspace** allows you to open a specific set of windows. Normally, this is the most recently used view.

Select **Autosave Workspace** on the **Workspace** menu to open the most recently used workspace whenever you start Chromeleon, for example, two ➢*Control Panels* showing the status of two different chromatography systems.

Of course, it is also possible to save or open any workspace view with a separate name. Chromeleon stores this information in WSP files. Use this option to save a workspace view for system control, a view for data processing, a view for peak purity analysis, etc.

If you want to save the settings of an individual window (for example, the on-screen report and the Printer Layout) without changing the workspace, you can use a ➢*Report Definition File (RDF)*. The appearance of the control panel is saved in the corresponding PAN file.

Combine single files with a workspace to create a customized representation of the screen. For more information, refer to **Basic Operation** 🔳 **User Profiles (Workspaces)**.

Xcalibur

Xcalibur (= XC) is the software data system of the Thermo Finnigan Corporation. You must install Xcalibur software to control a Thermo Finnigan ➢*Mass Spectrometer* with Chromeleon and to view mass spectra. Two XC setup programs are provided on the software installation CD. For installation details, refer to **How to …: Installing and Configuring Mass Spectrometers** **Installing MS Components** in the **Administrator Help** section.

To install the aQa mass spectrometer and to read aQa method files, install Xcalibur Rev. 1.2 on your PC.

To install the MSQ spectrometer, install Xcalibur Rev. 1.3.

Because Xcalibur software cannot control several mass spectrometers from one PC, a separate server PC is required for each mass spectrometer.

Xcalibur software allows you to define mass spectrometer programs in a ➢*PGM File*. You can create the control file in Chromeleon, using the Xcalibur Editor. (For more information, refer to **How to …: Using Mass Spectrometers** **Creating a PGM File for the aQa MS** and **Creating a PGM File for the MSQ**.**)**

Year 2000 Conformity

According to the document PD2000-1:1998, which was published by the British Standards Institution (BSI), "year 2000 conformity shall mean that neither performance nor functionality is affected by dates prior to, during, and after the year 2000."

In particular, this means that the following rules are adhered to:

1. *General Integrity:* No value for current date will cause any interruption in operation.

2. *Data Integrity*: Date-based functionality must behave consistently for dates before, during, and after year 2000.

3. *Explicit/Implicit Indication of the Century:* In all interfaces and data storage, the century in any date must be specified either explicitly or by unambiguous algorithms or inferencing rules.

4. *Leap Year:* Contrary to the year 1900, the year 2000 must be recognized as a leap year. (According to the Gregorian Calendar, the year 1900 was not a leap year.)

Chromeleon 4.20 and higher fulfill the BSI rules mentioned above. The Dionex hardware fulfills the BSI rules in the same way. Two instruments of the previous generation that are no longer available (M480 pump and GINA 160 ➢*Autosampler*) display the year 2000 as 1900. This, however, does not affect the functionality.

Please do not hesitate to contact Dionex Service for the corresponding Year 2000 Certificate.

Zoom

Draw a frame around a section of a signal plot or a 3D field that you wish to zoom while holding down the left mouse button. To undo the last zoom operation, select ➢*Unzoom* on the context menu. Select **Full size** to return to the original display (100% representation).

☞ *Tips:*

On the online signal plot, an ➢Overview Window is displayed in the upper right corner in addition to the enlarged section.

While you draw a zoom frame, you can cancel the operation by pressing <ESC> key or by right-clicking. Use this function to prevent a window, such as a 3D plot, from being redrawn if you have selected the wrong frame.

Index

E

H

K

L

M

P

Q

R

T

U

V

X

Y

Z