

CHROMELEON®

Chromatography Management System



Tutorial and User Manual

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A

AAA-*Direct*™ Amino Acid Analysis System

AAA-*Certified*™

AAES™

Acclaim® columns

Acrodisk® (Gelman Sciences, Inc.)

Acurate™ Flow Splitter

Advanced Computer Interface (ACI)

Advanced Gradient Pump (AGP)

AD20 UV/Vis Absorbance Detector

AES® Atlas Electrolytic Suppressor

Alliance® (Waters Corp.)

AminoPac® Columns

AminoTrap™ Columns

AMMS®-ICE, 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.)

Aroclor™ (Monsanto Corp.)

AS40, AS50 Automated Sampler

AS3500 Autosampler

ASE® 100, 200, or 300 Accelerated Solvent Extractor or Solvent Controller

ASI-100™ or ASI-100T™ Automated Sample Injector

ASI-100P™ or ASI-100PT™ Automated Sample Injector

ASRN™ Anion Self-Regenerating Neutralizer

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

Atlas® Suppressor

Auto OnGuard™ (but OnGuard®)

AutoASE® software

autoflex™ (Bruker BioSciences)

Autoion®

AutoNeutralization™ system or technology

AutoQ™

AutoRegen® System

AutoSelect™

AutoSuppression® device

AXIMA-QIT™ (Shimadzu Biotech)

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.)

Chemraz[®] (Greene, Tweed & Co.)

ChromatoCritters[™]

CH-2 Column Heater

CHX650 Column Temperature Controller

CHROMELEON[®] Chromatography Management Software

CMD[™] Carbohydrate Membrane Desalter

CMMS[®] Cation MicroMembrane Suppressor

Continuously Regenerating Anion Trap Column (CR-ATC)

Continuously Regenerating Cation Trap Column (CR-CTC)

CSRN[™] Cation Self-Regenerating Neutralizer

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

CD20 Conductivity Detector

CMMS[®] II Cation MicroMembrane Suppressor[®]

Cryptand columns

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 Electrochemical Detector

EG40, EG50 Eluent Generator

Electrochrom

EluGen[®]

EO1 Eluent/Solvent Organizer

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, GP50 Gradient Pump

GS50 Gradient Pump

H

HPICE[®] (mostly replaced by IonPac[®] ICE)

Hydromatrix[™] (Varian Associates, Inc.)

I

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

ICS-90, ICS-1000, ICS-1500, ICS-2000, ICS-2500

InkJet[®] (Hewlett-Packard)

IonPac[®] columns

IonPhor[™] Electrolyte Buffers

IonSep[®] reagents

IP20 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, 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.)

Micro Precolumn

MicroBead™ resin

MicroInjection valve

MicroMembrane™ Suppressor

Millennium® (Waters Corp.)

Milli-Q® (Millipore)

MMS™ MicroMembrane™ Suppressor

Monolithic capillary column

Mono Q® (Pharmacia LKB)

MonoStandard®

M Path™ (Thermo Electron Corp.)

MPIC® (Mobile Phase IC)

MS-DOS® (Microsoft Corporation)

MSQ™ Mass Spectrometer (Thermo Electron Corp.)

N

Nano precolumn

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 (Datensystem für 8200er-Serie)

ProPac[®] Columns

ProteinChips[®] (Ciphergen)

Q

QSTAR[®] (Applied Biosystems)

R

Reacti-Therm[™] (Pierce Chemical Company)

Reagent-Free[™]

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

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)
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™

Ultrex® (J.T. Baker)

UV-Booster

UVD 170S/170U UV/Vis Detector

UVD 340S/340U Photodiode Array Detector

U-Z View™ Capillary Flow Cell

V

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

VHP™ (The Separations Group, Inc.)

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

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:

➤ AIA	Analytical Instrument Association
BCD	➤ <i>Binary-Coded Decimal Code</i>
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	➤ <i>Backup</i> container/file (file extension)
CMS	Chromatography Management System
CS	Cluster Server
DHCP	Dynamic Host Communication Protocol
DNS	Domain Name Server
DX	Dionex
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 ➤ <i>License Server</i>
➤ LIMS	Laboratory Information Management System
LOD	<i>Limit of Detection</i>
MAC	Media Access Control address (unique address of each network interface card)
MS	➤ <i>Mass Spectrometer</i>
NFP	➤ <i>Network Failure Protection</i>
➤ ODBC	Microsoft Open Database Connectivity
➤ OQ	Operational Qualification
PAN	➤ <i>Control Panel</i> 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
➤ QNT	Quantification method for signal interpretation and evaluation (file extension)
QRF	➤ <i>Query</i> 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	➤ <i>Sequence</i> ; that is, the sequence of samples plus the corresponding files (file extension)
➤ SIM	Selected Ion Monitoring (MS channel for specific masses)
➤ SOR	Signed Off Results (File) = signed sequence
➤ SQL	Structured Query Language (computer language)
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	➤ <i>User-Defined Column</i>
USB	Universal Serial Bus
VCD	➤ <i>Virtual Channel Driver</i> (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.

Tutorial

Doc.: CM_Tutorial_660.doc

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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.

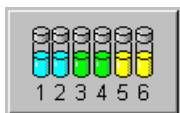
Table of Contents

I.



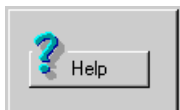
→Starting Chromeleon: If your computer is running under Windows NT; Windows 2000, or Windows XP, you can start Chromeleon by clicking Start > Programs > Chromeleon on the task bar.

II.



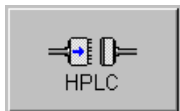
Managing Data: Chromeleon allows you to manage data in folders and directories similar to Microsoft Windows. The tool that helps you to handle your chromatography data is the **→Browser**.

III.



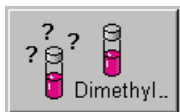
Online Help: The Tutorial can only deal with selected aspects of Chromeleon. For more information, refer to **→Online Help** and/or to the User Manual.

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. In addition, you can create a **→Control File (PGM File)** to control your system automatically.

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:

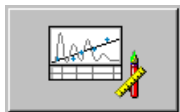
Use the **→Sequence Wizard** to include the samples to process in the sample list. The Wizard allows you to define the number of samples, the order of sample processing, the injection volumes, the sample type (analysis or standard), and the location where Chromeleon finds information about how to perform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

V.

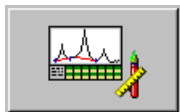
(cont'd)

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.

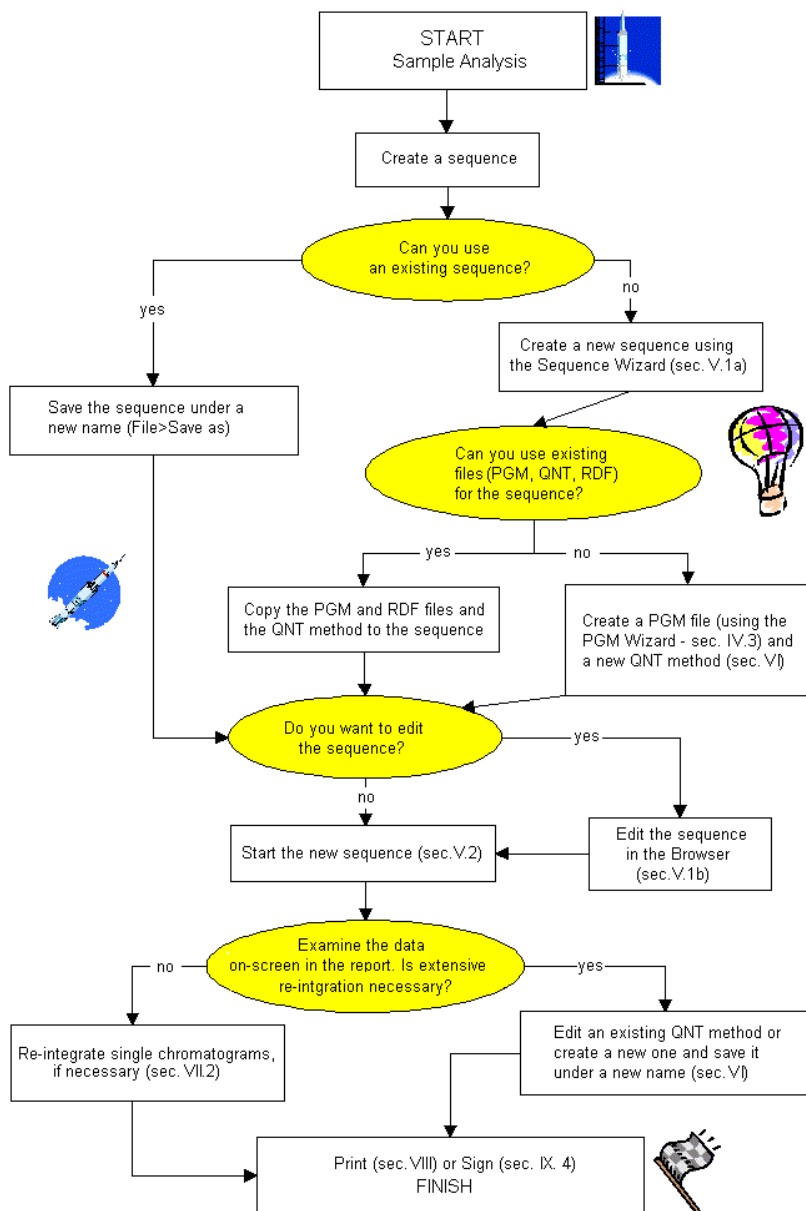
Printing Results: You can → *print* your analysis data immediately after data acquisition or at any time later. For perfect adjustment to your personal requirements, create your own report templates in the → *Printer Layout*, define your own result variables, or embed your company logo.

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*.

For an overview of the most important steps in Chromeleon, see → *Sequence - Flow Chart*

Sequence - Flow Chart

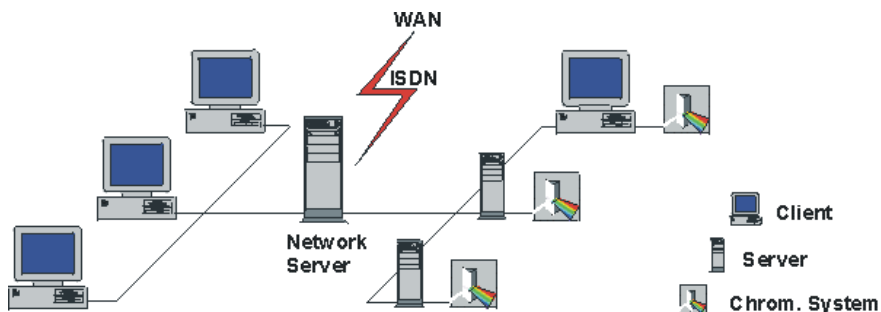


I. Starting Chromeleon

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).

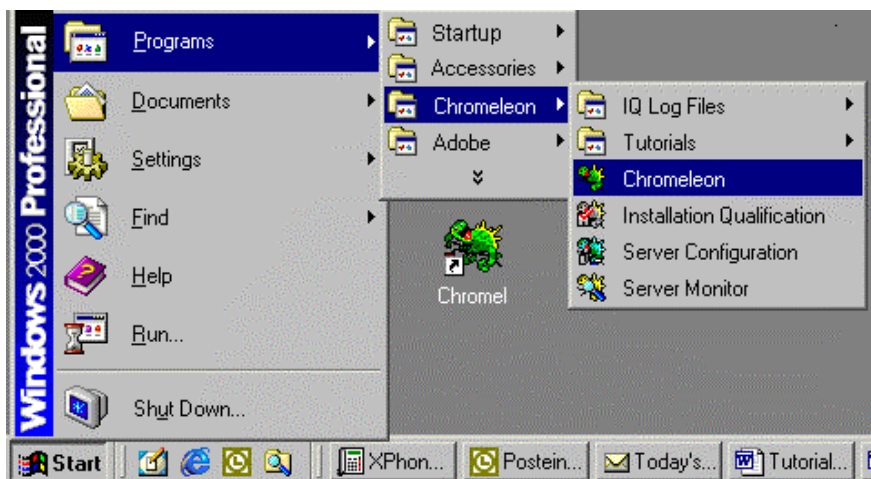
In addition, remote client PCs can be connected to the chromatography server on the company network. You can then perform client functions on a PC that is not connected directly to the chromatography system but to the server PC on the network.

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.

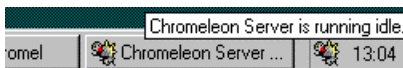
- Select **Server Monitor** to start the **Server Monitor** program. The Chromeleon icon appears on the Windows task bar next to the Windows system clock.



**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. The Browser

The Browser window loads automatically whenever Chromeleon is started.

Opening the Browser

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

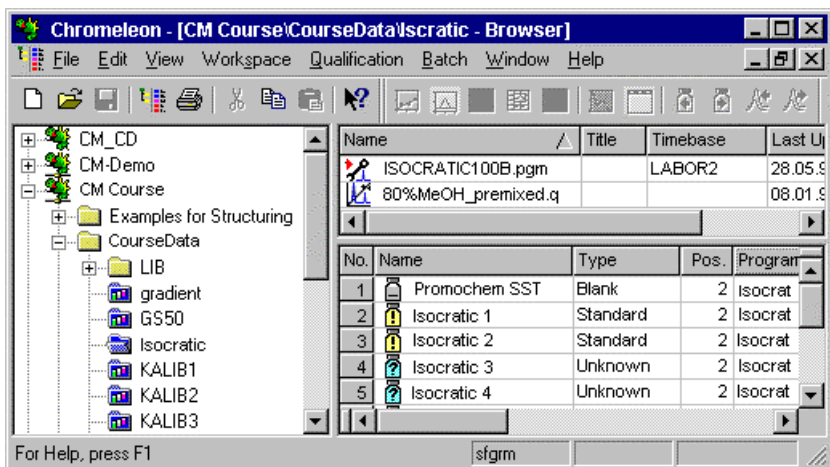
Browser Appearance and Functions

The Browser, which is the "control room" of Chromeleon, serves for data management, storage, and retrieval. The Browser's appearance and functions are similar to the Windows Explorer.

Caution:


Although the Browser is very similar to the Windows Explorer, you should not confuse these two windows! Do not use the Windows Explorer for operations within the Chromeleon datasources! Usually, your administrator will prohibit these operations!

The left window section shows a tree structure with different levels. The right window section shows detailed information about the currently selected item.







The → *Datasources*, for example, ComputerName_local , are the top level of the structure and serve to store data and results.

- Click the + or - character next to a datasource to expand or collapse the corresponding directory structure (yellow folders).








The lowest levels contain the → *Sequences* (blue folders: ) created by the user. During installation, one directory is created. 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** to display its subdirectories in the right window section.
- Select a **sequence** to view files and samples contained in the sequence.

When you click a sequence, the upper part of the right windows displays the files contained in the sequence. 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 lower part of the right window displays the sample list showing the individual samples of the sequence. The different icons indicate up to seven different 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

Opening Files

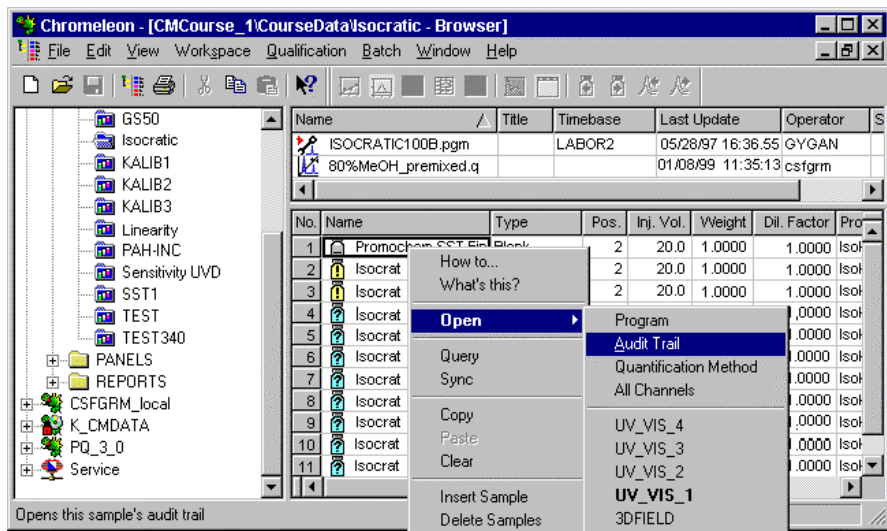
Double-click to open a file name in the Browser. Chromeleon loads the appropriate chromatographic environment according to the file type:

- 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, consider the following (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 of the **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, <Ctrl> <C>, to copy the element or <Ctrl> <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:

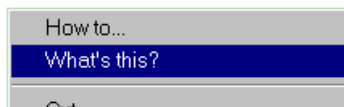
- → *Context-sensitive Help* answers your questions that may occur at the current cursor position.
- → *Systematic Help* allows you to
 - Select an interesting topic on the Contents tab page
 - Search the index for any required information
 - Search for specific words and phrases in help topics by searching 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 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

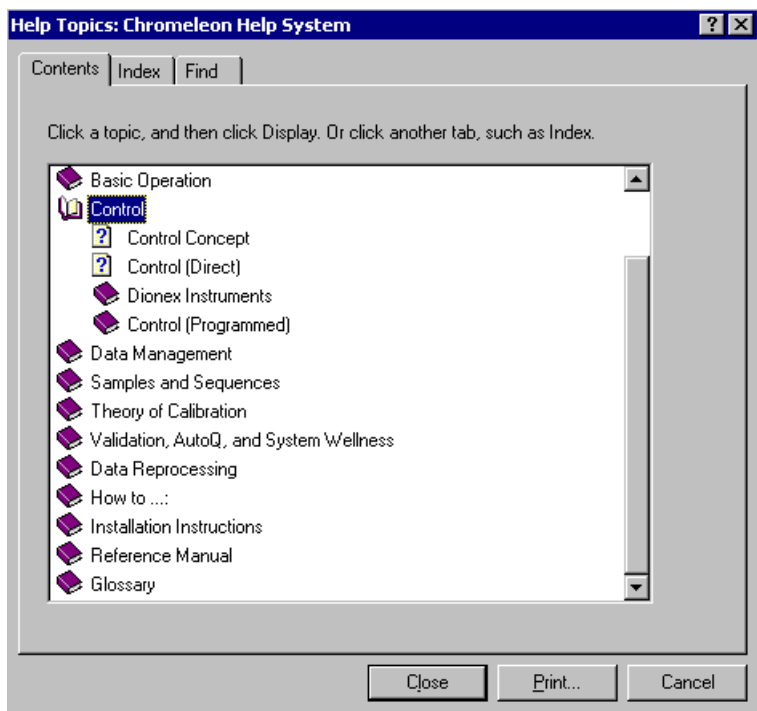
Select **Index** on the **Help** menu for an overview of the topics that are available in the online Help. The help window provides the following tabs:

- **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.



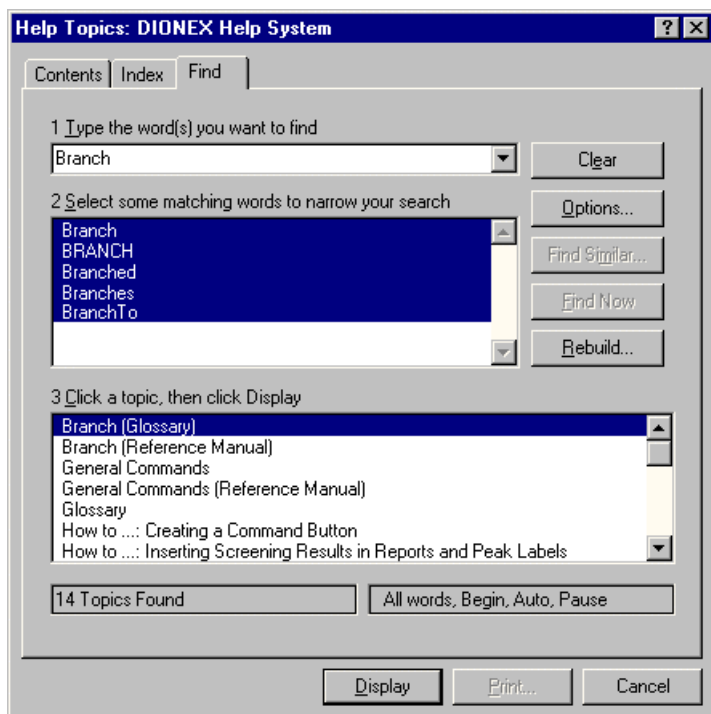
Index Tab

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 the desired 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:



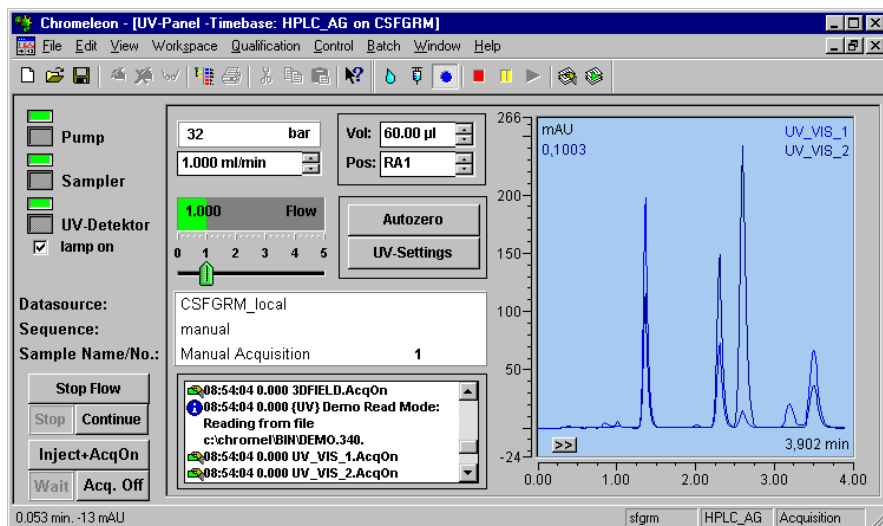
IV. Control

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.



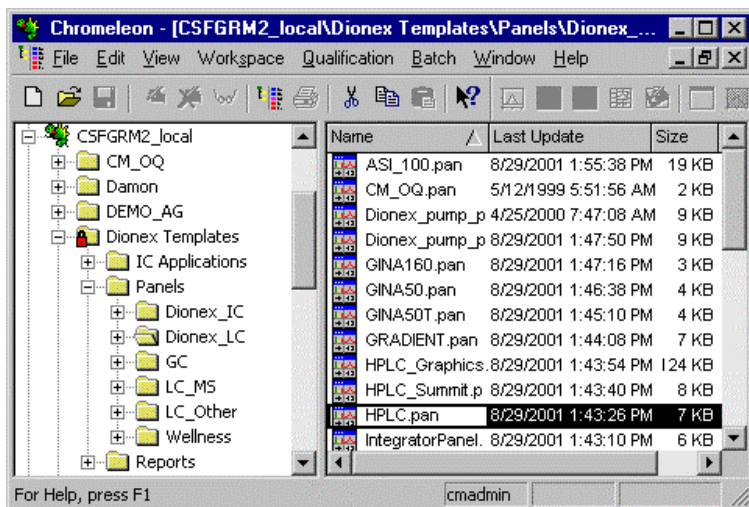
The above image shows a control panel for controlling the basic functions of a pump, an autosampler, and a two-channel UV detector. On the right, you see the detector signal.

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

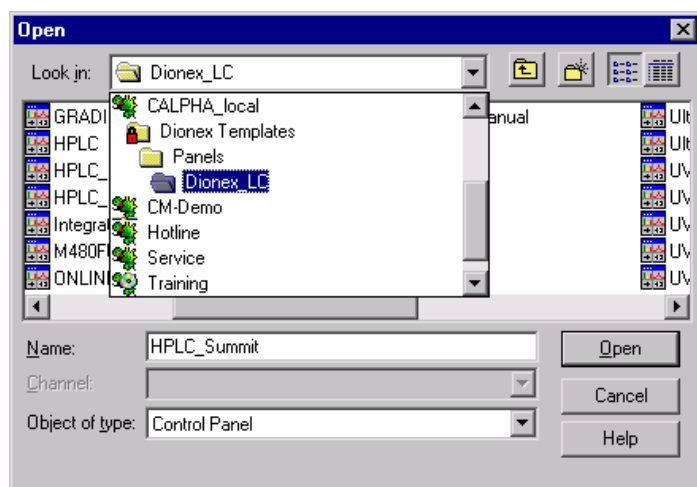
From the Browser

- 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.

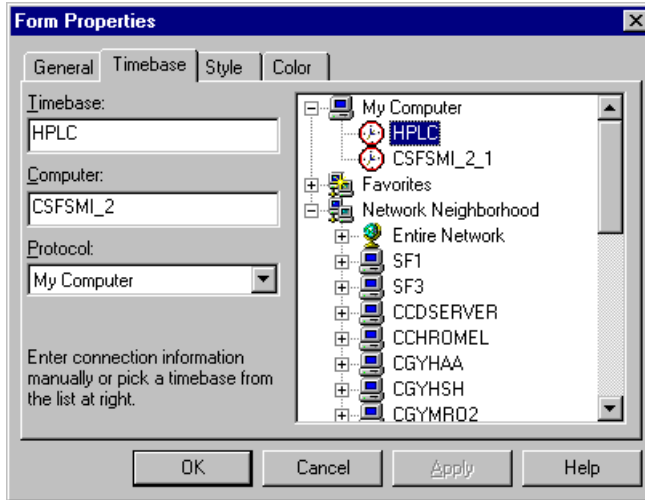
As soon as a control panel has been opened, Chromeleon tries to connect to the → *Timebase* that is linked to this control panel.

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 the desired 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, 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 "..."

⇒ 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 "..."

⇒ 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.



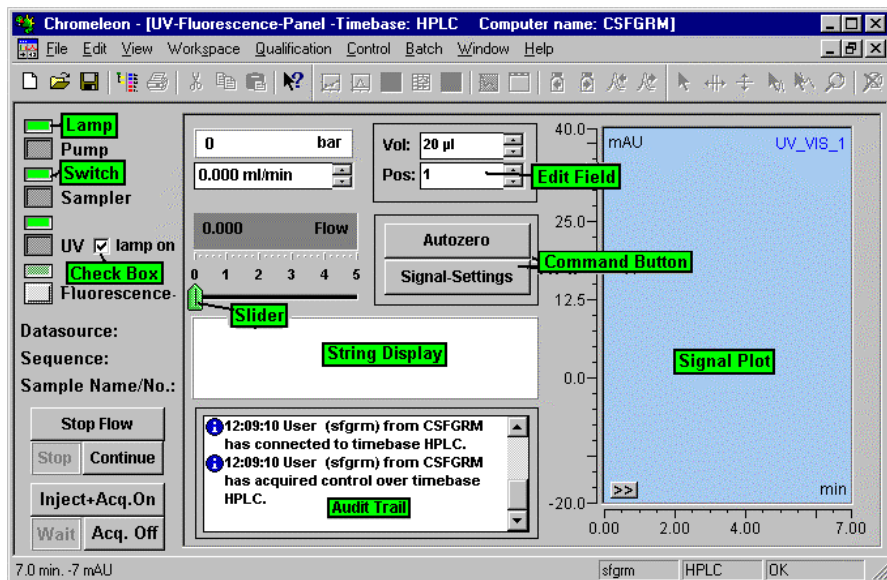
Tip:

*Have a look at the **Audit Trail** entries. They often provide precious 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 → *Control File (PGM File)*.

2. The Control File (PGM File)

Objectives of this chapter:

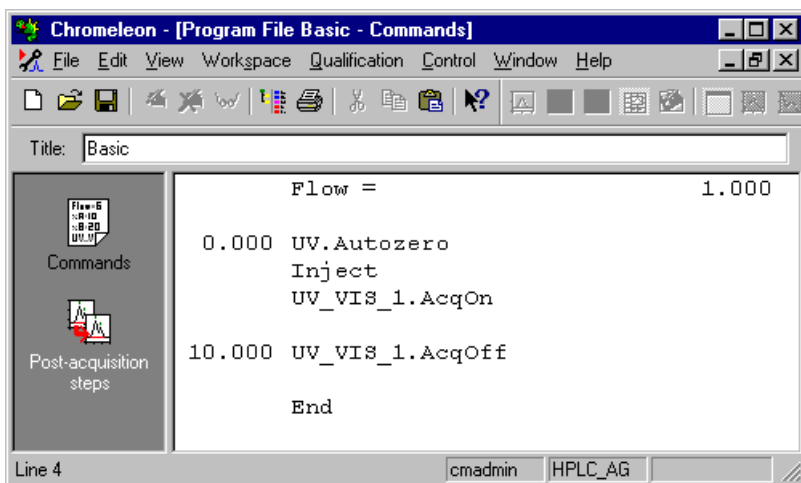
- To describe the definition and purpose of the program
- To introduce the Program Wizard that guides you through program creation
- To describe the functions for program reprocessing

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.

Control File Properties

For a very simple control file, the PGM Editor could look like this, for example:



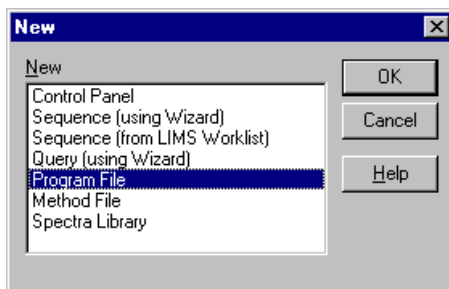
In the **Command** view, the various commands of the PGM File are listed on the right below the **Title** line. The commands are assigned to the corresponding retention time. For information about how to create a program, refer to the following sections.

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:

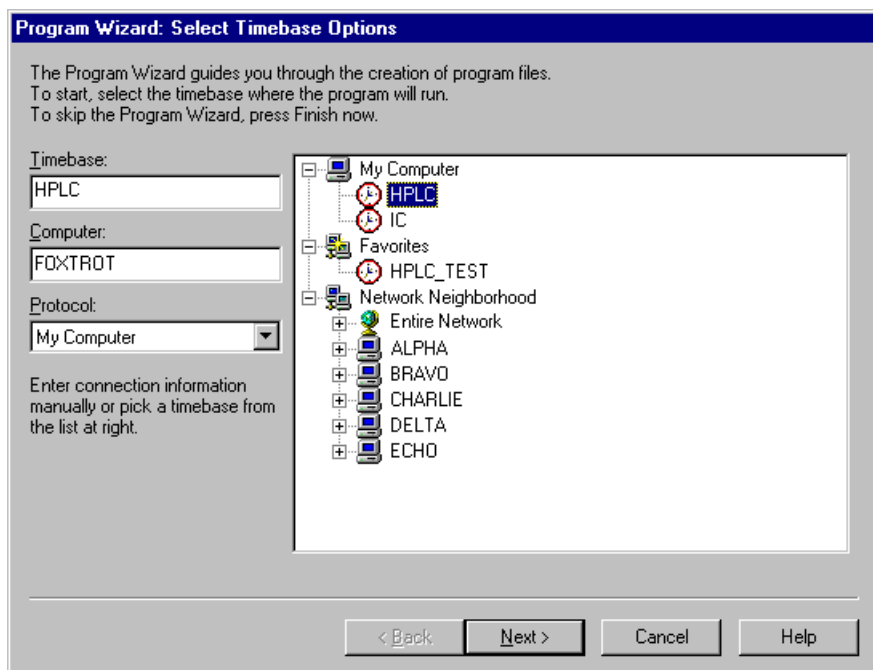
- P680 Pump
- ASI-100 Autosampler (without cooling option) or AS50 Autosampler
- TCC-100 Thermostatted Column Compartment
- UVD340U Photodiode Array Detector

For more information about each Wizard page, 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 the desired 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 made on the different wizard pages.

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

Use this Wizard page to set the nominal temperature and the upper and lower temperature limits:

Program Wizard: ColumnOven Options

☒ Use Temperature Control

Temperature: [5.0...85.0 °C]

Lower Limit: [5.0...85.0 °C]

Upper Limit: [5.0...85.0 °C]

Equilibration Time: [0.0...30.0 min]

Ready Temp Delta: [0.0...5.0 °C]

Leak Detection

Humidity Leak Sensor:

Gas Leak Sensor:

Column

MSV Position:

Active Column:

☒ Link MSV position changes and active column changes

Active Column: Column_A

Temp_LowerLimit: 5.0 °C

Temp_UpperLimit: 85.0 °C

Pressure_UpperLimit: 400.00 bar

ProductId: 059133

BatchNumber: 018-01-026

DateOfManufacture: 15 Apr 2003

Manufacturer: Dionex

Description: Acclaim 120 C

Diameter: 4.60 mm

Length: 150.00 mm

ParticleSize: 3.00 µm

PackingMaterial: (not defined)

VoidVolume: 1750.0 µl

pH_UpperLimit: 8.0

pH_LowerLimit: 2.0

DateOfExpiration: (not defined)

Injections_Limit: 50000

< Zurück Weiter > Abbrechen Hilfe

Step 3: Pump Options (here: Dionex P680)

Use this Wizard page to 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:

Solvents

Name	Type	Start	End
%A: <input type="text" value="%A"/>	<input type="text" value="Automatic"/>		
%B: <input type="text" value="%B"/>	<input type="text" value="Automatic"/>	<input type="text" value="0.0"/> %	<input type="text" value="0.0"/> %
%C: <input type="text" value="%C"/>	<input type="text" value="Automatic"/>	<input type="text" value="0.0"/> %	<input type="text" value="0.0"/> %
%D: <input type="text" value="%D"/>	<input type="text" value="Automatic"/>	<input type="text" value="0.0"/> %	<input type="text" value="0.0"/> %

Pressure Limits

Lower Limit: [0...400 Bar]

Upper Limit: [0...400 Bar]

Total Flow

Start: ml/min

End: 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, an additional Wizard page is opened when you click **Next**.

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:

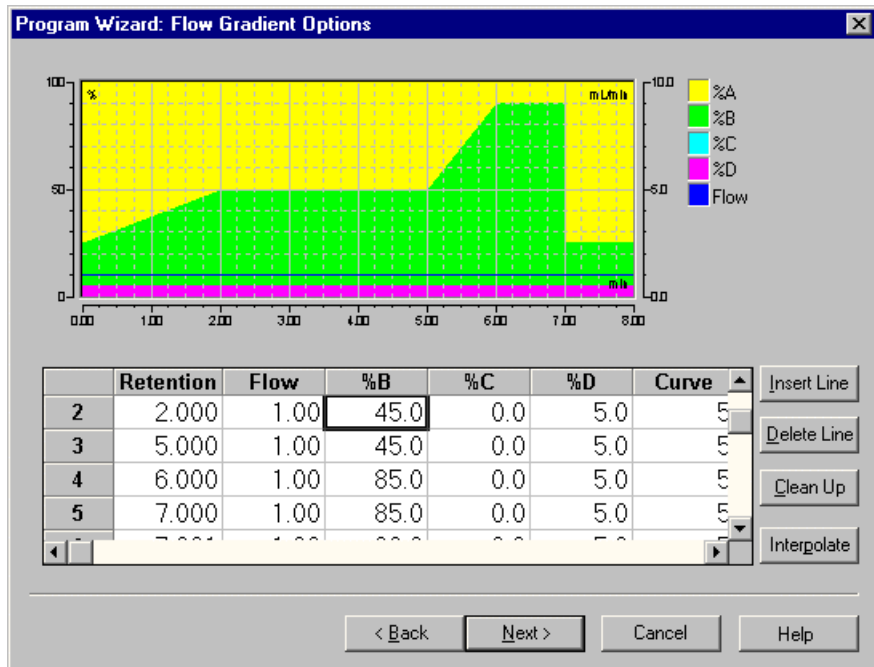
- Retention time
- Flow
- Solvent composition



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)

Use this page of the Wizard to determine the autosampler options:

Program Wizard: Sampler Options

Syringe speed and sample dependent parameters:

Dispense Speed:	10.00	[0.01...62.50 $\mu\text{L/s}$]	(default: 10.00 $\mu\text{L/s}$)
Draw Speed:	10.00	[0.01...62.50 $\mu\text{L/s}$]	(default: 10.00 $\mu\text{L/s}$)
Sample Height:	0.50	[0.00...99.00 mm]	(default: 0.50 mm)
Syringe Delay:	5	[0...300 s]	(default: 5 s)

Needle movement speeds:

Up Speed:	10.00	[0.10...25.00 mm/s]	(default: 10.00 mm/s)
Down Speed:	10.00	[0.10...25.00 mm/s]	(default: 10.00 mm/s)
Radial Speed:	20.00	[0.10...25.00 mm/s]	(default: 20.00 mm/s)

☒ Synchronize injection with pump.

< Back Next > Cancel Help

- 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:

Program Wizard: Sampler Options

Temperature

Column Temperature: 15

Tray Temperature: 10

☒ Wait for stable temperature

Cycle Time: 10

Syringe Speed: 3

Sample Needle Height: 2

Cut Volume: 0

Flush Volume: 100

- 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:

#	Function	Parameters
1	Pipet	CurrentVial 20.0 μ L to CurrentVial
2	Mix	CurrentVial 250.0 μ L x 5
3	Flush	250.0 μ L
4	Delay	0.5 min
5	Needle	2 mm
6	Dilute	CurrentVial 10.0 μ L + Reservoir_A
7	Dispense	Reservoir_A 1000.0 μ L to CurrentVial
...		

Function: Dispense

Source: Reservoir_A

Volume: 1000.0 μ L

Destination: CurrentVial

Buttons: Enter, Insert, Delete



Note:

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

- First, select the desired 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: especially Dionex UVD 340U)

Determine the signals to be recorded during sample processing.



Program Wizard: Acquisition Options

Acquisition Time: From: 0.000 min To: 10.000 min

Select the channels to acquire:

- ☒ ECD_1
- ☒ 3DFIELD
- ☒ UV_VIS_1
- ☒ UV_VIS_2
- ☐ UV_VIS_3
- ☐ UV_VIS_4

- 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: especially 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)).

Program Wizard: UV_VIS_1 Options

Wavelength: [200...595 nm]

Bandwidth: [1...395 nm]

Step: ☒ Auto
☐ Fixed at: [0.01...4.80 sec]

Average: ☒

Reference

Wavelength: [200...600 nm]

Bandwidth: [1...400 nm]

- 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.

Program Wizard: ECD - ECD_1 Options

Data Collection Rate: Hz

SRS
Current: mA

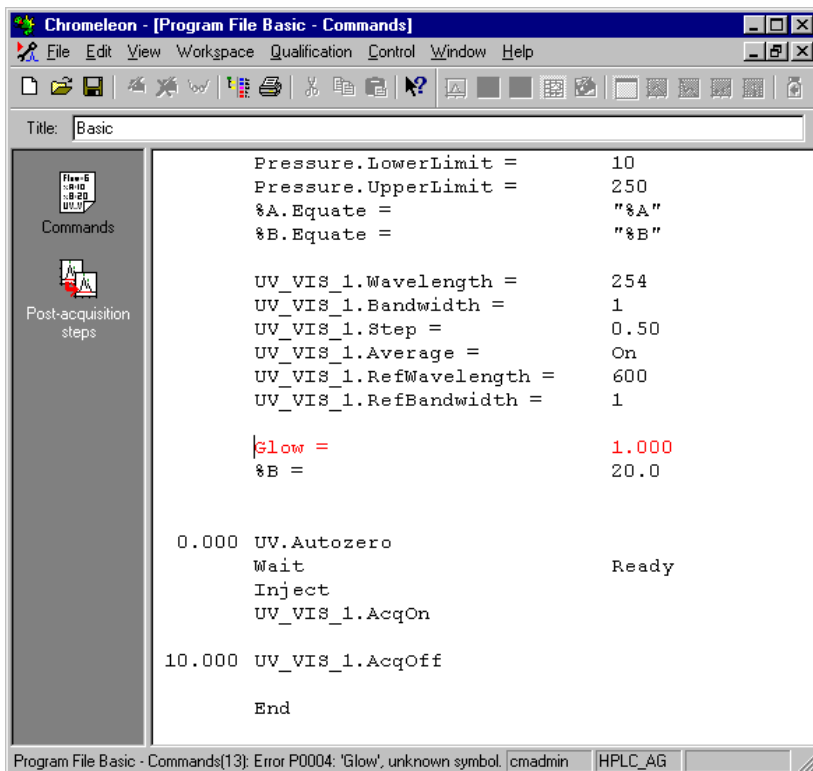
Cell

Temp Comp. %/°C

Cell Temp. °C

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 Control 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**.

The screenshot shows the Chromeleon software interface. The left pane displays a tree view of the file structure, with 'ISOCRATIC' highlighted under 'Seminars_Data'. The right pane is divided into two sections: 'Files' and 'Sample List'.

Files Section:








Name	Title	Timebase	Last Update	Operator	Size
80%MeOH.pgm		LABOR2	28/05/97 16:39:55	GYGAN	2 KB
DEFAULT.rdf			28/05/97 16:00:00		159 KB
ISOCRATIC.qpt					53 KB

Sample List Section:

No.	Name	Type	Pos.	Inj. Vol.	Program	Method	Status	Inj. Date/Time
1	Promochem SST	Blank	RA1	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 11:45:30
2	Isocratic 1	Standard	RA2	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 11:01:06
3	Isocratic 2	Standard	RA3	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 11:16:42
4	Isocratic 3	Unknown	RA4	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 11:27:18
5	Isocratic 4	Unknown					Finished	8/16/2000 11:37:54
6	Isocratic 5	Unknown					Finished	8/16/2000 11:48:30
7	Isocratic 6	Unknown	RA7	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 11:59:06
8	Isocratic 7	Unknown	RA8	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 13:09:43
9	Isocratic 8	Unknown	RA9	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 13:20:19
10	Isocratic 9	Validate	RA10	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 13:30:55
11	Isocratic 10	Validate	RA11	20.00	80%MeOH	ISOCRATIC	Finished	8/16/2000 13:41:31

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	<p>Sample type.</p> <p>Unknown: unknown sample (symbol in front of the sample name: )</p> <p>Standard: calibration sample ()</p> <p>Validate: sample for validating the calibration ()</p> <p>Blank: blank value (usually without injection) ()</p> <p>Matrix: blank value of the sample matrix ()</p> <p>Spiked: spiked sample ()</p> <p>Unspiked: unspiked sample ()</p>
Position	Sample position in the autosampler.
Inj. Vol.	Injection volume in µl
Program	→ <i>Control File (PGM File)</i>
Method	→ <i>Quantification Method File (QNT Method)</i>
Status	<p>Sample status</p> <p>Single: The sample has not yet been processed.</p> <p>Multiple: The sample can be analyzed several times (the old sample will be overwritten).</p> <p>Finished: The sample has already been processed.</p> <p>Running: The sample is currently being processed (green background).</p> <p>Interrupted: The sample was interrupted during processing.</p>
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.

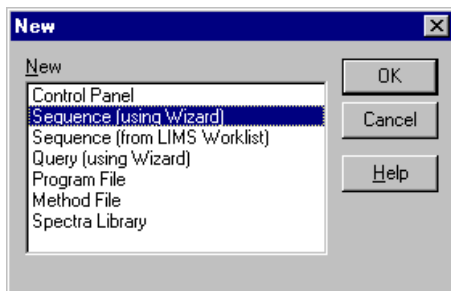
1.a) Creating a New Sequence

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

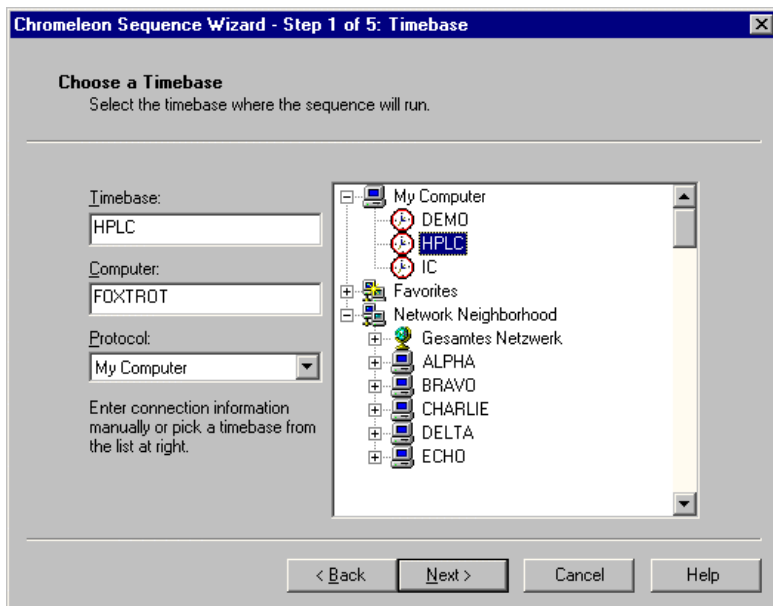
- Select **New** on the **File** menu.
- From the list, select **Sequence (using Wizard)**.
- Click **OK** to start the Sequence Wizard.



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.

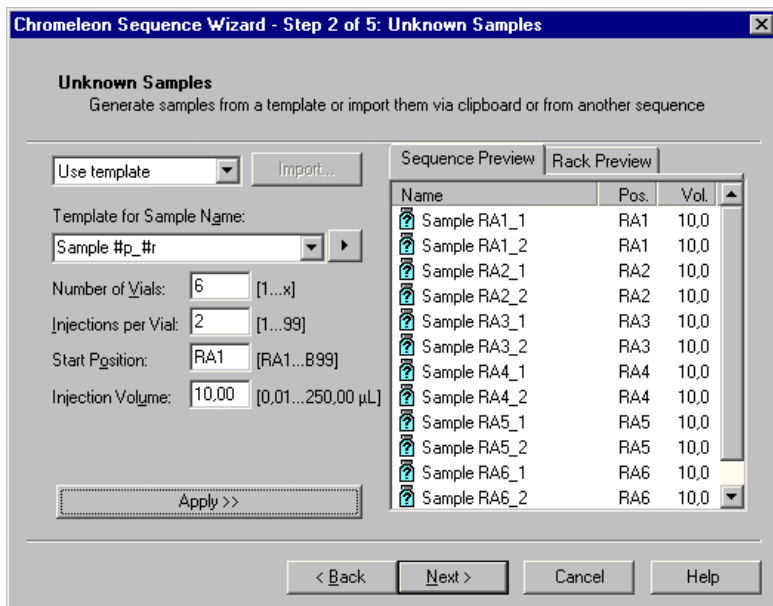
Step 1

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.

Chromeleon Sequence Wizard - Step 3 of 5: Standard Samples

Standard Samples
Generate standards from a template or import them via clipboard or from another sequence

Use template

Template for Sample Name:
Standard #p_#r

Number of Vials: 3 [1...x]

Injections per Vial: 2 [1...99]

Start Position: R99 [RA1...B99]

Injection Volume: 10.00 [0.01...250.00 µL]

☒ Variation: 1 Standard(s)
after each 4 Unknown(s)

Sequence Preview Rack Preview

Name	Pos.	Vol.
Standard R99_1	R99	10.0
Sample RA1_1	RA1	10.0
Sample RA1_2	RA1	10.0
Sample RA2_1	RA2	10.0
Sample RA2_2	RA2	10.0
Standard R99_2	R99	10.0
Sample RA3_1	RA3	10.0
Sample RA3_2	RA3	10.0
Sample RA4_1	RA4	10.0
Sample RA4_2	RA4	10.0
Standard G99_1	G99	10.0
Sample RA5_1	RA5	10.0

< Back Next > Cancel Help

- 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.

The screenshot shows the 'Chromeleon Sequence Wizard - Step 4 of 5: Methods & Reporting' dialog box. It has a title bar with a close button. The main area is titled 'Methods & Reporting' with a subtitle 'Specify a method or select a pre-defined template, and indicate reporting preferences.' There are three main sections: 1. 'Applications (includes method)' with a checkbox 'Use Application Template' and a dropdown menu. 2. 'Method Files' with 'Program:' and 'Quantification Method:' dropdowns, each with a 'Browse...' button. Below these is a note: 'The program and quantification files will be imported (copied) into the new sequence.' 3. 'Preferred Reporting Options' with 'Preferred Report:' and 'Preferred Channel:' dropdowns, each with a 'Browse...' button. Below these is a note: 'These settings are used when you print a report for the sequence or double-click a sample.' At the bottom are four buttons: '< Back', 'Next >', 'Cancel', and 'Help'.

Chromeleon Sequence Wizard - Step 4 of 5: Methods & Reporting

Methods & Reporting
Specify a method or select a pre-defined template, and indicate reporting preferences.

Applications (includes method)
☐ Use Application Template

Method Files
Program: CALPHA_local\Programs\gradient Browse...
Quantification Method: CALPHA_local\Methods\gradient Browse...
The program and quantification files will be imported (copied) into the new sequence.

Preferred Reporting Options
Preferred Report: CALPHA_local\REPORTS\defltdad Browse...
Preferred Channel: UV_VIS_1
These settings are used when you print a report for the sequence or double-click a sample.

< Back Next > Cancel Help

- 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:

Chromeleon Sequence Wizard - Step 4 of 5: Methods & Reporting

Methods & Reporting
Specify a method or select a pre-defined template, and indicate reporting preferences.

Applications (includes method)

☒ Use Application Template Suppressor Type: SRS

AminoPac PA10 Test Chromatogram
AminoPac PA10 Test Chromatogram
AS10 Production Test Chromatogram
AS11 Production Test Chromatogram
AS11 Production Test Chromatogram
AS12A Production Test Chromatogram
AS12A Production Test Chromatogram
AS14 Production Test Chromatogram

Browse...
Browse...

The program and quantification files will be imported (copied) into the new sequence.

Preferred Reporting Options

Preferred Report: CALPHA_local\REPORTS\default Browse...

Preferred Channel: ECD_1

These settings are used when you print a report for the sequence or double-click a sample.

< Back Next > Cancel Help

- 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.

The screenshot shows a Windows-style dialog box titled "Chromeleon Sequence Wizard - Step 5 of 5: Saving the Sequence". The main heading is "Saving the Sequence" with a subtitle "Specify where to save the sequence. Press Next to save it." Below this, there are two sections: "Name & Title" and "Location". The "Name & Title" section includes a note "Remember to enter a new sequence name, unless you want to overwrite an old sequence." and two dropdown menus: "Sequence Name" (set to "PDA_01_31_2002") and "Title" (set to "optional"). The "Location" section includes two dropdown menus: "Datasource" (set to "CM_Seminar") and "Directory" (set to "Sequences"), along with a "Browse..." button. At the bottom of the dialog are four buttons: "< Back", "Next >", "Cancel", and "Help".

- 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

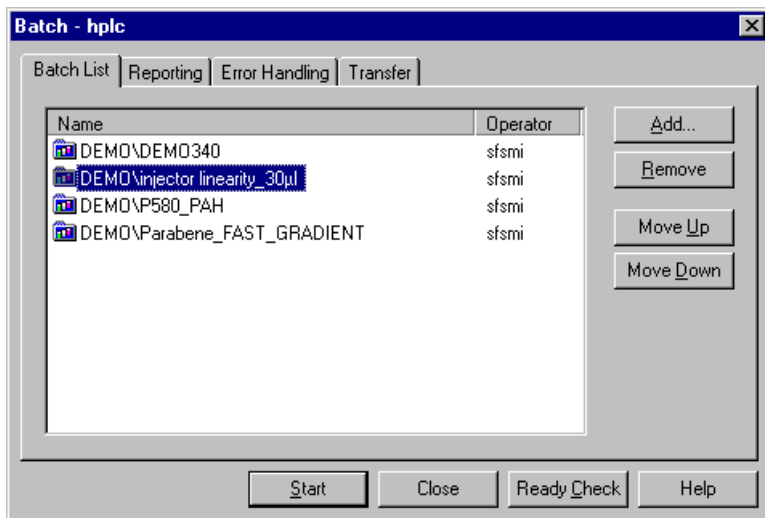
Analyzing one sample can be performed manually. For several samples, automatic sample processing is recommended. This requires a →PGM File, a →Sequence, and a →Quantification Method (if possible).

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 the desired 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

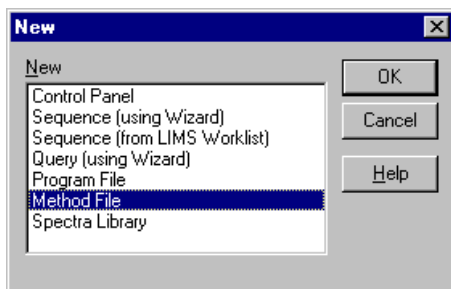
Introduction

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.

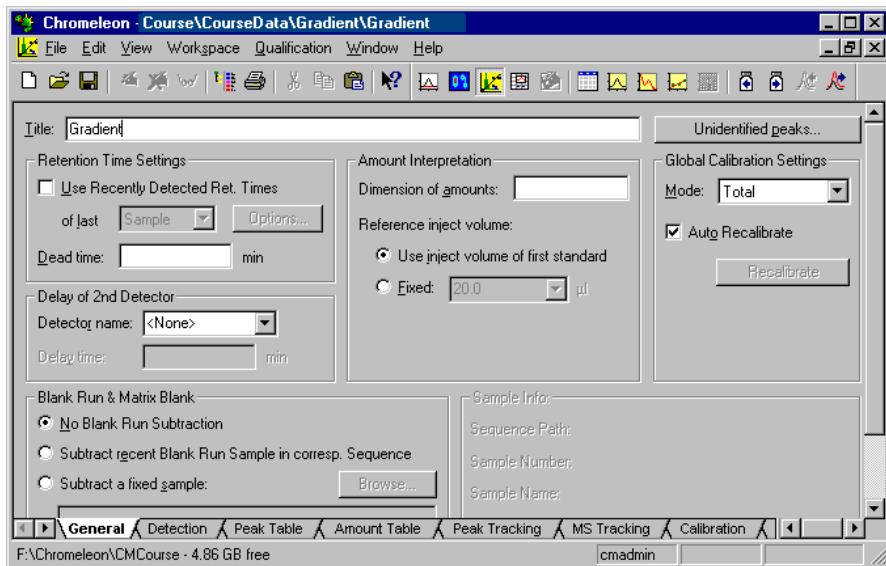
Creating a Quantification Method

- 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**, **MS Tracking**, **Calibration**, **Spectra Library Screening**, **SST**, and **MS**). Open the required worksheet by clicking the respective tab on the lower window bar.



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*) 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.



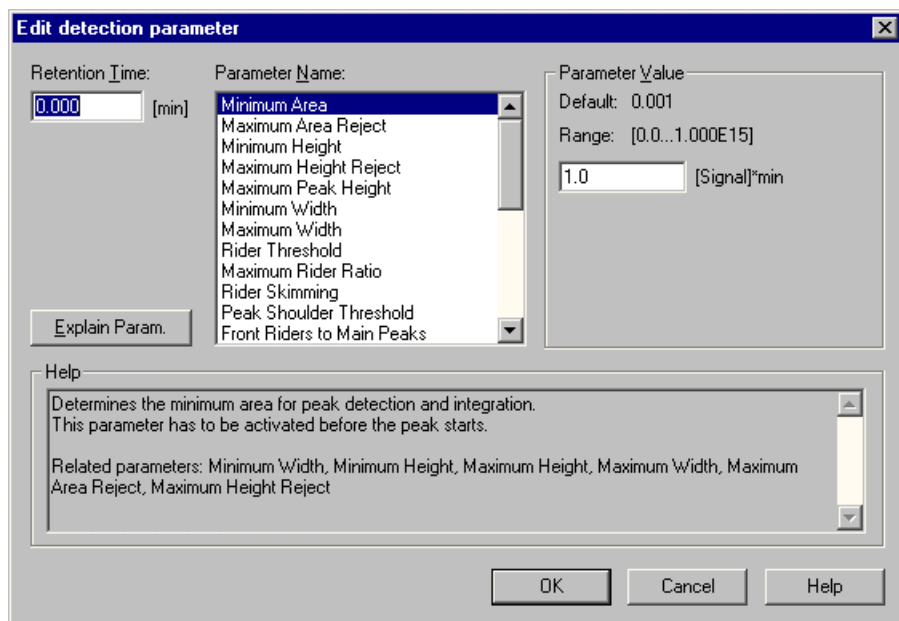
Caution:

*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:

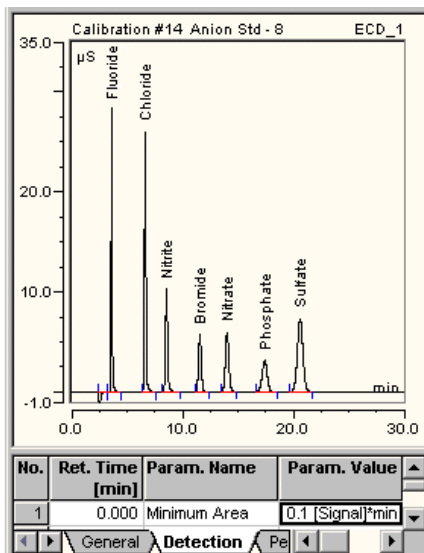
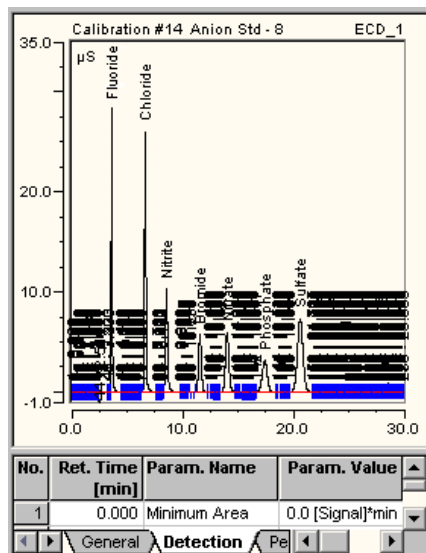


Frequently used Detection Parameter

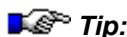
Minimum Area

Usually, the **Minimum Area** default parameter will be sufficient as filter. **Minimum Area** defines the area threshold, below which peaks are not identified 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.

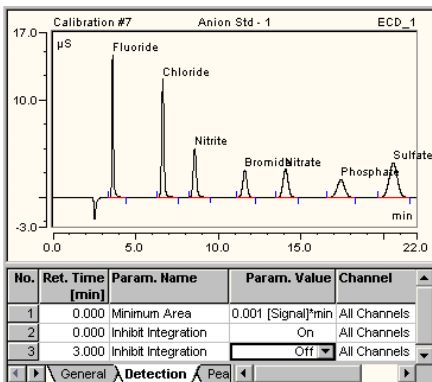
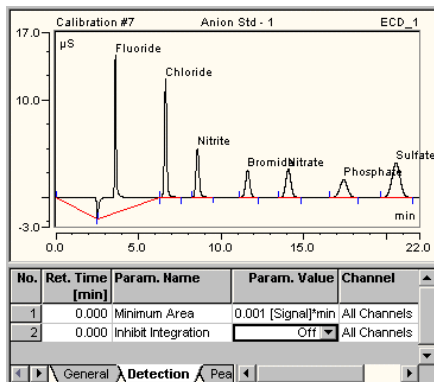


Tip:

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

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. Negative injection peaks may cause an undesired baseline; for example the **Water Dip** peak in ion chromatography. Enable the **Inhibit Integration** detection parameter to avoid this effect:

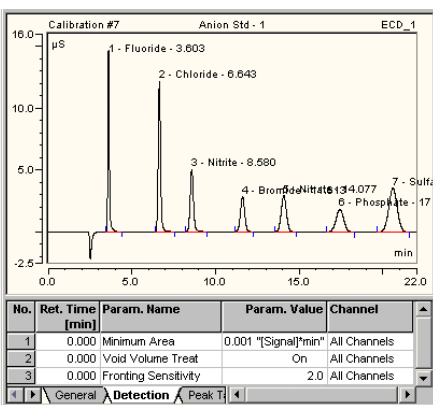
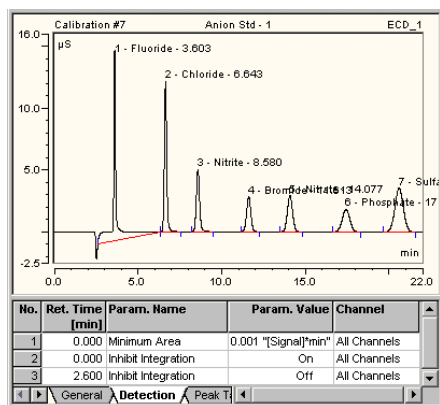


If the value is set to **On** before the first peak to be inhibited, peak detection is disabled until the parameter is disabled (**Off**), i.e., no peaks are recognized. The chromatogram is drawn on the screen but it is not integrated in this area.

Inhibit Integration 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 above example, the retention time of the negative peak is 2.513 min.)

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.

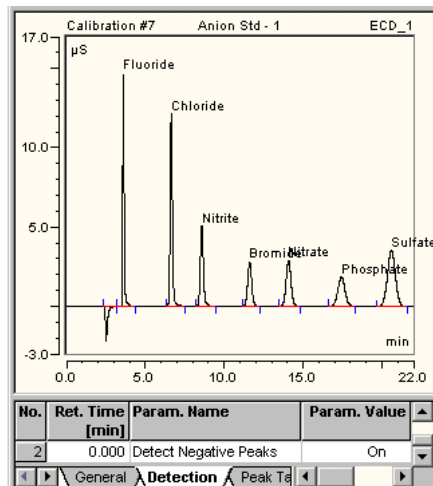
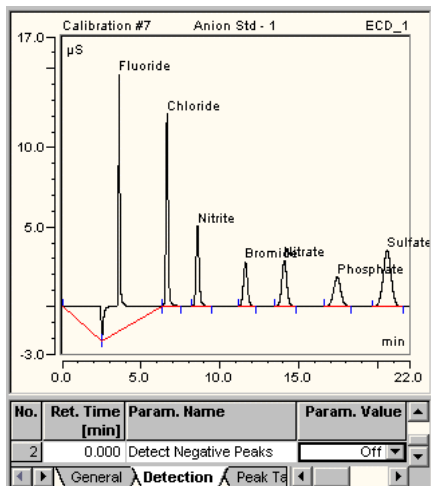
In this case, use the **Void Volume Treatment** parameter instead. If the peak start is still set too early, use the **Fronting Sensitivity** parameter to remedy the situation.

**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**.

**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.

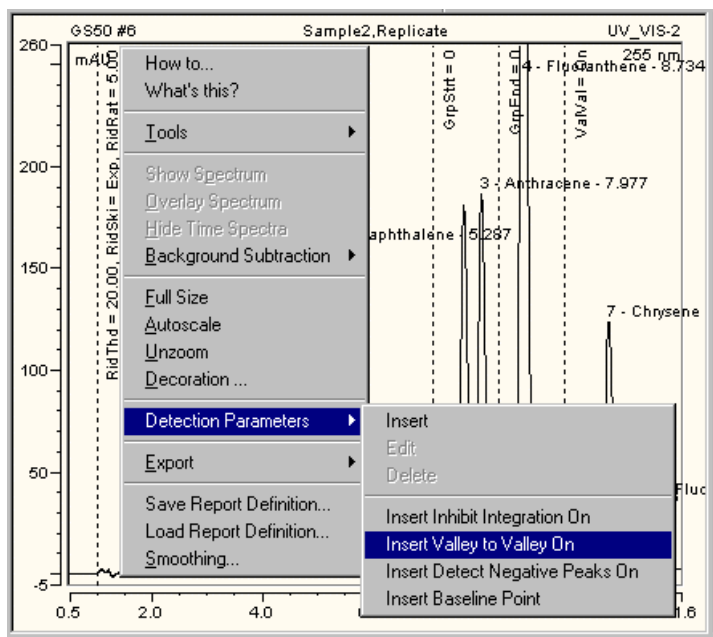
2. Graphical Input of Detection Parameters

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.

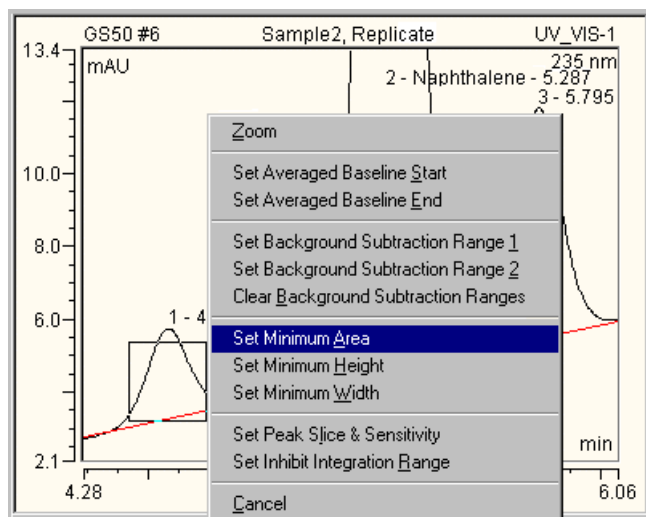
To insert a detection parameter at the position of the mouse cursor, select **Detection Parameters** on the context menu:



For example, this setting enforces valley-to-valley integration as from the selected time.

You can also enter detection parameters 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



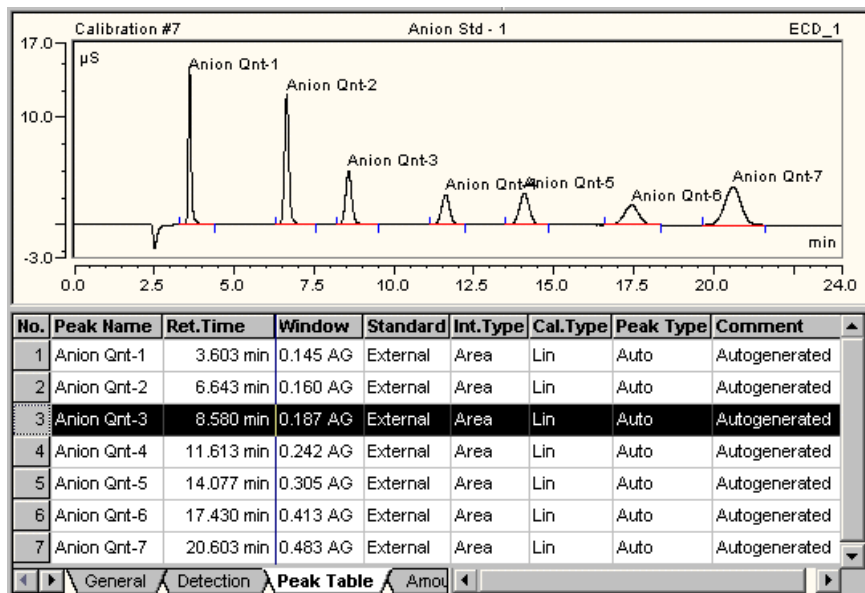
Tip:

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.

3. 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. (For more information, refer to → *Automatically generating the Peak Table*.)

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 not detected 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.)



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

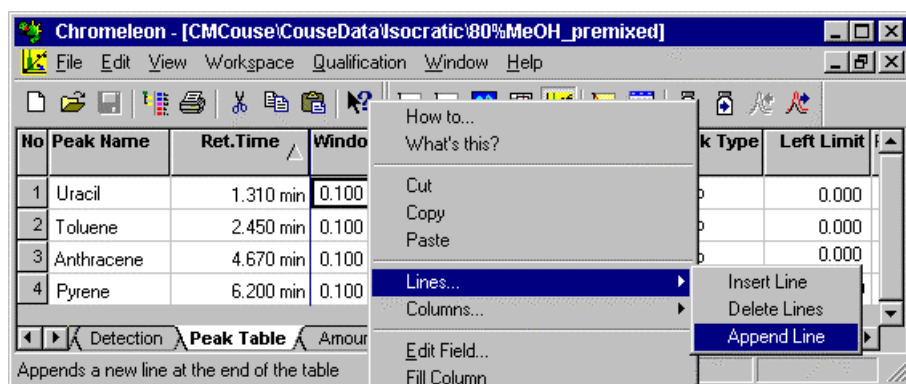
- The **Peak Names** are constructed from the name of the QNT Method, for example, in the Anion Qnt method, detected peaks will be assigned the names Anion Qnt -1, Anion Qnt -2, Anion Qnt -3, etc.
- The **Window** values are entered as absolute values (indicated by the suffix A).
- No **Group**.
- The **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).

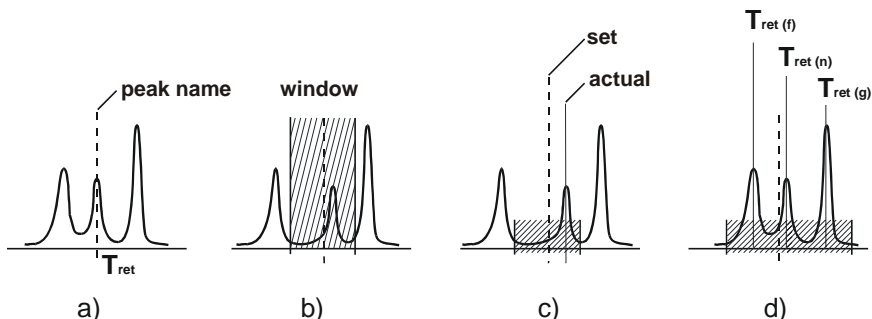
4. Identifying Peaks (Peak Table Tab)

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.



- 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.

5. 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 either a concentration value such as $\mu\text{g}/\mu\text{l}$ or an absolute value such as μg . (Enter the used dimension in the **Amount Interpretation** section on the **General** tab.)

Multi-Point Calibration

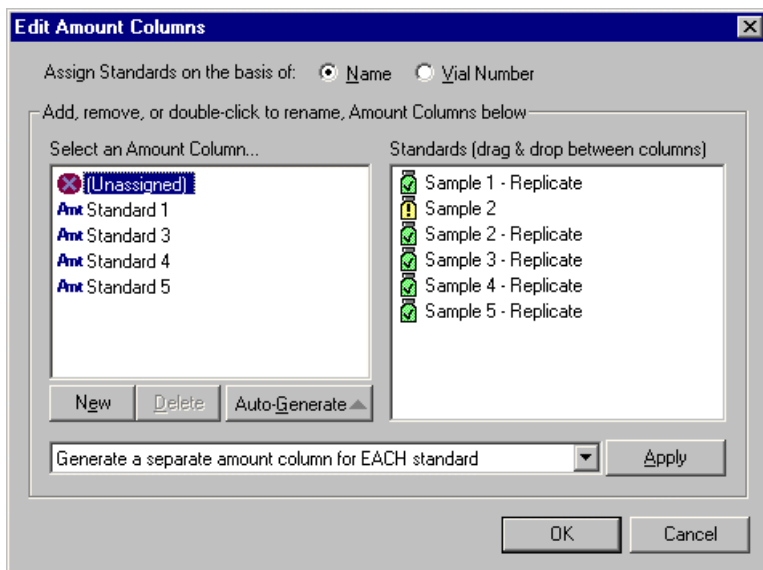
Multi-point calibration can be achieved by

- 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

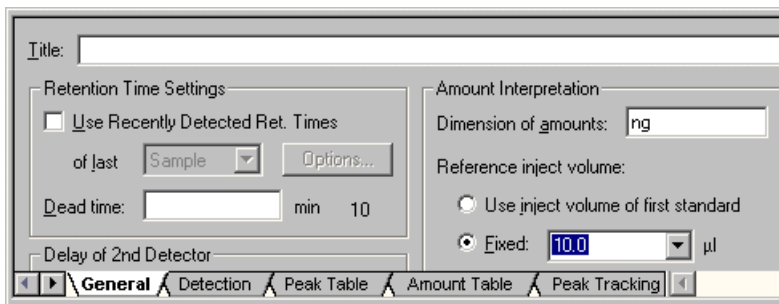
Injecting different volumes from a single sample vial

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. No *changes* are required.

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

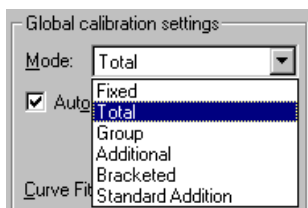
- **Calibration Type = Lin**
- **Integr. Type = Area**
- **Response Factor = 1,000**

6. 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:



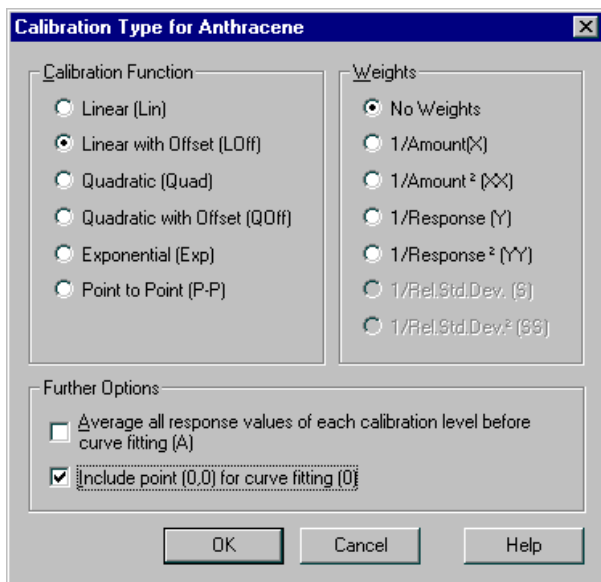
Select the **Total** mode to use all standard samples for calibrating all unknown samples. Use the **Fixed** mode to use certain standards (also from previous sequences) for calibrating all unknown samples. Select the **Standard Addition** mode to analyze unknown spiked or unspiked samples. For more information about the **Group**, **Additional** and **Bracketed** modes, see below:

Group	Name	Pos	Add.	Brack.
	Standard 1	1		
	Standard 2	2		
	Sample 1	3	←	
	Sample 2	4	←	
	Standard 3	5		
	Standard 4	6		
	Sample 3	7	←	←
	Sample 4	8	←	←
	Standard 5	9		
	Standard 6	10		

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 the desired 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/Amount²** and **1/Response²** 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:

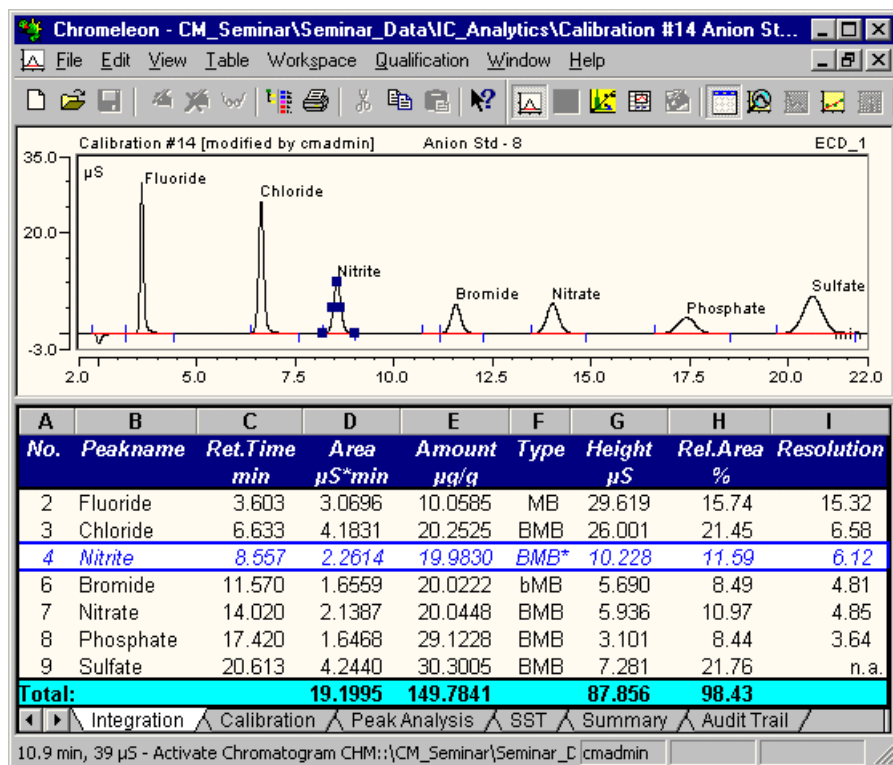
- Display a single chromatogram.
- Compare several chromatograms.
- Check the peak purity.
- Display calibration curves.
- Search single spectra in a spectra library.

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:

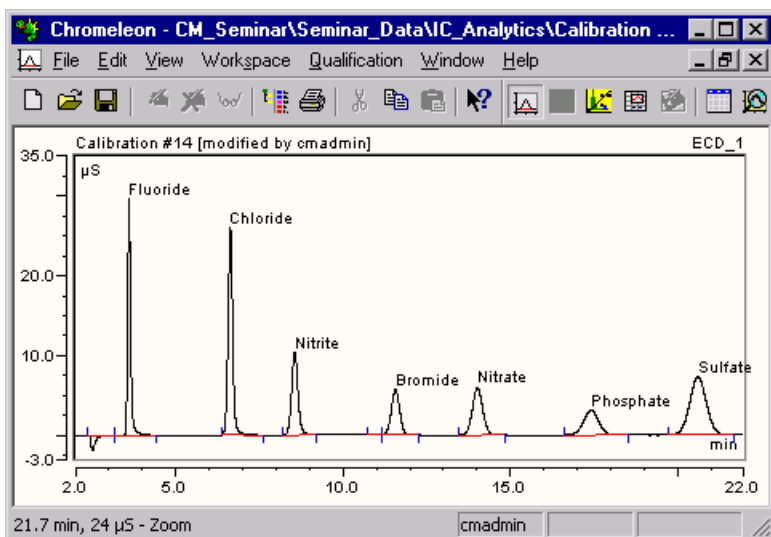
- Display the **Previous Chromatogram** or the **Next Chromatogram**
- Display the **Previous Channel** or the **Next Channel**

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:

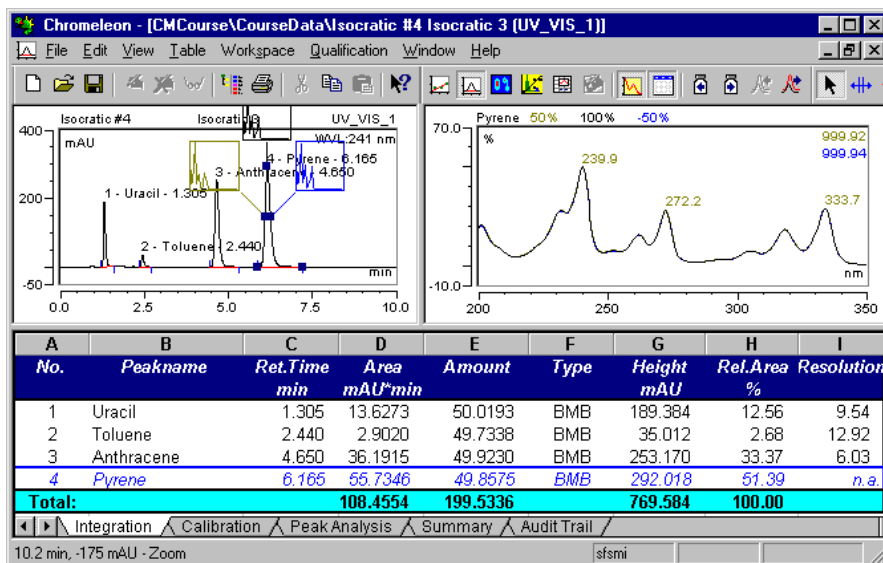


- Select **Decorations** on the context menu to change the appearance of the chromatogram. You can change the captions, axis ratios, and coloring as well as the actual chromatogram representation (display of peak heights, additional chromatograms, or grid).
- Double-click an individual peak to display its properties:

The figure is a screenshot of a dialog box titled "Properties of Peak No. 4". The dialog box contains a table of properties for a specific peak. The properties are listed on the left, and their values are shown in the right column. The values are: Component: Nitrite, Retention: 8.56 min, Width: 0.35 min, Peak Type: BMB*, Height: 10.26 μS, Area: 2.30 μS*min, and Amount: 20.056 μg/ml. On the right side of the dialog box, there are four icons: a pushpin, a magnifying glass, a trash can, and a printer.

Property	Value	Unit
Component:	Nitrite	
Retention:	8.56	min
Width:	0.35	min
Peak Type:	BMB*	
Height:	10.26	μS
Area:	2.30	μS*min
Amount:	20.056	μg/ml

- 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.

	Move left/right peak delimiter
	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 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	Area μS^*min	Height μS	Amount $\mu g/ml$	Type	Plates (EP)
		Sulfate ECD 1	Sulfate ECD 1	Sulfate ECD 1	Sulfate ECD 1	Sulfate ECD 1	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	PCID10608-1	20.573	4.279	7.363	30.55	BMB	8031
19	PCID10608-2	20.640	4.244	7.295	30.30	BMB	8074
20	PCID10608-3	20.587	4.057	6.962	28.96	BMB	7988
21	PCID10608-4	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:

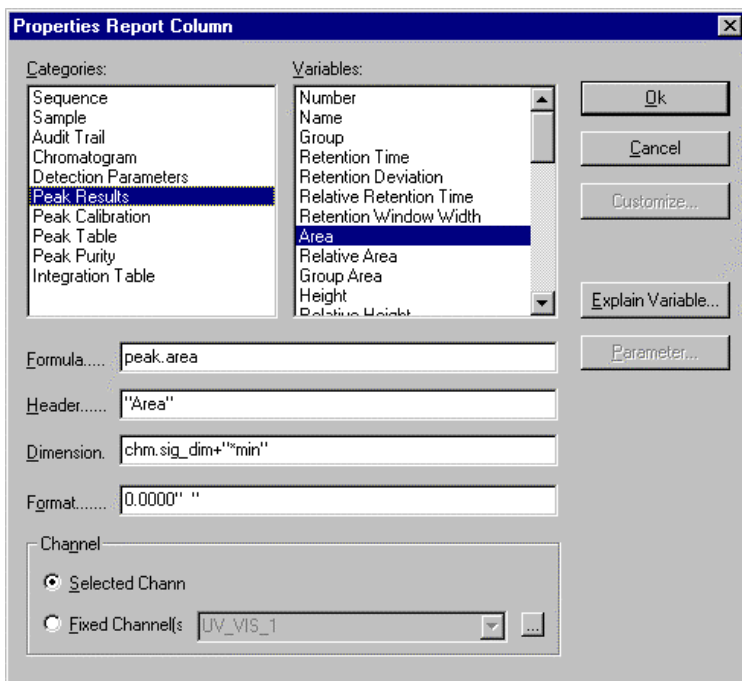
A	B	C
Day Time	Ret.Time	Command/Message
10:01:32	-2.400	Suppressor_Type = AES
10:01:32	-2.400	Suppressor_Current = 60
10:01:32	-2.400	Flow = 1.00
10:01:32	-2.400	%B = 0.0
10:01:32	-2.400	%C = 0.0
10:01:32	-2.400	%D = 0.0
10:01:32	-2.400	Curve = 5
10:01:32	-2.400	Pump_TTL_1.0v Duration = 5.00
10:03:56	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**.

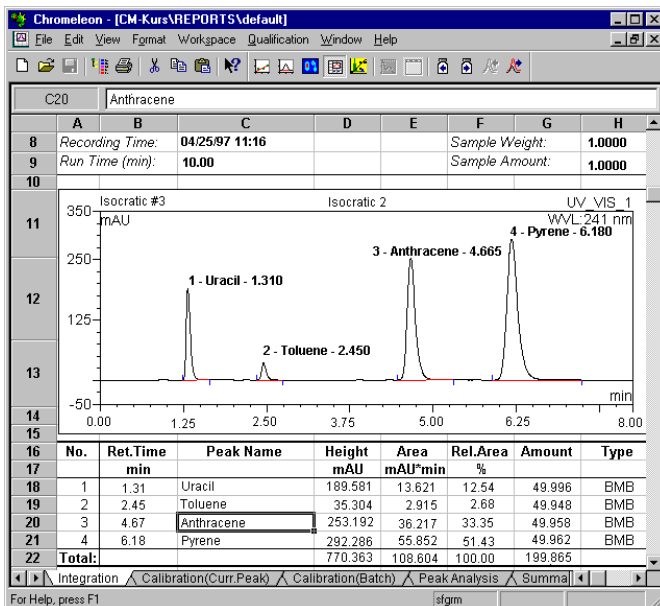


Tip:

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 DEFLTAD.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.

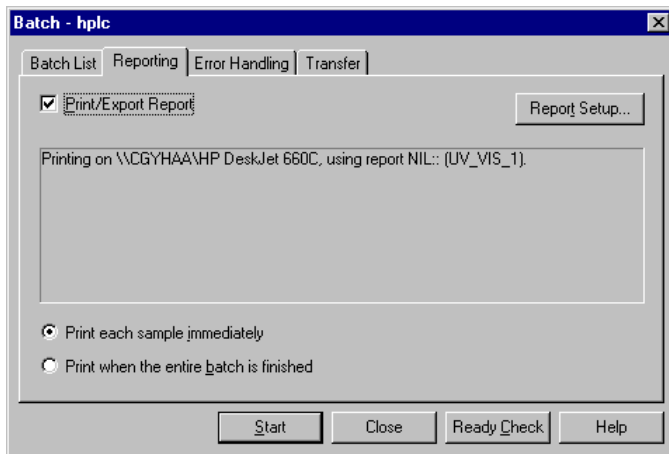
2. Printing

Printing in Automatic Batch Operation

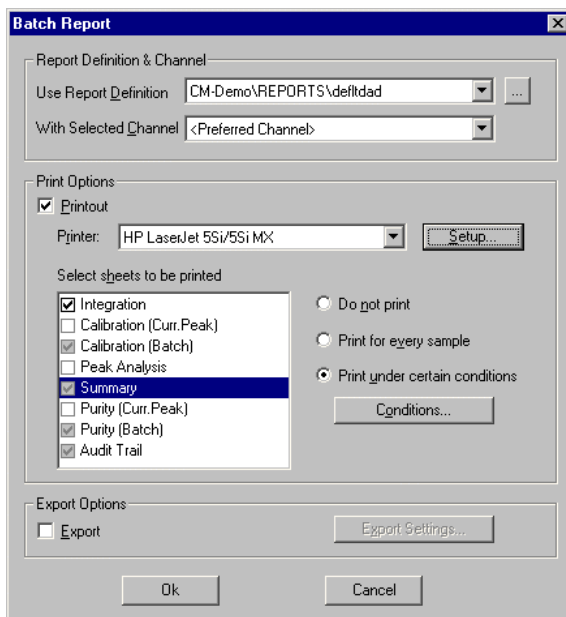
You can start printing the results during automatic sample processing (**Online Batch**). Make the corresponding settings in the **Batch** dialog box.

- 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 DEFLTAD.RDF, are available in the **Dionex Templates > Reports** directory.

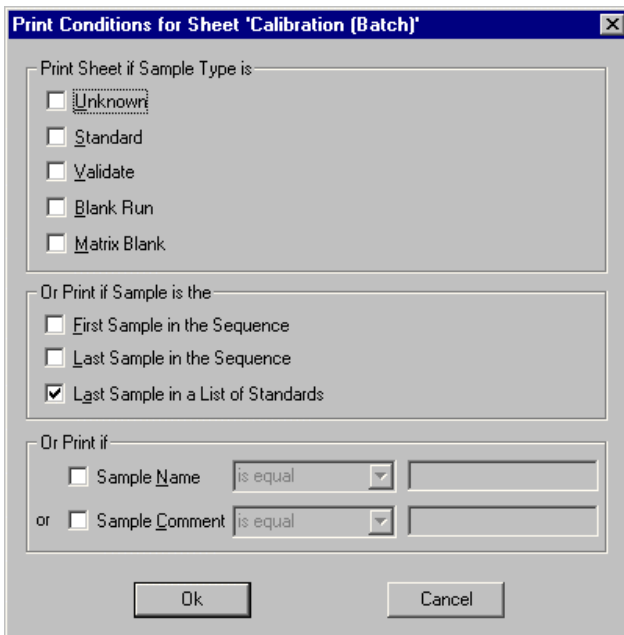
- From the **With Selected Channel** field, select the channel to print, for example, UV_VIS_1. If the field remains empty, the preferred channel is printed (see → *The Browser: Appearance and Function*).
- Select the **Printout** option to print the sheets.
- The **Printer** field shows the name of 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*

In 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>).

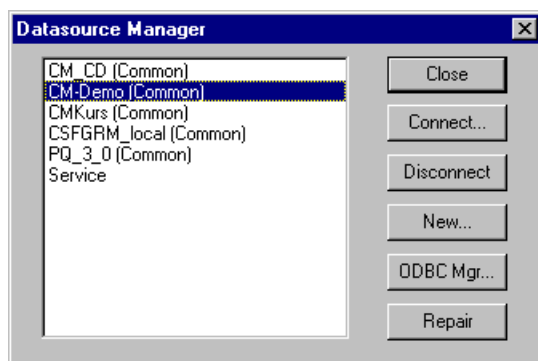
Creating additional datasources or accessing datasources on other computer in the network is often useful. Therefore, these options are supported as well. The section below describes how to create links to other datasources (For more information about how to create a new datasource,

refer to **How to ...: Actions in the Browser/Setting up a Datasource** in the User Manual and online Help).

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

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

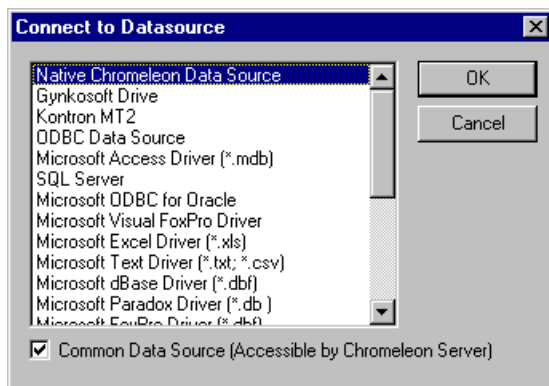
The **Datasource Manager** shows all datasources that the client can currently access.



Connecting an Existing Datasource ("Connect")

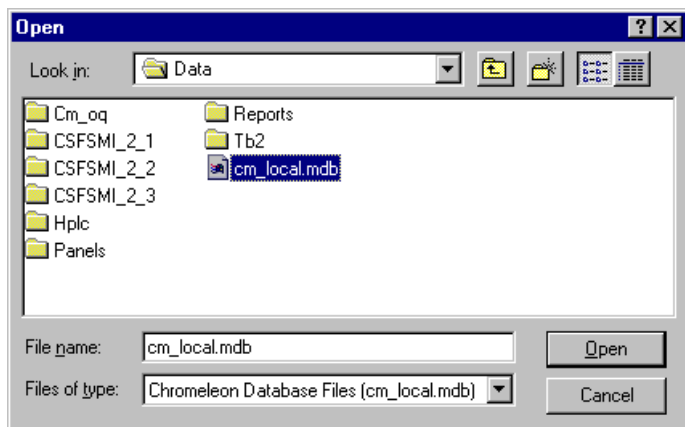
If you know the directory in which the other datasource is located, you can connect to this datasource.

- 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.



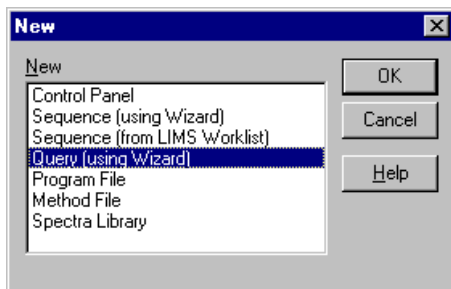
- Select the folder and click **Open**.

**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.

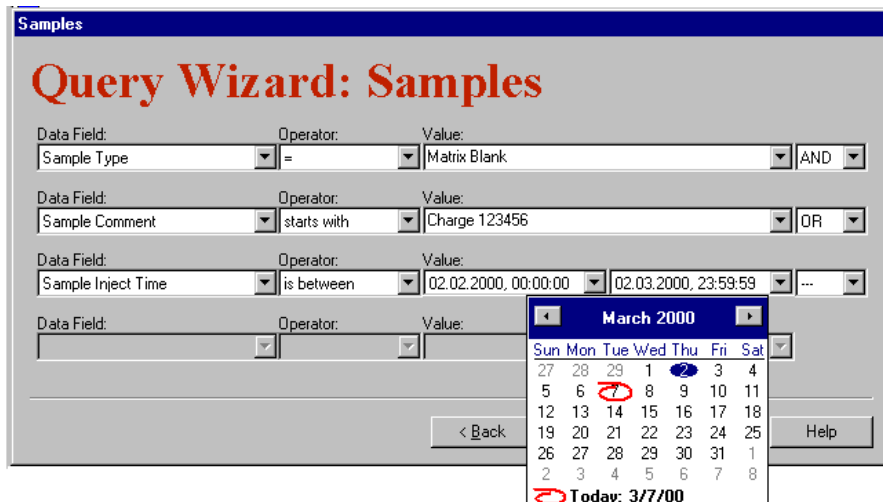
2. Finding Samples (Query)

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 to the right of 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:

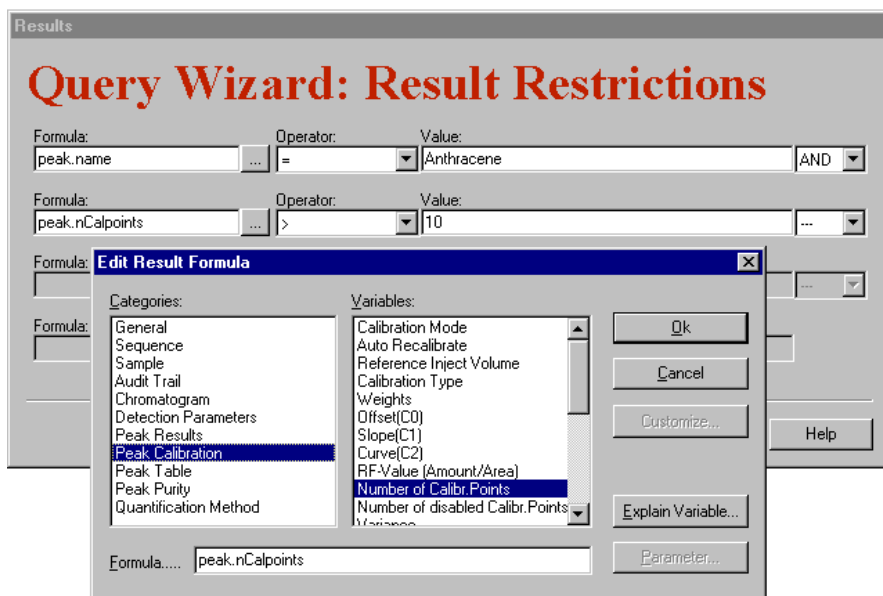


The following query searches for all samples with

- 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

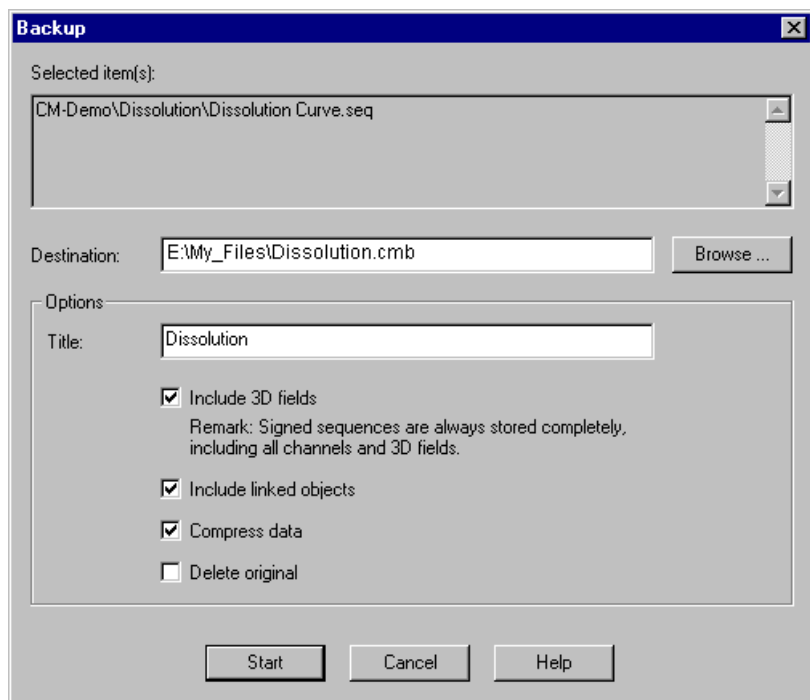


3. Backup and Restore

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.

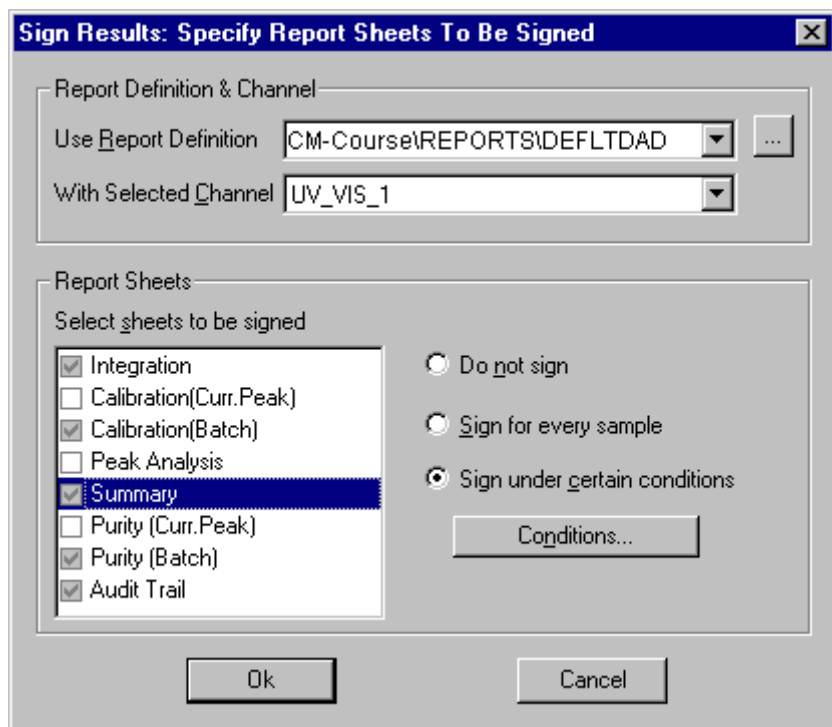


Note:

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.

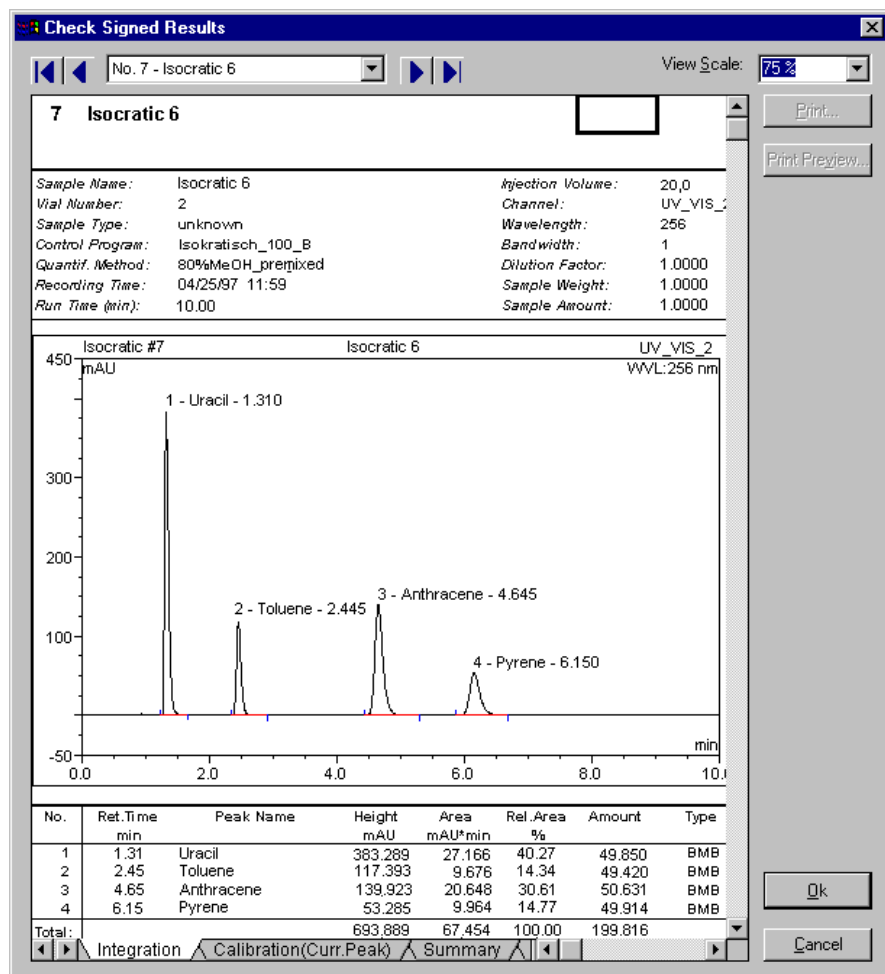
To sign a sequence proceed as follows:

- 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:

Submit Signature

Sequence
SEQ::\CDM_Seminar\Seminar_Data\Isocratic

Current Time: 25/07/2002 14:01:22 [Show Results...](#)

Signature

User ID

Signature Password [Change...](#)

Comment:

[OK](#) [Cancel](#)

- 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:

The screenshot shows a Windows-style dialog box titled "Properties of Sequence 'Isocratic'". It has four tabs: "General", "Statistics", "Signature" (which is selected), and "Report Columns". The "Signature" tab contains three sections: "Submit", "Review", and "Approve". Each section has a label, a text field for "Authorized users", a text field for the action (Submitted, Reviewed, or Approved), and a "User name" field. Each section also has an "Edit..." button and a "Details..." button. At the bottom of the dialog are four buttons: "OK", "Cancel", "Apply", and "Help".

Section	Status	Authorized users	Action	User name
Submit	Reviewed	User1,User2,Lab Manager	Submitted: 25/07/2002 14:06:57 by User2	User2
Review		Lab Manager	Reviewed: 25/07/2002 14:20:45 by Lab Manager	Lab Manager
Approve		Quality Officer	Approved:	

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.



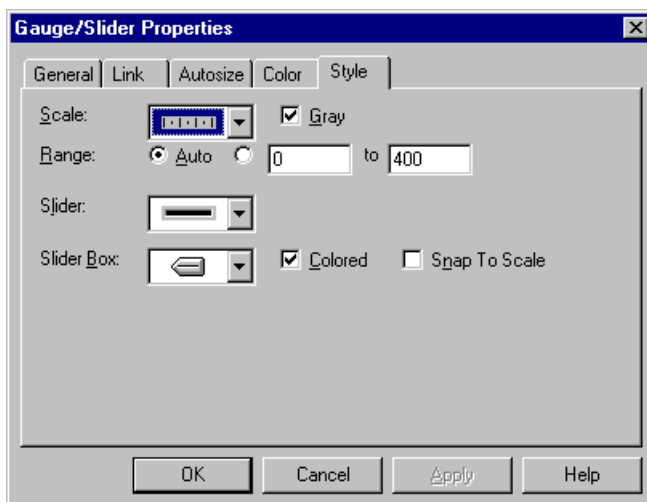
The Quick Info provides a brief description for the control on which the cursor is currently positioned.

- Select the desired 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.

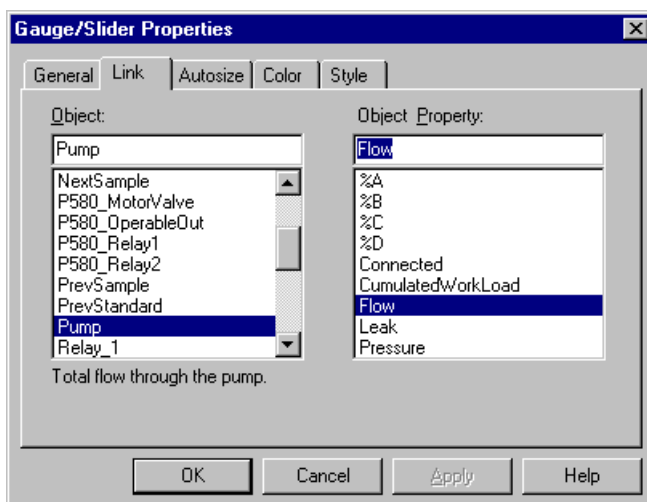
Functionality and appearance of the control

- 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:



- 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.



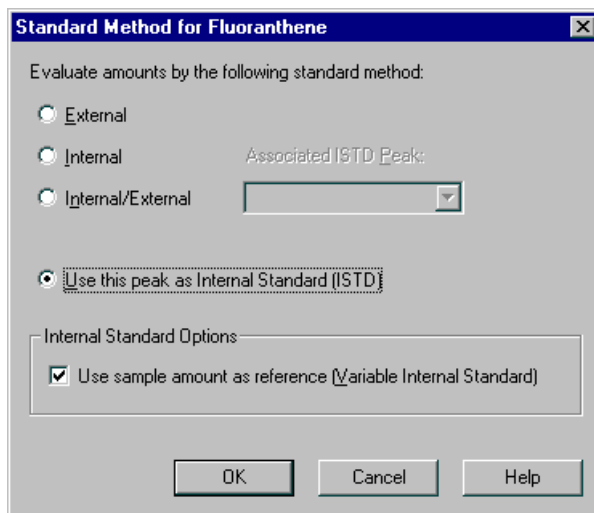
- From the **Object** list, select the instrument itself. From the **Object Property** list, select the instrument's function. Instrument and function selection depend on the components installed with the timebase.

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 Fluoranthene	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

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*.

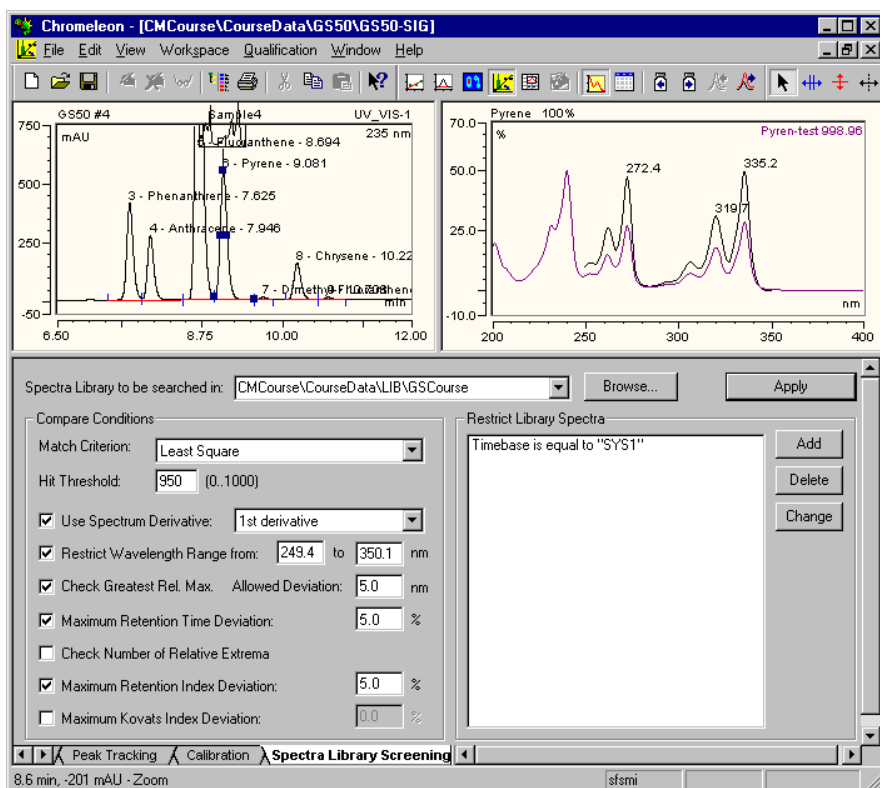
7. Spectra Library Screening

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, a message stating **No spectra library hits found!** appears.

**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.

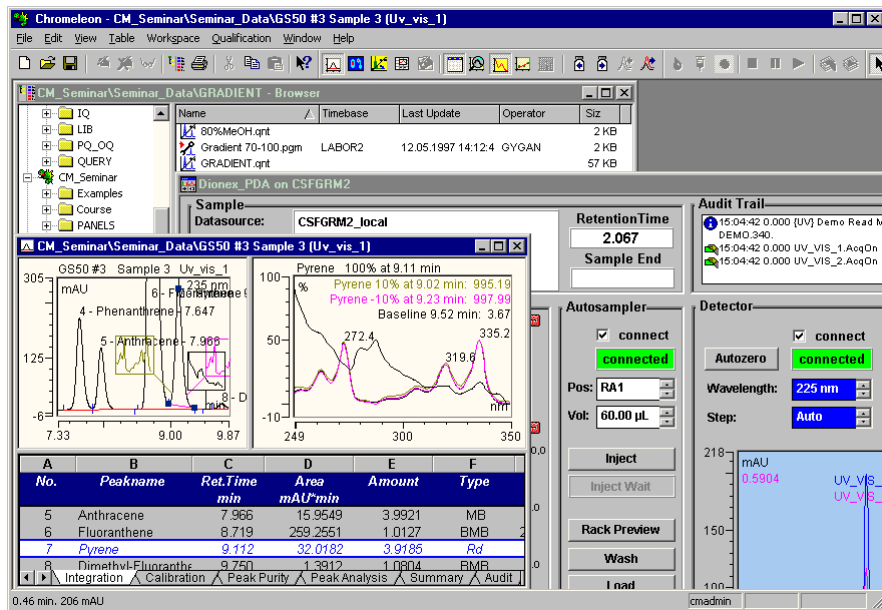
This facilitates opening single files or windows and allows you to work in whatever work environment you prefer.

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

Keyboard Shortcuts

Shortcuts are provided for many operations, especially in online control:

	Action	Where	Description
General	Right-click Ctrl + Tab	All Chromeleon windows	Opens the context menu. Changes to the next window.
	Esc	Browser	Aborts the drag or move action.
	F1		Opens the context-sensitive Help;
	F2		Enables the Edit mode.
	F3	Browser + QNT Editor	Renames files (after the Find command: Find Next).
	F4	Chromatogram	Brings you to the next sample.
	Shift + F4		Brings you to the previous sample.
	F5		Updates the window.
	F6		Brings you to the next partial window.
	F7	Browser	Optimizes the column width.
	Shift + F7	(F7 key only) + QNT Editor	Optimizes the line height.
	F8		Opens the dialog box.
	F10/Alt		Shows the hotkey underlines.
	Shift + F5	Browser	Displays the chromatogram.
	Alt + Enter	Browser + Control Panels	Opens the corresponding Properties .

	Action	Where	Description
General (cont'd)	Ctrl + Tab		Toggles between open windows.
	Ctrl	Signal plot in the Report and in the QNT Editor	Zooming (changes the cursors to the zoom cursor).
	Ctrl + N	Report + Printer Layout	Creates a new file.
	Ctrl + O		Opens the file.
	Ctrl + S		Saves the file.
	Ctrl + R		Opens the batch report.
	Ctrl + P		Prints your selection.
Edit	Ctrl + Z		Undoes the previous action.
	Ctrl + Y		
	Ctrl + X		Repeats the previous action.
	Ctrl + C		Cuts the selected object.
	Ctrl + V		Copies the selected object.
	Ctrl + F		Pastes the selected objects
	F3		
	Ctrl + H		Finds a string of characters.
	F9		Finds the next string of characters.
			Replaces the entry in the field.
			Fills the column or the selected cell(s) with the first value of the selection.
	Ins		Inserts the selected object.
	Del		Deletes the selected object.

	Action	Where	Description
Sample List	Ctrl + I		Inserts a sample.
	Ctrl + D		Deletes a sample.
	Ctrl + A		Adds a sample.
PPA	Alt + F4		Copies the spectrum to the clipboard.
Control	Ctrl + F		Changes the pump's settings
	Ctrl + I		Initiates injection.
	Ctrl + Break		Stops the pump.
	Break		Enables Hold mode.
	Ctrl + A		Starts data acquisition.
	Ctrl + B		Edits the batch.
Signal plot	Double-click...	<ul style="list-style-type: none"> - Overview window - Time axis - Signal axis - Plot range (- or else:...) 	Unzoom. Auto Plot Speed. Autoscale. Signals... Axis/decoration.
	Shift	<ul style="list-style-type: none"> - when zooming 	Retains scale ratio between signal and value axis (The shape of chromatograms is maintained).
Gauge/Slider	Ctrl key	Press when dragging	Toggles the Snap To Scale option.
Script Button	Click	Button hold down	Indicates whether the program is still running. Stops the program upon confirmation.

	Action	Where	Description
Edit Field	Tab/Enter		Sends the new value.
	Esc		Aborts the input.
Layout Mode	Alt + Click	In 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	In the Online Signal Plot of the panel	Opens the Properties...

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.

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CHROMELEON®







Chromatography Management System


















User Manual

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Introduction


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The User Help and Manual

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






- Refer to the user 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. We recommend that you read the first two sections and look up unfamiliar terms in the glossary.

In addition, a separate Administrator Help is available. For information about the topics described in the Administrator Help, refer to  **The Administrator Help and Manual**.




The Administrator Help and Manual

In addition to the User Help and the User Manual, a separate Administrator Help and an Administrator manual are available. The User Help/Manual provides useful information for the Chromeleon end user. The Administrator Help and Manual are intended for system administrators and Dionex Service. For an overview of the topics described in the User Help and/or Manual, refer to the Table of Contents.

The Administrator Help and Manual provide information about the different Chromeleon management tools and the hardware installation procedures:

-  **Software Installation and Communication**
-  **Validation and Qualification**
-  **Actions in the Browser and in the Operating System**
-  **Actions Related to Mass Spectrometers**
-  **Actions in the Server Configuration Program**

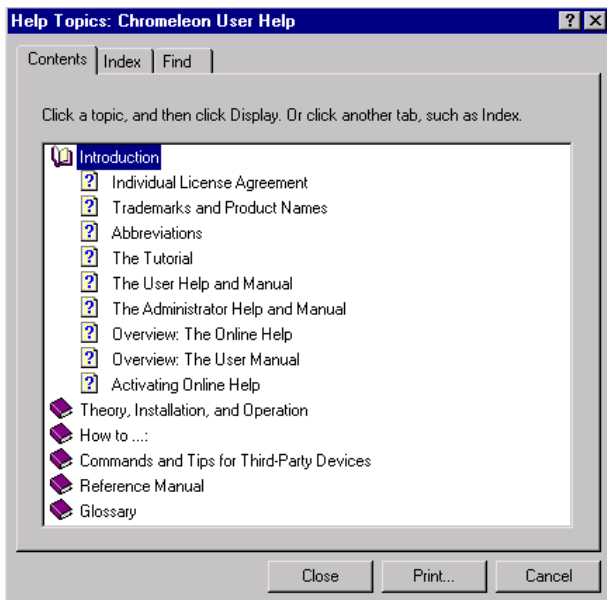
 **Actions in the Server Monitor Program** **21 CFR Part 11 and Electronic Signature** **Chromeleon User Management** (covering the *➤User Manager (CmUser) and Security Activation Tool (CmSecure)*)

 **Installation Instructions (Hardware)**. This topic provides among others the following sections:  **Installing Dionex Devices** and  **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.



Tip:

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.

The User Manual (Overview)

The User Manual is divided into different sections. In addition, the **Table of Contents** provides an overview of the various topics described in the manual. The **Index** allows you to search for specific terms.

There are many references to related topics. These references are indicated as follows:

	Jump term	References technical terms in the Glossary section.
	Jump term	References topics in the Reference Manual .
	Shortcut link	References topics in the Theory, Installation, Operation or How to sections.
	CmUser link	References topics in the Chromeleon User Management section of the Administrator Help.
	Installation link	References topics in the Installation Instructions of the Administrator Help.

1. Section: Theory, Installation, and Operation

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: Reference Manual

The reference manual describes commands, parameters, variables, and report Categories in table form.

Appendix: 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

Consult the index to locate information about a specific term. 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:

Command

Reconnect..... A-93

Wait..... A-147

Wash..... A-147

Comment..... A-30

Communication11; 21

Activating Online Help

There are several ways to access online Help.

➤F1 Key

Press the F1 key to display context-sensitive information.



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 the active window.

Help menu

Select ➤**Index** on the **Help** menu to display the table of contents of the online Help.

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



Click this icon on the toolbar to display a question mark appended to the mouse cursor. Then, click the item of interest for more information.



Click the **Help** button in a dialog box for more information about the dialog box.

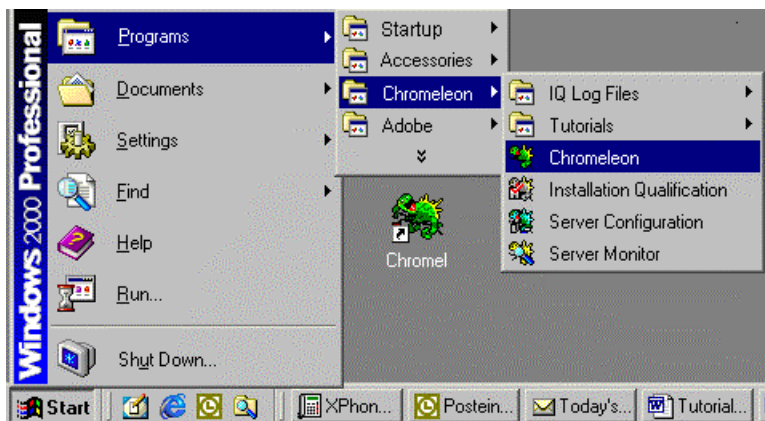
Online Help topics often include links that jump to other Help topics. To ➤*jump to another Help topic*, click the green underlined hyperlink or click a shortcut symbol.

Installation, 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 various sub-programs provide 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 Chromeleon\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:






To open the window of interest, click the associated icon:

Symbol	Description	Function
	➤ <i>Browser</i>	Data administration and storage
	Integration	Chromatogram display and reprocessing
	➤ <i>PPA</i>	Peak Purity Analysis: 3D Field (only for PDA data)
	QNT Editor	Quantification Method
	➤ <i>Printer Layout</i>	Printer Layout creation and/or modification
	Signed Results	Electronically signed sequence (➤ <i>SOR File</i>)


To open the following windows double-click the associated directory:

Symbol	Description	Function
	➤ <i>Panel</i>	Device control
	PGM Editor	Processing of a control file

The **Integration** window and **QNT Editor** provide different partial windows; click the associated icon on the Method toolbar:





Symbol	Description	Function
	Report	Display of various report tables
	➤ <i>Trend Plot</i>	Display of trends in data series
	Spectrum	UV spectrum (only for PDA data)
	Calibration Curve	Calibration curve
	MS Spectrum	➤ <i>Mass Spectrum</i>

For more information about the different windows, refer to:


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

Chromeleon Features

Chromeleon provides 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 (refer to the **Administrator Manual**  **Chromeleon 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 ...: Actions in the Browser**  **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 adjusted to the requirements of a specific application. In addition to the basic software package, various options are available from Dionex.

The purchased scope of performance is determined 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 the **Administrator Manual: Software Installation and Communication**  **The Software License**.)

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.

The following Chromeleon licenses are available from Dionex:

Server Features

Server License: The server license (without additional features) allows signal acquisition without spectra from one timebase for any number of synchronous channels.

Timebases Class 1 to 3: The *Timebase* licenses allow you to control chromatography systems. 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	Includes
Timebase Class 2	Timebase Class 1
Timebase Class 3	Timebase Class 1 and Timebase Class 2

Up to six timebases can be configured on one server PC. However, only two Timebase Class 2 or Timebase Class 3 are allowed.

Multiple Network Control: This license allows you to use Chromeleon on a network. In addition, this license supports the *Online Transfer Agent (OTA)* and *Network Failure Protection (= NFP)*. One separate license is required for each installed chromatography *Server*.

PDA License: Additional single-user license for *Photodiode Array Detectors* on a chromatography server. It supports digital data acquisition of a three-dimensional data field. The PDA license is required for spectra acquisition. One license is needed on each server.

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

IC Control SE: Additional control license for the DX-120, ICS-90, and ICS-1000 stand-alone ion chromatography systems and for the AD25, AS50, and UCI modules.

ICS-2000 Gradient Generation Additional single-user license for the ICS-2000. This supports creation of an eluent gradient.


Client Features

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


Server Control: Single-user license for data reprocessing on a network PC or a local PC, including control of any number of systems. In addition, for network control, make sure that the Multiple Network Control license is enabled on the chromatography server.



Note: This license is only available with a Client License.

Concurrent Clients:	Number of client licenses on a PC. A maximum of three Concurrent Clients can be established and licensed on one PC. For a ➤ <i>License Server</i> , the number of floating licenses allowed on one PC depends on the INI file (see the Administrator Manual: Installation Instructions  Installing the License Server). You can open a maximum of three clients on one PC.
Report Publisher:	This license extends the report and calculation features. In addition, it supports calculation using formulas and ➤ <i>Additional Functions</i> .
GLP Features License:	This license supports additional ➤ <i>GLP</i> features: <ul style="list-style-type: none"> • ➤File History • ➤Electronic Signature • User mode (see ➤User Management: User Manager and Security Activation Tool)
Library License:	Additional single-user license for access to special ➤ <i>Spectra Libraries</i> . These libraries must be purchased.
Virtual Column	Additional single-user license for the ➤ <i>Virtual Column</i> Tool
ASAP:	Additional single-user license for ➤ <i>SDK</i> applications.
Analyzer:	Additional single-user license for controlling the DX800 process analyzer.

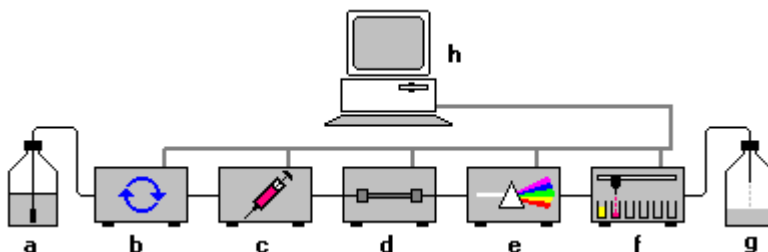
Installing Chromeleon

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

Components of a Chromatography System

Components of a Chromatography System

Modern HPLC or IC systems consist of the following components (HPLC: High Pressure/Performance Liquid Chromatography; IC: Ion Chromatography):



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). Using a thermostatted column compartment can 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. The signal whose profile corresponds to a Gaussian distribution is referred to as 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 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*, via serial interfaces, or via additional cards, e.g., via the A/D converter card.

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 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 Generator	EG40 or EG50
HPLC Pump	P680
IC Pump	GP40, GP50, GS50, IP20, IP25, IS25
Autosamplers	ASI-100, AS50, AS3500
Column Thermostats	TCC-100, LC25, LC30, AS50 Thermal Compartment
UV/VIS Detectors	
Single wavelength	AD20, AD25
Multiple wavelength	UVD 170U
Full-spectrum (PDA)	UVD 340U, PDA-100


Electrochemical Detectors	CD20, CD25, CD25A, ED40, ED50, ED50A
Fluorescence Detector	RF2000
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

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 the **Administrator Manual: Installation Instructions**  **Installing Dionex Devices**.






The Chromatography Data System

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




Tasks of a Data System

- It converts user input into time-precise control commands.
- It monitors the state of the connected chromatography instruments.
- It logs all user entries and modifications to the system.
- It saves and archives all data.
- It graphically represents data and allows you to check the system status and system results.
- It allows you to thoroughly check and evaluate data.



Components

- The basis for precise control is a real-time capable  **Operating System**.
- Installation and configuration is the task of the ➤ *Server Configuration Program*.
- The various  **Device Drivers** and other drivers, e.g.,  **Virtual Channel Drivers**, enable communication with the different instruments and device types (refer to the respective sections in the **Administrator Manual: Special Drivers**).
- Each operation, such as execution of a command, or the display 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

- Regarding the menu structure, screen elements, and operation, Chromeleon supports all typical Windows properties. 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. (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 with 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	MS and PDA Server
Service Pack	SP3	SP1	--
CPU	Pentium III	Pentium III 800	Pentium III 800
RAM	128 MB	256 MB	256 MB
Hard Disk	10 GB	20 GB	20 GB
Display	1024 x 768 x, minimum 256 colors		

For better software performance, we recommend a Pentium 4 with a CPU of at least 1 GHZ and 512 MB RAM.

For applications with several windows and control panels, e.g., in mass spectrometry, we recommend using a higher screen resolution.

The Operating System

Chromeleon supports the following operating systems:

- Windows XP SP1
- Windows 2000 SP3

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 the **Administrator Manual: Software Installation and Communication**  **Chromeleon and Windows Operating Systems.**

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 so-called ➤*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* has been started, 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.

Depending on whether the client and the server are located on the same PC or on different PCs in a network, a distinction is made between the following installation types: local client/server installation and network installation. For more information, refer to

Local Client/Server Installation

Network Installation

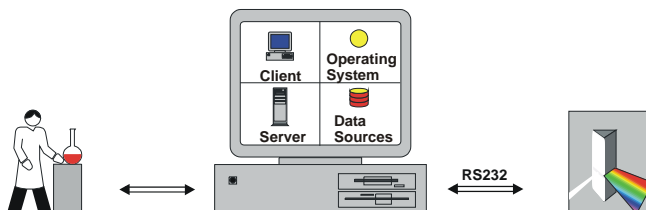
Local Client/Server Installation


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 to the PC, e.g., via RS-232 ports, a ➤ *DX-LAN*, or USB (Universal Serial Bus). Additional interface cards can supply a sufficient number of ports. Each chromatography server can control a maximum of 6 controlled ➤ *Timebases* (chromatography systems).

The data is saved on the local PC. During the 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.

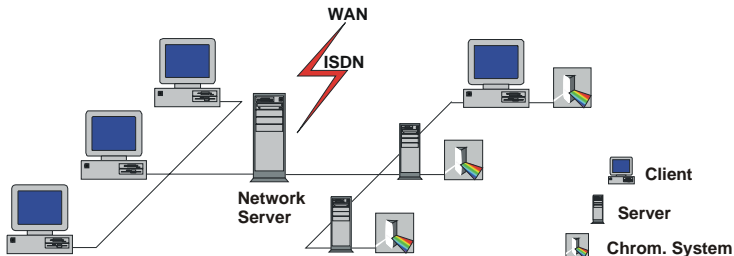


If the local PC is connected to a network, all options of a  **Network Installation** are available.

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 6 chromatography systems (➤*Timebases*).

The Client, Server, and Datasource are independent units on the network.



On each PC, a server, a client, and a 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 (TIME1/2/3) 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.

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 be continued, nevertheless. All data that are relevant for the Chromeleon server are locally stored on the server's hard disk, thus ensuring that the 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:

For more information, refer to the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System:**



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 hotkey letters. To open a menu via the hotkey, press **Alt** and the hotkey. To select a command or option on the menu, enter another hotkey. Alternatively, use the arrow keys to move to the desired 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, press **Alt**, and then press the **A** and **L** keys. Or else, press the **Alt** and **E** keys, and then select the desired option, using the **↓** and **→** arrow keys. Confirm your selection by pressing <Enter>.

In addition, important commands can be accessed via shortcuts. The shortcuts are displayed to the right of commands and menu options. Enter the key combination to directly execute the corresponding function.

Example: Press the **Control** and **C** (Ctrl+C) keys to copy a selected item.

For a list of available shortcuts, refer to **Operation**  **Keyboard Shortcuts**.


Keyboard Shortcuts

Shortcuts are provided for many operations, especially in online control:

General	Action	Where	Description
	Esc or right-click		Aborts the drag/move action.
	F1		Opens the context-sensitive Help;
	F2		Enables the Edit mode.
	F3	➤ <i>Browser +</i> ➤ <i>QNT Editor</i>	Renames files (following the Find command: Find Next).
	F4	Chromatogram	Takes you to the next sample.
	Shift + F4		Takes you to the previous sample.
	F5		Updates the window.
	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	Optimizes the column width.
	Shift + F7	(F7 key only) + QNT Editor	Optimizes the line height.
	F8		Opens the dialog box.
	F10 or Alt		Shows the hotkey underlines.
	Shift + F5	Browser	Displays the chromatogram.
	Alt + Enter	Browser + ➤ <i>Control Panels</i>	Opens the respective Properties .
	Ctrl + Tab		Toggles between open windows.
	Ctrl	Signal plot in the ➤ <i>Report</i> and in the QNT Editor	Allows zooming (the cursors is changed to the zoom cursor).
	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 + ➤ <i>Printer Layout</i>	Prints the selected object(s).

	Action	Where	Description
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.
Sample List	Ins		Inserts the selected object(s).
	Del		Deletes the selected object(s).
Sample List	Ctrl + I		Inserts a sample.
	Ctrl + D		Deletes a sample.
	Ctrl + A		Adds a sample.
PPA	Alt + F4		Copies the spectrum to the clipboard.
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
	Shift	- Plot range	Signals...
		(- or else:...)	Axis/decoration
		- when zooming	Retains the scale ratio between signal and value axis. (The shape of chromatograms is maintained.)
Gauge/Slider	Ctrl key	Press dragging	when Toggles the Snap To Scale option.

	Action	Where	Description
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 hotkeys are available. For information, refer to **How to ...: Actions in the Chromatogram**  **Using Hotkeys in the Chromatogram.**

Operation via the Mouse

Chromeleon features all windows-typical properties and capabilities of a two-button mouse.

The **left mouse button** serves to mark and select menus, menu commands and options, and icons, 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-hand mouse button (= left-click) to execute the selected function.

Simultaneously **left-click and press the SHIFT key** to select several cells in a table. Left-click the first cell, press the SHIFT key, and then select a new cell. All cells between these two cells will be selected.

Simultaneously **left-click and press the CTRL key** to select non-adjacent cells in a table. Left-click the first cell, press the CTRL key, and then select a new cell. Keep the CTRL key depressed and repeat 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, press the CTRL key while performing the Drag and Drop operation.

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

For more information, refer to your Windows manual.

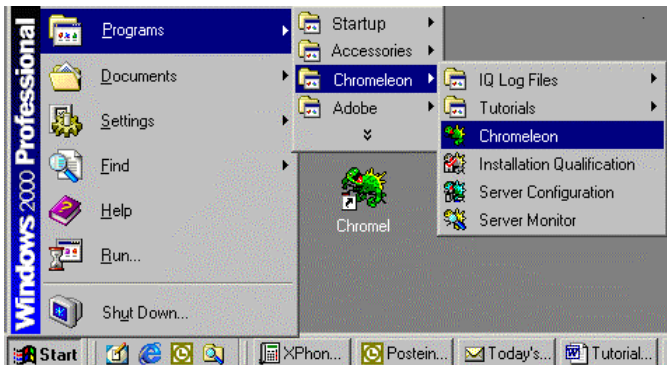
Undo/Redo Commands

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

Starting the Program

Chromeleon runs on client PCs equipped with Windows 2000 or Windows XP as operating system. To start Chromeleon:

- Click **Start** to open the **Start** menu. Move the mouse cursor to **Programs** and wait until the submenus display.
- Move the mouse cursor to **Chromeleon**. A submenu displays with several choices:



Client

- Select **Chromeleon** to start the Chromeleon software and to start data evaluation.
- If Chromeleon has been installed with integrated access control, the user must enter his/her 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 under 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 corresponding icon appears on the taskbar.

- If the icon does not appear on the taskbar, click **Start** and select **Server Monitor** on the **Chromeleon** menu to start the ➤ *Server Monitor Program*. The Chromeleon icon will appear next to the Windows system clock on the Windows taskbar.
- Place the mouse cursor on the icon. The **Chromeleon Server is not running** quick info message appears.
- Select the **Start Server** command 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 have been installed correctly, you can operate them online.



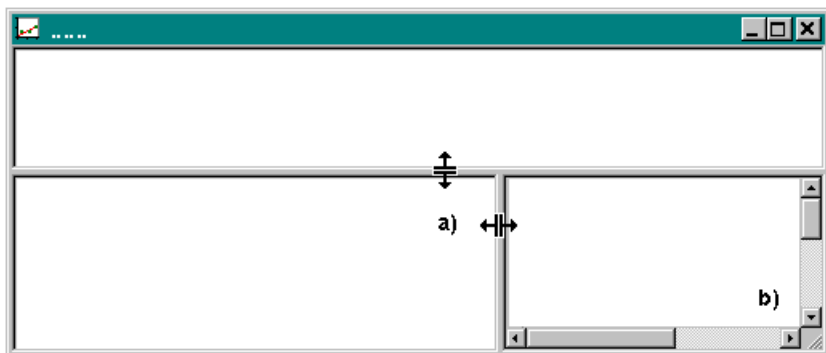
Tip:

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

Move the mouse cursor on one of the buttons to display its description. 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 of which the borders can be moved (a). If the window section is too small to display the entire information, display the hidden area, using the scroll arrows or scroll bars (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 cursor are indicated on the status bar.

Window Types

In addition to the Browser and the Control Panels, Chromeleon supports the following method windows:

- ⇒ *QNT Editor*
- *Integration*
- ➤ *Peak-Purity-Analysis (PPA)*
- ➤ *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 or 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!

Below please find two examples of samples with chromatograms. Specific detection parameters were used for integration and analysis.

Example 1: If the \Rightarrow *Minimum Area* detection parameter is corrected in the QNT Method window by entering a smaller value, the corresponding chromatogram is immediately adjusted. Peaks with an area smaller than the minimum area are not considered.

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.

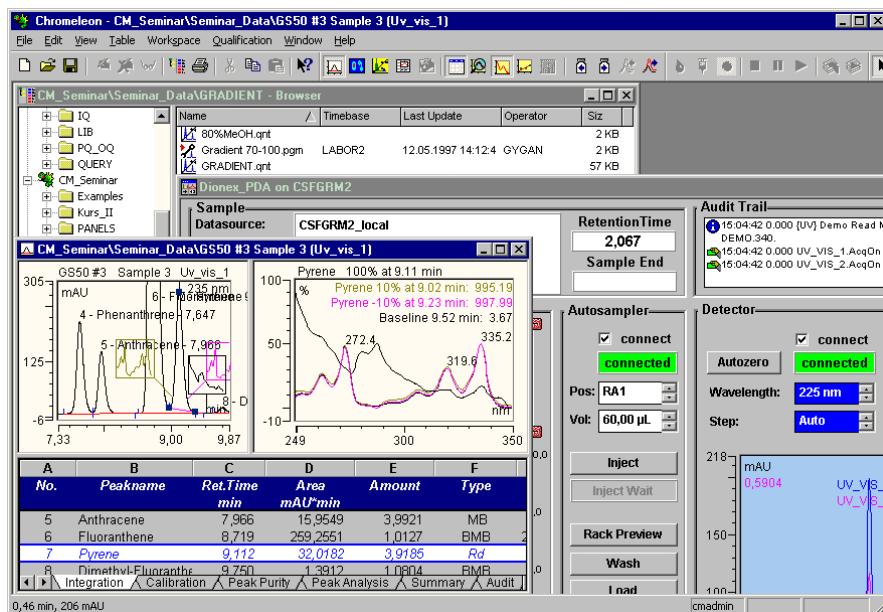
This facilitates opening single files or windows and allows you to work in whatever work environment you prefer.


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** on the **Workspace** menu 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 individual window is stored in the corresponding 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, the representation of chromatograms and spectra, the display of additional information or auxiliary lines, the setting of various fonts, font 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 located 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/Open Files, Windows, and Templates

There are several ways to open or create files, windows, and templates. Frequently, other information is required for this. For example, when a control panel is opened, it searches "its" timebase, that is, 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 are mainly caused because the user renamed timebases or datasources that were correctly installed before by the Dionex Service Representative.

Similar problems may occur if databases are located on a network PC to which the user currently cannot connect or for which (s)he has no share authorization.

*In this case, refer to the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Connecting a Datasource.***

Automatically Loading the Most Recent User Profile

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

Opening Existing File Types and Windows

Select the **Open** command on the **File** menu; 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* or select **Open** on the **File** menu.

Open Most Recent Files and Templates

The lower section of the **File** menu and the **Workspace** menu lists 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 process of creating a ➤ *Sequence* and lets you include the samples into the sequence.
- Select **Sequence (from LIMS Worklist)** to include data and a ➤ *Sequence* of a ➤ *LIMS* in the ➤ *Worklist* format.
- Select **Program File** to start the  **Program Wizard**. The Wizard guides you through the process of creating a  **Control Program** (PGM File). A ➤ *PGM File* includes all ⇒ *Control Commands* that must be transferred to the different chromatography devices to 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)* shall be used and which pages of the printer layout shall be printed for which sample type and for which channel.

The single pages are created in the Printer Layout: (See **Data 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 ...: Actions in the Printer Layout**  **Changing the Page Format.**)

Printing in 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 be started 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 corresponding **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)* by selecting **Print QNT Method** in the QNT Editor.



Control

Control Concept

The chromatographic equipment in an analytical laboratory usually comprises instruments from different manufacturers and of various generations. Depending on whether individual devices can be controlled via a PC, they are referred to as either controlled or non-controlled devices.

Controlled Devices

Controlling devices makes high demands on the adaptability of a modern chromatography data system, as operation of individual components must be easy and thus **device-independent**. This means that input and representation of a specific control command (for example the pump flow) must always be performed in the same way, regardless of whether the instrument is by manufacturer X or Y.

Chromeleon enables this with the Chromatography BIOS and the available device drivers. (For more information about these items, refer to the Administrator Manual: Device Communication  **Communication between the PC and the Chromatography System (Chromatography BIOS)** and  **Device Drivers**.)

The advantages of a uniform user interface are as follows:




- Short training time for inexperienced users.
- Uniform, device-independent operation: The same commands are used for all systems.
- Transfer of methods: Chromatographic methods can usually be transferred from one system to another. This is why the corresponding device functions must be available on both systems.
- Clear and uniform documentation.

Non-controlled Devices

In rare cases, non-controlled systems are used, and the individual instruments are operated manually. Chromeleon only records the data. Two conditions must be fulfilled:


- The injection time is communicated to Chromeleon by connecting the **Inject** contact of the injection valve with a ➤ *Remote Input* of the system. This guarantees time synchronization between sample start and recording time.
- Detector data is transferred to Chromeleon as digital or analog data (serial interface or ➤ *UCI Universal Chromatography Interface*).

In principle, there are two ways to control a chromatography system:

- Many samples are usually processed in a ➤ *Sequence*, via a program. For more information, refer to  **The Control Program**. To edit the program, refer to  **The PGM Editor**.
- To create a new chromatographic method, you can control the instrument directly from the ➤ *Control Panel*, i.e., the single devices and device functions are controlled directly and interactively from the control panel. (Also, see **Basic Operation**  **The Control Panel**.)

Control Requirements

The requirements for controlling devices are as follows:



- The appropriate  **Chromeleon License** must be available.
- The controlled instruments must be correctly installed and configured in the ➤ *Server Configuration* program.

Tips:

*Dionex Service or a network administrator usually performs installation. For more information, refer to **Administrator Manual: Installation Instructions***

Installing Dionex Devices

Installing and Controlling Third Party Devices

- A connection between the PC and the ➤ *Chromatography Server* must be established.
- A connection between the chromatography server and the chromatography system must be established via a serial interface, a DX-LAN, or any other interface (TCP/IP, GPIB, USB). (For more information, refer to the **Administrator Manual: Software Installation, Communication, and Validation**  **The Serial (RS-232) Interface** and/or  **The DX-LAN.**)

The Control Program

Modern analytical laboratories usually analyze many samples. These samples are grouped in ➤ *Sequences* and processed with a chromatographic control ➤ *Program*, 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 finished ➤ *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 Control Program (Details)



The Program Wizard




The PGM Editor



The Program Syntax

The Control Program (Details)

The control program (often referred to as the ➤*Program* or PGM File) includes a list of time-precise ➤*Control Commands*. The user creates the actual control program. The aim is the automatic and repeated execution of specific routine tasks such as processing samples automatically or conditioning a column by rinsing with various solvents. Monitoring certain parameters or limits, or triggering reactions when these limits are exceeded, can also be performed via a program.

A ➤*Wizard* (see  **Program Wizard**) assists you in creating a program by automatically converting your entries into the respective 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, open the program by double-clicking the program name in the Browser. This displays the view of the PGM Editor that you opened last.

- To edit the control settings for a device, click the corresponding device icon on the left pane. This view provides the corresponding pages of the Program Wizard. Enter the desired 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 to a ➤*Timebase* while the server is running. Therefore, first verify that the server is running, and then if necessary, start the server in the Chromeleon ➤*Monitor Program*. To connect the PGM File to a timebase, select **Connect to Timebase** on the **Control** menu.*

If the PGM File is connected correctly to a timebase, all icons corresponding to the devices for that timebase are available on the respective 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 ...**:

Actions in the PGM Editor  **Creating a Program**

Device Control  **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 of the information required to generate a basic program. Each step consists of a template in which the user enters or selects data. Depending on the installation, different 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 temperature settings (if supported)

Step 3: Define a flow system

Step 4: Determine a gradient profile (optional)


Step 5: Enter the autosampler settings (if supported)


Step 6: Determine channels and the duration of data acquisition

Step 7: Determine signal parameters for the individual channels

Step 8: Define the peak detection parameters for fraction collection (optional)
















Step 9: Complete the Program Wizard

Chromeleon completes the information entered in steps 1 to 9 by adding the ➤ *Inject* and ➤ *End* commands, thus creating an operable program, which is displayed in the **Commands** view of the PGM Editor. (For more information about the PGM Editor, refer to  **The PGM Editor**.)

To edit your basic program, use the  **Views in the PGM Editor**.

The PGM Editor (Overview)

The PGM Editor allows you to edit the control programs (➤ *PGM Files*). The PGM Editor includes different views, which are accessed via the corresponding icons on the left pane (the shortcut bar):


View	Icon	Description
Commands		Display of the program (see  The Commands View)
Post-acquisition steps		Determination of steps for data reprocessing (see  The Post-Acquisition Steps View)
Device		
<i>If the respective instrument is installed, the associated icon is available:</i>		
Finnigan AQA or Surveyor MSQ		Input of AQA or MSQ parameters (see  Surveyor MSQ or Finnigan AQA Views)
Column Oven		Input of thermostat parameters (see  Device Views)
Pump		Input of pump parameters (see Device Views above)
GC		Input of GC parameters (see Device Views above)
Sampler		Input of autosampler parameters (see Device Views above)
UV		Input of UV detector parameters (see Device Views above)
RI		Input of refractive index detector parameters (see Device Views above)
Emission		Input of fluorescence detector parameters (see Device Views above)
Relay and State Devices		Input of relay and condition parameters (see Device Views above)


**Note:**

The name of the single device views corresponds to the **Device Name** entered in the **Server Configuration** program. For example, if you have named your pump **HPLC pump**, the device view is also named **HPLC pump**; it is not named **pump**, as stated in the above table. The above table lists the default device names.

 **The Device Views**

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.


The device views contain the corresponding 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 special commands. Define only these commands in the **Commands** view. 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* and are used to specify the method that is used by the Surveyor MSQ or the 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 the **Help** button. 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 ...: Actions Related to 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 with the various commands in chronological order. To facilitate orientation within a program, control commands are displayed in **black**, comments in **green** and ➤ **Triggers** 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.


Press <F8> to open a dialog box that allows you to edit the program. The dialog box provides you with exactly those commands required for controlling the devices in the current timebase:

**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 print**.*

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 is displayed in **red print**.

- Press <F4> or <Shift> + <F4A> to browse through the errors and correct any wrong input.

If you are familiar with the  **Program Syntax**, you can change or extend your file directly via 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 ...**:

Actions in the PGM Editor  **Creating a Program**

Device Control  **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 ...: Actions in the PGM Editor**  **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, \Rightarrow *Control Commands* are always entered in English. The syntax for the \triangleright *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** as well as the different **Device Views** that guide you through program creation. For a list of general commands and commands for Dionex and for third-party devices, refer to  **Commands and Tips for Device Control**.


Time Value (Retention Time)

The time value is entered at the beginning of the control command. It determines when the command is to be executed. The information is entered 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. They can be recognized in the F8 dialog box by the device symbol (⊕ ). Each **Device** has a number of commands and/or properties.

As various instruments can have the same commands or properties, adding the device name in front of the command makes a distinction. 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 **Flow** command when only one pump is installed. The syntax is as follows:

```
Retention Time      Flow                          =      Value
```

Command

Commands are represented in the F8 edit box by an exclamation mark (). If a command can be clearly assigned to an instrument, the name is sufficient for identification:

```
2.500      NeedleUp
```

The ⇒ *NeedleUp* command exists for the Dionex Autosampler GINA 50, only. In this case, the device name can be omitted. This is in contrast to:

```
2.500      UV_VIS_1.AcqOn
```

The $\Rightarrow \text{AcqOn}$ command by itself is not unique (if there is more than one channel in the system). To address one specific channel, the channel name must be added to the command.


In addition, commands can be extended by additional parameters, for example:

```
2.500     $\Rightarrow \text{Inject}$            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

If a command cannot be assigned to a **Device**, it is listed in the F8 edit box by itself. This applies to the commands  $\Rightarrow \text{Branch}$, $\Rightarrow \text{Log}$, $\Rightarrow \text{Message}$, $\Rightarrow \text{Protocol}$, $\Rightarrow \text{Wait}$, $\Rightarrow \text{Delay}$, $\Rightarrow \text{Trigger}$, $\Rightarrow \text{EndTrigger}$ and $\Rightarrow \text{End}$.

For more information, refer to **How to ...: Device Control**






Trigger Commands









Mixed Commands

Property

Properties are distinguished by their value. An I/O-symbol () in the F8 edit box represents values predefined by the system. The  symbol 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 gives an actual value (for example, Pressure (bar), %A (%), Signal (mAU), etc.), this is indicated by a separate symbol (). Properties in connection with 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). Assigning a solvent name is via the same method ( %B,  Value, Equate). The corresponding syntax is:

```
2.500    pressure.UpperLimit    = 350
2.500    pressure.LowerLimit    = 20
2.500    %B.Value               = 30
2.500    %B.Equate              = "Methanol"
```

Here the same applies: 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. The comment lines are always started by a semicolon ";" and are displayed in green print:

```
;          The following program ...
```

Text must be entered in quotation marks if it is displayed on screen because of a command and is included in the audit trail (as with the \Rightarrow Protocol and \Rightarrow Message commands).

```
2.500    Protocol              "Test program"
```

If these commands are entered using the F8 edit box, the quotation marks are added automatically.

The Control Panel

You can directly control the different devices of a timebase using 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 the user 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. The appearance is determined by a combination of various default controls.

Control panels can include, for example:


- A slider to change the pump flow or another 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 active.
- A script button to execute the inject command.
- A signal plot to monitor the detector signal.
- The current ➤ *3D Field* and the current ➤ *Mass Spectrum*.
- The Audit Trail to follow the execution of an operation.

You can determine the number of available controls and their functionality, depending on individual requirements. Each user thus "designs" a personal interface. The available functions depend on the functionality of the analytical instruments that are combined in a timebase. For example, if a controllable column oven is part of your system, you can control the oven temperature.

The system administrator assigns user-specific *➤Privileges* in the *➤User Manager* (CmUser program) to determine whether a user is authorized to create his "personal" user interface. The organization of the panel allows you to lock certain functions on the user interface or to disable the display of 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.

If this type of screen arrangement seems confusing at first, the system provides several default control panels. It is not possible to modify these panels. They 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 ...:**  **Actions on the Control Panel.**

Control Panel: Function

➤Control Panels serve to control and monitor individual *➤Timebases*. The timebase that will be controlled from the control panel is specified when the panel is created. When you open the control panel, the system tries to connect to the specified timebase. If this is not possible, for example, because the corresponding timebase was renamed or because the corresponding chromatography *➤Server* is not running, a message appears. If this occurs, change the assignment manually:

- Select **Open** on the **File** menu to open the desired control panel.
- Select **Connect to Timebase** on the **Control** menu. Enter the name of the timebase to be connected with the control panel or select the 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 open the window the next time.


The currently selected timebase is displayed on the status bar.



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, are displayed online, i.e., during data acquisition. Different commands are available via the context menu (right-click). They allow you to define how the signals shall be displayed:

Autoscale	Each time when performed, the ➤ Autoscale command adjusts the scaling of the signal axis exactly to the open chromatogram or to a section thereof. Instead of executing the command from the context menu, you can also double-click the signal axis.
Auto Autoscale	The ➤ Auto Autoscale option 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	Select the Auto Plot Speed option to prolong the time axis automatically by the period defined on the Axis/Decoration tab page as soon as the end of the signal plot is reached.
Replot from Beginning	Selecting the ➤ Replot from Beginning command has the following effect: When the signal leaves the right border of the signal plot, the window is enlarged by the period defined on the Axis/Decoration tab page. Thus, the entire chromatogram is always displayed.



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.



After starting data acquisition, the Audit Trail window displays the start time and any subsequently performed commands (**⇒ AcqOn/Off**, **⇒ Inject**, etc.).

Of course, each event included in the Audit Trail window is stored. Storage is very precise and comprehensive. This makes it possible to determine later 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 ...: Actions in the Report Table**  **Displaying a 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: <ul style="list-style-type: none"> • 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.
Properties	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.**

Data Management

Data (Overview)

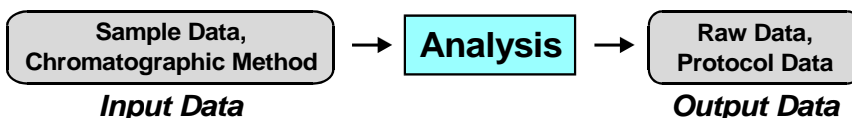
Input Data

For the analysis of a sample and for documentation and archiving purposes, various types of input data are required, which the user must determine or enter before starting the analysis.

These include, for example, the sample name, weight, injection volume, chromatographic conditions (solvent, flow, detection wavelength, connected devices, etc.), as well as the run time. A distinction is made 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 data serves as the basis for the analytical process.



Output Data

The data recorded during analysis is referred to as output data. Output data includes:

- Data provided by the analysis process itself (analysis and ➤ *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**).

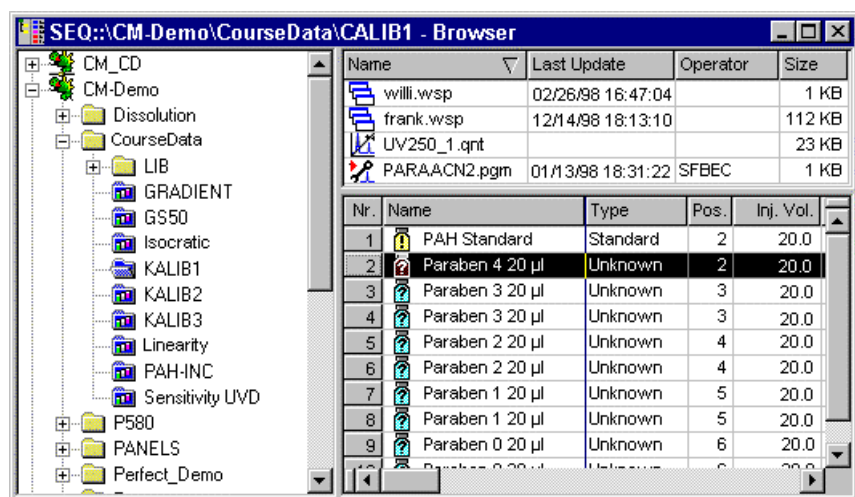
The Browser

The Chromeleon ➤ *Browser* is the tool for data management. It displays the directories in which chromatographic data is located and that can be accessed.




The Browser allows you to open, move, and delete chromatographic data, as well as search for specific data in various databases. 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

The window has two separate sections. As in the Explorer, the directory structure of all selectable directories is displayed on the left. Detailed information about files and *Sequences* is shown on the right. Operation is also identical to the Explorer:

- Click the + and - characters next to each directory name to expand or collapse the subdirectory structure.
- Select a directory to display its contents in the right-hand window section. Files and single samples are displayed.
- Double-click a file or sample in the right-hand window section to open the appropriate editor for a file or the corresponding chromatogram for a sample.

For convenient identification, each file type (sequence, *PGM File*, *Quantification Method*, ..) has a type-specific icon. Chromeleon automatically recognizes the *Type* (Sample Type) (standard sample, unknown sample) and/or the stored data format (3D field, etc.) and displays the data appropriately, i.e., in the corresponding chromatographic environment.

- Select a subdirectory, a sequence, or a file to drag it with the mouse to a different directory. If you drag the file while holding down the right mouse key, a context menu is opened. Copy or move the file to the new directory by selecting the corresponding option. If you drag the file while holding down the left mouse key, the action, which has been defined as default action (**Ask**, **Copy**, or **Move**), is performed.

Differences from the Windows Explorer

Structure

Only the chromatographic data that is part of a *Datasource* is presented in the Browser. (For more information, refer to the **Data** section.)

A datasource always represents the top level of the Browser hierarchy. The datasource can be created exclusively with the Browser and cannot be compared to the "normal" subdirectory in the Windows Explorer. A small icon indicates the type of data on which it is based (Chromeleon, GynkoSoft data, etc.). Users can only see those datasources for which they have the appropriate access rights.

Below the datasource level, there is the familiar Explorer directory structure (yellow file folder). The directory structure helps to manage the different ➤ *Sequences* in a datasource. A blue file folder represents a sequence. It is not possible to create a subdirectory below the sequence level.

Operation

- Select a datasource, a subdirectory, or a sequence to display its contents on the right pane of the browser.


In the case of the datasource or subdirectory, you see the usual list of all directories and files. If you select a sequence, the right-hand window half is divided in two horizontal sections. These include the header with general properties of the selected sequence and a list of all analysis and standard samples and their sample data (**Sequence Editor**).



Caution:

*The sample data can be edited directly here! GynkoSoft users recognize the **Sample (SMP) File**. Additional samples can be included in the sequence, existing sample data can be modified, or old samples can be removed. For a detailed description of the sequence editor, refer to the **Sequences** section.*

- Select **Query** on the context menu to search for specific sequences, data or samples. It is also possible to search several datasources simultaneously. This is a true ➤ *Query* that can also search for specific properties, for example, "all samples starting with PAK."

Moving, deleting, or copying directories, data, and files is very similar to these actions in the Microsoft Explorer. (For more information, refer to **The Browser**  **Common Features with the Windows Explorer**).



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

- Set up ➤ *Datasources* and create subdirectories, to copy or move files via **Drag & Drop**, or to delete files via the **Cut** command.
- Select a ➤ *Sequence* in the left window (blue folder) to display a list of its contents on the right.
- Double-click a sample name to display the corresponding 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 function for several files simultaneously.
- Select a file, right-click, and start a ➤ *Query* for several sequences and/or datasources. The result of the query, that is the different files or samples with at least one common feature, is displayed on the right pane of the Browser.

The Datasource

The term ➤ *Datasource* is used for the top level of the directory structure displayed in the ➤ *Browser*. The Browser is the tool for handling datasources. 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 the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Setting up a Datasource.**

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 the data that can be accessed. This simplified representation has the following advantages:

- Data is always accessed in the same way. The user does not have to worry about the data's actual storage location on the network. It is not necessary 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 the handling of 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. GynkoSoft and Chromeleon are easily recognized by their different symbols:



When Chromeleon is installed, a default datasource is automatically created on each client PC. The datasource name is derived from the computer name (assigned during the installation under Windows) and the addition **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. In this datasource, the raw data of each manually performed analysis is stored. Therefore, do not delete the <PC NAME_Local> datasource!

Directory Structure of a Datasource

Each datasource can have any number of hierarchically organized subdirectories. 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.

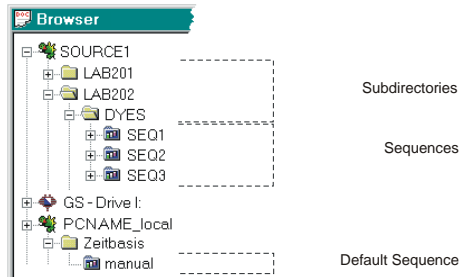


Caution:

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 and cannot be compared to or performed by the Windows Explorer! The representation of directories and data also differs considerably from the Explorer.

See the image below examples of datasources and their different directory structures:



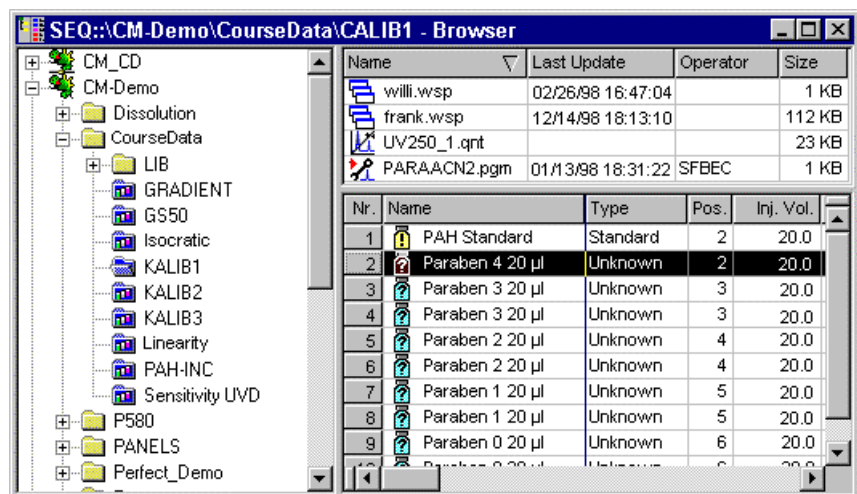
The **SOURCE1** datasource has the three subdirectories: LAB201, LAB202, and DYES. The **DYES** directory contains three ➤ *Sequences*: **SEQ1**, **SEQ2**, and **SEQ3**.

The default **PC Name_local** datasource has one subdirectory. The name of the subdirectory corresponds to the name of the timebase installed on the computer. The default sequence (named **manual**) is located in this subdirectory.

When a sequence is selected, its "inner life" is visible in the right Browser pane: the file structure (control ➤ *Program* (🔧), ➤ *Quantification Method* (QNT Method) (📄), ➤ *Report Definition Files* (RDFs), etc.), the sequence information, and the samples (standard samples (📁), and unknown samples (🔍), etc.)

For information about the representation, 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 in a datasource but also between datasources of different types. However, this copy process may also change the underlying database! If you want to actually move a sample, delete the original sample in the Browser after you have copied the sample to the new location.

Caution:

Execute **drag & drop** operations only in the ➤Browser. Drag & drop 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 deselect the **Locked** check box.

**Note:**

*Locking and sharing objects is subject to access control. (Refer to the **Administrator Manual: Software Installation and Communication**  **Access Control** for more information). Only users who have the corresponding privilege can lock and/or share objects.*

In addition to locking datasources and directories via 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 plays a very important role.

Data acquisition starts with the quality of the used detector, it comprises all components participating in the data flow and is concluded with processing the data 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. (Refer to the **Administrator Manual: Software Installation and Communication**  **The Serial (RS-232) Interface** for more information.) 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.

The 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 Dionex detectors, Chromeleon provides many drivers to control third-party detectors.

For an overview of the different manufacturers whose devices can be controlled under Chromeleon, refer to the **Administrator Manual: Installation Instructions**  **Installing and Controlling Third-Party Devices**.

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 the **Administrator Manual: How to ...: Actions in the Server Configuration Program**  **Adding, Configuring, or Deleting Components**

Data Storage

For fast and efficient access to specific data, intelligent storage and organization of the complete data is very important. Chromeleon solves this problem by storing data and files in different locations. Databases and sequence directories are available for this purpose. They are part of the




Datasource.

Storage in a Database

Data that can be compared across sequences is stored and managed in a relational, ➤*ODBC-capable database*. This applies to the entire ➤*Sample* and ➤*Sequence Data*. The advantage of this type of data management is not only the comfortable integration in other applications such as Excel, Access, dBase, etc., but also the efficient searching and sorting capabilities.

Perform a ➤*Query* to find all samples processed on a certain day, created by a certain user, and/or carrying a certain name.


Storage with the Sequence

The entire data describing the chromatographic treatment of a sample or data recorded during the analysis is stored in a ➤*Sequence*. This includes control files (➤*PGM File*) and evaluation parameters (QNT Method), but also 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 the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Tracking File Modifications (History)**.


Data Export

Chromeleon provides 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. Click the **Export Settings** button to open the **Export Wizard**. You can now export the corresponding report pages in five different formats:
 - a) ANDI/Chromatography - **➤A/A (*.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...: Actions in the Browser**  **Exporting Data During or After a Batch.**

2. In the **➤Printer Layout**, you can open a dialog box that corresponds 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...: Actions in the Browser**  **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 also export raw data in the AIA format. To do so, select **Export/Backup** on the **File** menu in the and then select the **ANDI/Chromatography (AIA)** option. (Also, refer to **Data Management**  **Raw Data Export**).
5. If you wish to send Chromeleon data to another laboratory, for example, via e-mail, we recommend that you execute 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 the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Using Chromeleon Data in an External Program.**

Backup

To avoid unforeseen data losses (for example, due to a defective hard disk), we recommend that you back up your saved data to a different data medium at regular intervals, using the **➤Backup** command on the **File** menu of the **➤Browser**.

Backup data is compressed; that is, 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 of the Browser.

The backup logs each single file that is copied and issues warnings if errors occur. The directory structure is maintained.

For more information, refer to **How to ...: Actions in the Browser:**




Creating Backup Files



Restoring Backup Files

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. The user is not allowed to change the raw data, e.g.:

- Sample data acquired on different channels (see **➤Signal**)
-  **Audit Trails**
- Injection times (see **⇒Inj. Date/Time**)
- **➤History**

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 extent and precision of the stored raw data depend on the selected
➤ *Sampling Rate* or \Rightarrow *Step*.

For more information, refer to



Raw Data Storage



Raw Data Compression

Raw Data Storage

Raw data storage refers to saving the signals received from a detector in digital form. Other important data (such as the analysis time, signal unit, number of data points, etc.) is also stored. If a detector is only supplied with an analog output, the data must be converted into digital signals. The A/D converter performs this task.

Storage Procedure

With conventional data systems, an analog value is digitized at a fixed time interval. For example, a digital value of defined accuracy is stored every second. The number of stored values per second is normally referred to as the ➤ *Sampling Rate*. The inverse of the sampling rate (the time interval between two data points) is referred to as \Rightarrow *Step*.

The higher the sampling rate, that is, the smaller the step, 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 a higher memory requirement. Chromeleon solves this problem by the step setting **step=auto**. This type of storage requires high algorithmic resources in real time (!), which is justified by the following advantages:

- Raw data files are as small as possible, as 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. The compression factor of 4:1 is thus achieved, making optimum use of the available storage capacity.

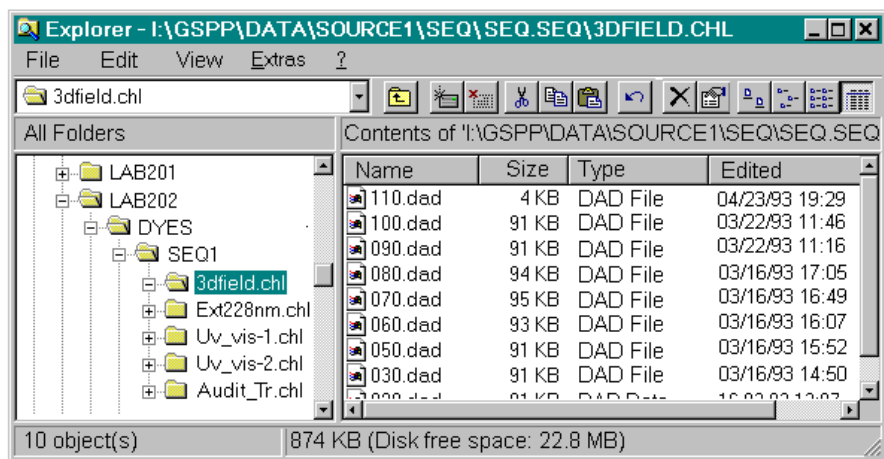
- Despite this minimal file size, maximum integration accuracy is ensured for the given chromatographic conditions, as the continuous signal is approximated to the optimum. Generally, more data points are stored below peaks than with conventional acquisition methods.
- The processing speed, for example, 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. For each channel that is specified during the Chromeleon installation, a separate subdirectory is created. In addition, an audit trail directory is created. The directories are not visible in the Browser.

The reason for this is: Chromeleon manages the entire raw data automatically. At no time, the user must access the raw data directly.

Viewing this type of data is only possible via the Windows Explorer provided the datasources are not locked. As, however, the datasource names are not displayed in the browser, you must follow the path to the corresponding sequence directory (see figure).



In this example, the **3dfield**, **Ext228nm**, **Uv_vis-1**, and **Uv_vis-2** channels were defined. The audit trail directory (**Audit_Tr**) was added. If a directory is expanded, the raw data of the corresponding channel is visible.

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! Therefore, we recommend that you protect your datasources to prevent that they are accessed via 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

Storing raw data automatically compresses the data. For the signal value, this is achieved by storing the difference to the next data point instead of storing each data point. Only from time to time, the actual value is stored. This way, the compression is increased by 50%. This effect is especially noticeable in the case of ➤ *3D Fields*.

The size of the raw data file of a 3D field increases with the number of recorded data points. These depend on the ➤ *Optical Resolution* of the detector, the field size (area between the upper and lower limit of the 3D field) as well as the selected ➤ *Sampling Rate*.

At a sampling rate of, for example, two spectra per second (step = 0.5) and an optical resolution of 2nm, this means that $2 \times 60 \times 70 = 8400$ data points per minute must be recorded for the UV range from 200 to 340nm. As each absorption value is recorded with an accuracy of 25bits, a hard disk storage capacity of $m \times (N+1) \times 4 = 70 \times ((2 \times 60) + 1) \times 4 = 33.88 \text{ kByte}$ per minute is required. The storage requirement for the 3D field of a "normal" (20-minute) chromatogram is thus 0.678 MByte!

However, by skillful data compression procedures, it is possible to reduce the required storage capacity by approximately 50-60%. This is possible by completely storing approximately each eighth spectrum. Of all other spectra, only the difference to the previous one is stored and is recalculated, 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. The 3D field thus contains the complete information provided by the detector. The data can be restored at any time.

Further, there are three ways to minimize storage capacity requirements:

- Limit the wavelength range to the necessary range.
- Reduce the sampling rate (step) so that no more than 10 to 20 spectra are below the narrowest peak, or select an automatic sampling rate (step).
- Use the possibilities of the \Rightarrow *Diode Bunching*.

Restoring a Chromatogram from Raw Data

When restoring a chromatogram from the raw data, equidistant data points are joined with straight lines. A diagram "resembling" the recorded analog signal is thus created.

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. When using a fixed sampling rate, the sampling rate must be set so that a minimum of 10 datapoints is stored during the smallest peak in order to integrate the smallest peaks of a chromatogram (generally the earlier peaks) with the same precision as the larger peaks. This results, however, in huge data volumes especially in the case of 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. That is few data points are stored during baseline sections, whereas many are stored below peaks. The local sampling rate is set according to the actual information volume such that the deviation between the resulting diagram and the actual analog signal is never greater (or smaller) than the actual noise component of the signal. This method ensures that neither too many nor too few data points are stored, but always the optimum. The \Rightarrow *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 into ➤ *AIA* and ASCII formats. In addition, you can export raw data as ➤ *Backup* file (*.cmb). Select **Export/Backup** on the **File** menu in the Browser to export the data as an AIA or backup file.

Format	Description
AIA Cat. 2	Samples and peak variables are stored in the AIA format.
AIA Cat. 1+2	In addition to samples and peak variables, the raw data of a chromatogram (each stored data point) is stored in the AIA format.
Chromeleon backup (*.cmb):	All data of a ➤ <i>Sequence</i> or a ➤ <i>Datasource</i> are stored in the cmb format.
ASCII	Raw data may be exported in the ASCII format as well. Select Batch Report on the File or context menu. Click Export in the dialog box and then select the ASCII export format. 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


Importing the following raw data is possible:


- ➤ *AIA* data (*.cdf)
- Agilent/HP ChemStation
- GynkoSoft data
- ➤ *LIMS*/➤ *Worklist* data (*.wle)
- PeakNet (up to version 5.2) data
- Files in the Chromeleon archive format (*.cmb)

On the **File** menu of the Browser, select **Import/Restore** and the corresponding option. Select **Import** to import any of the above raw data. Select ➤ *Restore* to import Chromeleon ➤ *Backup* data.

In addition, it is possible to 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 the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Connecting a Datasource**.

For details on importing PeakNet 5.2 or earlier data files, refer to **How to ...: Actions in the Browser**  **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, as a raw data autosave is performed continuously during sample processing. Autosave ensures that the raw data is stored on the hard disk in short intervals. The user can reduce the time intervals to approximately 30 seconds. In the case of a power failure, the maximum data loss is thus 30 seconds.

If a power failure interrupts the automatic sample batch, the *➤Power Failure Protection* and the power failure handling ensure that processing is continued at the same position after starting the system anew. In addition, you can run a power failure program before to reset the system to a defined state.

If processing is interrupted by warnings or error messages, it is possible to react with an appropriate *➤Emergency Program*. The system is then in a defined state that is recorded in the *➤Audit Trail*.

For information about how to develop appropriate programs for both cases, refer to:

How to ...: Actions in the PGM Editor:

 **Creating an Emergency Program**

 **Creating a Power Failure Program**

Audit Trails

In addition to raw data, Chromeleon also records the Audit Trail. An audit trail includes the following information:

Audit Trail entries for ➤ *Preconditions*:



Device settings before a sample run, such as the temperature of a column oven.



Note:

These entries can be displayed only in the daily audit trail and in the sample audit trail.

Audit Trail entries for a sample run:



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



Executed instruction. The color indicates the filter level. The message is displayed from this level on:

 Green: Normal

 Yellow: Advanced

 Red: Expert

This color code is not yet valid for manual and triggered commands.



Command executed manually from the control panel or the F8 box.



Triggered command



Fulfilled ⇒ *Trigger* condition



⇒ *Protocol*; comments program steps or describes chromatographic conditions



⇒ *Message* on the screen that must be confirmed by the user



⇒ *Log* (performed by either the user or the ➤ *Device Driver*)

- Each item is stored with the current time. Information gathered during recording the data and/or processing a sample batch, also contain the associated retention time.
- The Audit Trail information is stored continuously both over a whole workday and for the duration of processing a single file. Two different Audit Trails are available:



The Daily Audit Trail



The Sample Audit Trail

Audit trails can be displayed either in the Browser (as daily or sample audit trails), on a control panel, or in a report or Printer Layout (only as sample protocol).



To define the extent and the type of the audit trail display, select the display filter (**Normal**, **Advanced**, **Expert**, and **Error**, or **Warning**) on the context menu. The **Advanced** and **Expert** display options are available only for audit trails created with Chromeleon 4.0 or higher.

In addition, you can select one of the following options on the context menu:

- **Run only** to display only the entries for the sample run.
- **Preconditions only** to display only the conditions before a sample run
- **Preconditions and Run** to display all entries



The Daily Audit Trail

The daily  **Audit Trails** store the entire GLP-relevant data that is related to the status of a specific timebase. The information is displayed in the Audit Trail on the control panel (refer to **Control**  **The Audit Trail**). The Daily Audit Trail also includes the preconditions before the sample run.


When the Chromeleon ➤ *Server* is started, Chromeleon creates an **AUDIT** directory for the corresponding timebase in the server 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, 200403051027.slg is the file for the daily audit trail of March 5, 2004. A separate file is created for each day.

On the right, the Browser displays the daily audit trails indicating their names and the time of their last change. Double-click a file name to open the corresponding audit trail in a separate window. The information is the same as in the audit trail section of the control panels including the preconditions. It is also possible to print the contents of the daily audit trail file.


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.

In addition to the daily audit trail, a  **Sample Audit Trail** exists for each sample.

The Sample Audit Trail

The sample audit trail contains the entire data of the corresponding sample and is part of each default report (see  **Audit Trails**). For more information, refer to **How to ...: Actions in the Report Table**



Displaying an Audit Trail.

You can display the sample audit trail as follows:

- To display the audit trail data of the current sample, display a report in any method window and select the **Audit Trail** worksheet.
- You can also enable the sample audit trail in the **Browser**. Select the sample in the Browser, select **Open** on the context menu, and then select **Audit Trail**.

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 the chromatographic analysis. It can include simple procedures as weighing, solving, and diluting a sample, as well as more complicated physical (filtration, centrifugation etc.) and chemical separation procedures (liquid-liquid-extraction, fixed phase extraction). Generally, the careful performance contributes substantially to the quality and the reproducibility of chromatographic separations.

In addition, Chromeleon provides two correction factors: ➤ *Sample Weight Factor (Weight)* and ➤ *Dilution Factor*. They allow you both to use the "approximates 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 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 recognition may have priority in the latter 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** has a more specific meaning than in normal colloquial use.

Each injection is defined as an individual "sample"!


Multiple injections from the same sample vial under similar conditions are considered several samples.

Defining a sample means the process of determining how much of a substance is injected from which vial and under which conditions, and which evaluation parameters are used. A distinction is made between a single sample and a sample series.

Single Sample

A sample can be analyzed individually by entering all required information and user commands via the keyboard or the mouse.

The user selects the **Inject** command, enters the volume to inject, and performs the injection via a hand-operated valve. If an ➤ *Autosampler* is available, the user determines the sample location with the ➤ *Inject* command. Then, data acquisition is started (via the ➤ *AcqOn/Off* command). When the end of the sample is reached, the user completes data acquisition (**Acq Off** command) and specifies where to save the acquired data.


The recorded data is temporarily saved to the **manual** sequence of the default ➤ *Datasource* of the system. In network operation, this datasource is designated with the computer name of the user on the  **Network**.

As soon as the user completes data acquisition, the user is prompted to select the final storage location for the temporarily saved data.


Sequence/Batch

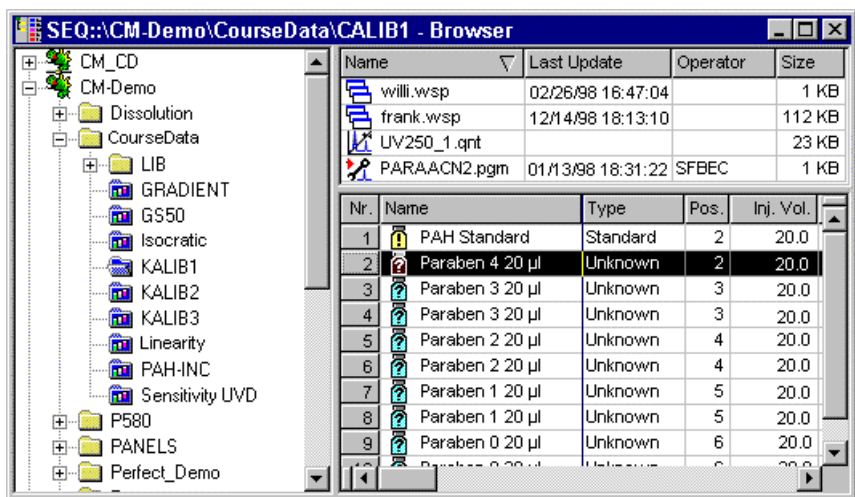
If several samples are to be processed successively, they are included in a sample list (sequence), together with the instrument control and evaluation information.

The samples are then 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. When a chromatographic analysis is 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:




Name	Last Update	Operator	Size
willi.wsp	02/26/98 16:47:04		1 KB
frank.wsp	12/14/98 18:13:10		112 KB
UV250_1.qnt			23 KB
PARAACN2.pgm	01/13/98 18:31:22	SFBEC	1 KB

Nr.	Name	Type	Pos.	Inj. Vol.
1	PAH Standard	Standard	2	20.0
2	Paraben 4 20 µl	Unknown	2	20.0
3	Paraben 3 20 µl	Unknown	3	20.0
4	Paraben 3 20 µl	Unknown	3	20.0
5	Paraben 2 20 µl	Unknown	4	20.0
6	Paraben 2 20 µl	Unknown	4	20.0
7	Paraben 1 20 µl	Unknown	5	20.0
8	Paraben 1 20 µl	Unknown	5	20.0
9	Paraben 0 20 µl	Unknown	6	20.0



A sample is characterized by various column entries. The entries are managed in a database and are referred to as **Sample Data**. For a short explanation of the column, press the F1 key.

Before the analysis, the user has to enter all samples to be processed and the characteristic sample data into the sample list. There are two options:

1. Change an existing sequence manually and then save it under a new name.
2. Create a new sequence automatically using the  **Sequence Wizard**.

For more information, refer to

How to ...: Actions in the Browser  **Creating a Sample List**.

In addition to "real" sample data (such as the sample name, the injection volume, the vial, and the 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 are added to a sequence, that is, the more sequences are created, 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*:

- Step 1: Select the timebase
- Step 2: Generate the analysis samples
- Step 3: Generate the standard samples
- Step 4: Determine the ➤*PGM File* and the analysis method
- Step 5: Save and name the sequence

Refer to the online Help for more information about the above steps.

Each step is performed on a separate wizard page. Clicking **<Back** or **Next>** takes you to the previous or next page.

In the fifth 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:**

*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 ...: Actions in the Browser  **Creating a Sample List.**

Automatic Batch Processing

The enormous technical complexity of modern chromatography systems, the resulting high purchasing costs and the constantly increasing number of samples in analysis laboratories make continuous operation even outside of regular working hours 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 of processing, the samples to be processed and in which order must be communicated to Chromeleon. This is performed in the sample list. The result is stored as a ➤*Sequence*.

Independently processing one or several sequences is known as ➤*Batch*. To start processing, the following steps are required:

Starting the Automatic Batch

After data input is completed, the analytical process can be started in an online batch.

- 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 process by clicking **Start**.

Online Batch: During the chromatographic analysis of the batch

As soon as the online batch is 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. Accordingly, more sequences are entered in the batch dialog (maximum 16). This 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: When starting the batch process, samples 1 to n of the first sequence, then samples 1 to n of the second sequence, etc. are analyzed.


Offline Batch: After the chromatographic analysis

The data acquisition results of the batch are saved with the individual samples. When the results are processed offline, e.g., printed, exported, signed, etc., after data acquisition is finished, a batch is called an offline batch.



Sample Evaluation

In spite of largely automated work processes and intelligent pre-settings, it is within the responsibility of each user to set the framework conditions for sample evaluation.


Calculations


Sample processing is performed based on a \Rightarrow *Program* (\Rightarrow *PGM File*) that was previously created and included in the sample list. Similar to this, the analysis results are calculated based on the evaluation method indicated in the sample list. The method itself is created in the \Rightarrow *QNT-Editor*. For more information, refer to **How to ...:**  **Actions in the QNT Editor**.

Result Output

The result of sample processing can be represented in graphics and tables, either on the screen or in a printed output. 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 ...:**  **Actions in the Report Table** and  **Actions in the Printer Layout**.

Overview of the Most Important Results in the Browser

To take 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 ...: Actions in the Browser**  **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 your 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 your results.



Note:

Electronic signature is only available for user databases that were 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 ...: Actions in the Browser**  **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 only determine the area (and height, respectively) below a peak, conversion into absolute amount or concentration units is possible only if a *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**
-  **Implementation**

At the end of a calibration, Chromeleon creates calibration curves from the available calibration points for each calibrated substance. Representing and evaluating the curves is performed in the calibration curve method window (see **Data Reprocessing**  **The Calibration Curve**).

Calibration Principle

The principle of the calibration is based on that one or several samples of known composition are analyzed by chromatography and a conversion factor amount (or concentration)/ area is calculated from the detected areas below the individual peaks and the known amounts or concentrations. This factor can then be used to multiply the area of the respective peak of an unknown sample. The result is the corresponding amount of the substance (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 c_1 applies:

$$S = c_1 * K$$

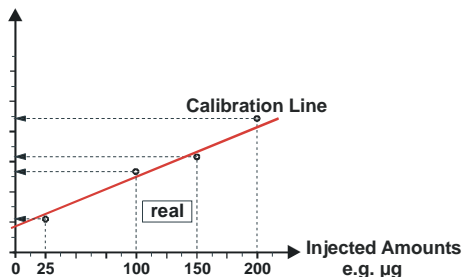
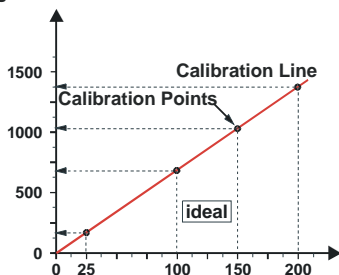
Under certain conditions, the area $F(x)$ corresponding to a certain amount (x) is proportional to the contained amount.


$$F(x) = c_1 * x$$

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 there is a direct ratio between the amount and the determined area. 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 which are above all caused by weight and/or dilution errors. This causes 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 and, usually, it does not go 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 \Rightarrow **Calibration Type** peak table variable, the user decides whether a linear or a non-linear calibration curve is calculated from the existing calibration points. Distinguish between the following calibration types:

 **Calibration Types (Linear)**

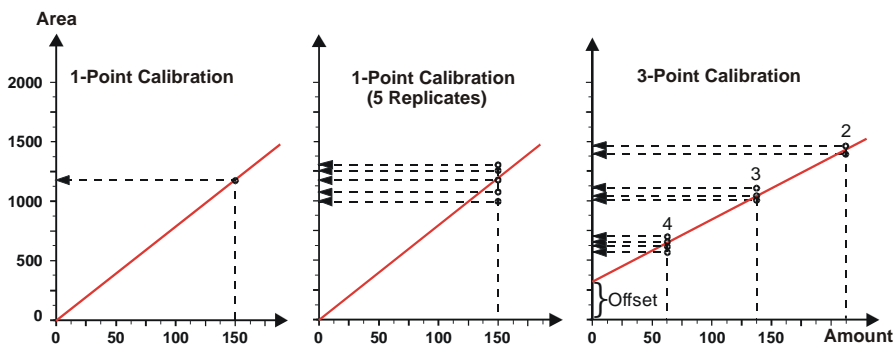
 **Calibration Types (Non-linear)**

Calibration Types (Linear)

If one calibration sample of a standard substance is analyzed for calibration only, the user enters exactly one concentration value in the first \Rightarrow *Amount* column of the peak table. The result is exactly one \Rightarrow *Calibration Point*. Connecting the calibration point with the origin then 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 c_1 (left partial fig.). C_1 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 available replicates, the impact of imprecision decreases after averaging. In spite of several replicates, only one amount/area ratio is determined. This is referred to as **multi-point calibration on one calibration level** (in the middle of the partial fig.).

The result is better secured if several concentrations are measured instead of one. 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 right partial fig.). The calibration curve does not necessarily have to go through the origin. The linear \Rightarrow *Calibration Function* is therefore corrected by an offset.

$$F(x) = c_0 + c_1 * x$$

**Caution:**

The decision whether a calibration type differing from the **linear** default is physically sensible, is within the responsibility of the user, not of Chromeleon!

**Note:**

If you calibrate using the ➤Standard Addition method and if no calibration points are available with Amount = 0 (only **Spiked** samples), Chromeleon calibrates with an offset.

If calibration points with Amount = 0 are available (also **Unspiked** samples) 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 using the **Linear with Offset** calibration method.

 **Calibration Types (Non-linear)**

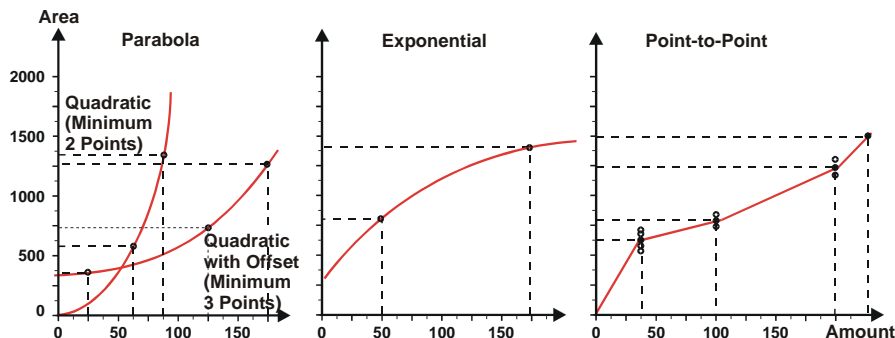
In (the more general) case of a non-linear calibration, more terms are added to the linear ➤Calibration Function.

Parabola-shaped curves are described as follows:

$$F(x) = c_1 * x + c_2 * x^2 \quad (\text{Quadratic})$$

$$F(x) = c_0 + c_1 * x + c_2 * x^2 \quad (\text{Quadratic with offset})$$

To calculate curves of this type, a minimum of two (quadratic) or three (quadratic with offset) calibration samples must be available (left partial fig.).

**Note:**

If you calibrate using the ➤Standard Addition method and if no calibration points with Amount = 0 are available (only **Spiked** samples), Chromeleon also calibrates with an offset when the **Quadratic** calibration method is used.

If calibration points with Amount = 0 are available (also **Unspiked** samples) and if you calibrate using the **Quadratic** 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 using the **Quadratic 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 (right partial fig.).

**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:

$$F(x) = \tilde{c} + c_0 * x^{c_1}$$

Distinguish between the following cases:

a) *If no calibration points with Amount = 0 are available (only **Spiked** samples), the expected offset is calculated using the **Linear with Offset** method, i.e.:*

$$\tilde{c} = c_0$$

b) *If calibration points with Amount = 0 are available (also **Unspiked** samples), 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, that is, 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:

*The decision whether a calibration type differing from the **linear** default is physically sensible, is within the responsibility of the user!*

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, that is, 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 for the range of the calibration samples only and not beyond it.

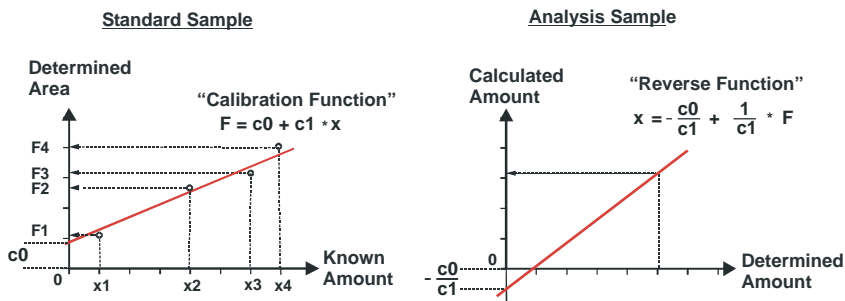
Using the Calibration Curve

If the calibration coefficients are known, the amount value can be calculated for any area value by inserting the coefficients in the formula of the respective calibration type. Within the range of the curve that is covered by the calibration points, it is possible to convert any peak area into the corresponding amount. This is shown in the following example:

Standard Sample

The user enters the amounts (x1 to x4) of the different standard samples and determines 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: c_0 , c_1).



Unknown Sample (Analysis Sample)

In the case of an unknown sample, the previously calculated calibration coefficient and one or several area values are known. The area value is now a known parameter and is thus drawn in x-direction. In the diagram, the two axes must be exchanged for one another. As a result, the calibration function must be converted in its inverse function. This is also performed by Chromeleon. Now, the amount can be calculated by inserting the calibration coefficients and area values.

Furthermore, the exclusion of outliers, the different weighting of calibration points, and the formation of "averaged" calibration points from one calibration level, are alternative ways of how to calculate the calibration curve.


Calculating the Calibration Curve


Calculation of the calibration curve (\triangleright *Calibration Function*) is based on the method of least squares. With a given calibration type (linear, linear with offset,...), the parameters of the calibration curve $F(a)$, that is, c_0 , c_1 , and c_2 , 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:

$$\sum_{i=1}^n w_i \cdot (x_i - F(a_i))^2 \rightarrow \min$$

$(x_i - F(a_i))$ refers to the distance of the x_i -value from the calibration curve $F(a)$, w_i is the selected weighting (see ➤ *Weights*, for example, $w_i=1$, $w_i=1/\text{Amount}$ or $w_i=1/\text{Amount}^2$) and x_i 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	<p>= 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.</p> <p>With an increasing number of different amounts, the area/amount ratio (=calibration curve) can be determined more exactly.</p> <p>If different amounts are obtained by diluting the original substance, a ➤ <i>Dilution Series</i> is resulting. The amount of each concentration is entered in a separate amount column in the corresponding line of the peak table.</p> <p>If the 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. The remaining amount values (for the different injection volumes) are calculated by Chromeleon.</p>
internal/ external	<p>Choose a substance as ➤ <i>Internal Standard</i> ("ISTD") whose retention time behavior is similar to the behavior of the substances to be analyzed. Before the sample preparation, an internal standard is added to all samples (unknown and standard samples) in exactly the same amount so that the 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.</p>

Standard	Description
internal	<p>In the pure internal standard method, calculation is via area and amount ratios instead of absolute areas and amounts. For this procedure, it is necessary to inject a constant amount of the ➤<i>Internal Standard</i> ("ISTD"). The internal standard is added before the sample preparation. In a dilution series, the standard does not have to be diluted (Const. Internal Standard). Due to the equivalent amount of added internal standard, the same ISTD result should be achieved for all samples. Forming the ratio of ISTD values allows you to draw conclusions about the precision of the analysis and calculating the actual result.</p> <p>Due to the intense experimental procedure, this type of calibration is rarely used in HPLC.</p>

For examples of the different standard methods, refer to

How to ...: Actions in the QNT Editor  **Calibration.**

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. Please 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, that is, calculating the \Rightarrow Amount values, the inverted form of the corresponding ➤*Calibration Function* F is used (=inverted function A). This means that the c0, c1, and c2 calibration coefficients form function A that is inverted for amount calculation. The result is F.

'External' Evaluation:

Calibration:

- $Y(i,k) = \text{RESPONSE}(i,k)$
- $X(i,k) = \text{AMOUNT_NOMINAL}(i,k) * (\text{WEIGHT}(k)/\text{DILFAC}(k)) * (\text{INJECTVOL}(k)/\text{REFINJECT})$

Evaluation: Calculation of the Amount peak variable for peak i in the sample x

- $\text{AMOUNT}(i,x) = F(\text{RESPONSE}(i,x)) * (\text{DILFAC}(x)/\text{WEIGHT}(x)) * \text{RSP-FACTOR}(i)$

Description: see below.

'Internal' Evaluation:*Calibration:*

- $Y(i,k) = 100 * (\text{RESPONSE}(i,k)/\text{RESPONSE}(\text{ISTD},k))$
- $X(i,k) = \text{AMOUNT_NOMINAL}(i,k) * (\text{WEIGHT}(k)/\text{DILFAC}(k))$

Evaluation: Calculation of the Amount peak variable for peak i in the sample x

- $\text{AMOUNT}(i,x) = F(100 * (\text{RESPONSE}(i,x)/\text{RESPONSE}(\text{ISTD},x)) * (\text{DILFAC}(x)/\text{WEIGHT}(x)) * \text{RSP-FACTOR}(i))$

The ISTD peak itself will not be evaluated!

Description: see below.

'Internal' Evaluation (with variable ISTD):*Calibration:*

- $Y(i,k) = \text{AMOUNT_NOMINAL}(\text{ISTD}) * \text{RESPONSE}(i,k)/\text{RESPONSE}(\text{ISTD},k)$
- $X(i,k) = \text{AMOUNT_NOMINAL}(i,k) * (\text{WEIGHT}(k)/\text{DILFAC}(k)) * (\text{INJECTVOL}(k)/\text{REFINJECT})$

Evaluation: Calculation of the Amount peak variable for peak i in sample x

- $\text{AMOUNT}(i,x) = F(\text{AMOUNT_NOMINAL}(\text{ISTD}) * (\text{RESPONSE}(i,x)/\text{RESPONSE}(\text{ISTD},x)) * (\text{DILFAC}(x)/\text{WEIGHT}(x)) * \text{RSP-FACTOR}(i))$

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'!
- $\text{FACTOR_IS}(x) = \text{AMOUNT_NOMINAL}(\text{ISTD})/\text{AMOUNT}(\text{ISTD},x)$
- $\text{AMOUNT}(i,x) = F(\text{RESPONSE}(i,x) * (\text{DILFAC}(x)/\text{WEIGHT}(x)) * \text{RSP-FACTOR}(i) * \text{FACTOR_IS}(x))$

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 (Sample Amount) is used. The sample weight of the ISTD peak is not considered.

- $Y(i,k) = \text{RESPONSE}(i,k)$
- $X(i,k) = \text{AMOUNT_NOMINAL}(i,k) * (\text{WEIGHT}(k)/\text{DILFAC}(k)) * (\text{INJECTVOL}(k)/\text{REFINJECT})$
- $X(\text{ISTD},k) = \text{AMOUNT_NOMINAL}(\text{ISTD},k) * (1/\text{DILFAC}(k)) * (\text{INJECTVOL}(k)/\text{REFINJECT})$

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.

- $\text{AMOUNT}(\text{ISTD},x) = F(\text{RESPONSE}(\text{ISTD},x)) * (\text{DILFAC}(x)) * \text{RSP-FACTOR}(\text{ISTD})$
- $\text{FACTOR_IS}(x) = \text{AMOUNT_NOMINAL}(\text{ISTD},x)/\text{AMOUNT}(\text{ISTD},x)$
- $\text{AMOUNT}(i,x) = F(\text{RESPONSE}(i,x)) * (\text{DILFAC}(x)/\text{WEIGHT}(x)) * \text{RSP-FACTOR}(i) * \text{FACTOR_IS}(x)$




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 peak table for the standard sample k
AMOUNT_NOMINAL(ISTD):	⇒ <i>ISTD Amount</i> for the sample k from the sample list
AMOUNT(ik):	Calculated amount of the peak i for the sample k
WEIGHT:	⇒ <i>Weight (Sample Weight Factor)</i> for a sample
DILFAC:	⇒ <i>Dil. Factor (Dilution Factor)</i> for a sample
INJECTVOL:	⇒ <i>Inj. Vol. (Injection Volume)</i>
REFINJECT:	Injection volume of the first sample in a calibration series
RSP-FACTOR(i):	⇒ <i>Response Factor</i> of the peak i from the QNT peak table


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, ..**).
- For a detailed description of the procedure, refer to **How to ...: Actions in the Browser**  **Creating a Sample List**.

Quantification Method (QNT Editor)

- Open the **General** worksheet and check the current settings. Define the calibration \Rightarrow *Mode* to be used.
- Enter the names and the retention times of the peaks to be determined on the **Peak Table** sheet. If a processed sample refers to a QNT File with an "empty" peak table, the peak table can be automatically filled with the retention times of the integrated peaks after the analysis by selecting the **Autogenerate Peak Table** command. Each peak contains a successively numbered default name.
- Enter the amount values of the standard substances in the amount columns. Determine the standard method, the calibration type, and the integration type for the calibration.
- Determine the standard method, the calibration type, and the integration type to be used for the calibration.
- For more information, refer to **How to ...: Actions in the Browser**  **Creating a Peak Table**.

Validation, AutoQ, and System Wellness

Validation and Qualification

Analysis data from various workstations or laboratories can only be compared 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.

When they are started, many instruments perform an automatic self-test 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*, 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 HPLC Devices

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. The following tasks can be automated with AutoQ test procedures.

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
- **Waters** HPLC modules (including the **Alliance 2690/2695** Systems and the 996/2996 PDA's)

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 you have validated the chromatography system, 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.




Tip:

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_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**.

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. Within the report, individual data sheets are very often accessed via references. 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 to make sure that, in the report, the data are read in and processed correctly. Select the sequence for which 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-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 \Rightarrow *QNT Editor*, define a \triangleright *System Suitability Test (SST)* to check whether the quantification method and the \triangleright *PGM File* are suitable for analyzing special samples. (For more information, refer to **How to ...: Actions in the QNT Editor**  **Defining the System Suitability Test**.) The System Suitability Test can already be performed while the chromatogram is recorded. The corresponding QNT Method needs to be available in the sample list (in the Browser). As **Fail Action**, select **Abort Batch** to automatically abort the sample \triangleright *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 \triangleright *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)

Device	Moduleware Version Required
Detectors	
AD25	1.02 (or higher)
CD20/ED40	3.05 (or higher)
CD25/ED50	1.05 (or higher)
CD25A/ED50A	1.00 (or higher)
PDA-100 (DX-LAN)	1.04 (or higher)
PDA-100 (USB)	1.0.0 (or higher)
➤Autosampler	
AS50	1.05 (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
- Pressure offset and degas calibration for pumps
- Flow rate calibration for pumps

For instructions on setting up and using System Wellness features, refer to

How to ...: Actions in the Browser  **System Wellness**

System Wellness for HPLC Devices (Overview)

Chromeleon provides several system wellness features for the Summit HPLC modules. 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
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
System	Documentation when new wear parts have been installed.	Tracking and troubleshooting
	Documentation when the solvent has been changed	Tracking and troubleshooting

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 partial window 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 is displayed 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 partial windows):



The QNT Editor



Integration



Reports



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** (2D channels)
- **Copy Channel**
- **Extract MS channel** (for extracting a *➤Mass Trace* - only possible if MS data is available)
- **Extract optimum integration path** (for extracting the *➤Optimum Integration Path* - only possible if a 3D field is available)
- **Extract UV channel** (for extracting a UV channel - only possible if a 3D field is available)
- **Smooth data** (for chromatogram *➤Smoothing*)

For more information, refer to **How to ...**:

Actions in the PGM Editor  **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 \Rightarrow *Amount* of the standard samples?
- Which ➤ *Calibration Function* is used for creating a calibration curve?
- Which peaks are identified by name?

Before the actual analysis, these evaluation parameters are defined in the Quantification method (QNT File).

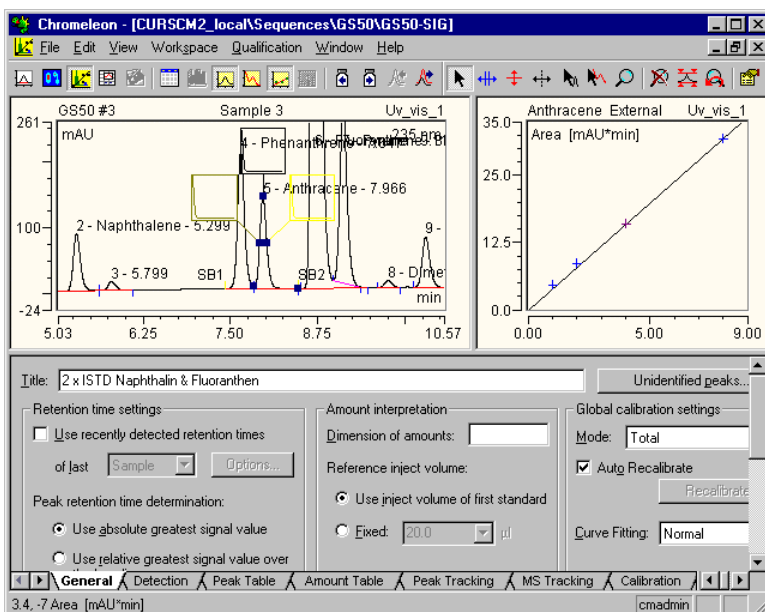
- Select **File > New > Method File** to generate a new QNT File.
- Alternatively, select a sequence in the Browser and double-click the corresponding QNT File to open it.
- To open the QNT Editor from a method file, click the **QNT Editor** icon  on the toolbar. This allows you to display the QNT File data of 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:

-  **The Trend Plot** (refer to the **Control** section; similar functionality as the control panel)
- UV spectrum (if available)
- *➤Mass Spectrum* (if available)

In addition, you can include report tables in the tab pages.

The QNT Editor is divided in two window sections. The upper section serves for information purposes. To create a QNT File, use only the lower window section.

Upper Pane

The upper pane serves to display additional plots:

- Select **Show Chromatogram** or **Show Calibration Curve** on the **View** menu to enable or disable the display of the chromatogram and/or the calibration curve.
- Press the F4 key or the Shift+F4 keys or select the **Next > Previous Chromatogram** option (on the **File** menu) to toggle between the chromatograms of the individual samples.
- Select the **Show Spectra** option to enable or disable the display of the spectrum of a single peak.

Lower Pane

The lower pane serves to determine the evaluation parameters.

- Select a specific tab to open a worksheet, similar to Microsoft Excel.
- You can freely select the names of the worksheets. Double-click the corresponding name, and enter a new name in the edit dialog box.

The following worksheets are available by default (unless you changed their names):



The General Tab Page



Detection Parameters



Peak Table, Amount Table, Peak Tracking, and MS Tracking



Calibration Settings



Spectra Library Screening



System Suitability Test




Mass Spectra (MS)



UV Spectra (UV)





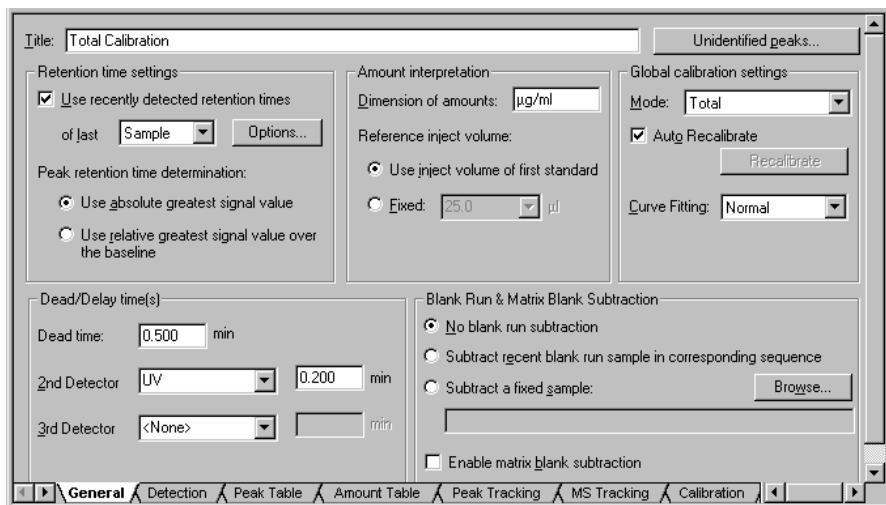
Tip:

*These topics describe the structure and functions of the individual worksheets. For detailed information about how to enter data, refer to **How to ...**:  **Actions in the QNT Editor.***

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 to quantify unidentified peaks.



The screenshot shows the 'General' tab page of the QNT Editor. The interface is organized into several sections:

- Title:** A text field containing 'Total Calibration'.
- Unidentified peaks...** A button in the top right corner.
- Retention time settings:**
 - ☒ Use recently detected retention times
 - of last: **Sample** (dropdown menu) **Options...** (button)
 - Peak retention time determination:
 - ☒ Use absolute greatest signal value
 - ☐ Use relative greatest signal value over the baseline
- Amount interpretation:**
 - Dimension of amounts: **µg/ml** (dropdown menu)
 - Reference inject volume:
 - ☒ Use inject volume of first standard
 - ☐ Fixed: **25.0** (text field) **µl** (dropdown menu)
- Global calibration settings:**
 - Mode: **Total** (dropdown menu)
 - ☒ Auto Recalibrate **Recalibrate** (button)
 - Curve Fitting: **Normal** (dropdown menu)
- Dead/Delay time(s):**
 - Dead time: **0.500** (text field) **min** (dropdown menu)
 - 2nd Detector: **UV** (dropdown menu) **0.200** (text field) **min** (dropdown menu)
 - 3rd Detector: **<None>** (dropdown menu) **min** (dropdown menu)
- Blank Run & Matrix Blank Subtraction:**
 - ☒ No blank run subtraction
 - ☐ Subtract recent blank run sample in corresponding sequence
 - ☐ Subtract a fixed sample: **Browse...** (button)
 - ☐ Enable matrix blank subtraction


The bottom of the window features a tab bar with the following tabs: **General** (selected), **Detection**, **Peak Table**, **Amount Table**, **Peak Tracking**, **MS Tracking**, and **Calibration**.

Retention Time Settings

- Select the **Use Recently Detected Ret. Times** check box to use the \Rightarrow Retention Time of the preceding sample to identify a peak via a retention time window (\Rightarrow 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 \Rightarrow 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.

- Use the **Peak retention time determination** section to specify how the retention time of peaks shall be 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.


Dead/Delay time(s)


- Enter the \Rightarrow *Dead Time* in the **Dead Time** field. The dead time is used for calculating the \Rightarrow *Capacity Factor k'* and the \Rightarrow *Kovats Indexes*.
- Select the name of any further detector from the **2nd Detector and/or 3rd Detector** drop-down list and enter the \Rightarrow *Delay Time* in the **min** field. (Also, refer to **How to ...: Actions in the QNT Editor**  **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 \Rightarrow *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 shall be calibrated and based on which \Rightarrow *Calibration Mode* calibration shall be performed. 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 ...: Actions in the QNT Editor**  **Calibration**.
- When **Auto Recalibrate** is enabled, each modification within a chromatogram such as moving peak delimiters results in automatic recalculation of the \Rightarrow *Calibration Coefficient* and all derived calibration data. Disable **Auto Recalibrate** in your peak table to include the c0, c1, and/or c2 columns. Perform recalibration by clicking the **Calibrate** button. Nevertheless, you can 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 ...: Actions in the QNT Editor**  **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. The chromatogram of the blank run sample is subtracted point by point from the active chromatogram.
- 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 ...: Actions in the QNT Editor**  **Subtracting a Blank Run Sample.**

Detection Parameters (Detection)

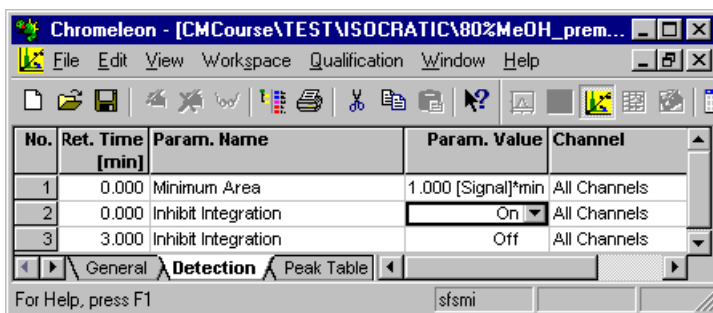
Detection parameters serve, for example, to recognize, classify, and suppress peaks as well as to determine the baseline.

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. Each parameter can be enabled, disabled, or changed in its value at a specific time. The time when the parameter shall change, the parameter name and its value are entered in the corresponding column of 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 $1 \times [\text{Signal}] \cdot \text{min}$ 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.

The \triangleright *Channel* column indicates whether the settings are valid for a specific channel (as in this example) or for all 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-Integrate** 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 \triangleright *Report Definition File*.

For more information and examples, refer to

How to ...: Actions in the QNT Editor 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 ...: Actions in the Chromatogram**  **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**. Individual columns and entire pages can be displayed and hidden.

The peak table is usually created manually; i.e., the user enters the names and retention times of the expected peaks. In addition, peaks serving as standard peaks must be labeled as such 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 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, the values in the **Retention Time** and **Window** columns are recalculated and default settings replace all other entries (see **How to ...: Actions in the QNT Editor**  **Autogenerating the Peak Table by Including Library Screening Results**).

 **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

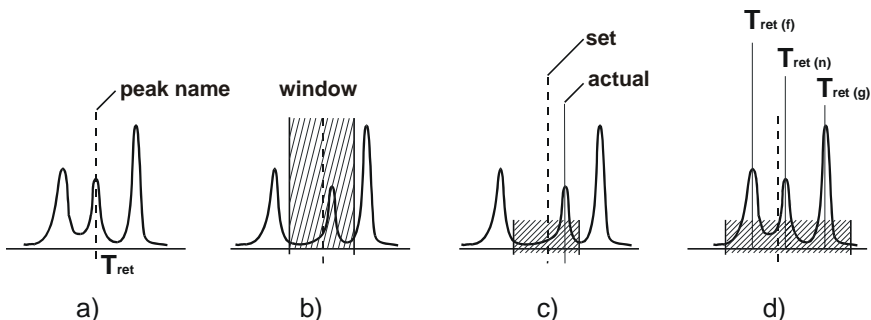
How to ...: Actions in the QNT Editor  **Creating a Peak Table**

Peak Identification/Peak Tracking and MS Tracking


Peak identification refers to the following procedure: A previously unknown peak is recognized due to peak-specific properties, such as the retention time or its UV spectrum, and is then 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 \Rightarrow 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 the *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).



The retention time value previously entered in the peak table is corrected accordingly!


Peaks can also be identified by the **UV spectrum**. You can use the **Spectrum** alone, or the spectrum and the retention time (**Spectrum and time**). This method requires a ➤*Photodiode Array Detector*, such as the Dionex UVD 340U. For each peak, the substance spectrum is recorded and compared to the library spectra, using certain criteria (see **How to ...: Actions in the QNT Editor**  **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 ...: Actions in the QNT Editor**  **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**. For a description of the columns and for details on the required column input, refer to **How to ...: Actions in the QNT Editor**  **Creating a Peak Table**.



Tip:

For background information about calibration, refer to **Theory of Calibration**  **Calibration (Overview)**. For information about how to perform calibration, refer to: **How to ...: Actions in the QNT Editor**  **Calibration**.

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 \Rightarrow *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 then excluded from the calculation. The corresponding calibration points are then highlighted in the curve by a different color.


For more information, refer to **How to ...: Actions in the QNT Editor**

 **Disabling Calibration Samples.**


Spectra Library Screening


To identify substances, spectra can be compared to library spectra. The spectra search can be performed via the **Spectra Library Screening** sheet of the **QNT Editor**. For more information about Spectra Library Screening, refer to **How to ...: Actions in the QNT Editor**  **Spectra Library Screening.**

The System Suitability Test


The aim and objective of the \triangleright *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 ...: Actions in the QNT Editor**  **Defining the System Suitability Test.**

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 ...:**  **Actions Related to 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 ...: Actions in the QNT Editor**  **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 ...: Actions in the QNT Editor**  **Processing Mass Traces.**

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 shall be aggregated into a total background spectrum that is then subtracted from the averaged peak spectrum.

For more information, refer to **How to ...: Actions in the QNT Editor**  **Processing UV Channels.**

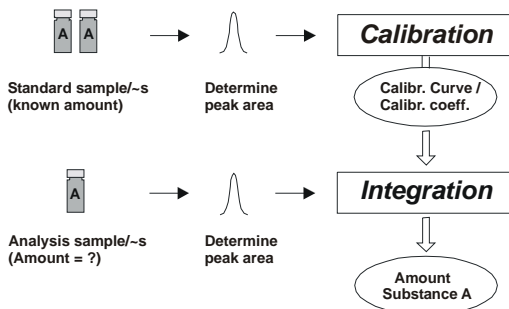
Integration


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**

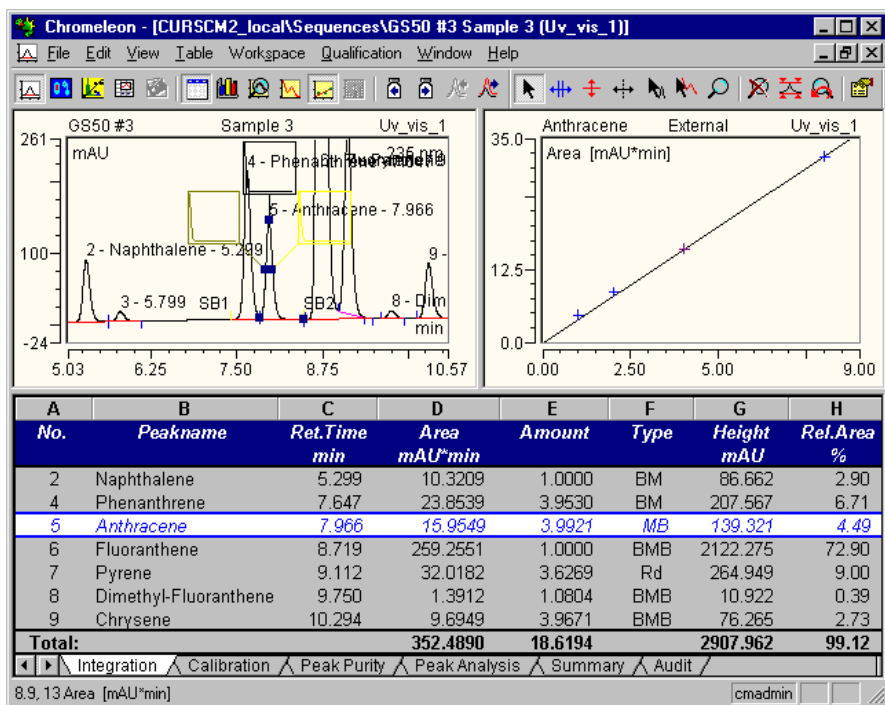
 **Reports Overview)**

 **The Calibration Curve**


The Integration Window


Chromatographic results are displayed in the **Integration** window. In addition, you can use the window to reprocess single chromatograms. Usually, the following items are displayed:

- The chromatogram of the current sample
- Various report tables



In addition, you may as well display:

- The calibration curve of the current peak (see the image above)
-  **The Trend Plot** (refer to the **Control** section; similar functionality as in the control panel)
- UV spectrum (if available)
- ➤ *Mass Spectrum* (if available)

Settings in the **Integration** window are saved together with the  **Printer Layout** in the \Rightarrow *Report Definition File* (RDF File).

For more information, refer to:

 **Opening a Sample**

 **Operation**

 **Manual Re-Integration**

 **Chromatogram Comparison**

 **Data Smoothing**

 **Peak Ratio**

 **The Spectra Plot**

 **The Mass Spectra 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 \triangleright *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.

Samples are usually opened after they have been completely processed. However, you may open samples while they are being analyzed, that is, while they are in the **Running** \Rightarrow *Status*, provided that a certain amount of data has already been acquired. Depending on the \Rightarrow *Step* and other detector settings, the sample must have run for several minutes. There are two ways to open a running sample:

- In the Browser, using the different options described above, or
- On a \triangleright *Control Panel*, by selecting **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.
- Select **Spectra Tool** on the context menu (or click the corresponding icon on the **Integration** toolbar) to display a spectrum from the chromatogram at the time *t*. As soon as the spectrum symbol is added to the mouse cursor, you can click any position to extract and display a spectrum on the spectra plot if the corresponding data exists. A minimized representation of the spectrum itself is displayed in the chromatogram. Repeat the operation while pressing the SHIFT key to overlay single spectra. Alternatively, select **Overlay Spectra** on the context menu. **Hide Time Spectra** will remove the overlaid spectra.

Opening Additional Window Sections



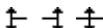







Select one of the following commands to display more window sections:

- Select **Split Zoom** on the **View** menu to split the window. By drawing a frame, you can zoom a window section. The upper half of the window shows the entire chromatogram and the frame, the lower half displays the zoomed section. Use the mouse to move and position the frame in the upper window section. The shape of the mouse cursor () indicates that this mode is enabled. Of course, you can draw a new frame at any time.
- Select **Report** on the **View** menu to display the integration report (see **Reports**  **The Integration Report**).
- Select **Show Spectra** on the **View** menu to display the peak spectrum of the currently selected peak (see  **The Spectra Plot**).
- 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 => <i>Detection Parameter</i>
	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 ...: Actions in the Chromatogram**  **Manual Re-Integration**.

Tip:

If manual modifications are performed, display the integration report to see the numerical results (see **Reports**  **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, an unlimited number of chromatograms can be displayed simultaneously in Chromeleon.

However, the presentation becomes confusing when many chromatograms are displayed. That is why Chromeleon prompts the user to confirm that more than 20 chromatograms shall be displayed. This is to prevent that too many chromatograms are displayed due to an operator error, which may result in a decrease of 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 specifically compare samples with certain properties.
- Simultaneously click the **Next Chromatogram** icon () and press the CTRL key to additionally display the chromatogram of the next sample.
- Simultaneously click the **Previous Chromatogram** icon () and press the CTRL key to additionally display the chromatogram of the previous sample.
- Simultaneously click the **Next Channel** icon () and press the CTRL key to additionally display the next channel of the same sample.
- Simultaneously click the **Previous Channel** icon () and press the CTRL key to additionally display the previous channel of the same sample.

For more information about how to select chromatograms and/or channels to be compared, refer to **How to ...: Actions in the Chromatogram**  **Performing a Chromatogram Comparison.**

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, other ways must be used to perform the comparison as exact as possible. This is achieved by adapting 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 ...: Actions in the Chromatogram**



Performing a Chromatogram Comparison.

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 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 ...: Actions in the Chromatogram**  **Performing Data Smoothing.**

For MS Chromatograms, refer to **How to ...: Actions Related to 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 desired 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: The resulting channel is calculated immediately.
- As ➤ *Post-Acquisition-Step* in the PGM Editor: The resulting channel is calculated immediately after data acquisition is complete.

Arithmetic combinations are not restricted to chromatograms in the strict sense. They can be used 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.

$$S = c1 \times K$$

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.

$$q = \frac{S1}{S2} = \frac{c1(\lambda1)}{c2(\lambda2)}$$

If q is entered against the time, the (theoretical) result for each peak of the sample is a horizontal line of the height $c1(\lambda1)/c1(\lambda2)$.

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.



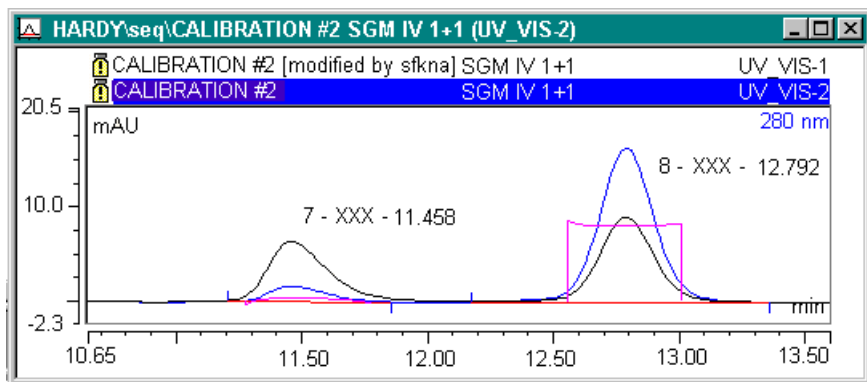
Note:

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 to numerically express the result of the peak ratio.

The 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 you to objectively compare two spectra of different concentrations. If spectra of the same peak, but with 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, determine the type of normalization that shall be applied to the spectrum.

Normalized spectra representation allows you to perform the following tasks:

- Comparing two spectra such as a standard and a sample
- Determining the number and position of minima and maxima
- Selecting an appropriate type of normalization

- Verifying the linearity of the Lambert-Beer law
- Deciding: Baseline correction Yes/No
- Determining exact integration limits by checking peak purity at various wavelengths and peak heights
- Identifying 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 enable **Spectra Tool** option or click the corresponding icon on the **Integration** toolbar. A spectra symbol that is added to the mouse points indicates that the mode has been 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 desired sample.
- 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 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 ...: Actions in UV Spectra and/or Mass Spectra**  **Starting a UV Spectra Search**.

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 objectively compare mass 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 fixed sample** option and click **Browse...** to navigate to the desired sample.

- 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**).

Reports

Reports (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*, together with the printer layout.

To open the report tables, either select **Show Report** on the **View** menu or click the following button: . 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.

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 ➤ <i>Mass Spectrometer</i> .
MS Status Log Report	Displays the mass spectrometer settings.
MS Tune Data Report	Displays the tune data of the ➤ <i>Xcalibur</i> raw data file.
MS Instr. Method Report (XRaw)	Indicates the MS method.

Spectra Results

MS Raw Report	Displays the raw data of the current ➤ <i>Mass Spectrum</i> .
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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	These reports combine data of the current sample. Displays all ⇒ <i>Detection Parameters</i> of the current sample.
Peak Table	Displays all variables that are required for displaying the peak table of the ⇒ <i>QNT Editor</i> .
SST Table	Lists variables of the ➤ <i>System Suitability Test</i> for the current sample.

PGM Tables

Commands	Displays the ➤ <i>Program</i> for the current sample.
Post Acq. Processing	Displays the ➤ <i>Post-Acquisition Steps</i> for the current sample.

Miscellaneous Tables

Database Query	Inserts any kind of database queries into the report.
History Report	Displays all variables required for creating a ➤ <i>History</i> 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 will be 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 ...:**

**Actions in the Report Table.****The Integration Report**

The Integration Report (or more precise, the Integration Report Table) is usually the first table of a report. For example, the **defltddad** \Rightarrow *Report Definition File* 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 \Rightarrow *Peak Variables*.
- The individual columns contain variables of the \Rightarrow *Peak Results* report category. Nevertheless, you can add more columns from other \Rightarrow *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 ...:**  **Actions in the Report Table.**



The Calibration Report

The Calibration Report (or more precise, 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 \Rightarrow *Peak Calibration* report category. Nevertheless, you can add more columns from other \Rightarrow *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 ...:**  **Actions in the Report Table.**

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 \Rightarrow *Sequence*.
- If **several samples** are selected in the Browser, these selected samples only will be part of the **Peak Summary** report table.
- If a \triangleright *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). Only when a peak is selected manually within a chromatogram, the Peak Summary Table receives entries. 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 5% of the detected retention time) is calculated for this peak. All peaks within this time window will be included in the summary. If two or more peaks are detected in the time window, the peak that is nearest to the specified retention time will be selected. If the peak is an identified peak, this will be indicated by **Ambiguous?** in the **Summary** header.

Creating a Summary

- Double-click one of the samples to open the Integration window.
- Display the Report and select the default **Summary** worksheet available Chromeleon. It contains a selection of the most important default \Rightarrow *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 ...: Actions in the Report Table**  **Adding and/or Renaming a Worksheet.**

As in all reports, the currently selected sample in the chromatogram window is highlighted in the Summary Table.

The History Report

The standard \Rightarrow *Report Definition Files* include both, an  **Integration Report** and a  **Calibration Report** but no history report table. However, you can easily include the History Report table (see **How to ...: Actions in the Report**  **Adding and/or Renaming a Worksheet**).




Contents

- By default, the history report displays the \triangleright *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 **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...
- The history mode includes a special layout mode. Enable **Layout Mode** on the **Layout** tab page.

Special Report Tables

The standard \Rightarrow *Report Definition Files* include both, an  **Integration Report** and a  **Calibration Report** but none of these special reports. However, you can easily include them (see **How to ... Actions in the Report Table**  **Adding and/or Renaming a Worksheet**).

MS Reports

MS Instrument Info Report	Information about the \Rightarrow <i>Mass Spectrometer</i> .
MS Instrument Method Report	MS method.
MS Raw Report	Raw data of the current \Rightarrow <i>Mass Spectrum</i> .
MS Status Log Report	Mass spectrometer settings.
MS Tune Data Report	Tune data of the \Rightarrow <i>Xcalibur</i> 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 \Rightarrow <i>Detection Parameters</i> .
Program Report	\Rightarrow <i>Program</i> of the current sample.
SST Report	\Rightarrow <i>System Suitability Test</i> .




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.

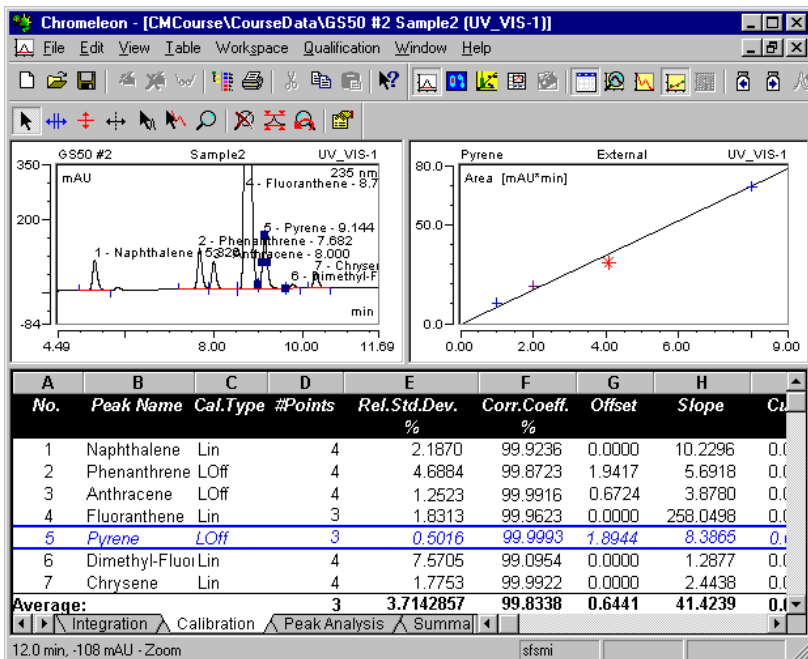
The Calibration Curve


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 else, 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 the F4 key or the Shift+F4 keys to change to the chromatogram of a different sample. Generally, all chromatograms of a sequence can be thus displayed one after the other.
- In the chromatogram, select the peak for which you want to display the calibration curve. The peak will be indicated by a different background color.
- The **Calibration** tab page of the **Report** window shows the most important calibration data, such as the \Rightarrow *Calibration Type*, the number of calibration points, the \triangleright *Relative Standard Deviation* (Rel.Std.Dev), the \triangleright *Correlation Coefficients* as well as the calibration coefficients \triangleright *Offset*, \triangleright *Slope*, and \triangleright *Curve* (offset c0, slope c1, curve c2). In addition, you can display, for example, the \triangleright *Variance* or the \triangleright *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 ...:**  **Actions in the Report Table**.

For more information about the calibration curve, refer to **How to ...:**  **Actions in the Calibration Curve**.

**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 (RDF)*. 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 ...:**




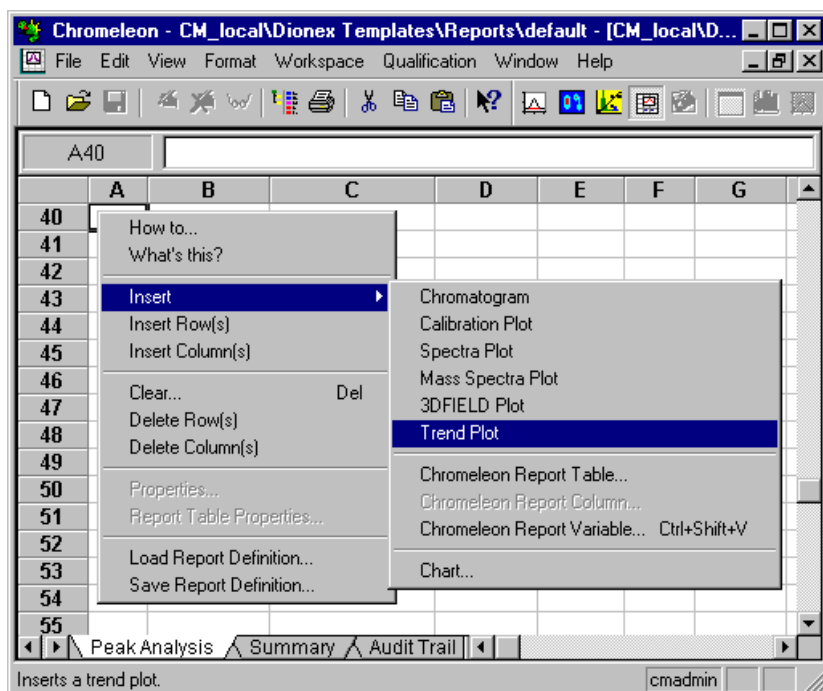
Actions in the Printer Layout. Also, refer to  **Specifying the Printout** for printing your results.



Appearance and Function

Important features of the Printer Layout are:

- The appearance and the structure are similar to Microsoft Excel. The functional scope is similar to Excel as well. To enter formulas and to create diagrams, use the ➤ *Report Publisher*, which is a Chromeleon add-on product.
- Toggle between the individual sheets of a Printer Layout by clicking the tabs provided on the bottom of the window bottom.
- Double-click the name of a tab to change its 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**.)



- 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 than 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 ...:**  **Actions in the Printer Layout.**

 **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.

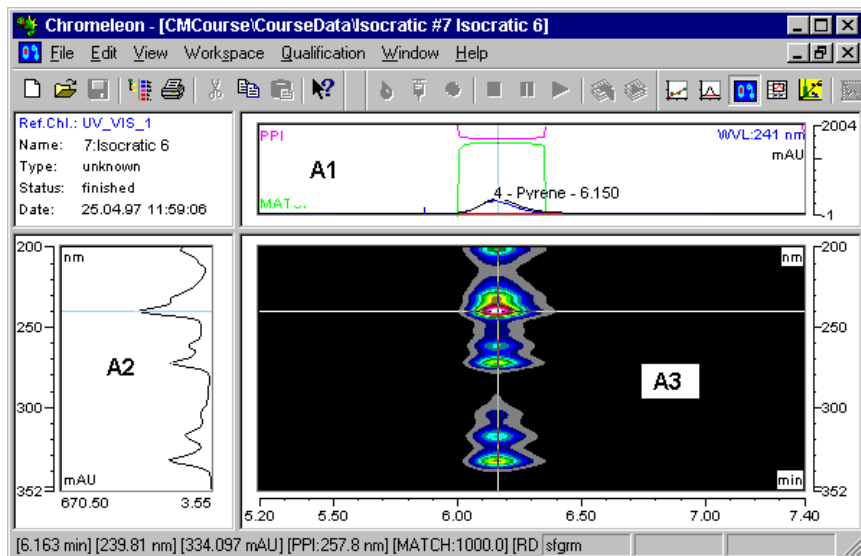
Use one of the following methods to display a sample (for which you have the corresponding 3d raw data) in the method PPA:

- Select the sample in the Browser and then select the **Open > 3DFIELD** on the context menu, or
- Change from a different method to the PPA method. Click the 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.



The display of the window sections A1 and A2 can be enabled or disabled via the **Show Chromatogram** or **Show Spectra** options. Optionally, an additional numeric report (PPA report) can be displayed (**Show Report**). The line of the currently selected peak is highlighted 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 the A3 windows to open the **3D field Decorations ...** dialog box.
- Right-click while the 3D field is redrawn to stop redrawing. This option is useful when the wrong zoom area was chosen.
- Double-click the time axis of the window to execute 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," so that it is still displayed even when moving the cross-wires. The spectrum extracted via the cross-wires is displayed in addition. If the operation is performed several times, any number of individual spectra can be displayed 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. This procedure can be performed 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 thus 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 ...: Actions in the PPA Window**  **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 ...: Actions in UV Spectra and/or Mass Spectra**  **Starting a UV Spectra Search**.

Function

Use the PPA method

- To analyze the peak purity (see **How to ...: Actions in the PPA Window**  **Checking Peak Purity**).
- To assign peaks interactively via the spectrum.
- To extract chromatograms, spectra, and the optimum integration path (see **How to ...: Actions in the PPA Window**  **Selecting the Optimum Integration Path** and  **Extracting Spectra, Chromatograms, and 3D Field Data**).

- 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

These methods are completed by various procedures and calculations for result interpretation and/or comparison. These include:

➤ *Baseline Correction*

➤ *Blank Run Subtraction*

➤ *Normalization*

➤ *PPI: Peak Purity Index*

➤ *PPI: Match Factor*

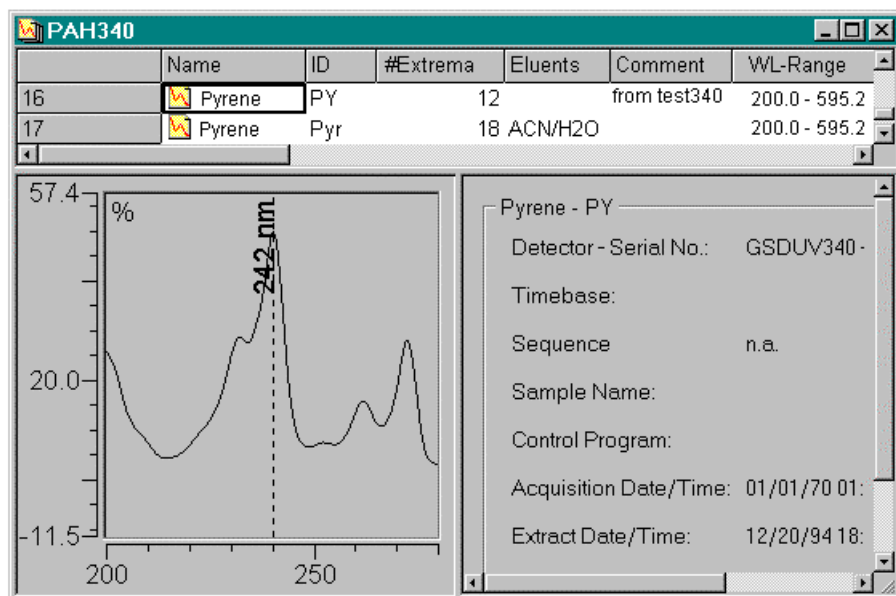
Spectra Libraries

Spectra Libraries (Overview)

The partial method **Spectra library** enables the comparison of ➤*Normalized* and ➤*baseline-corrected* individual spectra with spectra from various libraries. There are three sections:

Spectra Table


The upper section indicates all spectra contained in a ➤*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.



- As soon as you move the mouse cursor over the first column, the cursor changes to a horizontal arrow. Left-click to select a spectrum.
- Press and hold the CTRL key to select several spectra.

Spectra Plot

The spectra plot is shown in the lower left window section. It shows 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.**


Data Window

The lower right window section of the Spectra Library is reserved for representing the data. It shows important data regarding the spectrum currently selected in the spectra table.

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 ...: Actions in UV Spectra and/or Mass Spectra**  **Starting a UV Spectra Search.**

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. For more information about Spectra Libraries, refer to:



Creating a New Library



Comparing Spectra



Creating a New Library

Generating

Select the **File > New > Spectra Library** to open a new and empty spectra library.

Filling

There are two options for filling a spectra library:

- From the **PPA: Peak Purity Analysis** or **Spectra Plot** partial methods, copy a spectrum to the Windows clipboard using the **Copy** command, and then insert it in the open library using the **Paste Spectra** command. (Also, see **Data Reprocessing**  **PPA: Peak Purity Analysis** and/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 with each other, the spectrum and the reference spectrum should be recorded under identical conditions. The best search results are therefore obtained based on spectra that were recorded and saved by the user. It is highly recommended to write down the conditions under which the spectrum is recorded. The notes will be very useful when there are several spectra for the same sample.

Saving

Select **Save as** to save an open library under a different name to a directory of your choice. Libraries have the file extension **LIB**.

Comparing Spectra


To compare two spectra with each other, the curve of the normalized spectrum can be compared to those of individual library spectra. In some cases, better results are achieved when the first or second derivations are compared instead of the spectra themselves.

Via the comparison function (see \Rightarrow *Check Derivative*), the user determines the curve form to be used for the comparison.

In the next step, the user determines how the individual curves are compared with each other. This is by selecting one of three mathematical methods (\Rightarrow *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 \triangleright *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. Entering a minimum similarity value (threshold) displays only the hits above the threshold.

For more information about how to search spectra, refer to **How to ...: Actions in UV Spectra and/or Mass Spectra**  **Starting a UV Spectra Search.**



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 these hit lists. That is why \triangleright *Hit Criteria* are available as additional filters. Only the spectra fulfilling the selected criteria will be displayed.

How to ...:

Actions in the Browser

The Browser serves to manage, copy, move, and delete chromatographic data and files. For more information, refer to  **Moving and Copying Elements**. For general information about the Browser, refer to **Data Management**  **The Browser**.



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.

In the Browser, you can also create a sample list, set up datasources, or search and save data in a datasource.

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



System Wellness



Importing PeakNet Method Files (Release 4.5 Through 5.2)



Importing Agilent/HP ChemStation Data Files

For information about any actions that administrators can perform in the Browser, refer to the **Administrator Manual: How to ...**:



Actions in the Browser and in the Operating System

Moving and Copying Elements

Use the Drag & Drop function 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 the desired option under **When dragging item between folders**:

Ask	For each Drag & Drop action within a datasource, you are prompted to click the desired 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)	<p>There are two different cases:</p> <p>Within a datasource: For simple Drag & Drop actions, the selected element is moved to the new position and deleted from the original position.</p> <p>To a different datasource: The selected element is copied to the target position, but is not deleted from the original location.</p>

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 key	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:



Select the desired action (Copy, Move, or Cancel).

Copy elements via Copy & Paste

To copy the desired 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 either **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).


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 \Rightarrow *Program* and \Rightarrow *Method* columns, determine the \Rightarrow *Chromatographic Methods* to be used for the analysis.
- In the \Rightarrow *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., \Rightarrow *Weight* (Sample Weight Factor), \Rightarrow *Dil. Factor* (dilution factor) and \Rightarrow *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 the desired value or select the desired 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.
- To enter special values, create \Rightarrow *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 \Rightarrow *Sequence Report Columns* (see  **Creating a Sequence Report Column**).
- Save the sequence under a new name, selecting **Save** on the **File** menu.
- Check the sequence properties by pressing <Alt> + <Enter>. Or else, select the sequence, and then select **Properties...** on the context menu.

**Note:**

Of course, it is also possible to edit an existing sequence. Exception: It is not possible to edit a sample that is currently running (the corresponding line in the sample list is highlighted in a different color).

Automatically creating a sample list


- In the Browser, select **New** on the **File** menu. Select **Sequence (using Wizard)** from the list.
- Click **OK** to open the Sequence Wizard. (For information about the wizard, refer to **Samples and Sequences**  **The Sequence Wizard**.)
- 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.

**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 desired name is also an SQL keyword, we recommend 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 box:
- 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 ...: Actions in the Printer Layout**  **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 using user-defined columns, also see  **Creating a Sequence Report Column.**

Creating a Sequence Report Column

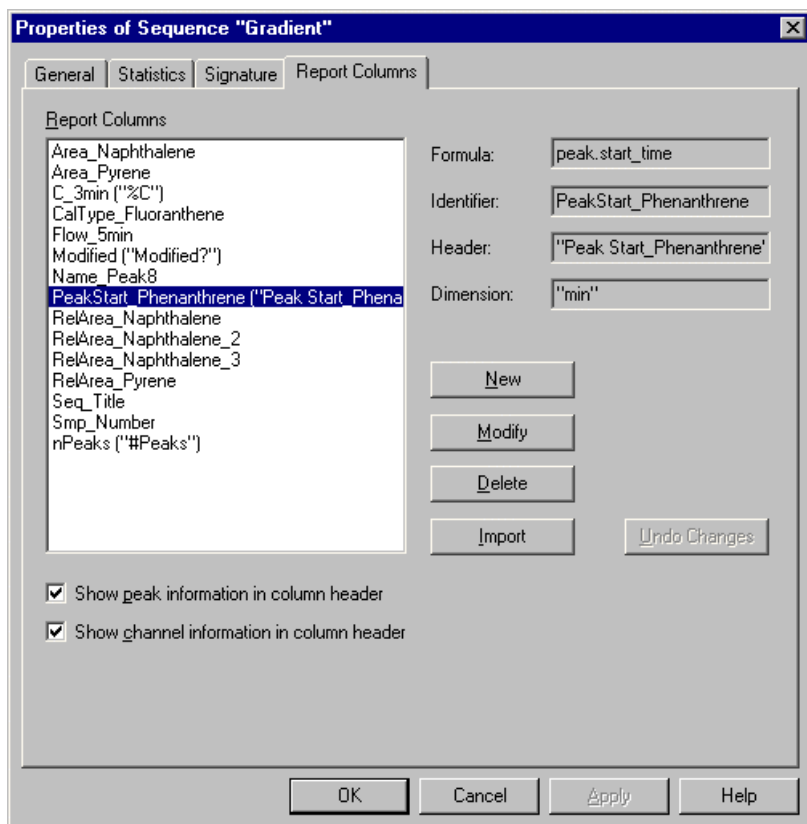
It often makes sense to have some sample results displayed in the sample list of a sequence. To do so, add sequence report columns to the sample list, in addition to the default columns and the ➤ *User-defined Columns*. Thus, the sequence report columns can provide an overview of the most important results 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**.

Create Sequence Report Column

Formula: ... (Alt + \')

Identifier: (unique name, e.g. used for column layout)

Header: (visible headline)

Dimension:

Format

Alignment: ☐ Left ☐ Center ☒ Right

Precision: (floating point digits) ☐ Digit Grouping (e.g. 1,234,567)

Peak

☒ Peak Name

☐ Peak Number

☐ Peak Table No.

Channel

☒ Preferred Channel

☐ Fixed Channel

Statistical Values

☐ Sum

☒ Average Value

☒ Relative Standard Deviation

OK Cancel Help

- First, select the desired report variable. All report variables of the different \Rightarrow *Report Categories* are available for selection. Click the '...' button, and then select the desired 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 shall refer. Select the desired option under **Peak**.
- 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 the desired 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	RelArea_N_2	RelArea_N_3	Ret.Time (det)	Area_P	Amount_P
					[%] Naphthalene Uv_vis_1	[%] Naphthalene Uv_vis_2	[%] Naphthalene Uv_vis_3	[min] Pyrene Uv_vis_1	[mAU*min] Pyrene Uv_vis_1	[µg/ml] Pyrene 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
\bar{x}	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 the desired 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 .


**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 the desired entry and press .

Undoing Changes

There are different ways to undo changes made for a sequence report column:

- In 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**.

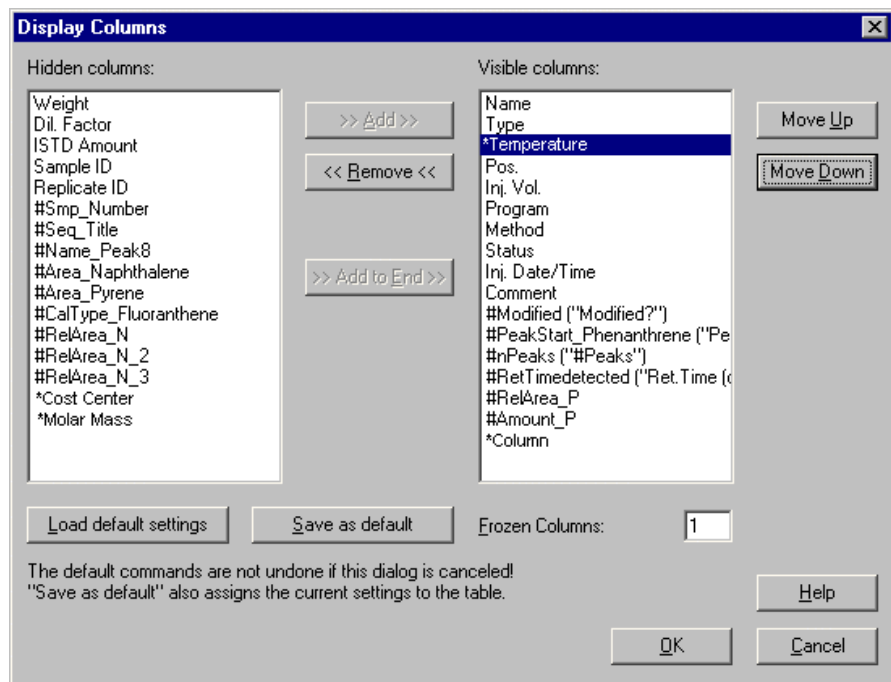
■ Displaying Sequence Columns

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 cursor to the right of the column separator until the cursor changes its appearance. Left-click and draw 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

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**

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, a ZIP drive, or a 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 desired datasource.
- 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 have connected to a datasource on a removable medium via the **Mount Datasource** command, do not remove the medium (diskette, Zip disk, CD-ROM) until you have correctly closed the connection, by selecting **Dismount Datasource** on the context menu. **If you do not close the connection in this way, the operating system will crash!***

Opening Audit Trails

In the Browser, you can open two types of Audit Trails (see **Data Management**  **The Audit Trail**):

**Daily Audit Trails****Sample Audit Trails**

In the ➤ *Standard Datasource*, the daily audit trails are listed in the AUDIT directory for the corresponding ➤ *Timebase*. Double-click to open a daily audit trail.

To display a sample audit trail, select the corresponding 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 corresponding sample, and then go to the **Audit Trail** tab page.

Creating Backup Files

Chromeleon provides a backup program for data storage and data exchange. The program allows you to create backup files that include all linked objects.

Before starting the **➤ Backup**, select the objects to archive in the **➤ Browser**. All selection options in the Browser are supported.

After selecting the desired objects, select **Export/Backup** on the **File** menu. You can back up the following objects:

Select a	To create a backup of
Directory	All subdirectories and the included files, sequences, etc.
Sequence	All samples and the files included in the sequence (QNT File and ➤ Program File (PGM File)).
Sample	The sequence information (name, directory, title, etc.), the selected samples, and the ➤ PGM and ➤ QNT Files used by these samples.
Other file	The corresponding files.

To save samples found in a **➤ Query**, the following options are available:

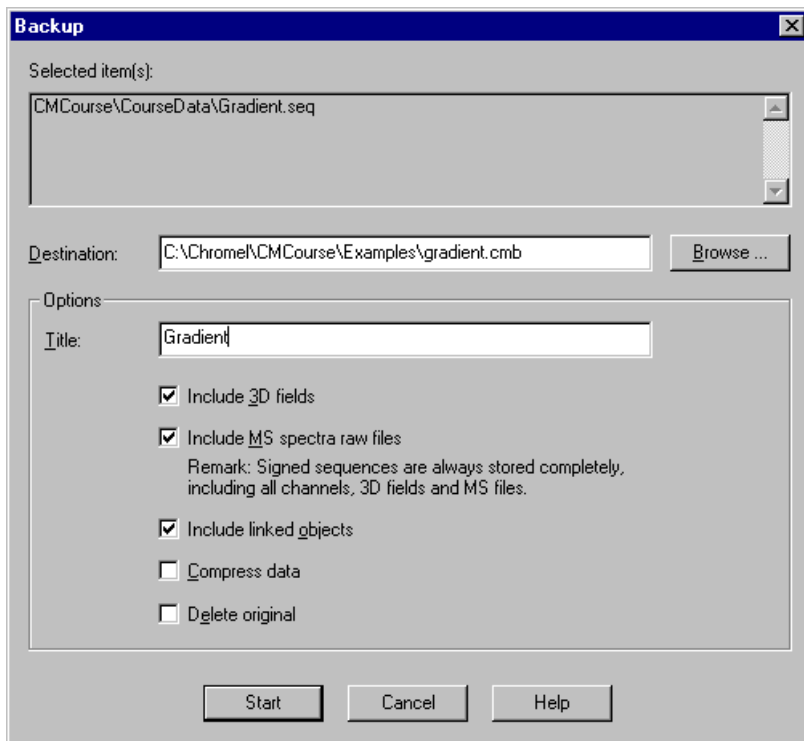
Select a	To save
Sample (from the sample list in the lower part of the right Browser pane)	The sequence information (name, directory, title, etc.), the selected samples, and the PGM and QNT Files used by these samples.
Sequence (from the sequence list in the upper part of the right Browser pane)	All samples contained in the query of the corresponding sequence.
Query (in the left Browser pane)	All samples contained in the query.

The following linked objects are copied for the backup:

Object	Link
Sequence	Preferred ➤ Report Definition file (RDF)
Peak table	Calibration list for the 'fixed' calibration mode; Blank run sample for 'fixed sample' blank run subtract mode; Spectra library for Spectra Library Screening.
Query	Preferred report definition file (RDF)
Samples	QNT File PGM File

If a link refers to an object included in the selected objects list, it is treated as a normal object.

Select **Backup** to open the **Backup** dialog box:

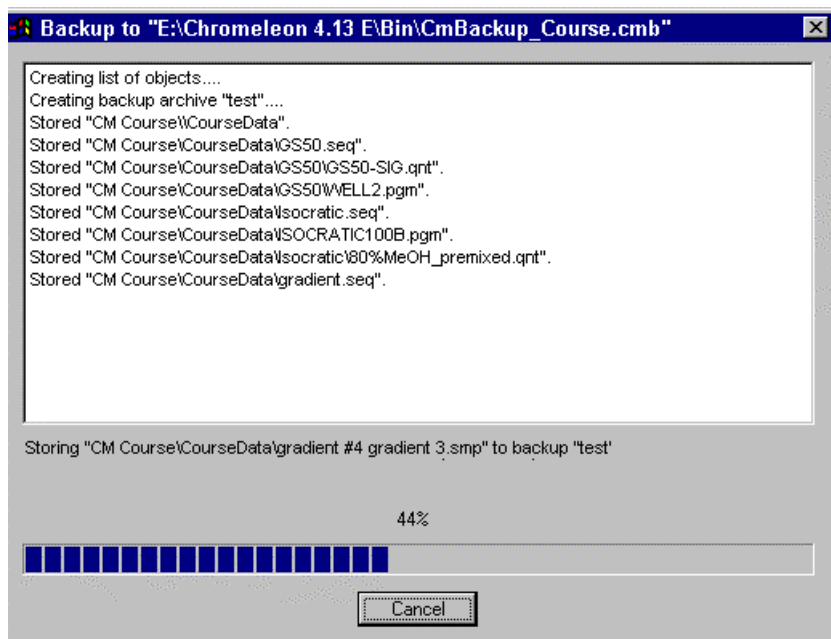


For a description of the individual fields, refer to **Backup** (in the online Help).

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 **PRIV_Backup** privilege for all selected objects (see ➤ *Privilege groups*). The backup is logged in the file history. To stop the backup, click **Cancel**. The backup then contains all objects stored up to this point.

You can also 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 about the history.

Backup files have the extension **.cmb**. For more information about how to restore backup files, refer to **How to ...: Actions in the Browser**

 **Restoring Backup Files.**

Restoring Backup Files

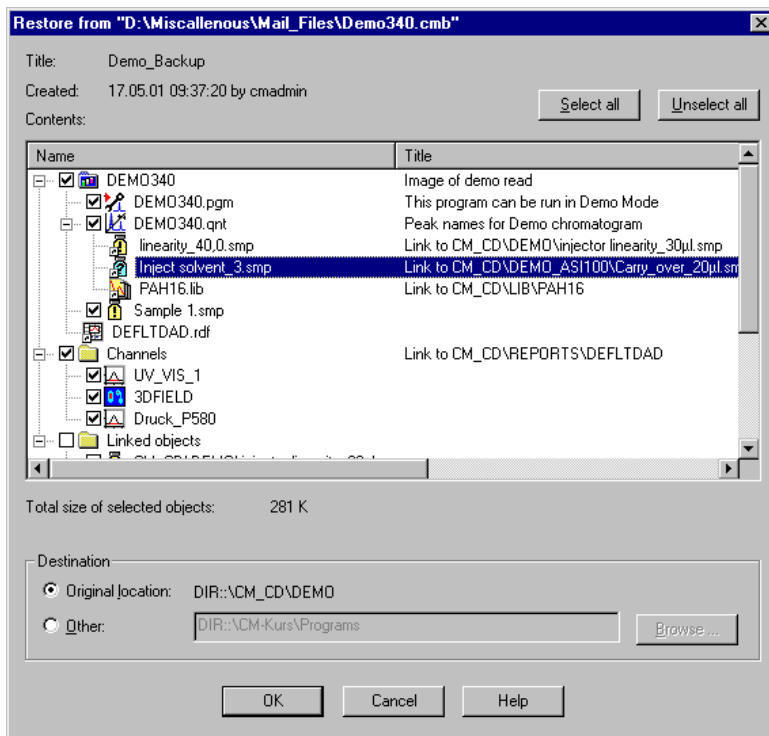
To restore backup files created via the **Backup** command, select **Import/Restore** on the **File** menu in the Browser, and then select **Restore** (also, refer to **How to ... Actions in the Browser**  **Creating Backup Files**).

Tip:

You can also select the backup file by double-clicking the file name in the Windows Explorer. Backup files are identified by their extension (.cmb).*

In the **Restore** dialog box, select the backup file you want to restore. 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 must insert the first medium now because it includes the list of contents. Chromeleon displays the list in the following dialog box:



For a description of the individual fields, refer to **Restore** (in the online Help).

The ➤ *Channels* and ➤ *Linked Objects* that are common to all samples are listed in separate groups. The linked objects are displayed under the corresponding objects.

Select the channels and the links to be restored from these groups. The default is that all channels are restored, but that no links are restored.

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, it is created by the system. Links are always restored to the original location. The corresponding sequence and datasource must exist. Missing directories will be created, if necessary.

If the destination object already exists, you are prompted whether to overwrite it. The following options are available: **Yes**, **Yes to all**, **No**, **No to all**, and **Cancel**. For each restored object, the corresponding entry is written to history.

To restore an object, you must have the **PRIV_Restore** privilege for the destination directory. To overwrite an existing object, you must have both the **PRIV_Restore** and **PRIV_Copy** privileges for the destination directory. To stop restoration, click **Cancel**.




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)

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:

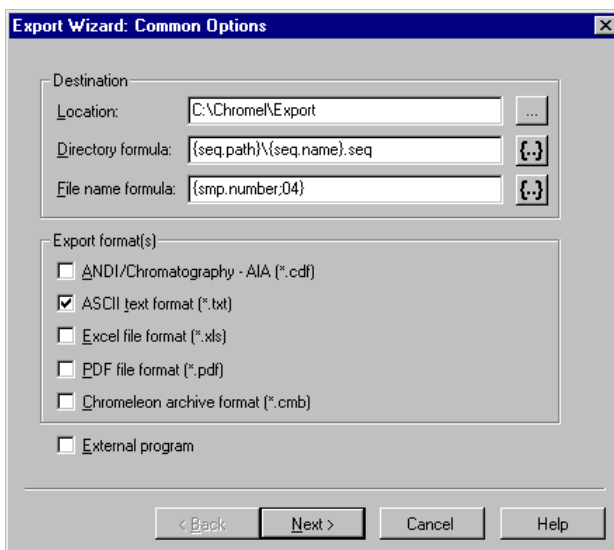
Exporting Data During or After a Batch

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 the **Export Settings...** button.)



The dialog box titled "Export Wizard: Common Options" contains the following elements:

- Destination section:**
 - Location:** A text field containing "C:\Chromel\Export" with a browse button (...).
 - Directory formula:** A text field containing "{seq.path}\{seq.name}.seq" with a formula button ({...}).
 - File name formula:** A text field containing "{smp.number;04}" with a formula button ({...}).
- Export format(s) section:**
 - ☐ ANDI/Chromatography - AIA (*.cdf)
 - ☒ ASCII text format (*.txt)
 - ☐ Excel file format (*.xls)
 - ☐ PDF file format (*.pdf)
 - ☐ Chromeleon archive format (*.cmb)
 - ☐ External program
- Navigation buttons:** "< Back", "Next >", "Cancel", and "Help".

- 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, we recommend 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 the desired export format.
- Click **Next** to go to the next wizard page. Select the desired 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.*

- 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** and  **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 the **Layout Mode** on the **Edit** menu.
- Double-click the header of the column of interest to open the report column properties. Select the desired peak, and then select **Save Report Definition...** on the **Workspace** menu to save the Report Definition File (RDF).

Tip:

*If the setting 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 **ASCII**, **Excel**, or **PDF** as the export format.
 - 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**.

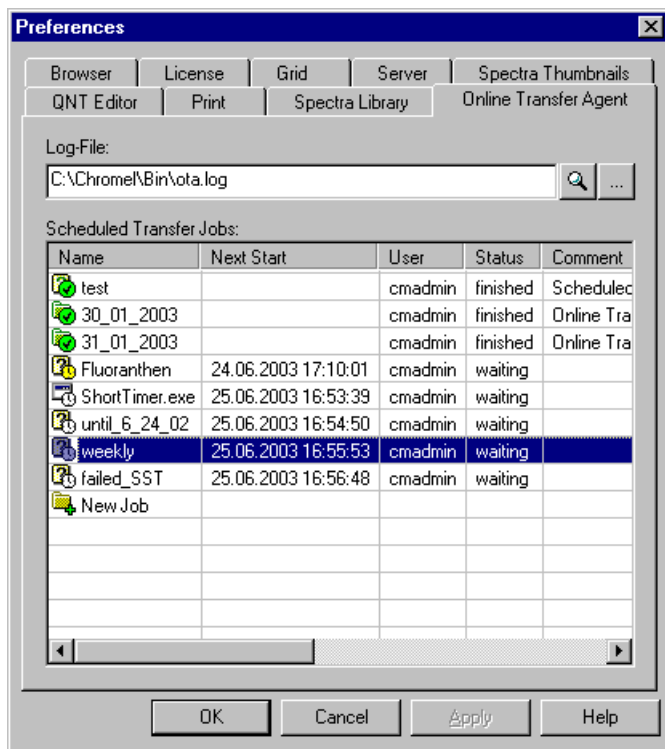
Transferring Sequences Automatically

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**.

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 the **Administrator Manual: Chromeleon User Management**  **Signature Privileges**



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:

The screenshot shows a dialog box titled "Properties of Sequence 'Gradient'". It has four tabs: "General", "Statistics", "Signature" (which is selected), and "Report Columns". The "Signature" tab contains three sections: "Submit", "Review", and "Approve". Each section has a "Status:" label, a text field for "Authorized users:", and buttons for "Edit..." and "Details...". The "Submit" section shows "Status: Not signed" and "Authorized users: User1;User2;Lab Manager". The "Review" section shows "Status:" (empty) and "Authorized users: Lab Manager". The "Approve" section shows "Status:" (empty) and "Authorized users: Quality Officer". At the bottom of the dialog are buttons for "OK", "Cancel", "Apply", and "Help".

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

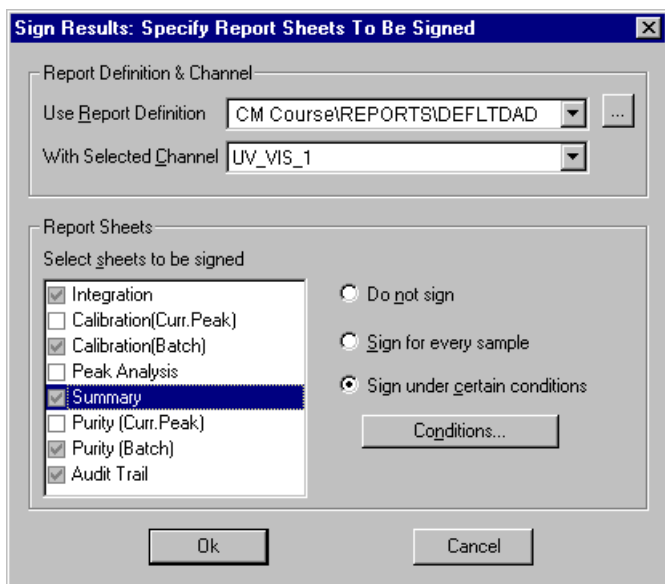
 **Individual Steps of Electronic Signature**

 **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**.



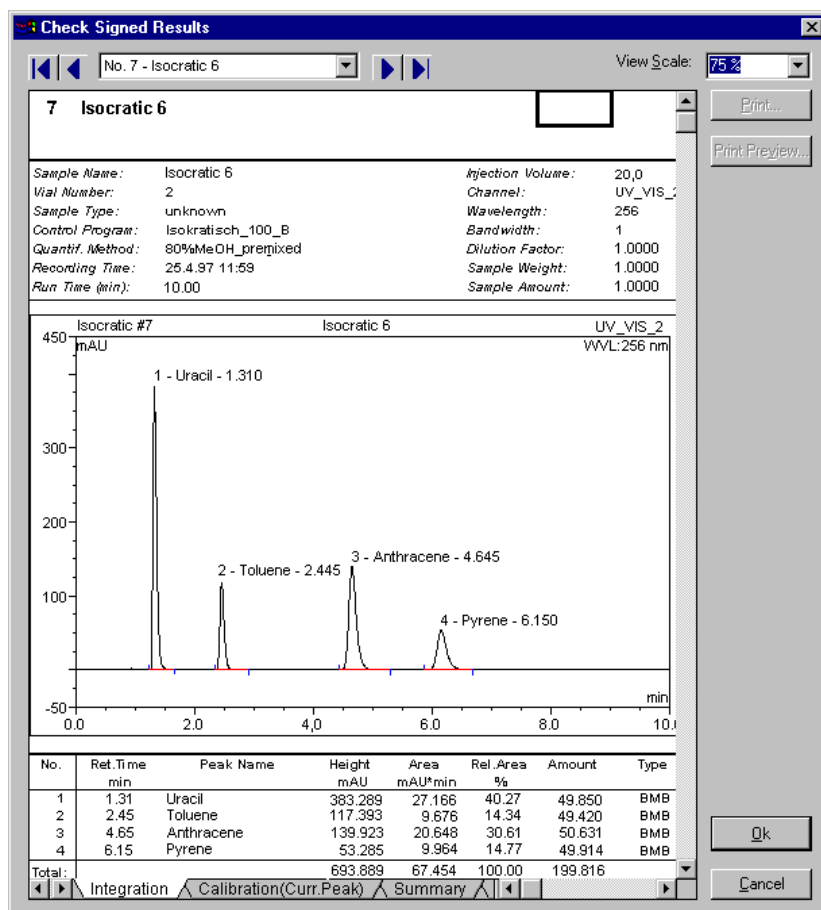
Select the **➤ Report Definition File (RDF)** to be used for displaying the sequence, select the channel for which you want to show the results, and select the report sheets to be signed.

**Tip:**

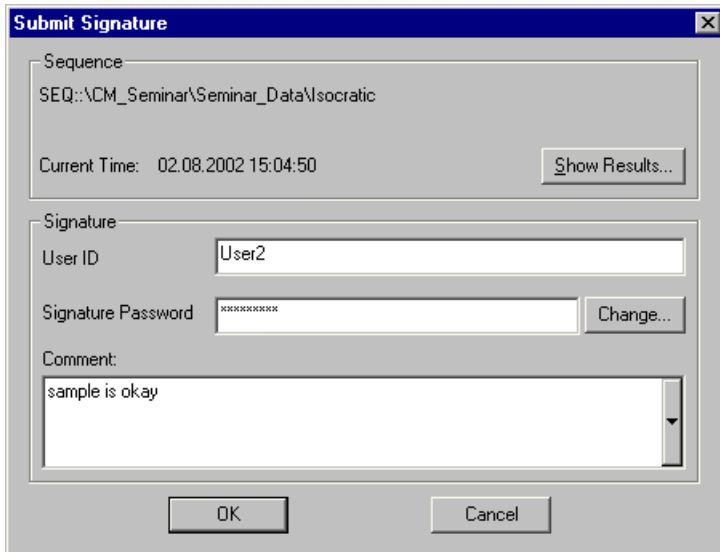
The settings saved in the Report Definition File are used as defaults for the worksheets to be signed and for the respective conditions. For more information, refer to **How to ...: Actions in the Printer Layout**

**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:

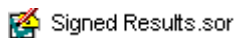


The **Submit Signature** dialog box contains the following fields and controls:

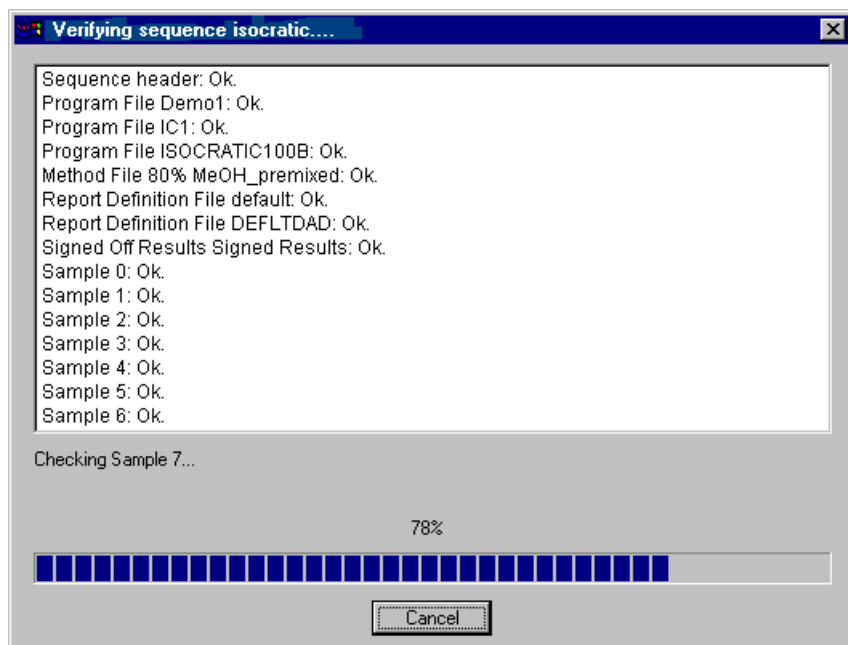
- Sequence:** A text field containing the path `SEQ::\CDM_Seminar\Seminar_Data\Isocratic`.
- Current Time:** A label showing `02.08.2002 15:04:50`.
- Show Results...:** A button located to the right of the Current Time label.
- Signature:** A section containing:
 - User ID:** A text field with the value `User2`.
 - Signature Password:** A text field with masked characters `XXXXXXXXXX`.
 - Change...:** A button to the right of the Signature Password field.
- Comment:** A text area containing the text `sample is okay`.
- OK** and **Cancel** buttons at the bottom.

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**.)

Properties of Sequence "Isocratic"

General | Statistics | **Signature** | Report Columns

Status: Reviewed

Submit

Authorized users: User1,User2,Lab Manager

Submitted: 25/07/2002 14:06:57 by User2

User name: User2

Review

Authorized users: Lab Manager

Reviewed: 25/07/2002 14:20:45 by Lab Manager

User name: Lab Manager

Approve

Authorized users: Quality Officer

Approved:

User name:

To check the sequence signature, select **Electronic Signature > Verify** on the **File** menu or click the following icon on the standard **Toolbar**:



 **Tip:**

If you use the sample \Rightarrow Types **Spiked** and/or **Unspiked**, please keep in mind that

- An \Rightarrow 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.

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 you through the process of 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 desired 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. Either select the **Selected datasource** option (the name of the currently selected datasource is indicated in brackets) or select **Fixed datasource** and then, select the desired datasource from the drop-down list box.

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 desired formula manually or click the "... " button and then, select the desired 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 the desired 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 desired Audit Trail variable. For example, to search samples that were recorded at a \Rightarrow LampIntensity > 500.000 counts/s, use the formula below:

`AUDIT.LampIntensity(0.0,"forward")>500000.`

Select an operator from the **Operator** field and enter the desired 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 the desired 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 desired criteria from the **Field** list box.

Name	Search Parameter
Sample Name	⇒ <i>Name</i>
Sample Number	⇒ <i>No.</i>
Sample Type	⇒ <i>Type</i>
Sample Status	⇒ <i>Status</i>
Sample Comment	⇒ <i>Comment</i>
Sample Replicate ID	⇒ <i>Replicate ID</i>
Sample Inject Volume	⇒ <i>Inj. Vol.</i>
Sample Inject Time	⇒ <i>Inj. Date/Time</i>
Sample Dilution Factor	⇒ <i>Dil. Factor</i>
Sample Weight	⇒ <i>Weight</i> (Sample Weight Factor)
Sample Amount	⇒ <i>ISTD Amount</i>
Sample Raw Data ID	Chromeleon sample ID.
Sample ID	Sample ID assigned by the user
Sample Program Name	⇒ <i>Program</i> used for sample processing
Sample Method Name	⇒ <i>Method</i> used for sample evaluation
*Sample x	➤ <i>User-defined Column</i> (column name)
Sequence Name	
Sequence Title	⇒ <i>Sequence</i>
Sequence Preferred Channel	
Sequence Preferred Report	
Sequence Directory	
Sequence Timebase	➤ <i>Timebase</i>
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.

Name	Search Parameter
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**, **Sample Position**, 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. It depends on the search criterion which operators are available:

Operator	Searches all samples...	Available for group no.
=	that are equal to the specified character string.	1, 2, 3, 4
< >	that are not equal to the specified character string.	1, 2, 3, 4
>	that are larger than the specified character string.	1, 2, 4
<	that are smaller than the specified character string.	1, 2, 4
>=	that are larger than or equal to the specified character string.	1, 2, 4
<=	that are smaller than or equal to the specified character string.	1, 2, 4
contains:	that contain the specified character string.	1
contains not:	that do not contain the specified character string.	1
starts with:	that start with the specified character string.	1
does not start with:	that do not start with the specified character string.	1
ends with:	that end with the specified character string.	1
does not end with:	that do not end with the specified character string.	1
is like:	that fulfill the specified wildcard condition.	1
is not like:	that do not fulfill the specified wildcard condition.	1

Operator	Searches all samples...	Available for group no.
is between:	that are between two values.	1, 2, 4
is not between:	that are not between two values.	1, 2, 4
during the previous:	that were 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 desired 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 the desired report variable (see \Rightarrow *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:

Samples

Query Wizard: Samples

Data Field: Operator: Value:

Data Field: Operator: Value:

Data Field: Operator: Value:

Data Field: Operator: Value:

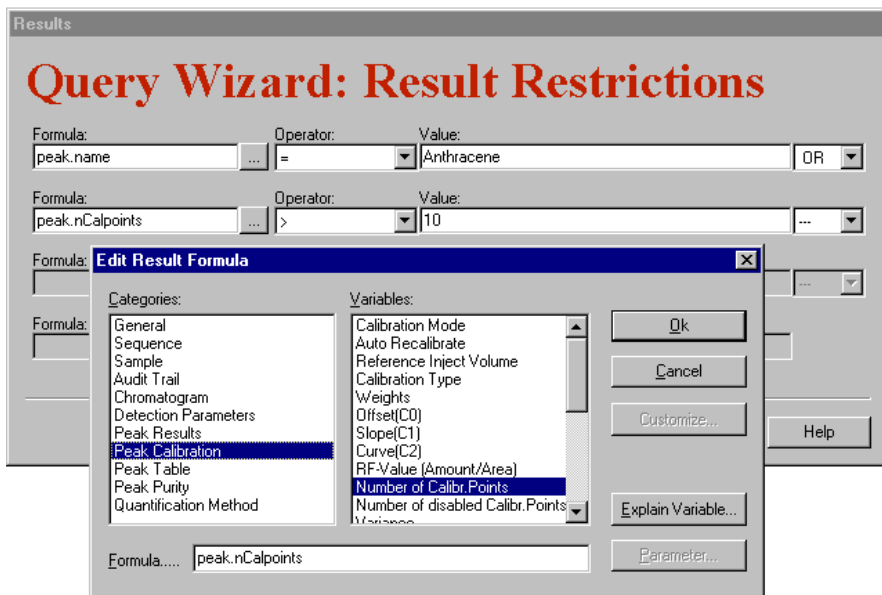
July 2003

Mo	Tu	We	Th	Fr	Sa	Su
30	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31	1	2	3
4	5	6	7	8	9	10

Today: 23/07/2003

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 box, 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 cursor 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 the desired formula. (For more information about the report variables and their categories, refer to ➤*Report Categories*). Enter the desired operator. (For information about which SQL operators are supported, refer to **How to ...: Actions in the Browser**  **Selecting Search Criteria for Samples and/or Sequences**.) Finally, type the desired 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

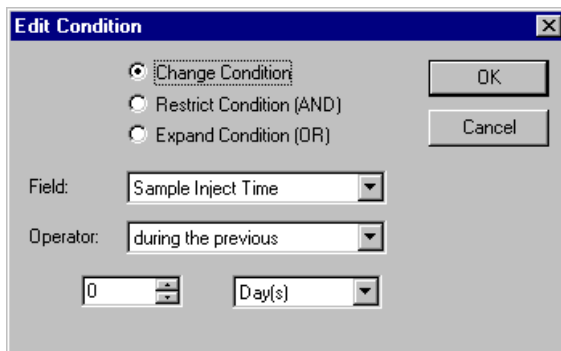
where *x* is the name of the user-defined column.

- 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)

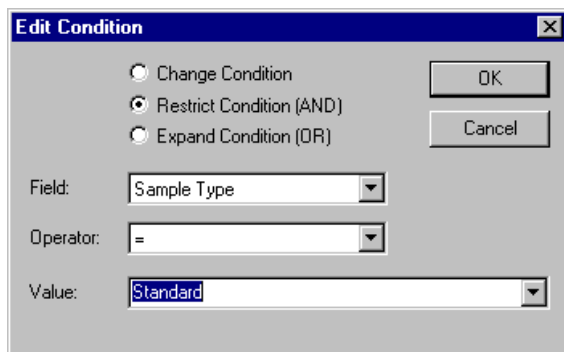
To search for all samples of the current day, set up the following ➤SQL query in the **Edit Condition** dialog box of the **SQL** tab page. (For information about how to open this dialog box, refer to  **Specifying the Sample Query Using the Dialog Box**.)



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:



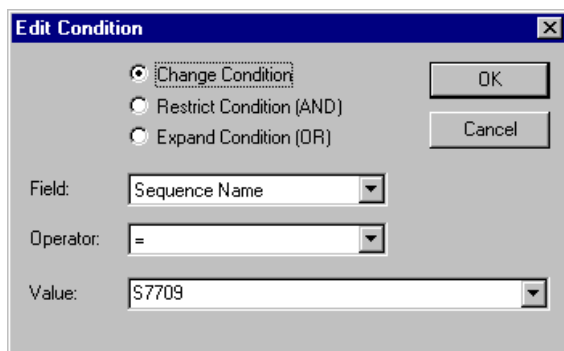
The 'Edit Condition' dialog box has a title bar with a close button. It contains three radio buttons: 'Change Condition' (unselected), 'Restrict Condition (AND)' (selected), and 'Expand Condition (OR)' (unselected). To the right of these are 'OK' and 'Cancel' buttons. Below the radio buttons are three input fields: 'Field:' with a dropdown menu showing 'Sample Type', 'Operator:' with a dropdown menu showing '=', and 'Value:' with a text box containing 'Standard' and a dropdown arrow on the right.

The following SQL statements are listed on the **SQL** tab page:

Samples.smp_inject_time >= CURRENT_INTERVAL '0' DAY
AND Samples.smp_type = 'S'

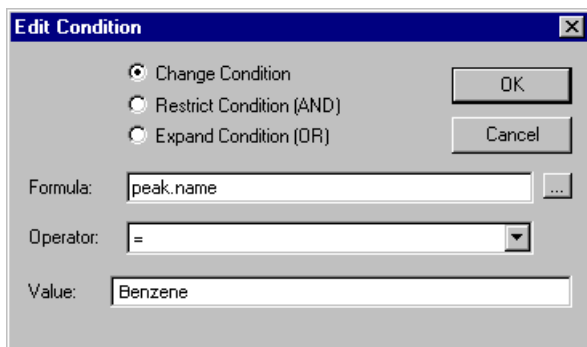
To search for all samples in a \Rightarrow *Sequence* named **S7709**, for which the **Benzene** peak or a peak of the **PAK** (PAH) peak group was identified, use the following SQL query:

Open the **Edit Condition** dialog box of the **SQL** tab page and enter the condition for the sequence name:



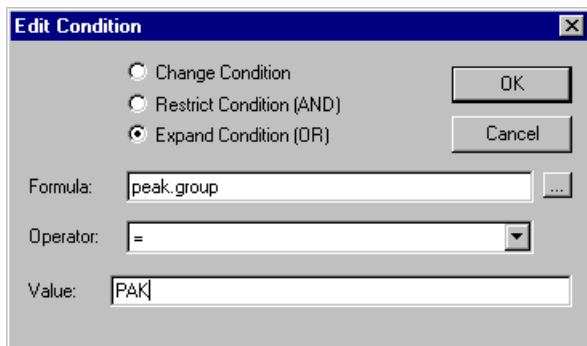
The 'Edit Condition' dialog box has a title bar with a close button. It contains three radio buttons: 'Change Condition' (selected), 'Restrict Condition (AND)' (unselected), and 'Expand Condition (OR)' (unselected). To the right of these are 'OK' and 'Cancel' buttons. Below the radio buttons are three input fields: 'Field:' with a dropdown menu showing 'Sequence Name', 'Operator:' with a dropdown menu showing '=', and 'Value:' with a text box containing 'S7709' and a dropdown arrow on the right.

Change to the **Result Restrictions** tab page and click the **Edit/Insert** button to open the **Edit Condition** dialog box. Enter the condition for the Benzene peak:



The 'Edit Condition' dialog box has a title bar with a close button. It contains three radio buttons: 'Change Condition' (selected), 'Restrict Condition (AND)', and 'Expand Condition (OR)'. To the right of these are 'OK' and 'Cancel' buttons. Below the radio buttons are three input fields: 'Formula:' with the text 'peak.name', 'Operator:' with a dropdown menu showing '=', and 'Value:' with the text 'Benzene'. There is a small button with three dots to the right of the 'Formula:' field.

Return to the **Edit Condition** dialog box and enter the condition for the peak of the **PAK** (PAH) group:



The 'Edit Condition' dialog box is shown with the same layout as the previous one. The 'Expand Condition (OR)' radio button is now selected. The 'Formula:' field contains 'peak_group', the 'Operator:' dropdown shows '=', and the 'Value:' field contains 'PAK'. The 'OK' and 'Cancel' buttons are still present.

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 \Rightarrow **Sequences**.

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

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. For more information, refer to the **Administrator Manual: How to Actions in the Browser and in the Operating System:**

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 if you are authorized to do so. (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: There is no default location where these files are saved. 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 ...:**  **Actions in the Browser** and the corresponding subtopics.

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 HPLC Devices (Overview)

System Wellness tasks for IC devices are performed from the Browser. 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 the **Administrator Manual: How to ...: Actions in the Server Configuration**  **Enabling and Disabling System Wellness Functions** for IC Devices.



Opening a Wellness Control Panel

➤ *Control Panels* for performing System Wellness functions are provided for various system configurations.

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 and 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.

Viewing and Restoring Calibration Data

Calibration data for a System Wellness supported IC device is displayed on the Wellness *➤ Control Panel* (see **How to ...: Actions in the Browser**

 **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 the **Administrator Manual: How to ...: Actions in the Server Configuration**  **Enabling and Disabling System Wellness Functions**).

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 to the right of 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 **How to ...: Actions in the Browser**  **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 given. 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

Pumps, Detectors, Autosamplers

Pumps

Calibration Procedures



Leak Detector



Flow Rate



Pressure Transducer Offset



Degas

Devices**Calibration Procedures**

Detectors



Wavelength (AD25/PDA-100)

Conductivity Cell
(CD20/25/25A, ED40/50/50A, IC20/25/25A)pH Reference Electrode
(ED40/50/50A Amperometry Mode)

AS50 Autosampler



Inject Port Volume

ICS-90 system



Pressure Transducer



Conductivity Cell

ICS-1000/1500/2000 systems



Flow Rate



Degas Pump




Conductivity Cell

 **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 \pm 1.4 MPa (2000 \pm 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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 1.0 mM KCl solution: Prepare by dissolving 0.07456 g of reagent grade KCl in one liter of 18 megohm deionized water
(Method B Only): 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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 $147.00 \pm 2 \mu\text{S/cm}$ 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 near zero, 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 **How to ...: Actions in the Browser**  **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 $147.00 \pm 2 \mu\text{S/cm}$. 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 $\mu\text{S}/\text{cm}$, 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 1.0 mM KCl solution: Prepare by dissolving 0.07456 g of
(Method B Only): 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 **How to ...: Actions in the Browser**  **Opening a Wellness Control Panel**).
2. Locate the **System Status** controls.
3. Click the **Details...** button 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 (see **How to ...: Actions in the Browser**  **Opening a Wellness Control Panel**).
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 $147.00 \pm 2 \mu\text{S/cm}$ 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 $\mu\text{S}/\text{cm}$, 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 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 **How to ...: Actions in the Browser**  **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.


Entering the AS50 Inject Port Volume

The inject port volume is the volume of tubing between the AS50 inject port and the injection valve. The AS50 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 AS50 operator's manual.

1. Open the System Wellness ➤ *Control Panel* for the autosampler (see **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**:

 **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **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 **How to ...: Actions in the Browser**  **Opening a Wellness Control Panel**).
2. Under **Device Parameters, sodium correction**, select **On**.

Importing PeakNet (Releases 4.5 Through 5.2) Method Files

Overview

In order to use Method files from PeakNet (releases 4.5 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, ED40, IC20, UI20, DX-120. 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 the 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, which were not in the first detector's data, are added to the table. This means that any reference component information for detectors other than the first 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 the **Add** button. 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 the desired 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 the desired 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 ...: Actions in the QNT Editor**  **Inverting Dependent and Independent Variables** for details.
8. Click the **Import** button. 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 (Release 4.5 Through 5.2) Data Files**

Importing PeakNet (Releases 4.5 Through 5.2) Data Files

Overview

In order to use data files from PeakNet (releases 4.5 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 into *➤PGM File* and *➤Quantification Method (QNT Method)* of Chromeleon, 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 the **Add** button. 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 the desired 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 ...: Actions in the QNT Editor**  **Inverting Dependent and Independent Variables** for details.
8. Click the **Import** button. The selected PeakNet 5 data files are imported into Chromeleon.

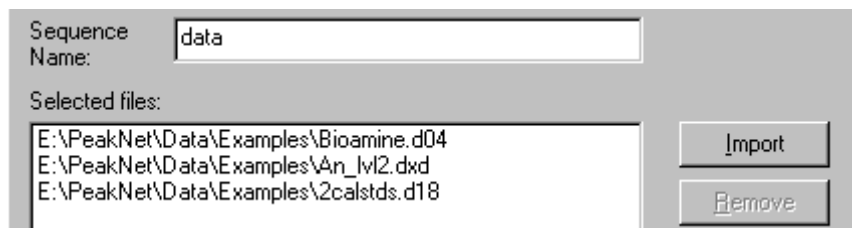
The following files are created:

- 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, which 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:

No.	Name	Type	Pos.	Inj. Vol.	Program	Method
1	biogenic amines/31.2ppm/TBAO Unknown	Unknown	0	10.0	cats001	cats001
2	biogenic amines/31.2ppm/TBAO Unknown	Unknown	0	10.0	cats002	cats002
3	Level 2 Standard	Unknown	0	10.0	as14003	as14003
4	Diabetic Candy Sample	Unknown	0	10.0	2sugars004	2sugars004










The following PGM and QNT Files were also created. These correspond to the programs and methods listed in the sequence:


Name	Title
2sugars004.pgm	PeakNet 5 Imported
as14003.pgm	PeakNet 5 Imported
cats001.pgm	PeakNet 5 Imported
cats002.pgm	PeakNet 5 Imported
2sugars004.qnt	PeakNet 5 Imported
as14003.qnt	PeakNet 5 Imported
cats001.qnt	PeakNet 5 Imported
cats002.qnt	PeakNet 5 Imported

Also, refer to  **Importing PeakNet (Release 4.5 Through 5.2) Method Files**

PeakNet (Release 4.5 Through 5.2) Translation Tables

See the following topics for how PeakNet (releases 4.5 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 Detectors**
-  **ED40 Detectors**
-  **IC20 Systems**
-  **DX-120 System**
-  **UI20 Module**

See  **Detector Component Table Translation** for how PeakNet (release 4.5 through 5.2) component table parameters are translated to Chromeleon QNT Method parameters.

AS50 Autosampler Setup and Timed Event Parameter Translation

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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 If Inject, command "Wait InjectState" is added	Setup & timed portions
Pipet function (Source/Volume/Destination)	Pipet (SourceVial/Volume/DestinationVial)	Setup portion
Mix function (Vial/Volume/Cycles)	Mix (SourceVial/Volume/NumberOfTimes)	Setup portion
Flush function (Volume)	FlushSP (Volume)	Setup portion
Delay function (Delay Time)	DelaySP (Time)	Setup portion
Needle function (Height)	SetNeedleHeight (Height)	Setup portion
Dilute function (Concentrate Source/Concentrate Volume/Diluent Source/Diluent Volume/Destination)	Dilute (SourceVial/ConcentrateVolume/SourceReservoir/ DiluentVolume/DestinationVial)	Setup portion
Dispense function (Source/Volume/Destination)	Dispense (SourceReservoir/Volume/DestinationVial)	Setup portion
Wait function	Wait (SamplePrep)	Setup portion

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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 (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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
Eluent percentage (%A)	Not converted	Calculated from %B, %C, and %D

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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 (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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
Mark (checked)	UV_Analog_out.Mark	Setup & timed portions

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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 (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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
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

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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 Setup and Timed Event Parameter Translation

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
Rate	Data_Collection_Rate	Setup portion
Time	AcqOff (at time=time of AcqOn + Time)	Timed portion
Range (μ S)	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
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

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
Plot Scales (Minimum/Maximum)	Not converted	
Detector Units	Not converted	
X-Y Data	Not converted	

ED40 Setup and Timed Event Parameter Translation

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command of	Comments
Rate	Data_Collection_Rate	Setup portion
Time	AcqOff (at time=time of AcqOn + Time)	Timed portion
Range (μ S)	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
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 (Off/50/100/300/500 mA)	SRS_Current (=Off/50/100/300/500)	Setup portion. Only for ED40/ED50c

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command of	Comments
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 Setup and Timed Event Parameter Translation

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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	
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 (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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
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 (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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

PeakNet (Release 4.5 Through 5.2) Method Parameter	Chromeleon PGM Command	Comments
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 Release 4.5 Through 5.2 (PN5) Component Table Parameter	Chromeleon QNT Method	Comments
Component Name	Peak Name	
Retention Time	Retention Time	<p>If peak Reference Component column = None then Retention Time options are set to Absolute.</p> <p>If peak Reference Component = <Component> then Retention Time options are set to Time Distance to Reference Peak and Reference Peak = <Component> is assigned</p>
Tolerance	Window	<p>If Tolerance = time then Window options are set to Absolute and Greatest.</p> <p>If Tolerance = % then Window options are set to Relative and Greatest.</p>
Reference Component	Retention Time Options	See Retention Time comments for rules.

PeakNet Release 4.5 Through 5.2 (PN5) Component Table Parameter	Chromeleon QNT Method	Comments
Internal Standard Component	Standard	<p>If global PN5 calibration options = External then all DDS Standard = External</p> <p>If global PN5 calibration options = Internal then</p> <ul style="list-style-type: none"> - 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.
Curve Fit Type	Calibration Type	<p>If Cubic in PN5 then set to quadratic.</p> <ul style="list-style-type: none"> - PN5 Fit Linear/quadratic with Origin = Force is Linear/quadratic in the DDS. - PN5 Fit Linear/quadratic with Origin = Ignore is Linear/quadratic with Offset in the DDS. - PN5 Fit Linear/quadratic with Origin = Include is Linear/quadratic with Offset and Include point (0,0) options in the DDS. - All DDS are set to No Weights. - No Average Response Factor in the DDS so set to Linear.
Origin Calibrate By	Calibration Type Calibrate By	<p>See Curve Fit Type for rules.</p> <ul style="list-style-type: none"> - 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.

PeakNet Release 4.5 Through 5.2 (PN5) Component Table Parameter	Chromeleon QNT Method	Comments
Groups	Groups	- For each peak in a group the DDS 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.	
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 to the DDS.

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:

- **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

Batch

Sequence

Method

Sample

Spectra Library

Chromeleon

One ➤ Sequence that has already been analyzed (not a ➤ Batch!)

Sequence

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.

Single sample

➤ Spectra Library

Depending on your selection, the following Agilent/HP ChemStation data are imported together with the selected data type:

Agilent/HP ChemStation

2D/3D raw data file

Instrument data such as pressure, flow, temperature, and gradient composition

Sample LOG File

Chromeleon

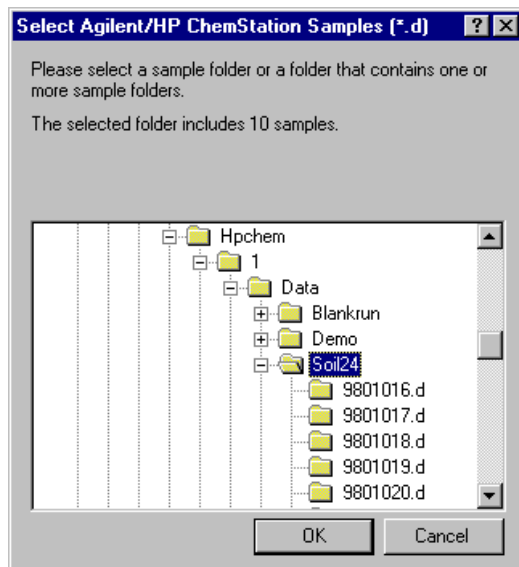
The corresponding channels; for example, UV and 3D channels.

➤ Audit Trail

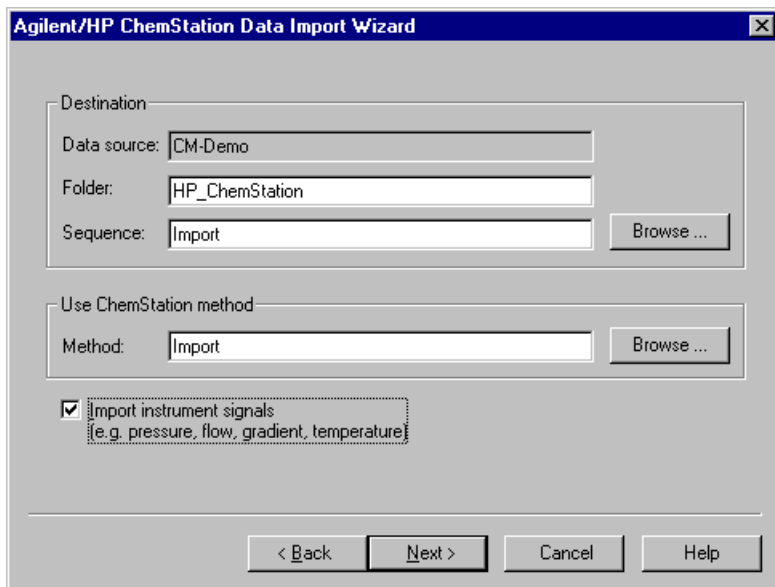
How To

- 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 the **Add...** button. 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 desired files 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):







The image shows a Windows-style dialog box titled "Agilent/HP ChemStation Data Import Wizard". It contains several input fields and buttons. Under the "Destination" section, there are three text boxes: "Data source:" with "CM-Demo", "Folder:" with "HP_ChemStation", and "Sequence:" with "Import". To the right of the "Sequence:" box is a "Browse ..." button. Below this is a section titled "Use ChemStation method" with a "Method:" text box containing "Import" and another "Browse ..." button. At the bottom of the main area is a checked checkbox labeled "Import instrument signals" with a sub-label "(e.g. pressure, flow, gradient, temperature)". At the very bottom are four buttons: "< Back", "Next >", "Cancel", and "Help".

- 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 the **Finish** button. The selected files are imported into Chromeleon.

For more information, 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:

- Cal Level: Used for ChemStation calibration; not used in Chromeleon. In Chromeleon, the corresponding information is available in the ⇒ *Amount* column of the ➤ *QNT Method*.
- Ini/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 and 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	⇒ <i>Name</i> (sample name)
Sample Type	⇒ <i>Type</i> (sample type)
Sample	Unknown,
Calibration	Standard,
Control Sample	Validation.
Location	⇒ <i>Pos.</i> (sample position)
Inj Volume	⇒ <i>Inj. Vol.</i> (injection volume)
-	⇒ <i>Program</i> (➤ <i>PGM File</i>)
Method Name	⇒ <i>Method</i> (quantification method)
	⇒ <i>Status</i> (sample status)
Date/Time of injection (evaluated from CS log file)	⇒ <i>Inj. Date/Time</i> (injection date and time)
Product of Sample Amount and Multiplier	⇒ <i>Weight</i> (Sample Weight Factor)
Dilution	⇒ <i>Dil. Factor</i> (Dilution Factor)
ISTD Amount	⇒ <i>ISTD Amount</i> (Amount of the ➤ <i>Internal Standard</i>)
Datafile (standards are named automatically - see below)	⇒ <i>Sample ID</i>
-	⇒ <i>Replicate ID</i>
Sample Info	⇒ <i>Comment</i>

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 not 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

Sample name information from a 2D/3D raw data file

*Cannot be evaluated from the CS sample data;
set to "unknown"*

Vial position information from a 2D/3D raw data file

Inj Volume from the Lcdiag.reg file

Not specified, no entry.

Method information from a 2D/3D raw data file

Set to "Finished"

Date/time of injection (from the LOG file)

Cannot be evaluated from the CS sample data, set to 1.0

Cannot be evaluated from the CS sample data, set to 1.0

Cannot be evaluated from the CS sample data, set to 1.0

Sample name (.d)*

-

Sample information from the "sample.mac" file

Chromeleon

⇒ *Name* (sample name)

⇒ *Type* (sample type)

⇒ *Pos.* (sample position)

⇒ *Inj. Vol.* (injection volume)

⇒ *Program* (➤ *PGM File*)

⇒ *Method* (quantification method)

⇒ *Status* (sample status)

⇒ *Inj. Date/Time* (injection date and time)

⇒ *Weight* (Sample Weight Factor)

⇒ *Dil. Factor* (dilution factor)

⇒ *ISTD Amount* (Amount of the
➤ *Internal Standard*)

⇒ *Sample ID*

⇒ *Replicate ID*

⇒ *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 = Off, 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	-	
Fixed Peak Width	Peak Slice	
Auto Peak Width	-	
Detect Shoulders	Peak Shoulder Threshold	Off = Off, 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 Window High		RT High - RT Low

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

File name of the imported library + hint "Imported CS library"

User who imported the spectra library

Creation date of the imported spectra library

Current user

Date/time of the import

Information on the imported library

Chromeleon

Name

Created by

Created

Last Update by

Last Update

Title



Actions on the Control Panel

For general information about control panels, refer to **Control**  **The Control Panel**.

The topics below provide information about how to open and/or modify a control panel and how to modify the control elements or change the timebase assignment:

-  **Opening a Control Panel**
-  **Connecting a Control Panel with a Timebase**
-  **Creating and Starting an Online Batch**
-  **Modifying a Control Panel**
-  **Modifying a Control**
-  **Linking a Control to a Device**
-  **Creating a Script Button**
-  **Creating a Command Button**
-  **Creating Hidden Windows**

In addition, you can simulate data acquisition and display sample and sequence information:

-  **Using/Recording Demo Data**
-  **Displaying Sample and Sequence Information**


Opening a Control Panel

To process unknown samples or to control an instrument by 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 with 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 ...: Actions on the Control Panel**  **Connecting a Control Panel with a Timebase**).



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 the desired 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.

Connecting a Control Panel with a Timebase

When you open a ➤ *Control Panel*, it is automatically connected with the assigned ➤ *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 indicates the name of the currently selected timebase, the **Computer** field indicates the name of the computer on which the corresponding ➤ *Server* is running, and the **Protocol** field indicates the currently used communication protocol. The list box on the right shows an icon for the local computer (**My Computer**) and, if the computer is connected to a network, also an icon for the network neighborhood (also, see **Chromatography Components: Hardware and Software**

Network Installation.)

- Click the + character 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 + character beside a computer name to display the Chromeleon servers underneath. If there is no active Chromeleon server, the entry is **Server not found**.
- Select the desired 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 with the selected timebase. If the connection is successful, you can control the connected instruments, using the control elements on the control panel.



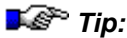
Tip:

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.



*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**, click **Settings > Control Panel > Network and Dial-up Connections > Configuration**. Select **Properties** on the context menu and then, select the **NWLink IPS/SPX/NetBIOS Compatible Transport Protocols** check box.

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.

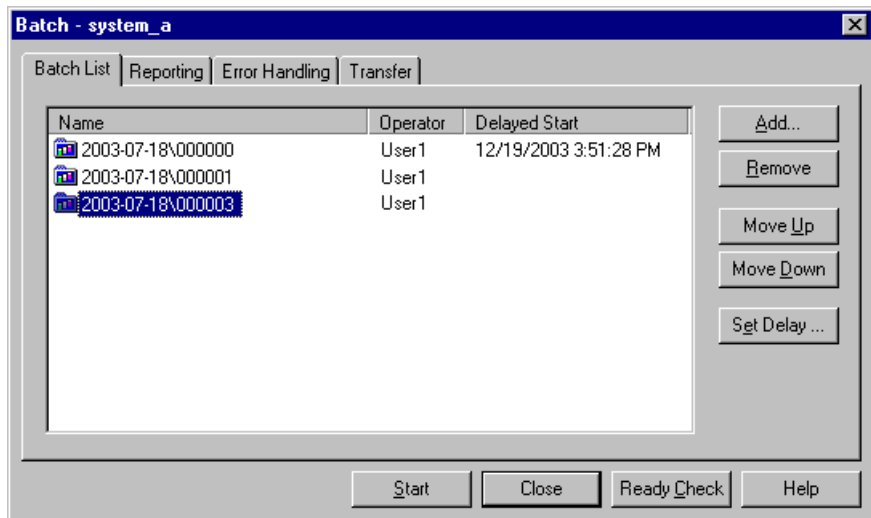
The **Monitor Only** status is also assigned when you attempt to access a local timebase that is currently controlled by an external user.



- Deselect **Monitor Only** on the **Control** menu to enable complete control over the timebase.


Creating and Starting an Online Batch

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*:

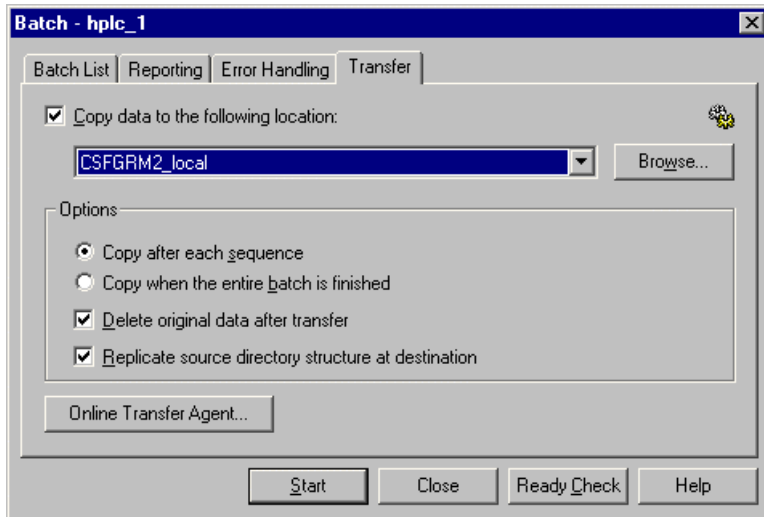
- Select **Edit** on the **Batch** menu. The **Batch** dialog box is opened.
- On the **Batch List** tab page, click **Add...** to add the sequences to be processed. (In the **Browse** dialog box, select **Sequence** as **Object of type** and navigate to the desired sequence(s). Click **Open** to confirm 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 ...: Actions in the Printer Layout**  **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 box or click **Browse** to navigate to the desired file. (For more information, refer to **How to ...: Actions in the PGM Editor**  **Creating 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 box or click **Browse** to navigate to the desired file. (For more information, refer to **How to ...: Actions in the PGM Editor**  **Creating a Power Failure Program**). In addition, determine how the system shall proceed after running the power failure program. Select the desired action from the drop-down list box.


- 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 the **Administrator Manual: How to ...: Actions in the Server Monitor Program**  **Setting Up the Online Transfer Agent for Network Access.**

- To align several controls, press the **Shift** key and left-click the desired controls. Select **➤Align** on the **Edit** menu and then, select the desired option. The control frame of the control selected first is used as the reference point and reference size for the other controls.

For information about how to change control properties, refer to **How to ...: Actions on the Control Panel**  **Modifying a Control**.

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 ...: Actions on 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.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
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									
Rack Display	X	X	X														
Online Mass Spectrum Plot	X		X	X													

For information about how to specify the property of a control, refer to **How to ...: Actions on the Control Panel**  **Linking a Control to a Device.**

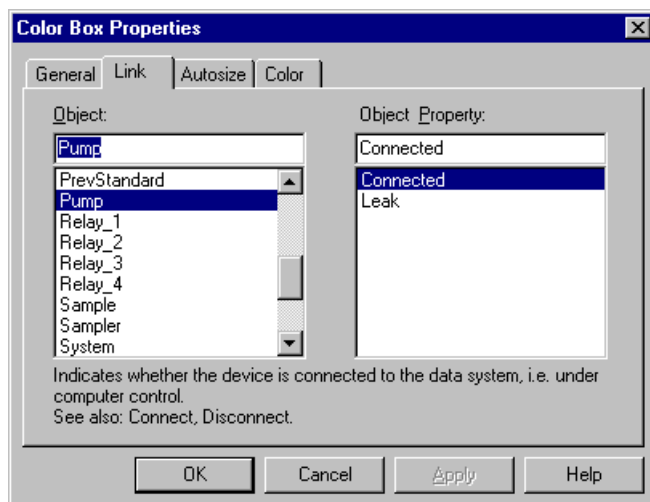
Linking a Control to a Device

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 the desired 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 desired 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 ...: Actions in the PGM Editor**  **Creating a Program** and **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 the desired property from the **Object Property** list box. Click **OK** to link the control to the object and the property.

Creating a Command Button

Chromeleon lets you assign a command to a command button so that you can execute the command by clicking the button. To create a command button, you must have the appropriate authorization.

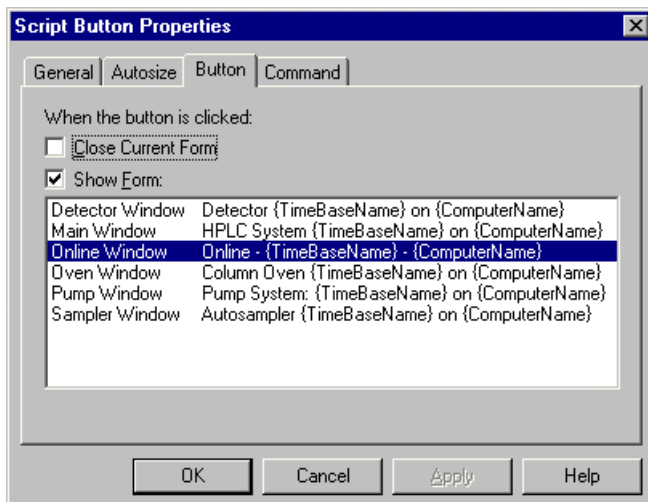
- Right-click the corresponding button and select **Properties...** on the context menu.
- Open the **Link** tab page.
- From the **Object** list box, select the object to which you want to link the command. From the **Command** list box, select the command to be linked to the selected object.
- Select 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.

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 is only few 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.



If you now click this button, the signal plot is opened.

Using/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 select the desired 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.

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 desired 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.

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.

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.

To display this type of information, link a ➤ *Control* (alphanumeric display) to one of the following functions (**Object Properties**):

Comment	Datasource	Program
Name	Sequence	ProgramMoniker
Number	SequencePath	Moniker
Type	SequenceMoniker	ID

For more information about controls, refer to the topics in **How to ...:**



Actions on the Control Panel.

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.

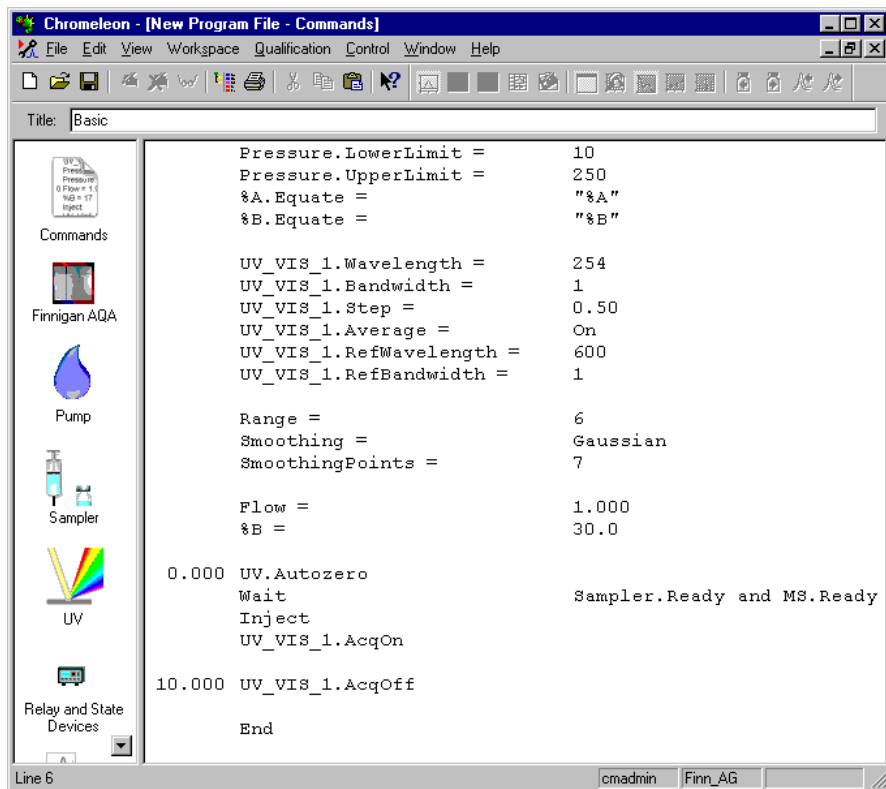
Question: Several control panels are listed on the **Window** menu. How can I delete an entry?



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 ...:**  **Actions on the Control Panel** and the corresponding subtopics.

Actions in the PGM Editor

Use the different views provided by the PGM Editor to edit your control programs (>*PGM Files*). To access a view, click the corresponding icon on the shortcut bar:





- Click **Commands** to edit a program if this is not entirely possible in the device view. For more information, refer to **How to ...: Actions in the PGM Editor**  **Creating a Program**.
- Click a device icon to edit the program for the device. (It depends on the installed devices which device views are available in the PGM Editor.) Also, refer to  **Editing PGM Files in the Device Views of the PGM Editor**.

- If installed, click **Finnigan AQA** (or **MSQ**) to create an MS method for an MS method for the corresponding spectrometer. For more information about the MS instrument method, refer to **How to ...: Actions Related to the Mass Spectrometers**  **Creating a PGM File for the aQa MS** or  **Creating a PGM File for the MSQ**.
- Click **Post-acquisition steps** to reprocess your data, e.g., to extract channels and smooth data. For more information, refer to **How to ...: Actions in the PGM Editor**  **Adding Post-Acquisition Steps**.




For general information about the PGM Editor, refer to **Control**  **The PGM Editor**.

Creating a Program


A **Program** is used to define precisely timed start conditions and **Control Commands**. You usually create the program using the  **Program Wizard** and edit the program later in the PGM Editor (see **Control**  **The PGM Editor**). The minimum entries required for an operable program are as follows:

- Signals to be recorded and their parameters
- \Rightarrow Flow rate and solvent composition (\Rightarrow %B, %C, %D - for controlled pumps, only)
- \Rightarrow Inject command
- Start of data acquisition (\Rightarrow AcqOn)
- End of data acquisition (AcqOff)
- End of the program (\Rightarrow End)


Verify that all relevant parameters are defined in the program. For parameters that are not explicitly defined, Chromeleon uses the settings of the last sequence.

We recommend to create the program automatically first (see  **Automatically Creating a Program**), and then edit the file via 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 Praxis)*, we recommend 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 **How to ...: Actions in the PGM Editor**  **Program Example**.


Automatically Creating a Program

Create the basic structure of a ➤ *Program* using the Program Wizard. (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.

When input is complete, click **Finish**. Based on your entries, Chromeleon creates an operable program. You are not required to pay attention to the syntax or other programming details.


You can then modify and/or extend this basic program later as described in  **Manually Editing a PGM File in the Commands View**.

For a simple example, refer to **How to ...: Actions in the PGM Editor**  **Program Example**.

For examples for special programs, refer to **How to ...: Actions in the PGM Editor**


Creating an Emergency Program

Creating a Power Failure Program

For an overview of the numerous programming capabilities, refer to **How to ...: Device Control**  **Overview**.

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.

- 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 corresponding page(s) of the  **Program Wizard** are opened.
- Enter the desired 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 pages of the Wizard 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 corresponding 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 (below called **Device**) for which you want to edit an instruction. A device can be any instrument of 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. A short help text is displayed in the **Help** section.
- Enter the retention time when the command shall be executed or when the status of a property shall 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 click **OK & Next** to change to the previous or to the next program line.
- Repeat the input procedure until you have changed all commands and/or properties of interest.

Adding command/property values to the audit trail

- Press the F8 key to open the **Commands** dialog box.
- Select the \Rightarrow 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 from the left window and in addition several read-only variables.

- 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 provides a short 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 \Rightarrow *Protocol* command from the commands list. Enter the desired 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 \triangleright *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 color. Check and correct the entry. If necessary, repeat this procedure, using the F8 box.


Adding comments

To enter a comment, place the cursor at desired position. Type the desired text, starting with a semicolon. Or else, press the F8 key to open the **Commands** dialog box and enter the desired comment in **Comment** field. Click **OK** to add the comment to the program.

Comments are displayed in green color, indicating that Chromeleon considers the text as a comment. Any comment entered in this way does not appear in the audit trail. It only serves to comment the 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**), we recommend 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**).

For a simple program example, refer to  **Program Example**.

For special program examples, refer to **How to ...: Actions in the PGM Editor**



Creating a Shutdown Program




Creating an Emergency Program



Creating a Power Failure Program


Also refer **How to ...: Actions in the PGM Editor**  **Using User-defined Columns in a Program**.

For an overview of the numerous programming capabilities, refer to **How to ...: 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 the **Administrator Manual: Installation Instructions**  **ASI-100 HPLC Autosamplers**.)

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.

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 **How to ...: Device Control**  **Program Examples for Virtual Channels.**

Relational and logical operators: ➤*Trigger Commands*


6. Use the following condition to combine, for example, fraction collection:

```
Trigger FRACTIONUV_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.

We recommend using the **Fraction Collection** device driver to control fraction collection. For more information, refer to **How to ...:**  **Actions Related to Fraction Collection.**


```
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 \Rightarrow *Inject*, \Rightarrow *AcqOn/Off*, and \Rightarrow *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 \Rightarrow *Flow* rate, eluent composition (\Rightarrow *%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 $t \leq 0.000$; for example, the detector lamp on command, here -10.000 min.
- You can omit the \Rightarrow *Lamp=On* command if you allow sufficient time for the lamp to reach the operating temperature.
- Any PDA-100 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 \Rightarrow *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 ...: Actions in the PGM Editor**

-  **Creating a Shutdown Program**
-  **Creating an Emergency Program**
-  **Creating a Power Failure Program**
-  **Program with Variable Run Time**

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:

We do 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

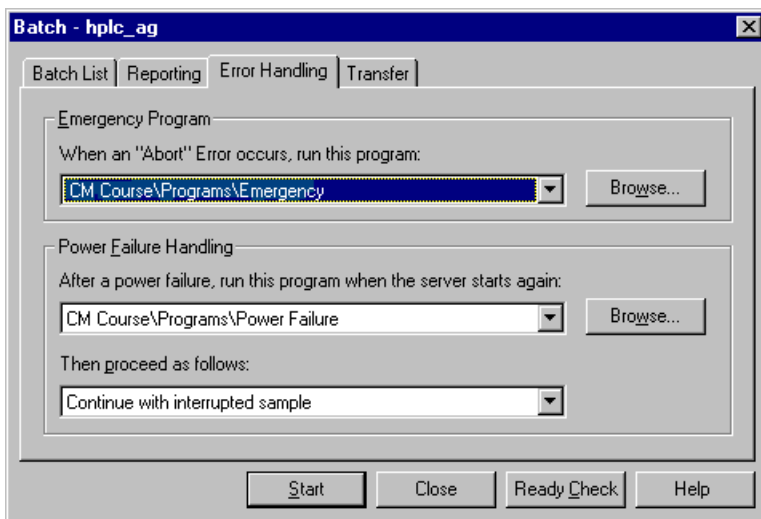
0.500 UV_Lamp = Off
      Flow = 0.100
      End
```

Also, refer to  **Creating an Emergency Program** and  **Creating a Power Failure Program**.

Creating 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 desired emergency program.



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 the **Administrator Manual: Installation Instructions**  **Agilent 1100 HPLC System: Program Tips**.

Also, refer to  **Creating a Shutdown Program** and  **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 desired power failure program.

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** and  **Creating an Emergency Program**.

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 Manual (see **Installation Instructions/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 ...: Actions on 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:



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/m ³	g/ml			
Flow rate	m ³ /s	ml/min	μl/min	μl/s	
Flow acceleration	m ³ /s ²	ml/min ²			
Fraction	None	%			
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/m ³	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	m ³	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:

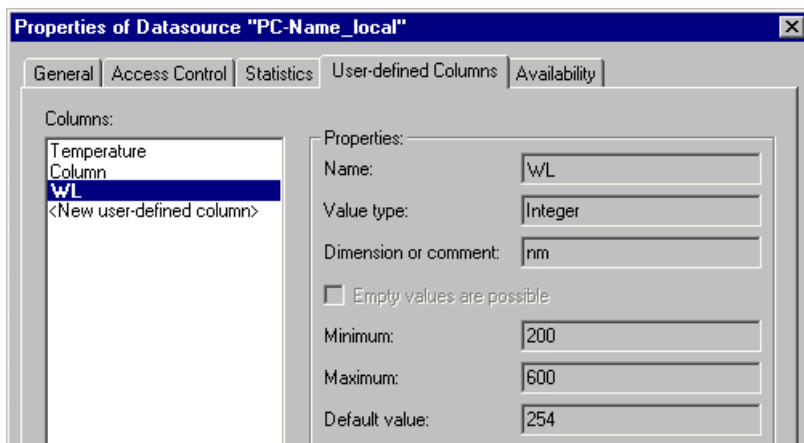
You can also use the values of the user-defined column of the next or previous sample (*NextSample / PrevSample.<UDC-Name>*) or of the previous standard (*PrevStand.<UDC-Name>*).

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 **How to ...: Actions in the Browser**  **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 ...: Actions Related to Fraction Collection**  **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:

- \Rightarrow Flow
- \Rightarrow %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. You can have these data reprocessing steps performed either online after data acquisition or offline of chromatograms, UV spectra, or [➤ Mass Spectra](#).

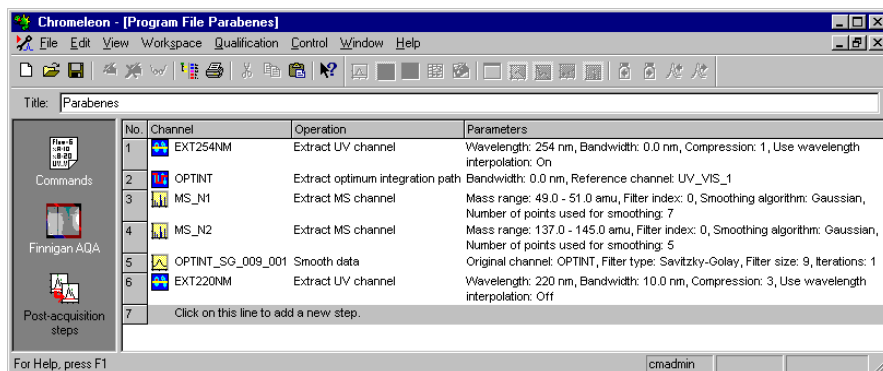
To open the **Post-acquisition steps** view, click the following icon on the shortcut bar:



Click the gray line to add a new post-acquisition step. A dialog box appears. Select the desired step. The following options are available:

- **Arithmetic combination of channels** (2D channels - see  **Combining Channels via Arithmetic Operations**)
- **Copy Channel** (see  **Copying a Channel**)
- **Extract MS channel** (for extracting a *Mass Trace* - only if MS data are available, see **How to ...: Actions Related to 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 ...: Actions in the Peak Purity Analysis (PPA) Window**  **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 ...: Actions in the Chromatogram**  **Performing Data Smoothing**)

For example, the following steps can be added:



In step 1 and step 6, two UV channels are extracted at 254 nm and 220 nm, respectively. The EXT254NM channel does not use compression; only data that were 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 step 3 and step 4, two MS channels (MS_1 and MS_2) are extracted. The first mass trace covers the mass range 49.0 to 51.0 amu, while the second trace covers masses from 137.0 to 145.0 amu. 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 each are used for creating one output data point.

To add additional steps, go to the bottom line. Or else, select **Insert Line** or **Append Line** on the context menu.

The server performs the post-acquisition steps when data acquisition of the last channel 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 Application 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 an application template is being used to create the program, 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.

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:

```
-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 \Rightarrow *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.

Actions in the QNT Editor

The settings selected in the quantification method determine which calibration, peak table, and \Rightarrow *Detection Parameters* are used to evaluate a peak or an entire chromatogram (see \Rightarrow *QNT Parameters*).

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 \triangleright *Mass Spectra*)











To determine which columns are displayed, select **Display Columns** on the **View** menu and determine the columns to be displayed in the **Display Columns** dialog box.

In addition to the tabs mentioned above, the QNT Editor provides the following tabs:

- **General** (general settings)
- **Detection** (detection parameters)
- **Calibration** (calibration parameters)
- **Spectra Library Screening** (spectra search parameters)
- **SST** (\triangleright *System Suitability Test* parameters)
- **MS** (\triangleright *Mass Spectrometry* parameters)
- **UV** (UV background subtraction)

To hide a sheet, select **Select Sheets** on the **View** menu and deselect the desired sheet in the **Select Sheets** dialog box. To rename a sheet, double-click its tab in the QNT Editor and enter the new name.

For more information, refer to:

-  **Creating a Peak Table**
-  **Defining Detection Parameters**
-  **Combining Peaks**
-  **Subtracting a Blank Run Sample**
-  **Disabling Calibration Samples**
-  **Calibration**
-  **Spectra Library Screening**
-  **Defining the System Suitability Test**
-  **Processing Mass Traces**
-  **Processing UV Channels**

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.

To determine which columns are displayed on the **Peak Table**, **Amount Table**, **Peak Tracking**, and **MS Tracking** tab pages, either 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**
-  **Entering Amount Values**
-  **Selecting the Standard Method**
-  **Selecting the Calibration Function**
(Columns: Cal. Type and Int. Type)
-  **Weighting and Averaging Calibration Points**
-  **Defining the QNT Method for Several Detectors**
-  **Entering Reference Spectra**
-  **Autogenerating the Peak Table**

Also, refer to **How to ...: Actions in the Browser**  **Creating User-defined Columns**

Identifying Peaks

(Peak Name, Retention Time, and Window columns)

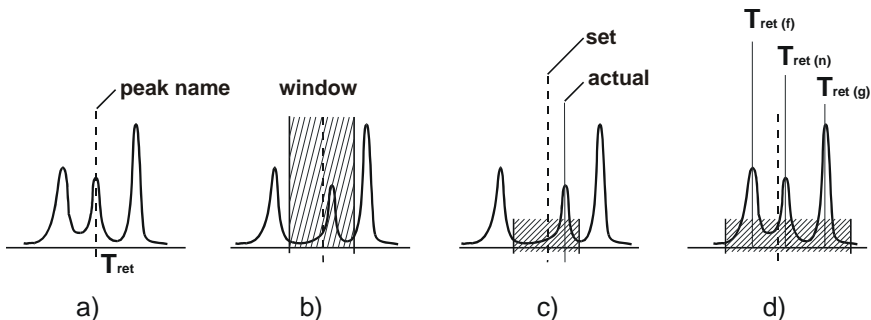
Most frequently, peaks are identified by their retention time.

- Enter the names of all peaks to be identified in the \Rightarrow *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 \Rightarrow *Retention Time* and \Rightarrow *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). Which peak is identified depends on the option selected in the **Peak Window** dialog box. In the **Window** column cell, the two letters after the time range indicated the selected option.



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 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 stated in the peak table only serve to identify a peak. The retention times indicated in a ➤Report are always the actual retention times (= 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 may happen that the peak leaves 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 also 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 ...: Actions in the QNT Editor**  **Entering Criteria for the Spectra Library Screening**.

**Tip:**

*If neither the minimum (**Min. WL**) nor the maximum wavelength (**Max. WL**) are set, the comparison is performed for the entire wavelength range of the reference spectrum.*

Enable peak tracking in the **Window** column. In the corresponding F8 dialog box (press the F8 key in any cell in the Window column or double-click), select **Spectrum** or **Spectrum and time** under **Peak Match**.

Peak Window for Naphthalin

Window Interpretation

☒ Absolute

☐ Relative

Peak Match

☐ First

☐ Greatest

☐ Nearest

☐ Spectrum

☒ Spectrum and time

Multiple hits

☒ Check the best hits only

Remark:
This option is common to all peaks using spectra match.

Mass spectrometry

☐ Check mass ratios

Remark:
This option is common to all channels.

Window width: 0.090 [min]

Range: [0.000 .. 999.999]

Default: 0.100

OK Cancel Help

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

4.1 min, 45 mAU - Select Peak 3 - Ethylparabene - 4.083

Double-click the respective cell or press the **F8** key to open the **Mass spectrometry conditions for ...** dialog box:

Mass spectrometry conditions for Ethylparabene

☒ Check mass ratios

Mass spectrum:

	m/Z [amu]	Intensity relative to peak 1
Peak 1:	165.0	100.0 %
Peak 2:	150.0	20.0 ± 10.0 %
Peak 3:	136.0	10.0 ± 5.0 %

Threshold: 5.0 % of base peak intensity

☒ Check retention times of mass spectra

Filter Mode:

☐ Automatic Use filter settings from active chromatogram

☒ Smart Use filter settings from active chromatogram if available, otherwise use fixed filter settings below

☐ Fixed Use fixed filter settings below

User Defined Filter Settings:

Polarity: Any

Start voltage: 100.0 [0 - 200 V] or empty

End voltage: [0 - 200 V] or empty

OK Cancel Help

If you have entered at least the mass of one mass peak, you can enable and disable peak identification via mass spectra by checking the **Check mass ratios** check box.


Under **Peak 1**, enter the mass of the substance (range: 1.0 to 2000.0 amu), which is expected at this peak. If you expect fragmentation and can estimate the fragments, which are expected, you can enter two of them with their expected intensities under **Peak 2** and **Peak 3**.

Select the **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** option to use also the retention times of all masses for peak assignment. All retention times must be within one scan.

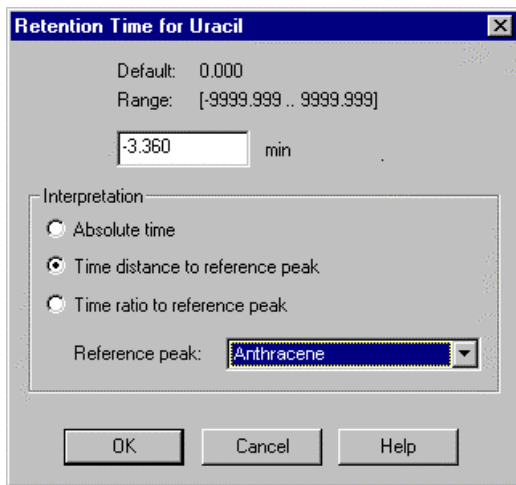
The settings made in the **Mass spectrometry conditions for...** dialog box are not active immediately. You can activate them later from the **Peak Table** tab page: Press the F8 key in the **Window** column or double-click to open the **Peak Window for...** dialog box. Click the **Check mass ratios** check box.

If you acquire data with a ➤ *Photodiode Array Detector*, you can identify peaks via their mass 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 to be interpreted for the respective peak. Double-click the corresponding peaks or press the F8 key in the **Ret. Time** column, to open the **Retention Time for...** dialog box.



The dialog box titled "Retention Time for Uracil" has a close button (X) in the top right corner. It contains the following elements:

- Default: 0.000
- Range: [-9999.999 .. 9999.999]
- An input field containing "-3.360" followed by the unit "min".
- An "Interpretation" section with three radio buttons:
 - ☐ Absolute time
 - ☒ Time distance to reference peak
 - ☐ Time ratio to reference peak
- A "Reference peak:" label followed by a dropdown menu showing "Anthracene".
- Three buttons at the bottom: "OK", "Cancel", and "Help".

On the **Interpretation** tab page, 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.

The **Reference peak** dialog box allows you to define one (or several) of the other peaks as reference peak. However, only peaks with absolute reference times can be used as reference peaks. They are indicated with a light blue background. It is not possible to delete reference peaks from a peak table.

The retention time is re-calculated automatically when the retention time interpretation is changed or when a different reference peak is selected.

Besides, you can also enter the retention time directly in the input field (**Peak Table** page in the **Ret. Time** column) in the following manner:

[<Reference Peak>] <Ret. Time> [<Unit>]

You are free to choose the order in which the fields are entered.

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 \Rightarrow *Ret. Index* (= RI) or \Rightarrow *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, ...

Kovats Index: 100*number of carbon atoms of the alkane.

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 \Rightarrow Dead Time on the **General** tab page of the QNT Editor.*

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 ...: Actions in the QNT Editor**  **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 \Rightarrow *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., \Rightarrow *Name* for the sample name, \Rightarrow *No.* for the sample (vial) number, or \Rightarrow *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; 🟢 = \Rightarrow *Validation Sample* or 🟣 = \Rightarrow *Spiked Sample*) from the **Standards** window to the new column.

-or-

Click **Auto-Generate**. Select the preferred option from the drop-down list box 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.

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 standard means that the calibration is performed based on one or several standard samples. (This is the default.)

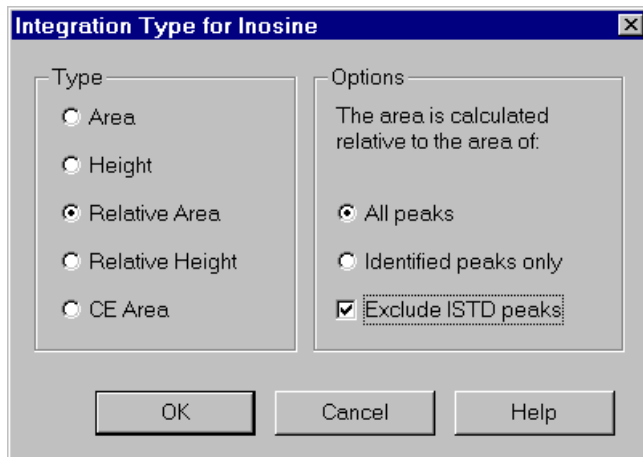
Using an **internal standard** substance means adding a standard to the unknown sample. This can be either before (**External/Internal**) or after (**Internal**) sample preparation. Either the standard (= ➤ *Internal Standard*) can be added to all samples or it can serve as a basis for a relative area 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, add the standard method for each peak to be calibrated (**external**, **internal/external Peak**, **internal Peak**).
- For the last two options, at least one peak of the peak table is used as the internal standard. This is achieved by assigning it the **Make current Peak to ISTD Peak: intern** or **Make current Peak to ISTD Peak: internal/external** option.
- 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)

Via the **Int. Type** (\Rightarrow *Integration Type*) column, 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:



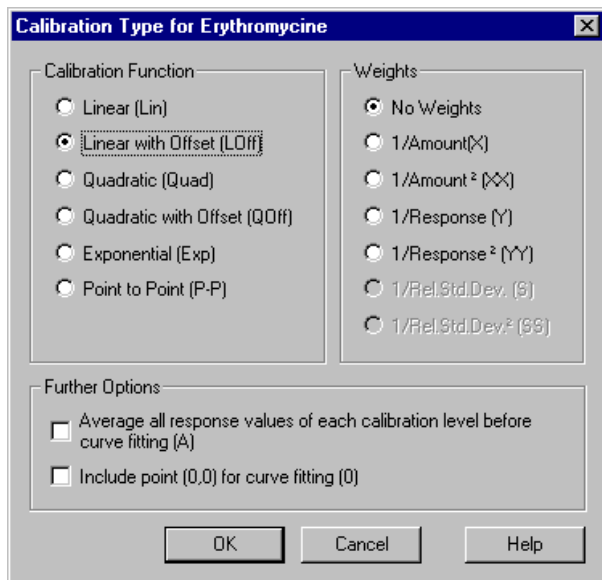
In addition to **Area**, **Height**, and **CE-Area**, you can select the relative area or the relative height as the reference for the evaluation (Integration Type).

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.



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 \Rightarrow *Calibration Type* determines which \triangleright *Calibration Function* is used for deriving a valid amount/area assignment for a larger range from the calibration points of the standard samples.

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...: Actions in the QNT Editor**  **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 **Include point (0,0) for curve fitting** 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

Chromeleon generally 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.

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.

First, enter the name of the second detector under **Detector Name** on the **General** tab page of the QNT Editor. Click the arrow key and then select the name from the list. Enter the measured delay time.

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

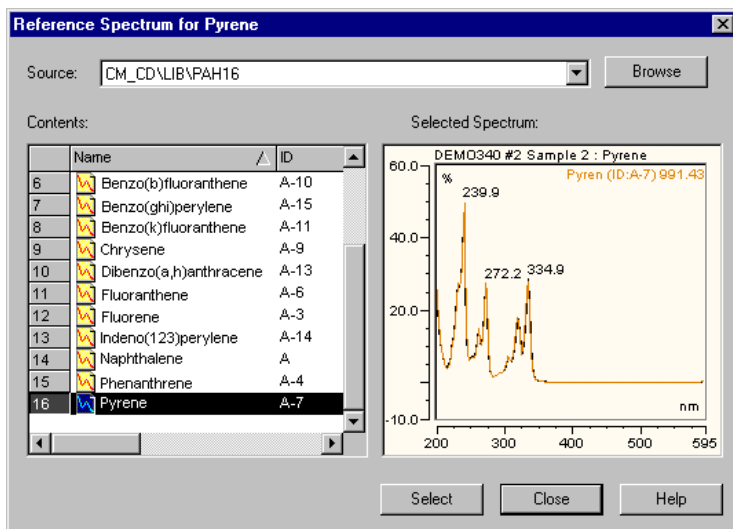
report, just leave the corresponding cell in the **Retention Time** column empty.

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: ...: Actions in the QNT Editor**  **Defining Detection Parameters (Detection).**

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 be 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 \Rightarrow *Comment*.

The peak names (\Rightarrow *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 \Rightarrow *Window* values are entered as absolute values; they represent one-third of the distance from the nearest peak.

No \Rightarrow *Group*.

The \Rightarrow *Amount* values and the \Rightarrow *Response Factor* are set to 1.0.

The peak type (\Rightarrow *Sample Type*) is Auto; that is, it is determined by Chromeleon.

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**).

Autogenerate Peak Table

Type

☐ Enumerate peaks of current chromatogram

☒ Use Spectra Library Screening results

Options

☒ Apply only to peaks with greater than %

☒ Apply only to peaks in current time interval

☒ Copy reference spectrum from

☒ Enable peak tracking using peak match type

Multiple hits

☐ Allow multiple best hits


☒ Unique identification ☒ Check the best hits only

OK Cancel Help

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 ...: Actions in the QNT Editor**  **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 to integrate the chromatograms, which are evaluated with the respective QNT Method, where to suppress peaks, how to recognize peak start and peak end, etc. This enables the user in many ways to define the integration 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 or define them graphically in the chromatogram.

In order to enter the parameters in the table on the **Detection** tab page of the QNT Editor, express the desired conditions via the columns **Parameter Name** and **Parameter**. They influence the integration of all chromatograms to which the respective QNT Method applies. There are 26 different *⇒Detection Parameters*, which can assume a new value at any time and whenever desired. Only the value, which was defined, last will take effect, however, only for the time of the chromatogram. The parameters are then reset to their default values. 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 will 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. Thus, in general about 90% of all chromatograms are automatically integrated correctly by the system. 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 the detection for defined periods. Many parameters can only assume the values **ON** or **OFF**. They are called **switches**. All other parameters are called **variables**. 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 image below:

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 ...: Actions in the Chromatogram**  **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 the **Autom. Re-Integerate** option 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:

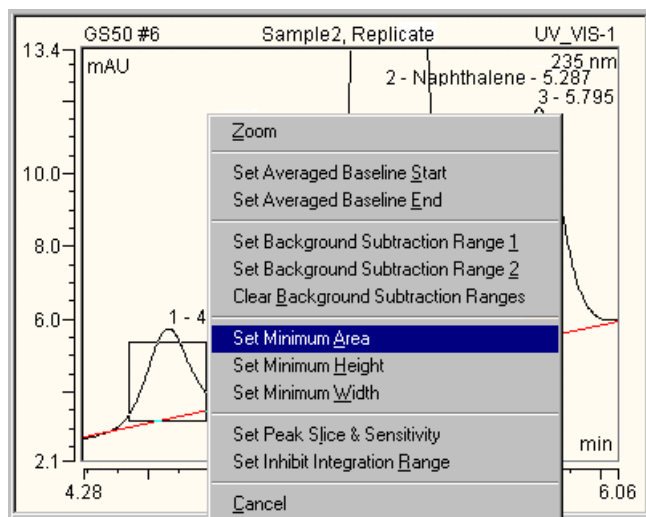
- Open a QNT File (select **Open** on the **File** menu) and click the **Detection** tab page.
- Look at the chromatogram and determine the minimum peak height to be evaluated. You may also click the smallest peak to be integrated. Then open the corresponding **Peak Properties** via the context menu. The dialog box then indicates the width, height, and area of the peak.
- Enter the name of the parameter to modify; for example, the \Rightarrow *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 an area smaller 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 desired peaks 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 (so-called spikes) occur in chromatograms due to, for example, air bubbles in the HPLC system. In such a case, the problem (= the air 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 \Rightarrow *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 \Rightarrow *Maximum Peak Height* parameter.
























Broad Peaks

If an unusually broad peak occurs in the chromatogram, it may be from a previous sample. Select the \Rightarrow *Maximum Width* parameter to define this peak as being unidentified.

Inhibiting Peak Integration

The \Rightarrow *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	

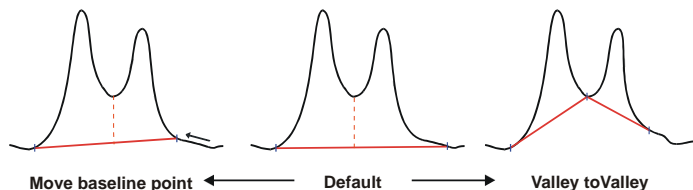
Peaks detected in this time span will not be integrated and will not be included in an integration report.

Modifying the Baseline

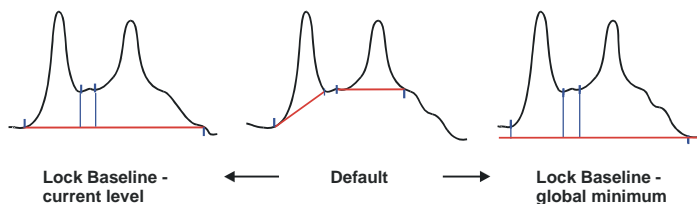
Modifying the baseline can be necessary especially for non-resolved peaks. The baseline is normally defined via a mathematical procedure. For calculating the individual peak areas, a perpendicular is dropped to the baseline from each local minimum (standard).

If you think the integration is started too early or the peak end is delayed, a better peak start or peak end can be forced by inserting a baseline point (\Rightarrow *Baseline Point* detection parameter).

If a series of non-resolved peaks can be recognized on an "absorption mound", for example, due to increased solvent absorption, the baseline can be forced from minimum to minimum using the \Rightarrow *Valley to Valley* detection parameter.



If single peaks are piled on a recognizable "absorption mound," these are integrated individually. To be able to integrate the entire area, the baseline can be fixed. The parameter \Rightarrow *Lock Baseline* extrapolates the baseline horizontally to the intersection with the signal curve. The **Global Minimum** option 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** option!



Tip:

In all of these actions, the classification criterion **Peak Type** of the peak table has priority! For a peak of the type **Baseline-Main-Baseline**, the peak limits always have baseline contact!

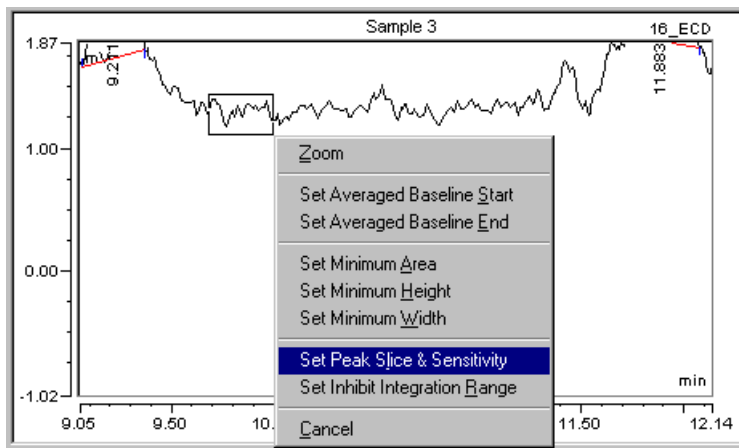
Modifying the Peak Recognition Algorithm

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 \Rightarrow *Peak Slice* and \Rightarrow *Sensitivity* detection parameters. Changing the parameters is required, for example, in chromatograms with unusually wide (many minutes) or very narrow ($< 0.1\text{sec}$) 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 \Rightarrow *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. Try 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 \Rightarrow *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 \Rightarrow *Peak Slice* (= about 20% of the smallest peak width) and, in addition, a higher \Rightarrow *Sensitivity*, if necessary.
2. For samples that have not been processed yet: Change the data acquisition \Rightarrow *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 \Rightarrow *Rider Threshold* and \Rightarrow *Maximum Rider Ratio* allow you to define which peaks shall be detected as \triangleright *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 \Rightarrow *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.

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 your 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 the desired 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 \Rightarrow 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.

Disabling Calibration Samples

The **Calibration** tab page provides information about the calibration and allows you to determine which sample shall be used.

It depends on the \Rightarrow *Calibration Mode* which sample \Rightarrow *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 \triangleright *Standard Addition* method, you can use the sample types **Unspiked**  and **Spiked**  (see \triangleright *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 ...: Actions in the QNT Editor**  **Calibration**

Calibration

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**

In addition, you may invert variables; see  **Inverting Dependent and Independent Variables**.

**Note:**

To apply an existing calibration to a new sequence consisting of one or several **Unknown** samples, set the \Rightarrow 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 \Rightarrow 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 \Rightarrow 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 μ l 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 ...: Actions in the Browser**  **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 \Rightarrow *QNT Editor*, enter the unit for all other entries in the \Rightarrow *Dimension of Amounts* field. (For this example, enter "mg/l"). In the **Global Calibration Settings** section, keep the setting for the \Rightarrow *Calibration Mode*, i.e., **Total**.

QNT Method/Peak Table Tab

Do not change the defaults in the **Standard** (External) and \Rightarrow *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 ...: Actions in the QNT Editor**



Calibration.

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 ...:**

Actions in the Browser  **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 \Rightarrow *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 \Rightarrow **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** in the drop-down combo box 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 \Rightarrow **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 ...: Actions in the QNT Editor**  **Calibration**.

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*.

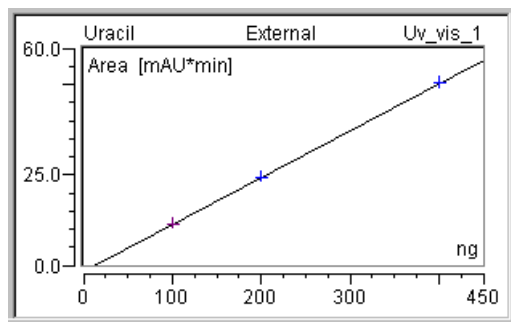
QNT Method/Peak Table Tab


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 ...: Actions in the QNT Editor**  **Calibration.**

Calibrating Using Standards of an Old Sequence

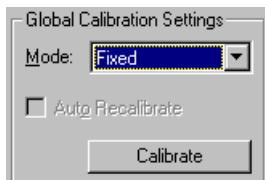
Calibration standards are often quite expensive. Thus, if the calibration curve remains constant for weeks or months, the standards of a sequence can be used for calibration for several weeks before the calibration constancy needs to be checked again.

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

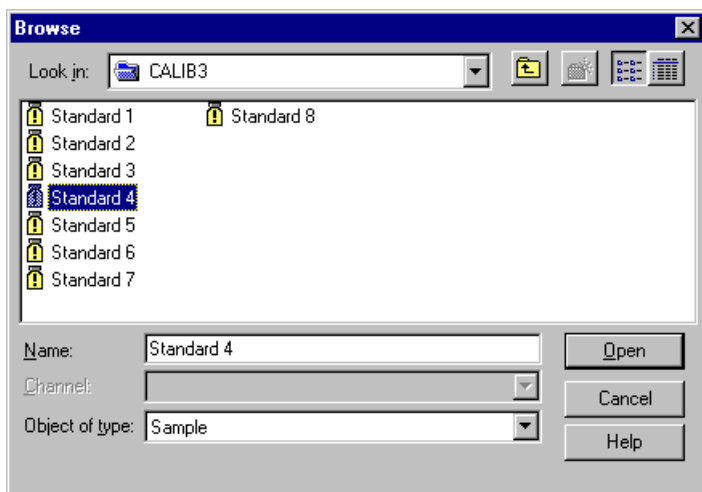
Select **Fixed** as *⇒ Calibration Mode* in the **Global Calibration Settings** section:



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 desired standard(s). The **Browse** dialog box is opened. Select the desired standard sample from any sequence:



The **Calibration** tab page then lists the desired standards:

No.	Enabled	Name	Sequence	Smp.No.	Pos.	Inj. Vol.	Inj. Date/Time
1	<input checked="" type="checkbox"/>	Standard 1	Parabenes\CALIB3.SEQ	1	6	4.0	04.12.00 12:51:27
2	<input checked="" type="checkbox"/>	Standard 2	Parabenes\CALIB3.SEQ	2	6	8.0	04.12.00 13:03:25
3	<input checked="" type="checkbox"/>	Standard 3	Parabenes\CALIB3.SEQ	3	6	12.0	04.12.00 13:15:24
4	<input checked="" type="checkbox"/>	Standard 4	Parabenes\CALIB3.SEQ	4	6	16.0	04.12.00 13:27:24
5	<input checked="" type="checkbox"/>	Standard 5	Parabenes\CALIB3.SEQ	5	6	20.0	04.12.00 13:39:23
6	<input checked="" type="checkbox"/>	Standard 6	Parabenes\CALIB3.SEQ	6	6	24.0	04.12.00 13:51:24
7	<input checked="" type="checkbox"/>	Standard 7	Parabenes\CALIB3.SEQ	7	6	28.0	04.12.00 14:03:22
8	<input checked="" type="checkbox"/>	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 ...: Actions in the QNT Editor**



Calibration.















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:

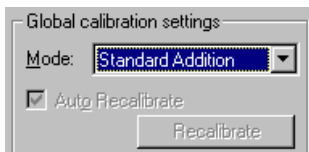
No.	Name	Type	Std. Add. Group	Ref. Amount Set
1	 Sample 1	Unspiked	1	
2	 Spiked 1_1	Spiked	1	Spike1
3	 Spiked 1_2	Spiked	1	Spike2
4	 Spiked 1_3	Spiked	1	Spike3
5	 Sample 2	Unspiked	2	
6	 Spiked 2_1	Spiked	2	Spike1
7	 Spiked 2_2	Spiked	2	Spike2
8	 Spiked 2_3	Spiked	2	Spike3
9	 Sample 3	Unspiked	3	
10	 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 \Rightarrow *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**.

We recommend spiking unknown samples always with the same amount of the same substances. In the \Rightarrow *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 \Rightarrow *Calibration Mode* in the **Global Calibration Settings** section:

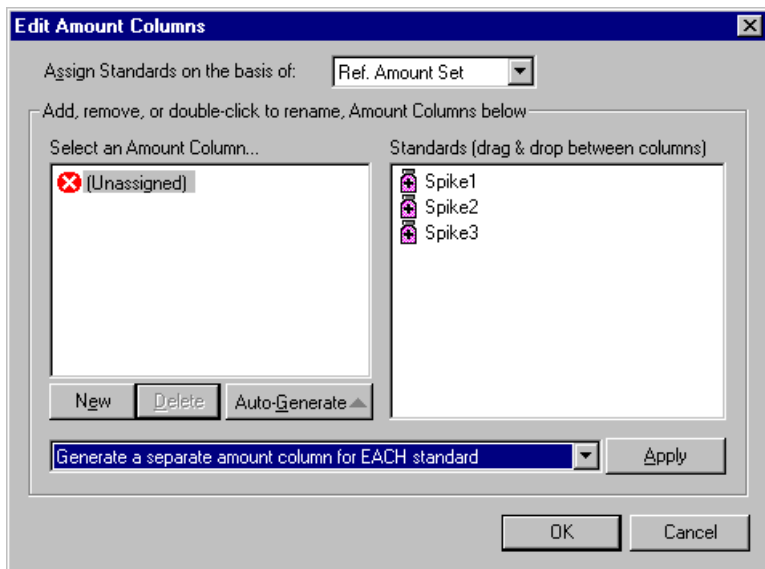


Re-calibration is performed automatically; it cannot be disabled.

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 ...: Actions in the QNT Editor**  **Calibration**.

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:

No.	Name	Type	Pos
1	 Standard 1	Standard	1
2	 Standard 2	Standard	2
3	 Sample 1	Unknown	3
4	 Sample 2	Unknown	4
5	 Standard 3	Standard	5
6	 Standard 4	Standard	6
7	 Sample 3	Unknown	7
8	 Sample 4	Unknown	8
9	 Standard 5	Standard	9
10	 Standard 6	Standard	10


QNT Method/General Tab


The bracketed calibration illustrated in the above figure has been achieved using the **Bracketed** \Rightarrow *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 \triangleright *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 (\triangleright *Virtual Channel Driver*) to record all chromatograms in this special channel as if the*

substance would not decay. For a program example, refer to **How to ...: 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 ...: Actions in the QNT Editor**  **Calibration**.

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**.

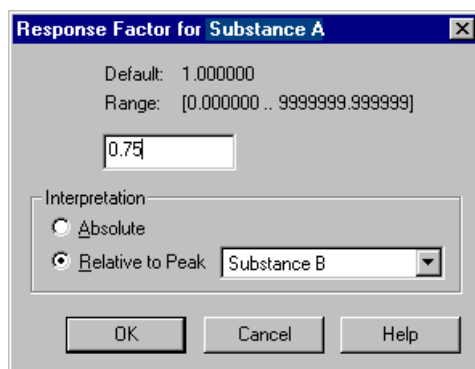
Then, create the ⇒Amount columns for your standards. As the pure substance A is not available and thus is not contained in the standards, the cells for substance A remain empty in the **Amount** table:

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

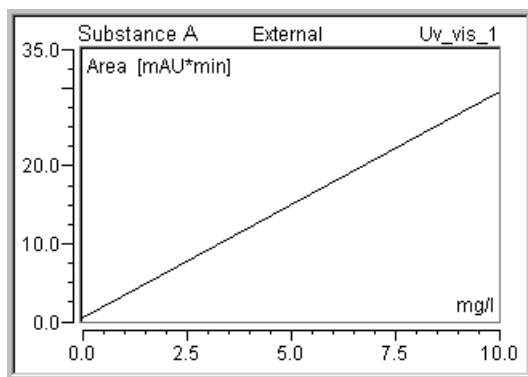
The entry made in the ⇒Response Factor column is the decisive entry. This column allows you to use the calibration of substance B for substance A. Double-click the selected cell (see above) to open the **Response Factor for Substance A** dialog box and enter the factor of the extinction coefficient at 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 the desired channel by clicking the **Add Channel** button.

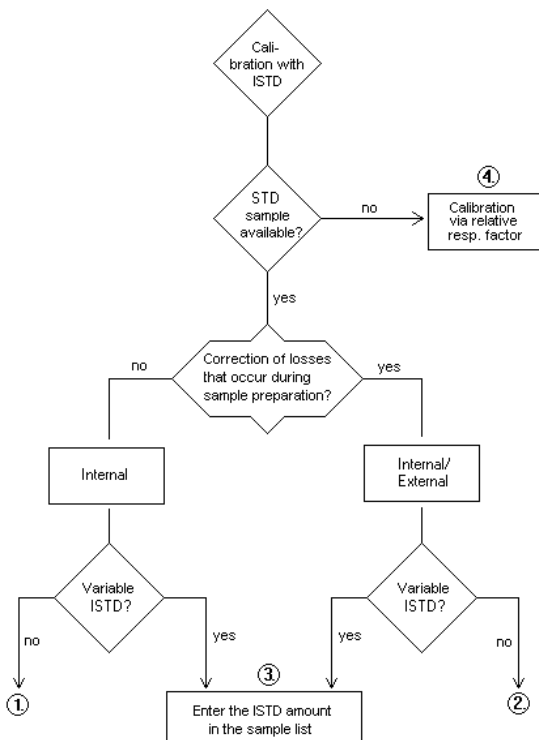
**Note:**

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 ...: Actions in the QNT Editor**

**Calibration.****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**

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 the option **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 ...: Actions in the QNT Editor**  **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	Amount1	Amount2
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 \Rightarrow 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 successively open the sample of a sequence. 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	<input type="checkbox"/>	STD 1	1	10.0	10/12/00 1:16:30 PM	Disabled
2	<input checked="" type="checkbox"/>	STD 2	2	20.0	10/12/00 1:29:05 PM	Ok

Peak Tracking MS Tracking **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 $x_{p,rel}$:

$$x_{(p,rel)} = \frac{Area_p}{Area_{ISTD}}$$

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 (c1). 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, c1 results as the y/x-quotient of each value pair **Amount p rel** to **x p rel**:

<u>Substance</u>	<u>Amount (p rel) / x (p rel)</u>	<u>c1</u>
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 c1 approximate value for each substance. If a different calibration type is selected, Chromeleon calculates the corresponding calibration coefficients (c0, c1, and c2).

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 c1 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:

<u>Sample I</u>	<u>Calculation: Amount/Amount ISTD</u>
Adrenaline	$2.000 \times (223/245) = 1.820$
Dopamine	$1.250 \times (150/245) = 0.765$
<u>Sample II</u>	<u>Calculation: Amount/Amount ISTD</u>
Adrenaline	$2.000 \times (178/255) = 1.396$
Dopamine	$1.250 \times (380/255) = 1.863$

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.

<u>Sample I</u>	<u>Calculation: Amount</u>
Adrenaline	$1.820 \times 50 = \mathbf{91.00}$ [ng]
Dopamine	$0.765 \times 50 = \mathbf{38.25}$ [ng]
<u>Sample II</u>	<u>Calculation: Amount</u>
Adrenaline	$1.396 \times 50 = \mathbf{69.80}$ [ng]
Dopamine	$1.863 \times 50 = \mathbf{93.15}$ [ng]

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to...: Actions in the QNT Editor**






Calibration.

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 and 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 the option **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 ... Actions in the QNT Editor**  **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 and 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 μ l sample + 20 μ l OPA reagent + 20 μ l 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 μ l of the standard solution (*➤Dilution Series*) and 10 μ l 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	

As the calibration of alanine and glycine is performed with only one (STD 1) standard concentration (same autosampler position in the sample list), only one amount value is entered for each peak. Then, 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 \Rightarrow 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 successively open the samples of a sequence. 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	<input type="checkbox"/>	STD1	1	10.0	10/12/00 1:16:30 PM	Disabled
2	<input checked="" type="checkbox"/>	STD1	1	20.0	10/12/00 1:29:05 PM	Ok

☐ Peak Tracking
 ☐ MS Tracking
 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:

<u>Name</u>	<u>Area Alanine</u>	<u>Area Glycine</u>	<u>Area Norvaline</u>
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:

<u>Substance</u>	<u>Area value</u>	<u>Amount value [1]</u>	<u>Amount value [2]</u>
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

A linear calibration curve through the origin (calibration type **Linear**) can already be described by one calibration coefficient (c1). 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, c1 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 \Rightarrow 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 amount values of glycine and alanine are calculated by means of the ➤*Formula for Amount Calculation*. In contrast to an external calibration, **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	$45 \times 1.111 \times 1.026 =$	51.30 (Alanine)
I	$90 \times 0.625 \times 1.026 =$	54.90 (Glycine)
II	$80 \times 1.111 \times 0.976 =$	86.75 (Alanine)
II	$160 \times 0.625 \times 0.976 =$	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 ...: Actions in the QNT Editor**  **Calibration**.




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. (Access to the option is 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

Except 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 ...: Actions in the QNT Editor**  **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 \Rightarrow *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 \Rightarrow *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 the option **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 the **Calibrate** button 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	C1	C2
					Uv_vis_1	Uv_vis_1	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 ...: Actions in the QNT Editor**  **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:

Group	Name	Pos	Add.	Brack.
	Standard 1	1		
	Standard 2	2		
	Sample 1	3	←	←
	Sample 2	4	←	←
	Standard 3	5		
	Standard 4	6		
	Sample 3	7	←	←
	Sample 4	8	←	←
	Standard 5	9		
	Standard 6	10		

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 c_0 (offset), c_1 (slope), and c_2 (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 ...: Actions in the Browser**

**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

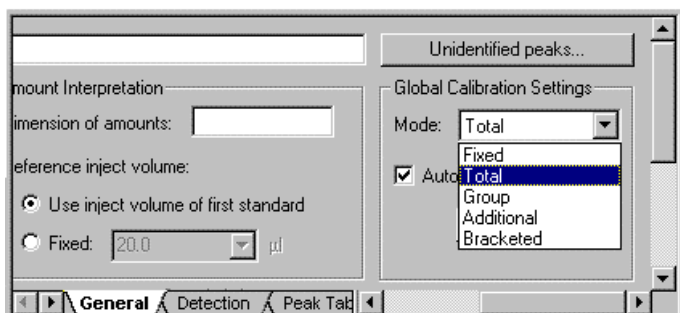
calibration with several replicates. If the injection volume varies (10 and 20 µl) as in this case, this is referred to as multiple-point calibration, that is, 2-point calibration. This is a special case of the *Dilution Series* ("concentration series").

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 ...: Actions in the QNT Editor**  **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** ⇒ *Calibration Mode*.



QNT Method/Calibration Tab

This page shows all standard samples (of a sequence) that are used for calibrating the current sample.

No	Enabled	Name	Smp.No	Inj. Vol.	Inj. Date/Time	Calib. Comment
1	<input checked="" type="checkbox"/>	 STD1	1	10.0	10/12/00 1:16:30 PM	Ok
2	<input type="checkbox"/>	 STD1	2	20.0	10/12/00 1:29:05 PM	Disabled

Press the F4 key or the SHIFT+F4 key combination to successively open all samples of a sequence. 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 on the basis of 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:

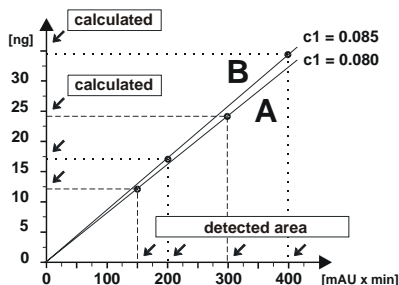
Name	Area Subst. A	Area Subst. B
STD 1 (first run)	150	200
STD 2 (second run)	300	400
Sample I	175	150
Sample II	95	180

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 c_1 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 (c_0 and c_2).

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	175×0.08	14.00	
I	150×0.085		12.75
II	95×0.08	7.60	
II	180×0.085		15.30

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to ...: Actions in the QNT Editor**  **Calibration**.

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

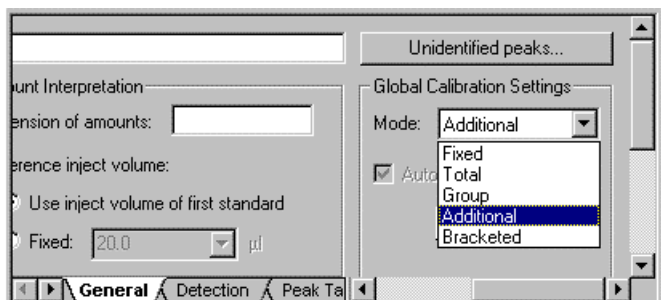
	Name	Type	Pos	Inj.Vol	Program	Method	Status
1	 STD 1	Standard	1	10.0	Control 2	QNT2	Single
2	 STD 1	Standard	1	20.0	Control 2	QNT2	Single
3	 Sample I	Unknown	2	10.0	Control 2	QNT2	Single
4	 Sample II	Unknown	3	10.0	Control 2	QNT2	Single
5	 STD 1	Standard	1	10.0	Control 2	QNT2	Single
6	 STD 1	Standard	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 successively open the samples. 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).

No	Enabled	Name	Smp.No	Inj. Vol.	Inj. Date/Time	Calib. Comment
1	<input checked="" type="checkbox"/>	STD 1	1	10.0	4/25/97 11:01:0	Ok
2	<input type="checkbox"/>	STD 1	2	20.0	4/25/97 11:16:4	Disabled
3	<input checked="" type="checkbox"/>	STD 1	5	10.0	4/25/97 11:48:3	Ok
4	<input checked="" type="checkbox"/>	STD 1	6	20.0	4/25/97 11:59:0	Ok
5	<input checked="" type="checkbox"/>	STD 1	9	10.0	4/25/97 1:30:55	Ok
6	<input checked="" type="checkbox"/>	STD 1	10	20.0	4/25/97 1:41:31	Ok

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 automatically corrected.

For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to ...: Actions in the QNT Editor**



Calibration.













Calibration Mode: Group

The **Group** \Rightarrow *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 successively open the sample of a sequence. 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 ...: Actions in the QNT Editor**



Calibration.

 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	QNT4	Single
2	STD 2	Standard	11	20.0	Control 4	QNT4	Single
3	Sample I	Unknown	1	10.0	Control 4	QNT4	Single
4	Sample II	Unknown	2	10.0	Control 4	QNT4	Single
5	STD 1	Standard	10	20.0	Control 4	QNT4	Single
6	STD 2	Standard	11	20.0	Control 4	QNT4	Single
7	Sample III	Unknown	3	10.0	Control 4	QNT4	Single
8	Sample IV	Unknown	4	10.0	Control 4	QNT4	Single
9	STD 1	Standard	10	20.0	Control 4	QNT4	Single
10	STD 2	Standard	11	20.0	Control 4	QNT4	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 \Rightarrow 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	Amount1	Amount2
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 \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 successively open the samples of a sequence. 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	<input checked="" type="checkbox"/>	STD 1	1	10	12/6/00 1:20:03	Ok
2	<input type="checkbox"/>	STD 2	2	11	12/6/00 1:24:05	Disabled
3	<input checked="" type="checkbox"/>	STD 1	5	10	12/6/00 1:27:03	Ok
4	<input checked="" type="checkbox"/>	STD 2	6	11	12/6/00 1:30:14	Ok

Calibration Spectra Library Screen

For the samples III and IV, the page will look as follows:

No	Enabled	Name	Smp.No	Pos.	Inj. Date/Time	Calib. Comment
1	<input checked="" type="checkbox"/>	STD 1	5	10	12/6/00 1:20:03	Ok
2	<input checked="" type="checkbox"/>	STD 2	6	11	12/6/00 1:24:05	Ok
5	<input checked="" type="checkbox"/>	STD 1	9	10	12/6/00 1:27:03	Ok
6	<input checked="" type="checkbox"/>	STD 2	10	11	12/6/00 1:30:14	Ok

Calibration Spectra Library Screen

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	167×0.2267	37.86
II	152×0.2267	34.46

For the samples III and IV, the result is as follows (taking K2 into account):

Sample	Calculation	Amount A
III	283×0.2261	63.99
IV	305×0.2261	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. Therefore, the results of a calibration with the **Bracketed** mode should be printed after completing all standard samples.

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 ...: Actions in the QNT Editor**

**Calibration.****Calibration Mode: Fixed**

To use any desired 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 also be created. It is not necessary to enter the \Rightarrow 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. Determine the standard samples to be used for calibration on the **Calibration** tab page.

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 ...: Actions in the QNT Editor**



Calibration.

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 \Rightarrow *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 ...: Actions in the QNT Editor**  **Calibration**.

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 ...: Actions in the Report Table**  **Defining the Contents of a Report**.

- Select the **Amount Deviation** variable in the \Rightarrow *Peak Results* report category. Click the **Parameter** button 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 \Rightarrow 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 desired confidence region to check whether your validation and standard samples are within the corresponding confidence interval (also, refer to **How to ...: Actions in the Calibration Curve**  **Indicating the Confidence Interval**).

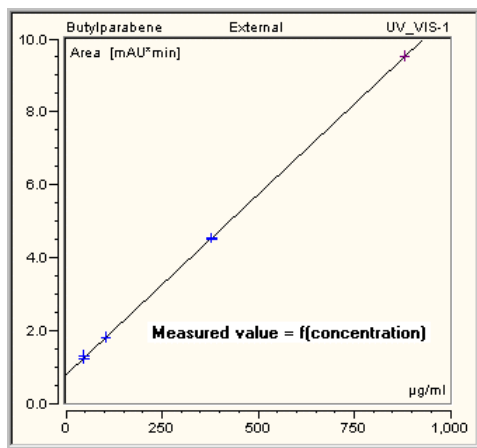
b) For single values, you can also indicate the limits for the respective \triangleright *Confidence Interval* in the Report using the **Upper/Lower Confidence Limit** variables of the \Rightarrow *Peak Calibration* report category.

For information about how to enter the concentration/amount, refer to **How to ...: Actions in the QNT Editor**  **Entering the Concentration/Amount of the Validation Sample**.

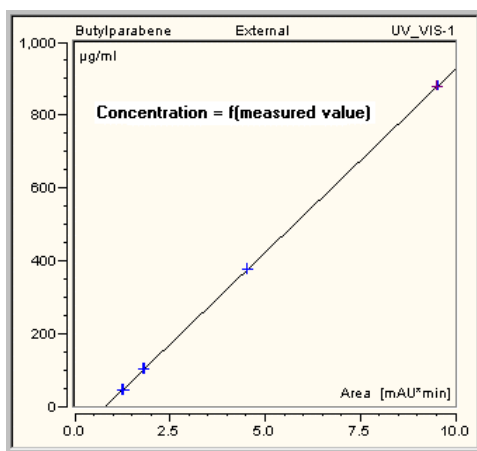
For an overview of the different calibration possibilities provided by Chromeleon, refer to **How to ...: Actions in the QNT Editor**  **Calibration**.

Inverting Dependent and Independent Variables

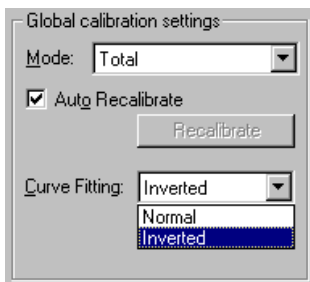
Normally, calibration curves are fitted using normal regression analysis, in which the known amount (or concentration) of the analyte is treated as the independent variable (x) and the corresponding response (peak area or peak height) is treated as the dependent variable (y):



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 inverted curve fitting is not supported by standard statistical theory, 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)
- ➤ *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)

Spectra Library Screening

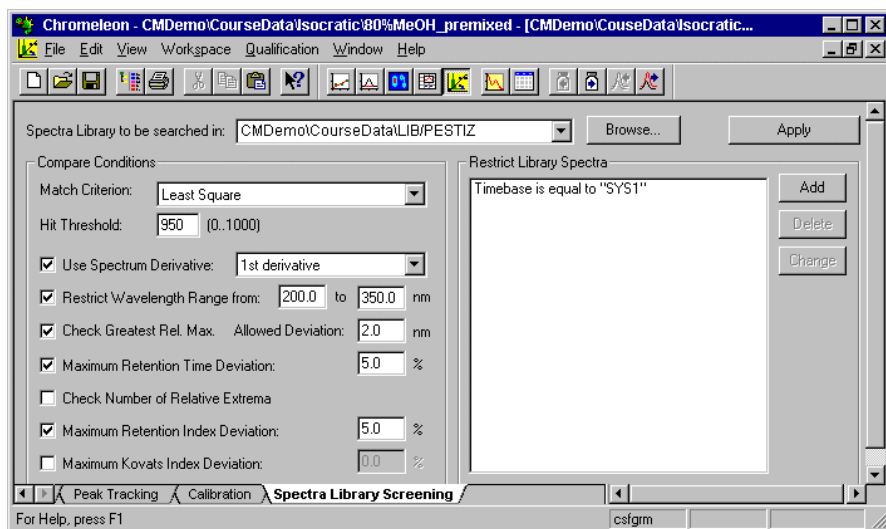
Use **Spectra Library Screening** page of the **QNT Editor** to search spectra libraries for reference spectra. The spectra search is also possible from the spectra plot, as described in **How to ...: Actions in UV Spectra and/or Mass Spectra**  **Starting a UV Spectra Search**.

For more information, refer to:

-  **Entering Criteria for the Spectra Library Screening**
-  **Starting Library Screening and Viewing Results**
-  **Integrating Screening Results in Reports and Peak Labels**

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 \Rightarrow *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 \triangleright *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 \Rightarrow *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, we recommended 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:**

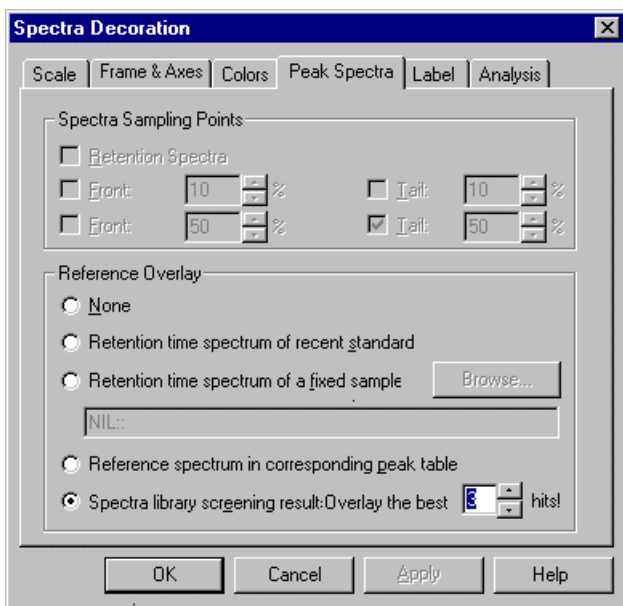
*If you want to search all libraries in a directory, simply enter the character * as the file name. The wildcard characters (known from MSDOS) * and ? are valid. Example: LIB:\CMDATA\LIB\D* searches all libraries starting with the letter **D** in the directory **LIB** of the datasource CMDATA.*

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 will be 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:

<u>Designation</u>	<u>Description</u>
Substance Name	
Match Factor	➤ <i>Match Factor</i>
Library Name	Name of the spectra library
Library Record	Opens the ➤ <i>Spectra Library</i> category
Number of rel. Extrema	
Solvents	
Comment	➤ <i>Comment</i>
Sequence Name	
Sequence Header Record	
Sample Name	
Sample Record	Branches to the ➤ <i>Sample</i> category
Acquisition Time	Acquisition date and time
Timebase	➤ <i>Timebase</i>
Program	➤ <i>PGM File</i>
Sample Rate	
Retention Time	➤ <i>Retention Time</i>
Lambda Min.	Minimum wavelength
Lambda Max.	Maximum wavelength
Lambda Range	Wavelength range
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 ➤ <i>Retention Index</i>
Kovats Index	➤ <i>Kovats Index</i>

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 reach the **Insert Variable into Peak Label Formula** dialog box. Select the **Number of SLS Hits** and **SLS Hit** variables (see **Report** 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 will receive the following **Formula** input on the **Peak Label** tab page of the **Chromatogram Decoration** dialog box:

The screenshot shows the 'Chromatogram Decoration' dialog box with the 'Peak Label' tab selected. The dialog has four tabs: 'Comparison', 'Events', 'Colors', and 'Peak Label'. The 'Peak Label' tab contains two sections: 'Identified Peaks' and 'Not Identified Peaks'. Both sections have a checked checkbox for 'Label' and a 'Formula' field. The 'Identified Peaks' formula field contains '{peak.hitSpec(1).name} - {peak.hitSpec(1).match}'. The 'Not Identified Peaks' formula field contains '{peak.hitSpec(1).name} - {peak.hitSpec(1).match}'. Below these sections is a 'Label Font' section with 'Arial' selected and a 'Change ...' button. To the right is a 'Label Elevation' section with an 'Angle' field set to '0' and a '<degree>' label, with a note 'Only valid in Portrait Model'. At the bottom are 'OK', 'Cancel', 'Apply', and 'Help' buttons.

Comparison	Events	Colors
Frame & Axes	Scale	Peak Decoration
Peak Label		

Identified Peaks

☒ Label Identified Peaks {...}

Formula: {peak.hitSpec(1).name} - {peak.hitSpec(1).match}

Not Identified Peaks

☒ Label Not Identified Peaks {...}

Formula: {peak.hitSpec(1).name} - {peak.hitSpec(1).match}

Label Font

Arial

[Change ...](#)

Label Elevation

Angle: 0 <degree>

Only valid in Portrait Model

OK Cancel Apply Help

**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**.

Defining the System Suitability Test

Use the **SST** page (see ➤ *System Suitability Test (SST)*) of the ⇒ *QNT Editor* to check your system's performance for individual samples. The number of tests is only limited by the capacity of your computer. Each test is defined in a separate line. Use the arrow key ↓ to append additional lines to the table. This action automatically opens the SST Wizard, which guides you through all further 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 and the QNT File has not yet 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 supports you in inserting a new ➤ *System Suitability Test* (SST). To open the SST Wizard, double-click a cell or press the F8 key. Or else, select **Lines... Append Line** or **Insert Line** on the context menu or press the ↓ key. (Note: To open the SST Wizard via the ↓ key, place the cursor on the bottom line.)

The SST Wizard provides the following pages:

- **Start**
- **Sample Condition**
- **Test Condition**
- **Aggregated (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.

If no other conditions are required, you can already complete the Wizard on this page by clicking **Finish**. Clicking **Next** takes you to the following Wizard pages. Use these pages to check and edit the predefined conditions as necessary.

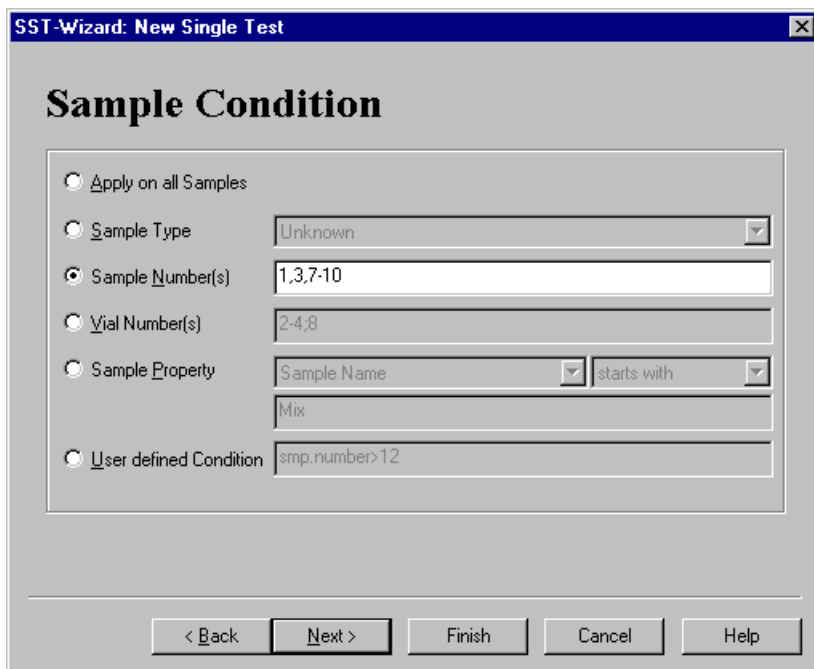
For more information, refer to:

 **SST Wizard: Sample and Test Conditions**

 **SST Wizard: Other Wizard Pages**

SST Wizard: Sample and Test Conditions

Use the **Sample Condition** page to specify for which sample(s) the test is performed. Select the desired option:



The screenshot shows a dialog box titled "SST-Wizard: New Single Test" with a "Sample Condition" section. It contains several radio buttons and input fields for specifying test conditions.

Sample Condition

- ☐ Apply on all Samples
- ☐ Sample Type: Unknown
- ☒ Sample Number(s): 1,3,7-10
- ☐ Vial Number(s): 2-4,8
- ☐ Sample Property: Sample Name starts with Mix
- ☐ User defined Condition: smp.number>12

At the bottom, there are five buttons: < Back, Next >, Finish, Cancel, and Help.

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 **Test Condition** tab page to enter the basic conditions for the single system suitability tests:

The screenshot shows a dialog box titled "SST-Wizard: New Single Test" with a "Test Condition" tab. The dialog contains the following fields and controls:

- Test Name:** A text input field containing "Peak Width (50%)".
- Test Condition:** A text input field containing "peak.width(50)". To the right of this field is a dropdown arrow and a button with three dots "...".
- Operator:** A dropdown menu showing "<=".
- Value:** A text input field containing "0.5".
- Check this box if you want to compute the test condition for more than the current sample.** A checkbox labeled "Use aggregation" is checked.
- Buttons:** At the bottom, there are five buttons: "< Back", "Next >", "Finish", "Cancel", and "Help".

First, enter a unique name in the **Test Name** input field. Specify the **Test Condition** by clicking the "..." (Browse) button. Select the desired variable from the **Edit Result Formula** dialog box. Select the **Operator** from the drop-down list box, 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
=	<i>equals</i> the entered string of characters.
< >	<i>does not equal</i> the entered string of characters.
>	<i>is larger</i> than the entered string of characters.
<	<i>is smaller</i> than the entered string of characters.
>=	<i>is larger than or equals</i> the entered string of characters.
<=	<i>is smaller than or equals</i> the entered string of characters.
contains:	<i>contains</i> the entered string of characters.
does not contain:	<i>does not contain</i> the entered string of characters.
starts with:	<i>starts with</i> the entered string of characters
does not start with:	<i>does not start with</i> the entered string of characters.
ends with:	<i>ends with</i> the entered string of characters
does not end with:	<i>does not end with</i> the entered string of characters.

**Note:**

If the operators >, <, >=, or <= apply to text variables, the lexicographical order is observed; for example, A<B.

The **Value** can be either a value or a report variable or formula. 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. (Refer to the online Help for information about the *Aggregate* tab page.)

Peak & Channel

This page is optional and appears only if the test condition requires that you enter a peak or channel. (Refer to the online Help for information about the *Peak & Channel* tab page.)

N.A. & Fail Action

Use this page to determine what the test result is if the test cannot be performed (**N.A.**). Also, determine which 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 provides the following tab pages:

- **Sample Condition**
- **Test Condition**
- **Aggregate**
- **Peak & Channel Condition**
- **N.A. & Fail Action**

These pages correspond to the individual SST Wizard pages. For more information, refer to **How to ...: Actions in the QNT Editor**  **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 the largest amount of the standard samples. You can check this using a System Suitability Test. Follow the description below:

Create a test that checks whether the current Amount is either equal or larger than the smallest Amount of all standard samples:

- If no System Suitability Test has been created yet, double-click the empty line on the SST tab page to open the SST Wizard. For an existing System Suitability Test, select the bottom line of the existing test and press the arrow down key on the keyboard. From the **Predefined Tests** list, select the **Minimum Peak Amount** test. The Wizard guides you through test creation. Clicking **Next>** takes you to the next Wizard page.
- On the **Sample Condition** page, specify for which samples the test is performed. The default setting is **Apply on all Samples**.
- You can accept the settings on the **Test Condition** page:

The screenshot shows a dialog box titled "SST-Wizard: New Single Test" with a close button in the top right corner. The main heading is "Test Condition". Below this, there are three input fields:

- Test Name:** A text box containing "Minimum Peak Amount".
- Test Condition:** A dropdown menu with "peak.amount" selected. To the right of the dropdown is a small button with three dots.
- Operator:** A dropdown menu with ">=" selected.
- Value:** A text box containing "peakTab.amount('min')". To the right of the text box is a small button with three dots.

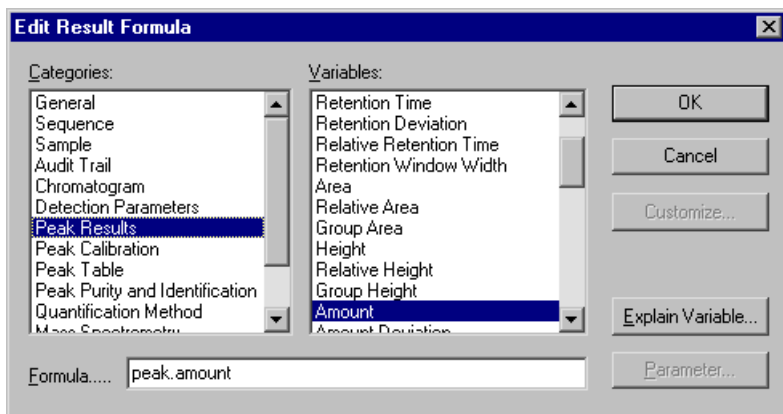
- Use the next Wizard page to determine the peak(s) and the channel for which the test is performed.
- Finally, determine what the test result is if the test cannot be performed and which action is taken if the test fails.

In the same way, create a second test that checks whether the current Amount is either equal 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. You can use a System Suitability Test to check whether the standard deviation is below a specified minimum value. Follow the description below:

- If no System Suitability Test has been created yet, double-click the empty line on the SST tab page to open the SST Wizard. For an existing System Suitability Test, select the bottom line of the existing test and press the arrow down key on the keyboard. The Wizard guides you through test creation. Clicking **Next>** takes you to the next Wizard page.
- On the **Sample Condition** page, specify for which samples the test is performed. The default setting is **Apply on all Samples**.
- On the **Test Condition** page, enter an appropriate name for the new System Suitability Test, e.g., Amount Deviation.
- Determine the **Test Condition**, e.g., **peak.amount >= 1**. (In this case, 1 is the maximum allowed deviation in percent.)
 - Click the "... " button behind the **Test Condition** input field. In the dialog box, select \Rightarrow *Peak Results* from the **Categories** list, and then select **Amount** from the **Variables** list.



- Click **OK** to confirm your selection. This returns you to the SST Wizard.
- In the **Operator** input field, select **>=**. In the **Value** input field, enter 1.

- Select the **Use aggregation** check box. Clicking **Next>** takes you to the **Aggregate** page.
- On the **Aggregate** page, select **RSD%** from the **Aggregate Function** drop-down list.
- Enter the **Maximum number of samples to aggregate**, e.g., **5**.
- 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.

The screenshot shows a window titled "SST-Wizard: New Single Test" with a sub-header "Aggregate". The window contains the following fields and controls:

- Test Condition:** peak.amount
- Aggregate Function:** A dropdown menu with "RSD %" selected.
- Maximum number of samples to aggregate:** A numeric input field with "5" entered, followed by the text "(including current sample)".
- Only aggregate samples with:** A section containing two dropdown menus separated by an equals sign. The first dropdown has "Replicate ID" selected, and the second dropdown has "smp.replicate" entered in its text field.

- Use the next Wizard page to determine the peak(s) and the channel for which the test is performed.
- Finally, determine what the test result is if the test cannot be performed and which 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 labeling 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:

<u>Variable</u>	<u>Description</u>
Number	
Name	
Sample Condition	
Test Condition	
Aggregate	
Operator	
Value	
Channel	
Peak	
N.A.	User-defined test result if the test is not performed
Fail Action	
Aggregated Samples	
Sample Condition Results	
Test Results	
Aggregated Sample List	
Aggregated Sample Result List	
Result of Test Condition or Aggregate	
Result of Compare Value	

The **Formula** field indicates your selection in short; for example, **sst.test_condition** for the test condition.

Processing Mass Traces

➤ *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 is finally 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 \Rightarrow QNT Editor, use the **MS** tab page to specify how mass spectra are formed from individual spectra. Select the **Enable Background Subtraction** check box to enable spectrum subtraction/bunching:

The screenshot shows the 'MS' tab in the QNT Editor. At the top, there is a checkbox labeled 'Enable Background Subtraction' which is checked. To its right is a 'Peak Spectrum Bunch' section with a 'Width' field set to '3' and the unit 'spectra'. An 'Apply' button is to the right of this section. Below these is the 'Background Subtraction' section, which has two radio buttons: 'Peak Dependent Background Subtraction' (selected) and 'Fixed Background Subtraction Ranges'. Under 'Peak Dependent Background Subtraction', there are three sub-sections: 'Left Region Bunch' with a 'Width' of '1' spectra, a central 'MS' plot showing a peak with a baseline, and 'Right Region Bunch' with a 'Width' of '1' spectra. Under 'Fixed Background Subtraction Ranges', there are two range boxes: 'Range 1' with a 'Width' of '0.681 ... 0.879 min' and 'Range 2' with a 'Width' of '12.303 ... 12.798 min'. At the bottom of the window, there is a navigation bar with tabs for 'Spectra Library Screening', 'SST', 'MS' (selected), and 'UV'.

In the combo box under **Peak Spectrum Bunch**, enter the number of single spectra that shall 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 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 mass spectrum. When the **Show Spectra** view is enabled, the newly calculated mass spectrum is displayed at once.



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.*

Processing UV Channels

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 then determined via linear interpolation of these two background spectra and is finally 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. The **Enable Background Subtraction** check box is enabled indicating that spectrum subtraction is active:

The screenshot shows the 'UV' tab of the QNT Editor. At the top, there is a checkbox labeled 'Enable Background Subtraction' which is checked. To its right is a 'Peak Spectrum Bunch' section with a 'Width' field set to '1' and the unit 'spectra'. An 'Apply' button is located to the right of this section. Below these is a 'Background Subtraction' section. It contains two radio buttons: 'Peak Dependent Background Subtraction' (which is selected) and 'Fixed Background Subtraction Ranges'. Under 'Peak Dependent Background Subtraction', there are two sub-sections: 'Left Region Bunch' and 'Right Region Bunch', each with a 'Width' field set to '1' and the unit 'spectra'. A small graph icon labeled 'UV' is positioned between these two sub-sections. Under 'Fixed Background Subtraction Ranges', there are two range selection boxes: 'Range 1' with a 'Width' field showing '2.519 ... 3.103 min' and 'Range 2' with a 'Width' field showing '12.770 ... 13.131 min'. At the bottom of the window, a tab bar shows 'Spectra Library Screening', 'SST', 'MS', and 'UV', with 'UV' being the active tab.

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 that shall 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.*

Actions in the Chromatogram

The window shows the integrated chromatogram of a sample. The peak areas are determined and integrated automatically, based on the \Rightarrow *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 Hotkeys in the Chromatogram



Manual Re-Integration



Manual Peak Assignment



Comparing Chromatograms



Performing Data Smoothing

Due to the increased noise, we recommend 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).

You can also generate additional channels after data acquisition, see:





Copying a Channel




Combining Channels via Arithmetic Operations

Also, refer to  **Displaying 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 \Rightarrow *Detection Parameters*, for example, because too small or unimportant peaks 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 \Rightarrow QNT-Editor on the **Detection** tab page (see **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**).

For more information about how to display a \triangleright Report or a spectra plot, refer to **How to ...:**

 **Actions in UV Spectra and/or Mass Spectra**

 **Actions in the Report Table**


Using Hotkeys in the Chromatogram

Often, it may be necessary to display specified chromatogram areas only. In this case, use the mouse to zoom out this area. In addition, so-called hotkeys are provided for user-friendly operation. These keys refer to the number and navigation area on the keyboard.

Scrolling

$\langle \leftarrow \rangle$ = $\langle \text{LEFT} \rangle$ scrolls all chromatograms to the right; $\langle \rightarrow \rangle$ = $\langle \text{RIGHT} \rangle$ scrolls them to the left. If you press the respective key, the chromatogram is scrolled by 5% of the window width.

$\langle \uparrow \rangle$ = $\langle \text{UP} \rangle$ ($\langle \downarrow \rangle$ = $\langle \text{DOWN} \rangle$) 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.

$\langle \text{HOME} \rangle$ ($\langle \text{END} \rangle$) brings you to the first (last) data point of the active chromatogram. The entry is ignored if the chromatogram is already displayed in full-size mode.

Zoom/UnZoom

Press the $\langle x \rangle$ (Multiply) key to zoom in horizontally by 20%. The anchor point is the center of the time axis; for example, 0.0 to 10.0 min \triangleright 1.0 to 9.0 min.

Press the <÷> (Divide) key to unzoom horizontally by 25%. The anchor point is the center of the time axis.

Press the <CTRL><x> or <CTRL><÷> key to display all chromatograms in full size. This action corresponds to an Autoscale of the time axis.

Press the <+> (Add) key 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 <-> (Subtract) 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 the <CTRL><+> or <CTRL><-> keys to autoscale the active chromatogram.

Press the <NUM5> key 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 the <CTRL><←> keys to select the previous peak of the active chromatogram. If no peak is selected, the first peak of the chromatogram will be selected.

Press the <CTRL><→> keys to select the next peak of the active chromatogram. If no peak is selected, the first peak of the chromatogram will be selected.

Press the <CTRL><POS1> keys to select the first peak of the active chromatogram.

Press the <CTRL><Home> keys 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 hotkeys are available for chromatogram ➤ *Overlay*:

Press the <Page Up ↑> or <CTRL><↑> keys to activate the next upper chromatogram; that is, the chromatogram whose caption is found above the active chromatogram.

Press the <Page Down ↓> or <CTRL><↓> keys to activate the next lower chromatogram; that is, the chromatogram whose caption is found below the active chromatogram.

Chromeleon provides many additional hotkeys and shortcuts. For a summary of the most important keys, refer to the table in **Basic Operation**



Keyboard Shortcuts.

Manual Re-Integration

All actions that can be performed directly on the chromatogram via the different icons on the Integration ➤ *Toolbar* are referred to as manual re-integration. They only affect 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, we recommend zooming the area in which you want to make the changes. Select **Automatic** on the context menu. All places in the chromatogram that can be changed manually will then be marked by the corresponding mouse cursor. The shape of the mouse cursor 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 cursor changes its appearance (if the **Automatic Tool** or the **Delimiter Tool** are selected). The  cursor indicates the peak start and the  cursor indicates the peak end.

Left-click and move the mouse cursor to the new peak start or peak end.

It is not possible to cross another peak delimiter. This peak delimiter, too, will then be moved in the same direction. After positioning, Chromeleon draws a new baseline. The modified peak properties (Area, Width, Amount, etc.) are immediately updated and displayed in the integration report.

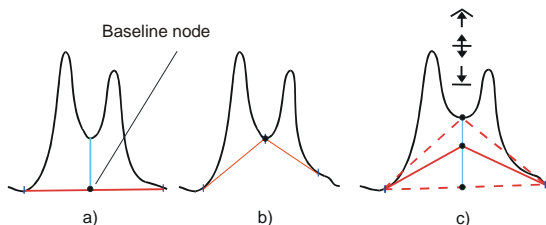


Note:

You can also move the peak delimiters of negative peaks.

Modifying the Baseline Manually

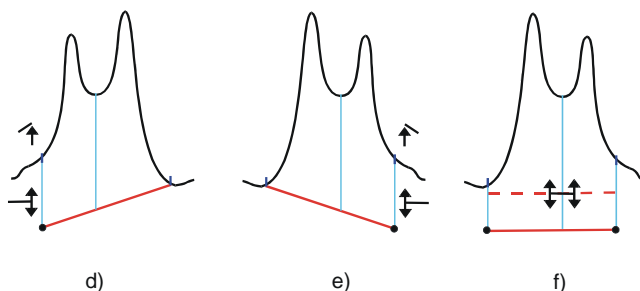
To individually integrate two peaks that are not completely separated in the chromatogram, drop a perpendicular line from the minimum between the two peaks to the baseline. The intersection with the baseline is referred to as "baseline node" (a).



To draw the baseline from peak end to peak end, enable the \Rightarrow *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 the desired position ("freely floating baseline node" (c)). If you approach state (a) or (b), the mouse cursor automatically clicks into place. The shape of the mouse cursor (\uparrow , \downarrow) 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 the mouse cursor is drawn, 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 cursor (\uparrow , \downarrow) indicates whether you can perform this action.

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.

If a small peak (\wedge) appears on the right side of the mouse cursor, you can insert a new peak at this position. If a warning sign (\otimes) appears instead, you cannot insert a new peak.


- Select **Insert Peak Tool** on the context menu to have the mouse cursor 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, place the mouse cursor on the peak.

Select **Delete** on the context menu to remove the peak's delimiter and its baseline.

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 cursor 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 cursor 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 cursor 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.

We recommend using the \Rightarrow **Peak Shoulder Threshold** to define shoulder shaping for the entire quantification method. Use the \Rightarrow **Rider Threshold** and \Rightarrow **Maximum Rider Ratio** detection parameters to define the peaks that will be detected as rider peaks. For more information, refer to **How to**

Actions in the QNT Editor  **Defining Rider Peaks.**

Changing the Peak Type

To change the peak type, position the mouse cursor on 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 \Rightarrow Rider Peak and vice versa. Single peaks are always interpreted as main peaks. That is why you cannot convert them into rider peaks. The context menu indicates: **Can't Change Peak Type**.

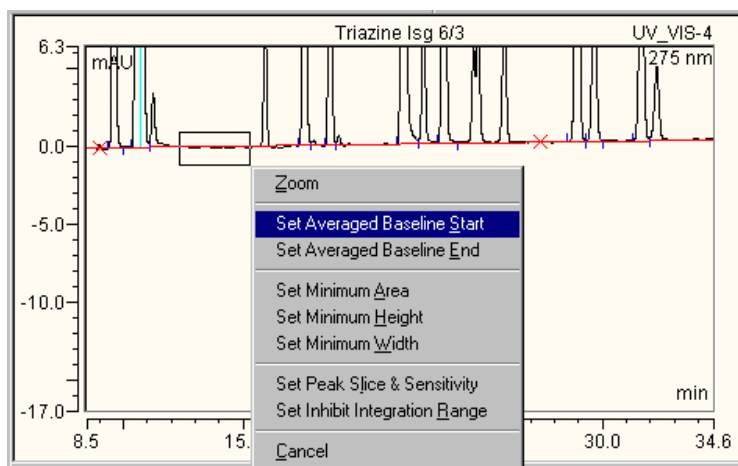
With automatic classification, the \Rightarrow Rider Threshold and \Rightarrow Maximum Rider Ratio parameters determine whether a peak is classified as a main peak or a rider.

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 \Rightarrow 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 will not be 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 will be 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 ...: Actions in the Chromatogram**  **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 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).

Properties of Peak No. 4

Component: Anthracene

Retention: ☒ Naphthalene +2.650 min
☒ Phenanthrene +0.295 min
☐ Tetracene [+0.000 min]

Width: ☒ Anthracene -0.28 %
☒ Fluoranthene -0.774 min
☒ Pyrene -1.167 min

Peak Type: ☒ Dimethyl-Fluoranthene -1.796 min
☒ Chrysene -2.317 min

Height:
Area:
Amount:

- 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.

Properties of Peak No. 5

Component: Phenol

Retention: 8.73 min





Width: 0.24 min

Peak Type: BMB^

Height: 1715.62 mAU

Area: 282.37 mAU*min

Amount: 49.268

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 (provided that 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



Selecting the Samples and Channels

Different alternatives are available to select samples and channels that are to be displayed as ➤ *Overlays*:

- Display the chromatogram of a sample in an integration window by opening the sample, for example, by double-clicking its name in the ➤ *Browser*.

Select one or several samples of one ➤ *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 cursor. When you release the mouse button, the chromatogram of the sample(s) is displayed. The same channel is used as for the reference sample.

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 **All Channels** on the context menu to compare all channels of a sample in a separate window. Press the F4 key or the Shift+F4 key combination 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 the F4 key or the Shift+F4 key combination to browse through the selected samples.



Or:

- Click the **Next/Previous Chromatogram** icons ( ) while pressing the Ctrl key to insert the chromatogram of the next or previous sample.

Or:

- Perform a **Query** over several sequences or **Datasources**. Select the **Compare** command 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:

- Click the **Next Channel** icon () while holding down the Ctrl key to insert the next channel of the same sample.
- Click the **Previous Channel** icon () while holding down the Ctrl key to insert the previous channel of the same sample.

Displaying Several Chromatograms

After adding an **Overlay** (= an additional chromatogram), you can define the display arrangement for the two chromatograms.

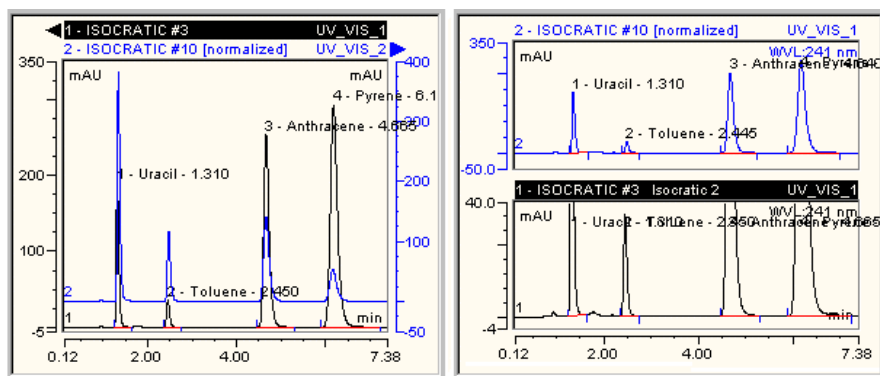
How To

- 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.

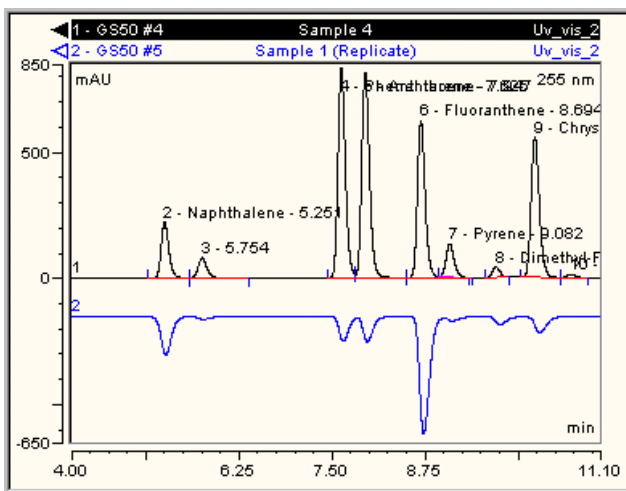
- Select the **Time** check box to move the active chromatogram in the x-direction.
- Select the **Signal** check box to move the active chromatogram in the y-direction.

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.

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 in the **Arrangement** section.
- You can mirror the chromatogram that was added first by selecting the **Mirror chromatogram** check box in the **Overlay** section.



If you have also selected the **Overlay with right signal axis** check box, this setting will be 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.

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 desired 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.

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 define data smoothing also in the ➤ *Post-Acquisition Steps* view of the PGM Editor (see **Control**  **The PGM Editor**). Please observe the corresponding hints below.

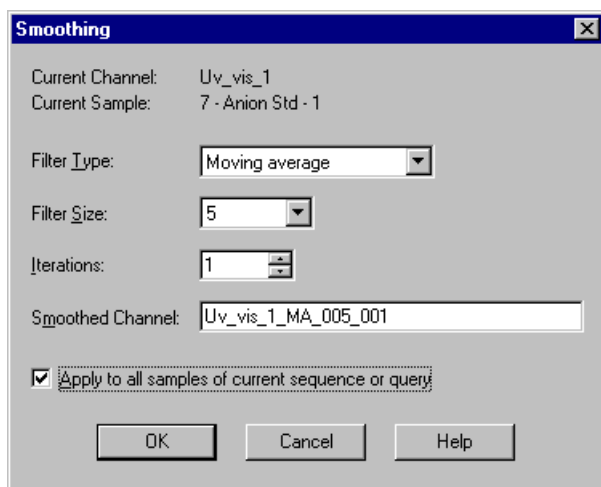
How To

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 data smoothing and select the **Post-acquisition steps** view, instead.*

2. Select **Smoothing** on the context menu. The **Smoothing** dialog box appears:




The Smoothing dialog box is shown with the following fields and controls:

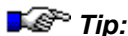
- Current Channel:** Uv_vis_1
- Current Sample:** 7 - Anion Std - 1
- Filter Type:** Moving average (dropdown menu)
- Filter Size:** 5 (dropdown menu)
- Iterations:** 1 (spin box)
- Smoothed Channel:** Uv_vis_1_MA_005_001 (text field)
- ☒ **Apply to all samples of current sequence or query**
- Buttons:** OK, Cancel, Help

 **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 **Smooth data** to open the **Smoothing** dialog box.*


3. Select the **Filter Type**, **Filter Size**, and **Iterations**. For more information, refer to  **Data Smoothing**.
4. In the **Smoothed Channel** box, enter the 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, and 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 of 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 ...: Actions Related to Mass Spectrometers**  **Extracting Mass Traces Afterward.**

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 contaminations 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**). 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 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:**

*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 **Copy Channel** to open the **Copy Channel** dialog box.*

3. In the **New Channel** box, enter the name for the new channel. The suggested name is the name of the current channel plus **_COPY**. However, you are free to enter any name of your choice as well.
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 of 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 desired 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:**

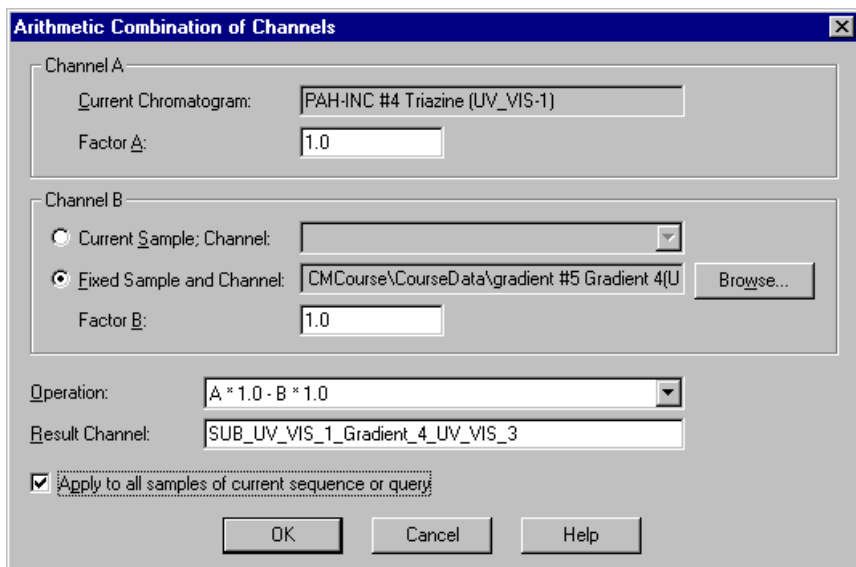
*You may also define the combination of two channels in the **Post-Acquisition Steps** view of the PGM Editor (see **Control**  **The PGM Editor**). Please observe the corresponding hints below.*

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.



The dialog box titled "Arithmetic Combination of Channels" contains the following elements:

- Channel A:**
 - Current Chromatogram: PAH-INC #4 Triazine (UV_VIS-1)
 - Factor A: 1.0
- Channel B:**
 - ☐ Current Sample; Channel: [dropdown]
 - ☒ Fixed Sample and Channel: CMCourse\CourseData\gradient #5 Gradient 4(U) [Browse...]
 - Factor B: 1.0
- Operation:** A * 1.0 - B * 1.0 [dropdown]
- Result Channel:** SUB_UV_VIS_1_Gradient_4_UV_VIS_3
- ☒ Apply to all samples of current sequence or query
- Buttons: OK, Cancel, Help

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 shall be multiplied.
5. Select the desired **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*, define the number of mass spectra or UV spectra to be used for the two ranges. (For more information, refer to **How to ...: Actions in the QNT Editor**



Processing Mass Traces and/or



Processing UV Channels.)

Fixed Background Subtraction for the Entire Chromatogram

At the beginning of the chromatogram, right-click and select the baseline range for which the mass spectra (UV spectra) will be subtracted. A context menu will appear. Select **Set Background Subtraction Range 1** on the context menu 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.*

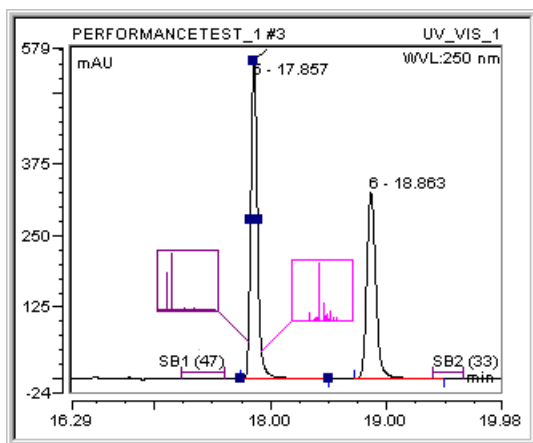
In the same way, select a baseline range at the end of the chromatogram. Select **Set Background Subtraction Range 2** on the context menu to define this range as the second range for which mass spectra (UV spectra) will be subtracted from the mass spectra (UV spectra) of the single peaks.



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, we recommend 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

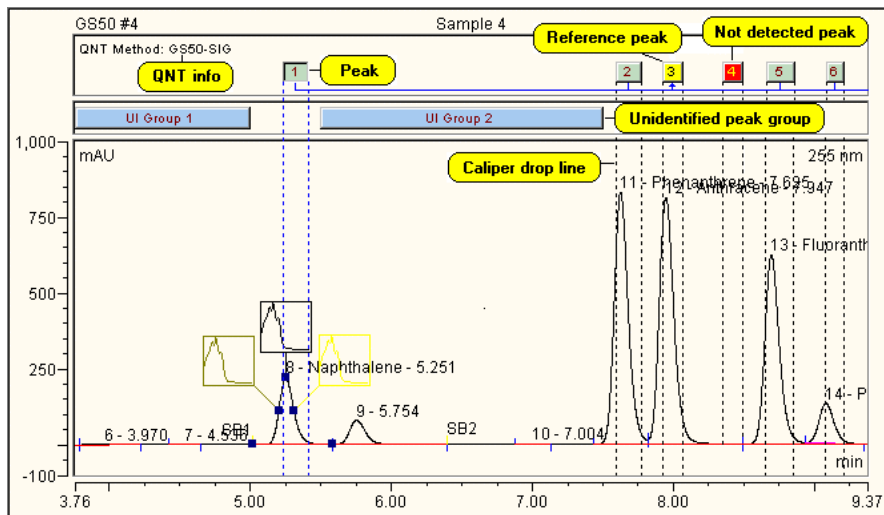
Chromeleon supports displaying the peak information in a separate area above the actual chromatogram:


- 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 \Rightarrow *Window* of the associated substance: **Show all caliper drop lines**.


The chromatogram looks as follows:




In addition, you 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 \Rightarrow Retention Time and the time \Rightarrow 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, place the cursor on the center of the associated peak caliper. The cursor 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, place the cursor on the edge of the caliper or on the associated dotted line. The cursor 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 the cursor is placed on the edge of a caliper or on a dotted line.

The cursor changes its appearance and looks as follows . 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 \Rightarrow *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 \triangleright *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 ...: Actions in the QNT Editor**  **Inhibiting Peak Integration**).

Insert Valley to Valley On or **Off** to enable or disable integration from valley to valley.

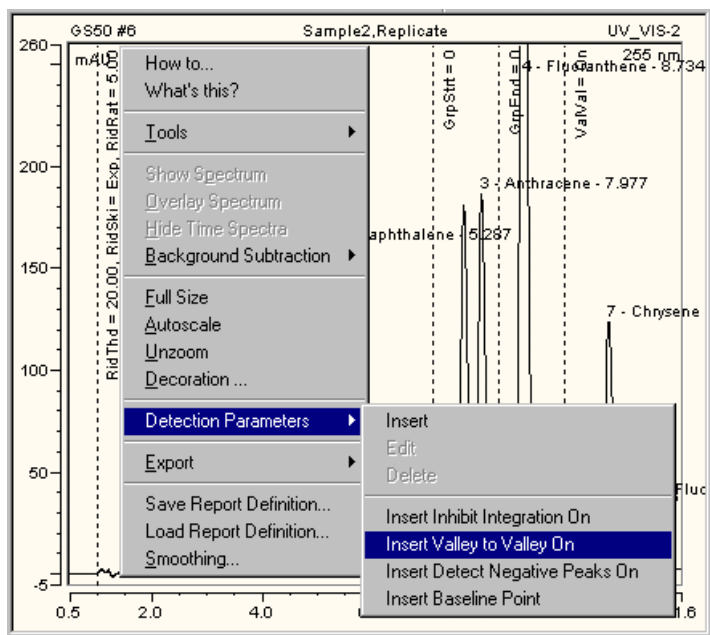
Insert Detect Negative Peaks On or **Off** to enable or disable integration of negative peaks.

Insert Baseline Point to insert a new baseline point (for more information about the last three commands, refer to **How to ...: Actions in the QNT Editor**  **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 on the next page corresponds to the table input in **How to ...: Actions in the QNT Editor**  **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 graphically, you also select a chromatogram area while holding the right mouse key. The context menu is opened automatically; the menu contains the following commands:

- **Set Averaged Baseline Start** and **Set Averaged Baseline Start End** to set an averaged baseline (see **How to ...: Actions in the Chromatogram** **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 ...: Actions in the QNT Editor** **Reducing the Number of Evaluated Peaks**).

- **Set Peak Slice & Sensitivity** to define the peak recognition algorithm (see **How to ...: Actions in the QNT Editor**  **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** sheet of the \Rightarrow **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 ...: Actions in the QNT Editor**  **Defining Detection Parameters**).

Actions in UV Spectra and/or Mass Spectra

To enable the **Spectra** plot, select **Show Spectra** on the **View** menu or click



The Spectra plot represents UV spectra and their identification by means of a spectra library.

The **Mass Spectra** plot is very similar to the **Spectra** plot. To enable the Mass Spectra plot, select **Show Mass Spectra** or click



For more information, refer to:



Displaying a Peak Spectrum (UV or MS)



Displaying and Overlaying Single (UV or MS) Spectra



Match Factor, Difference Spectra, 1st/2nd Derivatives of UV Spectra



Starting a UV Spectra Search

Also, refer to **How to ...: Actions Related to Mass Spectrometers**



Extracting Mass Traces Afterward.



Tip:

In order to represent spectra, make sure that the corresponding raw data is available. Obtain the raw data by recording a 3D field using a ➤Photodiode Array Detector.



Displaying a Peak Spectrum (UV or MS)


- In the neighboring chromatogram window, select the peak of which you want to display the UV or ➤*Mass Spectrum*.

The spectrum of the peak is visible. (Note: If the corresponding raw data is not available, an error message is displayed.)

- Select **Decorations..** on the context menu of the (Mass) Spectra window and determine the peak height at which to extract and display spectra via **Peak Spectra**.

Depending on the settings, up to five spectra of the same peak are 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 a criterion for peak purity.

Displaying and Overlaying Single (UV or MS) Spectra

In addition to peak spectra (= UV or  *Mass 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 Tool** on the context menu or click the corresponding icon on the **Integration** toolbar.



- The changed mode is indicated by a spectra symbol that is added to the mouse cursor.
- 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 objectively compare spectra of different samples, it is necessary to perform a chromatogram comparison.

- Compare two chromatograms by displaying an additional chromatogram in the Integration window (see **Integration**  **Chromatogram Comparison**).
- Enable the **Spectra 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. 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.

Match Factor, Difference Spectra, 1st/2nd Derivatives of UV 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 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 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 tool to extract the single UV spectra from the chromatogram, the spectrum that is first 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.

Starting a UV Spectra Search

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 cursor is positioned in a spectra window, select **Library Search ...** on the context menu to start the spectra search.

In the edit box, specify the library that should 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 \Rightarrow *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 will be 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 **1st 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 lose significance.
- Select **Hit Threshold** and enter a value between 0 and 1000, for example, 950. Only the spectra with a match above 950 will be displayed. Spectra with a lower match value will not be 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.



Actions in the Calibration Curve

The **Calibration Curve** displays the ➤ *Calibration Curve* calculated from the ➤ *Calibration Points*. 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) in the **Calibration** settings made in the QNT Editor, the corresponding calibration point is red. Calibration points of this type will not be used for calculating the calibration curve for which they were disabled. 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. (Also, refer to **How to ...: Actions in the QNT Editor**  **Disabling Calibration Samples.**)

Select **Decoration** on the context menu to perform the following actions on the different tab pages:

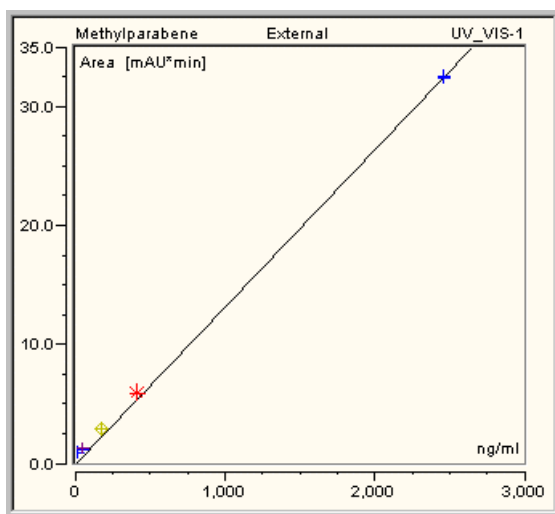
- Select the peak for which to show the calibration curve (**Peaks** tab page).
- Determine the axis scale (**Scale** tab page).
- Define the display and decoration of the axes, frames, and grids (**Frame & Axes** tab).
- Show validation samples (select **Draw validation sample points** on the **Options** 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.

■ 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*.) 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. The corresponding calibration point is then marked by a red asterisk: ✱. 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.



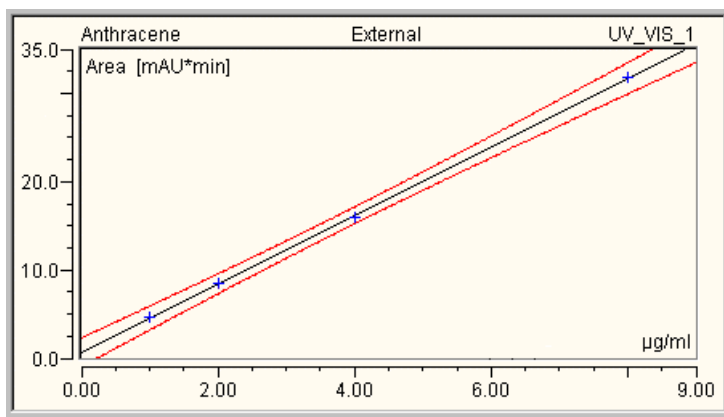
Indicating the Confidence Interval

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 ...: Actions in the Report Table**  **Setting Parameters for Variables (e.g., for the confidence interval).**

Actions in the Report Table

Chromeleon provides numerous options to work with reports. It is possible to use existing default reports or create special ➤ *Reports* to meet individual requirements. For more information, refer to:

-  **Displaying a Report**
-  **Defining the Contents of a Report**
-  **Defining the Appearance of a Report**
-  **Adjusting the Line Height Automatically**
-  **Saving a Report**
-  **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 each of the six method windows (Integration, PPA, QNT Editor, Spectra, Calibration Curve, and Spectra Calibration).

- Select **Report** on the **View** menu.

If you have not generated a report before, the default report (called **Default**) will be opened. 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 \Rightarrow *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; no additional input is necessary.

Defining the Contents of a Report

To change the *contents* of a \Rightarrow *Report Definition File*, select **Table** on the context menu or double-click a table column.

- 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:

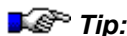
*A new column that has been created using the **Add Column** command has no format yet.*

- 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** to modify the column properties. Thus, the current column contents, for example, number of theoretical plates, can be replaced by a different variable.
- To change the formula of a cell, enable the **Layout Mode** on the **Table** 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 \triangleright *Additional Functions* of the \triangleright *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 ...: Actions in the Report Table**



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.

Adjusting the Line Height Automatically

Chromeleon allows you to adjust the line height automatically. 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 line marked in blue separating lines 6 and 7. This automatically adjusts the height of line 6 so that the entry in the **List of Aggr. Smp.** column is completely displayed.)

B8	Dimethyl-Fluoranthene			
	A	B	C	D
1	No.	Peakname	List of Aggr. Smp.	Test Result
2				
3	1	Naphthalene	#1: "Sample 1" #2: "Sample 2"	Passed
4	2	Phenanthrene	#1: "Sample 1" #2: "Sample 2"	Passed
5	3	Anthracene	#1: "Sample 1" #2: "Sample 2"	Passed
6	4	Fluoranthene	#1: "Sample 1" #2: "Sample 2"	Passed
7	5	Pyrene	#1: "Sample 1" #2: "Sample 2"	Passed
8	6	Dimethyl-Fluor	#1: "Sample 1" #2: "Sample 2"	Passed
9	7	Chrysene	#1: "Sample 1" #2: "Sample 2"	Passed

In the example in the picture, the heights of lines 3 to 5 are adjusted automatically. Thus, the list of aggregated samples is completely displayed in each line. The height of lines 6 to 9 is defined by a fixed value. In this case, the lists are not completely displayed.

Saving a New Report Definition File

You can save all modifications made in a report to a \Rightarrow **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 \Rightarrow *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 \Rightarrow *Report Categories*. You can copy the syntax by selecting the required formula and pressing the CTRL+C key combination. 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 \Rightarrow *Amount*, use the following expression:

peak.height / peak.amount

- You can also use the following operators to link expressions: **+**, **-**, **x**, and **^** (for powers)
- Enter the desired column header in the **Header** field.
- Click **Customize** to include newly defined variables in the report variables list.
- 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 \Rightarrow *Printer Layout* together with the \Rightarrow *Report Publisher*!*

Calculating the Peak Variable "Amount"

If you wish to check the indicated \Rightarrow 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:

$$f(y) = \frac{1}{c_1} y$$

Linear:

$$f(y) = -\frac{c_0}{c_1} + \frac{1}{c_1} y$$

Linear with offset:

$$f(y) = \frac{1}{2 * c_2} * \left(-c_1 \pm \sqrt{c_1^2 + 4 * c_2 * y} \right)$$

Quadratic:

$$f(y) = \frac{1}{2 * c_2} * \left(-c_1 \pm \sqrt{c_1^2 - 4 * c_2 * (c_0 - y)} \right)$$

Quadratic with offset:

$$f(y) = \left(\frac{1}{c_0} \right)^{\left(\frac{1}{c_1} \right)} * y^{\left(\frac{1}{c_1} \right)}$$

Exponential:



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.

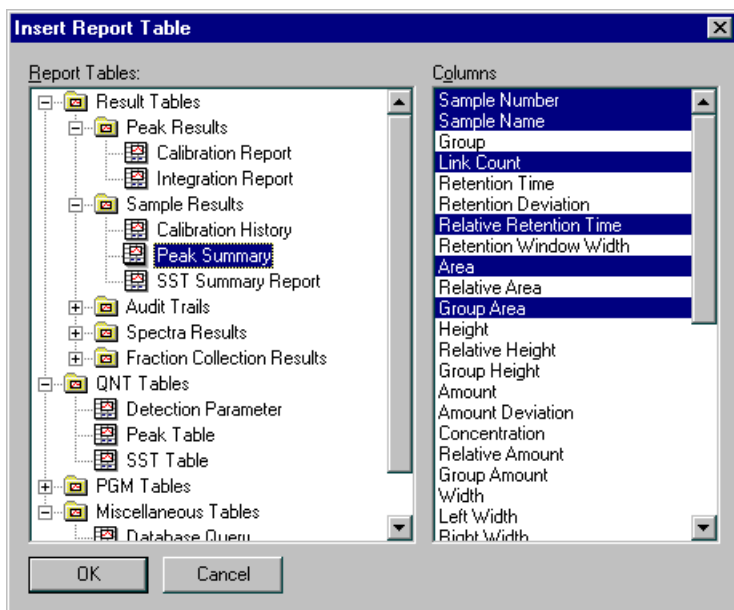
If it is not possible to calculate the expression (c1=0 for linear with or without offset, the radicant < 0 for quadratic with or without offset, or c0=0, or c2=0 for exponential), "n.a." is returned as amount.

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 \Rightarrow *Report Definition File* 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 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 \Rightarrow *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 ➤ <i>Mass Spectrometer</i> .
MS Status Log Report	Displays the mass spectrometer settings.
MS Tune Data Report	Displays the tune data of the ➤ <i>Xcalibur</i> raw data file.
MS Instr. Method Report (XRaw)	Displays the MS method.

Spectra Results

MS Raw Report	Displays the raw data of the current ➤ <i>Mass Spectrum</i> .
----------------------	---

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 ⇒ <i>Detection Parameters</i> 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 ➤ <i>System Suitability Test</i> .

PGM Tables

Commands	Displays the ➤ <i>Program</i> for the current sample.
Post Acq. Processing	Displays the ➤ <i>Post-Acquisition Steps</i> for the current sample.
MS Method	Displays information about the current method of the ➤ <i>Mass Spectrometer</i> .

Miscellaneous Tables

Database Query	Inserts any kind of database queries into the report.
History Report	Displays all variables required for creating a ➤ <i>History</i> 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 desired 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 **Reports**  **The Peak Summary Report**) to show certain results from all samples.

The required steps are described in **How to ...: Actions in the Report Table**:



Displaying a Report and



Adding and/or Renaming a Worksheet.

In contrast to the **Integration** and **Calibration** worksheets of the ⇒*Report Definition File* that list the data of the peaks from *one* sample, the **Summary** worksheet represents, e.g., data of a specific peak from *all* samples (generally from a ⇒*Sequence*).

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. 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 command shows the command itself, the text of a message, or an event.

To make further settings, select the **Table Properties** command on the context menu. The **Audit Trail Properties** dialog box is opened; define which the data shall be displayed.

In addition, you can select one of the following **Display** options:

- **Run only** to display only the entries for the sample run
- **Preconditions only** to display only the conditions before a sample run
- **Preconditions and Run** to display all entries

The display filter (**Filter Level**) defines the type and extent of the Audit Trail entries for a sample run.

Normal: Only the most important commands and properties are displayed.

Advanced: Normal and Advanced level commands and properties are displayed.

Expert: Normal, Advanced, and Expert level commands and properties are displayed (for experts only).

Errors and Warnings: Only error messages and warnings but no commands are displayed.

You can add the contents of the worksheet to other documents, using the **Cut** and **Paste** commands. It is also possible to print the entire audit trail in the ➤ *Printer Layout*.

Using Audit Trail Variables in Other Worksheets

Certain events (such as performing a \Rightarrow *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 is opened 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.


However, you can also search for the next associated entry in the Audit Trail. Starting point of the search is the entered retention time. Select **backward** as **Search Direction** to find the previous entry before the retention time. Select **forward** to find the next entry after the entered retention time.

Creating a History Report

The ➤*History* report is not part of a default report. Therefore, select **Insert Report** to add a history worksheet to a ⇒*Report Definition File* (see **How to ...: Actions in the Report Table**  **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 where you can change the settings:

- On the **History Objects** tab page, select the object for which 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 shall be 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 **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, thus, simplifying layout definition.

Displaying MS Reports

Similar to the ➤ *History* report, the different MS reports are not part of the default ⇒ *Report Definition Files*. Select **Insert Report** to add them as separate worksheets to a report definition file (see **How to ...: Actions in the Report Table**  **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 following message appears in the report: *"No MS Raw Data found"*.
- The **MS Status Log Report** shows the mass spectrometer settings. If there is no MS data, the following message appears in the report: *"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 following message appears in the report: *"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 dialog.

All MS reports comprise only the default columns. It is not possible to add more columns.

Selecting Other Special Reports

As with the ➤ *History* report, the following reports are not part of the default ⇒ *Report Definition Files* either. Select **Insert Report** to add them as separate worksheets to a report definition file (see **How to ...: Actions in the Report Table**  **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

- Select the **Database Query** report to integrate any kind of database query in the report. Select **Table Properties** on the context menu to open the table properties and specify the datasource in which the query shall be performed. Use **SQL** statements to define the properties for which the query shall 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.

Properties Report Column

Categories:

- Sequence
- Sample
- Audit Trail
- Chromatogram
- Detection Parameters
- Peak Results
- Peak Calibration**
- Peak Table
- Peak Purity and Identification
- Quantification Method
- Mass spectrometry
- Integration Table

Variables:

- Relative Standard Deviation
- Correlation Coefficient
- Coeff. of Determination
- DOF-Adj. Coeff. of Determination
- Calibration Point X
- Calibration Point Y
- Calibration Point Weight
- Evaluation of Cal.Function for X
- Residual for Cal.Point X
- Upper Confidence Limit**
- Lower Confidence Limit

Formula..... peak.confUpperLimit("Average", "95 %")

Header..... "Upper Confidence Limit "

Dimension..... peakTab.amount_dimension

Format..... 0.0000

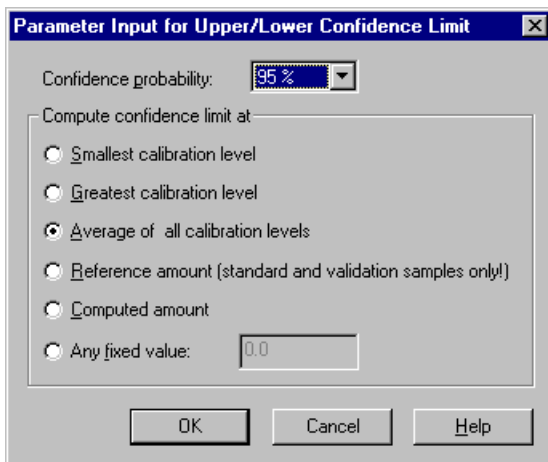
Channel

☒ Selected Channel

☐ Fixed Channel(s): UV_VIS_1

Buttons: Ok, Cancel, Customize..., Explain Variable..., Parameter...

Click the **Parameter** button 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 will be 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%.

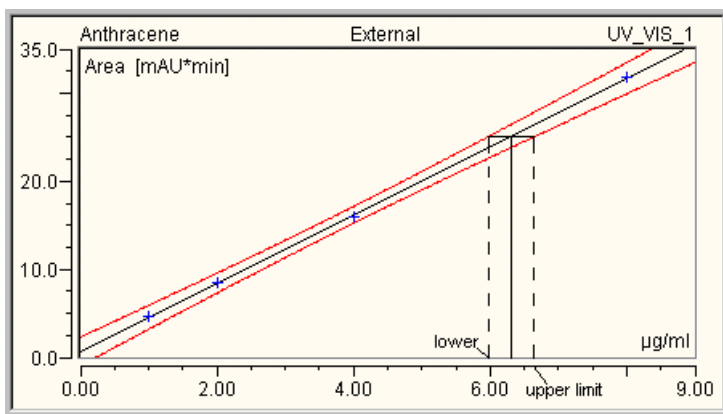
Compute confidence limit at

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 are given 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 ...: Actions in the Calibration Curve**  **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.)

Actions in the Printer Layout

Use the Printer Layout to prepare your data for the printout. It is saved in the \Rightarrow *Report Definition File* (= RDF). To assist you in preparing the first printout and in creating 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 printout more data that is not included in the default report definition files, Chromeleon provides the possibility to create individual worksheets via the \triangleright *Printer Layout* window. These worksheets can be combined and 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/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 a Sample Batch**
-  **Setting Print Area and/or Print Title(s)**
-  **Automatically Repeating the Output for the Selected Objects**
-  **Saving the Contents of a Printer Layout Page**

Users who have the \triangleright *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)**
-  **Creating Dynamic Columns That Contain Flexible Formulas**
-  **Creating Dynamic Links to Lines Other than the Current Line**

In addition, you can use the Printer Layout to create charts (see  **Creating Charts**).

For assistance to change the page format and to create 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 the desired sample and then change to the Printer Layout:

- Select and double-click the desired 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 the desired 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 is inserted before the open sheet.

For information about how to edit existing worksheets, refer to:

 **Inserting a Chromatogram**

 **Inserting a Trend Plot**


 **Inserting and Editing a Table**

 **Inserting a Column into an Existing Table**


 **Inserting and Deleting Individual Variables**

 **Inserting Text**

Inserting a Chromatogram

- Enable  *Layout Mode* on the **Edit** menu.
- Select **Insert > Chromatogram** on the **Edit** menu or context menu. The mouse cursor changes to 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 key or the Shift+F4 key combination to display the chromatogram of the next or previous sample and to check whether the selected settings are appropriate for all chromatograms.

Inserting a Trend Plot

- Open the Printer Layout (see  **Opening and Editing the Printer Layout**).
- Enable ➤ *Layout Mode* on the **Edit** menu.
- Select the worksheet to which you want to insert the trend plot, or add a new worksheet (select **Insert Sheet...** on the **Edit** menu).
- Select **Insert > Trend Plot** on the **Edit** menu or context menu. The mouse cursor changes to a + sign.
- Draw a rectangular frame of any size. The frame reserves this area for the trend plot.
- To indicate that the ➤ *Trend Plot* is a Chromeleon object all four corners of the plot are marked by a red triangle.
- To select the complete area, click inside the frame.
- You can now move the frame and reduce or enlarge it in size, as in Windows.
- Select **Trend Plot Properties** on the **Edit** menu or context menu. Define the data and format of the trend plot as desired on the corresponding tab pages of the **Trend Decoration** dialog box (**Statistics**, **Data Labels**, **Data**, **Caption**, **Frame & Axes**, etc.).

Inserting and Editing a Table

- 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 already 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, also see  **Inserting a Column into an Existing Table**.

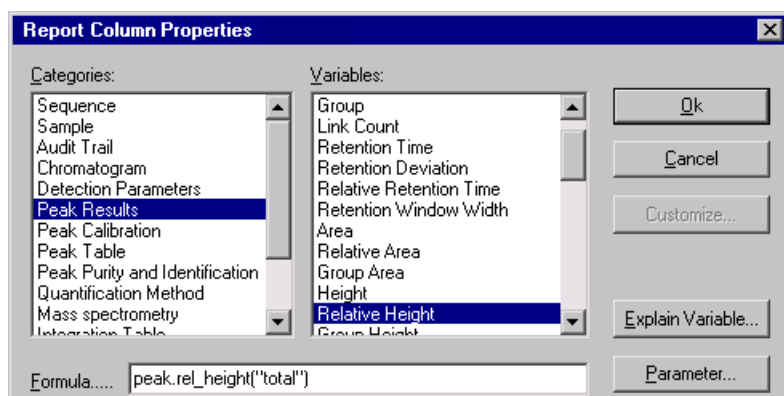
 **Inserting a Column into an Existing Table**

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	Peak Name	Height	Area	Rel.Area	Amount	Type
	min		mAU	mAU*min	%		
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 the desired 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**.

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 the desired 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. Follow the steps below:

- 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 edit line whenever the cell is selected, for example, *peak.height*. If the selected cell contains only text, the edit 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*) 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.

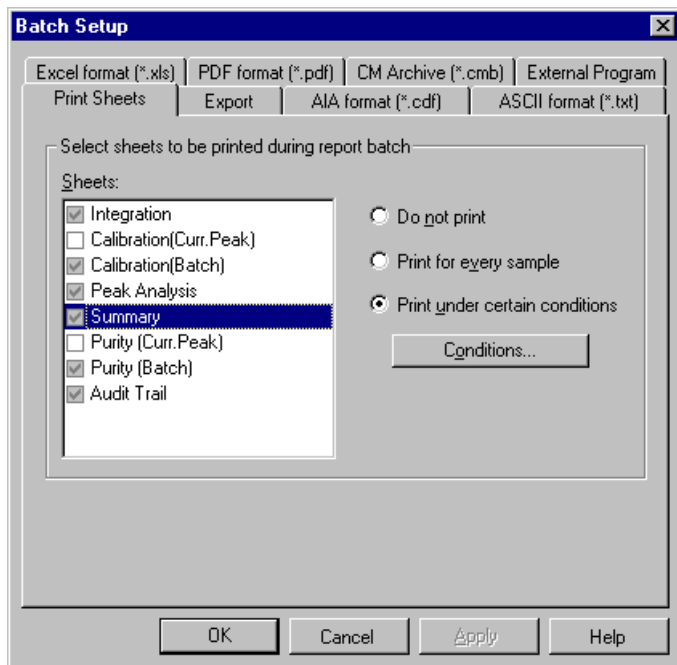
- Select **Save Report Definition** on the context menu to save the Report Definition File and its worksheets under either an existing or a new name.
- Select **Load Report Definition** on the context menu to open an existing Report Definition File.

**Tip:**

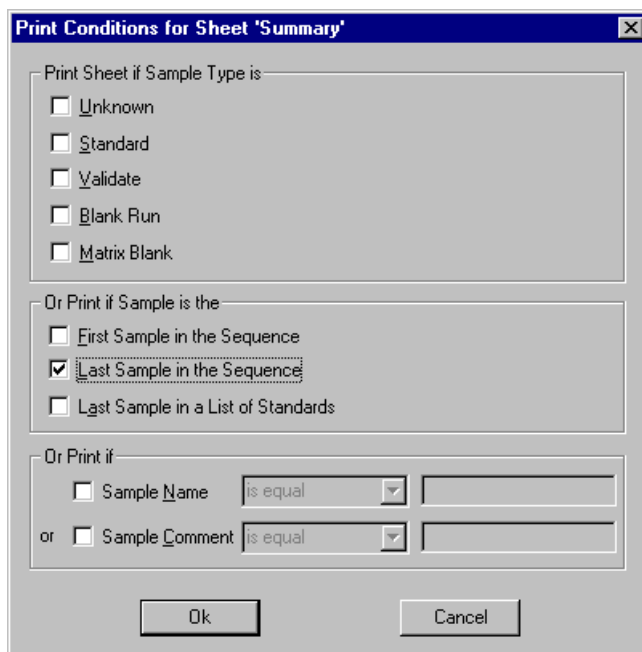
In addition, a Report Definition File contains various settings of the on-screen report, such as the window size, the type, and number of the columns in a report, axis captions, etc. When you save the Printer Layout, any modifications made to the on-screen report are saved as well.

Specifying the Pages to be printed

To specify which pages of the **Printer Layout** shall be printed, enable **Layout Mode** on the **Edit** menu. Select **Batch Report Setup** on the **File** menu to open the **Batch Report Setup** dialog box:



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:



The dialog box is titled "Print Conditions for Sheet 'Summary'". It contains three main sections for defining print conditions:

- Print Sheet if Sample Type is**: A list of sample types with checkboxes: ☐ Unknown, ☐ Standard, ☐ Validate, ☐ Blank Run, and ☐ Matrix Blank.
- Or Print if Sample is the**: A list of sample positions with checkboxes: ☐ First Sample in the Sequence, ☒ Last Sample in the Sequence, and ☐ Last Sample in a List of Standards.
- Or Print if**: Two rows for conditional printing based on sample name or comment. Each row has a checkbox, a text field, and a dropdown menu set to "is equal".
 - Row 1: ☐ Sample Name is equal []
 - Row 2: or ☐ Sample Comment is equal []

At the bottom are "Ok" and "Cancel" buttons.

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 default 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. on 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 desired 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). Having made these settings, a message box appears. Determine whether these settings shall 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 only apply 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 will 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 should be in black and white or in color. For more information, refer to **How to ...: Actions in the Printer Layout**  **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 ...: Actions in the Printer Layout**

 **Saving/Loading a Report Definition File**

 **Saving the Contents of a Printer Layout Page**

 **Printing the Results of a Single Sample**

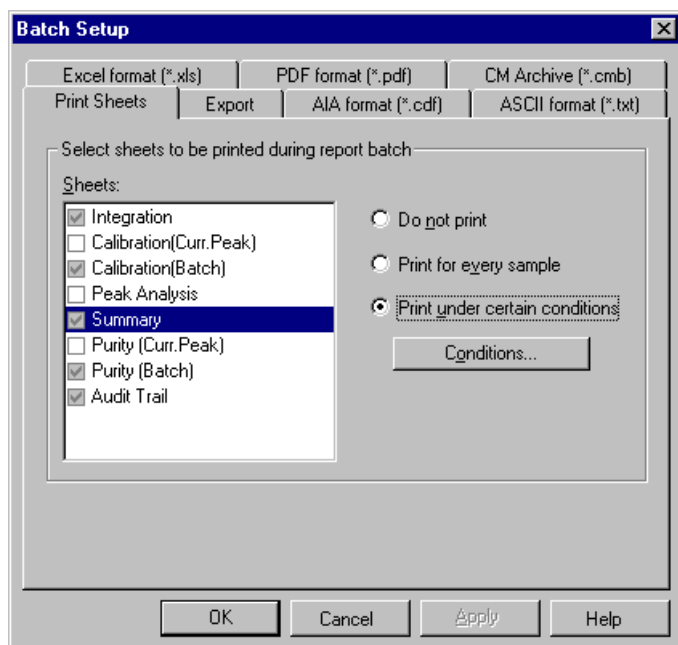
You can print the results of single samples from either the Printer Layout or the on-screen report (integration plot).

For each printout, the ➤*Printer Layout* pages saved in the ⇒*Report Definition File* 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:

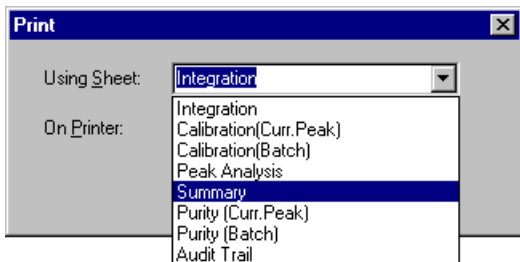


Select the corresponding settings on the **Print Sheets** tab page. The available worksheets are listed in the **Sheets** field. Define which pages shall be printed and under which conditions. Click **Conditions...** to define the print conditions.

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 box. 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:

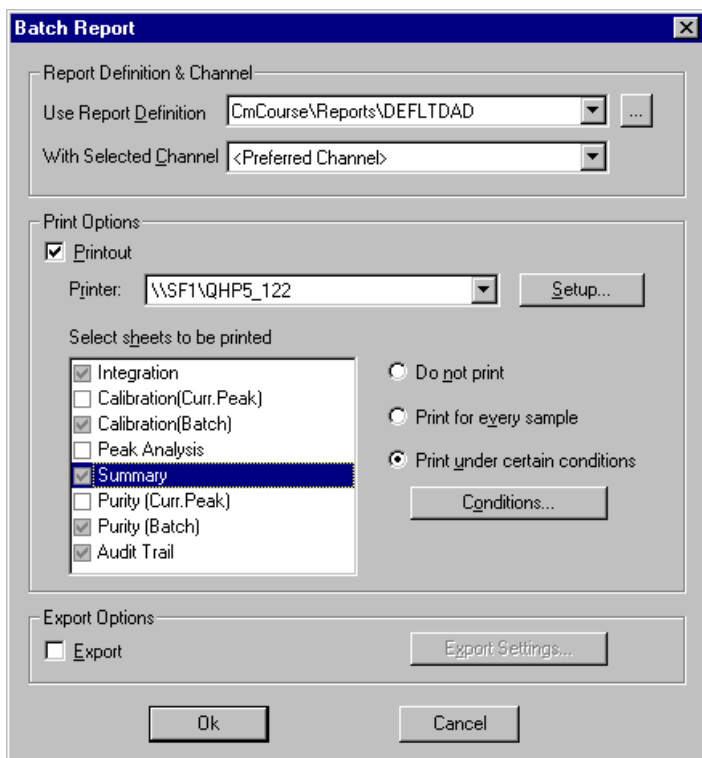
In the Printer Layout, you can only select peak spectra but no retention time spectra. If you want to print a retention time spectrum, open the Report, select the desired spectrum on-screen, and then print a worksheet that includes the spectrum.

Printing the Results of a Sequence or a 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 the desired Report Definition File from the **Use Report Definition** drop-down box. Or else, click the "..." button and navigate to the desired file.



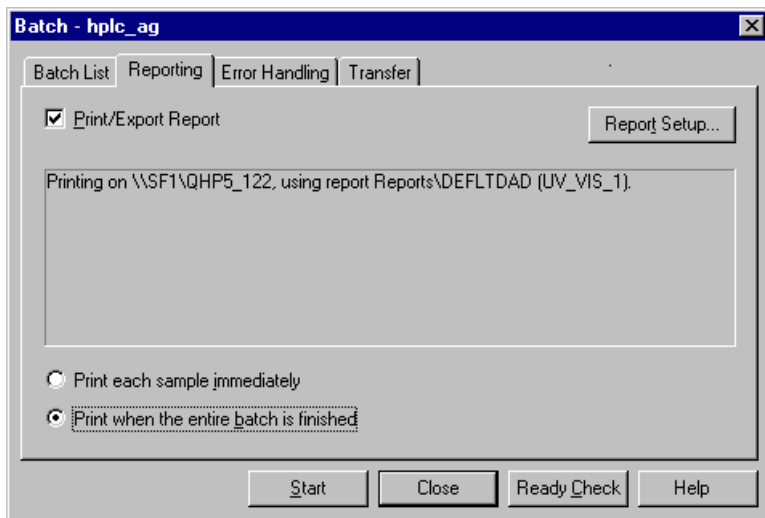
The worksheets defined in the Printer Layout of the Report Definition File are listed in the **Select sheets to be printed** field.

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 the desired 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 desired 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).*

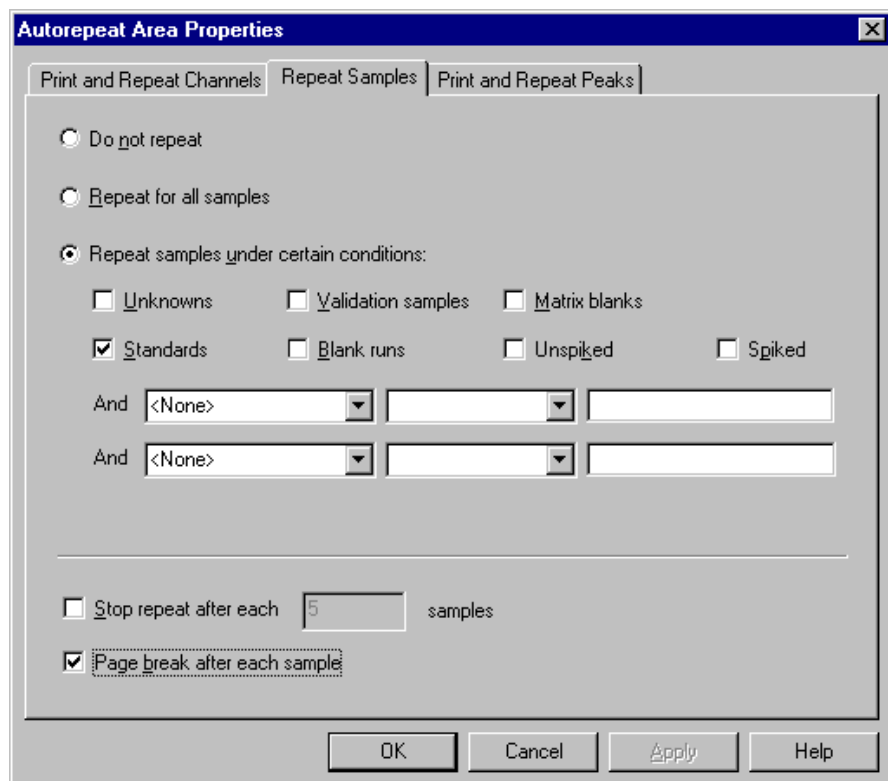
Automatically Repeating the Output for the Selected Objects: Example

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								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27	No.	Ret. Time	Peak Name	Height	Area	Rel. Area	Amount	Type
28		min		μS	μS*min	%	μg/g	
29	1	3.60	Fluoride	14.622	1.511	15.72	4.947	BMB
30	2	6.64	Chloride	12.137	1.994	20.75	9.656	BMB
31	3	8.58	Nitrite	5.036	1.144	11.90	9.910	BMB
32	4	11.61	Bromide	2.800	0.826	8.60	9.954	BMB
33	5	14.08	Nitrate	2.911	1.063	11.05	9.958	BMB
34	6	17.43	Phosphate	1.840	0.983	10.23	17.384	BMB
35	7	20.60	Sulfate	3.545	2.091	21.75	14.925	BMB
36	Total:			42.890	9.612	100.00	76.734	
37								

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:



The image shows a dialog box titled "Autorepeat Area Properties" with three tabs: "Print and Repeat Channels", "Repeat Samples", and "Print and Repeat Peaks". The "Repeat Samples" tab is selected. Inside the dialog, there are three radio buttons: "Do not repeat", "Repeat for all samples", and "Repeat samples under certain conditions:". The "Repeat samples under certain conditions:" option is selected. Below this, there are six checkboxes: "Unknowns", "Validation samples", "Matrix blanks", "Standards", "Blank runs", and "Unspiked". The "Standards" checkbox is checked. There are also two "And" labels followed by dropdown menus and text boxes. At the bottom, there are two more checkboxes: "Stop repeat after each" (with a value of 5 in a text box) and "Page break after each sample", which is checked. At the very bottom are four buttons: "OK", "Cancel", "Apply", and "Help".

Autorepeat Area Properties

Print and Repeat Channels | Repeat Samples | Print and Repeat Peaks

☐ Do not repeat

☐ Repeat for all samples

☒ Repeat samples under certain conditions:

☐ Unknowns ☐ Validation samples ☐ Matrix blanks

☒ Standards ☐ Blank runs ☐ Unspiked ☐ Spiked

And <None> <None> <None>

And <None> <None> <None>

☐ Stop repeat after each 5 samples

☒ Page break after each sample

OK Cancel Apply Help

With these settings, the following two pages are output via the **Print** command (**File** menu):

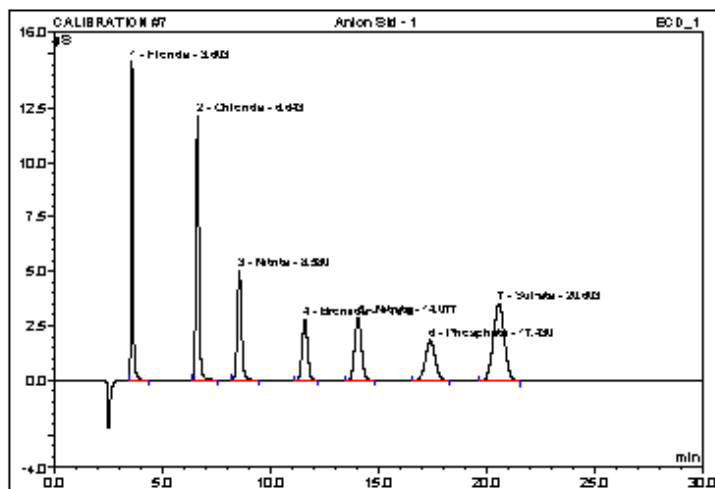
Operator: admin Timebase: WEST LAB NT_3_1 Sequence: CALIBRATION

Page 1-2

23.1.2004 4:16 PM

7 Anion Std - 1

Sample Name:	Anion Std - 1	Injection Volume:	25.0
Mat Number:	7	Channel:	ECD_1
Sample Type:	standard	Wavelength:	n.s.
Control Program:	Test Run1	Bandwidth:	n.s.
Quantify Method:	Anion Gnt	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.	RetTime min	Peak Name	Height μ S	Area μ S*min	Rel.Area %	Amount μ g/g	Type
1	3.60	Fluoride	14.622	1.511	15.72	4.947	BMF
2	6.64	Chloride	12.137	1.994	20.75	9.696	BMF
3	8.58	Nitrite	5.036	1.144	11.90	9.910	BMF
4	11.61	Bromide	2.800	0.826	8.60	9.954	BMF
5	14.08	Nitrate	2.911	1.063	11.05	9.968	BMF
6	17.43	Phosphate	1.840	0.983	10.23	17.384	BMF
7	20.60	Sulfate	3.545	2.091	21.75	14.925	BMF
Total:			42.890	9.612	100.00	76.734	

Operator: admin In Timebase: WEST LAB NT 3_1 Sequence: CALIBRATION Page: 2-2
23.1.2004 4:19 PM

8 Anion Std - 2							
No.	RetTime min	Peak Name	Height µS	Area µS*min	RelArea %	Amount µg/g	Type
1	3.59	Fluoride	4.039	0.426	15.91	1.396	MMB
2	6.62	Chloride	3.297	0.546	20.37	2.643	MMB
3	8.56	Nitrite	1.363	0.317	11.81	2.742	MMB
4	11.59	Bromide	0.809	0.242	9.03	2.918	MMB
5	14.06	Nitrate	0.832	0.309	11.51	2.891	MMB
6	17.44	Phosphate	0.436	0.234	8.74	4.144	MMB
7	20.66	Sulfate	1.020	0.606	22.63	4.330	MMB
Total:			11.817	2.680	100.00	21.064	

For general information, refer to  **Automatically Repeating the Output for the Selected Objects.**

Saving the Contents of a Report Definition File

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** 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.

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 equals 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 equals sign. Enter the SUM command required for adding cell values (see **SUM** in the Report Publisher online Help). Then 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**; all in the Report Publisher online Help).

**Tip:**

For an alphabetical list of the available formulas, refer to the ➤Additional Functions topic.

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. The fields to be calculated (D2 and D3) can then be calculated by simultaneously selecting 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 **Table Properties** on the context menu. On the **General** tab page of the **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. Press the F8 key to open the 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

Also, refer to **How to ...: Actions in the Printer Layout**  **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**.


The screenshot shows the 'Properties of Datasource "CM_Seminar"' dialog box with the 'User-defined Columns' tab selected. On the left, under 'Columns:', there is a list with '<New user-defined column>'. At the bottom left is an 'Import Columns' button. On the right, under 'Properties:', the following fields are visible: 'Name:' with the value 'concentration', 'Value type:' with a dropdown set to 'Floating point', 'Dimension or comment:' with the value 'µg/ml', a checked checkbox for 'Empty values are possible', and input fields for 'Minimum:', 'Maximum:', 'Default value:', and 'Precision:' (set to 3). At the bottom right is an 'Append Column' button.

(For more information, refer to **How to ...: Actions in the Browser**  **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:

The screenshot shows the 'Properties Report Variable' dialog box. On the left, under 'Categories:', a list includes 'General', 'Sequence', 'Sample' (highlighted), 'Audit Trail', 'Chromatogram', 'Detection Parameters', 'Peak Results', 'Peak Calibration', 'Peak Table', 'Peak Purity and Identification', and 'Quantification Method'. On the right, under 'Variables:', a list includes 'Vial Number', 'Replicate ID', 'Status', 'Date & Time Sample Run', 'Inject Volume', 'Dilution Factor', 'Sample Weight', 'ISTD Amount', 'Program', 'Quantification Method', and 'concentration' (highlighted). At the bottom, the 'Formula.....' field contains 'smp.concentration' and the 'Format.....' field contains '0.000'. On the right side, there are buttons for 'Ok', 'Cancel', 'Customize...', 'Explain Variable...', and 'Parameter...'.

2. Follow the description in the previous topic (see **How to ...: Actions in the Printer Layout**  **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						5fGrm		

Creating Dynamic Columns That Contain Flexible Formulas

Follow the description below to create a table column that contains flexible formulas and dynamically adapts to the number of existing peaks. (This procedure is only important if the table contains columns with report variables and if these columns are to the right of the newly added column.)

See the following example for information about how to create a column that allows you to check automatically whether the signal noise is below 0.005 mAU:

- Add a new column to the table. In the Printer Layout, new columns are always inserted to the left of the selected column. Select the first field below the header in the column that shall 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 the desired variable.
- Delete any existing entry in the **Formula** field.

Properties Report Column

Categories:

- Sequence
- Sample
- Audit Trail
- Chromatogram**
- Detection Parameters
- Peak Results
- Peak Calibration
- Peak Table
- Peak Purity and Identification
- Quantification Method
- Mass spectrometry
- Integration Table

Variables:

- Channel Name
- Raw Data File Path Name
- Number of Peaks
- Select Peak
- Count Peaks If...
- Sum Peak Results If...
- Start Time (relative to Inject Time)
- End Time (relative to Inject Time)
- Signal Value
- Signal Dimension
- Signal Noise**
- Sample Rate

Formula.....

Header..... "Noise "

Dimension. chm.sig_dim

Format..... 0.000

- Click **OK** to exit the dialog. This action automatically selects the first field in the new column.

- Enter the desired formula in the layout line without changing the selection in the report table. Press <Enter> to confirm your entry.

F5		=IF(E5<0.005;"Ok";"Not Ok")			
	A	B	E	F	I
1	Sample	Sample Name	Noise	Noise	Amount
2	No.		mAU	mAU	
3					Pyrene
4			Uv_vis 1	Uv_vis 1	Uv_vis 1
5	1	Sample 1	0.004	Ok	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

- 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 now displays as a solid black cross. Left-click and draw the formula into all lines of the selected report table column.
- Double-click 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:

Properties Report Column

Categories:

- Sequence
- Sample
- Audit Trail
- Chromatogram
- Detection Parameters
- Peak Results
- Peak Calibration
- Peak Table
- Peak Purity and Identification
- Quantification Method
- Mass spectrometry
- Summary Table

Variables:

Formula..... =IF(E5<0.05;"Ok";"Not Ok")

Header..... "Noise okay?"

Dimension.....

Format..... 0.000

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.

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 mouse cursor will change its shape to a small + 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 desired 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 desired 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 the desired 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 that is 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 the desired 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 desired 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 \Rightarrow Report Definition File
&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

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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 the desired 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 \Rightarrow Report Definition File 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 desired report definition.

The settings of this RDF are then always used when 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 \Rightarrow 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 ...:**  **Actions in the Printer Layout** and the corresponding subtopics.

Actions in the Peak Purity Analysis (PPA) Window

The PPA (Peak Purity Analysis) plot (see **Data Reprocessing**  **PPA (Peak Purity Analysis)**) 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 successively display all spectra of the active chromatogram.
- 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.

Apart from this simple spectra and chromatogram selection, use this method to perform the following operations:



Analyzing Peak Purity



Selecting the Optimum Integration Path



Extracting and Exporting Spectra, Chromatograms, and 3D Fields

Analyzing Peak Purity

Peak purity can be evaluated using the following methods:



Visual Check of Iso Line Plot



PPI and PPI Match Factor



Normalized Spectra Overlay



Peak Inhibition via Virtual Channels

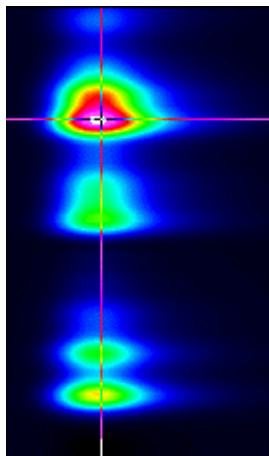


Multiple Ratio

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 $\text{Peak Purity Match Factor}$ 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 cursor 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 in the category **Peak Purity**.

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 $s_1(\lambda)$ and $s_2(\lambda)$ the following equation is true when the area below the baseline is ignored:

$$A(\lambda, t) = s_1(\lambda) \cdot c_1(t) + s_2(\lambda) \cdot c_2(t) \quad (1).$$

c_1 and c_2 stand for the time-dependant concentrations of the corresponding components in the flow cell. Then, two wavelengths λ_1 and λ_2 are selected and the following signal is created:

$$d(t) = A(\lambda_1, t) - K \cdot A(\lambda_2, t) = c_1(t)[s_1(\lambda_1) - K \cdot s_1(\lambda_2)] + c_2(t)[s_2(\lambda_1) - K \cdot s_2(\lambda_2)]. \quad (2),$$

The c_2 term disappears provided the appropriate expression was selected for K , that is:

$$K = s_2(\lambda_1) / s_2(\lambda_2) \quad (3)$$

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

$$K' = s_1(\lambda_1) / s_1(\lambda_2) \quad (4)$$

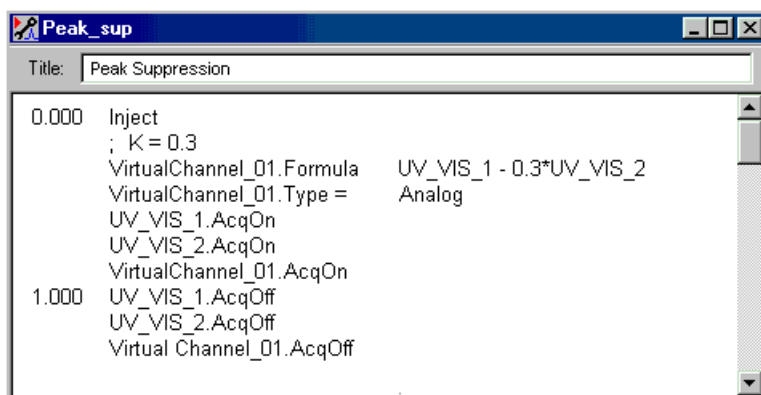
must be different from K . If not, the first term in equation (2) will disappear as well. Select the λ_1 and λ_2 wavelength in such a way that the difference between K and K' , that is:

$$\Delta K = |K - K'| \quad (5)$$

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*:



As already 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.



Note:

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 also be performed 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 automatically switched in the future.
- Enter a value for the **Bandwidth** if several chromatograms should 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 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.

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 \Rightarrow *Detection Parameters*, such as \Rightarrow *Minimum Area* or \Rightarrow *Inhibit Integration*?













Answer: Enter the parameters on the **Detection** tab page of the \Rightarrow *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).

Actions Related to Mass Spectrometers


For MS data acquisition, use the Thermo Finnigan aQa or MSQ ➤ *Mass Spectrometer* with Chromeleon. 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 aQa MS**
-  **Creating aQa MS Channels with the aQa PGM File**
-  **Creating a PGM File for the MSQ**
-  **Creating MSQ Channels with the MSQ 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**
-  **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)

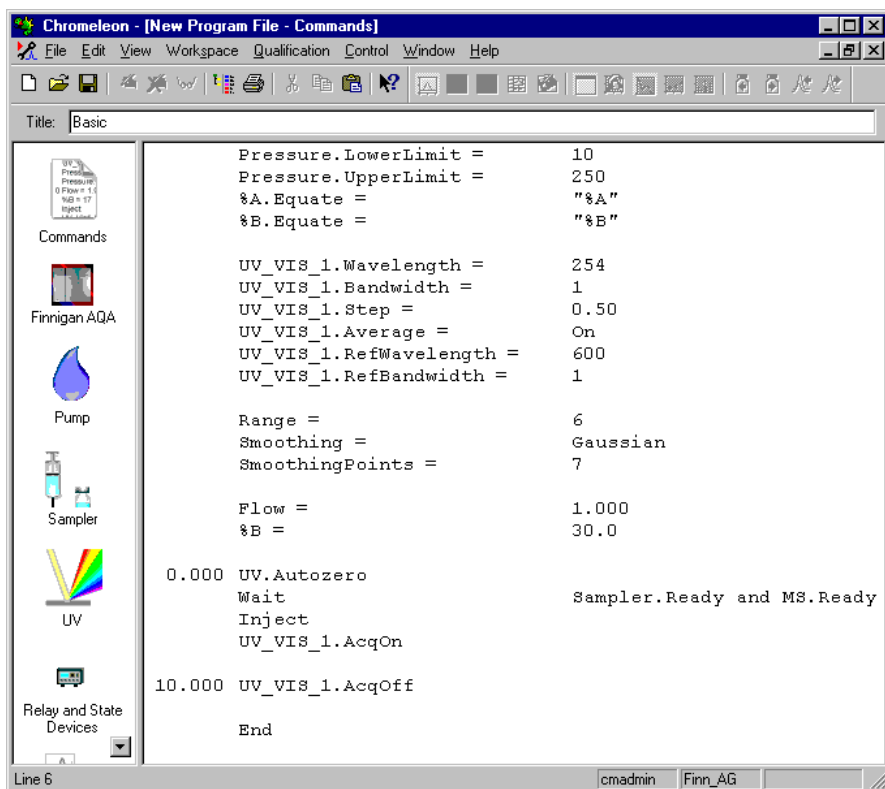
Create a new PGM File, using the Program Wizard (see **Programmed Control**  **The Program Wizard**). If a mass spectrometer is installed in the current timebase, select the data acquisition, using the MS channel option on the **Acquisition Options** tab page. This action opens the page for MS-specific 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 \Rightarrow *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 \triangleright *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.

- Using the Sequence Wizard: Enter the created PGM File in step 4.
- 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 **How to ...: Device Control**  **Starting Data Acquisition**). The current mass spectrum can be displayed on the \triangleright *Control Panel*.

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 Wait MS.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** (Electrospray Ionization) or **APCI** (Atmospheric Pressure Chemical Ionization) 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 the **Tune...** button.



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:

Chromeleon - [New Program File - Finnigan AQA]

File Edit View Workspace Qualification Control Window Help

Title: Parabene Analysis

Commands

Finnigan AQA

Pump

Sampler

UV

Relay and State Devices

Post-acquisition steps

Ionization Mode Analysis Acquisition

Type of acquisition:

☒ Full scan ☐ Selected ion monitoring (SIM)

Simultaneous acquisitions:

	Polarity	AQAmass (V)
1	+ve	100
2	-ve	100
3		0
4		0

☐ Advanced

Acquisition rate:

☐ LC peak width (sec): 20.0 @ Save Min scans per LC peak: 10 Actual scans per LC peak: 12

☒ Scans per second: 0.6

☐ Time per scan (sec): 1.67

Mass spectrum:

☐ Advanced Peak format: Centroid

Start: 20

2 amu

Mag mass range for this acquisition rate (amu): 316 1636 amu

End: 336

☐ Automatic background subtraction

Event control:

☒ Stop acquiring when LC run finishes

☐ Stop acquiring after a period of (min): 60.0

Help

Line 1 cmadmin

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 ...: Actions Related to 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 (➤ TICF 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.

**Tip:**

*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.*

**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 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 Actions Related to Mass Spectrometers**  **SIM Mass Lists for IC-MS.**

The first example creates two SIM channels plus the TIC channel:

Ionization Mode | Analysis | Acquisition

Type of acquisition:

☐ Full scan ☒ Selected ion monitoring (SIM)

SIM:

☒ LC peak width (sec): 20.0 @ Base Minimum points per LC peak: 10

☐ Minimum points per second: 5.00

☐ Advanced set-up

Retention Window (min) with Polarity, Dwell Time per Mass (sec) and Points per Second

RW: 0.00-60.00 Polarity: +ve Dwell: 0.370 Points/sec: 2.70					
MASS 1	100				
Voltage	100				
MASS 2	200				
Voltage	100				
MASS 3					
Voltage					
MASS 4					
Voltage					
MASS 5					
Voltage					
MASS 6					
Voltage					

Event control:

☒ Stop acquiring when LC run finishes

☐ Stop acquiring after a period of (min): 30.0


Help

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:

Ionization Mode | Analysis | Acquisition

Type of acquisition:
☐ Full scan ☒ Selected ion monitoring (SIM)

SIM:
☐ LC peak width (sec): 20.0 @ Base Minimum points per LC peak: 10
☒ Minimum points per second: 0.90  These settings result in retention windows with dwell times per mass above 0.370 sec. Where this is so, a dwell time of 0.370 sec will be used instead.
☐ Advanced set-up

Retention Window (min) with Polarity, Dwell Time per Mass (sec) and Points per Second

	RW: 0.00-60.00 Polarity: +ve Dwell: 0.370 Points/sec: 1.35	RW: 60.00-120.00 Polarity: +ve Dwell: 0.370 Points/sec: 1.35	RW: 120.00-180.00 Polarity: -ve Dwell: 0.370 Points/sec: 1.35	RW: 120.00-180.00 Polarity: +ve Dwell: 0.370 Points/sec: 0.90	
MASS 1	100	100	100	100	
Voltage	100	100	80	100	
MASS 2	200	300	200	200	
Voltage	100	100	100	100	
MASS 3				300	
Voltage				80	
MASS 4					
Voltage					
MASS 5					
Voltage					
MASS 6					
Voltage					
MASS 7					

Event control:
☒ Stop acquiring when LC run finishes
☐ Stop acquiring after a period of (min): 30.0

Help

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:

Ionization Mode | Analysis | Acquisition

Type of acquisition:
☒ Full scan
☐ Selected ion monitoring (SIM)

Simultaneous acquisitions:

	Polarity	AQAmx (V)
1	+ve	100
2	+ve	200
3	-ve	100
4		0

☐ Advanced

Acquisition rate:
☒ LC peak width (sec): 20.0 @ Base Min scans per LC peak: 10 Actual scans per LC peak: 10
☐ Scans per second: 0.5
☐ Time per scan (sec): 2.00

Mass spectrum:
☐ Advanced Peak format: Centroid
Start: 20 2 amu Max mass range for this acquisition rate (amu): 316 1636 amu
End: 336
☐ Automatic background subtraction

Event control:
☒ Stop acquiring when LC run finishes
☐ Stop acquiring after a period of (min): 30.0

Help

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 ...: Actions Related to Mass Spectrometers**  **Extracting Mass Traces Afterward**.)

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 the desired 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 Wait MS.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:

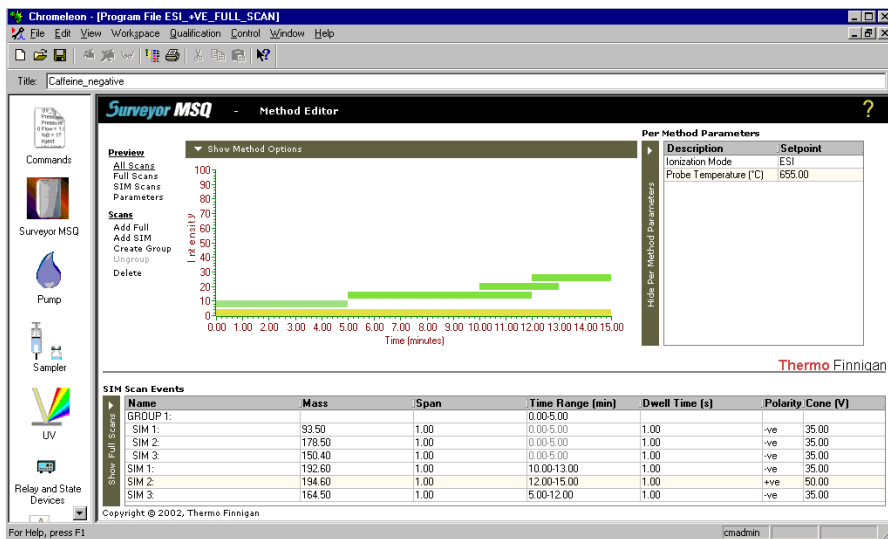
- All Scans to display all channels.
- **Full Scans** to display the ➤ *Full Scan* channels (orange) only.
- **SIM** to display the ➤ *SIM* channels (green) only.

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 the desired sample by clicking the "..." button under **Display**.

On the top right, in the **Per Method Parameters** section, select ESI (Electrospray Ionization) or APCI (Atmospheric Pressure Chemical Ionization) 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 desired 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 ...: Actions Related to 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 select the desired SIM channels by clicking.) Select **Ungroup** to undo this action. To delete a channel, select the channel, and then click **Delete**.

**Tips:**

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 ...: Actions Related to 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 amu, while the other channels record the same chromatogram at a mass of 85.00 amu (SIM2), 90.00 amu (SIM3), and 100.00 amu (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

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The channel assignment is as follows:

Xcalibur (CM program: MSQ)

Name	Mass [amu]	Span [amu]
GROUP 1: Retention time 0.00 to 2.00 min		
SIM 1	100	1
SIM 2	200	1
SIM 3	300	1
SIM 4	400	1

Chromeleon

Name	Mass Range [amu]
SIM_01	99.5 - 100.5
SIM_02	199.5 - 200.5
SIM_03	299.5 - 300.5
SIM_04	399.5 - 400.5

Xcalibur (CM program: MSQ)

Name	Mass [amu]	Span [amu]
GROUP 2: Retention time 2.00 to 3.00 min		
SIM 1	100	1
SIM 2	100	3
SIM 3	100	5
SIM 4	150	1
SIM 5	200	
Single channels:		
SIM 1	99	1
SIM 2	110	1

Chromeleon

Name	Mass Range [amu]
SIM_05	99.5 - 100.5
SIM_06	98.5 - 101.5
SIM_07	97.5 - 102.5
SIM_08	149.5 - 150.5
SIM_09	199.5 - 200.5
SIM_10	98.5 - 99.5
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 amu; retention time 0.00 to 1.00 min,

TICF_2:


FS 2, Mass range 60.00 to 400.00 amu; retention time 2.00 to 4.00 min,

TICF_3:

FS 3, Mass range 600.00 to 800.00 amu; retention time 0.00 to 2.00 min.

**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 ...: Actions Related to 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	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}^-$	
103	Hydroxybutyrate	$\text{CH}_3\text{CHOHCH}_2\text{COO}^-$	
103	Malonate	$\text{COOHCH}_2\text{COO}^-$	
113	Thiosulfate	HS_2O_3^-	113/115
113	Trifluoroacetate	F_3CCOO^-	
115	Maleate	COOHCHCHCOO^-	
115	Fumarate	COOHCHCHCOO^-	
117	Succinate	$\text{COOHCH}_2\text{CH}_2\text{COO}^-$	
127	Bromate	BrO_3^-	127/129
127	Dichloroacetate	$\text{Cl}_2\text{CHCOO}^-$	127/129
127	Iodide	I^-	
128	Selenite	SeO_3^-	128/126
131	Glutarate	$\text{COOH}(\text{CH}_2)_3\text{COO}^-$	
133	Malate	$\text{COOHCH}_2\text{CHOHCOO}^-$	
133	Tartrate	$\text{COOH}(\text{CHOH})_2\text{COO}^-$	
137	Bromoacetate	$\text{BrCH}_2\text{COO}^-$	137/139
144	Selenate	SeO_4^-	144/142
145	Adipate	$\text{COOH}(\text{CH}_2)_4\text{COO}^-$	
173	Bromochloroacetate	BrClCHCOO^-	173/171/175
183	Styrenesulfonate	$\text{CH}_2\text{CHC}_6\text{H}_4\text{SO}_3^-$	

Anion SIM Mass List for IC-MS (in water)

m/z	Anion	Detected As	Isotopes (decreasing frequency)
191	Citrate	$\text{HOOCCH}_2\text{COH}(\text{COOH})\text{CH}_2\text{COO}^-$	
191	Quinate	$\text{C}_6\text{H}_7(\text{OH})_4\text{COO}^-$	
191	Isocitrate	$\text{HOOCCHOHCH}(\text{COOH})\text{CH}_2\text{COO}^-$	
207	Dichlorobromoacetate	$\text{Cl}_2\text{BrCCOO}^-$	207/205/209/211
217	Dibromoacetate	$\text{Br}_2\text{CHCCOO}^-$	297/295/293/299
251	Dibromochloroacetate	$\text{Br}_2\text{ClCCOO}^-$	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	$1/2\text{Ca}_2^+$
23	Sodium	Na^+
39	Potassium	K^+
46	Ethylamine	$\text{CH}_3\text{CH}_2\text{NH}_3^+$
46	Dimethylamine	$(\text{CH}_3)_2\text{NH}_2^+$
60	Trimethylamine	$(\text{CH}_3)_3\text{NH}^+$
60	Guanidine	$(\text{NH}_2)_2\text{CNH}_2^+$
61	Ethanediamine	$\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+$
62	Monoethanolamine	$\text{OHCH}_2\text{CH}_2\text{NH}_3^+$
69	Imidazole	$\text{C}_3\text{H}_4\text{N}_2\text{H}^+$
74	Diethylamine	$(\text{CH}_3\text{CH}_2)_2\text{NH}_2^+$
74	Dimethylethylamine	$(\text{CH}_3)_2(\text{C}_2\text{H}_5)\text{NH}^+$
74	Butylamine	$\text{C}_4\text{H}_9\text{NH}_3^+$

Cation and Amine SIM Mass List for IC-MS (in water)

m/z	Cation	Detected As
75	Propanediamine	$\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+$
76	Methylethanolamine	$\text{HOCH}_2\text{CH}(\text{CH}_3)\text{NH}_3^+$
76	Dimethylmethanolamine	$\text{HOC}(\text{CH}_3)_2\text{NH}_3^+$
76	Propanolamine	$\text{C}_3\text{H}_6\text{OHNH}_3^+$
80	Pyridine	$\text{C}_5\text{H}_5\text{NH}^+$
85	Lysidine	$\text{C}_4\text{H}_8\text{N}_2\text{H}^+$
88	Morpholine	$\text{C}_4\text{H}_8\text{ONH}_2^+$
88	Methyldiethylamine	$\text{CH}_3(\text{C}_2\text{H}_5)_2\text{NH}^+$
89	Butanediamine (Putrescine)	$\text{H}_2\text{NC}_4\text{H}_8\text{NH}_3^+$
90	Dimethylethanolamine	$\text{C}_5\text{H}_{13}\text{NH}^+$
90	Methylpropanolamine	$\text{C}_4\text{H}_8(\text{OH})\text{NH}_3^+$
100	Cyclohexylamine	$\text{C}_6\text{H}_{11}\text{NH}_3^+$
102	Triethylamine	$(\text{CH}_3\text{CH}_2)_3\text{NH}^+$
103	Pentanediamine (Cadaverine)	$\text{H}_2\text{NC}_5\text{H}_{10}\text{NH}_3^+$
104	Dimethylamino-2-propanol	$(\text{C}_3\text{H}_6\text{OH})(\text{CH}_3)_2\text{NH}^+$
106	Diethanolamine	$(\text{OHCH}_2\text{CH}_2)_2\text{NH}_2^+$
118	Diethylethanolamine	$\text{C}_6\text{H}_{15}\text{ONH}^+$
120	Methyldiethanolamine	$\text{C}_5\text{H}_{13}\text{O}_2\text{NH}^+$
132	Diethylaminopropanol	$\text{C}_7\text{H}_{17}\text{ONH}^+$
146	Spermidine	$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_3^+$
150	Triethanolamine	$(\text{OHCH}_2\text{CH}_2)_3\text{NH}^+$
203	Spermine	$\text{C}_{10}\text{H}_{26}\text{N}_4\text{H}^+$

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:

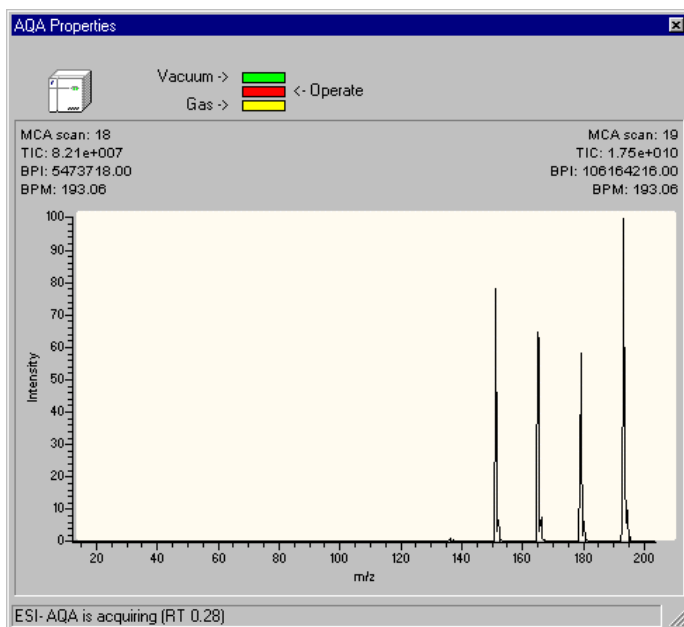
- Create a PGM File, using the Program Wizard (see **Programmed Control**  **The Program Wizard**).
- Verify that the PGM File includes an **Inject** command even if you do not use an autosampler. Otherwise, data acquisition cannot be started.
- In the PGM Editor (see **Control**  **The PGM Editor**), 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:

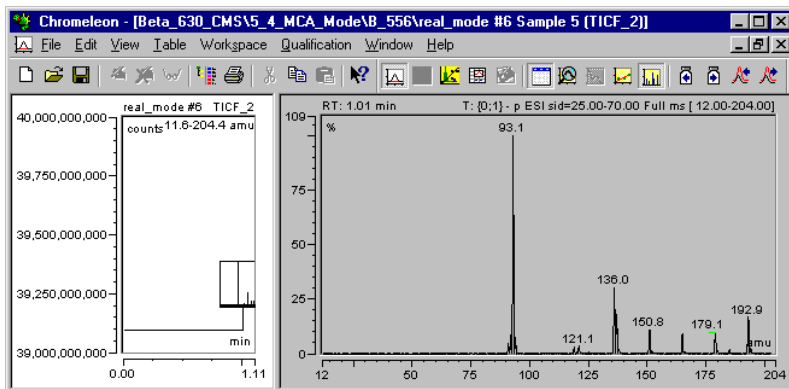
➤MCA data acquisition is not possible in demo mode!

The ➤ *Xcalibur* window below opens automatically when data acquisition is started:



The status bar indicates the time that passed since the data acquisition was started. At the top left and right, the results of the last two scans are given.

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 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 is possible online during data acquisition (online) but can be done later, as well (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.

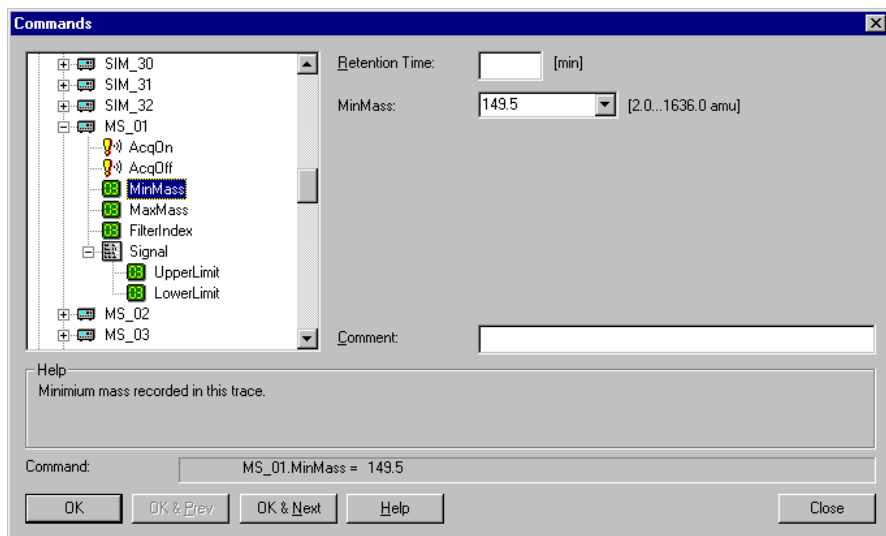
Create a ➤PGM File for data acquisition in ➤Full Scan mode using the Program Wizard (see **Programmed Control**  **The Program Wizard**). Each single MS channel needs its own ⇒AcqOn/Off command. Define as well the following parameters for each single MS channel:

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 amu (MSQ) 2.0 amu (aQa)	2000.00 amu (MSQ) 1636.0 amu (aQa)		Minimum mass of interval that will be extracted.

Parameter	Min.	Max.	Default	Usage
MaxMass	0.00 amu (MSQ) 2.0 amu (aQa)	2000.00 amu (MSQ) 1636.0 amu (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, we recommend 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* online 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 as well:

**Tip:**

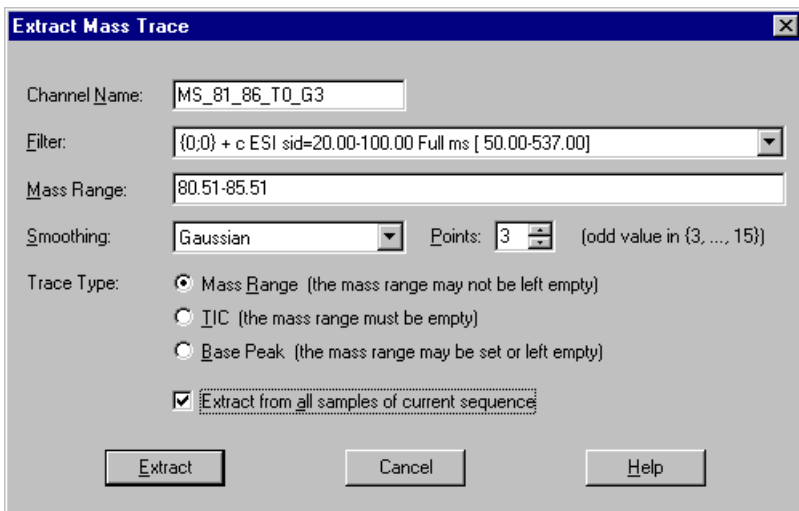
*To view a mass trace before finally extracting it, extract a temporary mass trace first (see  **Extracting a Temporary Mass Trace**).*

1. To extract a mass trace, open the ➤ *Mass Spectrum* from the **Integration** plot or the ⇒ *QNT Editor*.

**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 the desired filter and mass range, as well as the ➤ **Smoothing** type for the MS chromatograms and 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 amu) 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 provided in the **Extract Mass Trace** dialog box of the PGM Editor.*


6. Click **Extract** to make the new channel available for chromatogram 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 the **Administrator Manual: How to ...: Actions Related to Mass Spectrometers**  **Defining the Number of MS Channels**) or extract them as described above.





 **Note:**

*If you need help identifying found masses, refer to **How to ...: Actions Related to Mass Spectrometers**  **SIM Mass Lists for IC-MS**.*

Extracting a Temporary Mass Trace

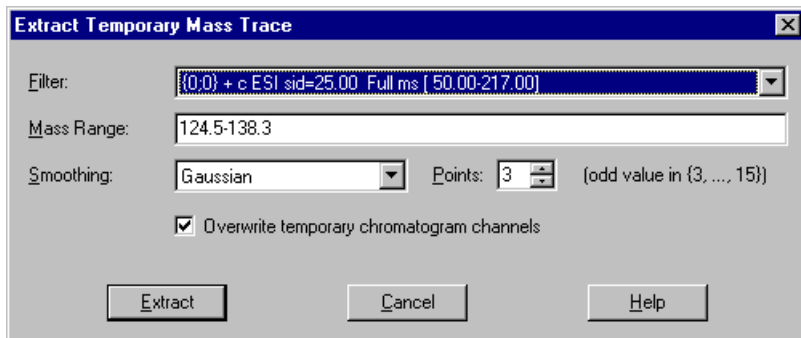
Before you extract a ➤ *Mass Trace* (see  **Extracting Mass Traces Afterward**), you can create it temporarily by just clicking the mouse. The following options are available:

Using the mouse cursor

Cursor	Activated by	What it does
	Placing the cursor 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.
	Placing the cursor 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 cursor between two mass range delimiters.	This action moves the mass range as desired.
	Placing the cursor 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:

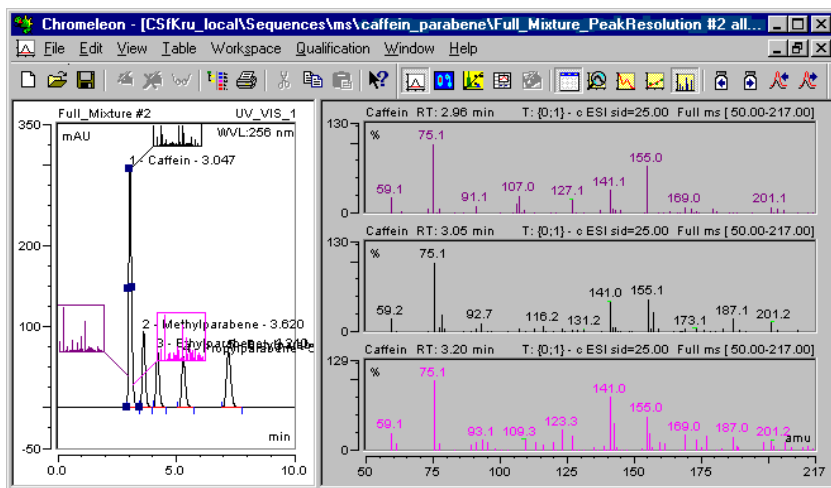




Showing Mass Spectra

The **Mass Spectra** view can be added to almost all Chromeleon plots (Integration, \Rightarrow *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:



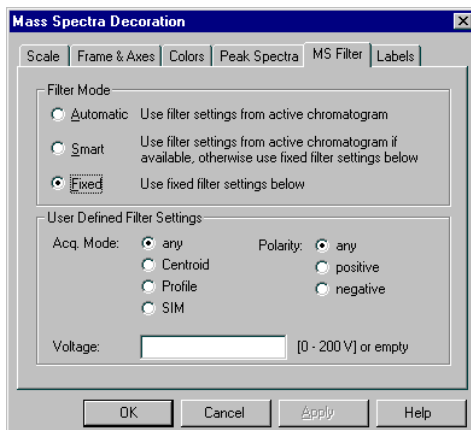
To add a mass spectrum to the *Printer Layout*, enable **Layout Mode** on the **Edit** menu. Select **Insert** on the View or context menu and then, select **Mass Spectra Plot**. The appearance of the displayed mass spectra may be quite different, depending on which the MS instrument method was used. For more information, refer to **How to ...: Actions Related to 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.

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 is given on the left. On the right, the acquisition mode is given: **Full ms** indicates *Full-Scan* mode; **SIM ms** indicates *SIM* mode. In the caption of full-scan mass spectra, the entire mass range is given in parentheses on the right. The fragmentation voltage that is given 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 are given 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 cursor 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 increase noise level. Especially with low signal intensity, they are considerably affected by the background spectrum. To use the information of mass spectra in the best possible way, we recommend that you reprocess them as described below. There are two ways:

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 \Rightarrow *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**.

In the chromatogram, define the range for which to display the entire **retention time spectrum**. Use the **Spectra Tool** to select the desired range while left clicking. You can perform this in a UV channel as well.

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 made on the MS tab page of the \Rightarrow *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 **How to ...**:

Actions in the QNT Editor  **Processing Mass Traces**

Actions in the Chromatogram  **Subtracting MS 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 page.
- 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.

Defining Further QNT Settings for MS

Handling the Retention Time Delay as against a second detector

Use the **➤ Delay Time** option on the **General** tab page to take the retention time difference into account that is due to 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 ...: Actions in the QNT Editor**



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 ...: Actions in the QNT Editor**



Identifying Peaks via Their Mass Spectra (MS Tracking) Detectors.

Actions Related to Fraction Collection

➤ *Fraction Collectors* are often used for fraction collection after the detector. Fractionation can be automated by the online interpretation of the signal.

On the one hand, fractions can be collected for preparative use. However, often it is also intended to analyze the single fractions more exactly after they have been collected. In both cases, 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.



Tip:

The Fraction Collection driver also allows you to collect fractions, independent of the signal, with a fixed volume at predefined retention times.

For more information about fraction collection, refer to:



Setting up Fraction Collection



PGM Wizard: Fraction Collection - General Options



Setting the Peak Detection Parameters



Checking the Fraction Collection Status on the Control Panel



Recognizing the Peak Start, Peak Maximum, and Peak End



Defining the Reactions to Certain Events



Tracking Fraction Collection in the Chromatogram



Tracking Fraction Collection in the Report



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

**Tip:**


The **Fraction Collection Automation** driver from earlier Chromeleon versions is obsolete and included in the distribution for compatibility reasons only. We recommend that you use the new **Fraction Collection** driver instead.

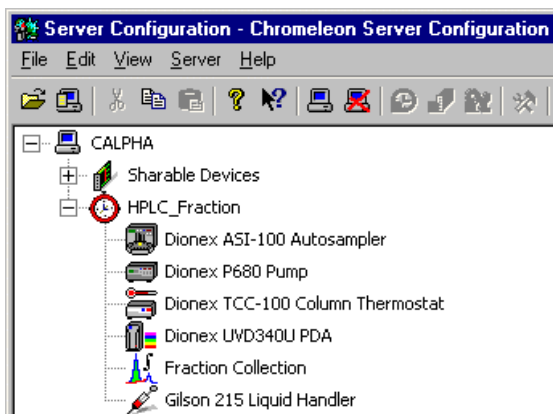
Setting up Fraction Collection

Before you can collect fractions, you have to set up fraction collection as follows:

Server Configuration



Install the **Fraction Collection** driver in the desired timebase. (Select **Add Device** on the context menu, select **General** 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 to collect the fractions, either in the Program Wizard (see **Programmed Control**  **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.
- On the **Fraction Collection - Channel Selection Options** page, determine the channel(s) for peak recognition during fraction collection.
- On the **Peak Detection Options** page, specify the peak detection algorithm for fraction collection (see  **Setting the Peak Detection Parameters**).

Standard Program Example

If you have not yet saved a FractionCollectionTemplate.pgm, the PGM Wizard creates the following standard program. (The program only includes the default fraction collection commands for a single detection channel plus the standard commands.)

```
Collect = On
CollectOutsidePeaks = No
;*****
;* 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
TotalNumberInstalled = 100
MaxTubesPerFraction = Unlimited
TubeWrapping = No
DelayTime = 0.0
OffsetTime = 0.0
DetectionChannel1.Name = "UV_VIS_1"
```

```

DetectionChannel1.PeakStartSlope =          0.500
DetectionChannel1.PeakStartThreshold =      10.00
DetectionChannel1.PeakMaxSlope =           0.000
DetectionChannel1.PeakEndSlope =           -1.000
DetectionChannel1.PeakEndThreshold =        10.00
DetectionChannel1.ThresholdNoPeakEnd =      2000.000
DetectionChannel1.BaselineOffset =          0.000
DetectionChannel1.BaselineDrift =           0.000
Flow = 1.00
%B = 0.0
%C = 0.0
%D = 0.0

0.000  Wait  Frac.Ready and Sampler.Ready
        Inject
        3DFIELD.AcqOn
        UV_VIS_1.AcqOn




10.000  3DFIELD.AcqOff
        UV_VIS_1.AcqOff

10.100  End    ;wait for all fraction events

```

**Note:**

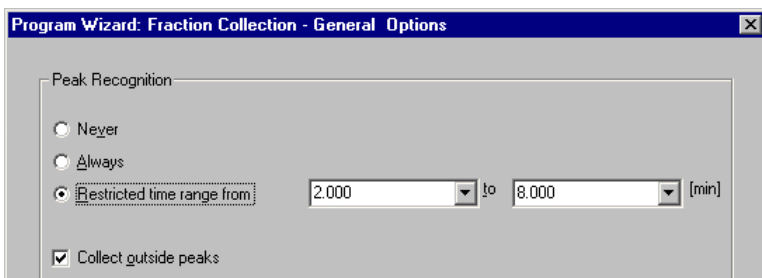
To make sure that the fractions are collected exactly, you have to enter and/or consider the actual delay time or the actual delay volume.

For more complex program examples, refer to  **Program Example (One Detection Channel)**,  **Program Example (Two Detection Channels)**, and  **Fraction Collection via an MS**.

For an overview of the fraction collection topics, refer to **How to ...:**  **Actions Related to Fraction Collection**.

PGM Wizard: Fraction Collection - General Options

Use the **Program Wizard: Fraction Collection - General Options** page (see **Programmed Control**  **The Program Wizard**) to determine the time range for fraction collection:



When you click **Next>**, more fraction collection Wizard pages are displayed. It depends on the settings made on this page, which pages are displayed and which program commands are generated:

Never

Select this option to disable fraction collection. In this case, no other fraction collection Wizard pages are displayed. The program includes the following commands:

```
Collect = Off
CollectOutsidePeaks = No
```

Always

Select this option to collect fractions over the entire chromatogram. The program looks as follows:

```
Collect = On
CollectOutsidePeaks = No ;(or Yes - see Note below)
;*****
;* Definition of triggers for fraction collection starts here.
;*****
; Definitions copied from <Timebase>\FractionCollectionTemplate!
Trigger FracStart FracStartDetected
EndTrigger
Trigger TubeChange FracTubeChange
EndTrigger
Trigger FracEnd FracEndDetected
EndTrigger
;*****
;* Definition of triggers for fraction collection ends here.
;*****
```

**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

Select this option to collect fractions during a specified period only. For example, for a time range from 2.000 to 8.000 min, the following commands are included in the program:

```
CollectFractions = No
CollectOutsidePeaks = No

;*****
;* Definition of triggers for fraction collection starts here.
;*****
; Definitions copied from <Timebase>\FractionCollectionTemplate!
Trigger FracStart FracStartDetected
EndTrigger
Trigger TubeChange FracTubeChange
EndTrigger
Trigger FracEnd FracEndDetected
EndTrigger
;*****
;* Definition of triggers for fraction collection ends here.
;*****

2.000 Collect = On
CollectOutsidePeaks = Yes ;(or No - see Note below)


8.000 Collect = Off
CollectOutsidePeaks = No
```

**Note:**

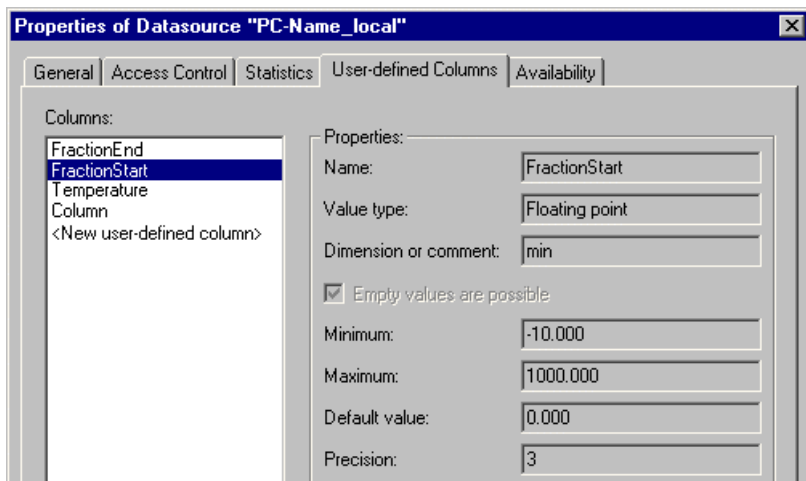
*It is also possible to collect fractions outside peaks (CollectOutsidePeaks = Yes). To do so, select the **Collect outside peaks** check box.*

Restricted time range & user-defined columns

You can use ➤ *User-defined Columns* in the sample list, to define when fraction collection shall start and when it shall stop:

- Create two user-defined columns (see **How to ...: Actions in the Browser**  **Creating User-defined Columns**) in the ➤ *Standard Datasource* of the server.

- Name the two columns, e.g., **FractionStart** and **FractionEnd**:



The screenshot shows a dialog box titled "Properties of Datasource 'PC-Name_local'". It has five tabs: "General", "Access Control", "Statistics", "User-defined Columns", and "Availability". The "User-defined Columns" tab is selected. On the left, under "Columns:", there is a list box containing "FractionEnd", "FractionStart" (which is highlighted), "Temperature", "Column", and "<New user-defined column>". On the right, under "Properties:", there are several input fields: "Name:" with "FractionStart", "Value type:" with "Floating point", "Dimension or comment:" with "min", a checked checkbox for "Empty values are possible", "Minimum:" with "-10.000", "Maximum:" with "1000.000", "Default value:" with "0.000", and "Precision:" with "3".

- Restart the server.
- On the **Fraction Collection - General Options** pages, select the **Restricted time range from** option. In the **from** field, select **FractionStart**; in the **to** field, select **FractionEnd**. The following triggers are generated:


```
Trigger FractCollectionOn Sample.FractionStart < System.Retention,
True=0.0,Hysteresis=0.0
Collect=                               On
CollectOutsidePeaks =                 Yes
EndTrigger
```


```
Trigger FractCollectionOff Sample.FractionEnd < System.Retention,
True=0.0,Hysteresis=0.0
Collect=                               On
CollectOutsidePeaks =                 No
EndTrigger
```

This program part 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.

Setting the Peak Detection Parameters

The **Fraction Collection** driver supports the parameters described in the tables below. They are available independently of the installed *➤Fraction Collector*. Use these parameters to start fraction collection in the *➤PGM File* or on the *➤Control Panel*.

Press the F8 key in the PGM File or on the control panel to open the **Commands** dialog box, and then enter the desired parameters. You can also establish controls with the desired functionality. (For more information, refer to **How to ...: Actions on the Control Panel**  **Modifying a Control Panel**.)

The tables below list the parameters in the order in which they appear in the **Commands** dialog box. (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 UV detector; here for a UV detector)

Parameter	Min.	Max.	Default	Description
Name	N/a	n/a	UV_VIS_1	Name of the signal channel used for peak detection (this may be a <i>➤Virtual Signal</i> , also).
OffsetTime	0.0 s	9999.9 s	0.0 s	Retention time offsets between the first detector and other detectors
OffsetVolume	0.0 µl	5000.0 µl	0.0 µl	Offset volume between the first detector and other detectors. (Based on the flow, the offset volume is converted into the corresponding OffsetTime.)
PeakStartThreshold	-1e10	1e10	10.00	The actual signal value must exceed the PeakStartThreshold value for the peak start to be recognized.
PeakStartSlope	0.000	1e10	0.500	The actual slope value must exceed the PeakStartSlope value for the peak start to be recognized.
PeakMaxSlope	-1e10	1e10	0.000	The actual slope value must be below the PeakMaxSlope value for the peak maximum to be recognized.

Parameter	Min.	Max.	Default	Description
PeakEndThreshold	-1e10	1e10	10.00	The actual signal value must be below the PeakEndThreshold value for the peak end to be recognized.
PeakEndSlope	-1e10	0.000	-1.000	The slope value must exceed the PeakEndSlope value for the peak end to be recognized.
ThresholdNoPeakEnd	-1e10	1e10	2000	The actual signal threshold value must be below the ThresholdNoPeakEnd value for the peak end to be recognized. (This parameter supports the PeakEndSlope parameter for peak detection.)
BaselineOffset	-1e10	1e10	0.0000	Offset of the baseline used to correct the signal value (see BaselineDrift).
BaselineDrift	-1e10	1e10	0.0000	Drift of the baseline used to correct the signal value. The specified thresholds (PeakStartThreshold, PeakEndThreshold, and ThresholdNoPeakEnd) are compared to: $\text{signal value} - (\text{BaselineOffset} + \Delta t * \text{BaselineDrift})$ 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.

Basic Parameters (directly under Fraction Collection)

Parameter	Min.	Max.	Default	Description
Collect	Off	On	No	Peak detection and thus, fraction collection is performed only if the variable is set to Yes . Note: Detected fractions will trigger related events after the delay time, even if Collect is Off at that time.
CollectOutsidePeaks	No	Yes	No	Determine whether fractions are collected outside peaks, also.

Parameter	Min.	Max.	Default	Description
➤ <i>DelayTime</i>	0.0 s	9999.9 s	0.0 s	Delay time between the first detector and the ➤ <i>Fraction Collector</i>
DelayVolume	0.0 µl	5000.0 µl	0.0 µl	Delay volume between the first detector and the fraction collector. (Based on the flow, the delay volume is converted into the corresponding DelayTime.)
PumpDevice			Pump	Pump whose flow is used to convert between time and volume.
TubePosition	1	9999	1	Position of the tube in the current fraction.
TubeMaxVolume	0.000000	1000.000000	Unlimited	Maximum filling volume of 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.


MaxTubesPerFraction	0	99	Unlimited	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.
TotalNumberInstalled	0	9999	100	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.



Parameter	Min.	Max.	Default	Description
TubeWrapping	No	Yes	No	If this option is selected, the TubePosition parameter is reset to 1 when TotalNumberInstalled is reached.
ChannelEvaluation	All	Any	All	Indicates how the results of the single detection channels are used for peak detection.


These properties cannot be changed during a peak, that is, while **PeakOn** is set to **Yes**. 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.


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  **Actions Related to Fraction Collection**.

Checking the Fraction Collection Status on the Control Panel

The **Fraction Collection** driver supports the various status variables that allow you to check the fraction collection status on a  *Control Panel*. The variables 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 a Lamp. (For more information, refer to **How to ...: Action on the Control Panel**  **Modifying a Control Panel** and the following topics.)


The tables below list the parameters in the order in which they appear in the dialog box. (For information about the parameters for active fraction collection control, refer to  **Setting the Peak Detection Parameters**.)


Parameters for the Single Channels

Status Variable	Description
PeakStartDetected	Yes when the peak start is detected. When the peak end is detected, the variable is reset to No . <i>The Delay Time does not delay a change.</i>
PeakMaxDetected	Yes when the peak maximum is detected. When the peak end is detected, the variable is reset to No . <i>(Not delayed)</i>
PeakEndDetected	Yes when the peak end is detected. When a new peak start is detected, the variable is reset to No . <i>(Not delayed)</i>
PeakOn	Yes when a peak start or a peak maximum is detected. <i>(Not delayed)</i>
PeakOnDelayed	Yes when a peak start or a peak maximum is detected. <i>(Delayed)</i>

Parameters for the Fraction Collection Driver

Parameter	Description
FracStartDetected	Set to Yes when a fraction start is detected. When the fraction end is detected, the variable is reset to No . <i>(Delayed)</i>
FracEndDetected	Set to Yes when a fraction start is detected. When the fraction end is detected, the variable is reset to No . <i>(Delayed)</i>
FracTubeChange	Set to Yes when a filled tube is detected. The variable is automatically reset to No . <i>(Delayed)</i>
FractionOn	Set to Yes during fraction collection. <i>(Corresponds to PeakOn, but delayed)</i>
FractionOnUndelayed	Set to Yes during fraction collection. <i>(Corresponds to FractionOn, but not delayed)</i>
TubeCntCurrentFracD	Number of filled tubes in the current fraction. <i>(Delayed)</i>
TubeFilling	Volume collected in the current tube. <i>(Delayed)</i>

In addition to these status variables, various parameters are provided for fraction collection control. For more information about these parameters, refer to  **Setting the Peak Detection Parameters.**

For an overview of the fraction collection topics, refer to  **Actions Related to Fraction Collection.**

Recognizing the Peak Start, Peak Maximum, and Peak End

To ensure that the *➤ Fraction Collector* fills the desired tube, the peak start, peak maximum, and peak end all must be correctly recognized.

Peak Start

The prerequisites for recognizing the peak start (and hence, the beginning of a fraction) are as follows:

- No peak start has been recognized so far.
- Fraction collection, and thus peak detection, is enabled via the **Collect** property.
- 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 condition is fulfilled for at least 1 second.

The **PeakStartThreshold** and **PeakStartSlope** variables influence the peak recognition sensitivity.

The **PeakStartSlope** variable can be changed within broad limits. The higher the value, the later the peak start is recognized. If the detector signal exceeds the **PeakStartThreshold**, the peak is recognized only if the slope threshold value is also exceeded.

Chromeleon remembers a peak start so that a peak maximum can be recognized next. Thus, a peak maximum can be recognized only if a peak start has been detected before.

Peak Maximum

The peak maximum is recognized only if

- A peak start has been detected before.
- No peak maximum has been detected yet.
- The signal slope is greater than the signal slope defined by **PeakMaxSlope**.
- The condition is fulfilled for at least 1 second.

A peak maximum can be recognized only if a peak start has been detected before. Thus, if no peak start has been detected, no maximum will be recognized 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** \Rightarrow *Trigger* will be executed.

Chromeleon remembers a peak maximum so that a peak end can be recognized next. Thus, a peak end can be recognized 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 recognized if:

- The signal slope is less than the signal slope defined by **PeakEndSlope** and
- The signal of the detection channel is less than the signal defined by **ThresholdNoPeakEnd** and
- A peak maximum has been recognized

OR:

- The signal of the detection channel is less than the signal defined by **PeakEndThreshold** and
- A peak maximum has been recognized

OR:

- A peak start has been recognized and
- Data acquisition on the signal channel has been finished

AND:

- The condition is fulfilled for at least 1 second.


The first group of conditions checks whether the signal is below the signal maximum, which is defined by the **ThresholdNoPeakEnd** variable. With heavily overloaded detector signals, there is a lot of signal noise so that a peak end and/or start would be recognized 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 the maximum for the detector signal, this part of the

condition will always be true so that 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 in any case.

The third group of conditions completes the current peak in case the data acquisition is disabled.

For more information, refer to  **Defining the Reactions to Certain Events**.

For an overview of the fraction collection topics, refer to  **Actions Related to Fraction Collection**.

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 a peak start or peak end has been recognized on a channel, the system does not necessarily start or stop collecting a fraction. Whether the system starts or stops collecting, depends on the setting of the **Channel Evaluation** parameter:

Option	Description
All	(Default) The system starts fraction collection when a peak start is detected in all detection channels. And: The system stops fraction collection when the end of the first peak is detected.
Any	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.

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 fraction collector.

Event	Condition	Action
FracStartDetected	A peak start, which means the start of a new fraction, "arrives" at the switching valve/ tube.	Switch to next fraction.* Switch to collect .

Event	Condition	Action
FracEndDetected	A peak end, which means the end of a fraction, "arrives" at the switching valve/ tube.	Switch to waste . Switch to next fraction.*
FracTubeChange	The tube is filled to the limit.	Switch to next tube.

- * 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.

The events are generated with a delay that takes the volume between the detector and the switching valve or tube into account. The following parameters are available:

Parameter	Min.	Max.	Default	Function
DelayTime	0 s	9999 s	0	Time delay between the detector and the switching valve or tube. This parameter cannot be used for flow gradients.


The driver is capable of tracking several peaks in the capillary between the detector and the fraction collector as it internally maintains a queue of peak recognition events.



Tip:

*Do not change the **DelayTime** and/or **PumpDevice** while collecting fractions. Usually this does not make sense either. Performing such a change in the program, for example, when a part of the peak is in the capillary between the detector and the fraction collector, will lead to unexpected results.*

For more information, refer to  **Recognizing the Peak Start, Peak Maximum, and Peak End.**

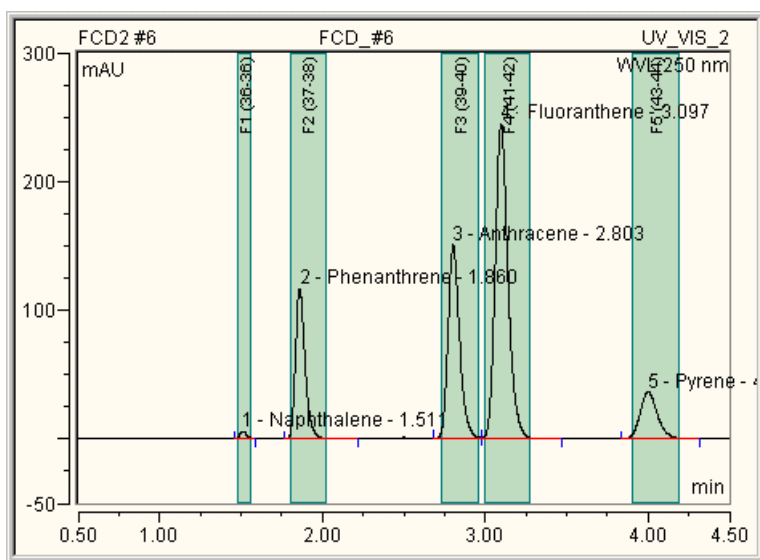
For an overview of the fraction collection topics, refer to  **Actions Related to Fraction Collection.**

Tracking Fraction Collection in the Chromatogram

After you have collected fractions during the analysis, it is important that you can later track which substances have been collected in which tube. This is possible in Chromeleon:

- In the chromatogram
- In the report (see  **Tracking Fraction Collection in the Report.**)

In the chromatogram, you can display which fractions have been collected at which time:



You can adapt the appearance of the display according to your requirements. Select **Decoration...** on the context menu. The **Chromatogram Decoration** dialog box appears.

On the **Fractions** tab page, select the desired options and thus, determine how the fractions are displayed in the chromatogram:

The screenshot shows the 'Chromatogram Decoration' dialog box with the 'Fractions' tab selected. The 'Fraction Visualization' section has 'Vertical stripes' selected with a radio button, and 'Fill stripes/rectangles' checked with a checkbox. 'Rectangles at top' and 'Rectangles at bottom' are unselected. 'Mark tubes inside fractions' is unchecked, and 'Mark tubes outside fractions' is checked. The 'Fraction Labels' section has 'Label Fractions' checked. The 'Formula' field contains: `F{frac.number} ({frac.tube(1).position}-{frac.tube(frac.nTubes).position})`. The 'Label Font' is 'Arial 8' with a 'Change...' button. The 'Label Elevation and Alignment' section has 'Angle' set to '90' degrees, and 'Horizontal and vertical alignment' set to 'Center' and 'Top' respectively. At the bottom are 'OK', 'Cancel', 'Apply', and 'Help' buttons.



Note:

The settings in the picture correspond to the settings used for the above chromatogram.

In the **Fraction Visualization** section, determine how the fractions shall be displayed. Usually, it makes sense to select the **Vertical stripes** and **Fill stripes** options. In this way, you can display the exact time assignment of the single peaks to the corresponding fractions.


In the lower section, determine how the fractions shall be labeled. In order to label fractions, select the **Label Fractions** check box first, and then determine the label in the **Formula** input field. The following formula has proved 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 the tubes 37 and 38. (Note: To mark the single tubes in the chromatogram by dotted lines, select the **Mark tubes inside fraction** or **Mark tubes outside fraction** check box.) The 'F' in the above formula serves to distinguish fraction labels from peak labels.

**Tip:**

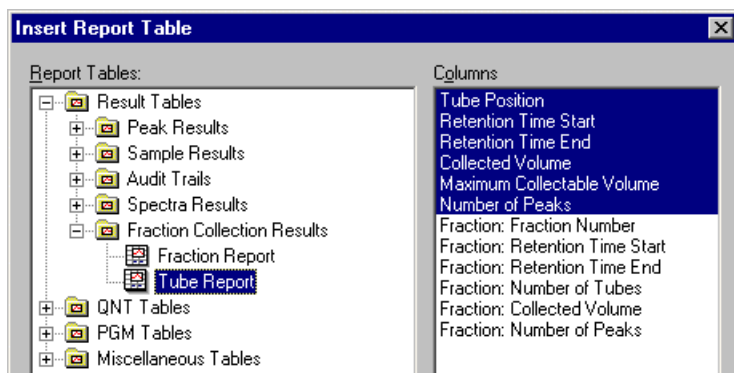
It is also possible to track fraction collection in the chromatogram during data acquisition. However, in this case, several complex settings are required that must be tailored to the individual installation. A description of these settings goes beyond the scope of this online Help. Therefore, if you need more information, please contact your local Dionex representative.

For an overview of the fraction collection topics, refer to  **Actions Related to Fraction Collection**.

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 the desired 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 Manual:

⇒ *'Fraction' Category*

⇒ *'Fraction Tube' Category*

⇒ *'Fraction Detection Parameter' Category*

For an overview of the fraction collection topics, refer to  **Actions Related to Fraction Collection**.

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

;Basic fraction collection parameters:
      Collect =                Off
      CollectOutsidePeaks =    No

;*****
;*Definition of triggers for fraction collection starts here
;*****
;      Definition                copied                from                template
<Timebase>\FractionCollectionTemplate!
Trigger FracStart FracStartDetected
      Z.Nominal =                Z.Nominal
      ;Moves the needle to the upper zero position
      Collect =                On
      ;Switches the fraction collector valve to "Collect" (= on)
EndTrigger

Trigger TubeChange FracTubeChange
      Collect =                Off
      ;Turns off the fraction collector valve
      Tube =                TubePosition
      ;Selects the next tube
      Z.Nominal =                Z.Nominal
      ;Moves the needle to the upper zero position
      Collect =                On
      ;Switches the fraction collector valve to "Collect" (= on)
EndTrigger

Trigger FracEnd FracEndDetcted
      Collect =    Off
      ;Turns off the fraction collector valve
      Tube =                TubePosition
      ;Selects the next tube
      Z.Nominal =                Z.Nominal
      ;Moves the needle to the upper zero position
EndTrigger
;*****
;*Definition of triggers for fraction collection ends here
;*****
```

```

;Maximum filling volume of a tube in ml:
    PumpDevice = "Pump"
    TubeMaxVolume = 10

;Maximum number of installed fraction tubes:
    TotalNumberInstalled = 240

;Basic fraction collection parameters:
    MaxTubesPerFraction = Unlimited
    TubeWrapping = No

;Delay time between detector output and switching valve or tube:
    DelayTime = 0.4
    OffsetTime = 0.0

;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

;Gradient program:
    Flow = 20.000
    %B = 10.0
10.000 %B = 90
13.000 %B = 90

13.000 UV_VIS_1.AcqOff

;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 of the Gilson 215 Liquid Handler whose relay has been named **Collect**. 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  **Actions Related to Fraction Collection**.

**Program Example (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

;Basic fraction collection parameters:
      Collect =                Off
      ChannelEvaluation =       Any
      CollectOutsidePeaks =     No

;*****
;*Definition of triggers for fraction collection starts here
;*****
;Definition                copied                from                template
<Timebase>\FractionCollectionTemplate!
Trigger FracStart FracStartDetected
      Z.Nominal =                Z.Nominal
      ;Moves the needle to the upper zero position
      Collect =                  On
      ;Switches the fraction collector valve to "Collect" (= on)
EndTrigger

Trigger TubeChange FracTubeChange
      Collect =                  Off
      ;Turns off the fraction collector valve
```

```

        Tube =                                TubePosition
        ;Selects the next tube
        Z.Nominal =                            Z.Nominal
        ;Moves the needle to the upper zero position
        Collect =                              Off
        ;Turns on the fraction collector valve
EndTrigger

Trigger FracEnd FracEndDetected
        Collect =                                On
        ;Turns off the fraction collector valve
        Tube =                                TubePosition
        ;Selects the next tube
        Z.Nominal =                            Z.Nominal
        ;Moves the needle to the upper zero position
EndTrigger
;*****
;*Definition of triggers for fraction collection ends here
;*****

;Maximum filling volume of a tube in ml:
        PumpDevice =                            "Pump"
        TubeMaxVolume =                        10

;Maximum number of installed tubes:
        TotalNumberInstalled =                240

;Basic fraction collection parameters:
        MaxTubesPerFraction =                  Unlimited
        TubeWrapping =                        No

;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

;Gradient Program:
      Flow =     20.000
      %B =      10.0
10.000 %B =      90
13.000 %B =      90

13.000  UV_VIS_1.AcqOff

;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 of the Gilson 215 Liquid Handler whose relay has been named **Collect**. 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 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  **Actions Related to Fraction Collection.**

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 ...: Actions Related to 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

;Basic fraction collection parameters:
    Collect =                      Off
    ChannelEvaluation =            Any
    CollectOutsidePeaks =          No

;*****
;*Definition of triggers for fraction collection starts here
;*****
;Definition          copied          from          template
<Timebase>\FractionCollectionTemplate!
Trigger PeakStart FracStartDetected
    Z.Nominal =                    Z.Nominal
    ;Moves the needle to the upper zero position
    Collect =                      On
    ;Switches the collection valve to "Collect" (= on)
EndTrigger
```

```

Trigger TubeChange FracTubeChange
    Collect = Off
    ;Turns off the fraction collector valve
    Tube = TubePosition
    ;Selects the next tube
    Z.Nominal = Z.Nominal
    ;Moves the needle to the upper zero position
    Collect = On
    ;Switches the collection valve to "Collect" (= on)
EndTrigger

Trigger PeakEnd FracEndDetected
    Collect = Off
    ;Turns off the collection valve
    Tube = TubePosition
    ;Selects the next tube
    Z.Nominal = Z.Nominal
    ;Moves the needle to the upper zero position
EndTrigger
;*****
;*Definition of triggers for fraction collection ends here
;*****

;Maximum filling volume of a tube in ml:
    PumpDevice = "Pump"
    TubeMaxVolume = 10

;Maximum number of installed fraction tubes:
    TotalNumberInstalled = 240

;Basic fraction collection parameters:
    MaxTubesPerFraction = Unlimited
    TubeWrapping = No

;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:
    Collect = On
    DetectionChannelN2.Name = "UV_VIS_01"
    ;Slope at peak start [mAU/s]:
    DetectionChannelN2.Name PeakStartSlope = 2.000
    ;Minimum signal height at peak start [mAU]
    DetectionChannelN2.Name PeakStartThreshold = 5000.00
    ;Slope after peak maximum [mAU/s]
    DetectionChannelN2.Name PeakMaxSlope = -5.000
    ;Slope at peak end [mAU/s]
    DetectionChannelN2.Name PeakEndSlope = -4.000
    ;Maximum signal height at peak end [mAU]
    DetectionChannelN2.Name PeakEndThreshold = 5000.00
    ;Signal mst be less than this threshold value
    ;before a new peak start can be recognized [mAU]

```

```

DetectionChannelN2.Name 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

;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

End

```

**Note:**

The trigger block used in this program example refers to the Gilson 215 Liquid Handler. In the example, DetectionChannel1 has not been used as the first detection channel because '1' and 'l' look identical when Courier is the selected font.

If the ➤ *Program* added below is used, fractions would be collected at the following retention times provided a peak occurs within the following mass traces:

Retention time-interval [min]	Channel	Mass [amu]	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 will occur 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 shall 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

How to ...:  **Actions Related to Fraction Collection.**

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 ...: Actions Related to 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 ...: Actions in the Browser**

 **Creating User-defined Columns**). With the program part quoted below, you read out the content of the corresponding user-defined columns during data acquisition and thereby 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 [amu]	Sample 1 /2 Mass [amu]	Sample3/4 Mass [amu]
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  **Actions Related to Fraction Collection**.

Actions in the Virtual Column Separation Simulator

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.

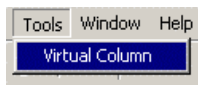


Note:

Results obtained with Virtual Column are intended only 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.

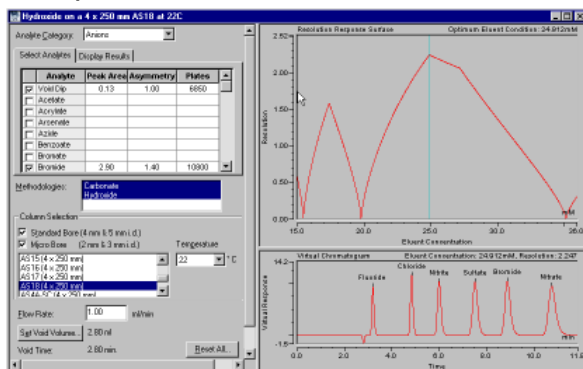
Using Virtual Column (Overview)

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.



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 Product Test Conditions



Manually Selecting an Eluent Condition



Saving and Reloading Virtual Column Settings



Note:

Parts of the Virtual Column software were developed jointly by the Australian Centre for Research on Separation Science (ACROSS) at the University of Tasmania, Australia and Dionex Corporation.



Selecting the Analysis Parameters

The left side of the ➤ *Virtual Column* window provides controls for selecting the desired 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.

	Analyte	Peak Area	Asymmetry	Plates
<input type="checkbox"/>	Void Dip			
<input type="checkbox"/>	Acetate			
<input type="checkbox"/>	Acrylate			
<input type="checkbox"/>	Arsenate			
<input type="checkbox"/>	Azide			
<input type="checkbox"/>	Benzoate			
<input type="checkbox"/>	Bromate			
<input type="checkbox"/>	Bromide			

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 **Plates** 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.

	Analyte	Peak Area	Asymmetry	Plates
<input checked="" type="checkbox"/>	Void Dip			
<input type="checkbox"/>	Acetate			
<input type="checkbox"/>	Acrylate			
<input type="checkbox"/>	Arsenate			
<input type="checkbox"/>	Azide			
<input type="checkbox"/>	Benzoate			
<input type="checkbox"/>	Bromate			
<input checked="" type="checkbox"/>	Bromide			

3. (Optional) Select a methodology.

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 highlight from the methodologies you do not want to use.

Methodologies: Carbonate Hydroxide

Column Selection

☒ Standard Bore (4 mm & 5 mm i.d.)

☒ Micro Bore (2 mm & 3 mm i.d.)

AS12A (4 x 200 mm)

AS14 (4 x 250 mm)

AS14A (3 x 150 mm)

AS15 (4 x 250 mm)

AS16 (4 x 250 mm)

Temperature: °C

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 use.

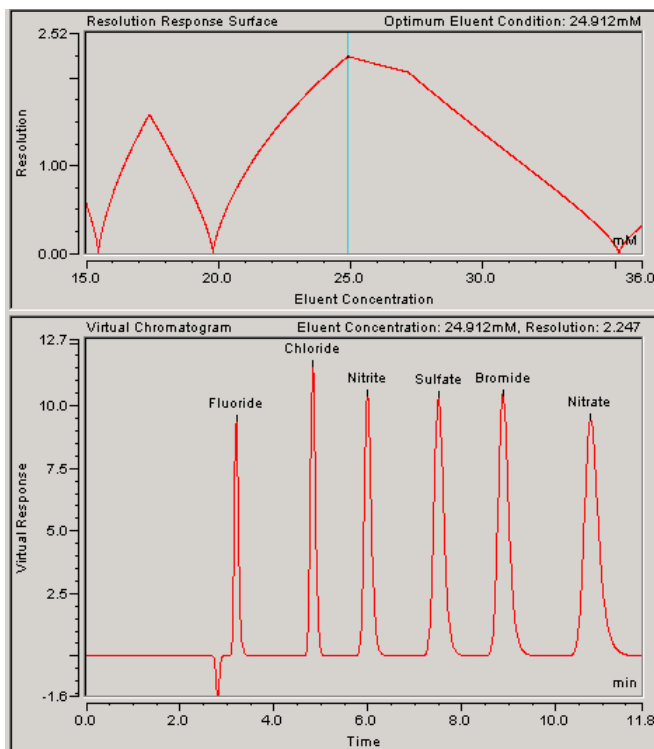
The screenshot shows the 'Methodologies' section with a list box containing 'Carbonate' and 'Hydroxide'. Below it, the 'Column Selection' section has two checked options: 'Standard Bore (4 mm & 5 mm i.d.)' and 'Micro Bore (2 mm & 3 mm i.d.)'. A list of columns is shown: AS14 (4 x 250 mm), AS15 (4 x 250 mm), AS16 (4 x 250 mm), AS18 (4 x 250 mm), and AS4A-SC (4 x 250 mm). To the right, there is a 'Temperature' field set to 22 °C.

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 screenshot shows the 'Column Selection' dialog box. The 'Standard Bore (4 mm & 5 mm i.d.)' option is checked, and 'Micro Bore (2 mm & 3 mm i.d.)' is unchecked. The column list now only shows columns with a 4 mm i.d.: AS15 (4 x 250 mm), AS16 (4 x 250 mm), AS18 (4 x 250 mm), and AS4A-SC (4 x 250 mm). The 'AS18 (4 x 250 mm)' column is highlighted. The 'Temperature' is set to 22 °C. Below the column list, the 'Flow Rate' is set to 1.00 ml/min, 'Set Void Volume...' is 2.80 ml, and 'Void Time' is 2.80 min. A 'Reset All...' button is at the bottom right.

The following example shows the plot and chromatogram for Fluoride, Chloride, Nitrite, Sulfate, Bromide, and Nitrate, when the AS18 column is selected.




6. (Optional) Select a Temperature from the list.

For some columns, you can select a different temperature to view the effect changing the temperature has on the Virtual Chromatogram.

7. (Optional) Enter Peak Area, Asymmetry, and Theoretical Plate Values

After you select the analytes and choose a column, the **Select Analytes** table displays the default *Peak Area*, *Asymmetry*, and *Theoretical Plates* 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**).

8. (Optional) Change the Flow Rate, Void Volume, and Void Time


After you select the analytes and choose a column, a default flow rate, void volume (also called \triangleright Dead Volume), and void time (also called \triangleright Dead Time) for the selected column is displayed. 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 Columnn, 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.

9. (Optional) View the retention data.

To view the calculated retention data, click the **Display Results** tab. See **How to ...: Actions in the Virtual Column Tool**  **Viewing the Results Table** for details.

Select Analytes		Display Results		
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		

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, asymmetry, and theoretical plates) and on the selected column, temperature, void time, and eluent conditions.

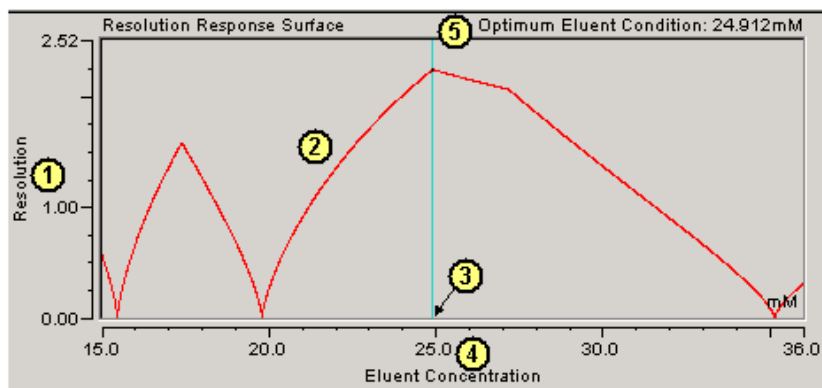
- *Retention Time* is the time (in minutes) since injection.
- *Retention Factor* (also called Capacity Factor) is the ratio of the net retention time to the void time (also called ➤ *Dead Time*).
- *Resolution* is the degree of separation between the current peak and the next peak in the chromatogram.

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

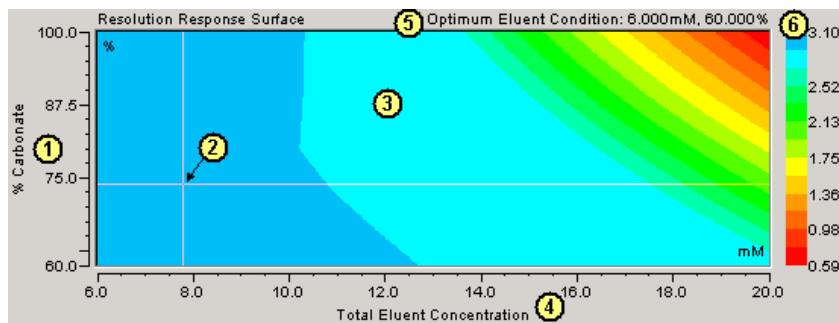
③ Currently selected eluent concentration (the concentration at which the Virtual Chromatogram is modeled)

④ Range of eluent concentrations available in the Virtual Column database for the selected column

⑤ Optimum eluent concentration, based on the current analyte, 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.

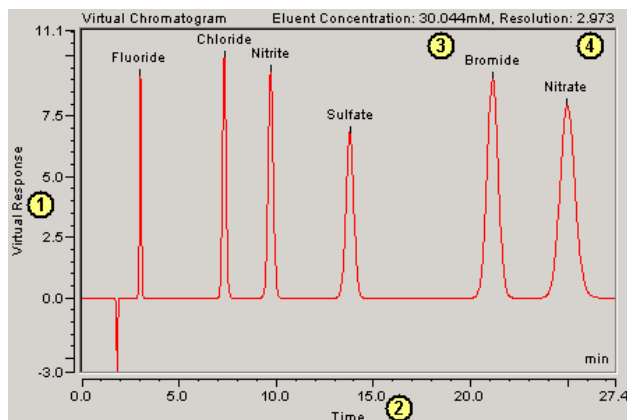


- | | |
|--|---|
| ① Percentage of carbonate in the eluent | ④ Range of eluent concentrations available in the Virtual Column database for the selected column |
| ② Currently selected eluent condition (the condition at which the Virtual Chromatogram is modeled) | ⑤ Optimum eluent condition, based on the current analyte, column, void time, temperature, and resolution criterion selections |
| ③ Plot of the lowest resolution value found at each eluent condition | ⑥ Range of resolutions (and the color assignment) of the least resolved peak pairs in the Virtual Chromatogram |

Viewing the Virtual Chromatogram

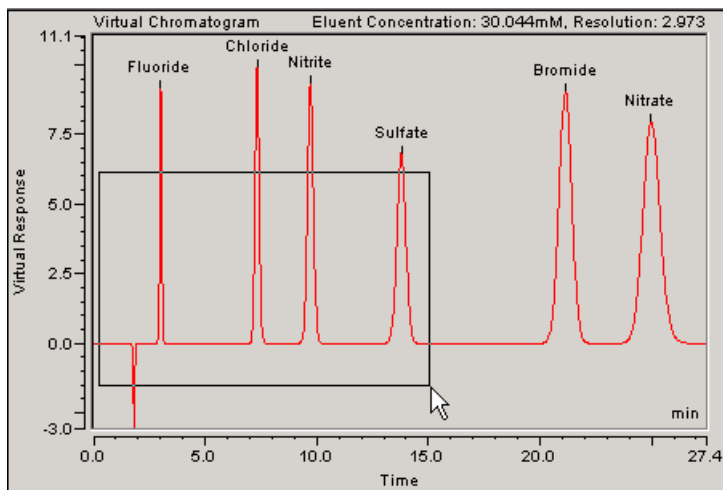
The Virtual Chromatogram simulates an actual analysis using the currently selected analytes, column, void time, temperature, resolution criterion, and eluent condition. The Virtual Chromatogram is updated whenever the selected analytes or other parameters are changed.

The figure below describes the features of the Virtual Chromatogram. In this example, the **Minimum Resolution** criterion is selected.

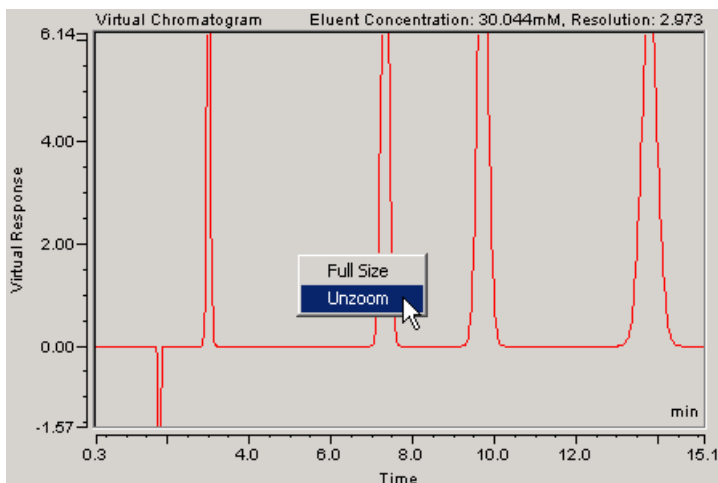


Zooming/Unzooming

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**, or select **Full Size** to display the full chromatogram in the pane.



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**

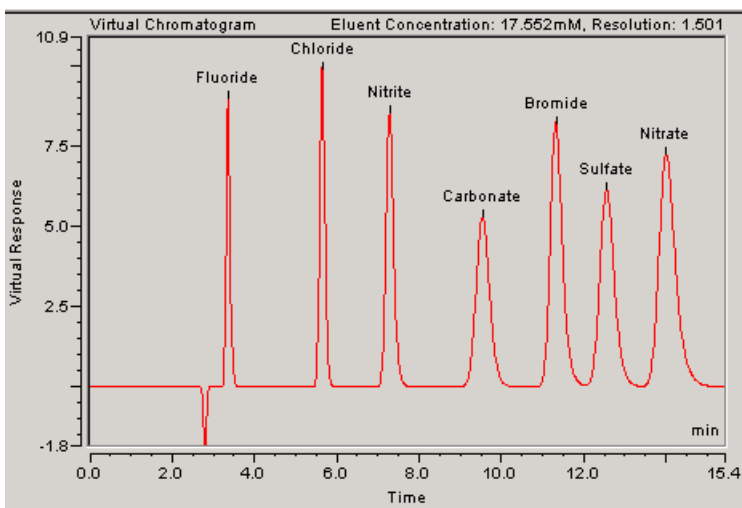
To select a resolution criterion, right-click on the **Resolution Response Surface** and select a criterion on the menu, or select on the **Criterion** menu on the Chromeleon menu bar.

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 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

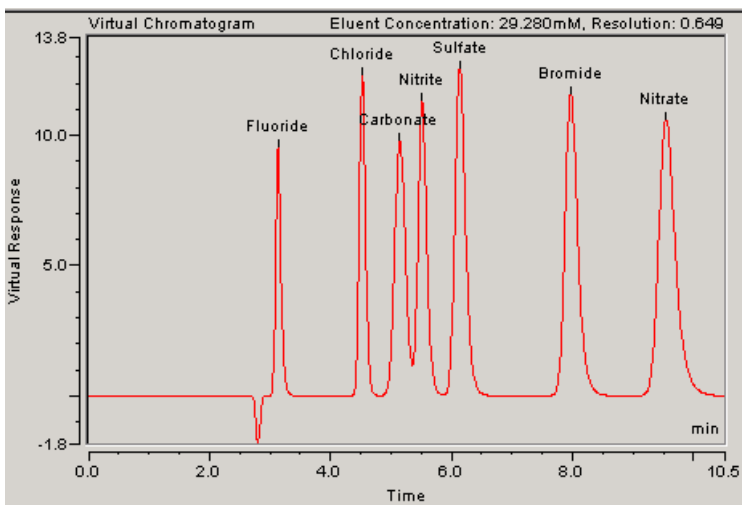
Product may find a chromatogram with evenly spaced peaks, but the peaks may not all be resolved.

The normalized resolution product (r) is defined by the following equation:

$$r = \prod_{i=1}^{n-1} \left(\frac{R_{s_{i,i+1}}}{\frac{1}{n-1} \sum_{i=1}^{n-1} R_{s_{i,i+1}}} \right)$$

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.

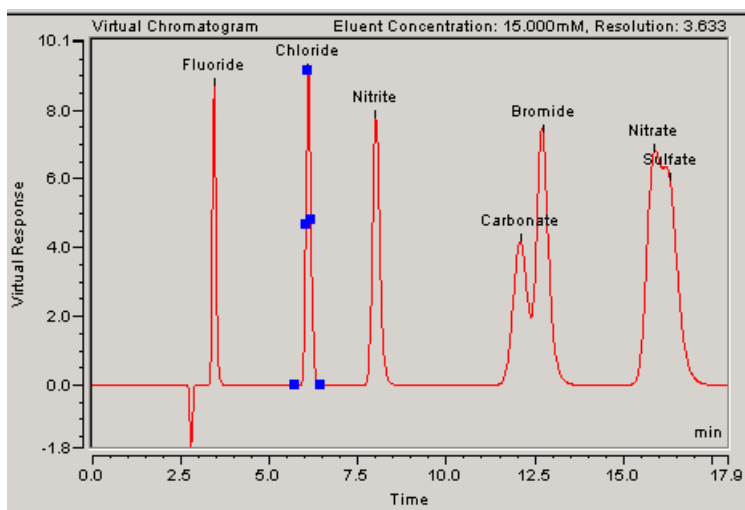


Resolution Optimized for Analyte

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.

Select Analytes		Display Results	
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:**

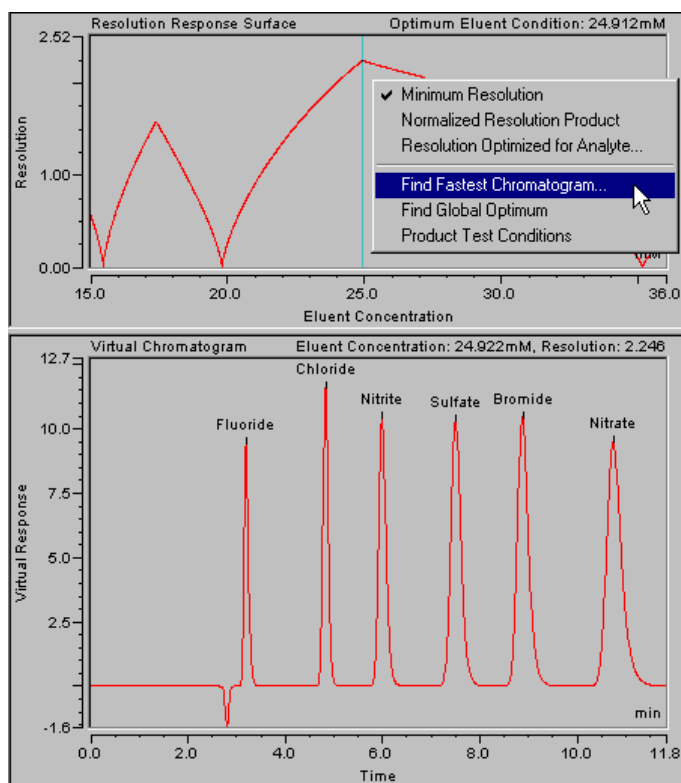
When **Resolution Optimized for Analyte** is selected, you can select a different analyte to be optimized by double-clicking the analyte peak on the Virtual Chromatogram.

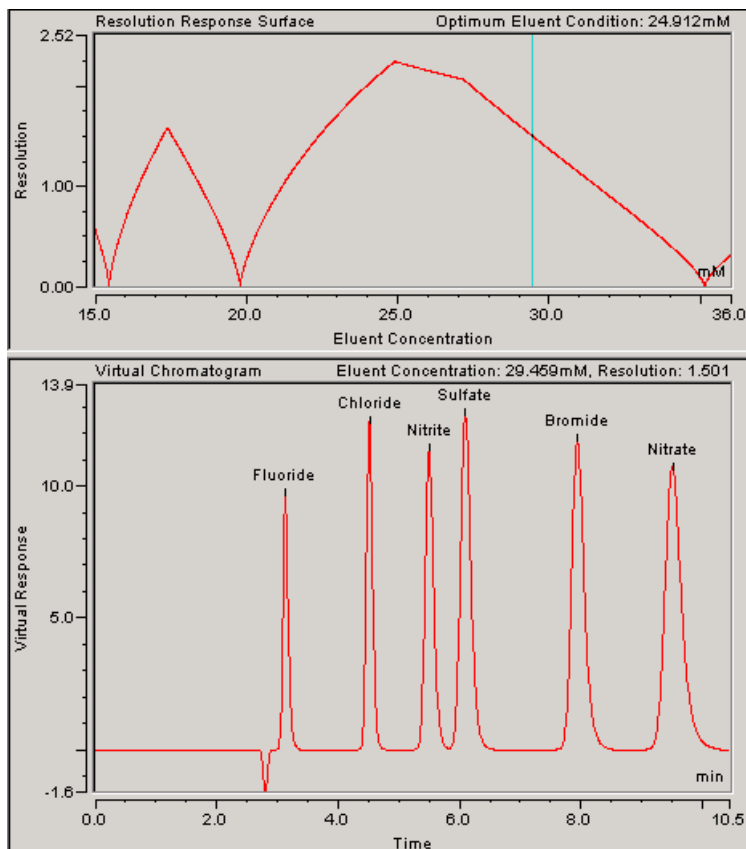
Finding the Fastest Chromatogram

➤ *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 Product Test Conditions


The  **Virtual Column Product Test Conditions** option sets the eluent composition to that specified in the production control test performed on every column before it is shipped. For details about the production test conditions, refer to the column manual. To select this option, right-click on the **Resolution Response Surface** and select **Product Test Conditions** on the menu, or select the option on the **Criterion** menu.



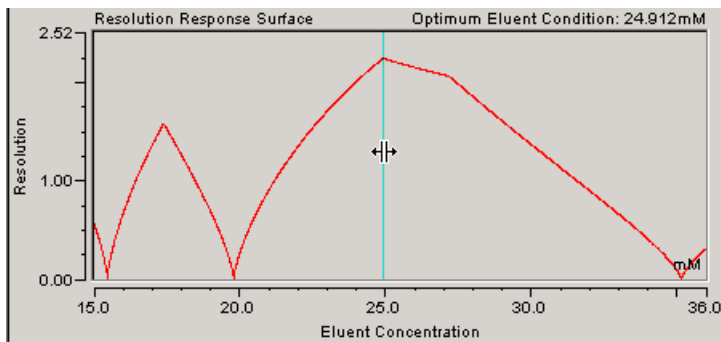
Note:

*The **Product Test Conditions** option only changes the eluent composition; it does not affect the analyte selections. If the production test chromatogram includes more analytes than those selected for display in Virtual Column, the missing analytes are not automatically added to the Virtual Chromatogram.*

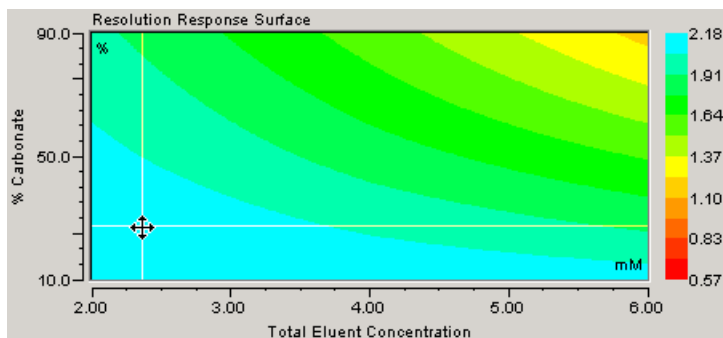
Manually Selecting an Eluent Condition

To determine the optimum eluent condition, you can select an option (**Find Fastest Chromatogram**, **Global Optimum**, or **Product Test Conditions**) on the  **Virtual Column Criterion** menu, or you can manually select the eluent condition on the Resolution Response Surface.

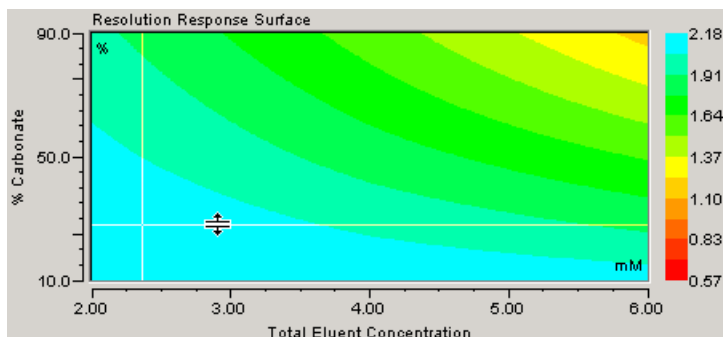
To manually select the eluent condition if you are working with a single-species eluent, drag the single vertical bar to the desired eluent concentration (see the example below), or click on the plot where you want to move the bar. The virtual chromatogram is updated to reflect the new concentration.



If you are working with a dual-species eluent, two eluent selection bars are provided: one for concentration and one for percent carbonate. 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 cursor 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 percentage of carbonate (see the example below) or drag the vertical bar to change the total 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, asymmetry, and theoretical plates
- Column name and size
- Temperature
- System void time

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 saved file is assigned a .vcol extension.



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**, **Asymmetry**, or **Plates**) that you do not want loaded.
4. Clear the **Void Time** check box if you do not want the void time loaded.




Note:

*If you clear a check box in the **Load Selected File** dialog box, Virtual Column loads data for the item from the embedded Virtual Column database instead of from the saved file.*

5. Click **OK**.

Device Control

Device Control (Overview)

In addition to the standard commands of the *Program*, which are easily created with the Program Wizard (see **Programmed Control**  **The Program Wizard**), Chromeleon supports various additional *Control Commands*.

For more information about these commands and the command syntax, refer to:



Control: Pumps



Control: Autosamplers



Control: Detectors



Control: IC



Control: GC and Temperature



Control: Component Controller



Control: Special Commands, Relays, and Others

- 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.

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:

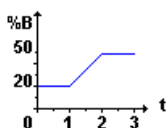
The pressure unit (bar, MPa, or psi) depends on the pump type.

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



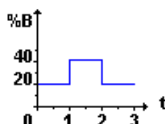
Tip:

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 $t=1\text{ min}$).

```
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
```

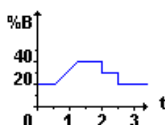
Rectangle Profile



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 $\Rightarrow \%B, \%C, \%D$) or the flow rate can be changed.

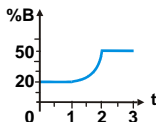
Creating a Non-Linear Gradient Ramp (Dionex GP40/GP50/GS50 pumps only)

A Curve command (also, refer to \triangleright *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

For non-controlled pumps, the \Rightarrow Flow rate is set directly on the instrument (also, refer to the Operating Instructions of the instrument). For controlled pumps, the flow rate can be 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 desired flow rate value 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 \triangleright Program (see **How to ...: Actions in the PGM Editor**  **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 \triangleright Flow Gradient. For a description on how to enter gradients, refer to **How to ...: Device Control**  **Determining a Gradient**.

Determining the Solvent Composition

Manually

For non-controlled pumps, solvent composition (see \Rightarrow %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 \Rightarrow Flow on the **Control** menu (also, see **How to ...: Device Control**  **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 ...: Actions in the PGM Editor**
 **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:

- ➔ *Flow* and ➤ *%* 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 the desired 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 **Programmed Control**  **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 of 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 wrong, 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 \gg *Program* syntax, you can directly determine the gradient in the program. Entering the \Rightarrow %B, %C, %D, \Rightarrow 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.

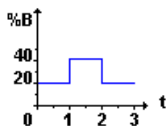


Tip:

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).

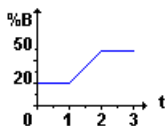
Example: The three different profiles are realized via the commands listed below the profiles.

Rectangle Profile



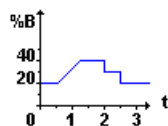
```
0.000 %B.Value = 20
1.000 %B.Value = 20
1.000 %B.Value = 40
2.000 %B.Value = 40
2.000 %B.Value = 20
```

Ramp Profile



```
0.000 %B.Value = 20
1.000 %B.Value = 20
2.000 %B.Value = 50
```

Multi-Step Profile




```
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
```

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 used control panel which control realizes this setting.


- Determine the pressure via the corresponding slider or type the value in the input field.

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 cursor. 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 ...: Actions on the Control Panel**  **Modifying a Control**).

If defined \Rightarrow *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 \Rightarrow *Audit Trail*.

It is also possible to determine the pressure limits by entering the following commands in the \Rightarrow *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 \Rightarrow *StopFlow* to stop the pump flow and the \Rightarrow *Gradient* formation. During a running \Rightarrow *Batch*, batch processing is stopped.
- Select \Rightarrow *Continue* to resume the pump flow, a running gradient program, or batch processing.



Tip:

The \Rightarrow Hold command interrupts gradient formation and automatic batch processing, but not the pump flow.

Holding the Pump Flow

- Select \Rightarrow *Hold* to interrupt \Rightarrow *Gradient* formation or a running batch. During a running \Rightarrow *Batch*, batch processing is stopped.

In **Hold** mode, the pump delivers a constant flow rate and solvent composition until a stop or a \Rightarrow *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, we recommend 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 **How to ...: Device Control**  **Program Examples for Virtual Channels.***

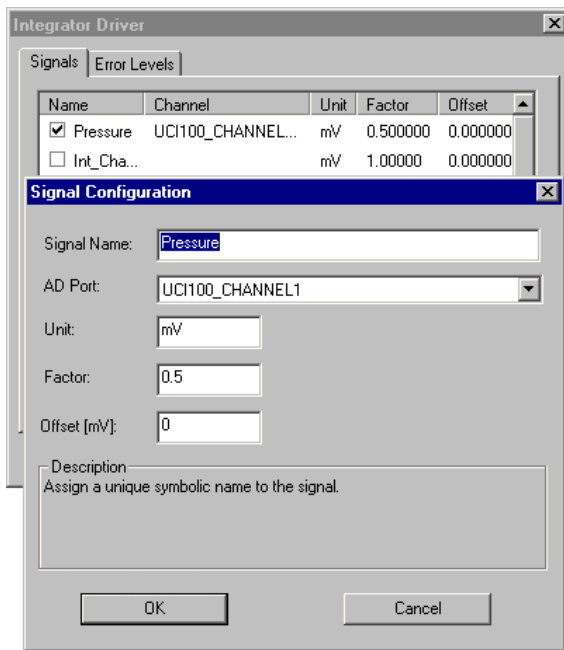
Server Configuration

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 the desired signal in the **Available Signals** field (that is, for the above example, select **Pressure**).
- When starting the data acquisition, select the **Pump_Pressure** channel and define the **Step** (see **How to ...: Device Control**  **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 ...: Actions on 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.

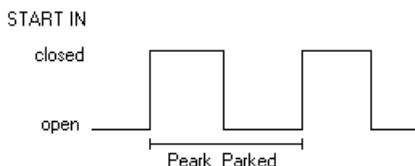
Select **ParkPercentage** to enable and disable peak parking. The following value disables peak parking:

```
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.

Control: Autosamplers

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 (AS50)



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 (AS50)



Overlapping Samples



Injecting Two Samples Simultaneously



Opening the AS50 Door during Operation



Monitoring the Status of the AS50



Autosampler Commands (GINA 50)

Suck (see \Rightarrow Draw)/ \Rightarrow 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 \Rightarrow 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     $\Rightarrow$ Inject
```

Description of the program part: First, the Autosampler GINA 50 draws 30 µl solution from \Rightarrow 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:

Only use the **Segment** command in combination with the **Dispense** command, as otherwise the sucked air volume is also injected.

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 \Rightarrow 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 \Rightarrow *NeedleUp* command to lift the sample needle. When lifting 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 the solvent flow through the sample loop again (for more information see \Rightarrow *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 or GINA 160 samplers, refer to **Control**  **Dionex GINA 50 and GINA 160 Autosamplers**. Also, refer to **How to: Device Control**  **Autosampler Commands (ASI-100 Series)**.

Autosampler Commands (ASI-100 Series)

Many commands of the Dionex ASI-100 \Rightarrow *Autosamplers* (ASI-100 T and ASI-100 PT= with temperature control) are similar to those of the GINA 50 (see **How to: Device Control**  **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 \Rightarrow *Positions* are indicated as follows:

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 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 actual 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 \Rightarrow *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    $\Rightarrow$ Inject         Position = PrepVial

```

Description of the program part: First, the autosampler draws 30 μ l of solution from the current sample vial, and, upon completion, 30 μ l 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 Device Control**  **Dionex ASI-100 Autosamplers**. Also, refer to **How to: Device Control**  **Autosampler Commands (AS50)**.

Autosampler Commands (AS50)

Sample Loading and Injection

In the AS50, 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 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 AS50 control panel sends both the **Load** and the **Inject** commands to the autosampler.

**Note:**

If the AS50 is in simultaneous injection mode, separate `Valve.LoadPosition` and `Valve.InjectPosition` commands are required (in addition to the `Load` and `Inject` commands). See the description of simultaneous injections below for details.

Flush the Inject Port

The **Flush** and **Wait** commands must be in the order shown and they must occur before any other ➤*Autosampler* commands, or after all other autosampler commands.

```
Flush      Volume = Value [µl]
Wait      FlushState
```

Autosampler Options

All 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 AS50 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 Prep

All AS50 autosampler sample prep commands (`Pipet`, `Mix`, `FlushSP`, `DelaySP`, `SetNeedleHeight`, `Dilute`, and `Dispense`) must be grouped together in the program. Place them at the beginning of the program, after the 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 only available if the AS50 is equipped with the sample preparation option.

In the example below, 20 μ l are pipetted from vial 1 and delivered to vial 10. 200 μ l are dispensed from reservoir A into vial 10. The contents of vial 10 are then mixed by drawing in and then expelling 100 μ l 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              SamplePrep
```

Also, refer to  **Defining Sample Preparation Steps.**

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 μ l 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 AS50 **Sample Overlap** option is enabled in the Server Configuration, the AS50 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**)

When the AS50 is preparing a sample, the **Status** field in the sequence displays **Preparing** and the sample line is highlighted in yellow. A message is also logged in the Audit Trail.

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 Injection

If the AS50 is equipped with the simultaneous injection mode option, 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, the AS50 is connected to two ICS-2000 systems. Each ICS-2000 is equipped with an injection valve, and there are no injection valves 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:

If the AS50 is not in simultaneous injection mode, separate Valve.LoadPosition and Valve.InjectPosition commands are not required. In this case, the valve position is switched automatically at the appropriate times when the Load and Inject commands are issued.

For an overview of the individual commands for the AS50 autosampler, refer to **Commands for Device Control**  **Dionex AS50 Autosampler**.

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 nl

```
;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
; creates 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
      ; creates 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
      ; creates 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 Device Control**



Dionex/LC Packings FAMOS Autosampler.

For information about the sample preparation commands supported by the FAMOS autosampler, refer to **Commands for Device Control**



Dionex/LC Packings FAMOS Autosampler: Sample Preparation.

Injecting a Sample

It depends on the laboratory equipment how the injection is performed:

- Manually
- Via an ➤ *Autosampler*
- Automatically (the injection is programmed).

Click the corresponding control on the control panel or select **Inject...** on the **Control** menu to open the **Inject** dialog box. Determine how much of a substance is injected (⇒ *Volume*) and from which Autosampler ⇒ *Position*.

Then, issue the ⇒ *Inject* command.

Reporting the end of the injection process to Chromeleon completes successful injection. If a hand-operated valve is used, this is via a contact closure relay. Modern autosamplers automatically send the message via the serial interface or a ➤ *DX-LAN*. When Chromeleon has received this message, the retention time is started.

Hand-operated valve (manual injection):


A µl-syringe is used to inject the sample into the needle seat of the hand-operated valve. In this way, it reaches the sample loop (**Load**). By switching the valve (**Inject**), the solvent flow is directed to the sample loop, and the sample enters the high-pressure circuit of the system. If correctly

connected (via a ➤*Remote Input*), switching the hand-operated valve triggers the **Inject** signal and thus the timer. Very exact operation is possible if the injected volume corresponds to the sample loop size. In this case, the sample loop is completely filled. When switching the valve, the exact volume of the sample loop is injected without any loss.

Autosampler (manual injection):

The autosampler can be operated via the input panel on the instrument or via the PC. Controlling the autosampler via the PC, is a very convenient method. However, this is only possible if the autosampler is connected with Chromeleon via an RS-232 interface, the DX-LAN, or USB (Universal Serial Bus).

- Select ➤*Inject* on the **Control** menu and determine the ➤*Volume* and the ➤*Autosampler* position for the injection.
- If your control panel provides the corresponding controls, enter the **Volume** and **Position** parameters directly in the corresponding edit box.

The time required by the autosampler to inject the sample can be indicated optically. 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**). In a controlled system, the pump is set to ➤*Hold* during injection.

Automatic injection:

- Enter the **Inject** command at the time $t = 0.000$ in the ➤*Program*.


If the command is executed by an autosampler that is controlled by Chromeleon, the autosampler returns a signal to Chromeleon when injection is completed. Then, the timer is started.



Tip:

With the AS50 autosampler, sample loading and injection are two distinct events. Therefore, include a **Load** command in the program at $t = 0.000$ and then the **Inject** command. See  **Autosampler Commands (AS50)** for details.

When injecting via a hand-operated valve or an autosampler that is not controlled by Chromeleon, the data system also waits for a signal before it starts the timer. For example, with a hand-operated valve, the signal is

returned after the valve has switched from **Load** to **Inject**, that is, 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 remote inputs (TTL or relay) and configuration of a remote inject device. See **How to ...: Device Control**  **Setting Up Remote Injection**.

**Tip:**

*As an **Inject** signal is not specified, there can only be one injection unit per timebase, that is, install either a hand-operated valve or an autosampler.*



Setting Up Remote Injection

If Chromeleon does not directly control the injection valve, a ➤*Remote Inputs* (remote start) device can be set up that communicates to Chromeleon that injection was performed. The setup procedure is as follows: Connect the remote input device via TTL or relay, install and configure a remote inject device in the ➤*Timebase*, and 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*. Only then, the device can communicate to Chromeleon that injection was performed. When injection is performed, the injection valve or autosampler sends a signal to the remote input device and the remote input device then communicates to Chromeleon that injection was performed.

Example Connections: AS40 and DX-120

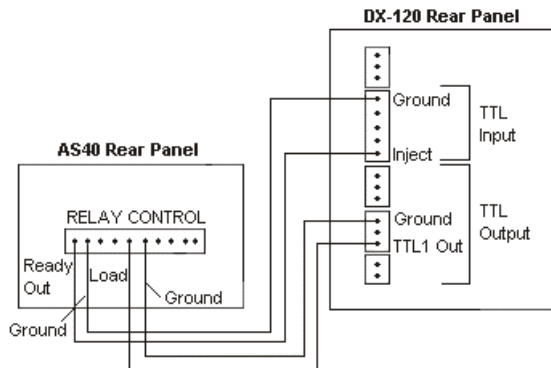


Tip:

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 and 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. First, the syringe is filled 5 times with washing liquid. Then, the procedure is repeated using eluent.

Before executing the PrimeSyringe command:

- Make sure that **the pump flow is off**. Else, the pump would deliver eluent to the wash vial when the injection valve switches into 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 μ L	250 μ L	1000 μ L*	2500 μ L*
WashSpeed [μ L/s]	5	10	25	25
DispSpeed [μ L/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 1000 μ l)	approx. 9 (5 x 2500 μ l)

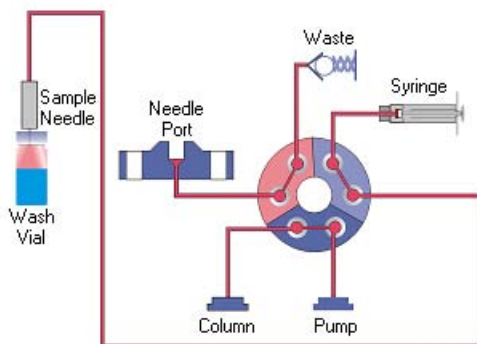
* Usually no PrimeSyringe command is required to remove air bubbles from syringes with a volume of 1000 or 2500 μ L. It is usually sufficient to inject the entire syringe volume of eluent.

- Fill the wash vial with the desired washing liquid. Enter the vial position in Chromeleon (**WashVial**).

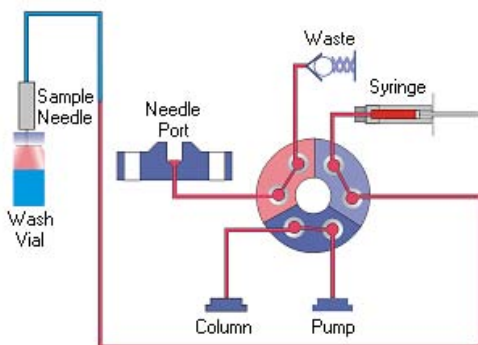
We recommend using isopropanol (2-propanol) for the PrimeSyringe cycle and repeating the cycle with the starting eluent of the next sample.

The ASI-100 automatically performs the following steps:

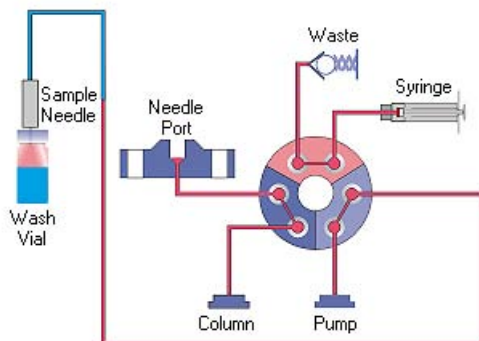
1. The injection valve is switched into the Load position. The eluent flow is from the pump through the valve directly to the column; the eluent does not get into contact with the needle or the syringe. In addition, the needle descends into the wash vial:



2. The syringe is completely filled:

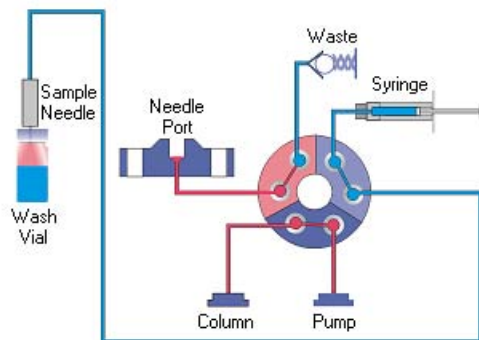


- The injection valve is switched into the Inject position; the needle remains in the wash vial and the contents of the syringe is dispensed into the waste:



- The injection valve is switched into the Load position

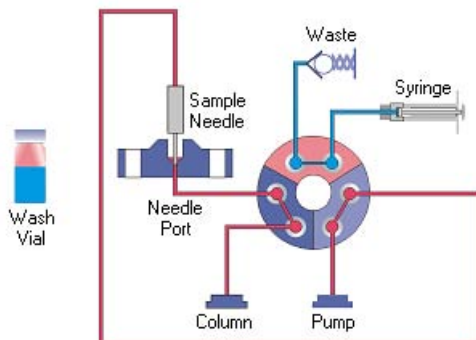
The above steps are performed five times. The washing liquid reaches the syringe, removes existing air bubbles, and exchange the liquid in the syringe:



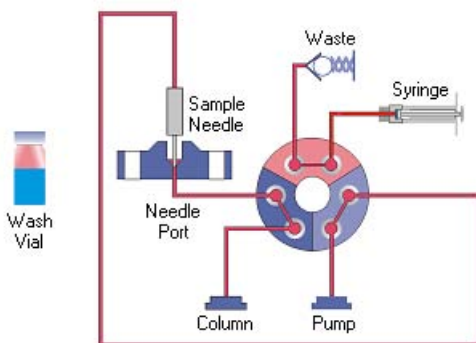
Tip:

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. After some time, the needle and the needle port are filled again with eluent. However, there is still washing liquid between the syringe and the waste:



Therefore, repeat the procedure using the eluent to precondition the valve and syringe with the eluent, too. (Note: Use the starting eluent without buffer additives to avoid deposits.)



Defining Sample Preparation Steps (AS50)

AS50 ➤ *Autosampler* sample preparation steps 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 **AS50 Sample Prep 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 the **Insert** button 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 the **Enter** button.



Tip:

To delete a step, select it, and click the **Delete** button.

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	Mixes 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

Sample Prep Functions	Description	Parameters
Delay	Pause sample preparation	Delay Time: the number of minutes to pause
Needle	Position needle above vial bottom	Height: the distance (mm) from the vial bottom
Dilute	(Available only with the AS50 Sample Preparation option) Dilute sample with reagent	<p>Concentrate Source: the vial that contains the concentrated sample</p> <p>Concentrate Volume: the amount of concentrated sample to be diluted</p> <p>Diluent Source: the reservoir that contains the diluent</p> <p>Diluent Volume: the amount of diluent to be used</p> <p>Destination: the vial into which the concentrated sample and diluent are added</p>
Dispense	(Available only with the AS50 Sample Preparation option) Dispense reagent to a vial	<p>Source: the reservoir from which reagent is dispensed</p> <p>Volume: the amount of reagent to dispense</p> <p>Destination: the vial into which reagent is dispensed</p>

Also, refer to:



Entering Sample Preparation Vial Positions



Overlapping Samples



Entering Sample Preparation Vial Positions

In AS50 autosampler sample preparation operations, 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 allow the currently running sample to finish. 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 \Rightarrow **Status Running**. The sample being simultaneously prepared has a yellow background and the status **Preparing**. This sample has not yet been injected. Therefore, the \Rightarrow **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
	 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.

Details about how overlapping samples is performed and which commands are required depend on the device.

For more information, refer to:

 **Autosampler Commands (AS50)**

 **Defining Sample Preparation Steps (AS50)**

 **Control: Component Controller**

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 AS50 equipped with the simultaneous injection option) 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 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, detector signals, TTLs, relays, 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 unknowns 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 **Control: Autosamplers**  **Autosampler Commands (AS50)** and **Actions in the QNT Editor/Creating a Peak Table**  **Defining the QNT Method for Several Detectors.**

Opening the AS50 Door during Operation

Normally, the AS50 *➤Autosampler* door must remain closed while the 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 AS50 front panel. If the AS50 is busy, a message in the *➤Audit Trail* window informs you that it is currently not safe to open the door.

When the AS50 is not 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 allow the timer to expire or press any button on the AS50'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 ...: Actions on the Control Panel***




Linking a Control to a Device.

Monitoring the Status of the AS50








The AS50 ➤ *Autosampler Status* control on a ➤ *Control Panel* displays the action currently being performed by the 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**.

Control: Detectors

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**
-  **Controlling a Suppressor**

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              =0.25 / Value [s]
```



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, this will use unnecessary disc capacity and the integration might become incorrect, especially with increased baseline noise. This may also happen when using the Step Auto.



Note:

To realize ➤Wavelength Switching, a new wavelength is defined 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
```

Switching Detector Lamps On/Off (AD25 and PDA-100 Detector)

```
0.000    Devicename.UV_Lamp             =On / Off
0.000    Devicename.Visible_Lamp        =On / Off
```

Selecting the Detection Mode (ED40/ED50/ED50A Detector)

```
0.000    Devicename.Mode                 =Conductivity / IntAmp / DCAmp
```

Turning the Amperometry Cell On/Off (ED40/ED50/ED50A Detector in Amperometry Mode)

0.000 Devicename.Cell =On / Off

Selecting the Electrode (ED40/ED50/ED50A Detector in Amperometry Mode)

0.000 Devicename.Electrode =pH / AgCL

Starting Data Acquisition

- Click the \Rightarrow 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, Autozero, 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 cursor to the signal plot of 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 **How to ...: Device Control**  **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 Param.	Description	Comment
⇒ <i>Average</i>	Averaging.	Use this parameter to improve the signal-to-noise ratio.
⇒ <i>Bandwidth</i>	Changes the bandwidth with which the signal is recorded.	If necessary, increase the bandwidth to improve the ➤ <i>Signal to Noise Ratio</i> .
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.
➤ <i>Polarity</i>	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 ➤ <i>Signal</i> Use UpperLimit and LowerLimit to set the signal acquisition limits.	If signal acquisition limits are not met, the running batch is stopped and the ➤ <i>Emergency Program</i> is started.
⇒ <i>Step</i>	Changes the step.	Select the step such that the narrowest peak contains approximately 20 datapoints
⇒ <i>RefBandwidth</i> (<i>Reference Bandwidth</i>)	Changes the reference bandwidth.	If necessary, increase the bandwidth to improve the signal-to-noise ratio.
⇒ <i>Wavelength</i>	Changes the recorded wavelength.	Note the possibilities for wavelength switching (see How to ...: Device Control  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 Param.	Description	Comment
⇒ <i>BunchWidth</i>	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.
⇒ <i>RefWavelength</i> (<i>Reference Wavelength</i>)	Changes the reference wavelength.	If necessary, increase the bandwidth to improve the ➤ <i>Signal-to-Noise Ratio</i> .
⇒ <i>RefBandwidth</i> (<i>Reference Bandwidth</i>)	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.
⇒ <i>Step</i>	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. All RF2000 spectra are added to this library.

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 ➤ <i>Filter</i> 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):															
		<table border="1"> <thead> <tr> <th></th><th>Polarity</th><th>AQAmx (V)</th></tr> </thead> <tbody> <tr> <td>1</td><td>+ve</td><td>100</td></tr> <tr> <td>2</td><td>-ve</td><td>100</td></tr> <tr> <td>3</td><td></td><td>0</td></tr> <tr> <td>4</td><td></td><td>0</td></tr> </tbody> </table>		Polarity	AQAmx (V)	1	+ve	100	2	-ve	100	3		0	4		0
	Polarity	AQAmx (V)															
1	+ve	100															
2	-ve	100															
3		0															
4		0															
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 ➤ <i>Signal</i> . UpperLimit and LowerLimit define the signal acquisition limits.	If signal acquisition limits are not met, the running batch is stopped and the emergency program is started.

Defining a Waveform

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 (see **Programmed Control**  **The Program Wizard**) or entered manually into the program (see **How to ...: Actions in the PGM Editor**  **Manually Editing a PGM File in the Commands View**).

Selecting a Preprogrammed Waveform from the Program Wizard

- 1 In the Select Waveform Options dialog box of the Program Wizard, select a preprogrammed waveform (any entry except **New**) from the list and click **OK**.

The selected waveform must match the electrode. Refer to the following table for guidelines.

Waveform	Working Electrode*	Reference Mode**	Comments
Carbohydrates (std. quad. potential)	Au (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.

Waveform	Working Electrode*	Reference Mode**	Comments
Amino Acids (pH reference)	Au (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 (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).
Carbohydrates (triple potential)	Au (P/N 044112)	Ag/AgCl	Discontinued waveform. DO NOT use with disposable gold electrodes; dissolves gold and can cause quantitation 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.

2 Click **Next>**.

The Waveform Options dialog box appears. The selected waveform's time, potential, and integration settings are displayed in the waveform table. If necessary, the settings can be modified now. The waveform can also be modified in the resulting program after completing the Program Wizard.


3 Select the **Reference Electrode Mode**.

4 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, refer to  **Selecting the Reference Electrode Mode with Alkaline Eluents** below.

Defining a Custom Waveform from the Program Wizard

- 1 In the Select Waveform Options dialog box of the Program Wizard, select **New** and click **OK**.

The Waveform Options dialog box appears.

- 2 In the **Time (Sec)** box, type or select the time to begin the first waveform step.
- 3 In the **Potential (V)** box, type or select the potential to apply between the reference and working electrode.
- 4 In the **Integration** box, select an  *Integration* option: **Begin** starts integration at this step, **End** stops integration, **No-Change** keeps the setting selected in the previous step.



Tip:

Each waveform must have one integration period. The integration period cannot begin or end at Time 0 or at the last step in the waveform.

- 5 Click the **Enter** button.

The step is added to the waveform table.

- 6 Repeat the above steps for each subsequent step in the waveform to create the potential vs. time plot.




Tips:

*To delete a step, select it in the waveform table, and click the **Delete** button.*

To edit an existing step, select it in the waveform table; change the time, potential, and/or integration settings; and press <Enter>.


Selecting the Reference Electrode Mode with Alkaline Eluents

The reference electrode inside the 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 two important consequences:

- The Ag/AgCl electrode is used in both reference modes (Ag and pH).
- The pH readout matters, even if the reference mode is Ag.

When used in 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 (for more information, refer to **How to ...: Actions in the Browser**  **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.



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, to 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.

Defining Step and Average

The time interval between two recorded data points is referred to as \Rightarrow *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, we recommend 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, we recommend that you select a fixed step because an automatic step may result in a faulty integration.

In combination with the \Rightarrow *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.*

The PDA-100 detector allows 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

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, and ICS-1000/1500/2000	SRS, AES

Controlling an SRS with a CD20, CD25, ED40, ED50, IC20, or IC25 Detector

Use the following command to set the current applied to the SRS suppressor:

SRS_Current = Off, 50, 100, 300, 500 (mA)

Controlling an AES or SRS with a CD25A, ED50A, IC25A Detector, or ICS1000/1500/2000

Use the following command to select the suppressor:

```
Suppressor_Type           =AAES,  CAES,  ASRS_4mm,  ASRS_2mm,
                           CSRS_4mm, CSRS_2mm, SRS-MPIC
```

Use the following command to select the current applied to the suppressor:

```
Suppressor_Current       =mA
```

For example:

```
Suppressor_Current       =100 mA
Suppressor_Current       =20  mA
```

The following table lists the operating current range for the suppressors.

Suppressor Type	Current Range
AAES	=0.0...150 (mA)
CAES	=0.0...150 (mA)
ASRS	=0.0...500 (mA)
ASRS	=0.0...150 (mA)
CSRS	=0.0...300 (mA)
CSRS	=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 one of these topics in **How to ...: Device Control**:



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 **Programmed Control**  **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 operation currents lead 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 Na ₂ CO ₃ /1.7 mM NaHCO ₃	2.00	36
2-mm AS4A-SC	1.8 mM Na ₂ CO ₃ /1.7 mM NaHCO ₃	0.50	9
4-mm AS9-HC	9.0 mM Na ₂ CO ₃	1.00	60
2-mm AS9-HC	9.0 mM Na ₂ CO ₃	0.25	15
4-mm AS14	3.5 mM Na ₂ CO ₃ /1.0 mM NaHCO ₃	1.20	32
2-mm AS14	3.5 mM Na ₂ CO ₃ /1.0 mM NaHCO ₃	0.30	8
3-mm AS14A	8.0 mM Na ₂ CO ₃ /1.0 mM NaHCO ₃	0.5	28
4-mm CS12A	22 mM MSA	1.00	72
3-mm CS12A-5 μ m	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:

$$\text{Current (mA)} = 2 \times 1.67 (\text{mA} \cdot \text{min} / \text{mN} \cdot \text{ml}) \times [\text{eluent (mN)}] \times \text{flow rate (ml / min)}$$

For anion separations:

$$[\text{eluent (mN)}] = 2 \times [\text{CO}_3^{2-} (\text{mM})] + [\text{HCO}_3^- (\text{mM})] + [\text{OH}^- (\text{mM})]$$

For cation separations:

$$[\text{eluent (mN)}] = 2 \times [\text{H}_2\text{SO}_4 (\text{mM})] + [\text{MSA} (\text{mM})]$$

(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 **Programmed Control**  **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 operation currents lead 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 Na ₂ CO ₃ /1.7 mM NaHCO ₃	2.00	26
2-mm AS4A-SC	1.8 mM Na ₂ CO ₃ /1.7 mM NaHCO ₃	0.50	7
4-mm AS9-HC	9.0 mM Na ₂ CO ₃	1.00	44
2-mm AS9-HC	9.0 mM Na ₂ CO ₃	0.25	11
4-mm AS14	3.5 mM Na ₂ CO ₃ /1.0 mM NaHCO ₃	1.20	24
2-mm AS14	3.5 mM Na ₂ CO ₃ /1.0 mM NaHCO ₃	0.30	6
3-mm AS14A	8.0 mM Na ₂ CO ₃ /1.0 mM NaHCO ₃	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

Column	Eluent	Flow Rate (ml/min)	Current (mA)
4-mm AS11	12 mM NaOH or KOH	1.00	30
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 H ₂ SO ₄ /9% Acetonitrile	1.25	74
2-mm CS15	10 mM H ₂ SO ₄ /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:

$$\text{Current (mA)} = 2.47 (\text{mA} \cdot \text{min} / \text{mN} \cdot \text{ml}) \times [\text{eluent (mN)}] \times \text{flow rate (ml / min)}$$

$$[\text{eluent (mN)}] = 2 \times [\text{CO}_3^{2-} (\text{mM})] + [\text{HCO}_3^- (\text{mM})] + [\text{OH}^- (\text{mM})]$$

For cation separations:

$$\text{Current (mA)} = 2.94 (\text{mA} \cdot \text{min} / \text{mN} \cdot \text{ml}) \times [\text{eluent (mN)}] \times \text{flow rate (ml / min)}$$

$$[\text{eluent (mN)}] = 2 \times [\text{H}_2\text{SO}_4 (\text{mM})] + [\text{MSA (mM)}]$$

(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 **Programmed Control**  **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 operation currents lead 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 Tetrabutyl ammonium Hydroxide	0.5-2.0	50/100	3-5	5-10
2.0-5.0 mM Tetrabutyl ammonium 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.

Control: IC

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 the desired 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 ...: Actions on 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 Device Control**  **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 **Programmed Control**  **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
;-0.100Message "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
;-0.100Message "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 Device Control**  **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 **Programmed Control**  **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 Generator** 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

Apart from the flow rate, the suppressor and cartridge types determine the maximum eluent concentration possible for a particular application. To determine the eluent concentration for your application, refer to the column manual. Dionex column manuals are included on the Dionex Reference Library CD-ROM. The concentration can be set manually or controlled via a **➤ Program**.



Manually Setting the Eluent Generator Concentration

1. Open the control panel for the **➤ Timebase**; see **How to ...: Actions on the Control Panel**  **Opening a Control Panel**.
2. Move the **Concentration** slider to select the desired value or enter the value into the edit field.

-or-

Enter the concentration from the **Commands** dialog box. To do this, select **Command...** on the **Control** menu. Under **EluentGenerator**, select the **Concentration** command.

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 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.

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)** is the percentage of ions 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 and the cartridge must be replaced before continuing operation.

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 operation with the cartridge after the expiration date, performance will 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 or the ICS-2000 that contains the eluent generator.
- 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.

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 ...: Actions on 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 front 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 ...: Actions on the Control Panel**:  **Modifying a Control Panel**,  **Modifying a Control**, and  **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.
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.

Control: GC and Temperature

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 HP 7673 and HP 7683 GC autosamplers support simultaneous injection of two samples. For more information, refer to

 **Injecting Two Samples Simultaneously (Dual Inject).**

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 **How to ...: Device Control**  **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.

Injecting Two Samples Simultaneously (Dual Inject)

If you wish to double the sample throughput, use the HP 7673 or HP 7683 GC autosampler under the HP 5890 system. This will allow you to simultaneously inject two samples from different sample positions.

Use the following command to enable or disable the Dual Injection option:

```
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 so-called 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
```

We recommend reserving the first and third quadrant for the Front Injector and the second and fourth quadrant 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 of the HP 7673 and HP 7683 autosamplers, refer to the **Administrator Manual: Installation Instruction**  **Agilent HP 7673 and HP 7683: Dual-Inject**.

For information about how to install the autosampler, refer to the **Administrator Manual**  **Agilent GC Autosamplers: Flow Chart**.

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 ...: Actions on the Control Panel**  **Linking a Control to a Device**). The actual temperature is set by an additional control (see **How to ...: Device 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 **How to ...: Device 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 ...: Actions on 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.
- Move the mouse cursor over 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.

(For more information, refer to **How to: ...: Device Control**  **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 Dionex TCC-100 Thermostatted Column Compartment and the Dionex STH585 Column Oven allow you to control the column temperature. The allowed temperature range is 5°C to 85°C.

The ICS-1000/1500/2000 Ion Chromatography Systems let you control the column temperature over a range of 30°C to 60°C. (The column heater is an option for the ICS-1000.)

The AS50 thermal compartment lets you control the temperature of the column to between 10°C and 40°C for PEEK systems, or 10°C and 80°C for stainless steel systems.

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 ...:**  **Actions on the Control Panel.**

It is also possible to integrate column temperature control into a ➤*Program*. If you have a TCC-100 or an STH-585, use the following 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.
```

If you have an ICS-1000/1500/2000 or an AS50 thermal compartment, use the following commands to integrate column temperature control into a program:

```
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.
```

Control: Component Controller

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

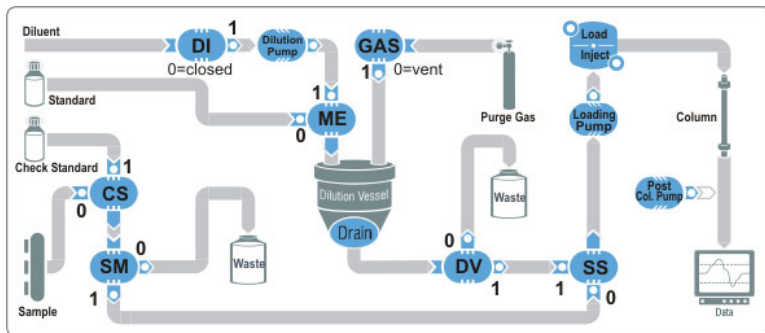


Valve Control for CC83: Dilution with Reagent

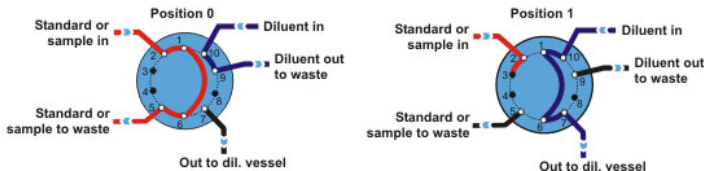


Valve Control for CC84: Concentration with Reagent

Valve Control for CC81: Concentration

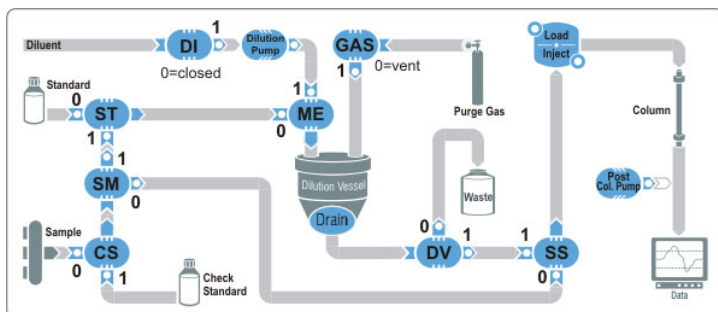


Metering Valve Detail

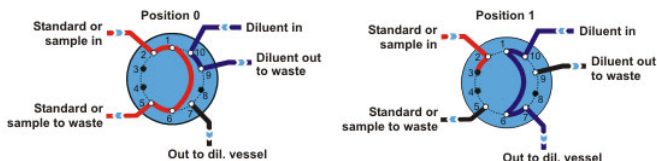


- | | |
|------------------------|--|
| Sample (SM) | waste (0) —directs output from the Chk Std (CS) valve to waste
SS valve (1) —directs output from the CS valve to the SS valve |
| Diluent (DI) | closed (0) —stops flow of diluent (typically DI water) to the dilution pump
open (1) —allows flow of diluent 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

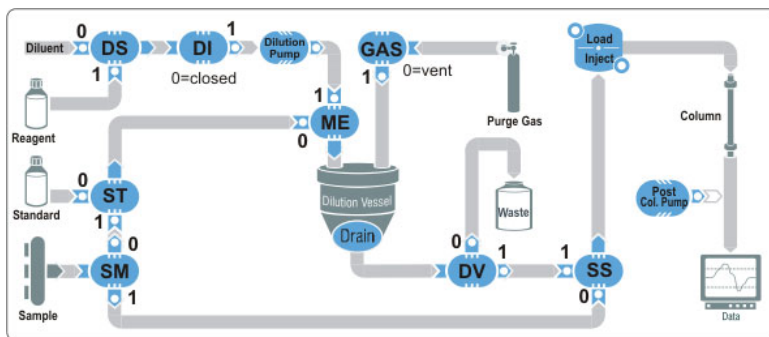


Metering Valve Detail

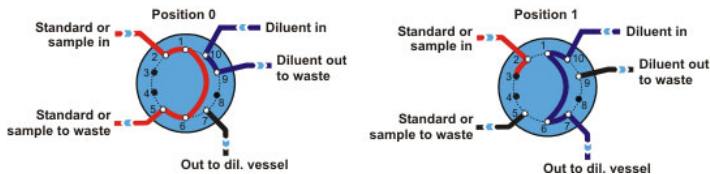


- Sample (SM)** **SS valve (0)**—directs output from the SM valve (sample or check standard) to the SS valve
- Diluent (DI)** **ST valve (1)**—directs output from the SM valve to the ST valve
- closed (0)**—stops flow of diluent (typically DI water) to the dilution pump
- open (1)**—allows flow of diluent 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

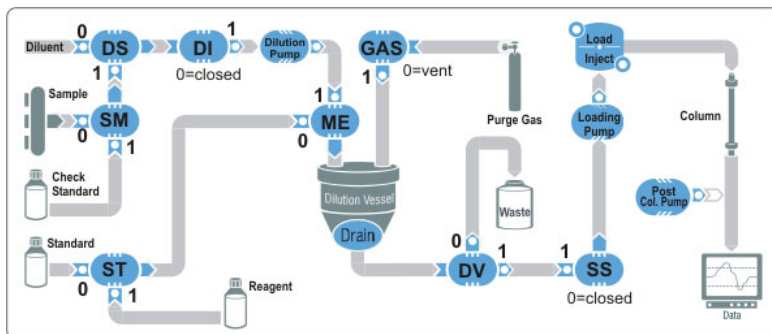


Metering Valve Detail

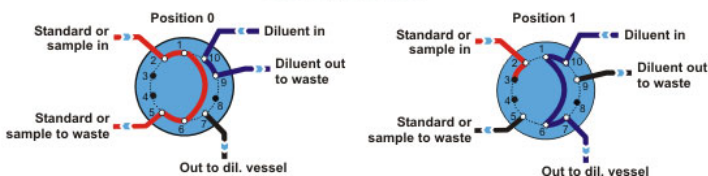


- Sample (SM)** **ST valve (0)**—directs sample to the ST valve
 SS valve (1)—directs sample to the SS valve
- Diluent (DI)** **closed (0)**—stops flow from the DS valve to the dilution pump
 open (1)—allows flow from the DS valve to the dilution pump
- Dil Vessel (DV)** **purge (0)**—directs output 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

Control: Special Commands, Relays, and Others

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 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 Sampling Step for Data Acquisition

```
0.000    VirtualChannel_01.SamplingStep    = Value [s]
```

Default setting: SamplingStep = 0.1 s. The input must be between 0.01 and 10 seconds.



Note:

SamplingStep defines the time step after which the virtual channel driver (= VCD) re-evaluates the expressions assigned to the channels. Only after re-evaluation will changes to the terms in the expression take effect on the virtual channel signal. Select a small sampling step to record all changes more precisely. However, this will increase the workload of the processor and slow down your computer.

Changing the Time Lag Threshold

0.000 VirtualChannel_01.LagThreshold = Value [s]

Default setting: LagThreshold = 0.01 s. The value should be between 0.001 and 100 seconds.



Note:

*The ➤Virtual Channel Driver (VCD) keeps the channel time deviation (number of generated data points multiplied by SamplingStep) from the actually passed time as small as possible. Comparing the two values performs this task. If the difference exceeds or falls below the time interval defined by **LagThreshold**, an additional data point is interpolated or a data point is omitted to approximate the channel time to the real time. With a larger interval, less data is falsified, but the time deviation increases (especially in the case of very long data acquisition periods!).*

All channels of a Virtual Channel Driver have the same sampling and monitoring rate. In order to define differing sampling and monitoring rates, you have to install two or more **Virtual Channel Drivers**.

Changing the Channel Type

To optimize signal recording, assign each virtual channel a type. Three types are currently defined (see the **Administrator Manual: Installation Instructions** 🔄 **Channel Types of the Virtual Channel Driver**:

0.000 VirtualChannel_01.Type = Analog, Digital or Fixed

Analog	Suitable for expressions that a) change quickly and/or b) are composed of other analog signals.	Examples: formula = a) pressure.value 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:

Even if the channel type is set to Analog, step gradient may occur for virtual signals that consist of other signals. The reason is that some detectors transfer data to Chromeleon only in certain intervals. For example, the HP1100 DAD transfers data every 1-3 (or more) seconds, depending on the setting.

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. For a fixed channel, same step rate should be identical to the **SamplingStep**. This ensures that the compressor will record only the originally sampled data points.


Defining the signal to record

Select **Formula** to specify the expression to be recorded. You can use the same expressions as for \Rightarrow *Trigger* conditions, i.e., arithmetic and logical links. The expression must be numeric. During program execution, the **FormulaCur** property indicates the value calculated (changes after each **SamplingStep**). **FormulaMin** and **FormulaMax** define the minimum and maximum values resulting from the expression.

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 **FormulaMin**, **FormulaMax**, and **FormulaCur** properties indicate 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 we recommend 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 \Rightarrow *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 **How to ...: Device Control**  **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 we recommend 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:**

Even if the channel type is set to Analog, step gradient may occur for virtual signals that consist of other signals. The reason is that some detectors transfer data to Chromeleon only in certain intervals. For example, the HP1100 DAD transfers data every 1-3 (or more) seconds, depending on the setting.

Relay Status

The status of a relay should be recorded as a virtual channel. For this rectangle signal, we recommend 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 we recommend 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 $t_{1/2}$ min
- The run time of the chromatogram is t_l 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 /  $t_{1/2}$ )
VirtualChannel_01.FormulaMin = -10000 / 2.718**
                                (-0.69314718*  $t_l$  /  $t_{1/2}$ )
VirtualChannel_01.FormulaMax = 10000 / 2.718**
                                (-0.69314718*  $t_l$  /  $t_{1/2}$ )
VirtualChannel_01.Type = Analog

UV_VIS_1.AcqOn
VirtualChannel_01.AcqOn

 $t_l$     UV_VIS_1.AcqOff
        VirtualChannel_01.AcqOff

```

In the program, replace the $t_{1/2}$ entry with the actual half-life. In addition, enter the actual run time of your program with three decimal places for t_l .

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 $\Rightarrow \Delta$, 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 \Rightarrow *Trigger* in the Reference Manual 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, that is, 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, as soon as...

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.

```
FoxyJr.Valve = Off
; switches the >Fraction Collector valve to the "Waste" position
0.000    Trigger STARTofPEAK          FoxyJr.Valve = 0 AND
                                           UV_VIS_1.Delta >1, True = 2

FoxyJr.Valve = On
; switches the valve to the "Collect" position
EndTrigger

Trigger TUBEFULL                      FoxyJr.Valve = 1,True = 60
Protocol "Tube full"
FoxyJr.Valve = Off
EndTrigger

Trigger ENDofPEAK                     FoxyJr.Valve = 1 AND
                                           UV_VIS_1.Delta > -1,
                                           True = 2, Delay = 5

FoxyJr.Valve = Off
; switches the valve to the "waste" position
FoxyJr.Tube = FoxyJr.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 ...: Actions Related to Fraction Collection:**



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 \Rightarrow *Sound* command.

Temperature Regulation for Column Oven

```
0.000    Temperature.Nominal  =Value [°C] (nominal value)
```

In combination with a \Rightarrow *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 **How to ...: Device Control**  **Control: Autosamplers**).

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 ...: Actions on 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 ...: Actions on 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, it can be controlled by Chromeleon.

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):

$$\text{Master Flow [ml/min]} = \text{CRP} * \text{System Flow [\mu l/min]}.$$

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
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 [\AA]
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 your requirements.

**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.

Commands and Tips for Device Control

Commands and Tips for Device Control

Chromeleon allows you to control several HPLC, IC, and GC devices from various manufacturers. To support the different device functions, you can issue 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

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
⇒ <i>AbortSample</i>	Stop data acquisition and sample; continue the ➤ <i>Batch</i> with the next sample.
⇒ <i>AbortBatch</i>	Stop data acquisition and batch processing.
ConsumableChanged	Log in the ➤ <i>Audit Trail</i> that a consumable has been changed.
⇒ <i>Continue</i>	Continue all operations that are in Hold mode.
EluentChanged	Log in the ➤ <i>Audit Trail</i> that the solvent has been changed.
⇒ <i>Hold</i>	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.

Command	Description
⇒ <i>Sound</i>	Generate a sound of selectable frequency and duration that is heard on the PC loudspeaker.
⇒ <i>StopFlow</i>	Stop pump flow, interrupt data acquisition, and stop batch processing.
<i>StopMode</i>	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
⇒ <i>Branch</i>	Completes the active ➤ <i>Program</i> and starts another one
⇒ <i>Delay</i>	Especially with trigger commands, execution of the next program command is delayed for the specified time.
⇒ <i>EndTrigger</i>	Continues all processes in hold mode.
⇒ <i>End</i>	Starts or stops data acquisition.
⇒ <i>Log</i>	Logs device variable values in the Audit Trail (see Data Management  Audit Trails).
⇒ <i>Message</i>	Displays a message to the user and interrupts the program until the message is clicked.
⇒ <i>Protocol</i>	Logs any message in the ➤ <i>Audit Trail</i>
⇒ <i>Trigger</i>	Freezes a running gradient program, stops data acquisition, stops sample processing.
⇒ <i>Wait</i> :	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 \Rightarrow *Flow* command is available for pumps and the \Rightarrow *Inject* command is available for autosamplers. However, some commands are available independent of the device type:

Command	Description
\Rightarrow <i>Connect</i>	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	Description
FirmwareVersion	Indicates the firmware version of the device (read-only).
ModelNo	Indicates the device type (read-only).
Ready	Indicates that the device is ready for operation, i.e., no Autozero is started, the temperature has been reached, no error has occurred (read-only).
\Rightarrow <i>Reset</i>	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 the Dionex devices. This section provides the special commands that Chromeleon supports for the corresponding 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 Autosamplers



Dionex Detectors




Dionex Ion Chromatography Systems



Dionex Eluent Generators




Dionex Thermostatted Column Compartments

Optimum support for all functions is ensured for Dionex devices. For a list of the third-party devices that are currently supported, refer to the **Administrator Manual: Installation Instructions**  **Installing and Controlling Third-Party Devices.**

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 controlling HPLC and IC pumps:

Command	Description
⇒ <i>Flow</i>	Enter the desired flow rate.
⇒ <i>Pressure.Lower/UpperLimit</i>	Sets the pressure limits.
⇒ <i>WasteLevel</i>	Enter the actual waste level before starting a sequence.

Besides, the following commands are available for controlling gradient pumps (P680, P580, GP40/GP50, and GS50):


Command	Description
$\Rightarrow \%B, \%C, \%D$	Changes the solvent composition, determines the gradient course.
$\Rightarrow \%A, \%B, \%C, \%D_Level$	Enter the amount of solvent that is available when you start the sequence.

The commands can be given either directly (via the toolbar or the menu bar or via 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 held as soon as the upper or lower pressure limits is exceeded. Changing the flow rate creates a ➤ *Flow Gradient*, changing the solvent composition creates a ➤ *% Gradient*. Flow and % gradients can be realized simultaneously.



Tip:

*Dionex GP40/GP50, IP20/IP25, IC20/IC25, GS50, 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 **How to ...: Device Control**  **Pump Commands** for additional information.*


Dionex M480, P580, and P680 Pumps

The Dionex M480, P580, and P680 pumps support automatic pre-compression control. The pumps are capable of adjusting to the compressibility of commonly used solvents. In addition, **Learn** and **Freeze** commands are available for the P580 pump to extend pre-compression control to unknown solvent types. Pre-compression control is fully automatic for the P680 pump. Therefore, the Learn and Freeze commands are not required.

For the user, it is irrelevant whether the system is a ➤ *Low-Pressure Gradient System* or a ➤ *High-Pressure Gradient System*. Command input is identical.

**Caution:**

Chromatography pumps are high-precision instruments! Dry operation or crystallization of buffer solutions within the fluidic system must be avoided. These problems occur especially when the pump flow is stopped while the detector lamp is still turned on. The flow cell heats up, and the solvent evaporates. Depositions of substances can result, for example, salt in a buffer solution.

For information about the special P680 commands, refer to  **Dionex P680 HPLC Pump.**

Dionex GP40/GP50, IP20/IP25, IC20/IC25, GS50, 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, see:




Dionex/LC Packings UltiMate Capillary/Nano HPLC Pump




Controlling Pumps Without a Separate Device Driver.


Dionex P680 HPLC Pumps

In addition to the standard pump commands (see  **Dionex Pumps**), the P680 pump supports the following commands and properties:

Command/Property	Description
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.
Degasser	Turns the integrated degasser on or off (LPG pumps only). The integrated degasser should always be on.
HeadType	Indicates the head type of the pump: Analytical or Semi-Prep (read-only).
Leak	Indicates the status of the central leak sensor: Ok or Leak (read-only).
MaximumFlowRamp	Upper limit for the flow rate acceleration (0.1 - 10,000.00 ml/min ²)
ModelVariant	Indicates the variant: Isocratic , LPG , or HPG (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 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 once per hour for five minutes. However, the drop sensor on the liquid reservoir is not active, i.e., monitoring the piston seals for tightness 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., the piston seals are monitored for tightness is enabled. Off turns the rear-seal wash system off.
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).

For information about how to install the P680 pump, refer to the **Administrator Manual: Installation Instructions**  **P680 HPLC Pump: Overview**.

Dionex/LC Packings UltiMate Capillary/Nano HPLC Pump

In addition to the standard pump commands (see  **Dionex Pumps**), the following commands and properties are available for controlling pumps in the UltiMate system:

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: $\int \text{Pressure dt}$
Pressure.LowerLimit	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)
Pressure.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 micropump 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 read out 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 on the Expert level.)
Ready	Indicates the state of the flow sensor: Ready or NotReady. (The property is visible only on 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 on the Expert level and is read-only.)

For information about how to install the UltiMate pump, refer to the **Administrator Manual: Installation Instructions**  **UltiMate Capillary/Nano HPLC System: Overview.**

Dionex/LC Packings UltiMate Pump: Columns

In addition to the pump commands (see  **Dionex/LC Packings UltiMate Pump**), the following properties are available for controlling columns in the UltiMate system:

Parameter	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 **How to ...:**

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.

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 the **Administrator Manual: Installation Instructions**

Installing and Controlling Third-Party Devices.


Besides, pumps can be controlled for which separate device drivers or serial interfaces are not available (for example, the former Gynkotec 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).

For more information, refer to the **Administrator Manual: Installation Instructions**:

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 the **Administrator Manual: How to ...: Actions in the Server Configuration Program**  **Adding, Configuring, or Deleting Components.**)

Chromeleon supports the following commands for voltage-controlled pumps (**DAC pumps**):

Command	Description
⇒ <i>Flow</i> :	Change the flow rate
⇒ <i>%B, %C, %D</i> :	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 Autosamplers

Automatic sample injection with a modern ➤ *Autosampler* has several advantages:

- Comfortable 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
⇒ <i>Dispense</i>	Dispenses the previously drawn amount of liquid.
⇒ <i>Draw</i>	Draws the specified amount of liquid.
⇒ <i>Inject</i>	Injects the sample.
Mix	Mixes the contents of a vial. (The mixing procedure depends on the autosampler.)
⇒ <i>Position</i>	Indicates the vial position.
⇒ <i>Volume</i>	Sets the volume to be injected.
⇒ <i>Wash</i>	Performs a wash cycle.

For information about the individual autosamplers and their specifications, refer to:



Dionex ASI-100 Autosamplers



Dionex AS50 Autosampler



Dionex/LC Packings FAMOS Capillary/Nano HPLC Autosampler



Dionex GINA 50 Autosampler

Dionex ASI-100 Autosamplers

In addition to the standard autosampler commands, the autosampler 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.

Special Commands and Properties:


Command/Property	Description
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 with which the contents of the syringe shall be dispensed.
DownSpeed	Specify the speed with which the needle moves down.
DrawSpeed	Specify the speed for filling the syringe.
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 of the autosampler.
Internal MSV	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.


Command/Property	Description
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.
PrepareNextSample	Starts the injection procedure for the next sample, if available. However, the injection valve is only switched at the next Inject command (Display level: Advanced).
PrepareThisSample	Starts the injection procedure for the current sample. However, the injection valve is switched only at the next Inject command (Display 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 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.
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	Relay3 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.
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 the P680A LPG pump. Select the pump for inject synchronization in the ➤ <i>Server Configuration</i> program.
Syringe	Indicates the volume of the syringe that is installed in the autosampler.
SyringeCounter	Indicates the number of syringe movements.
SyringeDelay	Specify the time the needle shall remain in the vial after loading.
⇒ <i>Temperature</i> (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.
⇒ <i>Wash</i>	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 **How to ...: Device Control**  **Autosampler Commands (ASI-100 Series)**

For information about how to install the autosampler of ASI-100 series, refer to the **Administrator Manual: Installation Instructions**  **ASI-100 HPLC Autosamplers: Overview**.

Dionex AS50 Autosamplers

In addition to the standard autosampler commands, the Dionex AS50 Autosampler provides many additional commands for controlling autosampler functions. The following commands are available:

Special Commands

Command	Description
DoorSafetyTime	Displays the amount of time the autosampler door can remain open without aborting the sequence.
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 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 AS50 is performing.
LoopSize	Displays the size (volume) of loop installed.
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.

Command	Description
SecondLoopSize	(For AS50 USB simultaneous injection mode only) Displays the size (volume) of loop installed on the second injection valve.
SerialNumber	Displays the autosampler serial number.
Status	Displays the current action being performed by the autosampler.
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 AS50s 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.
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 ➤ <i>Program</i> specified for each sample injection in a ➤ <i>Sequence</i> .
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 AS50s equipped with the sample temperature control option) Sets the temperature of the sample tray.
WaitForTemperature	(Only for AS50s 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 AS50 provides several commands for preparing the sample before injection. The standard AS50 supports six sample prep commands. If the AS50 is equipped with the Sample Preparation option, two additional commands are available.

Command	Description
Delay(SP)	Pauses sample preparation.
Flush (SP)	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.

The following commands are only available if the AS50 is equipped with the sample prep option:

Command	Description
Dilute	Dilutes the sample with reagent.
Dispense	Dispenses reagent to a vial.

Calibration Commands


Command	Description
DownloadCalibration	Downloads the selected set of calibration values to the autosampler.
DownloadCalibration Parameter	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 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.


Command	Description
TrayLeakDetTest	Performs a test of the autosampler tray leak detector.
TrayLeakDetTestData	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 **How to ...: Device Control**  **Autosampler Commands (AS50)**.


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, the Dionex/LC Packings FAMOS Autosampler provides additional commands for controlling autosampler functions. The following general commands are available:

Command/Property	Description
AlarmBuzzer	<p>When set to On, the buzzer will signal error conditions.</p> <p> Tip:</p> <p><i>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.</i></p>
CoolingOnTime ⇒Dispense	<p>Indicates cooling time (in total) of the autosampler (read-only).</p> <p>Dispenses a specified volume. The following options are available:</p> <p>To: Sample position to which to dispense the volume.</p> <p>Volume: Volume to dispense (0.0 to 25.0 µl).</p> <p>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)).</p> <p>SampleHeight: Height at which the sample is dispensed (0 to 40 mm - see below).</p>

Command/Property	Description
⇒ <i>Draw</i>	<p>Draws a specified volume. The following options are available:</p> <p>From: Sample position from which to draw the volume.</p> <p>Volume: Volume to dispense (0.0 to 25.0 µl).</p> <p>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).)</p> <p>SampleHeight: Height at which the sample is drawn (0 to 40 mm - see below).</p>
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).
⇒ <i>Inject</i>	<p>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.</p> <p> Note:</p> <p><i>Injection can be performed only if the autosampler is in Not running state. This is usually ensured by the WaitSamplerReady 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."</i></p>
InjectionCounter	Total number of injections (read-only).
InjectMode	<p>Injection mode (for more information, refer to the User Manual for the instrument):</p> <p>UserProg The autosampler executes the program or sample preparation steps defined by the user.</p> <p>Partial The syringe volume is injected in part only (at most 50% of the sample loop volume).</p> <p>FullLoop The syringe volume is injected completely.</p> <p>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.</p>
InjectValve	<p>Injection valve can be in the following positions:</p> <p>Inject or Load.</p>
LastTransportVial	Defines the vials to be used for µlPickUp injections. Used together with FirstTransportVial .

Command/Property	Description
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 the Administrator Manual: Installation Instructions  UltiMate Capillary/Nano HPLC System: Overview). 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 losing 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.

SyringeSpeedFactor	Factor used for scaling the syringe speed (see SyringeSpeed).
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Command/Property	Description
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 **How to ...: Device Control**  **Examples for User-Defined Programs for the FAMOS Autosampler (Dionex/LC Packings)**.

For installation instructions for the FAMOS autosampler, refer to the **Administrator Manual: Installation Instructions**  **FAMOS Capillary/Nano Autosampler: Overview**.


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 \Rightarrow *Inject* command.


Chromeleon supports the following sample preparation commands:

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)
\Rightarrow Dispense	<p>Dispenses a specified volume. The following options are available:</p> <p>To: Sample position to which to dispense the volume.</p> <p>Volume: Volume to dispense (0.0 to 25.0 μl).</p> <p>SyringeSpeed: Speed used to dispense the volume (Either select the desired option (Low, Normal, or High) from the drop-down list box or enter the any number between 1 and 9).</p> <p>SampleHeight: Height at which the sample is dispensed (0 to 40 mm).</p>
\Rightarrow Draw	<p>Draws a specified volume. The following options are available:</p> <p>From: Sample position from which to draw the volume.</p> <p>Volume: Volume to dispense (0.0 to 25.0 μl).</p> <p>SyringeSpeed: Speed used to draw the volume (Either select the desired option (Low, Normal, or High) from the drop-down list box or enter the any number between 1 and 9).</p> <p>SampleHeight: Height at which the sample is drawn (0 to 40 mm).</p>
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 9.999 μ l).

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: <i>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.</i>
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 **How to ...: Device Control**  **Examples for User-Defined Programs for the FAMOS Autosampler (Dionex/LC Packings)**.


Dionex GINA 50 Autosamplers


In addition to the standard commands, 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:

Special Commands


Command	Description
\Rightarrow 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)
\Rightarrow NeedleUp	Moves the needle up.
\Rightarrow 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 **How to ...: Device Control**  **Autosampler Commands (GINA 50)**.

For installation instructions for the GINA 50 autosampler, refer to the **Administrator Manual: Installation Instructions**  **GINA50 HPLC Autosampler: Overview**.








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
⇒ <i>AcqOn/Off</i>	Start/stop data acquisition.
⇒ <i>Autozero</i>	Automatic autozero.
➤ <i>AutoAutoscale</i>	Axis/scaling adjustment.
⇒ <i>Average</i>	Average all measured values over the Step interval.
⇒ <i>Delta</i>	Indicates the signal's slope, that is, the difference between the current value and the value one second ago; this is useful for ⇒ <i>Triggers</i> .
⇒ <i>Step</i>	Step width adjustment

For information about special commands and properties available for individual detectors, refer to:

-  **Dionex UVD 170/340 UV/PDA Detectors**
-  **Dionex PDA-100 Photodiode Array Detector**
-  **Dionex AD20/AD25 Absorbance Detectors**
-  **Dionex/LC Packings UltiMate Capillary/Nano HPLC UV Detector**
-  **Dionex RF2000 Fluorescence Detector**
-  **Shodex RI-101 Refractive Index Detector**
-  **Dionex CD20/CD25/CD25A, ED40/ED50/ED50A, and IC20/IC25/IC25A Electrochemical Detectors**

Dionex UVD 170/340 UV/PDA Detectors

In addition to the standard commands available for all detectors (see  **Dionex Detectors**), "special" control commands are also supported if the detector is connected to a server PC via an appropriate interface.

Special Commands

Command	Description
\Rightarrow Bandwidth	Sets the bandwidth of a UV-VIS channel.
\Rightarrow BunchWidth	For UVD 340U only: Averages photodiode signals for 3D field acquisition.
CheckWavelength	Checks wavelength calibration, using a \succ <i>Holmium-Oxide Filter</i> .
\Rightarrow 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.
\Rightarrow Lamp	Turns the lamp on or off.
\Rightarrow RefWavelength	Sets the reference wavelength.
\Rightarrow RefBandwidth	Sets the reference bandwidth.
\Rightarrow Wavelength	Sets the wavelength of a UV-VIS channel.

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:

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

For information about how to install the UVD 170 or UVD 340 detector, refer to the **Administrator Manual: Installation Instructions**




UVD 170S and UVD 340S HPLC Detectors: Overview



UVD 170U and UVD 340U HPLC Detectors: Overview

Dionex PDA-100 Photodiode Array Detectors

In addition to the standard commands available for all detectors (see  **Dionex Detectors**), the PDA-100 detector provides the following additional control commands.

UV_VIS Channel Commands

Command	Description
⇒ <i>Bandwidth</i>	Sets the bandwidth (the wavelength range at which the chromatogram is measured).
⇒ <i>MaxAutoStep</i>	Determines the maximum step if Step = Auto.
➤ <i>Offset_Level</i>	Sets the offset for the analog output signal.
➤ <i>Recorder_Range</i>	Sets the range of a full-scale recorder response (analog output).
⇒ <i>RefBandwidth</i>	Sets the bandwidth of the reference wavelength (if one is selected). If RefWavelength is Off , the RefBandwidth setting has no effect.
⇒ <i>RefWavelength</i>	Sets the reference wavelength.
⇒ <i>Wavelength</i>	Sets the wavelength.

3DFIELD Channel Commands

Command	Description
⇒ <i>BunchWidth</i>	Determines how many nanometers are averaged when collecting 3D data. Bunching (averaging) the signals of adjacent wavelengths reduces the size of the 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).
➤ <i>Data_Collection_Rate</i>	Sets the rate at which Chromeleon collects digital data points from the detector.
⇒ <i>LampIntensity</i>	Reports the deuterium lamp's intensity at 254 nm in counts/second.
ModuleAge	Reports (or resets) the number of hours the module has been on.

Command	Description
➤ <i>NegativeAbsorptionLevel</i>	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.
OpticalBenchTemp	Reports the temperature of the optical bench.
➤ <i>Rise_Time</i>	Sets how quickly the detector responds to a change in signal.
➤ <i>TTL_Input_Mode</i>	Sets the TTL input signal mode.
➤ <i>UV_Lamp</i>	Turns the deuterium lamp on and off.
UVLampAge	Reports (or resets) the number of hours the deuterium lamp has been on.
UVLampPowerOnSetting	Selects whether the deuterium lamp is turned on when the device power is turned on. The default setting is On.
➤ <i>Visible_Lamp</i>	Turns the tungsten lamp on and off.
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.
VisLampPowerOnSetting	Selects whether the tungsten lamp is turned on when the device 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 USB model of the PDA-100).
Intensity	Calibrates the intensity of the deuterium lamp.
LeakDet	Performs the leak detector calibration (not applicable to the USB model of the PDA-100).
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

Command	Description
LeakDetTest	Performs a test of the leak detector (not applicable to the USB model of the PDA-100).
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 ➤ <i>Holmium Oxide Filter</i> .
WavelengthVerTestDate	Reports the date of the wavelength verification.
WavelengthVerTestResult	Reports the result (pass or fail) of the wavelength verification.

For information about how to install the detector, refer to the **Administrator Manual: Installation Instructions**  **PDA-100 (DX-LAN) Detector: Overview** or  **PDA-100 (USB) Detector: Overview**.

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.

Special Commands

Command	Description
➤ <i>Data_Collection_Rate</i>	Sets the rate at which Chromeleon collects digital data points from the detector.
⇒ <i>Rise_Time_</i>	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.
➤ <i>UV_Lamp</i>	Turns the deuterium lamp to Off, Low, or High (AD20 detector), or Off or On (AD25 detector).
➤ <i>Visible_Lamp</i>	Turns the tungsten lamp to Off, Low, or High (AD20 detector), or Off or On (AD25 detector).
⇒ <i>Wavelength</i>	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.
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/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 "special" control commands.


Command/Property	Description
Adjust_Calibration	Adjusts the wavelength calibration to internal tests.  Tip: <i>The command stops the data acquisition!</i>
➤Data_Collection_Rate	The rate at which Chromeleon receives digital data points.
DemoFileName	Name of the file that is read in the ➤Demo Mode.
Description	Optional device description (read-only).
InstallationDate	Indicates the installation date (read-only).
⇒Lamp	Turns the lamp on and off.
LampNumber	Indicates the lamp number (read-only).
LastServiceDate	Indicates the date of the last service procedure (read-only).
LastServiceCode	Indicates the code of the last service (read-only).
LampStarts	Indicates the number of lamp starts (read-only).
LampType	Indicates the lamp type: Deuterium or Halogen (read-only).
LampWorkTime	Indicates the power-on time (read-only).
MotorWorkTime	Indicates the total motor power-on time (read-only).
TimeConstant	Used for noise suppression (values: 0.1; 0.2; 0.5; 1; 2; 5, or 10s)  Tip: <i>Most noise is suppressed if a time constant of 10s is used. However, for the acquisition of narrow peaks a lower time constant will be more appropriate.</i>
TotalLampTime	Read-only.
⇒Wavelength	Select the wavelength setting.

Tip:


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 information about how to install the UltiMate UV detector, refer to **Administrator Manual: Installation Instructions**  **UltiMate Capillary/Nano HPLC System: Overview.**

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
➤ <i>Emission</i>	Modifies the measuring wavelength.
➤ <i>Excitation</i>	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 **How to ...: 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 **Administrator Manual: Installation Instructions**  **RF2000 Fluorescence Detector: Overview**.

For information about the pin assignment of the RF2000 cable, refer to the **Administrator Manual: Installation Instructions (Appendix)**  **RF2000 RS Cable** in the appendix of the Installation Instructions.

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:

*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 refers to the time the detector requires to reach 98% of the full-scale deflection. The larger the chosen time interval is, the better is the ➤ *Signal-to-Noise Ratio*, but the lower is the resolution.


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 the ➤ *Emission* wavelengths. However, determination is not simultaneously. The procedure is described below for the emission wavelength as an example. For a corresponding ➤ *Program*, refer to the **How to ...: Device Control**  **Determining the Optimum Emission Wavelength (RF2000).**

Scanning

- Stop the pump flow via the **Flow = 0** command.
- Execute the **ScanEmission** command.

The duration of the Scan procedure depends on the wavelength range to be scanned (**Start/End Wavelength**) 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 optically indicate whether the detector is scanning.

After completing the actual scan process, the resulting spectrum is saved in the instrument 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.

- Determine an optimum excitation wavelength (excitation value) in the same way.



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.

How to form a difference spectrum

To receive a difference spectrum (= current spectrum minus background spectrum):

- Record a background spectrum as described above.
- Use the **Save Background Spectrum** command to save this spectrum also 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 behaves in the same way as if a Scan procedure has 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 **➤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: ScanSpeed**). 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**.

Shodex RI-101 Refractive Index Detector




Tip:

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:

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 μ RIU/V or 500 μ RIU/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)
Purge	Off	On	Controls the purge valve.


Property	Minimum	Maximum	Description
Recorder_Range	0.25 μ RIU	512.00 μ RIU	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 μ RIU	999 μ RIU	Validation span (The value should be between 487 and 537 μ RIU. 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 the **Administrator Manual: Installation Instructions**  **Shodex HPLC Refractive Index Detectors (RI-101, RI-102, RI-104): Overview.**

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. 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
 <i>Data_Collection_Rate</i>	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
➤ <i>Full_Scale</i>	Sets the full-scale analog out voltage.
➤ <i>Mark</i>	Sends a positive pulse to the detector's analog output as an event marker.
➤ <i>Offset_Level</i>	Sets the offset for the analog output signal.
➤ <i>Polarity</i>	Switches the analog output polarity (+/-).
➤ <i>Recorder_Range</i>	Sets the range of a full-scale recorder response.
➤ <i>Rise_Time</i>	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 ➤ <i>DS3</i> 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 ➤ <i>Suppressor</i> .
➤ <i>Temperature_Compensation</i>	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.
➤ <i>DC_Voltage</i>	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 type of electrode installed.
➤Waveform	Program 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 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:

Command	Description
Column	If the DX-120 is in ➤ <i>Column Mode</i> , 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.
➤ <i>Data_Collection_Rate</i>	Sets the rate at which Chromeleon collects digital data points from the detector.
Eluent	If the DX-120 is in ➤ <i>Eluent Mode</i> , the Eluent command selects the eluent reservoir (A or B).
Eluent Pressure	Turns the pressure to the eluent reservoir(s) on and off.
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 ➤ <i>Self-Regenerating Suppressor</i> (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.

Detector Commands


Command	Description
\Rightarrow AcqOn/Off	Starts/stops data acquisition.
\Rightarrow Autozero	Automatic autozero.

Pump Commands


Command	Description
Pump (on/off)	Turns the pump on or off.
\Rightarrow 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 \triangleright TTL), On (turns the TTL on), 5v (turns the TTL off), and Duration (toggles the TTL's state after the specified time).

In addition to the commands listed above, calibration and diagnostic commands are also available (see  **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.

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.
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
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.
<u>TotalConductivity</u>	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 > <i>TTL</i>), 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  **System Wellness** for details).

Dionex Eluent Generators

In addition to the  **General Device Commands**, the following commands are available for controlling the operating functions of Dionex eluent generators:

Command	Description
CartridgeExpirationDate	Reports the expiration date of the cartridge.
CartridgeNumber	Reports the serial number of the cartridge.
CartridgeRemainingLife Time	Reports the percentage of ions remaining in the cartridge.
CartridgeType	Reports the cartridge type currently installed.
Concentration	Sets the eluent concentration to be generated.
Curve	Sets the curve number for gradients.
SerialNumber	Reports the serial number of the eluent generator.

For additional information, refer to **How to ...: Control IC:**

 **Controlling the Eluent Generator Concentration**

 **Monitoring the Eluent Generator Cartridge Lifetime**


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.
LeakDet	Performs the leak detector calibration.

Diagnostic Commands

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 Thermostatted Column Compartments

In addition to the  **General Device Commands**, the following commands are available for controlling the operating functions of the Dionex Thermostatted Column Compartments:

Command	Description
Temperature	<p>Range: 5.0...80°C (41° - 186°F). For the STH585: 5.0...°F (41°...185°F). Click the "+" character beside the name to display the items underneath:</p> <p>Value-indicates the current temperature. Nominal-allows you to enter the nominal temperature. UpperLimit-allows you to enter the upper temperature limit LowerLimit-allows you to enter the lower temperature limit.</p> <p>If the nominal temperature is not in the limits, the command is not executed.</p>

For information about the special commands and properties available for the TCC-100 Thermostatted Column Compartment, refer to:

 **Dionex TCC-100 Thermostatted Column Compartment**

 **Dionex TCC-100 Thermostatted Column Compartment: Column ID**


For an example program, refer to **How to ...: Device Control**


 **Controlling the Column Temperature.**

Chromeleon supports no special commands or properties for the STH585 column oven. For information about how to install the STH585 column oven, refer to the **Administrator Manual: Installation Instructions**

 **STH585 HPLC Column Oven: Overview.**


Dionex TCC-100 Thermostatted Column Compartment

In addition to the commands supported for the different column compartments, such as Connect, Disconnect, and Temperature (see  **Dionex Thermostatted Column Compartments**), Chromeleon supports the following commands and properties for controlling the operating functions of the TCC-100:

Command/Property	Description
ActiveColumn	Determine for which column the column identification information shall be updated (A (1) or B (2)). The names are replaced with the names entered on the Configuration tab page in the Server Configuration program.
Column_A Column_B	The column names entered on the Configuration tab page in the Server Configuration program replace ColumnA and ColumnB . The various commands and properties under these commands allow you to enter and read out information about the column. For more information, refer to  Dionex TCC-100 Thermostatted Column Compartment: Column ID .
ColumnOven_TEMP	<p>This signal 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.) Use this command to record the temperature as a separate channel. The signal's name is replaced by the name entered on the Configuration tab page in the Server Configuration program.</p> <p>Click the "+" character beside the name to display the items underneath:</p> <p>Delta—indicates the signal's slope, i.e., the difference between the current value and the value one second ago. This is useful for triggers.</p> <p>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.</p> <p>AcqOff—terminates data acquisition.</p> <p>AcqOn—starts data acquisition.</p> <p>Retention—indicates the retention time of the signal (read-only).</p> <p>MaxAutoStep = Maximum step rate for Auto Step Mode; range: 0.1...5.1 s; default: 5.1 s</p> <p>Step—sets the step for data acquisition; range: 0.01...4.80 s; Auto selects the best step dynamically</p> <p>Average—averages all measured values over the step interval. Default: On. Off records only the last point of each interval.</p>
Door	Indicates whether the compartment door is open or closed (Open or Closed ; read-only).

Command/Property	Description
EquilibrationTime	Range: 0.0...30.0 min. Only if the deviation between the current temperature and the set point specified under ReadyTempDelta does not exceed the EquilibrationTime , the column compartment enters the Ready state (also, see ReadyTempDelta and Ready).
GasLeak	Indicates 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 the desired option from the list: Low , Standard , or High . To turn the sensor off, select Off .
HardwareVersion	Indicates the compartment'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 the desired option from the list: Low , Standard , or High . To turn the sensor off, select Off .
Leak	Indicates whether at least one leak sensor has detected leakage (Ok or Leak ; read-only; also see HumidityLeak and GasLeak).
LeakSensorState	Indicates whether the sensors are ready for leak detection (Ready , NotReady , or Error ; read-only).
MaxPosition	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 maximum number of position of the installed valve is indicated (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 valve is switched to the desired position (A (1) or B (2)).
Ready	Indicates whether the compartment is ready to operate (Ready or NotReady ; read-only; also see ReadyTempDelta and EquilibrationTime).
ReadyTemperatureDelta	Range: 0.0...5.0°C. 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 '0', the system does not check whether the temperature set point deviates from the actual temperature (also see Ready and EquilibrationTime).
SerialNo	Serial number of the column compartment (read-only).
TCC_INPUT_1	Indicates the state of digital input 1 (On or Off).
TCC_INPUT_2	Indicates the state of digital input 2 (On or Off).

Command/Property	Description
TCC_RELAY_1	<p>Relay 1. Click the "+" character beside the relay name to display the items underneath:</p> <p>State—indicates the state of the relay.</p> <p>Duration—when set, the relay toggles after the specified time.</p> <p>On—turns the relay on.</p> <p>Off—turns the relay off</p>
TCC_RELAY_2	<p>Relay 2. Click the "+" character beside the relay name to display the items underneath:</p> <p>State—indicates the state of the relay.</p> <p>Duration—when set, the relay toggles after the specified time.</p> <p>On—turns the relay on.</p> <p>Off—turns the relay off</p>
TempCtrl	Turns temperature control on or off .
Vendor	Indicates the device manufacturer (read-only).

For information about the column ID feature of the TCC-100, refer to  **Dionex TCC-100 Thermostatted HPLC Column Compartment: Column ID**

For an example program for the TCC-100, refer to **How to ...: Device Control**  **Controlling the Column Temperature.**

For information about how to install the TCC-100, refer to the **Administrator Manual: Installation Instructions**  **TCC-100 HPLC Thermostatted HPLC Column Compartment: Overview.**

Dionex TCC-100 Thermostatted Column Compartment: Column ID

The Dionex TCC-100 Column Thermostat device driver supports the following column ID properties:

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.


Secondary column properties

Having entered the following properties once, the user can later change them, if necessary:

Property	Description
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 ➤ <i>Batch</i> .
Description	Column description.
Diameter	Column diameter in [mm].
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	Upper and lower pH limits. If both limits are set to the same value, this means that no limit exists.
pH_UpperLimit	

**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: <i>Accept the default (0.0) if you do not want to have the system check the pressure.</i>
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



The following properties are determined by the system and/or updated during each injection. The user cannot change them:

Property	Description
DateOfFirstUse	Date of the first injection (read-only).
DateOfLastUse	Date of the last injection (read-only)
Injections_Count	Number of injections (read-only).
Pressure_Max	Maximum pressure measured while the column was in use (read-only).
Temp_Min	Minimum temperature measured while the column was in the column compartment (read-only).
Temp_Max	Maximum temperature measured while the column was in the column compartment (read-only).

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 Dionex TCC-100 Thermostatted Column Compartment, refer to  **Dionex TCC-100 Thermostatted Column Compartment**. For information about the standard commands supported for the different column compartments, refer to  **Dionex Thermostatted Column Compartments**.

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 the **Administrator Manual: Installation Instructions**  **Installing and Controlling Third-Party Devices**.

For many third-party devices, Chromeleon provides the same commands as for the Dionex devices. For more information, refer to **How to...: Device Control**  **Overview** and **Commands and Tips for Device Control**  **Commands for Controlling Dionex Devices**

In addition to the standard commands, several third-party devices support special commands. For information about these commands and other useful tips for the respective instruments, refer to:

 **Agilent**

 **CTC Analytics PAL Samplers**

Finnigan (see **ThermoFinnigan/TQ/TSP**)

 **Fisons AS800 Autosampler**

 **Gilson**

Hewlett Packard (see **Agilent**)


Hitachi (see **Merck Hitachi**)

 **Kontron**


 **Merck Hitachi**

 **Perkin Elmer Autosystem GC**

 **Rheodyne Valves**

 **Shimadzu LC-10A HPLC System**

 **ThermoFinnigan/TQ/TSP**





 **Valco (= VICI) Valves**

 **Varian**

 **Waters**













Agilent

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 1100 HPLC System

Refer to the following pages for information about the special commands supported for the Agilent 1100 HPLC System and for tips for practical operation:

-  **Agilent 1100 HPLC System: General**
-  **Agilent 1100 HPLC System: Pump**
-  **Agilent 1100 HPLC System: Autosampler**
-  **Agilent 1100 HPLC System: Wellplate-Sampler**
-  **Agilent 1100 HPLC System: Column Compartment**
-  **Agilent 1100 HPLC System: UV Detectors**
-  **Agilent 1100 HPLC System: Program Tips**
-  **Agilent 1100 HPLC System: Example for a Derivatization Program**
-  **Agilent 1100 HPLC System: Operating Modes for the Fluorescence Detector**
-  **Agilent 1100 HPLC System Fluorescence Detector: Tips for Lamp Commands**
-  **Agilent 1100 HPLC System: Checking the Solvent Liquid Level**
-  **Agilent 1100 HPLC System: Troubleshooting**

Agilent 1100 HPLC System: General

Property	Min	Max	Default	Description
Firmware	n/a	n/a	n/a	Firmware version of the module (read-only)
ModelNo	n/a	n/a	n/a	Module number (read-only)
NotReadyCauses	n/a	n/a	n/a	String to explain why the module is not yet ready for the next analysis (read-only)
SerialNo	n/a	n/a	n/a	Serial number of the module (read-only)

Command	Description
⇒ <i>Connect</i>	Connect the device.
<i>Disconnect</i>	Disconnect the device.
<i>Identify</i>	Identify the module. The module LED will flash.
⇒ <i>Reset</i>	Reset the device.

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

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:


 **Agilent 1100 HPLC System: Overview**

 **Agilent 1100 HPLC System: Installation**

Agilent 1100 HPLC System: Pump

Property	Min	Max	Default.	Description
⇒%B, %C, %D	0.0 %	100.0 %	100.0 %A	
Compressibility	autom. (-1), no compensation (0), 50..150	-	100	Compressibility [1e-6/bar] (Available only for the isocratic and quaternary pumps.)
Compressibility LeftPump	autom. (-1), no compensation (0), 50..150	-	50	Compressibility of the left pump [1e-6/bar] (Only for the binary pump)
Compressibility RightPump	autom. (-1), no compensation (0), 50..150	-	115	Compressibility of the right pump [1e-6/bar] (Available only for the binary pump.)
EmptySolventError	Off	On	Off	*
⇒Flow	0.000 ml/min	10.000 ml/min (quaternary) 5.000 ml/min (binary)	0.000 ml/min	
Pressure	0.0 bar	400.0 bar (5800 psi) (Flow < 5 ml/min) 200.0 bar (2900 psi) (else)	Min.: 0 Max. 400.0	
PrimaryChannel	autom. (0), A (1), B (2), C (3), D (4)	-	autom.	Automatic selects the channel with the maximum percentage. (Available only for the quaternary pump.)
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.)

Property	Min	Max	Default.	Description
SolventLeftPump	A1 (0), A2 (1)	-	A1	Eluent of the left pump. (Available only for the binary pump.)
SolventNotReady Limit	0.00 l	1000.00 l	0.00 l	*
SolventRightPump	B1 (0), B2 (1)	-	B1	Eluent of the right pump. (Available only for the binary pump.)
Stroke	autom. (-1), 20..100 µl	-	autom.	Pump stroke (Available only for the isocratic and quaternary pumps.)
StrokeLeftPump	autom. (-1), 20..100 µl	-	autom.	Stroke of the left pump. (Available only for the binary pump.)
StrokeRightPump	autom. (-1), 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.**

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions:**

 **Agilent 1100 HPLC System: Overview**

 **Agilent 1100 HPLC System: Installation**

Agilent 1100 HPLC System: Autosampler

Property	Min	Max	Default	Description
DispSpeed	10 µl/min <i>or</i> 90 µl/min	1000 µl/min	200 µl/min	Speed for ejecting the sample
DrawSpeed	10 µl/min <i>or</i> 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]
⇒ <i>Volume</i>	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
⇒ <i>Dispense</i>	Injects the sample into the loop.
⇒ <i>Draw</i>	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 lifted to the uppermost position.
InitiateChangePiston	Initiates the piston change by releasing the spring on the proportioning unit.
⇒ <i>Inject</i>	Performs an injection (see note below).
Mix	Mixes the sample using the syringe.
Needle Function=Up	Moves the needle to the uppermost position.
Needle Function=Seat	Moves the needle into the needle seat.
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: <i>Vial</i>	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: <i>Speed</i>	10 (or 90 µl) to 1000 µl/min Maximum Default (uses DispSpeed)	Speed for dispense or mix
Draw, parameter: <i>Source</i>	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: <i>Speed</i>	10 (or 90) to 1000 µl/min Maximum Default (uses DrawSpeed)	Speed for draw
Draw/Dispense, parameter: <i>Offset</i>	-2.5 to 35.0 mm SampleHeight	Needle draw/eject position offset for draw or dispense
Draw, Dispense, Mix parameter: <i>Volume</i>	0.0 to 100.0 µl (or 900.0 µl) Maximum InjectVolume	Volume for draw, dispense, or mix
Needle, parameter: <i>Vial</i>	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 \Rightarrow 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 given

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**

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 1100 HPLC System: Overview**

 **Agilent 1100 HPLC System: Installation**

Agilent 1100 HPLC System: Wellplate Sampler

The wellplate sampler supports some special commands in addition to the commands supported by the autosampler of the 1100 HPLC System (see  **Agilent 1100 HPLC System: Autosampler**). 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: <i>Vial</i>	SampleVial (current sample vial) Location (sample position No. n) Seat (needle seat)	Position of the vial for dispense
Dispense, Mix parameter: <i>Speed</i>	10-1000 µl/min Maximum Default (uses DispSpeed)	Speed for dispense or mix
DispSpeed	10-1000 µl/min	Speed for ejecting the sample
Draw, parameter: <i>Source</i>	SampleVial (current sample vial) Location (sample position No. n) Seat (needle seat) Air	Position of the vial for draw

Command	Values	Description
Draw, parameter: <i>Speed</i>	10-1000 µl/min Maximum Default (uses DrawSpeed)	Speed for draw
Draw/Dispense, parameter: <i>Offset</i>	-10.0 to 60.0 mm SampleHeight	Needle draw/eject position offset for draw or dispense
Draw/Dispense, Mix parameter: <i>Volume</i>	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: <i>Vial</i>	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

Special Wellplate Sampler Commands

Property	Min.	Max.	Default	Description
DelayVolumeReduction ²	Off	On	Off (read from device on connect)	
EquilibrationTime ³	0.0 s	100.0 s	0.0 s (sent to device on connect)	
FlushFactor	1.0	10.0	5.0 (read from device on connect)	
PrimePump	-	-	-	Primes needle for 5 seconds
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 (for user-defined inject programs)	<i>In</i> <i>In</i> = AsMethod, Flush, SampleVial or osition of sample	<i>Time</i> <i>Time</i> = 0.0 to 100.0 s	<i>Repeat</i> <i>Repeat</i> = 1 to 5	<i>Offset</i> <i>Offset</i> = -10.0 to 60.0 mm
Function	Defines the location of the wash solution	see WashTime required if <i>In</i> = Flush	see WashRepeat required if <i>In</i> > 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**

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 1100 HPLC System: Overview**

 **Agilent 1100 HPLC System: Installation**

Agilent 1100 HPLC System: Column Compartment

Property	Min	Max	Default	Description
AutoActivateSetpoint	0 (Off)	1 (On)	0 (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	0 (Combined)	1 (Separate)	0 (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	0 (Off)	1 (On)	Off	Turns temperature control on or off.
Valve	0 (Right Column)	1 (Left Column)	1 (Left Column)	Switches the column selector valve (option).

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 1100 HPLC System: Overview**

 **Agilent 1100 HPLC System: Installation**

Agilent 1100 HPLC System: UV Detectors

Property	Min	Max	Default	Description
AutoActivateUVLamp	Off	On	Off	If set to On , the deuterium lamp is automatically turned on right after power-up.
UVLamp	Off	On	Off, (On in ➤ <i>Demo Mode</i>)	Deuterium lamp state

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. (DAD only)
ReferenceDiodeExposure	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)
SampleDiodeExposure	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)
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. (DAD only)

Property	Min	Max	Default	Description
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 \Rightarrow Autozero. The command will be performed immediately when issued manually or at a \Rightarrow Program time <0.000 min. With a program time of 0.000, the command is executed before the injection program; with a program >0.000 , the command is executed 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

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.
\Rightarrow Bandwidth	2nm	400nm	channel dependent	Signal bandwidth
\Rightarrow 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)
\Rightarrow 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
PeakWidth	0	7	4	Expected minimum peak width (The setting affects the Step: see below).

Property	Min	Max	Default	Description
⇒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 3D micro slit.
⇒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 [s]	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 [s]	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.
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.

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:

 **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 $\triangleright Programs$ 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 $\triangleright Autosampler$ 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 given. (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 given that would result in another autozero. If necessary, this can be achieved via the **Wait UV.Ready** command. 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 be given 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.**

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.

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:



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 ; falls notwendig
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 \Rightarrow *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: Operating 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.
- **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**.*

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:



Agilent 1100 HPLC System: Overview



Agilent 1100 HPLC System: Installation



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`) 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 needs not 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.

Agilent 1100 HPLC System: Checking the Solvent Liquid Level

For the 1100 HPLC System, you can have Chromeleon check the liquid level for the single solvents and determine the system's reaction if a specified minimum volume is not met.

The following commands are available:


Command	Min.	Max.	Default	Description
SolventFillingXX	0.00 l	1000.00 l	n/a	Enter the volume in [l] with which the bottle is originally filled (XX = A1, A2, B1 or B2).
EmptySolventError	Off	On	Off	Determine how the system reacts when the SolventNotReadyLimit is not met: On = The pump is turned off immediately. (The red LED lights.) Off = The system waits until processing of the running sample is finished and then turns off the pump. The pump is in NotReady state; the yellow LED lights.
SolventNotReadyLimit	0.00 l	1000.00 l	0.00 l	Determine the minimum filling volume. If the actual solvent volume is below this value a warning appears.

A meaningful setting for **SolventNotReadyLimit** would be to enter a volume that is large enough so that the last sample can be completely processed in any case. If the **EmptySolvent Error** is set to **Off**, even the last sample can be processed when the **SolventNotReadyLimit** has been reached. The pump is stopped when sample processing is complete.



Tip:

*For older pump versions, e.g., with firmware version A.01.06, it is not possible to check the solvent liquid level. For these pumps, select the **Disable Solvent Limits Features** option on the **Options** tab page in the Server Configuration.*

*(For more information about the Options tab page and the Server Configuration, refer to **Options** the Administrator online Help and to the Administrator Manual: Software Installation and Communication  The Server Configuration Program.)*

For general information and installation instructions for the 1100 HPLC system, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 1100 HPLC System: Overview**


 **Agilent 1100 HPLC System: Installation**

Agilent 1100 HPLC System: Troubleshooting

- An error occurs if one of the single devices of 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)

In this case, re-start the Chromeleon server.

- If the pump stops delivering and the red LED lights, although no leak or any other obvious hardware problem could be detected, this may be due to the settings made 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 continues to light. For more information about these commands, refer to the **User Manual: Commands and Tips for Third-Party Devices**  **Agilent 1100 HPLC System: Checking the Solvent Liquid Level**.
- When connecting, the 1100 HPLC system sends different device notifications to the Audit Trail. Usually, these notifications are for information purposes only. They may facilitate troubleshooting in case of system failure.
- It may happen that the fluorescence detector remains in **Not Ready** state after the detector and the lamp have been turned on. In this case, the yellow LED on the detector is burning 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.
- We recommend performing blank runs with injection. Specify vial that contain solvent as position and then, specify the injection command as follows:

Inject Blank=Inject

The sampler of the 1100 HPLC system is running as well thus, improving synchronization of the entire system.

Agilent 1050 HPLC System: UV Detector



Device properties and commands

Property	Min.	Max.	Default	Description
Lamp	Off	On	Read from the device on connect (On in <i>> Demo Mode</i>).	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).	Time constant; indicates the time required by the detector to reach 98% of the full deflection.

Channel properties and commands

















Command/Property	Min.	Max.	Default	Description
\Rightarrow Delta	$-2 \cdot 10^4$	$2 \cdot 10^4$	Not available.	Signal slope [mAU/s].
\Rightarrow 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].
\Rightarrow Step	0.05	3.2	0.4	Data rate [s] for signal and spectra.
\Rightarrow Wavelength	190	600	Read from the device on connect (254 in Demo mode).	Signal wavelength [nm].

For general information and installation instructions for the HP 1050 HPLC system, refer to the **Administrator Manual: Installation Instructions**:

-  **Agilent HP 1050 HPLC System: Overview**
-  **Agilent HP 1050 HPLC System: Installation**

Agilent 6890/6850 GCs

For information about the special commands supported for the Agilent 6890/6850 GCs and for tips for practical operation, refer to:

-  **Agilent 6890/6850 GCs: General**
-  **Agilent 6890/6850 GCs: Detectors**
-  **Agilent 6890/6850 GCs: ECD**
-  **Agilent 6890/6850 GCs: FID and FPD**
-  **Agilent 6890/6850 GCs: NPD (Nitrogen Phosphorous Detector)**
-  **Agilent 6890/6850 GCs: TCD (Thermal Conductivity Detector)**
-  **Agilent 6890/6850 GCs: μ -ECD**
-  **Agilent 6890/6850 GCs: Column**
-  **Agilent 6890/6850 GCs: Inlet (General)**
-  **Agilent 6890/6850 GCs: Inlet**
(Purged Packed EPC, Cool On-Column EPC, ACI, PCM, Volatiles)
-  **Agilent 6890/6850 GCs: Inlet**
(Split/Splitless EPC, Gerstel PTV, PTV, CIS3, CSI4)
-  **Agilent 6890/6850 GCs: Inlet**
(Purged Packed, Cool-On-Column, Unknown)
-  **Agilent 6890/6850 GCs: Inject**
-  **Agilent 6890/6850 GCs: Auxiliary Devices**
-  **Agilent 6890/6850 GCs: Application**
-  **Agilent 6890/6850 GCs: PGM File - Entering Pressure or Flow?**

Agilent 6890/6850 GCs: General

Property	Min.	Max.	Default	Description
SerialNo	n/a	n/a	n/a	Serial number (20 characters; read-only).
ModelNo	n/a	n/a	n/a	Device number, i.e., 6850 or 6890 (20 characters; read-only).
FirmwareVersion	n/a	n/a	n/a	Firmware version (20 characters; read-only).
Ready	0 (Not Ready)	1 (Ready)	Depends on the Ready state of the instrument. Demo mode: 1	Indicates whether the GC is ready for injection (read-only).
Temperature	-	-	-	Properties related to the column compartment temperature.
Temperature. Value	Depends on the system and installed cryo type. For details, refer to the column compartment temperature section.		-	Current column compartment temperature (read-only).
Temperature. Nominal	Depends on the system and installed cryo type. For details, 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. For details, 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.
EquilibrationTime	0 min	999.99 min in 0.01 increments	² (Demo mode: 0.5)	After reaching the desired temperature, the GC waits for the equilibration time. When the equilibration time has passed, the GC is ready.
TempCtrl	0 (Off)	1 (On)	² (Demo mode: On)	
Oven Type	0 (Fast)	1 (Regular)	Fast	Regular or fast oven type.

Property	Min.	Max.	Default	Description
Cryo	0 (Off)	1 (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.)
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.)
QuickCool	0 (Off)	1 (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.)
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	0 (Off)	1 (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.)
CryoFault	0 (Off)	1 (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.)
RunState	0 (Idle), 1 (PreRun), 2 (Run), 3 (PostRun)		² (In Demo mode: 0)	GC state: Idle, PreRun, Run, or PostRun (read-only).

Property	Min.	Max.	Default	Description
RunLog	-	-	-	Writes the sample protocol in the Audit Trail.
PrepRun	-	-	-	Sets the instrument into the PreRun state.
ReportReady	-	-	-	Status of the instrument; reports whether the instrument is not ready for operation and why.

**Note:**

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:



Agilent 6890 GC: Overview



Agilent 6890 GC: Installation



Agilent 6850 GC: Overview



Agilent 6850 GC: Installation

Agilent 6890/6850 GCs: Detectors

Property	Min.	Max.	Default	Description
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 (33 characters, read-only)
Temperature	-	-	-	Properties related to the temperature of the heated zone. (The property is available only if Zone is installed on the Detector configuration tab page.)
Temperature. Value	0.0°C	450.0°C	-	Actual temperature of the heated zone (read-only).
Temperature. Nominal	0	450°C in increments of 1	² (Demo mode: 300°C)	Specifies the target temperature for the heated zone.
TempCtrl	0 (Off)	1 (On)	² (Demo mode: 0)	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.
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.
Range	0	13 in increments of 1	² (Demo mode: 0)	The property is available only for detectors with analog data acquisition mode.

Property	Min.	Max.	Default	Description
FastPeaks	0 (Off)	1 (On)	² (Demo mode: 0)	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).
MakeupMode	0 (Make-up)	1 (Combined)	² (Demo mode: 0)	Determine which flow shall be kept constant: Make-p or Combined. (The property is available only for FID; TCD, ECD, μ -ECD, NPD, and FPDs detectors.)
MakeupFlow (Detectors without EPC option)	0.0 ml/min	100.0 ml/min in increments of 1	² (Demo mode: 10.0 ml/min)	Makeup gas flow. In this form, the property is available for detectors without EPC option (see Detector configuration tab page).
MakeupFlow (Detectors with EPC option)	0.0 ml/min	100.0 ml/min in increments of 1	² (Demo mode: 10.0 ml/min)	Properties related to the makeup gas flow. In this form, the property is available for detectors with EPC option (see Detector configuration tab page).
MakeupFlow. Value	0.0 ml/min	100.0 ml/min	-	Current makeup gas flow (read-only).
MakeupFlow. Nominal	0.0 ml/min	100.0 ml/min	² (Demo mode: 10.0 ml/min)	Specifies the target makeup gas flow.
MakeupFlowCtrl (Detectors with EPC option)	0 (Off)	1 (On)	² (Demo mode: 0)	Turns the pneumatics on and off. In this form, the property is available for detectors with EPC option (see Detector configuration tab page).
CombinedFlow (Detectors without EPC option)	0.0 ml/min	1000.0 ml/min	-	Combined gas flow (makeup + inlet). In this form, the property is available for detectors without EPC option (see Detector configuration tab page).

Property	Min.	Max.	Default	Description
CombinedFlow (Detectors with EPC option)	0 (Off)	1 (On)	² (Demo mode: 0)	Properties related to the combined gas flow (makeup + inlet). In this form, the property is available for detectors with EPC option (see Detector configuration tab page).
Combined Flow.Value	0.0 ml/min	1000.0 ml/min	-	Indicates the current combined flow (read-only)
Combined Flow.Nominal	0.0 ml/min	1000.0 ml/min	-	Specifies the target combined flow.
⇒Autozero	-	-	-	Sets the current detector signal to 0.



Note:

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:



Agilent 6890 GC: Overview



Agilent 6890 GC: Installation



Agilent 6850 GC: Overview



Agilent 6850 GC: Installation

Agilent 6890/6850 GCs: ECD

Property	Min.	Max.	Default	Description
Pulser	0 (Off)	1 (On)	² (Demo mode: 0)	Turns the detector electronics on and off.
AdjustOffset	0 (Off), 1 (On), or 2 (Abort)		² (Demo mode: 1)	Adjusts the reference current until the desired output is reached as defined by the TargetOutput property.
TargetOutput	20	200 in increments of 1	² (Demo mode: 60)	Specifies the desired detector output for the AdjustOffset procedure.
RefCurrent	0.5 nA	5.0 nA in increments of 1	² (Demo mode: 1.5)	
MakeupGasType (Detectors with EPC option)	0 (Nitrogen), 3, Argon Methane, 7 (Unknown)		² (Demo mode: 0)	Makeup gas type. (The property is available only for detectors with EPC option, see the Detector configuration tab page.)
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. Value	0.0 ml/min	12.0 ml/min	-	Current anode gas flow (read-only).
AnodeFlow. Nominal	0.0 ml/min	12.0 ml/min	0.0 ml/min	Specifies the target anode gas flow.

Property	Min.	Max.	Default	Description
Anode FlowCtrl (Detectors with EPC option)	0 (Off)	1 (On)	² (Demo mode: 1).	Turns the pneumatics on and off. In this form, the property is available for detectors with EPC option, see the Detector configuration tab page.)
MakeupGasType (Detectors with EPC option)	0 (Nitrogen), 1 (Hydrogen), 2 (Helium), 3 (Argon Methane), 7 (Unknown)		² (Demo mode: 0).	Makeup gas type. The property is available only for detectors with EPC option, see the Detector configuration tab page.)

**Note:**

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 6890 GC: Overview**

 **Agilent 6890 GC: Installation**

 **Agilent 6850 GC: Overview**

 **Agilent 6850 GC: Installation**

Agilent 6890/6850 GCs: FID and FPD

Property	Min.	Max.	Default	Description
Electrometer	0 (Off)	1 (On)	² (Demo mode: 0)	Turns the detector electronics on and off.
Flame	0 (Off)	1 (On)	² (Demo mode: 0)	Turns the flame on and off.
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.
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.)
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)	0 (Off)	1 (On)	² (Demo mode: 0)	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.
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.

Property	Min.	Max.	Default	Description
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.Value	0.0 ml/min	100.0 ml/min		Current H2 gas flow.
H2Flow.Nominal	0,0 ml/min	100,0 ml/min in 1er Schritten	² (Demo mode: 30 ml/min)	Specifies the target H2 gas flow..
H2FlowCtrl	0 (Off)	1 (On)	² (Demo mode: 0)	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.)
OxidizerGasType	4 (Oxygen), 5 (Air), 7 (Unknown)			The property is available only for FPD detectors with EPC option, see the Detector configuration tab page.
MakeupGasType	0 (Nitrogen), 1 (Hydrogen), 2 (Helium), 3 (Argon Methane), 7 (Unknown)		² (Demo mode: 0)	Makeup gas type. The property is available only for FID detectors with EPC option, see the Detector configuration tab page.

Note:

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 6890 GC: Overview**

 **Agilent 6890 GC: Installation**

 **Agilent 6850 GC: Overview**

 **Agilent 6850 GC: Installation**

Agilent 6890/6850 GCs: NPD (Nitrogen Phosphorous Detector)

Property	Min.	Max.	Default	Description
PolVoltage	0 (Off)	1 (On)	² (Demo mode: 1)	Turns the detector electronics on and off.
BeadPower	0 (Off)	1 (On)	² (Demo mode: 1)	Turns the bead power on and off.
AdjustOffset	0 (Off), 1 (On), 2 (Abort)		² (Demo mode: 0)	Adjusts the reference current until the desired output is reached as defined by the TargetOutput property.
TargetOutput	20	200 in increments of 1	² (Demo mode: 50)	Specifies the target detector output for the AdjustOffset procedure.
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.
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.
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.
MakeupGasType (Detectors with EPC option)	0 (Nitrogen), 1 (Hydrogen), 2 (Helium), 3 (Argon Methane), 7 (Unknown)		² (Demo mode: 0)	Makeup gas type. The property is available only for detectors with EPC option, see the Detector configuration tab page.)
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.)

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	200.0 ml/min in increments of 1	-	Current air gas flow (read-only).
AirFlow.Nominal	0.0 ml/min	200.0 ml/min in increments of 1	² (Demo mode: 60)	Specifies the target air gas flow.
AirFlowCtrl (Detectors with EPC option)	0 (Off)	1 (On)	² (Demo mode: 1)	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.
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.Value	0.0 ml/min	30.0 ml/min	-	Current H2 gas flow (read-only).
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.

Property	Min.	Max.	Default	Description
H2FlowCtrl (Detectors with EPC option)	0 (Off)	1 (On)	² (Demo mode: 1)	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:**

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:



Agilent 6890 GC: Overview



Agilent 6890 GC: Installation



Agilent 6850 GC: Overview



Agilent 6850 GC: Installation

Agilent 6890/6850 GCs: TCD (Thermal Conductivity Detector)

Property	Min.	Max.	Default	Description
Filament	0 (Off)	1 (On)	1	Turns the filament on and off.
Polarity	0 (Positive)	1 (Negative)	² (Demo mode: 0)	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.
RefFlow.Value	0.0 ml/min	100.0 ml/min	-	Current reference gas flow (read-only).
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.
RefFlowCtrl (Detectors with EPC option)	0 (Off)	1 (On)	² (Demo mode: 1)	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 (FID Detectors with EPC option)	0 (Nitrogen), 1 (Hydrogen), 2 (Helium), 3 (Argon Methane), 7 (Unknown)		² (Demo mode: 0)	Makeup gas type. In this form, the property is available only for FID detectors with EPC option, see the Detector configuration tab page.



² **Note:**

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

Agilent 6890/6850 GCs: μ -ECD

Property	Min.	Max.	Default	Description
Pulser	0 (Off)	1 (On)	² (Demo mode: 1)	Turns the detector electronics on and off.
AdjustOffset	0 (Off), 1 (On), 2 (Abort)		² (Demo mode: 0)	Adjusts the reference current to the desired output as defined by the TargetOutput property.
TargetOutput	20	200 in increments of 1	² (Demo mode: 60)	Specifies the target detector output for the AdjustOffset procedure.
RefCurrent	0.5 nA	5.0 nA in 0.1 increments	² (Demo mode: 1.5 nA)	Reference current.
MakeupGasType (Detectors with EPC option)	0 (Nitrogen), 3 (ArgonMethane), 7 (Unknown)		² (Demo mode: 0)	Makeup gas type. The property is available only for detectors with EPC option, see the Detector configuration tab page.



Note:

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 6890 GC: Overview**

 **Agilent 6890 GC: Installation**

 **Agilent 6850 GC: Overview**

 **Agilent 6850 GC: Installation**

Agilent 6890/6850 GCs: Column

Property	Min.	Max.	Default	Description
Pressure	-	-	-	Properties related to the pressure.
Pressure.Value	0.0 bar	10.0 bar	-	Measured pressure value (read-only).
Pressure.Nominal	0.0 bar	10.0 bar in increments of 1	Emulation of the ramp.	Specifies the target pressure value.
Flow	-	-	-	Properties related to the flow.
Flow.Value	0.0 ml/min	1000.0 ml/min	-	Current flow value (read-only).
Flow.Nominal	0.0 ml/min	1000.0 ml/min in increments of 1	Emulation of the ramp	Specifies the target flow value.
Velocity	-	-	-	Properties related to the average linear gas velocity.
Velocity.Value	0 cm/s	1000 cm/s	-	Average linear gas velocity (read-only).
Velocity.Nominal	0 cm/s	1000 cm/s in increments of 1	-	Specifies the desired average target gas velocity. .
FlowMode	0 (Pressure-Ctrl)	1 (FlowCtrl)	0	Defines the pneumatics mode for the pressure or flow profile for the column.
Diameter	0.0 (Unknown) μ m	1000.00 μ m in 0.01 increments	250.00	Specifies the inner diameter of the column.
Length	0.0 (Unknown) mm	1000,00 mm in 0.01 increments	30.00 mm	Specifies the column length.
FilmThickness	0.0 (Unknown) μ m	1000.00 μ m in 0.01 increments	10.00 μ m	Specifies the film thickness of the column. .
Inlet	6890: 0 (Front), 1 (Back), 2 (Aux3), 3 (Aux 4), 4 (Aux 5), 5 (Unknown) 6850: 0 (Front), 2 (Aux3), 3 (Aux 4), 4 (Aux 5), 5 (Unknown)		6890: 0 for the front detector, 1 for the back detector 6850: 0	Inlet to which the detector is connected.

Property	Min.	Max.	Default	Description
Detector	6890: 0 (Front), 1 (Back), 2 (MSD), 3 (AED), 4 (Unknown) 6850: 0 (Front), 2 (MSD), 3 (AED), 4 (Unknown)		6890: 0 for the front detector 6850: 0	Detector to which the column is connected.
VacuumCorrection	0 (Off)	1 (On)	² (Demo mode: 0)	Set to On if the column is connected to the vacuum, i.e., if the column is directly connected to a mass spectrometer.
OutletPressure Correction	0 (Off)	1 (On)	² (Demo mode: 0)	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.
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.



Note:

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:



Agilent 6890 GC: Overview



Agilent 6890 GC: Installation



Agilent 6850 GC: Overview



Agilent 6850 GC: Installation

Agilent 6890/6850 GCs: Inlet (General)

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)
Temperature	-	-	-	Properties related to the temperature of the heated zone.
Temperature. Value	Depends on the inlet type (see Inlets temperature limits)		-	Current temperature of the heated zone (read-only).
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.
TempCtrl	0 (Off)	1 (On)	² (Demo mode: 0)	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:**

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?**

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:

**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)

Property	Min.	Max.	Default	Description
Flow	-	-	-	Properties related to the gas flow.
Flow.Value	0.0 ml/min	1000.0 ml/min	-	Measured gas flow (read-only).
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.
Pressure	-	-	-	Properties related to the gas pressure
Pressure.Value	0.00 bar	10.00 bar	-	Measured gas pressure (read-only).
Pressure.Nominal	0.00 bar	10.00 bar in 0.01 increments	² (Demo mode: 1.30 bar)	Specifies the target gas pressure.
PressureEquilibrationTime	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 0.00 min)	After reaching the desired gas pressure, the GC waits the time specified under PressureEquilibrationTime before signaling Ready.
FlowEquilibrationTime	0.00 min	999.99 min in 0.01 increments	0.00 min	After reaching the desired gas flow, the GC waits the time specified under FlowEquilibrationTime before signaling Ready.
GasType	0 (Nitrogen), 1 (Hydrogen), 2 (Helium), 3 (Argon), 7 (Unknown)		² (Demo mode: 0)	Gas type.



² **Note:**

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?

Agilent 6890/6850 GCs: Inlet (Split/Splitless EPC, Gerstel PTV, PTV, CIS3, CIS4)

Property	Min.	Max.	Default	Description
Flow	-	-	-	Properties related to the gas flow.
Flow.Value	0.0 ml/min	1000.0 ml/min	-	Measure gas flow (read-only).
Flow.Nominal	0.0 ml/min	1000.0 ml/min in 0.1 increments	² (Demo mode: 80.0 ml/min)	Target gas flow.
Pressure	-	-	-	Properties related to the gas pressure.
Pressure.Value	0.00 bar	10.00 bar	-	Measure gas pressure (read-only).
Pressure.Nominal	0.00 bar	10.00 bar in 0.01 increments	² (Demo mode: 1.30 ml/min)	Target gas pressure.
Pressure-equilibrationTime	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 0.00 min)	After reaching the desired gas pressure, the GC waits the time specified under PressureEquilibrationTime before signaling Ready
FlowEquilibrationTime	0.00 min	999.99 min in 0.01 increments	² (Demo mode: 0.00 min)	After reaching the desired gas flow, the GC waits the time specified under FlowEquilibrationTime before signaling Ready
GasType	0 (Nitrogen), 1 (Hydrogen), 2 (Helium), 3 (Argon Methane), 7 (Unknown)		² (Demo mode: 0)	Gas type.
ColumnDefined	0 (Off)	1 (On)	² (Demo mode: 0)	Indicates whether the column is defined (ready-only).
Mode	0 (Split), 1 (Splitless), 2 (PulsedSplitless), 3 (PulsedSplit), 4 (SolventVent/Vent)		² (Demo mode: 0)	Operation mode (split or splitless, with or without pulse).

Property	Min.	Max.	Default	Description
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.
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).	Time when the purge valve shall be opened (only valid in Splitless mode).
PulsePressure	0.00 bar	10.00 bar in 0.01 increments	² (Demo mode: 2.00 bar)	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 (only valid in Pulsed mode).
PulseTime	0.00 min	999.99 in 0.01 increments	² (Demo mode: 1.00 min)	Duration of the pressure pulse (only valid in Pulsed mode).
GasSaver	0 (Off)	1 (On)	0	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.
VentFlow	0.0 ml/min	1000.0ml/min in 0.1 increments	² (Demo mode: 0.0 ml/min)	Properties related to the flow from the split vent during the vent period (before the VentEndTime). The property is only valid in SolventVent mode.

Property	Min.	Max.	Default	Description
VentPressure	0.00 bar	10,00 bar in 0,01er Schritten	² (Demo mode: 0.00 bar)	Inlet pressure during the vent period. Set the property to 0 for the lowest possible pressure.
VentEndTime	0.00 min	999.99 min in 0.01 increments.	² (Demo mode: 0.00 min)	Time at which solvent venting ends. The time should be greater than the time needed for all injections (only valid in SolventVent mode).



Note:

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?

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:



Agilent 6890 GC: Overview



Agilent 6890 GC: Installation



Agilent 6850 GC: Overview



Agilent 6850 GC: Installation


Agilent 6890/6850 GCs: Inlet (Purged Packed, Cool-On-Column, Unknown)

Property	Min.	Max.	Default	Description
ModelNo	-	-	-	Model number (8 characters, read-only)
FirmwareVersion	-	-	-	Firmware version (6 characters, read-only)
StopInject	-	-	-	Stops the running injections.
EraseErrorLog	-	-	-	Deletes the sample error log.

Also, refer to:

Agilent 6890/6850 GCs: PGM File - Entering Pressure or Flow?

For general information and installation instructions for the HP 6890 and HP6850 GCs, refer to the **Administrator Manual: Installation Instructions**:

 **Agilent 6890 GC: Overview**

 **Agilent 6890 GC: Installation**

 **Agilent 6850 GC: Overview**

 **Agilent 6850 GC: Installation**

Agilent 6890/6850 GCs: Inject

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 GC				
ModelNo	-	-	-	Model number (8 characters, read-only)
FirmwareVersion	-	-	-	Firmware version (6 characters, read-only)
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).
Position	1	22 or 27 in increments of 1	1	Current sample position on the tablet.
State	Off	On	0	Indicates whether the autosampler has injected (read-only).
InjectWaitTime	0.0 s	1 E7 s	0.0 s	Time between the inject command and the inject response.
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).
Pre-a	0	15	0	Indicates how often the syringe is rinsed with solvent from the solvent reservoir A prior to the injection.

Property	Min.	Max.	Default	Description
Pre-b	0	15	0	Indicates how often the syringe is rinsed with solvent from the solvent reservoir B prior to the injection.
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 air bubbles and to enhance the reproducibility.
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.
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.
Speed	Fast	Slow	0	Average piston speed ³
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.
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.

Property	Min.	Max.	Default	Description
Depth	0.0 mm	1.0 mm in increments of 1	Default	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.
Waste	0 (A-only), 1 (B-only), 2 (A-and-B)		² (Demo mode: 0)	Specifies which waste bottles shall be used (alternating).
Fan	0 (Off)	1 (On)	² (Demo mode: 0)	Turns the injector fan on and off.



Note:

When connecting, Chromeleon reads the value from the instrument and accepts it as default.



Note:

*The syringe volume is selected in the Server Configuration program. The plunger speed that can be achieved with the **Speed** command depends on the selected syringe. Refer to the table below for details.*

Syringe (μl)	Plunger Speed (μl/min)	
	Fast	Slow
5	3000	150
10	6000	300
25	15000	750
50	30000	1500
100	60000	3000

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:

Agilent 6890 GC: Overview and **Agilent 6890 GC: Installation**

Agilent 6850 GC: Overview and **Agilent 6850 GC: Installation**

Agilent 6890/6850 GCs: Auxiliary Devices

Property	Min.	Max.	Default	Description
Temperature				Properties related to the temperature of the heated zone.
Temperature.Value	0.0°C	500.0°C	-	Current temperature of the heated zone.
Temperature.Nominal	0.0°C	500.0°C	Downloaded from the instrument.	Target operating temperature for the heated zone.
TempCtrl	0 (Off)	1 (On)	² (Demo mode: 0)	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.
Pressure	-	-	-	Properties related to the gas pressure.
Pressure.Value	0.00 bar	10.00 bar	-	Measured gas pressure (read-only).
Pressure.Nominal	0.00 bar	10.00 bar	Downloaded from the instrument.	Specifies the target gas pressure.
PresCtrl	0 (Off)	1 (On)	² (Demo mode: 0)	Specifies the state of the pressure control. The property is automatically set to On if an assignment is made in the Pressure field.



Note:

When connecting, Chromeleon reads the value from the instrument and accepts it as default.

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 corresponding Command dialog box or the Properties/Link box.

In Chromeleon, pressure is always stated in bar. Selecting a different pressure unit is not supported. 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
```

After this command, the column commands also control the inlet. In this case, the command given last applies.

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?**

Column

It is possible to enter column dimensions and connections. However, entering the calibration is currently not possible. If column dimensions and connections are not defined, certain commands, such as Velocity, MakeupMode = Combined, etc., cannot be executed

It is not recommended to enter the column dimensions and connections in a sample program. However, you should include the parameters in the *➤Audit Trail* using the *⇒Log* command.

Valves

Special valve types (Multiposition, Gas Sampling) are currently not supported. For more information, please contact Dionex Service.

Aux

For the 6850 GC, an auxiliary device can be installed. For the 6890 GC, a maximum of two auxiliary devices for temperature control are supported.

For both GC types, a maximum of three auxiliary devices for pressure control are supported.

Sampler

For the 6890 GC, the extended capabilities of the HP 7683 autosampler, such as solvent prewashes, are supported. The model 7673 did not support them.

When Chromeleon connects the 6890 GC or 6850 ALS for the first time (see \Rightarrow *Connect*), some (or many) error messages may appear starting with **Error log at**. These are previous errors recorded by the autosampler. You can simply ignore them. Future versions will show new errors only.

Barcode Reader (only for the 6890 GC): If **UseBCR = On** (see **UseBCR** in the User online Help), 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, using the **Flow** command on the **Control** menu (GC tab page) or by a \triangleright *PGM File*.

For the 6890 GC, a temperature profile can be entered with 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 so-called "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.

However, 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 Setting Commands

You can 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), using the following commands:

```
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
```

To set the temperature of the single modules, e.g., to 80°C, you have to use the following commands:

```
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
```

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 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 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 fast 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.*

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:



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	-	-	-	-
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	-	Flow	-	-
Cool On Column	Pressure	Pressure	Pressure	-
Split/Splitless	Pressure	Pressure	Pressure	-
PTV	Pressure	Pressure	Pressure	-
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.

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.

For general information and installation instructions for the 6890 and 6850 GCs, refer to the **Administrator Manual: Installation Instructions**:

 **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 so-called "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 \Rightarrow 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.*

For general information and installation instructions for the 5890 GC, refer to the **Administrator Manual: Installation Instructions**:




Agilent HP5890 GC: Overview



Agilent HP5890 GC: Installation

CTC Analytics

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

Apart from  **General Device Commands**, the CTC PAL Samplers support the following commands and properties:

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 (<i>read-only</i>).
CPUSerialNo	-	Expert	Indicates the CPU serial number of the connected sampler (<i>read-only</i>).
DeviceState	Ready/Busy/ Init(Error)/Unknown	Normal	Indicates the current device status (<i>read-only</i>).
StatusDescription	-	Normal	Indicates the current action of the sampler. If no action is performed, Idle is displayed (<i>read-only</i>).
Syringe	-	Normal	Indicates the installed syringe type (<i>read-only</i>).

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 (<i>only in HS_Progr injection mode</i>).
IncubTimeStart	0 - 1440 min	Normal	Incubation time for the first sample in a sequence. This value can only be set once per sequence (<i>only in HS_Progr injection mode</i>).
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 (<i>in LC_Inject, GC_Inject, and HS_Single modes</i>).
PostInjectDelay	0 - 99000 ms or Default *	Normal	Time between the injection and the removing of the needle from the injector (<i>in LC_Inject, GC_Inject, HS_Single, and HS_Progr modes</i>).
PreInjectDelay	0 - 99000 ms or Default *	Normal	Time between inserting the needle in the injector and injecting the liquid into the sample loop (GC inlet) (<i>in LC_Inject, GC_Inject, HS_Single, and HS_Progr modes</i>).
PreparationTime	0 - 1440 min	Normal	Preparation time. This value is set in HS_Single mode at the start time of a new sample (<i>read-only</i>). Corresponds to the remaining incubation time of the running sample (marked green), i.e., the time until the sample is injected.

Command	Value Range	Filter	Description
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 (<i>in GC_Inject mode and for the WashWithSample command</i>).
Tray	List of all tray objects of the installed PAL	Normal	Tray to be used for Inject (<i>for all injection modes except Custom</i>). 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

The 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**)

PAL Sampler for GC:

Standard GC injections -- **GC_Inject** injection mode

User-defined HPLC injections -- **Custom** injection mode

(see  **PGM File for Custom Inject Mode**)


Headspace injections -- **HS_Single** and **HS_Progr** injection modes

(see  **PGM File for HS_Progr Inject Mode**)

Depending on the injection mode, different parameters are required to define how the single steps shall be performed:


Step	Parameters for LC_Inject	Parameters for GC_Inject	Parameters for HS_Single and HS_Progr headspace modes
1. Preparation			IncubationTime <i>or</i> IncubStartTime/IncubOffset, GCCycleTime
2. Load	AirVolume FillSpeed FillStrokes	AirVolume FillVolume FillSpeed PullupDelay FillStrokes	AirVolume = 0 FillSpeed PullupDelay FillStrokes
3. Injection	PreInjDelay PostInjDelay InjSpeed Injector	PreInjDelay PostInjDelay InjSpeed Injector	PreInjDelay PostInjDelay InjSpeed 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 the **Administrator Manual: Installing and Controlling Third-Party Devices**  **CTC Analytics PAL LC/GC Samplers: Installation.**)
6. The needle is moved into the injector.
7. The sampler waits the time specified with **PreInjDelay**.
8. The injection valve is moved into Load position (only for **LC_Inject** mode).
9. The content of the syringe is dispensed into the sample loop or the GC inlet.

10. The sampler waits the time specified with **PostInjDelay**.
11. The injection valve is moved into Inject position (only for **LC_Inject** mode).
12. The needle is moved out of the injector.
13. The needle returns to Home position.

**Tip:**

Steps 4 and 5 and 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 could 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 sampler
  Wash WashStation=Wash1, WashCycles=1
  WashWithSample WashCycles=2
; Injection procedure
0.000 Wait Ready
  Inject
  UV.AcqOn
; Cleansing steps 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.

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 \Rightarrow *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 could look as follows:

```
; Pump/detector commands
...
; PAL commands
  InjectMode = Custom
; Preparation steps 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 sampler
  Wash WashStation=Wash1, WashCycles=1
    WashInjector WashStation=Wash2, Injector=GC_Inj1,
      WashCycles=1
10.00 UV.AcqOff

End
```

CTC Analytics PAL Samplers: Tips for Headspace Operation

Chromeleon supports two injection modes that allow you to perform headspace injections:

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 have to determine an incubation time (**IncubationTime** parameter) for every sample.

Contrary to **HS_Single** mode, you do not have to set the incubation time explicitly for the samples in a sequence in **HS_Progr** mode. You can increase the incubation time of the previous sample by a certain value (**IncubOffset**) instead. 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:

$$\text{IncubTimeStart} + (n-1) * \text{IncubOffset}.$$


Tip:

*You are not allowed to change the **IncubOffset** or **IncubTimeStart** parameters in a sequence, i.e., they can be set either 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 that this time is long enough for all actions of 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 position 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, we recommend 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, we recommend 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 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 processed, Chromeleon may attempt to place a vial on 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 of 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 a 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 (sample type **Blank** for which the inject command contains the **Skipped** parameter) and samples without an inject command: Sample preparation is only simulated, i.e., the system waits the desired 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 as well via the **HS_Progr** injection mode.

A typical program for headspace injections could look as follows:

```
Agitator_Temperature.Nominal = 100.0
Agitator_Temperature.Accuracy = 1.0
TrayName = "Tray1"
Injector = GC_Inj1
InjectMode = HS_Progr
FillStrokes = 3
PreInjectDelay = 3000
PostInjectDelay = 5000
; Incubation time for the first sample of a sequence
IncubTimeStart = 100
; Additional incubation time of the last sample
IncubTimeOffset = 50
; Minimum run time between two injections(buffer: 600s + 100s)
GCCycleTime = 700
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
```

CTC Analytics PAL Sampler: Troubleshooting

The following problems may occur when you operate the PAL sampler:

Missing vial:

If no vial is present at the position from which the sampler shall inject, the batch is aborted. Due to limitation of the firmware, the sampler may then remain in an inconsistent state.

Therefore, we recommend performing either 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 sampler while it is moving into z direction (e.g., because of an incorrect configuration), the sampler reports a z collision error.

First, solve the reason for the z collision error and then, perform a **Home** command. This will re-calibrate the motor in the directions x, y, and z. Only then, the driver will be able to perform movement commands again.



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 as described for a z collision error.

Aborting a sequence or abort error in headspace mode:

If a z collision error is not the reason for an abort error, the driver attempts to remove all samples that are still in the agitator. However, samples may be left in the (heated) agitator. For security reasons, we recommend 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.

Fisons AS800/ThermoQuest AS2000 Autosampler

Chromeleon supports the following special commands and parameters for these autosamplers. Also, refer to the information in the manual supplied with the instrument.

Command	Description
Inject	Injects the sample using the previously defined parameters (see below).
Clean	Cleans the syringe 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
Cleaning Cycles before Injection (Pre Inj Clean Cyc) [Solvent A/B/C/D]	Select this parameter to set pre-injection cleaning cycles with solvent A/B/C/D. Please note: Only the solvent that has been specified last is used.
Volume (Pre Inj Clean Vol)	Select this parameter to set the pre-injection cleaning volume (0,0 - 500,0 µl).
Clean Cycles	Select this parameter to determine the number of cleaning cycles before injection (0...15). Specify the volume with the FillingVolume parameter.
With Sample (Position)	Select this parameter to specify the sample that shall be used for cleaning (positions 0...15).
Pumps (Pullup Count)	Select this parameter to determine how often the syringe plunger is moved up and down before the sample is actually drawn up (0...15). By eliminating bubbles, this enhances reproducibility.
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 before (Pre Inj Delay Time)	Select this parameter to determine the delay the syringe remains in the injector before the injection (0 - 63 s).
Air Volume	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.
Filling Volume	Select this parameter to set the sample volume to be used for cleaning and eliminating air 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.

Parameter	Description
Pullup Speed (Aspiration Speed)	Select this parameter to determine how fast the sample is drawn (1 - 100 μ l/s).
Injection Speed	Select this parameter to determine how fast the sample is injected (1 - 100 μ l/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).
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
Cleaning Cycles after Injection (Post Inj Clean Cyc) [Solvent A/B/C/D]	Select this parameter to set the post-injection cleaning volume.

For more information, refer to the **Administrator Manual: Installation Instructions**



Fisons AS800 GC Autosampler




Thermo Finnigan/TQ/TSP AS2000 GC Autosampler



Fisons AS800/ThermoQuest AS2000: Program Example

After the \Rightarrow *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 ...: Actions in the PGM Editor**  **Creating a Program.**)

This might be a good starting point:

```
; Standard 10  $\mu$ l syringe.
; Injection Volume: 1.0  $\mu$ L.
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 information about how to install the instrument, refer to the **Administrator Manual: Installation Instructions**



Fisons AS800 GC Autosampler



Thermo Finnigan/TQ/TSP AS2000 GC Autosampler



Fisons AS800/ThermoQuest AS2000: 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	<p>The following messages appears on the instrument's display:</p> <pre>End Sample Total Injections 0</pre> <p>In addition, Chromeleon waits forever for an inject response.</p>	Abort the batch.
Bad vial	<p>After some retries, a message like this appears on the instrument's display:</p> <pre>PLG NDL INJ TUR CNTR --- BSY --- --- *</pre> <p>This happens quite easily if the vials are not perfectly crimped.</p>	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.

Error	Description	Remedial Action
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.

Gilson

For information about the special commands that Chromeleon supports for the different Gilson devices and for tips for practical operation, refer to:



Gilson 202 Fraction Collector (Ext.)/204 Fraction Collector



Gilson: Injection Modes



Gilson 333 and 334 Pumps





Gilson 202 Fraction Collector (Ext.)/ 204 Fraction Collector

Property	(Min/Max) Values	Filter	Purpose/Remarks
ModelNo	N/A	Advanced	Indicates the model number (for the Gilson 202 including the firmware version; (read-only).
FirmwareVersion	N/A	Advanced	Indicates the firmware version
ValvePosition	Possible values: Drain and Collect	Normal	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)	Normal	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	Normal	Moves the collect head to the specified position.
MovementMode	Possible values: normal and secure. Default: secure	Advanced	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	Filter	Purpose/Remark
Home	Normal	Moves the collect head to the Home position (drain outlet)
Next	Normal	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	Normal	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 an overview of the Gilson 202 fraction collector with the Gilson 202 Fraction Collector (ext.) device driver or the Gilson 204 fraction collector with the Gilson 204 Fraction Collector device driver, refer to the **Administrator Manual: Installation Instructions  Gilson 202 Fraction Collector (Ext.)/204 Fraction Collector: Overview.**

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 the **Administrator Manual: Installation Instructions  Gilson 202 Fraction Collector (Ext.)/204 Fraction Collector: Installation.**

Gilson: Injection Modes

Chromeleon supports the following injection modes:

- 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.

Total Loop:

(Cont'd):

$$V_S \leq F_I * V_S \leq F_S * V_S$$

V_S : Sample volume

F_I : IntoLoopFactor

F_S : Sample Factor

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 333 and 334 Pumps

Property	(Min/Max) Values	Filter	Purpose/Remarks
ModelNo	N/A	Advanced	Indicates the model number and firmware version of the master pump (read-only)
ChartOut	Possible values: Pressure, Flow, Percentage %A, %B, %C	Advanced	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	Normal	Determines which solvent is used when the Solvent Valve Block (SVB) is installed for the pump.

Property	(Min/Max) Values	Filter	Purpose/Remarks
Selection of predefined solvents (Cont'd)			
%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 333 pump.	Normal	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	Unit: 1/Mbar Range: 0 ... 2000	Normal	Specifies the empirical compressibility correction K0 for the selected solvent (for the Gilson 333 and 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 .
%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	Unit: 1/Mbar Range: 0 ... 2000	Normal	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 333 pump	Normal	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 .

Property	(Min/Max) Values	Filter	Purpose/Remarks
Maintenance Counters			
%A<x>Count_PistonSeal, <x>='R', 'L'	Unit: Hours 0: 65535	Expert	Maintenance counter for the right (R) or left (L) piston seal (read-only)
%A<x>Limit_PistonSeal, <x>='R', 'L'	Unit: Hours 0 -65535	Expert	Counter limit for the right (R) or left (L) piston seal (read-only)
%A<x>Count_Piston, <x>='R', 'L'	Unit: Hours 0: 65535	Expert	Maintenance counter for the right (R) or left (L) piston (read-only)
%A<x>Limit_Piston, <x>='R', 'L'	Unit: Hours 0: 65535	Expert	Counter limit for the right (R) or left (L) piston seal (read-only)
%A<x>Count_OutChkValve, <x>='R', 'L'	Unit: Hours 0: 65535	Expert	Maintenance counter for the right (R) or left (L) outlet valve (read-only)
%A<x>Limit_OutChkValve, <x>='R', 'L'	Unit: Hours 0: 65535	Expert	Counter limit for the right (R) or left (L) outlet valve (read-only)
%A<x>Count_InlChkValve, <x>='R', 'L'	Unit: Hours 0: 65535	Expert	Maintenance counter for the right (R) or left (L) inlet valve (read-only)
%A<x>Limit_InlChkValve, <x>='R', 'L'	Unit: Hours 0: 65535	Expert	Counter limit for the right (R) or left (L) outlet valve (read-only)

**Note:**

If one or two Gilson 334 valve pumps are installed, more maintenance counters (%B..., %C...) are added.

Further Information**Tip:**

For more information, for example, about the empirical K0 compressibility correction values for the Gilson 333 and 334 pumps, refer to the pump's User's Guide.

For information, refer to the **Administrator Manual: Installation Instructions**  **Gilson**,  **Gilson 333 and 334 Pumps: Overview** and  **Gilson 333 and 334 Pumps: Installation**.

Kontron

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

Chromeleon supports the following Kontron 465 commands for pre-column derivatization. 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	



Tip:

*The prerequisite for controlling this instrument is that the **Extended Device Control** feature must be licensed on your system.*

For more information, refer to the **Administrator Manual: Installation Instructions**  **Kontron Autosamplers**.

Kontron SFM25 Fluorescence Detector

Chromeleon supports the following special commands and parameters for the Kontron SFM25 Fluorescence Detector. Also, refer to the information in the manual shipped with the instrument.

Command	Description
Autoblack	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 Autoblack automatically calculates and stores the value.

For more information, refer to the **Administrator Manual: Installation Instructions**  **Kontron SFM25 Fluorescence Detector**.

Merck Hitachi

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 the **Administrator Manual: Installation Instructions**  **Merck Hitachi L6200/L6210 and L6250 Pumps: Overview.**

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

	AS2000	AS4000	L7250	L7200
InjectPortWashes	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
NeedleWashes	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 (100 µl) - 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
VolOptionalSyringe	Not supported	1 (100 µl) - 5 (5000 µl)	Not supported	Not supported
NumberOfDilutors	Not supported	1 or 2	Not supported	Not supported

	AS2000	AS4000	L7250	L7200
Dilutor	Not supported	1 or 2	Not supported	Not supported
RackCode	1 - 16	1 - 16	1 - 16	Not supported
⇒ <i>Volume</i>	1 - 500.0 µl	1 - 5000.0 µl	1 - 5000.0 µl	1 - 500.0 µl
⇒ <i>Position</i>	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

	AS2000	AS4000	L7250	L7200
⇒ <i>Connect (/Disc.)</i>	Supported	Supported	Supported	Supported
Disconnect	Supported	Supported	Supported	Not supported
Aspirate (see ⇒ <i>Draw</i>)	0 - 500.0 µl, Speed	Dilutor, 0 - 5000.0 µl, Speed	0 - 5000.0 µl, Speed	Not supported
⇒ <i>Dispense</i>	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 Y:0-68.0 mm Z:0-38.0 mm	X:0-244.0 mm Y:0-163.0 mm Z:0-57.0 mm	X:0-156.0 mm Y:0-156.0 mm Z:0-57.0 mm	Not supported
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
WashPumpPlunger	Not supported	Not supported	Supported*	Not supported

	AS2000	AS4000	L7250	L7200
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 Depending on the current value of the **Syringe** device property, three different programs are internally executed during processing an \Rightarrow *Inject* command. For **AS2000** and **L7250** the dilutor property always equals 1.

AS4000
L7250

```

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

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.

AS4000

L7250

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

For more information about the different autosampler types, refer to the **Administrator Manual: Installation Instructions**:

 **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

```

For more information about the different autosampler types, refer to the **Administrator Manual: Installation Instructions:**  **Merck Hitachi AS2000 and AS4000 Autosamplers: Overview,**  **Merck Hitachi L7250 Autosampler: Overview,** and  **Merck Hitachi L7200 Autosampler: Overview.**

Perkin Elmer Autosystem GC

For application examples for the Perkin Elmer Autosystem GC, refer to:



**Perkin Elmer Autosystem GC:
Split Valve Control for PSS and CAP Inlets**



Perkin Elmer Autosystem GC: Pressure and Flow Ramps



Perkin Elmer Autosystem GC: 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.

Perkin Elmer Autosystem GC: 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, we recommend 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

```

Rheodyne Valves

The **Rheodyne RV/EV Valve** driver supports the following commands:

Command	Min.	Max.	Default	Description
Position	1	Depends on the type.	Read out from the device on connect.	The current position of the valve. (The valve moves to the specified position, obeying the Direction property.)
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.)
Test				Tests the valve. (Moves the valve to position 1, steps to all positions, and returns the valve to position 1) Level: Expert.
ModelNo	Text string (64 characters) describing the valve type.		Read out from the device on connect.	Read-only - Expert level.
SerialNo	Text string (64 characters) indicating the serial number		Read out from the device on connect.	Read-only - Expert level.
FirmwareVersion	Text string (64 characters) indicating the firmware number		Read out from the device on connect.	Read-only - Expert level.

It is not possible to read out 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 LC-10A HPLC System

This section provides program examples for sample preparation, using a Shimadzu SIL-10ADvp preparative Autosampler as part of a Shimadzu LC-10A HPLC System:



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 HPLC System: Program Example for the Standard Injection Mode

The following program is an example for sample preparation 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



Program Example for the Dilution Injection Mode

Shimadzu LC-10A HPLC System: Program Example for the Reagent Injection Mode

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 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 μ l of air is automatically drawn first and then, the reagent volume, e.g. AVolume = 5 μ l, is drawn from the indicated position. The reagent volume + 10 μ l (here = 15 μ l) 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 cycles 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**

Shimadzu LC-10A HPLC System: Program Example for the Dilution Injection Mode

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 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 acquisition:
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**

Thermo Finnigan/TQ/TSP

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:



Trace GC



Surveyor MSQ Mass Spectrometer



aQa Mass Spectrometer



AS3500/AS3000 Autosamplers: Sample Preparation



UV1000 Detector



UV2000 Detector




UV3000 Detector



UV6000 PDA Detector



FL2000 and FL3000 Fluorescence Detectors

For the commands supported for the ThermoQuest AS2000 Autosampler, which corresponds to the Fisons AS800 autosampler, refer to  **Fisons AS800/ThermoQuest AS2000 Autosampler**.



Thermo Finnigan/TQ/TSP Trace GC

Hundreds of commands are available for the individual options of the Trace GC. Therefore, only the most important of them are described below. 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	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.)
TempCtrl	OFF	ON	ON	Turns the oven's temperature control on or off.
Temperature				
.Nominal	Note 1	Note 2	50°C	Nominal oven temperature.
.Value	Note 1	Note 2	N/A	Actual oven temperature (read-only).
.UpperLimit	70°C	Note 2	450°C	Upper limit for the oven temperature.
.LowerLimit	Note 1	50°C	30°C	Lower limit for the oven temperature.

Note 1: Depending on the cryo settings, the minimum temperature can be 30°C (86°F), -55°C (-131°F), or -99°C (-178.2°F).

Note 2: Depending on the cryo settings, the maximum temperature can be 450°C (842°F) or 500°C (932°F).

Injector(s)

Command	Min	Max	Default	Description
TempCtrl	OFF	ON	ON	Turns the injector's temperature control on or off.
Temperature				
.Nominal	30	450	50	Nominal temperature of the injector.
.Value	30	450	N/A	Actual temperature of the injector (read-only).
Mode	N/A	N/A	Split	Injector mode: Split or Splitless

Detector(s)

Command	Min	Max	Default	Description
⇒Autozero				Sets the signal output to 0.
TempCtrl	OFF	ON	ON	Turns the detector's temperature control on or off.
Temperature				
.Nominal	30	450	150	Nominal temperature of the detector.
.Value	30	450	N/A	Actual temperature of the detector (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 bar	7.00 bar	N/A	Nominal pressure in the column.
.Value	0.03 bar	7.00 bar	N/A	Actual pressure in the column (read-only).
FlowCtrl	OFF	ON	ON	Turns the column flow control on or off.
Flow				
.Nominal	0.0 ml/min	15.0 ml/min	N/A	Nominal flow in the column.
.Value	0.0 ml/min	15.0 ml/min	N/A	Actual flow in the column.

**Note:**

In Chromeleon, pressures are always displayed in bar. It is not possible to change the pressure unit.

Further Information

For an overview of the Trace GC, refer to the **Administrator Manual: Installation Instructions**  **Thermo Finnigan/TQ/TSP Trace GC: Overview**.

For information about how to install the Trace GC, refer to the **Administrator Manual: Installation Instructions**  **Thermo Finnigan/TQ/TSP Trace 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 receives a command, it switches to remote mode and locks the keypad. If no further communication takes place, 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 will not be 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, 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 so-called "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 the nominal temperature is 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 nominal temperature value 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.*

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:

Property	Min	Max		Description
⇒AcqOn/Off	-	-	MS_n only	Starts and stops data acquisition.
MinMass	0.00 amu	2000.00	Read-only	Minimum mass recorded in this trace. (SIM channels only)
MaxMass	0.00 amu	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 acquisition 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 defaults

Thermo Finnigan/TQ/TSP Surveyor MSQ: Troubleshooting

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.*

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, we recommend 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.

Thermo Finnigan/TQ/TSP aQa Mass Spectrometer

TIC and SIM Channel Properties and Commands

These channels are standard data channels with the following properties:

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 ➤ <i>Base Peak</i> 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.

Property	Min	Max	Default	Description
FirmwareVersion	n/a		Read-only	The spectrometer's firmware version. Note: This value is available only after the first raw data acquisition.
Command	Description			
⇒ <i>Connect</i>	Connects the device			
Disconnect	Disconnects the device			
⇒ <i>Reset</i>	Resets the device to the defaults			

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. The AS3500/AS3000 Basic Template example program (see below) lists the sample prep steps in the order they should be run and identifies the required steps.
- 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 make a delivery 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 only 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 ;optional
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

Thermo Finnigan/TQ/TSP UV1000 Detector

The detector driver supports the following commands and properties:

Property	Min	Max	Default	Description
AvailableLamps	None	UV & VIS lamp	UV-lamp only	Indicates which lamps are available (read-only).
Range	0.0005 AUFS	3.0000 AUFS	1.0000 AUFS	Amplifying factor for the output signal.
Response	0.0	5.0	1.0	Time constant. Indicates the time needed by the detector to reach 98% of full-scale.
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 \Rightarrow Autozero every time the wavelength is changed.

Command	Description
UV_LampOn	Turns the deuterium lamp on.
UV_LampOff	Turns the deuterium lamp off.
Visible_LampOn	Turns the tungsten lamp on.
Visible_LampOff	Turns the tungsten lamp off.

**Tip:**

After a \Rightarrow Reset command, it takes 90 seconds until the detector is ready to execute further commands.

Thermo Finnigan/TQ/TSP UV2000 Detector

The detector driver supports the following commands and properties:

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 98% of full-scale.

Property	Description
UV_LampOn	Turns the deuterium lamp on.
UV_LampOff	Turns the deuterium lamp off.
Visible_LampOn	Turns the tungsten lamp on.
Visible_LampOff	Turns the tungsten lamp off.

Channel-Specific Commands and Properties

Common to both channels

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 detector driver supports the following commands and properties:

Property	Min	Max	Default	Description
AcquisitionMode	Single Wavelength	Multi Wavelength	N/A	Changes the data acquisition mode.
Connected	Disconnected	Connected	N/A	Indicates whether the detector is connected.
UV_Lamp	Off	On	N/A	Turns the deuterium lamp on or off ($\lambda = 190 - 365$ 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 ($\lambda = 366 - 800$ 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 98% of full-scale.
\Rightarrow 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 **How to: 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 \Rightarrow 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.

AS350 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 the **Advanced** button 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 detector driver supports the following commands and properties:

Property	Min	Max	Default	Description
Data_Collection_Rate (For more information, refer to ➤ <i>Data Collection Rate</i> 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.
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 $\lambda = 190 - 340$ nm) or **Visible** (tungsten lamp: ($\lambda = 340 - 800$ nm)).

**Tip:**

When the **Data Collection Rate** command is executed, the default \Rightarrow 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
\Rightarrow Autozero	Sets the detector signal to 0.
\Rightarrow Connect	Connects the PDA detector to Chromeleon.
Disconnect	Disconnects the PDA detector from Chromeleon.
\Rightarrow Reset	Resets the PDA detector to the defaults.
XY_LampReset	Resets the XY_LampReplacedAt counter to now

Commands and Properties of UV Channels

Property	Min	Max	Default	Description
\Rightarrow Average	Off (0)	On (1)	Off	Allows signal averaging.
\Rightarrow Bandwidth	1 nm	121 nm	1 nm	(Odd values only)
			Read from the device on connect.	
\Rightarrow MaxAutoStep	0.01 s	5.10 s	5.10 s	Indicates the maximum step for Step = Auto
\Rightarrow Step	Auto, 0.01 s	5.10 s	Auto	Indicates the time interval between data points.
\Rightarrow 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
⇒ <i>Bandwidth</i>	1 nm	121 nm	1 nm (Read from the device on connect.)	Indicates the bandwidth for all wavelengths in the spectrum ² (odd values only)
⇒ <i>BunchWidth</i>	1 nm 2 nm 4 nm 5 nm 10 nm		1 nm (Read from the device on connect.)	Indicates the distance between the wavelengths in the spectrum ² .
MaxWavelength	190 nm	800 nm	800 nm	Indicates the upper limit of the measuring range.
MinWavelength	190 nm	800 nm	190 nm	Indicates the lower limit of the measuring range.
⇒ <i>Step</i>	0.05 s 0.10 s 0.20 s 0.25 s 0.50 s 1.00 s 2.00 s		1.00 s (Read from the device on connect.)	Indicates the time interval between recorded spectra.

² **Tip:**

For example, to scan the wavelengths between 400 nm and 500 nm with a BunchWidth of 10 nm, 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 driver for the fluorescence detector supports the following commands and properties:


Property	Min	Max	Description
AnOut_1_Offset, AnOut_1_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	➤ <i>Fluorescence</i> , ➤ <i>Phosphorescence</i>		Operating mode of the detector.

Property	Min	Max	Description
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)
NumberOfScans*	2 - 32 (in 2 nm increments)		This property is available only for the FL3000 . It specifies how often the monochromator shall repeat 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.)
ScanEndEmWavelength*	200 - 650 nm (or 800 nm; in 2 nm increments)		This property is available only for the FL3000 . Emission wavelength at the end of the scan. (ignored for ScanMode = Excitation) The difference between ScanStartEmWavelength and ScanEndEmWavelength must be a multiple of 10.
ScanEndExWavelength*	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.
ScanEndExWavelength (Cont'd)			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: <ul style="list-style-type: none"> • <i>Excitation</i>: The emission wavelength is constant; the excitation monochromator scans the wavelengths. • <i>Emission</i>: The excitation wavelength is constant; the emission monochromator scans the wavelengths. • <i>Delta</i>: Both monochromators perform the scans simultaneously; the difference between the wavelengths is constant.

Property	Min	Max	Description
ScanStartEmWavelength*	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)
ScanStartExWavelength*	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:**

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.) In this case, 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 during the run of a PGM File. 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: <i>The chromatogram is disturbed during the scan.</i>
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 <i>On</i> , 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.

Property	Min	Max	Description
EmWavelength	200 - 650 nm (or 800 nm; in 2 nm increments)		Emission wavelength
ExWavelength	200 - 650 nm (in 2 nm increments)		Excitation wavelength
Response	0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 s		Specifies the response time for the detector (see ➤Response).
Signal.UpperLimit Signal.LowerLimit	-1000	1000 FU	Upper and lower signal limit.  Tip: <i>If the actual value is above the upper limit or if it is below the lower limit, Chromeleon aborts the batch and starts emergency handling.</i>
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: <i>Run</i> (The lamp is turned on at the beginning of the sample run; it is turned off at the end of the run.) <i>Off@End</i> (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.)

Instrument	Chromleon	Description
Scan	Scan	This command corresponds to 'Scan' entry on the instrument's COMMANDS menu (manual scanning).
Scan Zero	ScanAutozero	<p>The Chromeleon command corresponds to the 'Scan Zero' command on the instrument's COMMANDS menu (manual baseline scanning).</p> <p>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.</p>
Autozero Time	Supported via the ScanAutozero command (see above)	
Auto Spectra: <i>On, Off</i>		Chromeleon sets this parameter always to Off because Chromeleon can perform automatic scanning more reliably by using ➤ <i>Trigger Commands</i> .
Zero on λ Change	AutozeroOnWChange	
Detection Type: <i>Fluor</i> <i>Phos</i>	DetectionMode: ➤ <i>Fluorescence</i> ➤ <i>Phosphorescence</i>	<p>In addition, it is possible to measure the</p> <p>➤ <i>Chemiluminescence</i>. Use one of the two modes and turn off the lamp.</p>

 **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

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 valve driver supports the following commands and properties:

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 FlipDelay 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 \Rightarrow 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 the **Administrator Manual: Installation Instructions**  **Valco Valves**.

Varian

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: Troubleshooting



Varian 3400 and Varian 3600 GCs



Varian 3400 and 3600 GCs: Different Names in Firmware and Chromeleon



Varian 3400 and Varian 3600 GCs: Detectors



Varian 3400 3600 GC Detectors: Different Names in Firmware and Chromeleon



Varian 3400 and 3600 GCs: Tips for Operation



Varian 3400 and 3600 GCs: Troubleshooting



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:

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).

Property	Min	Max	Default	Description
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 .
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
⇒ <i>Connect</i>	Connects the GC to Chromeleon.
Disconnect	Disconnects the GC from Chromeleon.
⇒ <i>Reset</i>	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.Nominal ³	0	100	40	Sets the nominal carrier gas flow [ml/min]
Flow.Value ³	0	100	N/a	Indicates the actual carrier gas flow [ml/min] (read-only).
Pressure.Nominal ³	0	60	15	Sets the nominal carrier gas pressure [psi].
Pressure.Value ³	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 the **Administrator Manual: Installation Instructions**  **Varian 3400 GC.**

For an overview of the Varian 3600 GC, refer to the **Administrator Manual: Installation Instructions**  **Varian 3600 GC.**

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 Out ²	CryoTimeout
div. fault codes	CausesForNotReady
Thermal Stabilize Time ²	StabilizationTime
Column Oven On/Off	TempCtrl
Column Temp Limit ³	Temperature.UpperLimit
Initial Column Temp	Temperature.Nominal

²  **Tip:**

*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

³  **Tip:**

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:

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
⇒ <i>Autozero</i>	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 the **Administrator Manual: Installation Instructions**  **Varian 3400 GC.**

For an overview of the Varian 3600 GC, refer to the **Administrator Manual: Installation Instructions**  **Varian 3600 GC.**

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 Limit ²	Temperature.UpperLimit
Detector Temp	Temperature.Nominal
Initial Atten	Attenuation

² **Tip:**

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, we recommend 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 desired 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 the **Administrator Manual: Installation Instructions**  **Varian 3400 GC**.

For an overview of the Varian 3600 GC, refer to the **Administrator Manual: Installation Instructions**  **Varian 3600 GC**.

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

For information about the special commands that Chromeleon supports for the different Waters devices and for tips for practical operation, refer to:

 **Waters Alliance 2690/2695 HPLC Modules**

 **Alliance 2790/2795 HPLC Modules**

 **Waters 996 and 2996 PDA Detectors**

 **Waters 2487 UV Detector**


For troubleshooting information, refer to:

 **Waters Instruments: Troubleshooting**

 **GPIB-Connected Devices: Troubleshooting**



Waters Alliance 2690/2695 HPLC Modules

In addition to the standard commands (refer to **How to ...: Device Control**

 **Control: Pumps**), the Waters 2690/2695 Separation Module device driver supports the following commands and properties:

Pump Commands and Properties

Property	Min	Max	Description
BubbleDetection ²	Off (0)	On (1)	<p>Checks the System for air bubbles in the flow path, can return an error message and perform a bubble reduction procedure.</p> <p>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).</p>

Property	Min	Max	Description
Curve	1 (convex)	9 (concave)	<p>➤ <i>Gradient Curve</i> (Default: 5 = linear)</p> <p> Note:</p> <p><i>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.</i></p>
MaxAcceleration ²	0.01 min	30.00 min	<p>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:</p> <p>MaxAcceleration = 10 / MaximumFlowRamp</p> <p>(Default: 0.01 min. The property is visible only on the Advanced level.)</p>
MaximumFlowRamp	0.34	1000.00	<p>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:</p> <p>MaximumFlowRamp = 10 / MaxAcceleration</p> <p>(Default: 1000.00 ml/min². The property is visible only on the Advanced level.)</p>
⇒ <i>Pressure.LowerLimit</i> <i>Pressure.UpperLimit</i> ²	0.0 bar	344.70 bar (= 5000 psi)	<p>Upper and lower pressure limits.</p> <p>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).</p> <p> Note:</p> <p><i>The instrument checks the lower pressure limit only during injection.</i></p>
PrimaryPressure	-3.45 bar	380 bar	<p>Sets the pressure after the first piston chamber. (The property is read-only and visible only on the Expert level.)</p>

Property	Min	Max	Description
StrokeVolume ²	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.

²  **Tip:**

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_D ²	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 desired 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 Mode ²	Normal (0) Continuous (1) Off (2)	Off	Normal: The degasser turns on and off, depending on the pressure. Continuous: The degasser is always on. Off: The degasser is always off.


²  **Tip:**

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:

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).
SampleHeight ²	0.0 mm	20.0 mm	Indicates the distance of the needle tip from the bottom of the sample vial (default: 0.0 mm).
SyringeSpeed ²	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.
WaitForTemperature ²	No (0)	Yes (1)	Specifies whether the autosampler waits for the sample temperature control.

Tip:

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 **How to ...: Device Control**  **Temperature Control (On/Off)** and  **Controlling the Temperature.**

In addition, the following property allows you to determine 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	<p>If the actual column temperature deviates from the expected temperature by more than the set value, an error message appears.</p> <p>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).</p>
⇒Ready ²	NotReady	Ready	Ready	<p>Indicates the state of the column heater. The heater is Ready:</p> <ul style="list-style-type: none"> 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.

Property	Min	Max	Default	Description
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 ready-only.)


**Note:**


For better understanding, [°C] is used as unit, even though [K] would be correct to indicate temperature differences.


**Tip:**

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 **How to ...: Device Control**  **Relay Commands**).

For information about how to install the Waters Alliance 2690 and 2695 HPLC modules, refer to the **Administrator Manual: Installation Instructions**  **Waters Alliance 2690/2695 HPLC Modules: Installation**.

For an overview of the Waters Alliance 2690 and 2695 HPLC modules, refer to the **Administrator Manual: Installation Instructions**  **Waters Alliance 2690/2695 HPLC Modules: Overview**.


Waters Alliance 2790/2795 HPLC Modules

The Alliance 2790/2795 Separation Module device driver supports several modules. For information about the corresponding commands and properties, refer to:

 **Waters Alliance 2790/2795: HPLC System**

 **Waters Alliance 2790/2795: Pump and Degasser**


 **Waters Alliance 2790/2795: Injector**

 **Waters Alliance 2790/2795: Column Heater and Sample Temperature Control**

 **Waters Alliance 2790/2795: Overlapping Sample Preparation**

Waters Alliance 2790/2795: HPLC System

In addition to the standard commands (refer to **How to ...: Device Control**

 **General Device Commands**), the Waters 2790/2795 Separation Module device driver supports the following commands and properties for the HPLC System:

Property	Values	Description	
ChartOut ²	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:	
		<i>Option</i>	<i>Module</i>
		VacuumLevel	Degasser
		SampleTemperature	Sample Heater
		ColumnTemperature	Column Heater

Property	Values	Description
LogStatus ²	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.)
PrecolumnVolume ²	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 the **Administrator Manual: Installation Instructions**  **Waters Alliance 2790/2795 HPLC Modules: Installation.**

For an overview of the Waters Alliance 2790 and 2795 HPLC modules, refer to the **Administrator Manual: Installation Instructions**  **Waters Alliance 2790/2795 HPLC Modules: Overview.**

Waters Alliance 2790/2795: Pump and Degasser

In addition to the standard commands (refer to **How to ...: Device Control**  **Control: Pumps**), the Waters 2790/2795 Separation Module device driver supports the following pump commands and properties:


Pump Commands and Properties

Property	Min	Max	Description
BubbleDetection ²	Off (0)	On (1)	Checks the system for air 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	1 (convex)	9 (concave)	➤ <i>Gradient Curve</i> (Default: 5 = 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
MaxAcceleration ²	0.01 min	30.00 min	<p>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.</p> <p>It is connected with the MaximumFlowRamp property:</p> <p>MaxAcceleration = 10 / MaximumFlowRamp</p> <p>(Default: 0.01 min. The property is visible only on the Advanced level.)</p>
MaximumFlowRamp	0.34	1000.00	<p>Maximum flow rate increase in [ml/min²]. This property serves to protect the column from major pressure changes.</p> <p>This property is connected with the MaxAcceleration property as follows:</p> <p>MaximumFlowRamp = 10 / MaxAcceleration</p> <p>(Default: 1000.00 ml/min². The property is visible only on the Advanced level.)</p>
⇒Pressure.LowerLimit Pressure.UpperLimit ²	0.0 bar	344.70 bar (= 5000 psi)	<p>Upper and lower pressure limits.</p> <p>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).</p> <p> Note:</p> <p><i>The instrument checks the lower pressure limit only during injection.</i></p>
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.)
StrokeVolume ²	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.

2 Tip:

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_D ²	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 desired 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 Mode ²	Normal (0) Continuous (1) Off (2)	Off	Normal: The degasser turns on and off, depending on the pressure. Continuous: The degasser is always on. Off: The degasser is always off.


2  Tip:

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 the **Administrator Manual: Installation Instructions**  **Waters Alliance 2790/2795 HPLC Modules: Installation.**


For an overview of the Waters Alliance 2790 and 2795 HPLC modules, refer to the **Administrator Manual: Installation Instructions**  **Waters Alliance 2790/2795 HPLC Modules: Overview.**


 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:

Property	Min	Max	Description
CheckPlateHeight ²	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.)
FullLoopOverfill ²	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.)
InjPortWashTime ²	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.)
InjectMode ²	PartialLoop; FullLoop		Determines the injection mode. (The default setting is FullLoop.) <i>FullLoop</i> The entire loop volume is delivered to the column. <i>PartialLoop</i> 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.)
NeedleWashTime ²	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.)
PrepDelay ³	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.)
PrepSubject ³	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.)
PrepVolume ³	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.
PurgeVolume ³	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
SampleHeight ²	0.0 mm	20.0 mm	Indicates the distance of the needle tip from the bottom of the sample vial (default: 0.0 mm).
SampleOverlapped ²	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.)
SeekWellBottom ²	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.)
SyringeSpeed ² :	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.
WaitForTemperature ²	No (0)	Yes (1)	Specifies whether the autosampler waits for the sample temperature control.
⇒ Wash			Flushes the autosampler.
			 Tip: <i>After priming the syringe, always perform a Wait Sampler.Ready command.</i>
WashCycle ²	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.)
WashFrequency ²	Never, EveryInject, EveryWell		Determines when a wash cycle shall be performed. The following options are available: <i>Never, EveryInject, EveryWell</i> . (The default setting is EveryInject. The property is visible only on the Advanced level.)
WashSequence ²	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
Draw ³	<p>Draws the PrepVolume from the PrepSubject (see above). (The command is available only on Advanced level.)</p> <p> Tips:</p> <p><i>Up to 16 Draw commands are allowed per injection.</i></p> <p><i>Always use a Mix command after a sequence of Draw commands.</i></p>
LoopOffline ³	<p>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.)</p> <p> Tip:</p> <p><i>Make sure to issue the command only after the previous sample has completely left the loop.</i></p>
Mix ³	<p>Starts sample preparation. (The property is visible only on the Advanced level.)</p> <p> Tips:</p> <p><i>No further Draw commands are allowed after a Mix command.</i></p> <p><i>Always use a Mix command after a sequence of Draw commands.</i></p>
PrimeSyringe	<p>Fills the syringe with the purge solvent. (The property is visible only on the Advanced level.)</p> <p> Tip:</p> <p><i>The command uses the values set for PurgeVolume and WashCycle.</i></p>

**Tip:**

It is not possible to change the setting during sample analysis.

For information the Waters Alliance 2790 and 2795 HPLC modules, refer to the **Administrator Manual: Installation Instructions** **Waters Alliance 2790/2795 HPLC Modules: Overview** and **Waters Alliance 2790/2795 HPLC Modules: Installation**.

Waters Alliance 2790/2795: **Column Heater and Sample Temperature Control**

For information about the standard commands for temperature control, refer to **How to ...: Device Control**  **Temperature Control (On/Off)** and  **Controlling the Temperature.**

In addition, the following property allows you to determine 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	<p>If the actual column temperature deviates from the expected temperature by more than the set value, an error message appears.</p> <p>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).</p>
⇒Ready ²	NotReady	Ready	Ready	<p>Indicates the state of the column heater. The column heater is Ready</p> <ul style="list-style-type: none"> • when temperature control (TempCtrl) is Off <i>or</i> • when the difference between the actual temperature and the target temperature is equal or less than 2°C for at least 30 minutes.
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 ready-only.)
TempCtrl ²	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:**

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 **How to ...: Device Control**  **Relay Commands**).

For information about how to install the Waters Alliance 2790 and 2795 HPLC modules, refer to the **Administrator Manual: Installation Instructions**  **Waters Alliance 2790/2795 HPLC Modules: Installation**.

For an overview of the Waters Alliance 2790 and 2795 HPLC modules, refer to the **Administrator Manual: Installation Instructions**  **Waters Alliance 2790/2795 HPLC Modules: Overview**.

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 preparation:
; 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 **How to ...: Control: Autosamplers**


**Overlapping Samples.**

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:

Property	Min	Max	Description
ExposureTime	0 (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: <i>Do not change the default ('Auto') unless absolutely required.</i>
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 (0)	On (1)	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
⇒ <i>BunchWidth</i>	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.)
MinWavelength	190.0 nm	MaxWavelength	190.0 nm	Indicates the lower limit of the measuring range. (The setting cannot be changed during data acquisition.)
⇒ <i>Step</i>		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: $190.0 \text{ nm} + n \cdot 1.2 \text{ nm}$ (where $n = \text{integer}$). 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 **How to ...: Device Control**  **Relay Commands**).

For an overview of the Waters 996 and 2996 PDA detectors, refer to the **Administrator Manual: Installation Instructions**  **Waters 996 and 2996 PDA Detectors: Overview**.

For information about how to install the Waters 996 and 2996 PDA detectors, refer to the **Administrator Manual: Installation Instructions**  **Waters 996 and 2996 PDA Detectors: Installation**.

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:

Command	Min	Max	Default	Description
Data_Collection_Rate (For more information, refer to ➤ <i>Data Collection Rate</i> 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 EventIn ²	On	Off	Off	If the property is enabled, autozeroing is performed whenever this is required by an event.
AutozeroOn WlChange ²	On	Off	On	If the property is enabled, autozeroing is performed whenever the wavelength changes.
⇒Average	Off (0)	On (1)	On	Allows signal averaging.
FilterTime Constant ³	0.0 s	99.0 s	1.0 s	Time constant for the selected noise filter.
FilterType	None (0)	RC (2)	Hamming (s)	Filter type used to reduce analog and digital signal noise.
Signal. Upper/ LowerLimit	-10 ⁴ 10 mAU	10 ⁴ 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 **AutozeroOnWICChange** 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 the **Administrator Manual: Installation Instructions**  **Waters 2487 UV Detector: Overview**.


For information about how to install the Waters 2487 UV detector, refer to the **Administrator Manual: Installation Instructions**  **Waters 2487 UV Detector: Installation**.

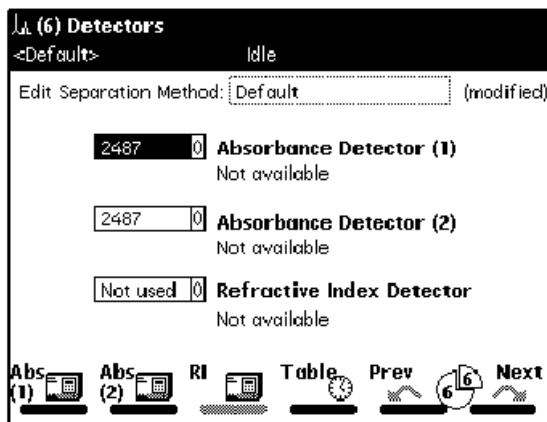
Waters Instruments: Troubleshooting

Waters 2690/2695 or 2790/2795 Separation Modules:

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. The reason is that both Chromeleon and the Separation Module attempt to control this instrument.

To remedy the situation, 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.


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  **PIB-Connected Devices: Troubleshooting.**

For an overview of the different Waters instruments for which device drivers are available in Chromeleon, refer to the **Administrator Manual: Installation Instructions**  **Waters.**

PIB-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.
- A wrong 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. (Refer to the **Administrator Manual: Installation Instructions**  **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.**

Reference Manual

The Sequence

A sequence is a list of samples that shall be processed and/or have already been analyzed. The sequence summarizes all sample data, for example, name, injection volume, dilution, etc. Details of sample processing are referenced by file names and stored in the corresponding ➤ *PGM Files* in the sequence.

To create a sequence, use the Sequence Wizard (see **Samples and Sequences**  **The Sequence Wizard**).

 **Tip:**

Do not use special characters, such as an umlaut, for new directory names or sequences, as this may cause problems in Novell networks!

The new sequence may be edited afterward in the ➤ *Browser*. Each line of the table represents a sample. Theoretically, the number of samples is not limited, but 100 sample entries should be exceeded in exceptions only. The more sequences are created, that is, the fewer samples are included in one sequence, the higher is the sample accessing speed. In addition, it is easier to keep track of the samples processed.


 **Tip:**

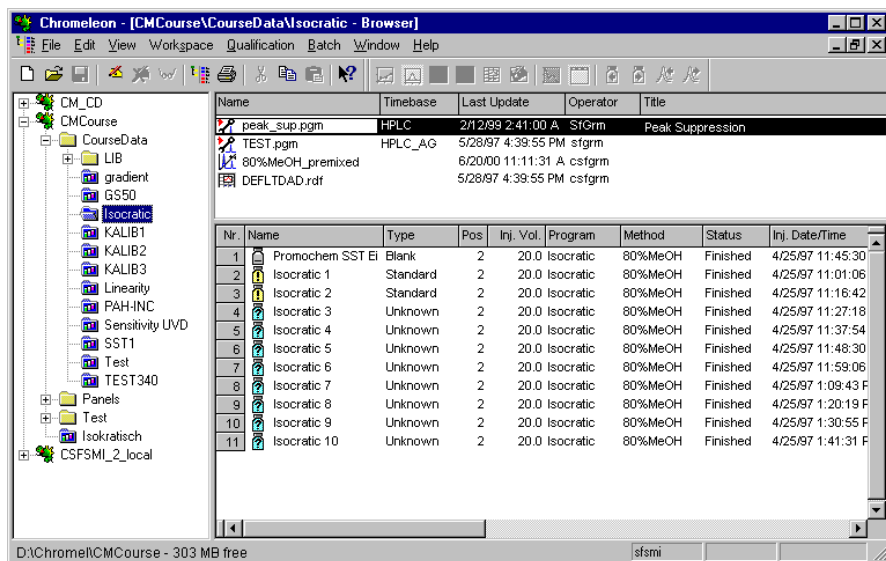
Injections that are made from the same vial are regarded as separate samples!

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

Usually, sequences are started from a ➤ *Control Panel*. However, they can be started directly in the Browser as well, if the sequence has already been assigned to the respective ➤ *Timebase*. Either select **Start** on the ➤ *Batch* menu or click the corresponding button on the **Online** ➤ *Toolbar*.

The entire data collected in connection with creating and processing a sequence, including raw data and protocol data recorded during the analysis, is saved in the sequence directory of a ➤ *Datasource* or in the underlying database. Of course, this includes the raw and protocol data recorded during the analysis.

Similar to datasources, sequences can be "locked." In this status, data and results can only be read, not modified or extended. For more information about how to lock datasources, directories, and sequences, refer to **Data Management**  **The Datasource**.




The screenshot shows the Chromeleon Browser window with the following data:


Name	Timebase	Last Update	Operator	Title
peak_sup.pgm	HPLC	2/12/99 2:41:00 A	StGrm	Peak Suppression
TEST.pgm	HPLC_AG	5/26/97 4:39:55 PM	stgrm	
80%MeOH_premixed		6/20/00 11:11:31 A	csfgrm	
DEFLTAD.rdf		5/28/97 4:39:55 PM	csfgrm	

Nr.	Name	Type	Pos	Inj. Vol.	Program	Method	Status	Inj. Date/Time
1	Promochem SST Ei	Blank	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 11:45:30
2	Isocratic 1	Standard	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 11:01:06
3	Isocratic 2	Standard	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 11:16:42
4	Isocratic 3	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 11:27:18
5	Isocratic 4	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 11:37:54
6	Isocratic 5	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 11:48:30
7	Isocratic 6	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 11:59:06
8	Isocratic 7	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 1:09:43 F
9	Isocratic 8	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 1:20:19 F
10	Isocratic 9	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 1:30:55 F
11	Isocratic 10	Unknown	2	20.0	Isocratic	80%MeOH	Finished	4/25/97 1:41:31 F

The screenshot shows the default configuration of the Browser, which can be reconfigured as required. You may add or delete columns or change their order. In addition, you can use **➤ User-defined Columns** and **➤ Sequence Report Columns** and thus, adapt the appearance of the table according to your specific requirements. The layout is saved as well. Therefore, if you reedit the sequence at later date, the representation will correspond to the one of your last access. The samples are entered in the table in the order in which they are to be processed. For information about the parameters that describe the individual samples, refer to the **⇒ Sample Variables** below.

For more information about how to create a sequence, refer to **How to ...: Actions in the Browser**  **Creating a Sample List (Sequence)** and the following topics.

Sample Variables (Overview)

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:

- ⇒ *Comment* (Sample Comment)
- ⇒ *Dil. Factor* (Dilution Factor)
- ⇒ *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*.

Comment (*Sample Comment*)

Type: **Text**

Dimension: ---

Value Range: **All printable characters**

Default: ---

Related Parameter: \Rightarrow *Name* (sample name)

Description: In addition to the sample name, further comments can be entered. Use F8 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: \Rightarrow Inj. Vol. (injection volume)

\Rightarrow Weight (Sample Weight Factor)

Description: The \Rightarrow 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.

Inj. Date/Time (*Time of Injection*)

Type:	Character String
Dimension:	Month/Day/Year Hours:Minutes:Seconds
Value Range:	---
Default:	---
Related Parameter:	Time
Description:	<p>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 \Rightarrow <i>Status (Sample Status)</i> M (multiple), the time of the last injection is entered.</p> <p>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.</p>
Function:	The kind of entry (empty or time value) indicates if the sample was processed and when it 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: \Rightarrow Dil. Factor (dilution factor)

\Rightarrow Weight (Sample Weight Factor)

Description: The \Rightarrow Sample Variable **Injection Volume** defines the injection volume in micro liters (μl). In automatic operation, the installed \Rightarrow Driver converts this value into a volume readable by the autosampler, then the value is sent to the \Rightarrow Autosampler. You can enter different injection volumes to create a \Rightarrow Dilution Series for multiple-point calibration (\Rightarrow 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. All additional calibration values, that is, the corresponding amounts of the subsequent standard samples (Type: **Standard**), are calculated by Chromeleon.



Note:

In order to minimize carry-over effects in such a multiple-point calibration, standard order should always 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*)

<i>Type:</i>	File name
<i>Dimension:</i>	---
<i>Value Range:</i>	---
<i>Default:</i>	Name of QNT File
<i>Related Parameters:</i>	⇒ <i>Program</i> (PGM File)
<i>Description:</i>	<p>The Method column (in the Browser) contains the name of the quantification method (see The QNT Editor  The Quantification Method (QNT Editor)).</p> <p>The quantification method includes all parameters that are used for evaluating a peak or the entire chromatogram.</p>
<i>Function:</i>	<p>The QNT Method serves as the basis for calculation for sample evaluation. It includes:</p> <p>⇒ <i>QNT Parameters</i> All parameters required for (qualitative) peak identification and for converting the determined peak areas into amount or concentration values (quantitatively)</p> <p>⇒ <i>Detection Parameters</i> All parameters regarding, for example, peak recognition and peak area evaluation</p> <p>➤ <i>Calibration Variables</i> All parameters regarding the type and performance of a calibration</p> <p>➤ <i>Blank Run Subtraction</i> Information about baseline subtraction</p> <p>Peak Tracking Parameters for comparing spectra to library or sequence spectra</p> <p>Spectra Library Screening Parameters for spectra library screening</p>

Function (Cont'd):

➤ *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: \Rightarrow No. (sample number)

\Rightarrow Sample ID

\Rightarrow Replicate ID

Description: The \Rightarrow Sample Variable **Name** serves to identify a sample and to label graphics and reports.



Note:

If the sequence contains \Rightarrow 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 \Rightarrow No.), the position (#p, refer to \Rightarrow Pos.), the replicate number (#r), and the injection volume (#i, s. \Rightarrow 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 (Cont'd):

Analysis-1_Repl.1

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:	⇒ Name (sample name) ⇒ 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.



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: Sample number

Related Parameter: Number

Description: The \Rightarrow *Sample Variable Pos.* determines the position of the sample in the \Rightarrow *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 segment type used, 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 describes 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).

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: ---

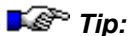
Value Range: All printable characters

Default: ---

Related Parameter: \Rightarrow Std. Add. Group

Description: The **Ref. Amount Set** \Rightarrow *Sample Variable* allows you to assign standards, \triangleright *Validation Samples*, and/or \triangleright *Spiked Samples* to the \Rightarrow *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 \triangleright Standard Addition method, we recommend adding identical amounts of the same substances to the same sample volume.



Function: To assign the samples to the desired **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 ...: Actions in the QNT Editor**  **Standard Addition**.

Replicate ID

<i>Type:</i>	Text
<i>Dimension:</i>	---
<i>Value Range:</i>	All printable characters
<i>Default:</i>	---
<i>Related Parameters:</i>	\Rightarrow <i>Name</i> (sample name) \Rightarrow <i>No.</i> \Rightarrow <i>Sample ID</i>
<i>Description:</i>	The Replicate ID column serves to mark samples as \Rightarrow <i>Replicates</i> .
<i>Function:</i>	<p>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.</p> <p>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 ...: Actions in the Browser  Performing a Query).</p>

Sample ID

<i>Type:</i>	Text
<i>Dimension:</i>	---
<i>Value Range:</i>	All printable characters
<i>Default:</i>	---
<i>Related Parameters:</i>	\Rightarrow <i>Name</i> (sample name) \Rightarrow <i>No.</i> \Rightarrow <i>Replicate ID</i>
<i>Description:</i>	The user or a help program enters a sample ID in the Sample ID column.
<i>Function:</i>	<p>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.</p> <p>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 ...: Actions in the Browser  Performing a Query).</p>

Std. Add. Group (Standard Addition Group)

Type: Text

Dimension: ---


Value Range: All printable characters

Default: ---

Related Parameter: \Rightarrow Ref. Amount Set

Description: The **Std. Add. Group** \Rightarrow *Sample Variable* allows you to assign ➤ *Spiked Samples* to unspiked unknown samples.

Enter the desired 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. Amount Group** variable is required to analyze several unspiked samples using the ➤ *Standard Addition* method. For more information, refer to **How to ...: Actions in the QNT Editor**  **Standard Addition.**

Status (*Sample Status*)

Type: Text

Dimension: ---

Value Range: **Single/Multiple/Finished/Interrupted/Running/Preparing**

Default: **Single**

Related Parameter: \Rightarrow *Inj. Date/Time (Time of Injection)*

Description: The \Rightarrow *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 \triangleright *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** The sample can be repeatedly acquired. The system just enters the \Rightarrow *Inj. Date/Time (Time of Injection)* in the **Time** column. Existing \Rightarrow *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 \Rightarrow *Power Failure*). In \Rightarrow *GLP* \Rightarrow *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 or 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 AS50 autosampler that has the **Sample Overlap** option enabled is preparing a sample. The Sample Overlap option is enabled in the AS50 Server Configuration Properties on the Options tab page.*

Also, refer to **How to ...: Control: Autosamplers**

**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:	<p>The Type \Rightarrow <i>Sample Variable</i> indicates the sample type:</p> <ul style="list-style-type: none"> • Select Unknown for an unknown analysis sample: . • Select Standard for a standard sample with known concentration: . • Select Validate for a \triangleright <i>Validation Sample</i>: . • Select Blank for a \triangleright <i>Blank Run Sample</i>: . If a sample is corrected by \triangleright <i>Raw Data</i> of a blank run sample, this is referred to as \triangleright <i>Blank Run Subtraction</i>. • Select Matrix for a \triangleright <i>Matrix Blank Sample</i>: . • Select Spiked for a \triangleright <i>Spiked Sample</i>: . • Select Unspiked for an unspiked unknown sample to be analyzed with Standard Addition method: .

Functions:

Blank

The injected sample 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 following sample, the **baseline chromatogram** may be subtracted before integration.

Unknown	The (unknown) sample shall be analyzed. It shall be determined whether the sample contains one or more substances.
Standard	The sample is a calibration standard . The corresponding \Rightarrow Amount values of the single peaks are listed in the corresponding Amount Table column.
Validate	Validation samples are treated as ordinary analyses. However, the type is written to the result file. The validation sample references to 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 indeed. Chromeleon automatically subtracts the peak areas or peak heights of the matrix blind 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.

**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 will not be 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 ➤ <i>Standard Addition</i> 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 <i>Standard Addition</i> ⇒ <i>Calibration Mode</i> , i.e., the ➤ <i>Standard Addition</i> method.

**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.*

Weight (*Sample Weight Factor*)

Type:	Floating point value
Dimension:	None
Value Range:	0.0001 ... 999999.9999
Default:	1.0000
Related Parameters:	⇒ <i>Dil. Factor</i> (dilution factor) ⇒ <i>Inj. Vol.</i> (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**.

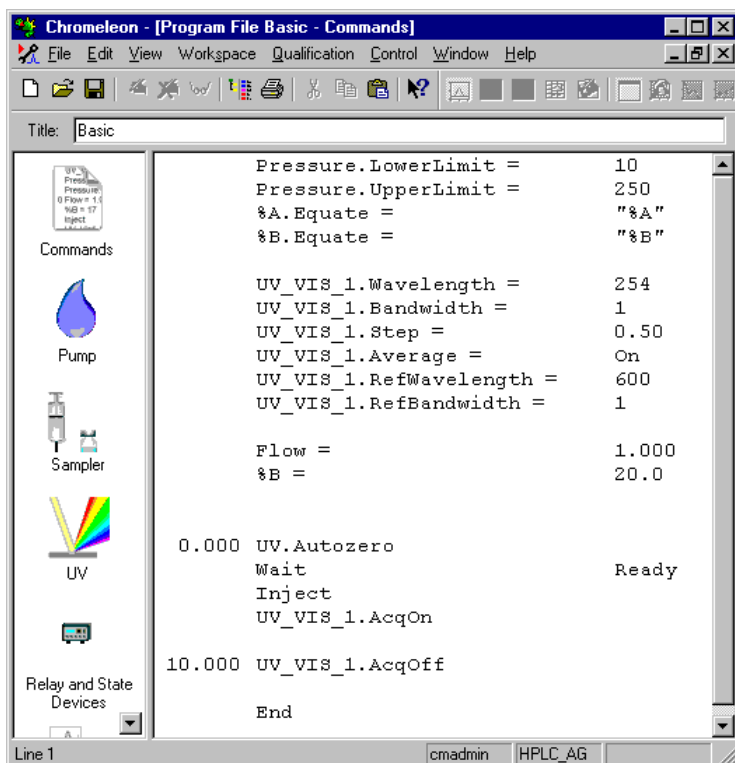
Function: The **Weight** parameter is implemented as a dimensionless multiplication factor or divisor:

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**).


The Program

The **control program** (or "program") is part of the PGM File and is displayed in the **Commands** view of the PGM Editor. It contains instrument commands and definitions for chromatographic sample processing. A control file for *➤Mass Spectrometers* additionally includes the device settings for the MS. The control file has the extension **PGM** and contains a chronological list of instructions (commands) for the chromatography modules or Chromeleon itself; for example, **End**. The commands are executed in chronological order.

Use the Program Wizard to create the basic structure of a PGM File. To further edit the basic structure afterward, double-click the PGM File. The PGM Editor is opened. (For more information about the wizard, refer to **Control**  **The Program Wizard**; for information about the Editor, refer to **Control**  **The PGM Editor**.)



**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 a time value at which the command should be executed. This value is in **Decimal Minutes**, that is, "2.500" indicates the time "2 minutes, 30 seconds". The actual program instruction, such as \Rightarrow AcqOn/Off, appears after the time.


You can both integrate control commands into a program and as well perform them separately via either the **Online** toolbar or the script buttons on the **control panels**.

If the program instruction requires the input of **parameters**, these are entered next in the text line. A comma separates them from instructions. If several parameters are required, they are specified in the following lines. Each parameter is identified by its parameter name, followed by "=" and then the parameter value. Example:

```
2.500 Pressure.LowerLimit = 10
      Pressure.UpperLimit = 250
```

Between instruction lines, you may also include any number of **comment lines**. Each of these lines starts with a semicolon.

**Note:**

*If you want to edit your program later in the **Device** views (see **Control/The PGM Editor**  **The Device Views**), we recommend 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:**

The injection, i.e., the Inject command, has the retention time 0 (zero), by definition. All preceding commands are assigned negative times, all following instructions positive times.

The advantage is that the PGM time corresponds to the **retention time** of data reduction, for example, 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.

When processing a program file with the Chromeleon program editor, the validity of instructions is checked automatically. You must only enter the commands and their respective parameters: Chromeleon takes care of the rest; i.e., Chromeleon

- Sorts 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 \Rightarrow *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 (warning)?
 - Is a corresponding **Acquisition Off** present for each **Acquisition On** (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 (otherwise the start times in the program file does not coincide with the actual start time)?
 - Is the final instruction an **end** command?



When creating program files with a word processor, you must carry out these checks yourself. It is particularly important to remember that the first line of the file is always interpreted as the header. If no header is required, you must leave a blank line at the beginning of the file.

Operation of the PGM Editor

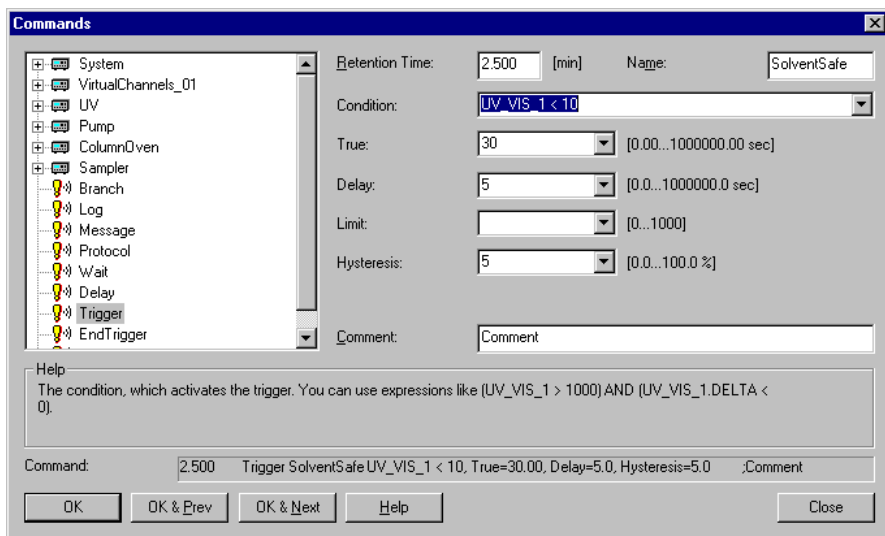
The PGM Editor includes different views that are opened via the associated icons on the left pane:

- Commands
- **Finnigan AQA** or **Surveyor MSQ** (only with PGM Files for a timebase with MS)

- Post-acquisition steps
- Different device views (if the associated devices are installed)

Below please find a description of the **Commands** view where you can enter individual control commands. For more information about the **Surveyor MSQ** view, refer to **How to ...: Actions Related to Mass Spectrometers**  **Creating a PGM File for the MSQ**. For more information about the **Finnigan AQA** view, refer to **How to ...: Actions Related to Mass Spectrometers**  **Creating a PGM File for the aQa MS**.

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) individually. The available instructions and instruments depend on the system configuration; they are listed for simple selection.



Commands

Retention Time: 2.500 [min] Name: SolventSafe

Condition: UV_VIS_1 < 10

True: 30 [0.00...1000000.00 sec]

Delay: 5 [0.0...1000000.0 sec]

Limit: [0...1000]

Hysteresis: 5 [0.0...100.0 %]

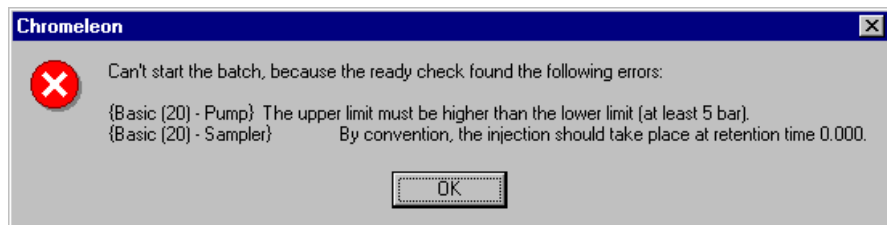
Comment:

Help
The condition, which activates the trigger. You can use expressions like (UV_VIS_1 > 1000) AND (UV_VIS_1.DELTA < 0).

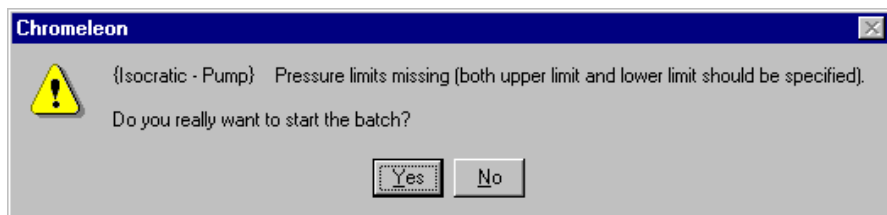
Command: 2.500 Trigger SolventSafe UV_VIS_1 < 10, True=30.00, Delay=5.0, Hysteresis=5.0 ;Comment

OK OK & Prev OK & Next Help Close

Before starting a program, a Ready Check is performed. The program is checked (as far as possible) for validity. In case of logical errors (for example, when Pressure.LowerLimit is greater than 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 is displayed. In this case, you can start the batch nevertheless:



You can sort all entries of the program file chronologically at any time during file generation by selecting the **Sort by Retention Time** command on the **Control** menu. Thus, you can enter all commands pertinent to a specific instrument simultaneously (e.g., all flow commands, all signal parameters, etc.) and later have them sorted chronologically.

For more information, refer to **Control**:



The Control Program



The Program Syntax

Control Commands (Overview)

On the following pages, the most important control commands 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*
- ⇒ *End*
- ⇒ *EndTrigger*
- ⇒ *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_WarningLimit*
- ⇒ *Connect/Disconnect*
- ⇒ *Delta*
- ⇒ *Flow*
- ⇒ *Freeze*
- ⇒ *ParkPercentage*
- ⇒ *Learn*
- ⇒ *Pressure.Lower/UpperLimit*
- ⇒ *WasteLevel*
- ⇒ *WasteWarningLimit*

Autosampler Commands

For more 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, refer to:

- ⇒ *AcqOn/Off*
- ⇒ *Autozero*
- ⇒ *Average*
- ⇒ *Bandwidth*
- ⇒ *Bunch Width*
- ⇒ *Connect/Disconnect*
- ⇒ *Delta*
- ⇒ *Lamp*
- ⇒ *LampIntensity*
- ⇒ *MaxAutoStep*
- ⇒ *RefBandwidth*
- ⇒ *RefWavelength*
- ⇒ *Step*
- ⇒ *Wavelength*

%B, %C, %D (Solvent Components)

Instrument Type: Pump(s)

Related Commands: \Rightarrow Flow

\Rightarrow %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 \Rightarrow %-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 \Rightarrow Timebase, one or more fluidic systems can be installed. They can be controlled independently, either manually or via a \Rightarrow 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 \Rightarrow Hold, \Rightarrow Continue, and \Rightarrow 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:**

We recommend 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: \Rightarrow %A, %B, %C, %D_WarningLimitI

\Rightarrow %B, %C, %D

\Rightarrow Flow

\Rightarrow WasteLevel

Description: This command monitors solvent consumption.

Function: Before starting a sequence, enter the existing volume for each solvent component. Based on the flow rate and the solvent composition, Chromeleon checks whether the available amount of solvent is sufficient for the sequence.

Parameters:

Value Actually available volume for the corresponding component in [I].

LowerLimit Lower limit for the corresponding component in [I]. 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:

The batch is aborted automatically, ensuring that neither the pump nor the detector cell run dry.



Tip:

Chromeleon checks the calculated demand for solvent during the ➤Ready Check. The corresponding warning appears if necessary.



Note:

This command is available for all pumps except for ion chromatography pumps.

%A, %B, %C, %D_WarningLimit

Instrument Type: **Pump(s)**

Related Commands: \Rightarrow %A,%B,%C,%D_Level

Description: This command allows you to enter a warning limit for monitoring the filling height in the associated solvent reservoir. Input is in percent and refers only to the initial filling height (**%X_Level.LowerLimit**).

Function: If the filling height in the solvent reservoir is below this percentage, 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 filling level reaches the %X_Level.LowerLimit, the batch aborted. This ensures that neither the pump nor the flow cell runs dry.



Note:

This command is available for all pumps except for ion chromatography pumps.

AbortBatch

Instrument Type:

System command

Related Commands:

\Rightarrow AbortSample

Description:

The **Abort Batch** command aborts a running sample batch. In combination with the \Rightarrow Trigger command, this command allows you to react, for instance, to external errors. Example:

```
0.000 Trigger Cond=RemErr
```

```
Abort Batch
```

```
EndTrigger
```

Function:

Abort batch terminates data acquisition, deletes all triggers, and aborts the current sample batch.

AbortSample

Instrument Type:

System command

Related Commands:

⇒ *AbortBatch*

Description:

The **Abort Sample** 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, we recommend observing the following command sequence:



Tip (Cont'd):

```
-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 \Rightarrow Hold and \Rightarrow StopFlow commands to interrupt data acquisition. Use the \Rightarrow 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 we recommend 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:

⇒ *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 ⇒ *Step* of 0.01 seconds or a ➤ *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 ➤ *Raw Data* file. This generally improves the ➤ *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: \Rightarrow *Wavelength*

\Rightarrow *RefBandwidth (Reference Bandwidth)*

\Rightarrow *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 \triangleright *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:

In Chromeleon versions without control option, the bandwidth cannot be modified during data acquisition.

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.



*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 ...: Actions on the Control Panel**  **Creating a Script Button**). Branch to the desired 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. To save PGM Files branched to via a **Branch** command, save them 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: \Rightarrow *Bandwidth*

Description: The distance between the wavelengths of a 3D field is referred to as BunchWidth. To enhance the \triangleright *Signal-to-Noise Ratio* of a \triangleright *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.



Tip:

*Averaging the signals improves the signal-to-noise ratio. However, at the same time, \triangleright *Optical Resolution* decreases.*

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 the instrument on. For all installed instruments, the **Connect** command is executed automatically when the Chromeleon server is started. Thus, if working in remote operation 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 operation 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: \Rightarrow *StopFlow*

\Rightarrow *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: **All**

Related Commands: \Rightarrow *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 a peak has been detected provided this peak is higher than 20 mAU.

Delta

Instrument Type:

All

Related Command:

⇒Trigger

Description:

Chromeleon is capable of recording and evaluating signals or output variables supplied by a detector, for example, UV/VIS channel, Temperature, Pressure, %A, Flow rate, etc.

Instead of the actual value (in mAU, Volt, °C, ml, etc.), the changes of a variable in a specific period can be calculated, that is, the first derivative of a signal. To do so, use the Delta signal property to calculate the difference between the current value and the value one second ago.

The Delta option 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 first derivative 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
```

Function (Cont'd):

With this program, an acoustic beep is given 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 ...: Device Control**  **Trigger Commands**.*

Dispense

Instrument Type: ➤Autosampler

Related Commands: ⇒Inject and ⇒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.

For more information, refer to **How to ...: Device Control**  **Autosampler Commands (GINA 50)**  **Autosampler Commands (ASI-100 Series).**

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 without bubbles.

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 to be specified. 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: **How to ...: 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:

⇒ *Trigger*

Description:

The **EndTrigger** 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 partial flows the actual eluent names. 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: $\Rightarrow \%B, \%C, \%D$
 $\Rightarrow \%A, \%B, \%C, \%D_Level$
 $\Rightarrow WasteLevel$

Description: The **Flow** command defines the total flow rate (in [ml/min]) through the column, i.e., the sum of all $\Rightarrow Partial\ Flows$ ($\%A+\%B+\%C+\%D=100\%$). 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 $\triangleright Ramps$, the total flow rate alters linearly with time, between two flow commands. By entering two flow commands at the same time, you can program $\triangleright Step\ Gradients$. 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 $\triangleright Program$ 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.

Function (Cont'd):

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:**

Chromleon 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).

**Tips** (Cont'd):


*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 Command: \Rightarrow *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 **How to ...: Device Control**  **Setting Automatic Pre-Compression Control.**)

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 Commands:

⇒ *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**.*

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 **Administrator Manual: Installation Instructions**  **FAMOS Capillary/Nano HPLC Autosampler**.)

-0.100 ; 6 seconds to process program

0.000 Inject

**Tip:**

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.

Lamp

Instrument Type:

All optical detectors

Related Commands:

Description:

The **Lamp On/Off** command turns the optical detector lamp on and off. UV/VIS detectors often have a separate lamp (deuterium/halogen) for each 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. Read out the \Rightarrow LampIntensity property, using the \Rightarrow 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 (\Rightarrow 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.*

LampIntensity

Instrument Type:

All optical detectors

Related Commands:

-

Description:

Detector lamps are subject to ageing. The **LampIntensity** property allows you to read out the intensity of the lamp using the \Rightarrow Log command. The value indicates the quality of the lamp. When you have installed a new lamp, periodically read out the lamp intensity using the **Log** command, and then compare the two values.

Function:

The **Log LampIntensity** command writes the lamp intensity for an optical detector at 254 nm to the \triangleright *Audit Trail*. The current intensity of the lamp is read out at the retention time when the command is given.



Tip:

Register the lamp intensity at a time when it is not affected by substances in the flow cell. Therefore, it makes sense to read the value at the beginning of data acquisition.



Tips:

*The lamp intensity can be included in the Report. Select the **LampIntensity** variable of the **Audit Trail** category.*

If you read out 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 read, and then indicates the lamp intensity that was measured at that time.

Or else, you may search for the retention time at which the lamp intensity was read from the data acquisition start:

AUDIT.LampIntensity(0.0,"forward").

b) If you know the retention time at which the intensity was read, you might as well 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: \Rightarrow 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 **How to ...: 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: \Rightarrow *Message*

\Rightarrow *Protocol*

Description: The **Log** command allows you to document the values of variables in the \triangleright *Audit Trail* at any time.

Function: All device settings are listed as \triangleright *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 \triangleright *Fraction Collectors*. For another example, refer to \Rightarrow *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

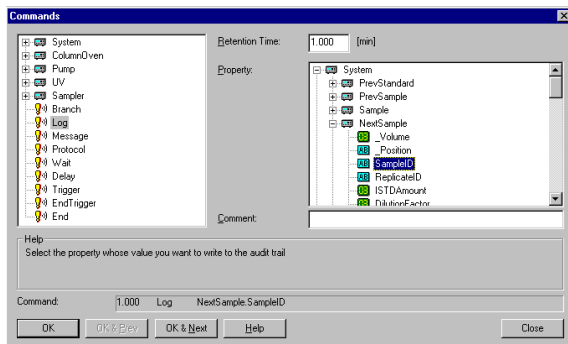
PrevStandard: the most recent standard sample

PrevSample: the most recent standard 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: \Rightarrow Wait

\Rightarrow Protocol

\Rightarrow 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, if 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.

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-50):

```
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 content of vial 10 is then mixed by drawing in and then expelling 30 µl of the vial contents. The mixing cycle is repeated 5 times.

You can also use the PrepSpeed (ASI-100) or SyringeSpeed (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 volume exactly and free of bubbles.

For more information, refer to **How to ...: Device Control**



Autosampler Commands (ASI-100 Series)



How to ...: Device Control



Autosampler Commands (AS-50).

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 pump only**

Related Commands: \Rightarrow Hold_
 \Rightarrow Continue
 \Rightarrow 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.

An external signal must be sent to the pump's START IN input.

Function: Peak parking is similar to the behavior of the \Rightarrow StopFlow command:

- The \Rightarrow Gradient program is interrupted.
- Usually, the flow is reduced (but not turned off).

However, unlike the behavior of the **StopFlow** command:

- Data Acquisition (see \Rightarrow Acquisition On/Off) is not interrupted.
- A running \Rightarrow Batch is not stopped.

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 \Rightarrow Mass Spectrometer in a more sensitive range.

Function (Cont'd):



Tips:

Peak parking freezes the gradient with its current composition and reduced flow while data acquisition continues. Please note the following:

- *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 \Rightarrow 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: \Rightarrow *Message*

\Rightarrow *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 is thus directly linked with the corresponding sample. The **Protocol** text can thus be used for commenting individual samples. The program run is not affected by a **Protocol** command.

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
```

Ready

Type: ➤Autosampler


Related Comm.: ⇒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:	<p>The reference bandwidth can be selected separately for each channel. The 3D field of a ➤Photodiode Array Detector also has its own reference bandwidth.</p> <p>Analogous to the conventional bandwidth of a channel, the reference bandwidth serves to average several photodiode signals of the Reference Wavelength.</p>
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:	<p>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.</p> <p>For PDA-100 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, 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.</p>

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" per definition. Should peaks be detected, nevertheless, select a different reference wavelength. You can deliberately use this as well to inhibit peaks.

Function (Cont'd):

If the reference wavelength is chosen in a light-deficient range, it is recommended to average the signals of several photodiodes by increasing the Reference Bandwidth and thus improve the signal to noise ratio.

For the Dionex Photodiode Array, 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 used for data acquisition: On the context menu, select **Extract** and then **Chromatogram to file**. Then, select the desired reference condition for the new channel.
2. Displaying the 3D field at a different reference wavelength: Select the **Decoration** option on the context menu to open the **Peak Purity Decoration**. Then, select the desired reference conditions on the **General** tab page.

**Tip:**

Do not confuse the reference wavelength with the ➤Reference Channel used for ➤Baseline Correction.

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 small 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.

**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.

*Settings (Cont'd)***Dionex PDA-100 Detector**

By default, the reference wavelength is disabled for the PDA-100. 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. The reference wavelength in this case 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 in 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 (\geq *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 given.

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: \Rightarrow *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.

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 \Rightarrow *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 **How to ...: 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


<i>Instrument Type:</i>	Detector
<i>Type:</i>	Fixed point value
<i>Value Range:</i>	Depends on the channel; for example, for a UV channel: 0.01 ... 4.8 [s] or Auto
<i>Default:</i>	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.

<i>Related Commands:</i>	⇒ <i>MaxAutoStep</i> ➤ <i>Sampling Rate</i> ➤ <i>Data Collection Rate</i>
--------------------------	---

<i>Description:</i>	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.
---------------------	---

<i>Function:</i>	Fixed Step [0.01 4.8 s] Every <i>step</i> 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: \Rightarrow Hold

\Rightarrow Flow

\Rightarrow 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 desired 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 status on the screen! Whether this is possible depends on the column oven connected.

Trigger

Instrument Type:

System Command

Related Commands:

\Rightarrow 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 \Rightarrow *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 (\Rightarrow *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 \Rightarrow AcqOn command of the program. The following command is executed every time the specified conditions are met (**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 given dependently from the time.

Even trigger commands can be triggered!

*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 Remotel, 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.*

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 micro liters (μl).

Function: 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).



Tip:

In order to minimize carry-over effects in multiple-point calibrations, you should always start with the sample with the highest dilution or the smallest injection volume.

Wait

Instrument Type: ➤ *Remote Input*

Related ⇒ *Message*

Commands: ⇒ *Inject*

⇒ *AcqOn/Off*

Description: The **Wait** command interrupts program execution until the specified remote input signal arrives.

Function: Wait stops the program time and the acquisition; the pump(s) are in Hold mode. For examples for how to use the **Wait** command, refer to **How to ...: Device Control**:



Control: Autosampler



Mixed Commands

Parameter:

Condition Condition for the realization of which the system waits (see ⇒ *Trigger*)

Wash

Instrument Type: ➤Autosampler

Related Commands: ⇒Inject

⇒NeedleUp

Value Range: **Depends on device and installed syringe**

Description: The **Wash** command serves for rinsing the autosampler.

Function: It causes the autosampler to lower the needle into the needle seat and to rinse the sample loop and needle with solvent in **Inject** state. This corresponds to the normal solvent flow following an **Inject** command.



Tip:

Select the **Wash** and **NeedleUp** commands 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 commands indicates the level for the solvent waste.

Function:

Before starting a sequence, enter the actual filling height in the waste container. Chromeleon checks whether the waste container has sufficient capacity.

Parameters:

Value

Actual waste level in [I].

UpperLimit

Upper limit for the waste level in [I]. If the level in the waste container reaches this value, the batch is aborted and following message appears:

[Abort] xx:xx:xx {Pump} The waste level is above the limit (x.xxx l)! Please empty the waste container.



Tip:

This avoids that the waste container runs over.



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.

WasteWarningLimit

Instrument Type: **Pump(s)**

Related Commands: \Rightarrow *WasteLevel*

Description: This command allows you to enter a warning limit for monitoring the filling height in the waste container. Input is in percent and refers to the upper limit (**WasteLevel.UpperLimit**).

Function: If the filling height in the waste container exceeds this limit, the following warning appears:

[Warning] xx:xx:xx {Pump} The waste level (actual level in [I]) will reach the upper limit (upper limit in [I]) in about 0.1 hours! Please empty the waste container.



Tip:

When the filling height reaches the upper limit, the batch is aborted. This avoids that the waste container runs over.



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.

The QNT Editor

Use the QNT Editor to define the method of quantification (in short: **QNT Method** or just method). The quantification method defines parameters that identify the peaks within a chromatogram (assign substances - peak identification) and others that enable the calculation of amount/concentration from the recorded peak areas (calibration).

Peak Identification

Chromeleon has two possibilities for identifying detected peaks, that is the assignment of substance names.

Generally, peaks are identified by their \Rightarrow *Retention Times*. If a peak elutes within a defined time window, it is assigned the corresponding name, calibration function, etc.

In the event that many peaks are obtained in rapid succession, or their order is altered by a change in chromatographic conditions (for example, pH value), identification by the **UV spectrum** or the \Rightarrow *Mass Spectrum* is considerably more reliable.



Note:

Peak identification by UV spectra requires use of a \Rightarrow Photodiode Array Detector, such as the Dionex UVD 340 or PDA-100, and the appropriate software configuration.

For mass spectra acquisition, a \Rightarrow Mass Spectrometer and the \Rightarrow Xcalibur software are required in addition to the Chromeleon software.

Calibration

The peak table contains all information for the calibration. Using the amount column and the recorded peak areas, the offset, slope, and curve parameters are calculated.



Tip:

With each new calibration the results are recalculated automatically for the report, as the calibration constants and the recorded integration values are entered to the peak table by the integration programs.

To save the user from having to determine the retention time of each peak manually, peak tables can be generated automatically. (For more information, refer to **How to ...: Actions in the QNT Editor**



Autogenerating the Peak Table.)

The QNT Editor provides up to ten tabs for parameter input:

- **General** (general settings)
- **Detection** (\Rightarrow *Detection Parameters*)
- **Peak Table** (general peak table)
- **Amount Table** (parameters to determine the amount)
- **Peak Tracking** (parameters for peak assignment via UV reference spectra)
- **MS Tracking** (parameters for peak assignment via \triangleright *Mass Spectra*)
- **Calibration**
- **Spectra Library Screening** (spectra search parameters)
- **SST** (\triangleright *System Suitability Test (SST)* parameters)
- **MS** (parameters for mass spectra evaluation)

The **General** tab page lists those parameters that apply to all peaks; for example,

- \Rightarrow *Use Recent Retention Time*: automatic correction of expected retention times during calibration runs.
- \Rightarrow (*Calibration*) *Mode*: Specification which sample is calibrated using which standard(s).
- \Rightarrow *Dimension of Amounts*

The peak table (on the **Peak Table**, **Amount Table**, **Peak Tracking**, and **MS Tracking** tabs) lists peak-specific parameters, as for instance:

- **Expected retention time**
- **Peak name**
- **Integration type (area/height)**
- **Calibration type (linear, exponential, etc)**


For more information about the general parameters and the peak table parameters, refer to the topics below.

QNT Parameters (Overview)

The QNT Editor allows you to define general, peak table, and detection parameters. For a description of the detection parameters, refer to *⇒Detection Parameters (Overview)*. The following topics provide information about the general parameters and the peak table parameters:

- ⇒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

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Table/Peak Results)
<i>Type:</i>	Floating point number
<i>Dimension:</i>	Determined by the Dimension of Amounts parameter on the General tab page
<i>Value Range:</i>	0.000001 ... 9999999.999999
<i>Default:</i>	1.000000
<i>Related Parameter:</i>	⇒ <i>Dimension of Amounts</i>
<i>Description:</i>	<p>The Amount parameter determines the content (amount, concentration) of a standard sample or a ➤ <i>Validation Sample</i> 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.</p>
<i>Function:</i>	<p>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: Actions in the Report Table  Calculating the Peak Variable "Amount"). The ➤ <i>Formula for Calculating the Amount</i> allows you to consider the response factor, dilution factor, weight, and a factor for the ➤ <i>Internal Standard</i>.</p> <p>In absolute amounts, the result always relates to the injected volume; for example, 17.6 µg per 20 µl injection volume. Select the ➤ <i>Concentration</i> 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.</p>

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 first standard. You can thus combine the amount values with the ➤*Dilution Series*!*

Even for multi-point calibration, normally only one peak table is required!

Calibration Mode

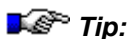
<i>Location:</i>	General tab page/ Report Variable (Peak Calibration)
<i>Type:</i>	Character
<i>Dimension:</i>	No dimension
<i>Value Range:</i>	Fixed/Total/Group/Additional/Bracketed/Standard Addition
<i>Default:</i>	Total
<i>Related Parameter:</i>	⇒ <i>Calibration Type</i>
<i>Description:</i>	<p>The Calibration Mode parameter determines the standards that are used for calibrating a specific sample of a sequence. Chromeleon provides six different calibration modes.</p> <p>Select the mode on the General tab page in the QNT Editor (see The QNT Editor  The General Tab Page).</p>
<i>Function:</i>	

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, and c2 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.

Group	Name	Pos	Add.	Brack.
	Standard 1	1		
	Standard 2	2		
	Sample 1	3	←	←
	Sample 2	4	←	←
	Standard 3	5		
	Standard 4	6		
	Sample 3	7	←	←
	Sample 4	8	←	←
	Standard 5	9		
	Standard 6	10		

**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 \Rightarrow *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

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Calibration)
<i>Type:</i>	Code
<i>Dimension:</i>	---
<i>Value Range:</i>	Linear (Lin)/Linear with Offset (LOff)/ Quadratic (Quad)/Quadratic with Offset (QOff)/ Exponential (Exp)/Point-to-Point (P-P)
<i>Combine with:</i>	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)
<i>Default:</i>	Linear
<i>Related Parameters:</i>	⇒ <i>Standard</i> ⇒ <i>Calibration Mode</i>
<i>Description:</i>	The Calibration Type parameter determines the ➤ <i>Calibration Function</i> and the ➤ <i>Weights</i> . 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. We recommend 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 .
<i>Function:</i>	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
(Lin)**

Minimum number of standards or ➤ *Spiked Samples*: 1

The model function presents a straight line passing the origin. (In ➤ *Standard Addition* calibration mode, the line is forced through the average of all Unspiked samples instead.) For more than one standard, the regression line is calculated. The calibration curve is based on the following equation:

Model function: $f(A) = c1 \times A$

Where A is the peak area and f(A) the calculated amount.

c1 (linear coefficient, slope) is calculated and stored.

**Linear with offset
(LOff)**

Minimum number of standards or spiked samples: 2

The calibration curve presents a straight line, crossing the ordinate at a finite value.

Model function: $f(A) = c0 + c1 \times A$

c0 and c1 are calculated and stored.

**Quadratic
(Quad)**

Minimum number of standards or spiked samples: 2

The calibration curve presents a parabola passing the origin. (In **Standard Addition** calibration mode, the line is forced through the average of all unspiked samples instead.) For more than two calibration samples, the regression parabola is calculated.

Model function: $f(A) = c1 \times A + c2 \times A^2$

c1 and c2 are calculated and stored.

Quadratic with offset (QOff)

Minimum number of standards or spiked samples: 3

The calibration curve presents a parabola crossing the ordinate at a finite value. For more than three calibration samples, the regression parabola is calculated.

Model function: $f(A) = c_0 + c_1 x A + c_2 x A^2$

c_0 , c_1 , and c_2 are calculated and stored.

Exponential (Exp)

Minimum number of standards or spiked samples: 2

The calibration curve presents an exponential function.

Model function: $f(A) = c_0 x A^{c_1}$

c_0 and c_1 are calculated and stored.

The following applies to the Standard Addition calibration mode:

Model function: $f(A) = \tilde{c} + c_0 x A^{c_1}$

with:

\tilde{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.

Weighting:**No Weights**

Default: higher weighting of higher amounts or signal values.

1/Amount (X)

Nearly cancels out the weighting of higher amounts.

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).
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, we recommend 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: \Rightarrow *Retention Time*
 \Rightarrow *Delay Time*
 \Rightarrow *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 \Rightarrow *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 \Rightarrow *Capacity Factors* and the \Rightarrow *Kovats Indexes*.

Delay Time

<i>Location:</i>	General tab page/ Report Variable (Quantification Method)
<i>Type:</i>	Floating point value
<i>Dimension:</i>	Minutes
<i>Value Range:</i>	-9999.999 ... 9999.999
<i>Default:</i>	Not selected
<i>Related Parameters:</i>	\Rightarrow <i>Dead Time</i> \Rightarrow <i>Retention Time</i>
<i>Description:</i>	The time needed by 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 .
<i>Function:</i>	Usually, a constant \Rightarrow <i>Flow</i> 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 \Rightarrow <i>QNT Editor</i> (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 \Rightarrow Flow Gradients does not make sense because the delay is not constant in this case. Follow the description in **How to: Actions in the QNT Editor**  **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: \Rightarrow 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 ...: Actions in the QNT Editor**  **Grouping Peaks**

Integration Type

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Table)
<i>Type:</i>	Selection
<i>Dimension:</i>	No dimension
<i>Value Range:</i>	Area/Height/Relative Area/Relative Height/CE Area
<i>Can be combined with:</i>	All peaks, identified Peaks only/Exclude ISTD peaks (only with Relative Area/Relative Height)
<i>Default:</i>	Area
<i>Related Parameters:</i>	⇒ <i>Calibration Type</i> ⇒ <i>Group</i>
<i>Description:</i>	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 $t_S = t'_S + t_0$

t_S = Retention time

t_0 = Dead time

n = Number of carbon atoms
(normally of n-alkane)

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 ...: Actions in the QNT Editor**  **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: \Rightarrow *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:

$$t - LL \quad \dots \quad t + RL$$

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*

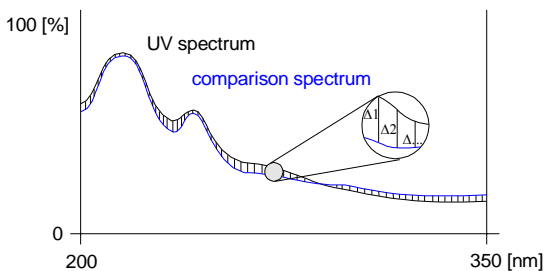
⇒ *Maximum/Minimum Wavelength*

⇒ *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 (λ_1 , λ_2 , ...). Since the square of the deviations is calculated as well, the direction of the subtraction does not matter.



<i>Function (Cont'd):</i>	The method provides a value of similarity (match) between completely different (= 0) and identical (= 1000). 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)

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Table)
<i>Type:</i>	String
<i>Dimension:</i>	no dimension
<i>Value Range:</i>	All printable characters
<i>Default:</i>	With automatic table generation: <Name of the QNT Method>x , where x is the current peak number. When appending a new cell: Peak-x
<i>Description:</i>	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.)
<i>Function:</i>	The substance name is taken for the report and the ➤ <i>Printer Layout</i> .
 Note:	<i>Only use letters, numbers, and punctuation marks, since the complete MS-DOS character set is not available on all printers.</i>

Reference Spectrum

Location: **Peak Tracking** tab page/
Report Variable (**Peak Table**)

Type: **Selection**

Dimension: **No dimension**


Value Range: ---

Default: **Empty**

Related Parameters: ⇒ *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 ...: Actions in the QNT Editor**  **Peak Tracking**.)



Tip:

Spectra acquisition is possible with the DAD license only.

Relative Maximum Deviation

<i>Location:</i>	Peak Tracking and Spectra Library Screening tab pages/Report Variable (Peak Table)
<i>Type:</i>	Floating point number
<i>Dimension:</i>	nm
<i>Value Range:</i>	Off; 0.0-100.0 nm
<i>Default:</i>	Off
<i>Related Parameters:</i>	Additional parameters for comparing spectra: ⇒ <i>Check Derivative</i> ⇒ <i>Check Extrema</i> ⇒ <i>Match Criterion</i> ⇒ <i>Maximum/Minimum Wavelength</i> ⇒ <i>Threshold</i>
<i>Description:</i>	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.
<i>Function:</i>	If the setting is Off , deviation is not checked.

Response Factor

Location: **Peak Table** tag page/
Report Variable (**Peak Table**)

Type: **Floating point number**

Dimension: ---

Value Range: **0.000000 ... 9999999.999999**

Default: **1.000000**

Related Parameters: \Rightarrow *Weight* (Sample Weight Factor)
 \Rightarrow *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** for calculating the peak in relation to a reference peak. As reference peak, all identified peaks with absolute response factor can be used. 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.

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; for example, once a year.

Relative To Peak
(Cont'd):

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 c_1 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 peak-specific!

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: \Rightarrow *Retention Time*

\Rightarrow *Dead Time*

\Rightarrow *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 ...: Actions in the QNT Editor
 **Defining the Retention Index and the Kovats Index.**

Retention Time

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Table)
<i>Type:</i>	Floating point value
<i>Dimension:</i>	Minutes
<i>Value Range:</i>	0.000 ... 9999.999
<i>Default:</i>	0.000 With Autogenerate Peak Table , the retention time of the active chromatogram is used.
<i>Can be combined with:</i>	Absolute time; Time distance/ratio to reference peak; Reference peak
<i>Related Parameters:</i>	⇒ <i>Window</i> ⇒ <i>Use Recently Detected Retention Times</i> ⇒ <i>Dead Time</i>
<i>Description:</i>	<p>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 ⇒ <i>Dead Time</i>).</p> <p>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 ➤ <i>PGM File</i> (file type .PGM) are compatible with the retention times, rather than being delayed by the injection time.</p> <p>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.</p>

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 highlighted 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.*



Note (Cont'd):



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

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Table)
<i>Type:</i>	String
<i>Dimension:</i>	---
<i>Value Range:</i>	External; Internal; Internal/External; Use this peak as internal Standard
<i>Default:</i>	External
<i>Can be combined with:</i>	Use sample amount as reference (only with Use this peak as internal Standard)
<i>Related Parameter:</i>	⇒ <i>Amount</i>
<i>Description:</i>	The Standard parameter indicates the standard method to be used for calibration. For a detailed description including numerical examples, refer to How to ...: Actions in the QNT Editor  Calibration .
<i>Function:</i>	
External	The calibration curve determines the area/amount ratio. During calibration, the calibration coefficients are calculated from mixtures of known components (see ➤ <i>Calibration Function</i> and Theory of Calibration  Standard Methods).
Internal	Calibration corresponds to the External Standard method. In addition, an ➤ <i>Internal Standard</i> 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 indicated 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 the desired ISTD peak in the **Associated ISTD Peak** check box.*

Entering Several Internal Standards

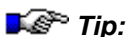
It is also possible to define several internal standards. Via the F8 box, define the respective peaks of the peak table in the Standard column of the peak table as ISTD peaks. A yellow background will highlight the corresponding line of the peak table. For the remaining peaks, enter the standard method 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.

Internal
(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 the desired 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)

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Table)
<i>Type:</i>	Alternatives
<i>Dimension:</i>	No dimension
<i>Value Range:</i>	Autodetect (Auto)/Rider (R)/Main (M)/Baseline- Main (B-M)/Main-Baseline (M-B)/Baseline- Main-Baseline (B-M-B)
<i>Default:</i>	Autodetect
<i>Related Parameters:</i>	⇒ <i>Rider Threshold</i> ⇒ <i>Maximum Rider Ratio</i> ⇒ <i>Valley to Valley</i> ⇒ <i>Lock Baseline</i>
<i>Description:</i>	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 ...: Actions in the QNT Editor  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 .
<i>Functions:</i>	
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.

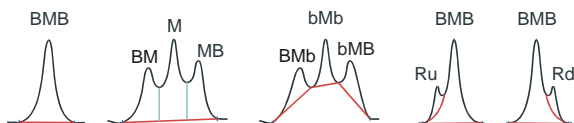
Rider (R)/Main (M) (Cont'd):

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.

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 (\Rightarrow 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

<i>Location:</i>	General tab page/ Use Prev. Retention Time (Peak Table) Report Variable
<i>Type:</i>	Check-box
<i>Dimension:</i>	---
<i>Value Ranges:</i>	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
<i>Defaults:</i>	Not marked Last sample Last value
<i>Related Parameters:</i>	⇒ <i>Retention Time</i> ⇒ <i>Window</i>
<i>Description:</i>	<p>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.</p> <p>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.)</p> <p>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.</p> <p>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).</p>

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 $1 \text{ min} + 9 \times 10 \text{ s} = 150 \text{ s}$ 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*)

<i>Location:</i>	Peak Table tab page/ Report Variable (Peak Table)
<i>Type:</i>	Positive floating point number or string
<i>Dimension:</i>	Minutes or %
<i>Value Range:</i>	0.000 ... 999.999 or 0 - 100% Absolute/Relative and First/Greatest/Nearest/Spectrum/Spectrum and time
<i>Default:</i>	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
<i>Can be combined with:</i>	Check the best hits only (with the peak match options Spectrum and Spectrum and Time only)
<i>Related Parameters:</i>	⇒ <i>Retention Time</i>
<i>Description:</i>	<p>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.</p> <p>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.</p>

Function: The dimension *W* of the time windows is calculated according to the following formula:

Absolute time window: $W = TW$

Relative time window: $W = T \times TW / 100$

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 $5.0 \text{ min} \pm 10\% = 5 \text{ min} \pm 0.5 \text{ min} = 4 \text{ min } 30 \text{ s to } 5 \text{ min } 30 \text{ s}$. 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!

QNT: Detection

Use the **Detection** page of the QNT Method to determine integration and detection parameters. They control peak detection, baseline treatment, peak classification, and inhibition (**integration parameters**). The values of these parameters are assigned time-dependently and channel-dependently; thus, they may be altered several times during a chromatogram.

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

Example of a detection table

Generally, about 90% of all chromatograms are integrated correctly by the system. In critical cases, such as **wavelength switching**, the user may influence the baseline or the peak type (for example, rider or main peak), or he can inhibit detection entirely for certain periods. The figure illustrates a detection table.

Besides, detection parameters can be defined graphically. This is extremely useful for the two parameters \Rightarrow *Sensitivity* and \Rightarrow *Peak Slice*. For more information, refer to **How to ...: Actions in the Chromatogram**



Defining Detection Parameter Graphically.

Each of the 26 differing parameters described in detail below, can be assigned a new value at any time. The last value is valid for the duration of a sample; the parameters are assigned their original default values. The assignment of a new value to a parameter is called an **event**. Events can be printed in the Report, with identification and a numerical value at the respective execution time.


Events need not be entered in chronological order. They are sorted automatically in chronological (ascending) order before being stored.

Parameter tables include the following columns:

No.	Line number, cannot be edited.
Ret. Time [min]	Retention time, specified in minutes within a range of 0.000 - 9999.999.
Param. Name	Parameter name ; dialog box (F8 or double-click) displays a list of all available parameters.
Param. Value	Parameter value ; contains detailed information concerning all permitted values of the selected parameter type.
Channel	Via the arrow or the dialog box (open via F8), all options available can be chosen. In addition to All Channels , the single channels that have been recorded can be chosen.

Many parameters can only have the values ON or OFF. They are called **switches**. All other parameters are called **variables**.


The parameters are described in detail on the following pages. (The order of description corresponds to the order in which they can be selected in the QNT Editor.)

For more information about how to define individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Detection Parameters (Overview)

Chromeleon provides 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 (PWStart/PPWEnd)*
- ⇒ *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: Actions in the QNT Editor**  **Defining Detection Parameters.**

Baseline Point


<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Command
<i>Dimension:</i>	---
<i>Value Range:</i>	---
<i>Default:</i>	---
<i>Related Parameters:</i>	⇒ <i>Lock Baseline</i> ⇒ <i>Valley to Valley</i> ⇒ <i>Inhibit Integration</i> ⇒ <i>Type (Peak Type)</i>
<i>Description:</i>	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 ...: Actions in the QNT Editor**  **Defining Detection Parameters.**


Detect Negative Peaks

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Switch
<i>Dimension:</i>	---
<i>Value Range:</i>	On; Off
<i>Default:</i>	Off ; that is, negative peaks are not detected.
<i>Description:</i>	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.
<i>Function:</i>	<p>Enabling detection of negative peaks automatically enables the \Rightarrow<i>Lock Baseline</i> 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.</p> <p>To correct the baseline without labeling the peaks or including peaks in the peak list, select the Don't label option.</p>
 Tip:	<i>In order to be effective, this switch must be activated before the peak start.</i>


For information about how to use individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.


Front Riders to Main Peaks

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Selection
<i>Dimension:</i>	-
<i>Value Range:</i>	Off, On
<i>Default:</i>	Off
<i>Related Parameters:</i>	⇒ <i>Rider Threshold</i> ⇒ <i>Maximum Rider Ratio</i> ⇒ <i>Type (Peak Type)</i>
<i>Description:</i>	Select the Front Riders to Main Peaks detection parameter to change rider peaks on the leading edge of a peak into main peaks.
<i>Function:</i>	Set the parameter value to On to change upward riders to main peaks even if riders are enforced; for example, ⇒ <i>Rider Threshold</i> = 0.00%, ⇒ <i>Maximum Rider Ratio</i> = 100.0%).
 Tip:	<i>This parameter has priority over the Rider peak type in the peak table (see ➤Rider).</i>

For information about how to use individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Inhibit Integration

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Switch
<i>Dimension:</i>	---
<i>Value Range:</i>	On; Off
<i>Default:</i>	Off
<i>Description:</i>	The Inhibit Integration detection parameter serves to fade out certain chromatogram areas. When set, peak detection is disabled.
<i>Function:</i>	<p>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.</p> <p>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.</p>
 Tip:	<i>To have any effect, set this parameter before the first peak to be inhibited starts.</i>

For information about how to use individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Lock Baseline

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Alternatives
<i>Dimension:</i>	---
<i>Value Range:</i>	Off; On; At current level / At global minimum
<i>Default:</i>	No
<i>Related Parameters:</i>	⇒ <i>Valley to Valley</i> ⇒ <i>Inhibit Integration</i> ⇒ <i>Baseline Point</i> ⇒ <i>Type (Peak Type)</i>
<i>Description:</i>	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.
<i>Function:</i>	
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 \Rightarrow 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 ...: Actions in the QNT Editor**  **Defining Detection Parameters**.


Maximum Area Reject

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Fixed point number
<i>Dimension:</i>	Signal dimension * Minute
<i>Value Range:</i>	0.000 ... 1,000,000.000
<i>Default:</i>	The default setting is Off ; i.e., all peaks are integrated.
<i>Related Parameters:</i>	⇒ <i>Minimum Area</i> ⇒ <i>Maximum Height Reject</i>
<i>Description:</i>	Select the Maximum Area Reject parameter to limit the number of integrated peaks. This parameter is very similar to ⇒ <i>Minimum Area</i> .
<i>Function:</i>	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 ...: Actions in the QNT Editor**  **Defining Detection Parameters**.


Maximum Height Reject

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Fixed point number
<i>Dimension:</i>	Signal dimension, such as mAU
<i>Value Range:</i>	0.000 ... 1,000,000.000
<i>Default:</i>	The default setting is Off ; i.e., all peaks are integrated.
<i>Related Parameters:</i>	⇒ <i>Minimum Height</i> ⇒ <i>Maximum Peak Height</i> ⇒ <i>Maximum Area Reject</i>
<i>Description:</i>	Select the Maximum Height Reject parameter to limit the number of integrated peaks. This parameter is very similar to ⇒ <i>Minimum Height</i> .
<i>Function:</i>	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 ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Maximum Peak Height (*MaxHght*)

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Fixed point number
<i>Dimension:</i>	Signal dimension, such as mAU
<i>Value Range:</i>	Off; 0.000 ... 1,000,000.000
<i>Default:</i>	The default setting is Off ; that is, all peaks are integrated.
<i>Related Parameters:</i>	⇒ <i>Minimum Height</i> ⇒ <i>Maximum Area Reject</i>
<i>Description:</i>	<p>Positive peak identification is performed using the ⇒ <i>Minimum Height</i> parameter. All peaks above this height value are identified.</p> <p>The Max. Peak Height detection parameter is used as a maximum criterion determining the height threshold above which peaks are not identified.</p>
<i>Function:</i>	<p>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.)</p> <p>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.</p>

For information about how to use individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Maximum Rider Ratio

Location: **Detection** tab page/
Report Variable (**Detection Parameters**)

Type: **Fixed point number**

Dimension: **Percent [%]**

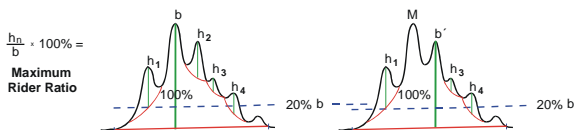
Value Range: **0 ... 99.99**

Default: **20**

Related Parameters: \Rightarrow *Rider Threshold*
 \Rightarrow *Type*

Description: If one or several peaks (h_1 to h_4) 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 \Rightarrow 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 ...: Actions in the QNT Editor**  **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 ...: Actions in the QNT Editor**  **Defining Detection Parameters**.


Minimum Area

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Fixed point number
<i>Dimension:</i>	Signal dimension * Minute
<i>Value Range:</i>	0.000 ... 1,000,000.000
<i>Default:</i>	The default setting is 0.001 ; that is, all peaks with an area > 0.001 [<i>Sig.dimension x minutes</i>] are integrated.
<i>Related Parameters:</i>	⇒ <i>Maximum Area Reject</i> ⇒ <i>Minimum Width</i> ⇒ <i>Minimum Height</i>
<i>Description:</i>	The Minimum Area detection parameter is used as a minimum criterion determining the area threshold below which peaks are identified during peak detection or integration.
<i>Function:</i>	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.



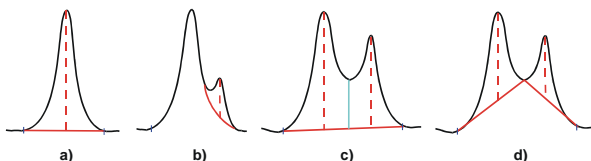
Tip:

*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 ...: Actions in the QNT Editor**  **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 [<i>Sig.dimension</i>] are integrated.
Related Parameters:	⇒ <i>Maximum Height Reject</i> ⇒ <i>Minimum Area</i> ⇒ <i>Minimum Width</i> ⇒ <i>Maximum Peak Height</i>
Description:	The Minimum Height detection parameter is used as a minimum criterion determining the height threshold below which peaks are identified.
Function:	The peak height is measured relative to the baseline (a). For ➤ <i>Rider Peaks</i> , the height measurement is relative to the profile of the carrier peak (b). The baseline can strongly influence these criteria (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

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Fixed point number
<i>Dimension:</i>	Minute
<i>Value Range:</i>	0.000 ... 1,000,000.000
<i>Default:</i>	The default setting is zero; that is, all peaks with the width > 0 [<i>minutes</i>] are integrated.
<i>Related Parameters:</i>	⇒ <i>Minimum Area</i> ⇒ <i>Minimum Height</i>
<i>Description:</i>	The Minimum Width detection parameter is used as a minimum criterion defining the minimum width below which peaks are ignored during peak detection. 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 ...: Actions in the QNT Editor**  **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 \Rightarrow 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 ...: Actions in the QNT Editor**  **Grouping Peaks**.

For information about how to use individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Peak Purity Start/End Wavelength (PPWISStart/PPWIEnd)

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Floating point number
<i>Dimension:</i>	nm
<i>Value range:</i>	190.0 - 900.0
<i>Default:</i>	Start: 190.0 nm; End: 900.0 nm
<i>Related Parameters:</i>	⇒ <i>Peak Purity Threshold</i>
<i>Description:</i>	The Peak Purity Start Wavelength and Peak Purity End Wavelength detection parameters limit the wavelength range for peak purity calculations.
<i>Function:</i>	For calculating the ➤ <i>Peak Purity Index (PPI)</i> and the ➤ <i>Peak Purity Match Factor</i> , only the wavelength range within the values limited by start and end is used.
 Note:	<i>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.</i>

For information about how to use individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Peak Purity Threshold (PPTrshold)

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Command
<i>Dimension:</i>	---
<i>Value range:</i>	0.00 ... 100.00
<i>Default:</i>	10.00
<i>Related Parameters:</i>	⇒ <i>Peak Purity Start/End Wavelength</i>
<i>Description:</i>	The Peak Purity Threshold detection parameter determines the threshold for the signal height above which spectra comparison is performed for the UV and ➤ <i>Mass Spectra</i> . 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.

<i>Function:</i>	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%.

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*.


Function (Cont'd):

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 \Rightarrow *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 ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

Peak Shoulder Threshold

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Fixed point number
<i>Dimension:</i>	-
<i>Value Range:</i>	Off; 0.01 ... 1000.00
<i>Default:</i>	Off ; that is, shoulders without relative maximum are not recognized as peaks. Only if value \neq Off, shoulders are detected.
<i>Related Parameters:</i>	\Rightarrow <i>Rider Threshold</i> \Rightarrow <i>Type (Peak Type)</i>
<i>Description:</i>	The Peak Shoulder Threshold parameter defines a threshold value for peak shoulder recognition.
<i>Function:</i>	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 \Rightarrow *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 (\Rightarrow Peak Group Start/Peak Group End) and the \Rightarrow 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 \Rightarrow <i>Sensitivity</i> !
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 and 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 to peak detection, not to integration. For area calculation, the original signal is used.

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 ...:Actions in the QNT Editor**  **Modifying the Peak Recognition Algorithm.***

Rider Skimming

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Selection
<i>Dimension:</i>	-
<i>Value Range:</i>	Tangential at lower peak end, Tangential at both peak ends or Exponential
<i>Default:</i>	Tangential at lower peak end
<i>Related Parameters:</i>	⇒ <i>Rider Threshold</i> ⇒ <i>Maximum Rider Ratio</i> ⇒ <i>Type (Peak Type)</i>
<i>Description:</i>	This parameter indicates how ➤ <i>Rider Peaks</i> are skimmed.
<i>Function:</i>	<p>There are three options:</p> <ul style="list-style-type: none">• 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.

**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 ...: Actions in the QNT Editor**  **Defining Detection Parameters.**

Rider Threshold

Location: Detection tab page/
Report Variable (**Detection Parameters**)

Type: Fixed point number

Dimension: Percent [%]

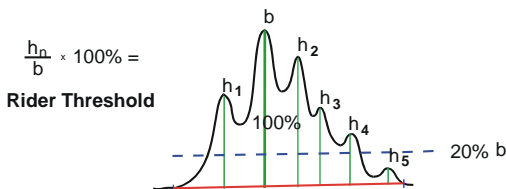
Value Range: 0.00 ... 100.00

Default: 10.00

Related Parameters: \Rightarrow Maximum Rider Ratio
 \Rightarrow Type (Peak Type)
 \Rightarrow 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 \triangleright Rider Peak (skimming peak) or as main peak.

Function: The ratio between the \triangleright 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.



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:

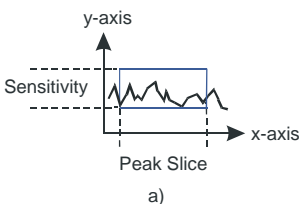
In order to be effective, the criterion must be enabled before the peak start!

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 ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

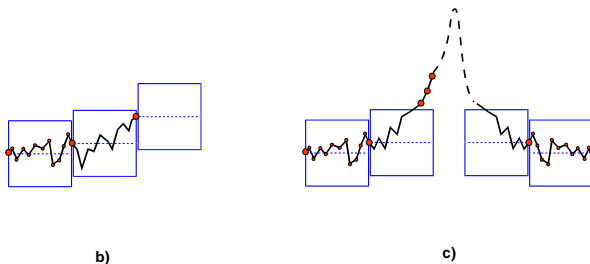
Sensitivity

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Floating point number
<i>Dimension:</i>	Signal dimension
<i>Value Range:</i>	0.0001 ... 10,000,000.0
<i>Default:</i>	0.0010
<i>Related Parameters:</i>	Always consider this parameter in combination with the \Rightarrow <i>Peak Slice</i> .
<i>Description:</i>	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.
<i>Function:</i>	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):



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):



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 to peak detection only, not to integration. For area calculation, the original signal is used.

*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.*

**Notes (Cont'd):**


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 ...: Actions in the QNT Editor**  **Modifying the Peak Recognition Algorithm**.*

For information about how to use individual detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.

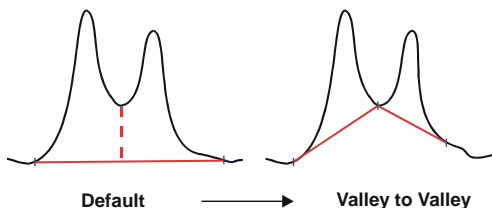
Tailing/Fronting Sensitivity Factor

<i>Location:</i>	Detection tab page/ Report Variable (Detection Parameters)
<i>Type:</i>	Floating point number
<i>Dimension:</i>	---
<i>Value range:</i>	Off; 1.0 ... 100.0
<i>Default:</i>	Off
<i>Related Parameters:</i>	Baseline Point, Lock baseline, Inhibit Integration
<i>Description:</i>	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.
<i>Function:</i>	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 ...: Actions in the QNT Editor**  **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.




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 ...: Actions in the QNT Editor**  **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.



Note (Cont'd):

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 ...: Actions in the Chromatogram**  **Defining Detection Parameters Graphically.**

The Report Definition File (RDF)

Report Definition Files (**RDFs**) have two functions: They serve to display and reprocess your results on the screen (on-screen report) on the one hand and to prepare the printout (➤ *Printer Layout*) on the other.

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 individual 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 window sections and views, window presentations and captions, color and size of individual elements, and the presentation of the sample results on-screen and in the printout. The information deals with 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 make up the report? How is the report formatted?
- Which columns does the worksheet include?


For information about the various Chromeleon report categories, refer to ➤ *Report Categories*. The following pages then provide tables for the individual categories with brief explanations on the individual report variables.

Report Categories

Follow the description in **How to ...: Actions in the Report Table**

 **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.

The report variables belong to 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 File (RDF)	pgm.*	➤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
⇒ <i>Spectra Library Screening</i>	sls.*	Variables for the settings in the QNT File regarding spectra library screening
⇒ <i>Mass Spectrum</i>	msspec.*	➤ <i>Mass Spectra</i> variables (accessible via the Select Spectrum variable of the Mass Spectrometry category)
⇒ <i>Spectrum Data</i>	peak.spectrum.*	Peak spectra variables (accessible via the Reference Spectrum variable of the Peak Table category)
⇒ <i>Hit Spectrum</i>	hitSpec.*	Variables concerning spectra of spectra libraries (accessible via the SLS Hit variable of the Peak Purity category)
⇒ <i>Spectra Library</i>	specLib.*	Spectra library variables (accessible via the Library Record variable of the Hit Spectrum category)
⇒ <i>User Information</i>	~.*	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.

"General" Category

The **General** category is only available in the ➤ *Printer Layout*. It contains the following variables:

Variable	Formula	Description
Version Number	gen.version	
Serial Number	gen.serialNo	
Key Code	gen.keyCode	➤ <i>Key Code</i>
Computer Name	gen.computerName	
Operator Name	gen.operator	User name - opens the ⇒ <i>User Information</i> 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 \Rightarrow *Sequence* category contains 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 \triangleright <i>Datasource</i>
Timebase	seq.timebase	\triangleright <i>Timebase</i> of last run
Title	seq.title	Sequence header
Number of Samples	seq.nSamples	
Creation Date & Time	seq.creation_time	(See \triangleright <i>Time</i>)
Creation Operator	seq.creation_operator	Opens the \Rightarrow <i>User Information</i> category
Last Update Date & Time	seq.update_time	
Last Update Operator	seq.update_operator	Opens the User Information category
Preferred RDF File	seq.prefRdf	Preferred \Rightarrow <i>Report Definition File</i>
Preferred Channel Sign Status	seq.prefChl seq.signStatus	Preferred \triangleright <i>Channel</i> Status of the \triangleright <i>Electronic Signature</i>
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
Review Comment	seq.reviewComment	Comment when checking the electronic signature

Variable	Formula	Description
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 \Rightarrow *Report Categories*.


"Sample" Category

The \Rightarrow *Sample* category contains the following variables:

Variable	Formula	Description
Sample Number	smp.number	\Rightarrow <i>No.</i>
Sample Name	smp.name	\Rightarrow <i>Name</i>
Raw Data ID	smp.id	
Sample ID	smp.ident	\Rightarrow <i>Sample ID</i>
Comment	smp.comment	\Rightarrow <i>Comment</i>
Sample Type	smp.type	\Rightarrow <i>Type</i> (sample type)
Vial Number	smp.position	See \Rightarrow <i>Pos.</i> (sample position)
Replicate ID	smp.replicate	\Rightarrow <i>Replicate ID</i>
Status	smp.status	\Rightarrow <i>Status</i> (sample status): Single/Multiple/ Finished/Interrupted/Running
Date & Time Sample Run	smp.time	See \Rightarrow <i>Inj. Date/Time</i>
Inject Volume	smp.inject_volume	\Rightarrow <i>Inj. Vol.</i> (injection volume)
Dilution Factor	smp.dilution_factor	See \Rightarrow <i>Dil. Factor</i>
Sample Weight	smp.weight	\Rightarrow <i>Weight</i> (Sample Weight Factor)
ISTD Amount	smp.amount	See \Rightarrow <i>ISTD Amount</i> (amount of the \Rightarrow <i>Internal Standard</i> (ISTD))
Program	smp.program	\Rightarrow <i>PGM File</i>
Quantification Method	smp.method	\Rightarrow <i>Quantification Method</i> (QNT Method)
Standard Addition Group	smp.spike_group	\Rightarrow <i>Standard Addition</i> group
Reference Amount Set	smp.refamount_set	ID for \Rightarrow <i>Amount</i> column in the QNT Editor
*x	smp.x	\Rightarrow <i>User-defined Column</i> (x = column name)

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *Report Categories*.

"Audit Trail" Category

Depending on the connected instrumentation, hundreds of variables are available in the Audit Trail (see **Data Management**  **Audit Trails**.) The list below contains only those 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.%A	Percentage of the respective component in the eluent (partial flow) (see \Rightarrow %B, %C, %D)
Average (Pump_Pressure)	AUDIT.Pump_Pressure.Average	Status of averaging (also, see \Rightarrow Average)
Equate (%A)	AUDIT.%A.Equate	Name of eluent component A (components B, C and D accordingly)
Flow	AUDIT.Flow	\Rightarrow Flow rate
Lower Limit (Pressure)	AUDIT.Pressure.LowerLimit	Lower pressure limit (see \Rightarrow Pressure.Lower/UpperLimit)
Step (Pump_Pressure)	AUDIT.Pump_Pressure.Step	Step for each channel (also, see \Rightarrow Step)
Upper Limit (Pressure)	AUDIT.Pressure.UpperLimit	Upper pressure limit (see \Rightarrow Pressure.Lower/UpperLimit)

UVD340

Variable	Formula	Description
Average	AUDIT.Average	Status of averaging: enabled or disabled (see \Rightarrow Average)
Bandwidth	AUDIT.BandWidth	\Rightarrow Bandwidth (for each UV channel)
Bunch Width	AUDIT.3DFIELD.BunchWidth	\Rightarrow Bunch Width of the diode bundle (3D-field only)
LampIntensity	AUDIT.LampIntensity	\Rightarrow LampIntensity [counts/s]
Max. Wavelength (3DFIELD)	AUDIT.3DFIELD.MaxWavelength	Maximum wavelength of the \geq 3D Field
Min. Wavelength (3DFIELD)	AUDIT.3DFIELD.MinWavelength	Min. wavelength of the 3D field
Ref Wavelength	AUDIT.RefWavelength	\Rightarrow RefWavelength (Reference Wavelength) (for each UV channel)
Ref Bandwidth	AUDIT.RefBandwidth	\Rightarrow RefBandwidth (Reference Bandwidth) (for each UV channel)
Step	AUDIT.Step	\Rightarrow Step
Wavelength	AUDIT.Wavelength	\Rightarrow Wavelength (for each UV channel)

"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 (see **Data Management**  **Audit Trails.**) The list below contains only 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	precond.%A	Percentage of the respective component in the eluent (partial flow) (see ⇒ %B, %C, %D)
Average (Pump_Pressure)	precond.Pump_Pressure.Average	Status of averaging (also, see ⇒ <i>Average</i>)
Equate (%A)	precond.%A.Equate	Name of eluent component A (components B, C and D accordingly) ⇒ <i>Flow rate</i>
Flow	precond.Flow	⇒ <i>Flow rate</i>
Lower Limit (Pressure)	AUDIT.Pressure.Lower Limit	Lower pressure limit (see ⇒ <i>Pressure.Lower/UpperLimit</i>)
Step (Pump_Pressure)	precond.Pump_Pressure.Step	Step for each channel (also, see ⇒ <i>Step</i>)
Upper Limit (Pressure)	precond.Pressure.Upper Limit	Upper pressure limit (see ⇒ <i>Pressure.Lower/UpperLimit</i>)

UVD340

Variable	Formula	Description
Average	precond.Average	Status of averaging: enabled or disabled (see ⇒ <i>Average</i>)
Bandwidth	precond.BandWidth	⇒ <i>Bandwidth</i> (for each UV channel)
Bunch Width	precond.3DFIELD.Bunch Width	⇒ <i>Bunch Width</i> of the diode bundle (3D-field only)
LampIntensity	precond.LampIntensity	⇒ <i>LampIntensity</i> [counts/s]
Max. Wavelength (3DFIELD)	precond.3DFIELD.Max Wavelength	Maximum wavelength of the ➤ <i>3D Field</i>
Min. Wavelength (3DFIELD)	precond.3DFIELD.Min Wavelength	Min. wavelength of the 3D field
Ref Wavelength	precond.RefWavelength	⇒ <i>RefWavelength (Reference Wavelength)</i> (for each UV channel)
Ref Bandwidth	precond.RefBandwidth	⇒ <i>RefBandwidth (Reference Bandwidth)</i> (for each UV channel)
Step	precond.Step	⇒ <i>Step</i>
Wavelength	precond.Wavelength	⇒ <i>Wavelength</i> (for each UV channel)

"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	
Raw Data File Path Name	chm.rawpath	See <i>➤Raw Data</i>
Number of Peaks	chm.nPeak	
Select Peak	chm.peak(<i>OPT</i>)	⇒ <i>Select Peak</i> selects a peak in the chromatogram according to input; opens the ⇒ <i>Peak Results</i> 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	
Signal Noise	chm.noise	[in mAU]
Sample Rate	chm.sig_step	
Modified?	chm.manipulated	(Yes/no)
Modification Time	chm.manip_time	Corresponding date/time
Modification Operator	chm.manip_operator	Corresponding operator - opens the ⇒ <i>User Information</i> category
XXXXX Start Value	chm.XXXXX	Start value of the signal parameter XXXXX when acquiring data.
Delay Time Value	chm.delayTimeValue	⇒ <i>Delay Time</i> between two detectors
Delay Time Detector	chm.delayTimeDetector	Name of the second detector
Average	chm.Average	⇒ <i>Average</i> (for UV channels only)
Step	chm.Step	⇒ <i>Step</i> (for UV channels only)
Wavelength	chm.Wavelength	⇒ <i>Wavelength</i> (for UV channels only)

Variable	Formula	Description
Bandwidth	chm.Bandwidth	⇒ <i>Bandwidth</i> (for UV channels only)
RefWavelength	chm.RefWavelength	See ⇒ <i>RefWavelength</i> (<i>Reference Wavelength</i>) (for UV channels only)
RefBandwidth	chm.RefBandwidth	See ⇒ <i>RefBandwidth</i> (<i>Reference Bandwidth</i>) (for UV channels only)
MassRange	chm.MassRange	Mass range of the ➤ <i>Mass Spectrometer</i> 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 ➤ <i>Filter</i> (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 contains the following variables:

Variable	Formula	Description
Minimum Area	det.minArea	⇒ <i>Minimum Peak Area</i>
Maximum Area Reject	det.maxAreaRj	⇒ <i>Maximum Area Reject</i>
Minimum Height	det.minHeight	⇒ <i>Minimum Height</i>
Maximum Height Reject	det.maxHeightRj	⇒ <i>Maximum Height Reject</i>
Maximum Peak Height	det.maxHeight	⇒ <i>Maximum Peak Height</i>
Minimum Width	det.minWidth	⇒ <i>Minimum Width</i>
Maximum Width	det.maxWidth	⇒ <i>Maximum Width</i>
Rider Threshold	det.riderMin	⇒ <i>Rider Threshold</i>
Maximum Rider Ratio	det.riderRatio	⇒ <i>Maximum Rider Ratio</i>
Rider Skimming	det.riderSkim	➤ <i>Rider</i> ⇒ <i>Skimming</i>
Peak Shoulder Threshold	det.shoulderThrshld	⇒ <i>Peak Shoulder Threshold</i>
Front Riders to Main Peaks	det.frontRiderToMain	⇒ <i>Front Riders to Main Peaks</i>
Detect Negative Peaks	det.negDetect	⇒ <i>Detect Negative Peaks</i>
Lock Baseline	det.lockBl	⇒ <i>Lock Baseline</i>
Valley to Valley	det.valval	Baseline from ⇒ <i>Valley to Valley</i>
Inhibit Integration	det.noInteg	⇒ <i>Inhibit Integration</i>
Sensitivity	det.noise	⇒ <i>Sensitivity</i>
Peak Slice	det.filter	⇒ <i>Peak Slice</i>
Fronting Sensitivity Factor	det.frontFac	⇒ <i>Fronting Sensitivity Factor</i>
Tailing Sensitivity Factor	det.tailFac	⇒ <i>Tailing Sensitivity Factor</i>
Part of a Peak Group?	det.isInGroup	See ⇒ <i>Group</i>
Peak Purity Threshold	det.ppThrshld	⇒ <i>Peak Purity Threshold</i>
Peak Purity Start Wavelength	det.ppStartWl	⇒ <i>Peak Purity Start Wavelength</i>
Peak Purity End Wavelength	det.ppEndWl	⇒ <i>Peak Purity End Wavelength</i>

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 contains the following variables:

Variable	Formula	Description
Number	peak.number	
Name	peak.name	⇒ <i>Name</i> (Peak Name)
Group	peak.group	⇒ <i>Group</i> (Peak Group)
Link Count	peak.linkCount	Number of substances assigned in one peak
Retention Time	peak.retention_time	⇒ <i>Retention Time</i> in [min]
Retention Deviation	peak.retention_deviation	Deviation in [min] or [%], depending on parameter (abs or rel)
Relative Retention Time	peak.rel_retention_time	
Retention Window Width	peak.retention_windows	Width of the Retention⇒ <i>Window</i>
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 ⇒ <i>Group</i> 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	⇒ <i>Amount</i> 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 ⇒ <i>Group</i> amount
Width	peak.width	Peak width (Parameters 0, 5, 10, 50 in % of signal height above baseline - 0 base width with tangent method)

Variable	Formula	Description
Left Width	peak.left_width	For the parameters, refer to Width
Right Width	peak.right_width	For the parameters, refer to Width
Peak Start Time	peak.start_time	In [min]
Peak Stop Time	peak.stop_time	In [min]
Signal Value at Peak Start/ Signal Value at Peak End	peak.start_value peak.stop_value	➤ <i>Signal Value at Peak Start</i> in [mAU]
Baseline Value at Peak Start/ Baseline Value at Peak End	peak.start_value_baseline peak.stop_value_baseline	➤ <i>Baseline Value at Peak Start</i> in [mAU]
Detection Code at Peak Start/ Detection Code at Peak End	peak.start_detection_code peak.stop_detection_code	➤ <i>Detection. Code at Peak Start/End</i>
Type	peak.type	Peak ⇒ <i>Type</i>
Modified	peak.modified	Modified peak?
Manually Assigned	peak.assigned	Manually assigned peak?
Resolution	peak.resolution	➤ <i>Resolution</i> (parameters EP and USP)
Asymmetry	peak.asymmetry	Peak ➤ <i>Asymmetry</i> , parameters EP and AIA
Theoretical Plates	peak.theoretical_plates	➤ <i>Theoretical Plates</i> (parameters EP and USP)
Skewness	peak.skewness	➤ <i>Skewness</i>
K'	peak.kValue	➤ <i>Capacity Factor</i>
Retention Index	peak.ri	Linear ⇒ <i>Retention Index</i>
Kovats Index	peak.ki	⇒ <i>Kovats Index</i>

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 contains the following variables:

Variable	Formula	Description
Calibration Mode	peak.calMode	⇒ <i>Calibration Mode</i>
Auto Recalibrate	peak.autoRelCal	Auto calibration On/Off
Reference Inject Volume	peak.reference_inject_volume	Reference Injection Volume
Calibration Type	peak.calibration_type	⇒ <i>Calibration Type</i>
Weights	peak.calibration_weight	➤ <i>Weights</i>
Offset (c0)	peak.offset	➤ <i>Offset c0</i>
Slope (c1)	peak.slope	➤ <i>Slope c1</i>
Curve (c2)	peak.curve	➤ <i>Curve c2</i>
RF-Value (Amount/Area)	peak.rf_value	(1/c1) (see ➤ <i>RF-Value (Amount/Area)</i>)
Number of Calibration Points	peak.nCalpoints	➤ <i>Number of Calibration Points</i>
Number of Disabled Calibr. Points	peak.nCalDisabled	➤ <i>Number of Disabled Calibration Points</i>
Variance	peak.variance	➤ <i>Variance</i>
Variance Coefficient	peak.variance_coefficient	➤ <i>Variance Coefficient</i>
Standard Deviation	peak.standard_deviation	➤ <i>Standard Deviation</i>
Relative Standard Deviation	peak.rel_standard_deviation	➤ <i>Relative Standard Deviation</i>
Correlation Coefficient	peak.correlation_coefficient	➤ <i>Correlation Coefficient</i>
Coefficient of Determination	peak.rQuadrat	➤ <i>Coefficient of Determination</i>
DOF-Adj. Coefficient of Determination	peak.rQuadratAdj	➤ <i>DOF-Adjusted Coefficient of Determination</i>
Calibration Point x	peak.calPointX	➤ <i>Calibration Point weighting</i>
Calibration Point y	peak.calPointY	
Calibration Point Weight	peak.calPointWeight	
Evaluation of Calibration Function	peak.calPointFX	
Residual for Cal. Point x	peak.calPointDist	
Calibration Point Status	peak.calPointStatus	
Upper Confidence Limit	peak.confUpperLimit	Upper limit of the ➤ <i>Confidence Interval</i>
Lower Confidence Limit	peak.confLowerLimit	Lower limit of the confidence limit

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to ⇒ *Report Categories*.

"Peak Table" Category

The **Peak Table** category contains the following variables:

Variable	Formula	Description
Number	peakTab.number	Peak number
Peak Name	peakTab.name	Peak \Rightarrow <i>Name</i>
Retention Time	peakTab.retention_time	\Rightarrow <i>Retention Time</i> in [min]
Retention Time Interpretation	peakTab.retention_type	Specification of retention times
Use Prev. Retention Time	peakTab.use_previous_retime	Status of corresponding variable in QNT File (see \Rightarrow <i>Use Recent Retention Time</i>)
Window	peakTab.window	Time \Rightarrow <i>Window</i> width
Standard Method	peakTab.standard_method	See \Rightarrow <i>Standard</i>
Integration Type	peakTab.integration_type	\Rightarrow <i>Integration Type</i>
Calibration Type	peakTab.calibration_type	\Rightarrow <i>Calibration Type</i>
Peak Type	peakTab.type	See \Rightarrow <i>Type</i>
Left Limit	peakTab.left_limit	\Rightarrow <i>Left Limit</i>)
Right Limit	peakTab.right_limit	See \Rightarrow <i>Right Limit</i>)
Peak Group	peakTab.group	See \Rightarrow <i>Group</i>
Response Factor	peakTab.response_factor	\Rightarrow <i>Response Factor</i>
Amount	peakTab.amount	\Rightarrow <i>Amount</i>
Amount Dimension	peakTab.amount_dimension	
C0 Value	peakTab.C0	Y \triangleright <i>Offset</i> of the calibration curve
C1 Value	peakTab.C1	\triangleright <i>Slope</i> of the calibration curve
C2 Value	peakTab.C2	Curvature of the calibration \triangleright <i>Curve</i>
Reference Spectrum	peakTab.reference_spectrum.x	\Rightarrow <i>Reference Spectrum</i> , opens the category \Rightarrow <i>Spectrum Data</i>
Match Criterion	peakTab.spec_compare_method	\Rightarrow <i>Match Criterion</i>
Check Derivative	peakTab.spec_derivative	\Rightarrow <i>Check Derivative</i>
Minimum Wavelength	peakTab.spec_min_wavelength	Lower limit of the compared wavelength range (see \Rightarrow <i>Minimum Wavelength</i>)

Variable	Formula	Description
Maximum Wavelength	peakTab.spec_max_wavelength	Upper limit of the compared wavelength range (see \Rightarrow <i>Maximum Wavelength</i>)
Threshold	peakTab.spec_threshold	\Rightarrow <i>Threshold</i> value for spectra search
Relative Maximum Deviation	peakTab.spec_relmaxdev	\Rightarrow <i>Relative Maximum Deviation</i> during spectra search
Check Number of rel. Extrema	peakTab.spec_check_extrema	Checking the number of extrema during spectra search (see \Rightarrow <i>Check Extrema</i>)
Comment	peakTab.comment	\Rightarrow <i>Comment</i>
Retention Index	peakTab.ri	Linear \Rightarrow <i>Retention Index</i>
Kovats Index	peakTab.ki	\Rightarrow <i>Kovats Index</i>
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	
Check of MS retention times	peakTab.ms_check_traces	
*x	peakTab.user_x	\Rightarrow <i>User-defined Column</i> (x = column name)

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *Report Categories*.

"Mass Spectrometry" Category

The **Mass Spectrometry** category contains the following variables:

Variable	Formula	Description
Background Subtraction Type	ms.spec_enhanced	Indicates the type of ➤ <i>Mass Spectra</i> 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 ➔ <i>Mass Spectrum</i> category
Instrument Information	ms.instrument	Information about the mass spectrometer
Instrument Method	ms.method	
Tune Data	ms.tune	Tune data of the ➤ <i>Xcalibur</i> 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 contains the following variables:

Variable	Formula	Description
Peak Purity Index	peak.ppi	Average \triangleright <i>PPI (Peak Purity Index)</i> 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 \triangleright <i>Peak Purity Match Factor</i> (0..1000) over the entire peak (from a specific height detection parameter)
RSD Peak Purity Match	peak.rsd_match	Corresponding \triangleright <i>Relative Standard Deviation</i>
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 \Rightarrow <i>Spectrum Data</i> category
Reference Spectrum Match	peak.refMatch	(See \triangleright <i>Match Factor</i>)
Number of SLS Hits	peak.nSlsHits	No. of reference spectra
SLS Hit	hitSpec.x	Opens the \Rightarrow <i>Hit Spectrum</i> category
Mass of n-th MS peak	peak.ms_peak_mass(n)	
Intensity of n-th MS peak	peak.ms_peak_intens(n,"peak1")	
Position of mass peak maximum	peak.ms_peak_maximum(n,"retTime")	
MS Peak Purity Match	peak.ms_match(x)	Averaged MS \triangleright <i>Peak Purity Match Factor</i> (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 \Rightarrow <i>Mass Spectrum</i> category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *Report Categories*.

"Quantification Method" Category

The **Quantification Method** category is only available in Summary tables and in individual ➤ *Printer Layout* cells. It contains 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	
Creation Operator	qnt.creation_operator.x	Opens the ⇒ <i>User Information</i> category
Last Update Date & Time	qnt.update_time	
Last Update Operator	qnt.update_operator	Opens the User Information category
Parent Sequence Name	qnt.seq_name	
Sequence Header Record	qnt.sequence.x	Opens the ⇒ <i>Sequence</i> category
Dead Time	qnt.deadTime	⇒ <i>Dead Time</i>
Delay Time Value	qnt.delayTimeValue	Also, refer to ⇒ <i>Delay Time</i>
Delay Time Detector	qnt.delayTimeDetector	Corresponding detector
Blank Run Subtraction	qnt.blankRun Mode	Status of ➤ <i>Blank Run Subtraction</i>
Blank Run Sample Record	qnt.blankRunSample.x	Opens the ⇒ <i>Sample</i> category
Number of Detection Parameters	qnt.nDet	
Number of Peaks in the Peak Table	qnt.nPeaks	
Select Peak in the Peak Table	qnt.peakTabLine("OPT")	⇒ <i>Select Peak in the Peak Table</i> selects peak in chromatogram according to the included option - opens the ⇒ <i>Peak Table</i> category
SST Result	qnt.sst-result	Total result of all single ➤ <i>System Suitability Tests (SST)</i>
SST Rows	qnt.sst_rows	Number of lines (= number of single System Suitability Tests) on the SST page in the QNT Editor

Variable	Formula	Description
Select SST	qnt.sst(n.)x	Opens the category: \Rightarrow 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 \Rightarrow <i>Spectra Library Screening</i> category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *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 contains 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	qnt.test_condition	
Aggregate Condition	qnt.aggregate	Aggregate function
Operator	qnt.operator	
Value	qnt.value	
Channel	qnt.channel	
Peak Condition	qnt.peak	
N.A.	qnt.n_a	User defined test result if the condition cannot be evaluated.
Fail Action	sst.fail_action	
Aggregated Samples	sst.agg_number_real	
Sample Condition Result	sst.sample_cond_result	
Test Results	sst.results	
List of Aggregated Samples	sst.agg_list	
Result List for Aggr. Samples	sst.agg_list_results	
Results of Test Condition or Aggregate	sst.test_cond_result	
Result of Compare Value	sst.value_result	

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 contains the following variables:

Variable	Formula	Description
Object Name	history.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 ➤ <i>Time</i>) of the modification.
Operator	history.operator	User - opens the ⇒ <i>User Information</i> category.
Operation	history.operation	Short description of the modification.
Comment	history.comment	
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 contains 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 contains the following variables:

Variable	Formula	Description
Name	pgm.name	Program name
Path	pgm.path	Directory where the ➤ <i>Program</i> is saved.
Title	pgm.title	Program title
Timebase	pgm.timebase	Name of the ➤ <i>Timebase</i> to which the program belongs.
Creation Time	pgm.creation_time	Creation date/time (see ➤ <i>Time</i>)
Creation Operator	pgm.creation_operator	Opens the ⇒ <i>User Information</i>
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 contains 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	pgm.dsn	Datasource where the report definition file (RDF) is saved.
Sheets	pgm.Sheets	Number of the sheets of the report definition file (RDF)
Current Sheet Number	pgm.CurSheetNo	The current sheet number.
Current Sheet Name	pgm. 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.

For an overview of the different report variable categories, including links to lists of variables for each category, refer to [⇒ Report Categories](#).

"Fraction" Category

The Fraction category provides different variables indicating how the fractions have been collected.



Note:

It is not possible to open the Fraction category from a Summary report. In the Printer Layout, the Fraction category is available only for report tables.

The Fraction category contains the following variables:

Variable	Formula	Description
Fraction Number	frac.number	
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	
Select Tube	frac.tube("OPT").x	Displays the selected fraction tube - opens the \Rightarrow <i>Fraction Tube</i> category
Collected Volume	frac.volume	Volume collected in the fraction
Channel Parameter	frac.channel.x	Displays the channel parameter selected for the fraction - opens the \Rightarrow <i>Fraction Detection Parameter</i> category.
Number of Peaks	frac.nPeaks("OPT")	Number of all peaks within the fraction
\Rightarrow <i>Select Peak</i>	frac.peak("OPT").x	Selects the peak from the fraction based on the selected parameter - opens the \Rightarrow <i>Peak Results</i> category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *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 \Rightarrow *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:

It is not possible to open the Fraction Tube category from a Summary report. In the Printer Layout, the Fraction Tube category is available only for report tables.

The Fraction Tube category contains 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
\Rightarrow <i>Select Peak</i>	tube.peak("OPT").x	Selects the peak from the tube based on the selected parameter - opens the \Rightarrow <i>Peak Results</i> category

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *Report Categories*.

"Fraction Detection Parameter" 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 \Rightarrow *Fraction* category.



Note:

It is not possible to open the Fraction Detection Parameter category from a Summary report. 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
Channel Name	frac.channel.name	Name of the channel used for peak detection.
Detection Channel	frac.channel.channel	Channel 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 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 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.
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.

Variable	Formula	Description
Baseline Drift	frac.baseline_drift	Start value for the baseline drift. This value is used to correct the signal value.
Baseline Offset	frac.baseline_offset	Start value for the baseline offset. This value is used to correct the signal value.

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 contains the following variables:

Variable	Formula	Description
Spectra Library	sls.library	➤ <i>Spectra Library</i>
Spectra Library	sls.library	
Match Criterion	sls.match	⇒ <i>Match Criterion</i>
Hit Threshold	sls.threshold	See ⇒ <i>Threshold</i>
Spectrum Derivative	sls.derivative	See ⇒ <i>Check Derivative</i>
Wavelength Range Minimum	sls.wavelength_min	See ⇒ <i>Maximum/Minimum Wavelength</i>
Wavelength Range Maximum	sls.wavelength_max	
Max. Allowed Deviation	sls.max_deviation	⇒ <i>Relative Maximum Deviation</i>
Max. Retention Time Deviation	sls.max_ret_deviation	See ⇒ <i>Retention Time</i>
Check Relative Extrema	sls.rel_extrema	See ⇒ <i>Check Extrema</i>
Max. Retention Index Deviation	sls.max_ret_idx_dev	See ⇒ <i>Retention Index</i>
Max. Kovats Index Deviation	sls.max_kov_idx_dev	See ⇒ <i>Kovats Index</i>
Spectra Library Restriction	sls.restriction	
Spectra Library Restriction Count	sls.restriction_count	

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 \Rightarrow *Peak Table* category. The Spectrum Data category contains 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	
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 \Rightarrow *Report Categories*.

"Mass Spectrum" Category

Open the **Mass Spectrum** category by selecting the **Select Spectrum** variable of the \Rightarrow *Mass Spectrometry* category. The Mass Spectrum category contains the following variables:

Variable	Formula	Description
Number of Data Points	mspec.mass_count	
Mass	mspec.mass	Mass [amu]
Intensity	mspec.intensity	Intensity [counts]
Relative Intensity	mspec.rel_intensity	Relative Intensity (%)
Minimal Mass	mspec.mass_min	Minimal mass [amu]
Maximal Mass	mspec.mass_max	Maximal mass [amu]
Mass Range	mspec.mass_range	Mass range [amu]
Spectrum Type	mspec.type	Spectra type (centroid/profile)
Resolution	mspec.resolution	Resolution of the \triangleright <i>Mass Spectrum</i>
Total Ion Current	mspec.TIC	Number of \triangleright <i>TIC</i> channels

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *Report Categories*.

"Hit Spectrum" Category

Open the **Hit Spectrum** category by selecting the **SLS Hit** variable of the \Rightarrow *Peak Purity and Identification* category. The Hit Spectrum category contains the following variables for the matching reference spectrum:

Variable	Formula	Description
Substance Name	hitSpec.name	
Match Factor	hitSpec.match	\triangleright <i>Match Factor</i>
Library Name	hitSpec.lib_name	Spectra library name
Library Record	hitSpec.specLib.x	Opens the \Rightarrow <i>Spectra Library</i> category
Number of rel Extrema	hitSpec.nExtrema	
Solvents	hitSpec.solvent	
Comment	hitSpec.comment	\triangleright <i>Comment</i>
Sequence Name	hitSpec.seq_name	
Sequence Header Record	hitSpec. sequence.x	Opens the \Rightarrow <i>Sample</i> category
Sample Name	hitSpec.smp_name	
Sample Record	hitSpec.sample.x	Opens the \Rightarrow <i>Sample</i> category
Acquisition Time	hitSpec.acqTime	
Timebase	hitSpec.timebase	\triangleright <i>Timebase</i>
Program	hitSpec.program	\triangleright <i>PGM File</i> used for recording the library spectrum.
Sample Rate	hitSpec.acqStep	
Retention Time	hitSpec.retention_time	\Rightarrow <i>Retention Time</i>
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	
Detector Serial No.	hitSpec.detectorSerNo	
Extract Time	hitSpec.extrTime	
Extract Operator	hitSpec.operator	Opens the \Rightarrow <i>User Information</i> category
Retention Index (lin)	hitSpec.ri	Linear \Rightarrow <i>Retention Index</i>
Kovats Index	hitSpec.ri	\Rightarrow <i>Kovats Index</i>

"Spectra Library" Category

Open the **Spectra Library** category by selecting the **Library Record** variable of the \Rightarrow *Hit Spectrum* category. The Spectra Library category contains 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	
Creation Date & Time	specLib.creation_time	(See \Rightarrow <i>Time</i>)
Creation Operator	specLib.creation_operator	Opens the \Rightarrow <i>User Information</i> category
Creation Software Version	specLib.creation_version	
Last Update Date & Time	specLib.update_time	
Last Update Operator	specLib.update_operator	Opens the User Information category
Last Update Software Version	specLib.update_version	
Retention Index (lin)	specLib.ri	Linear \Rightarrow <i>Retention Index</i>
Kovats Index (log)	specLib.ki	\Rightarrow <i>Kovats Index</i>

For an overview of the different report variable categories, including links to the lists for the respective categories, refer to \Rightarrow *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 \Rightarrow *Sequence* category. The User Information category contains 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 \Rightarrow *Report Categories*.

"Select Peak" Options

Use the **Select Peak** variable of the \Rightarrow *Chromatogram* category to select a peak in the chromatogram. The Select Peak variable opens the \Rightarrow *Peak Results* category. Enter the desired option in the **Formula...** field.



Note:

*Instead of using the **Formula...** field, you may as well click the **Parameter** button and select the desired option from the drop-down Select Peak 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 \Rightarrow *Group* (Peak Group) called CKW and then determines its \Rightarrow *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 \Rightarrow *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 \Rightarrow *Quantification Method* category to select a peak in the peak table of the QNT Editor. This variable opens the \Rightarrow *Peak Table* category. Enter the desired option in the **Formula...** field.



Note:

*Instead of using the **Formula...** field, you may as well click the **Parameter** button and select the desired option from the drop-down **Select Peak** 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 \triangleright <i>Reference Peak</i> (\triangleright <i>Retention Time Interpretation as Time Distance to ... or Time Ratio to...</i>) Example: qnt.peakTabLine ("Ref").name returns the name of the reference peak. |

Glossary

%B, %C, %D (Solvent Components)

The solvent and thus the \Rightarrow Flow usually consist of different individual components. The respective amounts of the single partial flows (solvent components) are indicated in percent of the flow. The total sum of all partial flows is 100% ($\%A + \%B + \%C + \%D = 100\%$), where %A is calculated from the remaining partial flows ($\%A = 100\% - (\%B + \%C + \%D)$). It is therefore sufficient to determine the values for %B, %C, and %D.

Changing the solvent composition during the analysis is referred to as \triangleright *%-Gradient*.

Also, refer to \Rightarrow %B, %C, %D (Solvent Components).

%-Gradient


See \triangleright (%-)Gradient.

3D Field

A \triangleright 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 \triangleright 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 \triangleright Sampling Rate, the detector's \triangleright 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)* partial method is available for this.

3D Field: Presentation Modes

The ➤ *3D Field* represents the relation between the retention time, wavelength, and absorption. For presenting these three-dimensional figures, two presentation modes have proved to be useful: the ISO plot and the 3D plot presentation. Use the **Decoration** dialog box of the ➤ *PPA (Peak-Purity Analysis)* window 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 the **Administrator Manual: Software Installation and Communication**  **Access Control**.)

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 a user account shall be locked 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 information about the significance of the individual formulas, refer to the Chromeleon online Help.

For an application example, refer to **How to ... Actions in the Printer Layout**  **Entering User-defined Formulas.**

For an overview of the available functions, refer to the following list:

ABS	EXACT	MATCH	SIGN
ACOS	EXP	MAX	SIN
ACOSH	FACT	MID	SINH
ADDRESS	FALSE	MIDB	SQRT
AND	FIND	MIN	STDEV
ASC	FINDB	MINUTE	STDEVP
ASIN	FIXED	MOD	SUBSTITUTE
ASINH	FLOOR	MONTH	SUM
ATAN	HLOOKUP	N	SUMIF
ATAN2	HOUR	NA	SUMSQ
ATANH	IF	NOT	T
AVERAGE	INDEX	NOW	TAN
CEILING	INDIRECT	ODD	TANH
CHAR	INT	OFFSET	TEXT
CHOOSE	ISBLANK	OR	TIME
CLEAN	ISERR	PI	TIMEVALUE
CODE	ISERROR	PRODUCT	TODAY
COLUMN	ISLOGICAL	PROPER	RIM
COLUMNS	ISNA	RAND	TRUE
CONCATENATE	ISNONTEXT	REPLACE	TRUNC
COS	SNUMBER	REPLACEB	TYPE
COSH	ISREF	REPT	UPPER

COUNT	ISTEXT	RIGHT	VALUE
COUNTA	LEFT	RIGHTB	VAR
COUNTIF	LEFTB	ROUND	VARP
DATE	LEN	ROUNDDOWN	VLOOKUP
DATEVALUE	LENB	ROUNDUP	WEEKDAY
DAY	LN	ROW	YEAR
DAYS360	LOG	ROWS	
DBCS	LOG10	SEARCH	
ERROR.TYPE	LOOKUP	SEARCHB	
EVEN	LOWER	SECOND	

AIA

AIA is the abbreviation for **Analytical Instrument 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 export format for chromatography data is known as **ANDI (ANalytical Data Interchange/Chromatography)**.

Since 1992, there are five data format categories (Category 1, ... 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*.

Also, refer to ⇒ *Amount*

Amount Deviation

The **Amount Deviation** peak table variable specifies the difference between the nominal \Rightarrow *Amount* included in the **Amount** column of the peak table and the value actually determined by area calculation (actual amount):

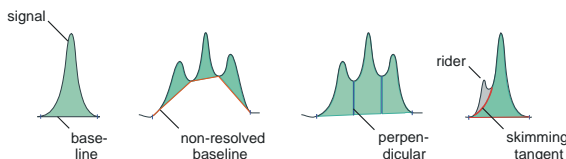
$$\text{Amount Deviation} = \text{Actual Amount} - \text{Nominal Amount}$$

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.

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

$$\text{Area} = (T2-T1)*((A1+A2)/2).$$

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

$$\text{Area} = (B_T2-B_T1)*((B_A1+B_A2)/2).$$

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 for peak fronting or tailing. The peak asymmetry helps evaluating the 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.

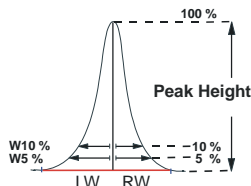
The parameter has no dimension and is defined differently, depending on whether using the USP, EP, or AIA standard:

Asymmetry

Calculation

USP/EP standard

$$A = \frac{RW_{5\%} + LW_{5\%}}{2 * LW_{5\%}}$$



AIA standard

$$A = \frac{RW_{10\%}}{LW_{10\%}}$$

With:

➤ *Right* and ➤ *Left* peak width in 5% and 10% of the peak height.

RW5% LW5%

RW10%, LW10%

For ideal peaks, asymmetry is $A = 1$. However, for real 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 in 10% of the peak height, this value is referred to as ➤ *Skewness*.

Select the column in the report, select **Column Properties** on the context menu, and then select the **Asymmetry** variable from the **Variables** list. Click the **Parameter...** button. In the **Parameter Input for 'Peak Asymmetry'** dialog box, specify whether the calculation is based on the USP/EP or AIA standard.



Note:

EP = European Pharmacopeia

USP = United States Pharmacopeia

Atlas Electrolytic Suppressor (AES)

See ➤ *Suppressor*

Audit Trail

The Audit Trail is a daily protocol that is kept independently by the system. It contains all ➤ *GLP* (Good Laboratory Practice) relevant operations, such as current and future commands, events, 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 the **Auto Autoscale** option to automate the performance of the ➤ *Autoscale* function.


During data acquisition, the **Auto Autoscale** option 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.

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
- **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 automating routine analysis. Depending on the autosampler type and model, a number of samples can be prepared and then processed (injected) in a ➤ *Batch* (automatic batch processing).

Distinguish loading the sample and injecting the sample.

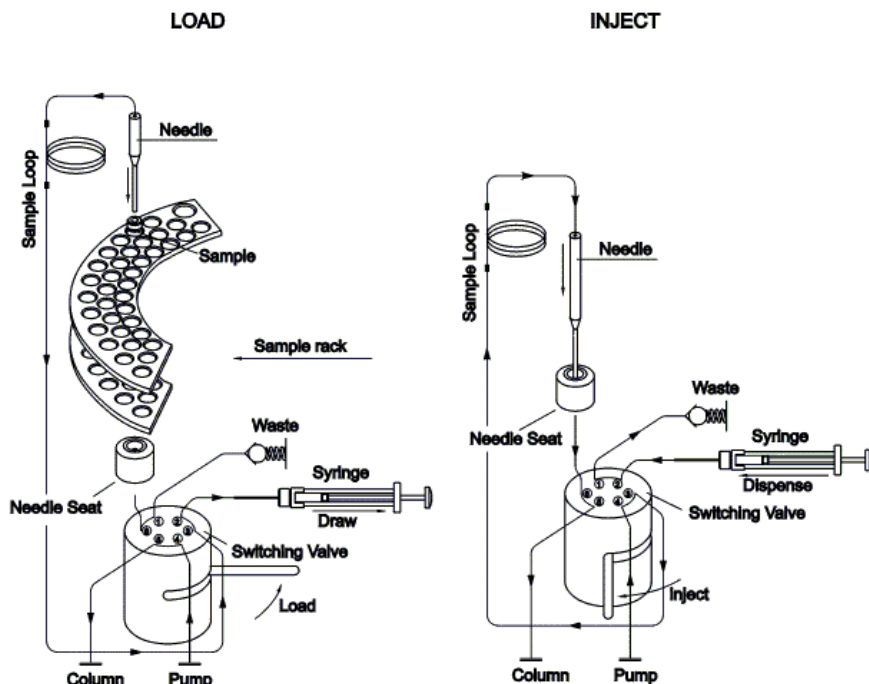
Loading the Sample

The fluid input and output are directly connected. The sample is transported from the pump through the autosampler 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 (AS50). The injection valve is switched, directing the solvent flow through the sample loop and the needle port or inject port to the column. Residues in the syringe are dispensed into the waste.

Example for a load/inject process (here: the Dionex ASI-100 autosampler):



The Dionex Gina 50, ASI-100, and AS50 autosamplers function on this basis. Apart from the standard model, 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 the ➤ *Auto Autoscale* option 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 100Hz. 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 ...: Actions in the Chromatogram**




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 ...: Actions in the Browser**  **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, diskettes 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 ...: Actions in the Browser**  **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.

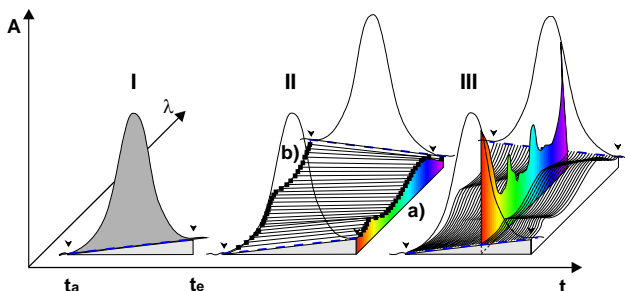
Baseline Correction

Automatic correction a spectrum by a calculated baseline spectrum allows you to compare single spectra even under different conditions. In this way, especially the peak spectra on a gradient or the spectra of ➤ *Rider Peaks* can be represented more precisely because a large "underground portion" must usually be considered, which is due to the gradient portion or the larger main peak.

When the spectra plot (**Show Spectra**) is displayed, it is always corrected by the baseline spectrum. If you enable the **BL Correction** option 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 (t_a and t_e). The ➤ *Baseline (I)* is drawn between the two delimiters.



This is performed for all detected wavelengths. The absorption values at all wavelengths measured at the time t_a and t_e 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 only be performed 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.

In principle, the PPA report is baseline corrected.

If you add a spectrum from the spectra plot to the spectra library using the Windows clipboard, it will always be baseline-corrected.

*If you add a spectrum from the PPA method to the spectra library using the Windows clipboard, it 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.) The **BL Correction** option only influences the representation of the PPA view.*

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 ...: Actions in the QNT Editor**  **Modifying Detection Parameters.**

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 always 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 chromatogram for which only the solvent absorption (= baseline) is recorded but no sample is injected is referred to as a **blank run**.

In the sample list, blank samples are labeled **Blank** and marked by the following symbol . The optional **Blank** parameter of the ➤ *Inject* command forces an injection for blank run samples if the **Inject** option is selected. Usually no injection is performed for these samples (default: **Skip**). Apart from this, blank samples are treated in the ➤ *Program* like normal integration samples.

Blank samples are stored together with the sequence. After a baseline or ➤ *Blank Run Subtraction* has been performed, it can be undone at any time.

Also, refer to ➤ *Matrix Blank Sample*

Blank Run Subtraction

If the ➤ *Raw Data* of a signal (channel or 3D field) is corrected by the raw data of a comparable ➤ *Blank Run* sample, this is referred to as blank run subtraction. 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 peak detection is disturbed due to a strongly ascending chromatogram; for example, gradient elution.

As 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, use this procedure in exceptions only.

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 desired 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 the **Properties...** option 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*.

c0

See ➤ *Offset c0*

c1

See ➤ *Slope c1*

c2


See ➤ *Curve c2*

Calibration Functions, Calibration Coefficients


In each calibration, a mathematical ratio 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 ratio 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)	$f(A) = c1 \times A$	min. 1 calib. point
Linear with offset (LOff)	$f(A) = c0 + c1 \times A$	min. 2 calib. points
Quadratic (Quad)	$f(A) = c1 \times A + c2 \times A^2$	min. 2 calib. points
Quadratic with offset (QOff)	$f(A) = c0 + c1 \times A + c2 \times A^2$	min. 3 calib. points
Exponential (Exp)	$f(A) = c0 \times A^{c1}$	min. 2 calib. points

The value of the corresponding calibration coefficient (c_0 , c_1 , or c_2) 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 c_0 , c_1 , and c_2 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 \Rightarrow *QNT Editor* (see **The QNT Editor**  **The General Tab Page**).

Also, refer to \Rightarrow *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 calibration point.

If the corresponding peak area is determined several times for the same \Rightarrow *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 \triangleright *Dilution Series* or by varying the injection volume.

Based on the calibration points, data system calculates the calibration coefficients of the \triangleright *Calibration Function* selected by the user.

For more information, refer to **Theory of Calibration:**  **Calibration (Overview)** and  **Calibration Principle** and **How to ...: Actions in the QNT Editor/Creating a Peak Table**  **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 and c_2 calibration coefficients. Also, refer to ➤*Calibration Type*

For more information about the calibration types, refer to **Theory of Calibration**:



Calibration Types (Linear)



Calibration Types (Non-linear)

Also, refer to **How to ...: Actions in the QNT Editor**



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 c_2*

➤*Number of Calibration Points*

➤*Number of Disabled Calibration Points*

➤*Offset c_0*

➤*Relative Standard Deviation*

➤*RF Value*

➤*Slope c_1*

➤*Standard Deviation*

➤*Variance Coefficient*

➤*Variance*

➤*Weights*

Capacity Factor (k')

The k' peak result variable refers to the capacity factor, that is, to the ratio of the net retention time to the \Rightarrow 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 \Rightarrow QNT Editor (see **The QNT Editor**  **The General Tab Page**).

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 the **Parameter...** button 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 quickly change to the desired window.

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:

- 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:

- Do all partial flows sum up to 100 %?
- Does the program contain an \Rightarrow *Inject* command at the time $t = 0.000$?
- Are the **Inject** \Rightarrow *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 \Rightarrow *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 automatically performed for each online command. If a problem is detected, an error message or warning is displayed.

The semantics check usually includes the syntax check. The ready check usually includes the semantics check.

Check Derivative

See ⇒ *Check Derivative*

Chemiluminescence

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, too, 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
➤ <i>Program</i> (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*


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 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:

$$R^2 = 1 - \frac{\sum_{i=1}^N W_i * (Y_i - F(X_i))^2}{\sum_{i=1}^N W_i * (Y_i - \bar{Y})^2}$$

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
	\bar{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 ...: Actions on 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. It depends on the connected column thermostat whether this is possible.

Comment

Chromeleon allows you to enter a comment at different places.

- In the \Rightarrow *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 \Rightarrow *QNT Editor*, enter any comments regarding the sample.
- Comments are often needed in a \Rightarrow *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 \Rightarrow *Datasource Properties* (select **Properties** on the context menu), specify that all modifications be logged in the \Rightarrow *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 \Rightarrow *Report Categories* in the Report and in the \Rightarrow *Printer Layout*.

Concentration

The **Concentration** peak result variable describes the quotient of the calculated \Rightarrow Amount and the injection \Rightarrow 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 $\mu\text{g}/\mu\text{l}$.

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 $\mu\text{g}/20 \mu\text{l} = 0.75 \mu\text{g per } \mu\text{l}$.

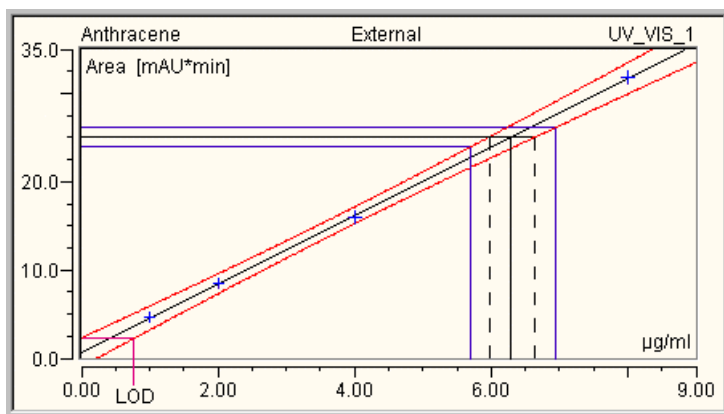
Confidence Interval/Confidence Range

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 is given 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). Besides, 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 ...: Actions in the Calibration Curve**  **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 ...: Actions in the Report Table**  **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**, you 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 automatically executed 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 shows 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 control and display elements that are combined in one window are referred to as the **Control Panel**. The control and display elements allow you to control and/or monitor a ➤ *Timebase*. The timebase that can be controlled is assigned when the control panel is created. Select **Connect to Timebase** on the **Control** menu to change the assignment. If you close the window, you can save the new assignment.

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).

Also, refer to **How to ...:**  **Actions on the Control Panel**.

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 $\pm 100\%$). 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:

$$\text{CorrCoeff} = \frac{\sum_{i=1}^N W_i * (X_i - \bar{X}) * (Y_i - \bar{Y})}{\sqrt{\left(\sum_{i=1}^N W_i * (X_i - \bar{X})^2 \right) * \left(\sum_{i=1}^N W_i * (Y_i - \bar{Y})^2 \right)}}$$

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
	\bar{X}	Average value of all x-values
	Y_i	Y-value of the standard sample no. i
	\bar{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.

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 **How to ...: 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.


This value is available only for the **Quadratic** and **Quadratic with Offset** functions.

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 the **Administrator Manual: Installation Instructions**  **Installing the 12-Bit DAC Card ("Pump DA Converter")**.)

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 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 to process than necessary.

In addition to the data collection rate, a step value is set. The default step value is the reciprocal value of the data collection rate. For example, if you select a data collection rate of 5 Hz, the step value is automatically set to 0.2 s. In general, we recommend using the automatically selected step value (see \Rightarrow *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:**

*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 want to use a different step.*

Datasource

A database that is mounted to the Chromeleon \triangleright *Client* is referred to as a datasource. To mount a datasource, select **Mount Datasource** on the **File** menu in the \triangleright *Browser* and then specify the format and the location of the database. For more information, refer to the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Setting up a Datasource**.

During the initial installation of Chromeleon, a local \triangleright *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 \triangleright *ODBC Capability* of Chromeleon allows you to use various other formats (dBASE, SQL, Oracle, etc.), as well. Both \triangleright *Sample Data* and \triangleright *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 \triangleright *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, 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.

For more information, refer to the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System:**



Setting up a Network Datasource




Saving Chromatography Data on the Network

Chromeleon also allows you to lock datasources, directories, or sequences. 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 the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Network Failure/Non-Availability**



Note:

*"Old" GynkoSoft data has the required database structure. That is why Chromeleon is capable of handling this data as it they were "real" Chromeleon data. Simply connect to the data stored under **Drive ...***

Data Smoothing

See ➤ *Smoothing*

DC Mode

DC Mode (direct current mode) is the basic operating mode of an electrochemical detector.

When you apply a fixed potential between reference and working electrode, you can observe a proportional current, depending on the concentration of an oxidable or reducible substance.

For optimum results, adjust the potential to the substance to be detected. This is performed in ➤ *SCAN Mode*.

If modifications occur during the analysis, for example, due to separating processes, the detector should be operated in ➤ *PULSE Mode*.

DC Voltage

The **DC Voltage** command for the 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 \Rightarrow *Dead Time* and the \triangleright *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

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 \Rightarrow *Delay* command specifies the time to pause before executing the next command(s). The **Delay** command is often used for \Rightarrow *Trigger* commands.

The 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 needed by a substance to travel from one position in the chromatography system to another is referred to as delay time.

On the **General** tab page of the \Rightarrow QNT Editor (see **The QNT Editor**  **The General Tab Page**), enter the \Rightarrow Delay Time needed by a substance to travel from the detector cell of the first detector to the detector cell of a second detector.

Use the **Fraction Collection Options** page of the Program Wizard (see **Control**  **The Program Wizard**) to enter the delay time between the detector and the \triangleright Fraction Collector. The Wizard only displays this page if you installed the Fraction Collection driver in the \triangleright Server Configuration program. If you additionally use several detectors, use the **Fraction Collection - Channel Selection Options** page to enter the delay time(s) between the detectors.




Tip:

It only makes sense to enter a fixed delay time when 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 \Rightarrow Delta signal property indicates the signal slope in a second.

This property is especially useful when creating \Rightarrow 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 **How to ...: 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 \triangleright Server Configuration program. With most \triangleright 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 ...: Actions on 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 ...: Actions in the QNT Editor**  **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 \Rightarrow *Peak Type* classification of Chromeleon. The classification described below complies with the \succ *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 user's PC instructions (via mouse click) 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 they can be read by Chromeleon and displayed 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. The extension **CDD** stands for Chromatographic Device Driver. For example, the device driver for the \succ *UCI Universal Chromatography Interface* is called **Uci100.cdd** and the device driver for the Dionex P580 pump is **P580RevA.cdd**.

During the Chromeleon installation, all device drivers are saved to the BIN directory and assigned a version ID. Select **Properties...** on the context menu, for example, in the Explorer, to display the ID.

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 the **Administrator Manual: How to ...: Actions in the Server Configuration Program**  **Controlling Devices without a Separate Device Driver**.) In special cases, you can use the generic device driver to create a user-defined device driver. (For more information, refer to the **Administrator Manual: Installation Instructions**  **The Generic Device Driver**.)

Digital Input

See ➤ *Remote Input*

Dilution Factor

The **Dilution Factor** \Rightarrow *Sample Variable* is a correction factor for diluting consecutive calibration samples.

Also, refer to \Rightarrow *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:

$$Amount = Am_{(Peak-Tab.)} * \frac{Inj.Vol_{unknownAmount}}{Inj.Vol_{knownAmount}} * \frac{Smp.Wght}{Dil.Factor}$$

**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 \Rightarrow Amount value (column 1) for each substance contained in the standard.

Dimension of Amounts

This peak table parameter defines the physical dimension of the \Rightarrow Amount values, i.e., either amount or concentration.

Also, refer to \Rightarrow Dimension of Amounts

Diode Array Detector

See \triangleright Photodiode Array Detector

Disconnect

Execute the **Disconnect** command to separate a user PC (client) from a \triangleright Timebase or to operate an instrument locally. The specified instrument is no longer controlled by Chromeleon nor can it be operated by Chromeleon. The instrument keyboard lock of the **Connect** command is no longer valid.

**Note:**

Select **Connect** to undo the operation.


Also, refer to \Rightarrow Connect/Disconnect

Dispense

The **Dispense** command causes the \triangleright Autosampler to dispense a specific quantity (volume) from the sample loop into a certain sample vial (\Rightarrow Position). The \triangleright Duration parameter determines the time that the autosampler may take for this operation.

After the operation has been completed, the autosampler returns the **Sampler.Ready** signal (for an ASI-100 Autosampler) to Chromeleon. The time between the **Dispense** command and the **Sampler.Ready** response signal can vary, depending on the instrument.

**Note:**

For AS50 autosamplers, the **Dispense** command is available only if the autosampler is equipped with the sample preparation option. The AS50 **Dispense** command dispenses reagent from a reservoir into a specified vial. See **How to...: Control: Autosamplers**  **Defining Sample Preparation Steps** for details.

Also, refer to \Rightarrow *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:

$$r_{DOF-adj}^2 = 1 - \frac{(N-1) * \sum_{i=1}^N W_i * (Y_i - F(X_i))^2}{(N-m-1) * \sum_{i=1}^N W_i * (Y_i - \bar{Y})^2}$$

With:	N	Number of standard samples involved in the calibration
	m	Number of degrees of freedom (= coefficients to be determined according to the \Rightarrow <i>Calibration Type</i> : 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
	\bar{Y}	Average of all Y-values.

Also, refer to \triangleright *Coefficient of Determination*

Dongle

A dongle is a software license device that is placed on the USB or parallel port (= LPT) of the PC. The free end of the dongle that can be placed on the LPT port is designed as a parallel interface connector, too.

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 the 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 Alladin Hardlock software does not provide a user interface. As it is not possible for you to make any settings, information about this software is not provided.

For more information, refer to the **Administrator Manual: Software Installation and Communication**  **The Software License and Installation Instructions**  **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*.

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 ...: Actions on 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 \Rightarrow *Relay On/Off* commands of the R1 relay.


Autosampler

The **Duration** parameter indicates the minimum time that the \rhd *Autosampler* may take to perform \Rightarrow *Draw* or \Rightarrow *Dispense* operations. To prevent evaporation of the sample load, do not draw or dispense more than 10 μ l 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 \Rightarrow *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. (Refer to the **Administrator Manual: Software Installation and Communication**  **The DX-LAN** for more information.)

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 \rhd *Raw Data*. This is an important aspect for quality assurance and \rhd *GLP*. When \rhd *User Mode* is enabled, you can sign and protect \rhd *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 ...: Actions in the Browser**  **Signing Sequences Electronically** and to the **Administrator Manual:  Chromeleon User Management.**

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).

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.

Chromeleon provides the following eluent generator control functions:

- Control of the generated eluent concentration (from 0.0 to 100 mM)
- Monitoring of the ions remaining in the EluGen cartridge
- Monitoring of the EluGen cartridge expiration date

Also, refer to: **How to ...: 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 ...: Actions on 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 differentiates between three types of errors:

- Fatal Error (**Abort** - The batch aborts.)
- Serious Error (**Error** - The batch continues and the error is corrected as far as possible.)
- Error (**Warning** - The batch continues and a warning appears.)
- Minor Error (**Ignore** - The batch continues. The error is only logged in the audit trail.)

An emergency program can be defined for the first type of error. In this case, the batch does not abort but the emergency program starts automatically instead.

The retention time continues while the emergency program is active; the times are interpreted as offset. Data acquisition and audit trail will continue as well. After the emergency program has run, an **End of Sample** entry is made in the audit trail.

For emergency program examples, refer to **How to ...: Actions in the PGM Editor**  **Creating 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.*

For more information, refer to the **Administrator Manual: Software Installation and Communication**  **The Software License** and **Installation Instructions**  **Entering the Software License**.

 **Note:** *Do not confuse the Evaluation Mode with the ➤Demo Mode of the respective instruments!*

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. Select the highest absorption maximum for which the wavelength is below the emission wavelength as the excitation wavelength. This is to allow the substance to be analyzed absorbing as much as possible of the irradiated energy. 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:

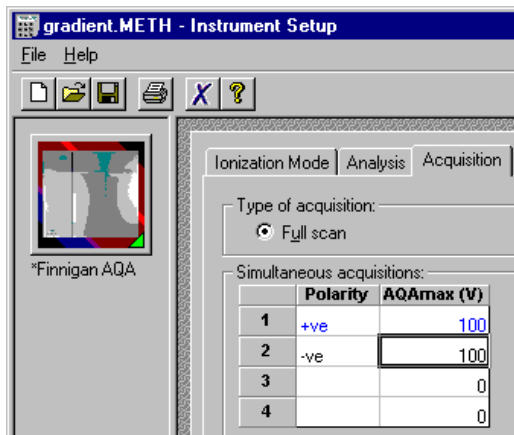
- Moving Average (Boxcar)
- Olympic
- Savitzky-Golay
- Gaussian


Data Display Filters

1. **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):




2. **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 only displayed 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.)

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 \Rightarrow Flow rate in a chromatogram are referred to as a flow gradient. This type of \triangleright Gradient is quite unusual in liquid chromatography. However, compared to the ordinary \triangleright %-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 (\triangleright Ramps) or immediately (\triangleright 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 $\Rightarrow\%B$, $\%C$, $\%D$ (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 \triangleright 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 \Rightarrow 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 higher 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 (S_0) and terminates in different excited vibrational levels of the next higher singlet state (S_1).

$$S_0 + h\nu_A \rightarrow S_1$$

where

h = Planck's constant

$$\nu_A = \text{light frequency at absorption} = \frac{c}{\lambda_A}$$

where:

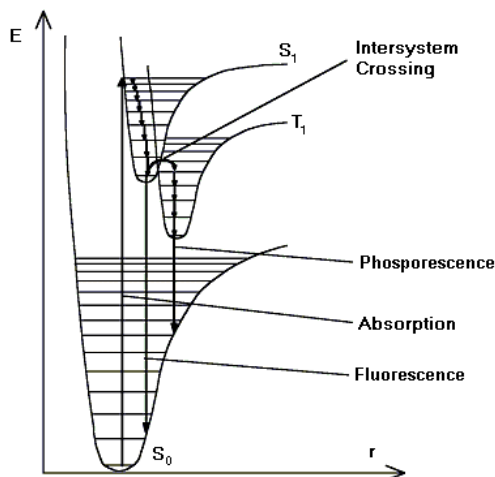
c = speed of light

λ = wavelength

Higher vibrational levels of S_1 undergo radiationless transitions to the lowest vibrational level of S_1 . Finally, fluorescence originates in the lowest vibrational level of S_1 and terminates in different excited vibrational levels of S_0 :

$$S_1 \rightarrow S_0 + h\nu_F$$

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., $S_0 \rightarrow S_2$), 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.

$$Amount_p = f_p(y_p) * Resp.Fact._p * ISTD\ Factor * \frac{Dil.Factor_n}{Weight_n}$$

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 ...: Actions in the Report Table  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.
\Rightarrow 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 \gg *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 ...: Actions in the Report Table  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.
\Rightarrow 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 ...:**  **Actions Related to Fraction Collection.**

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 ...: Actions in the QNT Editor**  **Defining Detection Parameters.**

Fronting Sensitivity Factor

See ➤ *Tailing Sensitivity Factor*.

Full-Loop Injections (AS50 Sample Prep Command)

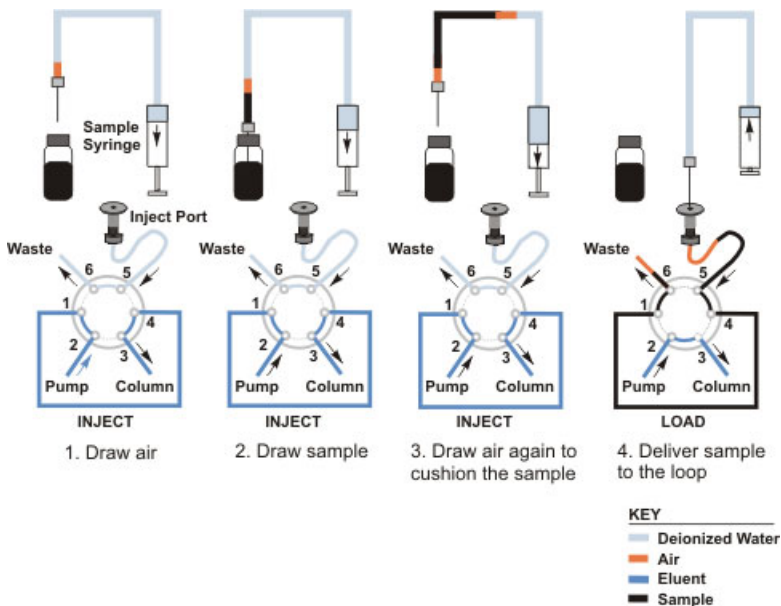
When performing a full-loop injection, the AS50 ➤ *Autosampler* draws four times the loop volume from the sample vial and delivers it to the injection valve. The middle portion of the sample is positioned in the loop and injected.

Tips:

For very small loop sizes (less than 17 μl), 2.5 times the loop volume plus 25 μl is delivered to the valve.

The maximum loop size for full-loop injections is 300 μl . If a larger loop size is used, sample can contaminate the flush bottle because the sample volume drawn is greater than the sample transfer line volume of 1200 μl .

Full-Loop Injection Sequence




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.

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 ...: Actions Related to the Mass Spectrometers**  **Extracting Mass Traces Afterward**.)

Also, refer to the **Administrator Manual: How to : Actions Related to Mass Spectrometers**  **Defining the Number of MS Channels**.

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.)

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 equation 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

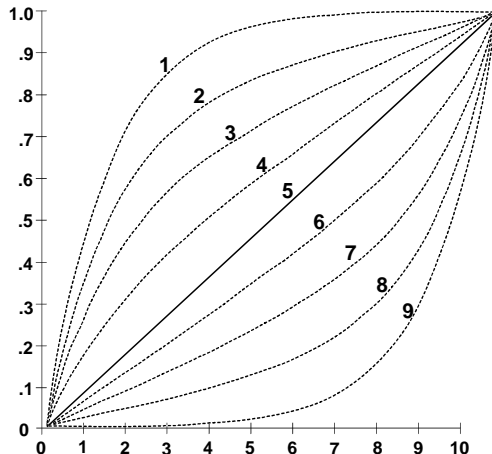
The Dionex GP40, GP50, and GS50 pumps let you specify linear or non-linear ➤*%-Gradient* profiles (gradient curves). ➤*Eluent Generators* can also deliver non-linear %-gradient profiles. For the mathematical description of the curve types, refer to ➤*Gradient Curves Equations*.

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:

$$V_e = V_f + (1 - k)(V_t - V_f) \left(1 - 2^{\frac{-10(T_e - T_f)}{(T_t - T_f)}} \right) + \frac{k(V_t - V_f)(T_e - T_f)}{(T_t - T_f)}$$

where: $k = 0, \frac{1}{4}, \frac{1}{2}, \frac{3}{4}, 1$ for curve numbers 1, 2, 3, 4, 5 respectively

For concave gradients:

$$V_e = V_f + (1 - k)(V_t - V_f) \left(2^{\frac{-10(T_t - T_e)}{(T_t - T_f)}} \right) + \frac{k(V_t - V_f)(T_e - T_f)}{(T_t - T_f)}$$

Where: $k = \frac{3}{4}, \frac{1}{2}, \frac{1}{4}, 0$ for curve numbers 6, 7, 8, 9 respectively

V_e	Current eluent value
V_f	Eluent value at the previous step time
V_t	Eluent value at the next step time
T_e	Current elapsed time
T_f	Time at the beginning of the gradient step
T_t	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*. 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 \Rightarrow *Group*

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 usually specified in mAU (milliabsorbance units).

Also, refer to \triangleright *Relative Height*

Help

See \triangleright *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 \triangleright *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 the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Tracking File Modifications (History)**.

Hit Criteria

If there are too many hits in a spectra comparison (see **How to ...: Actions in UV Spectra and/or Mass Spectra**  **Starting a UV Spectra Search**) due to the selected comparison function (see \Rightarrow *Check Derivative*) or \Rightarrow *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	\triangleright 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 so-called 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 \Rightarrow *Hold*

Holmium Oxide Filter

Recent Dionex UV/photodiode array detectors (the UVD 170U four-channel detector and the UVD 340U and PDA-100 ➤ *Photodiode Array Detectors*) feature holmium oxide filters.

For the UVD 170U and UVD U 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 detector, the holmium oxide filter is used to verify wavelength accuracy.



Note: *It is also possible to fit older UVD170S/340S instruments with holmium oxide filters. Please contact Dionex Service.*


Index (Button)

The **Index** tab page contains the alphabetical index list of online Help terms that are available in Chromeleon. Enter the initial letter of the topic and click **Display** to display help information about this topic.

Inhibit Integration

The **Inhibit Integration** detection parameter serves to shut off certain chromatogram areas.

Also, refer to ⇒ *Inhibit Integration*.

For information about how to apply detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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, you 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 peak table 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 (AS50 Autosampler)

Three types of sample injections are possible with an 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

Injection Type	Injection Volume*	Cut Volume**	Sample Volume Used	Volume Injected
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 AS50 (USB) is in simultaneous injection 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 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 ➤ *Device Driver* converts this value into a volume readable by the ➤ *Autosampler*, and then the value is sent to the autosampler.

By entering different injection volumes, a ➤ *Dilution Series* can be created in case of a multiple-point calibration (see ➤ *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 the **Administrator Manual: Validation and Qualification**  **Chromeleon Installation Qualification**.

**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 Comité 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.

EMA. The European Agency for the Evaluation of Medicinal Products is the European counterpart of the American FDA. Especially in Europe, the EMA 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.

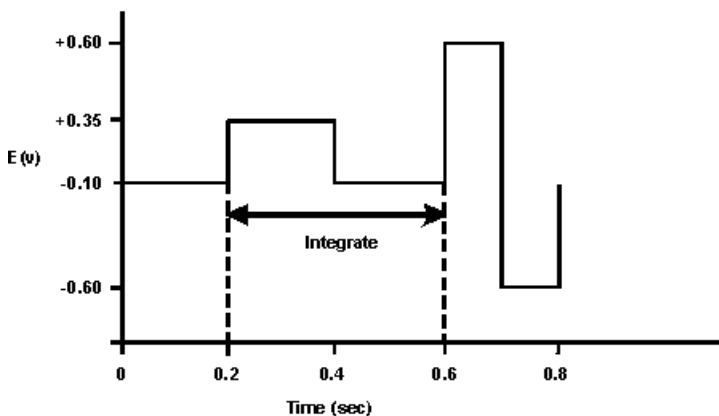
ISO. The International Organization for Standardization contains 130 national standardization institutes. Their task is to develop standards for the safer and more efficient development, manufacturing, and sales of products and services.

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 detection employs a repeating ➤*Waveform*. The cell current is ➤*integrated* during a specific section of the waveform. It is a more general technique than ➤*Pulsed Amperometric Detection* in that pulsed amperometry employs a repeating sequence of three potentials, while integrated amperometry can be any waveform.

With the integrated amperometry waveform shown below, the current is integrated twice - first, while the potential is swept across the metal oxide formation wave, and then 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 period.



Integration Parameters

In Chromeleon, integration parameters are referred to as **Detection Parameters**. For an overview, refer to ➤*Detection Parameters*.

Integration Period

In Integrated Amperometry detection, the integration period is the time during the ➤*Waveform* in which data is recorded. The integration period is also referred to as analysis or sample 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 \Rightarrow *Integration Type*.

Integrator Driver

Chromeleon does not directly process analog signals that are received via a Sharable Device, such as the \triangleright *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 \triangleright *Device Driver*.)

The advantage is that you do not have to rewrite all \triangleright *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 \triangleright *Sharable Device* to several \triangleright *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 **How to ...: Device Control**



Recording the Pump Pressure.

Intercept

See \triangleright *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 after the dilution! The dilution can therefore not 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 (provided that the same amount of the internal standard is actually contained in all samples). The results of the remaining substances of this sample can then be relativized.

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 *Amount* column of the sample list, not in the \Rightarrow *ISTD Amount* column of the peak table. For each sample, the amount of internal standard can thus be entered separately.

Also, refer to \Rightarrow *Standard*

IQ

See \triangleright *Installation Qualification (IQ)*

ISTD

See \triangleright *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 \triangleright *3D Field: Presentation Modes*

Jump to Another Help Topic

As soon as the mouse cursor is positioned on a link term, it changes into a pointing hand. All link terms are displayed in green.

Terms underlined by a dotted line, for example, \triangleright *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 only be operated if the serial number coded there matches the key code.

If a user expands the scope of Chromeleon functions by acquiring the corresponding licenses, (s)he receives a new key code. The extended scope of functions is made available as soon as the new code is entered.

Enter the key code is 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.

For more information, refer to the **Administrator Manual: Software Installation and Communication**  **The Software License** and **Installation Instructions**  **Entering the Software License**.

Kovats Index

The Kovats Index serves for achieving a uniform scaling of the \Rightarrow *Retention Time*. Define the Kovats Index on the **Peak Table** tab page of the \Rightarrow *QNT Editor*.

Also, refer to \Rightarrow *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 range (deuterium/halogen). To activate both lamps, the command must be entered and executed twice.

However, it is also possible to either switch both lamps simultaneously or turn on the second one automatically when the first lamp reaches a certain value.

Also, refer to \Rightarrow *Lamp*

LampIntensity

Select the `LampIntensity` property to read out the lamp intensity via the \Rightarrow `Log` command. Periodically reading out the value allows you to check the quality of the lamp and install a new one, if required.

Also, refer to \Rightarrow `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:

- \triangleright *Control Panels*
- \triangleright *Printer Layouts* (printouts)
- Reports (screen tables)
- \triangleright *History* reports

Control Panel

Enable **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 the functions of existing ones. 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 \triangleright *Layout Toolbar* allows you to select \triangleright *Control* (display and control elements). A \triangleright *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 ...: Actions on 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 deselect **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 deselect **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 the icons of 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 ...)		Switch
	Check Box		Entry Field (Edit Field)
	Color Box		Audit Trail
	String Display		Gradient Control
	Gauge Indicator		3D Plot



Script Button



Online Signal Plot



Group Box



Slider



Lamp

Rack Control (➤ Rack)

➤ Mass Spectrum



Command Button



Trend Plot

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 the **Parameter** button 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 the **Administrator Manual: Installation Instructions**  **Installing the License Server**

For more information, refer to the **Administrator Manual:**

Software Installation and Communication  **The Software License**

Installation Instructions  **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*). The LOD is reached 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 $b \neq 0$, 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 form, 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 ➤*A/A*. 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

Instead of being saved with a file, objects are often linked to 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 of 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

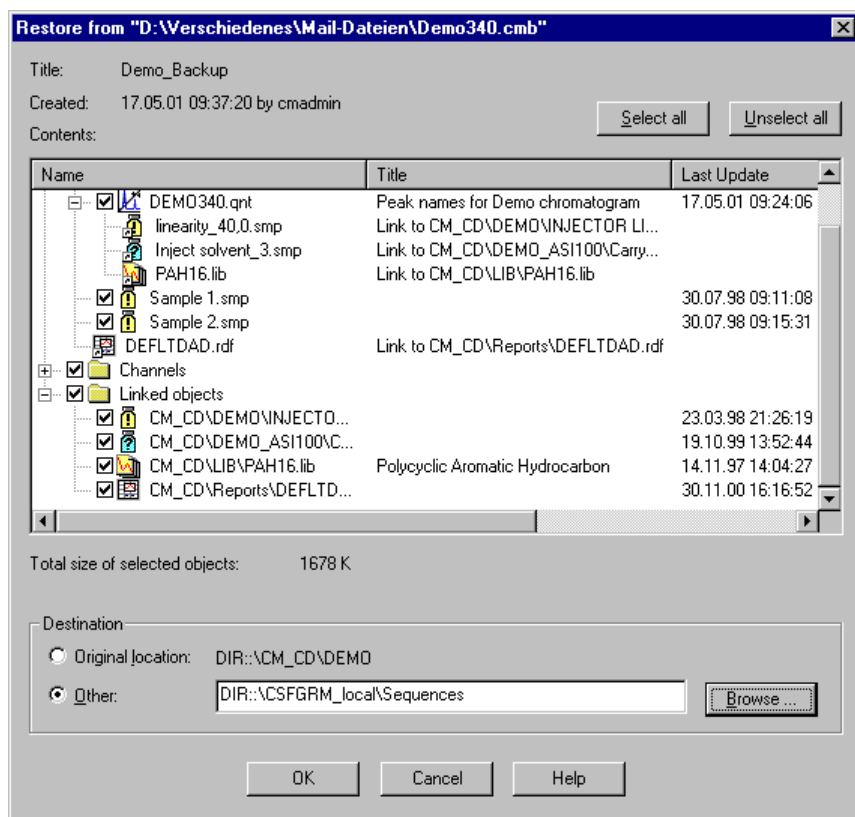
- **Spectra Libraries:** Use the **Spectra Library Screening** tab page in the QNT Editor to specify the spectra library to be used for peak identification.
- **Samples for Blank Run Subtraction:** Select a sample for blank run subtraction on the **General** tab page of the QNT Editor to determine which sample(s) shall be used for blank run subtraction.
- **Standard calibration samples** from a different sequence: On the **General** tab page of the QNT Editor, select **Fixed** as ➤*Calibration Mode*. To enter the desired standard (samples), change to the **Calibration** tab page and select **Append Standard** on the context menu.

Backup

When performing a ➤*Backup*, you can determine whether linked objects are saved as well. Select or deselect the **Include linked objects** check box as desired. We recommend saving the linked objects. This is to avoid that data are not be 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 \Rightarrow *Lock Baseline*

For information about how to apply detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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 \Rightarrow *Log*

Logon

The **Logon** dialog ensures access to the respective program. Access is permitted for authorized users only who are identified via a \triangleright *Password* in the logon dialog.

In Chromeleon, access to the \triangleright *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 \triangleright *User Mode* is enabled.

Loop Volume

Loop Volume is the sample loop volume obtained from the value entered in the Plumbing Configuration screen on the AS50 \triangleright *Autosampler* front control panel.

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. Also, 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 ...: Actions in the Chromatogram**  **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). Summing 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 (the difference between the integer and the actual mass is the mass defect):

^1H 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

So, when you want the mass of a compound you can calculate as follows:

Formula	Nominal Mass [amu]	Actual Mass [amu]	Defect [amu]
$\text{C}_5\text{H}_{10}\text{O}_5$	$5(12) + 10(1) + 5(16)$ = 150	$5(12.00) + 10(1.008) + 5(15.999)$ = 150.075	0.075
$\text{C}_{50}\text{H}_{102}$	$50(12) + 102(1) = 702$	$5(12.00) + 102(1.008) = 702.8$	0.8

Therefore, as you get to larger compounds with more atoms in it, you will sum all the "defects" of the individual atoms together and the difference can get to 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 with nitrogens and hydrogens in them and the only other element is carbon you end up with a positive mass defect. Since many hydrocarbon compounds have a lot of hydrogen, it can be a very large defect.

If you have in addition a substantial amount of oxygen, its negative mass defect can offset the nitrogen and hydrogen and the mass defect will not be as large. 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 **millimass unit** or one thousandth of a mass unit.

So 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 just works to "modify" the data so that people used to working with integer masses for their elements in a compound end up with what they expected.

Mass Spectrometer

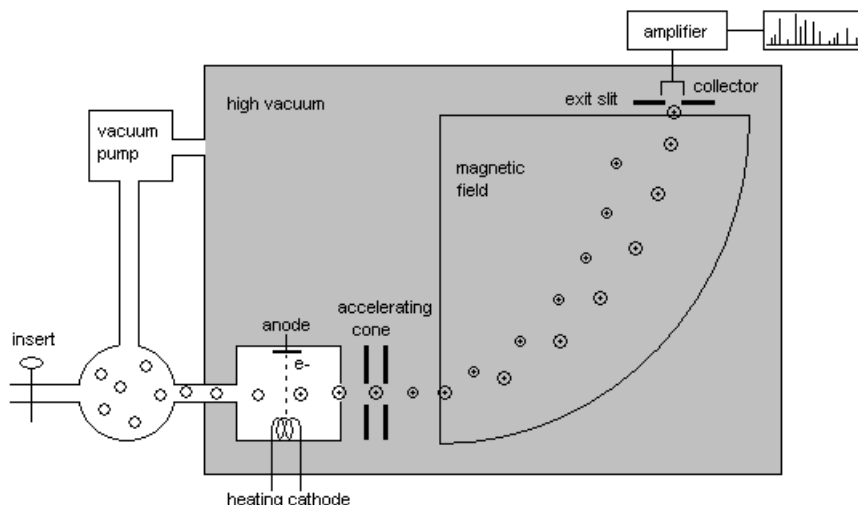
Mass spectrometer were originally developed as stand-alone instrumentation to analyze the structure of unknown, mostly organic compounds. In combination with chromatographic separation procedures, however, they also allow the qualitative and quantitative analysis of complex mixtures of substances.

While the GC/MS coupling has long been part of the standard equipment in analytical laboratories, HPLC-MS is not yet that widely spread because of its problematic coupling. However, acceptance is steadily growing as the HPLC/MS coupling has been decisively enhanced during the past years. Besides, with the MS Detector another detector allowing qualitative statements on individual substances of a chromatogram is available in addition to the ➤*Photodiode Array Detector*


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 ...:**  **Actions Related to Mass Spectrometers.**

Mass Spectrum

Use an MS detector (*>Mass Spectrometer*) to record a mass spectrum at any time *t* of a chromatogram. The two-dimensional view of a chromatogram (retention time and intensity) is extended by a third dimension (mass). In addition to the information about retention time and intensity information, each of the recorded data points also 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. Mass spectra representation with many needles is called **Centroid**. Contrary to the centroid representation, **Profile** representation combines the single needles to wide peaks.

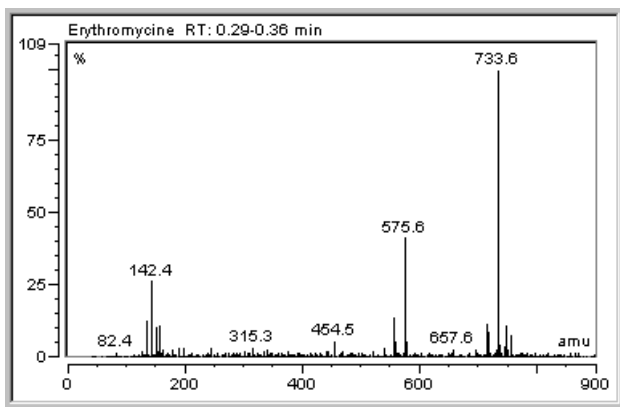
In the report, you can display the mass spectrum of a peak or the mass spectrum at any time of the chromatogram. You can also display the current mass spectrum on the control panel during data acquisition.

In addition, you may aggregate single mass spectra, thus, enhancing their **Signal-to-Noise Ratio** (see **How to ...: Actions in the QNT Editor**  **Processing Mass Traces**).


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 amu (atomic mass unit) where: $\text{amu} = m/z$ (mass/charge number). The base peak, that is the mass peak with highest intensity, is used as reference point for relative intensities. Thus, its relative intensity is always 100 %.



The base peak in the above example is the mass peak at 733.6 amu. The time when the mass spectrum was recorded is indicated as well. The mass spectra in the example were aggregated to the displayed total mass spectrum between 0.29 and 0.35 min.

For information how to record and process MS data, refer to **How to ...:**  **Actions Related to Mass Spectrometers**.

Mass Trace

Contrary to original ➤ *TIC* or ➤ *SIM* chromatograms that are recorded during data acquisition, a mass trace is a 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 ...:Actions Related to Mass Spectrometers:



Extracting Mass Traces Online



Extracting Mass Traces Afterward

Administrator Manual: How to ...: Actions Related to 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 chromatograms. 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*). This command is possible in windows or partial methods capable of displaying a spectrum. (For more information, refer to **Data 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 \Rightarrow *Maximum Area Reject*

For more information about how to use the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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 \Rightarrow *Maximum Height Reject*

For more information about how to use the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters.**

Maximum Peak Height (*MaxHght*)

Positive peak identification is via the \Rightarrow *Minimum Height* parameter. All peaks above this height value are identified.

Also, refer to \Rightarrow *Maximum Height Reject*

For more information about how to use the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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 ...: Actions in the QNT Editor**  **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 ...: Actions in the QNT Editor**  **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:

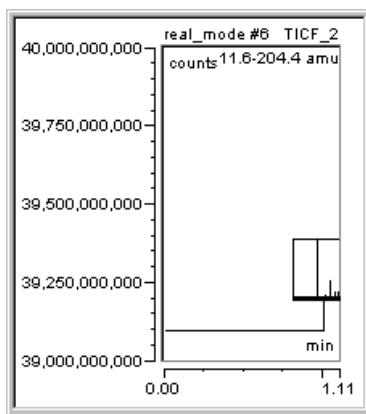
The ➤MCA mode is not available for the MSQ.

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 ...: Actions Related to 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 ...: Actions in the QNT Editor**  **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 ...: Actions in the QNT Editor**  **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 ...: Actions in the QNT Editor**  **Modifying Detection Parameters**.

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.

With the cable (3) with two 25-pin connectors, the remaining 16 pins are also mapped 1:1.

Dionex part numbers

160070: Cable (1): 9-pin (male) - 9-pin (female) (previous LC Packings part no. **ULT-S-COM** and **SW-S-COM**)

8914.0144A: Cable (2): 25-pin (male) - 9-pin (female), 5m

8914.0128: Cable (3): 25-pin (male) - 25-pin (male), 5m



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 respective instrument. (Also, refer to the **Administrator Manual: Installation Instructions** 🌐 **Installing and Controlling Third-Party Devices**).

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 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 we recommend 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, we recommend using an MS SQL or Oracle server as central database to protect your data even if the network fails.

Also, refer to the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Network Failure/Non-Availability**.

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.

(Signal) Noise

The **Signal Noise** parameter indicates the signal noise within a chromatogram calculated by Chromeleon. The value serves as the basis for the ➤*Signal-to-Noise Ratio* determined from the raw data at the start of the data acquisition.

Determining the Signal Noise (Default)

All datapoints recorded during the first 10 seconds of a chromatogram form the basis for determining the noise value. Chromeleon calculates a regression line using the method of least squares, then determines the maximum distance of two datapoints above *and* below the line. (When calculating the regression line, all datapoints are weighted with their

corresponding step unless the step is equidistant.) Adding both values supplies the first noise value.

The procedure is repeated for the last 10 seconds of a chromatogram. It returns a further noise value.

The lower value is indicated under **Noise** and used as ➤ *Sensitivity* for peak recognition.

Signal Noise in User-defined Range (Parameter Input)

You can also determine the signal noise for a specific range of the chromatogram. In the **Integration** view, for example, specify this 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. Mark **Chromatogram** in the **Categories** list, and then select **Signal Noise** in the **Variables** list. Click the **Parameter...** button to open the **Parameter Input for 'Signal Noise'** dialog box. Select the **Specific Range** check box and enter the desired range.



Tip:

*To define the **determination**, **identification**, and **detection** limit via the signal-to-noise ratio, always use the noise of the user-defined range! If you do not use the noise of the user-defined range, the determination, identification, and detection limits might be miscalculated.*

Normalization (Overview)

In the linear range, the height of a spectrum recorded at the time *t* is always proportional to the current substance concentration in the flow cell. An objective comparison of two spectra is therefore only possible if the concentration is identical.

As this condition is rarely fulfilled, and as it is not possible to convert a spectrum recorded at a specific concentration to another concentration, the height of one spectrum 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*).

- The noise portion depends on the wavelength and the solvent. Thus, at 200nm, not only the lamp energy of a deuterium lamp is considerably lower than at 250nm, but also the absorption of common solvents is especially high.
- Normalization should be performed where little influence on the above factors is expected and where a relative absorption maximum of the substances to be compared is observed.

Therefore, normalization can be performed by the ➤ *Absolute Maximum*, by the ➤ *Relative Maximum*, or by a fixed ➤ *Wavelength*.



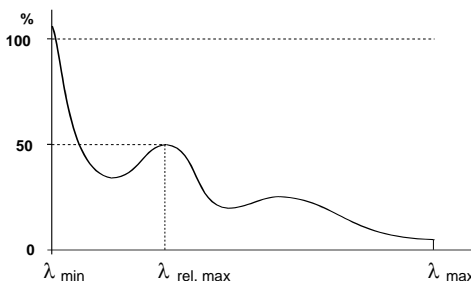
Note: *Chromatograms and peaks can also be compared. They are referred to as normalized chromatograms, areas, or amounts.*

Normalization (Absolute Maximum)

The absolute maximum of the spectrum is determined. The absorption maximum is chosen as reference point and it receives the absolute height 1 (=100%). Also, refer to ➤ *Normalization (Overview)*

Normalization (Relative Maximum)

The relative maximum of the spectrum is determined. This will be the greatest λ -peak of the spectrum. After normalization, this peak has the height **0.5** (50%). This prevents normalization with incomplete maxima on spectrum margins.



Tip:

Note that spectrum maxima can exceed the 100% line on the margin of the spectrum, 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)*

Null Modem Cable

Null modem cables are frequently used (crossover) standard cables for connecting instruments to the PC. The cable assignment 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

If there is an additional hardware handshake, the following assignments are also required (they are not realized for the Dionex cables):

4	7	-----	6	6
6	6	-----	7	4
7	4	-----	5	8
8	5	-----	4	7

**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:

9-pin to 9-pin:

- Dionex part no. 8914.0129 for RS cable RU, 2.5m
- Dionex part no. 8914.0130 for RS cable RU, 5m
- Dionex part no. 8914.0131 for RS cable RU, 10m

25-pin - 9-pin:

- Dionex part no. 8914.0103A for RS cable RU, 2.5m
- Dionex part no. 8914.0120 for RS cable RU, 5m
- Dionex part no. 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 respective instrument. (Also, refer to the **Administrator Manual: Installation Instructions** 🔄 **Installing and Controlling Third-Party Devices.**)

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 loop 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 eluent generator.


Online Help

The Chromeleon  **Online Help** provides on-screen help information in any situation.

- Click **Help** 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 display the **table of contents of the online Help**. Select **Using Help** for more information about how to use the online Help system.

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.

For installation details, refer to the **Administrator Manual: How to ...: Actions in the Server Monitor Program**  **Setting Up the Online Transfer Agent for Network Access.**



Tips:

*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**).*

If sequences of the same name already exist in the destination directory, these sequences may be overwritten. It is not possible to overwrite locked data, signed data, or sequences containing raw data.

*Sequences not containing raw data are overwritten; neither a warning appears before this operation nor are the ➤ *Privileges* (e.g., *DeleteSEQ*) checked.*



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 EUROACHEM, 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. Normally OQ is performed directly after the installation of a new device(s) or after repair or system service procedures have been performed.

On the **Qualification** menu, Chromeleon provides the **Instruments OQ** and **OQ Setup** commands. Select **Instruments OQ** to perform the 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 the **Administrator Manual: Validation and Qualification**  **Chromeleon Operational Qualification (OQ).**)



Tips:

*For more information regarding the Operational Qualification, refer to the **Operational Qualification/Performance Qualification** Operating Instructions that you can obtain from Dionex Service.*

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 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 look 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 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), we recommend 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.

For more information, refer to **How to ...: Actions in the Peak Purity Analysis (PPA) Window**  **Selecting the Optimum Integration Path**.

OQ

See **➤Operational Qualification (OQ)**

Origin

See **How to ...: Actions in the QNT Editor**  **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 the **Report** or in the ⇒ *QNT Editor* to overlay any 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: Actions in the Chromatogram**  **Performing a Chromatogram Comparison**

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 placed on another card, such as the A/D converter card or the pump control card, or is 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.

For more information, refer to the **Administrator Manual**:

Software Installation and Communication  **The Software License**

Installation Instructions  **Entering the Software License.**

Panel

See ➤ *Control Panel*

Partial Flows

See ➤ *%B, %C, %D*

Partial-Loop Injections (AS50 Autosampler)

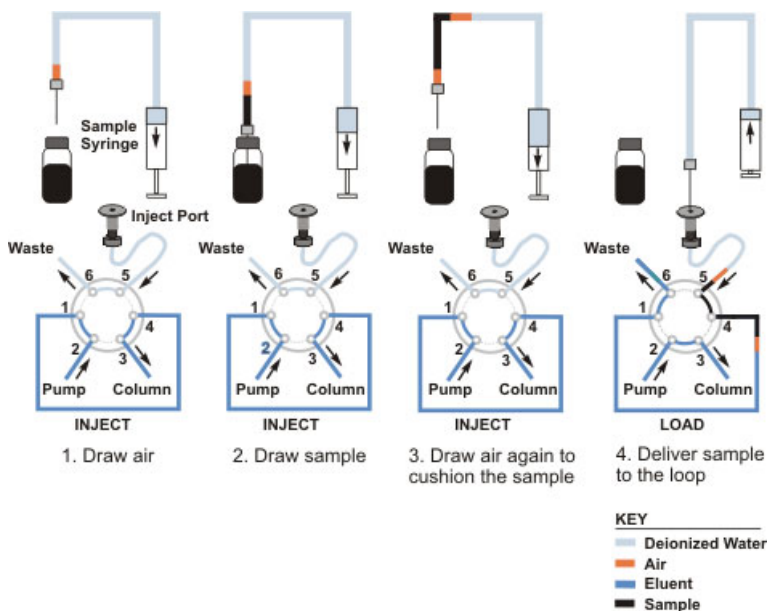
When performing a partial-loop injection, the AS50 autosampler draws the volume to be injected, plus two times the *cut volume* (a volume of sample discarded from each end of the aspirated sample), from the sample vial.



Tip:

*If the injection valve is installed externally or if an AS50 (USB) is in simultaneous injection mode, the autosampler can perform ➤ *Full-loop Injections* only.*

Partial-Loop Injection Sequence



Partial-Loop, Limited-Sample Injections (AS50 Autosampler)

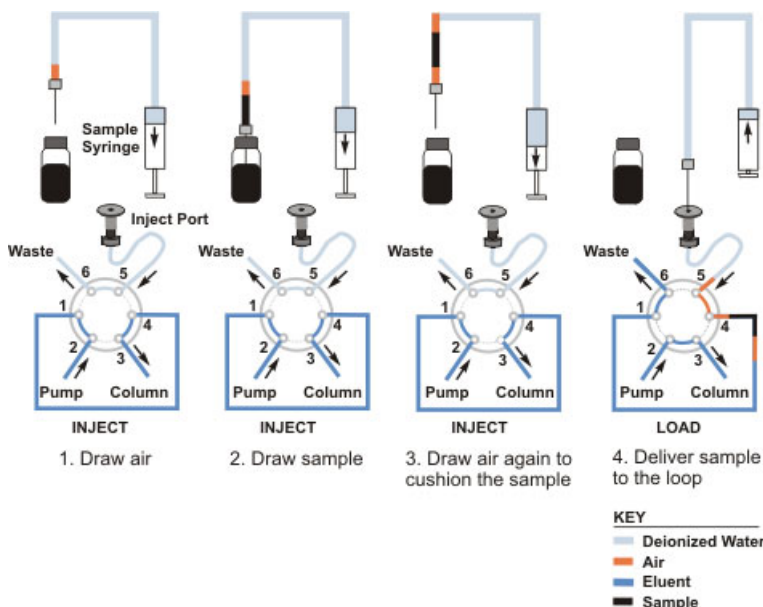
When performing a partial-loop, limited-sample injection, the AS50 autosampler draws only the volume to be injected from the sample vial.



Tip:

If the injection valve is installed externally or if an AS50 (USB) is in simultaneous injection mode, the autosampler can perform ➤Full-loop Injections only.

Partial-Loop, Limited-Sample Injection Sequence



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, the 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 \Rightarrow *Peak Group Start/End*

For more information about the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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 \Rightarrow *QNT Editor*. The peak name can be indicated as a separate peak variable in the report.

Also, refer to \Rightarrow *Name (Peak Name)*

Peak Number (No.)

See \triangleright *Number (No.)*

Peak Purity

See the following topics:

\triangleright *PPA (Peak Purity Analysis)*


\triangleright *PPI (Peak Purity Index)*

\triangleright *PPA Report*

Peak Purity End Wavelength (PPWEnd)

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 \Rightarrow *Peak Purity Start/End Wavelength*.

For information about how to apply detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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* 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*), 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 ...: Actions in the Chromatogram  **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.

$$RSD (Match) = \frac{SD}{Match \text{ Ø}} * 100\%$$

Peak Purity Start Wavelength (PPWStart)

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 ...: Actions in the QNT Editor**  **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 \Rightarrow *Peak Purity Threshold*

For information about how to apply detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Defining Detection Parameters**.


Peak Shoulder

See \triangleright *Shoulder*

Peak Shoulder Threshold

The **Peak Shoulder Threshold** parameter defines a threshold value for peak shoulder recognition.

Also, refer to \Rightarrow *Peak Shoulder Threshold*

For more information about how to use the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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 \Rightarrow *Sensitivity*!*

Also, refer to \Rightarrow *Peak Slice*

For more information how to use the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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 samples desired. The summary usually includes the samples of a sequence. However, you may also create a summary based on a ➤ *Query*.

For more information, see **Reports**  **The Peak Summary Report**.

Peak Table

The peak table is part of 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 the **Instrument PQ** command 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 contain up to three sections that correspond to the different views of the PGM Editor (see **Chromeleon (Overview)**  **The PGM Editor**):

- The actual **➤Program** (**Commands** view in the PGM Editor)
- The instruments method for the **➤Mass Spectrometer** (**Finnigan AQA** or **Surveyor MSQ** view in the PGM Editor - only with PGM Files for a timebase that includes a mass spectrometer)
- Extractions and data smoothing steps that are performed after data acquisition (**Post-acquisition steps** view in the PGM Editor)

Use the Program Wizard to create the PGM File (and especially the program). (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, enter the mass spectrometer method and the **Post acquisition steps** on the corresponding pages of the PGM Editor.

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.

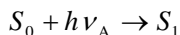


Note:

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 (S_0) and terminates in different excited vibrational levels of the next higher singlet state (S_1).



where:

h = Planck's constant

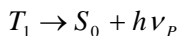
$$\nu_A = \text{light frequency at absorption} = \frac{c}{\lambda_A}$$

where:

c = speed of light

λ = wavelength

The excited vibrational states of S_1 transit via a radiationless intersystem crossing into the lowest triplet state (T_1). The transition from the triplet state into the ground state is spin prohibited. Therefore, it is temporally delayed and phosphorescent light is emitted:



**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, please refer to ➤ *Optical Resolution (Spectral Resolution)*.

Main Features

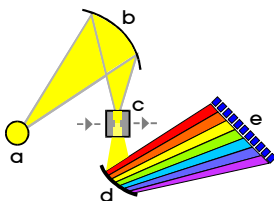
➤ *Photodiode Array Detectors* (PDAs) feature high sensitivity and a large linearity range, they are relatively independent from temperature fluctuations, and can be used for gradient elution (%-gradient). The simultaneous detection of all wavelengths at the time t is made possible by:

- Peak Purity Analysis
- Peak identification via 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 detector 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 $150 \text{ nm} / 150 \text{ photodiodes} = 1 \text{ nm per photodiode}$.

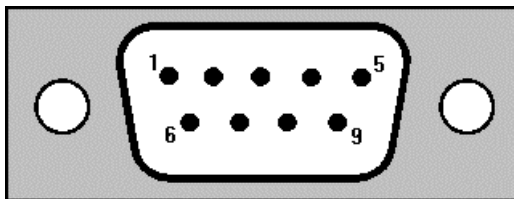
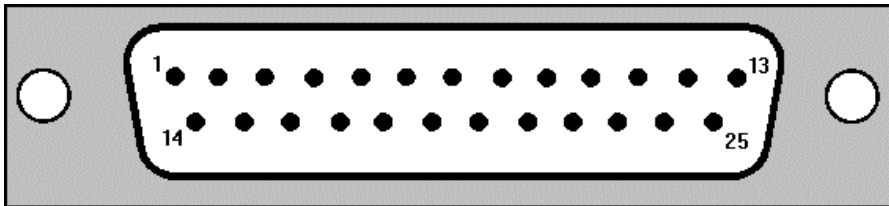
The ➤ *Optical Resolution* of the UV detector is determined by photodiode resolution and the optics (mirror, slit, grating, optical bandwidth).


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 the **Administrator Manual: Installation Instructions**  **Installing and Controlling Third-Party Devices**

Select the **Error Handling** command on the **Batch** menu and select the desired 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 ...: Actions in the PGM Editor**  **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 partial method of the offline module. It consists of a graphical section and the ➤ *PPA Report*.

For more information, see **Data Reprocessing**  **PPA: Peak Purity Analysis (Overview)**.

PPA Report

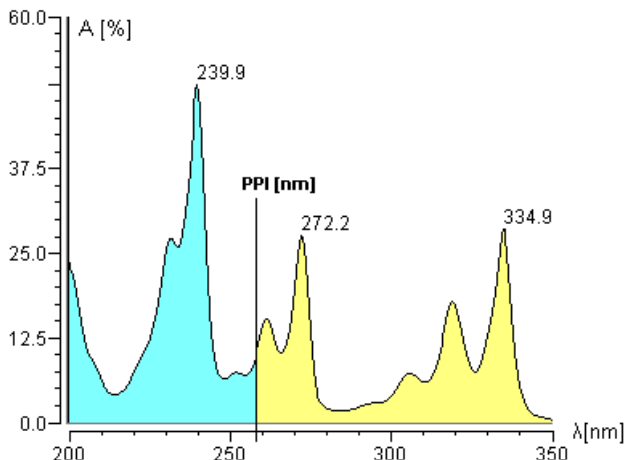
The PPA report represents the numerical addition to the partial method Peak Purity Analysis. In a table, this report combines variables characteristic for the peak purity analysis and variables freely selectable by the user.

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)

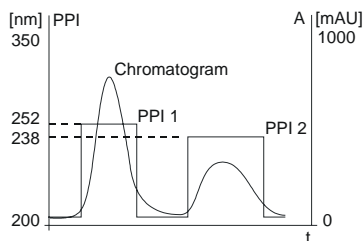
The peak purity index (PPI) represents the central wavelength of a spectrum. In simplified terms, this is the wavelength where the areas of the spectrum to the left and right are identical. The PPI is thus independent of the concentration.



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.

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.

$$SD = \sqrt{\frac{Q - Sum}{(n - 1)}}$$

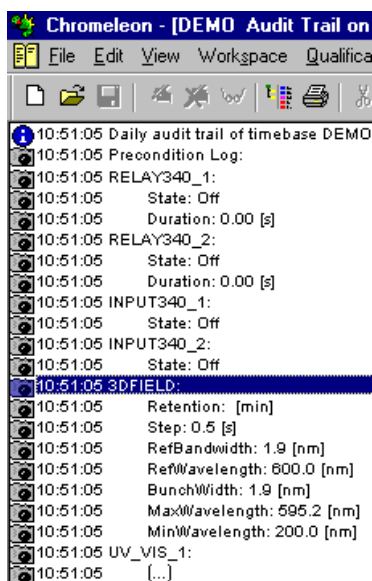
The relative standard deviation of the PPI (RSD (PPI)) in percent is obtained by dividing the standard deviation by the average of all PPI values (PPIØ), and by multiplying the result by 100%.

$$RSD(PPI) = \frac{SD}{PPI\bar{O}} * 100\%$$

Preconditions

The device settings before a sample run are referred to as **Preconditions**. The **Preconditions** are saved in the audit trail files and displayed in the Daily Audit Trail and the Sample Audit Trail behind the following icon: . (For more information about the Audit Trails, refer to **Data Management Audit Trails.**)

The device settings are listed in the Daily Audit Trail directly after the first message (**Daily audit trail of timebase X**).



PQ

See ➤ *Performance Qualification (PQ)*


Pressure Limits

Depending on solvent composition and the selected \Rightarrow 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 \Rightarrow Pressure.Lower/Upper.Limits

PrimeSyringe (ASI-100 Autosampler)


The **PrimeSyringe** command, which is supported for the ASI-100 Autosampler, allows removing air 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 **How to ...:**


Device Control  **Priming the Syringe (ASI-100 Series).**


Printer Layout

Use the Printer Layout to prepare your data for the printout. Enable \triangleright 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 \Rightarrow Report Categories)
- Chromatograms
- Calibration plots (calibration curves)
- Spectra plots (UV spectra)
- Mass spectra plots (\triangleright Mass Spectra)
- 3DFIELD plots (\triangleright 3D Fields)
- \triangleright Trend Plots
- Charts (graphical display of the values of the individual cells/columns)

The Printer Layout is stored in the \Rightarrow *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...:**  **Actions in the Printer Layout.**

For information about how to print your results, refer to **How to: Actions in the Printer Layout**  **Specifying the Printout.**

Privileges (User Rights)

When the \triangleright *User Mode* is enabled, the system administrator can assign user rights and thus, determine which actions a certain user group (= Privilege Group) is allowed to perform. The system administrator assigns the access rights in the \triangleright *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 the **Administrator Manual: Chromeleon User Management**  **Privileges (Categories).**


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 different 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 (in short: program) 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 completely automate the analysis 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 of the ➤ *Wizard* (also see **Control**  **The Program Wizard**) to create the basic structure of the program. The user input is automatically converted into uniform command syntax readable by Chromeleon. In this way, even new users can quickly create operable programs.

For more information, see **Control**  **The Control Program**. Also, refer to  **The Program Syntax** and ⇒ *Program*.

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, it is directly linked to the corresponding sample. Use **Protocol** text to comment individual samples.

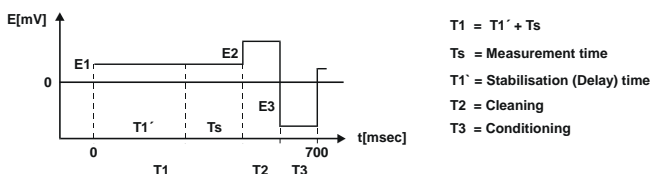
It is also possible to execute the **Protocol** command event-controlled.

Also, refer to ⇒ *Protocol*

Pulse Mode

In addition to the ➤*DC Mode*, most 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).

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.



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.

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 and





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: ⇒ *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 the **Query** command on the context menu or select **New > Query (using Wizard)** on the **File** menu. A wizard opens, which facilitates 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 ...: Actions in the Browser**  **Performing a Query**.

The result of a query can be saved as a new sequence via 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 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.

For the Dionex autosamplers, Chromeleon lets you display the rack for the desired sequence:

From an existing sequence

To display the rack indicating the ➤ *Status* of the single samples in the sequence:

- Connect the desired sequence to the corresponding timebase.
- Select **Rack** on the **View** menu.

When creating a sequence

When creating a new sequence, check your entries on the rack preview of the Sequence Wizard:

- Select **New...** on the **File** menu, and then select **Sequence (using Wizard)** from the list.
- After you have entered the samples in the corresponding step of the wizard, click the **Rack Preview** tab page.


The rack of the Dionex ASI-100 Autosampler 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 house up to 39 vials at 1.1 or 1.8 ml each + 1 vial at 4 ml.
Semiprep:	Select this option if you use the semi preparative segment. This segment can house up to 21 vials at 4 ml each.
Mini:	Select this option if you wish to analyze a large number of samples or if small sample volumes are available only. This segment can house up to 64 vials at 1.2 ml + 1 vial at 4 ml.
Eppendorf:	Select this option, if you are using the rack for Eppendorf vials. This segment can house up to 22 vials at 1.5 or 2 ml each + 1 vial at 4 ml.

If your system comprises an autosampler with rack cooling, such as the ASI-100T, you can determine the controllable temperature range. Exact temperature control is possible from +4°C to +45°C (39.2°F to 113°F). For example, the ASI-100T autosampler allows you

- to cool the rack by 18 K from ambient
- to heat the samples by up to 35 K from ambient.

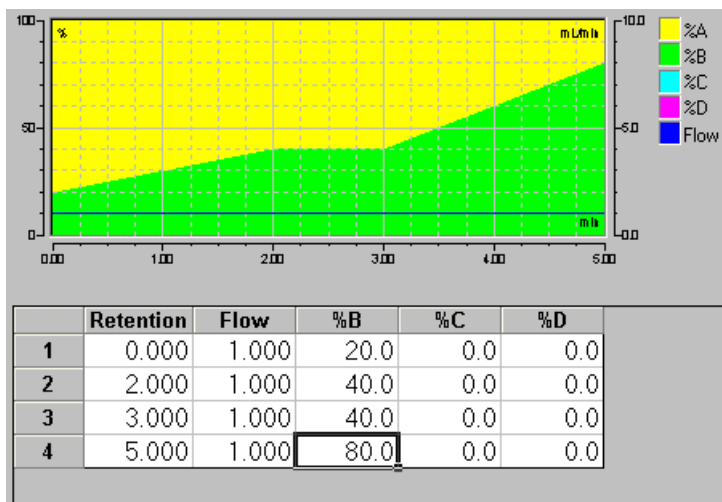
Fraction Collector

For fraction collectors, **➤ Trigger Commands** are used during the analysis to define when to start collecting the next fraction. Fraction collection control depends on many factors and is very complex. For more information, refer to **How to ...:**  **Actions Related to Fraction Collection.**

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):



Range

The **Range** parameter determines the factor by which the analog output signal of controllable detectors is amplified or reduced.



Notes:


The Dionex \triangleright UCI Universal Chromatography Interface ranges from -10V to +10V and thus covers virtually all detector output signals. It is possible to record the signal directly and adjust the size, using the **Factor** parameter of the signal configuration. (In the \triangleright Server Configuration, select the **Properties** of the fluorescence detector on the context menu, and then go to the **Signals** tab page.)

The **Range** parameter has a different meaning for the RF2000 fluorescence detector. The \triangleright Gain parameter is used, instead.

For \triangleright 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. Use the Ready Check to check whether the instruments in the chromatography system are ready to operate, i.e., whether they are turned on and connected, whether the detector lamp is burning, etc. In addition, the Ready Check verifies that all required files exist and that the storage capacity is sufficient.

As the batch list can be extended by further sequences during operation, the **Ready Check** command can also be performed during operation.



Tips:

Before a batch is started, Ready Check is performed automatically.

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 will be issued automatically.

Recorder Calibration

The **Recorder Calibration** commands 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 ➤ <i>Recorder Range</i> 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 given in microSiemens (μS). This command can be used to adjust the recorder output to accommodate larger or smaller peak heights.

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 \Rightarrow *Bandwidth* of a channel, the reference bandwidth serves to average several photodiode signals of the \Rightarrow *RefWavelength (Reference Wavelength)*.

Also, refer to \Rightarrow *RefBandwidht (Reference Bandwidth)*

Reference Channel

= Reference Channel for Baseline Correction

To performing automatic ➤*Baseline Correction* a reference channel is required.

As 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 desired 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 \Rightarrow *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 absolute time difference (in minutes) or as percent quotient (retention time substance peak/retention time reference peak). In addition, the reference peaks can be used for calculating the \triangleright *Relative Peak Area* and the \triangleright *Relative Peak Height*.

You can specify the reference peaks in the QNT File on the **Peak Table** tab page. Double-click the **Ret. Time** column to open the **Retention Time for ...** dialog box (see \triangleright *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 via the selector arrow.

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. It is not possible to delete reference peaks from the peak table. Besides, the retention time can be entered directly in the entry field in the following format:

[<Reference Peak>] <Ret. Time> [<Unit>]

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 absolute time, otherwise as relative time.

Either **min** or **%** can be selected as unit. If no entry is made, **min** is used. 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 \Rightarrow *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 \succ <i>Internal Standard</i> .
The corresponding Reference Peak	Amount portion relative to the amount of a \succ <i>Reference Peak</i> .

**Note:**

The values of options 2 to 4 can considerably exceed 100 %.

Select the respective column in the report, select the **Column Properties** command on the context menu, and select the variable in the selection box. Click the **Parameter** button 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 ➤ <i>Internal Standard peak</i>
The corresponding Reference Peak	The area of a peak is put in relation to a ➤ <i>Reference Peak</i> .

Select a column in the report, select the **Column Properties** command on the context menu, and select the variable in the selection box. Click the **Parameter** button 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 ➤ <i>Internal Standard Peak</i> .
The corresponding reference peak	The relative peak height is indicated in relation to the height of a selected ➤ <i>Reference Peak</i> .

Select the column in the report, select the **Column Properties** command on the context menu, and then select the variable in the selection box. Click the **Parameter** button 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 retention time \Rightarrow *Retention Time* and the retention time of the corresponding reference peak in percent:

$$\text{Relative retention time} = 100 * \frac{t_R}{t_{R, \text{ref}}}$$

Where:

t_R = Retention time (of the current peak)

$t_{R, \text{ref}}$ = Retention time of the corresponding reference peak

The ➤ *Reference Peak* is specified in the peak table of the \Rightarrow *QNT Editor*.

Clicking the **Parameter...** button opens the **Parameter input for 'Relative Retention Time'** dialog box. Select the desired 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 the desired 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). Usually, the relative standard deviation is expressed in percent.

The relative standard deviation (*relStdDev*) is calculated as follows from the absolute standard deviation (*StdDev*):

$$relStdDev = \frac{StdDev}{\bar{Y}}$$

With: \bar{Y} : Average value of all Y-values.

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 only be used 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 given.

Also, refer to ➤*Relay On/Off*

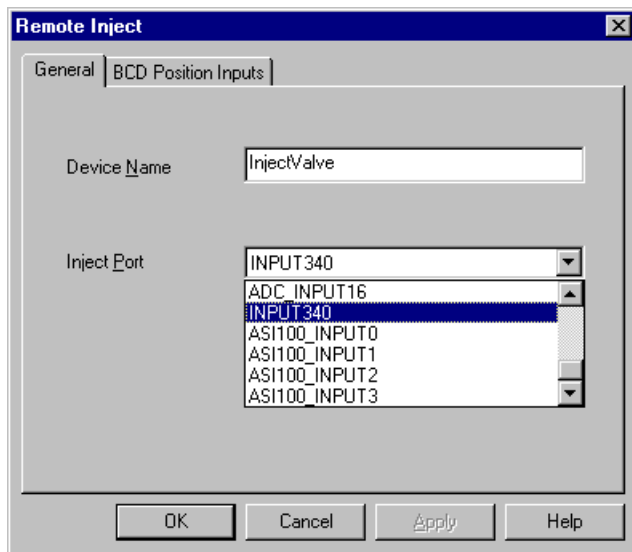
Remote Inject

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 (\Rightarrow *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 automatically replot 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*).

For more information, see ➡ *Report Definition File*.


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 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 the **Layout Mode** command on the **Edit** menu to display the edit line. Click the desired 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 ...:**  **Actions in the Printer Layout**.

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 absorption values recorded at the time t at different wavelengths using a photodiode array detector. Each absorption 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.
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 the degree of separation between two adjacent peaks. This resolution is a measure for the separating capability of the column. It is calculated based on the following formulas (EP and USP standards):

USP standard:

$$R = 2 * \frac{t_{next} - t_R}{BW_{next} + BW_R}$$

EP standard:

$$R = 1.18 * \frac{t_{next} - t_R}{W_{50\%,next} + W_{50\%,R}}$$

Where:

t_R	Retention time of the current peak
t_{next}	Retention time of the peak after the current peak
BW_R, BW_{next}	➤ Peak Widths of the two adjacent peaks
$W_{50\%,R}, W_{50\%,next}$	Widths of the two adjacent peaks at 50% of the peak height

The distance between the peak maxima is divided by the arithmetic average of the peak widths. Select the column in the report, select the **Column Properties** command on the context menu, and select the variable in the selection box. Click the **Parameter** button to define whether the calculation is based on the EU (European Pharmacopeia; EP formula) or the US (United States Pharmacopeia; USP formula) standard.

Response


Response is the time the detector requires for reaching 98% of the full deflection. 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*.

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 ...: Actions in the Browser**  **Restoring Backup Files**.

To restore data, select the **Import/Restore** command 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*

For information about how to enter the retention index, refer to **How to...: Actions in the QNT Editor**  **Defining the Retention Index and the Kovats Index**.

Retention Time

The retention time is used for peak identification. The **Retention Time** peak variable refers to the time (in minutes) that passed since the injection (also, refer to \Rightarrow *Dead Time*). The retention time of a peak is defined by the time of the data point with the highest absorbance value. (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 \Rightarrow *QNT Editor* as part of the peak table.
- In the Report and in the \triangleright *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 \Rightarrow *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 \Rightarrow *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 \Rightarrow *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 \Rightarrow *Retention Time* according to the peak table (in [minutes]).

Retention time deviation = $t_{R(\text{Soll})} - t_{R(\text{Ist})}$

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 the **Column Properties** command on the context menu, and then select the variable in the selection box. Click the **Parameter** button 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 \Rightarrow *Retention Time* in the peak table of the \Rightarrow *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 \triangleright *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 to 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) \Rightarrow Window are interpreted as percentage values of the absolute 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 \Rightarrow *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 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 \Rightarrow *Rider Threshold* and \Rightarrow *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 \Rightarrow *Rider Skimming*).

Rider peaks are different from \triangleright (*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, \triangleright *Rider Peaks* usually do have a distinct relative maximum of their own.

Rider Skimming

This parameter indicates how \triangleright *Rider Peaks* are skimmed.

Also, refer to \Rightarrow *Rider Skimming*

For information about how to apply the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Modifying Detection Parameters**.

Rider Threshold

The **Rider Threshold** detection parameter determines whether individual peaks in a series of non-resolved peaks are classified as \triangleright *Rider* or as main peak.

Also, refer to \Rightarrow *Rider Threshold*

For information about how to apply the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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 \triangleright *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 the **Column Properties** command on the context menu, and then select the variable in the selection box. Click the **Parameter** button 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 will be shifted.
- The peak height will be 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: $1/4 (5 \text{ s}) = 1.25 \text{ s}$. 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 AD25 and PDA-100 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 does not only include 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 that shall 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 \Rightarrow *Sample Variable* **Weight** has two functions. It serves to enter the sample weight, but it can also be used as a weight correction factor.

Sample Weight is implemented as a multiplication factor in the \triangleright *Formula for Amount Calculation* of not explicitly entered amount values (dilution series). It is without dimension.

Also, refer to \Rightarrow *Weight* (Sample Weight Factor)

Sampler.Ready

See \triangleright *Ready*

Sampling Rate


The number of stored signal values per second is referred to as sampling rate (or Data Collection Rate). The maximum of stored values corresponds to the number of values generated per second and depends upon the device, for example, Dionex UVD 170S/340S Detectors = 100, \triangleright *UC/ Universal Chromatography Interface* = 100, 3D field = 10, Dionex AD25 and PDA-100 Detectors = 10.

The reciprocal value, that is, the time interval between data points, is referred to as \Rightarrow *Step* (Dionex UV detectors = 0.01, UCI = 0.01, 3D field = 0.1, Dionex AD25 and PDA-100 detectors = 0.1).



Tip:

For Dionex detectors that are installed via the DX-LAN, a \triangleright *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.

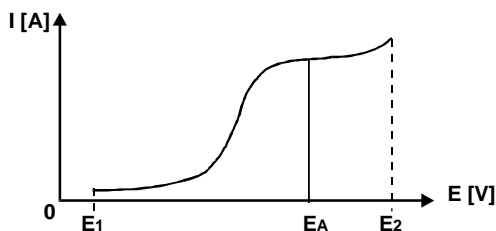
For more information, refer to **How to...: Device Control**  **Defining Step and Average.**

SCAN Mode

The SCAN Mode of an electrochemical detector serves to determine the analytical potential of a substance.

At a constant concentration in the flow cell that is, for example, realized by strongly reducing or by stopping the pump flow, the potential between reference and working electrode is changed linearly.

The change is either a linear increase or decrease between two values (E_1 and E_2 ; **Half scan**), or an increase with a subsequent decrease (**Full scan**). If the increase and decrease are constantly repeated, this is referred to as **Continuous scan**.



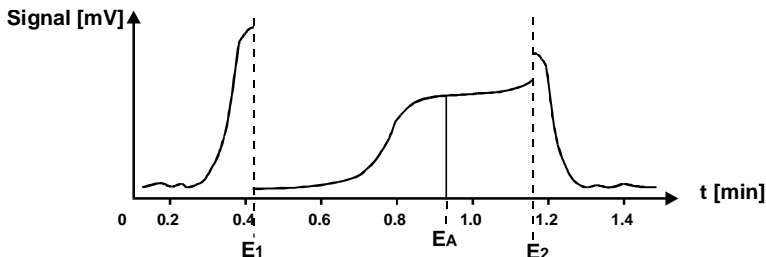
In all three cases, current/voltage curves (here represented by a **half scan**) are recorded. The working electrode potential ($= E_A$) here corresponds to the optimum analytical potential of a substance. You have to define this potential to ensure accurate analysis in ➤*DC Mode* and ➤*PULSE Mode*.

Practical Application with the Antec Detector DECADE

The analysis is performed in the DC mode or the PULSE mode. If the peak of a substance is detected for which the analytical potential is to be determined, stop the pump flow, switch to the SCAN mode, and slowly change the potential. When the final value is reached, return to the previous mode and reset the pump flow to its original value.

The entire process can be performed by a key click if the required commands are available on a script button.

On the signal plot of the ➤*Control Panel*, the following curve can be observed.



Unlike the pump, the chromatogram run time is not stopped. The retention times of the subsequent peaks will be shifted accordingly. However, the signal height of the peaks will be lower as part of the substance is "used up" during the **Scan** mode.

The optimum analytical potential cannot be directly read on the y-axis (time axis), but is determined with the printed chromatogram.

SDK (Software Development Kit)

A Software Development Kit (SDK) is provided 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 the **Administrator Manual: How to ...: Actions in the Browser and in the Operating System**  **Using Chromeleon Data in an External Program.**

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 always interpreted in the installed dimension; for example, in mAU.



Note: Always consider this parameter in combination with the \Rightarrow Peak Slice detection parameter!

For information about how to apply the detection parameters, refer to **How to ...: Actions in the QNT Editor**  **Modifying Detection Parameters** and \Rightarrow Sensitivity

Sensitivity (Signal Parameter)

The **Sensitivity** parameter adjusts Chromeleon to the \triangleright Signal-to-Noise Ratio of the detector. The RF 2000 fluorescence detector supports the values LOW, MED (default), and HIGH.



Sequence

A sequence combines \triangleright 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 \Rightarrow Sample Variables, e.g. Name, Inj. Date/Time or Type, etc.)
- \triangleright Sequence Report Columns to display sample results
- \triangleright 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 ...: Actions in the Browser**  **Creating a Sample List (Sequence)** and \Rightarrow 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)	Area_P	Amount_P
		Uv_vis_1	[min] Pyrene Uv_vis_1	[mAU*min] Pyrene Uv_vis_1	[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
⌘	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 ...: Actions in the Browser**




Creating a Sequence Report Column.


(Chromatography) Server

PCs connected to the components of a chromatography system via a serial interface 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. Besides, 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 have to configure the server. You can do this in the Chromeleon ➤ *Server Configuration* program on any client PC. (Also, refer to the **Administrator Manual: Software Installation and Communication**  **Chromeleon and Windows Operating Systems**.)


If the Chromeleon server software and the Chromeleon client software are installed on the same PC, this is referred to as local installation. If they are installed on different PC's, this is referred to as 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.

For more information, refer to the **Administrator Manual: How to ...: Actions in the Server Monitor Program**  **Setting Up the Chromeleon Server for Network Access**.

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 the **Administrator Manual: Software Installation and Communication**  **The Server Configuration Program**.)

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 the **Save Installation** command.

For more information, refer to the **Administrator Manual: How to ...:**  **Actions in the Server Configuration Program**.

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 the **Administrator Manual: Installation**  **The Server Monitor Program**.

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 that an individual card or interface has to be available for each device from which signals are recorded and to which signals are sent, respectively, the cards or the interface provides several channels.

The different devices need not necessarily be part of the same ➤ *Timebase*. To enable, for example, signal recording by detectors from different timebases the PC plug-in cards or the interface should not be assigned to one specific timebase. Instead, the devices of different timebases address the same card or interface; i.e., the card or interface is shared between these 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.

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 the **Add Device** command 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.

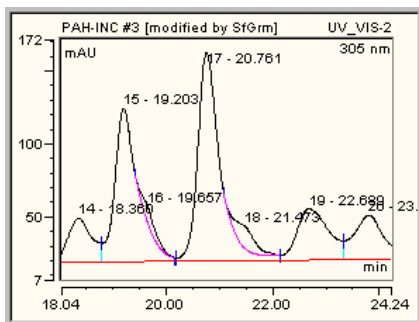
Each of the two tab pages indicates the current assignment of the relays and/or remote inputs that are currently used. If there is no entry on the list, either no **Sharable Device** component has been installed or the relays and remote inputs have not been assigned. Click **ADD** to install a new relay or remote input.

(Peak) Shoulder

A peak shoulder (or short: 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 their retention time. Similar to main peaks, the time of the signal maximum is defined as peak retention time for rider peaks. 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 have to correspond to the time of the signal maximum.

Chromeleon usually skims peak shoulders in an exponential way:



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

See ➤ *Noise*

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	⇒ <i>Average</i>	Fluorescence channel	<i>Average</i>
	⇒ <i>Bandwidth</i>		<i>EmWavelength</i> (see ➤ <i>Emission</i>)
	⇒ <i>RefBandwidth</i> (<i>Reference Bandwidth</i>)		<i>ExWavelength</i> (see ➤ <i>Excitation</i>)
	⇒ <i>RefWavelength</i> (<i>Reference Wavelength</i>)		➤ <i>Gain</i>
	⇒ <i>Step</i>		➤ <i>Response</i>
	⇒ <i>Wavelength</i>		➤ <i>Sensitivity</i>
			<i>Step</i>
3D field	⇒ <i>BunchWidth</i>	Electrochemical channel	<i>Average</i>
	➤ <i>Min/Max Wavelength</i>		➤ <i>Data_Collection_Rate</i>
	<i>RefBandwidth</i>		<i>Step</i>
	<i>RefWavelength</i>	Pressure channel	<i>Average</i>
	<i>Step</i>		<i>Step</i>
MS Channel	<i>FilterIndex</i> (see ➤ <i>Filter</i>)	Virtual channel (see ➤ <i>Virtual Signals</i>)	<i>Average</i>
	<i>Min/Max Mass</i>		<i>Formula</i>
	➤ <i>Range</i>		<i>Step</i>
	<i>Reset</i>		<i>Type</i>
	➤ <i>Smoothing</i>		
	<i>SmoothingPoints</i>		

(Also refer to: **How to ...: Actions Related to 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 are the possible signal variations that can be recorded. As a general rule, the average of a signal should be at least twice, better three times the standard deviation of the signal value.


The signal-to-noise ratio of the Dionex UVD 340U ➤ *Photodiode Array Detector* is 1,500 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 only be realized with an empty flow cell and a new but burnt-in lamp (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 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 ...: Actions in the Chromatogram**  **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 ...: Actions Related to Mass Spectrometers**  **Extracting Mass Traces Afterward**). If you record data in full-scan mode, you can later extract the desired 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 the **Administrator Manual: How to ...: Actions Related to Mass Spectrometers**  **Defining the Number of MS Channels**.

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.

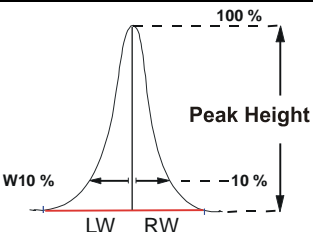
If calibration points on different levels determine the position of the calibration curve, this is referred to as multiple-point calibration; for example, two-point, three-point, etc. calibration. The number of calibration levels determines possible calibration types.

In both cases, the number of replicates is irrelevant for the number of calibration levels. Replicates only serve to statistically secure the measuring results.

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*.

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 ...:**

Actions in the Chromatogram  **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...: Actions Related to Mass Spectrometers**  **Extracting a Mass Trace**.

For more information about the different filter types and their parameters, refer to **Integration**  **Data Smoothing**.

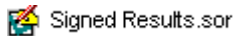
Snap to Grid

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:



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 serve for 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 are freely editable by the user and that 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 \Rightarrow <i>Bandwidth</i> 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 \triangleright <i>Retention Index</i> of the substance.
Kovats Index	If available, the column indicates the \triangleright <i>Kovats Index</i> of the substance.
Acq.Step:	This column indicates the Step with which the underlying \triangleright <i>3D Field</i> was recorded.
Detector:	This column indicates the detector with which the spectrum was recorded. The name is taken from the Server Configuration .
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.

Column	Description
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 ➤ <i>PGM File</i> 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 \Rightarrow *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 \Rightarrow *Std. Add. Group* column. In this column, the samples are assigned to a common standard addition group.

We recommend always spiking unknown samples with the same amount of the same substances. In the \Rightarrow *Ref. Amount Set* column, you can then assign the spiked samples to the same amount values in the **Amount Table** of the QNT Editor.

For more information, refer to **How to ...: Actions in the QNT Editor: Calibration**  **Standard Addition**.

SQL (Structured Query Language)

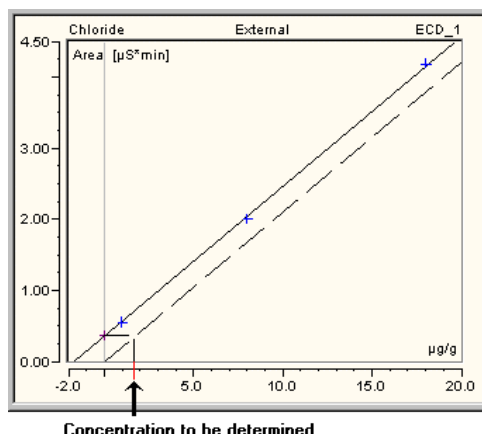
SQL is a computer language developed especially for queries in databases. Chromeleon uses an adapted SQL for ➤ *Queries* in the Browser (queries for specific samples/sequences), as well as for "Database Queries" in the report and in the ➤ *Printer Layout*.

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 more reliable results, you can spike the unknown sample 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.


For more information, refer to **How to ...: Actions in the QNT Editor: Calibration**  **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 > 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 the **Administrator Manual: How to ...: Actions in the Server Configuration Program**  **Adding, Configuring, or Deleting Components**).

To save chromatography data on the network, you can select a network datasource as standard datasource. For more information refer to the **Administrator Manual: How to ...: Action in the Browser and in the Operating System**  **Saving Chromatography Data on the Network** and  **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 Protocol\Server1

Network Datasource:Daily Protocol\Server2

Standard Deviation

The standard deviation is a measure for the deviation of single values from a mean value. The standard deviation of a random sample is defined as:

$$SD = \sqrt{\frac{n * \sum_{i=1}^n x_i^2 - \left(\sum_{i=1}^n x_i \right)^2}{n * (n - 1)}}$$

Where:

SD = Standard deviation

n = Number of elements for which the standard deviation is calculated.

x_i = Elements for which the standard deviation is calculated.


The square standard deviation is referred to as ➤ *Variance*.

Status Bar

In the status bar on the lower window margin, messages regarding the current system status are shown on the left, while the current timebase and the currently executed command are shown on the right.

The display of the status bar can be enabled and disabled on the **View** menu.

Step

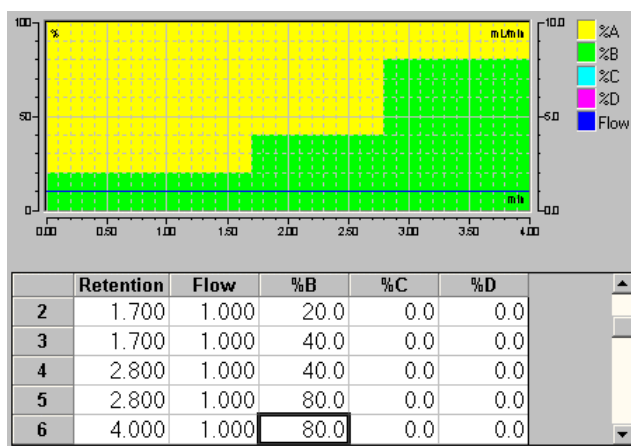
The time interval between two successively recorded data points is referred to as the step; the sum of all recorded data points is called raw data. The reciprocal value of the step value is the ➤ *Sampling Rate* (if the data is supplied by an A/D converter) or, by default, the ➤ *Data Collection Rate* (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:



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 \Rightarrow *StopFlow*

Suck

See \triangleright *Draw*

Sucked

See \triangleright *Ready*

Summit HPLC System

The Dionex HPLC product line is called Summit HPLC System. The system comprises the following devices:

Device	Name	Description
Pump	P680 P580	HPLC gradient pumps
Autosampler	ASI-100	Temperature control option available (ASI-100T)
Column	TCC-100	Temperature range: 5-85°C (41°-185°F)
Thermostats	STH-585	
Detectors	UVD 170 UV-VIS	\triangleright <i>UV Detector</i>
	UVD 340 PDA	\triangleright <i>Photodiode Array Detector</i>
	PDA-100	Photodiode array detector
	RF2000	Fluorescence detector
	(Shodex RI-101)	(Refractive index detector)
	(aQa MS, MSQ)	(\triangleright <i>Mass Spectrometers</i>)

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: **How to ...: 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. Many autosamplers support several syringe types and thus allow using a different type with a larger or smaller volume. In addition to the default types 25, 50, 100, 250, 500, 1000, and 4000 μl , using special syringes is possible. If the syringe type is changed, the new type must be specified in the Chromeleon **Server Configuration** program. In addition, when installing a new autosampler, the currently used syringe type must be defined by configuring the corresponding device driver.



Tip:

For the autosampler Dionex GINA 50, syringe types with the volumes 250 μl and 1000 μl are supported. If using different syringe types, the reproducibility stated in the instrument's specification cannot be guaranteed.

System Suitability Test (SST)


As defined in cooperation with EURACHEM, System Suitability Checking (SSC) or 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]. Aim and objective of System Suitability Testing is to ensure the performance of the operating system and the system.


SST or SSC can be applied to single measuring processes and thus may be part of the validation process. The System Suitability Test establishes for example that the operational conditions required for a specific measurement process are being achieved.



Tip:

*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!*

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 ...: Actions in the QNT Editor**  **Defining the System Suitability Test**.

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 Summit HPLC product line 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 Summit HPLC product line have been 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.


For more information about System Wellness, refer to **Validation, AutoQ, and System Wellness**:



System Wellness for IC Devices (Overview)



System Wellness for HPLC Devices (Overview)

For more information about how to perform System Wellness procedures for IC devices, refer to **How to ...: Actions in the Browser**  **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 ...: Actions in the QNT Editor**  **Modifying Detection Parameters**.

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 DEFLTAD.RDF).

Theoretical Plates

The **Theoretical Plates** peak result variable is a measure for the separating capability of the column. Theoretical plates are calculated from the peak width and the corresponding retention time. As with asymmetry, there are different USP and EP standards:

Name	Calculation	Comment
Theoretical Plates	$TP = 5.54 * \left(\frac{t_R}{W_{50\%}} \right)^2$	EP standard
Theoretical Plates	$TP = 16 * \left(\frac{t_R}{BW} \right)^2$	USP standard
Theoretical Plates	$TP = 5.55 * \left(\frac{t_R}{W_{50\%}} \right)^2$	JP standard

Where:

TP	= Number of theoretical plates
t_R	= Retention time
$W_{50\%}$	= Peak width at 50% height (half-width)
BW	= ➤ <i>Peak Width</i> on the base line.

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 says more about the quality of the currently used column. It gives the number of theoretical plates per column meter.

$$\frac{1}{H} = \frac{TP}{L}$$

Select the report column, select the **Column Properties** command on the context menu, and then select the variable in the selection box. Click the **Parameter** button to determine whether the calculation is based on the EU, US, or JP standard.

**Note:**


EP = European Pharmacopeia

USP = United States Pharmacopeia

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 ...: Actions Related to 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 the **Administrator Manual: How to ...: Actions Related to Mass Spectrometers**  **Defining the Number of MS Channels**.

**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 \Rightarrow *Inj. Date/Time* (Time of Injection)).

Timebase

All components 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. It can consist, for example, of two pumps, one \Rightarrow *Autosampler*, one column oven, two detectors that are switched in series, and one \Rightarrow *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 \Rightarrow *(Chromatography) Servers*. Each server can serve up to six timebases. A timebase can have only one \Rightarrow *Photodiode Array Detector* installed. A maximum of two photodiode array detectors are permitted per server.

Specify the name of a timebase and the assignment of the devices in the \Rightarrow *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 the **Toolbars** command 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 \Rightarrow *Control Panels* and \Rightarrow *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* (enables/disables the Status Bar)

Move the mouse cursor on an icon to display its 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 only available 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 is to avoid 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 the **Administrator Manual: Chromeleon User Management**  **Setting Up the Transaction Agent**.

Transmission

Transmission is 100%, when light passes through the UV detector flow cell without restraint. 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

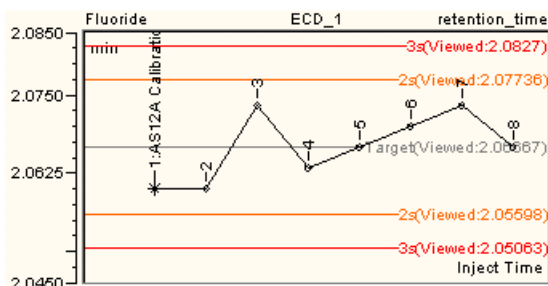
Chromeleon's trending feature provides a way for specific data to be graphically displayed from sample to sample. In many cases, you can more quickly view progressive changes, compare runs, or identify items of interest by looking at a graphical representation of different samples, rather than by examining 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 (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 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 PDA-100 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 device connected to the PDA-100 outputs a different signal type, select a different signal mode

The four input signal modes are:

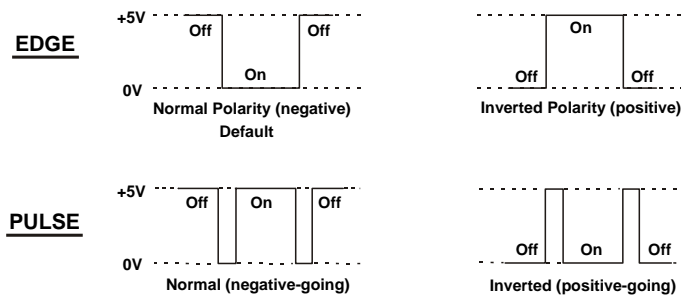
Normal Edge: In normal edge operation, the negative (trailing) edge of a signal turns on the function and the positive (leading) edge turns off the function.

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 of the PDA-100 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 \Rightarrow *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. We recommend using an instrument LAN for analog data acquisition (= installation of a second network interface card (= NIC; in the operating system = adapter) in the server PC; the **Administrator Manual: Installation Instructions**  **Connecting Dionex Devices via TCP/IP**).*

*Always connect instruments requiring a GSIOC cable, such as GILSON devices and VARIAN pumps, to the multi-serial 8-fold PCI interface card (Equinox 8-RS-232 Multi-COM card, Dionex part no. 5906.2095). (For more information about these devices, refer to the **Administrator Manual: Installation Instructions**  **GILSON** and the  **VARIAN**.) Otherwise, communication problems might occur with other RS-232 ports; for example,*

with the COM ports of the PC or the Dionex Universal Chromatography Interface (UCI). 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 the **Administrator Manual: Software Installation and Communication**  **The Dionex Universal Chromatography Interface (UCI)**

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.

Unspiked

Refer to ➤*Spiked Sempel*

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.

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*.

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 ...: Actions in the Printer Layout**



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, the Query Wizard allows you to select user-defined columns for the **Sample** field type. Click the arrow of 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 ...: Actions in the Browser**  **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 the **Import Column** command. This command imports an existing column from one datasource to another 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 ...: Actions in the PGM Editor**  **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 it is **not** automatically copied to the program directory.

The User Manager is password-protected. Only the system administrator or somebody with similar authority can open the program.



Tip:

*We recommend installing the Chromeleon User Management applications to a suitable network location 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 Manual also contains a section describing all steps and options for user management (refer to the **Administrator Manual**  **Chromeleon 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 **Datasource 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 **Administrator Manual: Software Installation and Communication**  **Access Control**.

UV Cutover

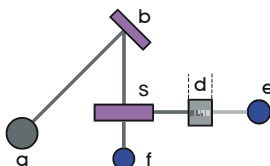
The **UV Cutover** command for the AD20 Absorbance Detector sets the wavelength above which the second order filter is inserted in the light path. Typically, the UV cutover wavelength is 380nm.

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 given 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 air bubbles in the solvent.
2. The solvent in the cell must not be fully absorbing in the wavelength range that will be 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 the **Disconnect** command, followed by the **Connect** command.

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, the ⇒Inject command should be placed 10 minutes (minimum) after the lamp is turned on. Note also that lamp(s) should never be turned off 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 \Rightarrow 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 will not be considered for calibration, though.

Validation samples can also be injected 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 ...: Actions in the QNT Editor**:  **Entering the Concentration/Amount of the Validation Sample** and  **Validating the Calibration Curve**.

Valley to Valley

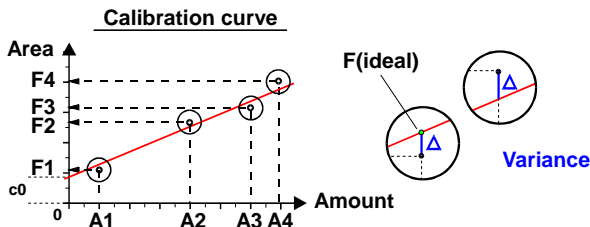
If the **Valley to Valley** detection parameter is enabled, the baseline is drawn from peak minimum to peak minimum (i.e., from peak end to peak end), below non-resolved peaks.

Also, refer to \Rightarrow Valley to Valley

For information about how to apply detection parameters, refer to **How to ...: Actions in the QNT Editor**  **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,
 X_i : X-value of the standard sample no. i ,
 Y_i : Y-value of the standard sample no. i ,
 W_i : Weight factor of the standard sample no. i , and
 Δi : $Y_i - F(X_i)$

Extracting the square root of the variance value results in the \triangleright **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** \triangleright **Variance** value. It is more meaningful 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 \triangleright **Coefficient of Determination** and in contrast to the \triangleright **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,


X_i: X-value of the standard sample no. *i*,


Y_i: Y-value of the standard sample no. *i*,



W_i: 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 **Administrator Manual: Installation Instructions**  **Installing the Virtual Channel Driver (VCD)**.

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 **How to ...: 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 at Dionex using an appropriate experimental design and then embedded into Virtual Column.

**Note:**

Results obtained with Virtual Column are intended only 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 licensed via a Chromeleon license bit. If you have not purchased the 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 ...:**

 **Actions in the Virtual Column Tool**

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 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 **How to ...: 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, the **⇒ Inject** command should be selected 10 minutes (minimum) after the lamp is turned on. Note also that lamp(s) should never be turned off during a sample batch.*

Void Time

See **➤ Dead Time**

Void Volume

See **➤ Dead Volume**

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: **How to ...: Device Control**:



Control: Autosampler



Special Commands, Relays, and Others

Also, refer to ⇒ *Wait*

Wash

The **Wash** command causes the autosampler to lower the needle into the needle seat and to rinse the sample loop and needle with solvent in the **Inject** state. This corresponds to the normal solvent flow following an ⇒ *Inject Command*.



Tip:

Use the **Wash** and ⇒ *NeedleUp* commands to wash the sample loop and thus prevent crystallization of substances in the sample loop.

Also, refer to ⇒ *Wash*

Waveform

A series of steps, defined as points on a plot of potential vs. time. A waveform must be defined when using the Dionex electrochemical detector in ➤ *Integrated Amperometry Mode*.

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 between 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. You can enter the wavelength also manually 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, which are fitted with a ➤ *Holmium-Oxide Filter*, perform a wavelength calibration after each *Lamp = on* command (see ⇒ *Lamp*) or ⇒ *Connect* command.

Dionex AD25 and PDA-100 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 the single 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 **How to ...: Device Control**  **Determining Wavelength Switching**.

Weight

Also, see ➤ *Sample Weight Factor (Weight)* and ⇒ *Weight* (Sample Weight Factor).

Weights

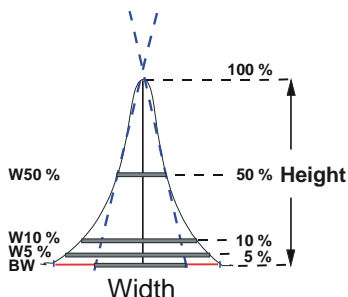
The **Weights** calibration variable indicates the weighting (see **How to ...: Actions in the QNT Editor**  **Weighting and Averaging Calibration Points** in the **Creating a Peak Table** section) assigned to the individual ➤ *Calibration Points* when creating the calibration curve.

Define the weighting in the ⇒ *Calibration Type* column of the ⇒ *QNT Editor*. The following options are available:

No weight	Default: higher weighting of higher amounts.
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 ➤ <i>Calibration Points</i> 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).

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**.



Chromeleon also determines the peak width at 5, 10, and 50% of the peak height (abbreviations: W5%, W10% and W50%).



Tip:

*In contrast to the base width **BW**, the peak widths at 5, 10, and 50% of the 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!*

Determining the peak width is only possible if the peak is resolved at least to half the height.

How to display the peak width in the report

- Select the column in the report
- Select the **Column Properties** command on the context menu.
- Select the **Width** variable in the selection box.
- Click the **Parameter** button 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. In addition, the value can be interpreted as absolute or relative value:

Also, refer to \Rightarrow *Window*

Wizard

Various input procedures such as creating a \triangleright *Sequence*, or a \triangleright *Program* or defining \triangleright *Query* conditions are facilitated by using **Wizards**. The system prompts the user to define conditions and to enter the required information. The Wizard then adds default elements and thus completes a basic structure. If required, the user can extend or modify this structure according to individual requirements.

Use the Wizards to avoid unnecessary typing, syntax errors in command entries and overlooking important information and parameters.

Worklist

The format for reading data (sequences) of a \triangleright *LIMS Chromeleon* is referred to as Worklist (WLE file). The worklist describes the contents and the structure of a sequence (also, refer to \triangleright *Worklist Format*).

Select the **New...** command on the **File** menu, and then select the **Sequence (From LIMS Worklist)** command to import an existing worklist.

If importing is possible, the imported sequence is listed in the Browser. The existing worklist is deleted to prevent importing it again.

If an error occurs during the import, Chromeleon reports an error describing the error and the error 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**. The incorrect worklist file is renamed to ***.err**.

Worklist Format

Worklists have the file extension **.wle** (Work List Export). The structure and the syntax of the ➤ *Worklists* are similar to the Windows **INI** files. There are various sections. Each section starts with a new line. The name of the section is written in brackets. There are one or several entries below the section name, with the syntax *name = value*. Comments start with a semicolon and finish at the end of the line.

Example of a valid worklist (+ comments - delete the comments to use the worklist in Chromeleon):

```
; 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 = pgm0815  
; default for the QNT Method column.  
qnt = qnt0815  
; 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=NOINT

; 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=NOINT

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 given in the section '[PGM File]' or '[QNT Files]'. 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 makes no difference 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 the Chromeleon. If there is a different entry here, the worklist will not be accepted by the Chromeleon. In this case, no log file is created, and the worklist is neither deleted nor 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 neither deleted nor 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 in *.err in the event of an error.

Character Set = Windows indicates the character set of the worklist. Any generated log file is also written with this character set. 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 the **Autosave Workspace** command 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. Two XC setup programs are provided on the software installation CD. For installation details, refer to the **Administrator Manual: How to ...: Actions Related to Mass Spectrometers**  **Installing MS Components**.

To install the aQa mass spectrometer and/or to display aQa data, install Xcalibur Rev. 1.2 on your PC.

To install the MSQ spectrometer and/or display MSQ data, 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 ...: Actions Related to 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.

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