ChromQuest™ 4.2 Chromatography Data System

User's Guide

CHROM-97202 Revision A April 2005

Analyze • Detect • Measure • Control™

Thermo ELECTRON CORPORATION

Software Version: ChromQuest 4.2

Contents

Preface

About This Guide

Welcome to ChromQuest™4.2. The ChromQuest chromatography data system is a member of the Thermo Electron family of LC data systems.

Whether you are a new ChromQuest 4.2 user or are upgrading from a previous version of ChromQuest, we think you will find the features of ChromQuest 4.2 both powerful and well organized. To make your use of ChromQuest 4.2 as easy as possible, this manual is designed to be a "How To" guide for the tasks you would normally perform using your data system. Technical details such as how integration is performed and equations used are located in the *ChromQuest Data System Reference Guide*.

In addition to this manual, ChromQuest 4.2 comes with a comprehensive on-line Help system that is at your fingertips.

If you are using ChromQuest 4.2 for the first time, you might want to perform the tutorial contained in Chapter 2.

If you are using ChromQuest 4.2 to control a Surveyor Plus instrument, you might want to perform the tutorials contained in the *Surveyor Plus Getting Started with ChromQuest* manual.

Related Documentation

In addition to this guide, Thermo Electron provides the following documents for the ChromQuest chromatography data system:

- Installation Guide
- Administrator's Guide
- Reference Guide
- QuickStart

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

IMPORTANT Highlights information necessary to avoid damage to software, loss of data, invalid test results, or information critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Helpful information that can make a task easier.

Contacting Us

There are several ways to contact Thermo Electron Corporation.

Assistance

For new product updates, technical support, and ordering information, contact us in one of the following ways:

Visit Us on the Web

www.thermo.com/finnigan

Contact Technical Support

Phone: 1-800-685-9535
Fax: 1-561-688-8736
E-mail: techsupport.finnigan@thermo.com

Find software updates and utilities to download at http://mssupport.thermo.com

Contact Customer Service

In the US and Canada for ordering information:
Phone: 1-800-532-4752
Fax: 1-561-688-8731
Web site: www.thermo.com/finnigan

Changes to the Manual and Online Help

To suggest changes to this guide or to the online Help, use either of the following methods:

- Fill out a reader survey online at www.thermo.com/lcms-techpubs
- Send an e-mail message to the Technical Publications Editor at techpubs.finnigan-lcms@thermo.com

Chapter 1 Basics of Operation

This chapter describes the basic operation of the ChromQuest 4.2 chromatography data system, its file structure, the features of the application windows, and the features of the chromatogram windows.

This chapter contains the following sections:

Main Menu Window

ChromQuest opens to the Main window shown below after you choose **Start > All Programs > Chromatography > ChromQuest** from the taskbar or click on the desktop shortcut icon. System administration and instrument configuration are performed from this window.

For information on administrating the ChromQuest chromatography data system, refer to the *ChromQuest Chromatography Data System Administrator's Guide*. For information on configuring your Thermo LC (SpectraSYSTEM LC) or your Surveyor Plus LC, refer to the relevant sections at back of this manual.

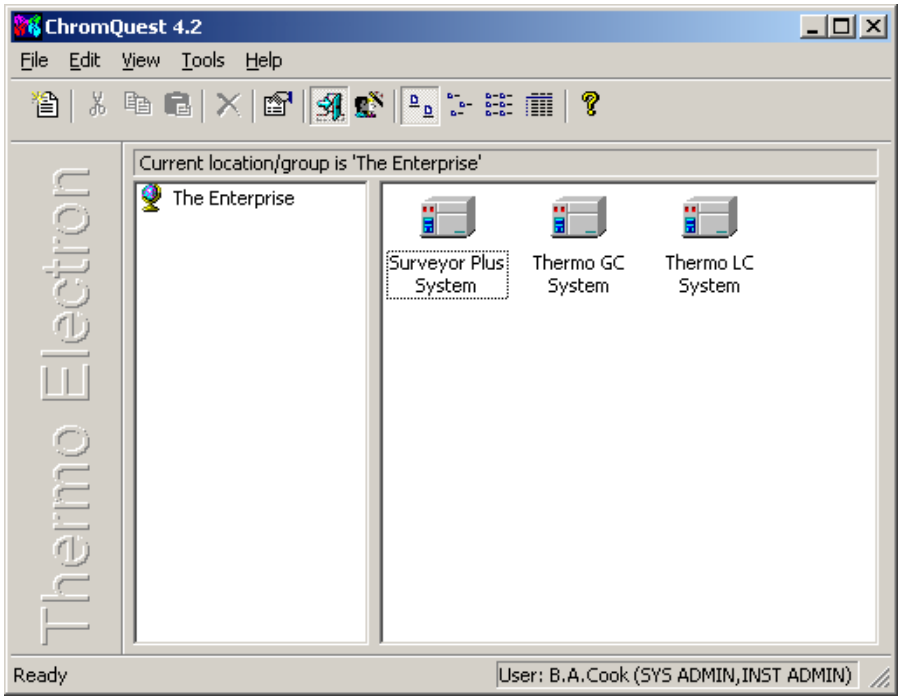


Figure 1. ChromQuest 4.2 main window

Instrument Wizard

Each time you start an instrument application (by double-clicking the instrument icon from the Main window), an **Instrument Wizard** will appear. This wizard is designed to direct you to the basic functions of the instrument window.

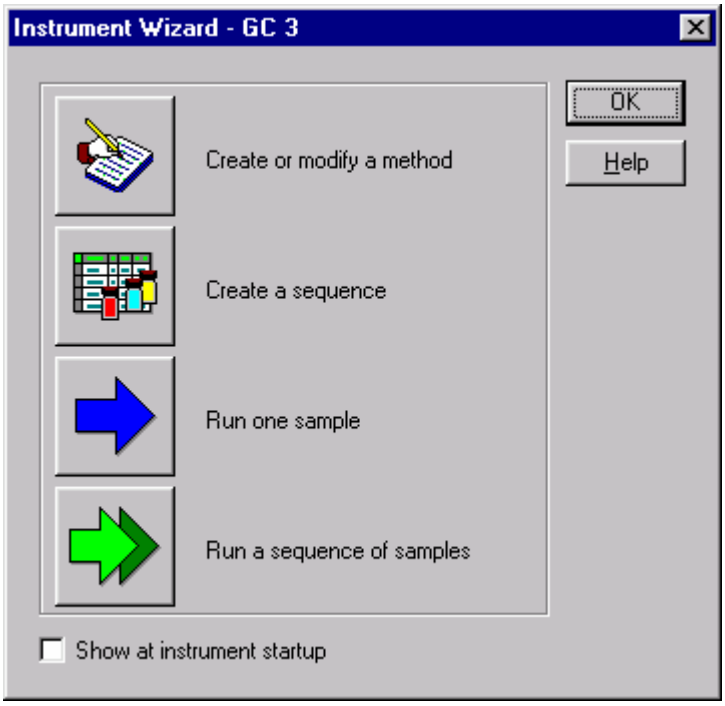


Figure 2. Instrument Wizard windw

- Create or modify a method

  This button starts the **Method Wizard** that will enable you to step through creating or modifying a method.

- Create a sequence

  This button starts the **Sequence Wizard** that steps you through creation of an acquisition or reprocessing sequence.

- Run one sample

  This button opens a dialog where you can use a stored method to run a single sample.

- Run a sequence of samples

  This button opens the Run Sequence dialog where you can start data acquisition using a stored sequence.

- Show at instrument startup

  If this box is selected, the Instrument Wizard will appear each time this instrument is started.

Offline Instrument Wizard

If you are using an instrument offline, the Instrument Wizard will display the following buttons, for creating methods, sequences, or processing a stored sequence.

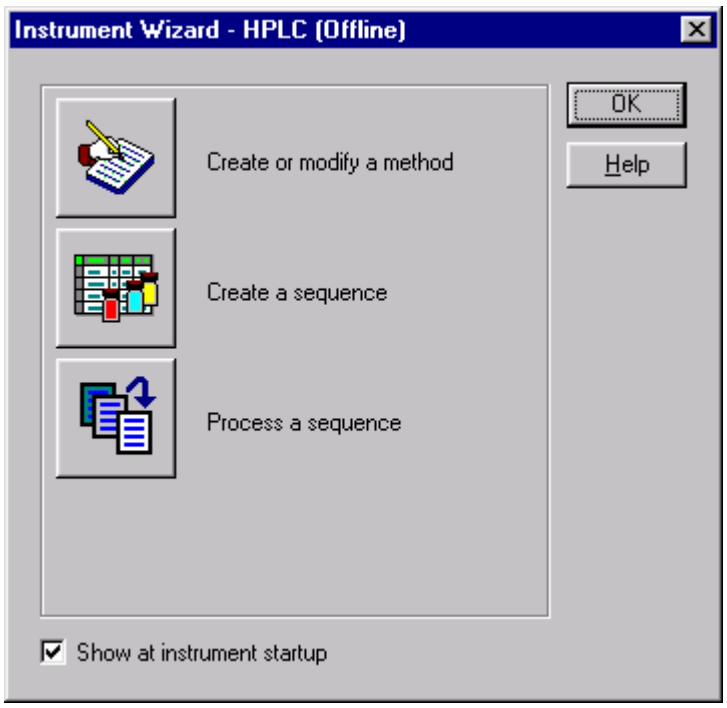


Figure 3. Instrument Wizard (Offline) window

Program Architecture and Data Structure

ChromQuest is divided into several application areas. Instrument application areas are used for development of methods and sample sequences when access to the instrument itself is required. This is also where you have access to the chromatograph and real-time data. The offline processing area is available for developing methods; creating and using sample sequences for reprocessing of stored data while the instruments are in use for sample acquisition, or method development. System Administration and Board Configuration are available for setting up system resources.

Method File Structure

A method is used whenever you acquire and/or reprocess a data file. It contains instructions for data acquisition (run time, sampling rate, and so on), integration, calibration and peak information, and reports, as well as optional functions such as data export and user programs. Each method is capable of acquiring multiple independent channels of data from a single chromatograph. Each channel can have its own complete independent parameters, including sampling rate, run time, integration events, external events, calibration, and reporting.

Although the method file is a separate file, the information contained in the method is saved in the raw data file at time of acquisition. This way, the original method can be reproduced, even if the method file was subsequently modified.

Data File Storage in Networked Operation

When running samples, the file is stored locally in a temporary file. At the end of each run, the file is copied to its final destination on the Windows 2000/XP Server. If this operation is not successful, an error log is generated. A watchdog program that periodically checks the network monitors this error log. As soon as the network is up the files queued on the local driver are copied to the Windows 2000/XP Server.

Note If the network is down, the file is not analyzed and no report is generated. After the network comes back up, the user will need to reprocess the data to get his/her reports.

Data File Structure

A data file is created on the designated drive whenever you acquire a sample using ChromQuest, or when you save a data file using the **Save As 32-bit** command. The file contains the following information:

- File Information Header

  This contains information such as the date and time of acquisition.

- Complete method parameters used to acquire and process the data (this is the "original" method saved only when the data is acquired).

  Because you can acquire multiple channels of data simultaneously on a given chromatograph, the method section may contain complete parameters for more than one channel.

- Raw data points for the run saved

  Multiple chromatograms may be present in a single data file, each of which represents a detector channel acquired for the run. The raw data points are saved in binary format.

- Results

  The original integration results are saved in the file and can be recalled later when the file is opened. In addition, the most recent analysis results and method are also saved in the data file and updated whenever you analyze. The Sample ID for the results is also saved, as are manual integration fixes.

- File Description

  If you entered a description for the file, this text information is stored with the file, and can be viewed under the Data File Properties or from the Open Data File dialog.

- Instrument Configuration

  The configuration of the instrument used to acquire the data file is saved.

- Data File Audit Trail

  An audit trail log is always saved in the data file that tracks analysis of the data.

  Data files are saved using the file name and extension you specify when you initiate the data acquisition. The limit on file name length is 255 characters, including path.

- Data File Checksum

  If Extended Security is enabled, a checksum is calculated for the entire file whenever the data file is closed. When the file is opened, its checksum is verified first. If the check fails, the file cannot be opened and an error message will appear in the instrument activity log. Checksum verification, when enabled, is enterprise-wide. The checksum feature is enabled from the **Tools > Options > General** dialog in the Main menu, and is labeled **Extended Security**. The default mode for this feature is **On.**

GLP (Good Laboratory Practices)

In order to adhere to good laboratory practices, ChromQuest does not normally let you over-write a data file. If you try to over-write an existing data file name, the system will either give you an error message, or trigger a failure action if encountered during a Sequence operation. If you need to re-use a data file name, use Windows 2000/XP utilities to rename the file and/or store it in an alternate location on your disk.

If, for some reason, you wish to ignore GLP and have access to over-writing of data files, your files must be located in a directory whose path contains the term "public". For example, if your data files are saved in a directory entitled "C:\Public\Data", the ChromQuest files saved in this directory can be overwritten.

Extended Security

This selection is ON by default. When this option is selected, causes a checksum to be calculated whenever a data file is closed. When the file is subsequently opened, its checksum is verified first. If the check fails (the calculated checksum for the file does not match the one previously calculated for the file) the file cannot be opened, and an error is posted in the instrument activity log. Checksum verification is enterprise-wide.

In addition, the Extended Security function provides additional security to the enterprise in the following ways:

- All system administrators have full access to everything.
- All non-system administrators will have read/execute rights to project directories for which they have rights.
- All non-system administrators will have read/write/execute rights to project subfolders for which they have rights.

This means that users without system administrator rights will not be able to create subdirectories or files under the project directory, and they will not be able to rename or delete files under the project subfolders. Directories can still be created in project subfolders, but only through ChromQuest.

Audit Trail

Each Method file can have an Audit Trail enabled. When this is enabled, changes to the method will be logged in the file and cannot be removed or overwritten. Once the method audit trail is enabled for a method, it cannot be turned off for that method. To view the changes logged in the Audit Trail for a method, use the **File > Method > Audit Trail** command to display the Audit Trail listing for the current method. If the audit trail option is turned on for the current method, this box will display the logged changes to the method.

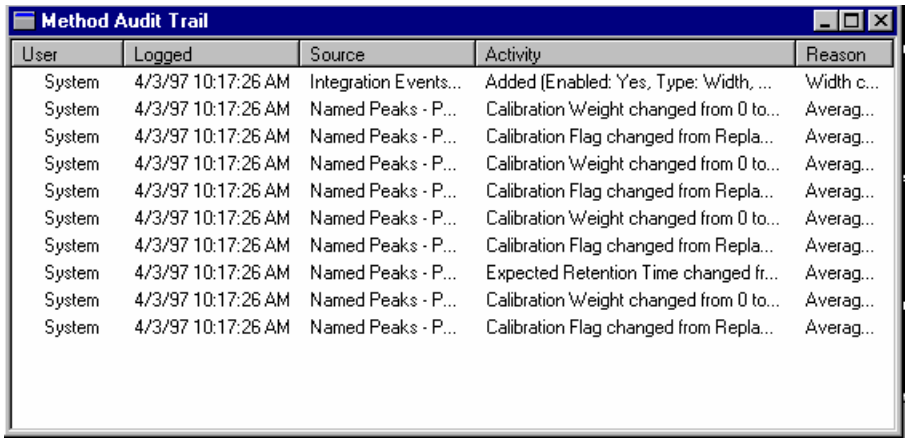


Figure 4. Method Audit Trail window

- User

  The user who was logged into the system at time of the change.

- Logged

  The time the change was logged into the system.

- Source

  The method location of the change – i.e. peak table.

- Activity

  The change that was made.

- Reason

  The reason for the change, if changes were logged.

To view the details of a given entry, click on the entry to highlight it, and then click the right-hand mouse button.

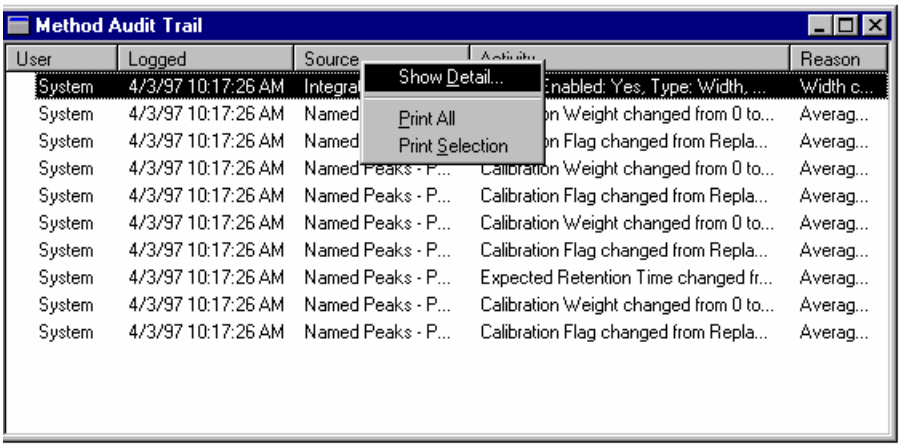


Figure 5. Method Audit Trail window - Show Detail menu

Click the **Show Detail** command. Details of the highlighted entry will appear.

Opening Data Files

Whenever you open a file using *ChromQuest,* you will be presented with a dialog box that allows you to not only open the file, but also specify parameters for searching, as well as previewing file contents. The **Open File** button ( ) on the command ribbon gives you access to the Open File menu.

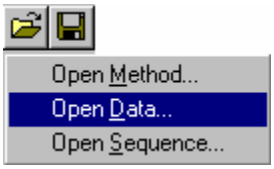


Figure 6. Open File menu

When you select one of the types of files to open, a dialog will appear where you can select the file from those on your hard disk.

The **Open Data File** dialog contains the most options for how you want to open and search for files. The list of files of the type selected (**Files of type**) is shown. As with most Windows NT applications, you can view these as a list, and show details by clicking the appropriate button at the upper right corner of the window. In addition, you can see a preview of the chromatogram in the data file by clicking on the **Preview Chromatogram** button, or view the file description by clicking on the **Description** button. You can also use the * "wildcard" character to view a list of certain file types.

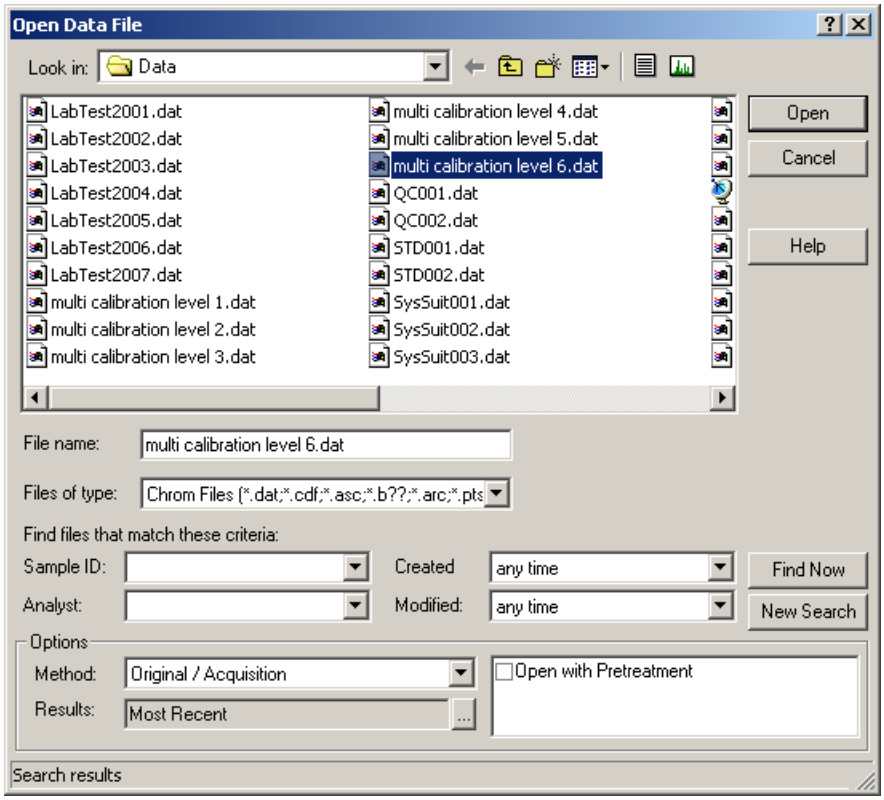


Figure 7. Open Data File dialog box

- Open Data File Options

  The Options box allows you to save time by loading additional information at the time the data file is opened.

  – Method

    If you select **Current**, the current method will not change when you open the data file. When one of the other Method options is selected, the method selected will be loaded at the time the data file is opened. **From Results** loads the method used to create the most recent results in the data file. **Original/Acquisition** loads the method used for the original acquisition of the data file. This method will replace your current active method.

  – Results

    When one of the Results options is selected, the data file will be opened along with the selected results. When a data file is opened with results, the integration and baselines that generated those results will be displayed automatically when the chromatogram is drawn on the screen. If **Previous Last Results** are selected, the data file will be opened with the results from the last time the chromatogram was analyzed. If the **Save all analysis**

results option is turned on (**Tools > Options > General** tab accessed from the Main Menu screen), a list of all analysis results will be available for you to open with the file.

– Open with Pretreatment

When this box is selected, the pretreatment file (if applicable) used at the time the data was acquired will be opened when the data file is opened.

- Searching for Data Files

If you are interested in specific data files, there are options that allow display of only the files of interest. Using the area titled "Find files that match these criteria", you can search for files that contain specific information. You can specify all or part of a **Sample ID**. You can search for files acquired by a designated **Analyst**. You can find files that were **acquired** during a specific time frame such as **Yesterday, Last 7 Days**, and **Today**. You can also search for files that were modified during a specific time frame. These criteria can be used one at a time or combined.

You can also include wildcards as part of the file name to search. To do the search, fill in the field of interest for files you want to search, and then click **Find Now**. For example, if you enter **Tester*** in the Sample ID field and click **Find Now**, all the files where the Sample ID is "Tester" followed by anything will be displayed. Click the **New Search** button if you want to clear the search settings and use new criteria for searching.

Opening Method and Sequence Files

The Open dialog boxes for Method and Sequence files are identical.

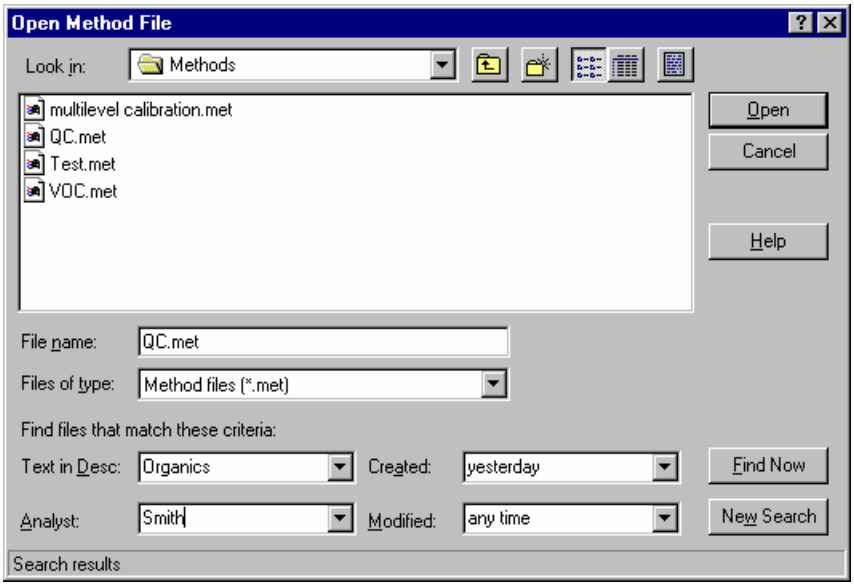


Figure 8. Open Method File dialog box

Searching for Method and Sequence Files

The criteria you can use to search for specific method and sequence files include selection of specific text found in the file description (**Text in Desc.**), **Analyst** name, and date **Created** or last **Modified**.

Saving Files

To save a current Method or Sequence file using the current method or sequence name, click the **Save** button and select the type of file you are saving. If you want to save a file using a new name, select **File** command and the type of file to save, then **Save As** and specify the new name.

Saving Data Files

The **File > Data > Save As 32-bit** command will save the current data file along with the current method in a single file. This command is only enabled when the current data file is not in 32-bit ChromQuest data format (such as converted files). In order to comply with good laboratory practices, you will not be allowed to **Save As 32-bit** using the same name as an existing data file, unless the file is located in a "Public" directory. A Public directory is a directory where the path contains the term "public". Data files in all other ChromQuest directories are protected from being over-written.

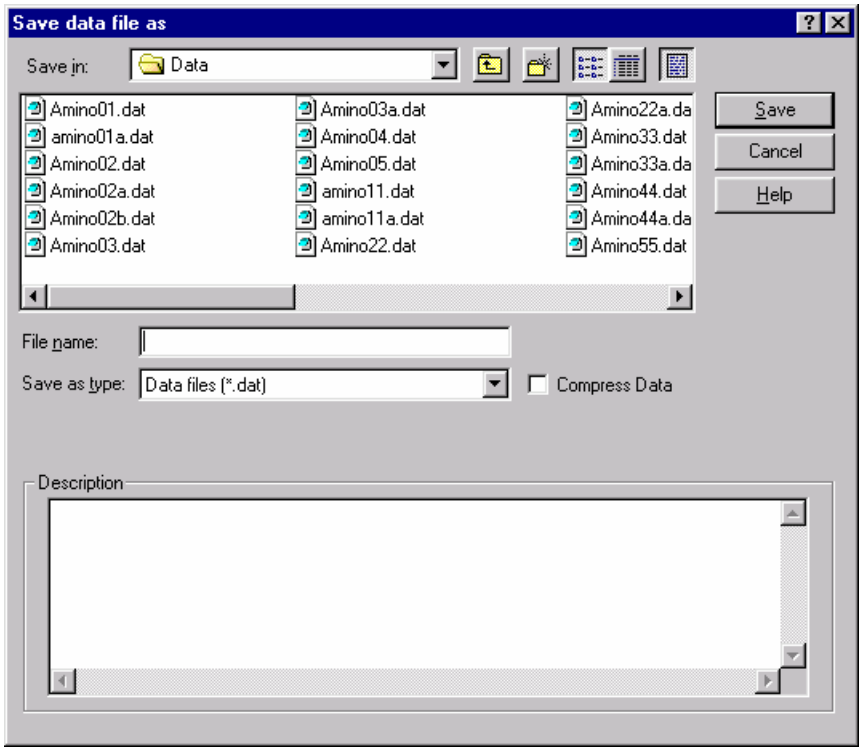


Figure 9. Save data file as dialog box

To save the current data in a new data file, type the name of the new data file in the **File name** field, then click on **Save**. Use the buttons at the upper right of the dialog box to view **details** of a highlighted file, or to view the **description** of a highlighted file. An entry "Saved from <FILENAME>" will be logged into the saved file as the first entry.

If the **Compress Data** box is selected, the file will be saved in a compressed format. Once saved in compressed format, it will automatically be "decompressed" whenever the file is opened. However, once a file is saved in compressed format, you must do a "save as" command to save it in decompressed format again.

Print Instrument Configuration

This will cause the current instrument configuration to be printed on the default printer.

Recent Method Files

When you choose **Files > Recent Method Files** the most recently used method files will appear in this list. You can change the number of recent files listed, or turn this feature off completely using the **View > Preferences > Files** tab.

Recent Data Files

When you choose **Files > Recent Data Files** the most recently used data files will appear in this list. You can change the number of recent files listed, or turn this feature off completely using the **View > Preferences > Files** tab.

Recent Sequence Files

When you choose **Files > Recent Sequence Files** the most recently used sequence files will appear in this list. You can change the number of recent files listed, or turn this feature off completely using the **View > Preferences > Files** tab.

Selecting a New Project

When the login and project management is turned ON, you must select a project when you log into an instrument. This project becomes the current project for your instrument. You can change the project using the **File > Select Project** command. When you select this command, the following screen will appear where you can select a project from a list of projects to which you have rights.

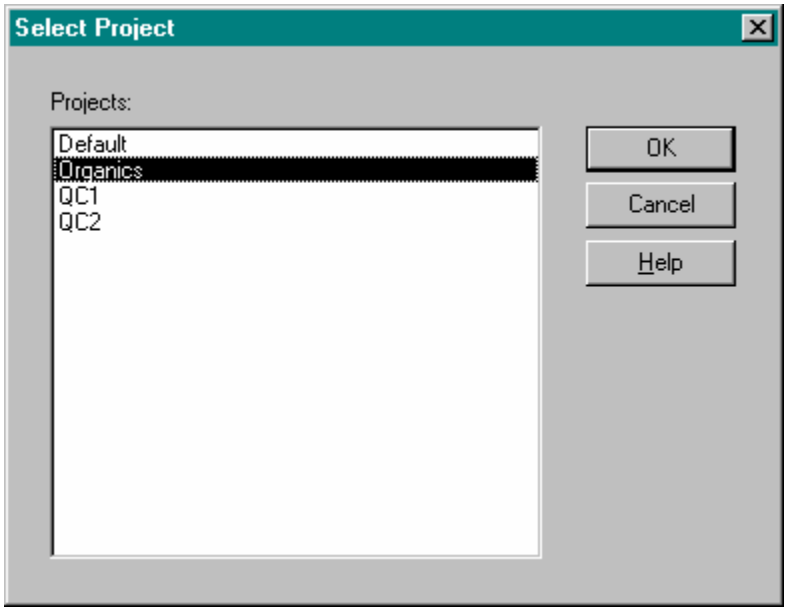


Figure 10. Select Project dialog box

Click on the desired project to highlight it, and then click on **OK**. The selected project will become the current project for the instrument.

Features of the Instrument Window

The application windows for Instruments and Offline Instruments are very similar, with certain aspects that are common to all. You can customize the appearance of the application window if you choose, adding or removing the Toolbars and Status information. However, these features are designed to make ChromQuest easier to use, and most users will prefer to have these turned ON.

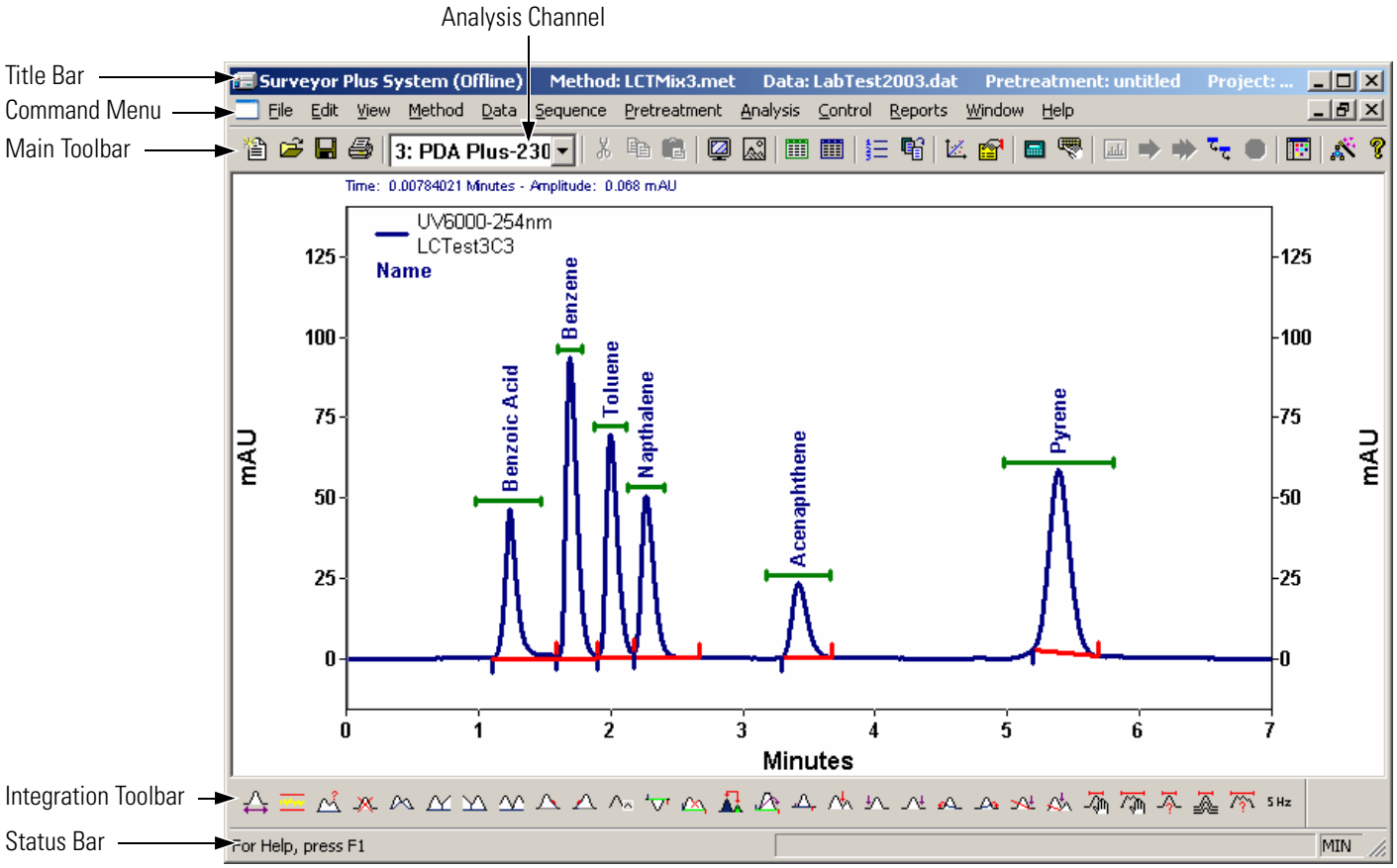


Figure 11. Instrument window

View Preferences

The appearance features are accessed with the **View > Preferences** command. The following dialog box will appear.

Preferences - General

This tab is used to set up general preferences in the instrument window.

- Toolbar options

  For each area of the window listed, you can turn on or off the Toolbar and Tooltips if available. Click on the toolbar area, and then check the **Show toolbar** and **Tooltips** boxes to enable your choices for that area.

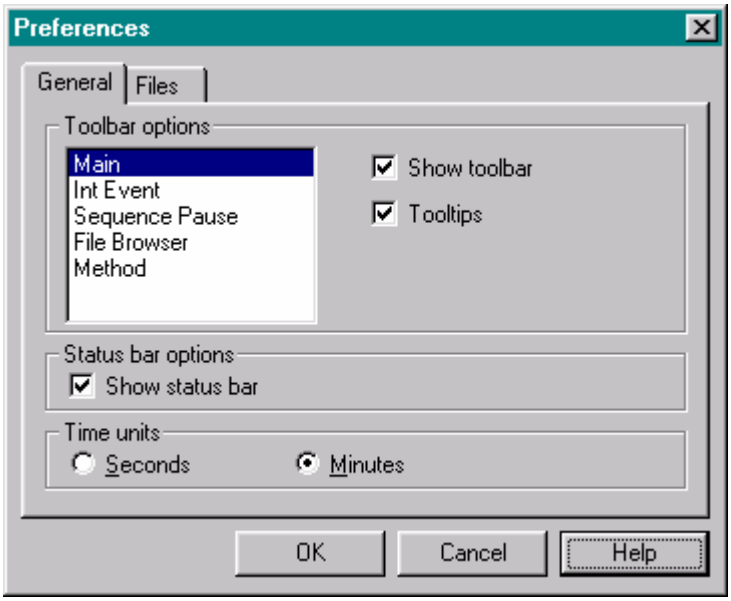


Figure 12. Preferences - General dialog box

- Status bar options

  Select the checkbox to turn on the status bar. The status bar provides brief information at the bottom of the instrument window, if enabled.

- Time units

  Select the time units for display of chromatographic information.

View Preferences Files

The Files tab is used to enter the maximum number of recent files to display when the **File > Recent Method Files (Method Files, Data Files, Sequence Files, Pretreatment Files)** command is selected. Select the file type, and then enter the number of files you wish to display in the **Max files** box. The **Clear Files** button will clear the max files number for the selected file type. The **Clear All Files** button will clear the max files for all file types.

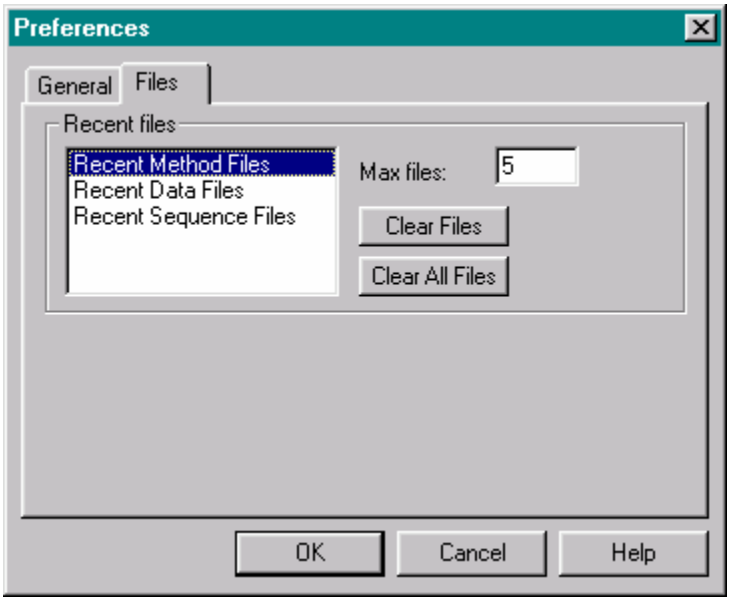


Figure 13. Preferences - Files dialog box

Right-mouse clicks

Throughout ChromQuest, you can use the right mouse button (or the alternate mouse button if you have switched the main mouse button for left-handed use) to access a menu showing commands for that region of the application window. For example, a right-mouse click executed inside the chromatogram window brings up a menu where you can set the properties of the chromatogram window and access other chromatogram operations.

Locking your screen

When you choose the **Window > Lock** command, all menu items will be "Locked" except the **Window** and **Help** menu. Locked commands will not be accessible until you unlock them again. This command is useful for multiple user labs, where you may want to lock your current work while you are temporarily away from the computer.

To unlock the screen, click on the **Window > Lock** command again. You will be required to log in your user name and password to unlock the screen.

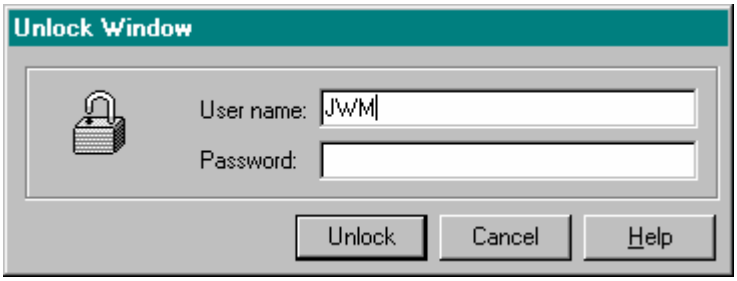


Figure 14. Unlock Window dialog box

The Chromatogram Window

Whenever there is data to be viewed, it is shown in a chromatogram window. Normally, one chromatogram will appear in each window, and multiple channel data files will display multiple chromatogram windows - one for each channel. However, it is possible to add multiple traces to a single chromatogram window and perform comparison and mathematical operations on them. To access specialized commands for the chromatogram window; click on the right-hand mouse button somewhere within the chromatogram window area. These commands allow you to add graphs or chromatograms to the window, change the appearance, annotations, and axes, perform mathematical operations on chromatograms, and view or change the properties of existing traces in the window.

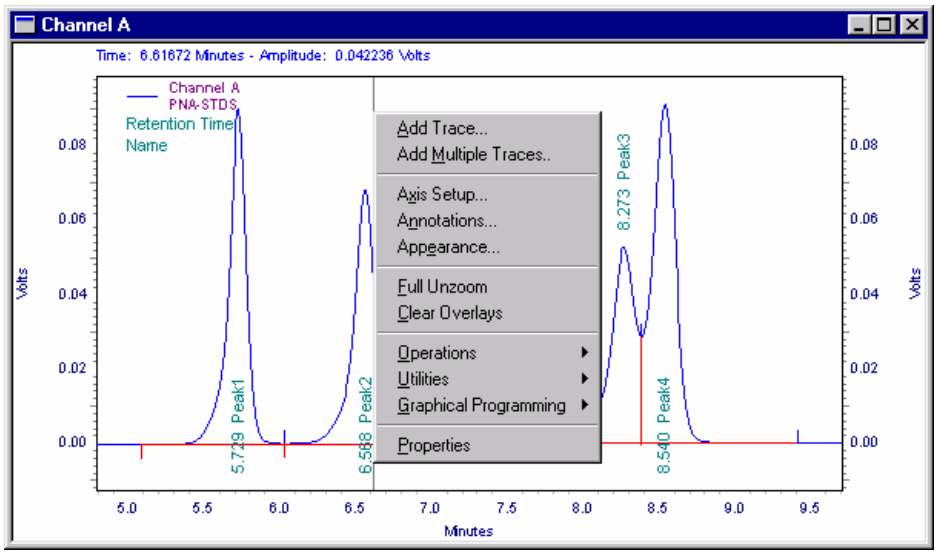


Figure 15. Chromatogram window

As data is being acquired, it is also displayed in a chromatogram window. At the end of a run the data becomes the "current data". You can change the appearance of the chromatogram and select annotations, fonts, and labeling. Utilities are available to print the current window view, copy it to a clipboard, or save it in a file.

View Tiled or Overlay Data

When viewing data from multiple channel methods, you can choose to view each channel in a separate window (**View > Tile Data**), or you can overlay all channels in a single window (**View > Overlay Data**). When all channels are overlaid in a single window, you can still zoom, and change the individual channel appearances, as described below.

When in **Tiled** mode, you can arrange how the windows are tiled on your screen by using the **Window > Cascade, Window > Tile Horizontally**, or **Window > Tile Vertically** command.

Zooming

You may want to examine a chromatogram in more detail, or zoom in on a portion of the chromatogram. To do this, drag a box around the area of interest by holding down the left mouse button and dragging the box until it highlights the section of interest. Then release the mouse button. To move quickly to the previous level of zoom, double-click on the chromatogram. To zoom to the full chromatogram again after multiple zooming operations, click on the right-hand mouse button anywhere in the chromatogram window, then select **Full Unzoom** from the menu displayed. You can also execute a full unzoom of your chromatogram with **Ctrl-Z** or **shift-double click** in the chromatogram window.

At the top of the chromatogram window is a display of **Time** and **Amplitude**. These values change as you move the cursor and reflect the time and amplitude of the trace where the cursor is located. If you have more than one trace, you can change the display to another trace by clicking on the chromatogram trace with the mouse. If the traces are displayed in different colors, the color of the Time and Amplitude display will reflect the color of the trace displayed.

Scrolling the chromatogram

Once you have zoomed in on a chromatogram, you can scroll the chromatogram to the right or left without losing the zoom. This is done by pressing the CTRL+SHIFT keys down and moving the mouse until the cursor changes to a "hand" and dragging it to the left or right.

You can also scroll the X- or Y- axis to view features that may be out of the range. To do this, press the CTRL+SHIFT keys down while the mouse cursor is outside the graph area, yet near the axis of interest. The cursor will change to an up/down arrow near the Y-axis, or a left/right arrow near the X-axis. Moving the mouse in this mode will scroll the graph up/down or left/right on the axis.

To restore the original view, do a right-hand mouse click in the chromatogram window, followed by the **Full Unzoom** command.

Adding a Trace (Viewing Multiple Chromatograms)

The chromatogram window is used to view data, either current data (real-time) in the instrument window, or data recalled from the disk. You can view multiple chromatograms in a single chromatogram window if you wish. These is convenient if, for example, you want to compare a past run with your current data, or overlay an oven or pump profile. To add a new trace, click on the **right** mouse button anywhere in the chromatogram window. The following menu will appear.

Figure 16. Chromatogram menu

Choose the **Add Trace** command. When you select this command, a dialog box will appear.

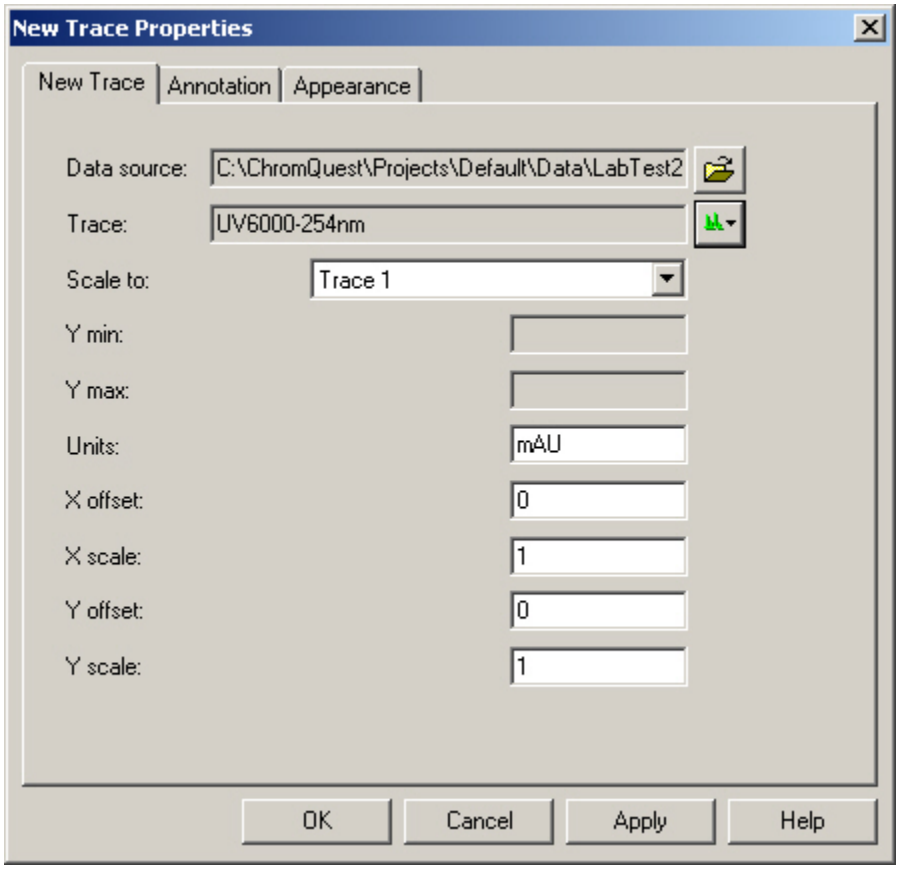


Figure 17. New Trace Properties - New Trace dialog box

Select the **New Trace** tab. Fill in the fields to add a trace to the chromatogram window and set its properties. These properties apply only to the trace selected and are not saved as part of the method. When a new trace is opened, the properties will be set to default values.

- Data source

  Enter the name of the file from which to get the trace. You can also click on the File button adjacent to the field and select a data source.

  – Current Data

    This selection allows you to select a trace from the current chromatography data.

  – Current Method

    This selection enables you to select a trace from your current method (if available). For example, you could load an oven temperature program from a Trace GC instrument method.

  – Open Data

    This allows you to select a stored data file from which you can select a trace for display.

- Trace

  Select the trace to be displayed. Click on the button to display available traces.

- Scale to

  Select one of the scaling options:

  – Trace x

    Scales to another trace in the window.

  – Autoscale to largest peak

    Scales such that the largest peak is on scale.

  – Autoscale to 2nd largest peak

    Scales such that the 2nd largest peak is on scale.

  – Autoscale to 3rd largest peak

    Scales such that the 3rd largest peak is on scale.

  - User Defined

    Allows you to enter a value for Y max and min.

  - Normalized

    Allows you to normalize one trace to fit on the graph.

- Y min

  If you have selected a User Defined scale, enter a minimum value for the Y-axis.

- Y max

  If you have selected a User Defined scale, enter a maximum value for the Y-axis.

- Units

  Select the units for display.

- X offset

  Enter a value in units for offset of the X-axis.

- Y offset

  Enter a value in units for offset of the Y-axis.

- Y scale

  If desired, enter a multiplier that will be applied to the entire trace here.

Clearing Overlaid Traces

You can clear all overlaid traces from the current chromatogram window by doing a right-mouse click, and then select the **Clear Overlays** command from the pop up menu.

Adding Multiple Traces

If you want to quickly add more than one chromatogram to your view, click on the right hand mouse button. Then, choose **Add Multiple Traces**. The Open File dialog box will appear where you can select the traces to be displayed by selecting them from the file list. To add a file either, click on the file name and then click on the Add button, or simply double-click on the filename from the list.

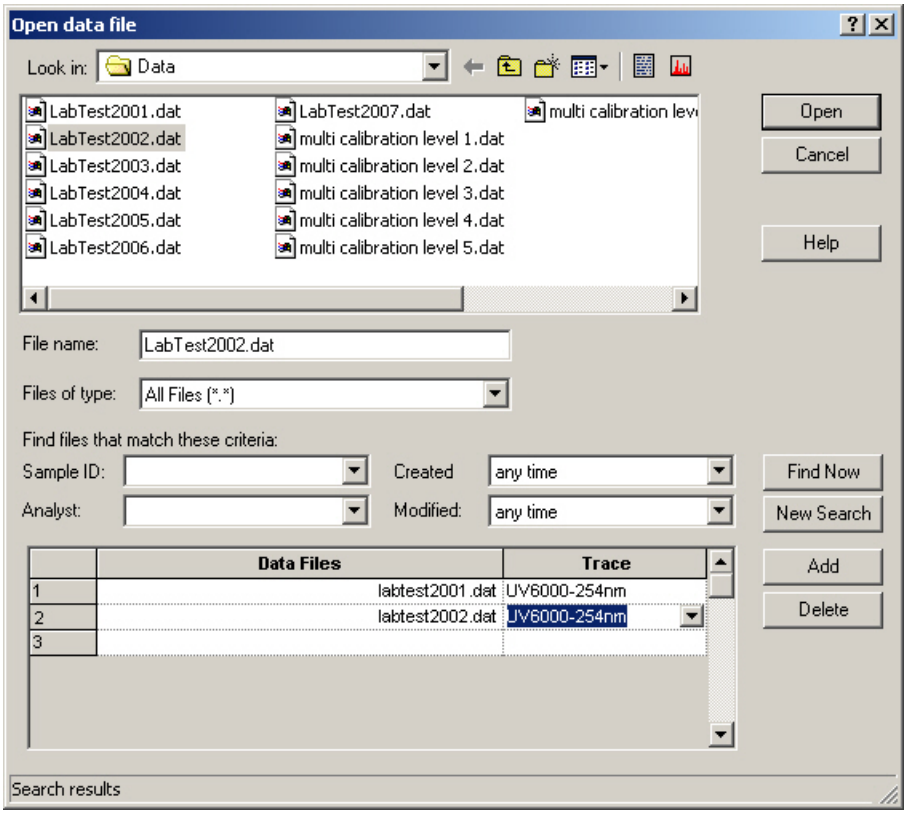


Figure 18. Open data file dialog box

Once you have added a data file to the list, you can select the channel by clicking on the **Trace** field, and then click on the down-arrow button. If multiple channels for that file are available, select the desired channel by clicking on it with the mouse. To delete a trace from the display list, click on its name or on its number, and then click on **Delete**. When you are ready to open the multiple traces, click on **Open.** The selected files/channels will appear in your chromatogram window.

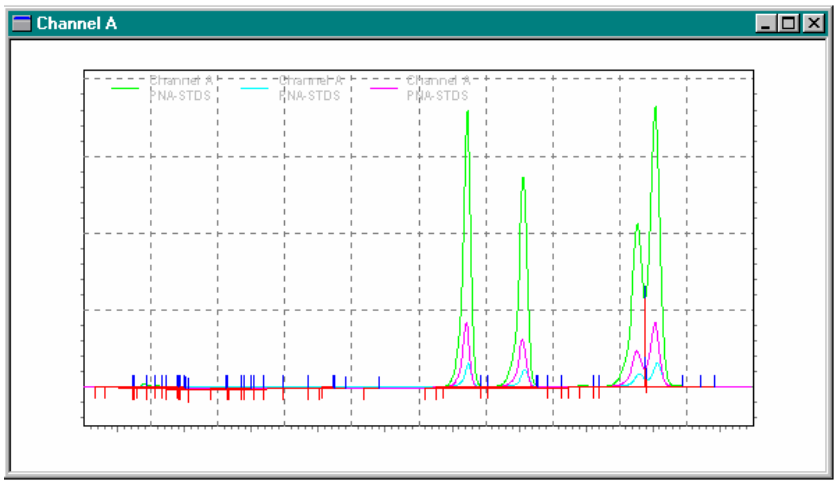


Figure 19. Chromatogram window with multiple traces

Chromatogram Annotation

Click on the **Annotation** button in the Data Graph Properties - Trace Setup box. (Or, do a right-hand mouse click in the chromatogram window and select the **Annotation** command - see below.) This brings forward a dialog where you can designate how you want the trace you are adding to be annotated.

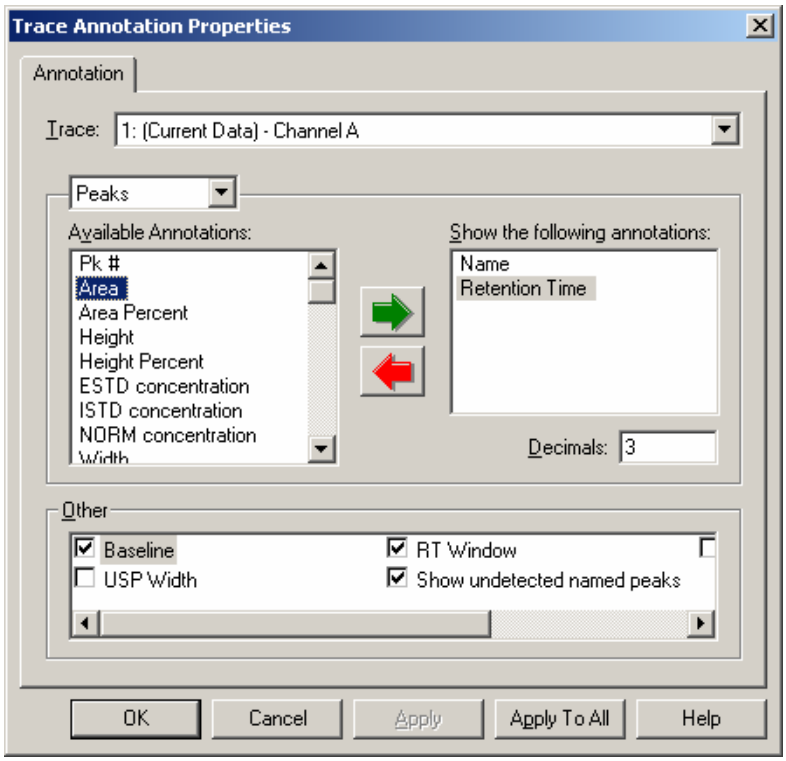


Figure 20. Trace Annotation Properties dialog box

Select the trace from the drop-down list. Then select what features you wish to annotate.

For the selected trace, click on an **Available Annotation**. When an annotation is highlighted, you can add it to the annotations to be shown by clicking the **Green** arrow key (pointing to the right). Double-clicking the selection can also do this.

For certain annotations, you can also designate the number of places to be displayed to the right of the decimal point. Enter this value in the **Decimals** box for the highlighted item.

Click on the check boxes to display the **Baseline, USP Width, Retention Time Windows**, or **Group Ranges** on the trace. With the SEC option installed, you will have access to additional SEC annotation features.

Note The Reference Peak window annotation displays the window set in the Peak Table. This window is not adjusted for relative retention time.

Continue to select as many annotations as you wish for this trace. When you have finished, click on **OK**.

You can select or change annotation for an existing trace by doing a right-mouse click in the chromatogram window, then select the **Annotation** command.

The selections you make will apply to all traces you open for this channel or until you change them (the **OK** or **Apply** button). If you want to apply the annotation changes to all open channels, click on **Apply To All**. Annotations are not saved as part of the method and are considered a function of the instrument application. If you close a method and re-open it, the current settings will apply.

Chromatogram Appearance

You can change the appearance of the trace (line type, color, etc.) from the **Appearance** tab in the Data Graph Properties box. Click on this tab to display the Appearance tab dialog.

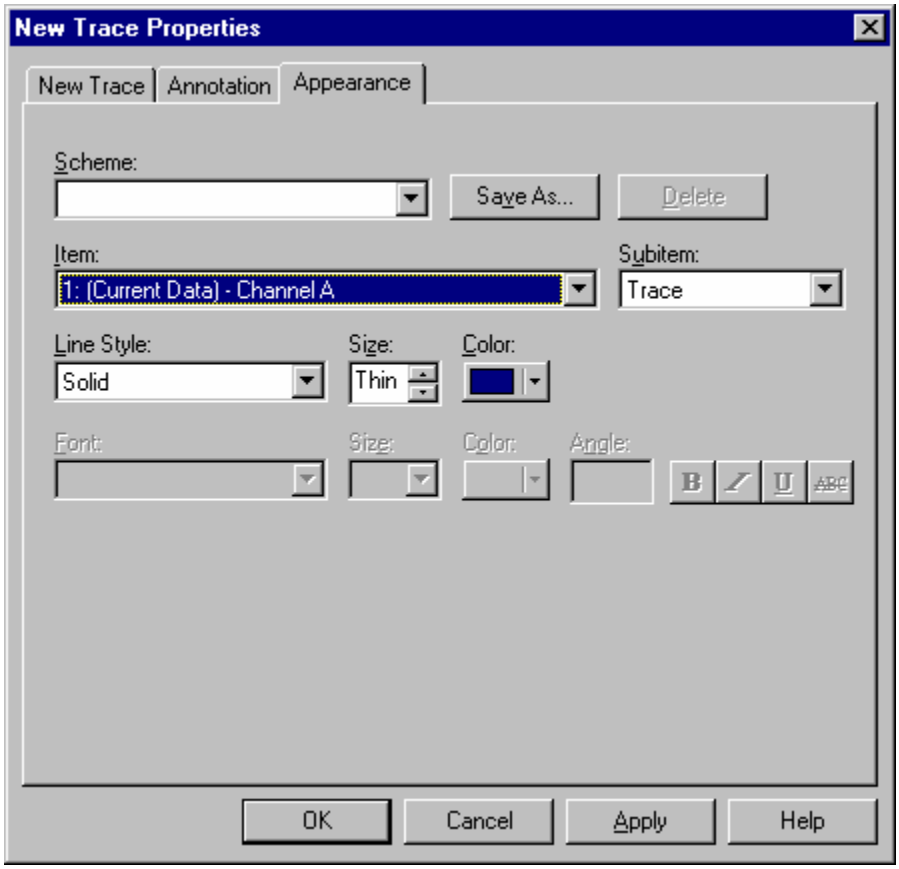


Figure 21. New Trace Properties - Appearance dialog box

- Scheme

  If you have previously saved an appearance scheme on disk, you can select it from this box. The **Save As** button allows you to save the existing appearance scheme on disk by giving it a name. The **Delete** button allows you to delete a scheme and start again.

- Item

  This drop-down list lets you select which part of the chromatogram window for which you wish to change the appearance. The choices will include the graph itself (including background and legends), and the available traces.

- Sub-item

  Select the sub-item you wish to modify. The choices for this will change based on the item you have selected. For example, if the Item selected is the Graph, you will have access to setting up appearances of sub-items including the background, axes and labels for the graph. If the item selected is a chromatogram data channel, you will have access to setting appearances of sub-items such as baselines, start and stop tic marks, and annotation. If the item selected is text, you will have access to the **Font** formatting commands as well.

  When a sub-item is selected, you will have access to fields appropriate to that item. For example, if you have chosen the **baseline** sub-item, you can choose the color and line type. If you have chosen the **annotation** sub-item, you can choose the font appearance and color.

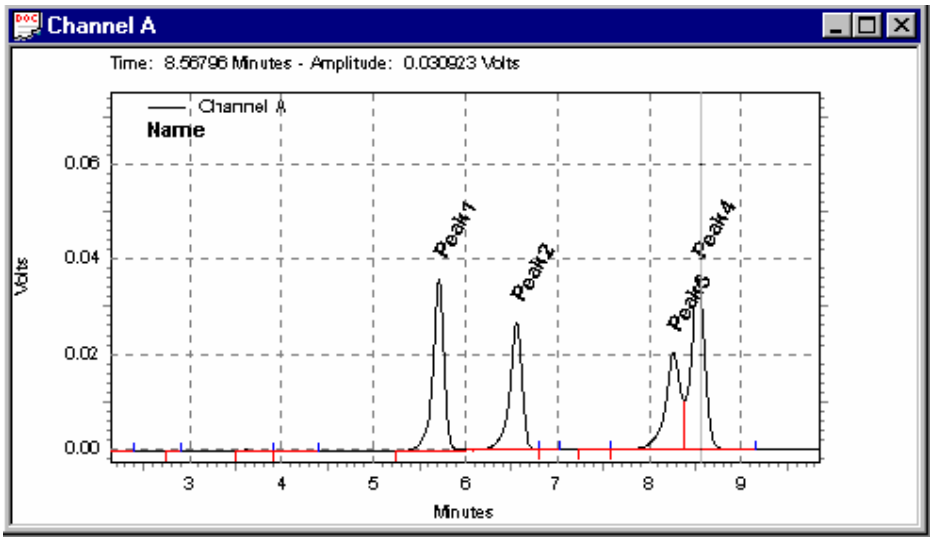


Figure 22. Chromatogram window with multiple traces

You can change the appearance of any trace without adding a new trace, by doing a right-mouse click in the chromatogram window, then selecting the **Appearance…** command. When you select this command, you will see an identical dialog to that shown above for the Appearance Tab.

Sub-items available in the Appearance tab are as follows:

Item	Sub-Item	Description
Graph	Background	Select the color of the graph background. Default is black.
Graph	Title	Select a color and font for the Title of the graph. There must be a Graph Title defined in the Axis Setup tab in order for it to appear in the window.
Graph	Left Y-Axis	Select a color for the left Y-Axis of the graph.
Graph	Left Y-Axis Major Ticks	Select a color for display of major unit marks on the Left Y-Axis.
Graph	Left Y-Axis Minor Ticks	Select a color for display of minor unit marks on the Left Y-Axis.
Graph	Left Y-Axis On/Off	Turns On or Off the Left Y-Axis
Graph	Right Y-Axis	Select a color for display of a right hand Y-Axis.
Graph	Right Y-Axis Major Ticks	Select a color for display of right Y-Axis major ticks.
Graph	Right Y-Axis Minor Ticks	Select a color for display of right Y-Axis minor ticks.
Graph	Right Y-Axis On/Off	Turns On or Off the right Y-Axis.
Graph	X-Axis	Select a color for the X-Axis display.
Graph	X-Axis Major Ticks	Select a color for display of major unit marks on the X-Axis.
Graph	X-Axis Minor Ticks	Select a color for display of minor unit marks on the X-Axis.
Graph	X-Axis On/Off	Turns On or Off the X-Axis.
Graph	Legend	Select a color and/or font for display of the graph legend. The legend indicates what traces are currently displayed in the window. The Legend is turned On or Off from the Axis Setup tab.
Graph	Grid	Select a color for display of the grid lines. Grid lines are turned On and Off from the Axis Setup tab.
Data	Trace	Select a color and/or line type for display of the selected trace.
Data	Annotation	Select a color and font for display of the trace Annotation(s). The items to be annotated for a trace are selected in the Annotations tab.
Data	Baseline	Select a color and/or line type for display of the baseline.
Data	Baseline Start Tick	Select a color and/or line type for display of baseline start ticks.
Data	Baseline Stop Tick	Select a color and/or line type for display of baseline stop ticks.
Data	USP Width	Select a color and/or line type for display of the USP Width, if calculated.
Data	RT Window	Select a color and/or line type for display of expected retention time windows for named peaks.
Data	RT Window (undet.)	Select a color for display of RT Window for expected peaks that were not detected.
Data	Group Range	Select a color, line type, and line weight for the group range bar. Select a color, font type, and font style to display the group range.

Axis Setup

The Axis Setup tab allows you to configure the appearance of the axis on your chromatogram. These settings apply to active traces. This tab is accessed using the **right-click > Axis Setup** command, or you can do a right-mouse click on the axis of the chromatogram, then choose the **Axis Setup** command.

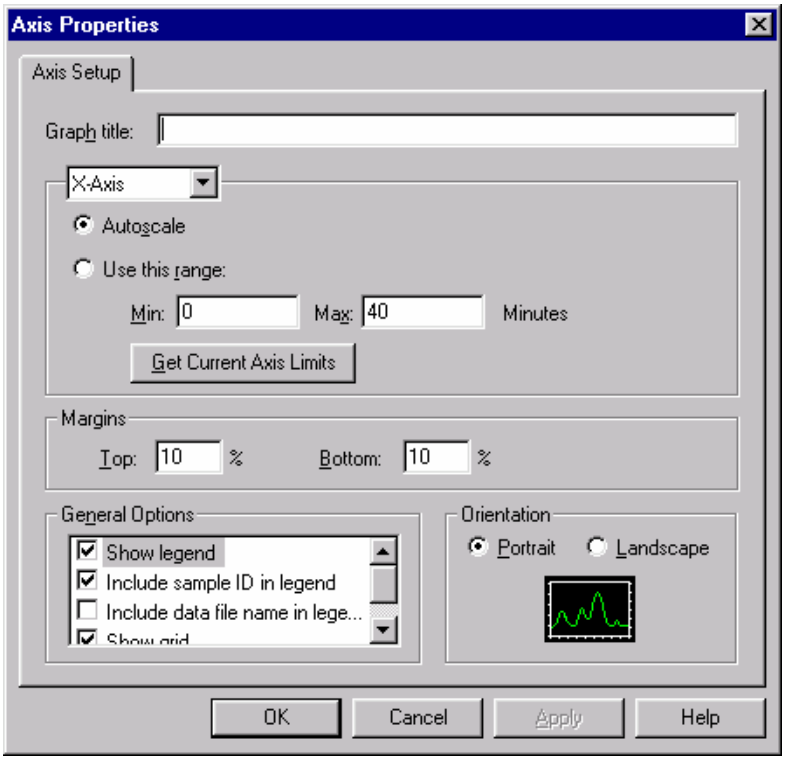


Figure 23. Axis Properties - Axis Setup dialog box

- Graph title

  Enter a title for the graph, if desired. This appears at the top of the graph.

- Axis

  Using the drop-down list, select the axis of interest: Left Y-Axis, Right Y-Axis, or X-Axis. Then for your selection, you can choose the limits for the axis.

  For Y-Axis selections, you may choose **Use limits of trace** to get the limits from one of the traces in the window, or you can select the **Manually set trace's limits to** box and set the Y-Axis limits to your desired range. If you choose None, no Y-Axis values will be displayed.

For the X-Axis, you may either choose to **Autoscale**, where the X-Axis is set to the longest trace. Or, you may set an absolute range for the X-Axis by clicking the **Use This Range** button, and then enter a minimum and maximum X-Axis value for the trace. Click on the **Get Limits** button to retrieve the X-Axis range from the current trace.

- Margins

  Enter a value for the trace margins, in percent, for top and bottom of the graph.

- General Options

  Select the check boxes to turn these graph annotations on and off. If the legend box is selected, the legend for a trace can be turned on or off from the Trace Properties spreadsheet.

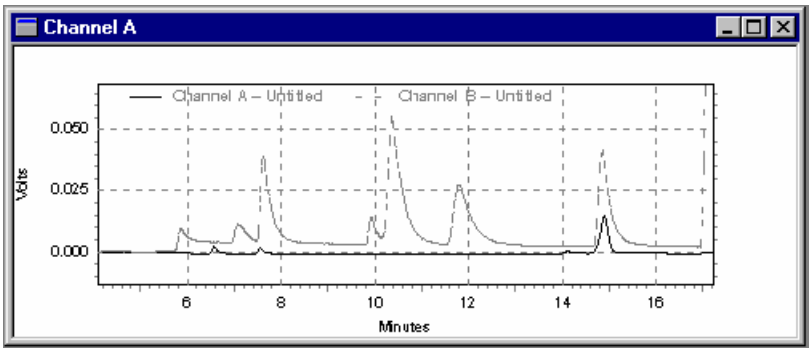


Figure 24. Chromatogram Window with Legend and Grid displayed

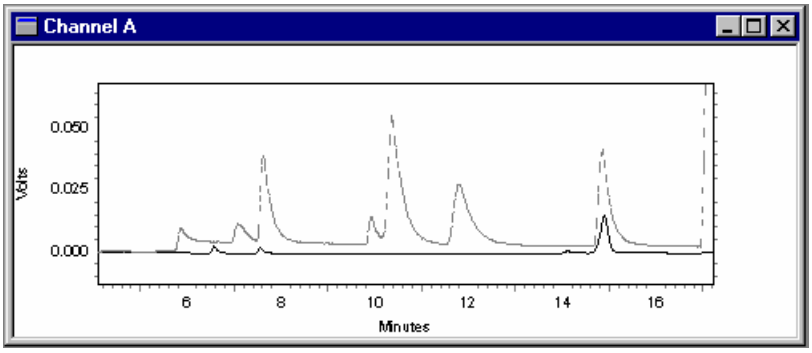


Figure 25. Chromatogram Window with no Legend or Grid displayed

- Orientation

  Select portrait or landscape orientation for your graph by clicking the appropriate button.

Data Graph Properties

The Data Graph Properties tabs are used to set the properties of the chromatograms or traces in the chromatogram window. The tabs available are Trace Setup, Axis Setup, and Appearance. Use these tabs to change chromatogram colors, annotations, and appearance, and to set specific chromatogram time/voltage ranges to display (if desired).

Trace Setup

This tab gives you access to adding/removing traces, and setting scaling options for the traces. Each row in the spreadsheet represents one of the traces currently in the chromatogram window. The details of the highlighted trace appear in the trace properties boxes in the bottom of the dialog box where you can view or change them.

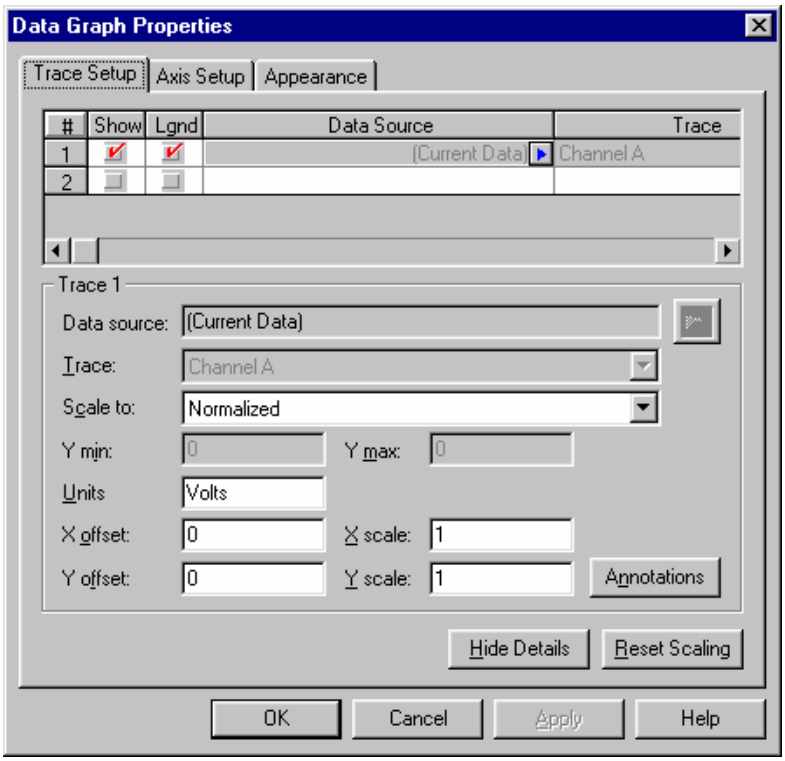


Figure 26. Data Graph Properties - Trace Setup dialog box

- Show

  Click this box to show the trace in the chromatogram window. De-select this box to remove the trace from the display (but leaving it open). This is a convenient way to temporarily remove a trace from the viewing window.

- Lgnd

  Click this box to show the legend for the trace. The legend appears in the upper right corner of the window and displays the name of the trace. De-select this box to remove the legend for this trace from the chromatogram window. Setup for the appearance of the legend (color, etc.) is done in the Appearance tab for the Graph item.

  Note If you have not turned on the Legend in the **Axis Setup** dialog, this box will have no effect.

- Data source

  Enter the name of the file from which to get the trace. You can also click on the File button adjacent to the field and select a data source. The data source can be a chromatogram or it can be a stored profile such as temperature or flow program.

  – Current Data

    This selection allows you to select a trace from the current chromatography data.

  – Open Data

    This allows you to select a stored data file from which you can select a trace for display.

  – Current Method

    This allows you to select a trace from the current method (if available). Examples of traces from a method include temperature profiles for instrument control, for example.

- Trace

  Select the channel to be displayed.

- Scale to

  Select one of the scaling options.

  – Trace x

    Scales to another trace in the window.

  – Autoscale to largest peak

    Scales such that the largest peak is on scale.

  - Autoscale to 2nd largest peak

    Scales such that the 2nd largest peak is on scale.

  - Autoscale to 3rd largest peak

    Scales such that the 3rd largest peak is on scale.

  - User Defined

    Allows you to enter a value for Y max and min.

  - Normalized

    Allows you to normalize one trace to fit on the graph.

- Y min

  If you have selected a User Defined scale, enter a minimum value for the Y-axis.

- Y max

  If you have selected a User Defined scale, enter a maximum value for the Y-axis.

- Units

  Select the units for display.

- X offset

  Enter a value in units for offset of the X-axis.

- Y offset

  Enter a value in units for offset of the Y-axis.

- Y scale

  If desired, enter a multiplier that will be applied to the entire trace here.

  Note You can set the X-axis range from the **right-mouse click > Axis Setup** menu selection.

- Annotations

  Click on this button to display the trace annotations dialog.

- Hide Details

  Click on this button to hide the current trace details and display only the spreadsheet.

- Reset Scaling

  Click on this button to reset the scaling values to their original values.

Removing a Trace

If you have multiple traces in your chromatogram window, and you want to remove one or more of them from the chromatogram window, click on the right-hand mouse button anywhere within the window, and select the **Properties** command. A spreadsheet will appear where the currently displayed traces are listed.

To completely remove a trace from the chromatogram window, select the row by clicking on the # number, then press the DELETE key on your keyboard, or select the **Edit > Delete** command. To temporarily remove the trace from the window, de-select the checkbox in the **Show** column. Click on **OK** to return to the chromatogram window.

Setting Limits for X-Axis and Y-Axis

Occasionally, you may want to set an absolute range for either the X-Axis or Y-Axis, or both. This is done using the Data Graph Properties tabs. Once you have set an absolute range for one or both of these axes, the designated chromatogram(s) will always be displayed in the chromatogram window using these ranges until you change or reset them.

Y-Axis ranges are set for each chromatogram using the **Trace Setup** tab. To set an absolute voltage range for all chromatograms, use the **User-Defined** option for the **Scale To** field. You must then enter a **Y-Min** (minimum Y-Axis value) and **Y-Max** (maximum Y-Axis value) for each chromatogram. If you want all chromatograms to be displayed using this same voltage scale, enter the same values for all chromatograms.

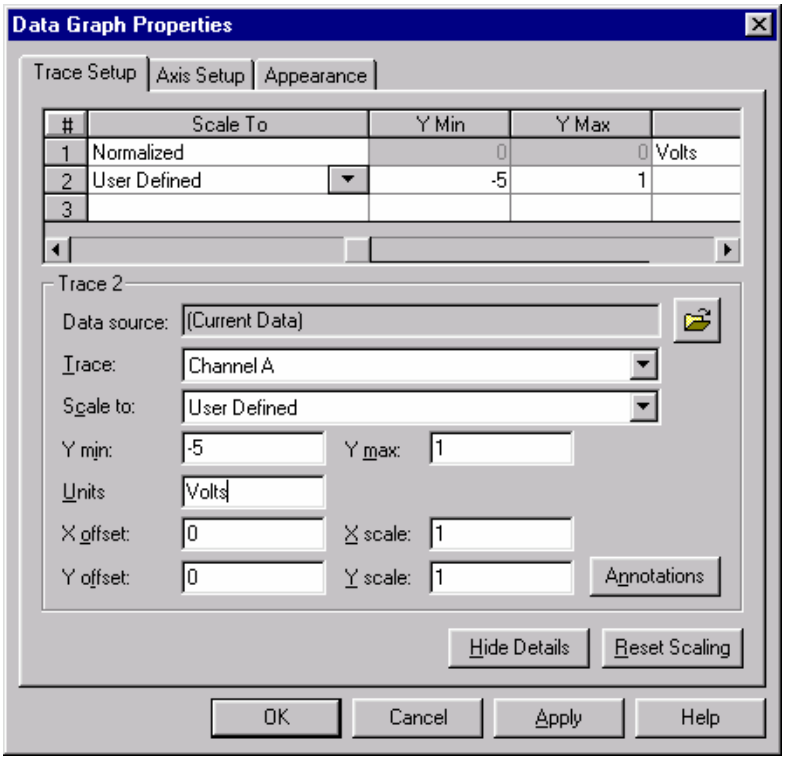


Figure 27. Data Graph Properties - Trace Setup dialog box

If you want to reset the scaling of all chromatograms to default values, click on the **Reset Scaling** button.

To set an absolute X-Axis range, use the **Axis Setup** tab. Here, you can choose to **Autoscale** the chromatograms, which sets the X-Axis range automatically to the range of the longest chromatogram, or select the **Use this range** option button to enter an absolute range in minutes. The **Get Current Limits** button brings in the X-Axis range from the current chromatogram window. This is useful because it allows you to use the zoom function to identify the desired region of the chromatogram and automatically enter the range values.

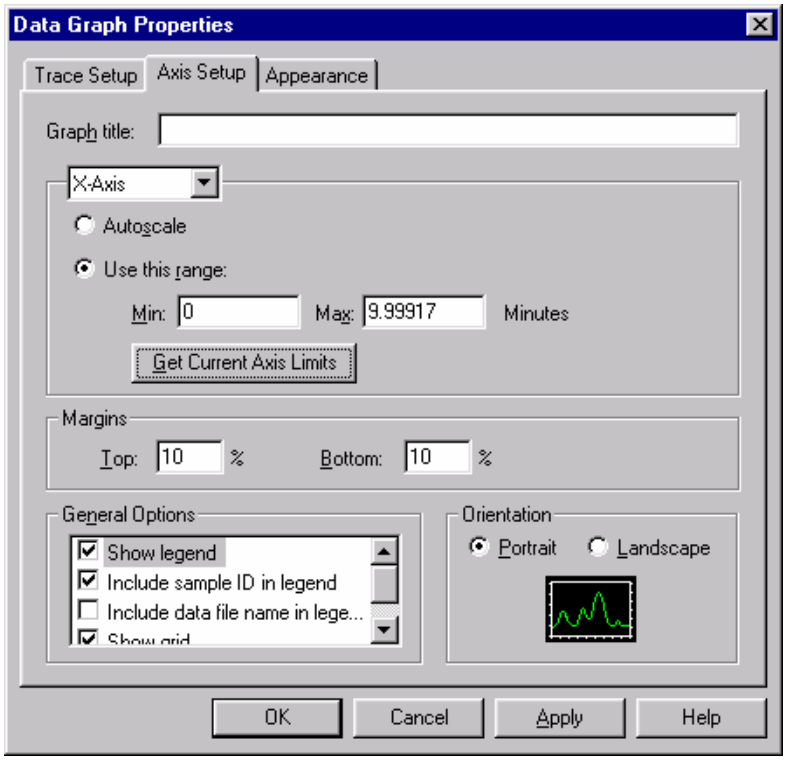


Figure 28. Data Graph Properties - Axis Setup dialog box

Once you have set a user defined X-Axis range, you can return to the normal mode by selecting the **Autoscale** option.

Chromatogram Operations

There are a number of chromatogram comparison and mathematical operations that are available from the chromatogram window. These are accessed using the **right-mouse click > Operations** command.

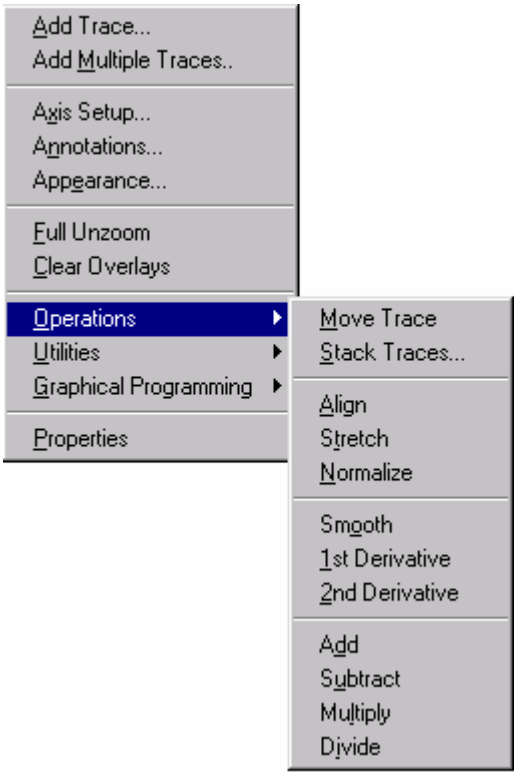


Figure 29. Operations menu

Moving a Trace

You can "grab" a trace and move it with your mouse using the **right-mouse click > Operations > Move Trace** command. When you select this command, and move your cursor over a trace, the cursor will change to a "move" icon. When this occurs, you can "grab" the trace by clicking the left mouse button and dragging the chromatogram to a new location. When you release the mouse button, the chromatogram will be placed where your cursor was located when you released the button. "Move Trace" will appear at the upper right corner of the window. You can continue to move traces. When finished, select the **right-mouse click > Operations > Move Trace** command again to turn off the move trace operation.

Stacking Traces

You can quickly change the X-axis and Y-Axis offset for a trace with the **right-mouse click > Operations > Stack Traces** command.

A dialog appears where you can enter a new X-axis and Y-axis offset, which will be applied to additional traces displayed in the chromatogram window.

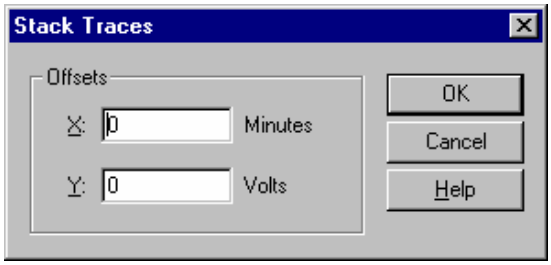


Figure 30. Stack Traces dialog box

Enter a value for X offset and Y offset for the additional traces, and then click **OK**. The chromatograms will be re-drawn using the offsets you entered.

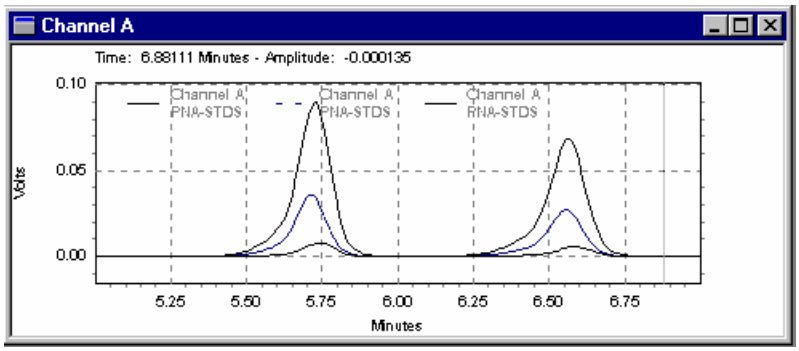


Figure 31. Chromatograms before stacking

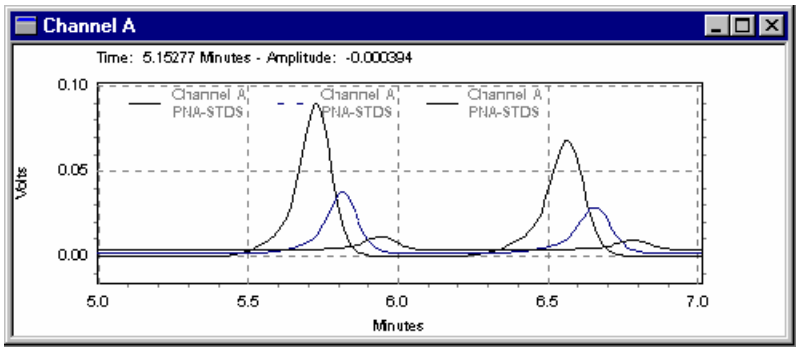


Figure 32. Chromatograms after stacking

To remove these offsets, use the **right-mouse click > Properties** command to view the trace spreadsheet. Click on the **Trace Setup** tab, and then scroll to the right to the X-axis and Y-axis offset columns where you can delete or change these settings. Click on the **Reset Scaling** button to restore ALL settings to their original values. Or, you can use the **Stack** command again, entering "0" for both stack parameters.

Align

To align one chromatogram to another, click on the **right-mouse click > Operations > Align** command. Click first on the point of the first chromatogram to which you wish to align, and then click on the peak (or point) of the second chromatogram that you wish to align to the first point. The second chromatogram will be adjusted such that the peak (or point) you clicked second will be aligned with the first point you clicked.

To remove the alignment, use the **right-mouse click > Properties** command to view the trace spreadsheet. Click the **Trace Setup** tab, then scroll to the right to the X-axis and Y-axis offset columns where you can delete or change these settings. Click on the **Reset Scaling** button to restore ALL settings to their original values.

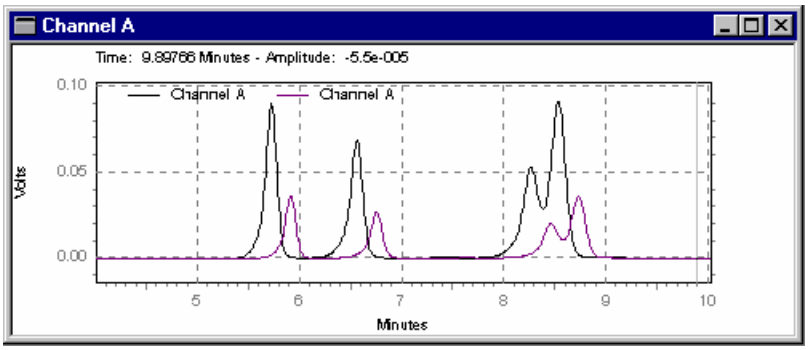


Figure 33. Chromatograms before alignment

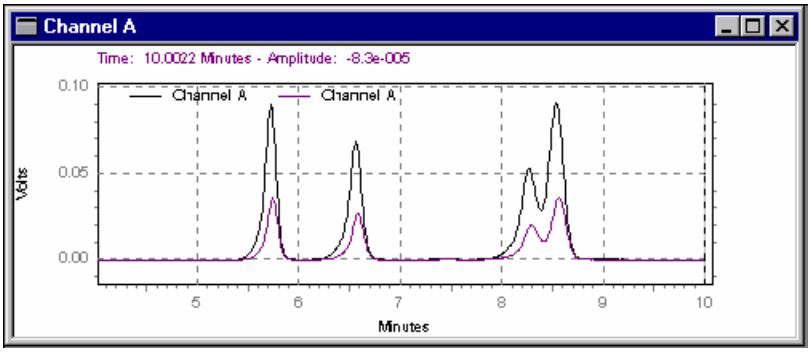


Figure 34. First peak of top chromatogram aligned to first peak on bottom chromatogram

Stretch

The stretch function allows you to perform a two-point contraction or expansion of chromatograms relative to another. First, you select points (or peaks) on the first chromatogram to which the second will be stretched (or contracted). Then, you select two points on the second chromatogram. The chromatogram between these two points will be stretched or contracted to fit the two points specified on the original chromatogram.

To un-do the stretch, use the **right-mouse click > Properties** command to view the trace spreadsheet. Click on the **Trace Setup** tab, and then scroll to the right to the X-axis and Y-axis offset columns where you can delete or change these settings. Click on the **Reset Scaling** button to restore ALL settings to their original values.

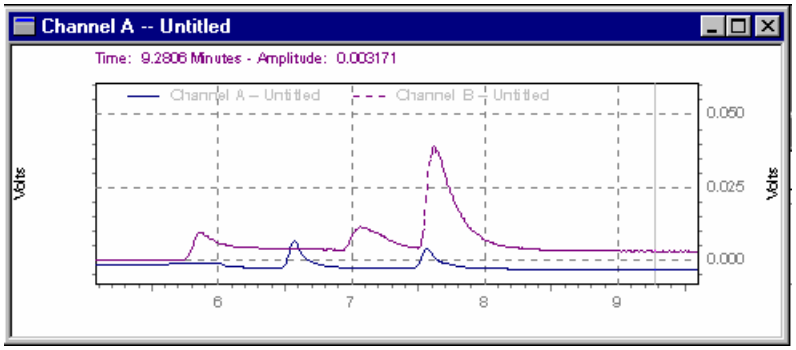


Figure 35. Chromatograms before stretching

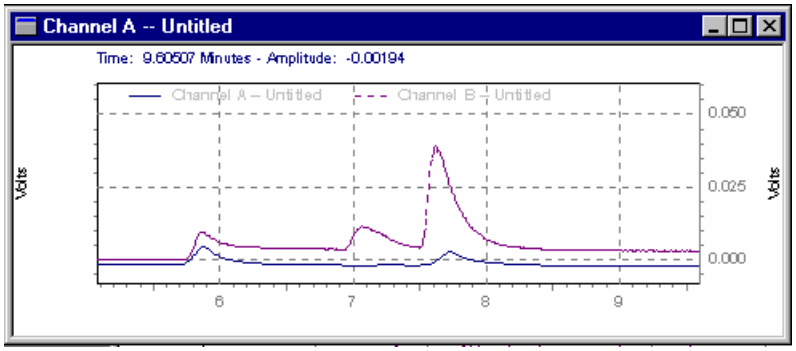


Figure 36. Bottom chromatogram stretched relative to top chromatogram

Normalize

This function allows you to normalize one or more chromatograms to the first chromatogram, adjusting the heights such that the apex height of a selected peak matches that of the peak selected on the first trace. Once you have selected this command, you will be prompted to select the start and then the apex of a peak in the first trace. Then you will be prompted to click on the start and apex of a peak in the second trace for normalization.

To un-do the normalization, use the **right-mouse click > Properties** command to view the trace spreadsheet. Click on the **Trace Setup** tab, and then scroll to the right to the X-axis and Y-axis offset columns where you can delete or change these settings. Click on the **Reset Scaling** button to restore ALL settings to their original values.

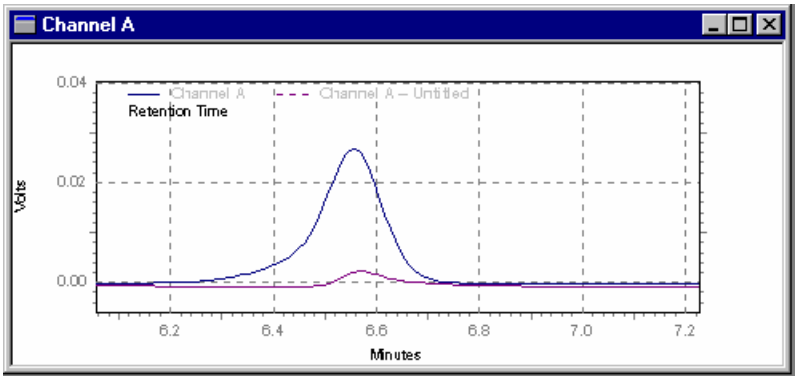


Figure 37. Chromatograms before normalization

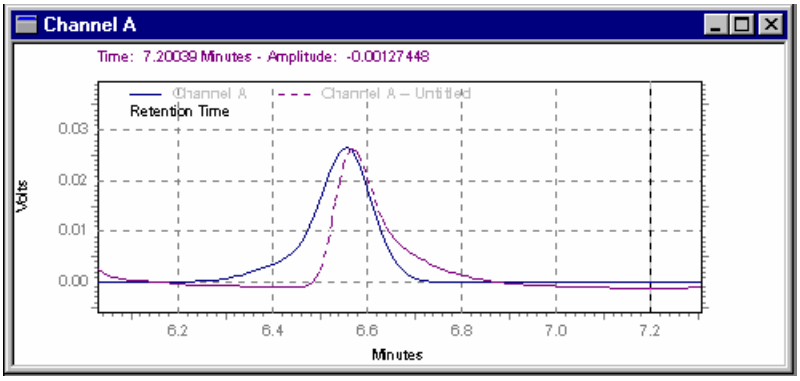


Figure 38. Chromatogram after normalization

Mathematical Operations

Performing mathematical operations on traces can be done from within the chromatogram window. In general, mathematical operations involved selecting the appropriate command from the **right-mouse click > Operations** menu (such as Add or Subtract), then follow the instructions in the upper-right corner of the chromatogram window to click on the desired traces in the window on which you wish to perform the operation. (To click on a trace, move the cursor so that any part of the cursor cross hairs is on the trace.) A "result" trace will appear in the window at the end of the operation.

For example, in order to add two traces, select the **right-mouse click > Operations > Add** command, and then follow the instructions in the upper right corner of the chromatogram window.

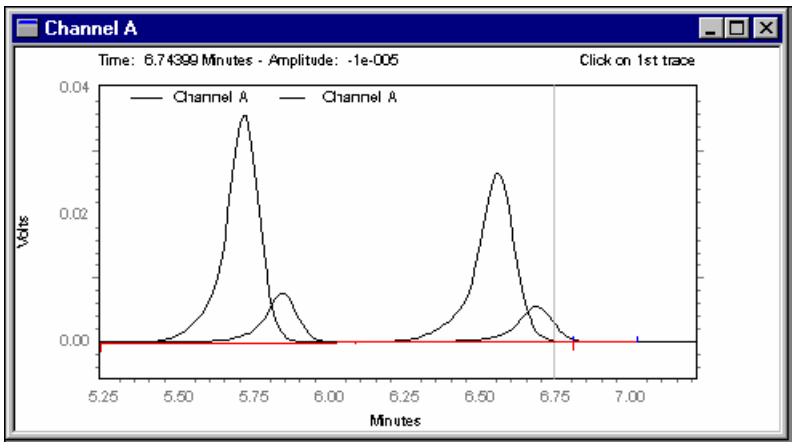


Figure 39. Chromatograms before addition

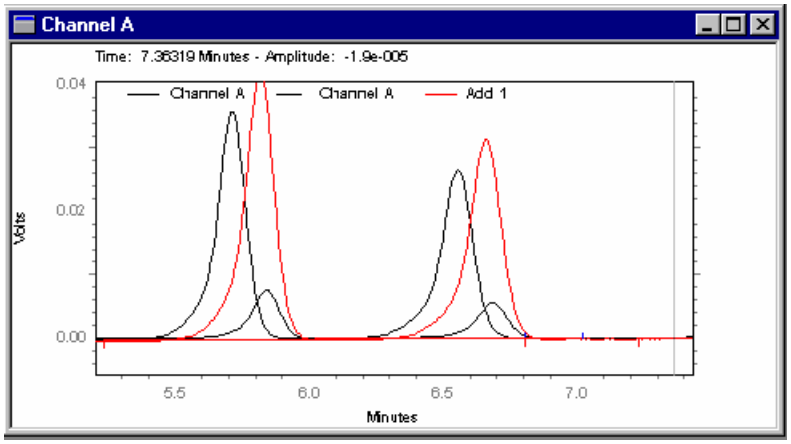


Figure 40. Result trace is displayed in window

Smoothing

To perform a 9-point Savitsky-Golay smoothing operation on a selected data file, click on the **right-mouse click > Operations > Smooth** command. A prompt will appear in the window instructing you to **Click on trace**. Click on the chromatogram to be smoothed. The result trace will appear in the window.

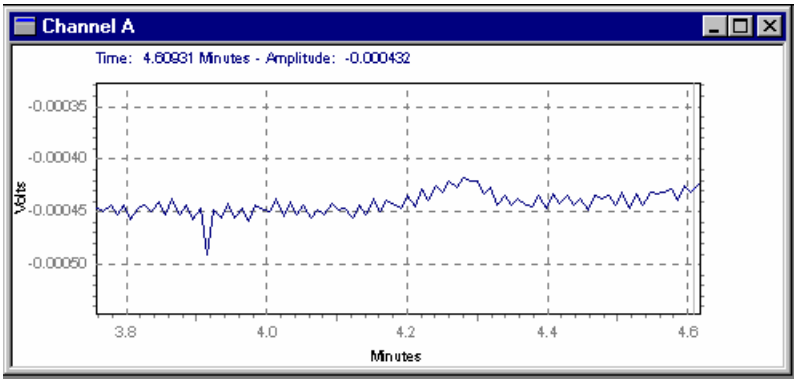


Figure 41. Chromatogram before smoothing

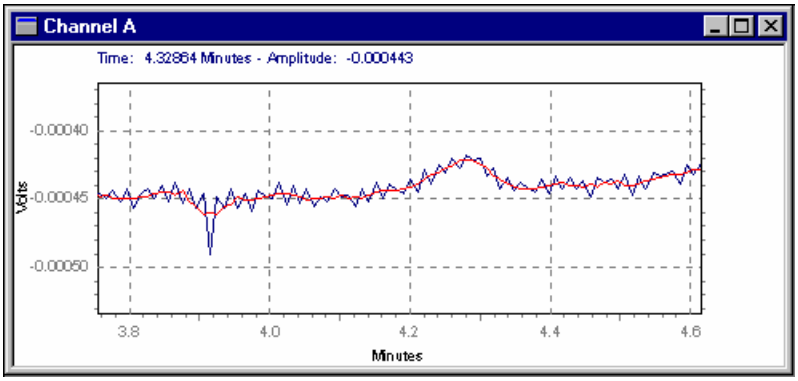


Figure 42. Smoothed result trace is displayed with original trace

Calculating Derivatives

To calculate and display the 1st or 2nd derivative of a chromatogram, click on the **right-mouse click > 1st Derivative or 2nd Derivative** command. A prompt will appear in the window **Click on trace**. Click on the chromatogram for which you wish to perform the operation. The result trace will appear in the window.

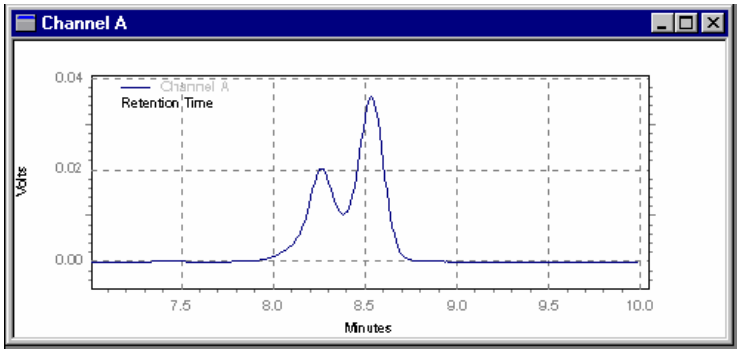


Figure 43. Trace before 1st derivative

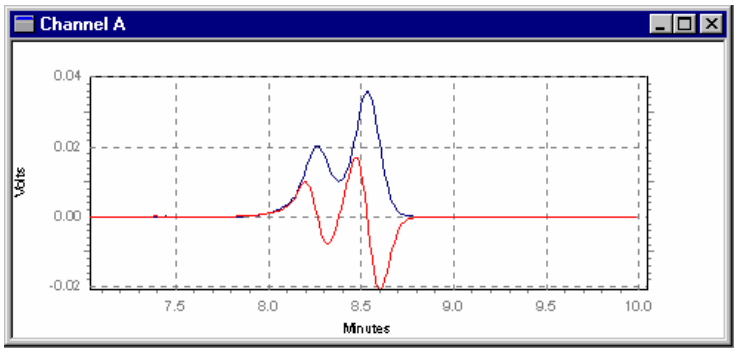


Figure 44. 1st derivative trace displayed with original trace

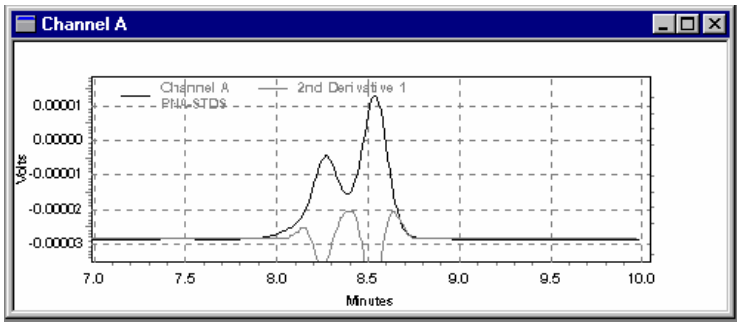


Figure 45. 2nd derivative displayed with original trace

Addition

To add two traces, click on the **right-mouse click > Operations > Add** command. A prompt will appear in the window **Click on 1st trace**. Select the first file by clicking the mouse on the chromatogram. A second prompt **Click on 2nd trace** will appear. Select the trace to be added to the first by clicking on the trace with the mouse. The result trace will appear in the window. Note that in order for this operation to be valid, both traces must have the same sampling frequency.

Subtraction

To subtract two traces, click on the **right-mouse click > Operations > Subtract** command. A prompt will appear in the window **Click on 1st trace**. Select the first trace by clicking the mouse on the chromatogram. A second prompt **Click on 2nd trace** will appear. Select the trace to be subtracted from the first by clicking on the trace with the mouse. The result trace will appear in the window. Note that in order for this operation to be valid, both traces must have the same sampling frequency.

Multiplication

To multiply two traces, click on the **right-mouse click > Operations > Multiply** command. A prompt will appear in the window **Click on 1st trace**. Select the first trace by clicking the mouse on the chromatogram. A second prompt **Click on 2nd trace** will appear. Select the trace to be multiplied by the first by clicking on the trace with the mouse. The result trace will appear in the window.

Division

To divide two traces, click on the **right-mouse click > Operations > Divide** command. A prompt will appear in the window **Click on 1st trace**. Select the first trace by clicking the mouse on the chromatogram. A second prompt **Click on 2nd trace** will appear. Select the trace to be divided into the first by clicking on the trace with the mouse. The result trace will appear in the window. The equation used to calculate the result trace is as follows.

$$p = \left(\frac{y_1}{\sqrt{y_1^2 + y_2^2}} \right) \div y_{mult}$$

where

p = the calculated point for the result trace at time t

y_1 = a point from the first trace at time t

y_2 = a point from the second trace at time t

y_{mult} = the y multiplier for the trace that converts it from microvolts to the trace's displayed units

Utilities

The Utilities menu gives you access to commands for saving, copying, or printing the current chromatogram window. Use the **right-mouse click > Utilities** menu for these features.

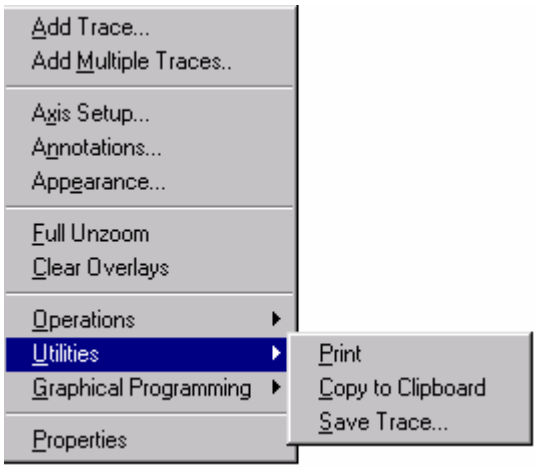


Figure 46. Utilities menu

Print

This command sends the current chromatogram window view to the printer.

Copy to Clipboard

This command copies the current chromatogram window to the clipboard as a metafile. From here, you can paste the view into a word processing document or other application that supports the clipboard. Note: To paste into Microsoft Word, you need to use the **Edit > Paste Special > Picture** command.

Save Trace

This command saves the current chromatogram window in a file. You must specify a name for the file.

Graphical Programming

This menu gives you access to the graphical method programming commands. This includes Integration Timed Events and commands to add peaks to a peak table. These commands are also available from the Toolbar, which can be turned on using the **Integration Tools** command.

A description of how to use these commands is provided under Method Development and Integration Timed Events.

Data Acquisition and Control

Commands that are available from the **Control** menu are related to data acquisition and control of the instrument. In general, there are two ways to acquire data: 1) single run acquisition, where you acquire data for a single injection and 2) sequence acquisition, where you acquire data automatically for a series of runs using a pre-programmed sequence that defines the number of injections, methods, file names, and calibration.

Stop Run

When you want to stop data acquisition during a run, click the STOP button that appears on the command ribbon when the run is in progress, or use the **Control > Stop Run** command. A dialog will appear that presents options for how you want to stop the run.

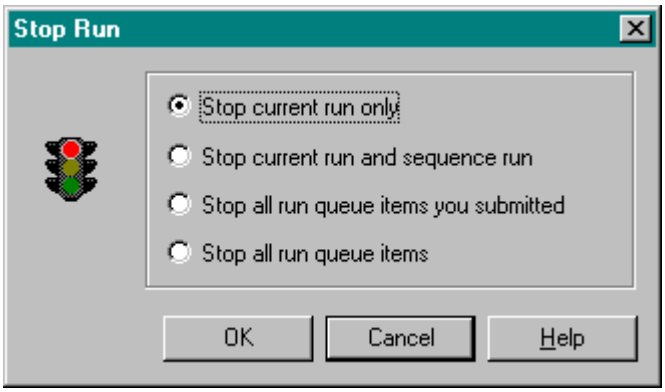


Figure 47. Stop Run dialog box

- Stop current run only

  Select this to end the run currently in progress. If the run is a part of a currently-queued sequence, the sequence will continue with the next run.

- Stop current run and sequence run

  This selection stops the run currently in progress, and terminates the sequence it is a part of. Other queued items will proceed.

- Stop all run queue items you submitted

  This selection stops the run currently in progress, and terminates all the items in the queue that were submitted by you. Queue items submitted by other users will be unaffected.

- Stop all run queue items

  This selection stops the run currently in progress, and terminates all items in the run queue.

Note

1. When a run is stopped, the data up to that point is saved in the data file. However, no analysis of the data will be performed. If you want to produce a report or view results from a run that was stopped, you must Analyze the data file.
2. If you are not the user that submitted the run or sequence or are using an instrument offline, you do not have access to the **Stop** command.

Extend Run

While a run is in progress, you can extend the data acquisition beyond the designated run time by using the **Control > Extend Run** command. When you select this command, a dialog will appear where you can enter the amount of time by which you wish to extend the run.

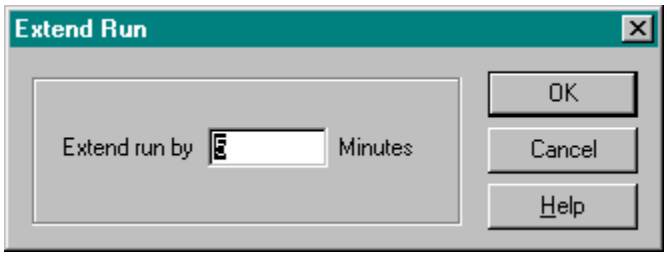


Figure 48. Expand Run dialog box

Enter the number of minutes you wish to extend the run, and then click on the **OK** button.

Note If you are not the user that submitted the run or sequence or are using an instrument offline, you do not have access to the **Extend Run** command.

Chapter 2 Tutorial

This chapter walks you through the basics of using ChromQuest. Follow the steps to set up a method and acquire a data file. Then, optimize the method for integration and set up a calibration. Use the Tutorial files provided with ChromQuest to "play" with the software and become comfortable with its features.

Note This Tutorial assumes that ChromQuest is installed with all instruments installed and properly configured.

The following list gives you a quick view of what the Tutorial contains. If you are just starting to use ChromQuest, perform all the topics in the Tutorial, in the order presented. If you are upgrading from a previous version of ChromQuest, follow the Tutorial to become familiar with how ChromQuest 4.2 is different from what you used before.

- Step 1: Using the Instrument Wizard
- Step 2: Creating an Acquisition Method (Optional)
  - Setting up the acquisition parameters
  - Running a preliminary sample
  - Setting integration parameters graphically
- Step 3: Creating a Single Level Calibration
  - Creating a peak table for the identification and quantitation of your chromatographic peaks
  - Running a Single-Level Calibration
- Step 4: Creating a Sequence Table
- Step 5: Running a Sequence
- Step 6: Using The Tutorial Files
  - Reviewing a Multi-level Calibration
  - Exploring a Peak Table
    - Examining a Custom Report
    - What IF…. (changing integration parameters)

Using the Instrument Wizard

If you are new to ChromQuest, use the built-in Instrument Wizard to locate and step through the dialog boxes necessary to acquire and process data.

To start this Tutorial, open the Instrument Wizard

1. From the desktop, double-click on the ChromQuest icon or choose **Start > Programs > Chromatography > ChromQuest**.

   ChromQuest opens to the Main Menu window shown on Figure 1 where the icons for the instruments in your Enterprise are displayed. You administrate and configure instruments from this window.

2. Double-click on the icon for your instrument. If required, login.

   ChromQuest displays the Instrument window. You control instruments and process data from this window.

3. If the Instrument window does not open with the Instrument Wizard displayed, click on the Instrument Wizard button in the command toolbar.

   The Instrument Wizard contains shortcuts to four main tasks.

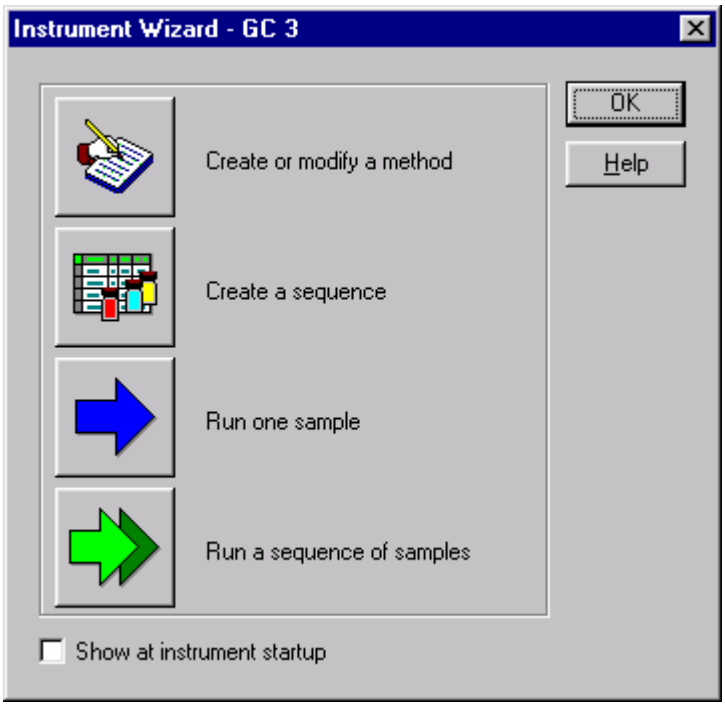


Figure 49. Instrument Wizard window

Creating an Acquisition Method

This procedure is a general case. Your instrument setup will depend on the type of instrument you have.

- For information on creating a data acquisition method for a SpectraSYSTEM (Thermo) instrument, refer to the *ChromQuest Chromatography Data System for Thermo LC User's Guide,* Chapter 3.
- For information on creating a data acquisition method for a Surveyor instrument, refer to the *ChromQuest Chromatography Data System for Surveyor User's Guide,* Chapter 2.

The first step toward acquiring a data file is to create a data acquisition method. To create a data acquisition method, perform the procedures contained in this topic in the following order:

1. Setting up the acquisition parameters
2. Running a preliminary sample
3. Setting Integration parameters graphically

Setting Up the Acquisition Parameters

To set acquisition run time and sampling rate

1. If the Instrument Wizard is not displayed, recall it by clicking on the Instrument Wizard button in the command toolbar.

2. In the Instrument Wizard, click on the Create Or Modify A Method button to invoke the Method Wizard displayed below.

Figure 50. Method Wizard window

3. In the Method Wizard, click on the Create A New Method button to start creating your method.

   The Method Wizard sets up a bank of buttons at the bottom of the Instrument window that allow you to "step" through all dialogs of method generation. A save button is also provided.

4. Open the Instrument Setup dialog box. This is the first dialog box displayed when you invoke the Method Wizard.

   The Instrument Setup dialog box appears.

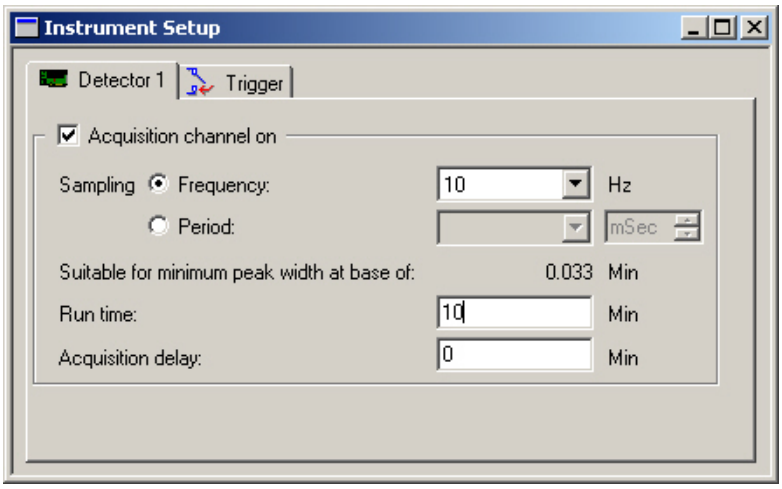


Figure 51. Instrument Setup - Detector 1 dialog box

5. Set up the acquisition parameters for your detector:

   a. Click on the Detector tab.

   b. Select the **Acquisition Channel On** checkbox.

   c. Leave the data sampling at its default. A sampling frequency of 10 Hz means that ChromQuest will collect 10 data points per s, which is adequate for peaks with a minimum width of 0.033 min at their base.

   d. Ensure that the **Run Time** is long enough for your last expected peak to elute. If you do not know how long it will take to elute, set the run time to a high number, such as 100 minutes. (You can stop the run manually after the last peak has eluted.) After your first run you can then adjust the run time to a more appropriate number.

6. Click on the Trigger tab and select the Trigger Type. (If no trigger is configured, this tab will not appear.) The trigger for each instrument is set up during configuration.

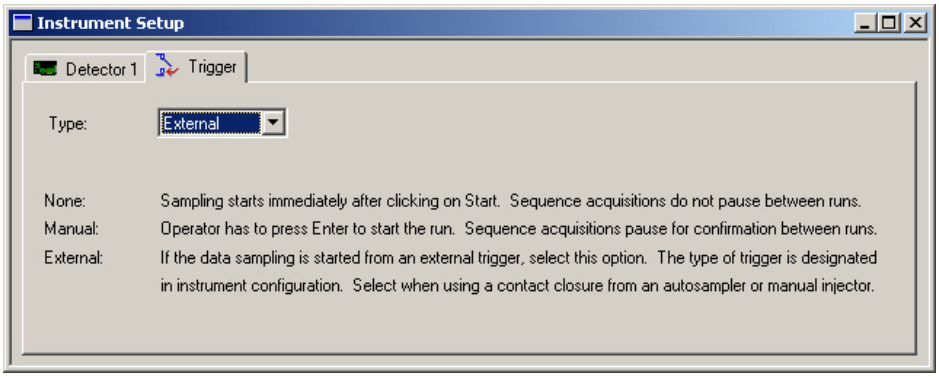


Figure 52. Instrument Setup - Trigger window

7. When you have completed the acquisition setup information, close the Instrument Setup dialog box.
8. Save your method:
   a. Choose **File > Method > Save As**.

      The Save Method File As dialog box appears.

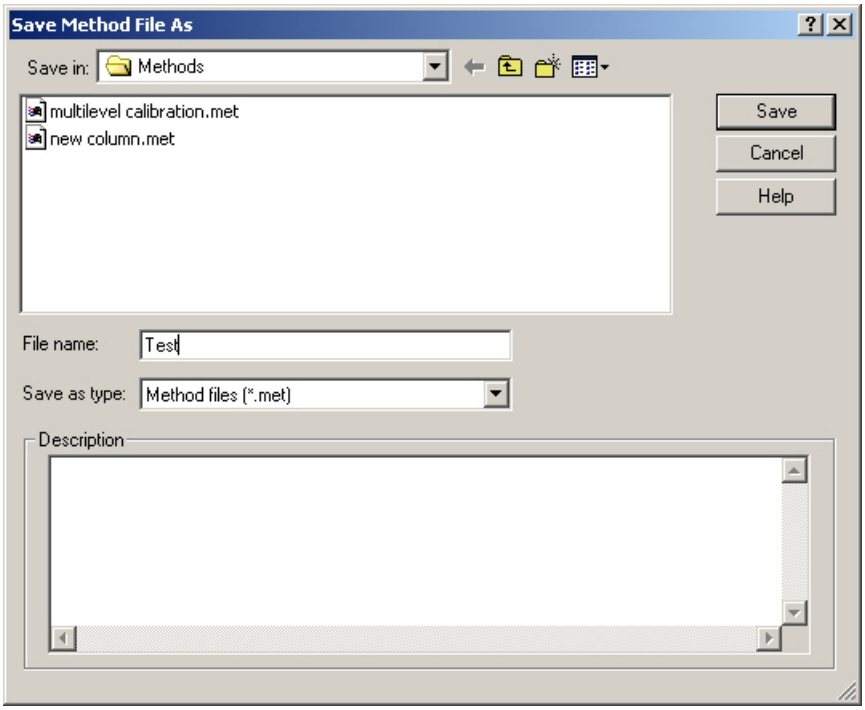


Figure 53. Save Method File As dialog box

   b. In the Save In text box, browse to the folder in which you want to save your file. For example, browse to the *Drive:\ChromQuest\Projects\Default\Methods* folder.

c. In the File Name text box, type **Test**.

d. Click on **Save**.

Running a Preliminary Sample

You will now use the method you just created to make your first data acquisition run. If your instrument contains an autosampler, place a vial in the sample tray.

To start a single run

1. Recall the Instrument Wizard by clicking on the Instrument Wizard button in the command toolbar. Then, click on the Run One Sample button. Alternatively, choose **Control > Single Run** from the menu bar.

   The Single Run Acquisition dialog box appears.

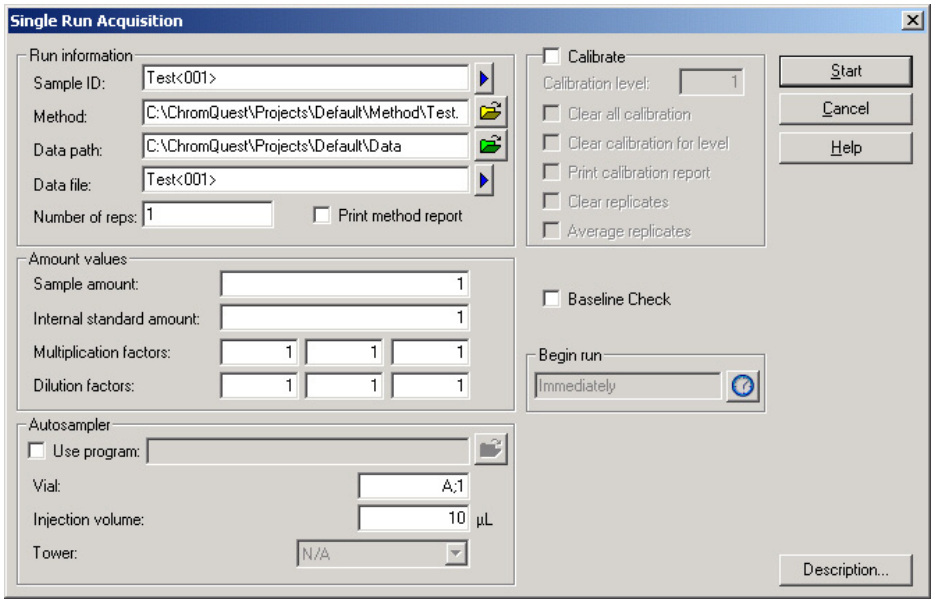


Figure 54. Single Run Acquisition dialog box

2. Type an identifying label for your sample in the Sample ID text box.
3. If the method you created is not listed in the Method text box, browse to an appropriate method by clicking on the open file button adjacent to the text box.
4. In the Data Path text box, type a path for storage of data files or browse to an appropriate directory by clicking the open file button adjacent to the text box.
5. In the Data File text box:

   a. Type **Test**.

b. Place your cursor at the end of the data file name. Press the spacebar. Click on the blue arrow to the right of the Data File text box. Then, choose **Increment Number** from the popup menu.

You must enter a unique file name in this field. Therefore, if you have performed this tutorial before, you must first delete this file from your disk or move it to a different directory before proceeding.

6. Leave the Amount Values at their default values of 1. Refer to "Single Run Acquisition" on page 105 for more details.
7. Click on **Start** to begin your run.

   You will see the data as it is acquired in the chromatogram window on your screen.

Setting Integration Parameters Graphically

ChromQuest uses default integration parameters that are appropriate for most HPLC applications. However, you may have certain peaks that require special integration treatment. Such special integration treatments are entered into your method as **Integration Timed Events**. These events can be placed at the beginning of the run to apply to all peaks, or they can be inserted at a certain place in the chromatogram such that only some peaks are affected.

Note You can perform this step using one of the multicalibration level.dat files provided with the software.

To add the integration event *Integration Off* to your method

1. If your recently acquired data file is not open, open it:
   a. Choose **File > Data > Open** to display the Open Data File dialog box.
   b. Browse to the appropriate directory. Then, select your data file from the list displayed. Alternatively, select one of the files supplied with ChromQuest.
2. Click on the Analyze button () to integrate the chromatogram and display the baselines.

   Note If you do not know what a button's function is, simply move the cursor over the button (don't click) and a Tool tip box will appear showing the button's name or function.

3. To add the **Integration Off** timed event, click on the Int Off button on the Integration Toolbar at the bottom of the Instrument window.
4. As instructed in the status bar at the bottom of the window, click your mouse once prior to a part of the chromatogram where you want to turn integration

off. (Select a section of chromatogram where one or more peaks elute.) Then click the mouse again at the point on the chromatogram where you want to turn integration on again.

The Integration Off dialog box appears.

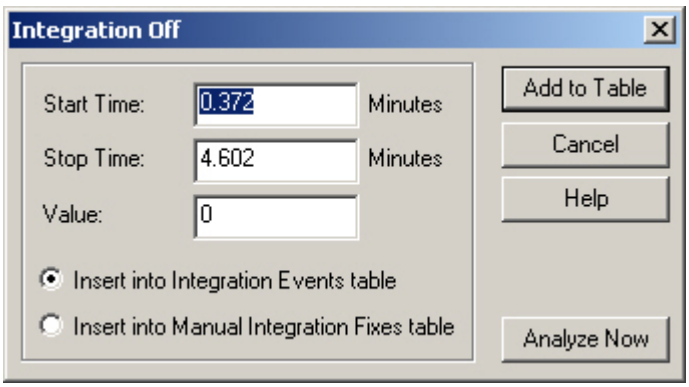


Figure 55. Integration Off dialog box

The points where you clicked your mouse are shown as **Start Time** and **Stop Time**. The integration will be turned off between these points on your chromatogram. The **Value** is set at zero, as no numeric value is required for this event.

5. Select the Insert into Integration Events table option button to add the event to the Integration Events Table of your method where it will be used on all chromatograms (for this wavelength channel if your method contains more than one acquisition channel) analyzed using this method.

   Note Events in the Manual Integration Fixes table are only applied to the current data file.

6. Click on **Analyze Now** to add the event to the method and re-integrate the chromatogram. (**Add to Table** will simply add the event to the integration timed events table without re-integration.)

   Your chromatogram will be re-drawn using the new integration event. Notice that the area you selected has no baselines drawn because the integration has been turned off for these peaks.

7. To view the Integration Events table, click on the Integration Events button on the command toolbar.

   The integration timed events table appears.

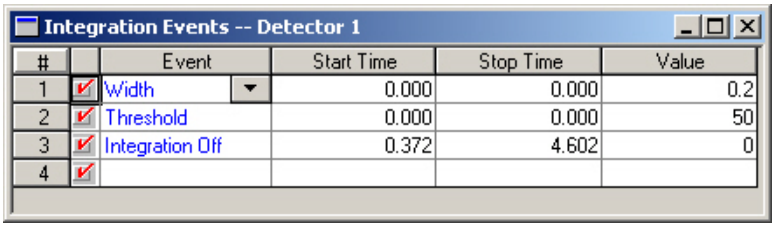


Figure 56. Integration timed events table

Notice the Integration Off event has been added to the table.

8. To remove the Integration Off event from your method, click on the **Integration Off** event name or the row number, then press DELETE. You can also delete the event using the **Edit > Cut** command or re-insert the event using the **Edit > Paste** command. To temporarily view the effect of removing an event without actually removing it from the table, click the check box adjacent to the event to de-select it. To re-select the event, click the check box once again.
9. When you are finished viewing the Integration Events table, close it and return to your chromatogram.

Creating a Single Level Calibration

If you are interested in determining the concentration of your analyte(s), you must set up your method for calibration. Refer to **Chapter 3: Method Development** for further details on how to set up multiple level calibrations. In this tutorial, you will set up a single level calibration.

Setting up any type of calibration involves the following steps:

- Identifying the Named Peaks and entering their concentration levels in the Peak Table section of the method
- Running the calibration standard(s) to enter peak areas into the calibration table

The easiest way to enter calibration information into the method is to run a calibration standard. Then, use the stored data file from this run to graphically define the peak(s) for your analyte(s).

If you have been following the Tutorial, you already have a stored data file named *Test001.dat*. If have not already run a preliminary sample, you can run a preliminary sample by following the procedure in the topic "Running a Preliminary Sample" on page 54 or you can select one of the data files provided with ChromQuest. These data files are located in the following directory: *Drive:\ChromQuest\Projects\Default\Data.* The "LabTest" data files contain scan data from a PDA detector. The "multi calibration level" data files contain one wavelength channel.

Creating the Peak Table

To create a peak table for the identification and quantitation of analytes

1. Open your stored data file by choosing **File > Data > Open**. Select your data file from the list or select one of the *ChromQuest* files.
2. Click on the Analyze button () to integrate the chromatogram and show the baselines.

3. Click on the Define Single Peak button on the Integration toolbar.

   The Define Single Peak dialog box appears. The retention time of the first detected peak is listed at the top of the dialog box.

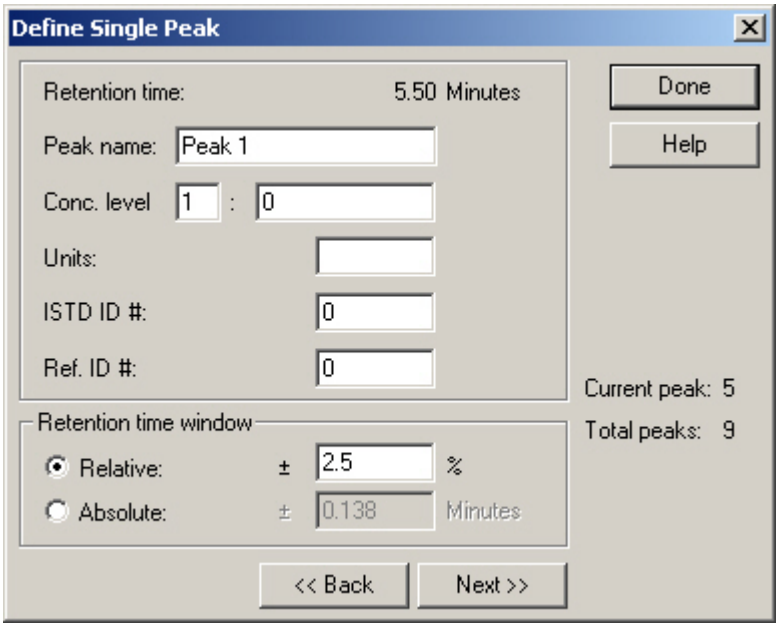


Figure 57. Define Single Peak dialog box

4. Check the retention time of the peak that is listed in the dialog box,
   - If you want to add the peak to the peak table, continue at step 5.
   - If you do not want to add this peak to the peak table, continue at step 6.
5. Make the appropriate entries in the dialog box for this peak:
   a. In the Peak Name text box, type the name of the peak.
   b. In the Conc. Level text boxes, leave the level of 1 at its default and type a numeric concentration value into the second text box.

      Note The Conc. Level text boxes allow you to enter the levels and the concentrations of your calibration standards. By default, the dialog box opens with a value of 1 in the first text box. To add a second concentration level for a peak, click on the peak a second time. Then, type **2** in the first text box and its associated concentration value in the second text box. Continue to click on the peak and enter the concentration levels and their associated values until you have entered all the concentration levels.

   c. (Optional) In the Units text box, type a unit of measurement.

      Note This unit label can be displayed in the method custom reports. The concentration levels in the Peak Table are unitless. Typically, you will enter the concentrations of your calibration standards in the same units as you want to report the samples.

d. In the ISTD# text box, leave the internal standard ID# set to its default of 0.

You are creating an external standard calibration. Refer to **Chapter 3: Method Development** for details on performing an internal standard calibration.

e. In the Ref ID# text box, leave the reference ID# set to its default of 0.

Refer to **Chapter 3: Method Development** for details on using reference peaks to adjust the expected retention times of your analytes.

f. In the Retention Time Window group box:

i. Select the Relative option button.

ii. Type **2.5** in the percentage text box.

This creates a relative retention time window of ± 2.5% for your analytes. In the chromatogram shown below, the retention time windows for the peaks are displayed as bars above the peak apexes.

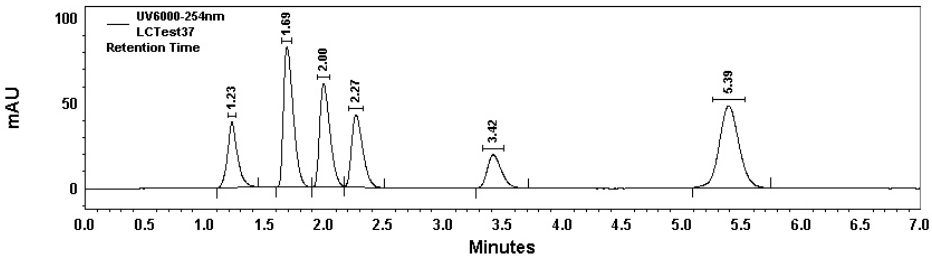


Figure 58. Chromatogram showing retention time

6. Click on **Next** to move to the next detected peak or click on **Back** to return to a previous peak.

- If you want to add more peaks to the peak table, return to step 4.
- Otherwise, continue at step 7.

The number of the current peak and the total number of peaks in the chromatogram are displayed on the right of the dialog box. When you have reached the last detected peak in the chromatogram, the Next button will be unavailable.

7. After you finish adding peaks to your peak table, click on **Done.**
8. To view the Peak Table:

a. Click on the Peak/Group Tables button in the command toolbar.

b. Click on the Named Peaks tab.

Each peak you defined will appear as a row in the Peak Table spreadsheet, along with its retention time and the other parameters you entered. Note that if you already had peaks in your peak table, the peaks you just defined will be added to those already present. Information in the Peak Table can be edited by scrolling through the rows and columns. Refer to the topic "Peak Table" on page 134 for details on the contents of the Peak Table.

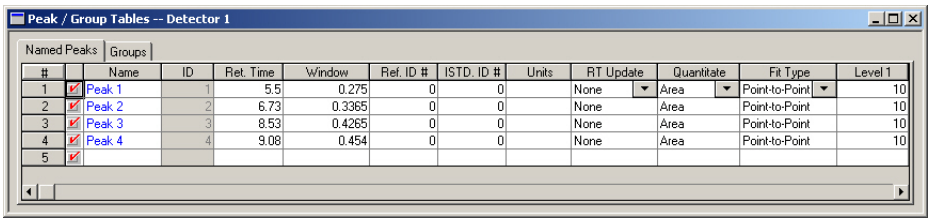


Figure 59. Peak/Group Tables

c. Do not enter information in the other columns of the Peak Table at this time. Close the Peak Table.

   Your method now contains a Peak Table for the identification and quantitation of your unknown sample types.

9. Before you proceed, save the method by choosing **File > Method > Save** from the menu bar. (If you want to save the method using a different name, use the **File > Method > Save As** command.)

Entering Peak Areas into the Method

Your method must contain calibration information for each analyte that you want to quantitate. To enter peak areas for your analyte(s) into the calibration table of the method, you can either run the standard sample again, designated as a calibration run, or you can re-analyze the stored data file that you created by performing the Running a Preliminary Sample task on page 54.

To create a calibration curve using a stored data file

1. If your data file is not open, open it:

   a. Choose **File > Data > Open** to display the Open Data File dialog box.

   b. Browse to the appropriate directory. Then, click on the appropriate data file in the list box.

   Note In the Files Of Type list box, select *All Files* if you did not add the .dat extension to your data file name.

   c. In the Options group box:

      i. From the Method list box, select *From Results*.

ii. From the Results list box, select the most recent date.

The method with the most recent data should contain the Peak Table that you created while performing the steps in "Creating a Single Level Calibration" on page 58.

d. Click on **Open**.

2. Choose **Analysis > Analysis Single Level Calibration** from the menu bar.

The Analysis / Single Level Calibration dialog box appears. The Sample ID for the data file appears in the Sample ID text box, the data path for the data file appears in the Data Path text box, and the name of the data file appears in the Data File list box.

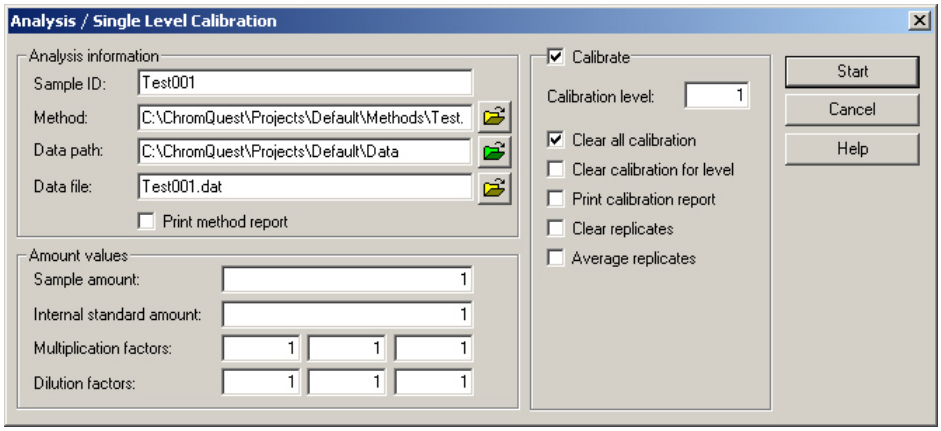


Figure 60. Analysis / Single Level Calibration dialog box

3. In the Method text box, browse to your method *Test.met*.

4. Leave the Amount Values set to "1". For details on how these values are used, see **Chapter 3: Method Development**.

5. Select the Calibrate checkbox.

6. Then, type **1** in the Calibration Level text box.

7. Select the Clear All Calibration checkbox.

Selecting the Clear All Calibration checkbox allows the absorbance values to be cleared from the calibration table as the calibration standard is analyzed.

8. Click on **Start**. When the analysis is complete, the chromatogram will be integrated and the areas for the named peaks will be entered into the Review Peak Calibration section of the method.

Creating a Sequence Table

If you are using an autosampler to inject samples, you must define the samples to be injected and how they are to be acquired and analyzed. This is done using a sample **Sequence**. A sample Sequence can be used to acquire both calibration and unknown samples. It can also be used to automatically re-analyze stored data files. Details on creating and using a Sequence are located in **Chapter 4: Sequence Operations**. In this part of the Tutorial you will create and use a simple sequence to acquire a calibration sample and three unknown samples.

To create a new sequence for the acquisition of data files

1. Recall the Instrument Wizard by clicking on the Instrument Wizard button in the command toolbar.

   Then, click on the Create A Sequence button. Alternatively, choose the **File > Sequence > Sequence Wizard** command from the menu bar.

   The Sequence Wizard – Method dialog box appears.

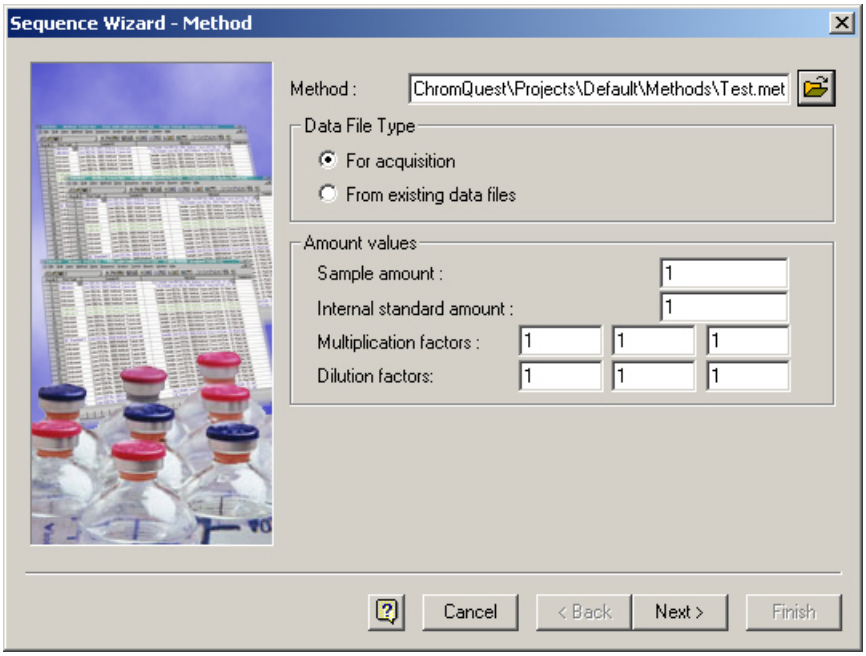


Figure 61. Sequence Wizard – Method dialog box

2. In the Sequence Wizard – Method dialog box:

   a. Type the method to be used for the acquisition or select the name from a list of available methods by clicking the File Open button. If you are following the Tutorial, enter *Test.met* as your method name.

   b. Select the For Acquisition button. This will cause the Sequence Wizard to prompt you for information required for data acquisition.

c. Leave the Amount values at their default values of 1.

3. Click on **Next** to continue to the next page of the Sequence Wizard.

   The Sequence Wizard – Unknowns dialog box appears.

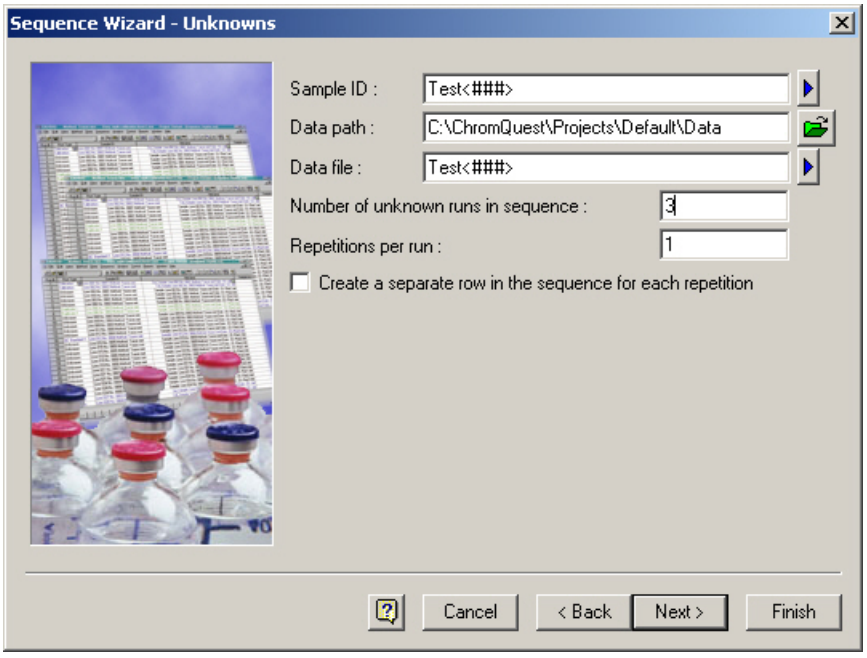


Figure 62. Sequence Wizard – Unknowns dialog box

4. Enter information about the "unknown" run types:

   a. In the Sample ID text box, type **Test**. Then, click on the blue right arrow and choose **Line number**. This will cause each sample to be identified with the sequence line number. You can use sample IDs to perform file searches from the ChromQuest datasystem.

   b. In the Data Path text box, browse to an existing path by clicking on the **File Open** button. For example, browse to the directory *Drive:\ChromQuest\Projects\Default\Data*. If other users have already performed this tutorial, place your data in a unique folder by adding another folder name to the end of the data path.

   c. In the Data File text box, type **Test**. Then, click on the blue right arrow and choose **Line Number**. This will cause each data file to be identified with the line number in the sequence table.

      Using a numbered identification ensures the data file name for each run is a unique name, preventing errors that will occur if you try to acquire data using an existing data file name.

   d. In the Number Of Unknown Runs In Sequence text box, type **3**.

e. Leave the other fields at default values.

5. Click on **Next** to continue to the next page of the Sequence Wizard. If you added your name to the end of the data path, ChromQuest asks you if you want to create a folder. Click on **Yes**.

   ChromQuest displays the Sequence Wizard – Autosampler page.

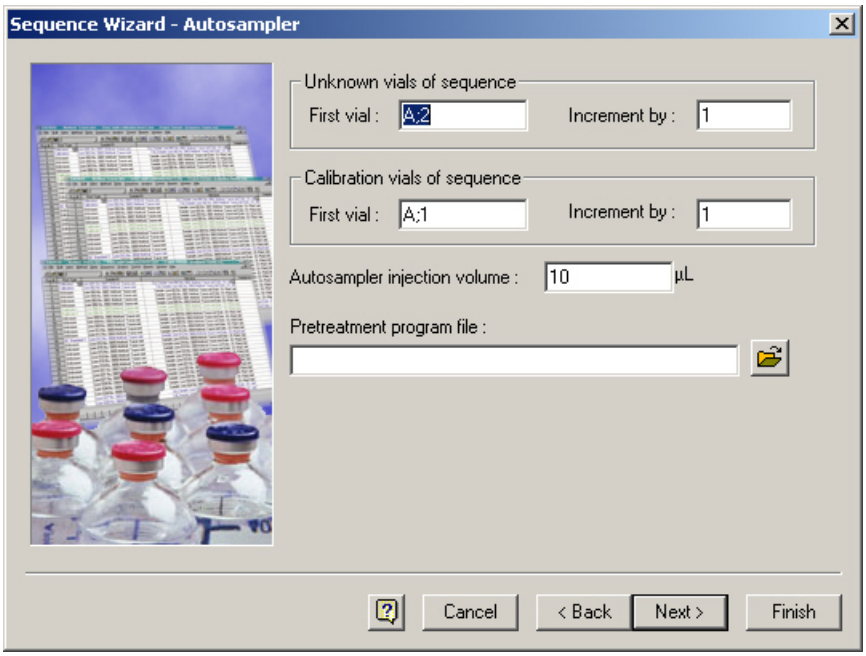


Figure 63. Sequence Wizard – Autosampler dialog box

6. In the Sequence Wizard – Autosampler dialog box, enter information that applies to the autosampler:

   a. Type the appropriate vial locations in the First Vial text box for the Unknowns and the First Vial text box for the calibration standards. Type the appropriate values in the Increment By text boxes.

      For example, in this Tutorial, place four standard 1.8 mL vials into your autosampler in positions A1 through A4.

      - For the Surveyor Autosampler, type A;2 in the First Vial text box for the Unknown Vials Of Sequence. Leave the Increment By value at its default of 1. Type A;1 in the First Vial text box for the Calibration Vials Of Sequence.
      - For a SpectraSYSTEM autosampler, type A02 in the First Vial text box for the Unknown Vials Of Sequence. Leave the Increment By value at its default of 1. Type A01 in the First Vial text box for the Calibration Vials Of Sequence.

b. Leave the value in the Autosampler Injection Volume text box at its default.

The value in the Autosampler Injection Volume text box is linked to the configuration of your autosampler.

c. Ensure that the Pretreatment Program File text box is empty.

7. Click on **Next** to continue to the next page of the Sequence Wizard.

ChromQuest displays the Sequence Wizard – Calibration page. The calibration ID and calibration file names are automatically set to the identifications from the Unknowns page.

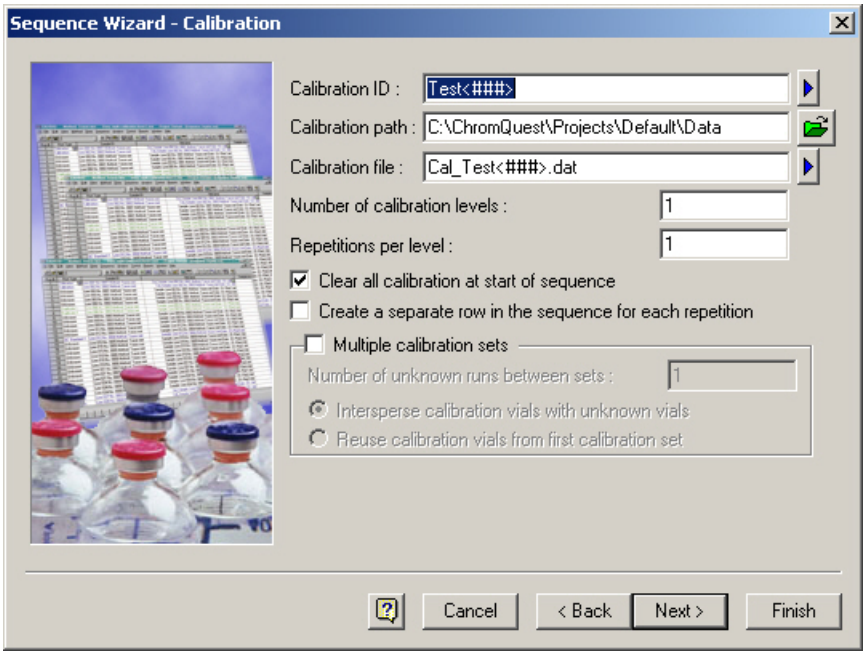


Figure 64. Sequence Wizard – Calibration dialog box

8. In the Sequence Wizard – Calibration page, enter information about the calibration standards:

a. In the Number Of Calibration Levels text box, type **1**. Leave the number of Repetitions Per Level at 1.

b. Select the Clear All Calibration At Start Of Sequence checkbox.

ChromQuest will clear the area values in the calibration section of the method at the start of the sequence run.

9. Click on **Next** to continue to the next page of the Sequence Wizard.

ChromQuest displays the Sequence Wizard – Reports page.

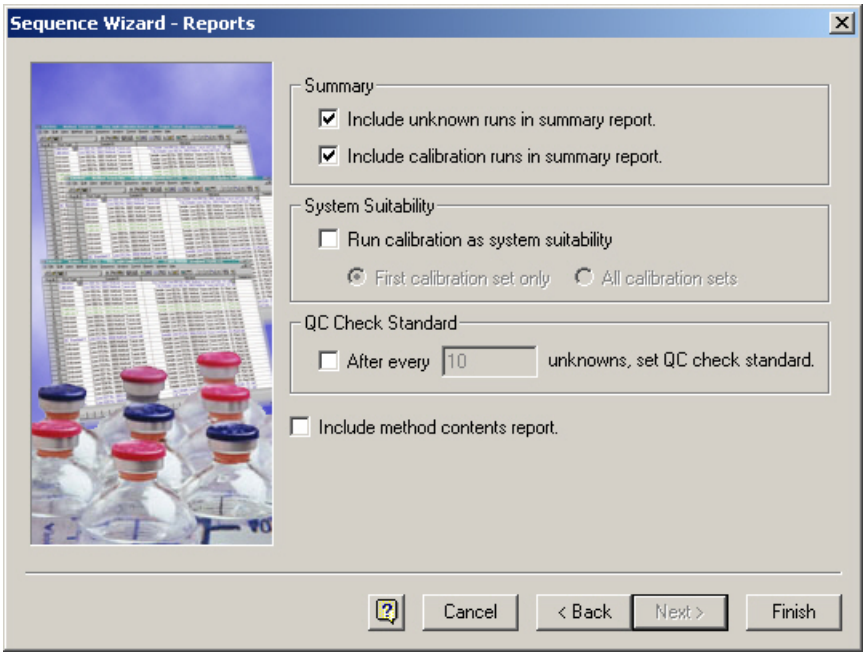


Figure 65. Sequence Wizard – Reports dialog box

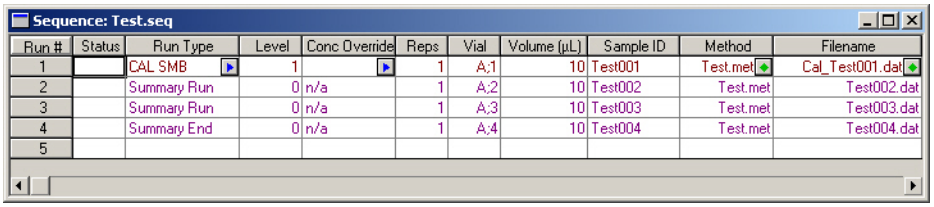
10. In the Summary group box of the Sequence Wizard – Reports page:

    - Select the Include Unknown Runs In Summary Report checkbox.
    - Select the Include Calibration Runs In Summary Report checkbox.

    By default, the Summary Begin run type, which is created by checking either of the Summary report checkboxes, uses the Summary.tpl template. This template reports the external standard concentrations of the sample peaks. The external standard concentrations for all the runs in the sequence will be reported in the Summary report after the sequence run is completed.

11. Click on **Finish**.

    The sequence table appears.



Run #	Status	Run Type	Level	Conc Override	Reps	Vial	Volume (µL)	Sample ID	Method	Filename
1		CAL SMB	1		1	A;1	10	Test001	Test.met	Cal_Test001.dat
2		Summary Run	0	n/a	1	A;2	10	Test002	Test.met	Test002.dat
3		Summary Run	0	n/a	1	A;3	10	Test003	Test.met	Test003.dat
4		Summary End	0	n/a	1	A;4	10	Test004	Test.met	Test004.dat
5										

Figure 66. Sequence table

At this point, the sequence is set up to run one calibration standard and three unknowns. Notice the Sample ID's and Data File names are incremented automatically to prevent duplication.

To run a calibration standard, you must enter its calibration level in the Level column of the sequence table. This has been done automatically by the Sequence Wizard. Unknown runs always have a Level of "0". The information in the Run Type field may be abbreviated if there is more than one run type designation. To view a list of possible Run Types, click on the blue arrow next to the run type. For details on each of these run types, see the Sequence section.

12. Save the sequence table:

    a. Choose **File > Sequence > Save As** from the menu bar.

    The Save Sequence File As dialog box appears.

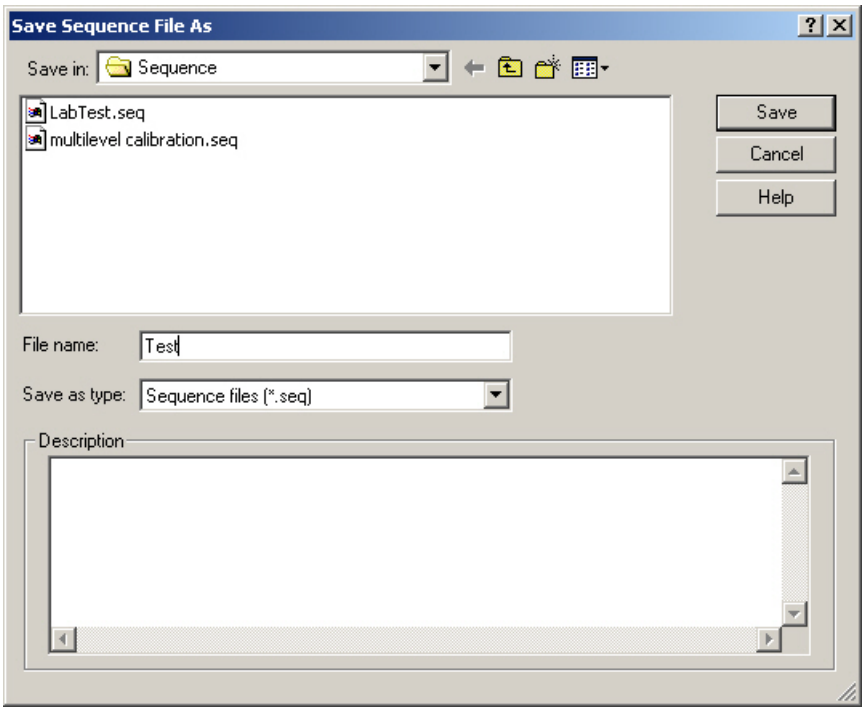


Figure 67. Save Sequence File As dialog box

    b. Type **Test** in the File Name text box.

    c. Click on **Save**.

Note By default, ChromQuest saves sequence files with the .seq extension.

Running a Sequence

Now that you have created a sequence table, you are ready to run a set of samples. If you are using an autosampler, place sample vials in the tray compartment at the vial locations indicated in the sequence table.

To start a sequence run

1. Recall the Instrument Wizard by clicking on the Instrument Wizard button in the command toolbar.

2. In the Instrument Wizard, click on the Run A Sequence Of Samples button. Notice that this button can also be found in the command toolbar.

   The Run Sequence dialog box appears.

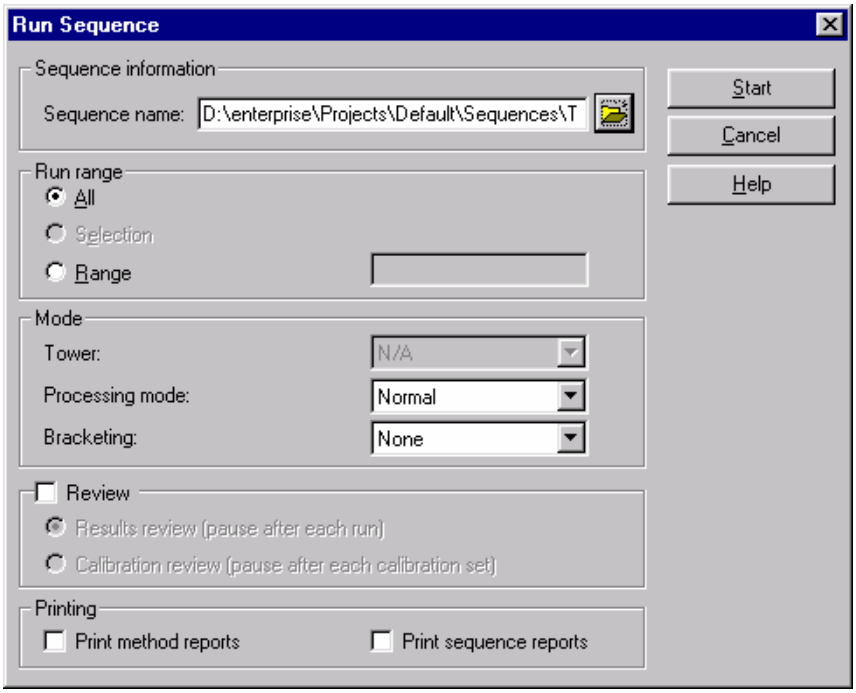


Figure 68. Run Sequence dialog box

3. Click on the File Open button next to the Sequence Name text box. Then, select the sequence that you created as you performed this Tutorial.
4. If you have a printer connected to your instrument, check the Print Sequence Reports checkbox.
5. Leave the other settings in the dialog box at their defaults.
6. If you have not already done so, prepare your autosampler to inject your standard sample, followed by 3 unknown samples.
7. Click on **Start**.

When the sequence is completed, you will have acquired data files for one standard and three unknown runs. In addition, you will have generated a sequence summary report for the sequence.

ChromQuest
Sequence Summary Report

Sequence name:	Test.seq
Analyst:	B. A. Cook

Channel A	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**
Data Filename	**ESTD**	**ESTD**	**ESTD**	**ESTD**
Cal_Test001.dat	10.00	10.00	10.00	10.00
Test002.dat	20.23	20.06	18.36	20.10
Test003.dat	29.86	29.70	26.12	29.99
Test004.dat	39.62	39.61	34.45	39.42
Min:	10.00	10.00	10.00	10.00
Max:	39.62	39.61	34.45	39.42
Mean:	24.93	24.84	22.23	24.88
Std Dev:	11.01	11.01	9.07	10.98
%RSD:	44.18	44.32	40.79	44.12

Figure 69. Sequence summary report

Using The Tutorial Files

In this section of the Tutorial, you will use the tutorial files provided to become familiar with additional features of the ChromQuest Chromatography Data System.

Reviewing Multi-level Calibration Curves

Once you have fully calibrated a method, the calibration curves and associated data can be viewed using the **Review Calibration** feature. In order to see a fully calibrated multi-level calibration, use the **multilevel calibration.met** file provided with ChromQuest.

To review calibration information in multilevel calibration

1. Open the **multilevel calibration.met** method file by choosing **File > Method > Open** to display the Open Method File dialog box. Select the **multilevel calibration.met** file from your disk. It is located in the *Drive:\ChromQuest\Methods* directory. Then, click on **Open**.

   Notice that the multilevel calibration.met method is listed in the title bar of the Instrument window.

2. Click on the Review Calibration button in the command toolbar or choose the **Method > Review Calibration** command.

   The following window appears.

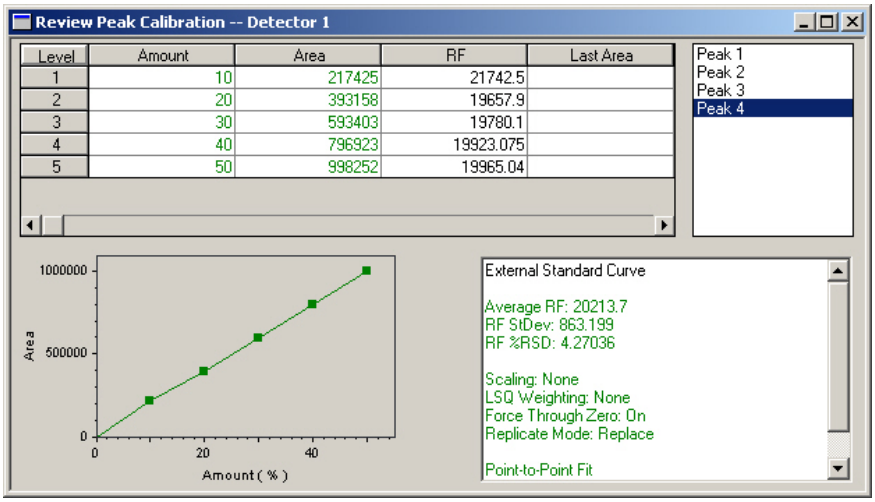


Figure 70. Review Peak Calibration window

The Review Peak Calibration window contains the following information:

- A table that includes information about the concentrations of the calibration standards and the peak areas generated by running the calibration standards
- A list of the named peaks in the Peak table

- A work tablet that contains information about the calibration curve
- A graphical display of the calibration curve

When you click on a peak name, its calibration information is shown in the bottom half of the window.

The calibration curve fit type by default is Point-to-Point. To overlay a different fit type, right-click in the calibration curve box to open a popup menu. Choose **Change Fit Type > Linear**. Notice the new linear calibration curve is overlaid on the Point-to-Point curve.

For additional details on using **Review Calibration**, refer to **Chapter 3: Method Development**. To close the window, click the X box at the upper right corner of the Review Calibration window.

Exploring a Peak Table

The Peak Table includes information about the calibration standards, check standards, and spike amounts. In this section, you will use a method provided with ChromQuest to examine a Peak Table and become familiar with what a completed peak table looks like.

To explore a Peak Table

1. Open the *multilevel calibration.met* method, which is located in the *Drive:\ChromQuest\Methods* directory, by choosing **File > Method > Open** to display the Open Method File dialog box. Then, select the multilevel calibration.met method from the list of methods on your disk.

2. After the *multilevel calibration.met* method is open, view its Peak Table by clicking the Peak Table button on the command ribbon.

   The following peak table appears.

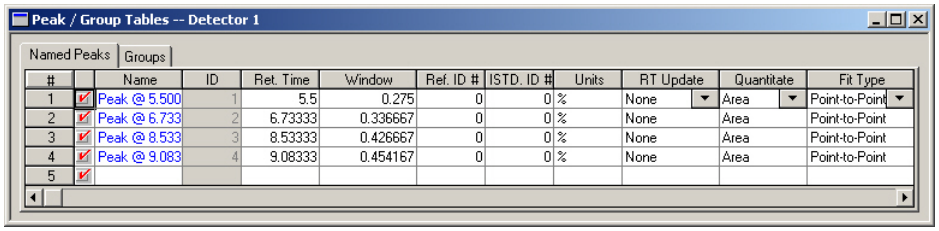


Figure 71. Peak Table

On the **Named Peaks** tab, you will find a table containing all of the calibration information for the calibrated peaks in this method. If you scroll to the right, you will see many different columns, each of which represent a parameter for the calibration, including the **Levels**, which contain the calibration amounts for each compound at each level of calibration.

3. To customize the Peak Table such that only parameters needed for a given calibration are displayed:

   a. Right-click on the Peak Table to display a popup menu.

   b. In the popup menu, choose **Properties** to open the Properties dialog box.

   c. To remove a column from the Peak table, deselect its checkbox. Then, click on **OK**.

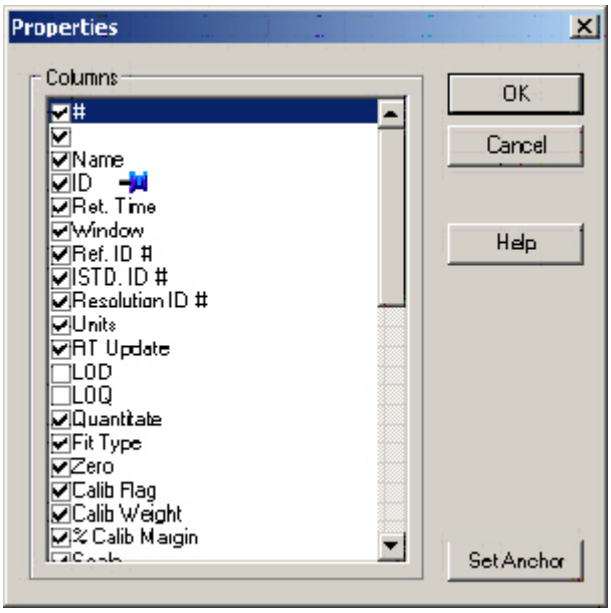


Figure 72. Properties dialog box

Details on what each column represents, along with how to customize the Peak Table, are given in **Chapter 3: Method Development**.

Examining a Custom Report

ChromQuest comes with a complete suite of report templates that can be used without modification to generate reports.

To view report

1. Open your data file by choosing **File > Data > Open** to display the Open Data File dialog box. Browse to the appropriate directory. Then, click on the appropriate data file in the list box. From the Method list box, select *From Results*. From the Results list box, select the most recent date.

   Note In the Files Of Type list box, select *All Files* if you did not add the .dat extension to your data file name.

2. Analyze your chromatogram to ensure that the baseline is displayed.

3. Choose **Reports > View > External Standard** from the menu bar

   The external standard appears on your screen.

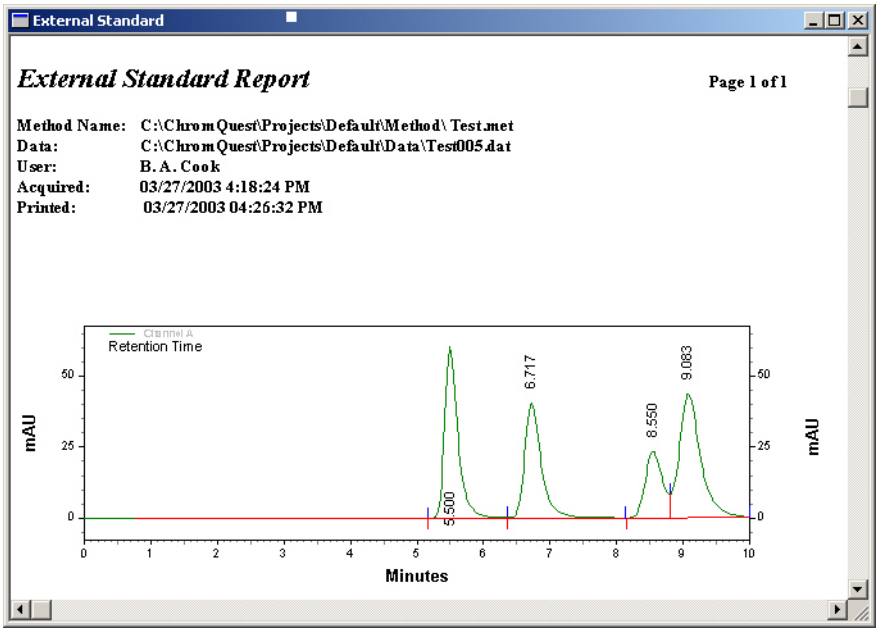


Figure 73. External Standard window

If you want, you can modify the standard report templates, or create entirely new reports using the Custom Report capability of ChromQuest. You can create custom method reports and / or custom Sequence reports. These are described in detail in **Chapter 5: Custom Reports**.

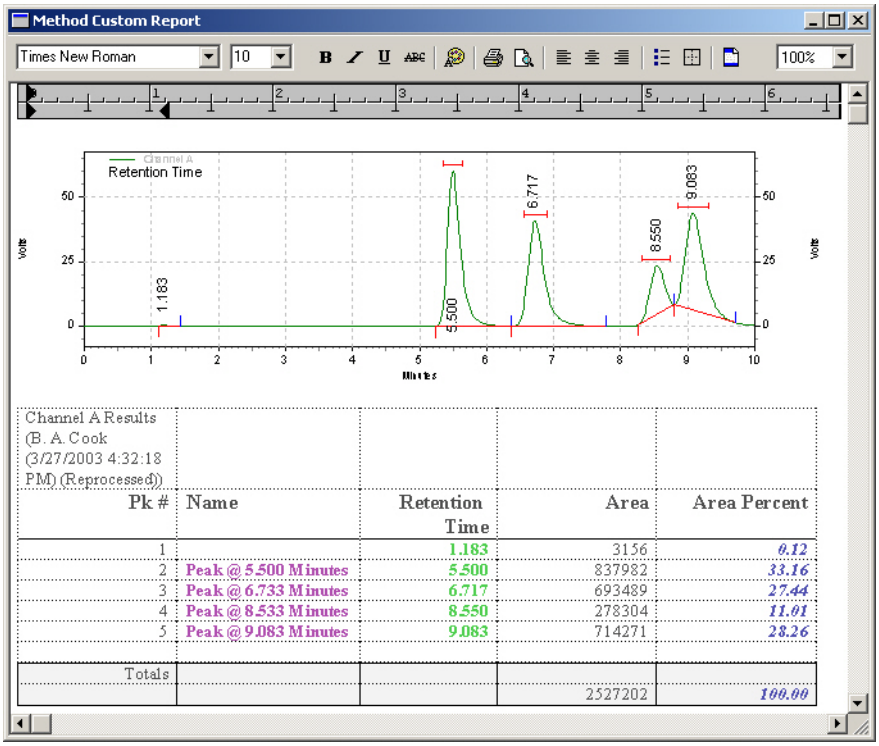
To view the custom report template in the multilevel calibration.met file

1. Open the file if it is not already open.

2. Click on the Edit Custom Report button on the command toolbar to open the method custom report editor.

   The current method custom report template appears.

   Examine the custom report template by scrolling through it using the scroll bars that appear on the bottom and side of the window. Before you attempt to edit or create your own custom report, thoroughly review **Chapter 5: Custom Reports**. To return to the method, click the **x** box in the upper right corner of the custom report window.



Pk #	Name	Retention Time	Area	Area Percent
1		1.183	3156	0.12
2	Peak @ 5.500 Minutes	5.500	837982	33.16
3	Peak @ 6.733 Minutes	6.717	693489	27.44
4	Peak @ 8.533 Minutes	8.550	278304	11.01
5	Peak @ 9.083 Minutes	9.083	714271	28.26
Totals			2527202	100.00

Figure 74. Method Custom Report window

What If … Changing Integration Parameters

Another important aspect of using a computerized data system is the ability to customize the integration using Integration Timed Events. In this part of the Tutorial, you will use the **multi calibration level 3.dat** data file provided to become familiar with how to enter integration timed events into your method, and to view the effects of some of these events. Refer to **Chapter 1: Examples of Integration Timed Events** of the *ChromQuest Chromatography Data System Reference Guide* for more information on integration events.

To familiarize with the graphical programming feature in ChromQuest

1. Open the multi calibration level 3.dat data file:
   a. Choose **File > Data > Open** to display the Open Data File dialog box.
   b. Browse to the *Drive:\ChromQuest\Data* directory on your computer.
   c. Select the in the list box.
   d. From the Method list box, select *Original / Acquisition*.
   e. Click on **Open**.
2. Click on the Analyze button to analyze the chromatogram and display the baselines.

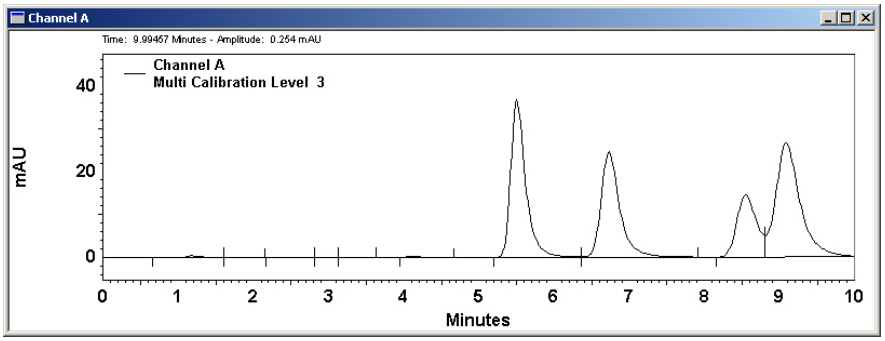


Figure 75. Chromatogram

Note The vertical line cursor moves with your mouse. The retention time where the cursor is located is shown at the top of the chromatogram window.

3. Add an annotation for Area to the chromatogram:
   a. Right-click on the trace to open a popup menu.
   b. In the popup menu, choose Annotations.

      The Trace Annotations Properties dialog box appears.

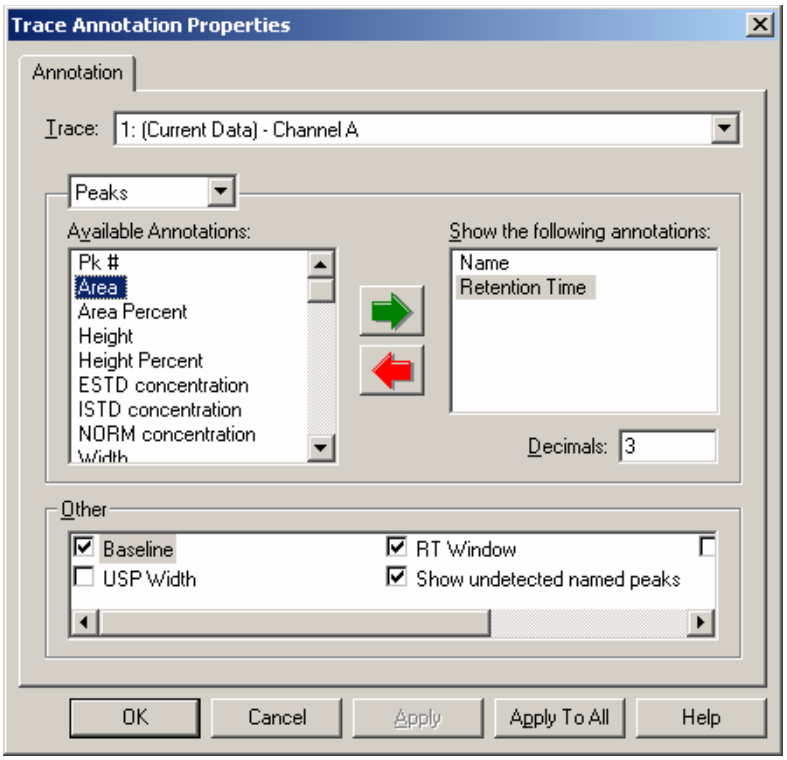


Figure 76. Trace Annotation Properties - Annotation dialog box

c. In the Available Annotations list box, double-click on the Area annotation.

d. Click on **OK**.

The trace is now annotated with peak areas.

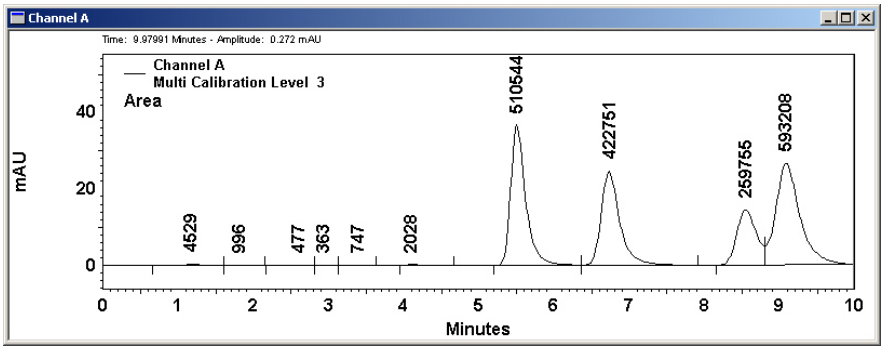


Figure 77. Chromatogram

4. Add the **Valley-to-Valley** timed event to integrate the cluster of 4 large peaks with valley-to-valley baselines:

a. Click on the **Valley** button in the Integration toolbar.

b. Follow the instructions in the status bar at the bottom of the Instrument window. These instructions tell you to "Select Start of Valley to Valley. Click on the trace just before the first large peak. After you click, the instructions tell you to "Select End of Valley to Valley". Click on the trace just after the last peak.

c. The Valley to Valley dialog box appears, displaying the start and stop points for the event.

d. In the Valley to Valley dialog box, click on **Analyze Now** to analyze the chromatogram and to add the event to the Integration table.

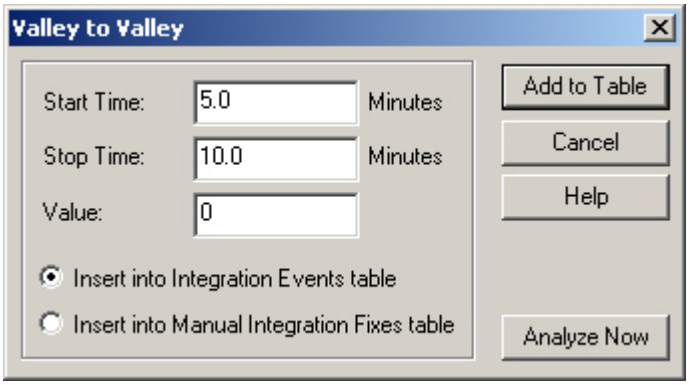


Figure 78. Valley to Valley dialog box

Notice the peaks within the region of the event are now integrated using the valley-to-valley event and baselines are adjusted accordingly.

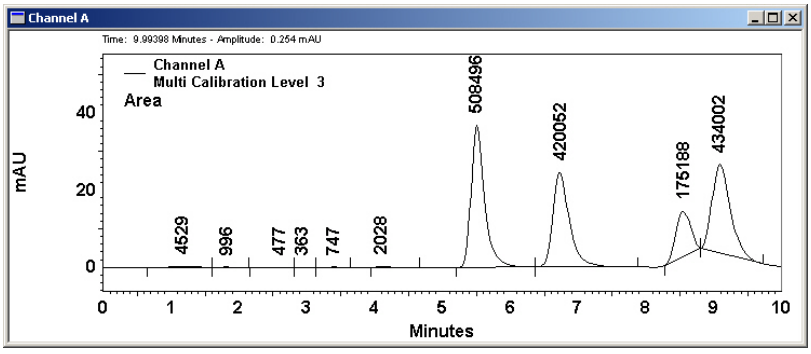
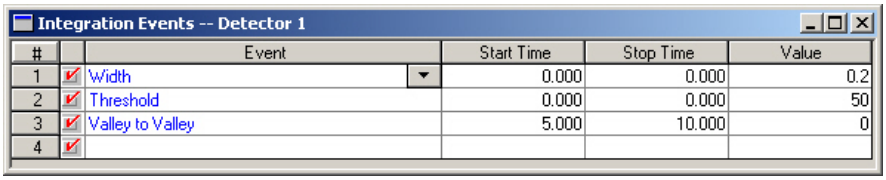


Figure 79. Chromatogram

5. Click on the Integration Events button in the command toolbar. Then, note the addition of the **Valley to Valley** event in the table.

Note If you are performing a multi-wavelength analysis, there is an integration table for each wavelength in the method. For scan data, there is an integration table for each discrete wavelength, each multi-chromatogram wavelength, and the spectrum max plot.



#	Event	Start Time	Stop Time	Value
1	Width	0.000	0.000	0.2
2	Threshold	0.000	0.000	50
3	Valley to Valley	5.000	10.000	0
4				

Figure 80. Integration Events window

6. Remove the Valley to Valley event from the Integration Events table by clicking on its number with the mouse, then choose **Edit > Delete** from the menu bar. You can also test integration without the event, yet leave it in the timed event table, by de-selecting the check box next to the Valley to Valley event and then re-integrating the chromatogram.

7. Close the Integration Events Table.

Practice adding and deleting integration timed events using the **multi calibration level 3.dat** data file until you feel comfortable with adding and deleting integration events.

You have completed the ChromQuest Tutorial. Detailed explanations on how to create multi-level calibrations, create custom reports, and create and use sample sequences are given in later sections. In addition, do not forget to use the extensive on-line Help as you work with the software.

Chapter 3 Method Development

A ChromQuest method contains complete information for acquisition, integration, and calibration of a chromatography run. A method has four main sections, as follows:

- Instrument Setup
- Integration Timed Events
- Calibration
- Custom reports

The method can also contain general data processing information such as data export options, valve programs, and user programs to run.

A method can be created from one of the Instrument windows, or when running an instrument **offline**. When you create a method from an Instrument window, you also have access to starting runs and viewing data acquisition. When you create a method **offline**, you do not have access to instrument control (starting or stopping runs), however you can view real time data. Offline, you can set up a complete method, including instrument configuration, which can be opened later and used for one of the Instruments on the system.

This chapter describes the steps required to prepare a method for data acquisition, optimize integration, and set up calibration. Later sections will detail report generation and sequence operations.

This chapter contains the following sections:

Creating an Acquisition Method

In order to acquire data and save it on the hard drive of your computer, you need to create a method that contains data acquisition information such as run time and sampling rate. To create a new method or edit an existing method, use the Method Wizard. You can also use the **File > Method > Method Wizard** command, then select the **Create New Method** button. This will over-write current method parameters with those of the current ChromQuest default method.

You can create a new method from the Instrument or Offline Instrument application areas of ChromQuest. However, you only have access to starting actual data acquisition when you are in one of the Instrument areas.

Method Wizard

The Method Wizard is started from the Instrument Wizard box by clicking the button labeled **Create or modify a method**.

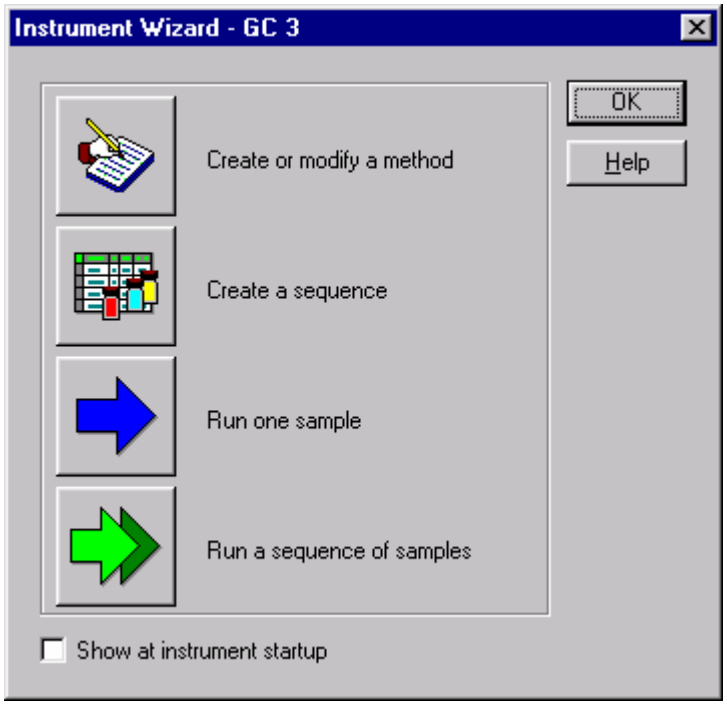


Figure 81. Instrument Wizard window

When you click this button, the Method Wizard will appear, allowing you to select how you want to use the wizard.

Figure 82. Method Wizard window

- Select **Create a new method** to start a new method with the system default method parameters.
- Select **Modify the current method** to step through the current method and make changes.
- Select **Modify a method on disk** to open a file saved on disk and step through it to make changes.

When you select one of these buttons, the Method Wizard sets up a bank of buttons in your application window that allow you to "step" through all dialogs of method generation. A save button is also provided.

Instrument Setup

The instrument setup portion of your method tells how you want to acquire the data coming from your chromatograph. This information is entered in the **Instrument Setup** dialog. Click on the **Instrument Setup** button, or select **Method > Instrument Setup** from the menu. A dialog box will appear, which will display the data acquisition parameters required for the type of data acquisition configured for this instrument.

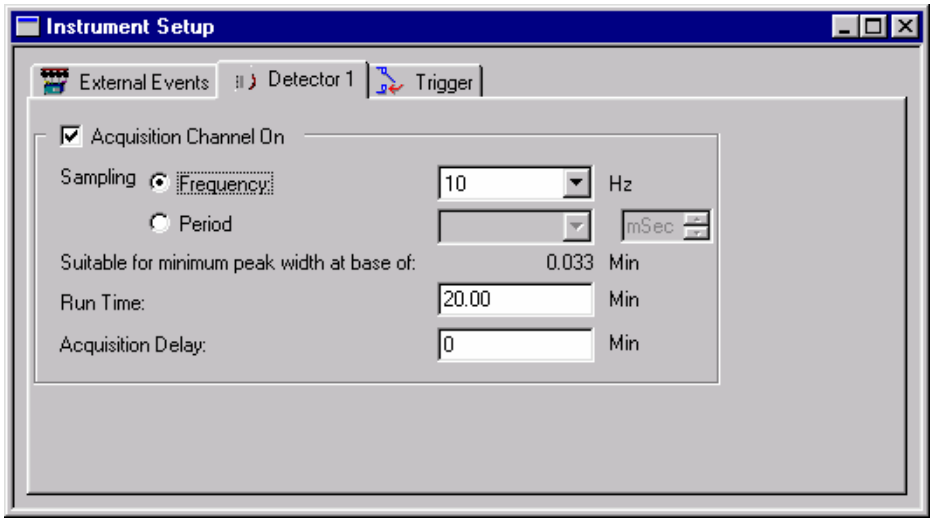


Figure 83. Instrument Setup - Detector 1 dialog box

Detector 1…x

For each detector channel (x) configured on the instrument, define the following acquisition information.

Acquisition Channel On

Select this box to turn the acquisition for this channel ON. If this box is not selected, no data will be acquired for this channel.

- Sampling

  Data will be sampled by the system at this rate. You can choose how you want to specify the sampling rate. When you select a sampling rate, a prompt will appear indicating the narrowest peak width for which this sampling rate will be adequate.

  Note It is recommended that you use the Graphical Events Programming to determine the optimum sampling rate for your chromatography.

  – Frequency

    This selection is in Hz (samples per second). This is the selection for most chromatography applications. Click on the down-arrow to get a list of the frequencies available for the configuration of your system.

  – Period

    When you select this type of sampling, you must select the number of seconds (or milliseconds) between data points. Enter the value, then select whether the period is in milliseconds (mSec) or Seconds.

- Run Time

  Run Time determines the length of time data will be sampled.

- Acquisition Delay

  Acquisition Delay is the interval between the start of run (Trigger) and the time when sampling starts for this channel.

Trigger

Select the Trigger tab to select the type of trigger for the instrument.

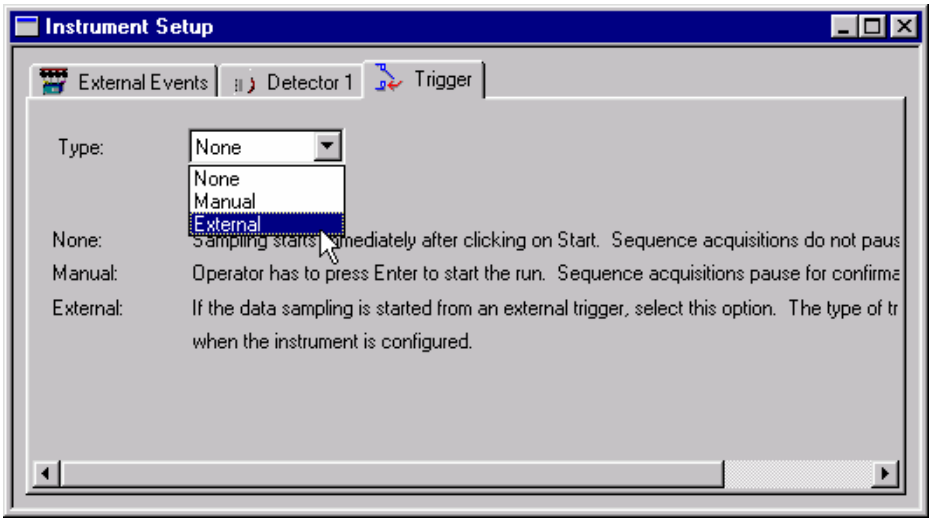


Figure 84. Instrument Setup - Trigger dialog box

Type

Determines how the data sampling is started.

- None

  Sampling starts immediately after clicking on Start. Sequence acquisitions do not pause between runs.

- Manual

  Operator has to press Enter to start the run. Sequence acquisitions pause for confirmation between runs.

- External

  If the data sampling is started from an external trigger, select this option. The type of trigger is designated when the instrument is configured.

When you have completed the acquisition parameters, click the X box in the upper right corner of the dialog box to exit the dialog.

Baseline Check

When the **Baseline Check** tab in configuration options for an instrument is enabled, the **Baseline Check Tab** will appear in Instrument Setup, where you can set the parameters to use when doing a baseline check while acquiring data using this method.

When a method containing baseline check parameters is used for data acquisition, a baseline check will occur when either:

- The *Perform Baseline Check* checkbox is checked in the *Single Run* dialog, or
- The Run Type of the current Sequence line includes *Baseline Check*.

When a baseline check is to occur as part of data acquisition, the software will first download the initial conditions from the method to the instrument. These initial conditions are then used to acquire the baseline check data before the normal data acquisition. If the baseline check data fails to meet the threshold for any channel, then the data acquisition is aborted.

If the baseline check results indicate a failure of the test, then the current run is aborted and the baseline check data and results are stored in the data file without any other acquisition data. If the run is aborted (by the user or because of a hardware error) prior to the completion of the baseline check data acquisition, then no data is saved.

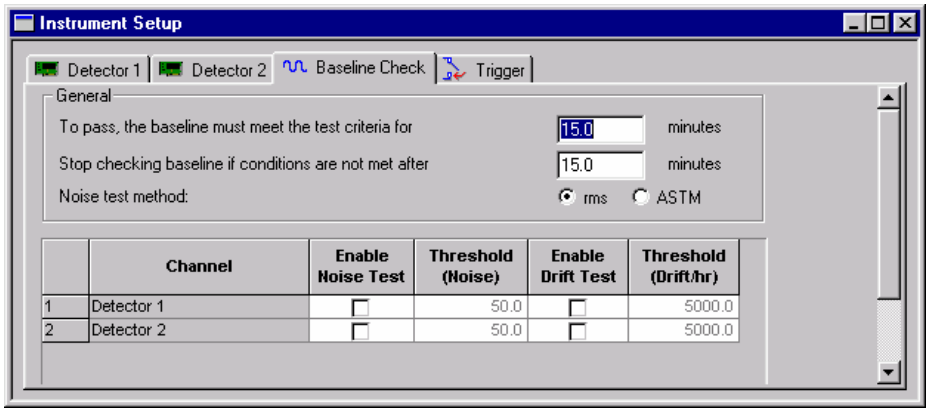


Figure 85. Instrument Setup - Baseline Check dialog box

- To pass, the baseline must meet the test criteria for

  This specifies the minimum time over which the baseline stability will be calculated.

- Stop checking baseline if conditions are not met after

  This specifies the maximum time that will be spent checking the baseline. If conditions are not satisfied in this time, then the baseline check fails.

- Noise test method

  This is used to specify the noise calculation method that should be applied to the acquired data.

- Test Spreadsheet

  This spreadsheet specifies the channels to be used for baseline calculations and the tests that should be performed on those channels. The number of rows of the spreadsheet equals the number of acquisition channels based on instrument configuration plus the number of Multichromatogram channels defined in the **Spectral Options** Window (if scanning detector is configured).

  – Channel

    Each entry in this column contains the name of an acquisition channel specified by instrument configuration or the Multichromatogram definitions. The entries cannot be edited.

  – Enable Noise Test

    Checking this box indicates that detector noise should be calculated on the corresponding channel.

    If this box is unchecked, the Threshold edit field to its right is disabled; otherwise, it is enabled.

  – Threshold (Noise)

    This is used to specify the maximum acceptable value for the calculated noise. If the calculated noise is greater than the Threshold, the baseline check is considered to have failed.

  – Enable Drift Test

    Checking this box indicates that detector drift should be calculated on the corresponding channel.

  – Threshold (Drift/hr)

    This is used to specify the maximum acceptable value for the calculated drift. If the calculated noise is greater than the Threshold, the baseline check is considered to have failed.

Aux Traces

This tab is used to acquire traces of instrument status parameters during normal data acquisition. Many instruments have the ability to report continuous monitoring data on status parameters such as flow rate and oven temperature. When this option is enabled, this tab appears, allowing you to designate which status parameters you wish to monitor during the run. The actual entries in this list will vary depending on the instrument configuration.

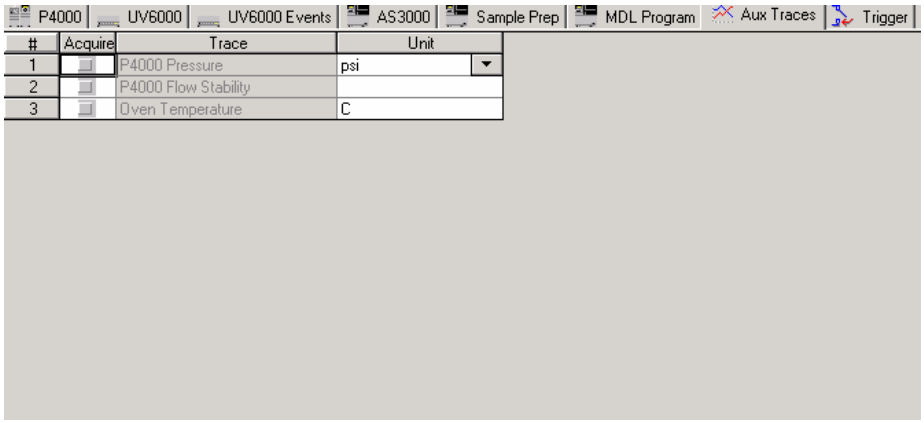


Figure 86. Aux Trace tab

To have status information logged for a parameter listed in this dialog, click on the check box for that row and select the appropriate units for that parameter. When data acquisition is performed, status information will also be acquired. The resultant data will be presented as an additional data channel whenever the data file is viewed.

Method Properties

Before you use a method to acquire data, be sure that the options for automatic post-run analysis of the data and other options (if desired) are turned on. To check these parameters, select the **Method > Properties** command from the menu. A tab box will appear where you set the properties for the method.

Description

Select the **Description** tab. A dialog box will appear where you can enter text information about your method. You can enter any information you wish. The description can be viewed from the Open File dialog, and therefore can be useful in sorting quickly through methods to find the right one.

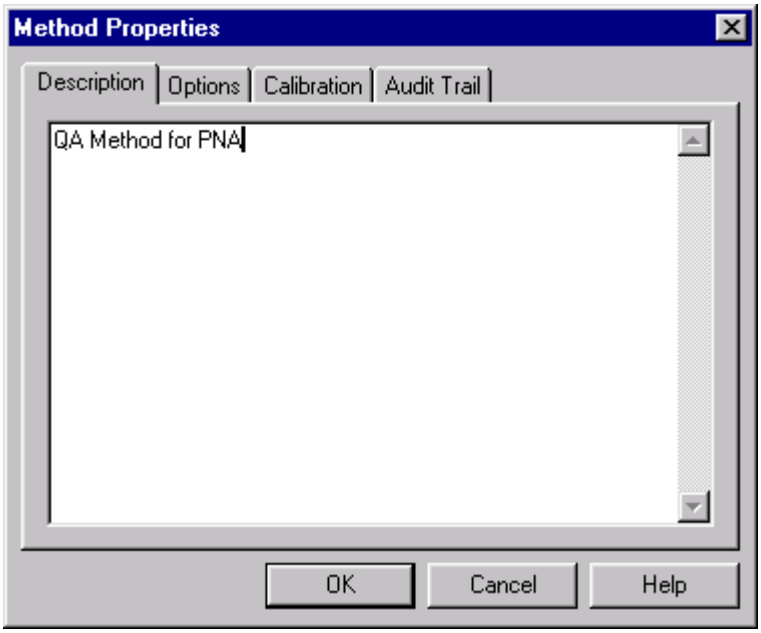


Figure 87. Method Properties - Description dialog box

Options

Select the **Method Properties > Options** tab.

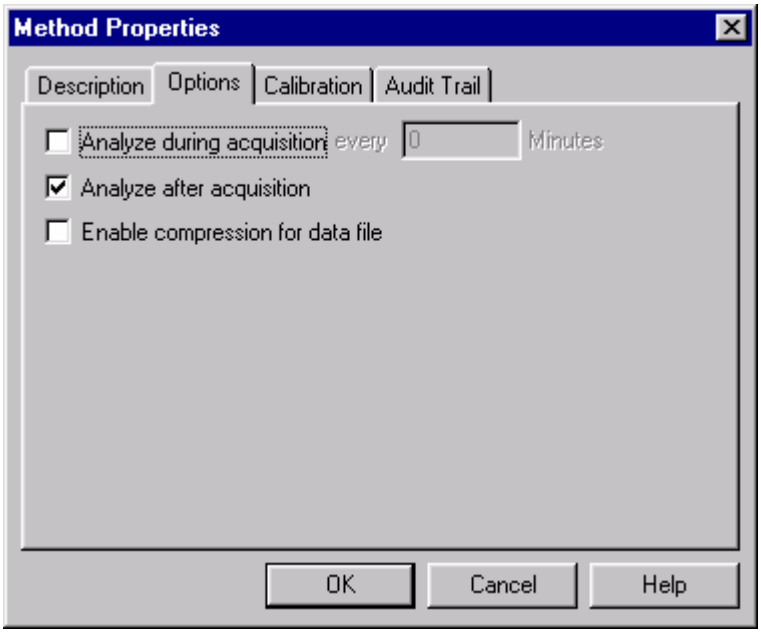


Figure 88. Method Properties - Options dialog box

- Analyze during acquisition

  If you select this box, the chromatogram will be automatically analyzed during the run at the interval you specify in the box.

- Analyze after acquisition

  If this box is selected, the chromatogram will be analyzed automatically after every acquisition. This is the ChromQuest default condition. If this box is not selected, you must either manually analyze the chromatogram by clicking the **Analyze** button, or analyze the sample as part of a sequence reprocessing after the run has been completed.

- Enable compression for data file

  When this box is checked, the data files acquired using the data system will automatically be compressed before saving. (Compressed data files are smaller but take longer to load.) Once a file has been saved using compression, the only way to decompress it is to save it as another data file with compression turned off.

Audit Trail

Select the **Audit Trail** tab. The **Enable Audit Trail** checkbox is an important box because, if checked, subsequent changes to the method will be logged in the method.

Note Once the Enable Audit Trail box is selected, it cannot be de-selected. The method will continue to have audit trail enabled unless you save the file under a new filename.

If you select the Enable Audit Trail box, the following warning will appear.

Figure 89. Enable Audit Trail warning window

If you select No, you will be returned to the Audit Trail tab and you can continue. If you select Yes, the Enable Audit Trail will be permanently checked for this method.

Once the **Enable Audit Trail** box is checked, the following options are available for documenting changes.

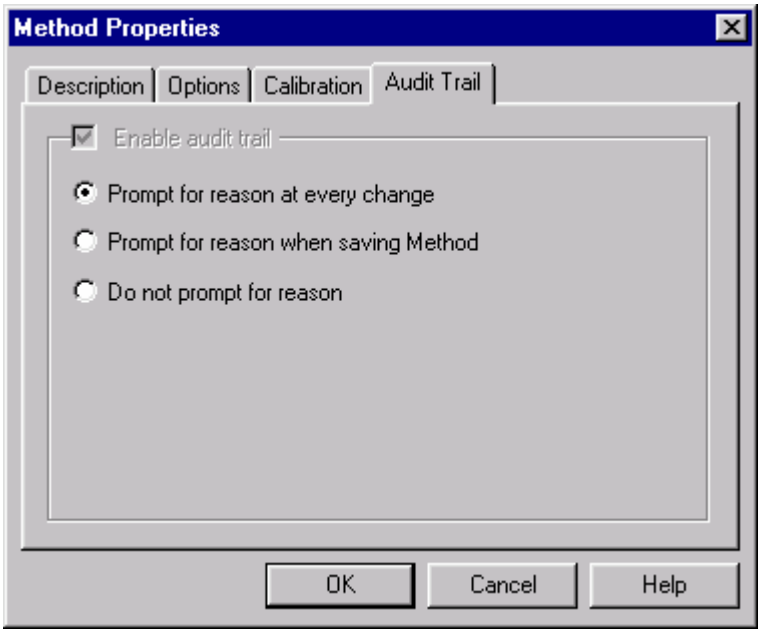


Figure 90. Method Properties - Audit Trail dialog box

- Prompt for reason at every change

  This option requires the user to enter a reason for every subsequent change to the method at the time the change is made.

- Prompt for reason when saving Method

  This option requires the user to enter a reason for each change when the Method is saved.

- Do not prompt for reason

  When this option is selected, changes are documented, but the user is not required to enter a reason for the changes.

Calibration This tab enables you to set calibration defaults.

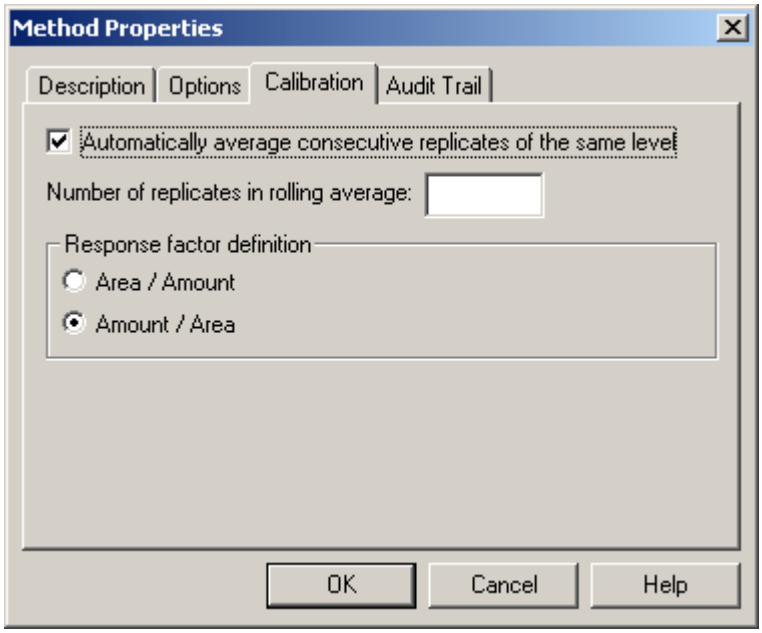


Figure 91. Method Properties - Calibration dialog box

- Automatically average consecutive replicates of the same level

  When this box is checked, calibration replicates (multiple injections of a single calibration level) will always be averaged. If this box is not checked, calibration replicates will not be averaged unless you so specify at the time of calibration.

- Number of replicates in rolling average

  If you wish to use a rolling average, enter the number of replicates per average here.

- Response factor definition

  Select how the response factors are to be calculated and displayed - **Area / Amount** or **Amount / Area**.

Audit Trail Reasons

When you have selected the Audit Trail Option for your method with reasons, ChromQuest prompts you for the reason for changes to the method, at the time of the change, or when the method is saved.

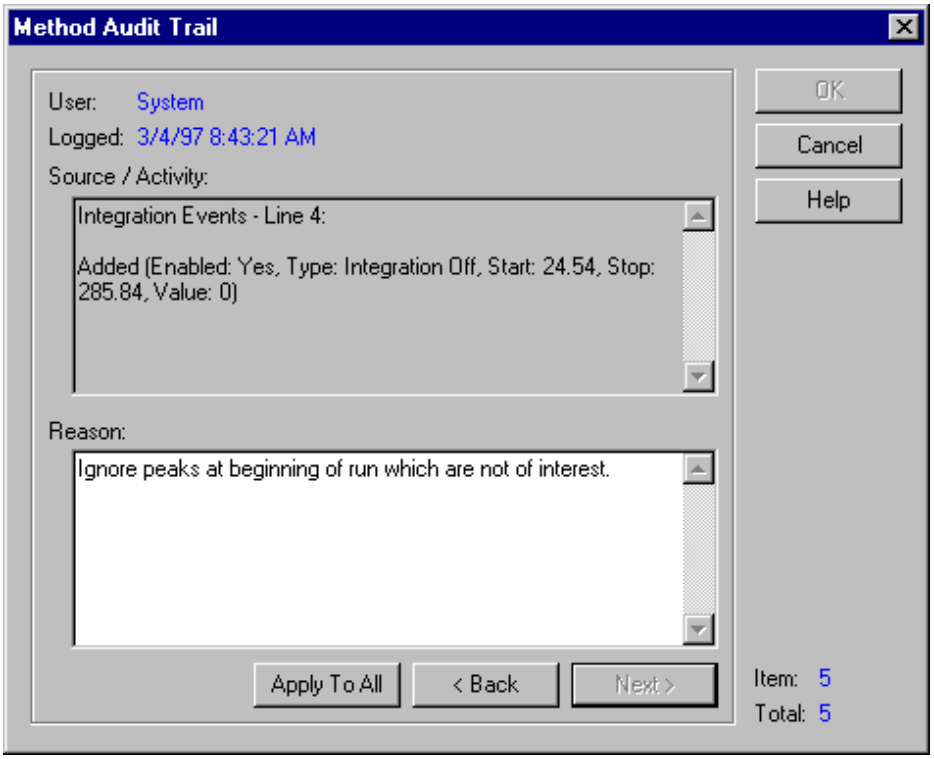


Figure 92. Method Audit Trail dialog box

If you have selected the option to enter a reason at every change, the dialog will appear each time you make a change to the method. A description of the change appears in the **Source/Activity** box. Type the reason for the change in the **Reason** box, and then click OK.

If you selected the option to enter a reason upon saving the method, the dialog will appear when you save the method and you must enter a reason for all changes to the method. Use the **Back** and **Next** buttons to view the descriptions of the changes. A change item number is displayed on the lower right of the window, along with the total number of changes in the method. If you want to enter one reason for all changes, type the reason, then click on the **Apply to All** button. After you have entered changes for all reasons, click the OK button.

Data Properties

The **Data > Properties** command presents tabs where you can view and change the description for the current data file, and turn on audit trail for the current data file.

- Description

  The information shown in the **Description** box is the description currently stored in the current data file. Because the description can be viewed from the Open File dialog, the description can be useful in sorting through the data files on your disk.

  Data file descriptions can be entered in the sequence at time of data acquisition, or can be entered from the **Single Run** dialog when running a single sample.

- Audit Trail

  Data files always have an associated audit trail. The **Data File Properties > Audit Trail** tab allows you to select whether or not you want the system to prompt you for reasons whenever a change is made, by selecting the appropriate button.

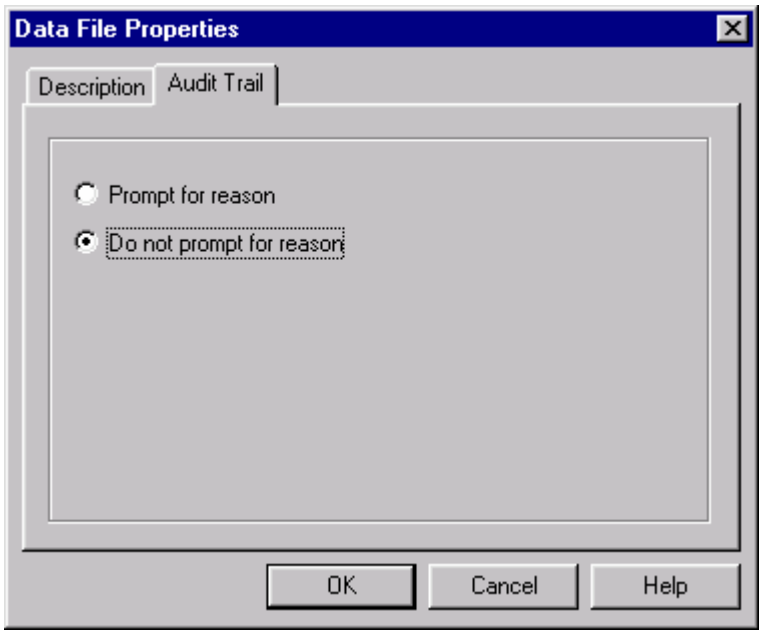


Figure 93. Data File Properties - Audit Trail dialog box

Electronic Signatures

Users whose privileges include data results sign-off can electronically "sign-off" results. To access the data results sign-off, choose **Data > Apply Electronic Signature** from the menu bar.

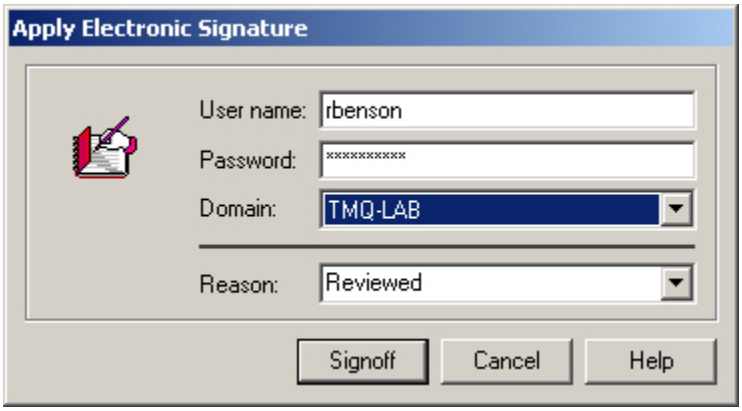


Figure 94. Apply Electronic Signature dialog box

Enter your name, password, and domain. You must also enter a **Reason** by either typing in a reason, or by selecting from the list displayed. Your "electronic signature "will be logged into the data file audit trail (if enabled). Available reasons include **Reviewed, Ready for Review, Approved, and Ready for Approval**. Once you have clicked the **Signoff** button, the data is locked to further analysis. Each data file can have multiple electronic signatures (no maximum).

Revoke Electronic Signature

Users with appropriate rights can also **Revoke** an electronic signature using the **Data > Revoke Electronic Signatures** command. No reason is required to revoke electronic signatures, although you may add one if desired. Once you have revoked the electronic signatures, all electronic signatures will be revoked for the data file and it can once-again be analyzed. When the electronic signature is revoked, an entry is made in the audit trail to that effect.

Figure 95. Revoke Electronic Signatures dialog box

Electronic signatures are available for printing on custom reports. See the Custom Report section.

Viewing the Audit Trail

Audit trail information is stored in the method or data file. You can view the current audit trail information as follows.

View the Method Audit Trail

To view the audit trail log for the current method, select the **File > Method > Audit Trail** command from the menu bar. A listing of the audit trail will appear that includes the User responsible for the change, Time Logged, method location (Source) of the change, what was changed (Activity), and Reason for the change.

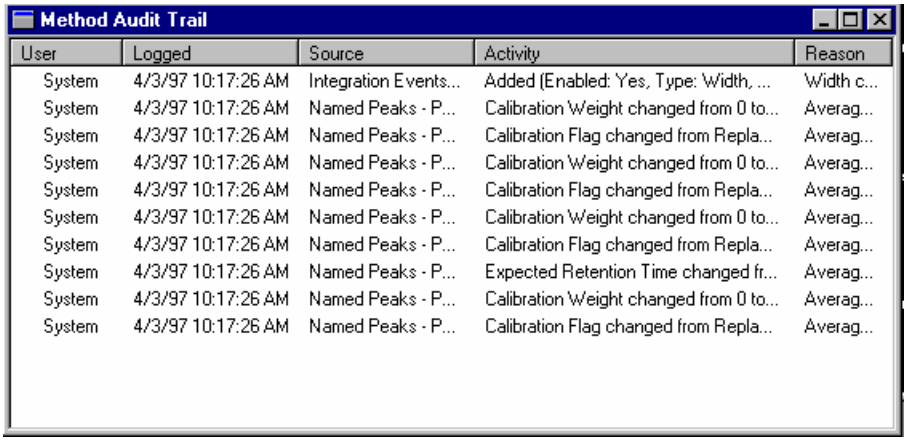


Figure 96. Method Audit Trail window

To see full information for a logged change, print one of the entries, or print all the entries, first select a row by clicking on it with the mouse to highlight it, then do a right-mouse click.

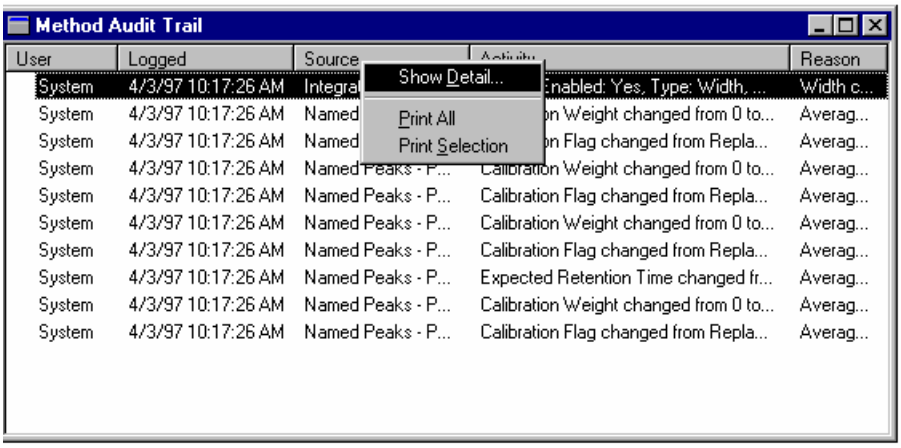


Figure 97. Method Audit Trail window - Show Detail menu

- Show Detail

  Select this option to view the full information on the selected activity.

- Print All

  Select this option to print all of the audit trail entries.

- Print Selection

  Select this to print only the highlighted entry.

View the Data File Audit Trail

To view the audit trail for the current data file, choose **File > Data > Audit Trail** and a listing of data audit trail events will appear. These include the name of the logged user and time activity was logged, the change or activity for the data file, along with the method used to analyze the data, MIF table changes, and changes to the data file description, along with reasons, if any.

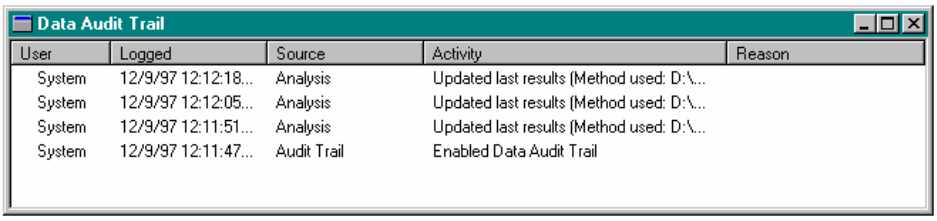


Figure 98. Data Audit Trail window

To see full information for a logged change, print one of the entries, or print all the entries, first select a row by clicking on it with the mouse to highlight it, then do a right-mouse click.

- Show Detail

  Select this option to view the full information on the selected activity.

- Print All

  Select this option to print all of the audit trail entries.

- Print Selection

  Select this to print only the highlighted entry.

External Events

Once you have configured external events for your instrument, you can program the events for your method using the **External Events** tab in **Method > Instrument Setup**. Note that this tab will not appear on your menu unless you have configured external events for your instrument. When you select this tab, a spreadsheet appears where you can select the events and designate when and how they activate during the run.

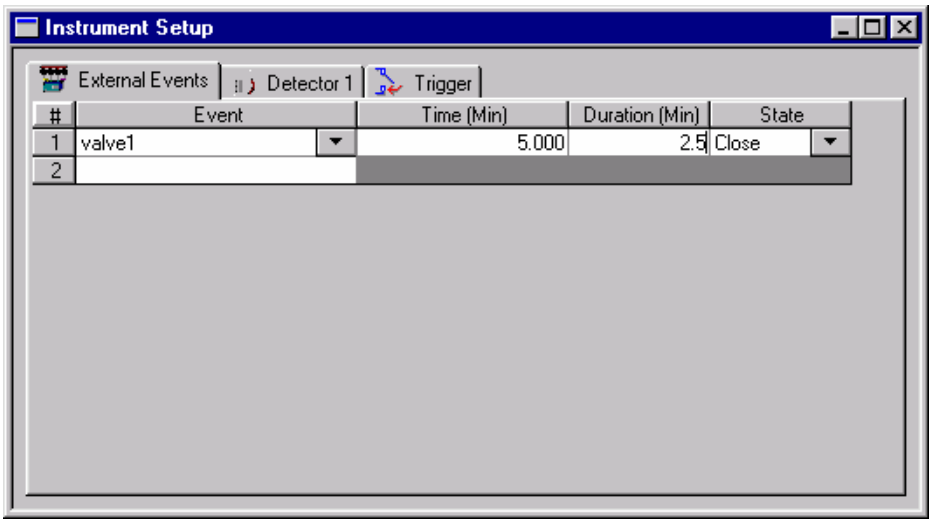


Figure 99. Instrument Setup - External Events dialog box

To program an event, select it from the drop-down list of configured events by clicking the **Event** field. Enter the time at which the event is to actuate, the length of time you wish the event to remain in the desired state (**Duration**), and the **State** of the event during the event (Close or Open).

You may configure up to 25 events for the run.

Instrument Status/ Event Direct Control

If you are using external events, you can directly access and control the configured events using the **Control > Instrument Status** command. When you select this command in an instrument where events are configured, a window will appear where you can view the current state of the event, and manually change it. The tabs in instrument status are dependent on instrument configuration so what you see may differ from this general case.

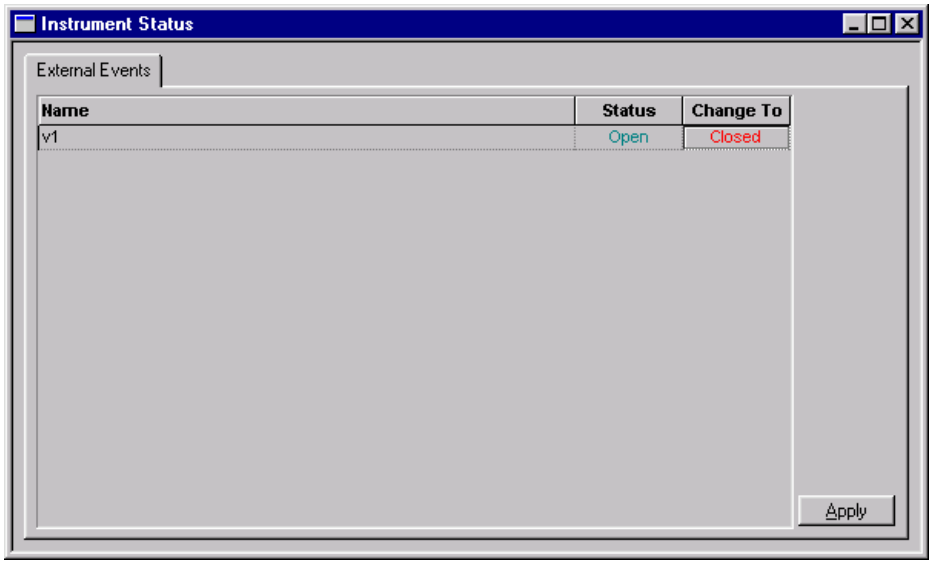


Figure 100. Instrument Status - External Events dialog box

On the External Events tab, each configured event will be displayed on a line, along with its current **Status**. To change the status, click on the field displayed in the **Change To** column. Each time you click the mouse; the "Change To" state will toggle. When you have selected the appropriate state, click the **Apply** button. The Status will be updated to reflect the change. If "Ignore" is selected in the **Change To** field for an event, pressing Apply will not change that event.

Instrument Activity Log

As you use a method to acquire data, a log of activity from the current instrument is kept. To view this log, choose the **File > Instrument Activity Log > Display Log** command. A window with the Instrument Activity Log appears. The window displays the User who used the instrument, the time the activity was logged into the Instrument Activity Log, and a description of the activity.

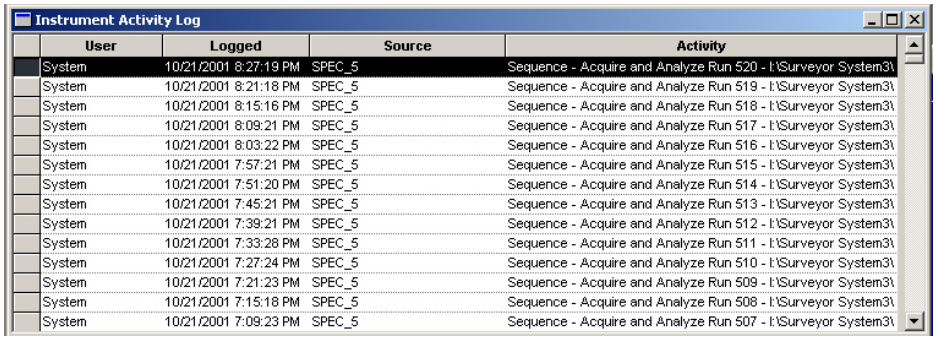


Figure 101. Instrument Activity Log window

To view details of any line in the instrument activity log, click on the line to highlight it, and then do a right-mouse click within the spreadsheet. From this pop-up menu, you can view details of the highlighted line, print it, or print the entire activity log.

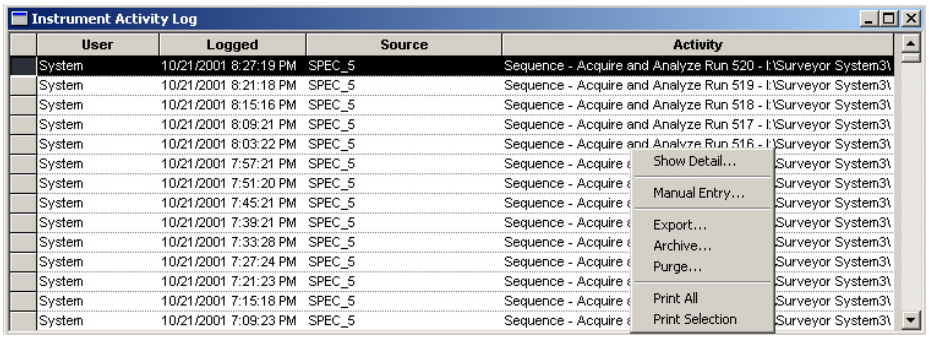


Figure 102. Instrument Activity Log window - Show Detail menu

Note Over time, the instrument activity log file may become large, so periodically you should archive the file to a floppy or another location and then purge it.

Instrument Activity Log Manual Entry

You can enter type a manual entry in the instrument activity log using the **File > Instrument Activity Log > Manual Entry** command.

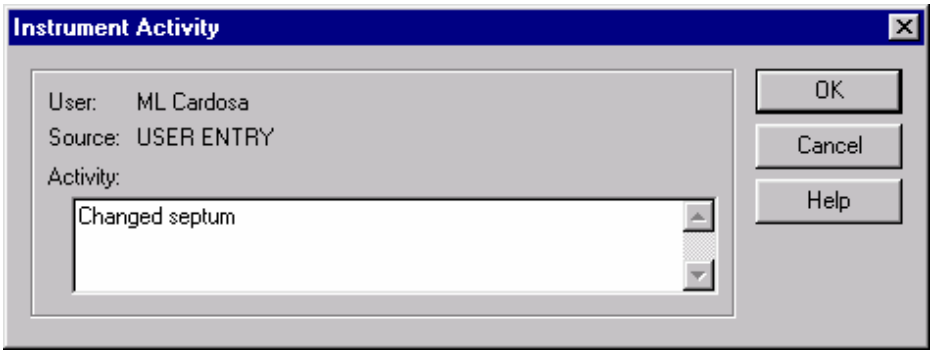


Figure 103. Instrument Activity dialog box

Type in the information you wish to enter in the instrument activity log, then click **OK**.

Instrument Activity Log Export

You can export the instrument activity log to a file using the **File > Instrument Activity Log > Export** command. The following dialog appears.

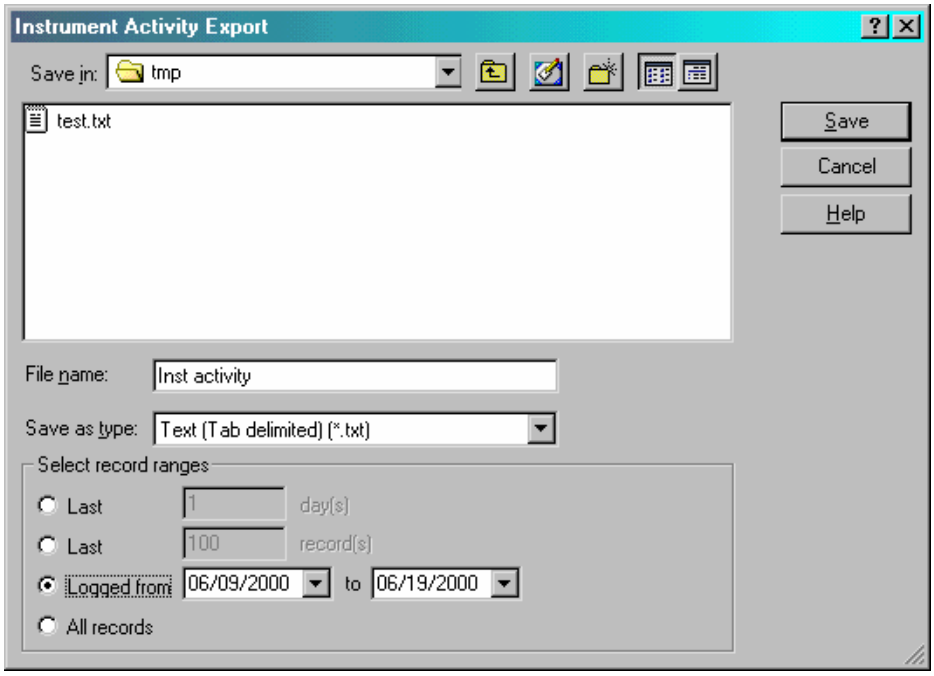


Figure 104. Instrument Activity Export dialog box

- File name

  Enter the name to be used to store the instrument activity export file.

- Save as type

  Select the type of file you wish to save from the choices displayed.

- Select record ranges

  Select the radio button next to the range desired.

Click the **Save** button to save the instrument activity log for the range selected in the file specified.

Instrument Activity Log Archive

To archive the instrument activity log for an instrument, choose the **File > Instrument Activity Log > Archive** command from the instrument window. A dialog will appear where you can select the location for the archive file. A default name is assigned, with the **.logarc** extension.

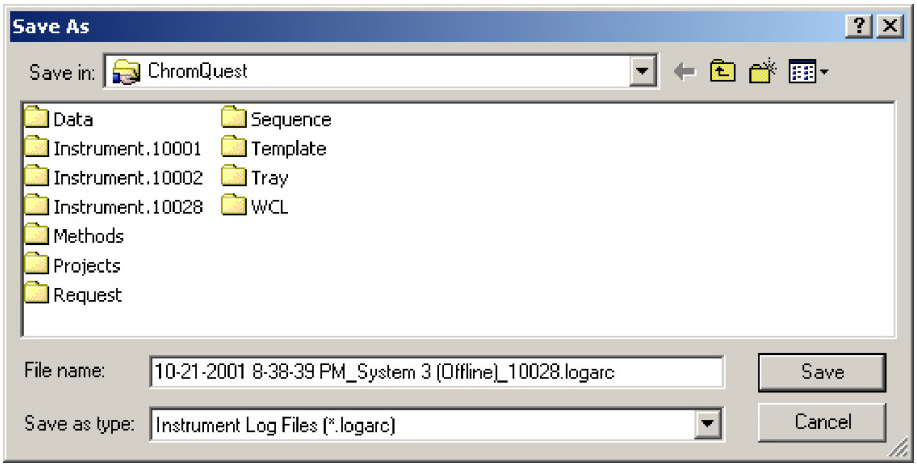


Figure 105. Save As dialog box

Instrument Activity Log Purge

To purge the current instrument activity log, use the **File > Instrument Activity Log > Purge** command from the instrument window. The following message will appear.

Figure 106. Instrument Activity Log message window

If you have set the enterprise options such that a purge is only allowed after archive, you will be presented with the Instrument Activity Archive dialog if the log has not been archived when you click either the Yes or the No button.

If you have not set the enterprise options such that a purge is only allowed after archive, you will be presented the Instrument Activity Archive dialog if you click **Yes**. If you click **No**, the instrument activity log will be purged.

Save Method As

When you have completed the sections for creation of a data acquisition method, save the file on your hard disk before you use it for data acquisition. To save the method, click the **Save** button, followed by **Method**, or select the **File > Method > Save As** command from the menu. A dialog box will appear where you can give your method a distinctive name and designate a disk folder (directory) where it will be saved.

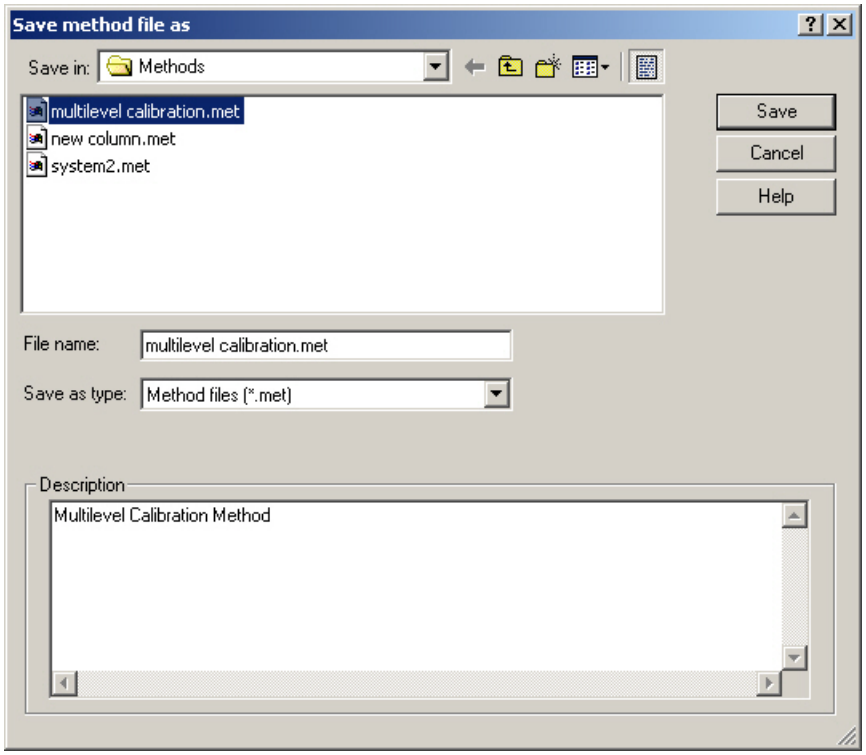


Figure 107. Save method file as dialog box

Select the directory where you want to save the method. Enter the name of the new method in the **File Name** field. You can review and change the method description using the **Description** box. To save the method, click on the **Save** button.

You are now ready to use your method for data acquisition.

Single Run Acquisition

There are two ways you can acquire data using ChromQuest. One way is with a sequence (for multiple runs), and the other way is to make a single run. To make a single data acquisition run, you need to specify the method to be used for analysis, and a file name for data storage.

Note To use a method (created for data acquisition from a digital to analog converter) for data acquisition, its instrument setup should have the acquisition channel turned On, and a sampling rate and run time designated.

To make a single run, click on the **Single Run** button, or select the **Control > Single Run** command from the menu. The following dialog will appear.

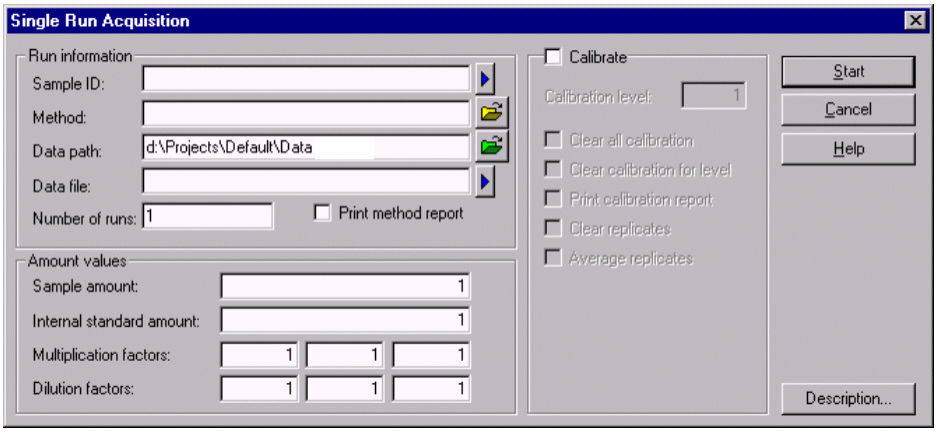


Figure 108. Single Run Acquisition dialog box

- Run information

  This section allows you to specify files for the run.

  – Sample ID

    Enter a Sample ID for the run. This can contain text and numbers, and is saved with the data file. You can also click the arrow to select from pre-defined Sample ID's.

  – Method

    Enter the name of the method to be used for data acquisition and processing. Include the entire path name if the method is not in your default method directory. You can select the method from a list of methods available on your disk by clicking the File button adjacent to the field.

– Data path

  Enter a path name where the data acquired for this run will be stored. Click the File button to select a path from a list of those on your disk.

– Data file

  Enter a file name to be used to save the data on disk. You can select a pre-defined data file name (such as sample ID) by clicking the arrow. It is not possible to use an existing file name, unless the file exists in located in a directory whose path contains the term "public". For example, if your data files are saved in a directory entitled "C:\Public\Data", the ChromQuest files saved in this directory can be overwritten. The software automatically appends a **.dat** file extension. You may change this if you wish.

– Number of runs

  Enter the number of runs you wish to make. The runs will automatically proceed without review until completed, incrementing each file name as designated. If the sequence of single runs is aborted, and the user then repeats the single acquisition without changing any parameter, the run number will start with the next number as if the sequence not been aborted. For example, setting 4 runs with starting run number of 101, then abort during run 102. When restarting, the next run number will be 105. If the Sample ID is also incremented, it will increment in parallel.

– Print Method Report

  When this box is checked, the method report (or reports) will be printed at the end of the run.

• Amount values

  In this section, you can enter values that affect how the concentrations are calculated. If you are making a single data acquisition prior to calibrating your method, simply leave these values at the default level.

– Sample amount

  The Sample Amount value is used as a divisor during calculation of concentrations. It is intended to compensate for differences between samples due to weighing and when percentages of the total sample are being calculated rather than the amount detected in an injection.

– Internal standard amount

For calibration runs, the Internal Standard Amount is taken from the method Peak Table. For unknown runs, enter the amount of the Internal Standard in your unknown sample.

– Multiplication factors

Enter one to three multiplication factors to be used for this run. All quantitated peaks will be multiplied by these factors.

– Dilution factors

Enter one to three dilution factors to be used for this run. All quantitated peaks will be divided by these factors.

• Calibrate

Select this box if the sample is to be a calibration sample. Once this box is clicked, the following fields and options will be available.

– Calibration level

Enter the number of the calibration level represented by this calibration standard. If this is a single level calibration, enter **1**.

– Clear all calibration

Click this box if you want to clear all existing calibration factors from your method before running the sample.

– Clear calibration for level

Click this box if you want to clear the existing response factors for this level only before running the sample.

– Print calibration report

Click this box if you want to print a calibration report after running the sample.

– Clear replicates

Click this box if you want to clear all existing replicates from the existing calibration level before running the sample.

– Average replicates

Click this box if you want to average the replicates for this calibration level.

When you have completed the Single Acquisition Run dialog box, click on **Start** to begin the acquisition. The current data will appear in the chromatogram window as it is acquired and stored on disk. At the end of the run, the chromatogram will be analyzed according to the method parameters, and a report generated if specified. If the sample is not analyzed at the end of the acquisition, click on the **Analyze** button if you wish to view the results.

Sample Description

When you click on the **Description** button, you can enter a text description for the sample that is saved in the data file. The description can be viewed from the Open File dialog, or when the file is open as the current data file using the **Data > Properties** command.

Submit

This button appears when data is currently being acquired using a sequence or single run. The **Submit** button allows you to submit a single acquisition to be run at the completion of the current run. The run is entered at the end of the Run Queue if you are currently running a sequence of runs.

Submit Priority

This button will appear when data is currently being acquired using a sequence. When you click on **Submit Priority** you can submit the single run to be executed immediately after the **current** sequence run record in the Run Queue. After this sample is finished, the sequence will resume.

Note If the chromatogram is not integrated at the end of the run, or if you were expecting a report and none was printed, check the **Method > Properties** section of your method to make sure data analysis is turned on for this method.

Stopping a Run in Progress

When you want to stop data acquisition during a run, click the STOP button that appears on the command ribbon when the run is in progress, or use the **Control > Stop Run** command. A dialog will appear that presents options for how you want to stop the run.

Note When clicking the STOP button on the command ribbon, do not release the mouse button until the STOP icon changes to the “depressed” appearance.

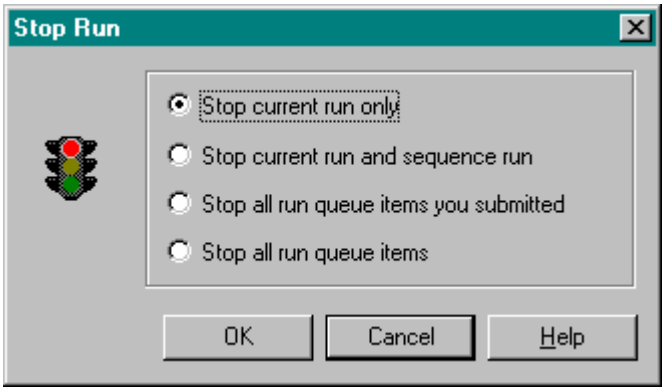


Figure 109. Stop Run dialog box

- Stop current run only

  Select this to end the run currently in progress. If the run is a part of a currently-queued sequence, the sequence will continue with the next run.

- Stop current run and sequence run

  This selection stops the run currently in progress, and terminates the sequence it is a part of. Other queued items will proceed.

- Stop all run queue items you submitted

  This selection stops the run currently in progress, and terminates all the items in the queue that were submitted by you. Queue items submitted by other users will be unaffected.

- Stop all run queue items

  This selection stops the run currently in progress, and terminates all items in the run queue.

Note When a run is stopped, the data up to that point is saved in the data file. However, no analysis of the data will be performed. If you want to produce a report or view results from a run that was stopped, you must Analyze the data file.

Baseline Check On Demand

To perform an on-demand baseline check, select **Control > Baseline Check**. A dialog will appear where you set the parameters for doing the baseline check.

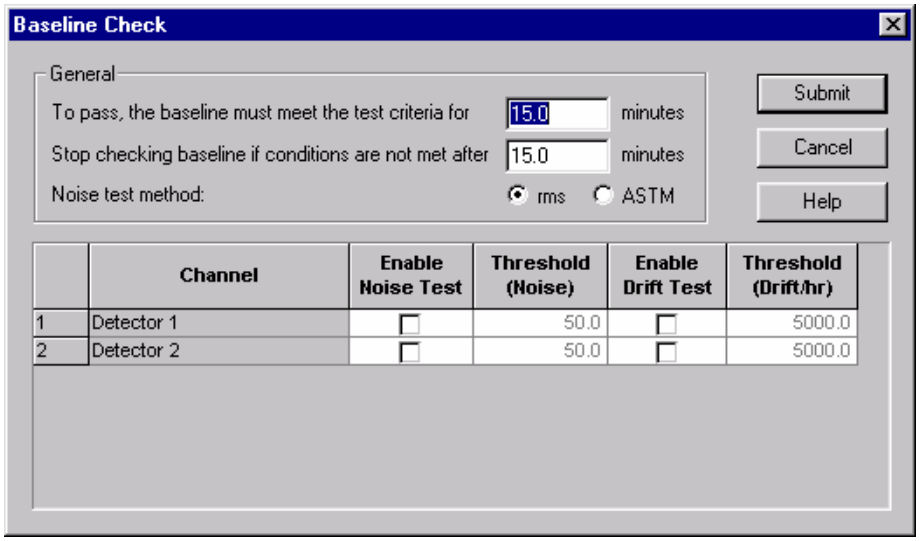


Figure 110. Baseline Check dialog box

- To pass, the baseline must meet the test criteria for

  This specifies the minimum time over which the baseline stability will be calculated.

- Stop checking baseline if conditions are not met after

  This specifies the maximum time that will be spent checking the baseline. If conditions are not satisfied in this time, then the baseline check fails.

- Noise test method

  This is used to specify the noise calculation method that should be applied to the acquired data.

- Test Spreadsheet

  This spreadsheet specifies the channels to be used for baseline calculations and the tests that should be performed on those channels. The number of rows of the spreadsheet equals the number of acquisition channels based on instrument configuration plus the number of PDA Multichromatogram channels defined in the **PDA Options** Window (if PDA is configured).

– Channel

  Each entry in this column contains the name of an acquisition channel specified by instrument configuration or the PDA multichromatogram definitions. The entries cannot be edited.

– Enable Noise Test

  Checking this box indicates that detector noise should be calculated on the corresponding channel.

  If this box is unchecked, the Threshold edit field to its right is disabled; otherwise it is enabled.

– Threshold (Noise)

  This is used to specify the maximum acceptable value for the calculated noise. If the calculated noise is greater than the Threshold, the baseline check is considered to have failed.

– Enable Drift Test

  Checking this box indicates that detector drift should be calculated on the corresponding channel.

– Threshold (Drift/hr)

  This is used to specify the maximum acceptable value for the calculated drift. If the calculated noise is greater than the Threshold, the baseline check is considered to have failed.

Integrating the Chromatogram

ChromQuest comes with a default method that is adequate for data acquisition and analysis of simple chromatograms. However, your chromatography may require more elaborate treatment of the data, or special integration of specific peaks. Integration events are normally entered in the Integration Timed Events Table. This section describes how to optimize a method for proper integration of your data files.

Basics of Integration - Required Integration Events

Two Integration events are required for each run: **Width**, and **Threshold**. These events are used to detect peak start, stop, and apex, and to distinguish true peaks from noise. The system uses default values of Width = 0.2 minute and Threshold = 50.

For details on setting Width and Threshold, as well as other integration timed events, see Integration.

Sampling Rate

The sampling rate used to acquire your data determines how much information the integration algorithm has for drawing and integrating the chromatogram. The sampling frequency is set in the **Instrument Setup** part of your method. To make sure you have the proper sampling rate, use the **Suggest Sampling Frequency** command in graphical programming. Slight over-sampling of data is corrected with the **Width** integration parameter and is not a problem. Try to avoid gross over-sampling of data, however, as it does not give better integration and it wastes space on the hard drive of your computer. More important, however, is not to under-sample, as there is no way to correct for data points that are not sufficient to define and integrate your peaks.

Graphical Integration Optimization

Optimizing integration using Graphical Programming is easy to do. With your chromatogram displayed in the chromatogram window, click anywhere in the window with the right mouse button. From the pop-up menu that appears, move the cursor to **Graphical Programming**. A list of available graphic events will appear. These events are also available from the Integration Toolbar if displayed.

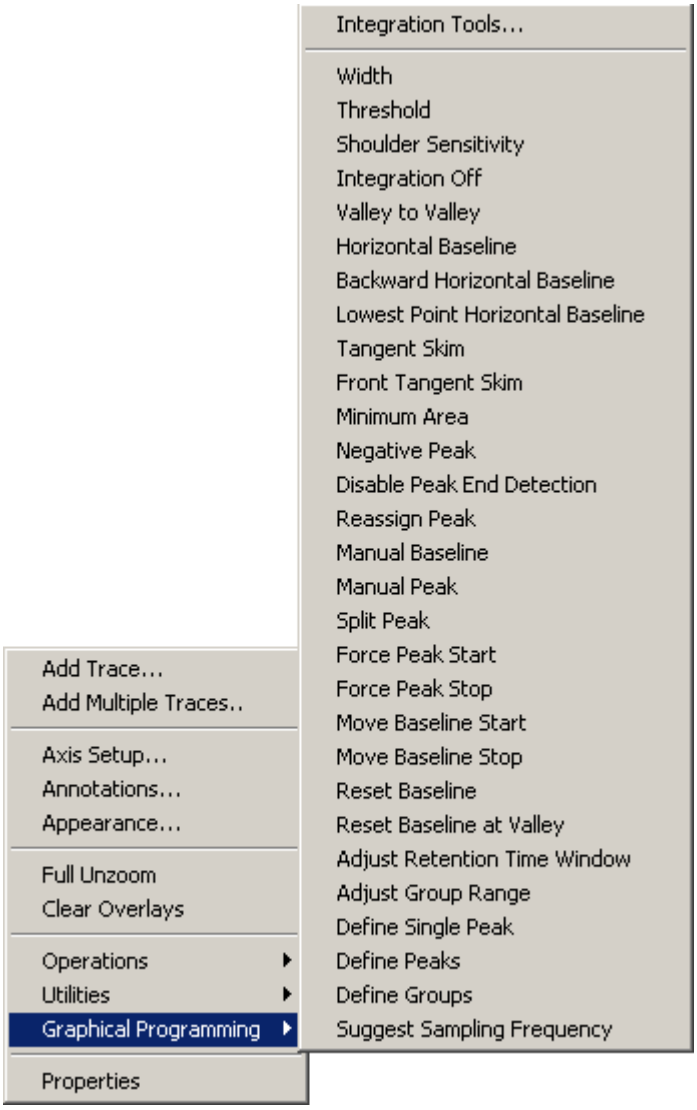


Figure 111. Graphical Programming options menu

Note Before attempting to add integration timed events, make sure your chromatogram has been analyzed using the current method. To make sure, look for baselines and start/stop tic marks on your chromatogram. Alternatively, click the **Analyze** button on the command ribbon.

- Graphically Setting Width

  From the graphical programming list, click **Width**. (Or click the **Width** button on the Toolbar.) Follow the instructions in the status bar at the bottom of the window to optimize the width integration parameter. Click once at the beginning of your narrowest peak, then once again at the end of the narrowest peak. ChromQuest will suggest a width value. Select **Analyze Now** to add it to

your method and reintegrate using the new width value. Click **Add to Table** if you want to simply add the event to your integration timed events table and go on without integrating the chromatogram at this point.

The Manual Integration Fixes table is used for special baseline integration and is described below. Do not select this option now unless you want this timed event to apply only to this sample and not become a regular part of the method integration.

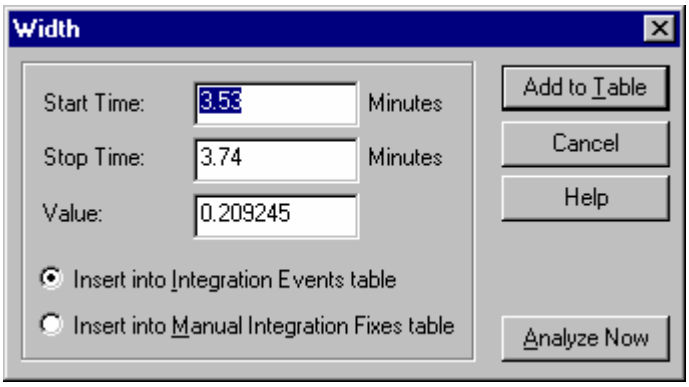


Figure 112. Width dialog box

- Graphically Setting Threshold

  Set the threshold using Graphical Programming next. This time, instead of using the pop-up menu, click the **Threshold** button on the integration toolbar. As instructed in the status bar at the bottom of the window, click the mouse once at the beginning of a section of your chromatogram where no peaks elute, then once again at the end of that section of chromatogram. ChromQuest will suggest a threshold value. Select **Analyze Now** to add the value to your method and reintegrate the chromatogram.

- Suggest Sampling Frequency

  Optimization of your sampling frequency can also be assigned graphically. Click on the **Sample Frequency** button, or the **right-mouse click > Graphical Programming > Suggest Sampling Frequency** command. Following the instructions on the status bar, click once at the beginning of your narrowest peak of interest, then once at the end of that peak. ChromQuest will suggest a sampling rate or period for acquisition of the sample. You can enter this value for your sampling rate using the **Instrument Setup** button.

- Adding and deleting integration timed events

  All of the integration timed events available in ChromQuest can be added to your method using the Graphical Programming technique. Each time you add an event, it will be stored in the Integration Timed Events Table or Manual Integration Fixes Table. To view the Integration Events Table, click the Integration Events button, or select **Method > Integration Events** from the menu.

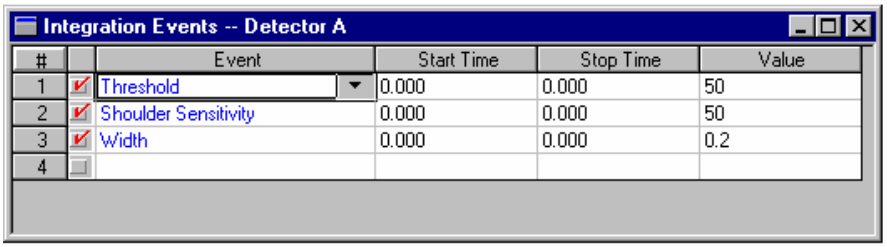


Figure 113. Integration Events window

Each row of the Integration Events spreadsheet represents an integration event in the method. To manually add an event, click on an empty row **Event**, and click the down-arrow. Select the event to enter, then enter a **Start Time, Stop Time**, and **Value** (if applicable) for the event.

You can temporarily remove an event from the table by de-selecting the check mark adjacent to the event. When the check mark is not displayed, the event will not be applied to the integration. To permanently delete an integration event, click the row of the event to select it. Click the right hand mouse button.

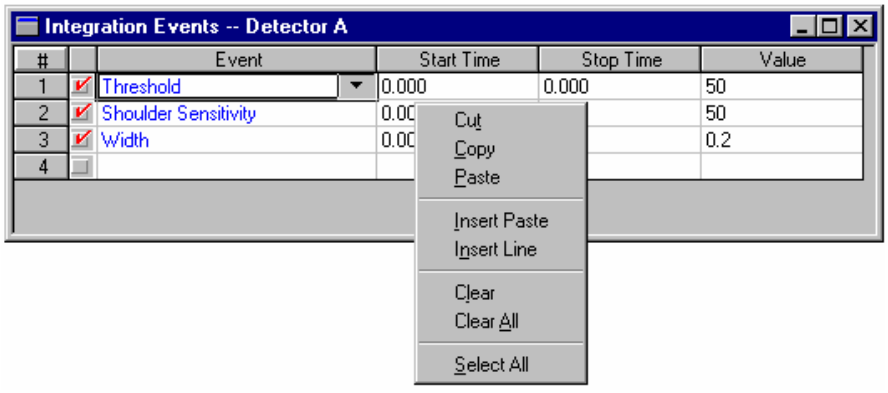


Figure 114. Integration Events window - Options menu

Select the **Cut** command. This will cut the event from the list. If you change your mind, you can reenter it using the **Paste** command. You can also use the right-mouse click commands to insert a line, clear a selected cell or row, clear all events, or select all events.

For a complete list of integration events, along with examples of how they work, see the **Examples of Integration Events** section.

Manual Integration Fixes

A Manual Integration Fix is an adjustment to integration required only for a particular chromatogram. Manual integration corrections that are created using graphical integration are logged into the **Manual Integration Fixes Table**. These manual changes can also be entered in the Manual Integration Fixes Table by selecting the desired integration change from the drop-down list, then entering a **Start** and **Stop** time, and a **Value** if appropriate. These integration changes are applied only to an individual chromatogram rather than becoming part of the method, and are saved in the data file only. The **Manual Integration Fixes** are saved in the data file so that the integration of the chromatogram can be re-created later.

When you choose the **Data > Manual Integration Fixes** command or click on the **Manual Integration Fixes Table** button, the Manual Integration Fixes table will appear, where you can view or edit the current Manual Integration events.

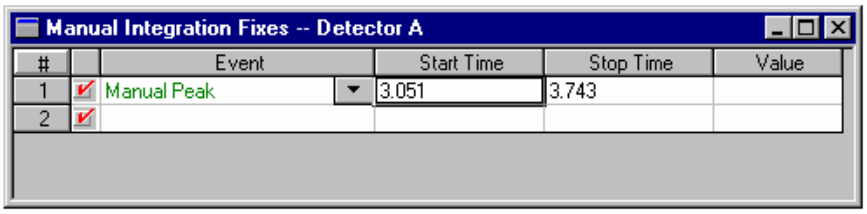


Figure 115. Manual Integration Fixes window

Calibration Setup

Before you can get accurate amounts calculated from the areas of unknown peaks, you must have a method that contains a calibration curve from which to calculate the answers. This involves setting up your acquisition method to receive the areas from calibrated standard peaks, then actually running the standards so that the standard areas are entered into your method. **Accurate results cannot be obtained until the method is completely calibrated**. In other words, standards for each level of calibration must be run to complete the calibrated method.

Calibration Theory

Calibration of chromatography instruments is usually necessary to obtain accurate results. The purpose of calibrating an instrument is to verify the response of a detector to a given component. The same detector may give different responses to equal amounts of different components under identical chromatographic conditions. Another reason for calibration involves the linearity of the detector. The rate of detector response to many compounds will decrease with increasing component concentration, therefore requiring calibration of the detector at varying concentration levels of the same component (a multi-level calibration).

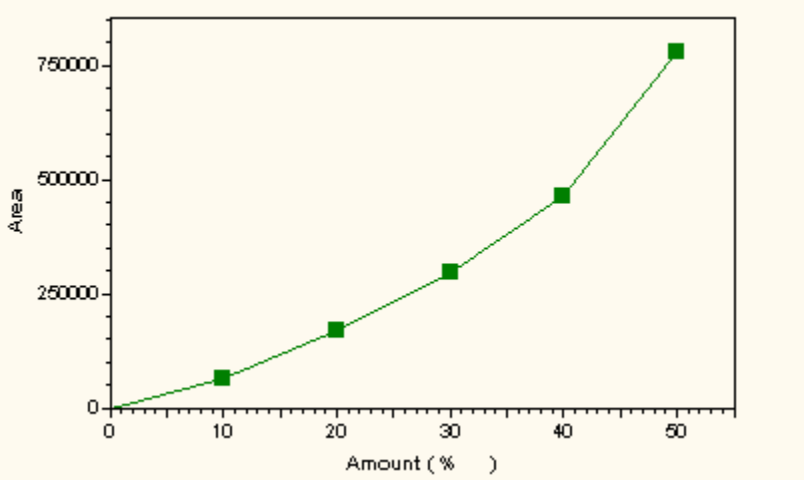


Figure 116. Multi-Level Calibration Curve

When all calibration standards have been run, each calibrated component will have its own calibration curve, representing the response of that compound to the detector over the concentration range. When an unknown sample is run, each component concentration is determined from the calibration curve by finding the amount corresponding to the component area (or height). There are two general techniques for calibrating samples: Internal Standard and External Standard.

- Internal Standards

  With the Internal Standard technique, each sample (both standards and unknowns) is spiked with a known amount of a known compound. When samples are subsequently run, the areas are adjusted using the internal standard. This technique is used to compensate for variations in sample work-up and injection technique.

- External Standards

  The External Standard technique does not use a spiked standard component. All unknown samples are compared to the standards without correction, and therefore it is important that the injection size is accurate and reproducible.

Single Level and Multiple Level Calibrations

A calibration curve can have as few as one level, or can have multiple levels. A single level calibration curve is created from running just one standard sample. The calibration curve for each peak then becomes a line through the origin and a point representing the area/amount relationship of the peak in the standard.

Running several (two or more) standard samples with different concentration amounts creates a multiple level calibration curve (also called Multi-level). The calibration curve for a given component then becomes a line between the points that represent the area/amount relationships of the compound at each concentration. In some cases, the calibration curve is “forced through zero”, which causes the line to use the origin as one of the points. This eliminates the possibility of negative concentrations being calculated for low area peaks. Several types of calculations are available for calculation of the actual curve (called “fit types”). The best-fit type would be the one where the calibration points most closely fit on the line.

For details on calibrations and the equations used to calculate results, see the Equations and Calculations section.

Replicates and Averaging Calibrations

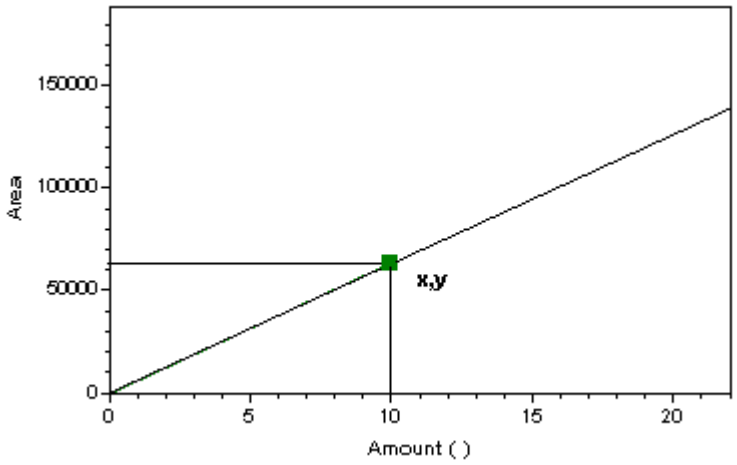
Initially, a method contains no calibration. When you run the first calibration standard, the areas/heights for each calibrated peak are entered into the method calibration. If you run a subsequent standard at the same calibration level (a **replicate**), you have a choice of how you want the data system to treat the new areas/heights for the calibration. Setting the Calib Flag in the peak table to either Replace or Wt Average for each calibrated peak makes this choice.

If you choose to **Replace** the existing calibration, the areas/heights for each replicate replaces the area/height for the previous replicate in the calibration. Only the most recent replicate areas/heights are used to create the calibration curve. Previous replicates are ignored.

If you choose **Wt Average**, each calibration point on the curve will be determined by performing the average of the current calibration with previous replicate areas/heights in the method. The following example describes how this is done.

Assume a starting method for a single-level calibration with no existing calibration data. After a series of "n" calibration replicate samples are run, the calibration curve is determined as shown below.

Level 1	Replicate	Area
	1	$Area_1$
	2	$Area_2$
	3	$Area_3$
	.	.
	.	.
	.	.
	n	$Area_n$



The resulting calibration curve is shown above, with the point (**x,y**) representing the calibration point for Level 1. The area, **y**, of this point is calculated by taking the average of the replicates at this level.

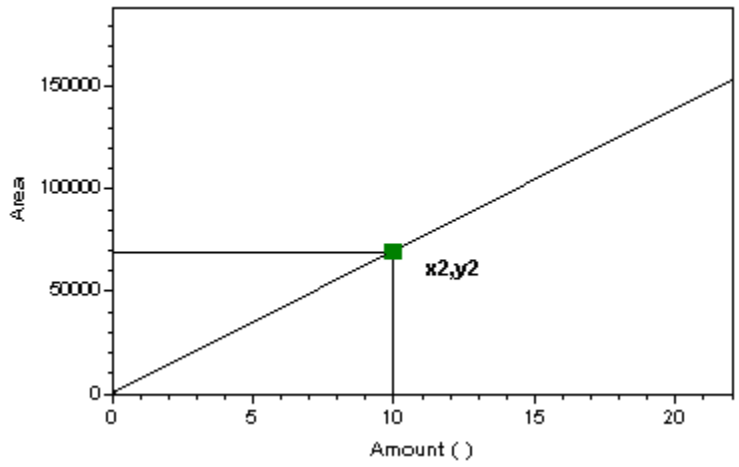
$$y = \frac{area_1 + area_2 + ...area_n}{n}$$

This average, y, is saved as the **last area** in the method. This value is used to calculate the weighted average when a new series of replicates for this level is run, as shown below.

Level 1	Replicate	Area
	1	$Area_1$
	2	$Area_2$
	3	$Area_3$
	.	.
	.	.
	.	.
	m	$Area_m$

Because this is the second time a series of replicates was run for this level, a weighted average is now calculated, using the Calib Weight (weighting factor) set in the peak table.

A new calibration curve is calculated.



The new calibration point (**x2,y2**) is used to draw the calibration curve, with the area, **y2**, calculated as follows.

$$y_2 = \left[\left(\frac{area_1 + area_2 + ...area_m}{m}\right) * W\right] + \left[y * (1 - W)\right]$$

where

y2 = area of calibration point x2,y2

W = Calib Weight, expressed as a percentage

y = Last area

Turning Calibration Averaging On or Off

Calibration averaging can be done in one of two ways. One way is to turn on automatic calibration averaging by selecting this item as part of the **Method > Properties**. The other way is to designate a calibration run type to **Average Replicates**. These selections designate how averaging is to be treated for the method. In order for a calibrated peak's areas to be averaged, you must also set the Calib Flag for that peak in the Peak Table to **Wt Average**. If you do not want a peak's areas to be averaged, set the Calib Flag for that peak to **Replace**.

Automatically Average Replicates

You can set up your method such that all calibrations are averaged using the **Method > Properties > Calibration** tab. Here, you can select the option to automatically average after every calibration. To turn averaging on, make sure there is a check mark next to the "**Automatically average replicates**" prompt. This is the default (recommended) selection. The default averaging is explained below.

If you wish to use a rolling average, you must enter the number of replicates to use in the rolling average. For details on how rolling average works, see the "Rolling Average Calibration" on page 124.

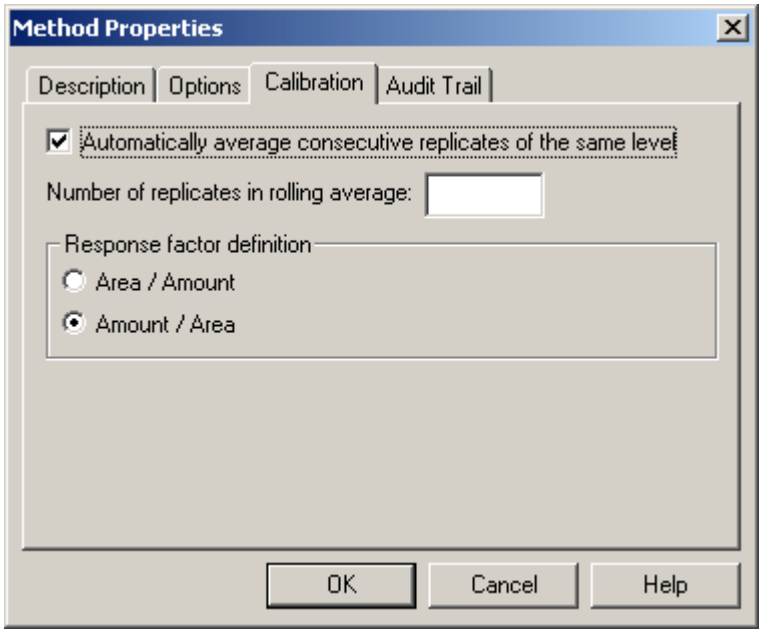


Figure 117. Method Properties - Calibration dialog box

When the automatic averaging of replicates is selected, averaging of all replicates for a given level will be performed until the replicates are cleared. If a sequence calibration (or sequence reprocessing calibration) is performed, automatic averaging of all replicates for a given level will occur automatically at the end of a series of calibration replicates for that level. The area/height average at that point will be saved in the method as "Last Area". When a new level is encountered, the replicates for the previous level will be cleared automatically from the method.

Note Averaging only occurs for peaks where the Calib Flag is designated as Wt Average.

If you turn the Automatic Averaging Off, be aware that for peaks whose Calib Flag is set to Wt Average, no results will be calculated until you force averaging by setting the Run Type to "Average Replicates" in either a single run or sequence run.

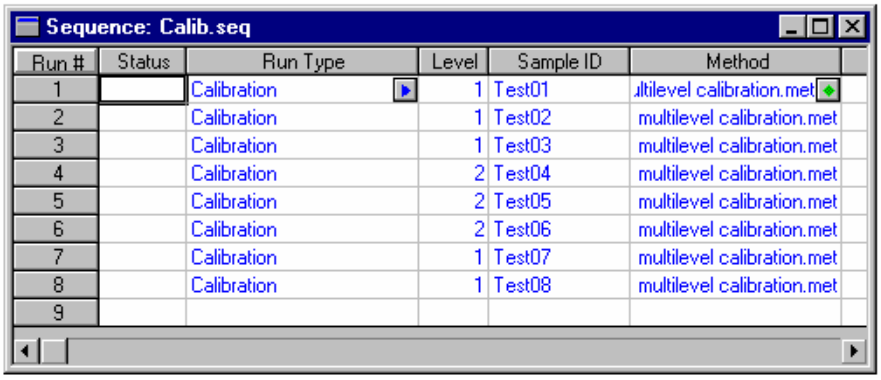
The following example demonstrates how replicates are treated during a calibration sequence using Automatic Averaging.

1. As each run is analyzed, the area for each named peak is saved in the method as the current area. At the end of Run 3, a true average will be calculated from replicates for Runs 1 - 3 for Level 1. This value is the calibration area/height for this level.
2. At Run 4, the calibration for Level 2 begins. Replicates for Level 2 will be saved for runs 4, 5, and 6.

3. At the end of Run 6, the replicates for Level 2 will be averaged and used to calculate the calibration point for Level 2. This area/height is saved as “Last Area” for Level 2.

4. At the beginning of Run 7, the existing replicates for Level 1 will be cleared and new Level 1 replicates will be saved for Run 7 and Run 8.

5. At the end of Run 8, the existing calibration area/height becomes the “Last Area”, the true average of Runs 7 and 8 is calculated and a weighted average is calculated using this value and the “Last Area” (see calculation in Calib Weight section). Note that if the Calib Weight is set to 100, the Last Area value is not taken into account.



Run #	Status	Run Type	Level	Sample ID	Method
1		Calibration	1	Test01	ultilevel calibration.met
2		Calibration	1	Test02	multilevel calibration.met
3		Calibration	1	Test03	multilevel calibration.met
4		Calibration	2	Test04	multilevel calibration.met
5		Calibration	2	Test05	multilevel calibration.met
6		Calibration	2	Test06	multilevel calibration.met
7		Calibration	1	Test07	multilevel calibration.met
8		Calibration	1	Test08	multilevel calibration.met
9					

Figure 118. Sequence: Calib. seq window

Automatic calibration averaging is a quick way to average calibration replicates that are grouped together, and is the most common use of averaging.

Rolling Average Calibration

If you have entered a number for replicates to be used in calibration in the **Method Properties > Calibration** dialog, a rolling average will be calculated for replicates as described below.

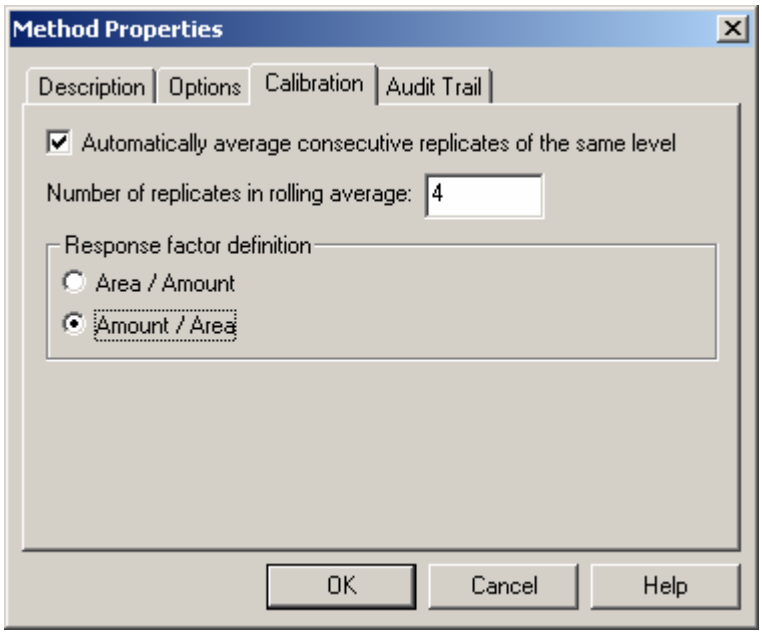


Figure 119. Method Properties - Calibration dialog box

The rolling replicate average is done as in the following example.

For an acquisition sequence, where S = Standard and U = Unknown, and the number of replicates in rolling average = 4,

S1, U1, S2, U2, S3, U3, S4, U4, S5, U5, S6, U6, S7, U7

In the above acquisition sequence, U1 would be quantitated using S1, U2 would be quantitated using the average of S1 and S2, U3 would be quantitated using the average of S1, S2, and S3, U4 would be quantitated using the average of S1, S2, S3, and S4, and U5 would be quantitated with the average of S2, S3, S4, and S5. In this example, every 4 calibration replicates will "roll" the average forward.

Calibration Averaging as Part of a Single Run or Sequence

Whether or not you are doing Automatic Calibration Averaging, you can designate averaging at the start of a single run, or as part of the **Run Type** of a sequence entry.

The Single Run dialog box has boxes for you to specify how you want to treat the calibration replicates. If you select the **Average Replicate** box, the replicates are averaged and the average is used in conjunction with the Calib Weight and Calib Flag to calculate a new calibration point.

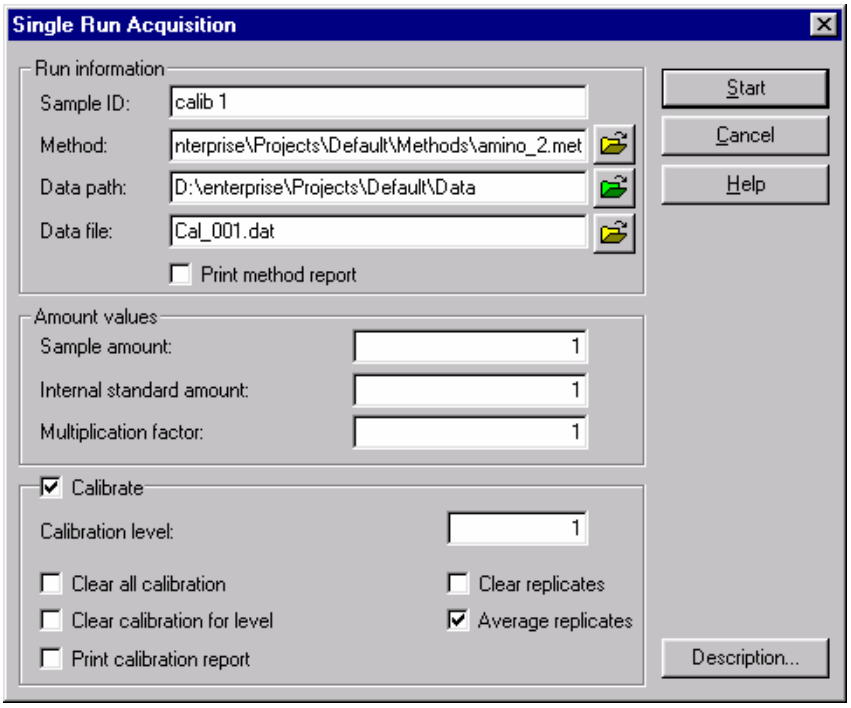


Figure 120. Single Run Acquisition dialog box

For a sequence, calibration averaging is designated as part of a run's **Run Type**. Click the **Run Type** field from the sequence spreadsheet to view the **Run Type** dialog.

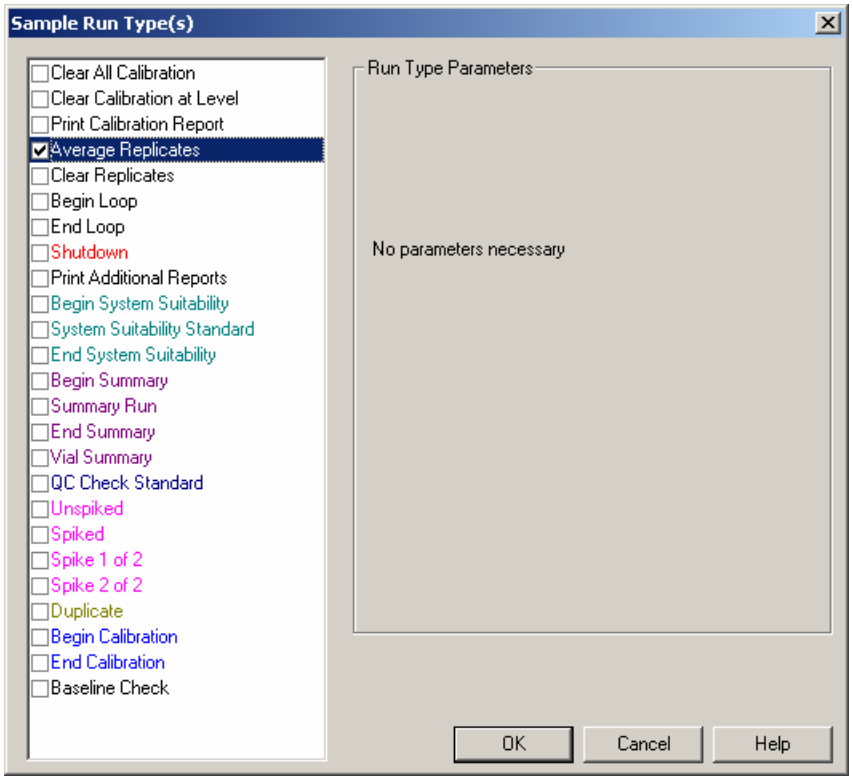


Figure 121. Sample Run Type(s) dialog box

If there is a check mark adjacent to the "Average Replicates" prompt, the replicates are averaged and the average is used in conjunction with the Calib Weight and Calib Flag to calculate a new calibration point.

You can quickly determine if a calibration run has an **Average Replicates** run type associated with it by looking at the sequence table. Runs that are designated for **Average Replicates** have the code **ARP** in the **Run Type** column.

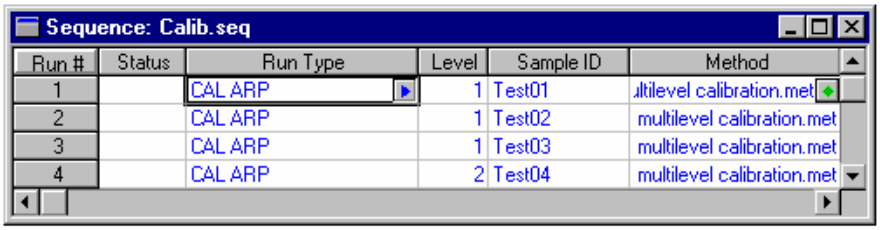


Figure 122. Sequence: Calib seq window

If you click on the Run Type for one of these runs, you will see the **Average Replicates** box has been checked for the Run Type of that sample. Click again on this check mark to remove the Average Replicates from the Run Type for this sample, if desired.

Note When Automatic Averaging is turned OFF, calibration replicates will continue to be saved in the method until you clear or average them at the beginning of a calibration run.

Steps for Creating a Calibration

To create a multi-level calibration

1. Using your data acquisition method, run one calibration standard, saving it on disk. Make sure your chromatography conditions and integration are correct.
2. Use your stored standard data file to graphically create your calibration peak table using the **Define Single Peak** or **Define Peaks** command. All the calibration parameters for each calibrated compound are entered in the Calibration Peak Table. The peak information is filled out graphically, and then specific information for each peak is entered manually. If you are doing multiple channel calibration, be sure to set your calibration parameters for **each** channel.
3. Complete your peak table by typing the peak names and concentration amounts, reference peak, internal standard numbers, and other necessary parameters for your samples.
4. Save your method.
5. Calibrate your method. The method can be calibrated using previously acquired standard data files or automatically as you run your standard(s). A calibration can be performed in the following ways:
   - Single Level Calibration
     - Update calibration using areas from a stored standard file.
     - Calibrate by running the standard sample.
     - Calibrate as part of a sequence of runs.
   - Multiple Level Calibration
     - Update using stored standard files (one level at a time, or sequence reprocess)
     - Update bracketed calibration using stored standard files (sequence reprocess using bracketed calibration).
     - Update as part of a sequence acquisition sequence.

Note Concentration results for an unknown run cannot be calculated until you have a complete calibration curve saved in your method.

Creating Calibrations Graphically

Before you can run a calibration standard, the method must contain the names of all the peaks you want to quantitate, their expected retention times for identification, and the amounts you will be injecting in the standard sample(s). These values are entered in a spreadsheet-like table called a Peak Table.

Define named peaks graphically

The most efficient and accurate way to enter this data into your method is to inject your first standard sample and save the data file on disk, then use the stored chromatogram to graphically enter most of the data you need.

Note The following steps assume the acquisition sampling rate and integration portion of the method have been optimized for the samples to be acquired.

If you have already acquired a calibration standard and it is saved on your disk, proceed to Step 2 on page 129.

To acquire the first standard sample

1. Select the **Control > Single Run** command from the menu, or click the **Single Run** button from the command ribbon. A dialog box will appear.

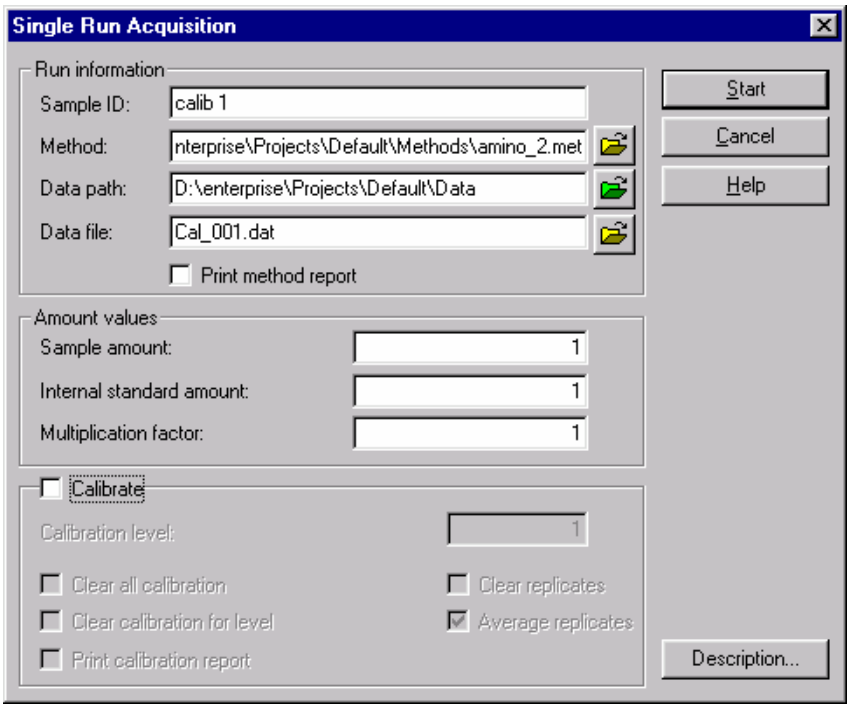


Figure 123. Single Run Acquisition dialog box

Fill in the information listed below. At this point, leave the **Amount Values** at the default values.

Run information

– Sample ID

  Enter a Sample ID for the run. The ID can contain both text and numbers. It is saved with the data file.

– Method

  Enter the name of the method to be used for data acquisition and processing. Include the entire path name if the method is not in your default method directory. You can select the method from a list of methods available on your disk by clicking the File Folder button adjacent to the field.

– Data path

  Enter a path location where the data file will be stored. If you wish, you can select a path by clicking the File button.

– Data file

  Enter a file name to be used to save the data on disk. It is not possible to over-write an existing data file. To use an existing data file name, you should use the Windows utilities to rename the existing file, or move it to another location first.

• Calibrate

  Do not select this box at this point, as you have not prepared your method for calibration yet!

When ready, click on **Start** to begin the acquisition of your sample.

2. When the run is completed, the chromatogram with baselines drawn should be shown on the screen. (If you are starting with an already-acquired data file, make sure the file is open and has been analyzed.)

If the baselines are not displayed, click on the **Analyze** button to make sure the chromatogram has been analyzed. If the baseline still does not appear, click the right mouse button within the chromatogram window, and select **Appearance**. Make sure the baseline is displayed in a color that is visible on the screen. You are now ready to define your calibrated peaks.

There are two ways to define calibration peaks using ChromQuest. Using **Define Peaks**, you add all of the peaks of interest to the peak table, then enter names and complete level information in the peak table. Using **Define Single Peaks**, you name and define each peak as you add it to the peak table.

Define Single Peak

To add peaks to the peak table one at a time, click on the **Define Single Peak** button from the Toolbar or choose **Define Single Peak** from the **right-mouse click > Graphical Programming** menu. A dialog box will appear for the first detected peak in the chromatogram.

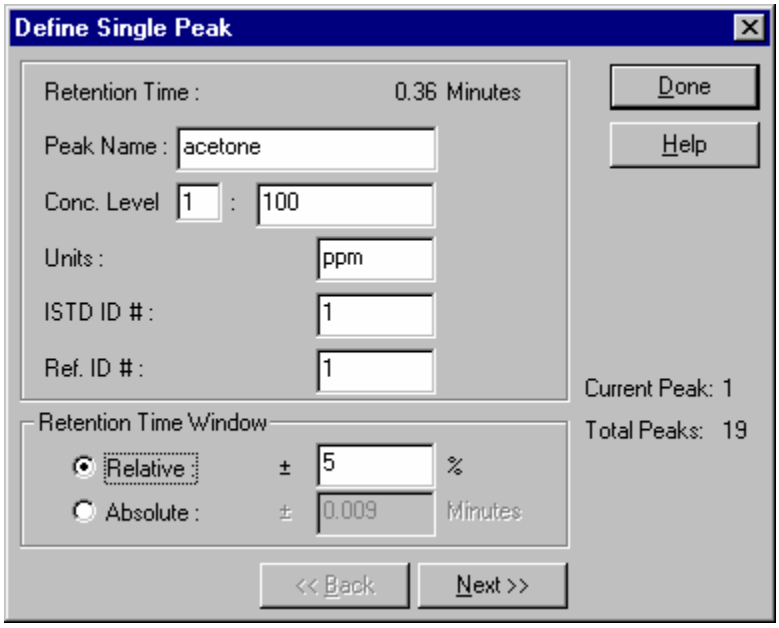


Figure 124. Define Single Peak dialog box

- Retention Time

  The retention time of the first detected peak will appear. If you want to add this peak to the peak table, complete the dialog for this peak. If you do not wish to add this peak to the peak table, click on **Next**. If you want to move to a specific peak in the chromatogram, click on that peak with your mouse. The retention time shown in the dialog will change to reflect the selected peak.

- Peak Name

  Enter the name of the compound in this field.

- Conc Level

  Concentration Level 1 is shown. Enter the amount of this compound for this concentration level. If you will be running more than one level for this compound, enter Concentration Level 2 and the amount for that level. Continue to enter level concentrations until you have completed the number of calibration levels desired. You can also enter or edit concentration level amounts from the method Peak Table.

- Units

  Enter the units to be used for display of results.

- ISTD ID #

  If you are doing internal standard calibration, enter the ID # for the internal standard peak for this compound. This is the peak ID number from the peak table. If you don't know it, you can add it in the peak table later.

- Resolution ID #

  Enter a peak ID# to be used for calculation of resolution, if desired. If this is left blank, resolution will be calculated for the peak of interest based on the detected peak preceding it. If the peak entered here is not detected, or if a peak refers to itself for the Resolution ID #, the resolution will not be calculated.

- Ref ID #

  Enter a retention time reference peak ID # to be used for this peak. This is the peak ID number from the peak table. If you don't know it, you can add it in the peak table later. Reference peaks are used to calculate Relative Retention Times.

- Retention Time Window

  The Retention Time Window value sets a window around the expected retention time of calibrated peaks. A retention time window is important because it allows a peak to drift slightly (within the window) and still be identified as a calibrated peak. If no retention time window is set, a calibrated peak must ALWAYS occur at exactly the expected retention time in order to be identified as the calibrated component. You can select a **Relative** Retention Time Window or an **Absolute** Retention Time Window.

  – Relative

    Relative retention time window is based on a % of the expected retention time of the component. By default, the Relative Retention Time Window is set to 5%, which means that the Retention Time Window for calibrated peaks will be set to 5% of their expected retention time. Later-eluting peaks will have larger time windows if a Relative Retention Time Window is used. Use a relative retention time window if your peaks tend to drift later in the run.

– Absolute

  Absolute retention time window sets up a retention time window that is the same for all calibrated peaks. You enter a value for the retention time window to be used for the peaks. An absolute retention time window does not vary with the retention time of the calibrated peak.

Click **Next** to move to the next detected peak. Click on **Back** to move to the previous detected peak in the chromatogram. To move directly to a specific peak, click on that peak in the chromatogram. The current peak and total peaks in the chromatogram is displayed on the right of the dialog box. When you are finished adding peaks to your peak table, click on **Done.**

Each peak you defined will become a row in your peak table. Note that if you already had peaks in your peak table, the peaks you just defined will be added to those already present. To view the peak table, click the **Peak/Group Tables** button from the command ribbon.

Define Peaks

The **Define Peaks** button allows you to create a row in the calibration peak table for each detected peak in a selected range. You can then edit each peak in the Peak Table. Click on the **Define Peaks** button from the Toolbar. Alternatively, click the right mouse button, followed by Graphical Events Programming then select the **Define Peaks** command.

Enter the peaks for your calibration by clicking the mouse once to the left of the first calibration peak in the chromatogram, then once to the right of the last calibration peak in the chromatogram. A dialog box will appear where you set up some of the parameters for the peaks that will be added to the peak table.

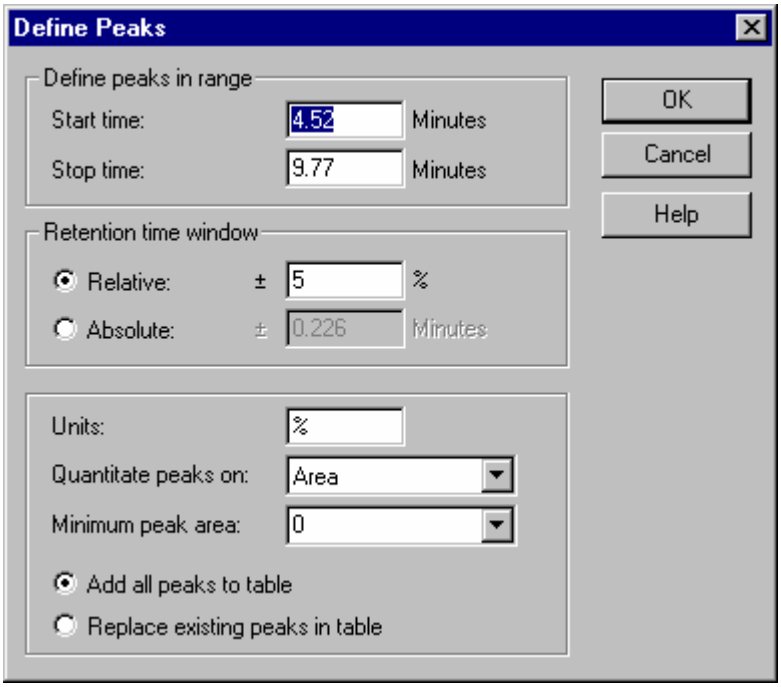


Figure 125. Define Peaks dialog box

- Define peaks in range

  All peaks detected within the range between the **Start Time** and **Stop Time** shown will be added to your peak table. This range was defined by your mouse clicks on the chromatogram. You may change these times manually in the boxes shown if you desire.

- Retention time window

  The Retention Time Window value sets a window around the expected retention time of calibrated peaks. A retention time window is important because it allows a peak to drift slightly (within the window) and still be identified as a calibrated peak. If no retention time window is set, a calibrated peak must ALWAYS occur at exactly the expected retention time in order to be identified as the calibrated component. You can select a **Relative** Retention Time Window or an **Absolute** Retention Time Window.

  – **Relative** retention time window is based on a % of the expected retention time of the component. By default, the Relative Retention Time Window is set to 5%, which means that the Retention Time Window for calibrated peaks will be set to 5% of their expected retention time. Later-eluting peaks will have larger time windows if a Relative Retention Time Window is used. Use a relative retention time window if your peaks tend to drift later in the run.

  - **Absolute** retention time window sets up a retention time window that is the same for all calibrated peaks. You enter a value for the retention time window to be used for the peaks. An absolute retention time window does not vary with the retention time of the calibrated peak.

- Units

  Enter the units to be used for labeling the concentration results using the calibrated peaks.

- Quantitate peaks on

  Select **Area** or **Height** for the basis of calculation of response factors.

  Note If you are doing both Peak and Group quantitation using internal standards, both Peaks and Groups must use the same quantitation measurement type (area or height).

- Minimum peak Area

  If you enter a minimum peak area, any peaks found within the defined peak range whose areas fall below this limit will not be considered calibration peaks, and will not be entered in the calibration Peak Table.

- Add all peaks to table

  Select this button to add the peaks in the current defined peak range to existing peaks in your calibration table.

- Replace existing peaks in table

  Select this button if you want to replace all existing peaks in the current calibration peak table with the peaks from the defined peak range.

Click **OK** to accept your selections.

A spreadsheet view of the **Peak Table** will appear with the retention time of each peak in the selected peak range displayed. Complete the peak table for each calibrated compound, as described below.

Peak Table

All of the information required to calculate concentrations for unknown peaks using a calibration is contained in the **Peak Table**. Each row of the peak table represents one of the calibrated components. Once you have graphically entered the peaks into the peak table, you must complete the peak table with information required to correctly identify and calculate unknown concentrations for each peak. You may not need to use all the columns of the spreadsheet. Enter information only for columns that will be required for calculation of amounts for each peak of interest.

Note Once you have become familiar with the parameter columns in the peak table, you can customize the appearance of the table to only display the columns of interest to you by doing a right-mouse click in the table and selecting “Properties”.

Calibrated Peak Parameters

The following columns in the Peak Table are used for calculating results for calibrated peaks.

- #

  Row number

- Name

  Type the name of the component that matches the retention time displayed.

- ID

  ChromQuest will automatically assign a peak ID# for each peak, starting with "1". This ID# is used to designate reference peak and internal standard peak number. If you are adding peaks to an existing peak table, you can renumber the new peak table automatically by doing a right-mouse click in the peak table, followed by **Renumber Peak ID#**.

  Note If you renumber the Peak ID#, make sure any custom parameter programs or other user programs that may use Peak ID# are updated to reflect the new Peak ID#’s.

- Ret. Time

  This is the calibrated peak's expected retention time. If you have used graphical events to enter the peaks into the peak table, Retention Times are automatically filled in using the retention times of the detected peaks in the standard file used to create the table.

- Window

  The width of the window is indicated and is centered on the retention time value. If a peak's retention time (adjusted for reference peak shift) falls outside this window, it is not identified as the calibrated peak. If more than one peak falls within the window, the peak closest to the center of the window is identified as the calibrated component. You can change the size of the window by entering a new value, in minutes.

- Resolution ID#

  Enter a peak ID# to be used for calculation of resolution, if desired. If this is left blank, resolution will be calculated for the peak of interest based on the detected peak preceding it. If the peak entered here is not detected, or if a peak refers to itself for the Resolution ID #, the resolution will not be calculated.

- Ref. ID#

  This is the ID# of the peak to be used as a reference peak for this component. A reference peak is used to adjust the expected retention time of a calibrated component to compensate for changes in the chromatograph such as flow rate. If more than one peak falls within the reference peak window, the largest peak in the window is used as the reference peak. Each peak can have its own reference peak.

  An ideal reference peak is one that is always present in the sample, and is well resolved from other peaks in the chromatogram. (Internal standards make excellent reference peaks.)

  If a named peak is assigned a reference peak, then its expected retention time is calculated as follows:

  Expected RT = (Actual Ref. Peak RT/Expected Ref. Peak RT) * Peak RT

- ISTD.ID#

  ID# of the peak to be used as internal standard for this component, if you are using an internal standard method.

  Note If you are doing an Internal Standard calibration, you must enter an ISTD ID# for each calibrated component. If the peak is an internal standard, enter its own ID# in the ISTD ID# column. If you are doing External Standard calibration, you should enter "0" in the ISTD ID# column for all components. You may designate more than one internal standard.

- Units

  Enter the concentration units to be used in reporting results, such as mg/ml or vol%.

- RT Update

  Select how you want to update the expected retention times in your Peak Table. The selection includes None, after every analysis (Run), after calibration runs only (Calib), or after every run and calibration (Run & Calib).

- LOD

  This parameter is used to calculate and report the ASTM LOD (Limit of Detection) value that is used to determine if the peak is within the Limits of Detection as previously determined for the method. The value entered here is the S/N ratio (signal to noise ratio) to be used for the calculation for this peak. This calculation is valid only for ESTD or ISTD methods.

- LOQ

  This parameter is used to calculate and report the ASTM LOQ (Limit of Quantitation) value that is used to determine if the peak is within the Limit of Quantitation as previously determined for the method. The value entered here is the S/N ratio (signal to noise ratio) to be used for the calculation for this peak. This calculation is valid only for ESTD or ISTD methods.

- Quantitate

  Choose whether the calibration and quantitation are to be based on peak height or peak area.

  Note If you are doing both Peak and Group quantitation using internal standards, both Peaks and Groups must use the same quantitation measurement type (area or height).

- Fit Type

  This option determines how the calibration curve will be fit to the data.

  Note You have five choices for how the calibration curves will be drawn: point-to-point, linear, and quadratic, cubic, and Average RF fits. You must have at least two calibration levels for a linear fit (not including zero), three levels for a quadratic and four levels for a cubic fit.

- Force Zero

  When this box is selected, the calibration curve will be forced through zero.

- Calib Flag

  The **Replace/WtAverage** selection allows you to determine how the calibration areas/heights for each peak in your method will be affected by running a standard: they will either be replaced, or averaged with the current replicate areas in the calibration method. If Calib Weight is set to a value other than 100, a weighted average of the areas/heights will be calculated. For details on calibration averaging, see "Replicates and Averaging Calibrations" on page 119.

Each time a calibration average is performed, the value is saved in the method as "Last Area", which is subsequently used for calculation of weighted averages.

- Calib Weight

  You can designate a "weight" for the average of the replicates. The weighting factor is applied to replicate injections as shown in the following example.

Current run value (area/height)	101
Replicate 1	104
Replicate 2	100
Replicate 3	102
Current Last Area Value =	102

  Using a weighting factor of 60, the new method average is:

  [((104 + 100 + 102) / 3) * .6] + [101 * .4] = 101.6

  Note For Internal Standard calibrations, each Replicate represents a ratio of the component area/height to internal standard area/height.

- % Calib Margin

  This selection creates an acceptance margin based on the % difference between the peak area or height found during calibration and the current peak area or height in the method. If the % difference between these values exceeds this limit, the peak will not be used to update the calibration.

- Scale

  This parameter allows you to apply a scaling factor to the calibration curve. This factor is applied to the entered amounts prior to computing the calibration curve. The purpose of using a scaling factor is to create a relationship between areas (or heights) and amounts that can be approximated by a polynomial fit. A scaling factor can be applied to any fit type. The available scaling operations are:

  None

  1/X

  $1/X^2$

  ln[X]

  1/ln[X]

  sqrt[X]

  x^2

- Weighting Method

  Select a LSQ Weighting Method to be used for calculation of least squares regression fits, either 1/Response, 1/Response2, 1/Amount, 1/Amount2 or none. Weighting gives increased importance to smaller concentrations and areas in the regression calculation. It can be applied to linear, quadratic, and cubic fit types only.

- Level

  Type in the exact amount of each compound (corrected for purity) in your first calibration standard in the column labeled "Level1". If you are doing a single-level calibration, you do not need to fill in any other "Level" columns. If you are doing a multiple-level calibration (e.g. you have more than one calibration standard mixture) you should repeat this process for each standard concentration level you plan to inject.

  For example, assume you have a multi-level calibration where component A is present in standard mixture 1 at 10 ppm, in standard mixture 2 at 20 ppm, and standard mixture 3 at 30 ppm. You would enter 10 in the **Level 1** column, 20 in the **Level 2** column, and 30 in the **Level 3** column.

  Note Occasionally, a situation may arise where one or more named components may not be present in the calibration level mixture. To avoid introducing error in your calibration curve, you can cause the component area to be ignored at that level by leaving the "Level" of interest blank for that component. (Click on the cell and press the delete key.)

- STD ID#

  If you wish to calculate the concentration of a peak for which you have no standard sample, you can designate that the calibration curve of a different peak be used. Using STD Mult (see below), you can multiply the result to arrive at a proportional number.

- STD Mult

  If you have designated another peak to be used to calculate concentration, enter a multiplier here to be used. The equation used to calculate the concentration of the peak of interest then becomes

$$Conc_i = f(Area_i) \, x \, \frac{Mult_S}{Mult_i}$$

  where

  f = the calibration curve equation for the standard peak according to the fit type selected (linear, quadratic, point to point, etc.)

  $Conc_I$ = concentration of peak of interest

  $Area_i$ = area of the peak of interest

  $Mult_S$ = STD Mult of designated STD peak

  $Mult_I$ = STD Mult of peak of interest

- Manual RF

  If you wish to assign a response factor to be used for a peak instead of the response factor calculated from the standard data, enter that value here. If a manual response factor is present in this field, it will be used to calculate amounts for this peak instead of using the calibration curve.

- Low Conc

  You can enter a concentration lower limit in this column. After each analysis, ChromQuest can compare the calculated concentration of the peak to this value. If the value falls below the lower limit value, in order for a message to be posted in the Instrument Activity log, a post-run sequence action must be selected.

- High Conc

  You can enter a concentration upper limit in this column. After each analysis, ChromQuest can compare the calculated concentration of the peak to this value. If the concentration value falls above the upper limit value, in order for a message to be posted in the Instrument Activity log, a post-run sequence action must be selected.

- QC Reports Parameters

  The following parameters in the Peak Table are used for calculation of QC Reports.

- Check Std 1…5 Conc

  Enter the amount of component present in the designated Check Standard. (Up to 5 check standards can be defined.)

- Check Std 1…5 %RD

  If you are generating a Check Standard Report, enter the value to be used for the percent relative difference limit here. When the run is made, the calculated concentration of the peak will be compared with the check standard concentration specified in the peak table (Check Std Conc). If the difference is less than the Check Std % RD, the component passes. If the difference is more than the Check Std % RD, the component fails, and the "failure action" for that line of the sequence will be taken. Up to 5 check standards can be defined.

- Spike 1 Amt

  For Spike Recovery Report, enter the amount(s) of the first component(s) used to spike the first spiked sample.

- Spike 2 Amt

  For Spike Recovery Report, enter the amount(s) of the second component(s) used to spike the second spiked sample (if you are spiking the sample twice).

- Low Spike Limit

  Enter the lower limit (in %) for spike recovery. The calculated spike recovery will be compared to this value. If it falls below the low spike limit, it fails.

- High Spike Limit

  Enter the high limit (in %) for spike recovery. The calculated spike recovery will be compared to this value. If it falls above the high spike limit, it fails.

- Dup % RD Limit

  Enter the value to be used for % relative difference for duplicate reports. If the % relative difference falls above this value, the sample "fails".

- RF % RSD Limit

  Enter the % relative standard deviation for calibration response factors. When a "Calib Start" and "Calib End" are designated in the sequence table, this value will be used to determine whether the calibration "passes" or "fails".

Peak Table Properties

If you are not using one or more of the parameters presented in the Peak Table, you can remove it from view using the **right-mouse click > Properties** command. When you select this command, a dialog box appears where you can select the columns that will appear in the peak table.

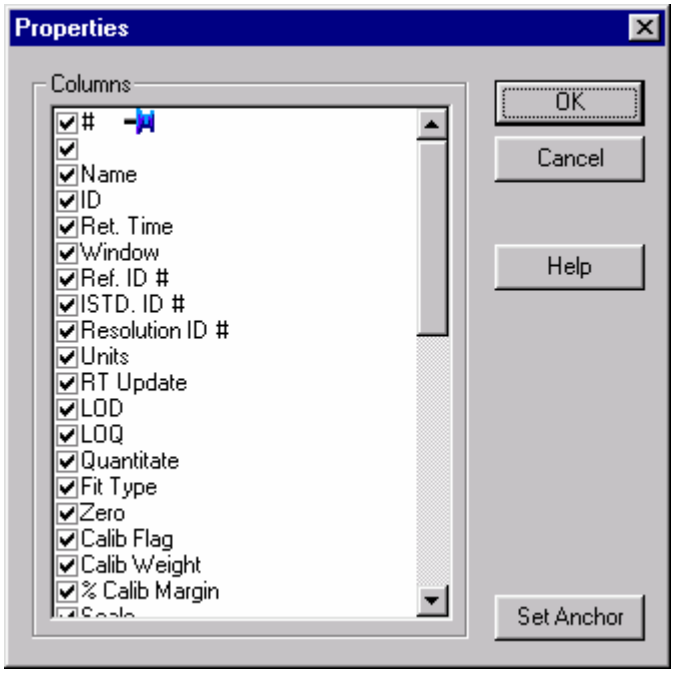


Figure 126. Properties dialog box

Columns with a check mark will be displayed in the Peak Table spreadsheet. Those without a check will not be displayed. To enter a maximum number of Levels to be displayed, double-click on the **Maximum # Levels** text. A box will appear where you can enter the number of calibration levels you want to have displayed in the peak table. Type the number of levels to be displayed, then press ENTER to accept.

The selections entered are saved on a per-instrument/per-user basis. This allows each user to set his/her own peak table parameters for each instrument.

Set Anchor

The blue "anchor" indicates what column will be used to anchor the right-left scrolling in your sequence spreadsheet. Once this anchor is set, columns to the right of the anchor will scroll to the right and left. Columns to the left of the anchor will not scroll. To change the anchor, click on the name of the column you wish to use as anchor, and click the **Set Anchor** button. The blue anchor will move to the designated anchor column.

Note When using the anchor, it is best to remove all unnecessary columns from the spreadsheet, and make the spreadsheet as wide as possible before you set the anchor. If you set the anchor to a column that does not currently show on the spreadsheet, you will not be able to scroll the spreadsheet.

Renumbering Peak ID's

Peak ID numbers are assigned to calibrated peaks in the order in which they are added. If you had edited your peak table by adding peaks, you may want to renumber the Peak ID's so the ID numbers are in order of the peak retention times. To do this, do a right-mouse click inside the peak table, and select the **Renumber Peak ID's** command. The Peak ID numbers in the peak table will be renumbered (including Ref and ISTD Peak ID numbers to reflect the changes).

Note Renumbering Peak ID#'s may require items identified by Peak ID# in custom parameters or custom reports to be re-defined using the new Peak ID#'s.

Calibrating Your Method (Running Calibration Samples)

Once the method is set up for calibration, the calibration is not completed until the areas for peaks in the standard samples are entered into the method. Updating the method with these areas is called "Calibration" or "Calibrating the Method". Calibration can be performed by updating the calibration in the method automatically as each standard sample is run, or it can be performed by sequence reprocessing using standard data files that were previously acquired and stored on disk.

For information on how to create a sequence either for running calibration samples, or for reprocessing stored data files, see **Chapter 4: Sequence Operations**.

You can also run calibration standards one at a time using the Single Run procedure described above. To calibrate a single level method using a single calibration standard that has been saved on the disk, follow the procedure outlined in Single Level Calibration Using a Stored Data File.

Single Level Calibration Using a Stored Data File

If you already have your calibration standard saved on your disk, you can calibrate your method using the areas from this file.

Choose **Analysis > Analysis / Single Level Calibration** from the menu to access the Analysis / Single Level Calibration dialog box.

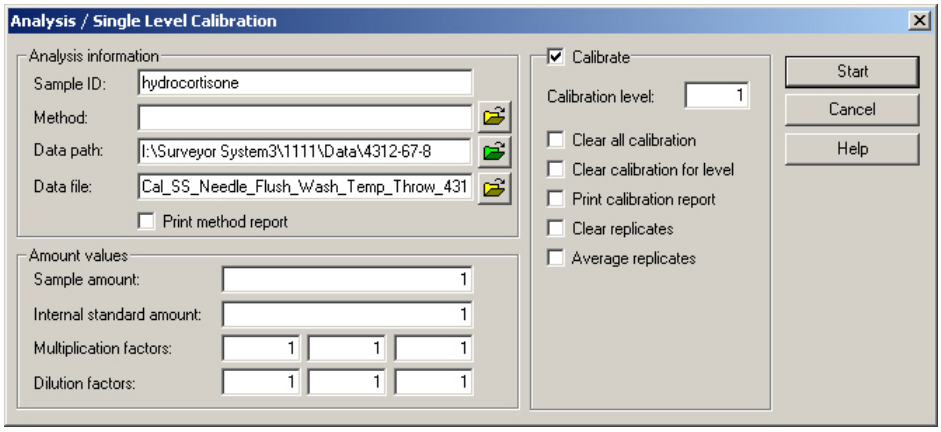


Figure 127. Analysis / Single Level Calibration dialog box

This dialog appears similar to the Single Run dialog used to acquire a single data acquisition sample. However, the **Analysis > Single Level Calibration** command does not initiate data acquisition. It updates the method specified using areas from the stored data file specified.

- Analysis information
  - Sample ID

    The sample ID for the data file selected is displayed here.

  - Method

    Enter the name of the method to be calibrated. Include the entire path name if the method is not in your default method directory. You can select the method from a list of methods available on your disk by clicking the File button adjacent to the field.

  - Data path

    Enter the path name where the data file is located. You can select the path name from a list by clicking the File button.

  - Data file

    Enter the name of the file to be used to calibrate the method. You can select a data file by clicking the File button adjacent to the field.

  - Print method report

    When this box is checked, the method report(s) will be printed at the end of the analysis.

- Amount values

  In this section, you can enter values that affect how the concentrations are calculated. If you are making a single data acquisition prior to calibrating your method, simply leave these values at the default level.

  – Sample amount

    The Sample Amount value is used as a divisor during calculation of concentrations. It is intended to compensate for differences between samples due to weighing and when percentages of the total sample are being calculated rather than the amount detected in an injection.

  – Internal standard amount

    For calibration runs, the Internal Standard Amount is taken from the method Peak Table. For unknown runs, enter the amount of the Internal Standard in your unknown sample.

  – Multiplication factors

    Enter up to three multiplication factors to be used for this analysis/calibration. All quantitated peaks will be multiplied by these factors.

  – Dilution factors

    Enter up to three dilution factors to be used for this analysis/calibration. All quantitated peaks will be divided by these factors.

- Calibrate

  Select this box to trigger calibration. Once this box is clicked, the following fields and options will be available.

  – Calibration level

    Enter the number of the calibration level represented by the stored calibration standard. If this is a single level calibration, enter **1**.

  – Clear all calibration

    Click this box if you want to clear all existing calibration factors from your method before running the calibration.

  – Clear calibration for level

    Click this box if you want to clear the existing response factors for this level only before running the calibration.

– Print calibration report

  Click this box if you want to print a calibration report after running the calibration.

– Clear replicates

  Click this box if you want to clear existing replicates from this level before running the calibration.

– Average replicates

  Click this box if you want to average replicates for this calibration level.

When you have completed the dialog box, click **Start**. ChromQuest will open the stored data file and use the areas for calibrated peaks to update the method specified.

Reviewing Calibration Curves

After you have completed running all of the calibration standards for your method, you can review the calibration curve and associated data by choosing **Method > Review Calibration** or by clicking on the **Review Calibration** button. A window will appear with the calibration information from your current method.

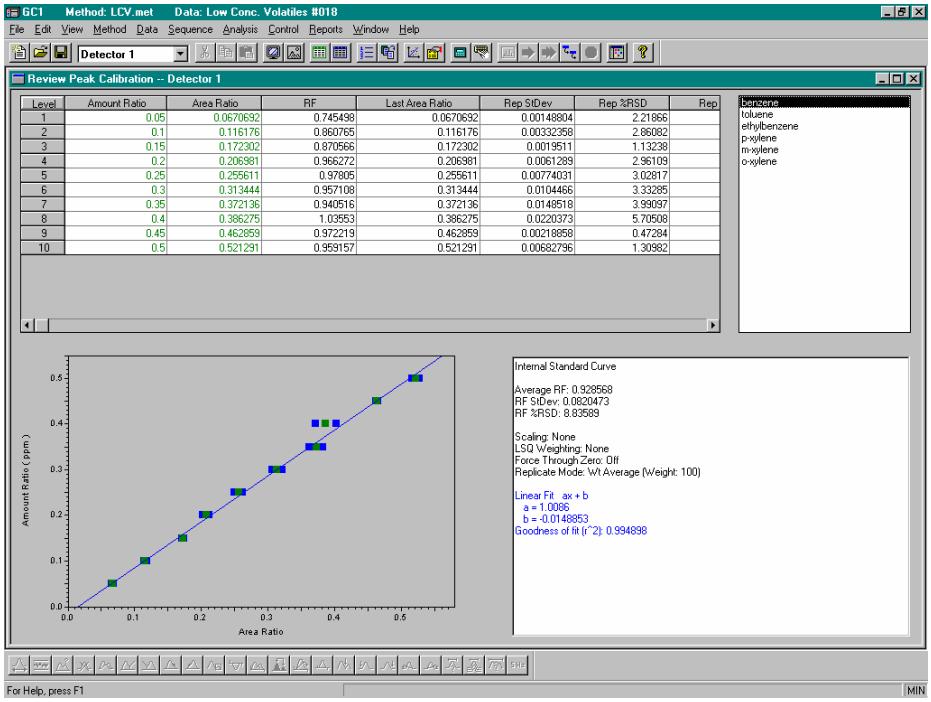


Figure 128. Review Peak Calibration window

From Review Calibration, you can:

- Look at the calibration curve for each calibrated peak.
- Change and overlay calibration curve fit types.
- Review the equations for curve fit types, and examine the R-squared (goodness of fit) value for each.
- Temporarily remove points from the calibration curve.
- View response factors, replicate areas, and standard deviation values.
- Use the concentration calculator to calculate amounts from manually entered areas.

Viewing the Calibration Curve

Calibrated peaks are listed in the **Peak List**. To view the calibration information for a given calibrated peak, click on the peak name from the Peak List. It will be highlighted, and the spreadsheet and calibration curve will be updated to include the current calibration information for that peak.

The calibration curve for the peak selected appears in the lower left corner of the window. When you move the cursor over one of the calibration point replicates on the curve, a "tool tip" will appear that gives you information about that calibration point.

The box at the lower right of the window displays parameters and calculation data for the calibration curve displayed. This includes Average Response Factor, the Response Factor Standard Deviation, the Response Factor % RSD, Scaling factor, LSQ Weighting factor, Force through zero On or Off, Replicate mode, and calculations for each fit type displayed, along with r^2 for the fit.

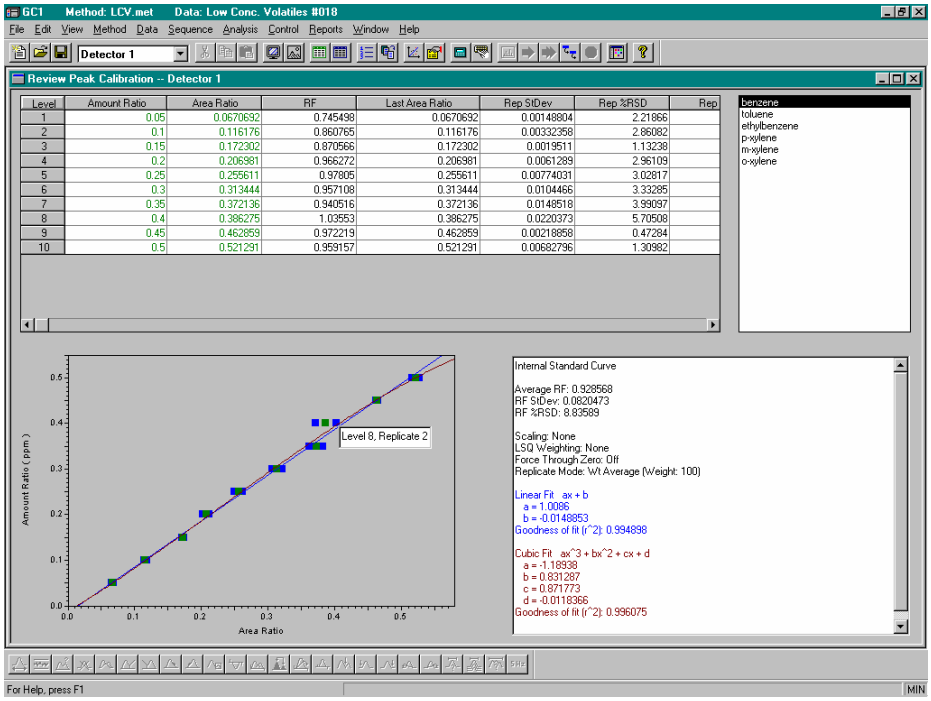


Figure 129. Review Peak Calibration window

Temporarily removing points from the calibration curve

If you want to see the effect of removing one of the points from your calibration curve, you can temporarily remove it from the calculation by clicking the point once with your mouse. The point will turn red, which indicates it is not currently used for the calculation of the calibration curve. The spreadsheet cells represented by the point will also turn red. To return the point to the calculation of the curve, click on the point again. Points can also be disabled from the spreadsheet by clicking the cell in the spreadsheet, then pressing the **Delete** key. To restore the point, click the **Delete** key again.

Using the right mouse button

As with other areas of ChromQuest, clicking the right mouse button give you access to quick menus. When you do a right-mouse click in the calibration curve region of the window, the following menu will appear where you can change the characteristics of the displayed calibration curve.

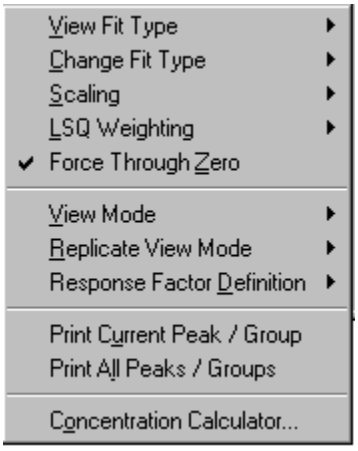


Figure 130. Calibration Curve options menu

- View Fit Type

  Select a new fit type to be displayed in the review peak calibration graph. The fit is overlaid with the current calibration curve.

- Change Fit Type

  Select a new fit type to be applied to the calibration curve. When you change the fit type, the change will also be entered into your peak table for this peak.

- Scaling

  Select a new scaling factor for the calibration curves. This scaling will be applied to all calibration fits displayed, and will be applied to the peak table for this peak.

- LSQ Weighting

  Select a least squares weighting factor to apply to the calibration curves. (Applies only to linear, cubic, and quadratic fits.) The LSQ Weighting selected will be entered in your peak table for this peak.

- Force Through Zero

  Select this to force the calibration curve(s) through the origin. Changing this will automatically change the peak table for this peak.

- View Mode

  Select **Peaks** or **Groups** for viewing.

- Replicate View Mode

  This selects how you want to view the replicate data in the calibration data spreadsheet. Select **Area/Height** to view the replicate area or heights. Select **Response Factor** to view the replicate response factors.

- Response Factor Definition

  Select how the response factors are to be calculated and displayed - **Area/Amount** or **Amount/Area**. If you select **Amount/Area**, you will have access to all fit types. If you select **Area/Amount,** all fits except cubic are allowed. The selection of **Area/Amount** or **Amount/Area** for your method is located in the **Method > Properties > Options** dialog.

  – Clear All Fits

    Selection of this will remove all fits to the calibration curve display.

  – Restore Original Fit

    This selection will restore the fit originally displayed for the calibration curve.

- Print Current Peak/Group

  Select this to print the currently selected peak or group information.

- Print All Peaks/Groups

  Select this to print all the peaks or group information.

- Concentration Calculator

  The Concentration Calculator lets you calculate amounts based on areas or heights you input.

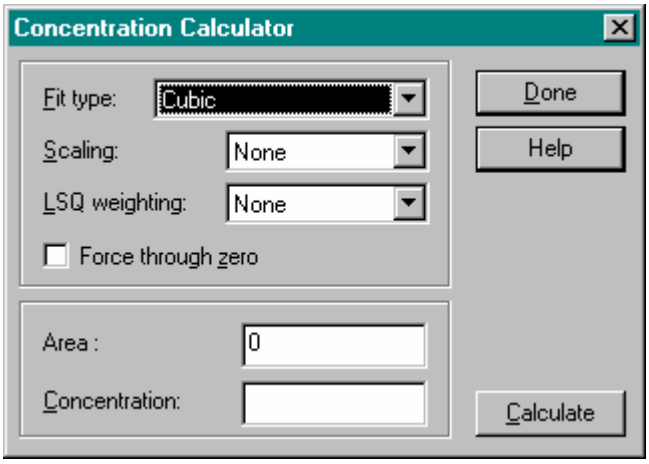


Figure 131. Concentration Calculator dialog box

– Fit type

  Select a fit type to be used to calculate the concentration or area.

– Scaling

  If desired, select a scaling factor to be used for the calculation.

– LSQ weighting

  Select a least squares weighting factor to be used, if desired.

– Force through zero

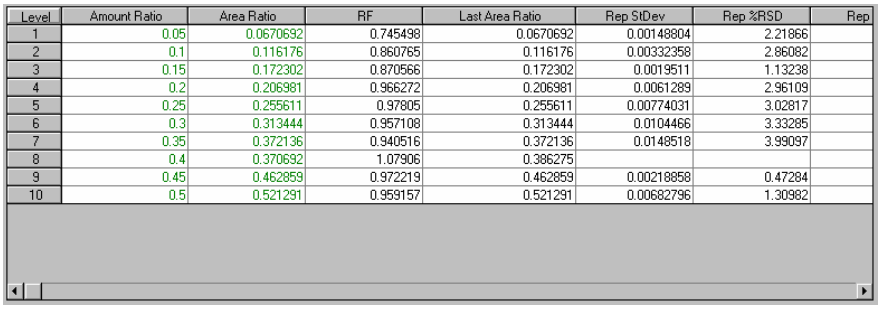
  Select this if you want the curve forced through the origin for the calculation.

– Area

  If you want to calculate an **Amount**, enter an area that represents the peak to calculate, then click on the **Calculate** button.

• Viewing Calibration Data

  When you select a peak from the peak list, the calibration data for that peak will be displayed in the spreadsheet at the top of the window. Each row in the spreadsheet represents a calibration level for that compound.



Level	Amount Ratio	Area Ratio	RF	Last Area Ratio	Rep StDev	Rep %RSD	Rep
1	0.05	0.0670692	0.745498	0.0670692	0.00148804	2.21866	
2	0.1	0.116176	0.860765	0.116176	0.00332358	2.86082	
3	0.15	0.172302	0.870566	0.172302	0.0019511	1.13238	
4	0.2	0.206981	0.966272	0.206981	0.0061289	2.96109	
5	0.25	0.255611	0.97805	0.255611	0.00774031	3.02817	
6	0.3	0.313444	0.957108	0.313444	0.0104466	3.33285	
7	0.35	0.372136	0.940516	0.372136	0.0148518	3.99097	
8	0.4	0.370692	1.07906	0.386275			
9	0.45	0.462859	0.972219	0.462859	0.00218858	0.47284	
10	0.5	0.521291	0.959157	0.521291	0.00682796	1.30982	

Figure 132. Calibration data spreadsheet

- Level

  A calibration level corresponds to a point (or points, if replicates are used) on the calibration curve, and represents the relationship of peak area(s) to calibrated amount. The number of calibration levels may be different for each component. ChromQuest software can handle an unlimited number of concentration levels per component. For each Level, the following fields are displayed.

  – Amount/Amount Ratio

    This is the component Amount (external standard) or Amount Ratio (internal standard) represented by this level.

  – Area (or Height)/Area Ratio (Height Ratio)

    This is the peak area or height (if external standard) or Area Ratio or Height Ratio (for internal standard) for the designated level.

  – RF

    This is the response factor for the peak at the designated level.

  – Last Area (ratio)/Height (ratio)

    If no replicate injections are made, this is the last area or height (or area ratio/height ratio) for the peak at the designated level. If replicate injections have been made, this is the old average of the replicates, which is used for weighting purposes.

  – Residual

    A residual is the difference between the user entered concentration and the concentration read back from the computed calibration curve.

  – Rep StDev

    This is the standard deviation for the replicates

– Rep %RSD

  This is the % relative standard deviation for the replicates.

– Rep 1…x Area (ratio)/ height (ratio)

  Each level can have multiple **replicates** (duplicate injections of a single standard level). Rep 1 is the first calibration replicate run, Rep 2 is the second replicate, and so on. When replicate standards are used, and the Calib Weight is set to 100, the response factor will be based on the true average of all replicates in the calibration level. In order for replicates to be used, you must select the **Wt Average** option in the Peak Table Calib Flag column. Otherwise, each injection at a given level will replace the current calibration areas/heights.

Level	Rep 2 Area	Rep 2 User	Rep 2 Data File	Rep 2 Sample ID	Rep 2 Calib. Time	Rep 3 Area
1	63056	Demo	C:\DATA\PNA_STDS10mu	PNA-STDS	12/4/97 11:28:02 AM	63056
2	171813	Demo	C:\DATA\PNA_STDS11mu	PNA-STDS	12/4/97 11:28:09 AM	171813
3	299360	Demo	C:\DATA\PNA_STDS12mu	PNA-STDS	12/4/97 10:57:54 AM	297813
4	462656	Demo	C:\DATA\PNA_STDS13mu	PNA-STDS	12/4/97 10:57:58 AM	461566
5	779320	Demo	C:\DATA\PNA_STDS14mu	PNA-STDS	12/4/97 10:58:01 AM	777863

Figure 133. Replicates Displayed in Review Peak Calibration Table

– Rep 1…x User

  This is the logged user at the time the calibration was last performed using this replicate.

– Rep 1…x Data File

  This is the data file where the replicate data is stored.

– Rep 1…x Sample ID

  This is the Sample ID for the replicate sample.

– Rep 1…x Calib. Time

  This is the date and time the last calibration was performed using this replicate.

Groups and Group Calibration

A "group" is a collection of peaks, which are somehow related. In ChromQuest, you can define as many groups as you want. Peaks in a group do not have to be contiguous peaks in the chromatogram. Groups may defined in one of three ways:

- Uncalibrated Range

  In this type of grouping, you define a time range. A manual response factor will be applied to determine concentration of uncalibrated peaks eluting within that time range.

- Calibrated Range (Group Calibration)

  Using this technique, you calibrate peaks together as a group, creating a common response factor for the group. Peaks in the Calibrated Range can be reported as a single peak using the common response factor for the group as a basis for calculation of concentration. You can also choose to include named peaks in a calibrated range group.

- Designate Named Peaks to be included in a group (Calibrated Peak Grouping)

  Using this technique, a group report gives the concentration summation of all calibrated peaks designated as belonging to that group. All peaks in the group can also be reported individually because they also appear in the Peak Table.

Defining a Group

Defining a group is similar to defining calibrated peaks. The group is defined manually by typing information into the Groups table, or it is defined graphically using an integrated chromatogram.

To define a group graphically, click on the **Define Group** button, or select the Define Group command from the **right-mouse click > Graphical Programming** menu. You will be prompted on the status bar to click the mouse to define group regions. Click once at the beginning and once at the end of a region of peaks you wish to add to the group. You may continue to add groups by clicking to define the group regions. When you are done defining the group regions, press the ESC key. A dialog box will appear where you further define the group.

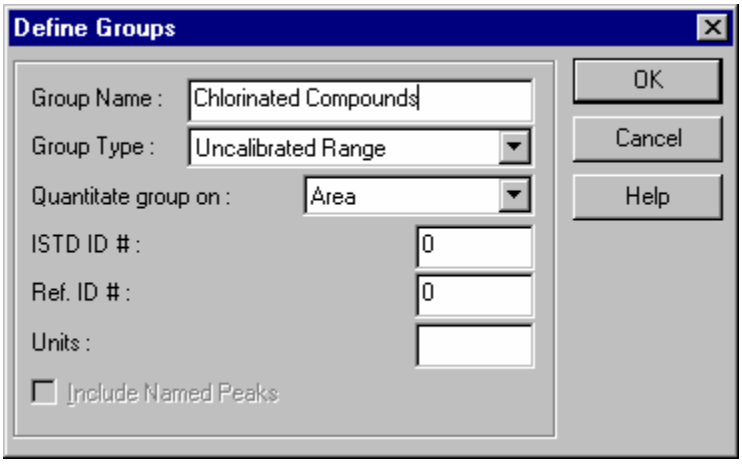


Figure 134. Define Groups dialog box

- Group Name

  Enter a name to be assigned to this group.

- Group Type

  Select the type of group you want to define.

  – Uncalibrated Range

    Select this if you want to define a group comprised of uncalibrated peaks eluting within a designated time range. You must enter a manual response factor (RF) in the Group Table that will be used to determine concentration of the peaks in the group range. Results will be reported as individual uncalibrated peaks that fall within the range, where the concentrations are calculated based on the manual response factor entered for the Uncalibrated Range group.

  – Calibrated Range

    Select this option if you want to create a group that will be calibrated as if it were one peak. See Group Calibration for details.

  – Named Peaks

    Select this option if you want to create a group comprised of named, calibrated peaks from the Peak Table. The group amount will be the sum of the amounts of individually calibrated peaks defined in the group. See Calibrated Peak Grouping for details.

Group Table Properties

To view the Group table, click on the **Peak/Group Tables** button from the command ribbon. A tab box will appear with the Named Peaks tab on top. Click on the **Groups** tab to view the Group Table. Each group region defined will appear as one row in the spreadsheet.

To customize the columns displayed in the Group Table, select the **right-mouse click > Properties** command.

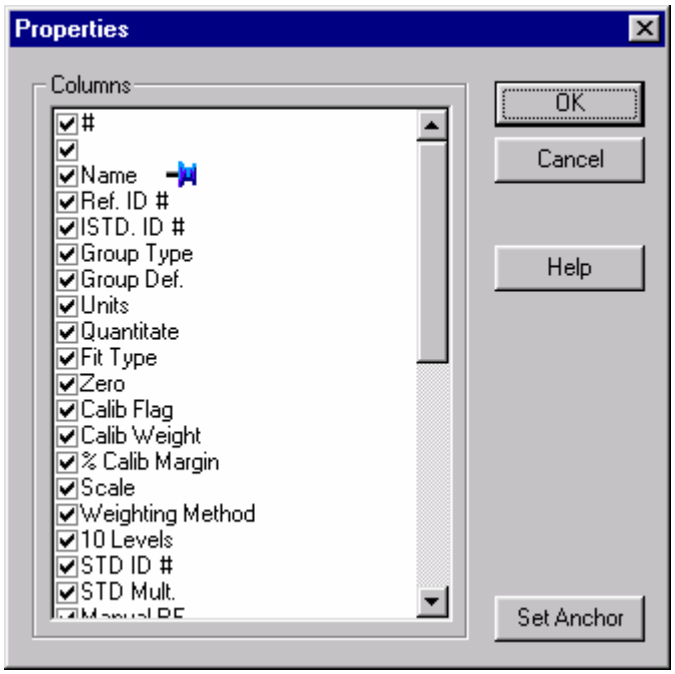


Figure 135. Properties dialog box

Select the columns to be displayed by clicking the adjacent boxes. To change the number of calibration levels displayed, double-click the **Levels** text. A box will appear where you can enter the **Maximum # Levels** to be displayed in the spreadsheet. Type the number of levels to be displayed, then press ENTER to accept.

Set Anchor

The blue "anchor" indicates what column will be used to anchor the right-left scrolling in your sequence spreadsheet. Once this anchor is set, columns to the right of the anchor will scroll to the right and left. Columns to the left of the anchor will not scroll. To change the anchor, click on the name of the column you wish to use as anchor, and click the **Set Anchor** button. The blue anchor will move to the designated anchor column.

Note When using the anchor, it is best to remove all unnecessary columns from the spreadsheet, and make the spreadsheet as wide as possible before you set the anchor. If you set the anchor to a column that does not currently show on the spreadsheet, you will not be able to scroll the spreadsheet.

Uncalibrated Range

The Uncalibrated Range group type is used to enter a response factor for uncalibrated peaks eluting within a specified retention time range. When the chromatogram is analyzed, the concentration of unnamed peaks eluting within the uncalibrated range will be calculated using the response factor entered for the uncalibrated range.

Defining an Uncalibrated Range group is done using a stored chromatogram. Since you are simply defining a range for uncalibrated peaks, it is not necessary for the chromatogram to be analyzed in order to create the group.

1. If you do not currently have the data file open, click on the **Open Files** button. Open the data file that contains the range(s) you wish to include in your group.
2. Click on the **Analyze** button. Make sure all the peaks you wish to include in the group are detected. (Optional)
3. Click on the **Define Group** button, or select the Define Group command from the **right-mouse click > Graphical Programming** menu.

   With this function, you define one or more group windows using the mouse. The regions defined by the mouse become the uncalibrated range(s) for the group. You can define any regions in the chromatogram, whether or not peaks are present.

4. Click the mouse once at a point on the chromatogram where you wish to define the beginning of the group region.
5. Click the mouse again to define the end of the group region.
6. If you want to add additional peak regions to the same group, repeat the above procedure as many times as necessary. When you are finished adding regions to the group, press the ESC key.
7. A dialog box will appear for the group. Select **Uncalibrated Range** for Group type, and enter a name for Group name.

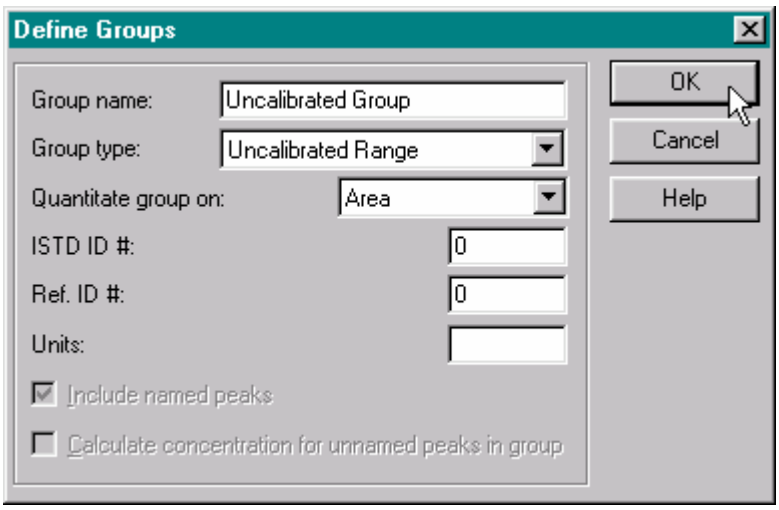


Figure 136. Define Groups dialog box

Because the concentration of the uncalibrated range is calculated from the manual response factor, it is not necessary to enter values for any other field, except **Units** for reporting.

8. When you have completed the dialog box, click on **OK**. A row will be added to your Group Table containing the information you entered.

To view the Group Table, click on the **Peak/Group Tables** button on the command ribbon. Click on the **Groups** tab to view the group information table.

Enter a response factor to be used for the uncalibrated range group in the **Manual RF** column for the group.

- Reference ID #

  If you are using a reference peak, enter its Peak ID number from the Peak Table for the peak to be used as a reference peak. The retention time of the reference peak will be used to adjust the start and end times of the group windows. Note that the reference peak must be identified in the Peak Table and given an expected retention time. The reference peak does not need to have calibration levels assigned to it.

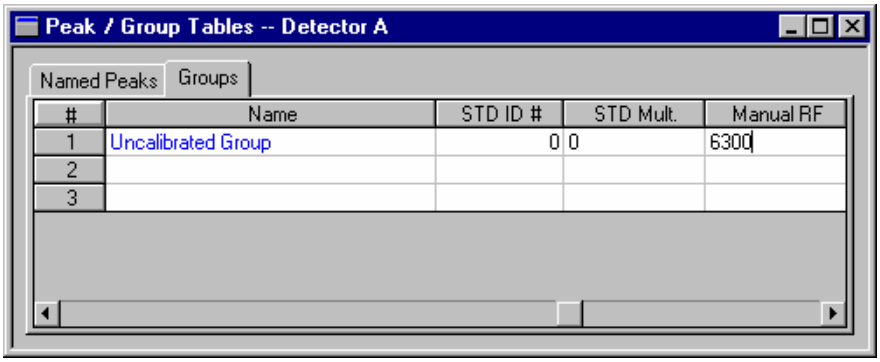


Figure 137. Peak/Group Tables - Groups dialog box

No other parameters are necessary. Close the Peak/Group Tables box. Be sure to save your method.

Group Calibration (Calibrated Range)

Calibrated Range grouping calibrates and analyzes peaks defined in the group as a single peak. A common response factor is calculated for the group, based on one or more calibration runs where concentration level(s) are defined for the group. In addition, you can choose to include calibrated (named) peaks in the group.

In the following example, we do not want to calibrate peaks individually. Instead, we want to combine peaks in certain regions of the chromatogram together and calibrate them in groups, creating a unique response factor for each group. Follow the steps to set up the groups.

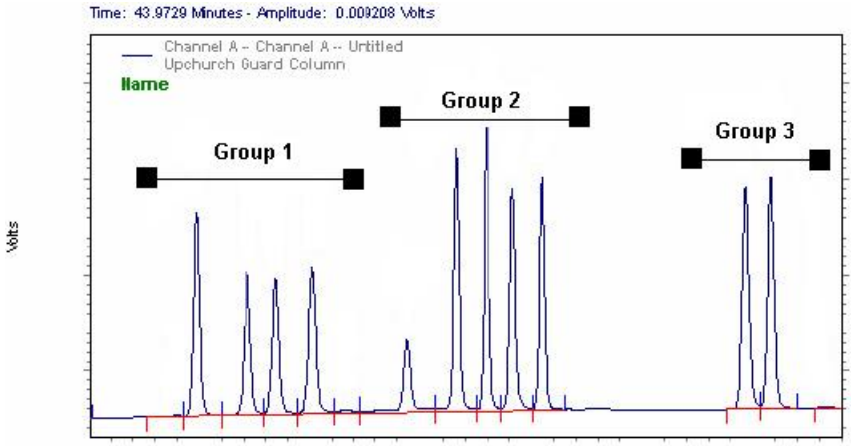


Figure 138. Group calibration

To define a group of peaks graphically using the stored chromatogram

1. If you do not currently have the data file open, click on the **Open Files** button. Open the data file that contains the peaks you wish to include in your group.
2. Click on the **Analyze** button. Make sure all the peaks you wish to include in the group are detected.
3. Click on the **Define Group** button, or select the Define Group command from the **right-mouse click > Graphical Programming** menu.

   With this function, you define one or more group windows using the mouse. All detected peaks within the group window(s) will become part of the group being defined. This allows you to create a group that contains non-contiguous peaks in the chromatogram.

4. Click the mouse once to the left of the first peak to be included in the group. This defines the beginning of a group window.
5. Click the mouse again to the right of the last peak to be included in the group window. This defines the end of the group window.
6. If you want to add additional peak regions to the same group, repeat the above procedure as many times as necessary. When you are finished adding peaks to the group, press the ESC key.
7. A dialog box will appear where you can give the group a name, number, and designate a reference peak and internal standard for the group.

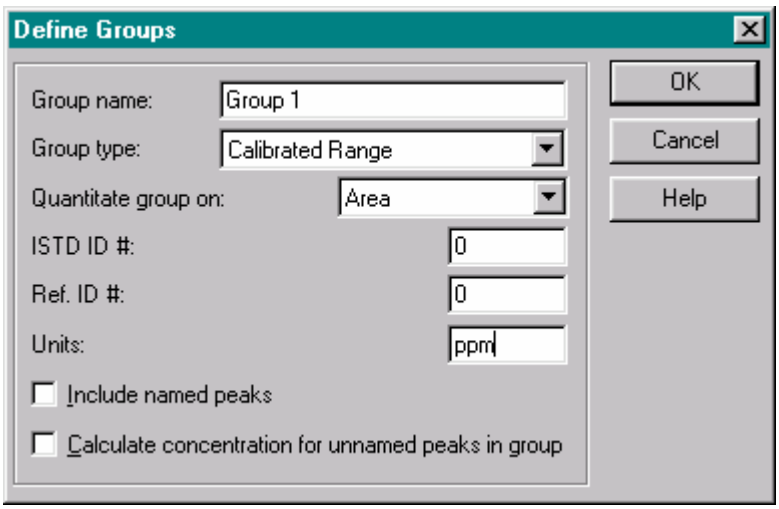


Figure 139. Define Groups dialog box

- Group name

  Enter a name to be assigned to this group.

- Group type

  Select **Calibrated Range** for the type of group you want to define.

- Quantitate group on

  Select whether you want to use Area or Height for calculation of response factors. Note: If you are doing both Peak and Group quantitation using internal standards, both Peaks and Groups must use the same quantitation measurement type (area or height).

- ISTD ID #

  If you wish to use an internal standard method for the group calibration, you must add to your sample a known amount of an internal standard. The internal standard must be entered in your Peak Table, and must have a calibration amount assigned to it. Enter the Peak ID number of the internal standard from your Peak Table in this field.

- Reference ID #

  If you are using a reference peak, enter its Peak ID number from the Peak Table for the peak to be used as a reference peak. The retention time of the reference peak will be used to adjust the start and end times of the group windows. Note that the reference peak must be identified in the Peak Table and given an expected retention time. The reference peak does not need to have calibration levels assigned to it.

- Units

  Enter the units to be used for reporting group concentration results.

- Include named peaks

  Click this box if you want to include any individually calibrated peaks (named peaks) in this calibrated group. The areas of the named peaks will be used in calculation of the group response factor.

- Calculate concentration for unnamed peaks in group

  Selecting the **Calculate concentration for unnamed peaks in group** box will cause the concentrations of the unnamed (uncalibrated) peaks in the group to be calculated using the group response factor and listed in the peak report. The peak report will include the unnamed peak concentrations, but will not report the group concentration as a "peak". The group concentration will be reported in a separate "group report" section.

Note When this box is checked, the total group area and height are not reported as part of the peak table, so the Area% and Height% columns in the peak report will add up to 100%. If Groups are reported in the run report, a separate report section will be generated that only includes groups. This will be the only place where the total group concentration using this option can be viewed. If this box is not selected, the group will be reported as part of the peak table, and the Area%, Height%, concentrations may add up to more than 100%.

For examples of reports using various Calibrated Range options, see the examples in "Group Calibration (Calibrated Range)" on page 159.

8. When you have completed the dialog box, click on **OK**. A row will be added to your Group Table containing the information you entered.

   After the group has been defined, you must enter calibration levels into the group table and then run the calibration sample(s) to determine the group response factors. See Group Table and Group Range Definition sections for information on completing the group calibration setup.

   After you have completed your group setup and calibration, the peaks in the defined groups will appear on your chromatogram if you have the "peaks" annotation on.

Group Table

To view the Group Table, click on the **Peak/Group Tables** button on the command ribbon. Click on the **Groups** tab to view the group information table.

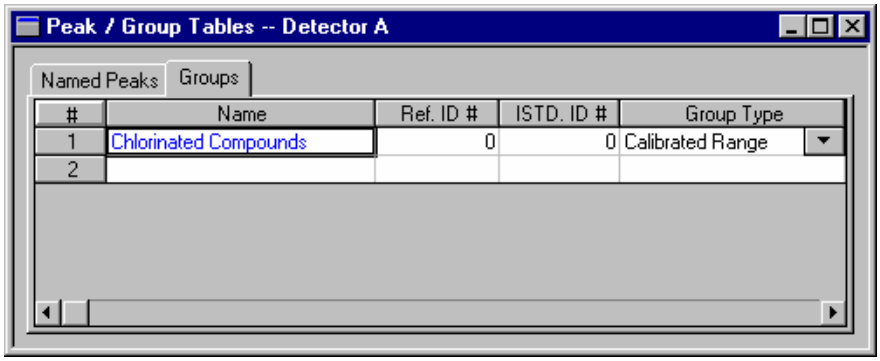


Figure 140. Peak/Group Tables window

The **Group Type** should indicate **Calibrated Range**. If you click the **Group Def** field, you will see the retention time range for the region(s) you selected using the mouse to define the group.

In order to calibrate the group(s), you need to enter calibration amount(s) for each group.

In the columns of the group table labeled **Level 1**, etc., enter the calibration amount to be used in calculation of the calibration curve for that group. Also, enter the concentration units for reporting group results in the column labeled **Units**.

In order to properly do group calibration, you need to designate the following parameters for each group to be calibrated:

- Fit Type

  This option determines whether the calibration plot is to be based on a point-to-point, linear, or quadratic, cubic, or Average RF fit to the data.

  Note You have five choices for how the calibration curves will be drawn: point-to-point, linear, and quadratic, cubic, and Average RF fits. You must have at least two points for a linear fit (not including zero), three points for a quadratic and four points for a cubic fit.

- Force Zero

  When this box is selected, the calibration curve will be forced through zero.

- Calib Flag

  The **Replace/WtAverage** selection allows you to determine how the calibration areas/heights for each group will be affected by running a standard: they will either be replaced, or averaged with the current replicate areas in the calibration method. If Calib Weight is set to a value other than 100, a weighted average of the areas/heights will be calculated. For details on calibration averaging, see "Replicates and Averaging Calibrations" on page 119.

  Each time a calibration average is performed, the value is saved in the method as "Last Area", which is subsequently used for calculation of weighted averages.

- Calib Weight

  You can designate a "weight" for the average of the replicates. The weighting factor is applied to replicate injections as shown in the following example.

Current run value (area/height)	101
Replicate 1	104
Replicate 2	100
Replicate 3	102
Current Last Area Value =	102

  Using a weighting factor of 60, the new method average is:

  [((104 + 100 + 102) / 3) * .6] + [101 * .4] = 101.6

  Note For Internal Standard calibrations, each Replicate represents a ratio of the component area/height to internal standard area/height.

- % Calib Margin

  This selection creates an acceptance margin based on the % difference between the group area or height found during calibration and the current group area or height in the method. If the % difference between these values exceeds this limit, the group will not be used to update the calibration.

- Scale

  This parameter allows you to apply a scaling factor to the calibration curve. This factor is applied to the entered amounts prior to computing the calibration curve. The purpose of using a scaling factor is to create a relationship between areas (or heights) and amounts that can be approximated by a polynomial fit. A scaling factor can be applied to any fit type. The available scaling operations are:

  None

  1/X

  $1/X^2$

  ln[X]

  1/ln[X]

  sqrt[X]

  x^2

- Weighting Method

  Select a Weighting Method to be used for calculation of least squares regression fits, 1/Response, 1/Response2, 1/Amount, 1/Amount2 or none. Weighting gives increased importance to smaller concentrations and areas. A scaling factor can be applied to linear, quadratic, and cubic fits.

- Level 1… x

  You must designate one or more calibration levels to be used in calculation of a group response factor. For each Level, enter the amount of the group in that calibration sample.

  Note Occasionally, you may want to calibrate one group with fewer levels than the other groups in your group table. (For example, when you have more than one calibration mixture for the same concentration level.) To avoid introducing error in your calibration curve, you can cause the group area to be ignored at that level by leaving the "Level" of interest for that group blank. To insert a blank, double-click on the cell and press the Delete key.

If you are performing QC checks, you should fill out the appropriate columns for your groups. See "Groups and Group Calibration" on page 154 for details on these columns.

Note As with the Peak Table, the Group Table can be "customized" to include only those parameter columns that you need for your group calibration. To select the group parameters, click on the upper left corner of the group spreadsheet, and select "Properties" from the drop-down menu. From the dialog box, click on those parameters you wish to include in the spreadsheet.

Be sure to save your method. To complete the group calibration, you must run a calibration standard at each level for which you have entered an amount. This can be done manually using stored data files, or as a part of a sequence. For details on how to calibrate, see "Calibration Setup" on page 117 and "Groups and Group Calibration" on page 154.

Group Range Definition

If you want to review or change the chromatogram time range(s) currently selected for the group, click on the **Group Def** field. A dialog appears with the currently defined range(s) for the group. You can manually change the range(s) if desired.

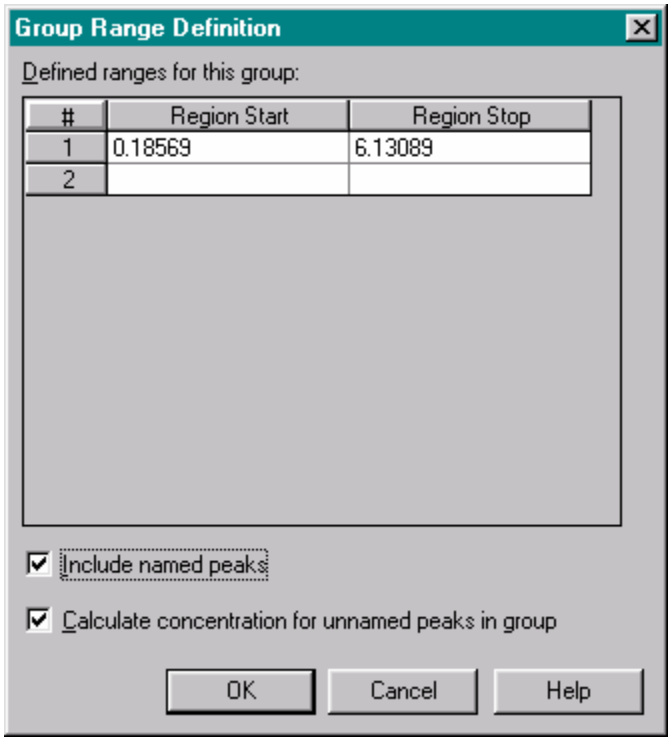


Figure 141. Group Range Definition dialog box

Click on the **Include Named Peaks** option if you want to include in the group any named (calibrated) peaks that fall within the range. If this is not selected, the named peaks eluting within this region will not be considered part of the group.

Selecting the **Calculate concentrations for unnamed peaks** option will cause the concentrations of the unnamed (uncalibrated) peaks in the group to be calculated using the group response factor and listed in the peak report. The peak report will include the unnamed peak concentrations, but will not report the group concentration as a "peak". The group concentration will be reported in a separate "group report" section.

Note When this box is checked, the total group area and height are not reported as part of the peak table, so the Area% and Height% columns in the peak report will add up to 100%. If Groups are reported in the run report, a separate report section will be generated that only includes groups. This will be the only place where the total group concentration using this option can be viewed. If this box is not selected, the group will be reported as part of the peak table, and the Area%, Height%, concentrations may add up to more than 100%.

For examples of reports using various Calibrated Range options, see the examples in Group Calibration (Calibrated Range).

- Calibrated Range Examples

  The following example reports were generated using the peak options for calibrated range groups, using the simple chromatogram shown below. In this example, there are four named (calibrated) peaks **Peak 1…Peak 4,** and one calibrated range group defined, **Group 1**, where Peak 2 falls within the time range defined for the group. In this situation, there are various options on how to treat the named peak as part of the group.

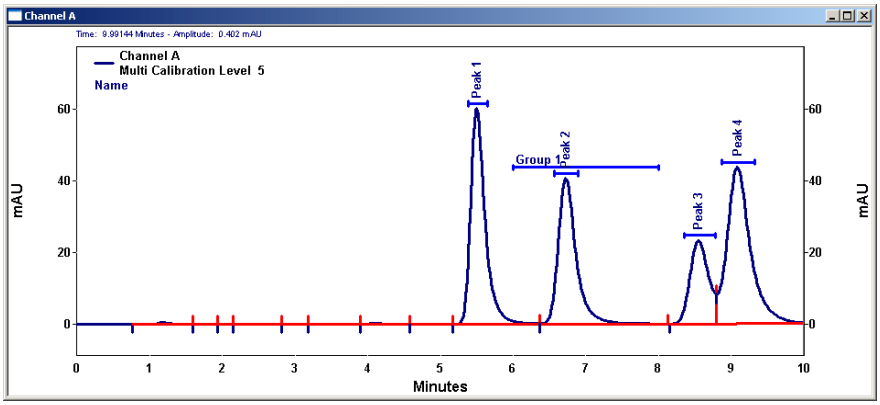


Figure 142. Calibration Range window

The various reports are generated based on the options selected in the **Group Range Definition** dialog. This appears when you define the group graphically, or when you click the group def field in the Group Table.

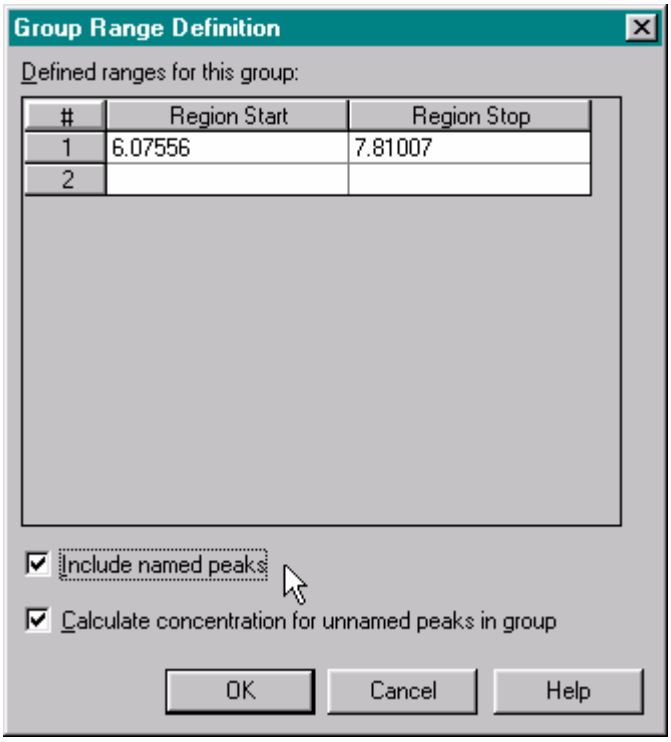


Figure 143. Group Range Definition dialog box

– Example 1

  In this example, the concentration of Peak 2 is calculated using its own response factor and its area is used in the calculation of the group response factor. (**Include named peaks** option is **On**.) The concentrations of unnamed peaks in the group range are calculated using the group response factor. The group concentration is calculated by summing the concentrations of the peaks defined for the group. The total group is not included in the individual peak report, but is listed separately in the "group report" section. Because the group total is not included as a separate line in the peak table, the Area% and Norm concentrations add up to 100%. (**Calculate concentration for unnamed peaks** option is **On**.)

– Example 2

  In this example, the concentration of Peak 2 is calculated using its own response factor and its area is used in the calculation of the group response factor. (**Include named peaks** option is **On**.) The concentrations of unnamed peaks in the group range are not calculated and reported individually. The group concentration is calculated using the total area of all defined group peaks and the group response factor. The total group concentration is included in the individual peak report. Because the group

total is included as a separate line in the peak table, the Area% and Norm concentrations do not add up to 100%. (**Calculate concentration for unnamed peaks** option is **Off**.)

Name	Retention Time	Area	Area Percent	ESTD Conc	NORM Conc	Response Factor
Peak1	5.719	462656	25.363	40.000	5.810	10405.54
Peak 2 (Group1)	6.558	394564	21.630	8.000	1.162	38653.03
Group 1	6.932	1750	0.096	0.000	0.000	705.93
Group 1	7.435	6360	0.349	0.000	0.000	705.93
Peak3	8.264	375582	20.589	30.000	4.358	10020.50
Peak4	8.530	583249	31.974	40.000	5.810	13135.42
Group 1		402674	22.074	570.414	82.859	705.93
Totals		2226835	122.074	688.414	100.000	

Figure 144. Group Range Definition report

– Example 3

In this example, the concentration of Peak 2 is calculated and reported using its own response factor. Its area is NOT used in the calculation of the group response factor. (**Include named peaks** option is **Off**.) The concentrations of unnamed peaks in the group range are calculated using the group response factor. The group concentration is calculated by summing the concentrations of the peaks defined for the group. The total group is not included in the individual peak report, but is listed separately in the "group report" section. Because the group total is not included as a separate line in the peak table, the Area% and Norm concentrations add up to 100%. (**Calculate concentration for unnamed peaks** option is **On**.)

Name	Retention Time	Area	Area Percent	ESTD Conc	NORM Conc	Response Factor
Peak1	5.719	462656	25.363	40.000	2.649	10405.54
Peak 2 (Group1)	6.558	394564	21.630	8.000	0.530	38653.03
Group 1	6.932	1750	0.096	300.343	19.892	5.83
Group 1	7.435	6360	0.349	1091.533	72.293	5.83
Peak3	8.264	375582	20.589	30.000	1.987	10020.50
Peak4	8.530	583249	31.974	40.000	2.649	13135.42
Totals		1824161	100.000	1509.876	100.000	
Group 1		8110	0.445	1391.876	92.185	5.83
Totals		8110	0.445	1391.876	92.185	

Figure 145. Group Range Definition report

– Example 4

In this example, the concentration of Peak 2 is calculated and reported using its own response factor. Its area is NOT used in the calculation of the group response factor. (**Include named peaks** option is **Off**.) The concentrations of unnamed peaks in the group range are not calculated and reported individually. The group concentration is calculated using the total area of all defined group peaks and the group response factor. The total group concentration is included in the individual peak report. Because the group total is included as a separate line in the peak table, the Area% and Norm concentrations do not add up to 100%. (**Calculate concentration for unnamed peaks** option is **Off**.)

Name	Retention Time	Area	Area Percent	ESTD Conc	NORM Conc	Response Factor
Peak1	5.719	462656	25.363	40.000	2.649	10405.54
Peak 2 (Group1)	6.558	394564	21.630	8.000	0.530	38653.03
Group 1	6.932	1750	0.096	0.000	0.000	5.83
Group 1	7.435	6360	0.349	0.000	0.000	5.83
Peak3	8.264	375582	20.589	30.000	1.987	10020.50
Peak4	8.530	583249	31.974	40.000	2.649	13135.42
Group 1		8110	0.445	1391.876	92.185	5.83
Totals		1832271	100.445	1509.876	100.000	

Figure 146. Group Range Definition report

Calibrated Peak Grouping (Named Peaks)

This technique assumes you have individually calibrated peaks in your chromatogram. You define the groups by designating in the Group Table the named peaks to be included in each group, and giving each group a name.

Note In order to define a peak group graphically, 1) make sure the current chromatogram is the chromatogram you wish to use, 2) you have already created a calibrated peak table in your method, and 3) you have analyzed the chromatogram.

1. If you have not already done so, open the data file containing the calibrated peaks you wish to include in your group. Click on the **Analyze** button to integrate the chromatogram and make sure the peaks are detected.
2. Define the peaks belonging to the group by graphically "defining" the group using a stored chromatogram. To do this, click on the **Define Group** button on the toolbar, or select the Define Group command from the **right-mouse click > graphical programming menu**. Click the mouse to the left of the first peak to be included in the group, then click the mouse again to the right of the last peak to be included in the group (if they are contiguous peaks).

You may continue to add peaks to the group by clicking the mouse to define peak regions. When you are done adding peaks to the group, press the ESC key on your keyboard. The following dialog box will appear:

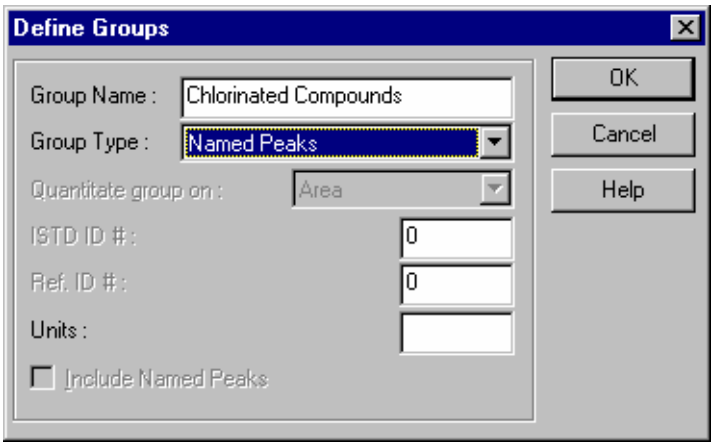


Figure 147. Define Groups dialog box

3. Type a name to be used for the group in the **Group Name** field.
4. Select Named Peaks for the Group Type.

   Click **OK** to accept and exit the dialog.

5. Click on the **Peak/Group Tables** button from the command ribbon. Click on the **Groups** tab to view the group table.

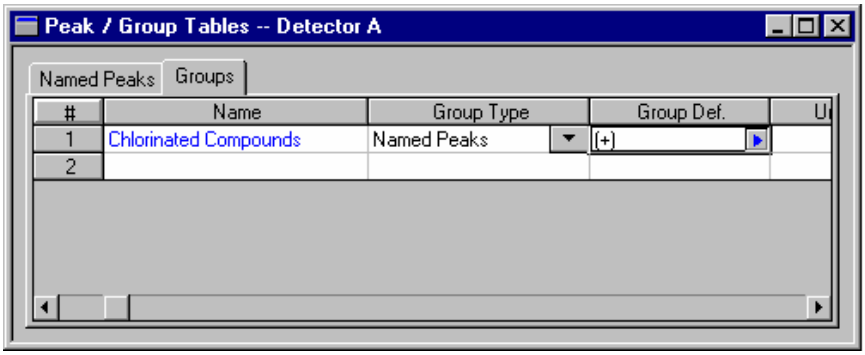


Figure 148. Peak/Group Tables dialog box

The **Group Type** field should show **Named Peaks**. Click on the **Group Def** arrow to review the calibrated peaks selected for this group. See Peak Group Definition for details on defining the named peaks for this group.

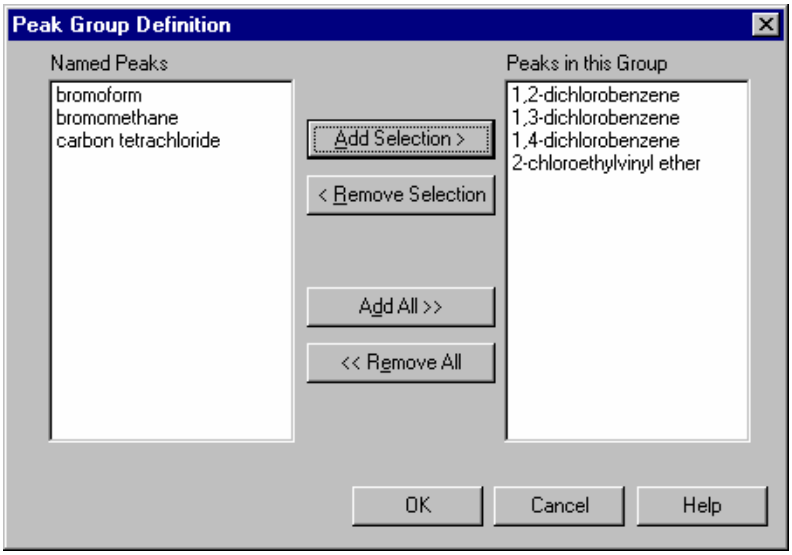


Figure 149. Peak Group Definition dialog box

6. Review the list of peaks on the right. If there is a peak that you do not want in the group, click on it with the mouse, then click on **Remove Selection**.

   To add a peak to the group, select it from the **Named Peaks** list, then click on **Add Selection**. You can quickly add or remove all of the peaks using the **Add All** or **Remove All** buttons.

   When you are finished, click on **OK**. The peaks in the box labeled **Peaks in this Group** will become the defined peaks for this group, and the Group Table will appear once more.

7. If you are using a calibrated peak group, you should not enter any **Level** information in the table, as the group total will be calculated using the calibrated amounts for the individual peaks in the group. To leave the group table, click on the upper left corner of the Group Table spreadsheet.

Once your peak group has been defined, it can be reported by selecting the **Report groups** option in the report properties.

Reporting Group Amounts

Group amounts can be reported in any report by selecting the Groups option in the Run Report box when adding a run report to your custom report. Or, you can do a right-mouse click inside an existing run report table in your report, then select the **Report Properties** command, and select the **Groups** box. To report peaks that are part of an Uncalibrated Range group, make sure the **Unnamed peaks** box is selected.

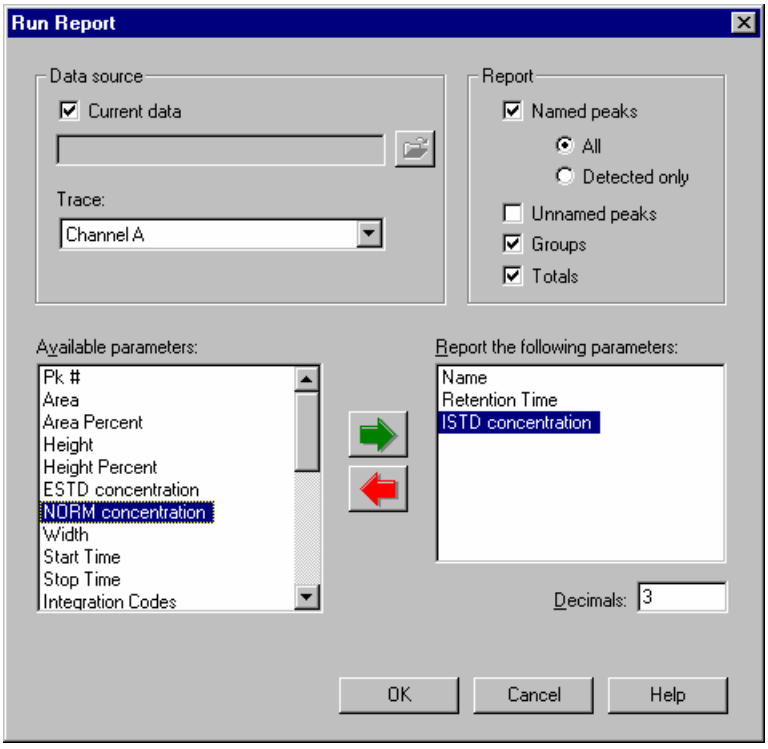


Figure 150. Run Report dialog box

Advanced Method Options

Advanced Method Options are available for users who have a need to go beyond the simple data acquisition and analysis provided by ChromQuest by customizing results or linking to external data systems or networks. When you select the **Method > Advanced** command from the menu, a tab box appears where you can set up various advanced options for your method.

Data Export

Select the **Export** tab to set parameters for exporting data to separate files on your disk. Click the **Export Enabled** box if you want the method to include data export.

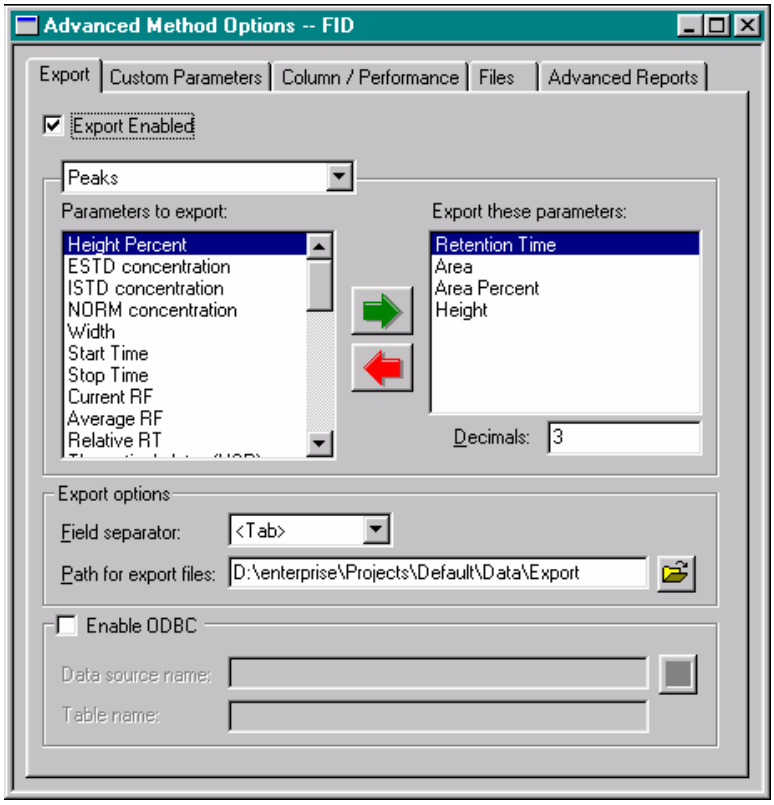


Figure 151. Advanced Method Options - Export dialog box

From the drop-down list, select the type of information to be exported. Choices include **Peaks** for export of peak information, and **Groups** for export of group information.

Note Microsoft Data Access Components (MDAC) 2.5 or higher is required for ODBC export. A version of this is on the ChromQuest CD-ROM under \Updates.

- Parameters to export/Export these parameters

  From the list of available export information, move the items you want to export into the box on the right side of the screen by first clicking the item to highlight it, then click the **Green** arrow key to move it to the right. You can also move several items at once by clicking the mouse and dragging it over the items to highlight them, then clicking the Green arrow key. Multiple items can also be selected by clicking the mouse on them one at a time while holding down the CTRL key. To remove an item from the list, select the item or items in the right box and click the **Red** arrow to remove it from the export list.

  If the item is numeric, you can designate the number of Decimal places to be displayed.

- Export options

  – Select a **Field separator** from the list. This will designate what separates each piece of information to be exported.

  – Enter a **Path for export files**, or select one from the available paths by clicking on the File button. Export files created will be stored in this folder (directory) on your disk.

- Enable ODBC

  Select this box if you want to utilize Open DataBase Connectivity for data export. When this is selected, you must then select or enter the name of your data source, or path name for the location of your database. Also, enter a name for the data table. If the table does not currently exist in your database, a new table will be created. The export parameters selected will become the field names in the table. If you are exporting to an existing table, the export parameters must all exist in the table, otherwise the export will fail. You must create a new table for ODBC export if you change the list of export parameters.

  Note Microsoft Data Access Components (MDAC) 2.5 or higher is required for ODBC export. A version of this is on the ChromQuest CD-ROM under \Updates.

Once this tab dialog is completed, it becomes part of the method. If the **Export Enabled** box is selected, the designated data will be exported every time an analysis is performed using this method.

Custom Parameters

A custom parameter is a user-defined calculation whose result is calculated by a Windows executable program. Custom parameters can be reported in a custom report, or exported as a result. The user supplies custom parameter programs. To set up custom parameters for the method, click on the **Custom Parameters** tab of the Advanced Method Options tab dialog box.

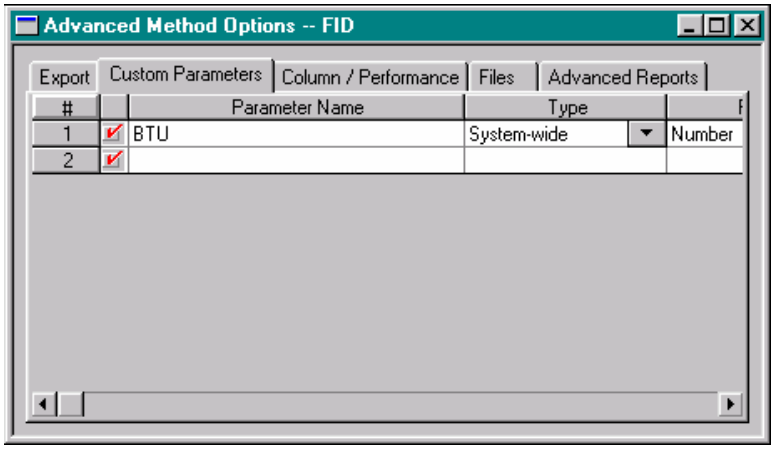


Figure 152. Advanced Method Options - Custom Parameters dialog box

1. To define a Custom Parameter, enter the title you want to use to report the result in the **Parameter Name** field.

2. Next, move the cursor to the **Type** field and double-click your mouse. You will be given a choice of **System-wide** or **Per-peak**.

   A **System-wide** parameter is one that is calculated once for the whole chromatogram. System-wide parameters can be displayed in a custom report, and will be printed at the bottom of the report. An example of a System-wide parameter would be a BTU calculation, where the program multiplies each peak by its BTU content, then sums the calculated BTU values providing a single value equal to the BTU content of the entire mixture.

   A **Per-peak** parameter is one that is calculated once for each peak in the chromatogram. Per-peak parameters can be displayed in a custom report column similar to the way the peak number or concentration would be displayed. Per-peak parameters can also be annotated on the chromatogram. An example of a Per-peak parameter would be HETP (height equivalent to a theoretical plate). For HETP, the Custom Parameter program would take the length of the column (in centimeters) and divide it by "n", the theoretical plates for the component that is already calculated by the system.

3. Select **Number** or **String** from the **Returns** drop-down list to select what type of parameter is returned by the parameter calculation.

4. Select the custom parameter file from the **Source File** dialog box. This program **must** be a Windows .DLL that satisfies all the requirements for a custom parameter program given in the User Program appendix of this manual.
5. Use the **Additional Parameters** field as necessary for your custom parameter application.

Once you have a custom parameter defined in your method, ChromQuest will attempt to run the Custom Parameter Program(s) designated whenever you analyze your chromatogram.

Column/Performance Parameters

The Column/Performance tab allows you to set up automatic calculation of system performance values such as Theoretical Plates, Capacity Factor, Resolution, or Peak Asymmetry.

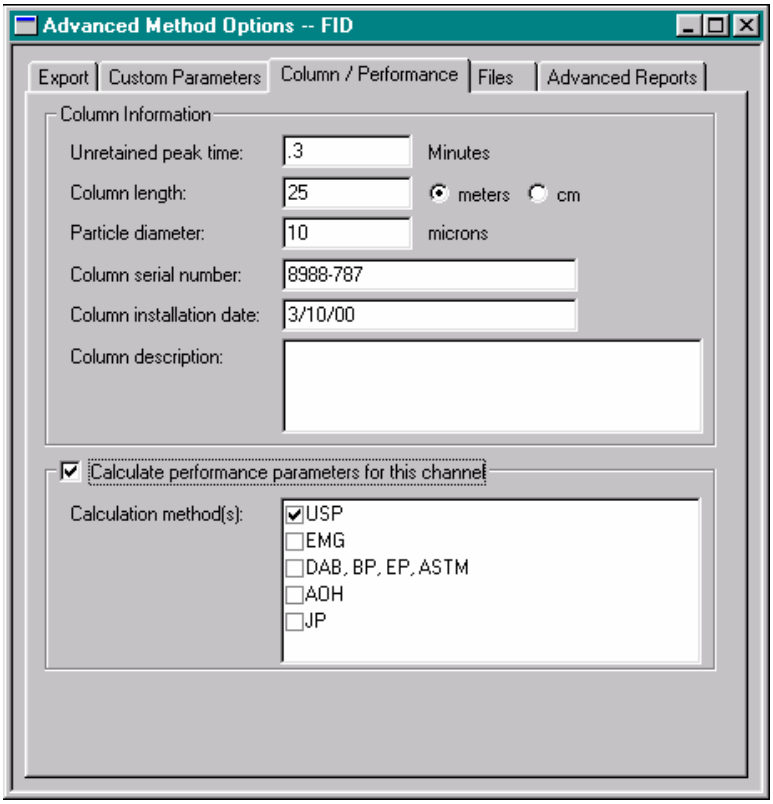


Figure 153. Advanced Method Options - Column Performance dialog box

Click the **Calculate performance parameters for this channel** box. Then fill in the information for the given fields required for the calculations.

- Column Information
  - Unretained peak time (min)

    Enter the retention time of an unretained peak for this column.

  - Column length (meters)

    Enter the length of the column, in meters.

  - Particle diameter (microns)

    Enter the particle diameter, in microns, for the column you are using (if applicable).

  - Column serial number

    Enter the serial number for the column (if applicable). This information will be saved with the data and available for reporting.

  - Column installation date

    Enter the date the column was installed in the instrument (if desired). This information will be saved with the data and available for reporting.

  - Column description

    Enter column description if desired. This information will be saved with the data and available for reporting.

- Calculate performance parameters for this channel

  Select one or more calculation methods. The choices include USP, EMG (Exponential Modified Gaussian), DAB (German Pharmacopeia), AOH (Area/Height), JP (Japanese Pharmacopeia), BP (British Pharmacopeia), EP (European Pharmacopeia), and ASTM calculation methods.

Once you have completed the Column/Performance tab, the system will calculate performance parameters after each analysis. These parameters can be exported, annotated on the chromatogram, and printed in a custom report.

Relative Retention Times

Relative retention times are calculated for a) named peaks with reference peaks and b) detected unnamed peaks that are members of uncalibrated or calibrated range groups if a reference peak is specified. The reference peak specified in the group is used to calculate the RRT for the unnamed peaks using the same equation used for named peaks.

$$\alpha = \frac{t_2 - t_a}{t_1 - t_a}$$

where

α = Relative retention.

t_2 = the retention time measured from point of injection

t_a = the retention time of an inert component not retained by the column, taken from "Unretained Peak Time" in the Performance Options section of the method.

t_1 = The retention time from point of injection for reference peak defined in the peak table. If no reference peak is found, this value becomes zero.

Files

The **Files** tab allows you to designate program files to associate with the method.

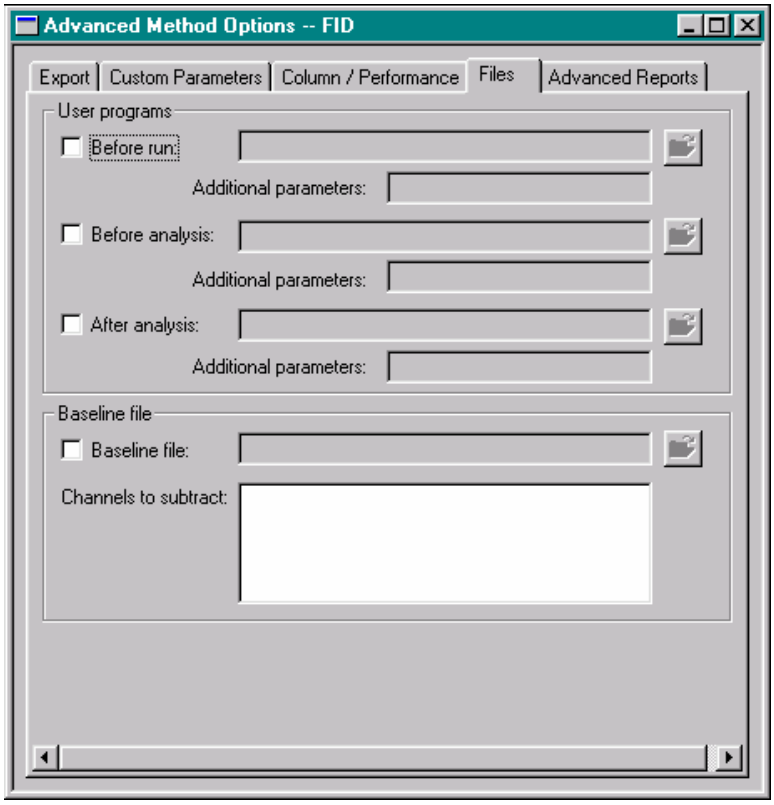


Figure 154. Advanced Method Options - Files dialog box

- User programs

  In this section, you can designate user programs to be run **Before** each run, **Before Analysis** of the chromatogram, or **After Analysis** of the chromatogram. Enter the user program name, or select it from a list by clicking the File button. For details on writing User Programs, see the User Programs appendix at the end of this manual.

- Baseline file

  If you want ChromQuest to automatically subtract a stored baseline file after each run, select the **Baseline File** box. Designate a baseline file name, and then check each channel from which the baseline file is to be subtracted. The chromatogram will be analyzed after the subtraction is performed.

Advanced Method Reports

This tab is used to specify one or more reports to be printed and/or exported when the method is used to analyze data.

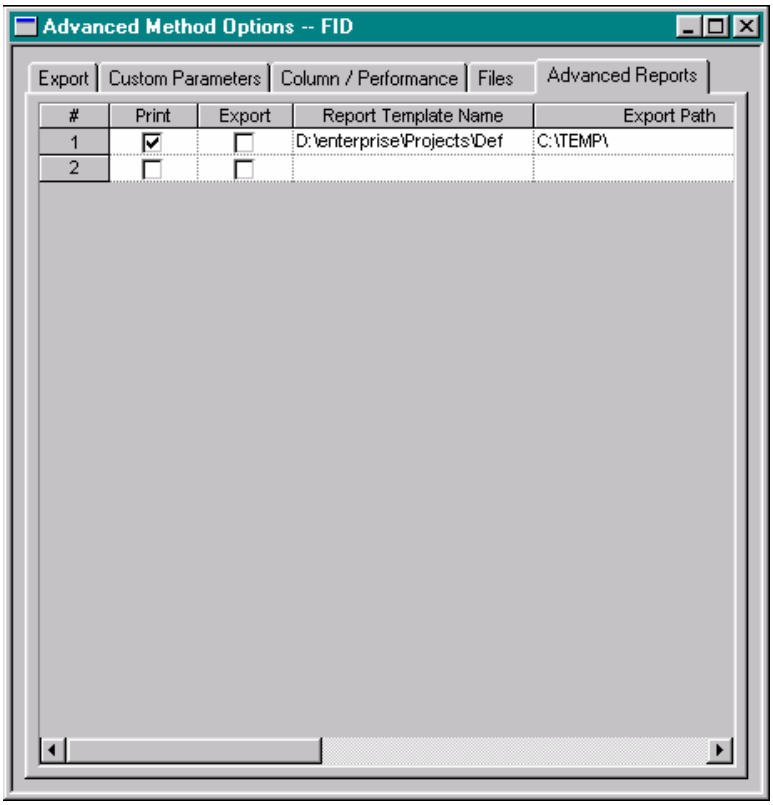


Figure 155. Advanced Method Options - Advanced Reports dialog box

- Print

  Select this box if you want to print the designated report when data is analyzed with this method.

- Export

  Select this box if you want to export the designated report when data is analyzed with this method. This results in tab-delimited export only.

- Report Template Name

  Enter or select the name of the report template file to be used to generate this report.

- Export Path

  If the report is to be exported, enter or select the path for the export file to be saved.

- Export File Name

  Select the file naming from the types presented in the list. The default file extension is .txt. The export file name will be created using the identification selected, or you can type in your own file name.

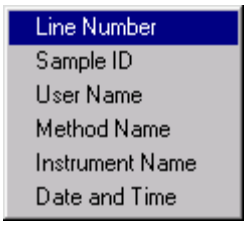


Figure 156. Line Number menu

Saving a Default Method

ChromQuest loads a set of default values whenever you create a new method. You can create a default method file for each instrument to include parameters that are frequently used in your laboratory. To save a default method, choose **Method > Save as Default**. The default method will be called **Instrument xxx Default Method.met** (where x = instrument ID). For example, the default method for Instrument 1005 would be **Instrument 1005 Default Method.met**. To view an instrument's ID number, do a right-mouse click on the instrument icon in the Main screen, and choose **Properties**. The assigned instrument ID is displayed. The default method files are stored in the project method directory, so you can have different default methods for each project.

A non instrument-specific default method can also be created and saved in the ChromQuest program directory that will be loaded whenever an instrument-specific default method cannot be found. If you want to create such a default method, name it Default.met, and save it in the ChromQuest program directory.

Creating Multi-Detector Methods

With ChromQuest, you can create a single method that will acquire and process data independently from multiple detectors simultaneously.

You need to complete the following sections of the method for each detector in your instrument configuration:

- Instrument Setup parameters
- Peak Table (select the appropriate detector from the Analysis Channel list)
- Advanced > Column/Performance page (select the appropriate detector from the Analysis Channel list)

For each channel in a method, you can set independent data acquisition, integration, reporting, calibration, and advanced options.

To create a multi-channel method, you must have the instrument configured for multiple detectors (channels).

If your instrument is configured for more than one detector, you can select the current channel by using the drop-down list on the menu bar. Once you select an Analysis Channel, the method parameters you select will apply to that channel, and will be saved separately in both the method file and in all data files acquired using this method.

Creating Multi-Wavelength Methods

You can collect data on more than one Analysis Channel from a single detector if your instrument contains one of the following detectors:

- SpectraSYSTEM UV2000 dual-wavelength detector
- SpectraSYSTEM UV3000 triple-wavelength and scanning detector
- SpectraSYSTEM UV6000 photo-diode array detector
- Surveyor UV/Vis dual-wavelength detector
- Surveyor PDA detector
- Surveyor PDA Plus detector

There is one Peak Table and one Column/Performance dialog box per detector. This means that you enter your peak identification and quantitation parameters in **one** Peak Table, and you enter your column performance parameters for system suitability testing in **one** Column/Performance dialog box. If you want to quantitate a peak on a second Analysis Channel, you must enter the peak information in a second row of the Peak Table, and then select the appropriate Analysis Channel in the Analysis Channel column.

There is one Integration Events table for each discrete wavelength and multi-chromatogram wavelength listed in the Instrument Setup and the Multi-Chromatogram sections of the method.

To edit an Integration Events Table, select the appropriate channel from the Analysis Channel list box. Then, click on the Integration Events Table button. Before you edit the table, size it (click the Restore Down button) such that you can see its title bar. The Wavelength field in the title bar of the Integration Events Table dialog box indicates which table you have open.

When you set up your custom reports, you can add a Data Graph and a Run Report for each trace that you want to review. You can also create custom advanced reports that allow you to report data from multiple analysis channels.

Offline Instruments

Starting and Instrument **Offline** allows you to create and edit methods and sequences, and do post-run data analysis, while data is being acquired on the instrument. It is also a convenient way to perform these operations or view real-time data acquisition from a network PC that may not be in the laboratory. All functions described in Method Development, Sequence Operations, and Custom Reporting are available when you are running an instrument offline. The only commands not available in Offline Processing are the Control commands that directly affect start and stop of data acquisition.

Starting Offline Processing

To open an instrument **offline**, click on the instrument icon from the ChromQuest Main Menu, and then select the **File > Open Offline** command. Alternatively, click on the instrument icon, then click the right mouse button, and select the **Open Offline** command.

Offline Instruments

Using an instrument offline is exactly the same as using one of the Instrument windows "online" with respect to method development, sequence development, and reprocessing of data. The Offline Processing window looks just like the Instrument window, except the prompt "Offline" appears at the top of the window, and control functions that relate to data acquisition are not available (appear grayed-out).

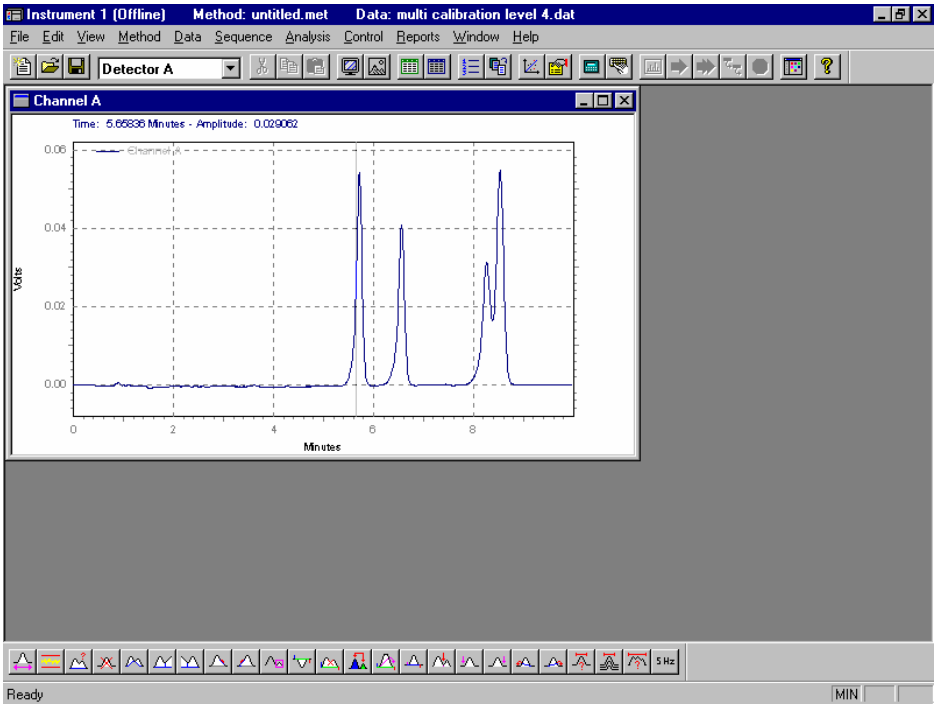


Figure 157. Offline Instrument window.

Once you have created and saved a method in an offline instrument, it can be used to acquire data by opening the method file from the Instrument window and proceeding as usual.

Monitoring an Instrument Offline

While you are in the offline instrument window, you can monitor the real-time data on the instrument using the **Control > Monitor Offline** command. Once you have selected this command, you will be able to view the real-time data in your offline instrument window as it is being acquired. You do not have access to start or stop of the data acquisition.

Pretreatment Files

An autosampler pretreatment (injector program) is comprised of a series of numbered lines, each of which defines an operation that the injector carries out sequentially. If your autosampler supports a pretreatment program, the **Pretreatment** command will appear on the menu bar. From this menu, you can create/edit an autosampler pretreatment program, and set up properties such as a description and audit trail for the pretreatment program.

Opening a Pretreatment File

To open an existing pretreatment file, from the Instrument Window menu bar, select **File > Pretreatment > Open**. The following dialog will appear where you can select a pretreatment file to open for the current instrument.

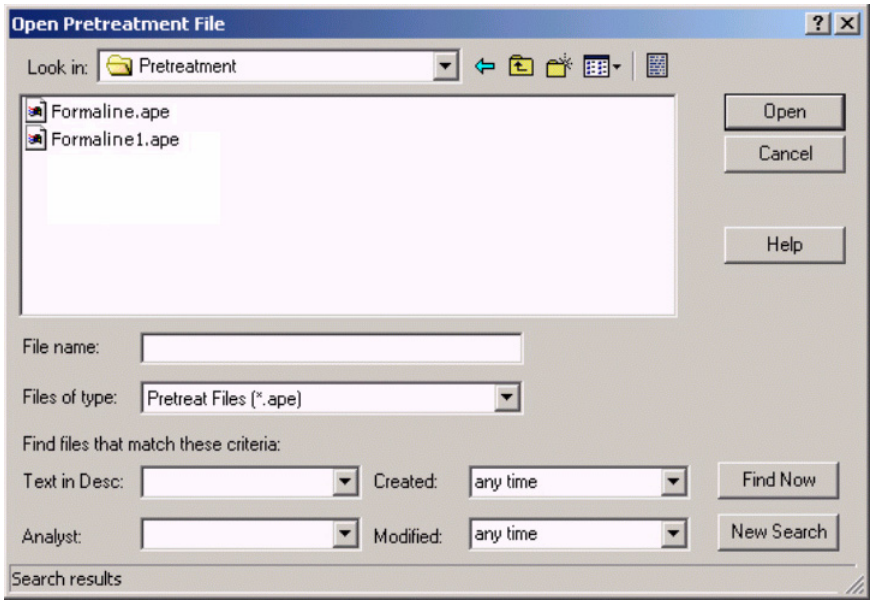


Figure 158. Open Pretreatment File dialog box

Select the file to open, or use the search boxes at the bottom to search for a file that matches criteria you select.

Saving a Pretreatment File

To save a pretreatment program in a file that can be used later, select **File > Pretreatment > Save As** from the Instrument Window menu bar.

View Pretreatment Audit Trail

If you have the pretreatment audit trail function enabled, you can view the pretreatment audit trail using the **File > Pretreatment > Audit Trail** command from the Instrument Window menu bar. The following audit trail dialog will appear where you can view the audit trail history for your pretreatment file.

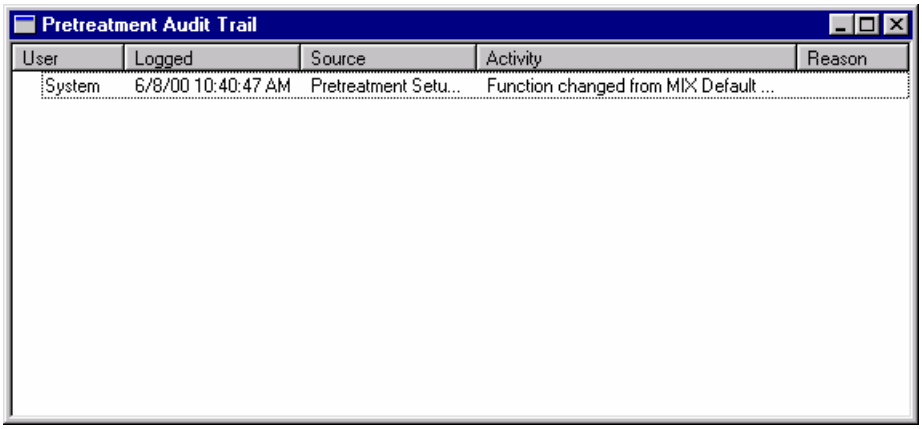


Figure 159. Pretreatment Audit Trail dialog box

A right-mouse click in the audit trail box gives you access to print functions. The **Show Detail** command will give more detailed information about the currently selected entry.

Pretreatment Properties Description

This is used to enter a text description of the pretreatment file. The description is saved in the file and can be viewed when opening or used for searching.

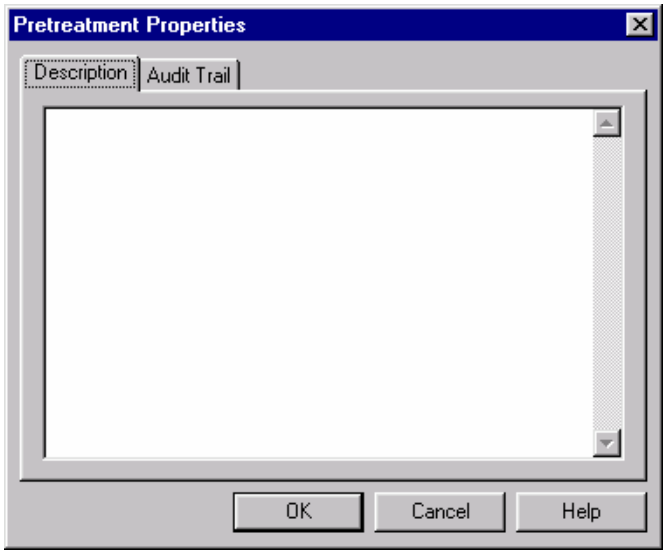


Figure 160. Pretreatment Properties - Properties dialog box

Pretreatment Properties Audit Trail

This tab is used to turn on the Audit Trail function for the pretreatment file. To enable the audit trail function, select the **Enable audit trail** box. You will be prompted with a warning that if you proceed, you cannot disable the audit trail once it is turned on.

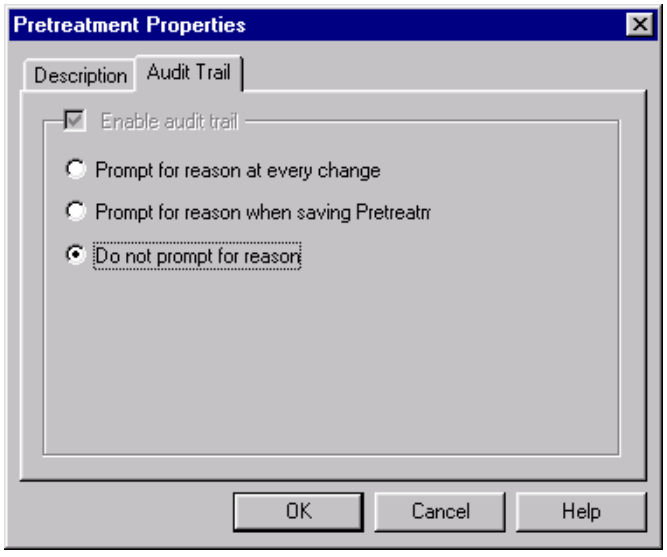


Figure 161. Pretreatment Properties - Audit Trail dialog box

- Prompt for reason at every change

  This option requires the user to enter a reason for every subsequent change to the pretreatment file at the time the change is made.

- Prompt for reason when saving Method

  This option requires the user to enter a reason for each change when the pretreatment file is saved.

- Do not prompt for reason

  When this option is selected, changes are documented, but the user is not required to enter a reason for the changes.

Chapter 4 Sequence Operations

Whenever you want to automate the acquisition and/or processing of chromatography data using ChromQuest, you will use a **sequence**. A sequence is a work list for ChromQuest that tells the order for runs to be acquired and/or processed. A sequence is normally used with an autosampler, however it can also be used for manual injections if they are injected in the exact order specified by the sequence.

This chapter contains the following sections:

Overview of Sequence Operations

The sequence is the cornerstone of automatic operation of the ChromQuest Chromatography Data System. With a sequence, you can automatically acquire, process, and store multiple runs. You can use a sequence to automate calibration, either at the time the runs are acquired, or post-acquisition by sequence reprocessing. You can set up run queues for automatic running of sequences, and you can trigger events based on results of a run in a sequence. Once a sequence is acquiring data, you can pause it, abort it, insert priority samples into it, or queue another sequence to start.

A sequence is displayed as a spreadsheet, with each row representing chromatography run or a file to be reprocessed. For each row, you designate a method, data file name, whether the sample is a calibration standard, along with various options for how you want the data to be processed. Details for each option of a sequence are given later in this chapter.

After you create a sequence, you can enable the Sequence audit trail feature.

Creating a Sequence Using Sequence Wizard

The Sequence Wizard is used to create a new sequence. To start the Sequence Wizard, choose **File > Sequence > Sequence Wizard** from the menu bar, or click on the **Create a Sequence** button from the Instrument Wizard.

Sequence Wizard – Method

Specify a method to be used for the sequence, or select it from a list of existing methods by clicking on the file open button.

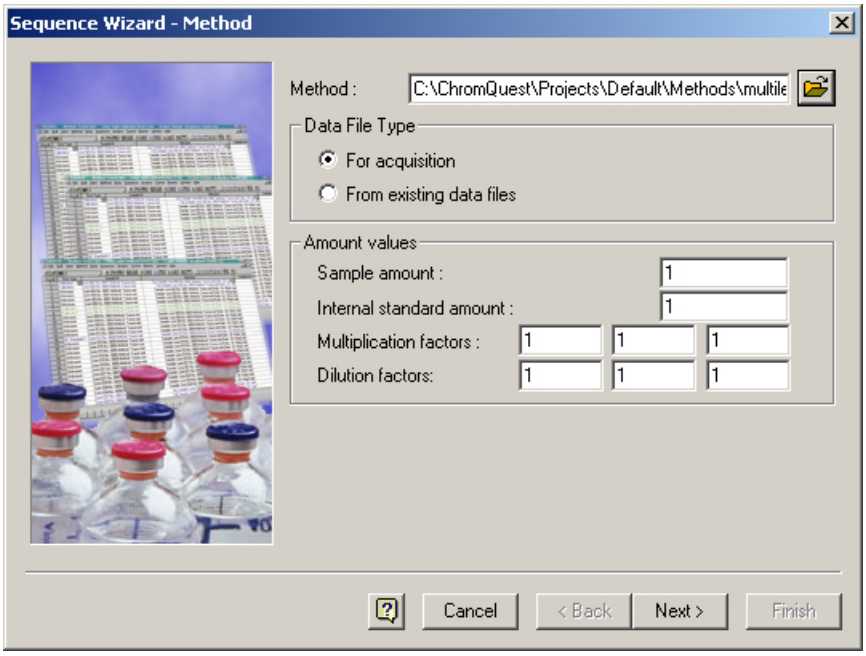


Figure 162. Sequence Wizard - Method dialog box

- Data File Type

  Select a **Data File Type** by clicking on the correct selection.

  – For acquisition

    Enables you to designate new file names to be used for acquisition.

  – From existing data files

    Enables you to select the data file names from a list of existing data files from your data directory. This is normally used to create a sequence to be used for reprocessing. Refer to "Creating a Reprocessing Sequence" on page 204 for details.

- Amount values

  In this section, you can enter values that affect how the concentrations of unknown samples are calculated.

  – Sample amount

    The Sample Amount value is used as a divisor during calculation of concentrations. It is intended to compensate for differences between samples due to weighing and when percentages of the total sample are being calculated rather than the amount detected in an injection.

  – Internal standard amount

    For calibration runs, the Internal Standard Amount is taken from the method Peak Table. For unknown runs, enter the amount of the Internal Standard in your unknown sample.

  – Multiplication factors

    Enter up to three multiplication factors to be used for these runs. All quantitated peaks will be multiplied by these factors.

  – Dilution factors

    Enter up to three dilution factors to be used for these runs. All quantitated peaks will be divided by these factors.

When you have completed this dialog, click on **Next** to continue.

Sequence Wizard – Unknowns

In this dialog, you enter information for data storage and sequence runs.

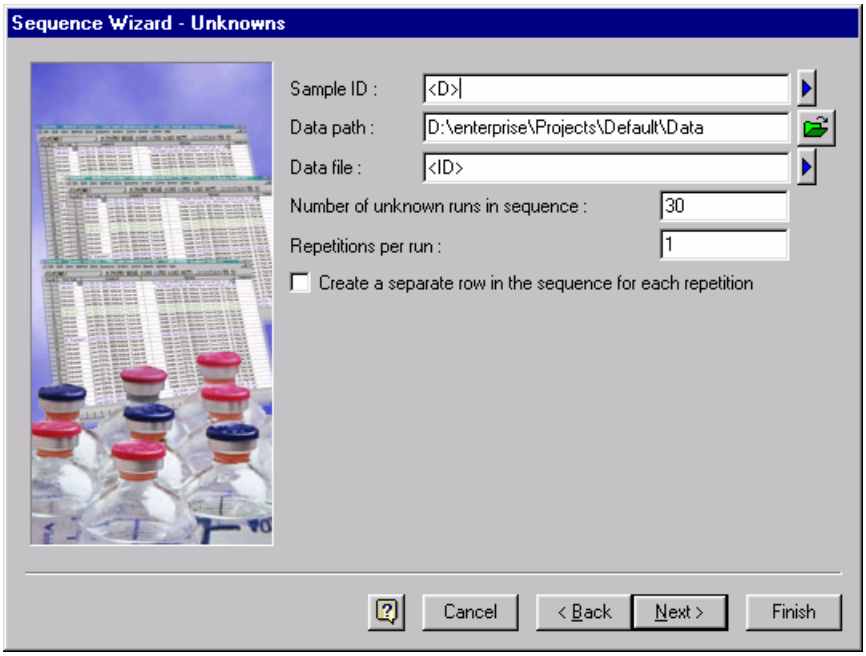


Figure 163. Sequence Wizard - Unknowns dialog box

- Sample ID

  Type a sample identification label. This can be numerical and/or text format, and will be saved with each data file.

  If you want the system to generate a Sample ID for you automatically, click on the blue arrow and select a parameter to be used for the basis of your Sample ID. You can select more than one parameter, which will be added sequentially to the Sample ID.

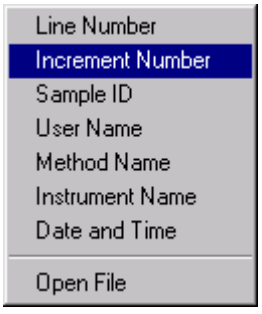


Figure 164. Sequence Wizard - Unknowns options menu

For example, if you select Line Number and Instrument name, the sample ID's generated will be the row number of the sequence followed by the instrument name: **3HPLC.**

To use the Increment Number option, enter a number to start the increment in angle brackets. For example, <33> would start numbering from 33. By default, the Increment Number option allows for three digits. To reduce or increase this number, adjust the digits in your brackets accordingly. For example, <0033> will give a 4-digit number.

- Data path

  Enter the path where your data files are to be stored. You can also select an existing path by clicking on the file button and then selecting a path from the paths that are accessible to you.

- Data file

  Enter a name for the data files. In order for unique data file names to be created automatically, you can have the system generate file names for you automatically, based on a system parameter. Click on the blue arrow and select a parameter to be used for the basis of your data file names. You can select more than one parameter. The software automatically appends the **.DAT** extension to the data file names, however you can change or remove this.

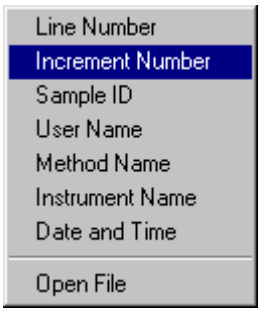


Figure 165. Sequence Wizard - Unknowns options menu

For example, you can use the Sample ID as the data file name.

Note In order to generate unique filenames automatically for each data file, you should make sure that the Line Number or Increment Number is included somewhere in the file name.

If you are creating a reprocessing sequence, click on the open file button, and select the file from the list of existing file names.

- Number of unknown runs in sequence

  Enter the number of unknown samples to be acquired or reprocessed with the sequence.

- Repetitions per run

  Enter the number of times each unknown will be repeated or re-injected.

- Create a separate row in the sequence for each unknown repetition

  Click this box if you wish to create a separate row in the sequence for each unknown repetition. If you do not select this box, unknown repetitions will not be displayed in the sequence spreadsheet, although individual data files will be created and stored for each repetition acquired.

When you have completed this dialog, click on **Next** to continue.

Sequence Wizard – Autosampler

If you have an autosampler control option, this screen of the sequence wizard will appear. It is used to set up the vial numbers for the first unknown and calibration of the sequence. A default injection volume can also be entered. This can be changed on a per-run basis in the sequence spreadsheet.

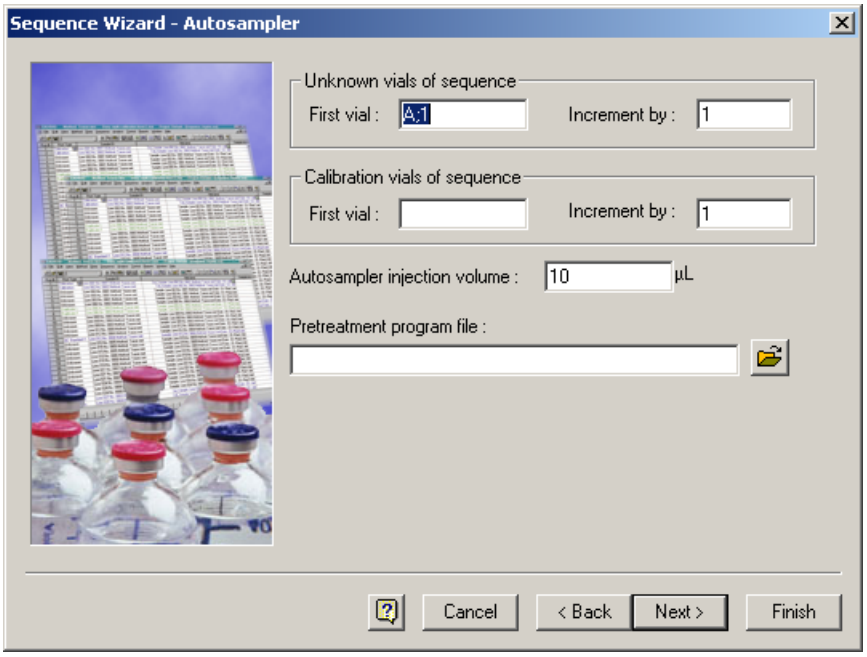


Figure 166. Sequence Wizard – Autosampler dialog box

Sequence Wizard – Calibration

This dialog allows you to set up calibration standards in your sequence.

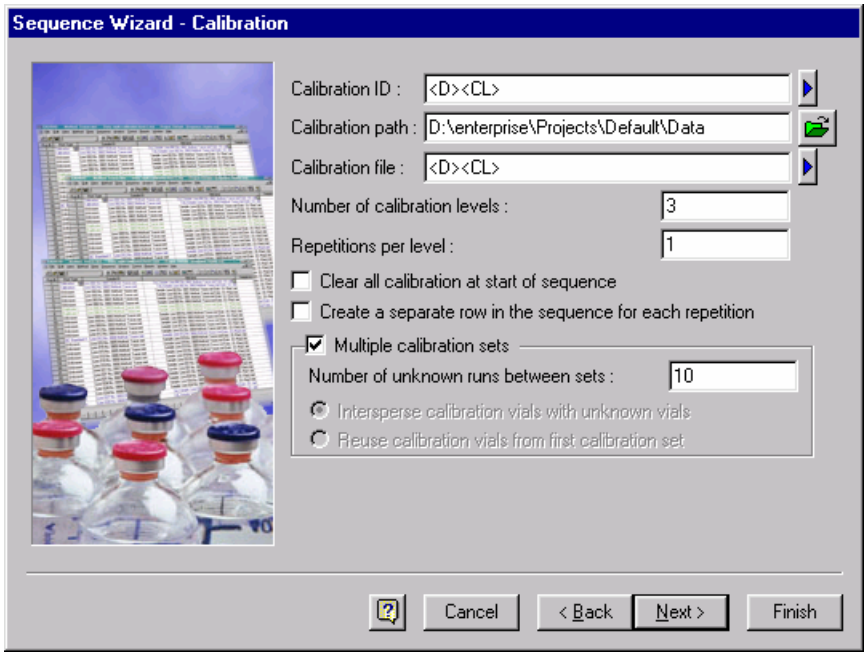


Figure 167. Sequence Wizard – Calibration dialog box

- Calibration ID

  Enter a calibration sample identification. This is a text value that is stored in the calibration data file. The identifiers from Sample ID are automatically entered in order to associate calibration IDs with your data file IDs. If you wish, you can have the system generate a different Calibration ID for you automatically based on a system parameter. Click on the blue arrow and select a parameter to be used for the basis of the Calibration ID.

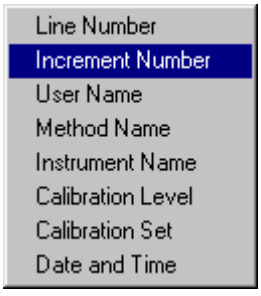


Figure 168. Sequence Wizard – Calibration options menu

- Calibration path

  Enter the path where the calibration files will be saved. You can select the path by clicking on the file open button and selecting an existing path from those displayed.

- Calibration file

  Enter a name for the calibration data files. The system automatically will apply the **Cal_** prefix to each calibration file in the sequence. You can change this if you wish. The identifiers from Data File are automatically entered in order to associate calibration file names with your data file names. You can change these if you wish by clicking on the blue button and selecting the parameter to be used for the basis of the calibration file name. You can select more than one parameter.

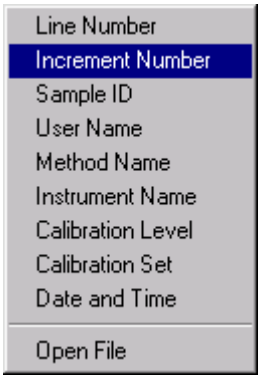


Figure 169. Sequence Wizard – Calibration options menu

Note In order to generate unique filenames automatically for each data file, you should make sure that the Line Number or Increment Number is included somewhere in the file name.

- Number of calibration levels

  Enter the total number of calibration levels to be run in the sequence.

- Repetitions per level

  Enter the number of repetitions or re-injections for each calibration level.

  – Clear all calibration at start of sequence

    Select this box if you want to clear all calibration information (response factors, replicates) before the first run of the sequence.

– Create a separate row in the sequence for each repetition

  Select this box if you want to create a separate row for each calibration repetition in your sequence. If you do not select this box, a separate data file will be created for each calibration repetition, however the repetitions will not appear in the sequence spreadsheet.

- Multiple calibration sets

  Select this box if you plan to run each calibration level (plus its replicates) more than once.

  – Number of unknown runs between sets

    If you have selected **Multiple calibration sets** box, this option will appear. Enter the number of unknown samples to be run between each calibration set.

    If you have an autosampler with control installed, the vials options will become available.

  – Intersperse calibration vials with unknown vials

    Select the Intersperse calibration vials with unknown vials option button if you want to run unknown samples between calibration sets.

  – Reuse calibration vials from first calibration set

    Select the Reuse calibration vials from first calibration set option button if you want to run calibration sets sequentially without unknown samples between.

When you have completed this dialog, click on **Next** to continue.

Sequence Wizard – Reports

Use this dialog to set up summary reports for your sequence.

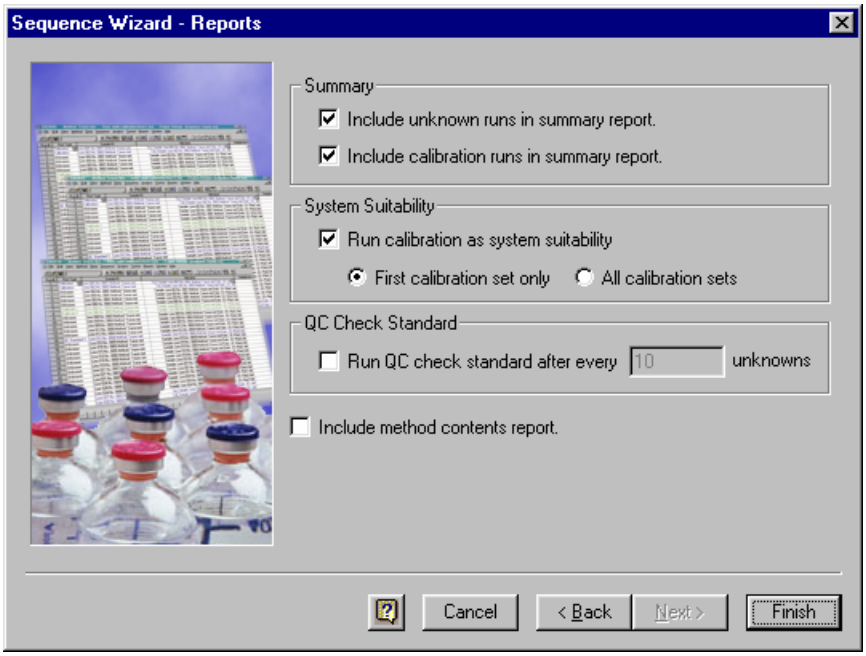


Figure 170. Sequence Wizard – Reports dialog box

- Summary

  These options allow you to designate summary runs.

  – Include unknown runs in summary report

    Select this box if you want your summary report to include unknown runs. These runs will have their Run Type set for a summary run.

  – Include calibration runs in summary report

    Select this box if you want your summary report to include the calibration runs. These runs will have the Run Type set to be included as a summary run.

- System Suitability

  These options allow you to designate system suitability runs.

  – Run calibration as system suitability

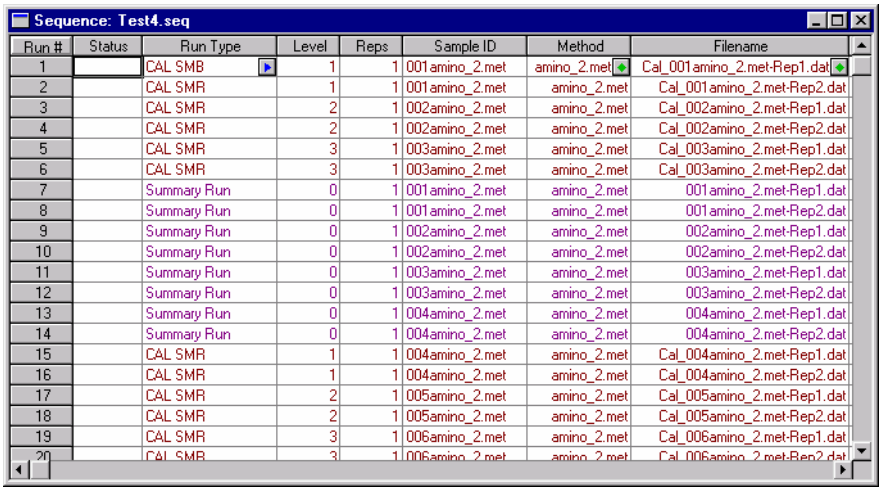
    Select this box if you have the system suitability software option, and you wish to designate calibration runs as system suitability types.

This also will cause display of system suitability parameters in the sequence spreadsheet.

- Include method contents report

  When this box is selected, a method contents report will be automatically generated whenever the method changes in a sequence. When this option is enabled, the sequence will automatically add a **Print Additional Reports** run type to each run where the method has changed from the previous run in the sequence. This can be changed or deleted for any given run in the Edit Sequence spreadsheet.

When you have finished this dialog, click on the **Finish** button. A sequence will be created using the information specified in the dialogs and displayed as a spreadsheet.



Sequence: Test4.seq

Run #	Status	Run Type	Level	Reps	Sample ID	Method	Filename
1		CAL SMB	1	1	001amino_2.met	amino_2.met	Cal_001amino_2.met-Rep1.dat
2		CAL SMR	1	1	001amino_2.met	amino_2.met	Cal_001amino_2.met-Rep2.dat
3		CAL SMR	2	1	002amino_2.met	amino_2.met	Cal_002amino_2.met-Rep1.dat
4		CAL SMR	2	1	002amino_2.met	amino_2.met	Cal_002amino_2.met-Rep2.dat
5		CAL SMR	3	1	003amino_2.met	amino_2.met	Cal_003amino_2.met-Rep1.dat
6		CAL SMR	3	1	003amino_2.met	amino_2.met	Cal_003amino_2.met-Rep2.dat
7		Summary Run	0	1	001amino_2.met	amino_2.met	001amino_2.met-Rep1.dat
8		Summary Run	0	1	001amino_2.met	amino_2.met	001amino_2.met-Rep2.dat
9		Summary Run	0	1	002amino_2.met	amino_2.met	002amino_2.met-Rep1.dat
10		Summary Run	0	1	002amino_2.met	amino_2.met	002amino_2.met-Rep2.dat
11		Summary Run	0	1	003amino_2.met	amino_2.met	003amino_2.met-Rep1.dat
12		Summary Run	0	1	003amino_2.met	amino_2.met	003amino_2.met-Rep2.dat
13		Summary Run	0	1	004amino_2.met	amino_2.met	004amino_2.met-Rep1.dat
14		Summary Run	0	1	004amino_2.met	amino_2.met	004amino_2.met-Rep2.dat
15		CAL SMR	1	1	004amino_2.met	amino_2.met	Cal_004amino_2.met-Rep1.dat
16		CAL SMR	1	1	004amino_2.met	amino_2.met	Cal_004amino_2.met-Rep2.dat
17		CAL SMR	2	1	005amino_2.met	amino_2.met	Cal_005amino_2.met-Rep1.dat
18		CAL SMR	2	1	005amino_2.met	amino_2.met	Cal_005amino_2.met-Rep2.dat
19		CAL SMR	3	1	006amino_2.met	amino_2.met	Cal_006amino_2.met-Rep1.dat
20		CAL SMR	3	1	006amino_2.met	amino_2.met	Cal_006amino_2.met-Rep2.dat

Figure 171. Sequence with repetitions as separate records

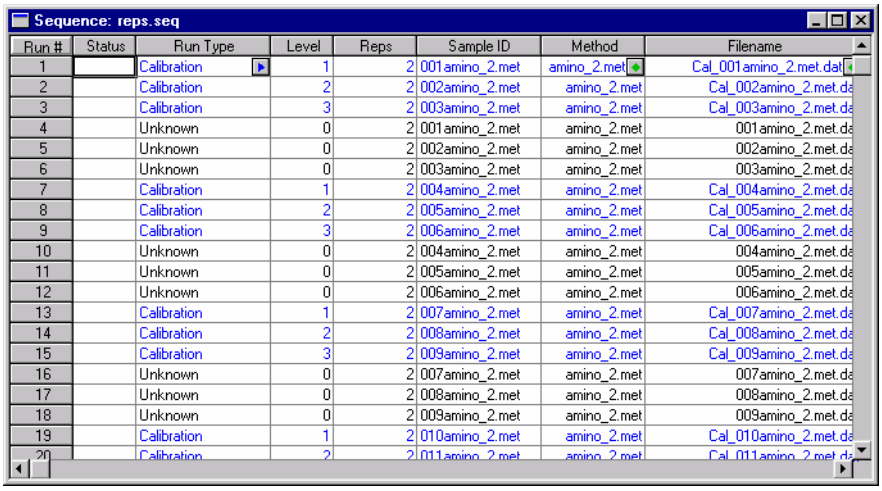


Figure 172. Sequence with repetitions combined

The new sequence will be displayed as “untitled”. To save your new sequence, use the **File > Sequence > Save As** command and type the name of the new sequence.

To close the sequence spreadsheet, click on the X box in the upper right corner of the spreadsheet. To open the current sequence spreadsheet, use the **Sequence > Edit Sequence** command, or click on the **Edit Sequence** button on the command ribbon.

Creating a Reprocessing Sequence

You can use the Sequence Wizard to create a sequence containing existing data files, for the purpose of reprocessing only. To create a reprocessing sequence, choose the **File > Sequence > New** command.

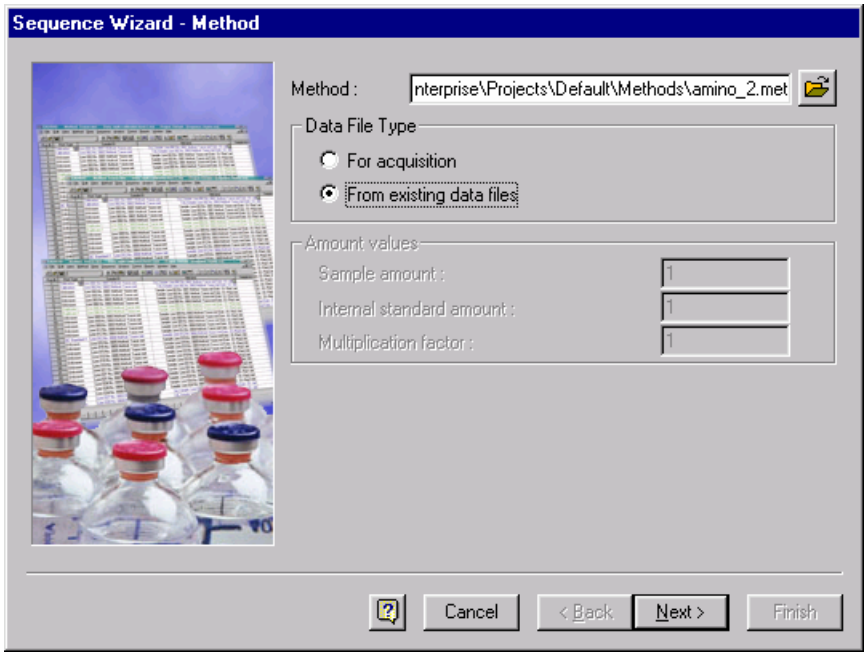


Figure 173. Sequence Wizard – Method dialog box

For **Data File Type**, select **From existing data files**. This will bypass the wizard screens necessary for creating an acquisition sequence and allow you to select data files to be reprocessed. In addition, amount values from the data files selected will be entered into the sequence. Click on **Next** to continue.

Sequence Wizard – Select Files

Files that are currently selected for inclusion in the reprocessing sequence are displayed.

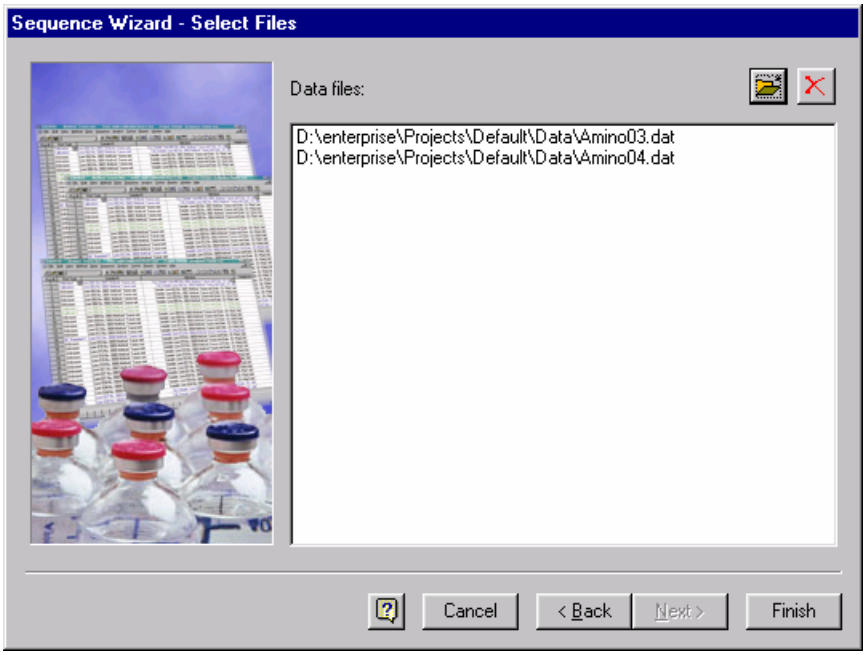


Figure 174. Sequence Wizard – Select Files dialog box

If no data files are selected, click on the **File Open** button to select the data files.

Sequence Wizard – Open data file

This screen is almost identical to the standard Open Data File dialog, but at the bottom displays a list of data files that are selected to be included in the sequence.

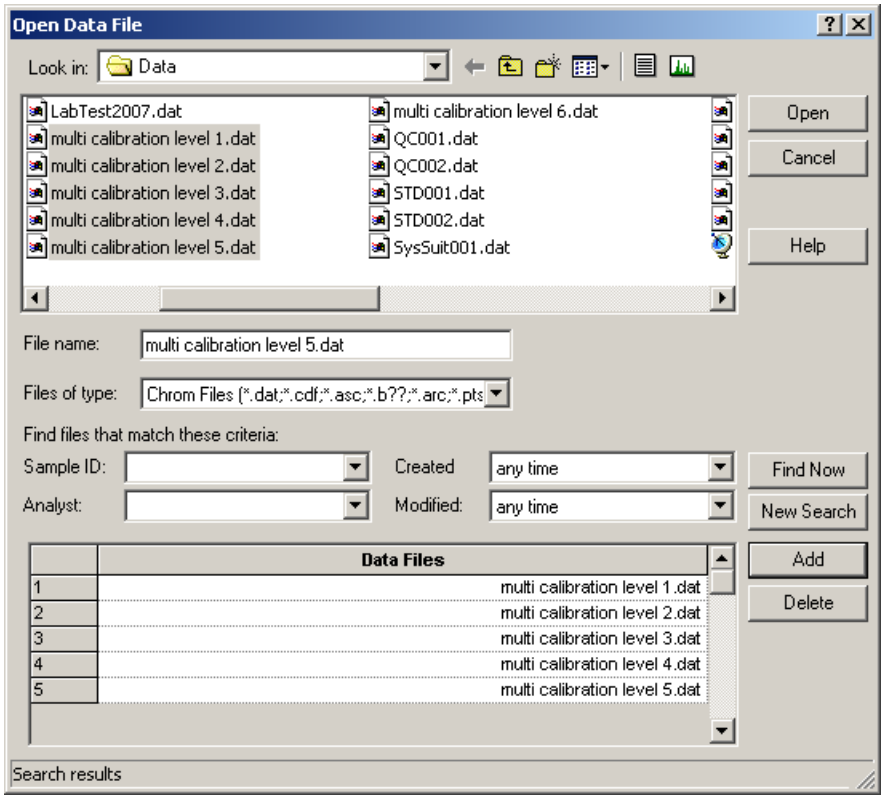


Figure 175. Open Data File dialog box

To select a data file, click on the file name with the mouse to highlight it, and then click on **Add**. Alternatively, double-click on the data file name. Each file you add will be added to the list of **Data Files** displayed at the bottom of the dialog. You may delete a file from the list by clicking on it, and then click on **Delete**. When you have completed the list of files, click on **Open** to return to the Sequence Wizard. To enter a range of data files, click on the last data file in your list, press SHIFT, and then click on the first data file in your list.

The selected files will be displayed in the sequence wizard dialog.

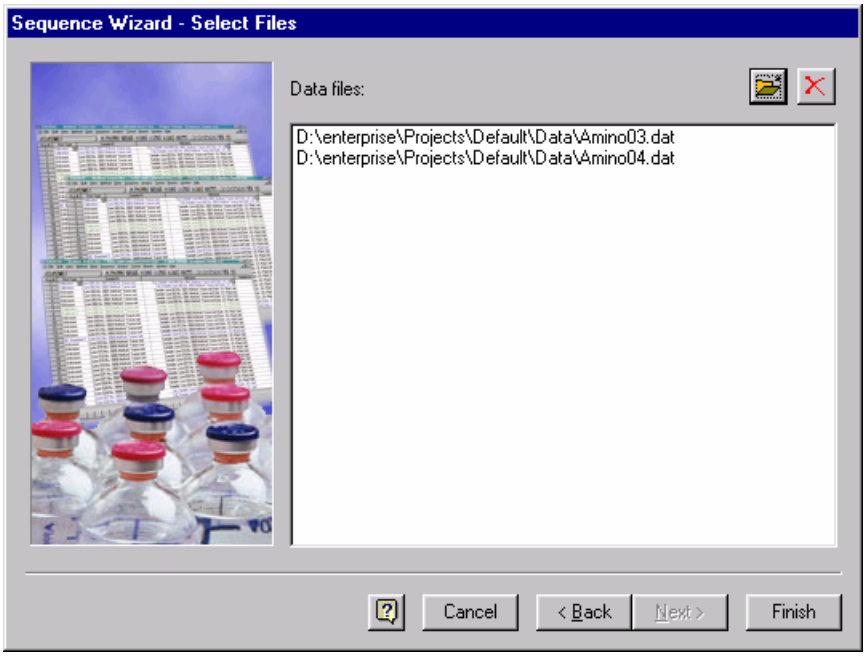


Figure 176. Sequence Wizard – Select Files dialog box

To add more files, click on the **File Open** box. Otherwise, click on **Finish**, and a reprocessing sequence spreadsheet will appear with the files selected appearing as rows.

Sequence Spreadsheet Basics

All sequences are displayed in a spreadsheet similar to the one shown below. Although the information in the fields will vary, the spreadsheets always support certain basic features.

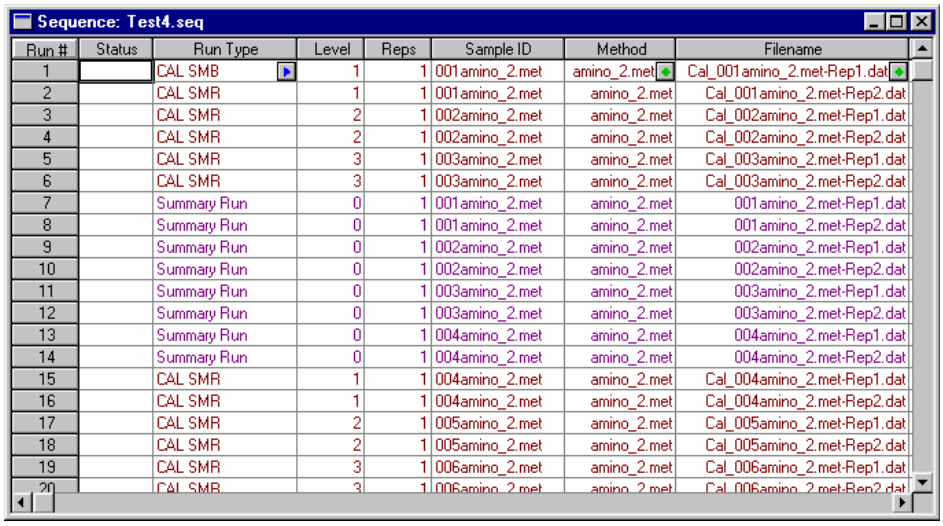


Figure 177. Sequence spreadsheet window

Each row is assigned a **run number**, followed by columns for information for each run in the sequence. Rows and field information can be cut, copied, pasted, and cleared. To access the menu for these commands, click on the **right** mouse button anywhere within the spreadsheet.

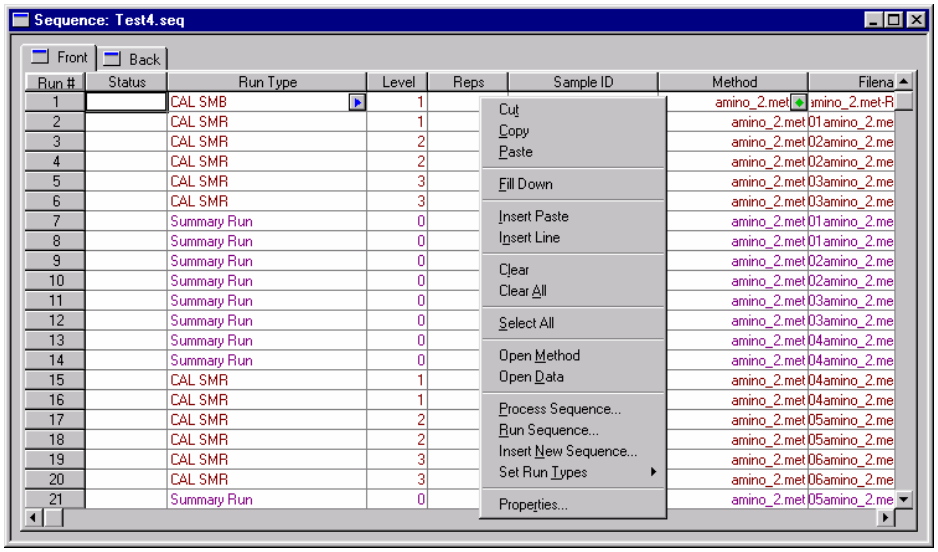


Figure 178. Sequence spreadsheet - options menu window

To select a field, click on that field to highlight it. To select a row, click on the **Run #** to highlight the entire row. To select the entire spreadsheet, use the right-click menu, and choose the **Select All** command.

Certain spreadsheet fields will have choices available for you to select. Fields with selections available will display a combo-box button when the field or row is selected. Click on the button to display the available choices.

Note Once you have Cleared or Deleted a row from your spreadsheet, the blank row will remain in the spreadsheet until you close the sequence spreadsheet and re-open it, or press the F5 key.

- Cut

  This command will cut the current selection and place it into the clipboard. You can subsequently paste the information to another application using the paste command, or move the selection to another location in the spreadsheet by selecting the location, then using the **paste** command.

- Copy

  Use this command to make an exact copy of the selection in the clipboard. Once you select **Copy**, you can paste the selection to another application, or copy the selection to a location in your spreadsheet.

- Paste

  This command is used to paste the information currently in the clipboard into the spreadsheet at the location of the cursor.

- Fill Down

  This enables you to automatically copy spreadsheet information from one field or row down through the rest of the spreadsheet.

- Insert Paste

  This command works like a combination of insert line and paste commands. The item currently in the clipboard will be pasted into a new line above where the cursor is located.

- Insert Line

  This command inserts a blank line in the spreadsheet above where the cursor is located.

- Clear

  Use this command to clear the information in the selected location. (You can also use the Delete key from your keyboard for this function.) The **F5** function key can be used to collapse rows that have been deleted from the spreadsheet.

- Clear All

  Use this command to clear the information from the entire spreadsheet.

- Select All

  Use this command to select the entire spreadsheet.

- Open Method

  This command will open the method associated with the currently selected run in the spreadsheet.

- Open Data

  This command will open the data file associated with the currently selected run in the spreadsheet. The data file will be opened with “last results”. If “last results” is not available, the data file will be recalled with the original results.

- Process Sequence

  This command opens the Process Sequence dialog and enables you to reprocess all or part of the current sequence.

- Run Sequence

  This command opens the Run Sequence dialog to enable you to start the sequence acquisition.

- Insert New Sequence

  This command will start the sequence wizard to create a new sequence, which will be inserted into your current sequence below the currently selected row.

- Properties

  This command opens the Sequence Properties dialog where you can add/edit the sequence description and select default paths for data and method files.

Note When copying or pasting spreadsheets from ChromQuest to other applications, “hidden” parameters that do not appear in the spreadsheet (such as action item parameters) will not be pasted.

Fill Down

The **Fill Down** command enables you to automatically copy spreadsheet information from one field or row down through the rest of the spreadsheet. To use this command, first select a field or a row by highlighting it with your mouse. Then do a right-mouse click and choose **Fill Down**. If you have selected a numeric field such as level or repetitions, the selected item will be automatically filled down the spreadsheet from where it is highlighted. If you select a row, a Filename, or a Sample ID, the following dialog will appear where you can designate a name and indicate whether you want to increment its associated numbering.

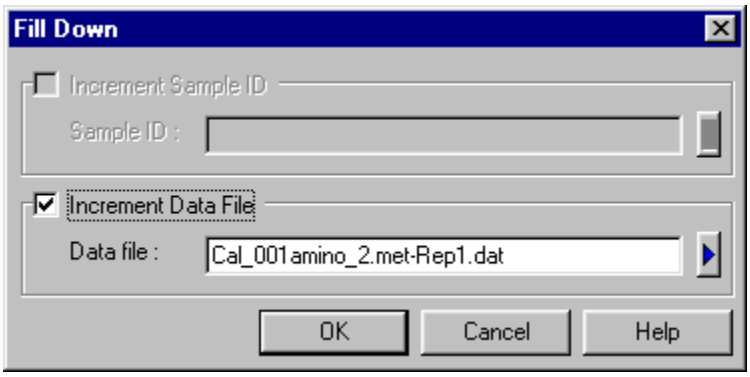


Figure 179. Fill Down dialog box

Increment

For Sample ID and Data File fields, you can opt to automatically increment the number or change the name by selecting the **Increment** check box. You can select parameters for the increment in a manner similar to that used in the sequence wizard by clicking on the blue arrow and selecting from the list. (Be sure to include the correct number of placeholders. For example, if you start at number <10>, but the number of files is between 100 and 1000, you should enter <010>.) Click on **OK** to fill the information down the spreadsheet.

Sequence Spreadsheet Properties

You can customize the sequence spreadsheet to include only the columns you will be using. Right-click in the sequence table and choose **Properties** from the popup menu. When you select this command, a dialog box appears where you can select and de-select the columns to be displayed in the spreadsheet.

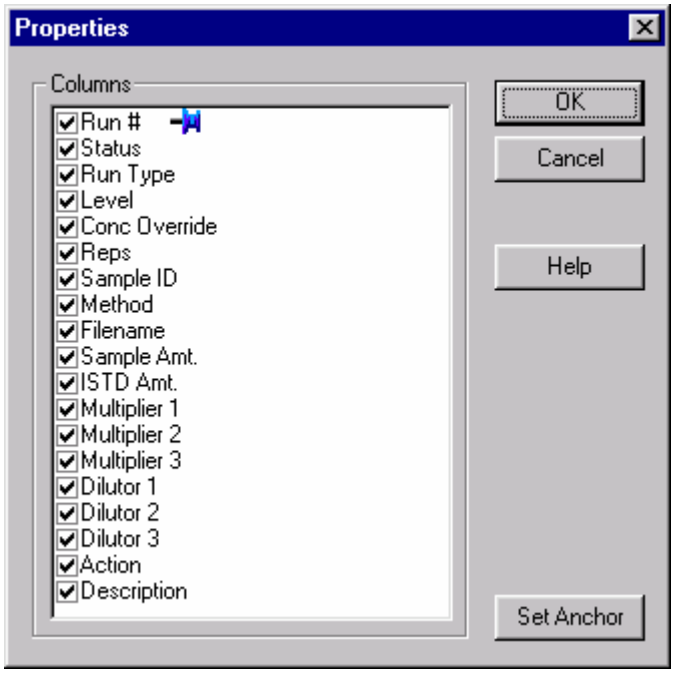


Figure 180. Sequence Spreadsheet Properties window

Columns with a check mark will be displayed in the spreadsheet. The selections are saved on a per-user/per instrument basis. That is, each user can set spreadsheet properties for each instrument.

Set Anchor

The blue "anchor" indicates what column will be used to anchor the right-left scrolling in your sequence spreadsheet. Once this anchor is set, columns to the right of the anchor will scroll to the right and left. Columns to the left of the anchor will not scroll. To change the anchor, click on the name of the column you wish to use as anchor, and click on the **Set Anchor** button. The blue anchor will move to the designated anchor column.

Note When using the anchor, it is best to remove all unnecessary columns from the spreadsheet, and make the spreadsheet as wide as possible before you set the anchor. If you set the anchor to a column that does not currently show on the spreadsheet, you will not be able to scroll the spreadsheet.

Process Sequence

This command allows you to process all or part of the currently displayed sequence, or to open a new sequence to process. This dialog is also accessed by the **Sequence > Process** command from the menu bar.

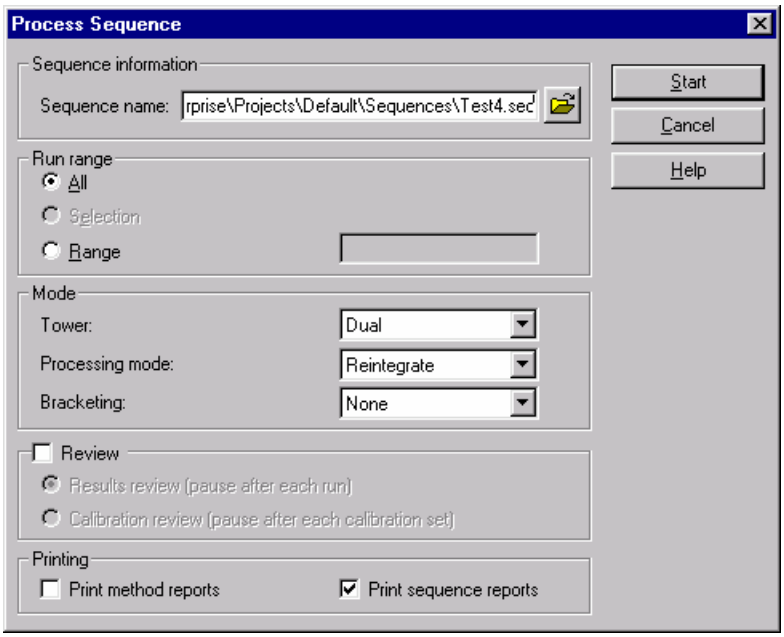


Figure 181. Process Sequence dialog box

- Sequence information

  The current sequence name will be displayed. If you want to open a new sequence to process, enter the name, or select it by clicking on the File button.

- Run range

  Select the range of the sequence to be run.

  – All

    Click on this to execute all runs in the sequence.

  – Selection

    If you have currently selected a series of runs in your sequence spreadsheet by highlighting them, click on this to run only the highlighted runs.

  – Range

    Enter a range of runs to be executed. For example, an entry of **4 - 6** will execute runs 4, 5, and 6 of the sequence. An entry of **4-** designates the fourth run through the end of the sequence.

- Mode

  Select the manner by which you want to handle data processing and bracketed calibration (if used).

  – Tower

    If your instrument is configured for Dual Tower, you can select the tower mode for reprocessing. Selections include Dual, Front, and Rear.

  – Processing mode

    Select a mode for running the sequence. **Normal** downloads the sequence one vial at a time. It is the correct setting for all HPLC runs and may be used with some GC runs. **High Throughput** performs two injections per vial for those GCs that support this mode of operation. **Full Download** downloads the entire sequence at the start of the sequence. This is the correct mode of line for systems that contain an HS2000 or an HS 850.

  – Bracketing

    Select the type of bracketing you wish to perform. (See Bracketed Calibrations section for details.)

    - None

    Select this if you do not wish to bracket calibrations.

    - Standard

    Select this if you wish to perform the standard mode of bracketing calibrations.

    - Std. w/Clear Calib

    Select this if you wish to perform the standard mode of bracketing calibrations, clearing the calibration before the start of each calibration set.

    - Sequence

    Select this if you want to perform the sequence mode of bracketing calibrations.

    - Seq. w/ Back Calc

    Select this if you want to perform the sequence mode of bracketing calibrations and back-calculate calibration runs.

- Review
  - Results review (pause after each run)

    Click this box if you want the sequence to pause between runs for you to review results.

  - Calibration review (pause after each calibration set)

    Click this box if you want the sequence to pause after each calibration set, where a calibration set is defined as one or more calibration runs that occur in a sequence.

- Printing
  - Print method reports

    Click this box if you want the custom report defined in the method to be printed for each run of the sequence.

  - Print sequence reports

    Click this box if you want sequence reports to be printed.

Note If you are currently reprocessing a sequence, you must have the **Results review (pause after each run)** option selected in order to submit an additional sequence to the queue. If you are reprocessing a sequence without this option selected, you must stop the sequence, select the **Results review (pause after each run)** option, then start it again. At this point, you can submit one or more sequences to the queue.

Run Sequence

This command allows you to run a sequence. It is the same as the **Control > Sequence Run** command.

Insert New Sequence

This command allows you to create sequence rows and insert them into your spreadsheet. This command calls the Sequence Wizard, which takes you through the process of defining the new sequence to insert. Once you have answered all the Sequence Wizard dialogs, the newly defined sequence rows are inserted into your current sequence spreadsheet.

Re-sizing Sequence Spreadsheet Columns

You can make the Sequence spreadsheet columns wider by graphically "dragging" the edge. To do this, move the cursor to the title area above the column you wish to size. The cursor will turn into two vertical lines with arrows. Click and drag the cursor until the column is the desired size, then release the mouse key.

The Sequence Spreadsheet

The following fields appear in the sequence spreadsheet. Although the New Sequence Wizard allows you to automatically create a sequence, you should review the sequence to make sure the information for each run is correct before it is run.

- Status

  This field becomes active when a sequence acquisition or processing is in progress. It indicates the current status of the run.

- Run Type

  Select a Run Type from the available types by clicking on the arrow button in the field. A dialog box will appear where you can select the run type.

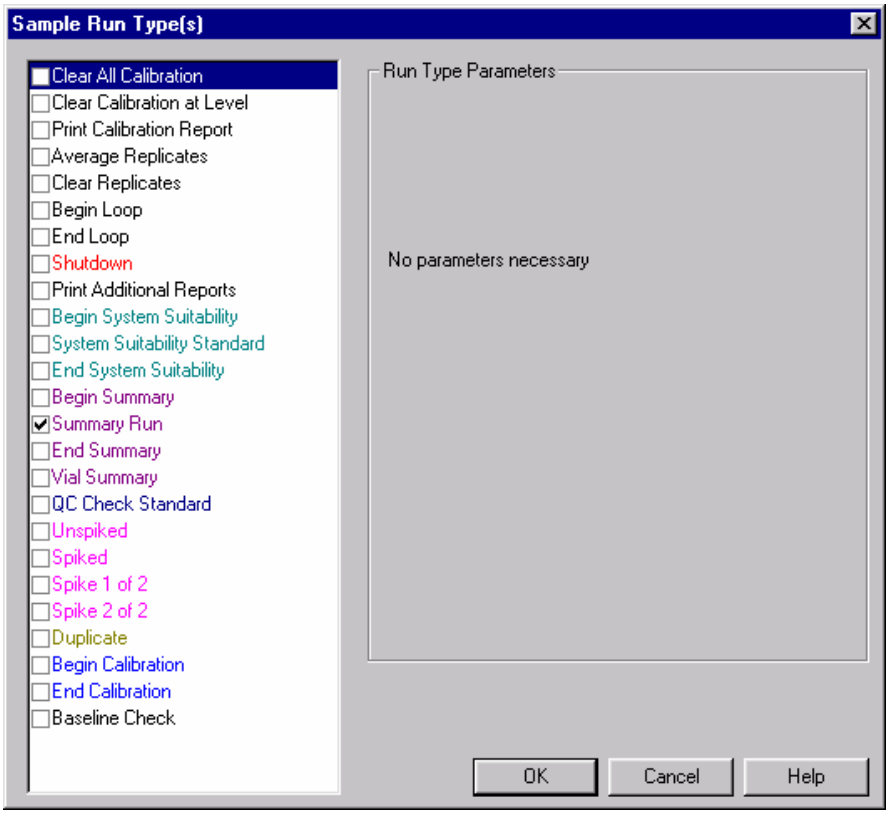


Figure 182. Sample Run Type(s) dialog box

  – Clear All Calibration

    Clears all calibration response factors and coefficients for all calibration levels.

  – Clear Calibration at Level

    Clears response factors and coefficients for current level only.

– Average Replicates

  Forces averaging of replicates.

– Clear Replicates

  Clears calibration replicates at this level before calibration.

– Begin Loop

  Flags the run as the start of an infinite loop. Runs between and including these flags will be continuously run until stopped.

– End Loop

  Flags the end of an infinite sequence loop.

– Shutdown

  Flags this sample as a shutdown sample.

– Print Additional Reports

  Allows you to specify one or more report templates and frequency for printing.

  Note Not applicable for the following report templates: Summary.brp, QC Check Standard.brp, Summary.tpl, Spike.brp, SysSuit.brp, Calibration report.crp, Calibration.brp, duplicate.brp. For details, see release notes.

– Begin System Suitability

  First run of a System Suitability sample set.

– System Suitability Standard

  System Suitability Standard sample between Begin and End System suitability.

– End System Suitability

  Last run of a System Suitability sample set.

– Begin Summary

  First run to be included in a sequence summary.

– Summary Run

  Run to be included in a sequence summary.

– End Summary

  Last run to be included in a sequence summary.

– Vial Summary

  Create a vial summary report for this row. A summary of all repetitions specified for this row of the sequence will be generated.

– QC Check Std

  Check standard sample. A check standard inserted in a sequence is used for generation of a Check Standard Report. It is a means for "checking" the chromatograph and conditions without calibrating.

– Unspiked

  Unspiked sample of a Spiked/Unspiked pair, used for Spike Report.

– Spiked

  Spiked sample used in single level spike analyses (unknown smp. & spiked smp.).

– Spike 1 of 2

  First spiked sample used for Spike Reports.

– Spike 2 of 2

  Second spiked sample used for Spike Reports.

– Duplicate

  Duplicate sample used for Duplicate Reports.

– Begin Calibration

  First calibration mixture to be used in Calibration Summary Report. When this run type is encountered, ChromQuest stores the current retention times of all named peaks. These are displayed in the reports as the "Old RT". After the End Calib sample is run, the updated retention times are stored and displayed as "New RT" in the Calibration Report. In addition, the average response factor for each peak is calculated, the % RSD is calculated, and compared to the expected % RSD from the peak table. Compounds falling above this % trigger the failure action.

– End Calibration

  Last calibration mixture to be used in Calibration Summary Report.

– Baseline Check

Enable baseline check.

- Run Type Parameters

For each run type selected, you may be prompted to enter parameters necessary for the run type to be used. In most cases, this involves selecting a template to be used for a report.

- Setting run types for multiple runs

You can quickly change the run type for multiple runs by highlighting the desired rows in the spreadsheet, then click on the right mouse button and select **Set Run Types**. When you select the desired run type from the displayed list it will be applied to the rows you have selected.

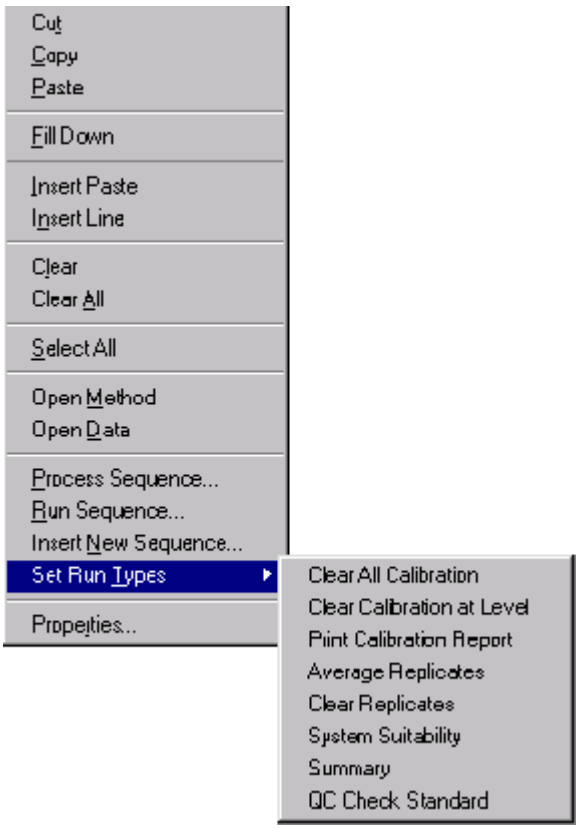


Figure 183. Sample Run Type options menu

– Level

For a calibration run, you must enter the level number for the calibration standard. (For example, if you have 5 calibration concentration levels, each calibration run will have a **level** number representing the calibration

concentration level being run. This number is NOT the concentration amount of the standards, however. Concentration amounts are entered in the peak table.)

– Sample ID

  Enter a sample identification here. This can be text and numeric information. If you entered a Sample ID in the new sequence dialog with a number in parentheses, the Sample ID will have a unique number attached to it. The Sample ID is saved in the data file.

– Method

  This is the name of the method to be used for data acquisition and processing. If you don't know the name of the method, click on the button to select it from a list of available methods on your disk.

– Filename

  Enter a filename to be used for storing the raw data from the run. If you entered a data filename in the New Sequence dialog, and designated a number in parentheses, the filename will already have a unique number appended to it.

– Sample Amt

  The Sample Amount value is used as a divisor during calculation of concentrations. It is intended to compensate for differences between samples due to weighing and when percentages of the total sample are being calculated rather than the amount detected in an injection.

– ISTD Amt

  For calibration runs, the Internal Standard Amount is taken from the method Peak Table. For unknown runs, enter the amount of the Internal Standard in your unknown sample.

– Multiplier 1,2,3

  Enter up to three multiplication factors to be used for this run. All quantitated peaks will be multiplied by these factors.

– Dilutor 1,2,3

  Enter up to three dilution factors to be used for this run. All quantitated peaks will be divided by these factors.

– Conc

Click on this to enter a concentration override for one or more peaks in the selected calibration run. This is not active unless the selected run is designated as a calibration run.

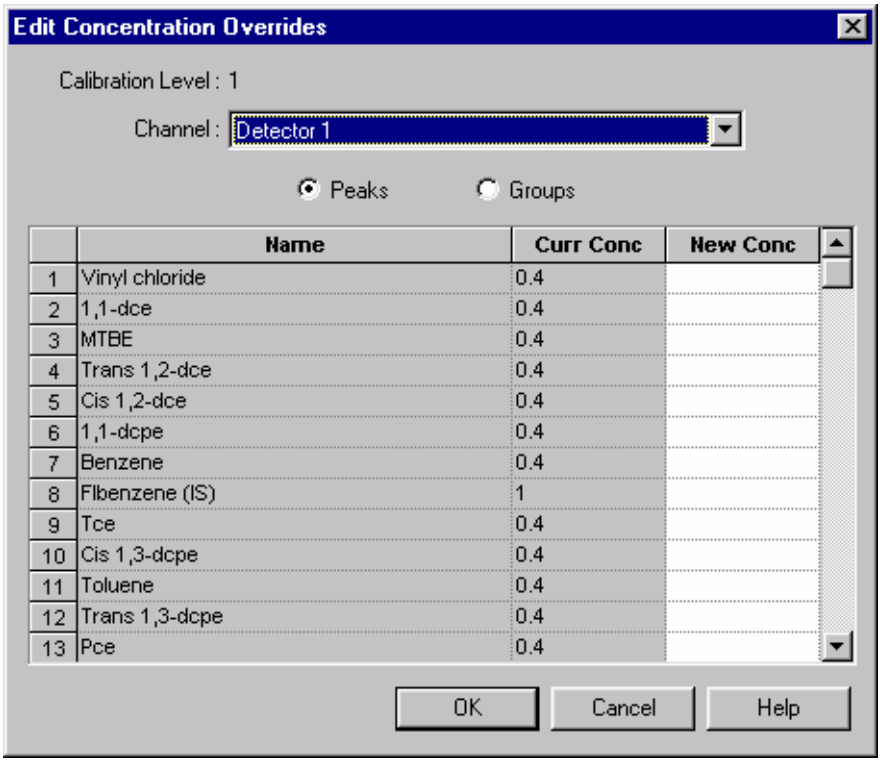


Figure 184. Edit Concentration Overrides dialog box

– Action

Brings up the run action dialog, where you can specify test/result/action combinations for each run.

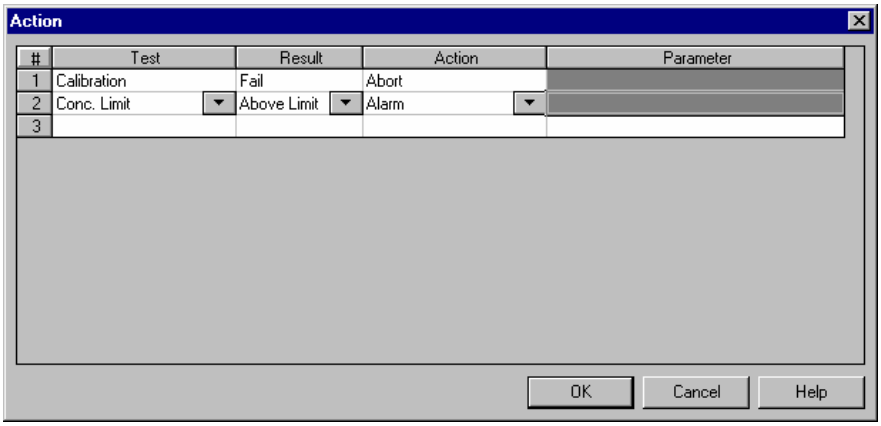


Figure 185. Action dialog box

- Test

  Select a test whose result will trigger the post run action selected. For each condition selected, specify a result and a post run action, if appropriate. Available conditions include:

  – Any

  – Calibration

  – QC

  – System Suitability

  – Hardware Status

  – Conc. Limit

- Result

  For the given test, select a result from the drop-down list. Results can include pass, fail, above limit, or below limit.

- Actions

  When you select an **Action**, you may be required to enter one or more **Action Parameters** for it.

  The following Actions require no Action Parameters:

  – Abort

    Abort the sequence.

– Pause

  Pause the sequence and wait for user to review and resume sequence.

– Alarm

  Trigger alarm.

– Run Shutdown

  On failure, the sequence will search for and execute the next run with a "shutdown" run type. If no "shutdown" run type is found, it will search from the beginning of the sequence.

The following Actions require you to select Action Parameters:

– Re-inject

  Specify the number of times you wish to attempt re-injection of the sample if the injection fails.

– Run Program

  Specify a program name to be run. You can also select a program name from the disk by clicking on the button in the field.

– Goto

  Specify a line in the sequence to “Go To”, and enter a “Repeat count” for the number of retries before going to the new line.

– Restart System Suitability

  Specify the number of times you wish to attempt re-starting system suitability.

• Description

  Enter a description for this sample. This is text information that will be stored in the raw data file.

Setting the Sequence Properties

When you create a new sequence, you can enter a description and designate the default directories for data and methods. To set these properties, select the **Sequence > Properties** command. A dialog box will appear.

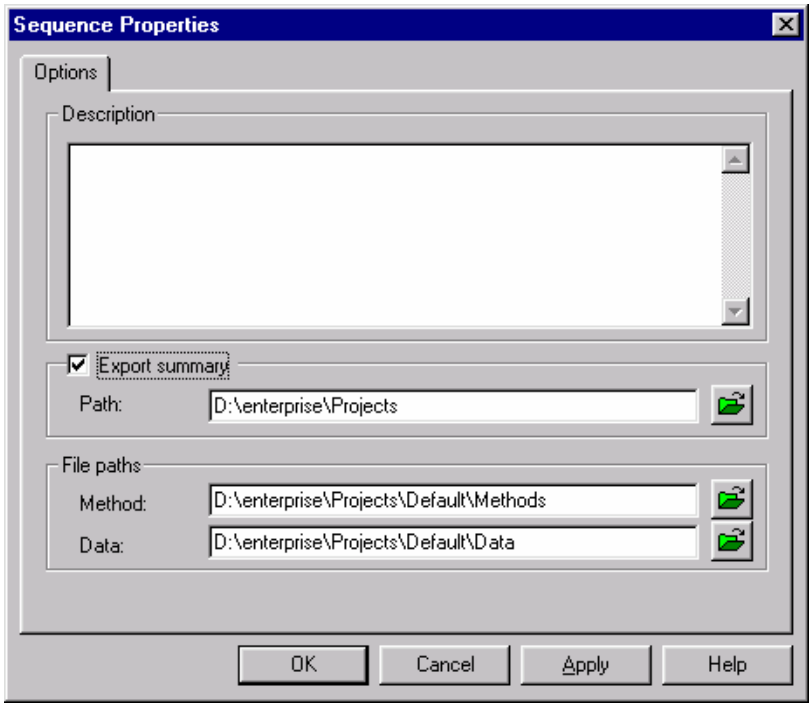


Figure 186. Sequence Properties - Options dialog box

- Description

  Enter a text description of the sequence, if desired. The description is saved in the sequence file and may be viewed from the **Open Sequence** dialog box.

- Export summary

  Click this box to export the summary report. Enter the path where you want the summary text file to be saved.

  The summary will be exported to a file entitled

  "Sequence Summary - 000005000.txt"

  Where the numbers represent

  nBatchTower, nReportRunTypeID, nReportNumber.

- File paths

  Select default paths to be used for methods, data, and autosampler, if appropriate. You can select a path from those available on the disk by clicking on the file button adjacent to the field. These paths are used in the event that a file is specified in the sequence table without a path.

- Print Sequence Reports

  Select this box to enable the printing of sequence reports during the running or reprocessing of a sequence.

Enabling the Sequence Audit Trail

To enable the audit trail for sequences, choose **Sequence > Properties** from the command menu. Then, click on the Audit Trail tab.

Note Once the Enable Audit Trail box is selected, it cannot be de-selected. The sequence will continue to have audit trail enabled unless you save the file under a new filename.

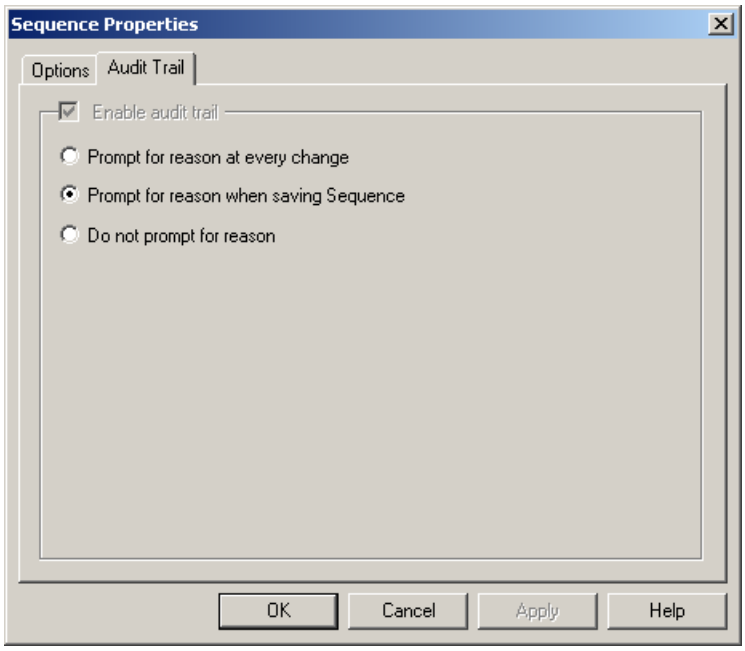


Figure 187. Sequence Properties - Audit Trail dialog box

Once the Enable Audit Trail box is checked, the following options are available for documenting changes:

- Prompt for reason at every change

  This option requires the user to enter a reason for every subsequent change to the sequence at the time the change is made.

- Prompt for reason when saving Sequence

  This option requires the user to enter a reason for each change when the Sequence is saved.

- Do not prompt for reason

  When this option is selected, changes are documented, but the user is not required to enter a reason for the changes.

Acquisition using a Sequence

Once you have created and saved a sequence, you can use it to acquire and process data. To start a sequence acquisition, click on the **Sequence Run** button, or select the **Control > Sequence Run** command. A dialog box will appear where you select the sequence to run.

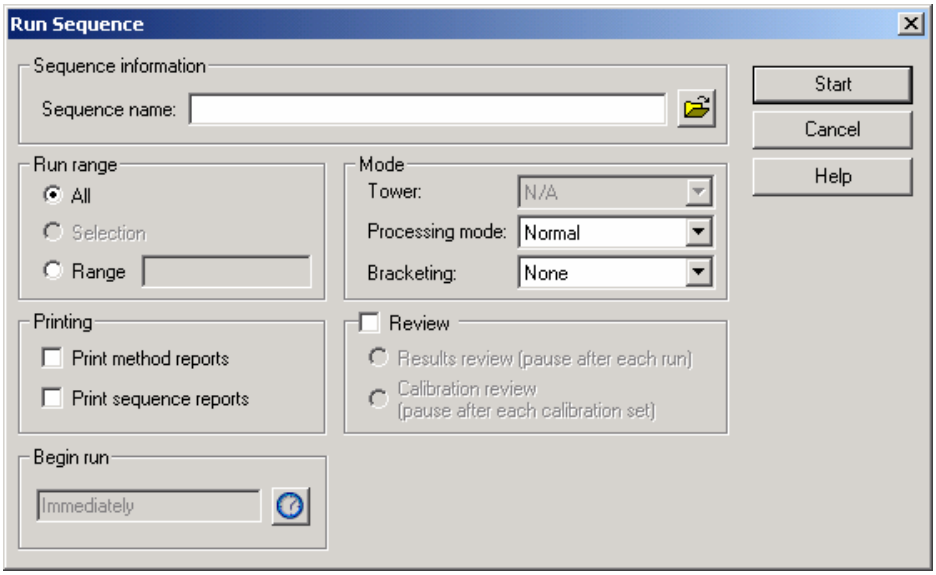


Figure 188. Run Sequence dialog box

- Sequence information

  Enter the **Sequence Name** to be used, or select the sequence file from a list of available sequence files by clicking on the File button.

- Run Range

  Select the range of the sequence to be run.

  – All

    Click on this to execute all runs in the sequence.

  – Selection

    If you have currently selected a series of runs in your sequence spreadsheet by highlighting them, click on this to run only the highlighted runs.

  – Range

    Enter a range of runs to be executed. For example, an entry of **4 - 6** will execute runs 4, 5, and 6 of the sequence. An entry of **4-** designates the 4th run through the end of the sequence.

- Mode

  Select the manner by which you want to handle data processing and bracketed calibration (if used).

  – Tower

    If your instrument is configured for Dual Tower, you can select the tower mode to be used for the sequence run. Selections include Dual, Front, and Rear.

  – Processing mode

    Select a mode for running the sequence. **Normal** downloads the sequence one vial at a time. It is the correct setting for all HPLC runs and may be used with some GC runs. **High Throughput** performs two injections per vial for those GCs that support this mode of operation. **Full Download** downloads the entire sequence at the start of the sequence. This is the correct mode of line for systems that contain an HS2000 or an HS 850.

  – Bracketing

    Select the type of bracketing you wish to perform. (See Bracketed Calibrations section for details.)

    • None

    Select this if you do not wish to bracket calibrations.

    • Standard

    Select this if you wish to perform the standard mode of bracketing calibrations.

    • Std. w/Clear Calib

    Select this if you wish to perform the standard mode of bracketing calibrations, clearing the calibration before the start of each calibration set.

    • Sequence

    Select this if you want to perform the sequence mode of bracketing calibrations.

    • Seq. w/ Back Calc

    Select this if you want to perform the sequence mode of bracketing calibrations and back-calculate calibration runs.

- Review
  - Results review (pause after each run)

    Click this box if you want the sequence to pause between runs for you to review results.

  - Calibration review (pause after each calibration set)

    Click this box if you want the sequence to pause after each calibration set, where a calibration set is defined as one or more calibration runs that occur in a sequence.

- Printing
  - Print method reports

    Click this box if you want the custom report defined in the method to be printed for each run of the sequence.

  - Print sequence reports

    Click this box if you want sequence reports to be printed.

- Begin run

  By default, run will start immediately. If you want to schedule the start of the sequence for a later time or date, click on the () button to open the **Schedule Run** dialog where you can enter or select the time to start the sequence.

When you have completed the dialog box, click on **Start** to initiate the sequence acquisition. You may see the data displayed in real time in the chromatogram window(s), if the "current data" is selected for viewing.

Schedule Run

This dialog appears whenever you start a single run or sequence. It allows you to designate when you want the run to start: immediately, after a designated amount of time (in minutes), or at a specific date and time.

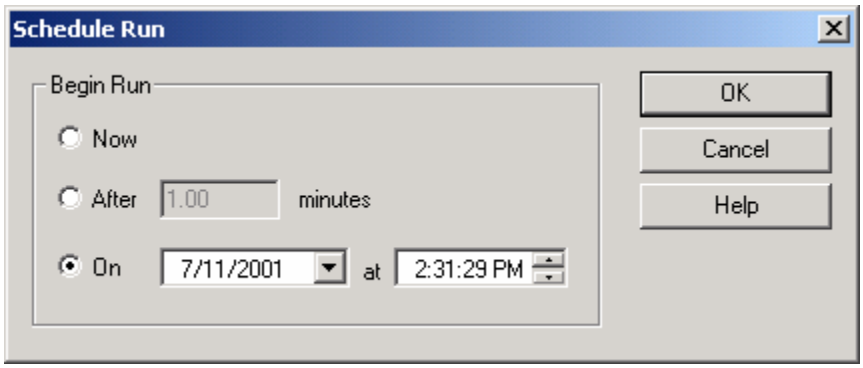


Figure 189. Schedule Run dialog box

Run Queues

ChromQuest uses a sample run queue to keep track of and schedule sequences and single acquisition runs. Once a sequence is initiated, it is entered into the run queue automatically.

Viewing the Run Queue

Click on the **View Run Queue** button to view the current run queue. If a sequence acquisition is in process, you will see a row in the run queue representing that sequence. The queue lists the sequence or single run **Type (single run or sequence), Name, Status**, and **Description**. Notice the **Status** in the sample queue displays the status of the sequence (or single run). If you are running a sequence, the **Status** shown in the sequence spreadsheet displays the status of an individual run in the sequence.

Adding to the Run Queue

Using the commands available from the right-mouse click, you can quickly add or delete items from your run queue.

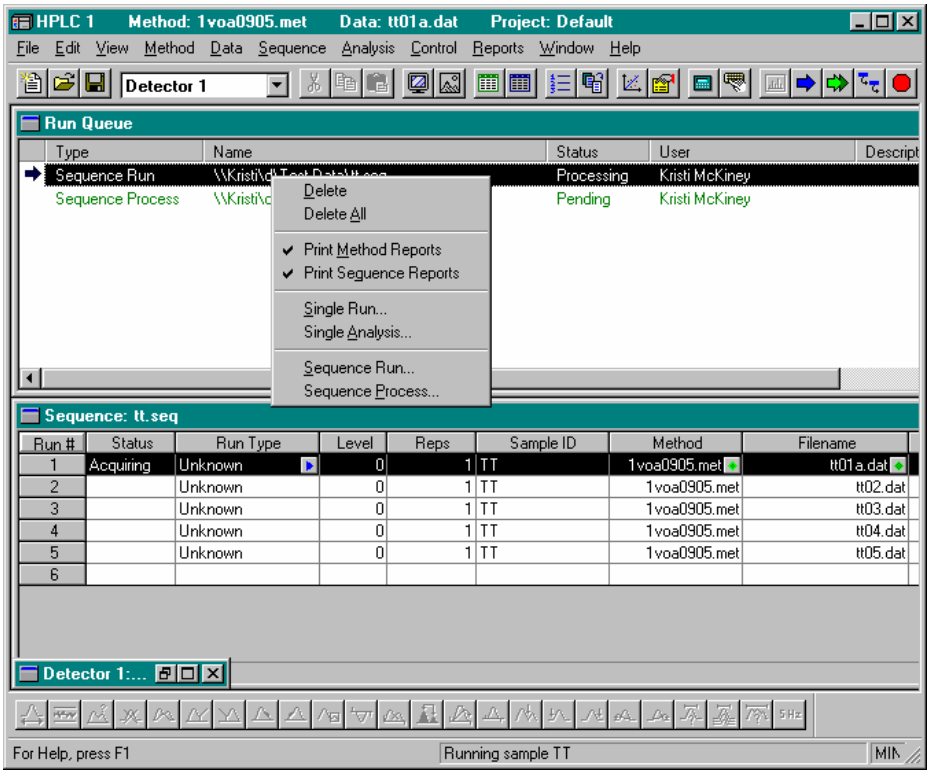


Figure 190. Run Queue dialog box

- Delete

  This command will delete the selected item from the run queue.

- Delete All

  This command deletes all items from the run queue.

- Print Method Reports

  When method report printing is turned ON for the highlighted queue entry, a check mark will appear. You can turn the Method reports printing function OFF for the selected queue entry by clicking on **Print Method Reports** to remove the check mark.

- Print Sequence Reports

  When sequence report printing is turned ON for the highlighted queue entry, a check mark will appear. You can turn the Sequence reports printing function OFF for the selected queue entry by clicking on **Print Sequence Reports** to remove the check mark.

- Single Run

  This command allows you to add a single run acquisition to the run queue.

- Single Analysis

  This command allows you to add a single run reprocessing (analysis/single level calibration) to the run queue.

- Sequence Run

  This command allows you to add a sequence run to the run queue.

- Sequence Process

  This command allows you to add a sequence for processing to the run queue.

Note If you are currently reprocessing a sequence, you must have the **Results review (pause after each run)** option selected in order to submit an additional sequence to the queue. If you are reprocessing a sequence without this option selected, you must stop the sequence, select the **Results review (pause after each run)** option, then start it again. At this point, you can submit one or more sequences to the queue.

If there is a sequence or single run acquisition in process, **Submit** and **Submit Priority** buttons will appear instead of a Start button.

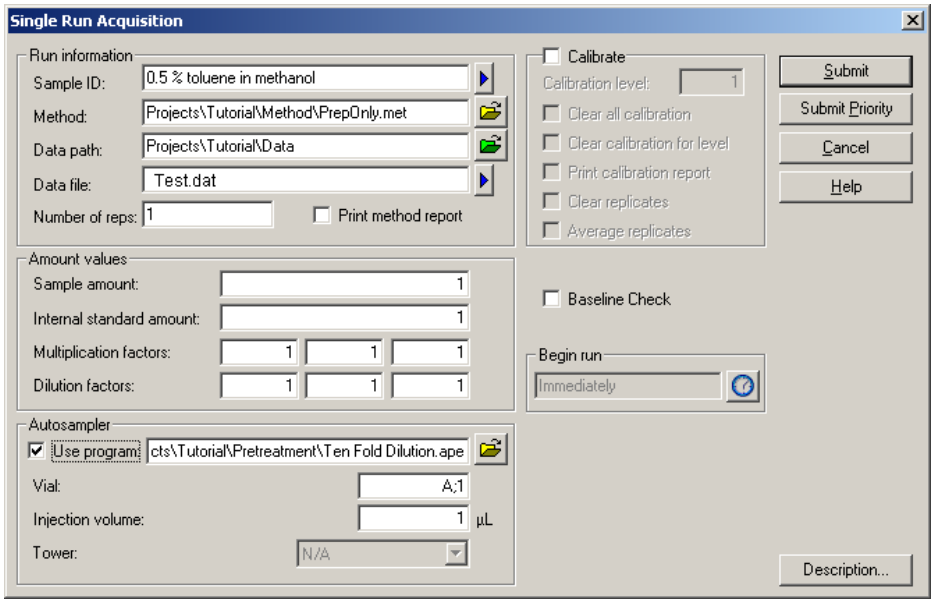


Figure 191. Single Run Acquisition dialog box

Click on the **Submit** button to add the sequence or single run to the bottom of the current queue.

Priority Samples

If you click on the **Submit Priority** button to add an item to the queue, the item you are submitting will be scheduled to run immediately after the current run is finished. After the priority item is complete, the previously running sequence will resume.

Bracketed Calibrations

The **Bracketing** option for starting a sequence acquisition allows you to process data using calibration replicates that are run in "brackets" around your samples in the sequence. This enables you to process all your data files using consistent response factors. When you choose a bracketing option, calculation of results will be handled such that the calibration standards will be processed before calculation of the unknown results, regardless of the fact that the calibration standards are bracketed around the unknown samples. Bracketing can also be performed as part of sequence reprocessing.

- Standard Bracketing

  If you choose this method of bracketing calibration, ChromQuest will calculate each group of unknowns based on the response factors determined by the calibration standards directly before and after the unknown group in the sequence.

- Standard Bracketing with Clear Calibration

  This method uses the Standard mode of bracketing calibrations, but clears the calibration before the start of each calibration set. This allows you to run each bracket independent of others.

- Sequence Bracketing

  If you choose this option for bracketing, the results for unknown samples will be calculated based on the response factors generated by the entire sequence of calibration standards, regardless of their position in the sequence. Calibration takes place after all samples and standards have been run.

- Sequence Bracketing with Back-Calculation

  This method of bracketing uses the sequence bracketing method, then "back-calculates" and reports amounts for the calibration runs by using the final calibration curves.

Example of bracketing calibrations

The following example demonstrates how bracketing works.

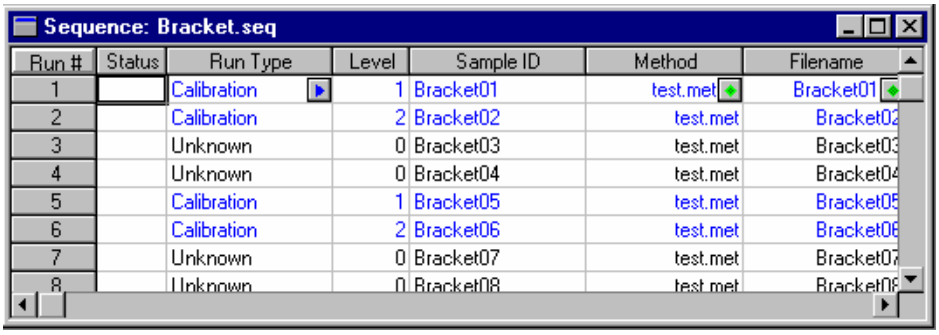


Figure 192. Sequence Bracket window

Using the above sequence as an example, using the **Standard** method of bracketing, unknown samples number 3 and 4 will be calculated using response factors generated after calibration runs 1, 2, 5, and 6 are completed. Unknown sample runs 7 and 8 will be calculated using response factors generated after running calibration sample runs 5, 6, 9 and 10.

For the same example, using the **Sequence** method of bracketing, all unknown samples will be calculated using response factors generated after all calibration standards are completed (1, 2, 5, 6, 9, and 10).

Sequence Reprocessing

In addition to using sequences for data acquisition, you can use a sequence to reprocess previously acquired data. A sequence can be used to calibrate multi-level calibrations using stored data files. It can also be used to generate reports post run.

To use a sequence to reprocess a run, do a right-mouse click in the sequence spreadsheet, and select **Process**, or select the **Sequence > Process** command. For additional details, see the **Process Sequence** section.

Data Acquisition without Processing

If you wish to acquire and store data on the hard disk, but postpone processing completely until you have reviewed the data, turn the option to analyze after acquisition off before you acquire the data. This is done in the **Method > Properties** section of your method.

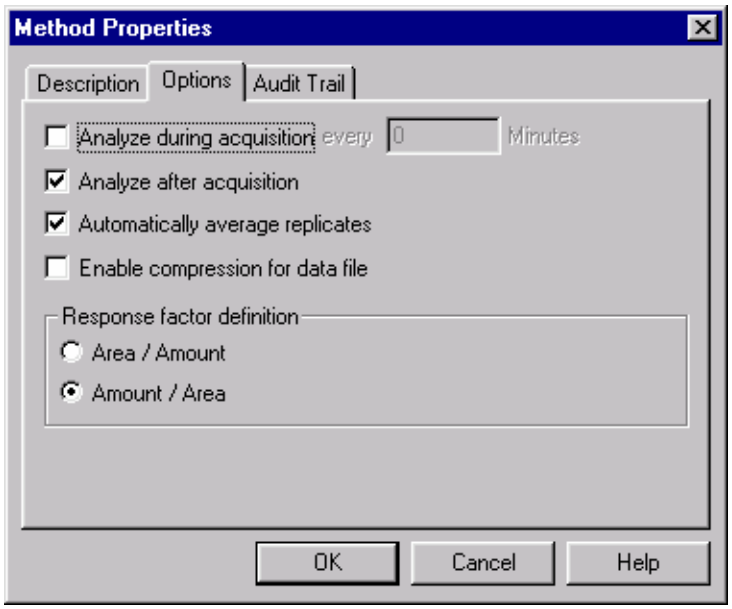


Figure 193. Method Acquisition - Options dialog box

If the check box for **Analyze After Acquisition** is **selected**, data files generated with this method will be integrated and results generated automatically after each acquisition. If you **de-select** this option in your method, no analysis will occur as you acquire runs with this method. The data will be saved on the disk, but no results will be generated.

Note The option to turn off analysis after acquisition is part of the method. If you want to postpone processing of a sequence until after data acquisition is completed, make sure all methods specified in your sequence have this option turned off.

Sequence Summary Reports

You can automatically generate reports that summarize the runs and results from a sequence. Sequence Summary Reports can be printed automatically at the end of the sequence, or they can be generated during sequence reprocessing. You can also summarize designated runs within a sequence.

A sequence summary report can be easily generated using the Advanced Report template **Summary.tpl**. To print this summary report at the end of your sequence, (or at any point during your sequence), click on the first run to be included in the summary, and select Run Type. When the Run Type dialog appears, select the **Begin Summary** run type, and enter the **Summary.tpl** template to be used. All designated Summary runs between and including the Begin Summary run and the End Summary run will be summarized by this report.

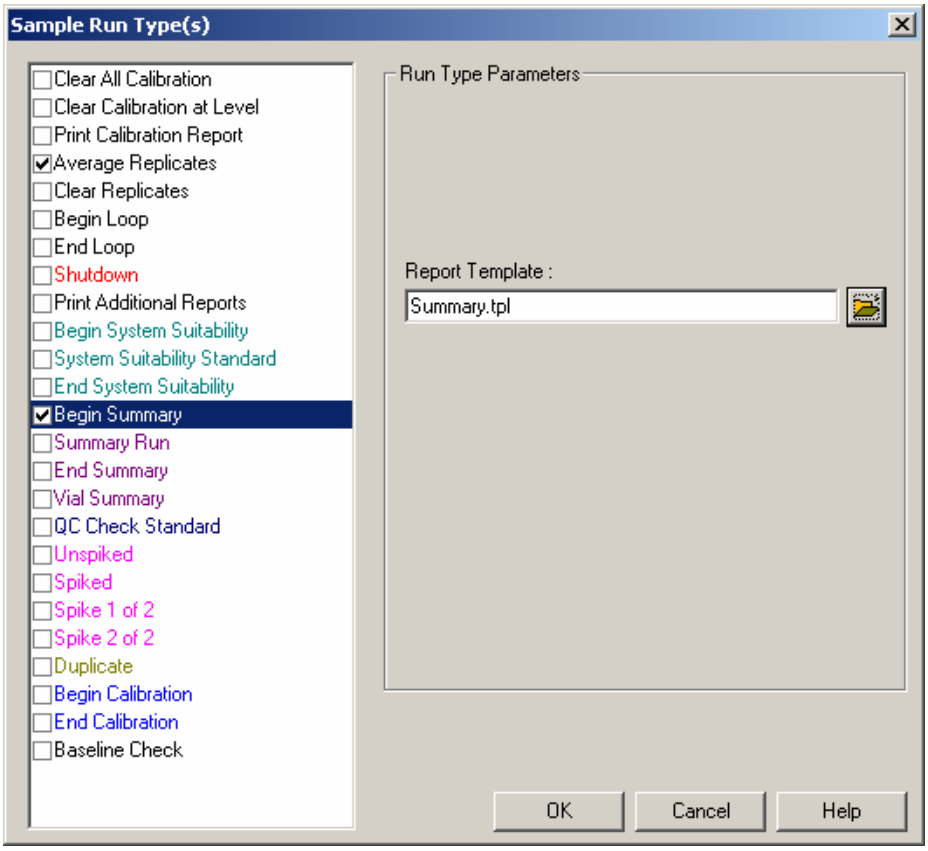


Figure 194. Sample Run Type(s) dialog box

To print sequence summary reports during the sequence, make sure you have selected the printing option when you start the sequence run.

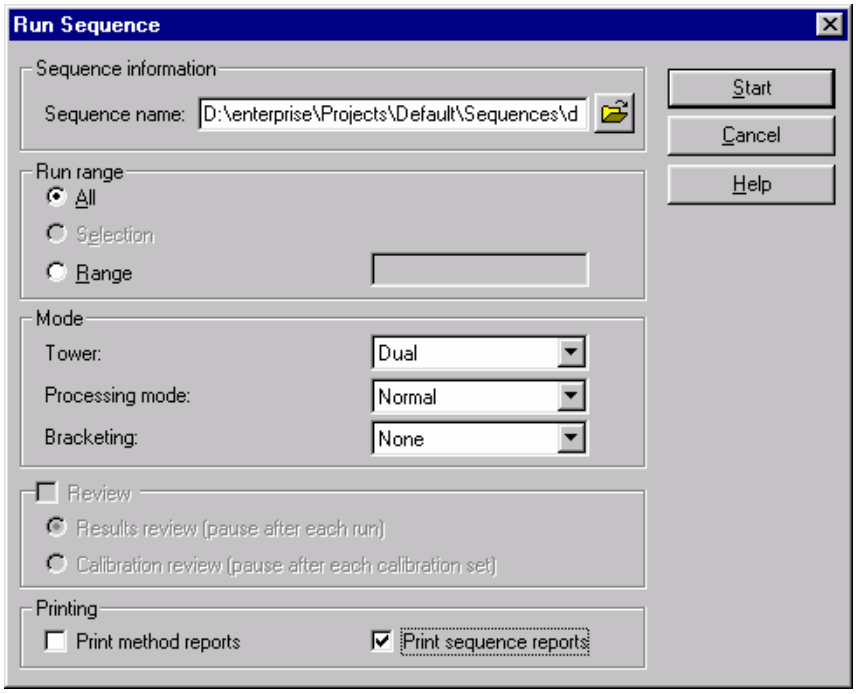


Figure 195. Run Sequence dialog box

- Export Summary

  If you wish to export the summary report information, select the **Export Summary** box in the **Sequence Properties** dialog. You must then designate a path for the summary export file.

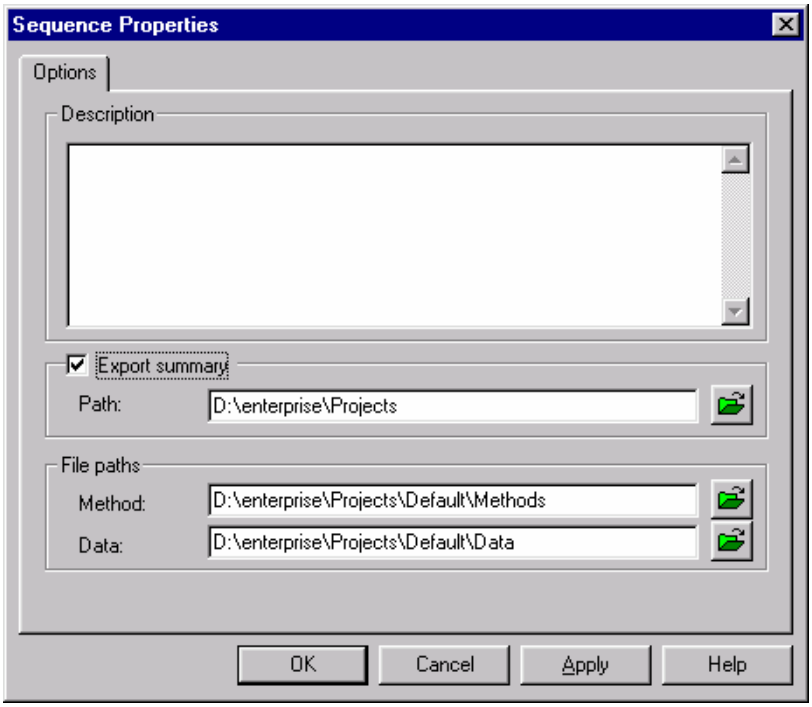


Figure 196. Sequence Properties - Options dialog box

The summary will be exported to a file entitled:

Sequence Summary - 000005000.txt

Where the numbers represent:

nBatchTower, nReportRunTypeID, nReportNumber.

Create a Sequence Summary Report Template

ChromQuest comes with a default sequence summary report template, SeqSummary.tpl, which can be used as-is or can be modified for your own use. To create or modify a sequence summary report template, use the **Advanced Report** generator.

Designating Sequence Summary Runs

In order for a run to be included in a sequence summary report, it must be designated as a **Summary Run** in the sequence. This is selected in the **Run Type** for each run. From the sequence spreadsheet, click on the **Run Type** button for a run. A dialog box will appear where you designate the type of run.

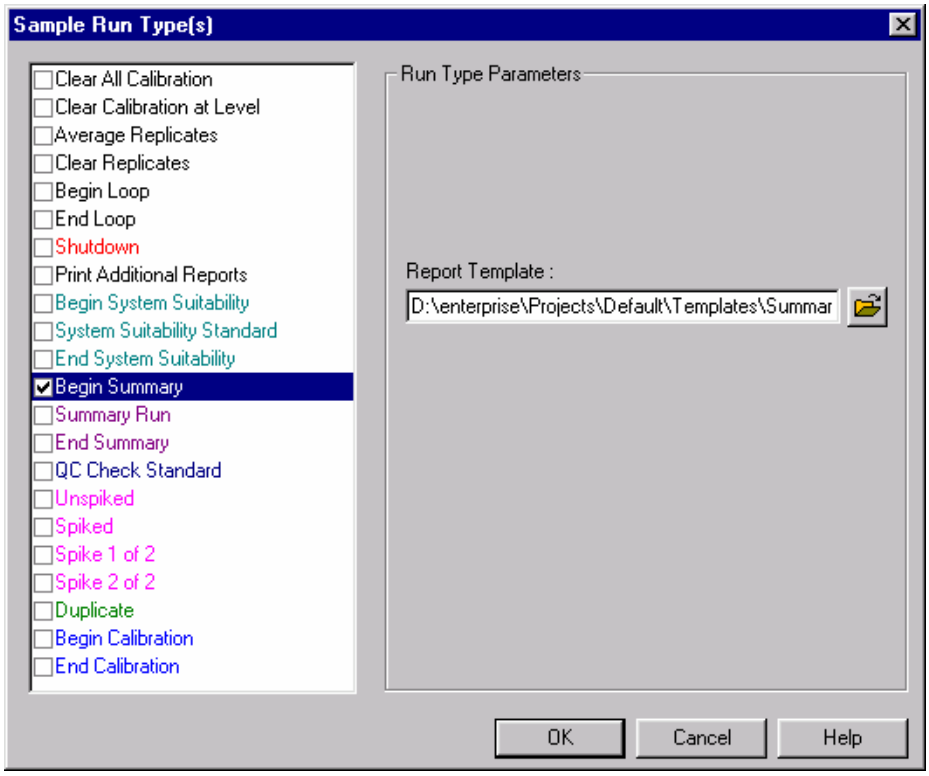


Figure 197. Summary Run Type(s) dialog box

If you want a run to be included in the summary report, click on the **Summary Run** box. If the run is the first run to be summarized, click on the **Begin Summary** box. When this box is selected, you must designate the name of the summary report template. If the run is the last run to be summarized, click on the **End Summary** box.

The Begin Summary and End Summary types allow you to generate more than one summary report within your sequence.

Turning on Sequence Summary Reports

Once you have completed the Sequence Summary dialog to set up the parameters to include in the Sequence Summary Report, turn on the sequence summary report printing by clicking on the **Print Sequence Reports** box in the **Process Sequence** or **Run Sequence** dialog.

See the Sequence Reports section for details on customizing sequence report templates. Sequence Reports will still be generated for viewing even if the **Print sequence reports** is not checked, however they will not be printed. To view the sequence reports, use the **Reports > View > Sequence Custom Reports** command. You can manually print the reports using the **Reports > Print > Sequence Custom Reports** command.

Saving a Sequence File

Once you have created or edited a sequence, you must save it on disk before you can use it to acquire or process data. To save the file using the current sequence file name, click on the **Save** button on the command toolbar. If you want to save the sequence using a new file name, use the **File > Sequence > Save As** command. A dialog will appear where you can either choose an existing sequence file name or enter a new filename to save the sequence.

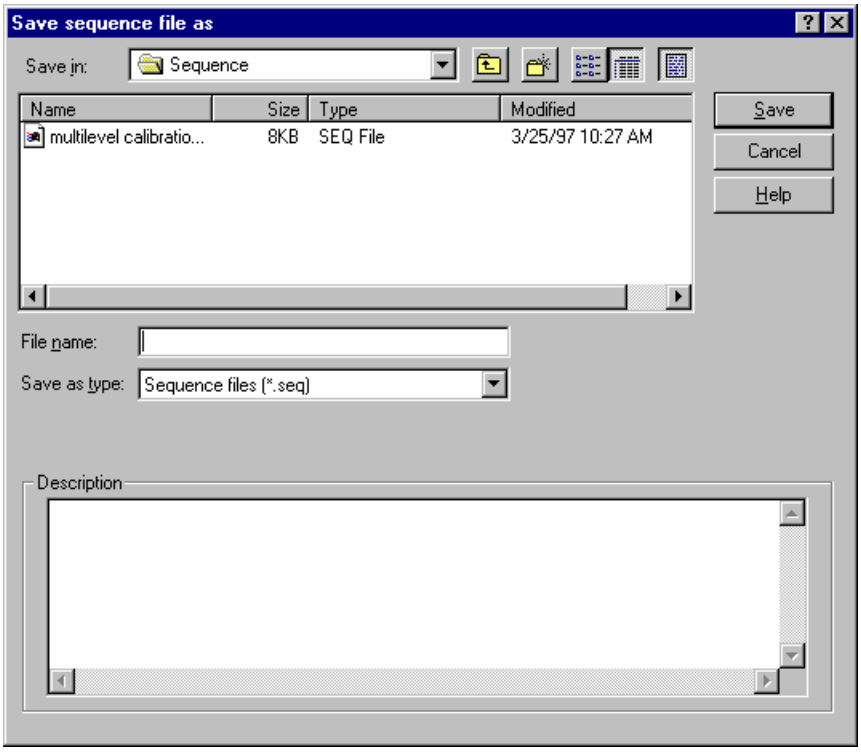


Figure 198. Save sequence file as dialog box

A listing of current sequence files is displayed. To view details of the files, click on the **Details** button at the top of the box. To view the description of the currently highlighted sequence file, click on the **description** button. To save your current sequence under a new filename, type the new name in the **File name** field and then click on **Save**. To save your current sequence using an existing filename, click on the file to highlight it. Then, click on **Save**.

Chapter 5 Custom Reports

One of the most important aspects of using a chromatography data system is getting the results into a useful format. While use of default standardized report formats is handy, often a standard report does not include enough information, or it contains information that is not necessary. The ChromQuest Custom Report editor allows you to create completely customized reports, or edit the standard reports to include only the information you want, presented in the most efficient way for your laboratory.

Using the Custom Report editor, you can create a custom report for your current method. The method custom report is saved as part of the method and will be used by default when you view or print the method custom report. The method Custom Report editor can also be used to create custom report templates which can be opened from other methods, making it easy to use the same report (or modify it) for other methods.

The Advanced Report editor lets you create advanced customized reports, including summary reports that can be viewed or printed. This advanced reporting editor uses a spreadsheet-like approach where formulas can be entered for creating completely customized display of simple statistics or complex mathematical computations for the data. A Wizard makes use of the Advanced Reporting features easy.

This chapter contains the following sections:

Anatomy of a Custom Report

A ChromQuest custom report can contain a wide variety of information, which can be displayed in any order. Because using the Custom Report editor is similar to using a word processing program, you can change fonts, colors, margins, insert chromatograms, graphics, system information, even multimedia sound and video clips.

In addition to free-form text, you can select a wide variety of information and objects to be placed in your report. These items are presented in three "categories" from which to choose: Field items, Graph items, Report items, and general Object items.

- **Field** items are individual fields of information related to the current data. File name, method name, and injection volume are examples of chromatography field items.
- **Graph** items generally contain groups of graphically related information that belong together. Chromatograms are examples of graph items. These items can be placed, moved, and edited independently of other items on the report.
- **Reports** are tables of information where the user defines the columns. A report table can include, for example, compound name, retention time, and concentration, and/or a variety of other sample-related items. Report tables can be formatted with fonts, centering, and decimal places. Reports are inserted as tables, because the number of rows is dynamic and can change from run to run.
- General **Object** items are non-chromatography objects that enhance or aid in the usefulness of your report. These objects can include bitmap graphics, Excel spreadsheets or graphs, audio or video clips.

Report Templates

Custom reports are templates for how data and objects are to be displayed and printed. A method custom report template is created and saved as part of a method, but it can also be saved as a template file that can be opened from within another method and used or edited.

ChromQuest comes with a suite of standard report templates for all types of reports. These can be used as-is or can be modified using the Method Custom Report editor and saved as new templates.

Sequence custom report templates are used for reporting data generated during batch sequence operations. For example, certain **Run Types** require a report template for reporting the data. When you designate a run as a **Begin Summary** run, for example, you will be required to designate a template name for the summary report. Other run types requiring templates include Suitability, QC Check Standards, and Duplicates.

Standard Report Templates

Standard templates for all reports are provided with ChromQuest, however you may wish to create your own or edit the templates provided. Standard report templates (Area %, External Standard, Internal Standard, and Normalization) are located in the *C:\ChromQuest* program directory and have the **.SRP** extension. You can create new standard report templates by saving your report template using the **.SRP** extension. Examples of the standard reports can be found in the **Standard Reports** section.

Sequence reports are created and saved using the Advanced Reports editor. Unlike method custom reports, sequence custom report templates are not saved as part of the sequence file, and therefore must be saved as a template file if you want to use a sequence report template to generate a report. Standard sequence report templates provided with ChromQuest (Calibration, Summary, Duplicate, QCCheckStd, Spike, and SysSuit), are located in the *C;\ChromQuest/Sequence* directory and have the **.BRP** or **.TPL** extension.

Method Custom Reports

A custom report is normally created and saved as part of developing a method. Once you have created a custom report within a method, it will be saved as part of the method. To create a **template** for the custom report, you must specifically save it as a template.

To access the custom report editor, click on the **Custom Report** button on the command ribbon, or choose the **Method > Custom Report** command. A custom report window will appear with either a "blank page" where you will create your report, or the current method custom report.

If you are familiar with using Microsoft Word, you will quickly become familiar with using the ChromQuest Custom Report generator. There are a number of features in this window to note.

- Ruler that shows position relative to the page. Black arrows that can be dragged to suit your needs indicate margins and indents.
- Command ribbon that contains frequently used commands for formatting. These buttons include bold, italic, underline, strike-through, color, left justify, center, right justify, view header/footer, add buttons, borders/shading, and zoom. When clicked, the formatting represented by these buttons will be applied to any text or item currently selected. Once a formatting button is clicked, it remains in effect for new text until the button is clicked again.
- Right-mouse click access to menus for inserting chromatograms, report objects, chromatography, method information, and electronic results signoff fields.
- Items inserted into the report must first be "activated" by clicking on them in order to edit or move them.
- Report tables can be removed quickly with a right-mouse click inside the table, followed by choosing the **Delete Table** command.

Your custom report can be generated by creating a new report, or by modifying an existing report. You can open an existing report template by choosing the **File > Report Template > Open** command, or by clicking on the **Open** button on the toolbar and then choosing the **Open Report Template** command. When you select a report template from the disk, it will over-write your existing custom report.

Short-Cuts for Custom Reports

There are a number of short-cut ways to customize the appearance of the information in your custom report. These include items such as setting various types of margins, forcing page breaks, and quick editing.

- Quick-Undo

  If you want to reverse, or "undo" an action you just performed, press CTRL+Z. The action (such as insert a field) most recently performed will be reversed. This is the same as doing a right-mouse click followed by the **Edit > Undo** command.

- Select all

  Press CTRL+A to select the entire custom report.

- Quick copy/cut/paste
  - Press CTRL+C to copy the selected item.
  - Press CTRL+V to paste the item.
  - Press CTRL+X to cut a selected item.

- Forcing a Page Break

  When you want to cause a certain element of your report to be printed always at the top of a new page, you should insert a "page break" before that item. To insert a page break on your custom report, position your cursor at the point where you want the new page to begin, then press CTRL+ENTER on your keyboard. This will force whatever follows in the report to be printed on a new page of paper when the report is printed. A page break is indicated on the custom report template by a dotted line that extends completely across the page. To remove a page break, move the cursor to just below the page break line, and press the BACKSPACE key until the page break is removed.

- Tab Stops

  Tab stops are used to align items on a page. By default, tab stops are set every ½ inch. However, you can move the tab stops so that there is room between items to accommodate changes (such as field items that change in length). To set a new tab stop, use your mouse to "insert" the tab onto the ruler bar at the top of the page, as described below. Once you have inserted a new tab stop, you can move it by clicking and "dragging" it to a new location on the horizontal ruler. To remove a tab stop, click on it and "drag" it completely to the left of the horizontal ruler.

  - Left Tab Stop

    Click on the left mouse button on the ruler at the location for the new tab. A left tab stop left-justifies tabbed text to that location.

– Right Tab Stop

  Click the right mouse button on the ruler at the location for the new tab. A right tab stop right-justifies tabbed text to that location.

– Center Tab Stop

  Hold the shift key down and click on the left mouse button on the ruler at the location for the new tab. A center tab stop center-justifies tabbed text to that location.

– Numeric Tab Stop

  Hold the shift key down and click on the right mouse button on the ruler at the location for the new tab. A numeric tab stop aligns the tabbed text to the decimal point.

Sequence Custom Reports

A sequence custom report is normally created and saved as part of developing a sequence. Sequence reports differ from method custom reports in that they report information generated as a result of running a sequence of runs instead of just a single run. Examples of sequence reports include Sequence Summary reports and Sequence QC reports. Because the report template used in a sequence custom report contains information that is relative only to a sequence, it is created using the Sequence Custom Report editor.

To access the sequence custom report editor, select the **Sequence > Custom Report** command. A custom report window will appear with either a "blank page" where you will create your report, or the current sequence custom report template will appear.

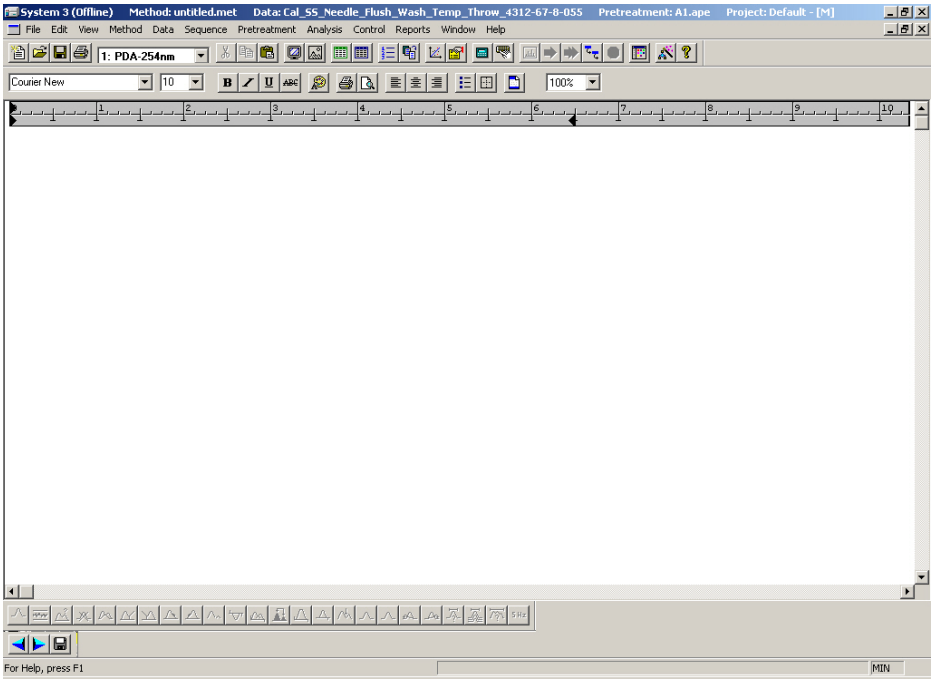


Figure 199. Sequence Custom Report window

If you are familiar with using Microsoft Word, you will quickly become familiar with using the Sequence Custom Report generator. There are a number of features in this window to note.

- Ruler that shows position relative to the page. Margins and indents are indicated by black arrows that can be dragged to suit your need.
- Command ribbon that contains frequently used commands for formatting. These buttons include bold, italic, underline, strike-through, color, left justify, center, right justify, add buttons, borders/shading, view header/footer, and zoom. When clicked, the formatting represented by these buttons will be applied to any text or item currently selected. Once a formatting button is clicked, it remains in effect for new text until the button is clicked again.
- Right-mouse click access to menus for inserting chromatograms, report objects, chromatography and method information.
- Items inserted into the report must first be “activated” by clicking on them in order to edit or move them.
- Report tables can be removed quickly with a right-mouse click inside the table, followed by selecting the **Delete Table** command.

Your custom report can be generated by creating a totally new report, or by modifying an existing report. You can open an existing report template using the **File > Report Template > Open** command, or click on the **Open** button on the toolbar followed by **Open Report Template**. When you select a report template from the disk, it will over-write your existing custom report.

General Text Information

Enter general text information on your report by simply typing it on the page. Use the formatting buttons on the ribbon to customize fonts and appearance of the text.

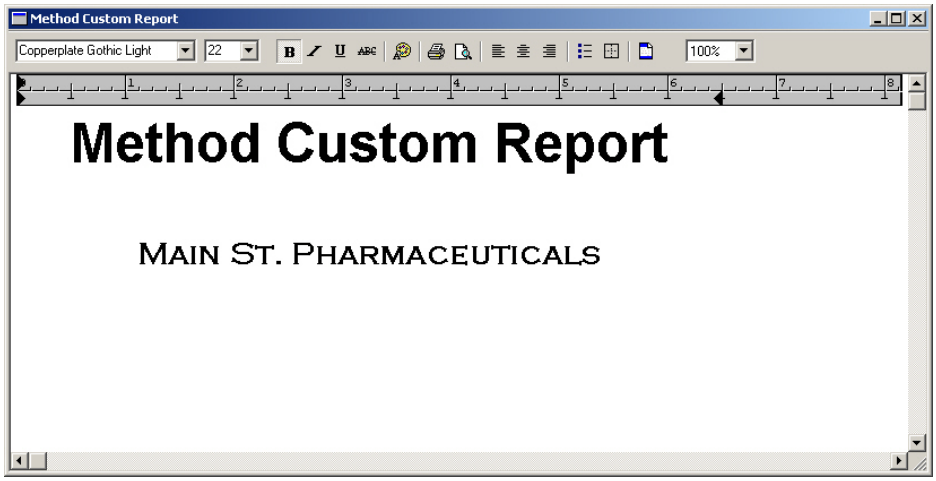


Figure 200. Method Custom Report window

General text information typed onto the page of the report will appear on the report in the location that it was typed. If you want information to appear as a header or footer (appearing on all pages of the report), you should enter the text information as a header or footer, as described below.

Setting Default Font

Fonts can be changed in one of two ways. If you want to set a default font that will be used unless you specifically change the formatting for a selected range of text, click on the right mouse button, followed by **Edit > Text**. Choose the **Font…** command to set the default font for the text you enter in your report.

The font selection is also available on the command ribbon. You can use the drop-down font selection to change the current font, or to quickly change a section of highlighted text without changing the default font. Use the color button, along with the Bold, Italic, Underline, and strike-through buttons to quickly format these aspects of the text.

Setting Justification

To set default justification for text, click on the right mouse button followed by **Edit > Text > Left Justify**, **Center Justify**, or **Right Justify** to set default justification for text on the report. These commands are also available on the command ribbon.

Adding Bullets

You can automatically add bullets to text by clicking on the **Bullets** button on the command ribbon, or by doing a right-mouse click and choosing the **Edit > Text > Bullets** command. When this button is activated, each new text paragraph will be added with a bullet.

Adding Borders and Shading

If you want to highlight an item on the report with a border or by shading it, select the item, then click on the **Borders and Shading** button, or do a right-mouse click, then choose the **Edit > Text > Borders and Shading** command. A short dialog will appear where you can designate where the border or shading will appear, and the %shading to apply.

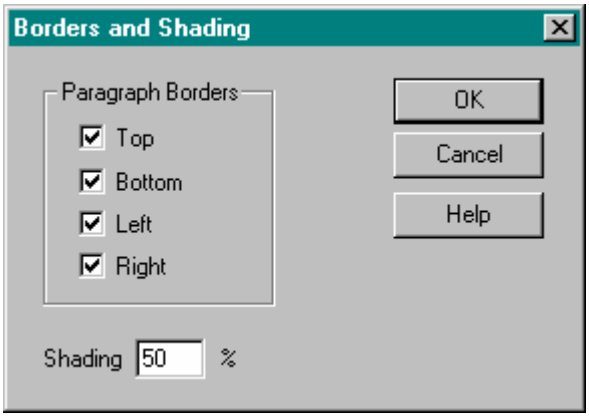


Figure 201. Borders and Shading dialog box

Setting Default Margins

To set margins for the report, click on the right mouse button. Then choose **Margin Setup** from the shortcut menu. A dialog will appear for you to enter the units (inches or centimeters) and margins for the page (right, left, top, and bottom).

Zoom Levels

Select the level of "magnification" for viewing your report by clicking on the **Zoom** button on the command ribbon, then selecting the %zoom level. You can "zoom in" to get a close-up view of your document, (increase %zoom) or "zoom out" to see more of the page at a reduced size (decrease %zoom).

Drawing Lines and Boxes

You can add emphasis to your report by drawing lines and boxes for important areas.

- Drawing a line

  To add a line to your report, click on the right mouse button. Then choose **Drawing > Line**. A line will appear at the location of your cursor. Activate the line by clicking on it with the mouse. A box will appear at each end of the line and the cursor will change to a "+". You can click and drag the line handles to change its size and orientation on the page (angle). You can also drag and drop the line by selecting it with your mouse, holding down the mouse button and dragging the line to a new location on the report.

- Drawing a box

  You can draw a box on your report using the **right-mouse click > Drawing > Rectangle** command. A rectangle will appear on the report where your cursor was located. To size the box, click on it, then drag the edges to the size desired. To move the box, click on the box to activate it, then move the cursor to just **outside** the box, where the cursor becomes a "+". Then hold down the mouse button and drag and drop the box in its new position

- Creating a Text Box

  If you want to add a box of text to your report, use the **right-mouse click > Drawing > Text Box** command. A rectangle will appear with a cursor where you can type text. This tool creates an independent text box in the location of your cursor. To move the text box, click on the text box to activate it, then move the cursor to just **outside** the text box, where the cursor becomes a "+". Then hold down the mouse button and drag and drop the box in its new position.

- Positioning Graphs Next to Tables

  You can use the Text Box function to move a chromatogram or other graph to a position next to a report table. To do this, first create a text box. Then click on inside the text box and insert a graph. Once the graph is inserted in the text box, you can use the "drag and drop" feature of the text box to move the chromatogram or graph to a position adjacent to a report table on your custom report.

- Undo Insert/Delete

  Once you have inserted an item onto your report (field, report, graph), you can "Undo" the action by pressing CTRL+Z on your keyboard.

Headers and Footers

If you want to enter information that will appear on every page of your report, click on the **View Header/Footer** button or click on the right mouse button anywhere in the report area, and then choose **Header/Footer**. This will allow you view and edit the header and footer area of your report. To edit the header or footer, click on your mouse in the displayed header or footer area and enter the information you want to include. Note that this area can contain free text as well as chromatography fields and objects. It can not include report tables or drawing objects.

When you have finished formatting the header and/or footer, you can turn off the display of the header and footer by again clicking on the **View Header/Footer** toolbar button, or the right mouse button, followed by **Header/Footer**. Note the check mark next to **Header/Footer** is turned off.

Cut, Copy and Paste Report Items

Once you have inserted an item onto your custom report, you can use the **Cut, Copy**, and **Paste** functions to move the item to another location on your report, or to another Windows application that supports the clipboard functions. These commands are located in the **Edit** menu on the menu bar.

Adding Fields to a Report

1. Click on the right mouse button anywhere in the report area. The following menus will appear that allow you to create specific information areas on your report.

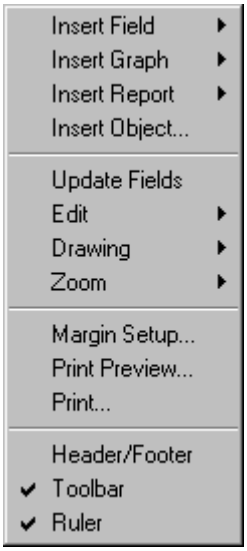


Figure 202. Report options menu

2. Choose **Insert Field**. The following menu of Field items will appear.

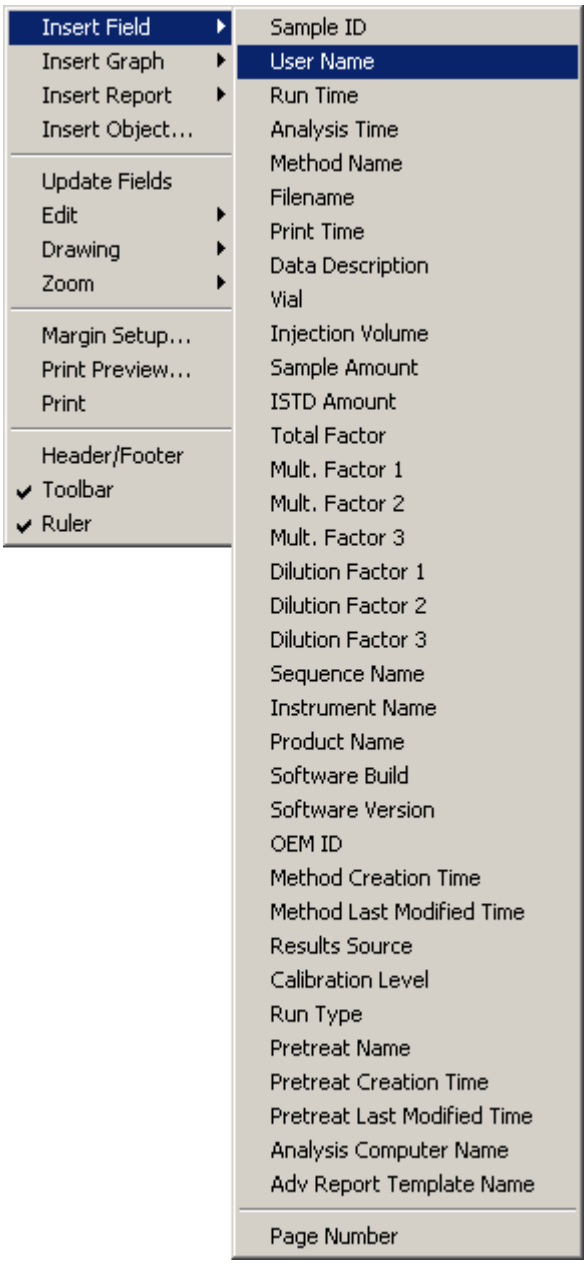


Figure 203. Report - Insert Field options menu

When you choose any of the Field items, it will be placed on your report at the current location of your cursor. These items can be placed individually, or they can be grouped together to create a custom area of chromatography information for your report. These items are updated to reflect current data information whenever a report is printed or viewed. They can also be updated by choosing the **Update Fields** command from the right-mouse click menu, or by pressing the **F9** key to update the current field.

Note The directory path shown for data files in reports is the originally specified directory path, even if the data has been subsequently moved.

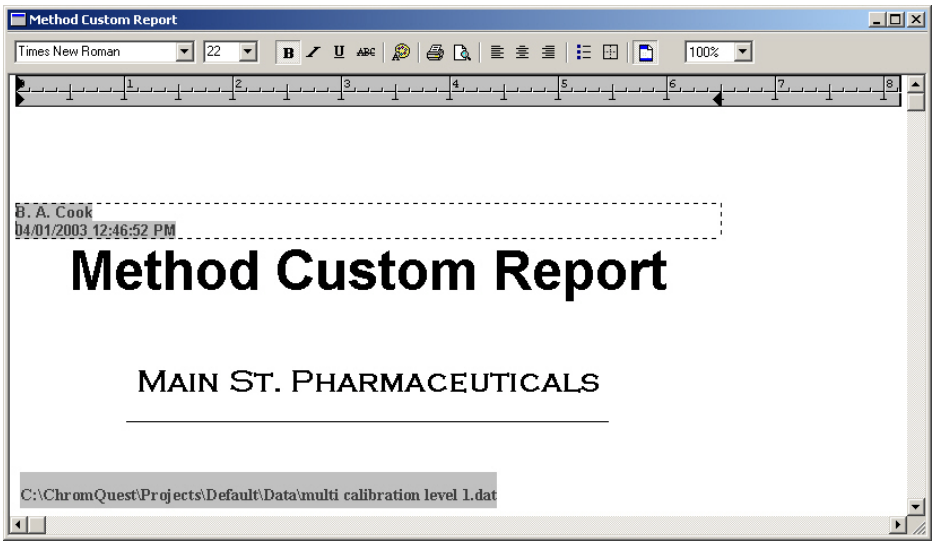


Figure 204. Method Custom Report window

Once a field is added to the custom report, you can move it, change the font, and the appearance. The formatting will remain constant, however the field information will change as each report is printed to reflect the current information.

Adding a Chromatogram to the Report

You can include as many chromatograms in your report as you wish. Each chromatogram can appear in its own region, or you can display multiple chromatograms, pump profiles, or graphs in the same region. You can include chromatograms that have been saved on disk, and you can include current data that will change as each run is acquired.

1. Move your mouse cursor over the **Insert Graph** command.

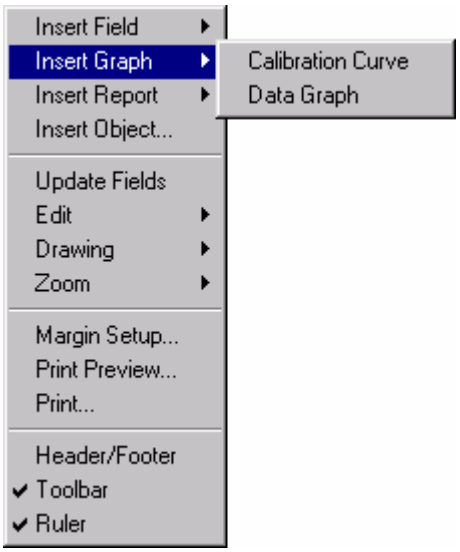


Figure 205. Report - Insert Graph options menu

2. Click on the **Data Graph** option to insert a chromatogram on your report where your current cursor is located. The following tab box will appear.

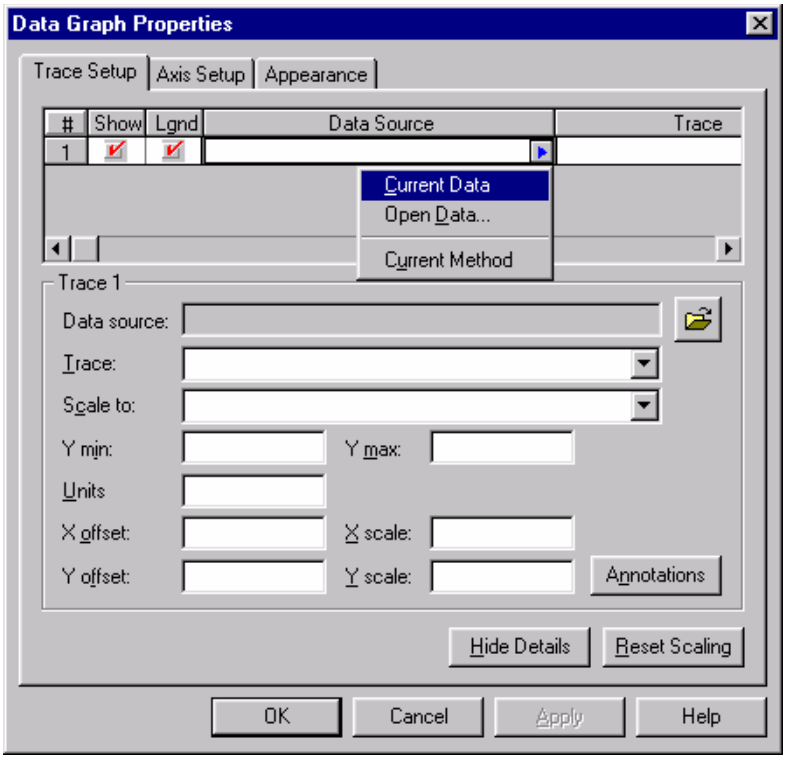


Figure 206. Data Graph Properties - Options menu dialog box

3. The spreadsheet allows you to add chromatograms or other traces to the current graph. Each row represents a trace that will appear in this graph. For each row, you must designate how you want the data to be displayed.

   • Data Source

     Click on the button adjacent to the field and select a source from the available data sources listed.

   – Current Data

     This selection allows you to select a trace from the current chromatography data.

   – Open Data

     This allows you to select a stored data file from which you can select a trace for display.

   • Trace

     Use the drop-down list to select the type of trace you want to display. Available traces from the data source specified will be presented from which to select.

     Use the **Show** and **Lgnd** check boxes to turn ON or OFF display of the trace or the legend.

   • Scale to

     Select one of the scaling options. Note that these work only after the trace has been analyzed.

     – Trace x

       Scales to another trace in the window.

     – Autoscale to largest peak

       Scales such that the largest peak is on scale.

     – Autoscale to 2nd largest peak

       Scales such that the 2^{nd} largest peak is on scale.

     – Autoscale to 3rd largest peak

       Scales such that the 3^{rd} largest peak is on scale.

  - User Defined

    Allows you to enter a value for Y max and min.

  - Normalized

    Allows you to normalize one trace to fit on the graph.

- Y min

  If you have selected a User Defined scale, enter a minimum value for the Y-axis.

- Y max

  If you have selected a User Defined scale, enter a maximum value for the Y-axis.

- Units

  Enter the units for display.

- Annotation

  Select the items you wish to have displayed as annotations on the graph.

- X offset

  Enter a value in units for offset of the X-axis.

- Y offset

  Enter a value in units for offset of the Y-axis.

- X scale

  Enter a multiplier, which will be applied to the X-Axis, if desired.

- Y scale

  If desired, enter a Y-scale multiplier, which will be applied, to the entire trace.

- Reset Scaling

  This button resets the scaling items in the trace setup tab to their default values.

You can select more than one item to be displayed (for example, multiple channels of data or a chromatogram with stored pump or oven profile). Each trace will appear as one row in the Trace Setup spreadsheet. Use the **Show** and **Legend** check boxes to turn on or off display of the trace itself or the legend.

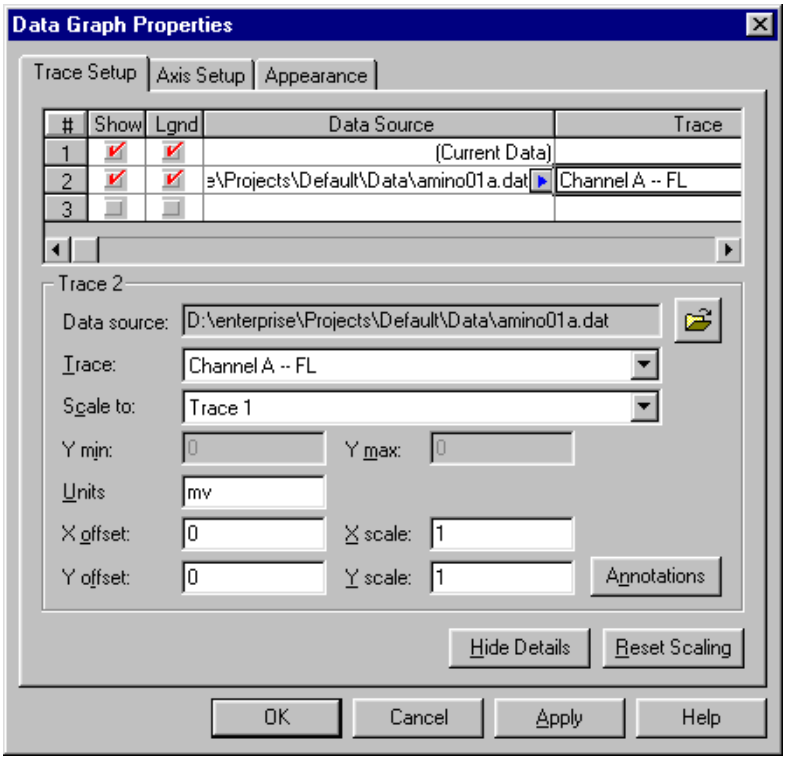


Figure 207. Data Graph Properties - Trace Setup dialog box

4. Click on **Axis Setup**. This tab allows you to designate a graph title and general options for all traces to be displayed.

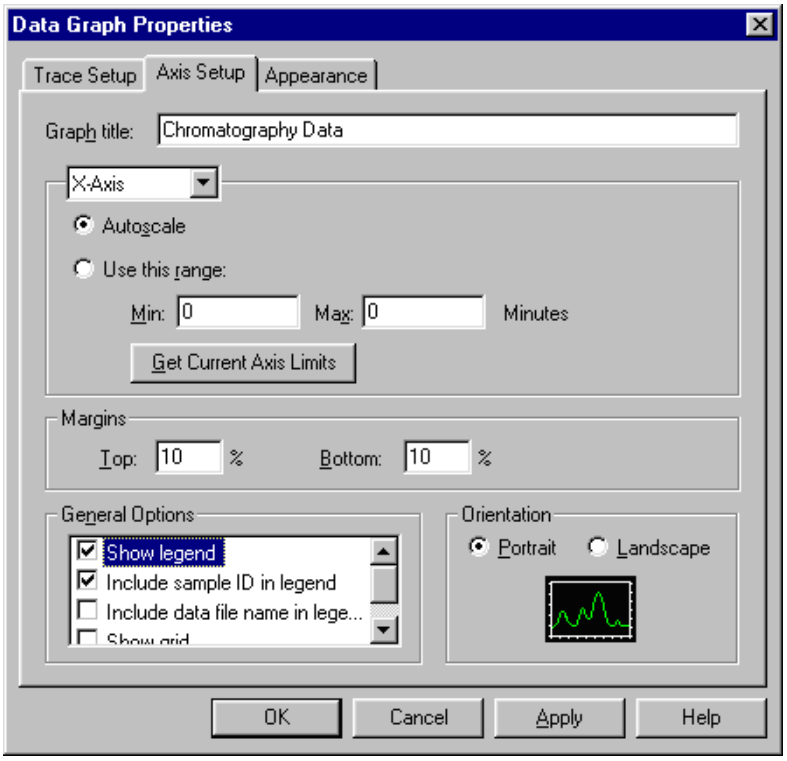


Figure 208. Data Graph Properties - Axis Setup dialog box

- Graph title

  Enter a title, if desired for the graph. This will appear at the top of the trace region on the report.

- Axis information

  From the drop-down list, select the axis to configure. You can select Left Y-Axis, Right Y-Axis, or X-Axis. For Y-Axis setup, you select a trace to use for the limits. If more than one trace is listed in the Trace Setup tab, you can select one of the traces to automatically use the Y-Axis limits from that trace for the graph.

  For X-Axis, you can choose to Autoscale (the entire trace shown), or you can use a specific range for display.

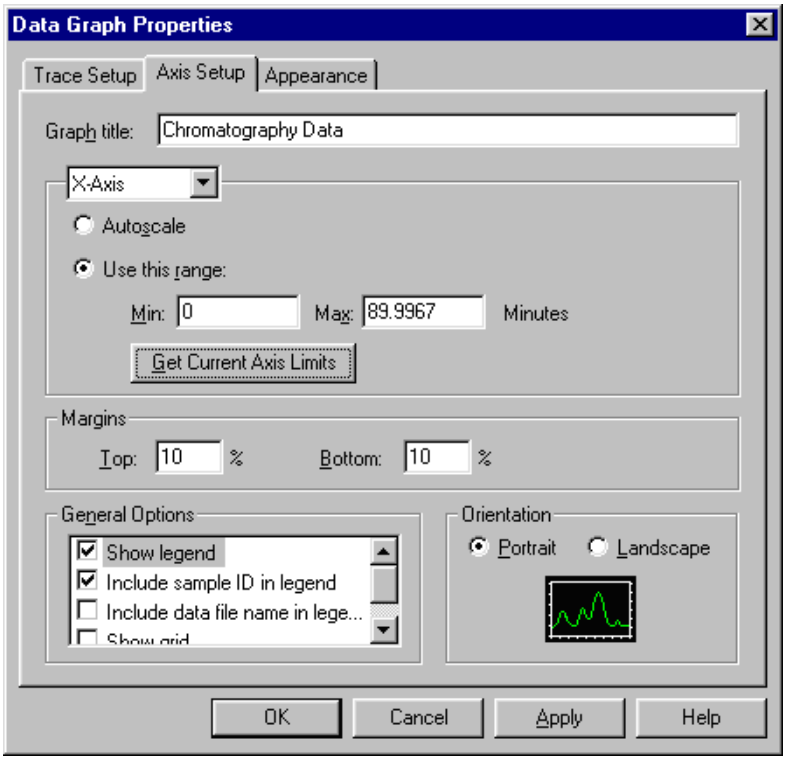


Figure 209. Data Graph Properties - Axis Setup dialog box

You can also click on the **Get Limits** button to bring in the limits for the data currently displayed in your method. (For example, if you have zoomed a region of the chromatogram and always want that region displayed.)

- Margins

  Enter a margin, in %, for top and bottom display of the graph.

- General Options

  Turn On or Off the **desired settings** by checking the adjacent boxes. These act as a master switch for legends and can be turned off for each trace using the check boxes in the **trace properties** tab.

- Orientation

  Select whether you want the graph printed in a **Landscape** or **Portrait** orientation relative to the page. This does not alter the orientation of printing the report on your printer - it affects only the selected graph.

5. Click on the **Appearance** tab to set the look of the graph.

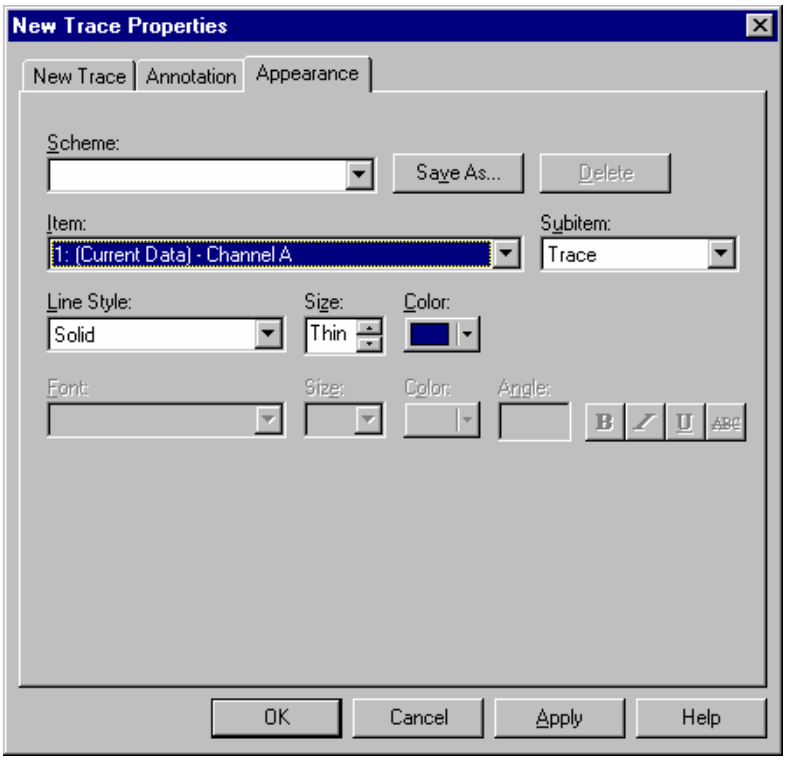


Figure 210. New Trace Properties - Appearance dialog box

- Scheme

  If you have previously saved an appearance scheme on disk, you can select it from this box. The **Save As…** button allows you to save the existing appearance scheme on disk by giving it a name. The **Delete** button allows you to delete the scheme and start again.

- Item

  This drop-down list lets you select which part of the chromatogram window for which you wish to change the appearance. The choices will include the graph itself (including background, axis setups, and legends), and the available traces.

- Sub-item

  Select the sub-item you wish to modify. The choices for this will change based on the item you have selected. For example, if the item selected is the Graph, you will have access to sub-items that include the background, axes and labels for the graph. If the item selected is a chromatogram data channel, you will have access to sub-items such as baselines, start and stop tic marks, and annotation.

When a sub-item is selected, you will have access to fields appropriate to that item. For example, if you have chosen the **baseline** sub-item, you can choose the color and line type. If you have chosen the **annotation** sub-item, you can choose the font appearance and color.

For details on items and sub-items, refer to **Chapter 1: Basics of Operation**.

To go back to the **Trace Setup** or **Annotation** tab, click on the top of the desired tab. When you have completed the tab dialogs, click on **OK** to add the selected traces to the current trace window on your report.

If you wish to modify any of these items again, click on the right mouse button anywhere in the trace box, and select the desired area from the menu shown.

Figure 211. Trace options menu

- Zooming the Chromatogram

  Once you have inserted a chromatogram onto your report, you can adjust the view by clicking on it to activate it, then zooming in the same manner as you can in the chromatogram window. To go back to the previous zoom level, double-click on the mouse in the chromatogram region on the report.

- Scrolling the Chromatogram

  Once you have zoomed in on a chromatogram, you can scroll the chromatogram to the right or left without losing the zoom. This is done by pressing the CTRL+SHIFT keys down and moving the mouse until the cursor changes to a "hand" and dragging it to the left or right.

You can also scroll the X- or Y- axis to view features that may be out of the range. To do this, press the CTRL+SHIFT keys down while the mouse cursor is outside the graph area, yet near the axis of interest. The cursor will change to an up/down arrow near the Y-axis, or a left/right arrow near the X-axis. Moving the mouse in this mode will scroll the graph up/down or left/right on the axis.

To restore the original view, do a right-mouse click in the chromatogram window, followed by the Full Unzoom command.

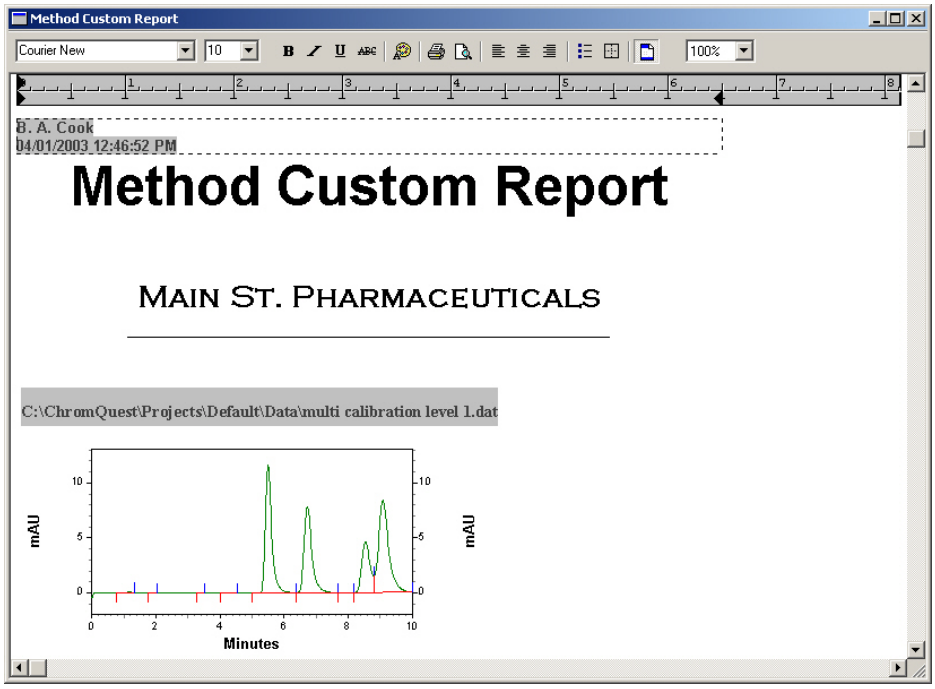


Figure 212. Method Custom Report window

Note If you insert Current Data into your report, it will be updated whenever your current data changes. Therefore, you can see the effects of integration by clicking the Analysis button while you are in the custom report screen.

Insert Report

There are a variety of report tables you can add to your custom report. To add a report table to your custom report, position your cursor on your report template at the location you wish to insert the report table, then do a right-mouse click, followed by **Insert Report…** A list of available report tables will be displayed.

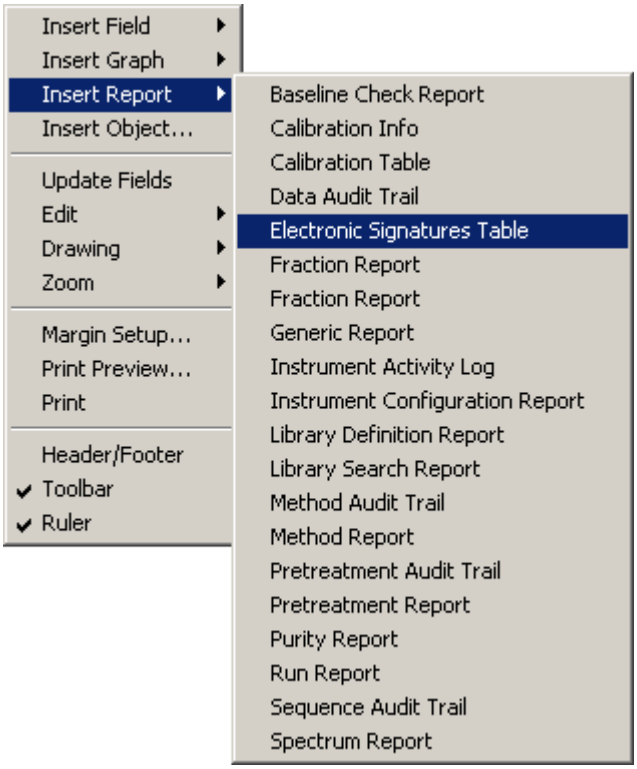


Figure 213. Insert Report options menu

- Calibration Info

  Use this command to insert a table containing details of your calibration.

- Calibration Table

  Use this command to insert a table containing the peak calibration details into your report.

- Data Audit Trail

  Use this to insert the data file audit trail table into your report.

- Electronic Signatures Table

  Use this command to insert the electronic signatures table into the report.

- Generic Report

  Use this command to insert a report table file that was created by a user program. A dialog requests you to enter the filename for the generic report file. You can also select the file by clicking the Open button.

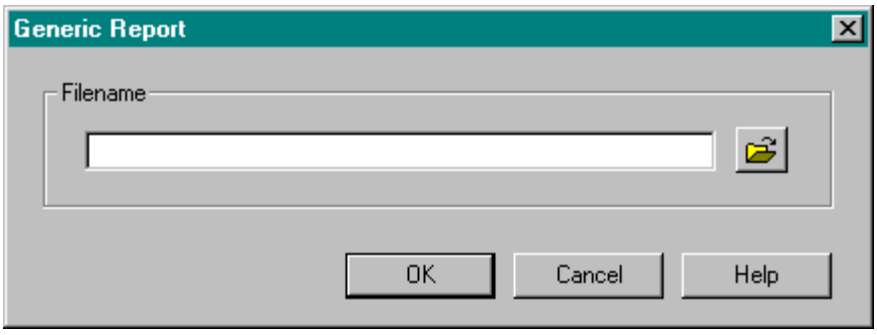


Figure 214. Generic Report dialog box

  For details on the required format for generic reports, contact Thermo Electron.

- Instrument Activity Log

  Use this command to create or update the template for viewing and printing Instrument Activity logs (LogViewInstActReport.lrp). If you insert this into a custom report, no activity log will be printed. The only way to print an Instrument Activity log is by choosing **File > Instrument Activity Log > Print All** from the command menu bar.

- Instrument Configuration Report

  Use this command to insert a configuration report for the instrument into your report.

- Method Audit Trail

  Use this command to create or update the template for viewing and printing the Method Audit Trail (LogViewMethodAuditReport.lrp). If you insert this into a custom report, no method audit trail will be printed. The only way to print a method audit trail is by choosing **File > Method > Audit Trail** to open the Method Audit Trail window. Then, right-clicking in the Method Audit Trail window and choosing **Print all**.

- Method Report

  Use this command to insert a report containing method details into your report.

- Pretreatment Audit Trail

  Use this command to insert the audit trail for the autosampler pretreatment file into your report.

- Run Report

  Use this command to insert a table containing chromatography results into your report.

- Spectrum Report

  Use this command to insert the spectrum report into your custom report.

Method Reports

To add details about the current method to your report, right-click in the method custom report to open a shortcut menu. From the shortcut menu, choose **Insert Report > Method Report**. A dialog box appears where you select the items from the method you wish to include in your report.

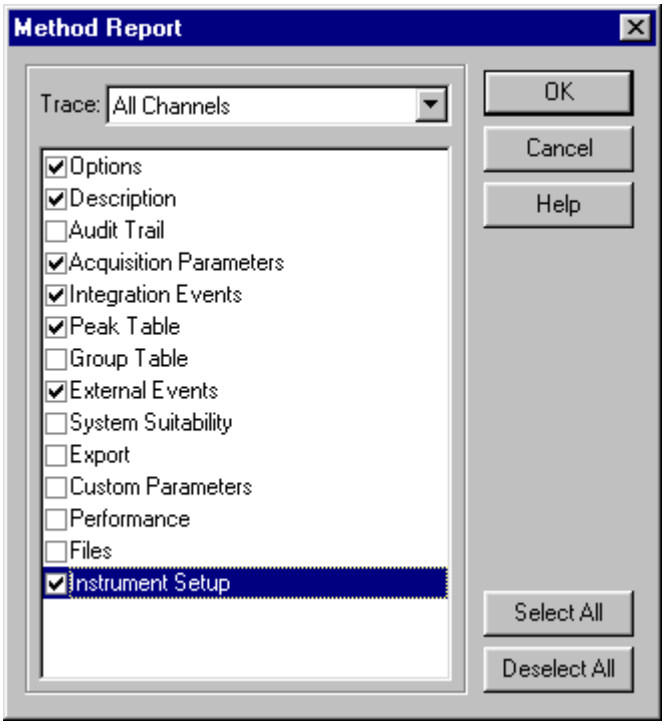


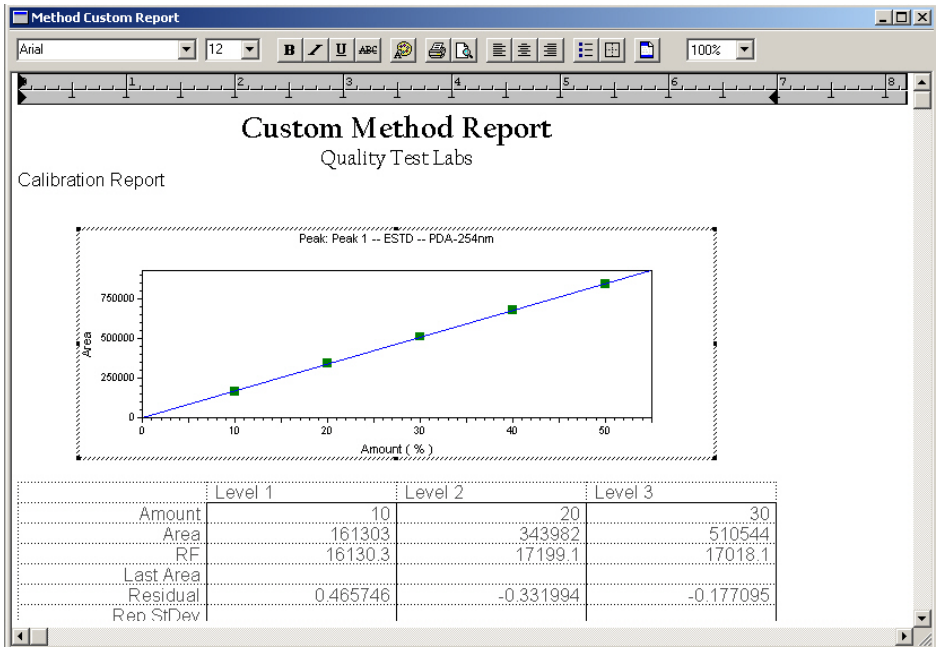
Figure 215. Method Report dialog box

Use the drop-down menu to select the channel(s) from which to display method information. Then select the method items to display by clicking the check-boxes adjacent to the desired items. When finished, click on OK.

A method report can be deleted from the custom report template by a right-mouse click inside the table, followed by the **Delete Table** command.

Calibration Reports

ChromQuest provides a standard calibration report template that will be used whenever you request a printout of calibration information. Therefore, it is not necessary to create a calibration custom report in order to print or view this information. The calibration report template, **Calibration.crp** contains Calibration Information, Calibration Curve, and Calibration Table sections. This report template can be opened using the **Open** button or the **File > Report Template > Open** command.



	Level 1	Level 2	Level 3
Amount	10	20	30
Area	161303	343982	510544
RF	16130.3	17199.1	17018.1
Last Area			
Residual	0.465746	-0.331994	-0.177095
Rep StDev			

Figure 216. Method Custom Report - Calibration Report window

You can also add any of the three calibration report sections independently to your method custom report.

Note These sections are not available in Sequence Custom Reports.

Adding a Calibration Curve to a Custom Report

1. Click on the right hand mouse button anywhere in the Method Custom Report window. Choose the **Insert Graph > Calibration Curve** command.

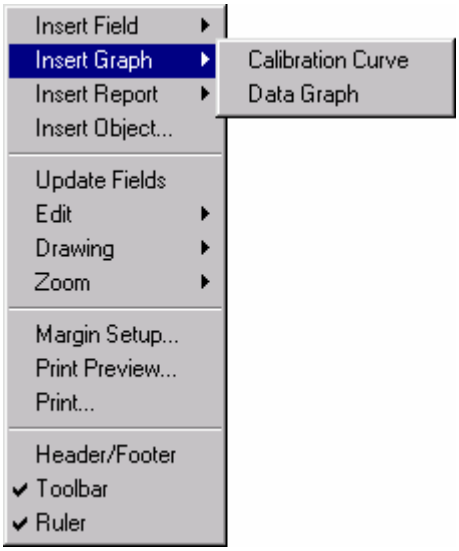


Figure 217. Method Custom Report - Insert Graph options menu

The following dialog box will appear.

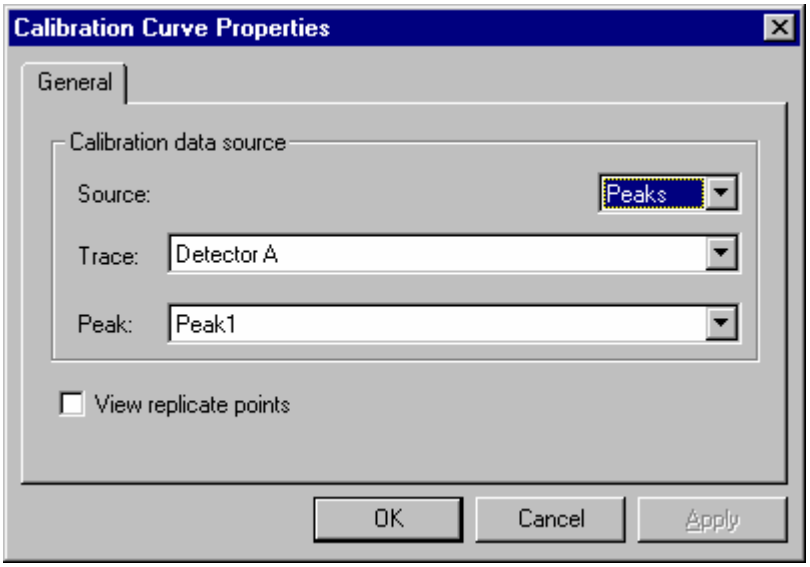


Figure 218. Calibration Curve Properties - General dialog box

2. In the Calibration Curve Properties dialog box, make the appropriate selections.

   - Calibration data source

     Select where you want the data for the calibration curve to come from.

– Source

Select the source of the calibration curve data from the **Calibration Data Source/Source** drop-down list. Select **Peaks** or **Groups** to select what calibration peak table to use.

– Trace

For the calibration curve trace, select the specific data to be displayed. When you select a data channel for the trace, you must then select a **Peak** from the list. This peak will always be displayed.

You should select **Template** only if you are editing a calibration report template. If you select this option within a method report template, the information will not be printed. If you select **Template**, calibration curves for all peaks in the calibration will automatically be printed as defined in the **.crp** calibration report template file.

- View replicate points

  Click on this box if you want replicate data points to be displayed on the report calibration curve.

3. Click on **OK** to enter the calibration curve into your custom report.

Adding Calibration Information to a Custom Report

You can add just the calibration information into your custom report using the right-mouse click **Insert Report > Calibration Info** command. When you select this command, a dialog will appear where you can specify what information to show.

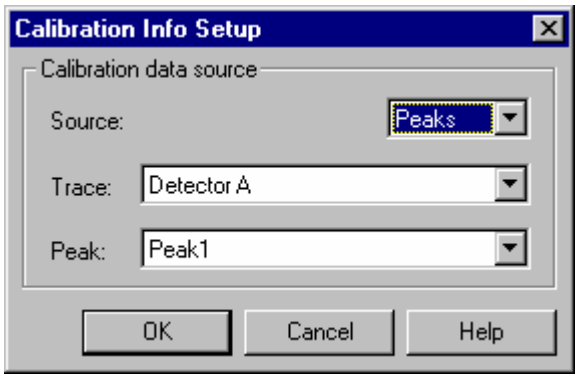


Figure 219. Calibration Info Setup dialog box

- Calibration data source

  Select where you want the data for the calibration information to come from.

– Source

  Select the source of the calibration information from the **Calibration Data Source/Source** drop-down list. Select **Peaks** or **Groups** to select what calibration peak table to use.

– Trace

  Select the specific data to be displayed. Select a data channel for the trace, then select a **Peak** from the list. The calibration information for the selected peak will always be displayed, as in the example below.

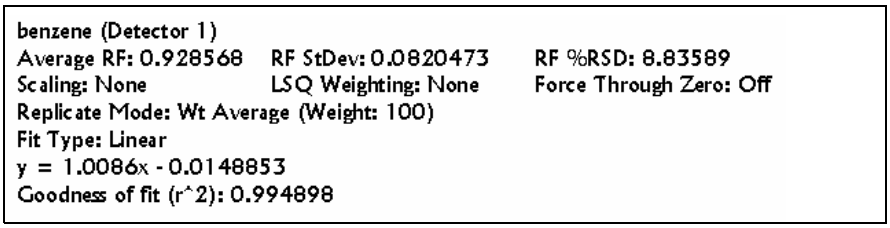


Figure 220. Calibration information for the selected peak

  Select **Template** only if you are creating or editing a calibration report template. If you select this for a method custom report, the information will not be printed. When you select **Template**, calibration information for all peaks in the calibration will automatically be printed, as defined in the **Calibration.crp** calibration report template file.

Click on **OK** to add the calibration information to your custom report.

Adding a Calibration Table to the Custom Report

The **right-mouse click > Insert Report > Calibration Table** command brings up a dialog box where you designate information to be included in a Custom Report calibration table.

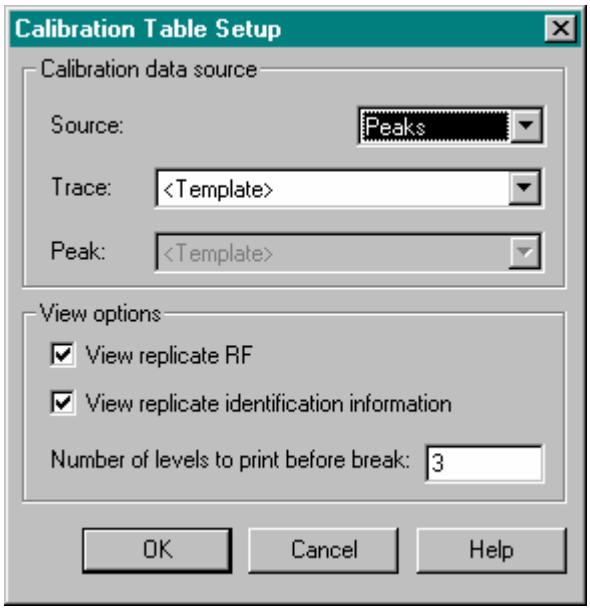


Figure 221. Calibration Table Setup dialog box

- Calibration data source

  Select where you want the data for the calibration table to come from.

  – Source

    Select the source of the calibration table from the **Calibration Data Source/Source** drop-down list. Choose **Peaks** or **Groups** to select what calibration peak table to use.

  – Trace

    Select the specific data to be displayed. Select a data channel for the trace, then select a **Peak** from the list. The calibration table for the selected peak will always be displayed, as shown in the following example.

	Level 1	Level 2	Level 3
Amount Ratio	0.05	0.1	0.15
Area Ratio	0.0670692	0.116176	0.172302
RF	0.745498	0.860765	0.870566
Last Area Ratio	0.0670692	0.116176	0.172302
Rep StDev	0.00148804	0.00332358	0.0019511
Rep %RSD	2.21866	2.86082	1.13238
Rep 1 Area Ratio	0.066017	0.118526	0.170922
Rep 2 Area Ratio	0.0681214	0.113826	0.173681

Figure 222. Calibration data for the selected peak

Do not select **Template** unless you are creating or editing a calibration report template. If you select **Template**, calibration tables for all peaks in the calibration will automatically be printed as defined in the .**crp** template.

- View options
  - View replicate RF

    Click on the **View Replicate RF** box if you want to display response factors for all replicates in the calibration table.

  - View replicate identification information

    Click on this box if you want to display the User, Sample ID, and Calib Time for each replicate.

  - Number of levels to print before break:

    Enter the number of calibration levels to print on each line.

Click on **OK** to accept and enter the calibration table on your custom report template. A calibration table can be removed quickly with a right-mouse click inside the table, followed by selecting the **Delete Table** command

Run Reports

Results can be added to your method custom report by inserting a **Report**. To add a report to your custom report, click on the right mouse button, and choose **Insert Report**. Note that the available reports for the sequence custom report are different that those available for the method custom report.

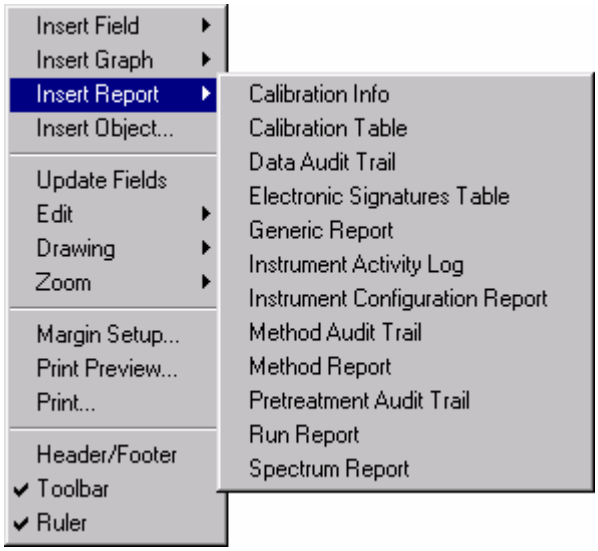


Figure 223. Method custom report - Insert Report options menu

Choose **Run Report**.

Report Properties

The Run Report dialog box appears where you can designate items for your report.

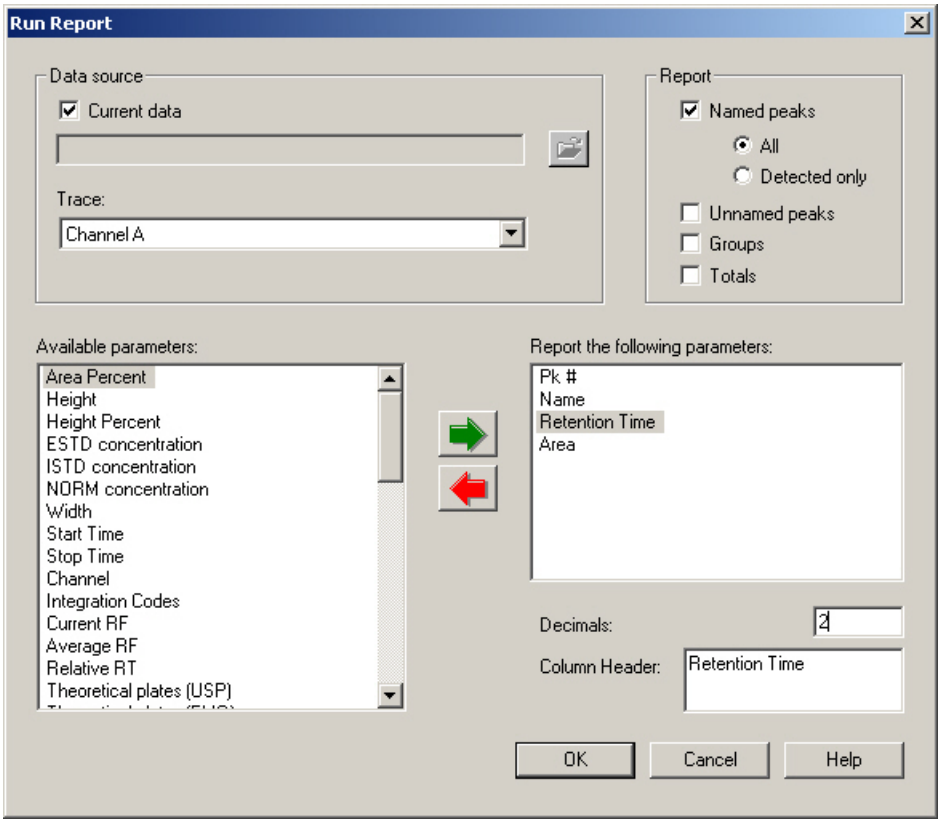


Figure 224. Run Report dialog box

- Data source

  Select the Data Source (current data or stored data file) and the Channel from which you want to display information.

- Report

  Use the check boxes to select what peaks you want to report.

  – Named peaks

    Choose **All** if you want to report all calibrated peaks, whether detected or not. Choose **Detected Only** if you want to report only the calibrated peaks detected in the sample.

  – Unnamed peaks

    Click here if you want to include uncalibrated peaks in your report.

- Groups

  Click here if you want to include defined groups in your report.

- Totals

  Click here if you want to include totals for numeric columns in your report.

- Available parameters

  A list of the possible parameters to report is displayed in this box. To add one of the parameters to your report, double-click on it, or click on it with the mouse to select it, and then click on the Green arrow to move it to the **Report the following parameters** box. You may select one or more of the parameters at a time by holding down either the SHIFT key (to select contiguous parameters), or the CTRL key (to select **non-contiguous parameters**.)

- Decimals

  For each numeric parameter, you can enter the number of decimal places to be displayed. Click on the parameter in the **Report the following parameters** box, then type the number of decimals to be shown for that parameter. Default decimals will appear for all numeric parameters.

- Column Header

  Use this box to enter or edit the title that will appear at the top of the column.

Click on **OK** to insert the report table into your custom report at the current location of your cursor. The report table will appear on your custom report page, with column headings and data placeholders shown.

Report tables can be removed quickly with a right-mouse click inside the table, followed by selecting the **Delete Table** command.

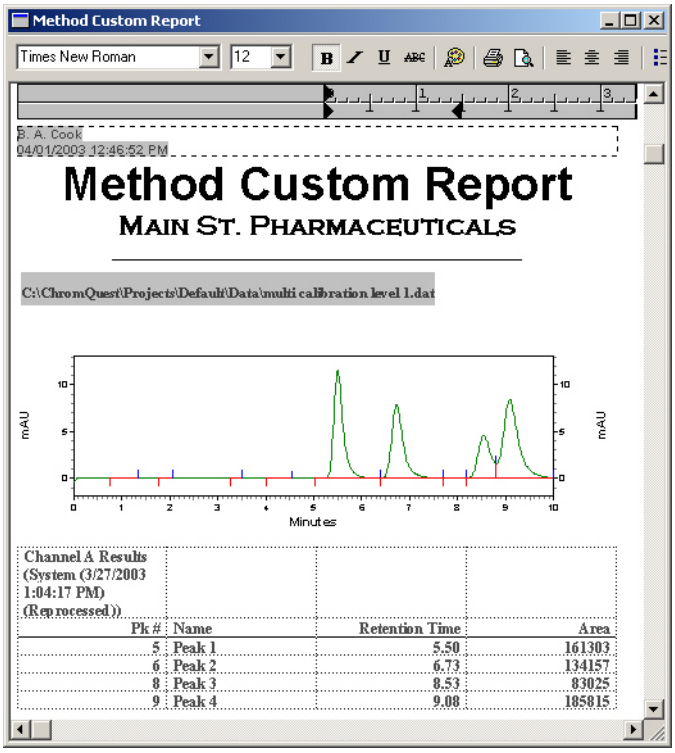


Figure 225. Method Custom Report window

You can format the fonts and appearance of the report table by modifying the appearance of the table on the page.

- Change Column Width

  To change a column width, move the cursor over the border of the column until double vertical lines appear. Hold down the mouse button and drag the column border to the width you require.

- Change Fonts

  To change a font for text in the table, select the text by highlighting it with the mouse, or click once in the field. Then choose one of the formatting buttons to change the formatting to desired appearance. You can change formatting for both column headings and sample data areas.

- Change Column Titles/Headers

  ChromQuest will enter default titles for each parameter selected for the report. You can change the column titles by editing them in the **Column Header** box in the **right-mouse click > Report Properties** dialog. You cannot change the data fields other than formatting changes.

The right-mouse click gives you access to commands for modifying the report table. A right-mouse click executed in the report title row gives the following menu.

Figure 226. Method custom report - Report Properties options menu

Change Report Parameter

This command allows you to change a report parameter without re-defining the report. Right click the mouse on the title of one of the report parameters. Select the **Change Parameter** command to delete the current parameter and select a new parameter to take its place in the report.

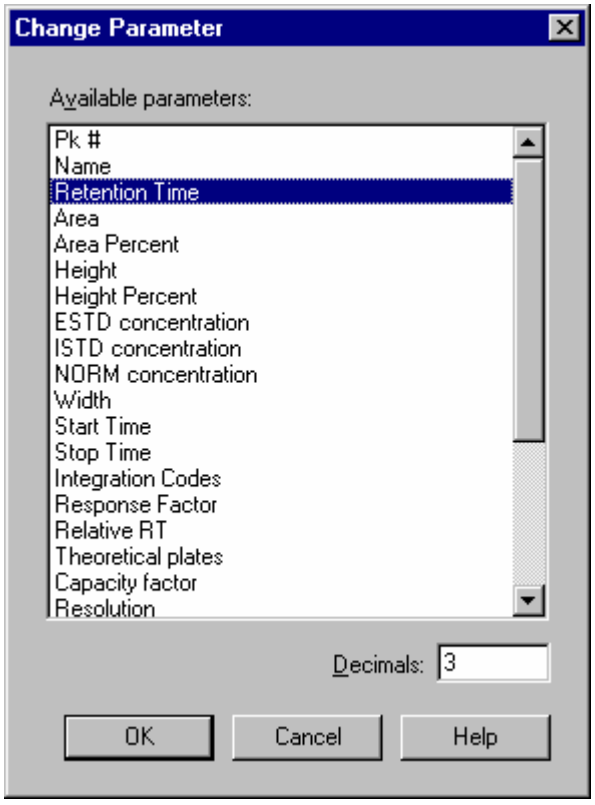


Figure 227. Change Parameter window

The current parameter is selected. To change it, simply click on another parameter in the list, and then click on **OK**.

Text This command gives you access to changing the default report table font, and justification for the report.

Show Report Data at Design Time By default, ChromQuest displays template fields when you create report tables in a custom report, without the actual data displayed. You can view the actual data using the **right-mouse click > Print Preview** command. If you prefer to always view the report table data as you develop your custom report, select the **right-mouse click > Show Data at Design Time** option. When this is enabled, the data for the report table will always be displayed.

Delete Table This command removes the current table from the custom report template.

Report Properties… This command brings up the Run Report dialog box. You can use it to re-define or add items to your report.

Placing a Graph Next to a Report

To insert a chromatogram (or other graph) adjacent to a report on your custom report template

Name	Retention Time	Area
Peak1	5.729	779320
Peak2	6.568	667324
Peak3	8.283	633854
Peak4	8.540	994037
Totals		
		3074535

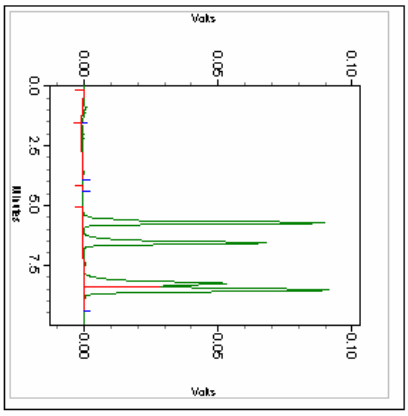


Figure 228. Graph placed next to a report

1. Draw a text box on your report using the **right-mouse click > Drawing > Text Box** command.
2. Use the **right-mouse click > Insert Graph >Data Graph** command inside the text box to insert a chromatogram (or other graph) into the box.
3. To move the text box with the chromatogram/graph inside, click on the border of the text box until the black rectangular “handles” appear. Then move the cursor just outside the border until the cursor turns to crosshair “+”. Then hold down the left mouse button and drag the text box with the graph a new location (for example, next to a report) and then release the mouse button.

4. To edit the chromatogram/graph (annotations, etc.), double-click the mouse within the chromatogram/graph. Make sure you have selected the chromatogram and not the text box. To re-size the chromatogram, select the text box (look for the text box "handles"), then click and drag the handles to the desired size.

Note You can not place two reports side-by-side.

Inserting an Instrument Activity Report

To add a copy of the method instrument activity log, select the **right-mouse click > Insert Report > Instrument Activity Log** command. A table for the instrument activity log will be inserted on your custom report. This table is a template placeholder and will not display the actual instrument activity report until the custom report is printed.

User	Logged	Activity
Demo	01\02\99 12:34:56	What was changed and how

Figure 229. Instrument activity log table

Inserting an Instrument Configuration Report

To add a listing of the current instrument configuration, choose the **right-mouse click > Insert Report > Instrument Configuration Report** command. A table for the instrument configuration report will be inserted onto your custom report template. The instrument configuration information can be viewed using the **right-mouse click > Print Preview** or when the report is printed.

```
Instrument Configuration
========================
  Instrument Name: Instrument 1
  Instrument Type: Generic System

  External Events:
    Name          Source      Configuration
    ____          ______      _____________
    Trigger       SS420       Board: 0, In0, State: Closed

Detector Name: Detector A - Channel A
Detector Model: Analog
Acquisition Source:      SS420
        Board: 1, Analog Input: Chan A
Y-Axis Units:    Volts
Y-Axis Multiplier:       0.00
```

Figure 230. Instrument configuration information

Inserting a Method Audit Trail Report

You can insert a listing of the current method's Audit trail using the **right-mouse click > Insert Report > Method Audit Trail** command. A table template for the audit trail report will be inserted on your custom report template. This table is a template placeholder and will not display the actual audit trail report until the custom report is printed.

User	Logged	Source	Activity	Reason
Demo	01\02\99 12:34:56	Source of change	What was changed and how	Why it was changed

Figure 231. Audit trail report table template

Electronic Signatures in Custom Reports

To add an electronic signature table to your report, do a right-mouse click in the report, then choose **Insert Report > Electronic Signatures Table**. This command will insert a table that contains the electronic signatures for this data file into your report.

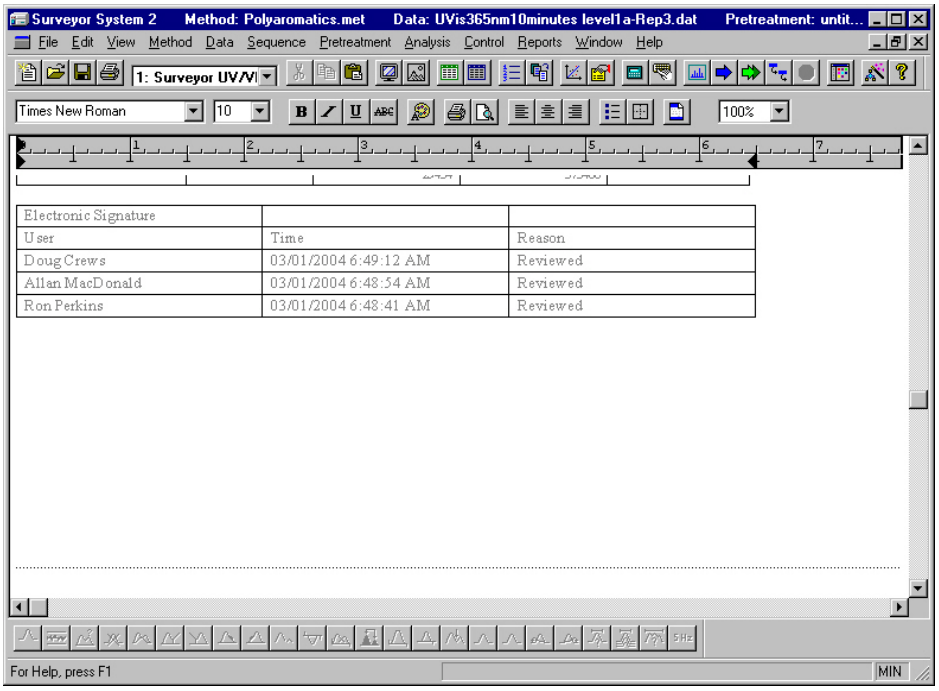


Figure 232. Electronic Signatures in Custom Reports window

Sequence Reports

To create a custom report that contains sequence information, such as a Sequence Summary report, use the Advanced Reports function (**File > Advanced Reports > New**). The Advanced Reports application enables you to create a Sequence Summary template (.tpl file) that can be used to generate a standard report for any sequence. Because the template is not saved as part of a sequence, it can be used independently for summarizing data from any sequence.

For details on how to create a Sequence Summary template, refer to Table Wizard topic in "Advanced Reporting" on page 299.

Viewing a Sequence Report

To view or print a complete sequence report, you must first process a sequence where one or more of the files in the sequence have Run Types designated as **Begin Summary** and **Summary Run**. The first run of to be included in the summary report is designated as a **Begin Summary** run, and indicates the summary report template to be used for the summary report.

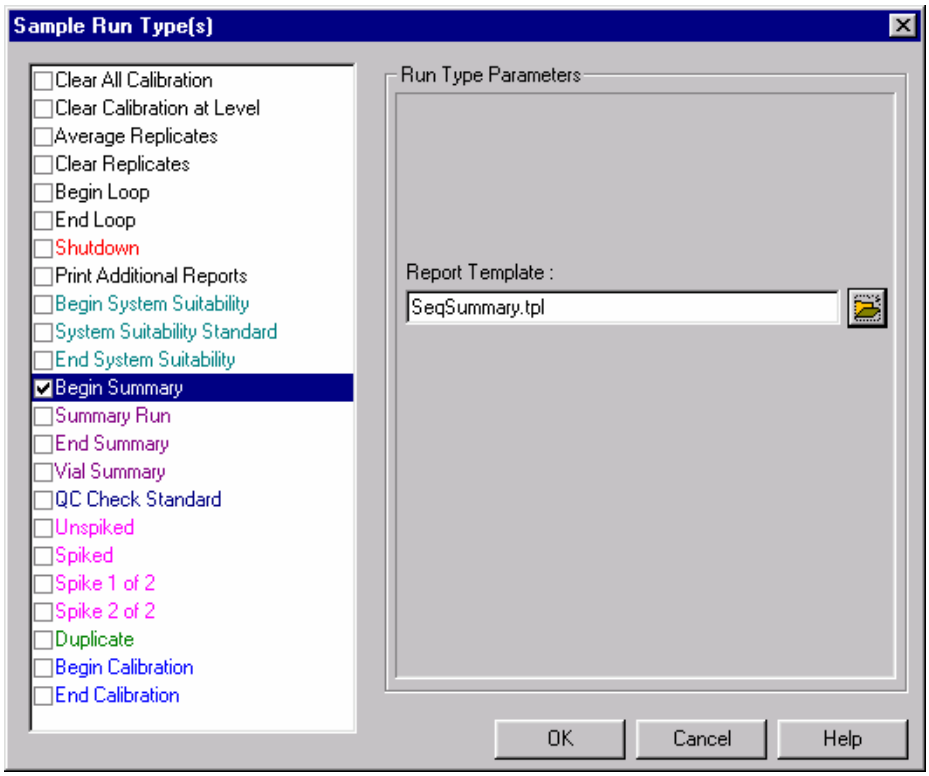


Figure 233. Sample Run Type(s) window

Runs after the **Begin Summary** run with **Summary Run** types will be included in the same summary run until another **Begin Summary** type is encountered in the sequence.

Once the sequence has been processed, you may view the current summary report by choosing the **Reports > View > Sequence Custom Report** command. A box appears where you can select from available custom reports (if more than one summary report has been generated).

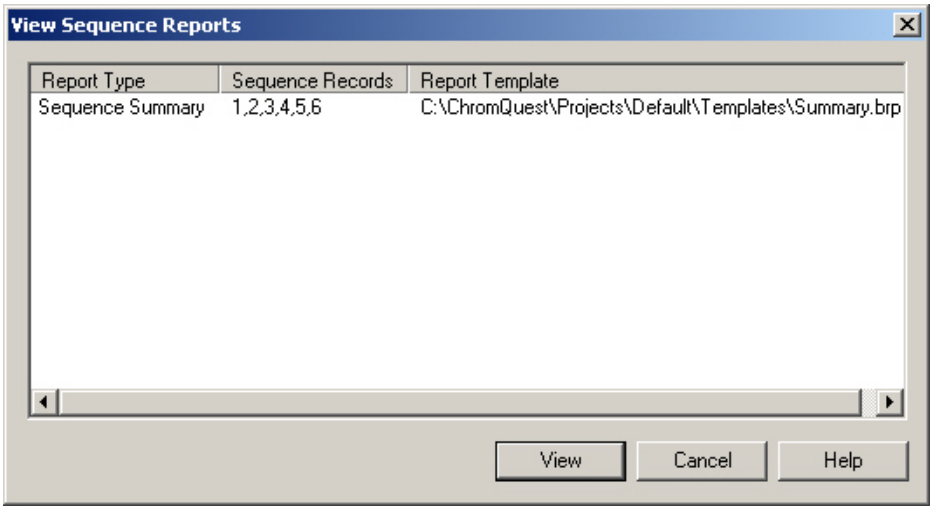


Figure 234. View Sequence Reports window

Select the report you wish to view (or print). The report then is displayed.

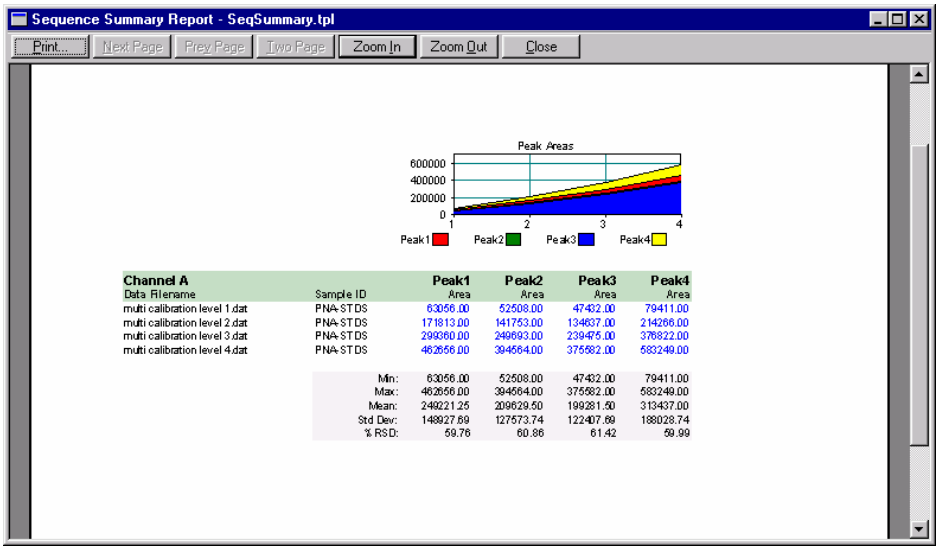


Figure 235. Sequence Summary Report window

Note In order to display a **Sequence Summary** report, you must have defined a sequence summary advanced report template, and set the Run Type to include Summary in the Sequence Table for the sequence runs to be included in the report. Designate the correct sequence summary template file to be used for generating the report.

While in the Summary Report viewing window, buttons at the top of the window allow you to print the report, display additional pages, zoom, or close the report view.

Automatically Printing Sequence Reports

In order for sequence reports to be printed automatically during the running or reprocessing of a sequence, you must specifically request the reports to be printed. Select the **Print Sequence Reports** box at the start of a sequence acquisition or processing.

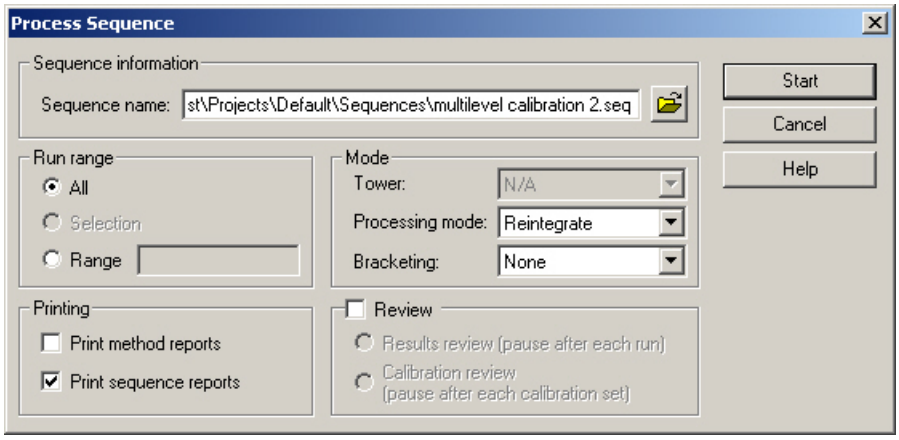


Figure 236. Process Sequence dialog box

Sequence Print - Adding a Sequence List to a Custom Report

To include details of a sequence in your sequence custom report, use the right-click shortcut menu command **Insert Report > Sequence Print**. This command will add a Sequence area to your template that will contain current sequence information when you Print or View the report. This command is only available from the Sequence Custom Report editor.

Inserting a Sequence Calibration Report

To add a sequence calibration report to your sequence custom report template, right-click shortcut menu command **Insert Report > Sequence Calibration**. A table template will be inserted into your sequence report for the sequence calibration report. Note that this table contains placeholder information only - it does not contain data from your system.

Channel A						
Compound	Old RT (Min)	New RT (Min)	RF Average	RF %RSD	RF %RSD Limit	Status
Peak 1	44.350	43.758	231.123456789	21.123456789	25.00	Passed
Peak 2	44.350	43.758	231.123456789	21.123456789	20.00	FAILED

Figure 237. Sequence calibration report

In order for your sequence report to contain valid Calibration Report information when it is printed, you must make sure your method and sequence are set up to create a sequence calibration report. Make sure your **Method Peak Table** contains RF %RSD Limit values for peaks in your calibration. Also, make sure the sequence table contains correct Run Types for **Begin Calibration**, **End Calibration**, and **Print Calibration** at the appropriate runs in the sequence.

OLE (Object Linking and Embedding)

ChromQuest custom reports have full OLE support. This means you can add objects that are linked to other Windows 2000 / XP applications, such as Excel. You can even add a video clip or voice recording to your report. These items are added as **Objects** to your report.

Adding Graphs, Charts, Bitmaps, Logos, Word Documents

To add an object to your report, position your cursor on the custom report at the location where you want to insert the object. Click on the right mouse button, and then choose **Insert Object** from the menu. A dialog box will appear where you can select the type of object you want to add.

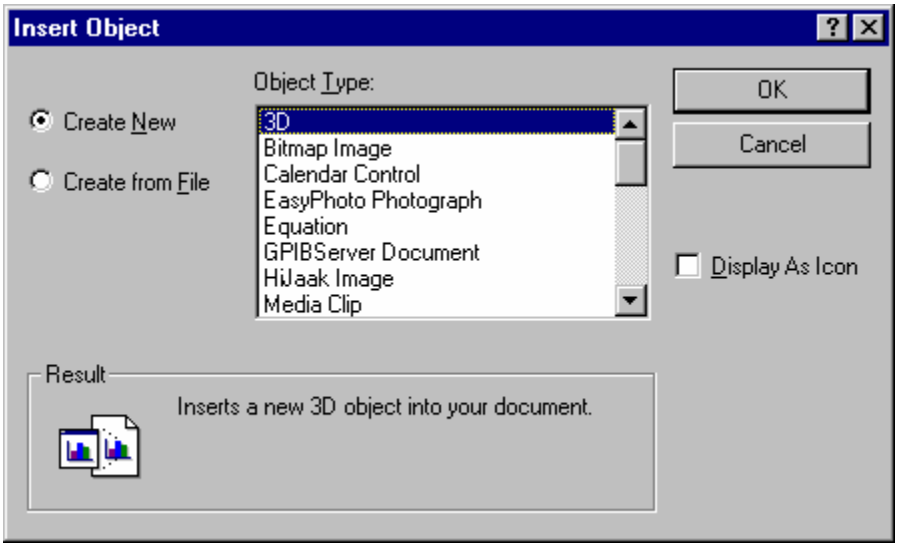


Figure 238. Insert Object dialog box

There are a variety of object types to insert, based on the applications you have installed on your computer. For many selections, inserting an object will cause an application to "run" within your report editor, allowing you to modify and edit the object. To exit the application and return to the custom report, simply click anywhere outside the object area on the report. You can create a new object to insert, or you can open an existing object file to insert.

In the example shown below, a Microsoft Excel chart is being inserted as an object into the custom report. When the Excel chart is inserted, the menus and ribbons from Microsoft Excel become available and are active for you to edit and modify the object.

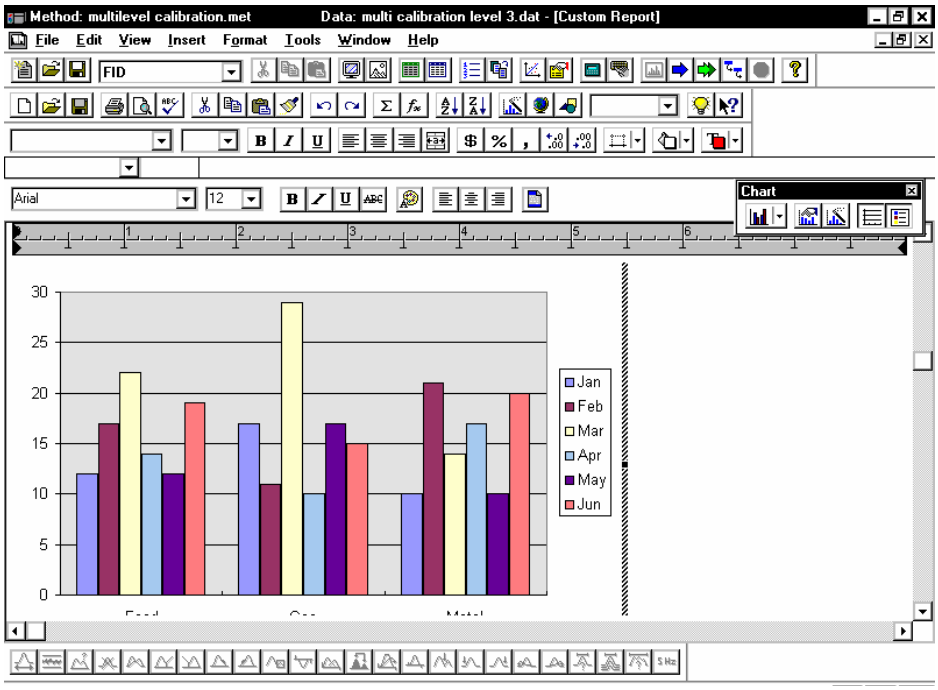


Figure 239. Custom Report - Microsoft Excel chart window

If an OLE object, such as a Microsoft Excel chart, is inserted as a linked object, it will be updated automatically in the custom report if the chart changes. This creates a very efficient way of combining data for reports.

Saving the Custom Report Template

To save your report template for use with other methods, choose **File > Report Template > Save As** and give the report template a name (by default, ChromQuest gives report template files the .rep extension). To open a report template from the Custom Report editor, choose **File > Report Template > Open** and select the template desired from the list.

Once a custom report has been created, click the X box at the top right corner of the custom report editor to return to the ChromQuest application in which you are working. To view or print the report, choose **Reports > View** or **Reports > Print** and then choose the **Custom Report** option.

Viewing and Printing the Custom Report

Once the custom report has been created, it will be updated whenever the files it reports are integrated. You can view the current custom report without printing it in one of two ways. One way is to use the right-click shortcut menu command **Preview** from the custom report editor screen. The other way is to choose the **Reports > View Custom Report** command from the menu bar.

Note In order to view a report that includes current data, the data must be **analyzed** before the report will contain information.

Using Print Preview to View Custom Reports

You can preview the current custom report by clicking on the **Print Preview** button on the command ribbon, or by choosing the right-click shortcut menu command **Print Preview**. The Print Preview screen allows you to examine one or two pages of the current report at a time.

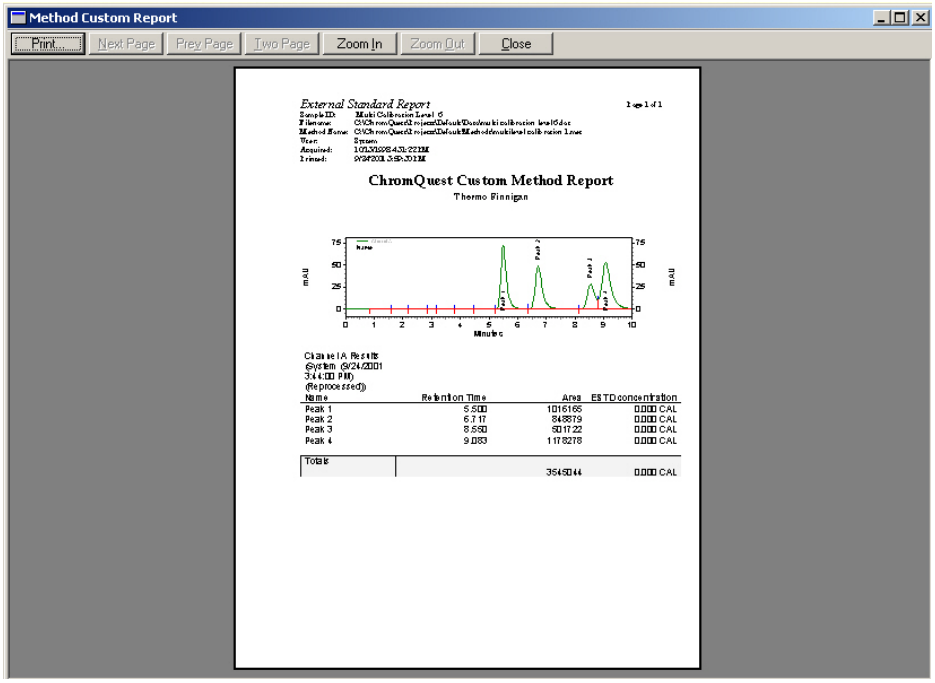


Figure 240. Method Custom Report - Print Preview window

When you are in the print preview screen, the cursor temporarily turns into a "magnifying glass" image. You can click on a location of the report to zoom in for details.

- Print

  Click this button to print the report.

- Next Page

  Click this button to view the next page of the report.

- Prev Page

  Click this button to view the previous page of the report.

- Two Pages

  Click this button to view two pages of the report at a time.

- Zoom In

  Click this button to zoom in on the chromatogram. This has the same effect as clicking on the report when the cursor has a magnifying glass shape.

- Zoom Out

  Click this button to zoom out one level.

- Close

  Click this button to return to the custom report editor.

Viewing Method Custom Reports

Another way to view the custom report is by choosing the **Reports > View > Method Custom Report** command from the menu bar. This command is available even when you are not editing the custom report. The current method custom report will appear in a window for viewing only. You cannot edit the report from this window.

Viewing Sequence Reports

Once you have processed a sequence that has one or more sequence reports designated, you can view the sequence report(s) on your screen choosing the **Reports > View > Sequence Custom Report** command. A list box appears with the sequence reports for the current sequence listed. For each sequence report, the Report Type is listed, along with the sequence rows included in the report, and the report template used to produce the report. To view a report on-screen, click on it with the mouse to highlight it, and then click on **View**.

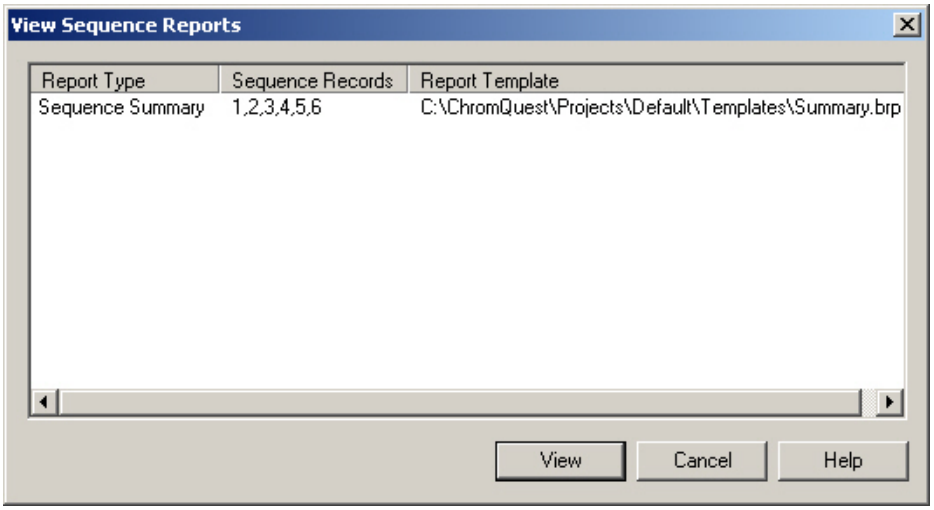


Figure 241. View Sequence Reports window

Printing Custom Reports

You can print custom reports in one of four ways:

1. From within the custom report editor. To print a report from the custom report editor, use either the Print button in **Print Preview**, or print the report directly using the right-click shortcut menu command **Print**.
2. To print the current custom report, choose the **Reports > Print > Custom Report** command from the menu bar.
3. As a part of data acquisition or reprocessing. A custom report can be printed at the end of each analysis, at the end of a data acquisition run, during or at the end of a sequence. Selecting the Print Method Reports option when starting a run, sequence acquisition, or reprocessing determines whether or not a method custom report is printed. No reports are printed unless this option is selected. Sequence reports are not printed unless the **Print Sequence Reports** option is selected.
4. From a sequence run. In the Run Type, select the **Print additional reports** checkbox. This will allow you to select report templates to print, and to specify when you want the report(s) to print.

Note When opening an instrument **on-line**, the configured printer is remembered for that instrument regardless of the user. When opening an instrument **off-line**, the configured printer is remembered for the user of that instrument.

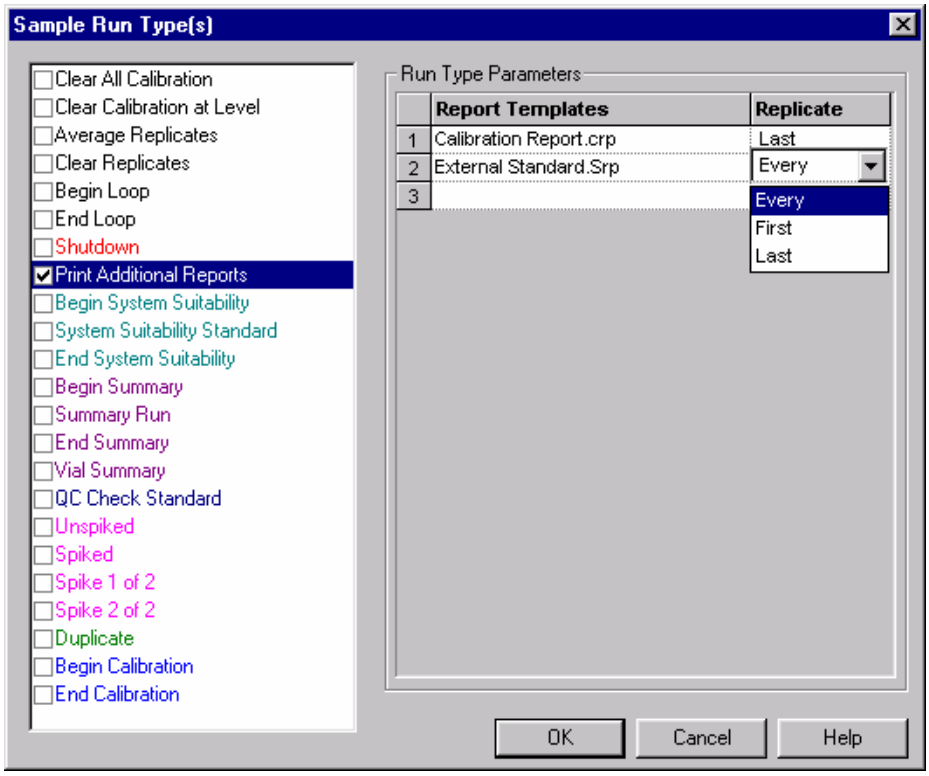


Figure 242. Sample Run Type(s) window

Printing Sequence Custom Reports

To manually print a sequence custom report, choose the **Reports > Print > Sequence Custom Report** command from the menu bar. A list box appears with the sequence reports for the current sequence listed. For each sequence report, the Report Type is listed, along with the sequence rows included in the report, and the report template used to produce the report. To print a report, click on it with the mouse to highlight it, and then click on the **Print** button.

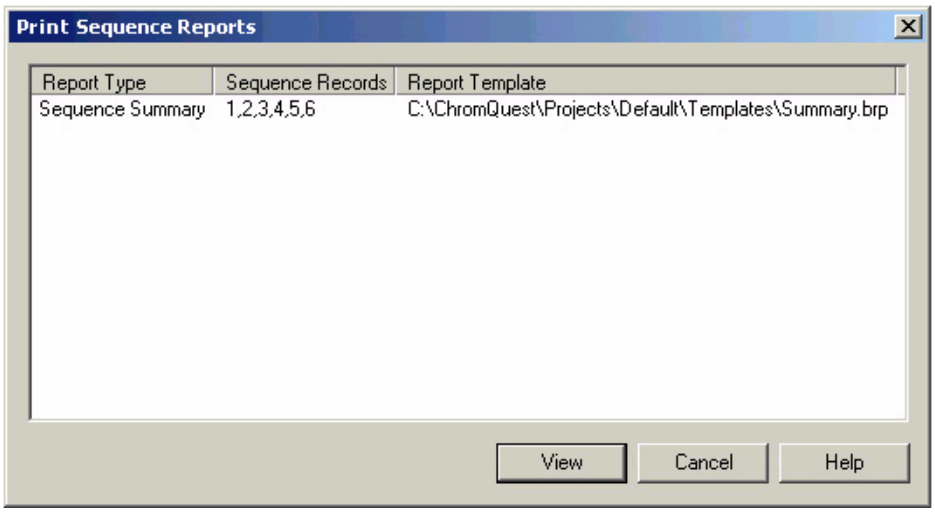


Figure 243. Print Sequence Reports window

Note In order for sequence custom reports to appear in the Print Sequence Reports list box, you must have already processed a sequence in which sequence reports are designated.

Creating QC Reports

A number of Quality Control reports can be generated automatically with ChromQuest. These require specific information that is entered into the Sequence spreadsheet for the runs involved in the report.

These different types of QC Reports are selected in the Run Type column of the Sequence. The values used to test the results of a Sequence analysis are set in the Peak Table of the Method for each component in the analysis.

Sequence Spike Report

If you want your sequence report to include a Sequence Spike report, use the right-click shortcut menu command **Insert Report > Sequence Spike**.

Select the **Spike/Unspike** option button if the report is to cover a spiked sample followed by an unspiked sample. Select the **Spike 2 of 2** option button if you want a QC Spike report where the unspiked sample is followed by the first spiked sample, followed by the second spiked sample.

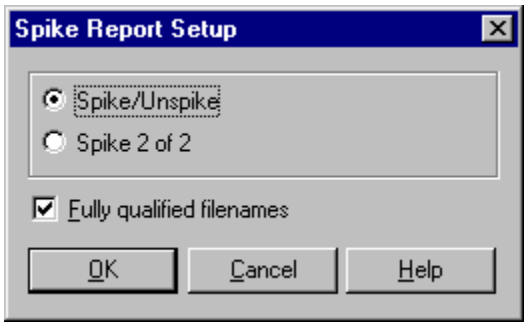


Figure 244. Spike Report Setup dialog box

Select the **Fully qualified filenames** checkbox if you want to include full paths in the file names. A table template will be inserted into your sequence report for the sequence Spike report. Note that this table contains placeholder information only - it does not contain data from your system.

Type	File	Sample ID	Acquired				
UnSpiked	D:\CHROM\Data\Multical.001	Multi 1	1/30/97 9:28:38 Monday				
Spiked	D:\CHROM\Data\Multical.001	Multi 1	1/30/97 9:28:38 Monday				
Channel A							
Compound	Unspiked Conc.	Spiked Amount	Spike Conc.	Spike Recovery	Low Limit	High Limit	Status
Peak 1	30.094	30.000	40.034	33.133	25.000	45.000	OK
Peak 1	30.094	30.000	40.034	33.133	45.000	55.000	Low
Peak 1	30.094	30.000	40.034	33.133	5.000	25.000	High

Figure 245. Sequence Spike report

In order for your sequence report to contain valid spike information when it is printed, you must make sure your method and sequence are set up to create a sequence Spike report.

In the Peak Table of the Method, entries are made for the Spike 1 Amount and Spike 2 Amount (if two spiked samples are to be run), the Low Spike Limit and the High Spike Limit. The Low and High Spike limit values are entered as a percentage recovery used to determine whether the test passes or fails.

In the Sequence, one sample must have an **unspiked** Run Type. This is the sample that is not spiked. This sample must be followed by the **spiked** sample. This sample is identical to the **unspiked** sample, except that it has been spiked with the standard component(s).

After the unspiked and spiked samples are analyzed, a report is printed, showing the calculated concentration of the compound(s) in the unspiked sample, the amount(s) entered for the spiked amount(s), the calculated concentration(s) for the spiked sample(s) and the percentage(s) of the spiked amount(s) that is recovered. The recovery percent value is compared to the Low and High Limit values, and the status of the results (Low, High or OK) is printed.

The following is an example of a QC Spike report (template Spike1.brp), where the unspiked sample is followed by the spiked sample.

Type	File	Sample ID	Acquired
UnSpiked	C:\ChromQuest\data\multi calibration level 3.dat	Multilevel Calibration Level 3	11/26/90 8:49:21 PM
Spiked	C:\ChromQuest\data\multi calibration level 4.dat	Multilevel Calibration Level 4	11/26/90 8:49:34 PM

Channel A

Compound	Unspiked Conc.	Spiked Amount	Spike Conc.	Spike Recovery	Low Limit	High Limit	Status
Peak1	30.000	10.000	40.000	100.000	90.000	110.000	OK
Peak2	7.000	1.500	8.000	66.667	75.000	125.000	OK
Peak3	25.000	4.500	30.000	111.111	90.000	110.000	OK
Peak4	30.000	10.000	40.000	100.000	90.000	110.000	OK

Figure 246. QC Spike report

The following equation is used in this QC Spike report:

$$\text{Spike Recovery} = \frac{100 \times \text{Spiked Conc.} - \text{Unspiked Conc}}{\text{Spiked Amt}}$$

The following is an example of a QC Spike report (template Spike2.brp), where the unspiked sample is followed by the first spiked sample (1), followed by the second spiked sample (2).

Type	File	Sample ID	Acquired
UnSpiked	C:\ChromQuest\data\multi calibration level 3.dat	Multilevel Calibration Level 3	11/26/90 8:49:21 PM
Spike 1	C:\Chromquest\data\multi calibration level 4.dat	Multilevel Calibration Level 4	11/26/90 8:49:34 PM
Spike 2	C:\ChromQuest\data\multi calibration level 5.dat	Multilevel Calibration Level 5	11/26/90 8:51:56 PM

Channel A

Compound	Unspiked Conc.	Spike1 Amount	Spike1 Conc.	Spike1 Recovery	Spike2 Amount	Spike2 Conc.	Spike2 Recovery	Relative Diff.	Mean Recovery	Low Limit	High Limit	Status
Peak1	30.000	10.000	40.000	100.000	20.000	50.000	100.000	0.000	100.000	90.000	110.000	OK
Peak2	7.000	1.500	8.000	66.667	2.000	9.000	100.000	0.400	83.333	75.000	125.000	OK
Peak3	25.000	4.500	30.000	111.111	10.000	35.000	100.000	0.105	105.556	90.000	110.000	OK
Peak4	30.000	10.000	40.000	100.000	21.000	50.000	95.238	0.049	97.619	90.000	110.000	OK

Figure 247. QC Spike report

The following equations are used in this QC Spike report:

$$\text{Spike Recovery} = \frac{100 \times \text{Spiked Conc.} - \text{Unspiked Conc}}{\text{Spiked Amt}}$$

$$\text{Mean Recovery} = \frac{\text{Spike 1 Recovery} + \text{Spike 2 Recovery}}{2}$$

$$\text{Relative Diff} = \frac{|\text{Spike 1 Recovery} - \text{Spike 2 Recovery}|}{\text{Mean Recovery}}$$

Sequence Duplicate Report

If you want your sequence report to include a Sequence Check Standard report, use the right-click shortcut menu command **Insert Report > Sequence Duplicate**. You will be prompted whether you want to include Fully Qualified file names. Check the box if you want to include full paths in the file names. A table template will be inserted into your sequence report for the sequence duplicate report. Note that this table contains placeholder information only - it does not contain data from your system.

Type	File	Sample ID	Acquired			
Dup 1	D:\CHROM\ Data\Multical .001	Multi 1	1/30/97 9:28:38 Monday			
Dup 2	D:\CHROM\ Data\Multical .001	Multi 1	1/30/97 9:28:38 Monday			
Channel A						
Compound	Dup 1 Conc.	Dup 2 Conc.	Mean Conc.	%RD Actual	Dup %RD Limit	Status
Peak 1	15.000	20.000	17.500	28.571	30.000	Passed
Peak 2	15.000	20.000	17.500	28.571	25.000	FAILED

Figure 248. Sequence Duplicate report

In order for your sequence report to contain valid duplicate report information when it is printed, you must make sure your method and sequence are set up to create a sequence duplicate report.

In the Peak Table of the Method, the % relative difference limit for the calculated concentrations between duplicate samples is entered as the Dup % RD value.

In the Sequence, select Duplicate in the Run Type column for the samples of interest. For each duplicate, you must also designate the **Duplicate Level** for that sample. (For example, Duplicate 1 and Duplicate 2.)

After Duplicate samples are analyzed, the QC Duplicate Report is printed showing the calculated concentrations of the two samples, the mean value of the concentrations, calculated % relative difference between the two, the % relative difference limits entered in the Peak Table and the Pass/Fail status of the results.

The following is an example of a QC Duplicate report.

Type	File	Sample ID	Acquired
Dup 1	C:\ChromQuest\data\multi calibration level1.dat	Multilevel Calibration Level 1	11/26/90 8:48:53 PM
Dup 2	C:\ChromQuest\dalat\multi calibration level2.dat	Multilevel Calibration Level 2	11/26/90 8:49:09 PM

Channel A

Compound	Dup 1 Conc.	Dup 2 Conc.	Mean Conc.	%RD Actual	Dup %RD Limit	Status
Peak1	10.000	20.000	15.000	66.667	50.000	FAILED
Peak2	5.000	6.000	5.500	18.182	60.000	Passed
Peak3	15.000	20.000	17.500	28.571	50.000	Passed
Peak4	10.000	20.000	15.000	66.667	50.000	FAILED

Figure 249. QC duplicate report

The following equations are used in this QC Duplicate report:

$$\text{Mean Conc} = \frac{\text{Dup 1 Conc} + \text{Dup 2 Conc}}{2}$$

$$\%\text{RD} = \frac{|\ \text{Dup 2 Conc} - \text{Dup 1 Conc}\ | * 100}{\text{Mean Conc}}$$

Sequence Check Standard Report

If you want your sequence report to include a Sequence Check Standard report, use the right-click shortcut menu command **Insert Report > Sequence Check Standard**. You will be prompted whether you want to include Fully Qualified file names. Check the box if you want to include full paths in the file names. A table template will be inserted into your sequence report for the sequence check standard report. Note that this table contains placeholder information only - it does not contain data from your system.

File	Sample ID	Acquired			
D:\CHROM\Data\Multical.001	Multi 1	1/30/97 9:28:38 Monday			
Channel A					
Compound	Expected Conc.	Actual Conc.	%RD Actual	%RD Limit	Status
Peak 1	24.000	25.232	4.883	5.000	Passed
Peak 2	24.000	25.232	4.883	4.000	FAILED

Figure 250. Sequence Check Standard report

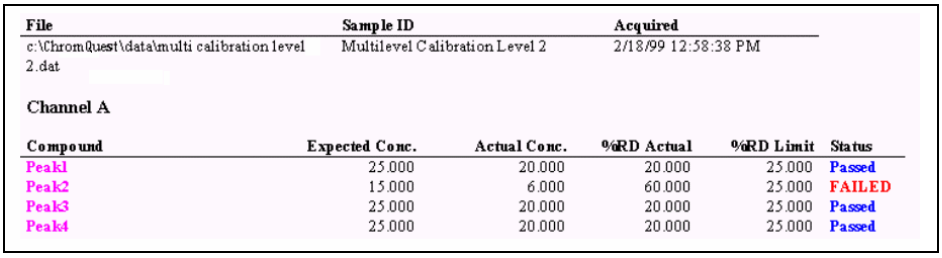
In order for your sequence report to contain valid check standard information when it is printed, you must make sure your method and sequence are set up to create a sequence check standard report.

In the Peak Table of the Method, the concentration of the compound in the check sample is entered as the Check Std Conc value. The % relative difference limit between the actual amount and the calculated amount is entered as Check Std % RD.

In the Sequence, select QC Check Std in the Run Type column for the samples to be used as QC check standard.

After the QC check samples have been analyzed, the QC Check Standard Report is printed showing the expected concentration value from the Peak Table, the calculated concentration, the calculated % relative difference for the sample, the % relative difference limit from the Peak Table and the Pass/Fail status of the result.

The following is an example of a QC Check Standard report.



File	Sample ID	Acquired
c:\ChromQuest\data\multi calibration level 2.dat	Multilevel Calibration Level 2	2/18/99 12:58:38 PM

Channel A

Compound	Expected Conc.	Actual Conc.	%RD Actual	%RD Limit	Status
Peak1	25.000	20.000	20.000	25.000	Passed
Peak2	15.000	6.000	60.000	25.000	FAILED
Peak3	25.000	20.000	20.000	25.000	Passed
Peak4	25.000	20.000	20.000	25.000	Passed

Figure 251. QC Check Standard report

The following equation is used in this QC Standard report:

$$\%RD = \frac{\text{Abs} \mid \text{Expected Conc} - \text{Actual Conc} \mid * 100}{\text{Expected Conc}}$$

Sequence System Suitability Report

If you want your sequence report to include a Sequence System Suitability report, use the right-click shortcut menu command **Insert Report > Sequence Suitability**. A table template will be inserted into your sequence report for the sequence System Suitability report. Note that this table contains placeholder information only - it does not contain data from your system.

		SYSTEM IS NOT SUITABLE					
Channel A	Compound	Parameter	Min	Max	%RSD		
	Peak 1	Area	1234.23	1468.99	0.094		
Sample ID	Compound	Parameter	Average	Low	High	%RSD	Status
	Peak 1	Area	1287.84	1178.23	1397.45	0.067	
Samp 1		1397.45					Passed
Samp 2		1178.23		FAILED			FAILED
Channel A	Sample ID	Test	Start(Min)	Stop(Min)	Threshold	Result	Status
	Samp 1	Noise	3.2	4.2	234.34	232.23	Passed

Figure 252. Sequence System Suitability report

In order for your sequence report to contain valid check standard information when it is printed, you must make sure your method and sequence are set up to create a sequence System Suitability report. For details on how to do this, see **Chapter 6** in the *ChromQuest Chromatography Data System Reference Guide*, .

Advanced Reporting

The Advanced Reporting feature uses a "spreadsheet format" to make it easy to create complex customized reports. A variety of standard report templates is provided for you that can be easily modified to suit your application. In addition, you can create completely customized report templates that extract virtually any data or file information and apply mathematical functions to it. For example, you can create reports that combine peak data from multiple channels into a single report. Or, you can take the results from a sequence of runs and calculate statistical analysis on them.

Each advanced report begins as an advanced report template. Once the report template is created and saved, it can be used to create and print reports from sequence runs or sequence reprocessing by designating the report template from the Run Type of the run in the sequence. Unlike the Custom Method and Sequence reports, Advanced Reports are not saved as part of the method or sequence.

Note For details on advanced reporting functions, see the **Guide to Advanced Templates.doc** file located in the Manual folder of the program CD.

Creating a new report template

To create a new advanced report template, choose **File > Advanced Reports > New**. A blank spreadsheet template will appear. The report template is created by inserting data series, formulas and text into the cells of the spreadsheet. You can also define a header and footer, insert charts of data, and format the cells as described in the following sections.

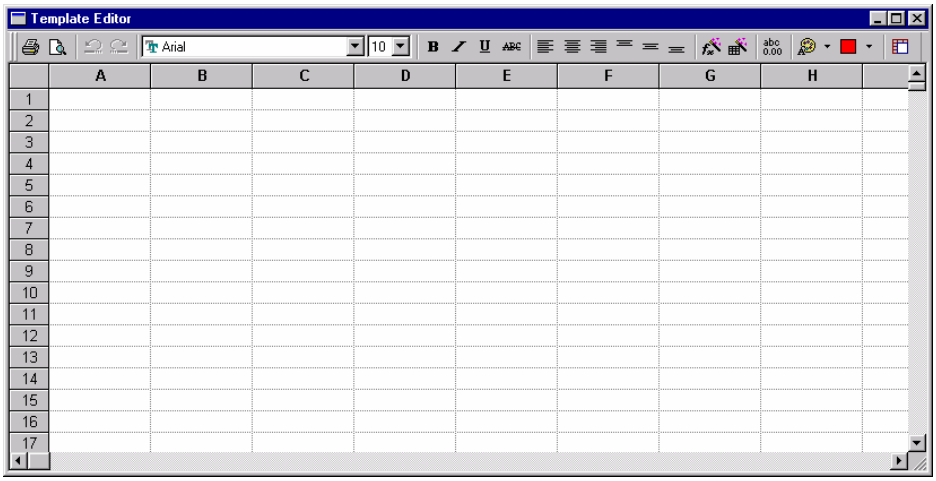


Figure 253. Template Editor window

Insert Text

To insert text on the template, simply click on the cell where you want the text to appear, and type the text. You can format the text using the buttons on the toolbar, changing font, bold, italic, underline, and justify. You can also change the color of the text.

Format Cell Style

There is a wide variety of ways to automatically format the style of the selected cell (or cells). Click on the **Cell Style** button and a dialog will appear where you can select the appropriate style for the currently selected cell(s).

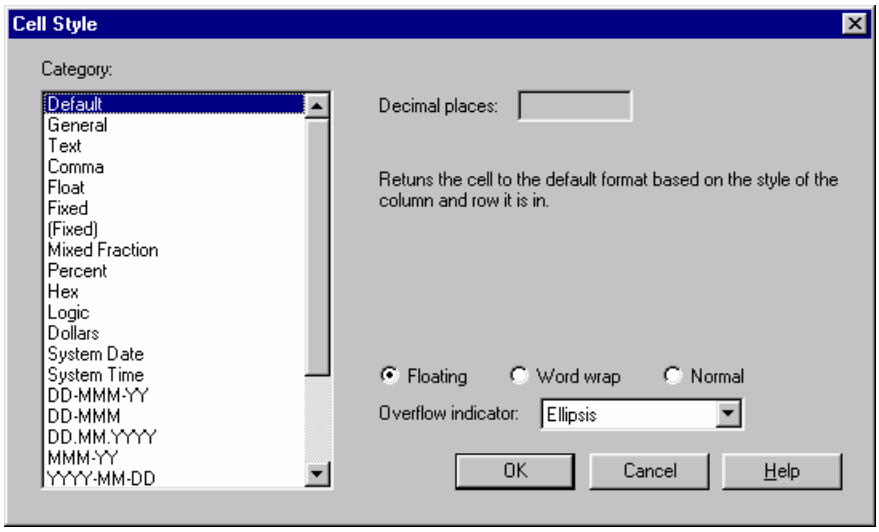


Figure 254. Cell Style dialog box

Select the cell style desired in the **Category** list. Based on your selection, fields on the right may be come active to further define the cell style.

- Decimal places

  Enter the number of decimal places to be displayed for numeric data.

- Floating

  If this is selected, text or numeric data that exceeds the width of the cell will automatically "float" into the next cell (if empty). If this selection is **Off**, the text or data that extends beyond the limits of the cell width will be either truncated, or displayed with an "overflow indicator" selected.

- Overflow indicator

  Select the way cells that overflow are to be displayed: with no indicator, with ellipsis (e.g. **cell overflow is…**), or with pound sign (#########).

- Word wrap

  When this button is selected, information entered into a cell will be "wrapped" to the next line when the end of the cell is encountered.

- Normal

  When this button is selected, information will be displayed in the default mode.

Define an Advanced Report Header/Footer

To define the header and/or footer for the report, click the right mouse button on the spreadsheet and choose the **Header/Footer** command. A dialog will appear where you define the contents of your header and footer.

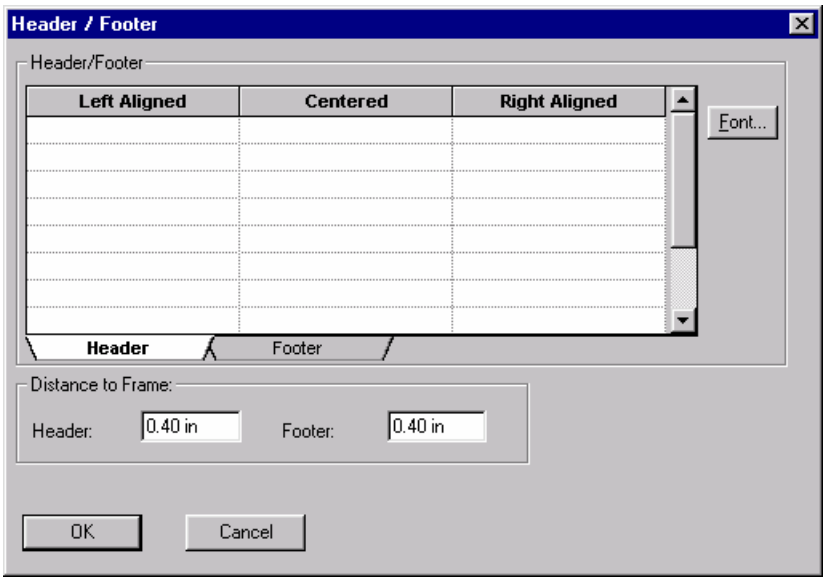


Figure 255. Header/Footer dialog box

Click in one of the fields of the “Left Aligned”, “Centered”, or “Right Aligned” columns. You can type text that will appear with the indicated alignment, or you can insert field codes for automatic insertion of items such as date, time, and page numbers. When you have a cell selected, you can change the font by clicking on **Font** and selecting the desired font and characteristics.

Note Font colors are not supported in the header and footer sections.

- Field codes that can be inserted in a header or footer

  $F Report Template Name

  $P Page number

$N Number of pages

$SEQNUM Sequence page number

$D Date – you can specify the date formatting with an additional parameter, as for example $D{%h:%m}

- Date Format Codes:

%a Abbreviated weekday name

%A Full weekday name

%b Abbreviated month name

%B Full month name

%c Date and time representation appropriate for locale

%d Day of month as decimal number (01 - 31)

%H Hour in 24-hour format (00 - 23)

%I Hour in 12-hour format (01 - 12)

%j Day of year as decimal number (001 - 366)

%m Month as decimal number (01 - 12)

%M Minute as decimal number (00 - 59)

%p A.M./P.M. indicator for 12-hour clock

%S Second as decimal number (00 - 59)

%U Week of year as decimal number, with Sunday as first day of week (00 - 51)

%w Weekday as decimal number (0 - 6; Sunday is 0)

%W Week of year as decimal number, with Monday as first day of week (00 - 51)

%x Default Date representation

%X Default Time representation

%y Year without century, as decimal number (00 - 99)

%Y	Year with century, as decimal number
%z, %Z	Time-zone name or abbreviation; no characters if time zone is unknown

- Distance to frame

  The values you enter here determine how far the header and footer are placed from the edge of the paper. This value is independent of the paper margin, and if set incorrectly can result in overlap with the body of the report.

When you click the OK button, the header and footers defined will become part of your template, however they will not appear. To view the header and footer, click on the **Print Preview** button and zoom in to see the details.

Formatting the spreadsheet

To format the general characteristics of your advanced report, click the right mouse button in the spreadsheet, and choose **Grid Properties**. A dialog will appear where you can customize the grid size, margins, page order, and default cell style.

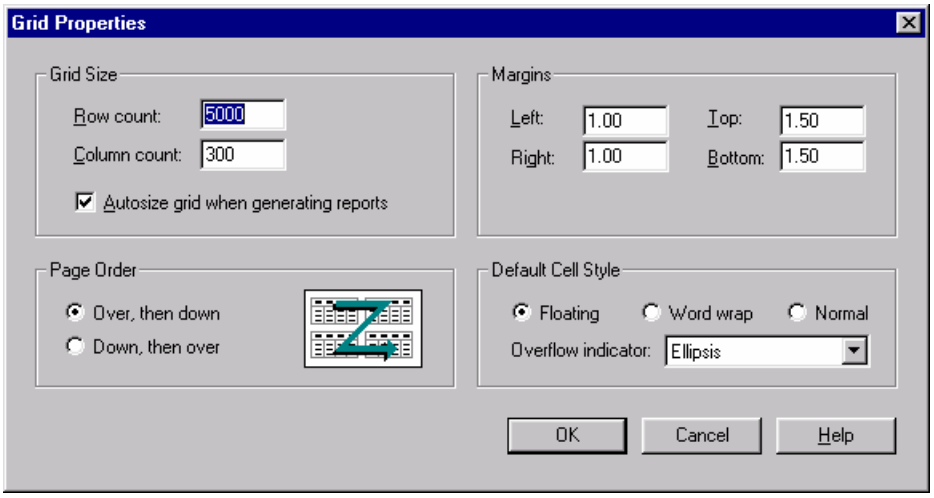


Figure 256. Grid Properties dialog box

- Grid Size

  You can limit the size of the report spreadsheet to make it more manageable.

  – Row count

    To limit the number of rows in your report, type in a number here.

  – Column count

    Enter a number of columns for your report.

- Autosize grid when generating reports

  When this is selected, the spreadsheet grid will automatically be reduced to include only the number of rows and columns required for your report when printing. If this is not selected, empty rows and columns will be printed.

- Page Order

  Select the order for printing pages.

- Margins

  Enter the page margins in the fields indicated. This will establish the margins used for printing the body of the advanced report. Note that these margins are independent of the “distance to frame” setting in header and footer.

- Default Cell Style

  Select the default setting for your cell style. Any cell or range of cells can be changed from this independently.

  - Floating

    If this is selected, text or numeric data that exceeds the width of the cell will automatically “float” into the next cell (if empty). If this selection is **Off**, the text or data that extends beyond the limits of the cell width will be either truncated, or displayed with an “overflow indicator” selected.

  - Word wrap

    When this button is selected, information entered into a cell will be "wrapped" to the next line when the end of the cell is encountered.

  - Normal

    When this button is selected, information will be displayed in the default mode.

  - Overflow indicator

    Select the way cells that overflow are to be displayed: with no indicator, with ellipsis (e.g. **cell overflow is**), or with pound sign (#########).

Function Wizard

Data and information are brought into the report for display using system functions that extract the desired information from the data system. To make it easy, the Function Wizard enables you to select the type of information to be displayed and lets you define how it will appear on the report. The result of your choices becomes a formula (a combination of functions) that is placed in the currently selected spreadsheet cell.

To insert a formula into the template, select a cell of the spreadsheet where you want the data to appear. Then click on the **Function Wizard** button at the top of the spreadsheet.

The Function Wizard will step you through some dialogs that will help you define the information to be inserted in the selected cell.

Functions

Functions are used to define what data are to be displayed and the source of the data, as well as for application of mathematical formulas. Functions do not appear on the spreadsheet unless they are being edited. (To view the function in a cell, double-click on that cell.) The results of defined functions do not appear on the spreadsheet, but will appear in Print Preview or when the report is printed, provided the data is available for the function to work. (For example, sequence summary tables will not contain valid data until after a sequence has been acquired and processed or reprocessed.)

In cases where data is not available for the function to work on, an error may be displayed on your spreadsheet. If this happens, click OK to close the error, then click the Print Preview button. If the function is technically correct, but has no data available, no error messages will appear on the print preview. If an error message appears on the print preview, your function has not been entered correctly.

Detailed descriptions of all functions available in the Advanced Reporting application are beyond the scope of this manual. For a complete list of these functions, see the **Guide to Advanced Template Reporting.doc** file available in the Manual folder of the ChromQuest CD.

Dynamic Data

When Advanced Report is used to display dynamic data, such as data from a sequence of runs, or peaks from every run where the number of peaks may change, the report uses Dynamic Data Functions. This type of function enables the report to expand or contract by repeating the function for all data that meet the specified requirements.

Function Wizard Select Function

Begin by specifying whether or not this function will be used in a sequence report. If so, additional screens will appear for required information.

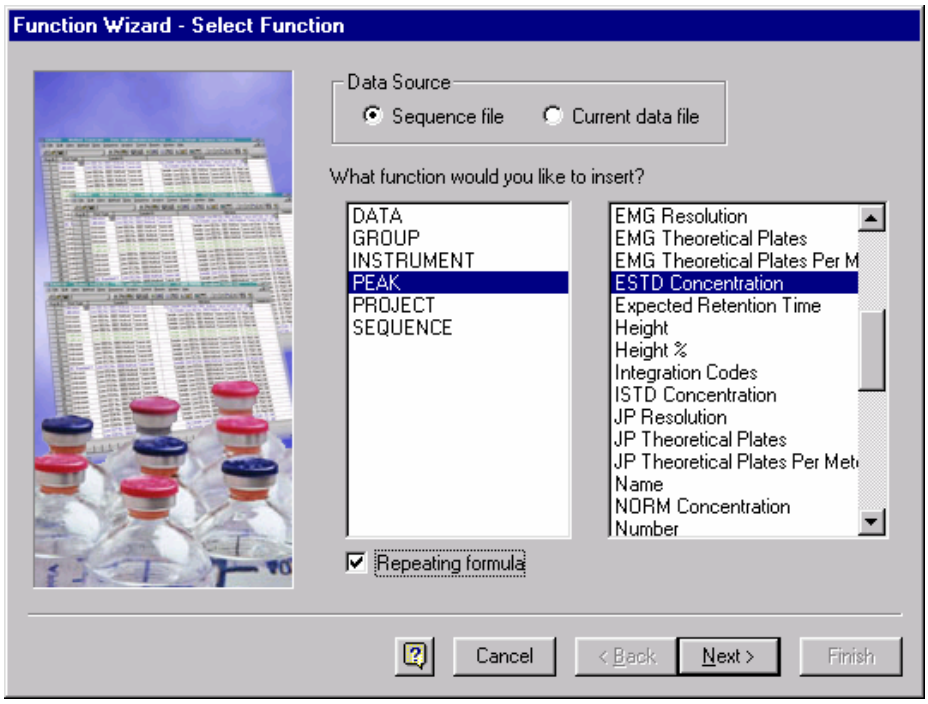


Figure 257. Function Wizard - Select Function dialog box

- Functions

  You can select a system custom function for your advanced report by choosing a category on the left, then selecting a function from the category function list on the right. When you have made your selection, click on **Next**.

  The available categories and the types of functions they contain are as follows:

  – Data

    Data and parameters available in a data file

  – Group

    Information from the group table

  – Instrument

    Instrument parameters (ID, Name, User Name)

  – Peak

    Information from the peak table

  - Project

    Project name and associated paths

  - Sequence

    Sequence names and run numbers

- Repeating formula

  Select this box if the formula is to be repeated for a series of peaks or files.

Function Wizard Identification

If a peak function is specified, this dialog allows you to designate which peak to report.

- Trace index

  Select the trace number (if multi-channel files), where the first trace = 1, second trace = 2, etc.

- Peak index

  If you select this button, enter the peak index number for the peak of interest, where the first peak = 1, second peak = 2, etc.

- Peak ID

  If you select this button, enter the Peak ID number from the peak table for the peak of interest.

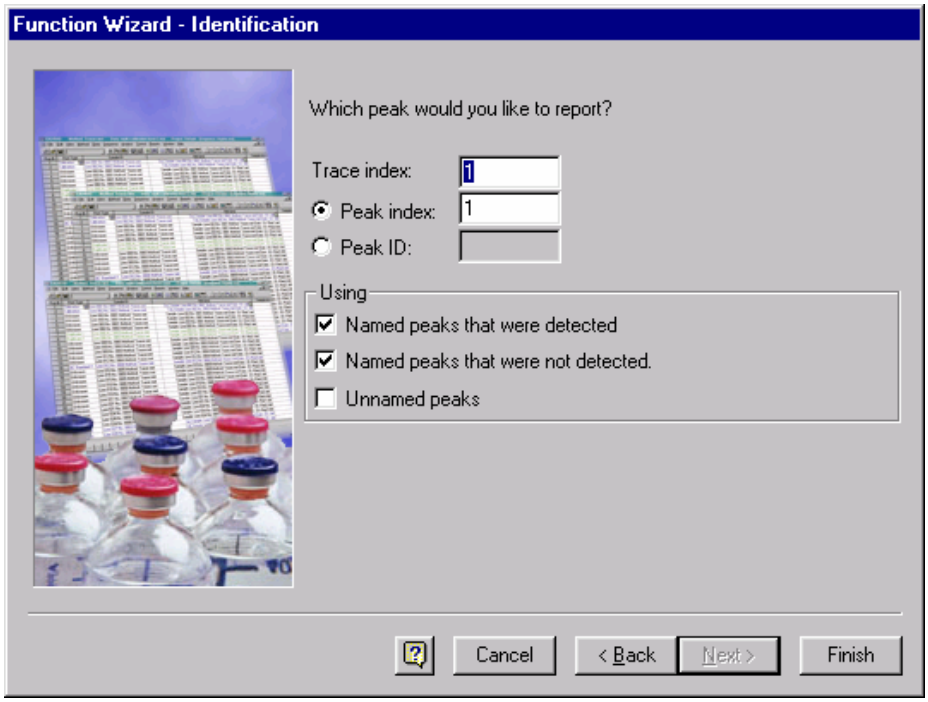


Figure 258. Function Wizard - Identification (Peak) dialog box

- Using
  - Select the boxes for the type of peaks to include.
  - If a group function is specified, this dialog allows you to designate which group to report.

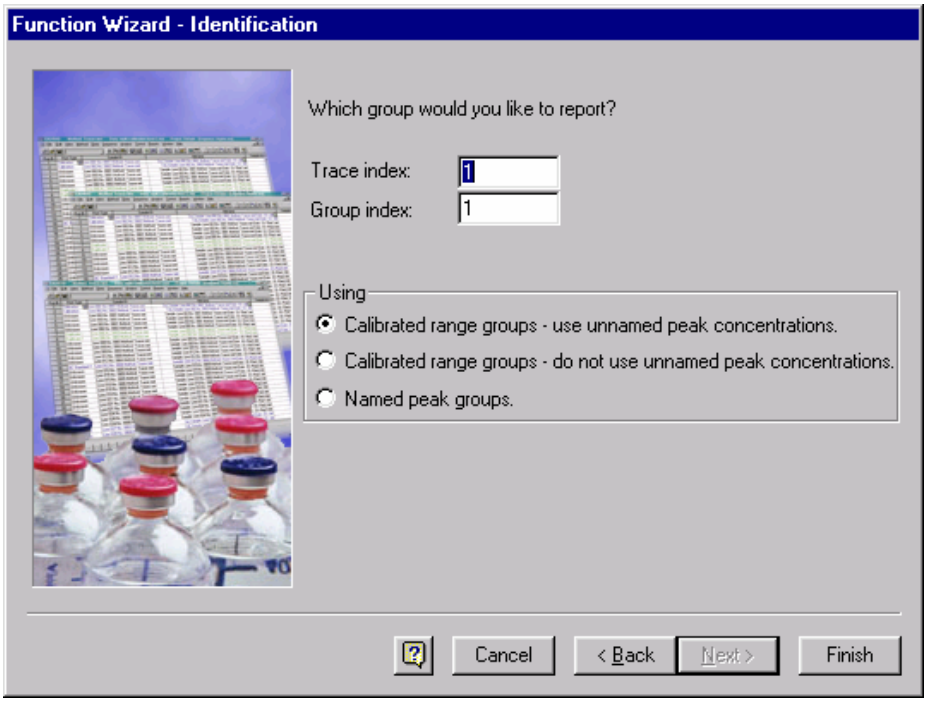


Figure 259. Function Wizard - Identification (Group) dialog box

- Trace index

  Select the trace number (if multi-channel files), where the first trace = 1, second trace = 2, etc.

- Group index

  If you select this button, enter the group index number for the group of interest, where the first group = 1, second group = 2, etc.

- Using

  Select the type of group to be reported. Calibrated range and named peak groups are supported. For Calibrated range groups, if you have defined your group to calculate concentrations for unnamed peaks you can include these in your group reporting by selecting the appropriate button.

Function Wizard Dynamic Data

This screen only appears if you have selected the **Repeating formula** box. The questions here enable you to specify the source data for repeated formulas.

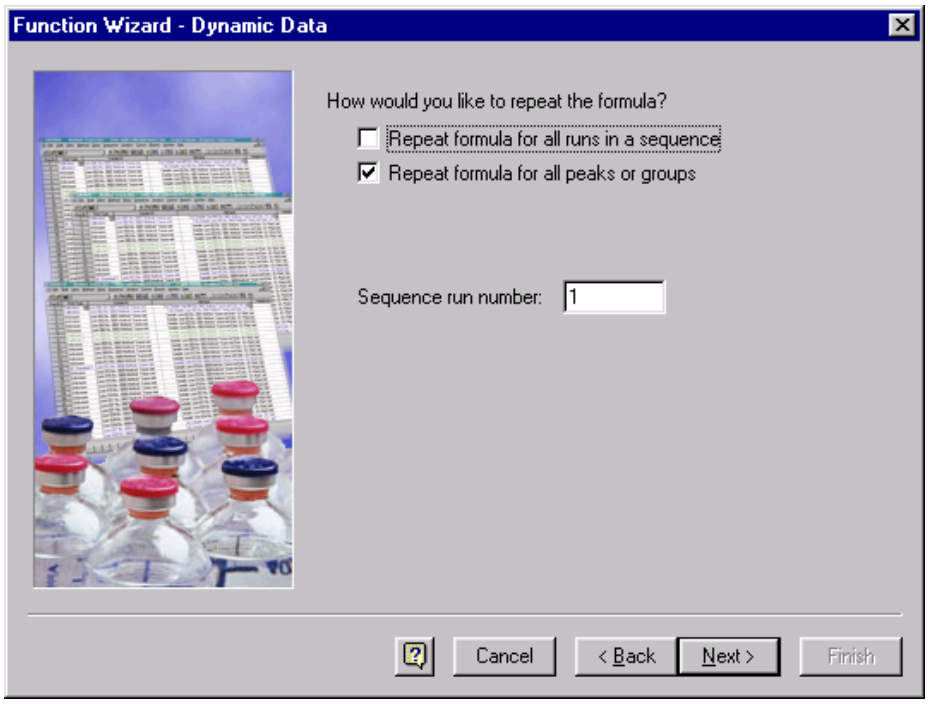


Figure 260. Function Wizard - Dynamic Data dialog box

- Repeat formula for all runs in a sequence

  Select this box to extract the designated data from all runs of the sequence. For example, if the selected function is peak area from a sequence file, this option would enable you to select one peak from a trace of the sequence and then report its area for each run in the sequence.

- Repeat formula for all peaks or groups

  This option will extract the designated information for all peaks or all groups of a sequence run you select. (Sequence run number prompt appears when this option is chosen.) For example if the selected function is peak area from a sequence file, this option would extract the area for all peaks or groups of the chosen sequence run.

- Repeat formula for all runs in a sequence and Repeat for all peaks or groups

  If you select **both** options, the formula will be repeated for all runs and all peaks/groups of the sequence. For example, if the selected function is peak area from a sequence file, the combination of these options would produce a listing of areas for all peaks in every sequence run.

Function Wizard Data Direction

This screen asks how you want the data series to be displayed - either across the row or down a column.

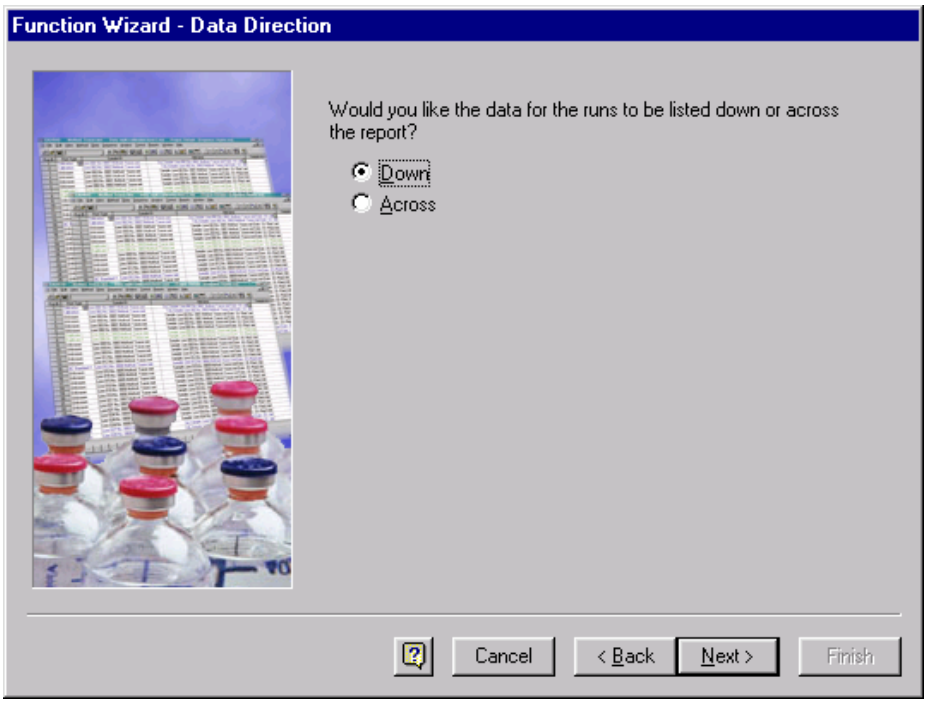


Figure 261. Function Wizard - Data Direction dialog box

Function Wizard Types

This dialog allows you to select the peak or group information.

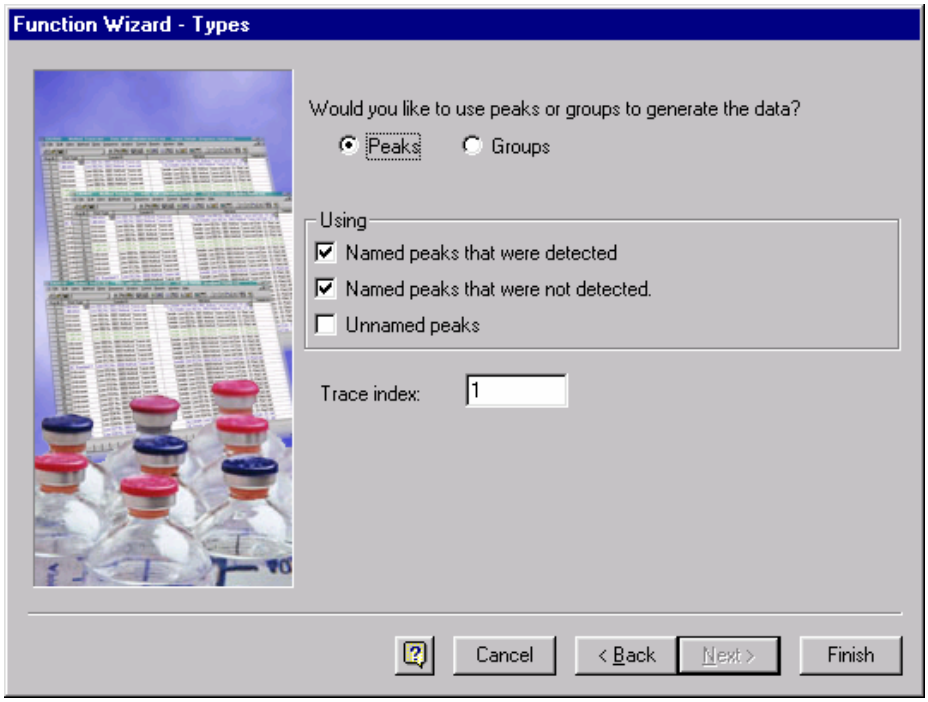


Figure 262. Function Wizard - Types (Peaks) dialog box

Peaks

- Using

  Select the boxes for the type of peaks you will be using.

- Trace index

  Select the trace number (if multi-channel files), where the first trace = 1, second trace = 2, etc.

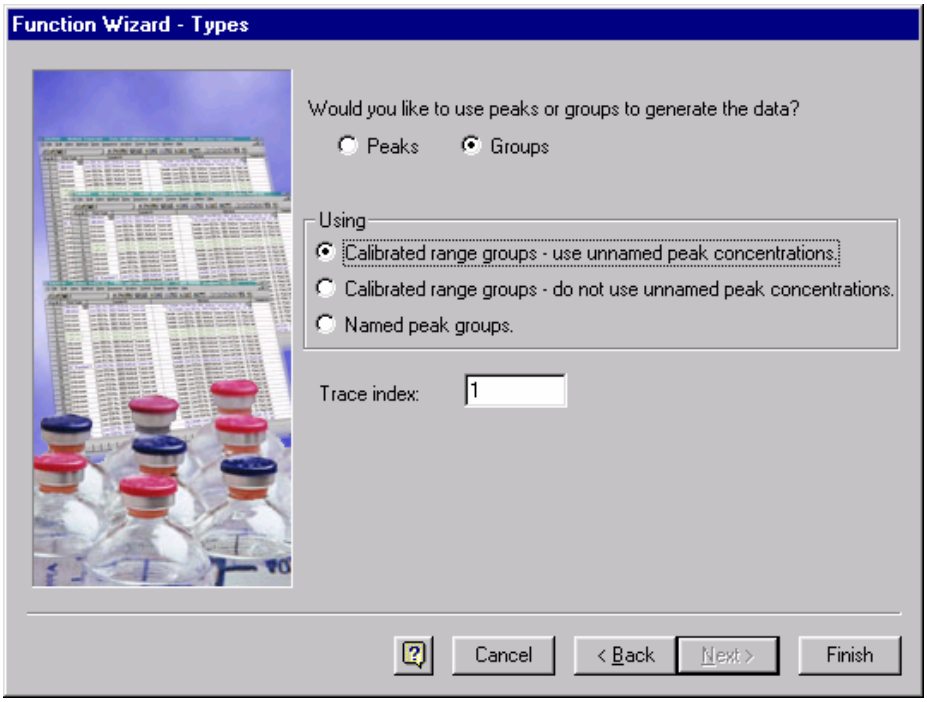


Figure 263. Function Wizard - Types (Groups) dialog box

Groups

When you select groups to generate the data, the following choices appear.

- Using

  Select the type of group to be reported. Calibrated range and named peak groups are supported. For Calibrated range groups, if you have defined your group to calculate concentrations for unnamed peaks you can include these in your group reporting by selecting the appropriate button.

- Trace index

  Select the trace number (if multi-channel files), where the first trace = 1, second trace = 2, etc.

  When you are done with this dialog, click on **Finish**. The function defined by your answers to the Function wizard will be inserted in the currently selected cell.

Table Wizard

The Table Wizard is used to create tables of information in your report. Use this to create a sequence summary report. Click on the Table Wizard button to start the wizard.

Table Wizard Table Type

Select the type of table from those available. This may be expanded in the future to support other types of tables.

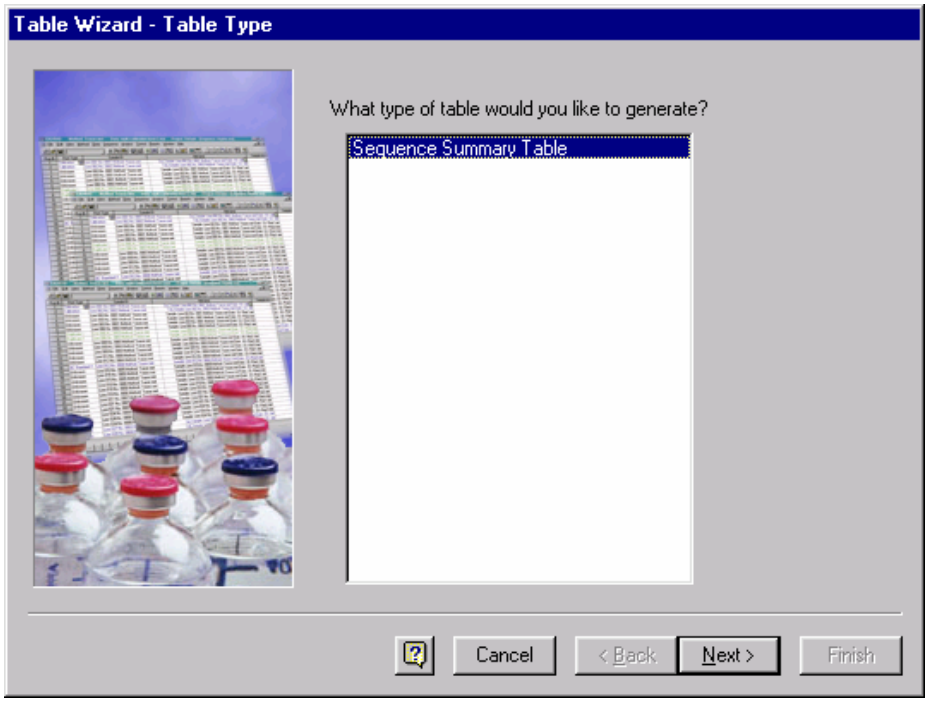


Figure 264. Table Wizard Table Type dialog box

Table Wizard Parameters

Use this dialog to select the parameters you want to include in your table. The selections will change depending on whether you choose **Peaks** or **Groups**.

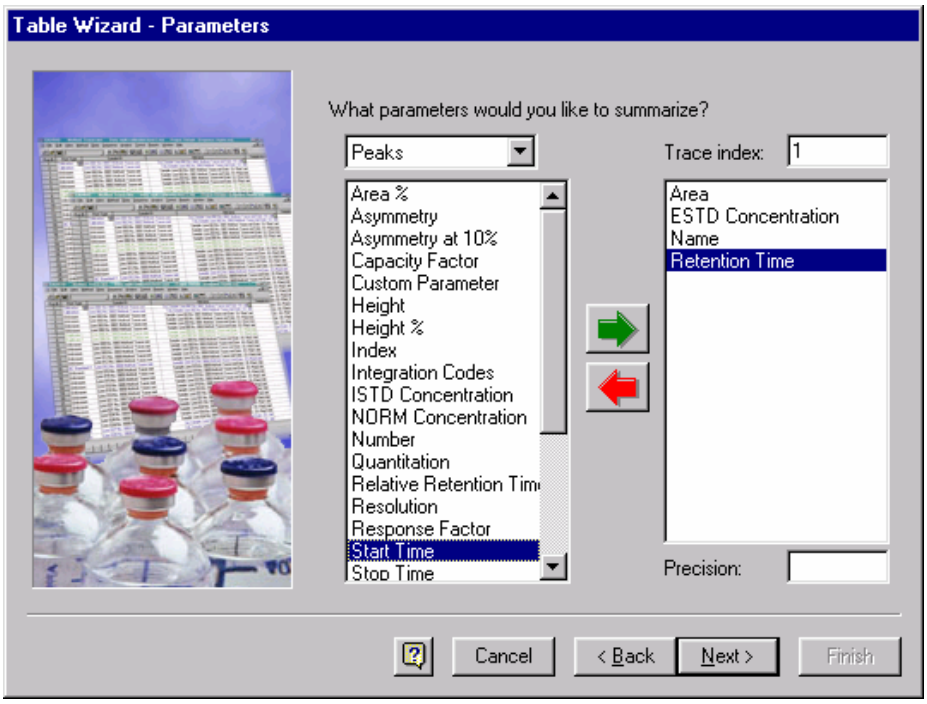


Figure 265. Table Wizard - Parameters dialog box

Select the **Trace index** of the channel you want to include (if using a multichannel file), where 1 = first trace, 2 = second trace, etc.

Select **Peaks** or **Groups** from the drop down list, then select a parameter you want to summarize from the list on the left by double-clicking it. You may select as many parameters as you wish. If the parameter is numeric, you can enter a value for Precision, or accept the value presented.

Table Wizard Types

Select the types of peaks to be included in your summary by checking the box(es). A different set of parameters appears if you have selected **Groups**.

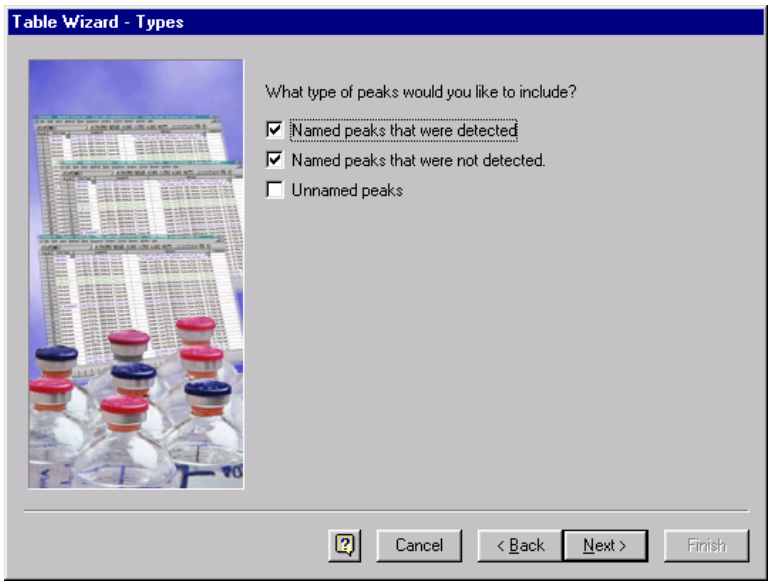


Figure 266. Table Wizard - Types (Peaks) dialog box

Select the types of peaks you want to include.

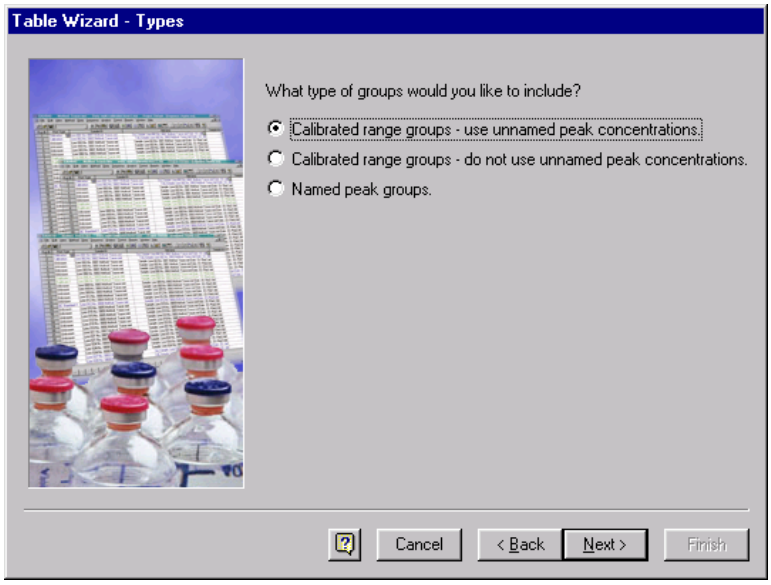


Figure 267. Table Wizard - Types (Groups) dialog box

For groups, select the type of groups to include in the table.

Table Wizard Run Parameters

Double click each parameter you want to include for each run in the summary table.

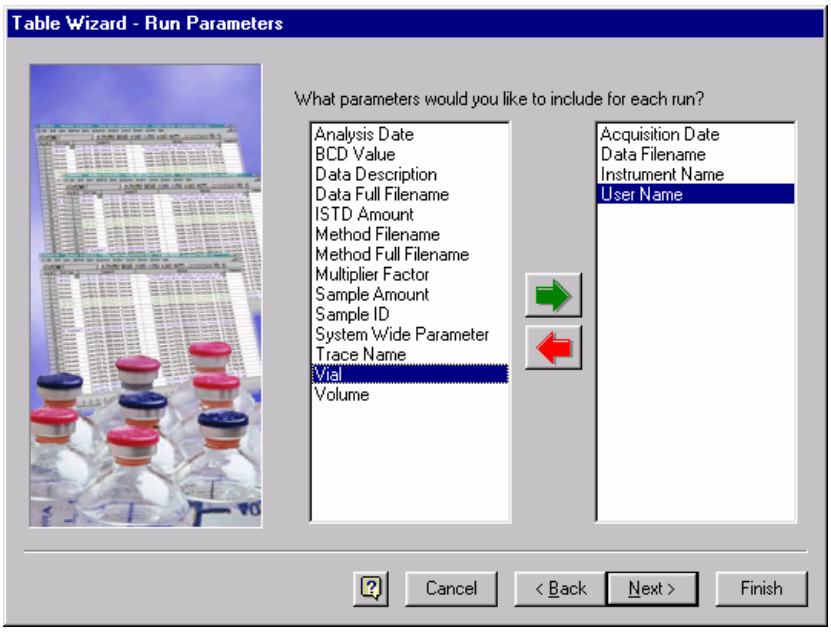


Figure 268. Table Wizard - Run Parametes dialog box

Table Wizard Run Direction

Select which direction you want the runs to be displayed on the table.

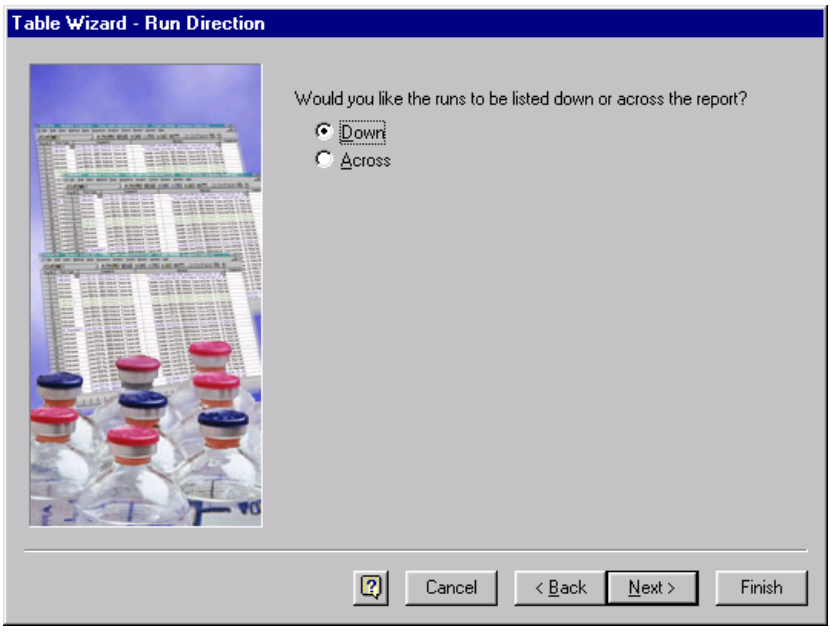


Figure 269. Table Wizard - Run Direction dialog box

Table Wizard Statistics

Click yes if you want to include a statistics section in your table. Calculated statistics include Min, Max, Mean, Std. Deviation, and %RSD.

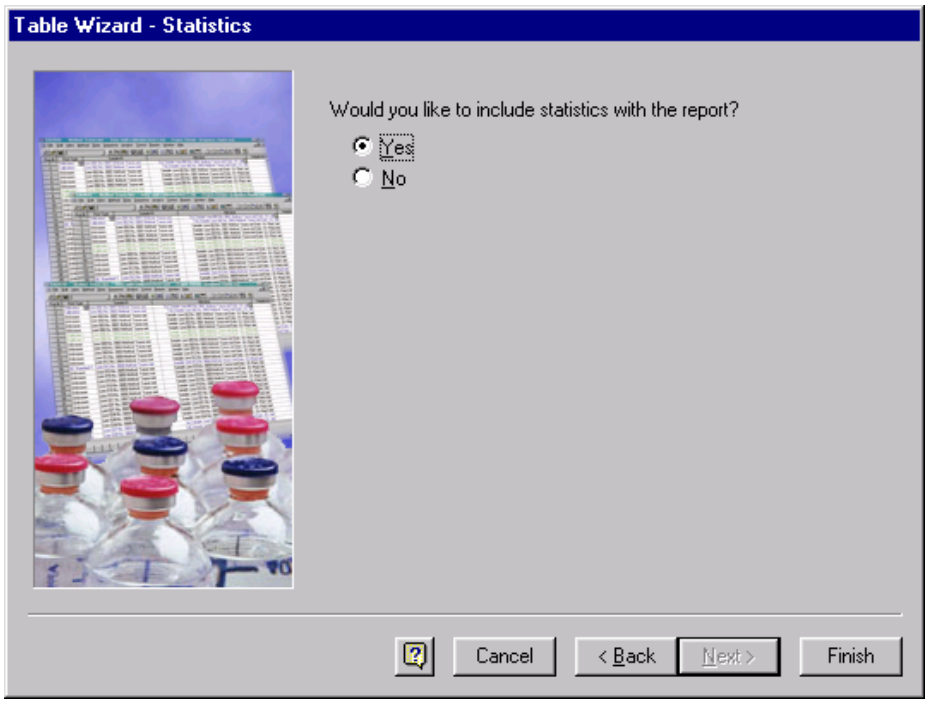


Figure 270. Table Wizard - Statistics dialog box

When you are done with this dialog, click Finish. A sequence summary table will be inserted in your Advanced Report at the current cell location.

Adding a Chart

You can add a chart to graph data in your advanced report. First, you must define an area on your report for the chart to appear. To do this, hold down the mouse button on the spreadsheet and drag it over an area where you want the chart to appear.

Next, click the right mouse button in the cover cell area you just defined, and choose **Insert > Chart**. A dialog will appear where you can define your chart.

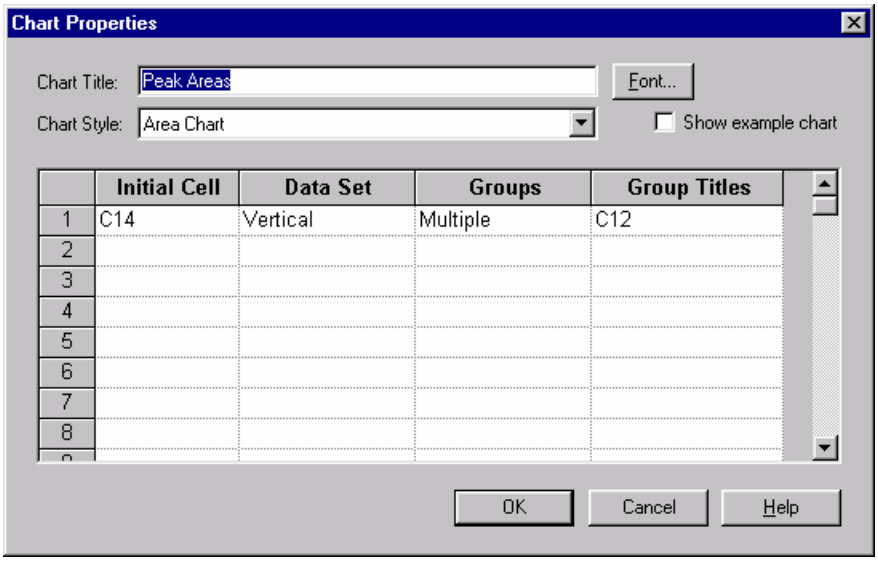


Figure 271. Chart Properties dialog box

- Chart Title

  Enter a title that will appear at the top of your chart.

- Chart Style

  Select the style for your chart from the drop-down list.

- Font

  Click this button to change the font for your chart title.

- Initial Cell

  Enter the cell where the initial data is located (the first cell of a series, for example). In many cases, the cell location you want to enter is the cell where the formula for the data series is defined.

- Data Set

  Select whether the data series extends vertically or horizontally on the spreadsheet.

- Groups

  Select **Single** to display one series of data or **Multiple** to display more than one series of data. For example, in a sequence summary report where the summary table contains areas from 4 peaks in 5 runs, you would choose Single to chart areas from one peak, or Multiple to chart areas from all 4 peaks.

- Group Titles

  Enter a cell reference to be used for titles (legends) for the charted data series, or type in text to be used.

- Display Sample Data

  Select this checkbox if you want to view an example of the chart in your advanced report spreadsheet. (Note: This is not the actual data. To view the actual data in the chart, click the print preview button.)

Cover Cells

This button appears at the top of the Advanced Report spreadsheet window. It is used to expand or shrink the size of a chart, or to create text that spans several cells. To use the cover cells button, drag your mouse to highlight an area on the spreadsheet where you want the cover cells to appear, and then click on the **Cover Cells** button. The highlighted area will appear as a single cell into which text or charts can be added. An example is given below.

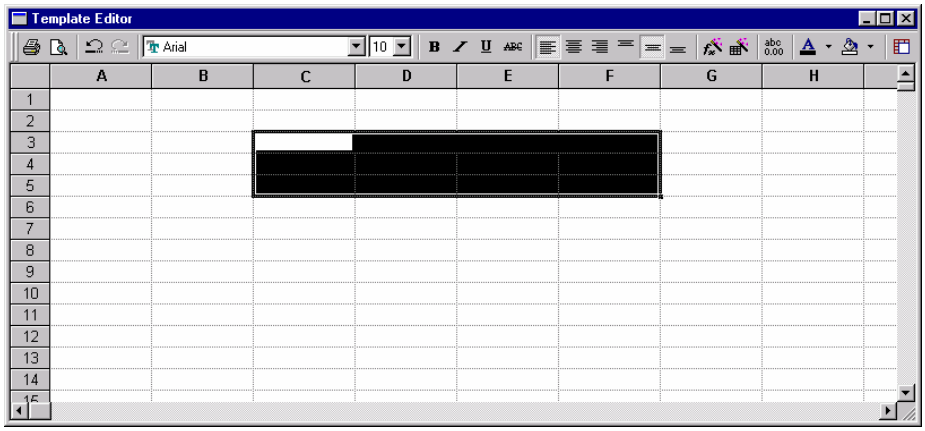


Figure 272. Drag mouse to highlight cells

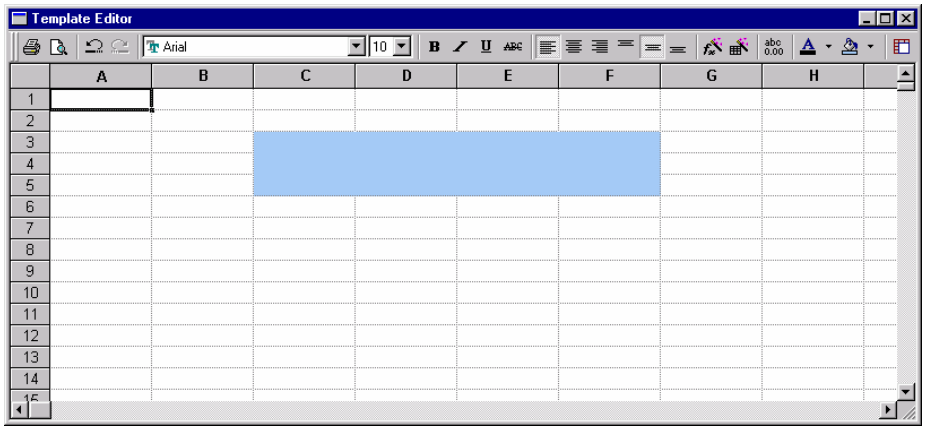


Figure 273. Click Cover Cells button and change color if desired

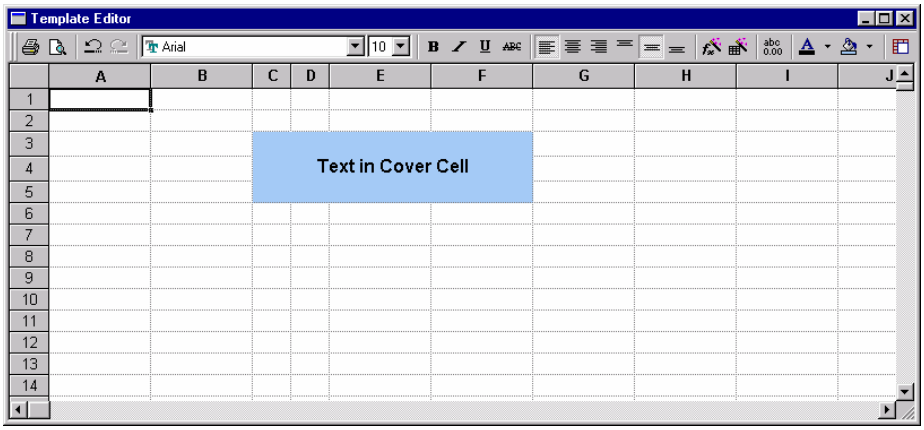


Figure 274. Enter text or chart

Once cover cells have been defined, clicking on the Cover Cells button inside the defined cover cells range will undo the cover cells. You can resize the cover cells by dragging the size of the cover cells box, then clicking the Cover Cells button again. This will only resize the cover cells box if the size of the new area is both wider and longer than the existing cover cell. See the example below.

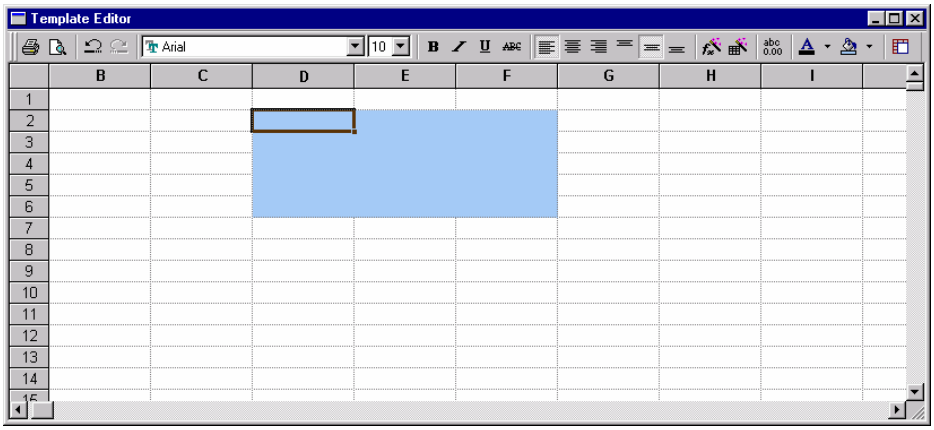


Figure 275. Cover Cells defined

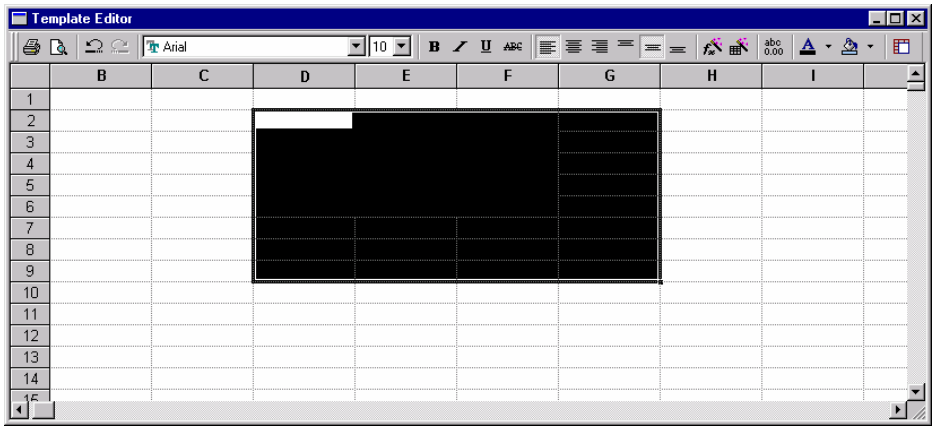


Figure 276. Drag to new cover cell size (longer and wider)

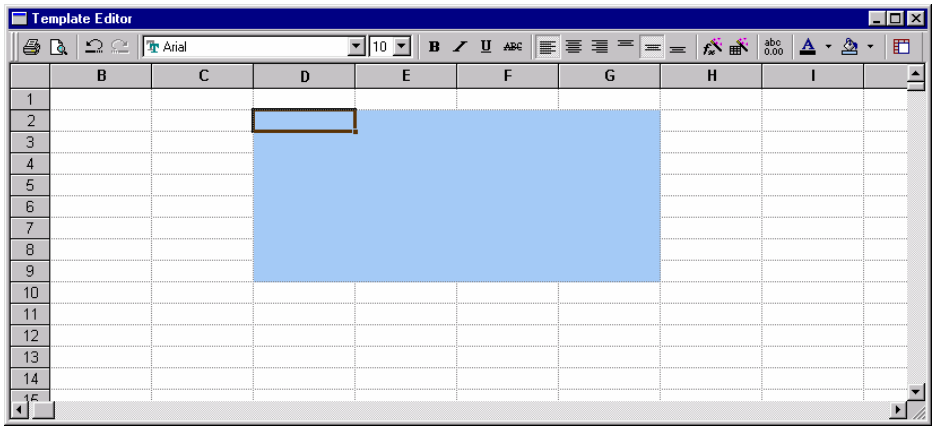


Figure 277. Click Cover Cells button to create new cover cell

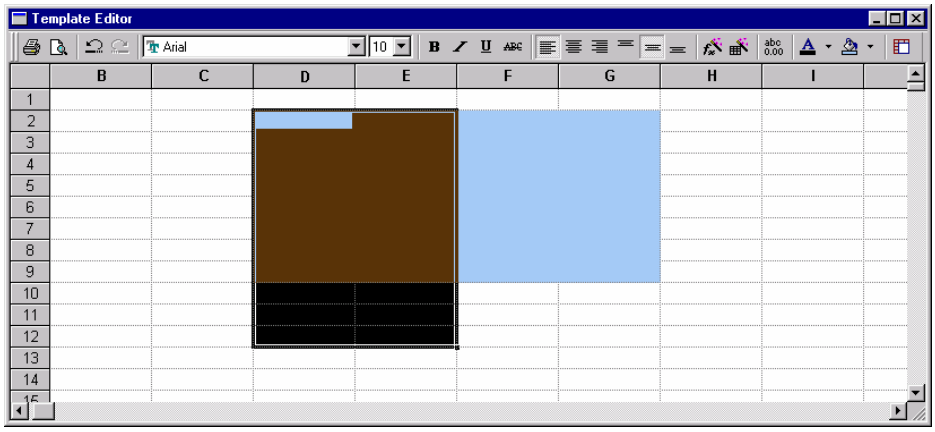


Figure 278. Dragging cover cell definition longer (or wider) only has no effect

Preview Custom Report

Whenever you use the **View > Sequence Report** command to preview a custom report (sequence or advanced report), the report will appear in a preview window where you can examine the report.

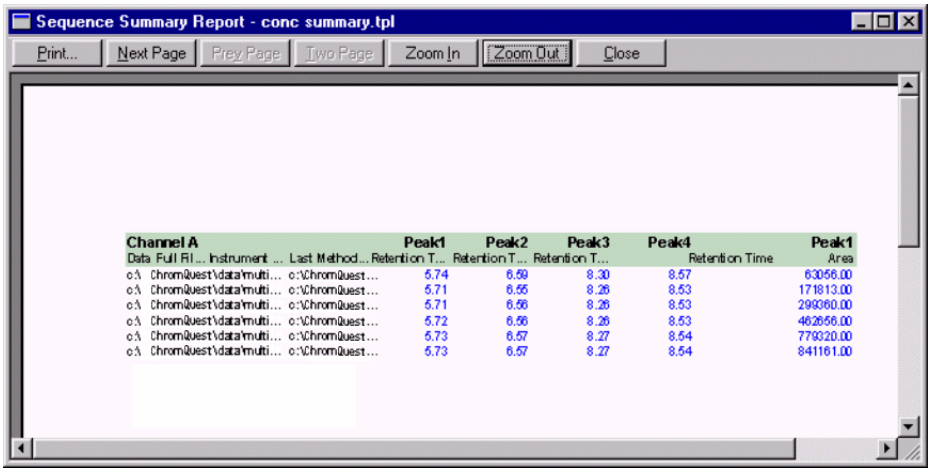


Figure 279. Preview custom report - Sequence Summary Report window

The buttons at the top of the screen enable you to manipulate the view and print the report.

- Print…

  Click this button to print the report. You can choose which printer to send the report to.

- Next Page/Prev Page

  These buttons let you navigate to the next or previous page in a multi-page report.

- Two Page

  This button lets you see two pages at a time if the report has multiple pages.

- Zoom In/Zoom Out

  These buttons let you zoom in for a closer report or zoom out to view more of the report. The **Zoom in** functions the same way as clicking the mouse on the report when the cursor looks like a "magnifying glass".

- Close

  This button closes the report preview window.

Index

A

B

C

D

E

F

G

I

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z