HUMAN HEALTH

ENVIRONMENTAL HEALTH

USER'S GUIDE

Chromera® Chromatography Data System

PerkinElmer®

Chromera User's Guide

Release History

Part Number	Release	Publication Date	Software Version (Minimum)
09931095	B	July 2013	4.1.0

Any comments about the documentation for this product should be addressed to:

User Assistance
PerkinElmer, Inc.
710 Bridgeport Avenue
Shelton, Connecticut 06484-4794 U.S.A.

Or emailed to: info@perkinelmer.com

Notices

The information contained in this document is subject to change without notice.
Except as specifically set forth in its terms and conditions of sale, PerkinElmer makes no warranty of any kind with regard to this document, including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.
PerkinElmer shall not be liable for errors contained herein for incidental consequential damages in connection with furnishing, performance or use of this material.

Trademarks

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are protected by law.

PerkinElmer is a registered trademark of PerkinElmer, Inc.

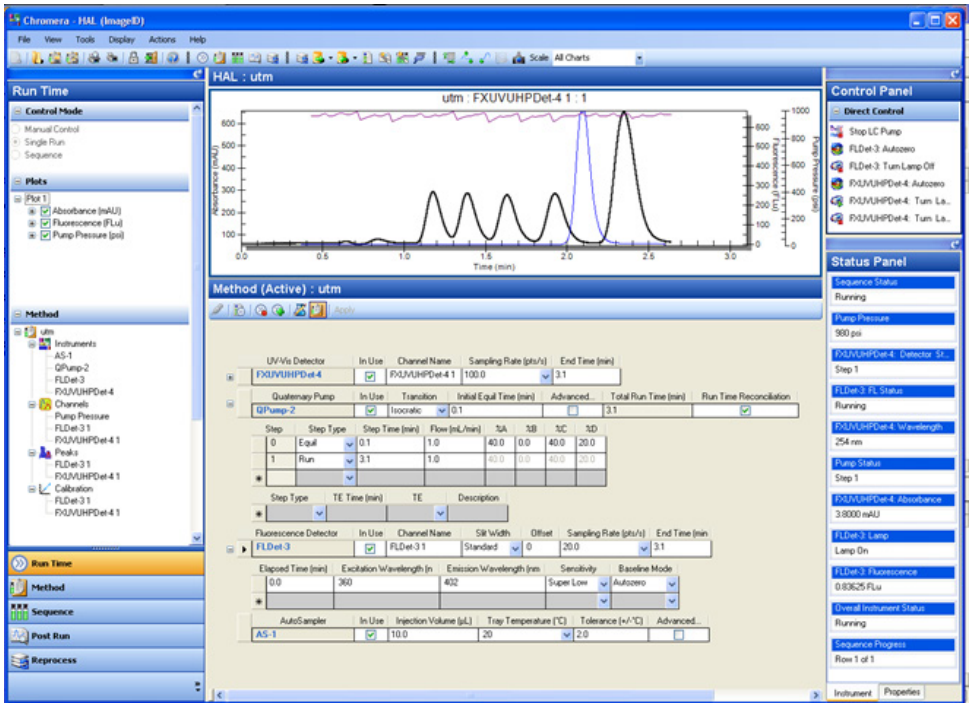
Table of Contents

Chromera Overview

Chromera Overview

Chromera is a powerfully-easy data system for liquid chromatography. It provides highly configurable and responsive LC instrument control for multi-detector systems, combined with an elegantly simple user interface for interactive processing, and flexible, multi-channel quantitation and reporting. Chromera is designed to display all of the necessary information on the screen to give you complete control of your system. The screen layout consists of a Navigation pane on the left that contains buttons and tree lists to select various views within the software. The views are composed of different combinations of controls contained within separate panes or frames. In many cases a control contained within one pane will interact with other controls contained in other panes. The views are pre-determined but as you become more familiar with Chromera, you are able to construct views of your own, save them, and recall them on demand.

The main Chromera window includes standard elements such as a title bar, a menu bar, and a toolbar. The main area of the window includes a navigation pane on the left-hand side, Status and Control panels on the right-hand side, and a main display in the center whose contents vary depending on the selected environment. You can hide the Navigation pane, Control panel, and Status panel to provide maximize usable main display screen space The Status panel and Control panel will not be displayed in an offline instance of Chromera.



When more than one frame is displayed in the main display area (as shown in this example where it contains two frames: a plot and a parameter section) you are able to adjust the relative sizes of the frames by means of a **splitter bar** between the two frames. The main window title bar will always display **Chromera**. In an online instance **Chromera** is followed by **–** the **instrument name** followed by **–** the **user name** in parenthesis. For example:

Chromera – Instrument 1 (GolayM)

An offline instance associated with an instrument will look essentially the same but with **Offline** following the instrument name. An offline instance not associated with a particular instrument will show Offline and then just the user name:

Chromera – Instrument 1 Offline: (GolayM)

The main environments in Chromera are Run Time, Method, Sequence, Post Run, Reprocess and Reports.

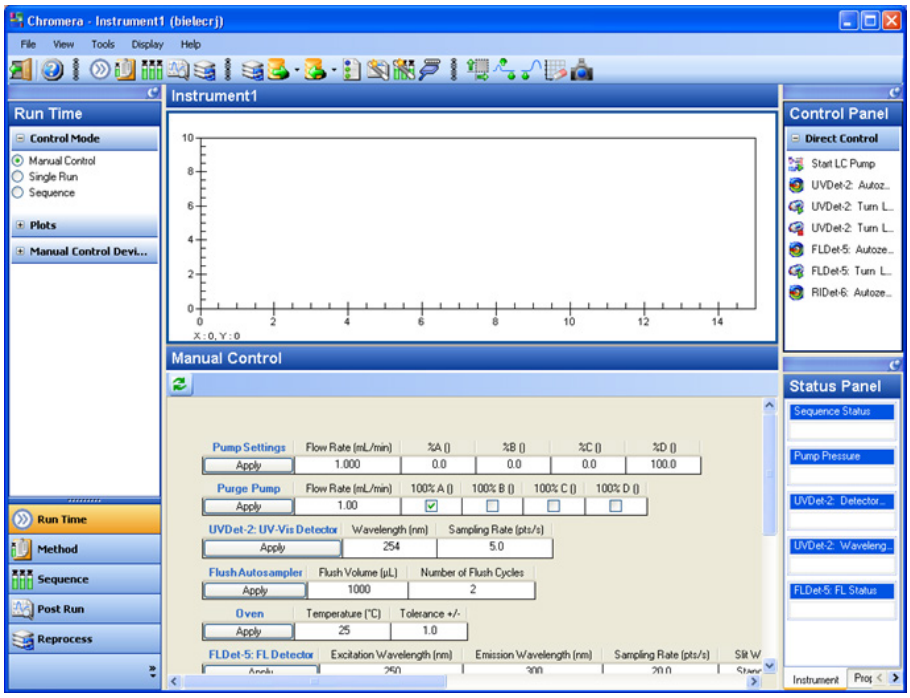
About Fast User Switching

Fast User Switching, is a feature of Windows XP Home Edition and Windows XP Professional (when it is not joined to a domain) that makes it possible to switch between users without actually logging off from the computer. Multiple users can share a computer and use it simultaneously, switching back and forth without closing the programs they are running.

Operation of Chromera is **not compatible** with Fast User Switching and therefore **Fast User Switching must be turned off** under User Accounts in any workstation on which Chromera will be run.

Run Time

The Run Time environment consists of three views, one for each of the three modes of operation: **Manual Control**, **Single Run**, and **Sequence**. Each of these views contains two frames: a graphics frame for displaying the signal from one or more channels, and a frame that displays control parameters relating to the mode of operation. The active mode of operation is selected via the set of radio buttons in the upper section of the navigation pane.

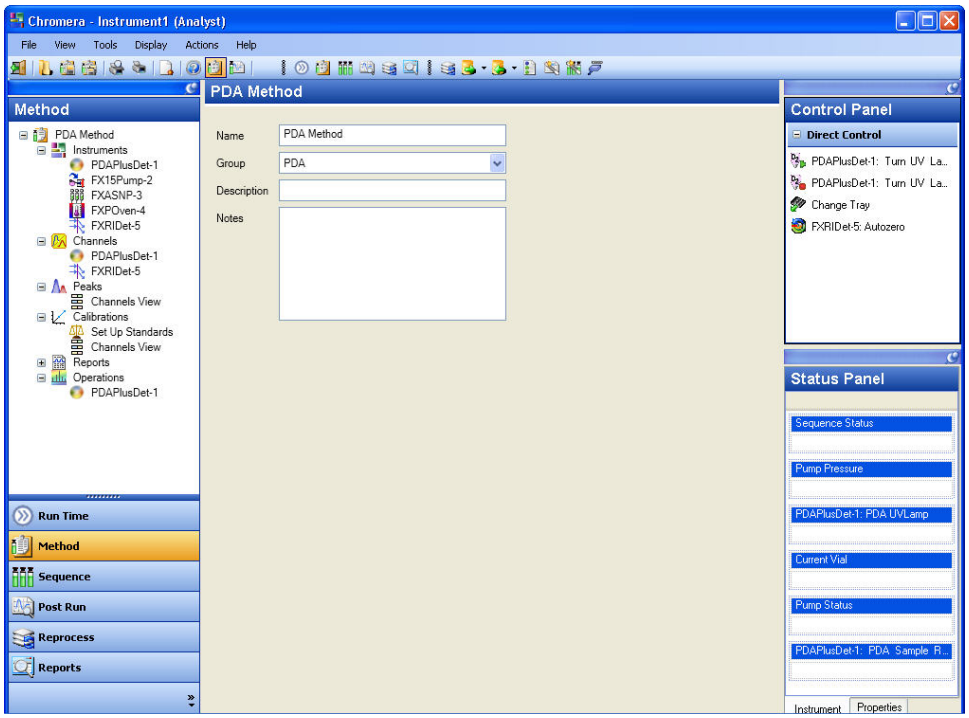


Manual Control	Displays settings to provide immediate control of the instrument devices.
Single Run	Displays the controlling method or run parameters such as sample name and vial number.
Sequence	Displays the sequence or the method from the current row (or a selected row when sequence is not running)

For full details see **Run Time**.

Method Editor

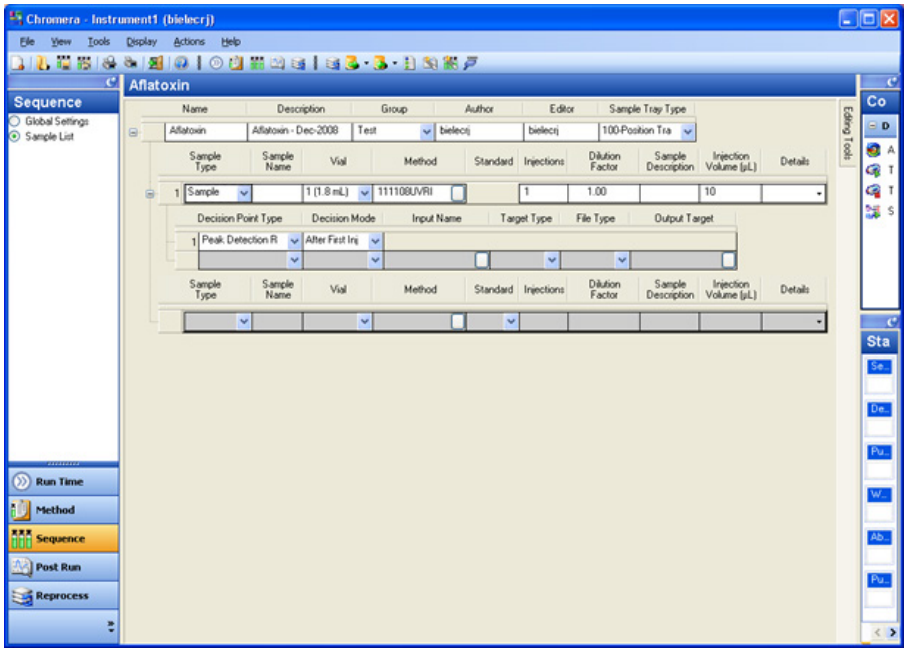
In the Method Editor environment sections of the method are displayed in the center of the screen. The specific section displayed depends on the selection in a tree control shown in the upper section of the Method pane. You can select and display the Instruments, Channels, Peak Detection, Calibration, or Reports section.



For full details see **Method Editor.**

Sequence Editor

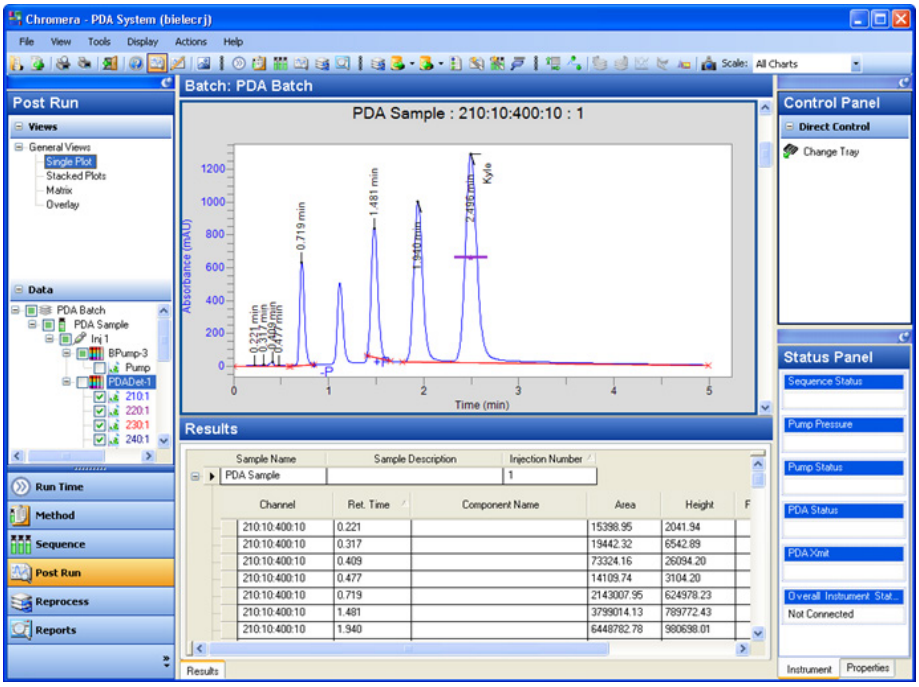
In the Sequence Editor environment the main display area contains a single frame that displays a view of the sequence. The specific view displayed depends on the selection made via radio buttons in the upper section of the navigation pane. You can select **Global Settings** or **Sample List**.



For full details see **Sequence Editor.**

Post Run

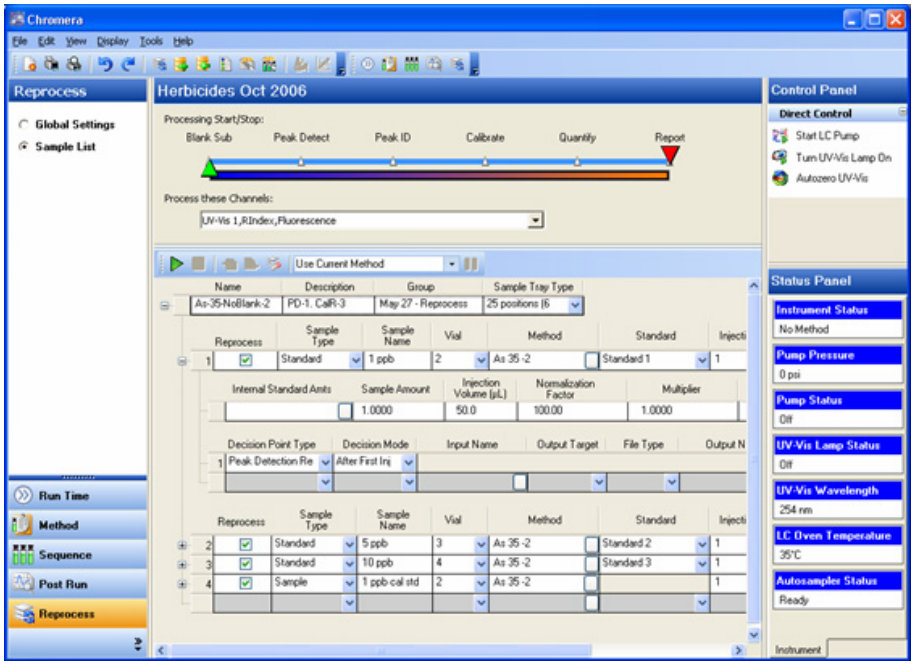
The Post Run environment contains two frames, one for graphics and one for result data. You may maximize one or the other. The selection in the **Views** tree determines the configuration of the chromatograms frame, while the selection in the **Data** tree determines the selected chromatogram and the contents of the results frame.



For full details see **Post Run Environment**.

Reprocess

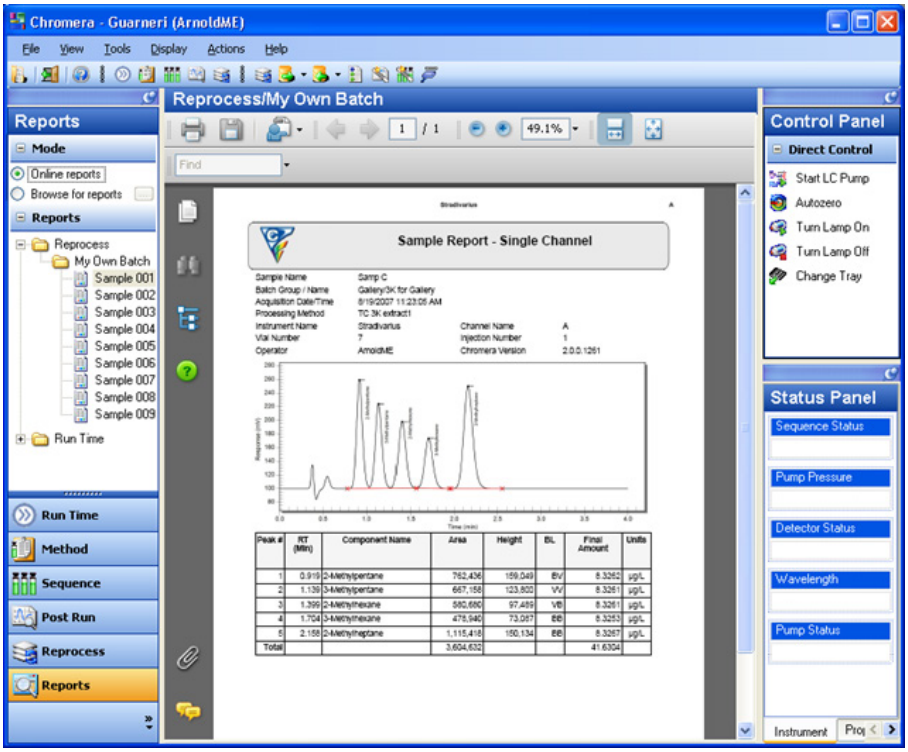
The Reprocess environment contains two frames. The upper frame contains controls for setting up the reprocessing functions and the lower frame both displays the batch to be reprocessed and allows selection of the sample in the batch to be reprocessed.



For full details see **Reprocess**.

Reports

The main area of Report Viewer is a PDF viewer. It provides the basic requirements of page selection, a variable magnification display, and printing. In addition it provides some search capabilities.



For full details see **Report Viewer**.

Navigation Pane

The Navigation pane consists of two main panels: the upper ***Navigation Panel*** and the lower ***Button Panel***. The Button Panel contains context-switching buttons that provide access to the major environments of the software. The Navigation Panel contains secondary navigation and selection controls specific to the current environment. The contents of the upper panel of the Navigation Pane depend on the selected environment.

The buttons available in the Navigation Pane are: Run Time, Method, Sequence, Post Run, Reprocess, and Reports. They can be displayed as buttons or icons. They can be displayed as buttons or icons. A selected button is highlighted to indicate the current environment. Moving the mouse pointer over a button will temporarily highlight it. You can resize the button panel by dragging the divider above the buttons. As the size is reduced, buttons are removed from the bottom of the list. Hidden buttons are displayed as icons displayed on the bottom section of the panel (the icon bar), which is always visible.

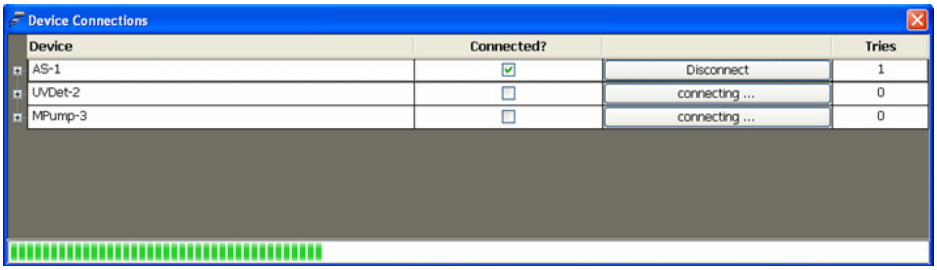
The title at the top of the navigation pane is the same as the text on the currently selected button. The icon bar at the very bottom of the button panel is always displayed. Clicking on the icon at the right hand side of the icon bar displays a popup menu. When there are no buttons the popup menu icon will still be available. The iconized buttons are:

Button	**Icon**
Run Time	
Method	
Sequence	
Post Run	
Reprocess	
Reports	

The Navigation pane can be pinned in place at the left hand side of the screen or set to the **AutoHide** mode, so that it appears when the Navigation tab is selected from the fly-out panel and then disappears a few seconds after the mouse pointer is moved elsewhere on the screen.

Device Connections

The Device Connections dialog appears when you first start Chromera to show the instruments connected, indicated by a checkbox in the **Connected** column. You can also connect or disconnect devices while running Chromera by selecting **Device Connections** from the Control Panel and clicking on the **Connect/Disconnect** button.

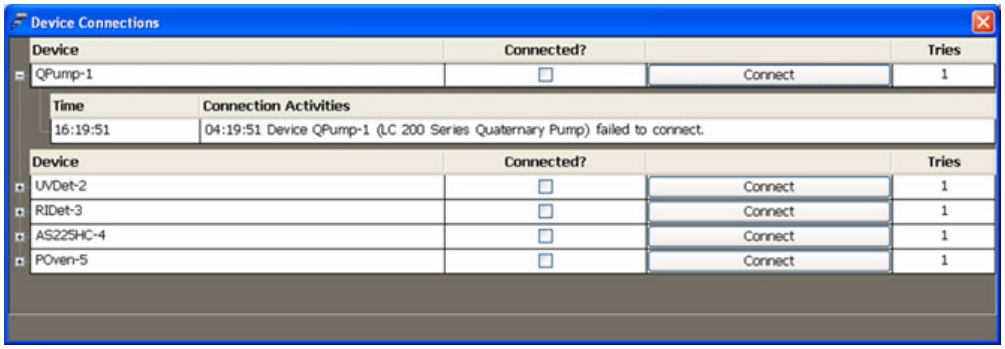


When starting Chromera, if a Device does not connect (as indicated in the Device Connections dialog) turn the device off (power down the device), wait about 30 seconds, then turn it back on. This usually establishes the connection. If not, click the **Connect/Disconnect** button for that device.

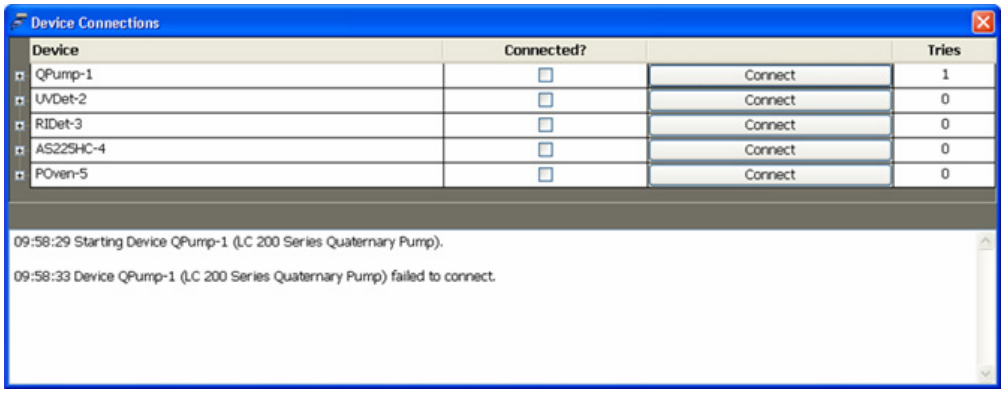
Control	**Description**
Dialog	A row is displayed for each instrument module configured on the system. The module is identified by the name given to the device by the analyst during configuration.
Device	The name given to the device by the analyst during configuration
Connected?	A check box indicating the current connection state of the module. The box is checked if the software is currently in communication with the instrument. You are not able to directly change the state of this control by clicking on it.
Connect/Disconnect	Click the button to make the software attempt to connect to the module or to disconnect from it (depending on the current connection state). In the case of the ICP-MS, the software attempts to communicate via the NEXUS interface, or terminate communications, as applicable. For the other modules, if the software is currently controlling the module it will release it. If the software is not currently controlling the module it will attempt to communicate with it (and seize it where applicable).
Tries	Displays the number of attempts the software has made to connect to the device.

Messages

If there are messages associated with the current session of the Device Connections dialog then they are displayed associated with the appropriate device and are viewed by expanding the device row by clicking on the  sign.



When the dialog is opened from the Tools menu and you initiate an action (e.g. by clicking on the Connect button for a device), a message is displayed below the display to confirm the action. Similarly, if an error results from that action then an error message is displayed in the same area. This ensures you will see the messages, even if the row for that device is not expanded.



All device messages are cleared when the Device Connections dialog is closed (although errors will be retained in the Error Log and can be viewed at any time). Whenever the Device Connections dialog is opened from the Tools menu, it will initially contain no device messages (unless one happens to be generated coincidentally).

Common Menus

There are menu items common to every environment (**File**, **Edit**, **View**, **Tools,** and **Help**). Since the contents of the **File** menu is customized for every environment that menu is described in the sections specific to each environment and not here (but see Exit Dialog for details of the Exit dialog).

For example, a set of functions you might want to access from any environment are gathered into the **Tools** menu. Each of these common tools is independent of any specific environment and it operates in a separate window. These functions include: (Batch) Reprocess, Export, Import, Preferences, Report Format Wizard, and Sequence Wizard.

Menu	Command	Description
View	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Reprocess environment. The same action as clicking the Reprocessing button in the Navigation Pane.
	Reports	Switches to the Report Viewer.
	Log Viewer	Opens a special mode of the Data Selector which displays a Microsoft Windows Event Viewer styled listing of the current events recorded in the database.
	Toolbars ► View	Toggles display of the View toolbar.
	Tools	Toggles display of the Tools toolbar.
	Graphics	Toggles the display of the Graphic toolbar.
	Show Small Icons	When selected the toolbar icons displayed will be 16x16
	Show Large Icons	When selected the toolbar icons displayed will be 24x24
Tools	Export ► Chromera Results…	Displays the Database export dialog.
	Methods…	Displays the Method export dialog.
	Sequences…	Displays the Sequence export dialog.
	Report Templates…	Displays the Report template Export dialog.

	Import ► TotalChrom Data… Chromera Results… Chromera Methods… Chromera Sequences… Chromera 2.0 Data... Report Templates…	Displays the Import window. Displays the Import Results dialog. Displays the Import Methods dialog. Displays the Import Sequences dialog. Displays the Import Chromera 2.0 data dialog. Displays the Report template Import dialog.
	Preferences	Displays the Preferences window, showing the preferences associated with the current user (see Setting Preferences).
	Report Format Wizard	Displays the Report Format Wizard in New Report mode (see Report Format Wizard).
	Sequence Wizard	Displays the Sequence Wizard.
	Device Connections	Displays the Device Connections dialog.
	View Current Session Logs	Displays the Data Selector - Event Viewer with session log information.
	Error Log	Displays the Error Log dialog.
	Dictionary Editor	Displays the Dictionary Editor dialog
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector (see Batch Reprocess). It is disabled in an online instance when data acquisition is in progress, or a sequence is running.
	Batch Builder	Displays the Batch Builder dialog.
	View Retrieved Archived Logs	Displays a dialog to select a retrieved event logs database to display in the Data Selector - Retrieved Session Logs Viewer.
Help	Topics	Opens the Chromera Help window.
	Consumables and Accessories	Opens the PerkinElmer Consumable and Accessories Catalog
	About Chromera	Displays the copyright and version dialog.

Common Toolbars

There are two common toolbars displayed in every environment: **View** and **Tools**. A Standard toolbar will always appear but the contents will change depending on the environment. The standard toolbar is defined within the sections describing the individual environments.

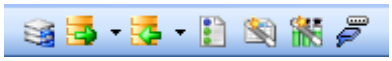
View Toolbar

This toolbar simply mirrors the buttons in the Navigation Pane and the commands in the **View** menu.

Icon	Command	Description
	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Batch Reprocess environment. The same action as clicking the Batch Reprocessing button in the Navigation Pane.
	Reports	Switches to the Report Viewer.

Tools Toolbar

This toolbar includes the most commonly used commands from the Tools menu.



Icon	Command	Description
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector in single batch selection mode.
	Export ► Chromera Results… Methods… Sequences…	 Initiates the Export of Chromera results by displaying the Database export dialog. Initiates the Export of Chromera methods by displaying the Database export dialog. Initiates the Export of Chromera sequences by displaying the Database export dialog.
	Import ►	

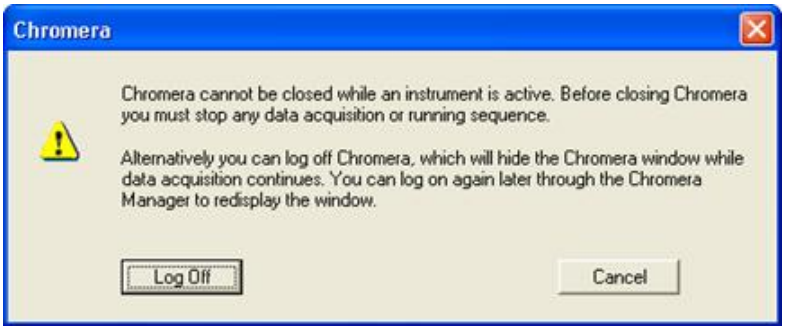
	TotalChrom Data… Chromera Results… Chromera Methods… Chromera Sequences…	Displays the Import window. Displays the Import Results dialog. Displays the Import Methods dialog. Displays the Import Sequences dialog.
	Preferences	Displays the Preferences window, showing the preferences associated with the current user.
	Report Format Wizard	Displays the Report Format Wizard in New Report mode.
	Sequence Wizard	Displays the Sequence Wizard.
	Device Connections	Displays the Device Connections dialog.

Exit Dialog

The one **File** menu command that is common to all environments is **Exit**. If you select Exit in an online instance of Chromera when either a Single Run mode run is in progress or a sequence is running, then one Exit dialog displays if User Management is active and a different dialog displays if User Management is not active.

User Management Active Version

If User Management is active the dialog includes Log Off and Cancel buttons and the message text is:



Chromera cannot be closed while an instrument is active. Before closing Chromera you must stop any data acquisition or running sequence. Alternatively you can log off Chromera, which will hide the Chromera window while data acquisition continues. You can log on again later through the Chromera Manager to redisplay the window.

If you click the **Log Off** button, the instance of Chromera is locked and hidden, while data acquisition continues. If you click the **Cancel** button then the Chromera window remains open and visible.

Without User Management

If User Management is not active the dialog includes just a **Close** button and the message text is:

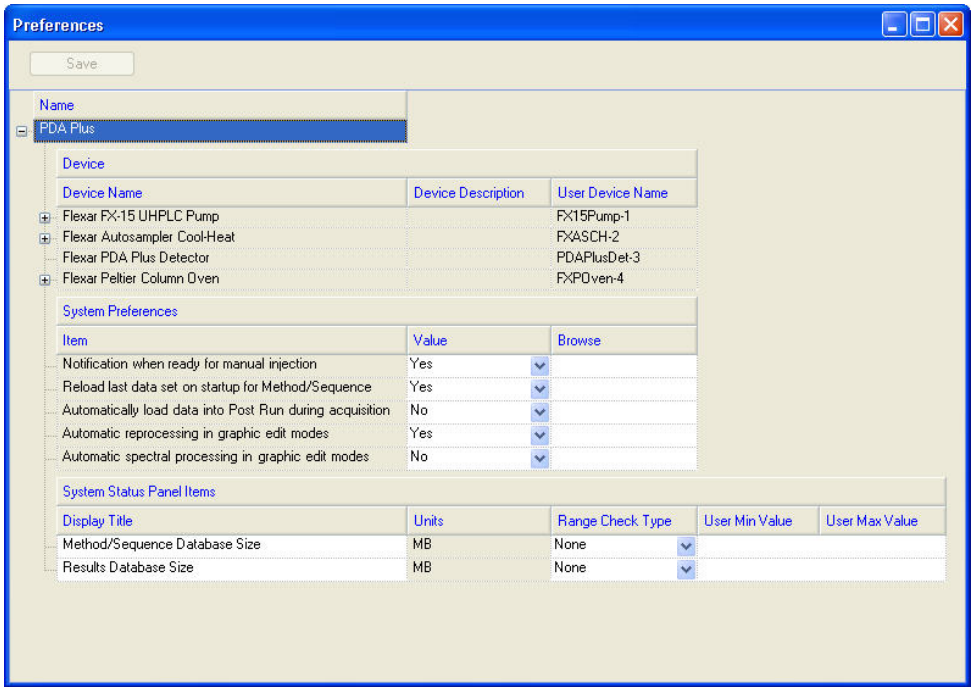
Chromera cannot be closed while an instrument is active. Before closing Chromera you must stop any data acquisition or running sequence.

When you click the **Close** button the Chromera window remains open and visible.

Setting Preferences

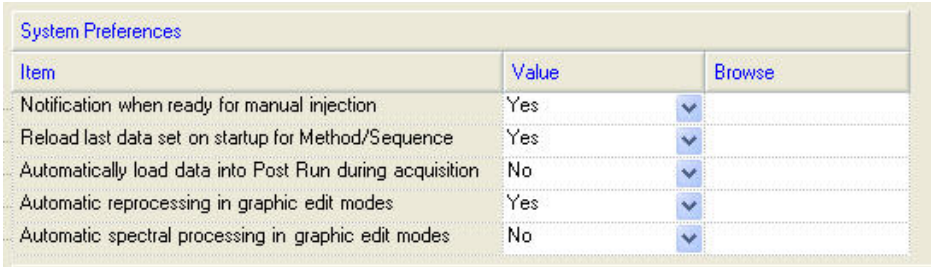
About Setting Preferences

User Preferences are typically set within the **Preferences** window. Also, additional user preferences are set within the environment with which they are associated. The user interaction associated with implicit preferences is described within the environment (e.g. Plot Layout) or control (e.g. Plot Styles). Within an instance of Chromera, only the preferences for the current instrument and current user displays.



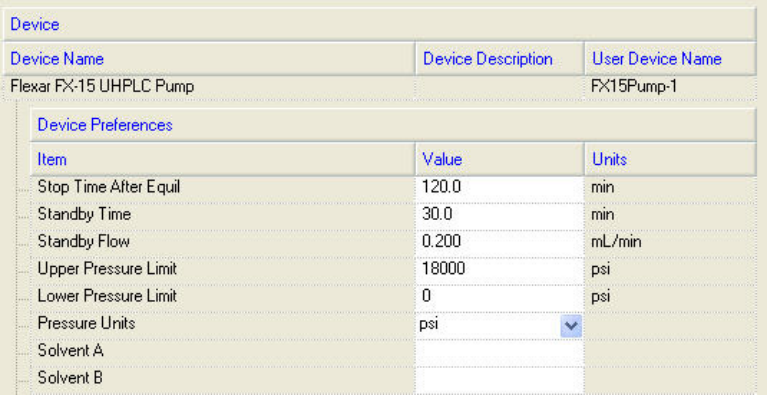
To set preferences, select **Preferences** from the **Tools** menu in Chromera.

1. Set the **System Preferences** associated with a device. These are Yes/No flags that set certain behaviors of user interaction.



2. Set the preference settings associated with each hardware device that includes default method settings.

   In addition, some devices have settings enabling you to define limits for status panel items. Icons are displayed in the status panel when the current value for such an item is above or below the defined range.



Device

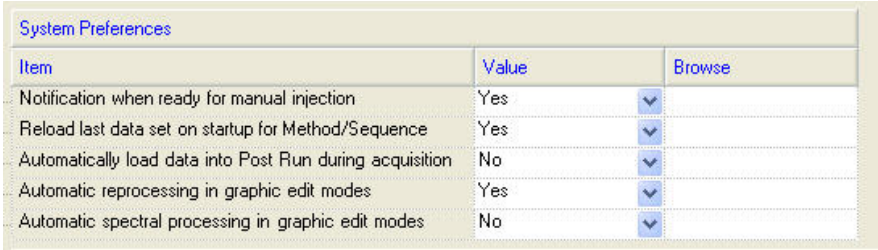
Device Name	Device Description	User Device Name
Flexar FX-15 UHPLC Pump		FX15Pump-1

Device Preferences

Item	Value	Units
Stop Time After Equil	120.0	min
Standby Time	30.0	min
Standby Flow	0.200	mL/min
Upper Pressure Limit	18000	psi
Lower Pressure Limit	0	psi
Pressure Units	psi	
Solvent A		
Solvent B		

System Preferences

You can set following System Preferences:



System Preferences

Item	Value	Browse
Notification when ready for manual injection	Yes	
Reload last data set on startup for Method/Sequence	Yes	
Automatically load data into Post Run during acquisition	No	
Automatic reprocessing in graphic edit modes	Yes	
Automatic spectral processing in graphic edit modes	No	

Preference	Description
Notification when ready for manual injection	Select **Yes** or **No** from the drop-down list whether or not an Outlook-style notification message is displayed in the lower right-hand corner of the screen when a system without an autosampler becomes ready for injection. See Manual Injection.
Reload last data set on startup for Method/Sequence	Select **Yes** or **No** from the drop-down list whether or not the data set displayed in each environment at the time the user closes an instance of Chromera is reloaded when an instance of that type is reopened.
Automatically load data into Post Run during acquisition	Select **Yes** or **No** from the drop-down list whether data generated by the active sequence is automatically displayed in the post run environment as they are generated.
Automatic reprocessing in graphic edit modes	Select **Yes** or **No** from the drop-down list if the data are automatically reprocessed with each change made in the graphic editors, or if reprocessing only occurs on user command.
Automatic spectral reprocessing in graphic edit modes	Select **Yes** or **No** from the drop-down list if the spectral data are automatically reprocessed with each change made in the graphic editors, or if reprocessing only occurs on user command.

Implicit Preferences

The term implicit preferences is used only to indicate that you will not enter these settings in the Preferences window. However, you enter many of the settings in dialogs while using the environment with which they are associated.

Implicit preferences are:

Plot options	Control the display of chromatograms (and other channel graphics). They are set within a dialog and are described in detail in Plot Styles.
Plot layouts	Defines layout of plots in Run Time and Post Run. They are set within dialogs, described in detail in sections (Run Time Graphic Plot Layout Dialog, Stacked Plots View, and Matrix View). There are separate settings for Run Time, Post Run - Stacked View, Post Run - Matrix View.
Control Panel display	State (floating, pinned or auto-hide) and width. Set by popup menu commands and/or direct manipulation of the window.
Status Panel display	Contents, state (floating, pinned or auto-hide) and width. The content are set within the Status Panel Setup dialog. Also, other behavior set popup menu commands and/or direct manipulation of the window.
Navigation Panel display	State (floating, pinned or auto-hide) and width. Also button display state (full or iconized). It is set by popup menu commands and/or direct manipulation of the window.
Main display area layout	The relative sizes of each pane.

Pump Preferences (Flexar or Series 200)

Set the following Pump Preferences:

Control	Description
Lower pressure limit	Set lower limit of the pressure below which the pump will shut off. Refer to your pump specifications to determine an appropriate value.
Upper pressure limit	Set the upper limit of the pressure, above which the pump will shut off. Refer to your pump specifications to determine an appropriate value.
Stop Time After Equil (min)	Set the number of minutes that the pump remains in a READY state, after which it will shut down. (The range is 15.0 min to 999.0 min in 0.1 min increments.)
Solvent: A Solvent: B Solvent: C Solvent: D	Define the name of solvent in reservoir A, B, C, and D using up to 25 characters. **NOTE:** This is the solvent name and it should not include % but it is required for correct operation of the Status Panel.
Standby Time (min)	Set the number of minutes that the pump will remain in the Ready state before changing to the standby flow rate. (The range is 15 to 999 min.)
Standby Flow (mL/min)	Set the flow rate to be set after the Standby Time has elapsed. 0.00 to 3.00 mL/min for microbore pumps 0.00 to 10.0 mL/min for other Series 200 pumps
Pressure Units	Select the pressure units (psi, bar, or MPa) from a drop-down list to be used throughout the user interface for this instrument. When the units are changed, the values for the Upper and Lower pressure limit preference settings are converted and displayed in the new units.

NOTE: *The actual maximum value when units are bar or MPa will be the equivalent to 6100 psi based on the conversion factors used.*

Pump Preferences (Flexar SS Pumps)

Set the following Flexar SS Pump Preferences:

Control	Description
Lower pressure limit	Set lower limit of the pressure below which the pump will shut off. Refer to your pump specifications to determine an appropriate value.
Upper pressure limit	Set the upper limit of the pressure, above which the pump will shut off. Refer to your pump specifications to determine an appropriate value.
Stop Time After Equil (min)	Set the number of minutes that the pump remains in a READY state, after which it will shut down. (The range is 15.0 min to 999.0 min in 0.1 min increments.)
Solvent reservoirs used	Select from the drop-down list the pair of solvent reservoirs (A1/B1, A1/B2, A2/B1, A2/B2) to be used in defining the mobile phase composition during the run.
Solvent A: 1 Solvent A: 2 Solvent B: 1 Solvent B: 2	Select a name from the solvents dictionary (which populates the drop-down list), or enter a new solvent name, for reservoir A1, A2, B1, and B2.
Compressibility	Select from the drop-down list a compressibility factor that most closely matches the selected solvent, for reservoir A1, A2, B1, and B2. The compressibility values are: Water: 46, Acetonitrile: 115, Chloroform: 100, Ethyl Acetate: 104, Hexane: 150, Isopropanol: 100, Methanol: 121, THF: 95, Toluene: 87
Standby Time (min)	Set the number of minutes that the pump will remain in the Ready state before changing to the standby flow rate. (The range is 15 to 999 min.)
Standby Flow (mL/min)	Set the flow rate to be set after the Standby Time has elapsed. 0.00 to 3.00 mL/min for microbore pumps 0.00 to 10.0 mL/min for other Series 200 pumps
Pressure Units	Select the pressure units (psi, bar, or MPa) from a drop-down list to be used throughout the user interface for this instrument. When the units are changed, the values for the Upper and Lower pressure limit preference settings are converted and displayed in the new units.

NOTE: *The actual maximum value when units are bar or MPa will be the equivalent to 6100 psi based on the conversion factors used.*

Autosampler Preferences (Flexar or Series 200, 225, or 275)

Set the following Autosampler Preferences.

Control	Description
Flush Volume (µL)	Set the volume of flush liquid used for each flush cycle. (The range is 1 to 2500 µL in 1 µL increments with a default setting of 1000.)
Flush Speed	Select the speed at which the pump will flush the system. (The selections are Very Slow, Slow, Medium, Fast and Very Fast with a default set to Fast.)
Number of Pre-Injection Flushes	Set the number of flush cycles before each sample injection. (The range is 0 to 9 with a default setting of 0.)
Number of Post-Injection Flushes	Set the number of flush cycles after each sample injection. (The range is 0 to 9 with a default setting of 1.)
Temperature Control	Select On or Off from the drop-down list to set whether the tray temperature control will be active or not. This is not displayed for No Peltier models.
Tray Temperature (°C)	Set the temperature to be maintained when the Peltier autosampler tray is installed. (The range is 4 to 60 °C (and -1 = Off) with a default setting of 20.)
Tolerance (± °C)	Set the deviation from set temperature allowed for the Ready condition. (The range is 0.1 to 60.0 with a default setting of 2.0.)
Loop Size (µL)	Set the sample loop size. (The range is 0 to 2500 µL with a default setting of 200.)
Air Cushion (µL)	Set the air cushion between sample and flush solvent. (The range is 0 to 99 µL in 1 µL increments with a default setting of 10.)
Sample Syringe Size (µL)	Set the volume of the syringe used to dispense sample. (The range is 50 to 2500 µL with a default setting of 250.)
Sample Speed	Select from the drop–down list the speed at which you want sample dispensed. (The selections are Very Slow, Slow, Medium, Fast and Very Fast with a default set to Fast.)
Injection Delay Time (min)	Set the time after the autosampler has sent the Start signal to the other instrument devices that the injection is actually made. (The range is 0.00 to 99.99 min with a default of 0.00.)

NOTE: *The Series 225 and 275 have a similar set of preferences to the Series 200 except Excess Volume and Fixed Mode are not present for Series 225/275.*

Autosampler Preferences (Flexar DS)

Set the following Autosampler Preferences.

Control	Description
Flush Volume (µL)	Set the volume of flush liquid used for each flush cycle. (The range is 1 to 2500 µL in 1 µL increments with a default setting of 1000.)
Flush Speed	Select the speed at which the pump will flush the system. (The selections are Very Slow, Slow, Medium, Fast and Very Fast with a default set to Fast.)
Number of Pre-Injection Flushes A	Set the number of flush cycles with solvent A before each sample injection.
Number of Pre-Injection Flushes B	Set the number of flush cycles with solvent B before each sample injection.
Number of Post-Injection Flushes A	Set the number of flush cycles with solvent A after each sample injection.
Number of Post-Injection Flushes B	Set the number of flush cycles with solvent B after each sample injection.
Temperature Control	Select On or Off from the drop-down list to set whether the tray temperature control will be active or not. This is not displayed for No Peltier models.
Tray Temperature (°C)	Set the temperature to be maintained when the Peltier autosampler tray is installed. (The range is 4 to 60 °C (and -1 = Off) with a default setting of 20.)
Tolerance (± °C)	Set the deviation from set temperature allowed for the Ready condition. (The range is 0.1 to 60.0 with a default setting of 2.0.)
Loop Size (µL)	Set the sample loop size. (The range is 0 to 2500 µL with a default setting of 200.)
Air Cushion (µL)	Set the air cushion between sample and flush solvent. (The range is 0 to 99 µL in 1 µL increments with a default setting of 10.)
Sample Syringe Size (µL)	Set the volume of the syringe used to dispense sample. (The range is 50 to 2500 µL with a default setting of 250.)
Sample Speed	Select from the drop–down list the speed at which you want sample dispensed. (The selections are Very Slow, Slow, Medium, Fast and Very Fast with a default set to Fast.)
Injection Delay Time (min)	Set the time after the autosampler has sent the Start signal to the other instrument devices that the injection is actually made. (The range is 0.00 to 99.99 min with a default of 0.00.)

UV/Vis Detector Preferences (Flexar or Series 200)

Set the following UV/Vis Detector Preferences:

Control	Description
Wavelength (nm)	Set the wavelength value in nm.
Data Rate Parameter	A drop-down list to set the parameter that appears in the method for setting data acquisition rate. (The selections are: Sampling Rate (pts/s) and Peak Width (s))

Column Oven Preferences (Flexar or Series 200)

Set the following Oven Preferences:

Control	Description
Temperature (°C)	An edit field to set the oven temperature. (The range is 5 °C to 90 °C with a default setting of 25.)
Tolerance (± °C)	An edit field to set an acceptance value. (The range is 1 °C to 90 °C with a default setting of 1.)

Fluorescence Detector Preferences (Flexar or Series 200a)

Set the following Fluorescence Detector Preference:

Control	Description
Lamp Life Limit (hrs)	Hours of use before the detector will flag a lamp error.

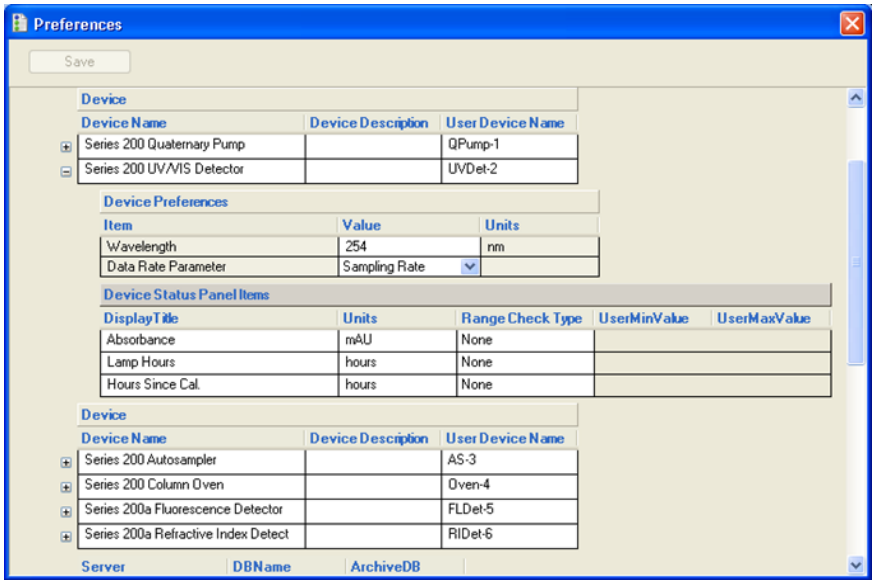
ICP-MS Preferences

Set the following ICP-MS Preferences:

Control	Description
Dead Time (ns)	Enter a value (from 0 to 100) for dead time to be used in reprocessing data from the ICP-MS.
Short Settling Time in Use	Select **On** or **Off** from a drop-down list to use the Short Settling Time in place of the Normal Settling Time. This is applicable to Pulse mode only.
Sweeps	Enter the number of sweeps (from 0 to 100) through the mass spectrum that is averaged to yield each data point.

Status Panel Limits

The Device Status Panel Item preferences enable you to define warning limits for certain key parameters, such that if the value fails outside the defined range a warning icon will appear in the Status Panel.



Control	Description
Display Title	Enter the name of the parameter in the Status Panel.
Units	Display the units of the parameter, where applicable.
Range Check Type	Select from the drop-down list None, Min/Max to define if range checking is to be performed.
User Min Value	Enter the minimum allowed value for the parameter.
User Max Value	Enter the maximum allowed value for the parameter.

The parameters for which such range checking is available are defined below, for each device.

Pump Status Panel Items

- Pump Pressure
- Pump Flow

Autosampler Status Panel Items

- Peltier Tray Temperature

Detector Status Panel Items

- Absorbance
- Lamp Hours
- Hours Since Cal.

Fluorescence Detector Status Panel Items

- EX Wavelength
- EM Wavelength
- Fluorescence
- EX Energy
- EM Energy
- Xe Lamp Hours
- Hg Lamp Hours

Refractive Index Detector Status Panel Items

- Temperature offset
- Lamp Voltage
- Lamp Hours
- Drift
- Noise
- Refractive Index
- Temperature

Oven Status Panel Items

- Oven Temperature

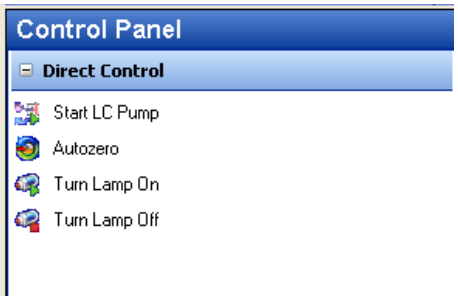
ICP-MS Status Panel Items

- Vacuum Pressure
- Nebulizer Gas Flow
- ICP RF Power
- Lens Voltage
- Main Water Temperature
- Interface Water Temperature
- Torch Box Temperature
- Cell A Gas Flow
- Cell B Gas Flow
- MGF Gas Flow

Control Panel

About the Control Panel

The Control Panel provides convenient control of the instrument functions in Real Time and lets you control certain instrument settings after you have set up an instrument. The actual parameters available depend on the configuration setup. The Control Panel is available at all times and contains **Start/Stop Items** and **Adjustments.**



The panel may be **pinned in place** at the right hand side of the screen or it may be set in **Auto Hide** mode so that it appears when the Control Panel tab is selected from the fly-out panel and then disappears a few seconds after the mouse pointer is moved elsewhere on the screen. The Control Panel and Status Panel share the right hand edge of the screen. When both are pinned the control panel appears at the top and the status panel at the bottom, with a slider bar between them, allowing resizing of the relative space occupied by each.

- You can float or dock the Control Panel anywhere in the application. If you move the Control Panel out, double-click on it to return it to its default position.
- You can also hide/unhide the Control Panel by clicking the thumbtack in the upper right corner of the dialog.

The instrument configuration determines the commands available in the **Control Panel** since each command is associated with a device. The displayed string will be exactly as shown below when only a single device of that role (e.g. pump or detector) is configured, but will be preceded by the user name for the device when more than one device of that role is present. For example, Autozero would be displayed if a single UV-Visible detector was configured but if a UV-Visible and a fluorescence detector were configured the commands might be:

My UV-Vis: Autozero
My FL: Autozero

When the mouse pointer hovers over a Control Panel button, in addition to the button being highlighted, a tool tip indicates the formal model name of the device associated with the command (e.g. Series 200 Quaternary Pump).

Using the Control Panel

The Control Panel is a convenient tool for conditioning a column (performing the equilibrium between the mobile phase and the column). It defaults to the conditions of the last active method. The panel allows flushing of the Autosampler based on the last active method. The LC and ICP-MS controls easily accessible are: Start/Stop Sequence, Start Peristaltic Pump, Start the Plasma, Start/Stop LC Pump, control the Switching Valve, PCI Valve, Manual Control, Monitor Baseline, and Device Connections.

NOTE: *Any change that is made on the Control Panel **will** affect the current active method.*

The **Control Panel** displays on the right side of the screen; however, it can float or dock anywhere in the application.

Control	**Description**
Start/Stop Sequence	Allows you to start or stop the sequence at any time.
Start/Stop Peristaltic Pump	Allows you to start the ICP-MS Peristaltic pump. This is necessary for draining the spray chamber.
Start/Stop Plasma	Allows you to start or stop the plasma.
Start/Stop LC Pump	Allows you to start or stop the LC pump.
Switching Valve	Directs the output from the LC to either the ICP-MS or waste.
PCI Valve	Controls the state of the post–column injection (PCI) valve.
Manual Control	Displays the Manual Control view. Through this dialog you have the following LC instrument control; pump, purge, autosampler, Peltier tray, and column oven.
Monitor Baseline	Allows the baseline to be monitored for a selected method. No injections are made or data stored in this window.
Device Connections	Displays the connected devices and provides the ability to Connect or Disconnect a device.

Control Panel

The following icons are examples of what may be displayed in the Control Panel:

Icon	Function	Instrument	Description
	Turn PDA Vis Lamp On	Detector	Sends a PDA Vis lamp On command to the detector
	Turn PDA Vis Lamp Off	Detector	Sends a PDA Vis lamp Off command to the detector
	Turn PDA UV Lamp On	Detector	Sends a PDA UV lamp On command to the detector
	Turn PDA UV Lamp Off	Detector	Sends a PDA UV lamp Off command to the detector
	Start LC Pump	Pump	Sends the start command to the pump module.
	Stop LC Pump	Pump	Sends the stop command to the pump module.
	Turn Lamp On	Detector	Sends a lamp on command to the detector (UV /Vis and/or Fluorescence detector)
	Turn Lamp Off	Detector	Sends a lamp off command to the detector (UV/Vis and/or Fluorescence detector)
	Autozero	Detector	Sends an autozero command to the detector (UV/Vis, RI and/or Fluorescence detector).
	Start Peristaltic Pump	ICP-MS	Sends a start peristaltic pump command to the ICP-MS software.
	Stop Peristaltic Pump	ICP-MS	Sends a stop peristaltic pump command to the ICP-MS software.
	Start Plasma	ICP-MS	Sends a Start Plasma command to the ICP-MS software.
	Stop Plasma	ICP-MS	Sends a Stop Plasma command to the ICP-MS software.
	Switching Valve	Valve	Changes the state of the switching valve. (one icon per state)
	PCI Valve	Valve	Changes the state of the Post Column Injection valve. (one icon per state)

Pump Commands (Flexar or Series 200)

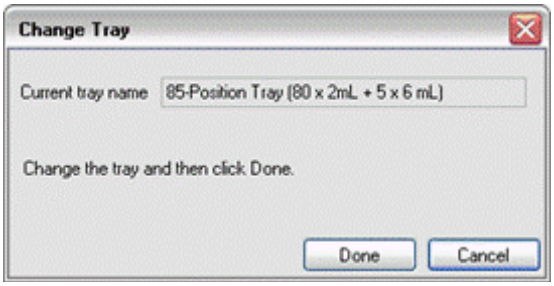
Start/Stop LC Pump Clicking **Start** sends the start command to the pump module and then (assuming the command is accepted) changes the Control Panel to show **Stop LC Pump**. If the instrument includes more than one pump there is a separate command for each pump, distinguished by a numeric suffix. The tooltip associated with the command shows the full model name for the pump module.

Autosampler Controls (Flexar or Series 225/275)

The Flexar or Series 225/275 Autosampler will not automatically recognize a change of sample tray under all situations and hence this procedure must be directed by the software in order to be successful.

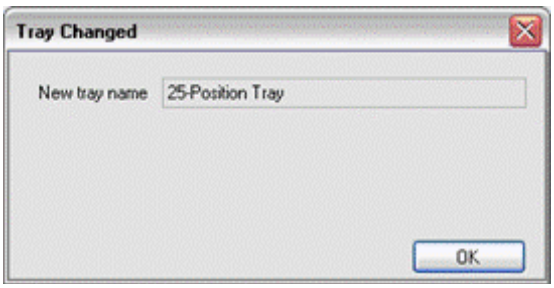
1. To begin this process, click the **Change Tray** button in the **Control Panel**.

   Change Tray enables you to change the sample tray installed in a Flexar or Series 225/275 Autosampler. The software will then display a dialog indicating the current tray and instructing the user what to do next.



2. After changing the tray, click **Done.**

   The software will read the identity of the new tray and display a second dialog, confirming the change has been successfully recognized:



UV/Visible Detector Commands (Flexar or Series 200)

Turn Lamp On Sends a lamp on command to the detector. Since the state of the lamp cannot be read by Chromera the **Turn Lamp On** and **Turn Lamp Off** commands will always be shown together.

Turn Lamp Off Sends a lamp off command to the detector. Since the state of the lamp cannot be read by Chromera the **Turn Lamp On** and **Turn Lamp Off** commands will always be shown together.

Autozero Sends an autozero command to the detector.

ELSD Commands

Autozero ELSD Issues an Autozero signal to the ELSD.

Turn ELSD Gas On Issues the Gas On command to the ELSD. Only displayed when ELSD is in Active mode.

Turn ELSD Laser On Issues the Laser On command to the ELSD. Only displayed when ELSD is in Active mode.

ELSD to Standby Turns off Gas, Laser, Drift Tube Temperature and Spray Chamber Temperature. When changes to Standby state, controls for Gas and Laser are hidden.

(ELSD to Active) (Turns on Gas, Laser, Drift Tube temperature and Spray Chamber Temperature.) When changes to Active state, controls for Gas and Laser are displayed and set to 'Turn ELSD Gas[/Laser] Off'.

PDA Detector Commands

Turn ON/Off Vis Lamp Sends a visible Lamp On or Lamp Off command to the detector.

Turn ON/Off UV Lamp Sends a deuterium Lamp On or Lamp Off command to the detector.

Fluorescence Detector Commands (Flexar or Series 200a)

Turn Lamp On (Off) Sends a Lamp On command to the detector. The command will change to Lamp Off when the software determines that the lamp is on.

Autozero Sends an Autozero command to the detector.

Refractive Index Detector Commands (Flexar or Series 200a)

Autozero Sends an autozero command to the detector.

ICP-MS Commands

Start Peristaltic Pump Sends a Start Peristaltic Pump command to the ICP-MS software. Since the state of the pump cannot be read by Chromera the Start and Stop commands will always be shown together.

Stop Peristaltic Pump Sends a Stop Peristaltic Pump command to the ICP-MS software. Since the state of the pump cannot be read by Chromera the Start and Stop commands will always be shown together.

Start (Stop) Plasma Sends a Start Plasma command to the ICP-MS software. The command will change to Stop Plasma when Chromera determines it has been successful.

Switching Valve Commands

Switching Valve Commands change the current state (position) of the valve using **Direct Control**. Chromera uses the LC/UHPLC Rheodyne flow switching and column selector valves.

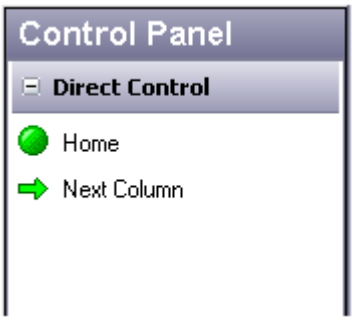
The commands are **Home**, **Next Column** and **Previous Column**.

Home – moves the valve to the home position. This moves the valve to position 1 very slowly.

Next Column – moves the valve one position clockwise. Does not go past position 6.

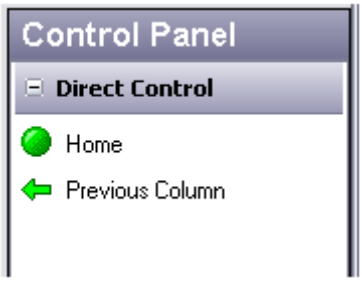
Previous Column – moves the valve one position one position counterclockwise. Does not go past position 1.

Here is Direct Control with the valve at column 1: Note that Previous Column is not available.



Here is Direct Control for columns 2-5:

Here is Direct Control for column 6: Note that Next Column is not available.



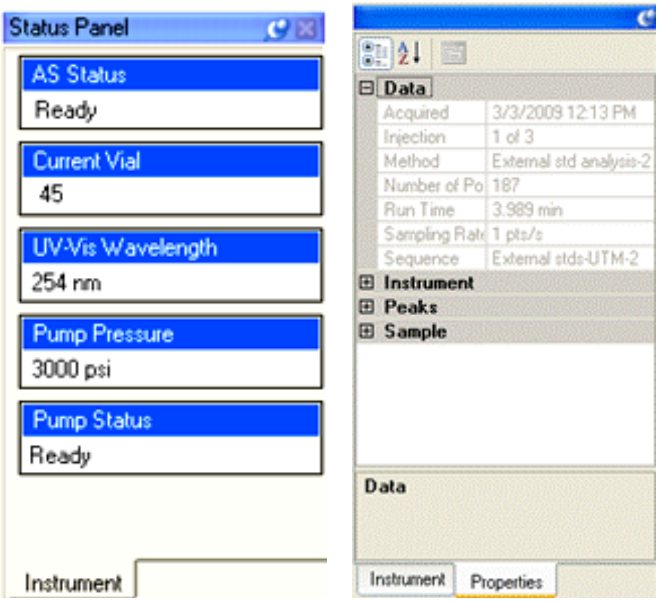
Post Column Injection Valve Commands

Post Column Injection Valve Commands change the current state (position) of the PCI valve.

Status Panel

About the Status Panel

The Status Panel displays the general status of all instruments currently configured on your system. Since the Status Panel also displays information other than instrument status in some environments, there are tabs on the Status Panel so that you can select the information to be displayed. The **Instrument** tab is always available, although the tab itself may be suppressed when no other tabs exist, to maximize the available display space. A second tab, Properties, displays information relevant to your analysis. For example when in Post Run information about the selected plot is provided.



The Status Panel may be pinned in place at the right hand side of the screen or it may be set in Auto-Hide mode so that it appears when the Status Panel tab is selected from the fly-out panel and then disappears a few seconds after the mouse pointer is moved elsewhere on the screen.

About System Status Items

There are different types of system statues items, such as:

- **Overall Instrument Status.** This includes settings such as **No Method**, **Not Ready**, **Ready** and **Running**. The full set of status messages depends on the configuration and is derived from the Instrument Descriptor definitions.
- **Database Status.** This provides an indication of the amount of space available, for example as **% Free Space**. Since the data store consists of multiple databases, this status shows the state of the results database, which is the one most likely to become filled.
- **Database Size.** This reports the total space occupied by all the Chromera databases.
- **Injection Number.** Displayed as 'n of m' where n is current injection and m is number specified in the sequence.

NOTE: *The Injection Number will always be **1** in Single Run mode.*

About Instrument Status

- All status information supplied by each device is available for display.
- You are able to select from the available status values those to be displayed, on an instrument-by-instrument basis.
- The display of each status item indicates whether or not it is at the set value (where applicable).
- The display of each status item indicates if the value is out of range.
- In addition to individual device status items, an overall instrument status (Not Ready, Equilibrating, Ready, Running, etc) is provided.
- The combination of status items selected for display for each instrument is saved as User Preferences.
- Where available, diagnostic information is available as status items.

Overall Instrument Status

This panel provides an overall view of the instrument status, by displaying the state of each device configured on the instrument. The following status is displayed:

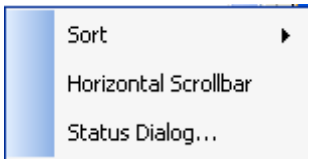
Not Connected	One or more devices in the configured instrument are not connected to the software
No Method	The devices are currently not under control of a method (although Manual Control may be in use)
Not Ready	One or more devices are not yet at the initial conditions set in the controlling method
Equilibrating	All devices are at set conditions but an equilibration stage is in progress
Ready	The instrument is at set conditions and equilibration is complete
Running	An analytical run is in progress
Monitor	Baseline monitoring is in progress (only on instruments that require specific monitor mode)
Error	An unrecoverable error has occurred on the instrument

NOTE: *If the controlling sequence has been paused then you are notified of this by appending it to the above status. For example: Ready — Paused*

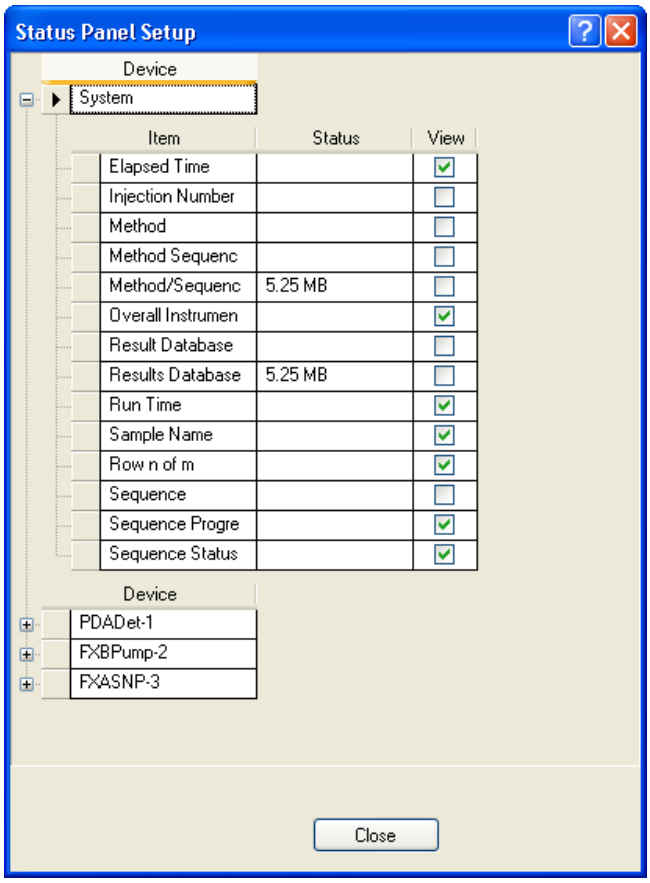
How to Setup Parameters in the Status Panel

To configure the parameters displayed in the Status Panel:

1. Right-click on the **Status Panel** to display the **Status Panel** popup menu and select **Status Dialog**.



2. The Status Panel Setup dialog consists of an expandable list that shows all the parameters available to be displayed, along with the current status (for quick summary checking).



3. Select a **Device**. Each **Device** contains a list of status **Items**.

4. Each status **Item** has a checkbox, to select the parameters you want to display in the Status Panel. When an item is checked or unchecked to **View**, the Status Panel is updated immediately to reflect the change.

5. Right-click in the **Status Panel Setup** dialog to display a popup menu for additional control.

6. Click **Close** to close the dialog and the **Status Panel** will be updated.

Database Status

Database status is reported as the % of free space available in the results database. This is because, in the multi-database structure, this individual database is most likely to reach capacity.

Of course, given enough time, any of the other databases could reach capacity (except for raw data, which will utilize a database chaining scheme to provide indefinite capacity limited only by disk space). Therefore, for all databases (including results), if any of them should reach 95% capacity then this is treated as an error condition and an error is displayed.

You also can view total database size (encompassing all elements of the database set), to see the amount of space required for a backup (the backup should always require less space than this value since it is a compressed format).

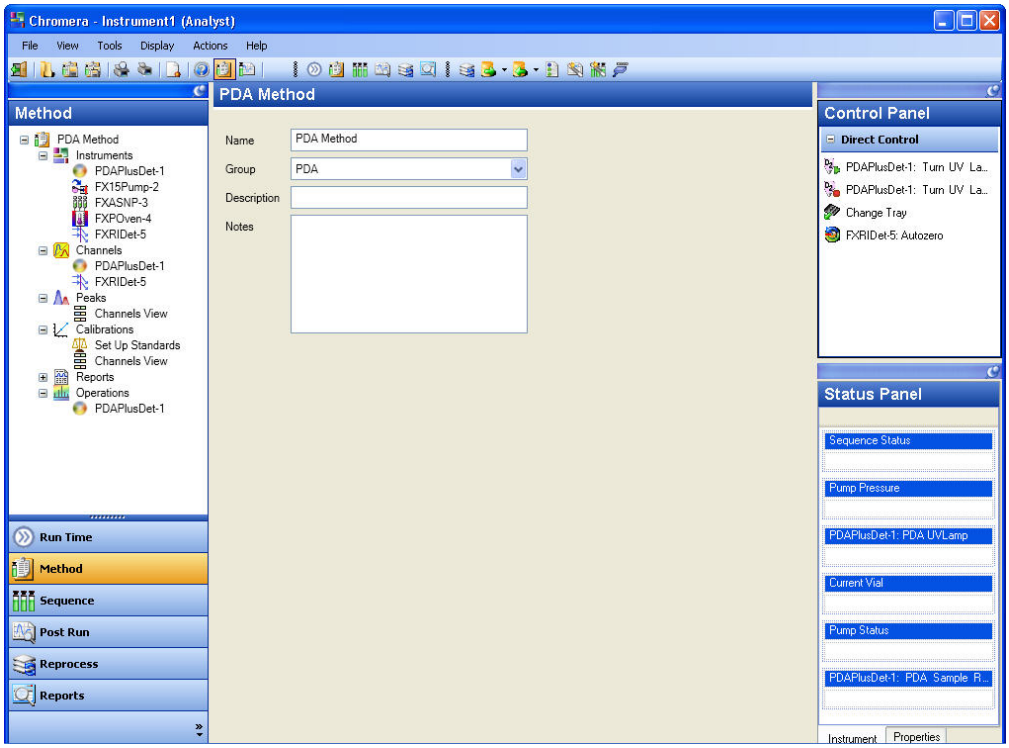
Method

About the Method

A method is a collection of parameters that determine how Chromera acquires and analyzes data from your chromatography equipment, based on the specific requirements of your instrument and your sample. Each method contains the following major sections:

- **Instruments:** Setup and control of the functions and parameters of the devices making up the instrument
- **Channels:** Defines the chromatographic data channels from which data is acquired
- **Peaks:** Parameters defining processing of the acquired data streams and definition of specific components to be analyzed in the sample
- **Calibration:** Calibration information to convert processed data to component amounts
- **Reporting:** Contains the Channel specific plotting options

Clicking the **Method** button in the Navigation Pane displays the method tree and the first page of the method.



The upper section of the Navigation Pane displays the major sections of the Method whose contents are based on the configuration of the displayed method (that is, the configuration of the associated instrument), while the main display area (the large area in the middle of the screen) displays the part of the method associated with your selection.

The major sections of a method: **Instruments, Channels, Peaks, Calibration,** and **Reporting** always appear in the Navigation Pane. You can reorder these sections (by right-clicking and selecting Move Up/Move Down commands) but they will remain at the same

level. With a configured PDA detector the child nodes of the second level nodes of the method tree: Instrument, Channels, Peaks, Calibration and Reporting will each reflect the presence of the PDA detector.

A device name appears under **Instruments** for each device configured. The text displayed is the name you give to the device, as defined in the instrument configuration. Each device that generates a data stream that is subject to peak detection (e.g., a detector) also appears under Peaks, Channels, and Calibration. The text displayed for is the name of the channel, as defined in the Channels section.

The part of the method displayed in the main display area of the screen depends on the selection in the navigation Pane. For example:

Method name	A unique name to identify the method
Instrument	Instrument settings for all devices
Device node	Instrument settings for selected device only
Channels	Channel parameters for all devices
Detector (Devices) node	Channel parameters for selected detector only
Data Streams	Data stream for the selected detector
Peaks	Peak Detection parameters and component information for all detectors
Detector node	Peak Detection parameters and component information for selected detector only
Calibration	Calibration information for all detectors
Device node	Calibration information for selected detector only
Reporting	Channel specific plotting options

Method editing consists of modifying existing method parameters. This includes a modification to the method name, since in Chromera the name is a method parameter like any other (the method is uniquely identified by a database index that is not visible to a user). After making changes and saving the method, the previous version of the method is replaced by the modified version. Only a single version of a method is saved in the database. Also, a method cannot be saved with the same name as another method in the Group. To create a copy of an existing method but with a different name, leaving the original method unchanged, use the **Save As** command.

Method Menu Commands

The following menu items appear in the **File** menu when you are in the method environment.

Menu	Command	Description
File	New Method	If the current method has been modified then you will first be asked if the modified method should be saved. Displays a new default method. For an online instance the method configuration is that of the connected instrument. In the case of an offline instance the user is prompted to select or create a configuration.
	Open Method…	If the current method has been modified then you will first be asked if it should be saved. Displays the data selector in **single sample result** selection mode. When you has selected a method, it is displayed.
	Save Method	Save the current method replacing the existing version in the database.
	Save Method As…	Displays a dialog to enter a Name and a Group for the new method, which will then be saved under a new identifier (index) in the database.
	Extract Method from Results…	If the current method has been modified then you are first asked if it should be saved. Displays the **Data Selector** in **single sample injection result** selection mode. When a single sample has been selected the method parameters (instrument, channels, peaks and calibration) is read from the results and displayed in the editor. The Method Name and Group fields on the Identification page of the method will also be filled in (and Description and Notes will be copied from the results if they exist). You may be required to change the Name or enter a new Group name before the method can be saved, if a name conflict occurs.
	Print Preview Method	Displays the Print Method dialog. When you select the required options in the Print Method dialog (and click OK) the method displays in a print preview method in accordance with the current print options.
	Print Method…	Displays the Print Method dialog. When the user has selected required options in the Print Method dialog (and clicked OK) the method is printed.

	Lock	Only displayed when user authentication/tracking is active. Minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	**Online instance:** If data acquisition or a sequence is running then you are prompted that the instrument must be in an idle state before the application can be closed. If the instrument is not active then the following procedure for an offline instance will apply. **Off line instance**: If the current method has been modified then you are first asked if it should be saved. If unsaved data are present in any other environment then you are prompted to save each in turn. Closes the application window.
View	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Reprocess environment. The same action as clicking the Reprocessing button in the Navigation Pane.
	Toolbars ► View	Toggles display of the View toolbar.
	Tools	Toggles display of the Tools toolbar.
	Show Small Icons Show Large Icons	Toggles the size of the icons in the Graphic toolbar.
Tools	Export ► Chromera Results… Methods… Sequences… Report Templates…	Displays the Database export dialog. Displays the Method export dialog. Displays the Sequence export dialog. Displays the Report template Export dialog.

	Import ► TotalChrom Data… Chromera Results… Chromera Methods… Chromera Sequences… Report Templates…	Displays the Import TotalChrom data dialog. Displays the Import Results dialog. Displays the Import Methods dialog. Displays the Import Sequences dialog. Displays the Report template Import dialog.
	Preferences	Displays the Preferences dialog, showing the preferences associated with the current user (see Setting Preferences).
	Report Format Wizard	Displays the Report Format Wizard in New Report mode (see Report Format Wizard).
	Sequence Wizard	Displays the Sequence Wizard (see Sequence Wizard).
	Device Connections	Displays the Device Connections dialog.
	View Current Session Logs	Displays the Data Selector - Event Viewer with session log information.
	Error Log	Displays the Error Log dialog
	Dictionary Editor	Displays the Dictionary editor dialog
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector (see Batch Reprocess). It is disabled in an online instance when data acquisition is in progress, or a sequence is running.
	Batch Builder	Displays the Batch Builder dialog.
	View Retrieved Archived Logs	Displays a dialog to select a retrieved event logs database to display in the Data Selector - Retrieved Session Logs Viewer.
Actions	Standard Method Editor	Displays the standard configuration of the method editor, as shown in About the Method Editor (that is a single frame in the main display and the method tree in the navigation pane.
	Edit Method Graphically	If no data has yet been associated with the current method for this session then the data selector is displayed in select sample results mode. When you select one or more sample results, the window is displayed in the graphic method editing configuration.

	Copy Components ► Between Channels… From TotalChrom Method…	 Displays the Copy Components Between Channels dialog. Displays the Copy Components From TotalChrom Method dialog.
	Update Search Windows…	Displays the Update Component Search Windows dialog.
Help	Topics	Opens the Chromera Help window.
	Consumables and Accessories	Opens the PerkinElmer Consumable and Accessories Catalog
	About Chromera	Displays the copyright and version dialog.

Method - Standard Toolbar

This toolbar displays in the standard Method Editor mode:

Or when user authentication active this toolbar displays:

The following table describes the icons:

Icon	Command
	Opens a New Method
	Open Method - Displays the Data Selector
	Save Method
	Save Method As
	Print Preview Method
	Print Method
	Lock (Only when user authentication active)
	Exit Chromera
	Standard Method Editor
	Edit Method Graphically
	Opens Help

Common Toolbars

There are two common toolbars displayed in every environment: **View** and **Tools**. A Standard toolbar will always appear but the contents will change depending on the environment. The standard toolbar is defined within the sections describing the individual environments.

View Toolbar

This toolbar simply mirrors the buttons in the Navigation Pane and the commands in the View menu.

Icon	Command	Description
	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Batch Reprocess environment. The same action as clicking the Batch Reprocessing button in the Navigation Pane.
	Reports	Switches to the Report Viewer.

Tools Toolbar

This toolbar includes the most commonly used commands from the Tools menu.

Icon	Command	Description
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector in single batch selection mode.
	Export ► Chromera Results… Methods… Sequences…	 Initiates the Export of Chromera results by displaying the Database export dialog. Initiates the Export of Chromera methods by displaying the Database export dialog. Initiates the Export of Chromera sequences by displaying the Database export dialog.
	Import ► TotalChrom Data… Chromera Results… Chromera Methods… Chromera Sequences…	 Displays the Import window. Displays the Import Results dialog. Displays the Import Methods dialog. Displays the Import Sequences dialog.
	Preferences	Displays the Preferences window, showing the preferences associated with the current user.
	Report Format Wizard	Displays the Report Format Wizard in New Report mode.
	Sequence Wizard	Displays the Sequence Wizard.
	Device Connections	Displays the Device Connections dialog.

Method Tree Popup Menu

Right-clicking anywhere within the method tree control in the Navigation pane will display a popup context menu. The contents of the menu will always be the same but which commands are enabled will depend on what specific part of the tree is clicked.

Command	**Description**
Expand	Displays the child nodes of the node clicked on.
Collapse	Hides the child nodes of the node clicked on.
Expand All	Fully expands the tree to show all nodes.
Collapse All	Hides all nodes in the tree other than the root node (the method name).
Move Up	Moves the clicked node, and its children, up one position in the tree. This allows you to position method sections, the instrument modules within the Instrument section, and channels within other sections of the method in the desired order.
Move Down	Moves the clicked node, and its children, down one position in the tree. This allows you to position method sections, the instrument modules within the Instrument section, and channels within other sections of the method in the desired order.

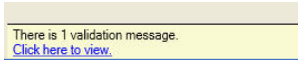
NOTE: *If you click in empty space within the control and not on a node, then the only commands enabled are* ***Expand All*** *and/or* ***Collapse All****, as applicable.*

How To Create a New Method

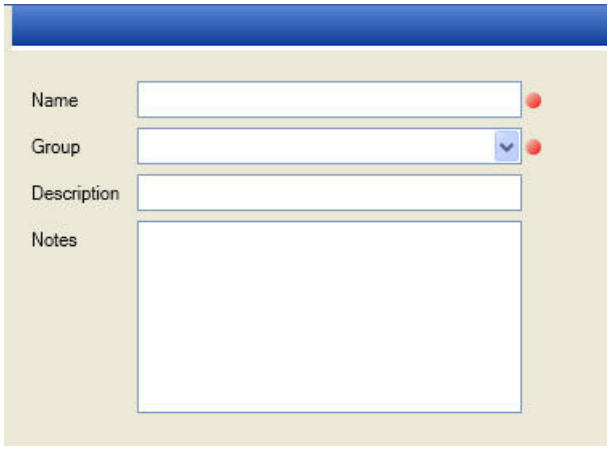
To create a new method:

1. Click the **Method** button 

or icon  to open the Method window.

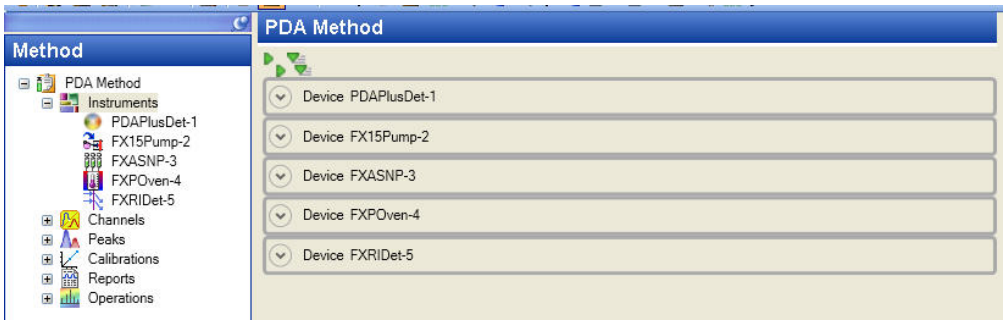
2. Choose the **New Method** command from the **File** menu. A blank Method dialog appears with a message bar 

on the bottom of the screen.

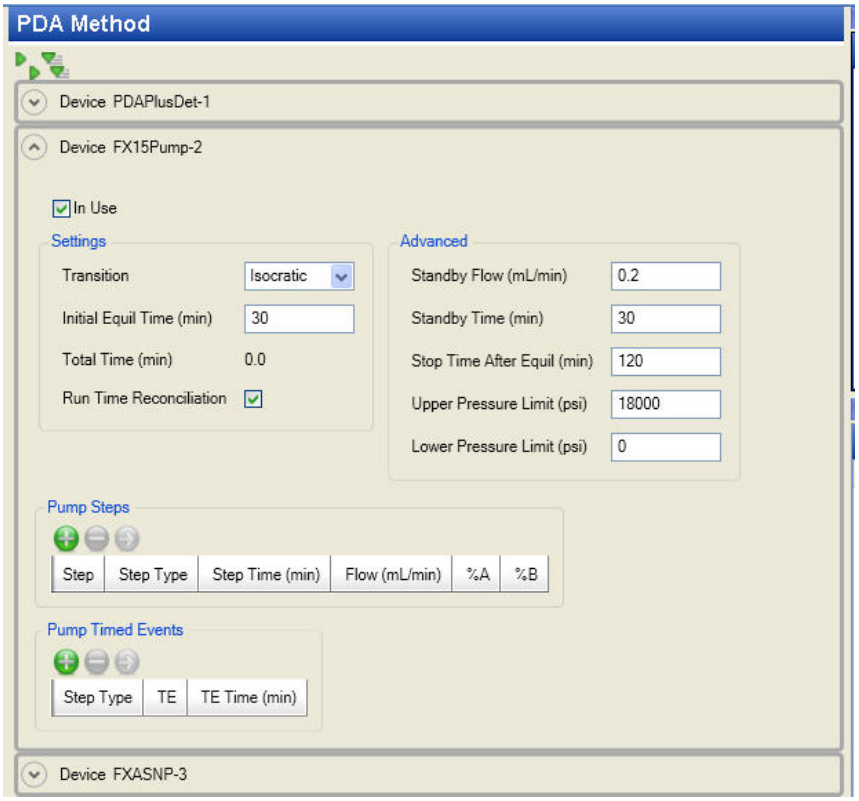


3. In the top level row of the method screen, enter a **Method Name** (this is the name the method will be saved as in the database), the name of the **Group** (virtual folder) where the method will be stored, a **Description** of the method, and **Notes** about the method.

4. Click on Instruments in the Method tree to display the instruments that are controlled by this method.



5. Click the button  to **Expand** each instrument section to show the parameter settings for each device (instrument) configured on the system.



6. For each device (instrument) enter or edit parameter settings as required.

Device Parameter Setting	Description
LC Pump Settings	Enter the LC pump analytical program and pump parameters.
LC Autosampler Settings	Enter the LC autosampler settings.
LC Detector Settings Fluorescence, UV/Vis, or PDA	Enter the LC detector settings.
LC Column Oven Settings	Enter the LC column oven temperature.
ICP-MS Settings (for Speciation)	Enter the ICP-MS parameters; analytes, species, etc.
PCI Valve (Post Column Injection for Speciation)	Enter the valve settings to control the injection of a standard into the mobile phase after the chromatographic column and before it enters the ICP-MS nebulizer.

In a device (instrument) row is an **Advanced** checkbox. Checking this box expands the method row to display the parameters normally set in Preferences. You can set the parameter values here so that they override the Preference values only for this method.

7. Save the method by choosing the **Save Method/Save Method As...** command from the **File** menu.

NOTE: *After making a change to the method you must save the method to retain the changes made.*

How to Modify a Method Used for Spectral Processing

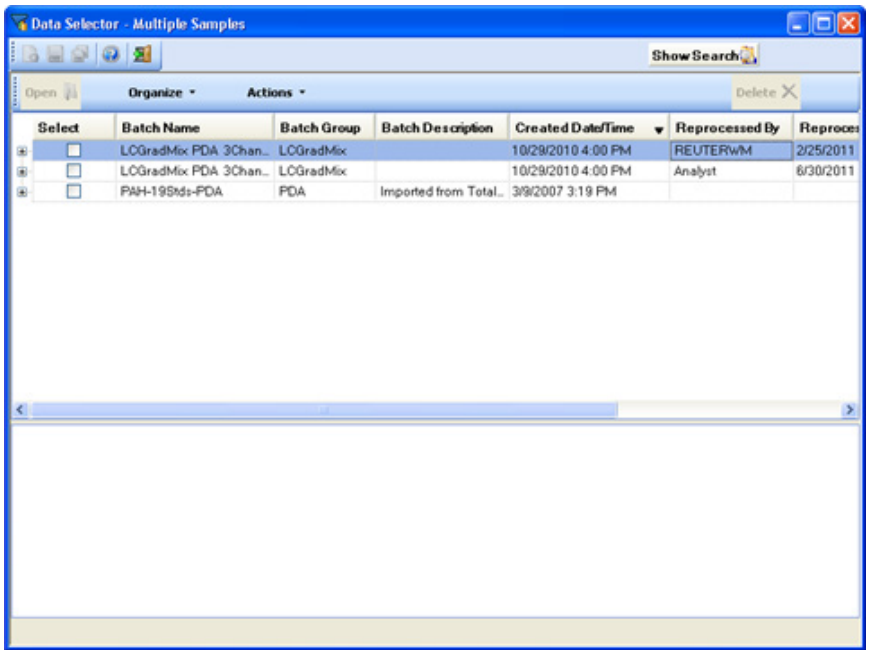
Methods used with a PDA interact with the Spectral Processing Window (SPW). The following procedure outlines the interaction between Chromera and the SPW to modify a method. You can modify the method three ways:

- Create a new channel
- Optimize operations
- Modify the Component List

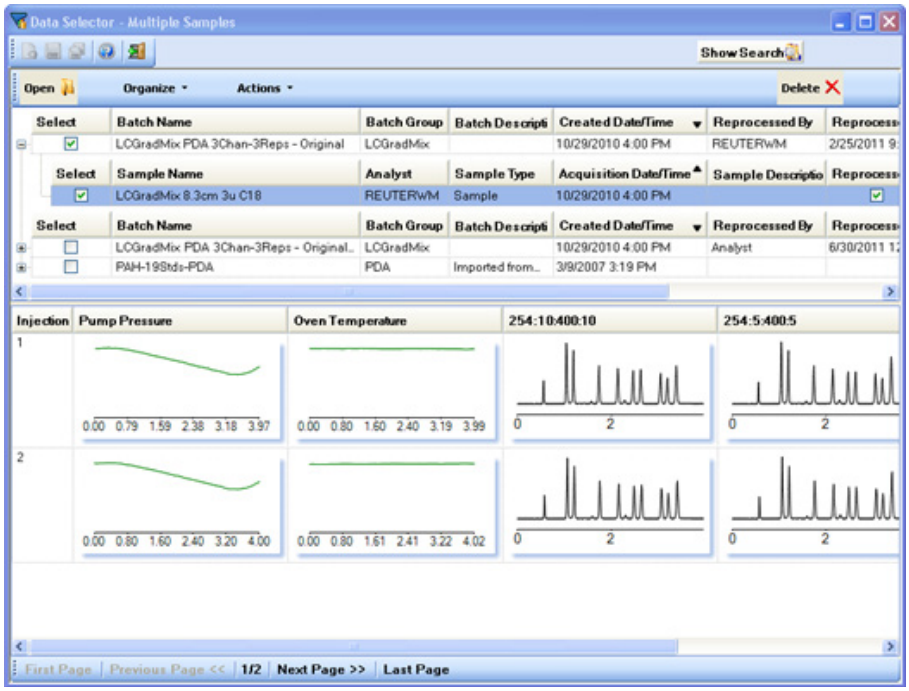
To modify a method used for spectral processing:

1. Open your method that is used with the PDA.

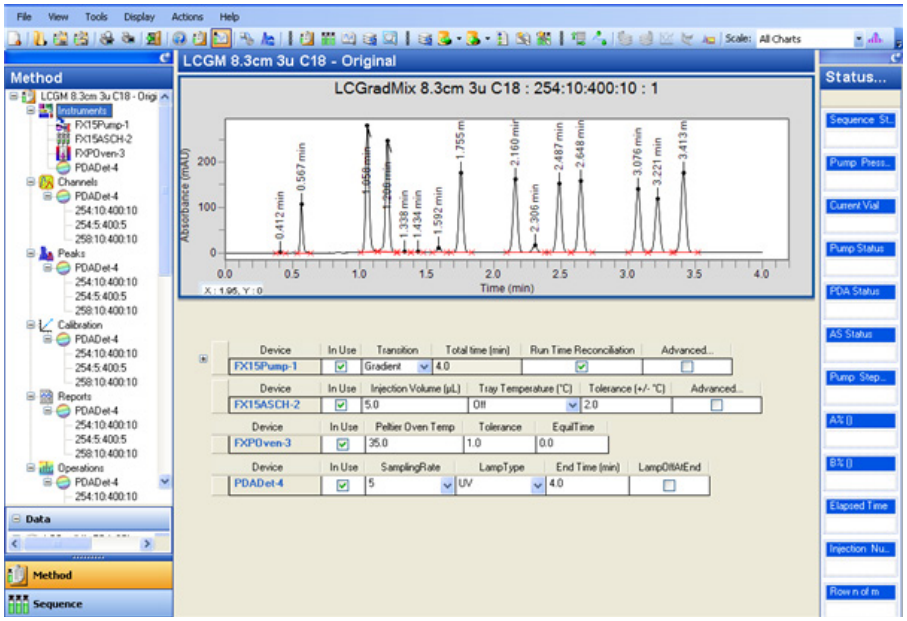
2. Select **Edit Method Graphically** from the **Actions** menu.
   The **Data Selector** opens.



3. Click the check box to the left of the **Batch Name** you want to open, click the plus sign  to expand the batch, click the **Sample Name** check box, then click the **Open** button.



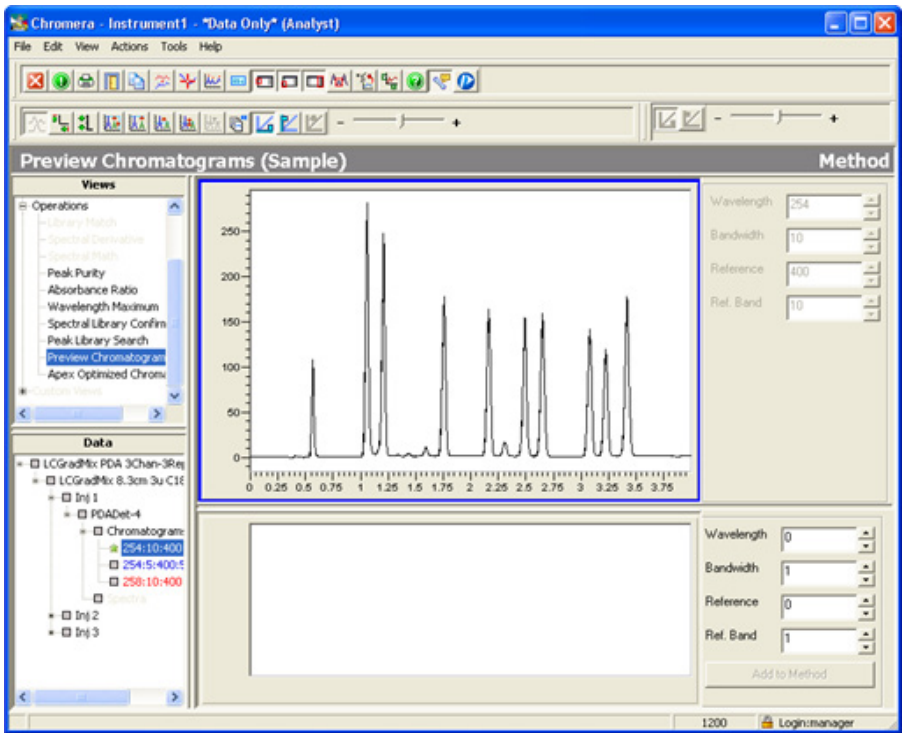
The Method opens in Graphic method Editor (GME). This is the template chromatogram.



To Create a New Channel

1. If you want to create a new channel select **Channels** in the Method tree, then from the **Actions** menu, select **Create channel definition from spectral data**.

   The SPW window opens in **Preview Chromatogram**.



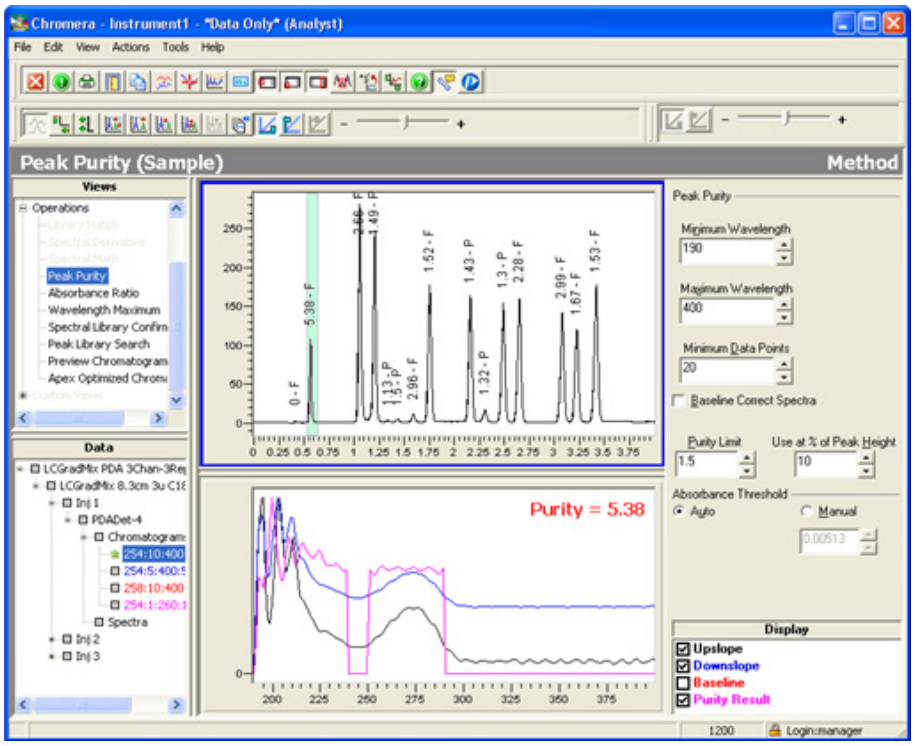
2. Here you can perform SPW Operations on your channel.

3. When done you can add this new channel to the method clicking the **Add to Method** button in Preview Chromatograms.

4. Save this method by selecting **Save Method** or **Save Method As...** from the Chromera File menu.

To Optimize the Operations

1. To optimize a channel in the Method tree (in this example 254:10:400:10 is selected), select **Edit Spectral Operation Graphically** from the **Actions** menu. The SPW window opens in Peak Purity.



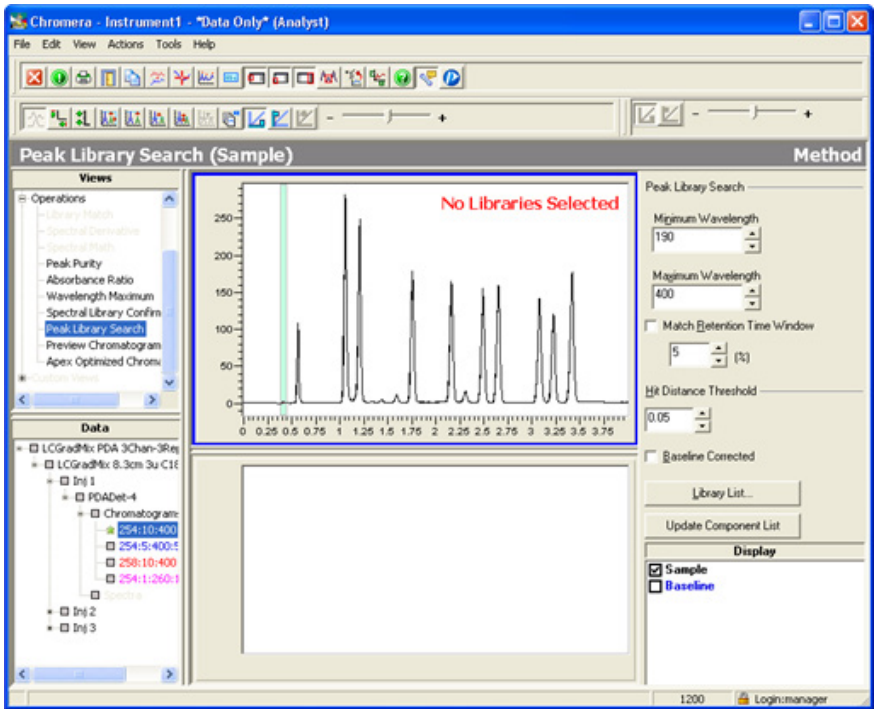
2. Now you can optimize the different operations in SPW for this channel.

3. When done save this method by selecting **Save Method** or **Save Method As...** from the Chromera **File** menu.

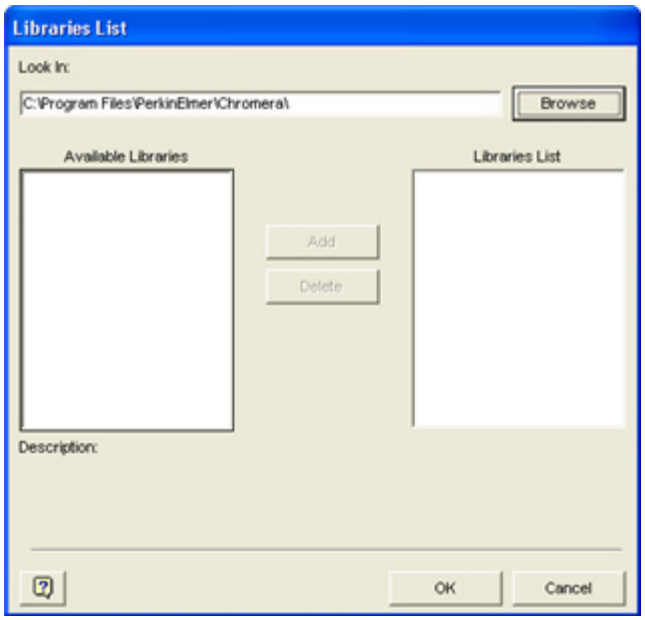
To Modify the Component List

1. To modify the component list select **Peaks** in the Method tree (in this example 254:10:400:10 is selected), select **Component list using spectral library search matches** from the **Actions** menu.

   The SPW window opens in Peak Library Search.



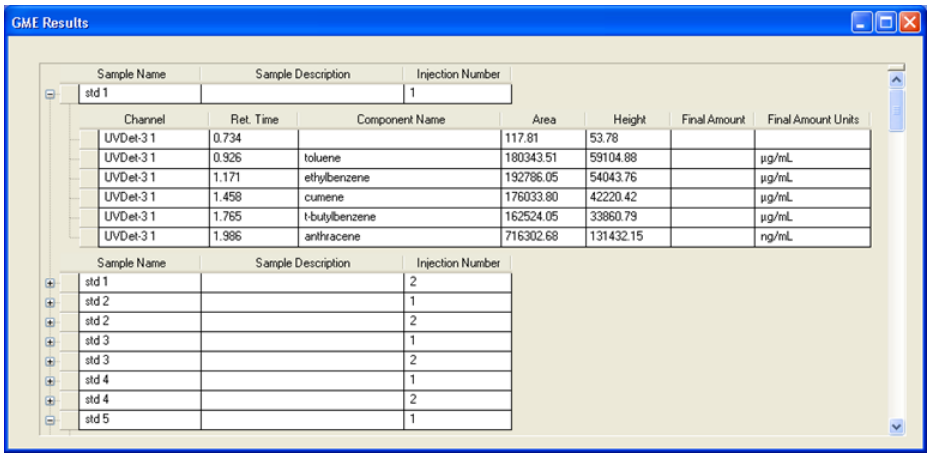
2. Click the **Library List** button to open the **Library List** dialog and browse for a library.



3. When done use the spectra to update the component list in the method by clicking the **Update Component List** button.

An updated list identifies where the name comes from (for example, _PLS is Peak Library Search)

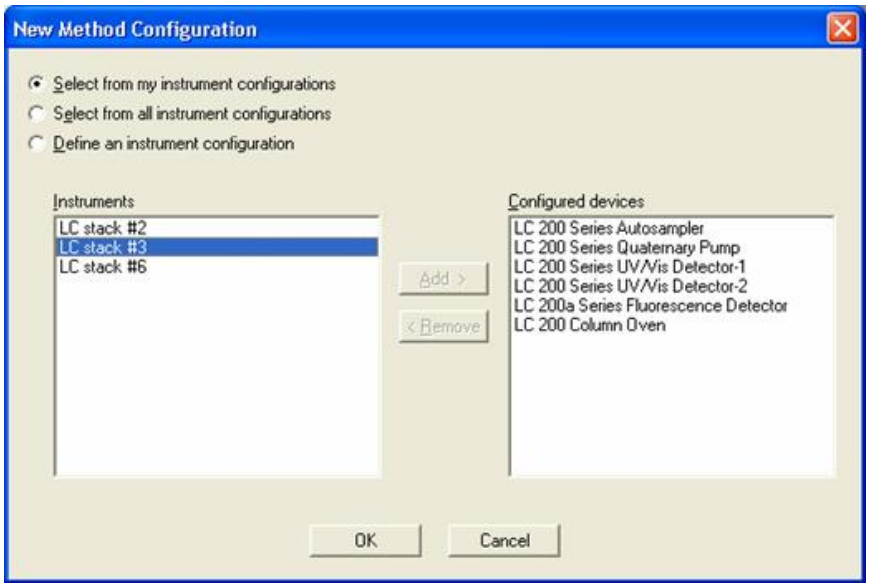
4. You can see the **GME Results** when you select the **Show Results** command from the **Actions** menu.



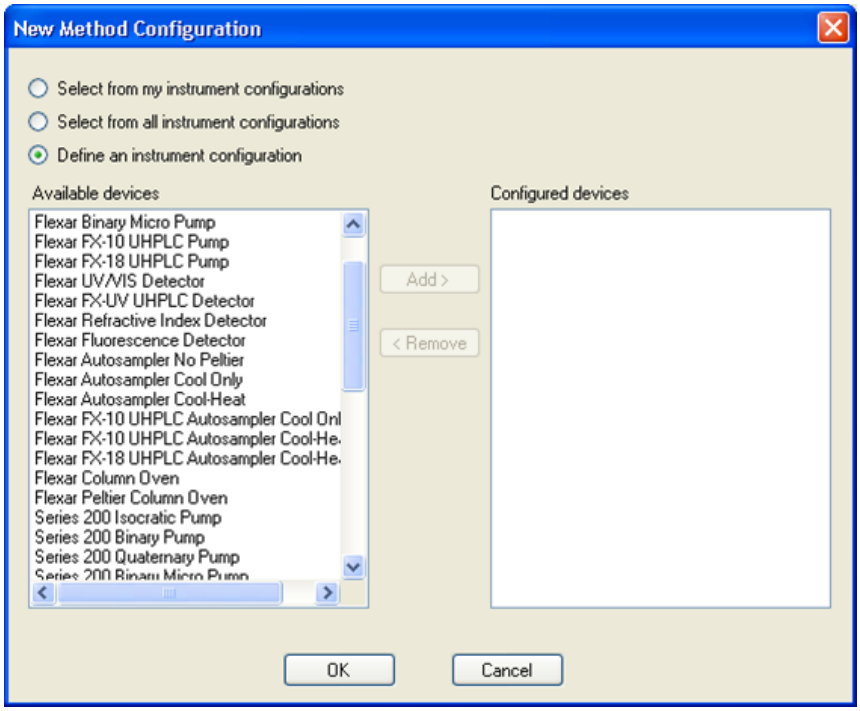
5. When done save this method by selecting **Save Method** or **Save Method As...** from the Chromera **File** menu.

New Method Configuration Dialog (Offline Instance)

The New Method Configuration dialog is displayed when you select the **New Method** command from the **File** menu in an offline instance of Chromera. It enables you to define the instrument configuration to be used for the new method. You may select an existing configuration or define an instrument structure specifically for use with the new method. The default selection is the current instrument configuration with the current devices displayed.



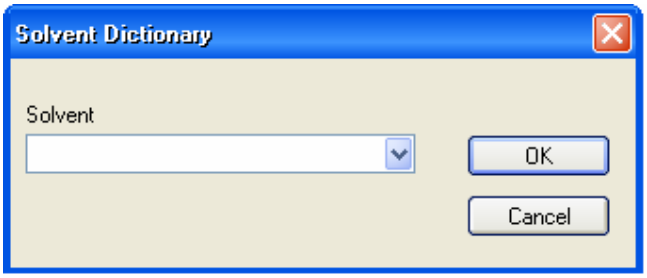
When you want to Define an instrument configuration a list of available devices displays. Select the devices you want and click the **Add** button.



Control	Description
Select from my instrument configurations	Select to indicate that an instrument configuration assigned to the current user will be selected for the new method.
Select from all instrument configurations	Select to indicate that an instrument configuration from the full system collection will be selected for the new method.
Define an instrument configuration	Select to indicate that you will construct an instrument configuration for the new method.
Instruments	Displays existing instrument configurations for you to select to define the new method structure. The list displays all of your existing defined configurations or all of the instrument configurations defined on the system (depending on the radio button selection).
Available Devices	Displays the devices comprising the instrument for the new method. It is only displayed when the third radio button option is selected. The list displays all instrument devices supported by the Chromera software (manufacturer's name displayed).
Configured Devices	Displays the devices making up the instrument. If an existing instrument is selected from the Instruments list box then the devices assigned to that instrument will be displayed in this list box. If an instrument configuration is being defined then the list box contains those devices assigned to the instrument so far. When **Define an instrument configuration** is selected: Displays the device names added to the configuration, in the order in which they were added. This order also defines the order in which they will appear in the method editor tree. Multiple selections are allowed in this mode. Existing configuration option selected: Displays the names of devices making up the selected instrument configuration, in the order they appear in the configuration. This order also defines the order in which they will appear in the method editor tree
Add >	When a device name is selected in the **Available Devices** list, clicking this button copies the device name to the **Configured Devices** list. If a device of that type already exists then a numeric identifier will be appended, so that each occurrence of the device can be distinguished. When items from the **Available Devices** list are added to the **Configured Devices** list, the selected items in the **Available Devices** list will be deselected/unhighlighted.
< Remove	Selecting one or more device names in the **Configured Devices** list, clicking this button, removes the device name(s) from the list. All items will then be deselected in the **Configured Devices** list.

Selection from a Dictionary

Chromera maintains several dictionaries to facilitate the consistent entry of component names and solvent descriptors into methods, and standard names and units associated with calibration. The functionality of these dictionaries is identical. Each dictionary is made available via a combo box. This combo box is used directly (such as in the Component column of the component list in the Peaks section of the method), otherwise the combo box is contained in a selection dialog displayed as a result of some command.

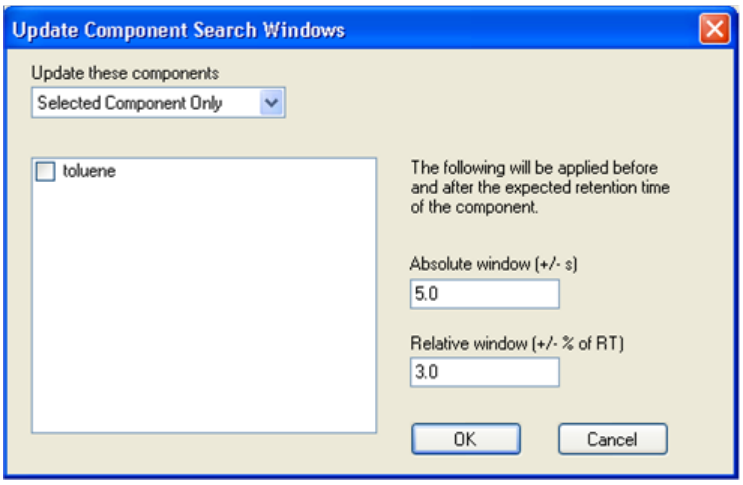


When you type an initial character in the edit field of the combo box, if one or more entries starting with that character are found within the dictionary then the first entry found will be displayed in the combo box. As you type in more characters of the desired component name, as long as a match is found within the dictionary then an entry will be displayed. If entry cannot be found in the dictionary then the autocomplete action is discontinued and only your entry will be displayed in the edit field. When you accept the entry by leaving the Component field then the contents of the field will be added to the dictionary.

At any time a dictionary item is displayed in the combo box you can use the up/down arrow keys to select the previous/next item in the dictionary. The dictionary display is always ordered alphabetically (with a sort mode suitable for the current locale).

Update Component Search Windows

This dialog is displayed when the **Update Search Windows**… command is chosen from the **Actions** menu in the Method Editor.



Control	Description
Update these components	Select from the drop-down list the scope of the changes to be made to the component search windows. The choices are: **All Components**, **Internal Standards Only**, **Reference Peaks Only**, or **Selected Component Only**
<check list box>	Select the components whose search windows are to be updated. Displayed are all components from the method, regardless of the channel. This is only enabled when **Selected Component Only** is selected in the **Update these components** drop-down list.
Absolute window (± s)	Enter a value to set the component search window size, in seconds. (The range is 0.0 to 999.9 s.)
Relative window (± % of RT)	Enter a value to set part of the component search window size, as a percentage of the expected retention time for the component. (The range is 0.0 to 500.0.)

Clicking the **OK** button updates the search windows for all the specified components on the **basis of the settings in the dialog. This update process can only produce search windows that** are centered on the expected retention time of the component. For asymmetric search windows the user must enter values manually or define the window graphically.

The search window values generated for each specified component are:

$$\text{Peak Search Start} = \text{Expected Retention Time} - \left(\frac{\text{Absolute window}}{60} + \frac{\text{Expected Retention Time} \times \text{Relative window}}{100} \right)$$

$$\text{Peak Search End} = \text{Expected Retention Time} + \left(\frac{\text{Absolute window}}{60} + \frac{\text{Expected Retention Time} \times \text{Relative window}}{100} \right)$$

Active Method

The Active Method is the set of parameters currently controlling the various devices (pump, autosampler, detector, oven) of the instrument. The active method is available by default within the **Run Time** environment but you can also display it within the Method environment.

NOTE: *These two environments are functionally distinct; that is, they can be displaying different methods at any given time.*

Display

Because the behavior of editing the active method is radically different from that of editing an offline method the appearance of the editor is different to help you see which mode is in effect.

When an instrument is running and the active method is displayed, there are attributes applied to the parameter display to indicate which parameters can be changed and which cannot. This display is updated as the run progresses.

For example, if Step 1 of a UV/Vis detector wavelength program is being executed then that step is shown as read-only but subsequent steps (if any) are shown as editable. But when Step 1 is complete and the detector begins executing Step 2, that step will become read-only and only subsequent steps are shown as editable.

NOTE: *Some parameters may always be shown as read-only in the active method display, if the device does not permit the value to be changed during a run. For other parameters it will depend on the current state of the run (i.e. position in device program steps), while other parameters may be accessible at all times.*

Changes

A change made to the method will not be immediately downloaded to the instrument; this occurs when you explicitly request that changes made be applied to the instrument (see Run Time environment for details of the user interaction). The instrument control software is responsible for determining the appropriate control structure to send to the device. For example, a change to the wavelength of a UV/VIS detector program will require downloading the complete program step.

Changes to other sections of the method (channels, peak detection, and calibration) will take effect the next time they are used. This includes use of peak detection parameters for the purpose of real-time integration as well as normal end-of-run processing and reporting.

All such changes to the active method generate a log entry associated with the acquired data, indicating:

- The device the change was made to (and the channel if applicable)
- The change to parameter value or setting that was made
- The date and time of the change
- The person who made the change (the Full Name defined in the OS if available, otherwise the user account name)

For example - Channel BBC1: Step 2: Wavelength: From: 254 To: 285 At: 02:34:55 PM November 25, 20087 By: Gene

In addition to the log entry the method name associated with the data is flagged as being **Modified during the run**.

Persistence of Changes

Sequence Mode

By default the changes made to an active method will persist only for the current sequence row. It is important to note that this could mean for subsequent replicate injections specified for that row as well as just for the active analysis.

If you take no other action, prior to the next sample row the stored copy of the method specified for that row is downloaded to the instrument and the modified copy of the method for the prior row is discarded. If, after modifying the active method, you **Save** it, then the modified version replaces the version stored in the database and this modified version is used for all subsequent rows that specify that method.

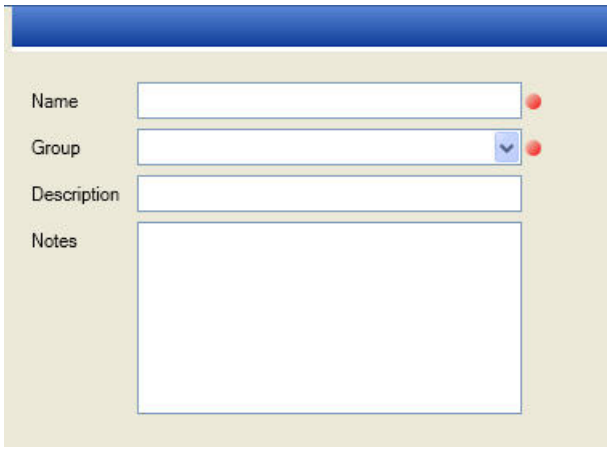
Single Run Mode

The situation here is different from that when a sequence is run. The active method in single run mode continues to define the conditions at the instrument for as long as the session persists (this could be across several Chromera sessions). This is true whether or not the method is saved. Thus in single run mode the 'active method' is an entity of some permanence. For further details see Run Time - Single Run and Run Time Method States.

Method: Identification Section

The **Identification** section of the method is used to avoid redundancy and potential confusion of having the **Method Name, Group,** and **Description** fields repeated in every section. It also contains a **Notes** section where you can enter more detailed information about the method, such as column information or sample preparation steps.

1. Begin by entering the following information, starting with **Method Name**, into the dialog to identify your method.

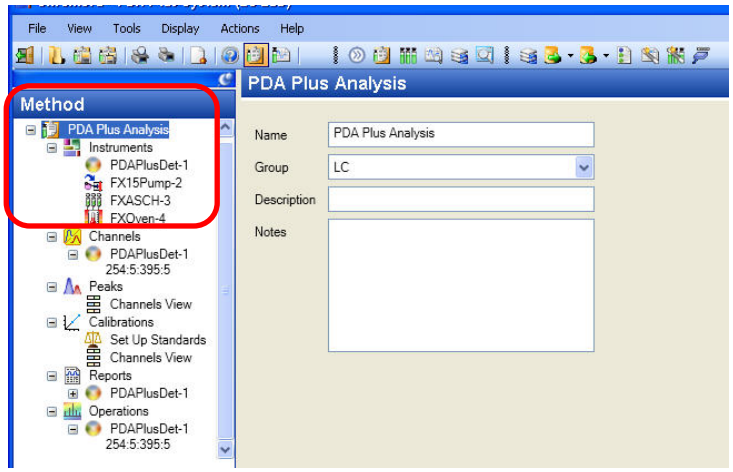


Control	Description
Name	Type a name for the method. You must enter a method name (using up to 25 characters) before the method can be saved and the name must be unique within the Group. After typing a name and pressing Enter, the name also appears in the title bar of this dialog. **NOTE:** Changing the name in this field it does not create a new method. It renames the existing one.
Group	This is a way to categorize (or group) your information. Here you assign the method to an existing group (using up to 25 characters) or to a new group. To use an existing group you can type in the group name or select it from the drop-down list. To create a new group and assign the method to it type a group name that does not already exist.
Description	Type a description of the method using up to 50 characters.
Notes	You can type more extensive details of the method.

2. When done, click on **Instruments** in the Navigation pane to display the instrument parameters.

Instrument Parameters

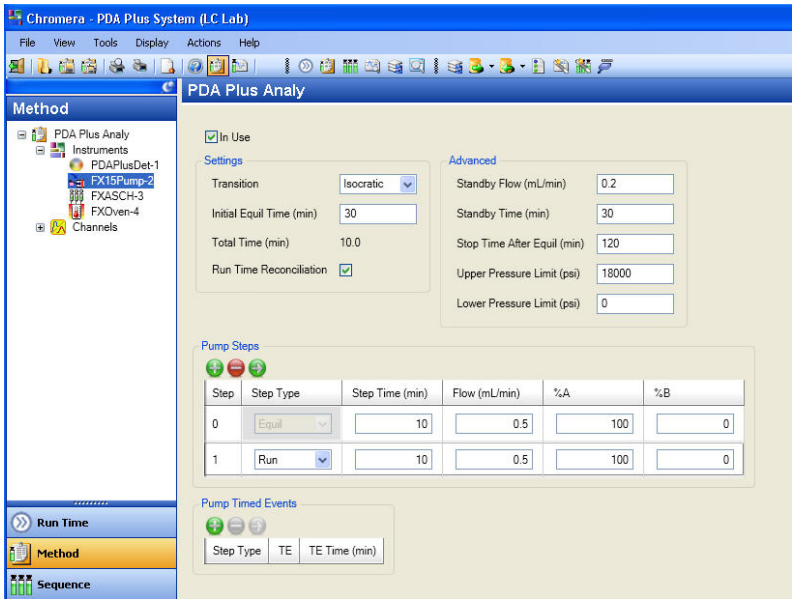
The content of the display when the Instrument (in this example the instrument is PDA Plus Analysis) is selected depends on the configuration of the associated instrument. For example, you can have an LC instrument consisting of a pump, autosampler, PDA detector, and oven.



When the **Instruments** node is selected all instrument bands are displayed. The order in which the bands are shown on the screen depends on the order they appear in the Method tree. By default the devices appear in the order in which they were entered at configuration. You can reorder the devices in the tree (and hence on the screen) using Move Up and Move Down commands in the context menu associated with the tree.

NOTE: *The **In Use** check box when checked indicates that the device is controlled by Chromera when the method is used for an analysis. When the box is unchecked the method will not be downloaded to that device but the display of status from the device will continue. Unchecking the **In Use** box will not stop Chromera from trying to connect to a device when an instrument instance is started. Since the software has no idea what method you plan to use it must attempt to connect to all configured devices on startup.*

When a device node is selected, only the parameters associated with that device are displayed.



Associated Instrument

Each method in the database has an instrument configuration associated with it. This instrument configuration determines what sections are displayed when the method is loaded in the Method Editor. This means that the method will appear exactly the same when it is imported into a different Chromera system, even one that has no instruments configured.

However, in order to accommodate changes to an existing instrument configuration, a check is made before a method is loaded for use in an acquisition as to whether the associated instrument is currently configured on the system and, if it is, whether its present configuration matches that stored with the method. If it does then the method is loaded and displayed. If there is a mismatch between the configurations then the user is informed and is given the option to allow the software to update the method to the current configuration or to have the method displayed in conformance with the stored configuration. If the latter option is chosen the method can be edited and saved but it cannot be set up on the instrument.

When a new method is created within an online instance of Chromera (see Offline/Online) the method will assume the configuration of the associated instrument. When a new method is created within an offline instance of Chromera, you are able to select the instrument configuration to be used. This may be an existing configuration defined on the system or an 'ad hoc' configuration made up of devices selected from the system device pool. See New Method Configuration Dialog for the user interaction associated with this process.

Run Time Reconciliation

When a method is saved the software will compare the total run time of the pump to the end time for each detector configured on the system. If the pump run time and the detector end times are all equal then the method is saved without comment. Any other condition will (optionally) generate a message.

Pump Run Time less than detector End Time

If the end time specified for any detector is longer than the pump run time then the following message is displayed:

The pump run time is less than the data acquisition end time for the following devices:
<first detector with end time exceeding pump time>
…

Do you want the end time of the above to be reduced to be the same as the pump run time?

Yes and **No** buttons are provided. Clicking **Yes** then adjusts the detector end time to be equal to the pump run time. If necessary, the detector program is adjusted to conform to this end time, by eliminating any program change that occurs after the new end time. Clicking **No**, saves the method with the current settings.

Pump Run Time exceeds detector End Time

If the pump run time is longer than the end time specified for any of the detectors then the following message is displayed:

The pump run time exceeds the end time for the following devices:
<first detector with end time exceeding pump time>
…

Do you want the end time of the above to be extended to be the same as the pump run time?'

Yes and **No** buttons are provided. Clicking **Yes** extends the detector end time to be equal to the pump run time. Clicking **No**, saves the method with the current settings.

Disabling Run Time Reconciliation

Run time reconcilation is enabled for new methods however for those cases where the difference between pump run time and detector end time is intentional, it is possible to disable run time reconcilation for a method simply by unchecking the Run Time Reconcilation check box at the end of the main pump band.

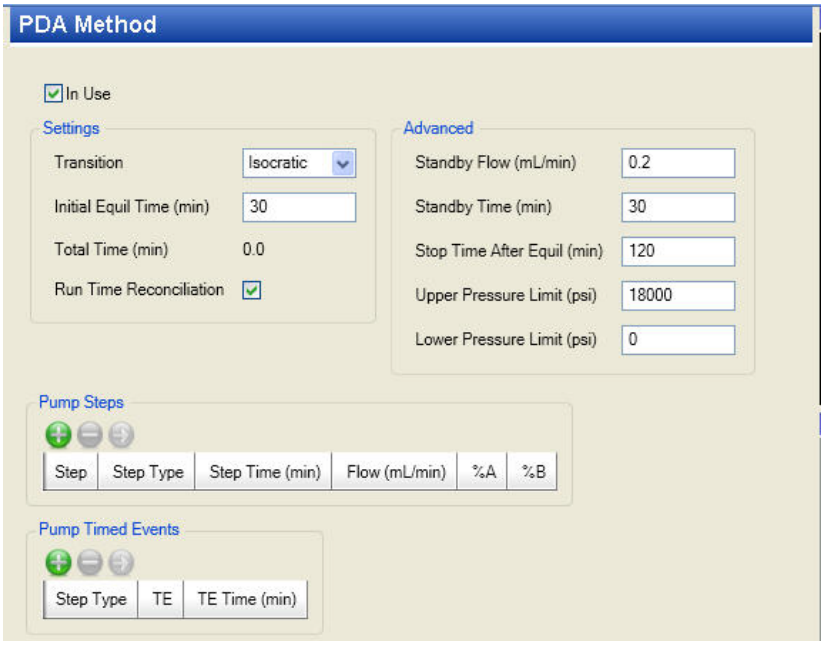
Method: Pump Settings (Flexar or Series 200)

The LC Pump Settings portion of the method is used to define the pump parameters; more specifically, you define the pump program which consists of steps, step type, step time, flow rate, mobile phases, mobile phase composition, composition curve, timed events, and pressure limits.

NOTE: *When **configuring more than one pump** in an LC system, please make sure that each pump's **upper pressure limit** is properly set within the method. This will depend on the individual pump's role in the system; be it for column re-equilibration, post-column functions or otherwise.*

When a method is created, the default **solvent names** are taken from the instrument preferences, and you have the ability to change them. The solvent names will be stored within the method and subsequently will never change (regardless of what modifications to names are made in preferences), unless you explicitly change them within the method.

1. Enter your initial LC Pump Settings.



2.

Parameter	Description
Transition	Defines the type of analysis: • Isocratic – Constant mobile phase composition throughout the analysis. • Gradient – Varying mobile phase composition throughout the analysis.
Initial Equil Time (min)	The equilibration time prior to the first isocratic analysis run in a sequence. This field is not displayed when the Transition type is set to Gradient. For an Isocratic run, there are no changes to the solvent composition. Therefore, the compositions are set in Step 0 and maintained throughout the Pump Program. The equilibration time required between runs is therefore minimal but equilibration is required prior to the first run. This value is used for the initial equilibration but it is replaced by the Equil Time in Step 0 for subsequent runs.
Total Run Time (min)	Displays the total run time of the pump program (that is, the combined step times for steps 1 to n (where n is the final step). This value is used for the purpose of run time reconciliation with the detectors.
Run Time Reconciliation	When a method is saved the software will compare the total run time of the pump to the end time for each detector configured on the system. If the pump run time and the detector end times are all equal then the method is saved without comment. Any other condition will generate a message.
Standby Time (min)	Enter the number of minutes (15 to 999 min) that the pump will remain in the Ready state before changing to the standby flow rate.
Standby Flow (mL/min)	Enter the flow rate (0.00 to 10.0 mL/min) to be set after the Standby Time has elapsed.
Stop Time After Equil (min)	Enter the length of time the pump will wait for an external command before shutting the pump down.
Upper pressure limit	Enter the upper limit of the pressure, above which the pump will shut off.
Lower pressure limit	Enter the lower limit of the pressure, below which the pump will shut off.

3. Enter your initial LC Pump Steps.

Step	The steps in the pump program. **Step 0** – Is the equilibration step. All programs must begin with Step 0. **Step 1 through 19** – Are the steps in the pump program during data collection. For an ICP-MS method, the sum of the times in Steps 1 - 19 should equal the run time in the ICP-MS portion of the method. **Wash** step is always last step, where the column is cleaned. No data is collected during this step.

Step Type	**Equilibration** for Step 0, allows time for the column to equilibrate prior to data collection. **Run** for subsequent steps indicates data collection. **Wash** is where the column is cleaned and no data is collected.
Step Time (min)	The duration of the current step in minutes.
Flow (mL/min)	The flow rate of the mobile phase (solvents) in mL/min.
%A, %B, %C, %D	The percentage of each of the four solvents comprising the mobile phase. The percentage are entered by the user. Percentage of D is calculated (100 – %A –%B – %C) However, D can never be less than zero. If a user entry would cause this to happen, then the amount by which D would go negative is subtracted from the next available solvent. For example, if you increase B by 20% and D is only 10% then 10% would be subtracted from C (or from C and A if necessary). Similarly if you enter a value in D, solvent C (and/or B and/or A) is adjusted as necessary to maintain a total of 100%.
Curve	The solvent gradient curvature (change in concentration) during the step. This parameter is only displayed when Gradient is selected as the Transition type. A Curve of 1 is linear. A Curve of 0 is a step gradient. Curves -9.9 to 9.9 (in 0.1 increments) are concave/convex profiles.

NOTE: *If a Wash step is used, it must be the last step for both the Isocratic and Gradient curves since no data is collected during this step.*

4. Enter your initial **LC Pump Timed Events**.

Parameter	**Description**
Step Type	Select the Step from a drop–down list indicating whether the event will occur during Equilibration, Run, or Wash.
TE Time (min)	The time into the run at which the event will occur.
TE	Select the event relay to be activated. There are two relays available: TE1 and TE2.

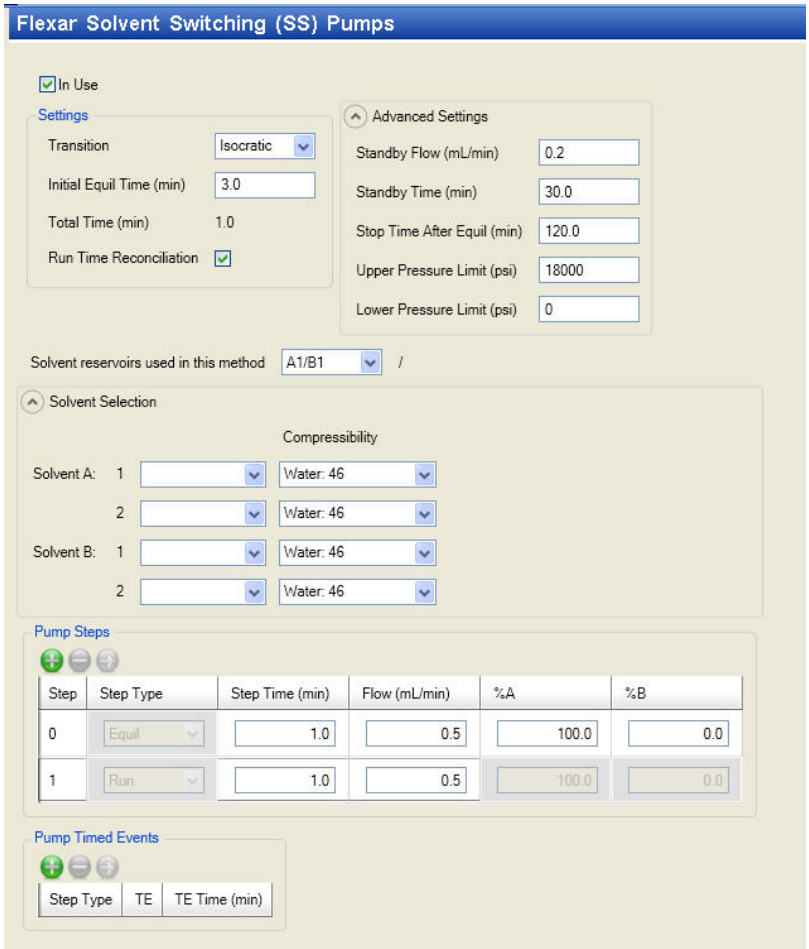
Method: Flexar SS Pump Settings

The LC Solvent Switching Pump Settings portion of the method is used to define the pump parameters; more specifically, you define the pump program which consists of steps, step type, step time, flow rate, mobile phases, mobile phase composition, composition curve, timed events, and pressure limits.

NOTE: *When configuring more than one pump in an LC system, please make sure that each pump's upper pressure limit is properly set within the method. This will depend on the individual pump's role in the system; be it for column re-equilibration, post-column functions or otherwise.*

When a method is created, the default **solvent names** are taken from the instrument preferences, and you have the ability to change them. The solvent names will be stored within the method and subsequently will never change (regardless of what modifications to names are made in preferences), unless you explicitly change them within the method.

1. Enter your initial LC Pump Settings.



<table>
<tr><th>Parameter</th><th>Description</th></tr>
<tr><td>Transition</td><td>Defines the type of analysis:
Isocratic – Constant mobile phase composition throughout the analysis.
Gradient – Varying mobile phase composition throughout the analysis.</td></tr>
<tr><td>Initial Equil Time (min)</td><td>The equilibration time prior to the first isocratic analysis run in a sequence. This field is not displayed when the Transition type is set to Gradient.

For an Isocratic run, there are no changes to the solvent composition. Therefore, the compositions are set in Step 0 and maintained throughout the Pump Program. The equilibration time required between runs is therefore minimal but equilibration is required prior to the first run. This value is used for the initial equilibration but it is replaced by the Equil Time in Step 0 for subsequent runs.</td></tr>
<tr><td>Advanced Settings expander</td><td>Displays the additional pump parameters for which defaults are set in Preferences.</td></tr>
<tr><td>Total Time (min)</td><td>Displays the total run time of the pump program (that is, the combined step times for steps 1 to n (where n is the final step). This value is used for the purpose of run time reconciliation with the detectors.</td></tr>
<tr><td>Run Time Reconciliation</td><td>When this checkbox is checked, when a method is saved the software will compare the total run time of the pump to the end time for each detector configured on the system. If the pump run time and the detector end times are all equal then the method is saved without comment. Any other condition will generate a message.</td></tr>
<tr><td>Solvent reservoirs used in this method</td><td>Define the pair of solvent reservoirs to be used in defining the mobile phase composition during the run by making a selection from the drop-down list (A1/B1. A1/B2, A2/B1, or A2/B2).</td></tr>
<tr><td>Solvent Selection expander</td><td>
<table>
<tr><th>Parameter</th><th>Description</th></tr>
<tr><td>Solvent A: 1
Solvent A: 2
Solvent B: 1
Solvent B: 2</td><td>Select a name from the solvents dictionary (which populates the drop-down list), or enter a new solvent name, for reservoir A1, A2, B1, and B2.</td></tr>
<tr><td>Compressibility</td><td>Select from the drop-down list a compressibility factor that most closely matches the selected solvent, for reservoir A1, A2, B1, and B2.

The compressibility values are: Water: 46, Acetonitrile: 115, Chloroform: 100, Ethyl Acetate: 104, Hexane: 150, Isopropanol: 100, Methanol: 121, THF: 95, Toluene: 87</td></tr>
</table>
</td></tr>
</table>

Advanced LC Pump Settings

The following parameters display when you click the Advanced expander.

Parameter	Description
Standby Flow (mL/min)	Enter the flow rate to be set after the Standby Time has elapsed. **Range:** 0.00 to 3.00 mL/min for Series 275 HiRes Micro, Flexar Binary Micro, FX-10 0.00 to 5.00 mL/min for FX-15/FX-20 0.00 to 10.0 mL/min for all other pumps
Standby Time (min)	Enter the number of minutes (15 to 999 min in 0.1 min increments) that the pump will remain in the Ready state before changing to the standby flow rate.
Stop Time After Equil (min)	Enter the length of time the pump will wait for an external command before shutting the pump down.
Upper Pressure Limit	Enter the upper limit of the pressure, above which the pump will shut off.
Lower Pressure Limit	Enter the lower limit of the pressure, below which the pump will shut off.

The allowed overall range of pump pressure limits is shown the the table below.

	Flexar FX-15/FX-20	Flexar FX-10 and Series 275 HiRes Micro	All other pumps
Range in psi	0 to 18000	0 to 10000	0 to 6100
Range in bar	0 to 1241	0 to 689	0 to 420
Range in MPa	0 to 124	0 to 68	0 to 42

NOTE: *The Pressure Units are set in Preferences.*

The Standby Time range is the same for all of the Flexar/Series 200 pumps supported in this release.

The Standby Flow range is the same for all of the Flexar/Series 200 pumps supported in this release, with the exception of the microbore pumps, for which the range is 0.00 to 3.00 mL/min.

Pump Steps	Description
Step	The steps in the pump program. **Step 0** – Is the equilibration step. All programs must begin with Step 0. **Step 1 through 19** – Are the steps in the pump program during data collection. For an ICP-MS method, the sum of the times in Steps 1 - 19 should equal the run time in the ICP-MS portion of the method. **Wash** step is always last step, where the column is cleaned. No data is collected during this step.

Step Type	**Equilibration** for Step 0, allows time for the column to equilibrate prior to data collection. **Run** for subsequent steps indicates data collection. **Wash** is where the column is cleaned and no data is collected.
Step Time (min)	The duration of the current step in minutes.
Flow (mL/min)	The flow rate of the mobile phase (solvents) in mL/min.
%A, %B, %C, %D	The percentage of each of the four solvents comprising the mobile phase. The percentages are entered by the user. Percentage of D is calculated (100 – %A –%B – %C) However, D can never be less than zero. If a user entry would cause this to happen, then the amount by which D would go negative is subtracted from the next available solvent. For example, if you increase B by 20% and D is only 10% then 10% would be subtracted from C (or from C and A if necessary). Similarly if you enter a value in D, solvent C (and/or B and/or A) is adjusted as necessary to maintain a total of 100%.
Curve	The solvent gradient curvature (change in concentration) during the step. This parameter is only displayed when Gradient is selected as the Transition type. A Curve of 1 is linear. A Curve of 0 is a step gradient. Curves -9.9 to 9.9 (in 0.1 increments) are concave/convex profiles.

NOTE: *If a Wash step is used, it must be the last step for both the Isocratic and Gradient curves since no data is collected during this step.*

Pump Timed Events	**Description**
Step Type	Select the Step from a drop–down list indicating whether the event will occur during Equilibration, Run, or Wash.
TE Time (min)	The time into the run at which the event will occur.
TE	Select the event relay to be activated. There are two relays available: TE1 and TE2.

Method: Autosampler Settings (Flexar or Series 200/225/275)

When an autosampler is configured as part of an instrument, the vial number, injection volume and number of injections settings in the sequence row act as control parameters for the autosampler. These are in addition to the control parameters in the instrument method (in the case of injection volume the sequence value over-rides the value from the method).

The autosampler instrument method includes the Injection Delay parameter. This causes the autosampler to delay sample injection for a set period of time after the run has started (that is, after the autosampler has sent the 'start' signal to the other devices making up the instrument). Chromera also supports the Fixed Mode of injection for the Series 200 autosampler. This is applicable to loop sizes of 200µL or less.

Chromera also supports the Flexar or Series 225 and Series 275 autosamplers, which are largely compatible with the Series 200, with the exception of the parameters in the following table:

Parameter	Series 200	Flexar/Series 225/275
Excess Volume (µL)	Yes	N/A
Fixed Mode	Yes	N/A
Mode	N/A	Yes (Partial Loop/Fixed Loop/µPickup)
Needle Level	Yes (0 to 100)	Yes (2.0mm to 6.0 in 0.5mm increments)

Each autosampler supports specific trays:

Series 200 Autosampler:

Series 200 Autosampler Tray Description	Display Name
25-Position 6mL vial tray	25-Position Tray
80-Position Sample Tray, Dilution Kit; for serial dilutions	80-Position Tray w/ Dilution Tank
85-Position Sample Tray; (80) 2mL vials plus (5) 6mL reagent vials (for pre-column automated sample derivatization)	85-Position Tray (80 x 2mL + 5 x 6mL)
100-Position 2mL vial tray	100-Position Tray
205-Position sample tray; (195) 0.2mL micro-vials plus (10) 2mL vials	205-Position Tray (195 x 0.2mL + 10x2mL)
225-Position 0.2mL micro-vial tray	225-Position Tray
2 x 96-well 'shallow' (200-300L) micro-titer tray; w/ hold-down mechanism	96-Well "Shallow" Microtiter tray
2 x 96-well 'deep' (0.8-1 mL) micro-titer tray; w/ hold-down mechanism	96-Well "Deep" Microtiter tray
100-Position 2mL vial Peltier Temperature-Controlled Tray Accessory (100 standard 2mL vials). Temperature controlled from 4 to 60° C within ± 1° C	100-Position Tray w/ Peltier
2 x 96-well "shallow" Micro-titer Peltier Temperature-Controlled Tray Accessory (supports 2 "shallow" (200-300L) or "medium" (400-500 L) depth microtiter plates). Temperature controlled from 4 to 60° C within ± 1° C	96-Well "Shallow" Microtiter Tray w/ Peltier
2 x 384-well "shallow" Micro-titer Peltier Temperature-Controlled Tray Accessory (supports 2 "shallow" (100L) depth microtiter plates). Temperature controlled from 4 to 60° C within ± 1° C	384-Well "Shallow" Microtiter Tray w/ Peltier

Flexar/Series 225/275 Autosampler:

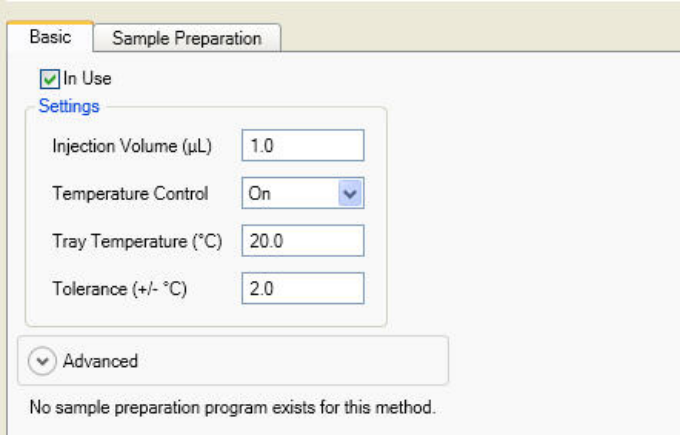
Flexar/Series 225/275 Autosampler Tray Description	Display Name
25-Position 6mL vial tray	25-Position Tray
80-Position 2mL vial tray with 60mL dilution tank (for serial dilution applications)	80-Position Tray w/ Dilution Tank
85-Position tray; (80) 2mL vials plus (5) 6mL reagent vials (for pre-column automated sample derivatization)	85-Position Tray (80 x 2mL + 5 x 6mL)
100-Position 2mL vial tray	100-Position Tray

205-Position tray; (200) 0.2mL micro-vials plus (5) 2mL calibration vials	205-Position Tray (200 x 0.2mL + 5 x 2mL)
96-well 'shallow' adaptor (1 adaptor is required to use the Series 225/275 autosampler with either one or two 96-well 'shallow' micro titer plates)	96-Well "Shallow" Microtiter Plates
96-well 'deep' adaptor (1 adaptor is required to use the Series 225/275 autosampler with either one or two 96-well "deep" micro titer plates)	96-Well "Deep" Microtiter Plates
384-well adaptor (1 adaptor is required to use the Series 225/275 autosampler with either one or two 384-well micro titer plates)	384-Well Microtiter Plates

Flexar or Series 200/225/275 Method Values

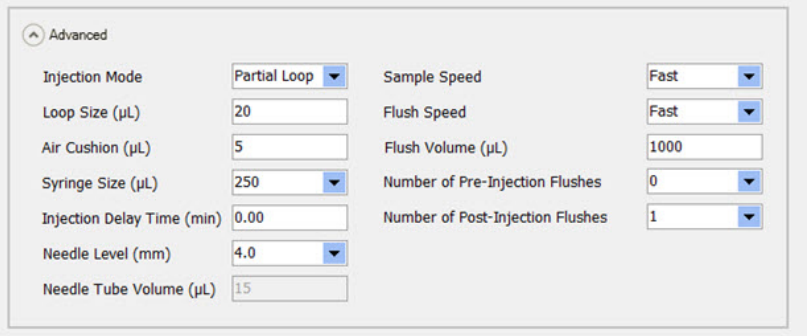
➢ Enter method values for the following Autosampler parameters.

You use this row to setup and configure the method for the **Autosampler** settings. The primary setting is the Injection Volume, but additional parameters are accessible in the **Advanced** settings.



Parameter	Description
Injection Volume (µL)	The amount of sample injected.
Temperature Control	Select On or Off from the drop-down list to set whether the tray temperature control will be active or not. This is not displayed for No Peltier models.
Tray Temperature (°C)	The temperature to be maintained when the Peltier autosampler tray is installed.
Tolerance (± °C)	The deviation of the Tray Temperature from set temperature allowed for the Ready condition.

Advanced Autosampler Settings



Advanced Band	**Description**
Injection Mode	Select from the drop-down list the operational mode of autosampler.
Loop Size (µL)	The size of the installed sample loop.
Air Cushion (µL)	The volume of the air cushion between the sample and flush solvent. 10 µL is recommended.
Flush volume (µL)	The volume of flush solvent used to flush the injector needle for each flush cycle.
Syringe Size (µL)	The volume of the syringe used to withdraw and inject sample.
Injection Delay Time (min)	Define the time (from 0.00 to 99.99 min) after the autosampler has sent the Start signal to the other instrument devices to when the injection is actually made.
Needle Level (mm)	Select from the drop-down list the position of the sampling needle in the sample vial (2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6 mm)
Needle Tube Volume (µL)	Enter the volume used to center the sample within the sample loop in µL (1 to 20) – Pickup mode.
Sample Speed	The rate (Very Slow, Slow, Medium, Fast and Very Fast) at which the sample syringe fills the injection loop.
Flush Speed	The speed (Very Slow, Slow, Medium, Fast, and Very Fast) at which you want the pump to flush the system.
Flush volume (µL)	The volume of flush solvent used to flush the injector needle for each flush cycle.
Number of Pre-injection flushes	The number of flush cycles you want before each sample injection.
Number of Post-injection flushes	The number of flush cycles you want after each sample injection.

NOTE: *The method for the* ***Series 225/275*** *autosamplers is slightly different.*

The Series 225/275 autosamplers do not use the Excess Volume parameter and exhibit differences from the Series 200 in the **Mode** and **Needle Level** parameters.

Method: Autosampler Settings (Flexar DS Autosampler)

When an autosampler is configured as part of an instrument, the vial number, injection volume and number of injections settings in the sequence row act as control parameters for the autosampler. These are in addition to the control parameters in the instrument method (in the case of injection volume the sequence value over-rides the value from the method).

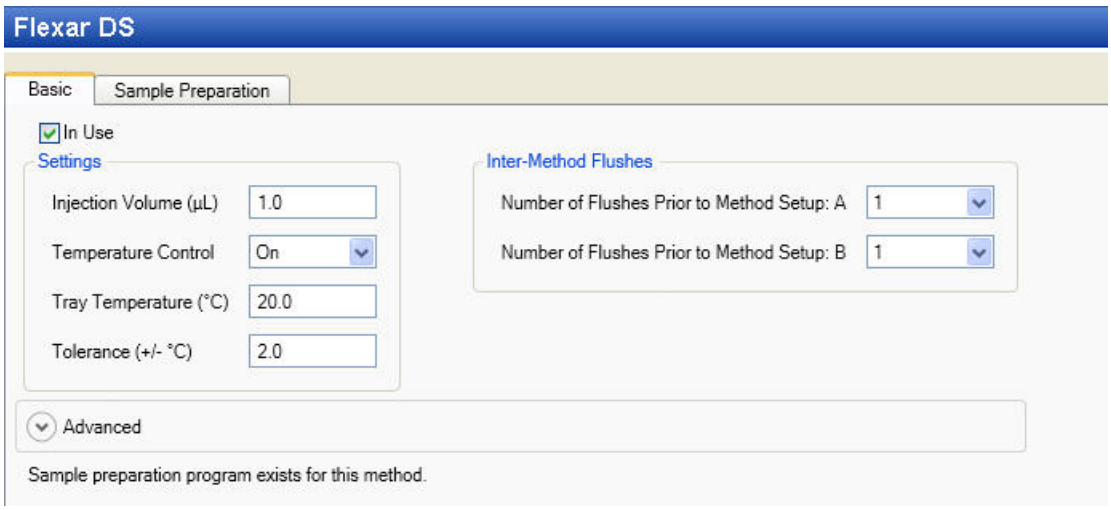
The autosampler instrument method includes the Injection Delay parameter. This causes the autosampler to delay sample injection for a set period of time after the run has started (that is, after the autosampler has sent the 'start' signal to the other devices making up the instrument).

Flexar/Series 225/275 Autosampler Tray Description	**Display Name**
25-Position 6mL vial tray	25-Position Tray
80-Position 2mL vial tray with 60mL dilution tank (for serial dilution applications)	80-Position Tray w/ Dilution Tank
85-Position tray; (80) 2mL vials plus (5) 6mL reagent vials (for pre-column automated sample derivatization)	85-Position Tray (80 x 2mL + 5 x 6mL)
100-Position 2mL vial tray	100-Position Tray
205-Position tray; (200) 0.2mL micro-vials plus (5) 2mL calibration vials	205-Position Tray (200 x 0.2mL + 5 x 2mL)
96-well 'shallow' adaptor (1 adaptor is required to use the Series 225/275 autosampler with either one or two 96-well 'shallow' micro titer plates)	96-Well "Shallow" Microtiter Plates
96-well 'deep' adaptor (1 adaptor is required to use the Series 225/275 autosampler with either one or two 96-well "deep" micro titer plates)	96-Well "Deep" Microtiter Plates
384-well adaptor (1 adaptor is required to use the Series 225/275 autosampler with either one or two 384-well micro titer plates)	384-Well Microtiter Plates

Flexar Autosampler DS Method Values

- Enter method values for the following Autosampler parameters.

You use this row to setup and configure the method for the **Autosampler** settings. The primary setting is the Injection Volume, but additional parameters are accessible in the **Advanced** settings.



Parameter	Description
In Use	Click in the check box to use the autosampler when running this method. When not checked it ignores the autosampler when running a method.
Injection Volume (µL)	The amount of sample injected.
Temperature Control	Select On or Off from the drop-down list to set whether the tray temperature control will be active or not. This is not displayed for No Peltier models.
Tray Temperature (°C)	The temperature to be maintained when the Peltier autosampler tray is installed.
Tolerance (± °C)	The deviation of the Tray Temperature from set temperature allowed for the Ready condition.

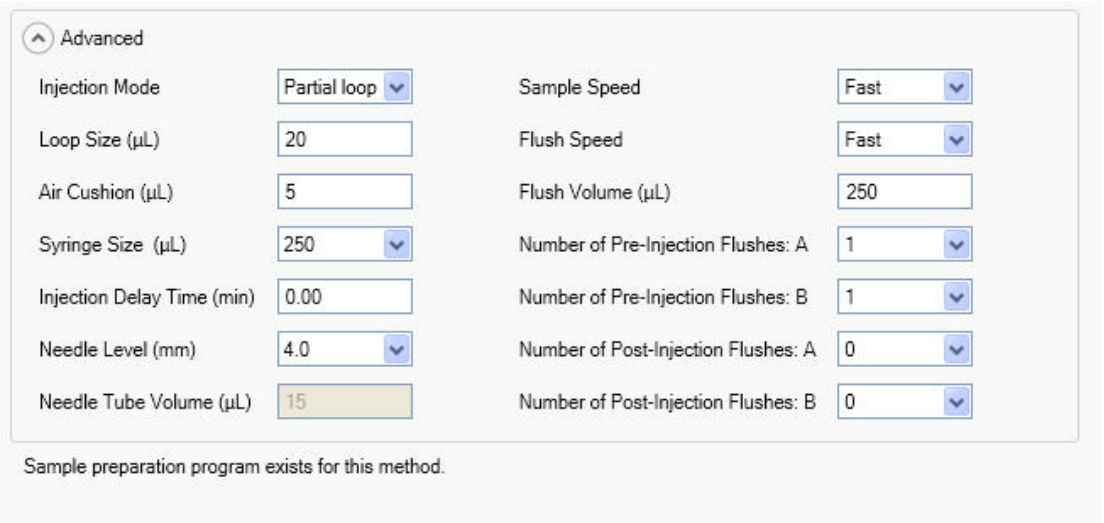
Inter-Method Flushes

Select the following values. Note that these flushes are in addition to any pre-injection flushes defined for the method but they occur only at initial set up of the method.

Control	Description
Number of Flushes Prior to Method Setup: A	Select from the drop-down list the number of flush cycles (from 0 to 9) with solvent A when the method is first set up.
Number of Flushes Prior to Method Setup: B	Select from the drop-down list the number of flush cycles (from 0 to 9) with solvent B when the method is first set up.

Advanced Autosampler Settings

Select the **Advanced** expander to display the additional autosampler parameters for which defaults are set in Preferences.



Advanced expander	**Description**
Injection Mode	Select from the drop-down list the operational mode of autosampler.
Loop Size (µL)	The size of the installed sample loop.
Air Cushion (µL)	The volume of the air cushion between the sample and flush solvent. 10 µL is recommended.
Flush volume (µL)	The volume of flush solvent used to flush the injector needle for each flush cycle.
Syringe Size (µL)	The volume of the syringe used to withdraw and inject sample.
Injection Delay Time (min)	Define the time (from 0.00 to 99.99 min) after the autosampler has sent the Start signal to the other instrument devices to when the injection is actually made.
Needle Level (mm)	Select from the drop-down list the position of the sampling needle in the sample vial (2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6 mm)
Needle Tube Volume (µL)	Enter the volume used to center the sample within the sample loop in µL (1 to 20) – Pickup mode.
Sample Speed	The rate (Very Slow, Slow, Medium, Fast and Very Fast) at which the sample syringe fills the injection loop.
Flush Speed	The speed (Very Slow, Slow, Medium, Fast, and Very Fast) at which you want the pump to flush the system.
Flush volume (µL)	The volume of flush solvent used to flush the injector needle for each flush cycle.
Number of Pre-injection flushes A	The number of flush cycles with solvent A before each sample injection.
Number of Pre-injection flushes B	The number of flush cycles with solvent B before each sample injection.

Number of Post-injection flushes A	The number of flush cycles with solvent A after each sample injection.
Number of Post-injection flushes B	The number of flush cycles with solvent B after each sample injection.

NOTE: *The method for the **Series 225/275** autosamplers is slightly different.*

The Series 225/275 autosamplers do not use the Excess Volume parameter and exhibit differences from the Series 200 in the **Mode** and **Needle Level** parameters.

Method: Autosampler Sample Preparation (Derivatization or Dilution)

The Sample Preparation tab for Derivatization or Dilution enables you process each sample and then inject the resultant. The sample preparation step applies to a row of the sequence, not to an injection. That is, the sample preparation is performed for the current row and then each specified injection and acquisition for that row will follow, before execution proceeds to the next row.

A new Status item is used with Sample Preparation – Sample Vial. This is specifically the vial from which sample is taken for the purposes of derivatization or dilution. This is (or may be) distinct from the vial from which the injection is made (the target vial), which is what will be shown for the existing Status items 'Current Vial' and 'Injection Number' (this is how these items work). The item 'Sample Vial' will always show the vial number from the current sequence row. Having this in Status along with the target vial (as shown by 'Current Vial' and 'Injection Number') allows you to see the full sample preparation picture in one place.

The following examples show Derivatization and Dilution settings.

Derivatization Example

This is a typical example of derivatization, indicating that more than one reagent can be used. The sample preparation steps to take place when the program is run are:

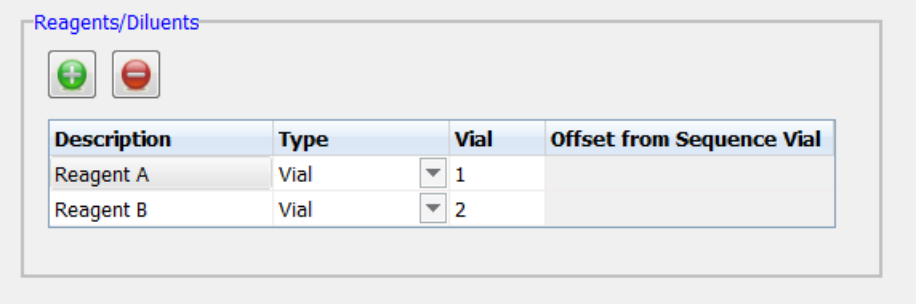
1. 500 µL of sample is transferred from the sample vial to the target vial – which is offset by 50 from the sample vial (the vial defined in the current row of the sequence, where applicable).

2. 50 µL of reagent A is transferred from vial 1 to the target vial. Mixing will be performed to ensure a homogeneous mixture is obtained. A wait time of 1 minute will then be allowed to elapse before the next transfer.

3. 60 µL of reagent B is transferred from vial 2 to the target vial. Mixing will be performed to ensure a homogeneous mixture is obtained. A wait time of 2 minutes will then be allowed to elapse before the injection proceeds.

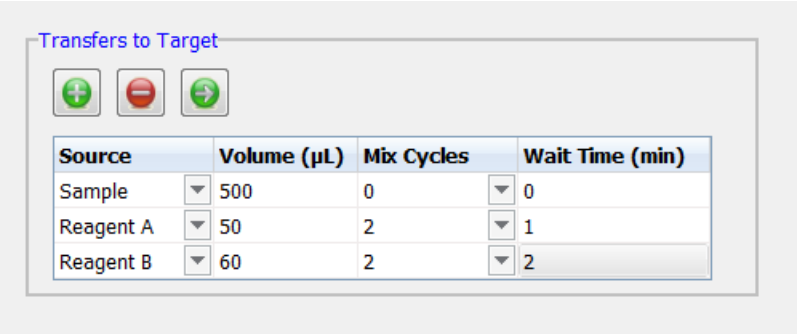
Following the preparation process, the amount specified for the Injection Volume in the current row of the sequence will be injected from the target vial.

This derivatization process requires 'Offset of Target Vials from Sample Vials' to be set to 50 and two items in the Reagents/Diluents:



The fact that the reagent transfers are to be made from a pair of specific vials (rather than a vial series) suggests that either the 85-Position tray (80 x 2mL + 5 x 6mL) or the 205-Position tray (200 x 0.2mL + 5 x 2mL) is likely to be required, if any significant number of samples is to be derivatized. This is not an issue during the creation of the Method but it will be discussed further under Sequence.

The sample preparation steps for this example derivatization are defined in the Transfers to Target:



Dilution Example

The sample preparation steps to take place when the program is run are:

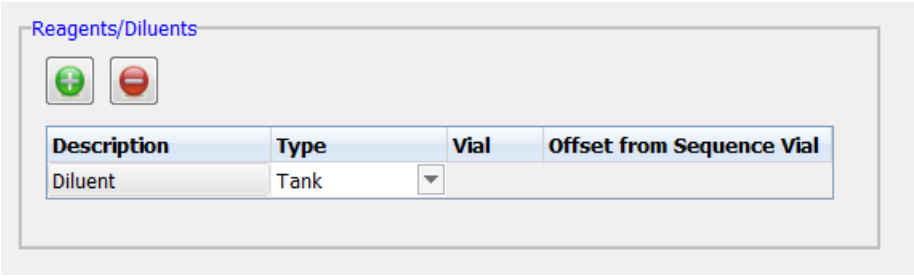
1. 500 µL of diluent is transferred from the Diluent Tank to the target vial – which is offset by 40 from the sample vial (the vial defined in the current row of the sequence, where applicable.

NOTE: *Applicable depends on a couple of factors. Firstly, the sample preparation method is not executed for rows that do not involve injection (e.g. Background, Wash). Secondly, if the 'Execute sample preparation for 'Sample' row types only' flag is checked, the sample preparation will not be executed for 'Standard', 'Matrix', 'Calib' or other non 'Sample' rows.*

2. 100 µL of sample is transferred from the sample vial to the target vial. Mixing will be performed to ensure a uniform diluted sample is obtained.

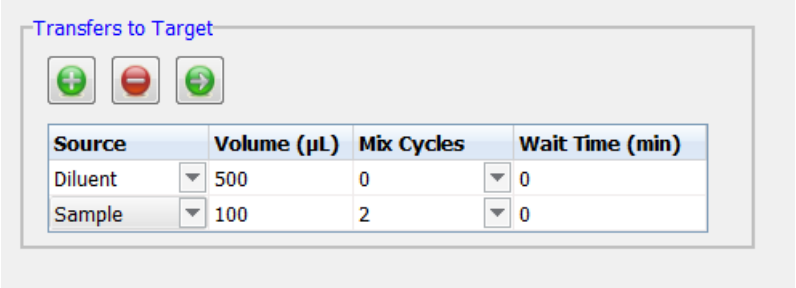
Following the preparation process, the amount specified for the Injection Volume in the current row of the sequence will be injected from the target vial.

This dilution process requires just a single item in the Reagents/Diluents grid:



The use of the Tank implies use of a specific autosampler tray (80-Position tray – with dilution tank). This is not an issue during the creation of the Method but it will be discussed further under Sequence.

The sample preparation steps shown above are defined in the Transfers to Target table:



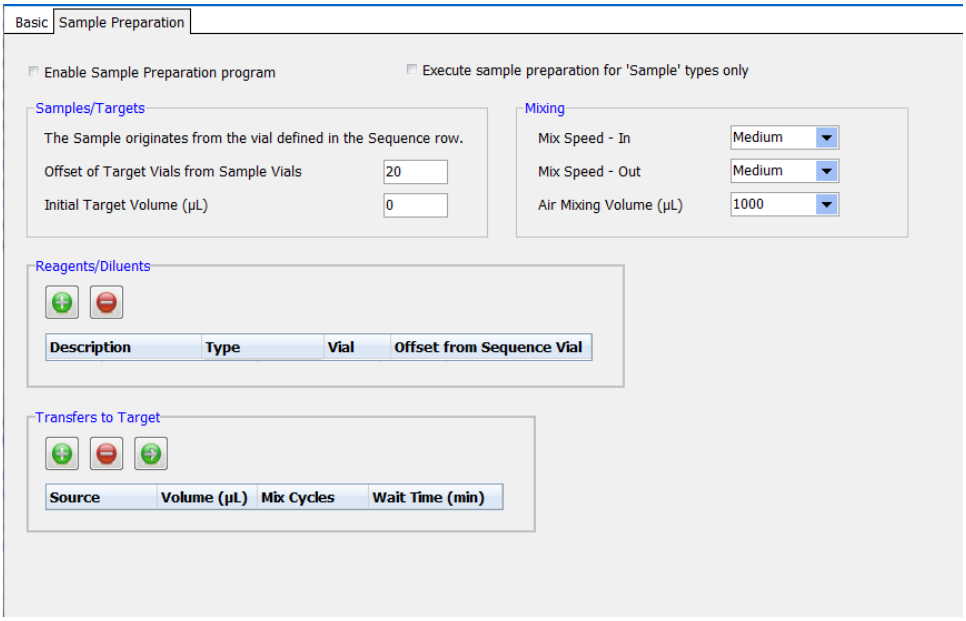
Note that neither the Source 'Sample' nor the Target has to be defined in the Reagents/Diluents table. The Sample is a default item that is always present (effectively the item with index=0). The Target is defined by the value of 'Offset of Target Vials from Sample Vials' (in this case a value of 40 would be entered as indicated in step 1 above).

Sample Preparation

The existing autosampler parameters will appear on the first tab – labeled 'Basic' . A new static text label and the bottom of the view will indicate whether or not an active sample preparation program exists in the method.

By default the Sample Preparation tab will appear as shown below:

NOTE: *Air Mixing Volume control is only for the Series 200 autosampler. For all other autosamplers (Series 225, 275 and Flexar) the Air Mixing Volume control will not appear.*



Click the check box to set each of the two options at the top of the tab that affect the general behavior of the sample preparation program.

Control	**Description**
Enable Sample Preparation program	A check box that determines whether or not the sample preparation defined on this page will be executed when the method is used for acquisition.
Execute sample preparation for 'Sample' row types only	A check box that determines whether the sample preparation defined on this page will be executed for all (injection-type) rows of the sequence or only for rows of type 'Sample'.

NOTE: *Although checking 'Enable Sample Preparation program' is not really valid when no program exists, this control will <u>not</u> be disabled if there is no program. The reason for this is a consequence of the fact that the logical place for this control is at the top of the page. To be forced to leave this setting until a program has been entered and then have to remember to go back and check the (now enabled) control would be annoying and open to error. If the method download code will have a problem with a program of zero steps then we should implement a method validation rule that will stop the method from being saved if there are no program steps while 'Enable Sample Preparation program' is checked.*

Samples/Targets Group

This group box contains three items related to the definition of the source and target vials.

Control	Description
Samples originate from the vials defined in the Sequence rows.	A static text field indicating that the location of the current sample (source) vial is taken from the current sequence row.
Offset of Target Vials from Sample Vials	An integer numeric field that enables the user to define the starting position of a target vial (the vial from which the injection will be made) relative to the source (sample) vial defined in the sequence tray.
Initial Target Volume (µL)	A numeric field that defines the initial volume of liquid present in each target vial (meaning the vial [series] from which the injection will be made).

Mixing Group

This group contains the parameters related to sample mixing. The Air Mixing Volume parameter only appears for the Series 200 autosampler.

Control	Description
Mix Speed – In	Liquid mixing: The speed of the flush syringe when it picks up sample plus reagent (or diluent) from the target vial for mixing. Air mixing: The speed of the flush syringe when it draws in air.
Mix Speed – Out	Liquid mixing: The speed of the flush syringe when it ejects up sample plus reagent (or diluent) back into the target vial for mixing. Air mixing: The speed of the flush syringe when it expels the air into the target vial for mixing.
Air Mixing Volume (µL) (Series 200 autosampler only)	The volume of air drawn into the syringe for mixing.

Reagents/Diluents

This section contains definitions of the 'ingredients' to be used in sample preparation – excluding the original sample itself. Up to 6 'ingredients' can be defined.

Control	Description
	This button adds a new row at the end of the Reagents/Diluents/Target Vials grid.
	This button deletes the currently selected row of the Reagents/Diluents/Target Vials grid.
Description	Type a description to identify the item. Any string of characters that can be entered via the keyboard. Ideally there should be no length restriction but testing does not need to exceed 50 characters (which can be used as a limit by the software if necessary).
Type	Select from the drop-down list the kind of source item being defined.
Vial	Enter a number in the edit box to identify the vial in the autosampler tray to be the source of an item of 'Type' = 'Vial'. Enter from 1 to 225 (384/768-well microtiter plates are not supported for sample preparation.)
Offset From Sequence Vial	Enter a number (from 0 to 100) to define the starting position of a 'Vial Series' relative to the source vial defined in the sequence tray.

Transfers to Target

The rows define the specific liquid transfers to be made as part of the sample preparation prior to injection. Up to 10 rows may be used.

Control	Description
	This button adds a new row at the end of the Transfers table.
	This button deletes the currently selected row of the Transfers table.
	This button inserts a row above the currently selected row of the Transfers table.
Source	Use this drop-down list to define the source of the current transfer operation. Sample (meaning the vial defined in the current sequence row) plus all items defined in the Reagents/Diluents/Target Vials table.
Volume (µL)	Enter a number (from 1 to Syringe Size) to define the amount of liquid that will be transferred from the Source to the Target. **NOTE:** For the Series 200 this is the Sample Syringe size (from the Basic settings tab). For other autosamplers this is the Syringe Size from the Basic settings tab
Mix Cycles	Select from the drop-down list a number of mixing cycles (from 0 to 9) that will be performed for the current transfer operation.
Wait Time (min)	Enter a number (from 00.00 to 99.99) to define the time that will be allow to elapse following completion of the current transfer before the next transfer (if any) or the sample injection (if no further transfers) will be initiated.

Method: ELSD Settings

The Evaporative Light Scattering Detector (ELSD) does not itself recognize a Not Ready state, it goes directly to Ready when set from Standby into active mode, even if the various thermal zones are not yet at their setpoints. To ensure the detector is in a stable state Chromera will monitor various elements of the ELSD when it is set to Active mode and only post the Ready status when all are within specified limits. The specific elements monitored and the conditions for Ready defined below:

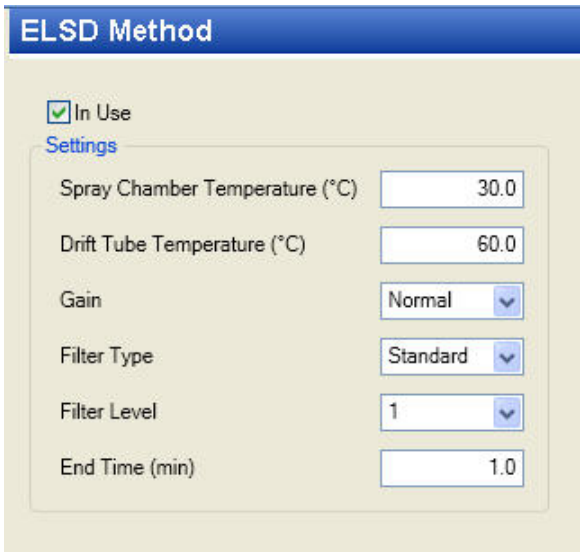
Drift tube and spray temperatures are stable at setpoint (within 1.5 degrees)

Gas On and Pressure

- Gas On and pressure >= 30 psi
- Signal response > 10 mV and < 1 V
- Laser On

If any of the above conditions is not met the ELSD status will be reported as Not Ready.

➢ Enter your initial method parameters for the ELSD:



Settings	Description
In Use	Click in the check box to use the ELSD when running this method. When not checked it ignores the ELSD when running a method.
Spray Chamber Temperature (ºC)	Enter a temperature (from 10 to 60 ºC) for the spray chamber.
Drift Tube temperature (ºC)	Enter temperature (22 to 120 ºC) for the drift tube.
Gain	Select from the drop-down list the gain (Normal or Low) of the amplifier.

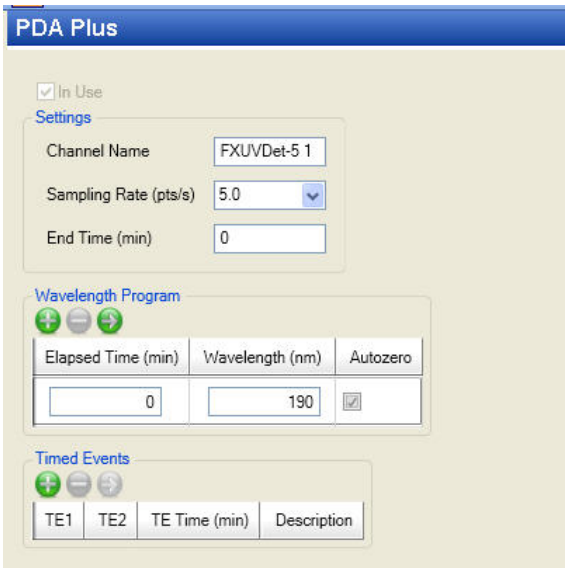
Filter type	Select from the drop-down list the type of filtering (Standard or Baseline).
Filter Level	Select from the drop-down list the degree of standard or baseline filtering (Off, 1 to 10).
End Time (min)	Set the length of time (from 0.0 to 999.9 min) for which data is acquired in the run (starting at time 0.0). Must be greater than (or equal to) the highest setting for Elapsed Time in the wavelength program table.

Method: UV/Vis Detector Settings (Flexar or Series 200)

The UV/Vis detector is a single channel, programmable wavelength device.

NOTE: *Chromera also supports the Flexar or Series 200 HS UV/Vis detector, which is identical to the standard detector, except for the addition of a sampling rate of 100 pts/s (equivalent to Peak Width of 0.05s).*

1. Enter your initial method parameters for the UV/Vis Detector:



Parameter	Description
Channel Name	Enter a name (from 1 to 25 characters) to be used as an identifier for the data channel in the Channels, Peak Detection and Calibration sections of the method. The Series 200 UV/Vis detector only supports one channel but this field is required in the future for multi-channel detectors. A name must be entered before the method can be saved. The name must be unique within the channels of the method.

Sampling Rate (pts/s)	The data acquisition rate from the detector. Select the rate (0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, pts/s) from a drop-down list. The UHPLC version has a 100 pts/s selection. The detector front panel provides three modes of access to this setting: Data Rate, Rise Time and Peak Width. See notes below.
Peak Width (s)	Select one of the peak width at half height settings (25.00, 10.00, 5.00, 2.50, 1.00, 0.50, 0.25, 0.10) supported by the detector from the drop-down list. See notes below.
End Time (min)	Set the length of time (from 0.0 to 999.9 min) for which data is acquired in the run (starting at time 0.0). Must be greater than (or equal to) the highest setting for Elapsed Time in the wavelength program table.

NOTES: *(1) The Series 200 UV/VIS detector supports a different sampling rate for each wavelength program step of the method, as would the Chromera data acquisition software. However, the normal processing of wavelength programmed runs is to concatenate the data streams and perform peak detection on the complete chromatogram. Since the current peak detection algorithms do not support multiple sampling rates within a single analysis, the sampling rate is set for the channel as a whole. (2) Either Sample Rate or Peak Width is displayed, depending on the user preference setting.*

2. Enter Detector Wavelength Program parameters (1 to 20 steps can be created).

Detector Wavelength Program Parameter **(1 to 20 steps can be created)**	**Description**
Elapsed Time (min)	The time (from 0.0 to 999.9 min) into the run at which the settings are to take place. Time is always 0.0 for the mandatory first program step and cannot be edited. For subsequent steps, if you enter a value that is larger than the current End Time setting then End Time is set equal to that value.
Wavelength (nm)	The wavelength to be monitored for this step. Deuterium lamp: 190 to 700 nm Tungsten lamp: 360 to 700 nm
Autozero	A check box indicating whether the detector should perform an autozero (yes) at the beginning of the step.

3. Enter the Timed Event parameters (Up to 20 steps can be created).

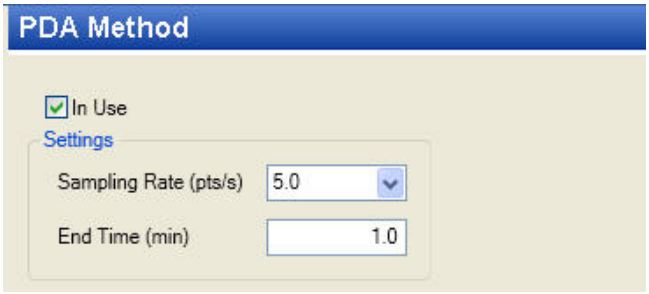
Timed Event Parameters	**Description**
TE Time (min)	The elapsed time (from 0.00 to 999.99 min) into the run at which the specified event should occur. Up to 20 events may be specified.
TE1	Select No Action, Open, Close, or Momentary from a drop-down list to specify the action to be performed with event #1. No Action = leave in current state. Momentary = close for 2 seconds and then reopen.
TE2	Select No Action, Open, Close, or Momentary from a drop-down list to specify the action to be performed with event #2. No Action = leave in current state Momentary = close for 2 seconds and then reopen.
Description	Enter a comment about the event using up to 50 characters.

Method: PDA Detector Settings

The PDA detector is a multi-channel, programmable wavelength device.

NOTE: *The lamp is automatically selected based on the wavelength selected for the channel*

1. Enter your initial method parameters for the PDA Detector:



Parameter	**Description**
In Use	This check box enables you to use or ignore the PDA detector when running the method. If the box is not checked then the PDA detector will be ignored when the method is applied to the instrument.
Sampling Rate (pts/s)	Select from the drop-down list the data acquisition rate from the detector. Chromera will use a single value for the entire run. Sampling Rate (pts/s) 1000, 500, 333, 250, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2 pts/s.

End Time (min)	Set the length of time (from 0.0 to 999.9 min) for which data is acquired in the run.

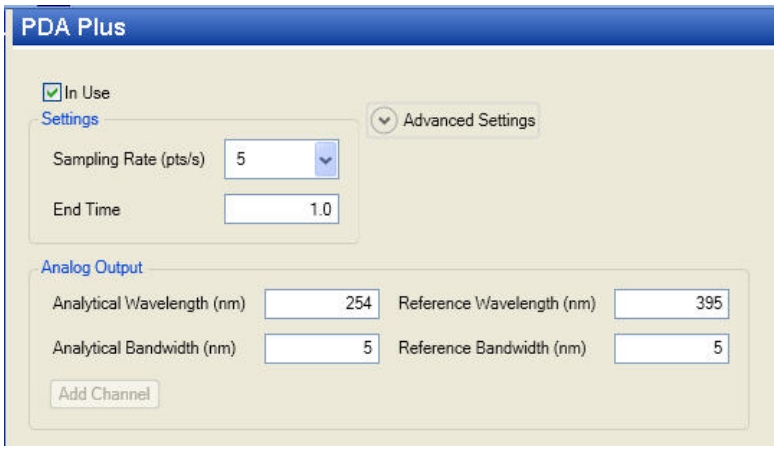
2. After entering your method parameters, select **Channel** in the Method tree and enter the Channel parameters for this method.

Method: PDA Plus Detector Settings

The Flexar PDA Plus detector is a multi-channel, programmable wavelength device.

NOTE: *The lamp is automatically selected based on the wavelength selected for the channel.*

1. Enter your initial method parameters for the PDA Plus Detector:



Settings	Description
In Use	This check box enables you to use or ignore the PDA Plus detector when running the method. If the box is not checked then the PDA Plus detector will be ignored when the method is applied to he instrument.
Sampling Rate (pts/s)	Select from the drop-down list the data acquisition rate from the detector. Chromera will use a single value for the entire run. Sampling Rate (pts/s): 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2 pts/s
End Time (min)	Set the length of time (from 0.0 to 999.9 min) for which data is acquired in the run.

<table>
<tr><th>Advanced Settings
(For advanced users)</th><th>Description</th></tr>
<tr><td>Pixel Read Time (ms)</td><td>Enter the time for which signal from each pixel of the array will be read to generate a data point. Only integer values may be entered (from 2.0 to 100.0 ms).

The Pixel Read Time field only accepts an entry of numeric characters (0 to 9) and a decimal point. If the Pixel Read Time value entered is to more than 1 decimal place, it will be rounded to 1 decimal place.

NOTE: Selecting Pixel read times longer than 5 ms will limit the fastest selectable sampling rates.

Pixel Read time and Sampling Rate are inter-related. With a Pixel Read Time of 5.0 ms (the default) or less, all sampling rates (from 0.2 to 200pts/s) will be available. At higher Pixel Read Times, some of the faster rates will become unavailable.

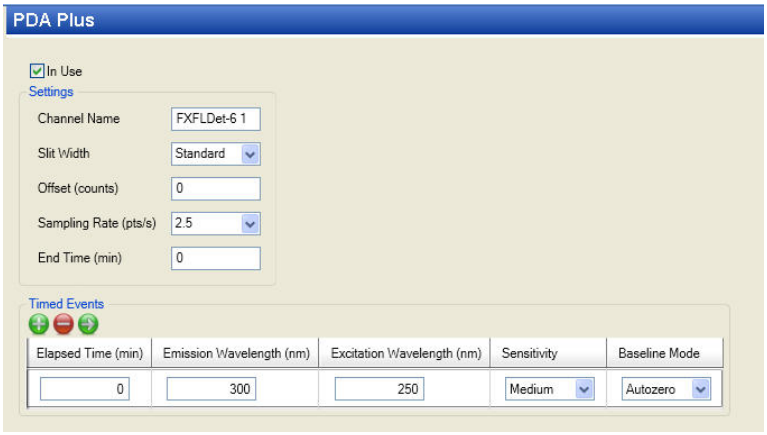
<table>
<tr><th>Pixel Read Time (ms)</th><th>Valid Sampling Rates (pts/s)</th></tr>
<tr><td>2.0 to 5.0</td><td>0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0</td></tr>
<tr><td>5.1 to 10.0</td><td>0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0</td></tr>
<tr><td>10.1 to 20.0</td><td>0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0</td></tr>
<tr><td>20.1 to 50.0</td><td>0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0</td></tr>
<tr><td>50.1 to 100.0</td><td>0.2, 0.5, 1.0, 2.0, 5.0, 10.0</td></tr>
</table>
</td></tr>
</table>

Analog Output Settings	**Description**
Analytical Wavelength (nm)	Enter the central analytical wavelength (from 190 to 790) for the analog output. The default is 254.
Analytical Bandwidth (nm)	Enter the wavelength range around the central analytical wavelength for the analog output (from 1 to 25; odd numbers only).
Reference Wavelength (nm)	Enter the central reference wavelength (from 190 to 790) for the analog output. The default is 395.
Reference Bandwidth (nm)	Enter the wavelength range around the central reference wavelength for the analog output (from 1 to 25; odd numbers only).
Add Channel	Click the button to create a Chromera data channel from the currently displayed Analog Output settings. When the Analog Output parameters all contain valid values the Add Channel button will be enabled. When the button is clicked a Chromera data channel will be created with these values (even if a channel already exists with these values).

2. After entering your method parameters, select Channel in the Method tree and enter the Channel parameters for this method.

Method: Fluorescence Detector Settings (Flexar or Series 200a)

The Fluorescence Detector portion of the method is used to define the Fluorescence detector parameters.



1. Enter your initial **Fluorescence Detector** settings.

Parameter	**Description**
Channel Name	A text field (from 1 to 25 characters) that is used as an identifier for the data channel in the Channels, Peak Detection and Calibration sections of the method. Note that the Series 200 Fluorescence detector only supports one channel but this field is required in the future for multi-channel detectors.
Slit Width	Select Standard or Wide from a drop-down list to define the detector's Emission Bandwidth value.
Offset	Enter an Offset value (from 0 to 1000) to which detector output is set by an autozero operation.
Sampling Rate (pts/s)	Select the Sampling Rate from the drop-down list that specifies the data acquisition rate (of 20, 10, 5, 2.5, 1.25, 0.625, 0.3125) from the detector. The actual detector parameter is Sampling Period but this is mapped to points per second in the GUI, for consistency with other Chromera detectors. The detector's Time Constant parameter is set to conform to the sampling rate defined (see below).
End Time (min)	An edit field specifying the length of time for which data is acquired in the run (starting at time 0.0 and continuing to 999.9 min). Must be greater than (or equal to) the highest setting for Elapsed Time in the wavelength program table (see below). See Run Time Reconciliation.

Sampling Rate to Time Constant mapping:

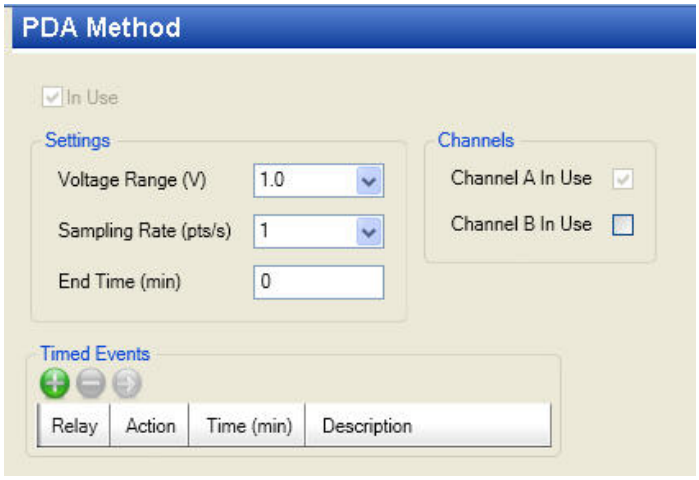
Sampling Rate (pts/s)	Time Constant (ms)
20	0.05
10	0.1
5	0.1
2.5	0.5
1.25	1.0
0.625	2.0
0.3125	4.0

2. Enter your **Detector Program** settings (1 to 20 steps can be created).

Detector Program Parameter (1 to 20 steps can be created)	**Description**
Elapsed Time (min)	An edit field defining the time (from 0.0 to 600.0 min) into the run at which the settings are to take place. Time is always 0.0 for the mandatory first program step and cannot be edited. For subsequent steps, if the user enters a value that is larger than the current End Time setting then End Time is set equal to that value.
Excitation Wavelength (nm)	Set the excitation wavelength (from 200 to 850 nm) for this program step.
Emission Wavelength (nm)	Set the emission wavelength (from 250 to 900 nm) to be monitored for this program step.
Sensitivity	Define the detector's PMT Voltage parameter (Previous, Super High, High, Medium, Low, Super Low) from the drop-down list. (Previous means that the Sensitivity value is not changed by this step, so it remains as it was.)
Baseline Mode	A drop-down list to set how the baseline level is set (Autozero, Hold, or None) at the beginning of each program step.

Method: Flexar USB A/D Settings

The Flexar USB A/D are analog to digital (A/D) interfaces that acquire data from a chromatograph. You may perform a run either via manual injections or with an autosampler. The NCI 902 contains two A/D converters where the second A/D converter allows you to collect data from two detectors during a single run.



1. Enter your initial method parameters for the Flexar USB A/D Settings:

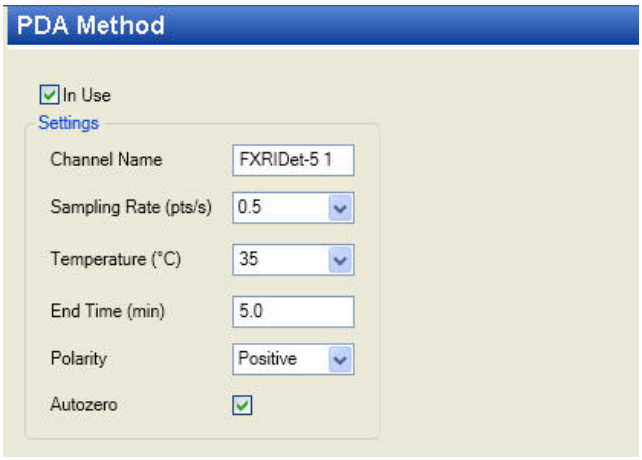
Parameter	Description
In Use	Check this box to use or ignore the A/D when running the method. If the box is not checked then the A/D will be ignored when the method is applied to the instrument.
Settings	
Voltage Range	Select the voltage range from the drop-down list for the detector. Chromera will use a single value for the entire run. Unipolar -0.125v to +1.250v Bipolar -1.250v to +1.25v
Sampling Rate (pts/s)	Select the data acquisition rate from the drop-down list for the detector. Chromera will use a single value for the entire run. Sampling Rate (pts/s) 200 (single channel), 100, 50, 25, 20, 15, 10, 5, 2, 1, 0.5, 0.2 pts/s
End Time (min)	Set the length of time (from 0.0 to 999.9 min) for which data is acquired in the run.
Channels	
Channel A In Use	Channel A is always selected.
Channel B In Use	Click this box to use Channel B.

Timed Events	
Description	Enter a description for this relay action.
Relay	Select a Relay 1 through 6 from the drop-down list.
Action	Select On or Off from the drop-down list.
Time (min)	Enter a time for this action to occur.

2. After entering your method parameters, select Channel in the Method tree and enter the Channel parameters for this method.

Method: Refractive Index Detector Settings (Flexar or Series 200a)

The Refractive Index Detector portion of the method is used to define the RI detector parameters.



1. Enter your initial **RI Detector** settings**.**

Parameter	**Description**
Channel Name	Enter an identifier (from 1 to 25 characters) for the data channel in the Channels, Peak Detection and Calibration sections of the method. The Series 200a Refractive Index detector only supports one channel.
Temperature (°C)	Set the temperature (Off, 30 to 50°C) of the RI cell.
Sensitivity	Set the detector sensitivity/dynamic range. Select from a drop-down list **High** (125 µRIU/V) or **Low** (500 µRIU/V)

Sampling Rate (pts/s)	Set the data acquisition rate from the detector. The actual detector parameter is Response Time but this are mapped to points per second (i.e. 1 / response time). Select the Sampling Rate (pts/s) values from the drop-down list: 10, 5, 2, 1, 0.66, 0.5
End Time (min)	Set the length of time (0.0 to 999.9 min) for which data is acquired in the run (starting at time 0.0). Must be greater than (or equal to) the highest setting for Elapsed Time in the wavelength program table.

NOTE: *The Flexar or Series 200a Refractive Index detector has no internal run clock, so the End Time and program steps are implemented by the Chromera software.*

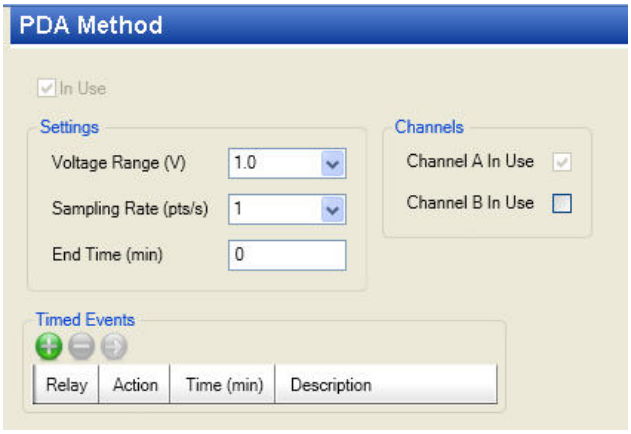
2. Enter your **Detector Program** from 1 to 20 steps can be created**.**

Parameter	Description
Elapsed Time (min)	Enter the time into the run at which the settings are to take place. Time is always 0.0 for the mandatory first program step and cannot be edited. For subsequent steps, if you enter a value that is larger than the current End Time setting then End Time is set equal to that value.
Polarity	Set the polarity by selecting from the drop-down list (**+, -)** for this step.
Autozero	A check box indicating whether the detector should perform an autozero at the beginning of the step.

Method: NCI 901/902 Settings

The NCI 901 and NCI 902 are analog to digital (A/D) interfaces that acquire data from a chromatograph. You may perform a run either via manual injections or with an autosampler. The NCI 902 contains two A/D converters; the NCI 901 contains one. On the NCI 902, the second A/D converter allows you to collect data from two detectors during a single run.

1. Enter your initial method parameters for the NCI 902 or NCI 901.



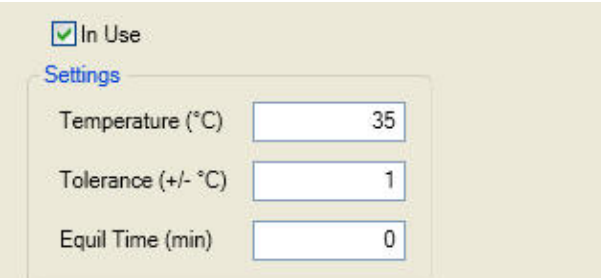
NCI 902 Display with Channels

NCI 901 Display without Channels

Parameter	Description
In Use	Check this box to use or ignore the A/D when running the method. If the box is not checked then the A/D will be ignored when the method is applied to the instrument.
Settings	
Voltage Range (V)	Select the voltage range from the drop-down list for the detector. Chromera will use a single value for the entire run. Voltage Range (V) 10.0, 2.0, 0.1 1.0 V
Sampling Rate (pts/s)	Select the data acquisition rate from the drop-down list for the detector. Chromera will use a single value for the entire run. Sampling Rate (pts/s) 50, 20, 10, 5, 2, 1, 0.5, 0.2 pts/s
End Time (min)	Set the length of time (from 0.0 to 999.9 min) for which data is acquired in the run.
Channels (NCI 902 only)	
Channel Name	If you have an NCI 902, it Displays A or B.
Channel A or B In Use	Click this box to use or ignore the channel A or B. If you ignore channel B, the NCI 902 behaves like an NCI 901.
Timed Events	
Description	Enter a description for this relay action.
Relay	Select a Relay 1 through 7 from the drop-down list.
Action	Select On or Off from the drop-down list.
Time (min)	Enter a time for this action to occur.

2. After entering your method parameters, select Channel in the Method tree and enter the Channel parameters for this method.

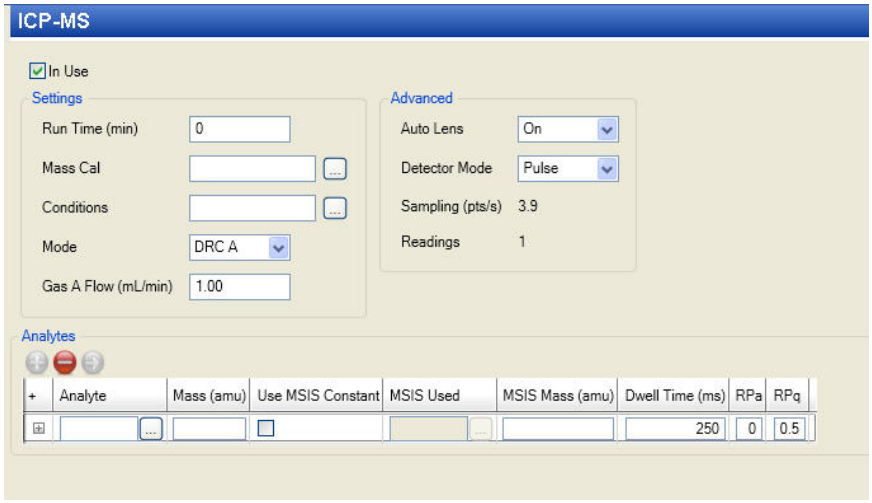
Method: Column Oven Settings (Flexar or Series 200)



Parameter	Description
In Use	Check this box to use or ignore the oven when running the method. If the box is not checked then the oven will be ignored when the method is applied to the instrument.
Temperature (ºC)	The temperature to which the column oven is heated.
Tolerance (+/- ºC)	Set an accepted the + / - range for your over temperature.
Equil Time (min)	Set the length of time for the oven to achieve set temperature.

Method: ICP-MS Settings

The ICP-MS section of the method is used to define the ICP-MS parameters is used for speciation analysis.



Parameter	Description
Run time (min)	The entire chromatogram (data collection) run time. This is set on a per-method basis.

DRC Mode (this only appears when DRC is configured) or Cell Mode	The operational mode of the Dynamic Reaction Cell (DRC). Select from the drop-down DRC–A, DRC–B, Standard. When you select DRC-A or B the following two fields appear. **Gas A or Gas B Flow** - Displays the flow rate for the reaction gas chosen in the DRC mode. **RPq** - Displays the RPq value applied to all the analytes.
Tuning File or Mass Cal	Specifies the name of the ICP-MS tuning file that is used for calibration of the ICP-MS.
Optimization File or Condition	Specifies the name of the ICp-MS DAC file that will be used for the ICP-MS parameters.
Dwell Time (min)	Determines the length of time spent measuring the analyte during a single sweep. It is the total time spent at the mass corresponding to the individual analyte listed.
Auto Lens	The spectrometer permits the ion lens to be synchronously scanned with the actual quadrupole mass scan, permitting dynamic adjustment of the lens voltage for optimum ion throughput. This functionality can be turned on or off.
RPa	Only in DRC: -A or B, it displays the RPa value for all the analytes.
Detector Mode	Specify which detector signal should be used during a determination. Selected per method. **Pulse** - (Default setting) Specifies use of the detector's pulse counting signal option only. This option is applicable if you are performing a measurement on a sample likely to produce a low signal level. **Analog** - Specifies use of the detector's analog signal measurement option. This option is applicable if you are performing a measurement on a sample likely to produce a very high signal level. **Dual** - The detector measuring both the pulse count and analog signals.
Use Short Settling Time	This option applies to Standard mode elements only for DRC series instruments, and to all elements for the ELAN 6000, 6100, and 9000 instruments. By selecting this option, the settling time for the quadrupole becomes much shorter (for example, 200 microseconds). This disables the Extended Dynamic Range feature; only the pulse stage of the detector is used.
Sampling (pts/sec)	Value calculated by the software will be provided. It is calculated by Sample Frequency = 1 / (Dwell Time * # Isotopes).
Readings	This entry defines how many individual readings will be acquired during the course of a single sample injection. Readings = total run time / (Dwell Time * # Isotopes)

Analytes

Parameter	**Description**
Analyte	The element to be analyzed. Values may be typed in or selected. To select an analyte click the button to display a pop–up window containing the Periodic Table, from which you can select the element

	and isotope.
Mass	Populated with the mass of the selected isotope. You may also type a mass in this field.
Use MSIS Constant	Select Yes or No from a drop down list indicating whether a constant–level Mass Spectral Internal Standard (MSIS) will be used. This element is typically spiked in the mobile phase to produce a constant signal during a chromatogram.
MSIS Used	Select the analyte Used. The button displays a pop–up window containing a depiction of the Periodic Table, from which the element (and isotope, where applicable) can be selected.
MSIS Mass	The isotope mass may be typed in or, if the pop–up Periodic Table dialog was used to select the standard, the value will be entered automatically.
View Equation	A check box that displays the Interference Correction Equation entry field.
Equation In Use	Indicates if a correction equation is being used.

Method: Valves

Chromera uses the following LC/UHPLC Rheodyne flow switching and column selector valves:

- **Rheodyne 6-column selector valve (6000psi limit)**

  This column selector valve, optional on Peltier column ovens, allows you to select one of up to 6 columns for each method

- **Rheodyne 750 6-port/2-postion valve (6000psi limit)**

  Depending on how it is configured in Chromera, this valve can be used as a general purpose LC flow switching valve, as well as either a diverter or post-column ISTD injection valve for LC-ICPMS applications.

- **Rheodyne MX II valve module - includes TitanHT valve (18000psi limit)**

  Depending on how it is configured in Chromera, this valve, available as either a 6-port or 10-port valve, can be used for a multiple of LC/UHPLC tasks. To list a few:

  - General purpose LC flow switching valve (6 or 10-port)
  - 2-column selector valve (6-port; must be configured as such)
  - Equilibrating one column while running another (requires 10-port valve and second pump)
  - Pre-concentration/purification (6-port)

Method Parameters for Various Valve Configurations

Rheodyne MX II/TitanHT 2-Position UHP Column Selector and the Rheodyne 6-Column Selector

- Enter following column selector parameters.

Parameter	Description
Column	Select the Column (2-position: Column 1 or 2; 6-position: Column 1 through Column 6) from the drop–down list.

Description	Type a description for the selected column.

Rheodyne MX II/TitanHT 2-Position UHP Switching Valve and the Rheodyne 750 2-Position 6k psi Switching Valve

- Enter following switching valve parameters.

Parameter	Description
Time (min)	The time into the run at which the post–column injection is to take place.
Valve Position (1 or 2)	Select 1 or 2 from the drop–down list.
Description	Type a description for the valve position.

Method: Post-Column Injection

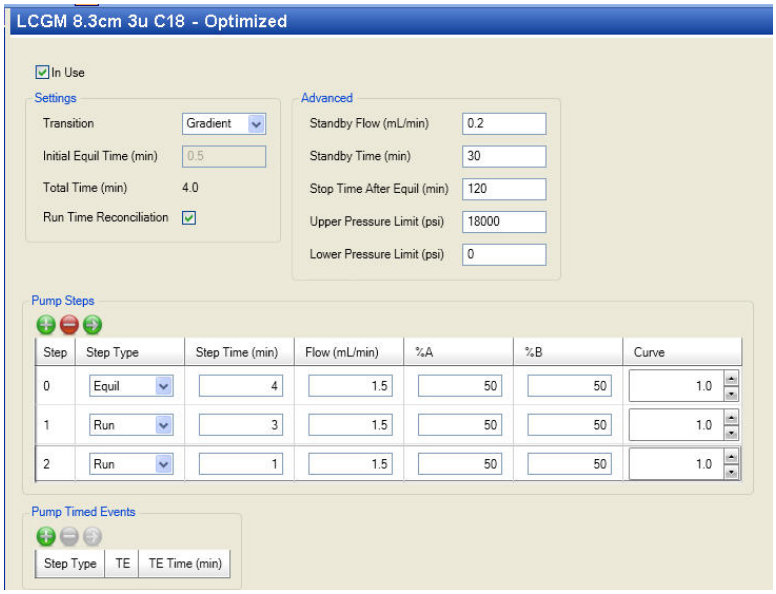
The Post-Column Injection section controls the injection of a standard into the mobile phase after the chromatographic column and before it enters the ICP–MS nebulizer. The injection is carried out using a sampling valve dedicated to this procedure. Use the Post Column Injection (PCI) Valve only with CIS Blanks.

➢ Enter values for the following Post-Column Injection parameters.

Parameter	Description
Start Position	A drop–down list indicating the fill position of the sampling valve.
Duration (sec)	The length of time the valve will remain in the inject position.
Start Offset Time (min)	The time into the run at which the post–column injection is to take place.

Method Switching between Gradient and Isocratic Modes

The rules defined below will apply when a pump method is switched between gradient method and isocratic method.



When a **gradient** method is switched to **isocratic** the action will be:

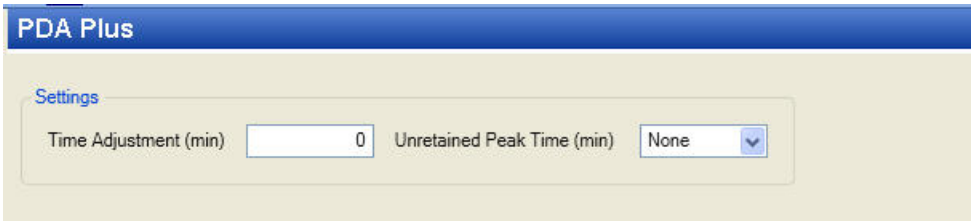
1. All Pump steps will assume the composition settings of step 0.
2. The curve field will be set to zero for all rows, and it will be hidden.
3. All subsequent pump steps (1 and higher) will be disabled, including any new rows inserted.

Conversely, when an isocratic method is switched to a gradient method the actions will be:

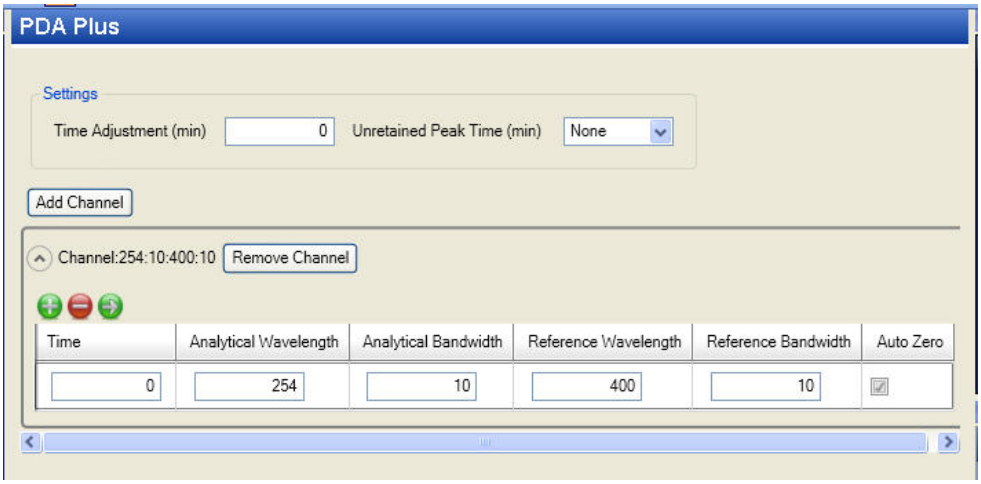
1. Curve values will be displayed (all will be initially set to zero).
2. Any disabled rows present will be enabled.

Channel Parameters

A Channel Name band appears for each data channel defined in the hardware. The **Channel Name** is just an identifier for the channel and cannot be edited in this section. Bands will also appear for subsidiary channels.



An example with a UV-Vis Detector



An example with a PDA Detector

Example Channels section with Channels node selected in method tree.

General Data Channel Parameters Band

Parameter	Description
Time Adjustment (min)	An adjustment to be applied to the data from this channel, to correct for difference in mobile phase transit time between detectors plumbed in series. To correct for difference in mobile phase transit time between detectors enter an adjustment (0.000 to 5.000 min).
Unretained Peak Time (min)	Enter or select from the drop-down list the time taken by an unretained peak to elute from the chromatographic column. (User entry: 0.000 to 9999.999 min. List items: **Use first peak**)

When a PDA is configured as a device you can identify specific PDA channels and enter additional parameters.

PDA Chromatographic Data Channel Parameters

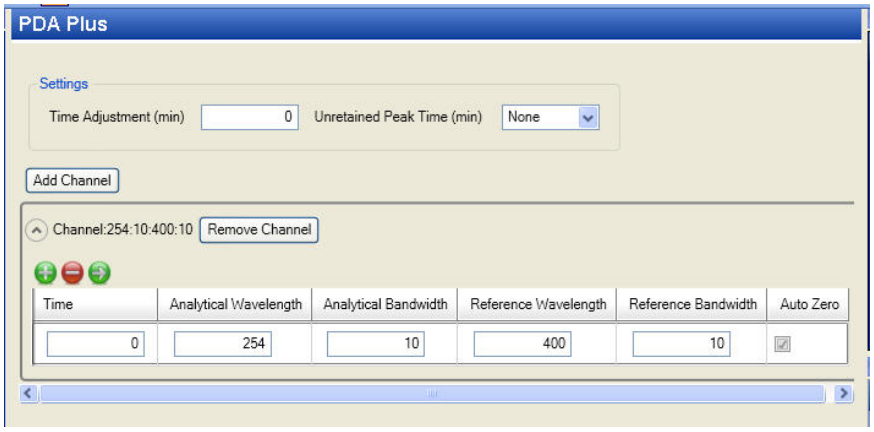
The PDA detector differs from most other detectors in that no chromatogram channels will be available from the detector and only spectral data will be downloaded. All chromatograms will be derived, extracted from the Spectral data.

NOTE: *The lamp is automatically selected based on the wavelength selected for the channel.*

For the PDA detector, the channel parameters consist of:

- **Channel Name** - An identifier for the derived/extracted chromatographic channel. This name will be created from the user-entered or derived channel parameters. This Channel Name will appear for Channels, Peak Detection, Calibration and Reporting sections of the method.
- **Time** - The time of the wavelength change.
- **Analytical Wavelength** - This specifies the central analytical wavelength. This is the wavelength used to monitor the chromatographic channel.
- **Analytical Bandwidth** - This specifies the wavelength range around the central analytical wavelength. The width is in nm of that monitoring channel.
- **Reference Wavelength** - This specifies the central reference wavelength. This wavelength is used to correct the chromatographic channel for lamp instability and drift.
- **Reference Bandwidth** - This specifies the wavelength range around the central reference wavelength. The width is in nm of the correcting information.

You can define the derived Channels to generate on the Channels section of the method.



Derived Channels	Description
Channel Name	An identifier of the new derived data channel. The name is created automatically as the derived channel parameters are being entered.

Analysis and Reference Wavelengths	Description
Time (min)	Enter the time (>0 to Pump Time) into the run at which the settings are to take place. Time is always 0.0 for the mandatory first program step and cannot be edited. Times greater than the pump time will not be accepted. If the pump time is modified after this field

	has been entered and Run Time reconciliation has been checked this field will be automatically updated.
Analytical Wavelength (nm)	Enter the central analytical wavelength (190 - 700). Setting or modifying a wavelength will automatically set the required Lamp Used flag.
Analytical Bandwidth (nm)	Enter the wavelength range around the central analytical wavelength. The range is Calculated on the current Analytical Wavelength.
Reference Wavelength (nm)	Enter the central reference wavelength (190 - 700). The reference channel eliminates background chromatographic or lamp drift. No limitation is placed on the allowed entry, providing it is within the instrumental range. It is therefore possible to set it equal to the Analytical Wavelength!
Reference Bandwidth (nm)	Enter the wavelength range around the central reference wavelength. The range is Calculated on the current Reference Wavelength
Autozero	A check box to specify if an autozero should be performed at the start of this segment. By preference, the checkbox should not be present for the first step. It is never editable for the first step.

NOTE: *End time for a PDA channel is defined on the Instrument: PDA section of the method. Since all PDA channels are extracted from a single spectral array, all channels must have the same end time.*

Other Parameters

Time Adjustment (min)

A time adjustment can be applied to the data from this channel to correct for a difference in mobile phase transit time between detectors plumbed in series (or unequal parallel arrangements). If this value is greater than zero then the raw data acquired from the start of the run up to the time adjustment value is ignored for processing and display purposes.

NOTE: *The data <u>is</u> saved with the raw data set in the database and could be viewed by a subsequent modification to the time adjustment setting. The effect of this is that peaks in the adjusted channel will appear to have retention times less than indicated by their position in the complete raw data stream.*

For the PDA data this time adjustment is applied to the spectral array and not to the individual derived chromatograms to ensure that we maintain synchronicity between the chromatographic and spectral data. This also ensures that all the derived channels will have exactly the same time adjustment.

So that an adjusted data channel does not display less data than an unadjusted channel, the user-entered End Time is extended by the amount of the Time Adjustment. Although this means the actual elapsed time between the start of the run and end of acquisition for the adjusted detector is slightly longer than specified in the method, the net data acquisition time (after adjustment) is what the user has requested. (This automatic correction does assume that the user is not so clever as to specify a slightly longer End Time himself in order to take account of the Time Adjustment setting!)

Clearly, although the Time Adjustment value can be adjusted post run, the amount of data acquired cannot be. Therefore a post-run modification to the Time Adjustment value will lead

to display of chromatograms of different length between channels, even if they had the same nominal End Time.

The Time Adjustment value entered by the user must be automatically updated if the pump flow rate is modified in the method. This is straightforward for isocratic analyses:

$$TA' = TA \times \frac{\text{New flow rate in Step 1}}{\text{Old flow rate in Step 1}}$$

Where:

TA'	Updated Time Adjustment value
TA	Original Time Adjustment value

A gradient program however provides the user with the option of setting a different flow rate for each step. Since such a program would cause the transit time between detectors to vary during the run, which simply cannot be handled by the simple time adjustment functionality described here. Therefore, in the case of gradient programs, the Time Adjustment value is updated based solely on changes made to the flow rate in step 1. If this behavior is not suitable for the application the user has the option of disabling the automatic update via a user preference.

Unretained Peak Time (min)

This is the time taken by an unretained peak to elute from the column. This value is used in Relative Retention and System Suitability calculations.

In addition to being able to enter a specific time value to be used, the user will have the option of selecting 'Use first peak', which will cause the software to use the retention time of the first peak detected in the run for that channel as the unretained peak time.

The Unretained Peak Time must be updated (assuming it is an entered value and not set to 'Use first peak') if the pump flow rate is modified in the method, in the same manner as is done for Time Adjustment. As with Time Adjustment, the update will only be performed when a change is made to flow rate for step 1. (Principally because that is often the only step that will affect the unretained peak time!)

Derived/Extracted Channel Name

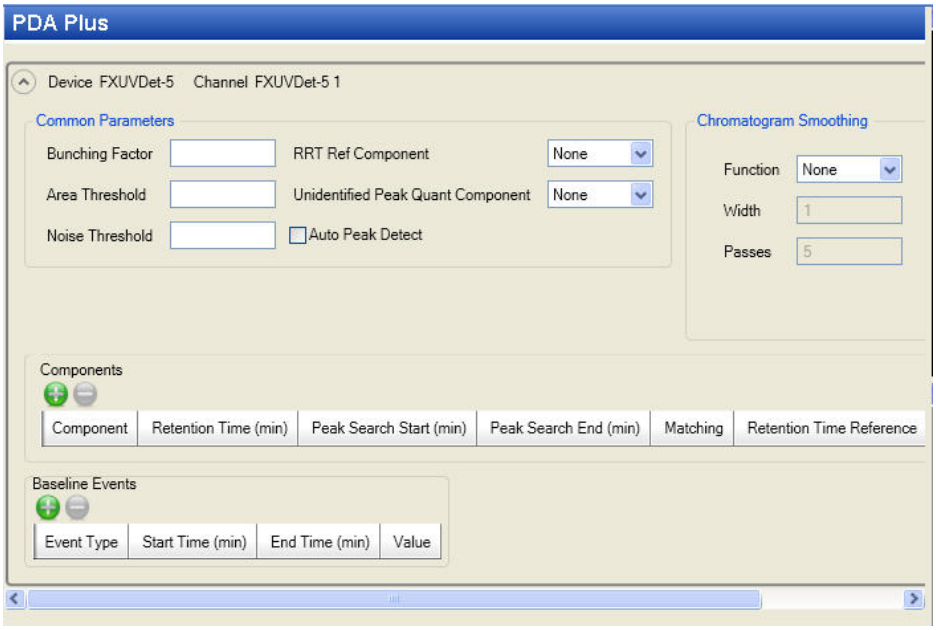
Sub-Channel Name is the new derived data channel name. The name is created automatically as the derived channel parameters are being entered. You may however overwrite the default name.

The name is created automatically from the four parameter fields, Analytical Wavelength, Analytical Bandwidth, Reference Wavelength and Reference Bandwidth in that order, using ":" as separators. If the band defines the start of a wavelength program then the postfix "P" is added to the created name. The name should be created/modified as the four controlling fields are edited. Thus, starting with all four fields empty, you are able to observe the name being built up. You may replace the default name with your own name.

Editing of any of the four fields, Analytical Wavelength, Analytical Bandwidth, Reference Wavelength and Reference Bandwidth, replaces any existing name, user or automatic, with the default automatic name. Adding a Band 3 entry, i.e. creating a wavelength program, will simply add a "P" to the end of the current name plus the number of the parent band with which it is associated. It's important that the sub-channel names should be unique and if the names created are similar to the one already existing, then the name should increment with a next higher number at the end of it.

Peak Detection Parameters

The **Peaks** section of a method shows the general layout; in particular the ordering of the parameters in the different bands. This example is a dual-detector, single channel per detector for an LC Application, where the channel name is used as the identifier (as opposed to using Mass as the identifier for an LC/ICP-MS application).



The whole structure shown under **Device** (in this example a PDA detector), the Component and component Event bands, are repeated for each active channel that requires integration. If you are using an LC system (i.e. no ICP-MS configured) the term **Component** displays and if you have an ICP-MS system the term **Species** displays.

The available integration events are described in the Integration Timed-Events section.

Common Parameters	**Description**
Device	Displays the current detector.
Channel Name	Enter an identifier for the data channel in the Channels, Peak Detection and Calibration sections of the method.
Common Parameters	
Bunching Factor	An edit field containing the bunching factor value used for integration of the analyte chromatogram.
Area Threshold	An edit field containing the area threshold value used for integration of the analyte chromatogram.
Noise Threshold	An edit field containing the noise threshold value used for integration of the analyte chromatogram.

RRT Ref Component	Select from the drop-down list the component to be used for the calculation of Relative Retention. The RRT reference component must be from the current channel. Note that this setting is not related to peak identification in any way; it is just used for the calculation of RRT values. See Relative Retention Time (RRT).
Unidentified Peak Quant Component	Select from the drop-down list the component whose calibration curve is used to calculate all unidentified peaks detected on the channel. The component should be from the current channel.
Auto Peak Detect	Click in the check box to turn Auto Peak Detect On or Off. Auto Peak Detect is an algorithm that will automatically search for and integrate peaks when selected. If this box is checked, all manual peak detection parameters and timed events for this channel will be grayed out and unavailable. Method Preferences are used to set the default selection for this parameter, but can be overwritten here.

Chromatogram Smoothing	**Description**
Function	Select a desired function: Savitzky-Golay, Mean, or Gaussian from the drop-down list. **Savitzky-Golay** takes an average of the intensities weighted by a quadratic curve. This tends to enhance peak and valley shapes, as well as preserving the height of the peaks better than the Moving Mean. However, Savitzky Golay does tend to produce small artifacts on either side of the real peaks. **Mean:** For each data point in the source curve the processed curve is calculated as the average of the data points within the specified window. **Gaussian** sets how well a bell curve mathematically correlates with the displayed peak.
Size	The number you specify is the half-width of the smoothing window in scans.
Passes	Enter the number of times the smoothing algorithm should be applied to the window specified.
Order	Select the Polynomial Order curve fit to apply from the drop-down for Savitzky-Golay. (This selection will only appear when the Savitzky-Golay smoothing is selected.)

Component/Species	Description
Component (Species for ICP-MS)	Enter a component (species) name (from 1 to 50 characters), select an existing name from the list, or select a name from the component dictionary via the drop-down list. Editing this field in this section reflects to changes made to the Calibration section also.
Retention Time (min)	Displays the mass of the analyte from the method.
Peak Search Start (min)	Enter the time in minutes or seconds or select a point or range in the graph.
Peak Search End (min)	Enter the time in minutes or seconds or select a point or range in the graph.
Matching	Select from the drop-down list what process will be used for matching a component when more than one peak falls within the peak search window. Use Tallest or use Closest.

Component Type	Description
Type	Select from the drop-down list the type of component being defined. This depends on the application. Multiple type properties can be defined but a component cannot specify incompatible options (see note below). **Uses a CIS** - Chromatography Internal Standard ratio where every peak in a chromatogram is compared to this peak. **Uses a Retention Time Reference** - Accounts for peak shifts in a chromatogram.
Reference Peak	Select from a list populated from the retention time reference peaks included in the method (or defined here).

NOTE: *A component that specifies* ***Uses a Retention Time Reference*** *cannot also specify* ***Is a Retention Time Reference****. Similarly a component that specifies* ***Uses an Internal Standard*** *cannot also specify* ***Is an Internal Standard****.*

Baseline Events	Description
Event Type	Commands that affect the peak detection and/ or integration at specific times in a chromatogram. Select the event type from the drop-down list.
Start Time	The time at which the event is to occur (for an instantaneous event) or start (for a event of specific duration).

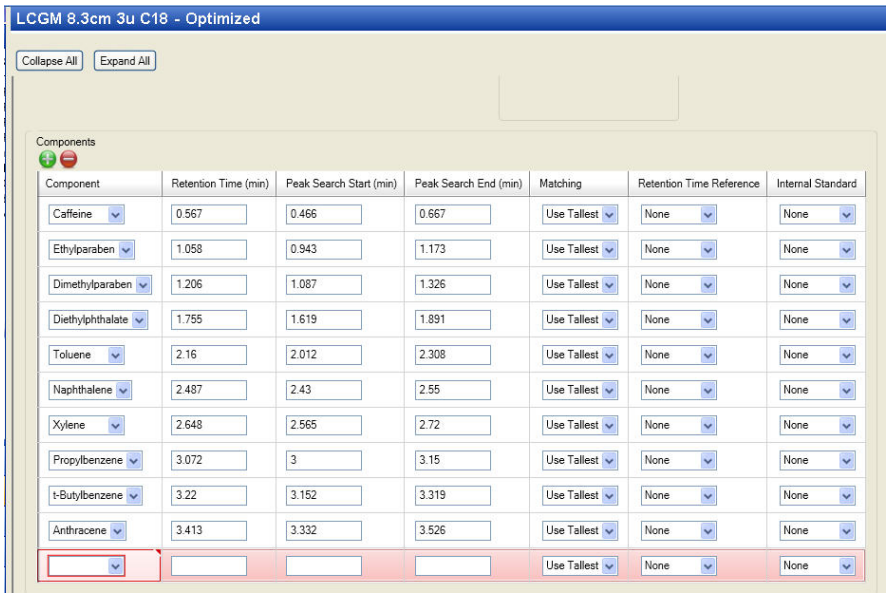
End Time	The time at which the event will end (for a event of specific duration).
Value	The value associated with the baseline event (where applicable).

- For values that have a Start Time and End Time, you can draw a box in the chromatogram to define the region (Start/Stop times) where the events will be implemented.

- For values that have just a Start Time or an End Time, you can click on the box, then click on the desired point in the chromatogram to set that time value.

Entry of a New Component

You can enter new components in this section. Click the plus button  to add a blank line at the end of the component list can be used to define a new component (or species) rather than just select one defined elsewhere. This is the primary point of entry for Species in an ICP-MS system.



Component	Retention Time (min)	Peak Search Start (min)	Peak Search End (min)	Matching	Retention Time Reference	Internal Standard
Caffeine	0.567	0.466	0.667	Use Tallest	None	None
Ethylparaben	1.058	0.943	1.173	Use Tallest	None	None
Dimethylparaben	1.206	1.087	1.326	Use Tallest	None	None
Diethylphthalate	1.755	1.619	1.891	Use Tallest	None	None
Toluene	2.16	2.012	2.308	Use Tallest	None	None
Naphthalene	2.487	2.43	2.55	Use Tallest	None	None
Xylene	2.648	2.565	2.72	Use Tallest	None	None
Propylbenzene	3.072	3	3.15	Use Tallest	None	None
t-Butylbenzene	3.22	3.152	3.319	Use Tallest	None	None
Anthracene	3.413	3.332	3.526	Use Tallest	None	None
				Use Tallest	None	None

Entry of a **Component** name acts to create a new component record, with appropriate default values where applicable.

NOTE: *A component name can also be selected from the component dictionary via a drop-down list.*

Scope of Component List

The method effectively contains only one component list (regardless of the underlying database structure). Thus any change to the component list (such as editing of a component name or addition of a new component) in the Peaks section will automatically be reflected in the component list in the calibration section, and vice versa. While the component information on the other tab may be incomplete (for example, a component newly added in the Peaks section will not show any standard levels or calibration data when first viewed in the Calibration section) the component names comprising the list will always match in the two sections.

How to Copy Components Between Channels

This dialog is displayed when the Between Channels… command is chosen from the **Copy Components** submenu of the **Actions** menu within the Method Editor.

Set the following in this dialog:

Control	Description
Copy from channel	Use the drop-down list to set the source of the components to be copied. All data channels n the method that have components defined.
Copy to channel	Use the checkbox list to set the data channel(s) the components are copied to. The list displays all data channels in the method except the **Copy from** channel. This list contains nothing until the **Copy from** channel is selected. The list is then populated will all other data channels from the method. You can copy the component to more than one channel by selecting the required channels within this control.
Copy these components	Use the drop-down list (select **All**, **All but internal standards, Internal standards only)** to set which components are copied.
Include integration events	A check box (**Yes or No**) to set the integration events from the source channel should also be copied to the destination channel.
Include calibration standards	A check box (**Yes or No**) to copy the calibration standard amounts with the other component information.

Clicking the **OK** button copies the components from the source channel to the destination channel. In general, the copied components are merged with the existing components and does not replace them. However, if a component with exactly the same retention time exists in the destination channel then that component will not be copied.

Calibration Parameters

The Calibration View is used for viewing and interpreting calibration curves generated from the measurement of your standard solutions. The Calibration View allows you to evaluate the quality of the calibration by viewing both the graphic plot of the calibration points and by reviewing statistical information on the curve fit. You can also evaluate the effect of eliminating individual calibration points or replicates and changing the origin treatment.

In the calibration graphs the results from each replicate injection is plotted and not the average. This also means that the ability to exclude a calibration point will mean exclusion of a single replicate and not an entire level. You are able to select whether each replicate is included in the calibration curve using the **In Use** checkbox displayed for each replicate.

The Calibration section contains two sections **Setup Standards** and **Channels View** (which contains the Summary, and Details tabs).

The **Set Up Standards** screen is designed to provide an easy approach to creating standards and entering standard amounts for all components.

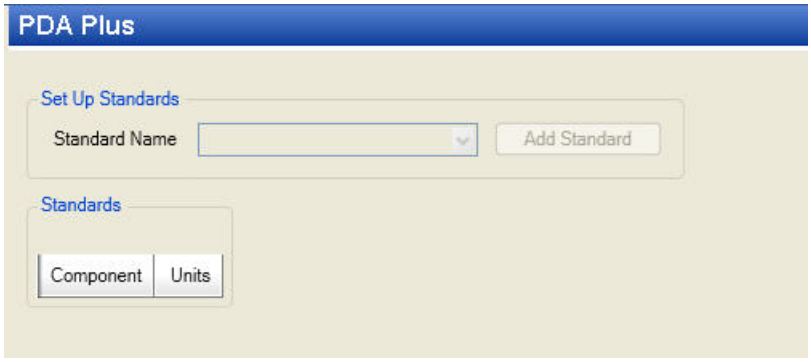
The **Summary** tab shows thumbnails displays of the curves for all components/species; for all detector devices or a single device, depending on the method tree selection.

The **Detail** tab shows details of the calibration for a selected component. Double-clicking on a curve on the Summary tab will cause the Detail tab to be displayed with that component selected.

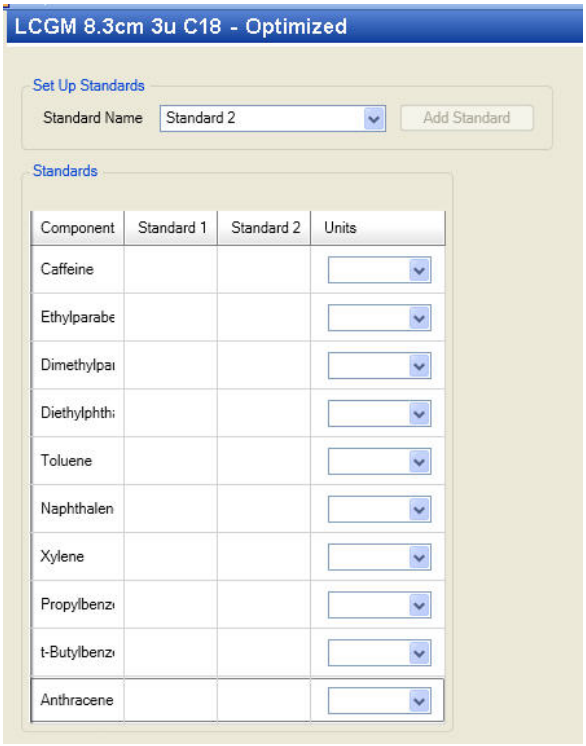
Set Up Standards

The Set Up Standards screen is designed to provide an easy approach to creating standards and entering standard amounts for all components.

Setup Standards has a top level entry for the **Standard Name** followed by a table display containing a row for each **Component** defined in the method. The displayed component list will always be the same as the component list in the Peaks section and the Calibration-Detail section. Unlike the **Details** view, the new Standards view does not have sub-bands for the calibration standard levels but instead has a column for each level.



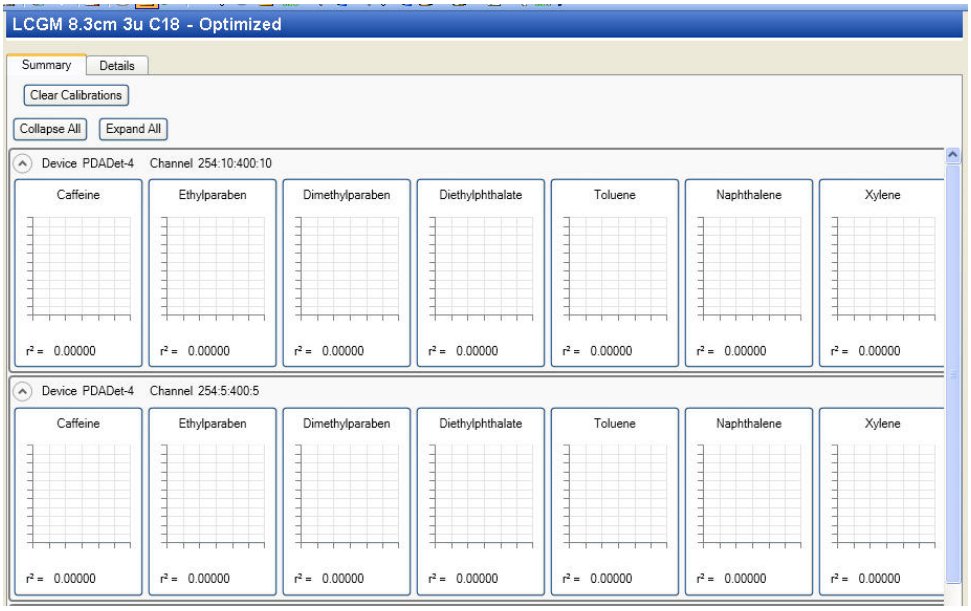
Since the entry of values in these columns is the primary reason for having this view, these are the first columns shown following the **Component** name column. Additional columns for new **Standards** can be added. A column for **Units** is to accommodate the Units Dictionary function.



Any edits made on this tab will be immediately reflected in the other calibration tabs. You can reorder the columns by using drag-and-drop.

Summary Tab

The **Summary** tab shows thumbnails displays of the curves for all components/species; for all detector devices or a single device, depending on the method tree selection.

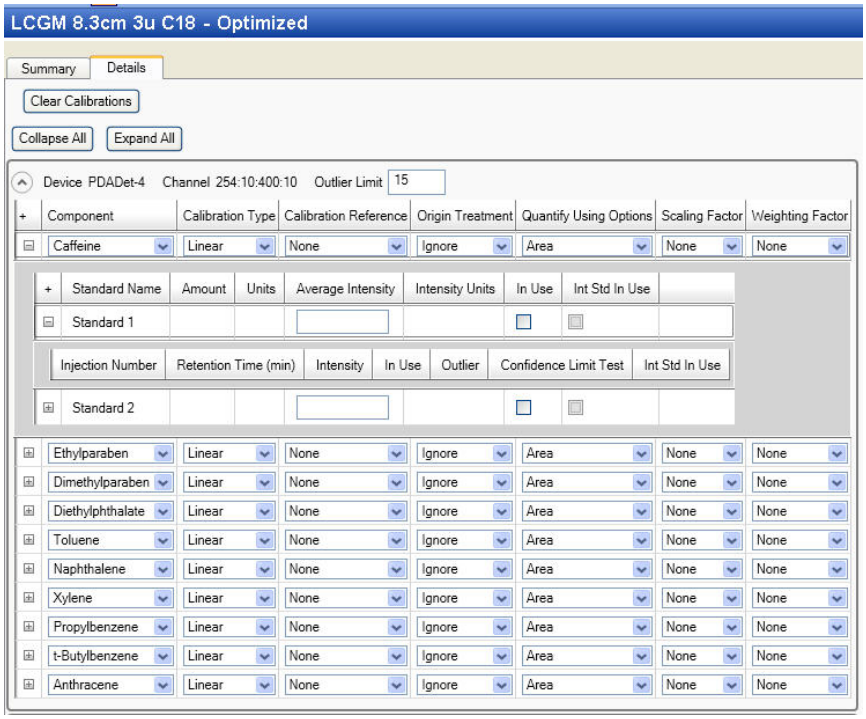


NOTE: *This example is for a pure LC instrument; for an ICP-MS instrument* ***Analyte Name*** *are used in place of* ***Channel Name****.*

Detail Tab

Calibration Parameters provides an overview of calibration.

The **Detail** tab shows details of the calibration for a selected component. Double-clicking on a curve in the Summary tab displays the Detail tab with that component selected.



The above example of an LC Application shows the channel name used as the identifier (as opposed to the Analyte name for the LC/ICP-MS application). The whole structure shown above is repeated for each active channel that includes calibration. You can reposition columns in the display using drag-and-drop.

Identification	Description
Device	Displays the name of the device
Channel Name	Enter an identifier for the data channel in the Channels, Peak Detection, and Calibration sections of the method.
Outlier Limit (%)	Set the maximum permitted deviation between replicate calibration results. The range is 0.0 to 10000.00

Components List - One row per component

Component	Description
Component (Species for ICP-MS)	Enter a component (species) name (from 1 to 50 characters), select an existing name from the list, or select a name from the component dictionary via the drop-down list.
Calibration Type	Select from the drop-down list the type of calibration curve used (Average Calibration Factor and Linear).

Calibration Reference	Select from the drop-down list the component (or None) to reference.
Origin Treatment	Select from the drop-down list whether or not the origin is considered when fitting the calibration curve (Ignore, Include, or Force).
Quantify Using Options	Select from the drop-down list the response mode (Area or Height) used for quantitation for the component.
Scaling Factor	Select from the drop-down list (None, 1/X, 1/(X*X), log(X), 1/log(X)) to set the type of scaling to be applied to the plotted amounts.
Weighting Factor	Select from the drop-down list (None, 1/X, 1/Y, 1/(X*X), 1/(Y*Y)) to set the weighting to be applied to the calibration points.
Y-Intercept	Displays the y-intercept value from the curve fit.
Slope (X)	Displays the slope (Linear) or x coefficient from the curve fit.
r-squared	Displays the r^2 value, reflecting the *goodness of fit* of the calibration equation to the standard sample results.

NOTE: *If the calibration type is **Average Response Factor** then the ONLY fields in this band are the component name, the calibration type, and the average response factor value.*

Standards List - One row per calibration level

Standards	**Description**
Standard Name	Enter the name of the standard level.
Amount	Set the amount (0.000000 to 999,999.999999) of the current component in the current standard.
Units	An display of the units of the previous field. The units are set on the Setup Standards tab.
Avg Area	Displays the average area from the individual replicate injections.
Avg Height	Displays the average height from the individual replicate injections.
In Use	A check box to indicate whether the standard level is included in the calibration curve.
Avg Area/Ht Ratio	Displays the average area/height ratio from the individual replicate injections.

Replicates Data - One row per injection of the standard

Replicates	Description
Injection Number	A counter field indicating the replicate number.
Area	Displays the peak area from the standard injection.
Height	Displays the peak area from the standard injection.
Area/Ht Ratio	Displays the peak area/height ratio average area/height ratio from the standard injection.
In Use	A check box to indicate whether the replicate data is included in the calibration curve.
Int Std In Use	A check box to indicate whether the internal standard is included in the calibration curve.

How to Perform a Calibration

The following steps provide an overview on how to perform a calibration.

1. Make calibration standards, usually consisting of a blank and 2-5 standards covering the expected concentration range of species in the samples.

2. Place the calibration standards in the LC autosampler.

3. Create a **Method** and define the species and calibration standards in the Method.

4. Create a **Sequence** and define the calibration standards in each line of the Sequence in the Sample Type column.
   a. For a calibration blank, the appropriate **Sample Type** is **Blank (pt by pt).**
   b. For a calibration standard, the appropriate **Sample Type** in **Standard.**

5. In the **Sample Name** column of the Sequence, type a unique sample name for the calibration standards.

6. In the **Vial** column of the Sequence, define the autosampler locations of the calibration standards.

7. In the **Method** column of the Sequence, select the appropriate Method (where the calibration standard amounts were defined).

8. In the **Standard** column of the Sequence, select which calibration standard in the method corresponds to the calibration standard in that row.

   For example, if in the Method, 10 µg/L was defined as Standard 2, select "Standard 2" from the drop-down menu in the Standard column of the line defining the 10 µg/L standard in the Sequence. (Note: the entries in the Standard column are linked to the method specified in the Method column).

9. In the **Injections** column of the Sequence, define how many times each sample should be run (i.e., the number of times it is injected).

NOTE: *If more than 1 injection is performed, the average of the injections will be reported.*

10. After the last Sample Type calibration standard in the Sequence, define a Calibration Review decision point (clicking on the  next to the last Standard and selecting **Calibration Review** under **Decision Point Type**.)

11. Save the Sequence (from the **File** menu > **Save Sequence**).

12. Start the Sequence (from the **Control Panel** > **Start Sequence**).

13. When the **Species Calibration** review window appears, review and make any necessary changes. When the calibration appears as desired, click the **Close** button to continue the sequence.

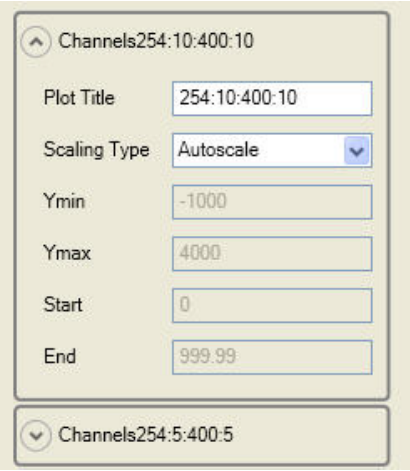
Reporting Parameters

Plot Parameters

These settings define the chromatogram image file(s) that are to be generated from the channel data; for use in reports.

Plot Main Band - multiple records can be created

The plot parameters define the number and format of plots to be generated for each run on the identified channel. In addition to all plots defined on this tab, overlay plots consisting of all the plots defined for all channels are generated.



Parameter	Description
Plot Title	Enter a set of plot parameters (using up to 50 characters).
Scaling Type	Select from the drop-down list the scaling option (Autoscale, Auto Baseline, or Manual) to be applied to the plot.
Y Units	The units in which the Y-axis is to be plotted. The units are detector dependent.
Y min	Set the Y-axis minimum (in the units specified). This value must be less than Y max. **NOTE:** The minimum and maximum values for the plot are determined by the specific device. The allowed range is defined in the Device Descriptor (aka Instrument Descriptor) for each applicable supported device and will not be duplicated here.
Y max	Set the Y-axis maximum (in the units specified). This value must be greater than Y min. **NOTE:** The minimum and maximum values for the plot are determined by the specific device. The allowed range is defined in the Device Descriptor (aka Instrument Descriptor) for each applicable supported device and will not be duplicated here.
Start Time (min)	An edit field indicating the setting of the X-axis minimum.

End Time (min)	An edit field indicating the setting of the X-axis maximum.
Sample ID	A check box indicating whether the Sample ID will appear on the printed plot.
Baselines	A check box indicating whether baselines are drawn in on the printed plot (using the user's preferences for color and line type).

Plot Annotations Band

Band 2	**Description**
RTs	A check box indicating whether peak retention times will appear on the printed plot (using the user's preferences for text properties).
Peak Names	A check box indicating whether peak names will appear on the printed plot (using the user's preferences for text properties).
Timed Events	A check box indicating whether time event annotations (integration and instrument events) will appear on the printed plot (using the user's preferences for text properties).
Wavelengths	A check box indicating whether wavelength changes will appear on the printed plot (using the user's preferences for instrument event text properties).
X Axis Label	A check box indicating whether the X-axis label Time (min) will appear on the printed plot.
Y Axis Label	A check box indicating whether the Y-axis label (Response (mV) or Response (mAU), according to Y units setting) will appear on the printed plot.

Other Device Channels Bands

These bands are also associated with scaling of plot data - the subsidiary data channels that can be overlaid on chromatograms. The only applicable channels are pump pressure and oven temperature. Below the **Other Device Channels** band there are bands for each applicable device.

Pump Band	**Description**
Device	Displays the user name for the pump.
Pressure Units	Displays the pressure units currently in effect for the pump.
Pressure Plot Min	Set the Y axis minimum to be used for the pressure plot. 0 to 6100 psi; 0 to 420 bar; 0 to 42 MPa
Pressure Plot Max	Set the Y axis maximum to be used for the pressure plot. 0 to 6100 psi; 0 to 420 bar; 0 to 42 MPa

Scaling Type

The available options are:

- Automatically scale the chromatogram to the height of the largest peak.
- Scale both the X and Y axes to user-supplied values.
- Scale the Y axis to a user-supplied full scale setting above an automatically determined baseline offset.
- Allow the y axis to be scaled in any units applicable to the detector.

Additional Scaling Parameters

Y min	Setting of the Y axis minimum
Y max	Setting of the Y axis maximum
Start Time (min)	Setting of the X axis minimum
End Time (min)	Setting of the X axis maximum

Annotations

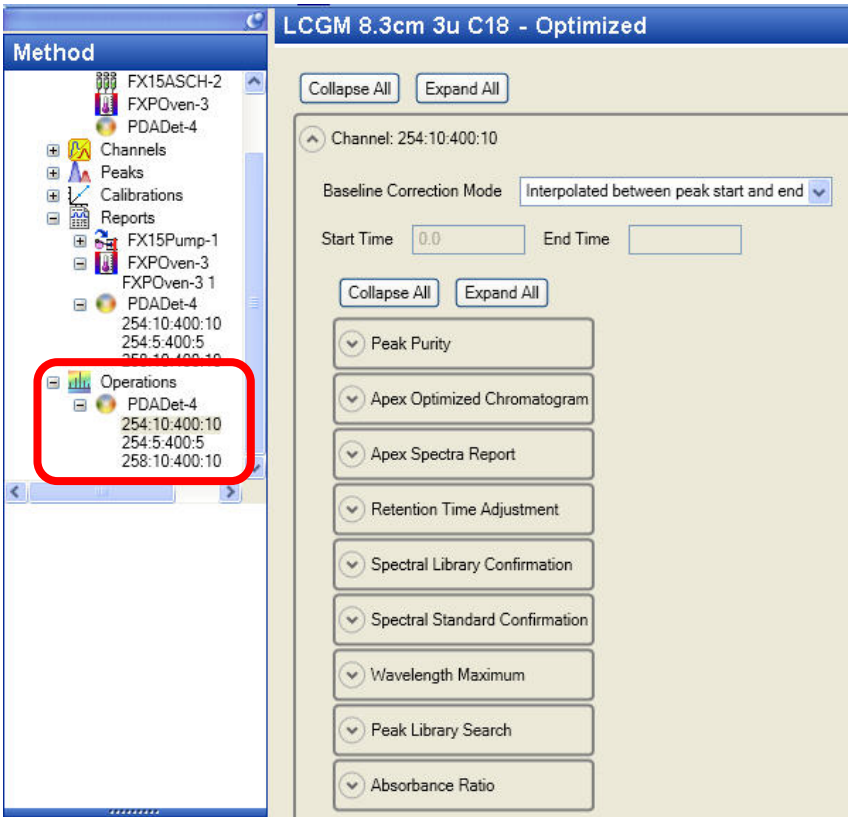
Any combination of the following may be specified to appear on the plot:

Sample identification	The sample name from the sequence will appear at the top of the plot.
Baselines	The baselines (including drop lines and skim lines) are drawn in.
Retention times	Peak retention times are shown.
Component names	Names of identified peaks are displayed.
Timed events	Integration and instrument timed events are marked.
Wavelength settings	Changes of detector wavelength are marked.
X-axis label	The label **Time (min)** will appear on the X axis.
Y-axis label	The label for the units defined for the channel will appear on the Y axis.

Operation Parameters

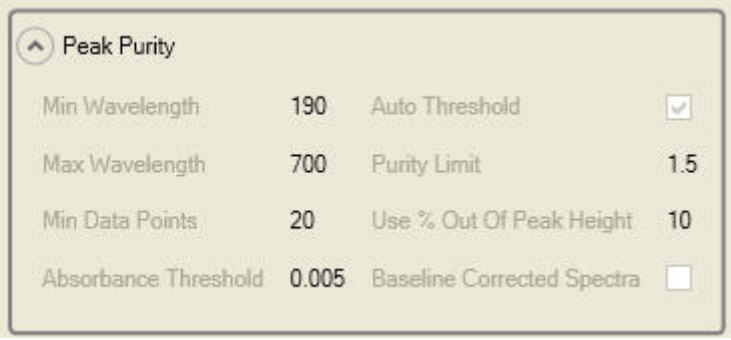
The PDA Operations functions and parameters in the Chromera method are all copied from the similarly named Operations views within the Spectral Processing window (SPW). The Spectral Processing method is a container to hold these operations parameters developed and applied within the spectral processing window. The Operations parameters are viewable from within the Chromera method but they are not editable. They will only be editable from within the Spectral Processing window.

Spectral Operations is channel based and each channel may have a different set of Spectral Operations controlling parameters. In reality, all Spectral Operations are based on the use of spectra which, of course, are injection based not channel based. Therefore results obtain from one channel will be identical to all other channels derived from the same spectral data.



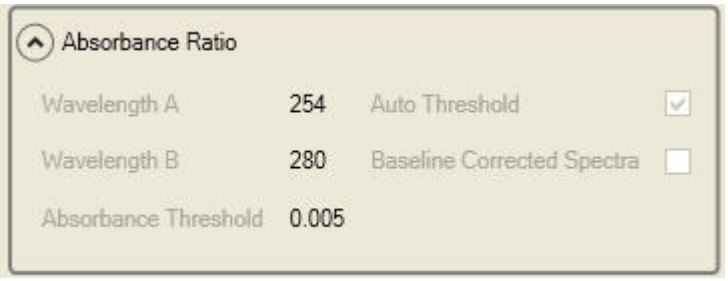
They are, Peak Purity, Absorbance Ratio, Wavelength Maximum, Spectral Standard Confirmation, Spectral Library Confirmation, Peak Library Search, Retention Time Adjustment, Apex Optimized Chromatogram, and Printing.

Method Operations - Peak Purity



Parameter	Description.
Minimum Wavelength	Displays the minimum wavelength to be used in the purity calculation.
Maximum Wavelength	Displays the maximum wavelength to be used in the purity calculation.
Minimum Data Points	Displays the minimum number of data points that must be used for the result to be valid.
Baseline Corrected Spectra	A check box to use baseline corrected spectra for the calculations.
Purity limit	Displays the maximum limit for an acceptable match.
Use at % of Peak Height	Displays where on the peak the results are calculated.
Auto Threshold	A checkbox that specifies if the Absorbance Threshold used in the calculation is calculated automatically or manually.
Absorbance Threshold	Displays the minimum absorbance value to be used in the calculation when set manually.

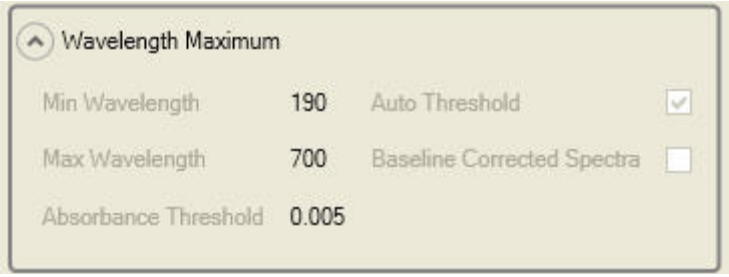
Operations - Absorbance Ratio



Parameter	Description.
Wavelength A	Displays the first wavelength to be used.
Wavelength B	Displays the second wavelength to be used.

Baseline Corrected Spectra	A checkbox that uses the baseline corrected spectrum for the calculation.
Auto Threshold	A checkbox that specifies whether to set the minimum absorbance value used in the calculation automatically.
Absorbance Threshold	Displays the minimum absorbance value to be used in the calculation.

Method Operations - Wavelength Maximum



Parameter	**Description.**
Minimum Wavelength	Specifies the minimum wavelength to be used.
Maximum Wavelength	Specifies the maximum wavelength to be used.
Baseline Correct Spectra	A checkbox to use baseline corrected spectra for the calculations.
Auto threshold	A checkbox that specifies if the Absorbance Threshold used in the calculation is calculated automatically or manually.
Absorbance Threshold	Displays the minimum absorbance value to be used in the calculation when set manually.

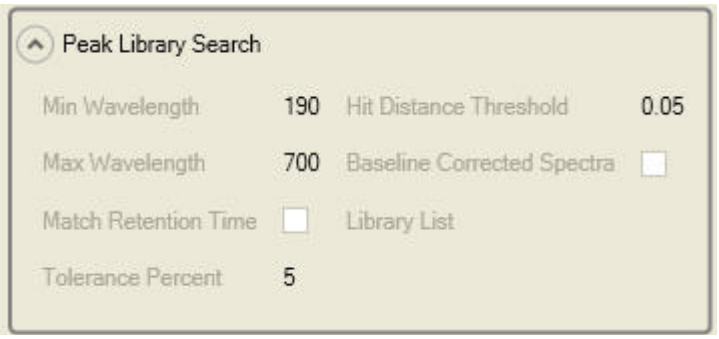
Operations - Spectral Standard Confirmation

Parameter	Description.
Minimum Wavelength	Displays the minimum wavelength to be used in the Spectral Standard Confirmation.
Maximum Wavelength	Displays the maximum wavelength to be used in the Spectral Standard Confirmation.
Baseline Correct Spectra	A checkbox to use baseline corrected spectra for the calculations.
Minimum Data Points	Displays the minimum number of data points that must be used for the result to be valid.
Pass Threshold	Displays the maximum value set for a match.
Sample Auto Threshold	A checkbox that determines the Absorbance Threshold used in the calculation for the sample should be calculated automatically. If checked the minimum absorbance value used in the calculation is to be determined automatically.
Sample Absorbance Threshold	Displays the minimum absorbance value for the sample to be used in the calculation.
Standard Auto Threshold	A checkbox that determines the Absorbance Threshold used in the calculation for the standard should be calculated automatically. If check the minimum absorbance value used in the calculation is to be determined automatically.
Standard Absorbance Threshold	Displays the minimum absorbance value for the standard to be used in the calculation.
Standard Chromatogram	Displays the name of the method selected for use in acquiring and processing data in the sequence. The method is selected by clicking on the browse button following the field.

Method Operations - Spectral Library Confirmation

Parameter	**Description.**
Minimum Wavelength	Specifies the minimum wavelength to be used in the Spectral Library Confirmation.
Maximum Wavelength	Specifies the maximum wavelength to be used in the Spectral Library Confirmation.
Hit Distance Threshold	Displays the maximum value for which the result is considered a pass.
Baseline Correct Spectra	A checkbox to use baseline corrected spectra for the calculations.
Library List	Click the drop-down button to display a card view that contains a check list of the available libraries to be matched against.

Method Operations - Peak Library Search



Parameter	**Description.**
Minimum Wavelength	Specifies the minimum wavelength to be used in the Peak Library Search.
Maximum Wavelength	Specifies the maximum wavelength to be used in the Peak Library Search.
Match Retention Time	A checkbox that sets the retention time of the spectrum as one of the search criteria Will use the retention time of the spectrum as one of the search criteria
Tolerance %	Displays a window around the retention time of the spectrum using the percentage value as a plus and minus for the retention time match

Hit Distance Threshold	Displays the maximum value for which the result is considered a pass.
Baseline correct spectra	A checkbox to use baseline corrected spectra for the calculations.
Library List	Click the drop-down button to display a card view that contains a check list of the available libraries to be matched against.

Method Operations - Retention Time Adjustment

Parameter	**Description.**
Minimum Wavelength	Specifies the minimum wavelength to be used in the Retention Time Adjustment.
Maximum Wavelength	Specifies the maximum wavelength to be used in the Retention Time Adjustment.
Hit Distance Threshold	Displays the maximum value for which the result is considered a pass.
Baseline Correct Spectra	A checkbox to use baseline corrected spectra for the calculations.
Library List	The drop-down button displays a card view that contains a check list of the available libraries to be matched against.

Method Operations - Apex Optimized Chromatogram

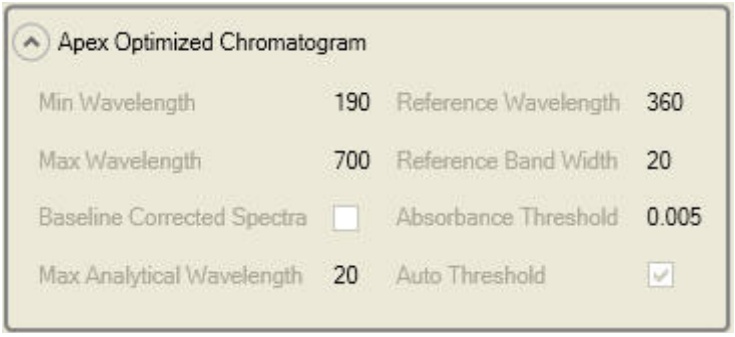
This operation can be used to create an optimized version of the current chromatogram or to build an optimized method for future chromatograms. The Apex Optimized Chromatogram operation yields parameters used in creating additional derived chromatograms using a wavelength program. The results of this operation can be used to add a wavelength program to the derived channels section of the Chromera PDA method for the current chromatogram as well as creating a wavelength program in a PDA method for use with future chromatograms. Multiple wavelength programs can exist in the PDA section of the method, each program being optimized for specific compounds being analyzed.

In addition to the ability to create an optimized wavelength program for future use, the Apex Optimized Chromatogram operation within Spectral Processing Window is an automated process, containing a set of controlling parameters that allow an Apex Optimized Chromatogram to be created automatically during the running of a sequence. (In this respect it is similar to the Base Ion Chromatogram function used in an LC Mass Spec.) As with the other Spectral Processing Window operations, these parameters will need to be stored in the hidden Spectral Processing Window section of the Chromera method for recall during automated operation.

The wavelength program created by the Apex Optimized Chromatogram operation and passed to the Chromera PDA method consists of multiple bands each band contain the following parameters.

Time - Analytical Wavelength - Analytical Bandwidth - Reference Wavelength - Reference Bandwidth

This set of parameters is repeated for each wavelength changed required in the method.



Parameter	**Description.**
Minimum Wavelength	Displays the minimum wavelength to be used in the Peak Library Search.
Maximum Wavelength	Displays the maximum wavelength to be used in the Peak Library Search.
Maximum Analytical Bandwidth	Displays the maximum analytical bandwidth to be used.
Reference Wavelength	Displays the reference wavelength to be used.
Reference Bandwidth	Displays the reference bandwidth to be used.
Absorbance Threshold	Displays the minimum absorbance value to be used in the calculation.
Baseline Corrected Spectra	A checkbox to use baseline corrected spectra for the calculations.
Auto Threshold	A checkbox that specifies if the Absorbance Threshold used in the calculation is calculated automatically or manually.

Editing a Method

Method editing consists of modifying existing method parameters. This includes a modification to the method name, since in Chromera the name is a method parameter like any other (the method is uniquely identified by a database index that is not visible users). After making changes and saving the method, the previous version of the method is replaced by the modified version. Only a single version of a method is saved in the database. Also, a method cannot be saved with the same name as another method in the Group.

To create a copy of an existing method but with a different name, leaving the original method unchanged, use the **Save As** command.

Method Access

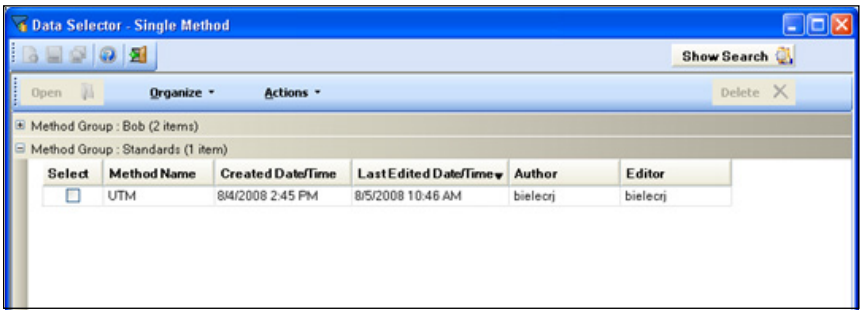
A method in use (meaning it is on any row of a running sequence) in an online instance of Chromera cannot be edited and saved as the same method in any other instance of Chromera. Use of the Save As command to create a copy of the method will however be permitted.

How to Edit a Method

To edit a method:

1. Select the **Method** window and choose the **Open Method** command from the **File** menu. The Data Selector - Single Method dialog appears.

2. Expand  the Method Group to see a list of the available methods.



3. Click in the box in the **Select** column to select the method; then click **Open**.

4. In the top level row of the method screen, modify the **Method Name**, a **Description** of the method and the name of the **Group** (virtual folder) where the method will be stored.

5. Click on **Instruments** in the Navigation pane.

6. Click the **Expand All** button to open all devices to edit the parameters.

7. For each device (instrument) enter or edit parameter settings as required.

In a device (instrument) **Advanced** section, you can set the parameter values here so that they override the Preference values only for this method.

8. Save the method by choosing the **Save Method/Save Method As...** command from the **File** menu.

NOTE: *After making a change to the method you must save the method to retain the changes made.*

Online vs. Offline

Within an **Online** instance of Chromera only methods configured for the associated instrument can be created. If you choose to open a method created for a different instrument the software offers to update it to the configuration of the current instrument. Updating is performed on a device type basis; that is, if the configured instrument has a device type that is not in the method, then a set of default parameters are provided for that device. Devices referenced in the method that are not present in the instrument configuration are eliminated from the method. No mapping of parameters from one device type to another is attempted. When a method is updated in this way, the **Method/Save** command will not be available (only **Save As** will), so that the original method is not destroyed.

If you choose not to update the method to the configuration of the current instrument you can still view the method but it is recommended that you do not edit it in this situation.

A different situation applies in **Offline** (Data Only) mode. In this case it is possible to generate a method for the associated instrument, any other configured instrument or for an arbitrary configuration defined by selecting items from the pool of supported devices. It is also possible to edit any method stored in the database, regardless of its instrument configuration, (provided it is not in use). Of course in Data Only mode the Run Time environment does not exist and hence no conflicts can arise.

Extracting a Method from Results

A copy of the full set of method parameters used to acquire and process the data are stored with each and every result data set (that is, the data collected and calculated from a single injection). This set of method parameters will often simply be a copy of the method specified on the sequence row defining the analysis but it may also be an edited version of a method resulting from graphic reprocessing. That is, the results may contain a method that never existed as an independent entity in the database. For this reason (and also because the result data set may have been imported so the method never existed on the system at all) it

is possible to extract a method from a result data set and save it as a new method in its own right.

How to Copy Components from TotalChrom

This dialog displays when you select the **From TotalChrom Method...** command from the **Copy Components** submenu of the **Actions** menu within the Method Editor.

Control	Description
Copy from TotalChrom method	Displays the source of the components to be copied. The field is populated by selecting a file from a standard File Open dialog displayed when the browse button is clicked.
	A browse button that displays files with .mth extension in a standard Windows File Open dialog, enabling you to select a TotalChrom method file.
Copy to channel	A checkbox list to set the data channel(s) the components will be copied to. The list is populated with all data channels from the method. You can copy the components to more than one channel by selecting the required channels within this control.
Include integration events	Check this box to copy the integration events from the TotalChrom method to the destination channel.
Include calibration standards	Check this box to copy the calibration standard amounts with the other component information.
<component list>	A display listing the components found in the TotalChrom method, together with their retention times.

NOTE: *The **OK** button is disabled until you select a TotalChrom method and at least one destination channel (as required).*

When the **OK** button is clicked the components from the TotalChrom method are copied to the destination channel(s). In general, the copied components are merged with the existing components and will not replace them. However, if a component with exactly the same retention time exists in the destination channel then that component will not be copied. All components will be copied; any selection made in the component list has no impact on the component copied.

How to Copy Components Between Channels

This dialog is displayed when the Between Channels... command is chosen from the **Copy Components** submenu of the **Actions** menu within the Method Editor.

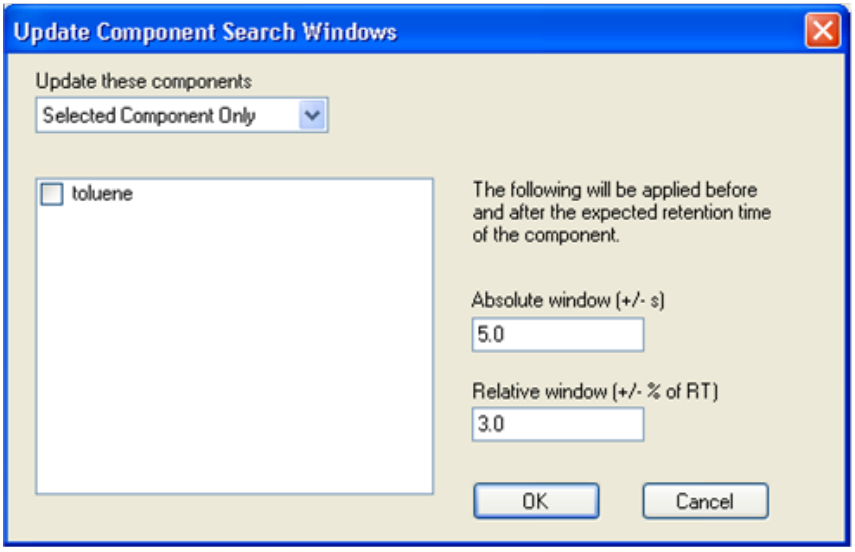
Set the following in this dialog:

Control	Description
Copy from channel	Use the drop-down list to set the source of the components to be copied. All data channels n the method that have components defined.
Copy to channel	Use the checkbox list to set the data channel(s) the components are copied to. The list displays all data channels in the method except the **Copy from** channel. This list contains nothing until the **Copy from** channel is selected. The list is then populated will all other data channels from the method. You can copy the component to more than one channel by selecting the required channels within this control.
Copy these components	Use the drop-down list (select **All**, **All but internal standards, Internal standards only)** to set which components are copied.
Include integration events	A check box (**Yes or No**) to set the integration events from the source channel should also be copied to the destination channel.
Include calibration standards	A check box (**Yes or No**) to copy the calibration standard amounts with the other component information.

Clicking the **OK** button copies the components from the source channel to the destination channel. In general, the copied components are merged with the existing components and does not replace them. However, if a component with exactly the same retention time exists in the destination channel then that component will not be copied.

Update Component Search Windows

This dialog is displayed when the **Update Search Windows**… command is chosen from the **Actions** menu in the Method Editor.



Control	Description
Update these components	Select from the drop-down list the scope of the changes to be made to the component search windows. The choices are: **All Components**, **Internal Standards Only**, **Reference Peaks Only**, or **Selected Component Only**
<check list box>	Select the components whose search windows are to be updated. Displayed are all components from the method, regardless of the channel. This is only enabled when **Selected Component Only** is selected in the **Update these components** drop-down list.
Absolute window (± s)	Enter a value to set the component search window size, in seconds. (The range is 0.0 to 999.9 s.)
Relative window (± % of RT)	Enter a value to set part of the component search window size, as a percentage of the expected retention time for the component. (The range is 0.0 to 500.0.)

Clicking the **OK** button updates the search windows for all the specified components on the basis of the settings in the dialog. This update process can only produce search windows that are centered on the expected retention time of the component. For asymmetric search windows the user must enter values manually or define the window graphically.

The search window values generated for each specified component are:

$$\text{Peak Search Start} = \text{Expected Retention Time} - \left(\frac{\text{Absolute window}}{60} + \frac{\text{Expected Retention Time} \times \text{Relative window}}{100} \right)$$

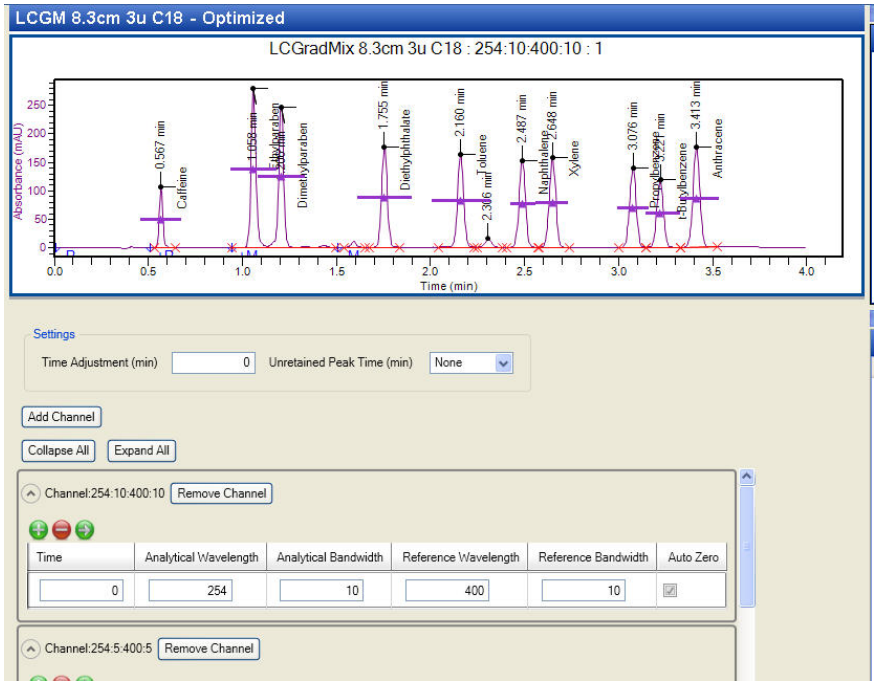
$$\text{Peak Search End} = \text{Expected Retention Time} + \left(\frac{\text{Absolute window}}{60} + \frac{\text{Expected Retention Time} \times \text{Relative window}}{100} \right)$$

Graphic Method Editing (GME)

Features of the graphic method editing environment include:

- The same basic presentation of the method parameters as within the standard method editor.
- All parameters in the method are available for editing within the graphic method editing mode.
- The graphics are highly interactive (e.g., drag-and-drop of integration events, component search windows).
- Reviewing the effect of the modified method on different raw data sets are quick and convenient.

The main display area is divided into two frames, with the controls in the lower frame and a plot showing the selected chromatogram in the upper frame (this is also referred to as **the chart)**. The tree control used to select the method section will be reduced in area to make room for a tree control showing the selected data set. This could be a single sample run, multiple samples or a complete batch.



Interaction with the Spectral processing Window

The selection of the channel for the template chromatogram is from the Method Tree rather than the Data Tree. When you enter the Spectral Processing window from the Chromera GME environment, if no instance of the GME mode of the Spectral Processing window exists, it will be created, even if a GRE/Post-Run instance of the Spectral Processing window exists. (An existing, GRE, instance will not be terminated and will be accessible through either the GRE/Post-Run environment within Chromera.)

When the Spectral Processing window is accessed from Graphic Method Edit within Chromera, the data tree passed is generated from the Batch, Sample, Injection nodes displayed within the current Chromera data tree combined with the channel data present in the current Chromera method tree. The currently selected channel within Chromera will be

passed to the Spectral Processing window together with the memory resident copy of stored version of the referenced method (The memory resident copy of the method is used since you may have modified any parameters within the method before it is passed to the Spectral Processing window. In order to retain these earlier changes, the Spectral Processing window passes back any changed parameters to this memory resident version of the method). Operations within the Spectral Processing window will initially use the spectral operations parameters contained in this passed method. These parameter values may be modify to optimize your results.

Within one channel, you are able to move freely between spectral operations and the Spectral Processing window is responsible for updating Chromera with any changed results and/or method parameters.

Since, in Graphic Method Edit, the displayed chromatogram is simply a template and not the object which is being modified, you are able to move freely between any chromatograms present in the data tree. The Spectral Processing window is responsible for ensuring that Chromera is updated with any information required for maintaining any spectral annotations that may be being displayed in Chromera in the Graphic Method Edit environment.

How to Access Graphic Method Editing

Graphic Method Editing can be accessed in either of two ways:

- From within the **Method Editor** – by choosing the **Edit Method Graphically** from the **Actions** menu and then selecting a suitable data set to be used as the basis for graphic editing.
- From within the **Post Run** environment – by choosing the **Go to Graphic Method Editor** command from the Actions menu. This switches context to the Method Editor and uses the data set from Post Run mode as the basis for graphic editing.

In either case, the specified data set (or at least the currently selected injection) is immediately reprocessed with the current version of the method (that might be a modified version of the stored method if graphic editing was reached from the Method Editor).

Main Menu Commands

The menus in graphic editing mode are basically the same as in standard display mode, with some minor changes as described in the table below.

Menu	Command	Description
The following are inserted into the File menu.		
File	Open Data…	Replaces all existing data in the data tree with new data. Displays the data selector in multiple batch/samples' selection mode. When the user has selected one or more data sets, the data tree will be populated with those items and the first chromatogram in the data set will be selected, processed with the method and displayed. If unsaved data exist you are prompted if the current unsaved data should be saved prior to display of the data selector.

	Add Data…	Adds new data sets to the end of the data tree. Displays the data selector in 'multiple batch/samples' selection mode. When the user has selected one or more data sets, the data will be added at the end of the data tree and the first newly added data set will be selected, processed with the method and displayed.
Display	Rescale	Displays the Rescale Axes dialog.
	Auto Rescale	Redraws the contents of the active plot frame at default scaling.
	Radar Window	Toggles display of the radar window on and off. Radar Window is only enabled according to options set in Plot Options (not displayed/displayed in all single plot views/displayed in graphic edit only).
	Plot Styles	Displays the Plot Styles dialog.
Actions	The following is appended to the Actions menu in graphic editing mode:	
	Peak Integration Wizard	Displays the **Peak Integration Wizard** dialog.
	Reprocess Current Chromatogram	Reprocesses the currently displayed chromatogram with the current (displayed) version of the method. This command is only used when automatic updating after each change to the method is disabled in preferences (see Preferences).
	Show Results	Displays the **Results** window.
	Signal to Noise Measurement	Displays the **Noise Measurement** dialog so you can enter values to measure the signal to noise.
	Edit Spectral Operation Graphically	Displays the Spectral Processing window in the Peak Purity Spectral Operation view with the same chromatogram you were viewing in the Graphic Method Edit environment. This chromatogram contains the parameters taken from the Peak Purity parameters in the Operations section of thr corresponding method. **This command is visible only if the user is in the Peaks or Operations section of the Method**. It will be hidden for all other method sections. Enabled only when a PDA channels is being displayed. Disable for all non-PDA channels.

	Current Chromatogram and Spectral Reprocessing	Reprocesses the chromatogram and the spectra. Enabled only when a PDA channel is being displayed. Disabled for all non-PDA channels.
	Component List using Spectral Library Search Matches	Creates or extends the component list in a method using the results obtained from a Spectral Library Search. This will display the **Spectral Processing window** in the Peak Library Search Operation view, displaying the same chromatogram you were viewing in the Graphic Method Edit environment with the parameters taken from with the Peak Library Search parameters in the Operations section method being displayed in the Graphic Method Edit environment within Chromera. **This command is visible only if you are in the Peaks section of the Method.** It will be hidden for all other method sections. Enabled only when a PDA channels is being displayed. Disabled for all non-PDA channels.
	Create Channel definition from Spectral Data	Displays the Spectral Processing window in the Preview Chromatograms view with the same chromatogram you were viewing in the Graphic Method Edit environment. The channel definition of the displayed channel is shown in the top section of the view. The last, user specified channel definition is used for the default preview channel. **This command is visible only if you are in the Channels section of the Method.** Hidden for all other sections. Enabled only when a PDA channels is being displayed. Disabled for all non-PDA channels.

	Edit Spectral Operation Graphically	Displays the Peak Purity Spectral Operation mode with the same chromatogram you were viewing in the Graphic Method Edit environment with the parameters taken from with the method being displayed in the Graphic Method Edit environment within Chromera. A new GME instance is created through this command. If Spectral Processing is already open in GME mode, the new Data is passed on to the open instance of Spectral Processing. **This command is visible ONLY when the Method Tree contains visible PDA nodes.** Since this command is intended to examine the spectra of the chromatogram you are currently viewing this command will be enabled only if a PDA chromatogram is currently being display in the chart region. If execution of the command fails to launch Spectral Processing, error dialogue is displayed and an entry is made into the Error Log.

NOTE: *The display menu is restricted in Graphic Method Editing.*

The following item exhibits a change in behavior in graphic edit mode.

Open Data... If unsaved data exist you are prompted if the current unsaved data should be saved prior to display of the data selector.

Graphic Method Editing Standard Toolbar

This toolbar adds two buttons for **Action** menu items (Optimize Peak Detection and Reprocess Current Chromatogram) to those available in the standard Method Editor.

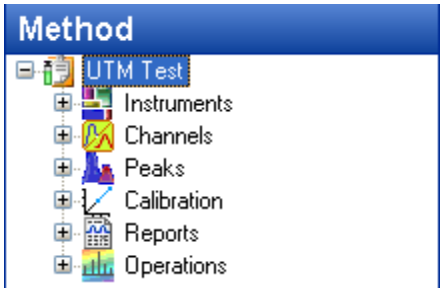
Or when user authentication is active:

Icon	Command
	File/New Method
	File/Open Method
	File/Save Method
	File/Save Method As
	File/Print Preview Method

	File/Print Method
	File/Lock (Only when user authentication active)
	File/Exit
	Actions/Standard Method Editor
	Actions/Edit Method Graphically
	Actions/Edit Spectral Operation Graphically
	Actions/Create Channel definition from Spectral Data
	Actions/Reprocess Current Chromatogram
	Help/Topics

Method Tree

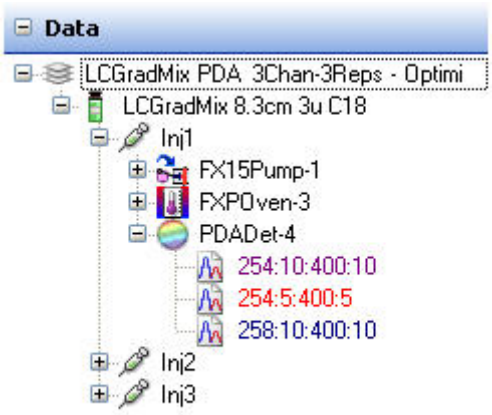
The form of this tree in the navigation pane is unchanged from that in the standard method editor (other than the reduced space it occupies).



The currently selected node is indicated even when the control does not have focus. During graphic editing the method tree affects the display in the plot frame and results tab, in addition to the method parameters display (see Interactions between Controls for details).

Data Tree

The form of this tree is the same that displays in the Post Run environment but it has a somewhat different behavior. Graphic Method Editor always displays one chromatogram and the chromatogram node selected in the data tree is always checked and displayed. The check boxes are effectively superfluous.



Synchronization with the Spectral Processing Window Data Tree

1. The data tree in the Spectral Processing window shows the Batch, Sample, and Injection of the Chromera Data Tree and the channels, from the method section in Chromera.

2. If you select a channel from anywhere in the Chromera method it will be displayed in the graphic plot in Chromera and the same channel will be selected in the Spectral Processing window data tree and displayed in the client region of the Spectral Processing window.

3. When a channel is selected from the Method section, the corresponding chromatogram is displayed in the graphic pane in Chromera. The corresponding data tree is also passed to the Spectral Processing window which then displays the appropriate chromatogram in the graphic pane of the Spectral Processing window.

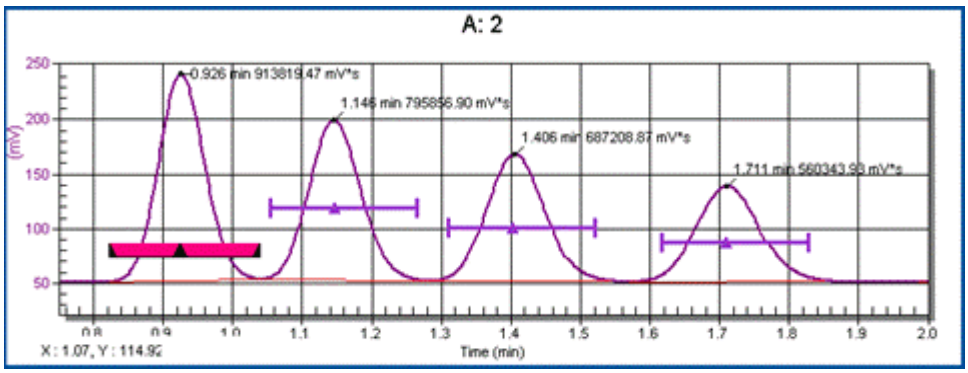
4. If you delete a channel from the Method Section in Chromera, the corresponding channels in the Data Tree in the Spectral Processing window will be deleted.

5. If the deleted channel was being displayed in the Spectral Processing window the plot will also be cleared.

Chromatogram Control

Annotations on the chromatogram are determined by the **Plot Styles** settings. The plot exhibits the full range of interactive behavior described in Plot Control.

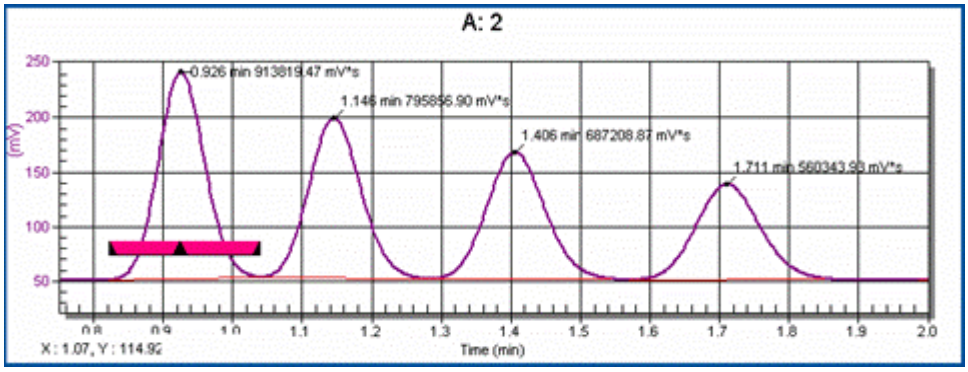
In graphic editing only one chromatogram is displayed at a time. That is, a single plot control (no stack) and no overlaid chromatograms. This is to make it clear as to which chromatogram the integration and component settings apply to.



When a sample injection includes data from multiple channels, the chromatogram displayed depends on the current selection in the method tree.

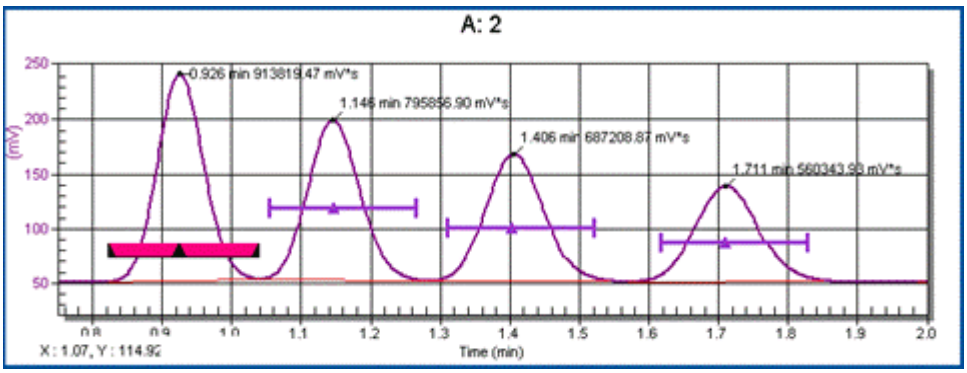
Component Search Window Tool

This is a graphic element that enables you to set the expected retention time and the search window for a component. The tool initially appears (assuming that Component Search Windows are selected as displayed Annotations) when you click on the chart to indicate the peak to be identified as the component. The selected form of the tool is similar to the Bunching Factor tool but it has an additional triangle marker indicating the expected retention time of the component within the search window. You can drag this central triangle to a new position within the search window, as well as repositioning the whole tool and resizing the tool. Whenever you make a change to the position or size of any element of the tool, the Retention Time, Peak Search Start and Peak Search End fields will be updated in the parameters grid of the selected channel.



When the mouse pointer is positioned over any of the triangular handles the cursor changes to ↔, indicating that the width of the object can be changed and the central marker can be moved.

When multiple component tools are displayed, the single selected component is indicated by its different form as well its different color. The colors used for the body of the tool in the case of selected and non-selectable components are user-selectable within Plot Options. The handles for resizing the gadget are always black.

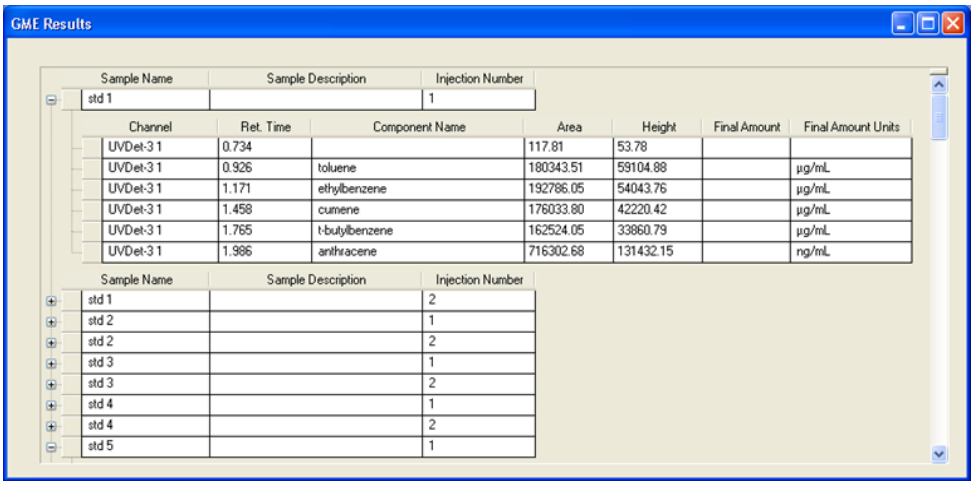


Method Parameters

The contents of the method parameter display are determined by the current setting in the method tree, in exactly the same way as in the standard method editor. The contents of the Method parameter display can be edited in the usual way. Certain parameters can also be edited interactively as described elsewhere in Graphic Method Editing.

Results Window

The Results window displays when you select the **Show Results** command from the **Actions** menu. The Results window displays in an Always on top mode.



The results displayed are those from the sample injection associated with the currently displayed chromatogram. However, the data from all channels will be displayed, rather than just the displayed channel, so that you can make comparisons if necessary.

The results are updated when you reprocess the chromatogram or when a different chromatogram (injection) is selected. If the selected chromatogram has not been processed and has no results, then a blank display is shown (rather than leaving the results for the last chromatogram displayed).

Results Window Popup Menu

The area within the Results window has the same popup menu as in the Results area within the Post Run Environment. In general the behavior of the commands are the same as in Post run but with one significant exception: The **Save to Spreadsheet** commands will save only the results for the displayed injection and not the whole batch.

How to Develop a New Method

Develop a New Method

The basic principle of this environment is that the annotations on the currently displayed chromatogram always reflect the results of the processing of that chromatogram by the currently displayed method.

The following procedure describes the steps that would typically be performed when developing a method to process an acquired chromatogram or set of chromatograms. This example assumes that you are starting with a completely new method, or at least a method in which no entries have been made within the Peaks or Calibration section, and that the method is open in the Method environment.

1. Choose **Edit Method Graphically** from the **Actions** menu or click the  toolbar button.

2. Select one or more sample data sets from the **Data Selector**. For the purposes of this workflow it is assumed that the channels in the data sets correctly match the channels defined in the method. The consequences when this is not the case will be discussed later.
   By default, the chromatogram from the first injection of the first sample opened will be selected in the data tree but which channel is displayed depends on the current selected in the method tree. If a channel-specific section is selected (e.g. Peaks/Chan A), then the chromatogram from that channel will be displayed in the graphics frame. If the method tree selection does not define a specific channel then the chromatogram from the first data channel will be displayed.

3. The display in the frame remains unchanged up to this point and simply reflects the current selection in the method tree, in the usual way.

4. The displayed chromatogram is processed with the method as it exists, regardless of the current selection in the method tree and which section of the method is displayed. Because the method contains no values for Bunching Factor, Area Threshold, or Noise Threshold (as stated at the beginning of this workflow), the software automatically determines suitable values for these parameters (by assessing the intrinsic signal noise) as the first stage of processing. These values will be assigned to the appropriate fields in the Peaks section for the selected channel, even if that section is not currently visible.

5. Annotations on the chromatogram are updated based on the processing results and the current on/off settings for annotations.

6. At this point the most likely course of action is to select the Peaks node in the method tree (assuming it, or a child node, is not already selected). The display displays the Peaks section for all channels. If you select the specific channel node associated with the displayed chromatogram then only the parameters for that channel will appear. If you select a different channel within the Peaks section then the chromatogram for that channel is displayed in the graphics frame, it will be processed (i.e. return to step 5) and its parameters (only) will be displayed.

7. The situation where the Peaks node is selected and parameters for all channels are displayed, while a single chromatogram is displayed in the graphics frame, will not persist for long. As soon as you click on a field within the display, the channel

associated with that field becomes the selected channel and all elements of the window will update to reflect that. That is, the method tree shows that channel selected under the Peaks node, the data tree will show that channel selected and the graphics frame will display the chromatogram from that channel. Also, the display updates to show only the parameters from the selected channel. This same basic procedure occurs when you select the Channels or Calibration node, and subsequently selects a channel-specific parameter.

NOTE: *You are not prevented from selecting these top-level nodes, since that would seriously violate behavior that is normal in the standard method editing mode.*

8. After selecting the Peaks section for a specific channel, you can examine the chromatogram integration and decide if the Bunching Factor and Threshold values determined by the software are satisfactory. If they are not you can update them.

9. Define components and map them to detected peaks.

10. Select a different data channel within the method tree then that chromatogram will be displayed and processed as described above. If BF, AT and NT values do not currently exist in the Peaks section of the method for the newly selected channel they will be calculated as described in step #5 above.

11. Select a different chromatogram within the data tree then that chromatogram will be displayed and processed (using the currently displayed version of the method) as described above.

Updating Bunching Factor, Area Threshold, and Noise Threshold Values

The following procedure can be used to update these parameters.

1. Select the **Bunching Factor** field in the method parameter area for the selected channel. This causes the prompt below the parameter band to be displayed.

2. Scale the chromatogram as required to zoom in on a peak of interest.

3. Hold down the Control key and draw a box around the base of the peak of interest. As you release the mouse button a new BF value is calculated (and shown in the parameter display field) based on the size of the drawn box. The Bunching Factor field remains selected.

4. Repeat steps 2 and 3 until a satisfactory value has been obtained.

5. Select either the Area Threshold or the Noise Threshold field. The Thresholds prompt displays below the parameter band.

6. Scale the chromatogram as required to zoom in on a section of baseline.

7. Hold down the Control key and draw a box encompassing the required section of baseline. As you release the mouse button, new Area and Noise Threshold is calculated based on the signal characteristics in the defined time region.

8. Repeat steps 6 and 7 until satisfactory values have been obtained.

Adding a Component

To define a new component in the selected data channel:

1. Select the Name field in the **New Component** row within the Peaks section of the method for the selected channel. A name for the component can be defined either by selecting an existing value from the component dictionary contained in the drop-down list, or by typing in a new name (which will then be added to the dictionary).

2. When a name has been defined, the retention time field will be automatically selected in the grid and the mouse pointer on the chart will change to an upward pointing arrow, indicating that the expected retention time of the component can be set by clicking on the chart.

3. Click on the chart (it will not matter whether or not you hold down the Ctrl key) to enter a value into the RT field for the component and the search window sets to default size (defined by Start and End values entered into the appropriate fields in the display. The value set for the RT depends on where you click and on the current state of the chromatogram. If the chromatogram is integrated and you click within a peak envelope (between start and end times) then the calculated crest of that peak (based on curve fit, where applicable) will be entered as the component expected RT. If any other conditions apply then the time at which you clicked on the chart will be entered as the component expected RT.

4. The component search window tool displays on the chart as soon as you define the component RT and the default search window start and end times have been calculated.

5. After clicking on the chart to define the component RT, the chart cursor reverts to the standard mouse pointer and the chart is once again in zooming mode.

6. Adjust the size of the component search window as required by drag-and-drop using the start and end handles of the component search window gadget. Updated values for these times will be entered into the grid upon each drop.

Adding/Deleting Channels

You have the ability to view a modified or new channel in the Chromatogram pane when it is added to the Channel section of the Method. In addition the new or modified channel and will be added to the Method Tree.

Adding Channels

Behavior within Chromera

The basic behavior of adding a channel in GME is the same as in the Standard Method Edit. If a PDA injection has been associated with a method in GME, then, when that new channel is selected in the data tree, the new channel will be extracted from the spectral data, displayed in the graphic pane and processed.

Behavior within the Spectral Processing window

Adding a channel within the Chromera method while in GME with the Spectral Processing window currently running will update the Spectral Processing window data tree and the added channel will be added to every injection within the Spectral Processing window data

tree. The new chromatogram will be displayed in the Spectral Processing window when selected in the method tree within Chromera.

Adding a channel to a method in Chromera from the Spectral Processing window

Channels may also be added to the channels section of the Chromera method while in GME from the Preview Chromatograms operation within the Spectral Processing window

If you transfer the Previewed Channel to the Chromera method, it will be added to the Chromera Method section in exactly the same manner as if added manually from within Chromera. The channel added to Chromera will then be added to the Spectral Processing window data tree.

If the channel name is already present in Chromera then the channel name will auto-incremented.

Deleting Channels

Behavior within Chromera

The behavior is the same as in the Standard Method Edit, the only difference being that the channel will also be deleted from the Spectral Processing window.

Behavior within the Spectral Processing window.

The data tree within the Spectral Processing window, which consists of the Batch, Sample, Injections nodes from the selected batch or batches with Chromera concatenated with the channels nodes from the current version of the open method will be update and the deleted channel deleted from every injection within the Spectral Processing window data tree.

Adding an Integration Event

To define a new integration event in the selected data channel.

1. Select the required timed event from the Type drop-down list in the **new event** row within the Peaks section of the method for the selected channel, or click the required event button within the integration event toolbar (if applicable).

2. If the event type requires a single time entry, the mouse pointer on the chart will change to an upward pointing arrow, indicating that the time of the event can be set by clicking on the chart. If the event requires start and end times then the mouse pointer will change to crosshairs, to indicate that the times can be set by drawing a box.

3. Click or drag on the chart and the event annotation(s) appear on the chart at the designated time(s).

4. The event start time is displayed in the display (as well as the end time where this is applicable).

5. After clicking (or dragging) on the chart to define the event time (or times), the chart cursor reverts to the standard mouse pointer and the chart is once again in zooming mode.

6. If the event type requires a value you can enter this in the Value cell of the row.

7. Once the event is complete the chromatogram will be reprocessed and the chart will be redisplayed to show the new integration.

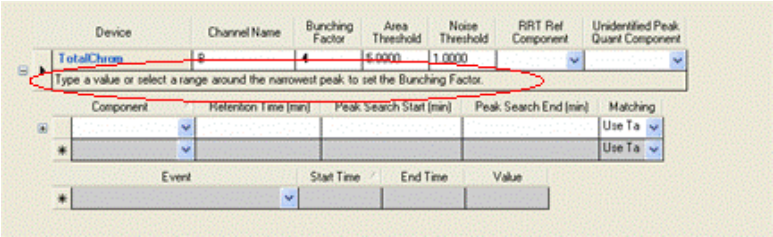
Interactive Field Prompts

When a field that can be set interactively is selected a prompt displays below that line. There are three basic cases.

NOTE: *These prompts only appear in the GME mode and not in standard Method editor mode (when no chart is displayed).*

Integration Parameters

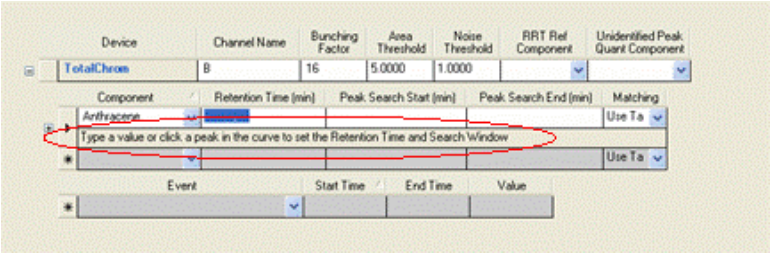
When the Bunching Factor, Area Threshold, or Noise Threshold field is selected a prompt displays.



Field selected	Prompt
Bunching Factor selected	To set the Bunching Factor, hold down the Ctrl key and draw a box around the narrowest peak on the plot.
Area Threshold or Noise Threshold	To set the Area and Noise Thresholds, hold down the Ctrl key and draw a box around a region of baseline on the plot.

Component Parameters

The prompt displayed for a component row depends on the state of the row; for example, when the Retention Time field is selected during component addition.

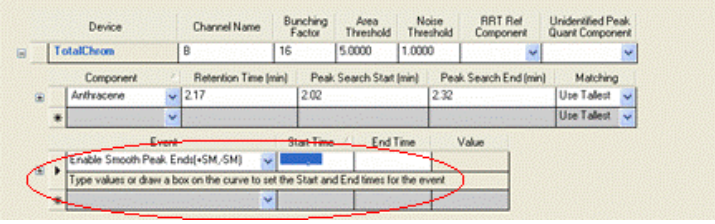


State	Field Selected	Prompt
Adding New Component	Retention Time	Type a value or click a peak in the curve to set the Retention Time and Search Window.
Editing Component	Retention Time	Type a value or move the component marker to modify the Retention Time.
Any	Peak Search Start	Type a value or resize the component marker to adjust the Peak Search Start time.
Any	Peak Search End	Type a value or resize the component marker to

		adjust the Peak Search End time.

Integration Events

As in the case of components, the prompts associated with integration events are different depending on whether the event is being added or edited. In this case the type of event being added also affects the prompt displayed. The prompt are displayed in the usual location, when a time field is selected.



Events which require a single time value

State	Field Selected	Prompt
Adding New Event	Start Time	Hold down the Ctrl key and select a point on the curve to set the event time.
Editing Event	Start Time	Type a value or move the event annotation to modify the time.

Events which require start and end time values

State	Field Selected	Prompt
Adding New Event	Start Time or End Time	Hold down the Ctrl key and draw a box on the plot to set the Start and End times for the event.
Editing Event	Start Time or End time	Type a value or move the event annotation to modify the time.

Action with Mismatched Channel

The purpose of Graphic Method Editor is to optimize a method for acquisition or processing using one or more example files of data acquired from the same instrument. The normal situation will be that the channels defined in the method match (in name and data type) those that exist in the data set. However, since it cannot be guaranteed that the user will always make an appropriate selection of data, the consequences of mismatched channels must be defined. There may also be legitimate cases where the method has more channels defined than exist in the data set, or vice versa.

The data channel in the method must be of the same type (i.e. the same basic detector type) and have the same name as a channel in the sample data set in order to be edited graphically. In this context, all ICP-MS models are of the "same detector type" and the standard and "HS" models of the Series 200 UV/Visible detector are also of the "same detector type."

Two basic mismatch cases exist:

1. The method includes a channel for which no matching channel exists in the data set.

   In this case, whenever that channel is selected in the method (i.e. from the method tree or by selection in the grid frame), the chart will be blanked.

2. The data set includes a channel for which no matching channel exists in the method.

   In this case, whenever a chromatogram from that channel is selected in the data tree, the grid frame will be blanked.

Peak Integration Wizard

The Peak Integration Wizard provides an easy way to set the bunching factor and area and noise thresholds for your method.

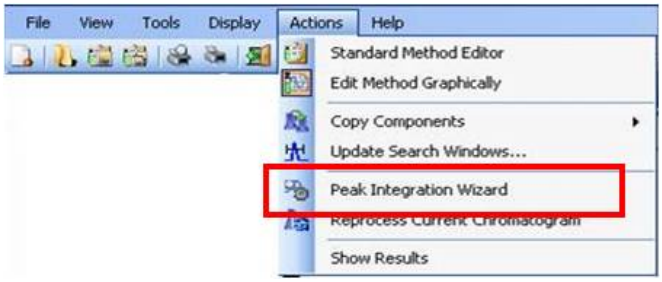
NOTE: *You cannot enter values in the Wizard. They are calculated when you perform the described action.*

The Peak Integration Wizard appears within the **Graphic Method Editor**. Since it is a wizard the instructions are included on every screen.

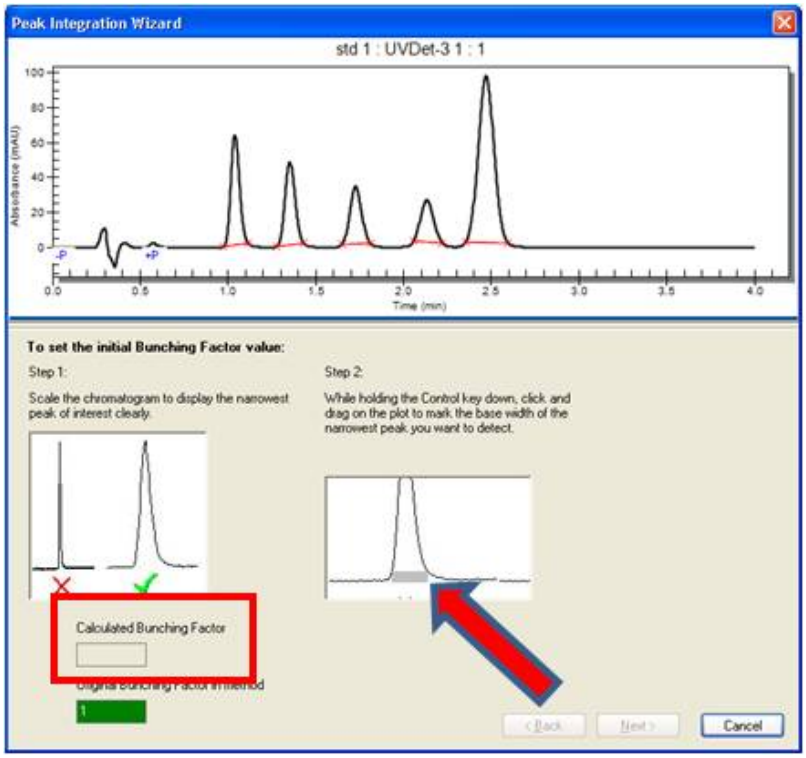
NOTE: *Care must be taken in the use of this wizard to assure that you select the appropriate regions of the chromatogram for evaluation. The functions for setting the Bunching Factor, Noise Threshold, and Area Threshold for a chromatogram are exactly the same as those accessible from the Menus and Toolbar in Graphic Method Edit and in Post Run. Suggested values for these parameters are calculated upon the specific baseline noise and peak width selections made by the user. The primary purpose of this wizard is to facilitate the process of setting these values, rather than to make those determinations automatically for you using the current chromatogram.*

How to use the Peak Integration Wizard

1. Select Peak Integration Wizard from the **Actions** menu.

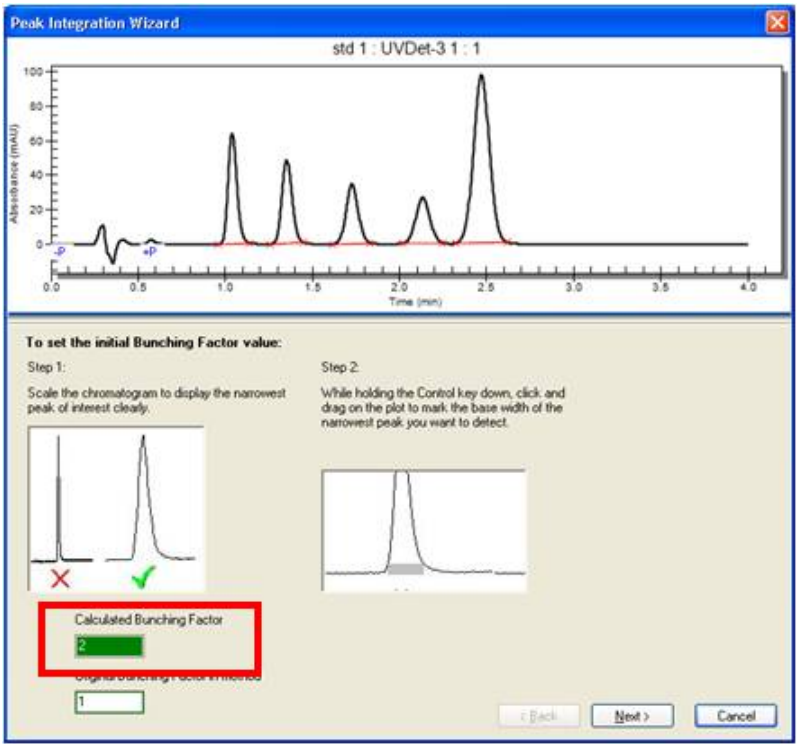


2. On the initial screen the **Calculated Bunching Factor** field will be blank until you draw a box on the plot to indicate the peak width at base of the peak for which the bunching factor should be calculated.



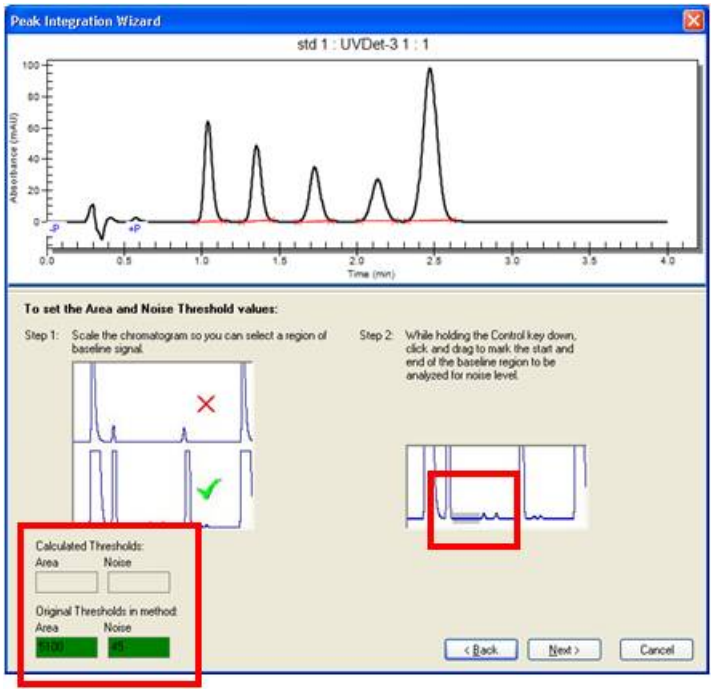
This is intended to be the narrowest peak that is desired to be integrated. You do not need to select the entire peak because only the starting and ending times of the region selected will be used. However, the appropriate width for this peak is critical for the calculation, because drawing the box too wide or too narrow may result in the suggested Bunching Factor being too high or too low. And if the sampling rate is too slow for adequate integration of the peak initially, adjusting the Bunching Factor will have little or no effect.

The chromatogram will be reprocessed (using the updated Bunching Factor value) each time you make a new selection for the width of the narrowest peak. The Calculated Bunching Factor is shown highlighted to indicate that it is the value used for the current integration display.



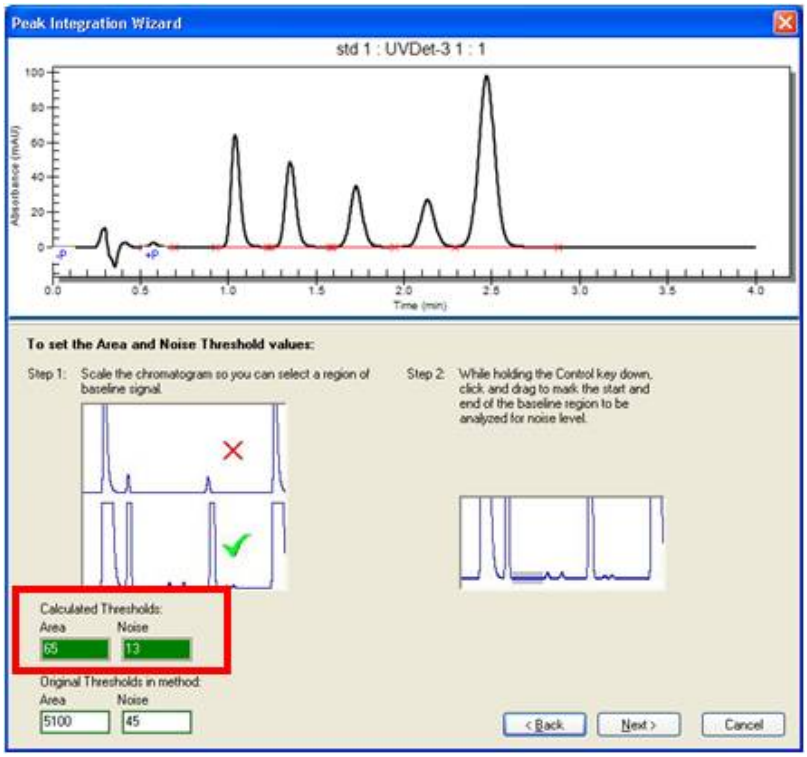
NOTE: *You cannot directly edit any of the fields in the Wizard. You can affect what is displayed in the Calculated Bunching Factor field by outlining a peak width on the plot but cannot enter numbers directly, here or elsewhere.*

3. When you click the **Next** button the next page of the Wizard will be displayed, enabling you to set optimum initial **Area and Noise Threshold** values.



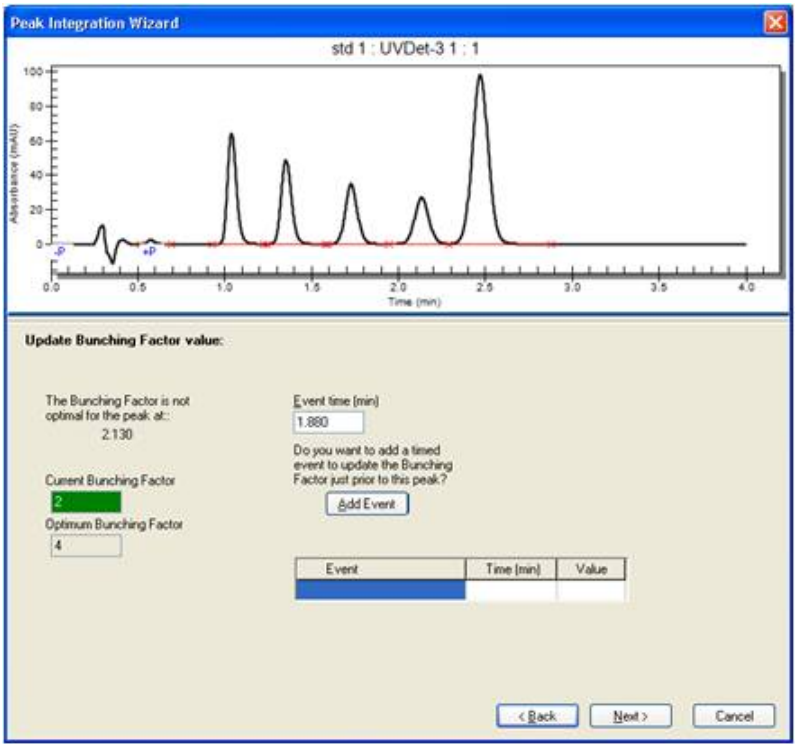
The **Calculated Thresholds** fields will be blank until you draw a box on the plot to indicate the region of baseline to be used for Noise and Area Threshold calculation. The height of the box drawn is not important. Only the data between the starting and

ending times of the region selected will be used. However, the selection must be made carefully to ensure that only data considered by the user to be noise is included in the calculation. If true peaks are included in this region, then the determination of Noise and Area Thresholds may be erroneous, and improper integration of peaks may result. The chromatogram will be reprocessed (using the updated parameters) each time a new selection for noise determination is made, and the calculated values will be displayed in the appropriate read-only fields. The **calculated fields are now highlighted** to indicate that these are the values employed for the displayed integration:



4. When you click the **Next** button the software will determine if the Bunching Factor (BF) should be increased by a the application of a BF Timed Event later in the chromatogram, for peaks that are at least double in width compared to the peak used for the initial determination. The procedure for this is described at the end of this document (see **Determination of Bunching Factor Timed Events**).

   If the software determines a Bunching Factor event should be added the following screen will be displayed:

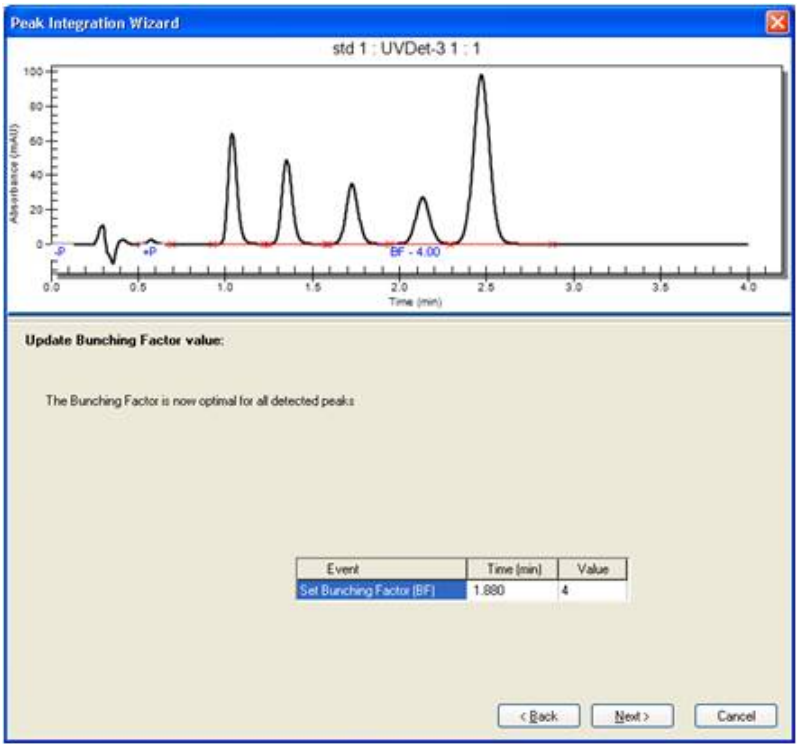


NOTE: *This screen will not appear if no BF events are found to be necessary.*

If you click the **Add Event** button the event will be added and the chromatogram will be reprocessed to show the updated integration.

If the software determines another **BF** update is required later in the chromatogram (because of another doubling of peak width), the above screen will be redisplayed but with the appropriate time and bunching factor values added. The table will also display the BF event added previously.

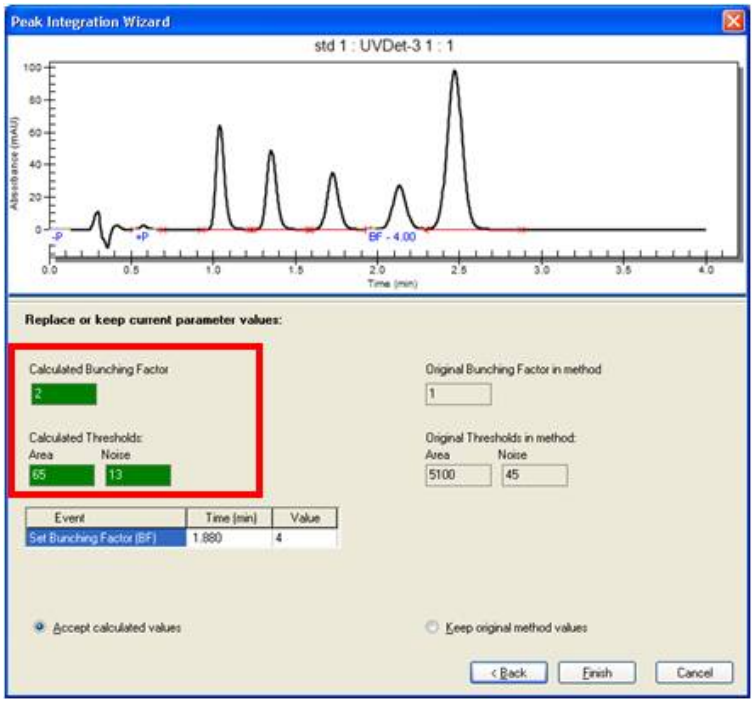
When you determine that no further BF events are required, the following summary screen is displayed, listing all the events added.



NOTE: *As with the other fields in the Wizard, the timed events list is not directly editable by a user.*

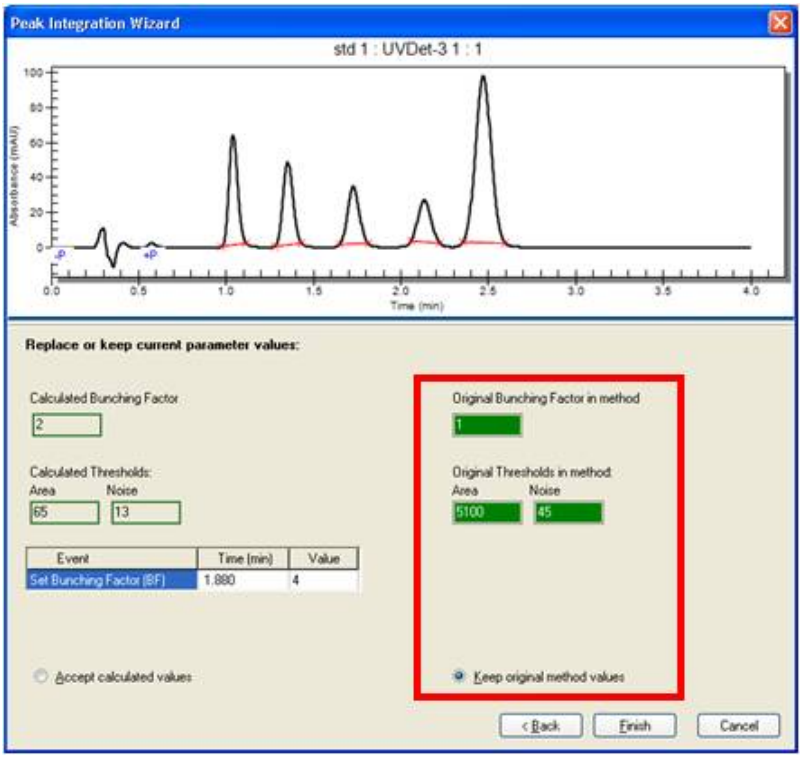
5. Having reviewed the events and the integration, you can click **Next** and proceed to the next stage. Alternatively, you could click **Back**, to repeat the timed event process from the beginning (i.e. with no timed events in the list). This is a way for you to undo the entry of the timed events if so desired.

6. When you click **Next**, the final review screen will be displayed:



Initially the calculated parameter values will be highlighted and the chromatogram display will reflect the integration produced from those values.

If you click the Keep original method values radio button, then the original parameter fields will be highlighted and the chromatogram will be reprocessed with the original conditions. In this way you can compare the two sets of parameters before deciding on which set to use in the method.



7. When you click **Finish**, the selected set of parameters will be entered into the Peaks section for that channel. Clicking **Cancel** will have the same effect as selecting the **Keep current method values** option.

Determination of Bunching Factor Timed Events

The input to this procedure will initially be the results derived from processing the chromatogram using the bunching factor and the area and noise thresholds accepted by the user in the first two screens of the Wizard. These results include the suitability values (specifically the Peak Width @Base values) calculated for the detected peaks.

8. For each detected peak the software will calculate:

   Peak Width @Base / Width of current bunch

   where: width of current bunch = Current BF in effect / Sampling Rate

9. If the calculated value is greater than 60 then the software will propose adding a Bunching factor event just prior to the start of the peak (say peak start time – ½ Peak Width @Base), with a value equal to double the current Bunching Factor.

If you accept the events, then the chromatogram will be reprocessed with the revised conditions and the procedure will start again with the updated peak list – beginning at the peak following the peak that triggered the BF event.

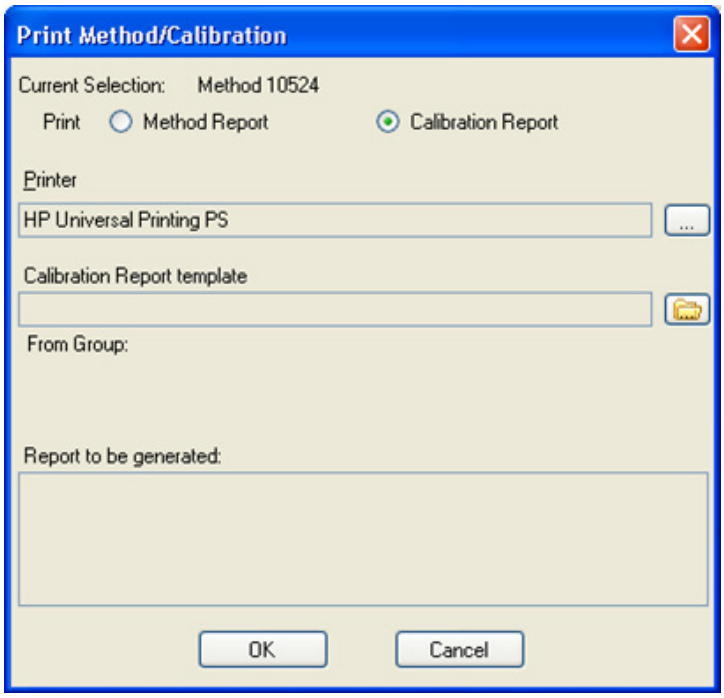
If you decline to add the event then no further action will take place; you must either proceed to the next step or cancel the whole process.

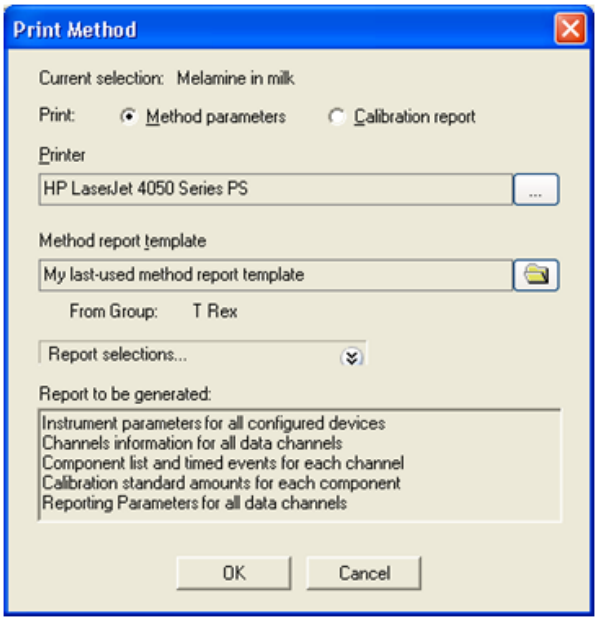
Differences between GME and Post Run Versions

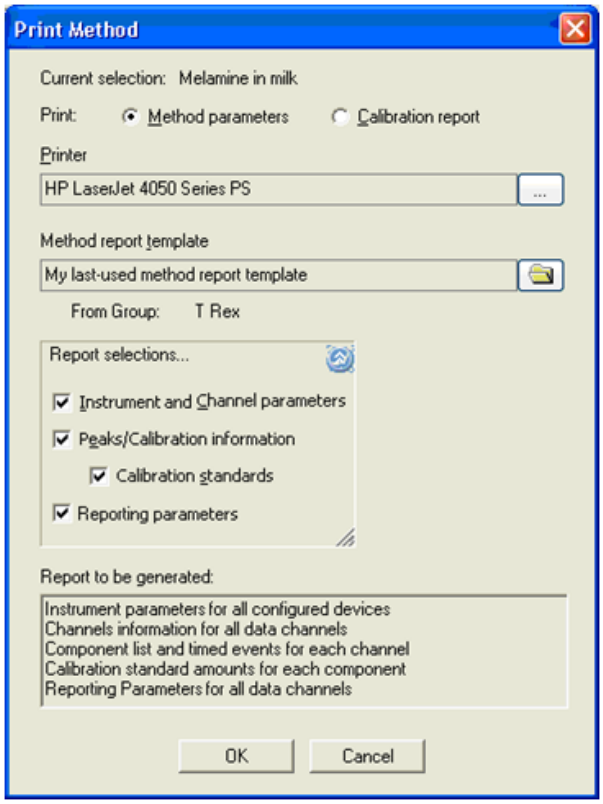
Functionally the Wizard will operate in exactly the same manner in the two environments. The only differences will be in labeling, to clarify as to what is being modified. Specifically, the words "in method" will not appear in the Post Run version, since the actual method is not being modified. The term 'Original' is used throughout for the values in use when you entered the Wizard. The term 'Current' is restricted to the active Bunching Factor at various times during the run in the part of the Wizard where timed events are suggested.

Print Method/Calibration and Print Preview Method/Calibration

Selecting the **Print Method/Calibration** or **Print Preview Method/Calibration** command from the File menu within the Method environment displays the Print Method dialog. It opens with the most recently used report template associated with the currently selected option (i.e. method or calibration – method by default when the dialog is opened). If no most recently used template exists for this user/instrument, then the field will be blank. In this latter case the **Report to be generated:** field will also be blank. The dialog allows you to define what sections of the method are printed, on which printer the report is printed, and report template to be used. If you select the Calibration report option the dialog changes.



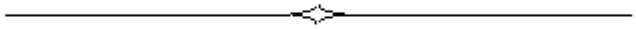




Set the following in this dialog:

Control	Description
Current Selection	Displays the name of the current method.
Print: Method parameters	Select to generate and print a standard method report listing the method parameters.
Print: Calibration report	Select to generate and print a calibration report from the calibration data in the method, rather than a standard report listing the method parameters.
Printer	Click this browse button ... to display the standard Windows Select Printer dialog and select a printer to be used to output the method report.
Method/Calibration Report template	To generate the method or calibration report, click this browse button, to display the standard report template selection dialog. The templates available for selection are filtered, so that only templates appropriate to the currently selected option are shown. **Method parameters** selected: The method template most recently used by the current user on this instrument (within this dialog). **Calibration report** selected: The calibration report template most recently used by the current user on this instrument (within this dialog).

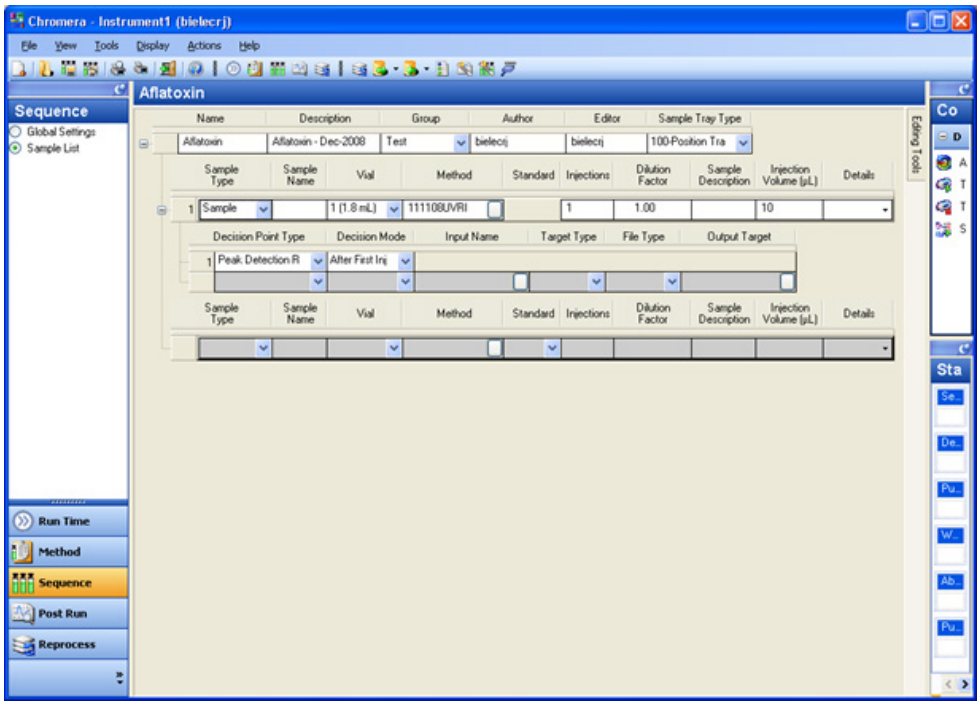
From Group:	Displays the name of the Group the currently selected report template is assigned to.
Report selections…	Clicking the plus sign next to Report Selections expands the dialog. This control is hidden when the **Calibration report** option is selected.
Instrument and Channel parameters	A check box to include the Instrument and Channels section of the method in the printout.
Peaks/Calibration information	A check box to include the Peaks and Calibration sections of the method in the printout.
Calibration standards	A check box to include the list of standards and associated amounts for each component in the printout.
Reporting Parameters	A check box to include the Reporting section of the method in the printout.
Report to be generated:	When the report template field is populated, the software examines the template to determine its basic characteristics. This information about the selected template is displayed in the multi-line, **Report to be generated: field.**



Sequence

About the Sequence

A Sequence is a list of samples to be run that is created prior to an analysis. A sequence controls the acquisition and analysis of chromatographic data produced by a series of injections in Chromera. Each row in the sequence contains, directly or by reference, all information relevant to the collection, processing and reporting of one sample. This includes:



- Sample identification information (Sample name, description and type; plus standard name for a calibration sample)
- The method to be used for instrument control, data acquisition, processing and quantitation.
- The vial number, injection volume and number of injections to be made from the vial
- The type of sample, which determines the nature of the processing and calculations to be performed on the sample data
- Sample-specific values used in quantitation (such as sample amount, dilution factor, internal standard amounts)
- Decision points, during the execution of the sequence, that enable the user to examine results and take appropriate actions. For each row it is possible to define a Decision Point Type, which is an action associated with the row. Examples of Decision points are as follows:
  - Peak Detection Review - Displays peak integration results for review and modification
  - Calibration Review - Displays calibration plot(s) and data for review
  - Data Review - Displays integrated chromatograms and results
  - Report - Sends results to the printer, screen or file
  - Shutdown - Only available with the Sample Type Wash

You can create a sequence in he following ways:

- use the **Sequence Wizard**, to generate long sequences based on a fixed pattern of standard and samples quickly and easily.
- build new sequence in the Sequence Editor
- edit an existing sequence.
- Import an existing Chromera sequence.

Building a Sequence (About Sequence Parameters)

When you build a sequence, you must specify a **Sample Type** from the provided drop-down list. Another key entry on each row of a sequence is the analysis **Method**. The default method is the method currently in the method editor, but you can specify a different method by clicking the button in that cell and choosing a method using the Open Method dialog.

When an autosampler is in use the tray position to be sampled is indicated in the **Vial number** field. This number will automatically increment by one as rows are added to the sequence. You can also specify the number of times you want to inject a sample **(Injections)** from the Vial using the conditions of this sequence row. Each time you inject from the vial you will run through the conditions set in that row.

For each row it is possible to define a **Decision Point Type**, which is an action associated with the row. Examples of Decision points are as follows:

- **Peak Detection Review** - Displays peak integration results for review and modification
- **Calibration Review** - Displays calibration plot(s) and data for review
- **Data Review** - Allows review of the integrated chromatograms and results (without editing)
- **Report** - Sends results to the printer, screen or file
- **Shutdown** - Only available with the Sample Type Wash

Any row can be removed from the sequence without affecting any other item.

NOTE: *After making a change to the sequence you must **save** the sequence to retain the changes made. **Only a single version of a sequence is saved in the database.** When you make changes and select the **Save** command, the previous version of the sequence is replaced by the modified version. This includes a modification to the sequence name, since in Chromera the name is a sequence parameter like any other (the sequence is uniquely identified by a database index that is not visible to the user).*

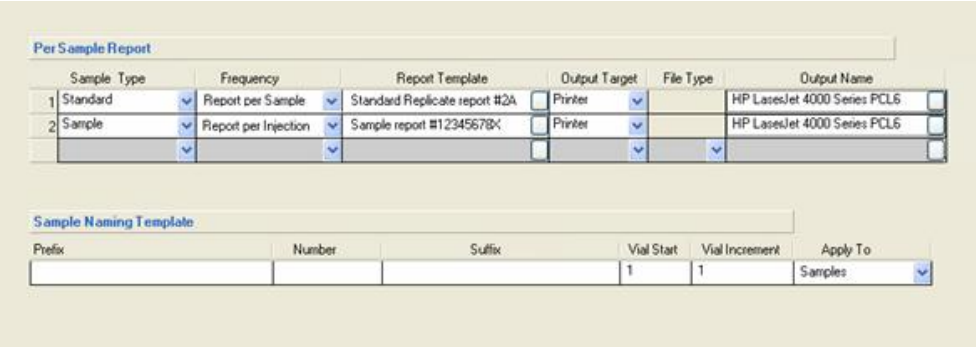
To create a copy of an existing sequence but with a different name, leaving the original sequence unchanged, the **Save As** command is used.

NOTE: *A sequence running in an online instance of Chromera cannot be edited and saved as the same sequence in any other instance of Chromera. Use of the Save As command to create a copy of the sequence will however be permitted.*

Sequence Editor - Global Settings

Global Settings can be set on a per sequence basis. In this way, if you have different reporting needs for different analyses you won't have to edit preferences constantly or pretend to be someone else half the time.

The Global Settings are displayed when the **Global Settings** view is selected in the Navigation pane.



Per Sample Report Band - Up to 10 rows may be defined.

Control	Description
Sample Type	Select application-specific sample types from the drop-down list of application-specific sample types that control processing of the acquired data. LC Sample Types: Calib: Replace, Calib: Average, Sample, Matrix, Background, No Injection, Wash Speciation Sample Types: Blank, Standard Blank, Standard, CIS Blank, Reagent Blank, Sample, Wash
Frequency	Select the frequency as to when to generate the report for a row with multiple injections from the drop-down list. The selections are Report per Sample and Report per Injection.
Report Template	A selection field to name the report template. Select the template from a database selector dialog by clicking on the button.
Output Target	Select from the drop-down list the way you want the report output. The selections are File or Printer.
File Type	Select from the drop-down list the type of file to be created as output for a report. The selections are Adobe Acrobat (pdf) or Microsoft Excel (xls).

Output Name	Target Type = File - the path where the report output files will be saved (under names constructed automatically from the sample name). Selected via a standard Browse for Folder dialog. Target Type = Printer - the name of the printer where the report is printed. Selected via a standard Print dialog.

NOTES: *(1) The Per Sample Report allows multiple rows rather than only two (per sample/per replicate). This provides more flexibility in the global general report definitions. (2) The term* ***Report per Injection*** *is used as an option for Frequency since this is more consistent with the use of the* ***Injections*** *field in the sequence itself.*

Sample Naming Template Band (at least one row per sample type can be defined)

Control	Description
Prefix	Type (up to 25 characters) a prefix to define the beginning of each Sample Name for the added rows. The Sample Name consists of the Prefix, Number (incremented by one for each sample), and Suffix concatenated, without intervening spaces.
Number	Type the initial value of the incrementing number appearing in the Sample ID. The range is 0 to 9999 with as many digits as entered (e.g. 0000 is a valid entry).
Suffix	Type (up to 25 characters) a suffix to define the end of each Sample ID for the added rows.
Vial Start	Type the vial number for the first row.
Vial Increment	Type a number using values 1 to 9 to define how the vial number will change for each row added.
Apply To	Select from the drop-down list the Sample Type(s) the naming template is applied to.

How the Sample Naming Template Works

NOTE: *The software will cease looking for matching rows within the Sample Naming Template rows as soon as one match is found. If you enter more than one row for any sample type only the first will ever be used. Similarly if you enter a row with* ***Apply To*** *set to* ***All*** *now rows following that will ever be used, since that is a match for all sample types.*

Adding a Row

Selecting a **Sample Type** in the last row of the display causes the software to determine if a matching sample type exists in the global **Sample Naming Template** list type (note that all

sample types will match a row with **Apply To** set to **All**). If a match is found then the sample name defined by that template will be constructed, taking account of the current state of the Number count, and entered into the sample name cell – provided that cell is currently blank. Note that an automatically generated sample name will never overwrite an existing entry.

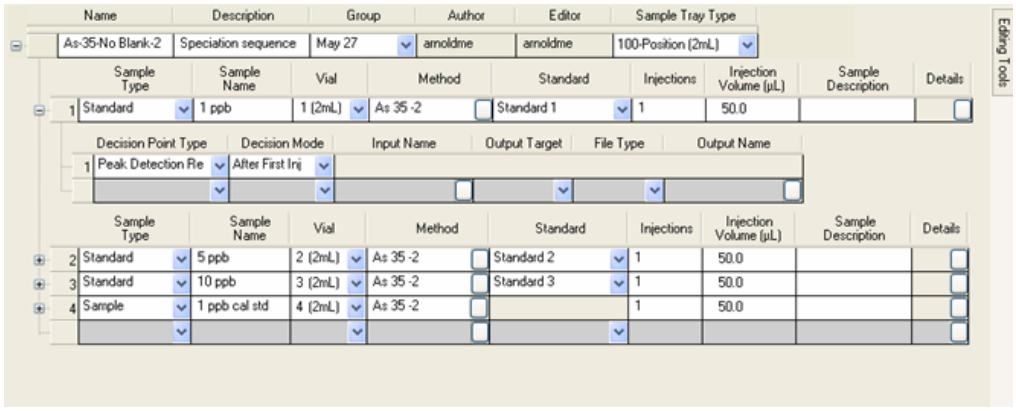
If this is the first row of the specified sample type then the vial number field will be initialized as defined within the Sample Naming Template row.

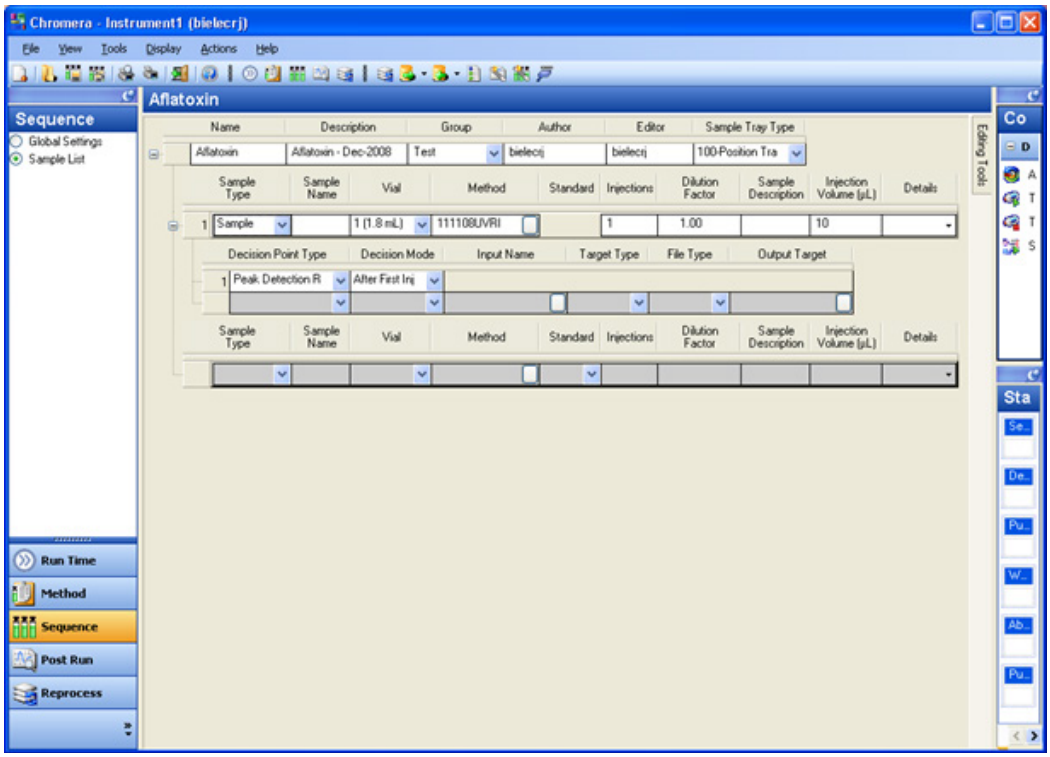
Adding Rows via Dialog

Selecting a **Sample Type** in either the Add Rows or Insert Rows dialog causes the software to determine if a matching sample type exists in the global **Sample Naming Template** list type (note that all sample types will match a row with **Apply To** set to **All**). If a match is found then the fields in the dialog will be filled from the settings in the matching Sample Naming Template row. This action will only occur when you change the **Sample Type** setting in the dialog; the initial default of **Sample** does not cause the fields to be filled.

Sequence Editor - Sample List

The Sample List parameters for the LC sequence editor or speciation sequence editor are described below.





Identification (Band 1)

Parameter	Description
Name	Type the name associated with the sequence. A name must be entered before the sequence can be saved and name must be unique within the group specified below. Changing the name in this field does not create a new sequence it merely renames the existing one.
Description	Type a description of the sequence.

Group	Assign the sequence to an existing group or to a new group. To use an existing group type the group name or select it from the drop-down list. To create a new group and assign the sequence to it, simply type a group name that does not already exist.
Author	Displays the user name of the person who created the sequence. Once this field has been populated it will never be changed.
Editor	Displays the user name of the person who last saved changes to the sequence. This field will be updated each time the sequence is saved.
Sample Tray Type	Select the autosampler tray type to be used for the sequence from the drop-down list. This field is not present (or will be disabled) when the associated instrument configuration does not include an autosampler. The contents of this list depends on the autosampler configured.

NOTE: *This band can also include fields displaying the Date/Time the sequence was created and the Date/Time is was last edited (and saved) but these are not displayed by default.*

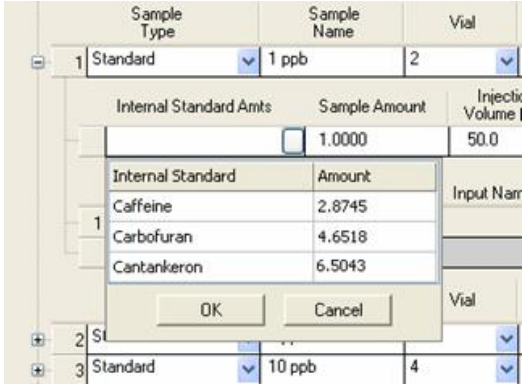
Sample Information (Band 2)

Parameter	**Description**
Sample Type	Select from the drop-down list of application-specific sample types that control processing of the acquired data. LC Sample Types: Calib: Replace, Calib: Average, Sample, Matrix, Background, No Injection, Wash Speciation Sample Types: Blank, Standard Blank, Standard, CIS Blank, Reagent Blank, Sample, Wash
Sample Name	Enter a unique description of the sample (using up to 50 characters). This field is used as an identifier (together with channel name and injection number) when the chromatogram(s) acquired are displayed.
Vial	Set the vial position from which the current sample will be taken. The display indicates the size of the vial in addition to its position. This cell will be blank and disabled if no Sample Type has been defined for the row. The allowed range depends on the selection for Sample Tray Type.
Internal Standard Amts	Single ISTD in method: Enter a number (greater than zero to 999,999.999) to specify the amount of the internal standard in the sample. Multiple ISTDs in method: A pop-up editor field (see below), enabling you to enter an amount for each internal standard in the sample. You can enter a single number if the method defines only one internal standard, or it will display a button to provide access to a pop-up editor if more than one internal standard is defined.

Sample Amount	Enter a number (greater than zero to 999,999.999999) to set the actual amount of the sample used in the sample preparation from which the injection was made, excluding the amount of internal standards. This value is used to convert absolute amounts calculated for unknown samples to concentration units.
Injection volume (µL)	Enter a number to set the volume injected by the autosampler. This cell will be blank and disabled if the row does not have a Vial number defined.
Normalization Factor	Enter a number (greater than zero to 999,999.999999) to set the factor by which Area % values are multiplied by when reporting Normalized % (or Final Amount values when reporting Normalized Amount). Normalized % = Area % x Normalization Factor / 100. Normalized Amount = Final Amount x Normalization Factor / 100.
Multiplier	Enter a number (greater than zero to 999,999,999.99) to set a value by which the software multiplies preliminary amounts to generate final amounts.
Divisor	Enter a number (greater than zero to 999,999,999.99) to set a value by which the software divides preliminary amounts to generate final amounts.
Addend	Enter a number (greater than zero to 999,999,999.99) to set a value which the software adds to the preliminary amounts to generate final amounts.
Details	A button that displays a popup window containing a card view of the parameters not displayed in the main sample band.

NOTE: *The defaults values shown above for numeric parameters apply to the first row in a sequence. Rows added subsequently will use the value from the previous row as default.*

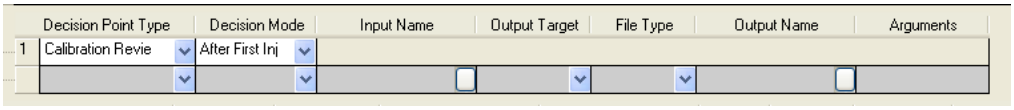
When the button in the Internal Standards field is clicked, a pop-up editor is displayed, listing the internal standards defined in the method. Here you can enter the individual amount for each internal standard present in the sample.



Decision Point Row (Band 3)

NOTE: *Different fields will be editable or de-selected depending upon which* ***Decision Point Type*** *is chosen.*

- Enter the following Sequence Decision Point information.



NOTE: *The Standard report template is displayed in the* ***Report View*** *window. You will need to maximize this window to view the Report. The report will only be viewable for the current run. You can save it in the Microsoft Excel format for later viewing.*

Parameters	**Description**
Decision Point Type	Select one of the following action for the sequence row from the drop–down list: Calibration Review, Peak Detection Review, Data Review, and Report. If your Sample type is Wash, Shutdown appears.
Decision Mode	Select one of the following from the drop down list. This field is for selecting on which injection(s) the decision point should occur: After First Injection, After Last Injection, and After Each Injection (where Injection refers to the number of injections specified in the main row).
Input Name	Select the name of the Report Template desired for this sample by clicking on the button and selecting the file from a File Open dialog.
Target Type	Enter the file type (Printer, File, or Email).
File Type	Select the type of file to be created as the output for a report. Adobe Acrobat (pdf) or Microsoft Excel (xls).
Output Target	Select where the report will be sent. Select either File or Printer.

Main Menu Commands-Sequence

The menu commands shown in the table below appear in the sequence environment.

Menu	Command	Description
File	New Sequence	If the current sequence has been modified, then you are first asked if it should be saved. New Sequence displays a new default sequence. For an online instance the sequence configuration is that of the connected instrument. In the case of an offline instance you are prompted to select a configuration (see New Sequence Configuration Dialog).
	Import Sample List ...	Opens the Import Sample List Wizard so you can import a sample list into a sequence.
	Open Sequence …	If the current sequence has been modified then you are first asked if it should be saved. Open Sequence displays the data selector in 'single sequence' selection mode. When you select a sequence it is displayed.
	Save Sequence	Saves the current sequence, replacing the existing version.
	Save Sequence As…	Displays a dialog enabling you to enter a Name and a Group for the new sequence, which will then be saved under a new identifier in the database.
	Print Preview Sequence ...	Displays the current sequence in a print preview method in accordance with the current print options (see Print Sequence Dialog).
	Print Sequence …	Displays the Print Sequence Dialog. After selecting the required options in the Print Sequence dialog (and click OK) the sequence is printed.
	Lock	Only displayed when user authentication/tracking is active. It minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	Online instance: If a single run is in progress or a sequence is running then you are prompted that the instrument must be in an idle state before the application can be closed (see Exit Dialog). If the instrument is not active then the following procedure for an offline instance will apply. Off line instance: If the current sequence has been modified then the user will first be asked if it should be saved. If unsaved data are present in any other environment then you are prompted to save each in turn. Closes the application window.

View	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Reprocess environment. The same action as clicking the Reprocessing button in the Navigation Pane.
	Toolbars ► View	Toggles display of the View toolbar.
	Tools	Toggles display of the Tools toolbar.
	Graphics	Toggles the display of the Graphic toolbar.
Tools	Export ►	
	Chromera Results…	Displays the Database export dialog.
	Methods…	Displays the Method export dialog.
	Sequences…	Displays the Sequence export dialog.
	Report Templates…	Displays the Report template Export dialog.
	Import ►	
	TotalChrom Data…	Displays the Import TotalChrom data dialog.
	Chromera Results…	Displays the Import Results dialog.
	Chromera Methods…	Displays the Import Methods dialog.
	Chromera Sequences…	Displays the Import Sequences dialog.
	Chromera 2.0 Data...	Displays the Import Chromera 2.0 data dialog.
	Report Templates…	Displays the Report template Import dialog.
	Preferences	Displays the Preferences window, showing the preferences associated with the current user (see Setting Preferences).
	Report Format Wizard	Displays the Report Format Wizard in New Report mode (see Report Format Wizard).
	Sequence Wizard	Displays the Sequence Wizard (see Sequence Wizard).

	Device Connections	Displays the Device Connections dialog.
	Error Log	Displays the Error Log dialog
	Dictionary Editor	Displays the Dictionary editor dialog
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector (see Batch Reprocess). It is disabled in an online instance when data acquisition is in progress, or a sequence is running.
Display	No Commands	
Action	No Commands	
Help	Topics	Opens the Chromera Help window.
	Consumables and Accessories	Opens the Consumable and Accessories Catalog
	About Chromera	Displays the copyright and version dialog.

Sequence Editor Standard Toolbar

This toolbar displays in the Sequence Editor:

Or when user authentication active:

Icon	Command
	File/New Sequence
	File/Open Sequence
	File/Save Sequence
	File/Save Sequence As
	File/Print Preview Sequence
	File/Print Sequence
	File/Lock (Only when user authentication active)
	File/Exit
	Help/Topics

Common Toolbars

There are two common toolbars displayed in every environment: **View** and **Tools**. A Standard toolbar will always appear but the contents will change depending on the environment. The standard toolbar is defined within the sections describing the individual environments.

View Toolbar

This toolbar simply mirrors the buttons in the Navigation Pane and the commands in the View menu.

Icon	Command	Description
	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Batch Reprocess environment. The same action as clicking the Batch Reprocessing button in the Navigation Pane.
	Reports	Switches to the Report Viewer.

Tools Toolbar

This toolbar includes the most commonly used commands from the Tools menu.

Icon	Command	Description
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector in single batch selection mode.
	Export ► Chromera Results… Methods… Sequences…	 Initiates the Export of Chromera results by displaying the Database export dialog. Initiates the Export of Chromera methods by displaying the Database export dialog. Initiates the Export of Chromera sequences by displaying the Database export dialog.
	Import ► TotalChrom Data… Chromera Results… Chromera Methods… Chromera Sequences…	 Displays the Import window. Displays the Import Results dialog. Displays the Import Methods dialog. Displays the Import Sequences dialog.
	Preferences	Displays the Preferences window, showing the preferences associated with the current user.
	Report Format Wizard	Displays the Report Format Wizard in New Report mode.
	Sequence Wizard	Displays the Sequence Wizard.
	Device Connections	Displays the Device Connections dialog.

Import Sample List

The Import Sample List Wizard is designed to make creating a Chromera sequence from external data quick and easy. The wizard remembers and displays the settings from the previous session for the current user on the current instrument, so the process can be even simpler on subsequent occasions.

On the initial page of the Wizard you can select the file from which the sample list information is to be imported, indicate how the mapping of columns from the source file to the Chromera sequence will be managed, and select the Chromera Method to be specified for each imported row. To select a new source file the click on the Browse button, which displays a standard Windows file selector. The **Files of type** option available in the **Sequence File Selector** dialog is a Delimited Text (CSV) file. After selecting a file and closing the dialog, the selected file name will appear in the edit field within the Wizard.

The Sample List Import Wizard process will add samples defined in a delimited text file (CSV) to a new sequence constructed for the current instrument.

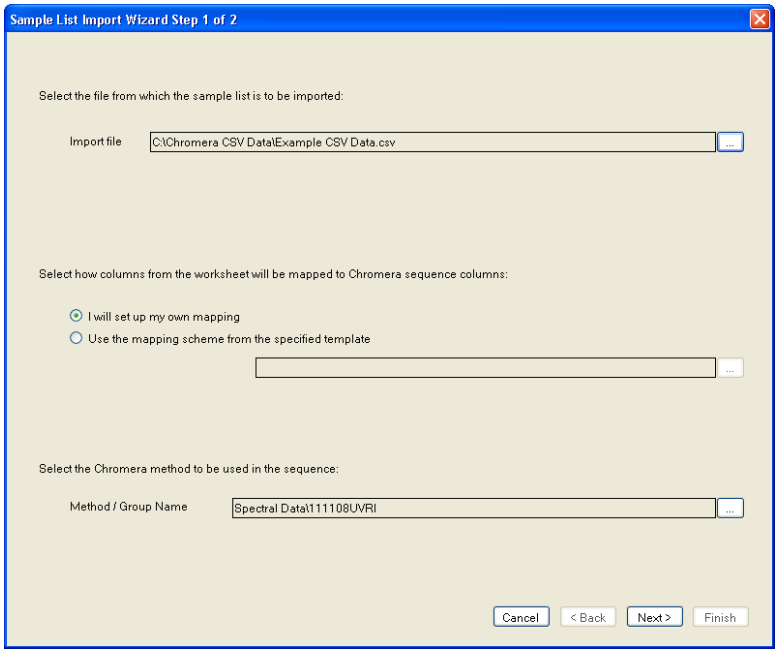
How to Import a Sample List

To import a sample list/table into a Chromera sequence, follow these steps:

1. Select **Sequence** or **Run Time** (Sequence mode) environment.
2. Open a sequence or create a new sequence.
3. Select **Import Sample List** from the **File** menu.

NOTE: *In Run Time this command will be disabled if the sequence is currently running. It will be available when the sequence is stopped or paused.*

The Import Sample List Wizard starts displaying Step 1 of 2 (the first dialog of the Wizard). **The Wizard will display the settings from the previous session for the current user on the current instrument.**

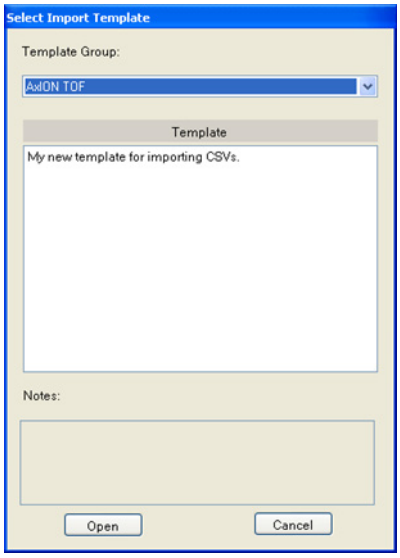


4. To **Select the file from which the sample list is to be imported**, click the **Browse** button to display the **Sequence File Selector** dialog and navigate to the directory containing your files.

5. Select a delimited text file (for example, .csv) from which to import a sample table.
   The first row of the delimited text file must contain the names of the columns within the file.

6. Click the **Open** button.
   The file name displays in the **Import file** text field.

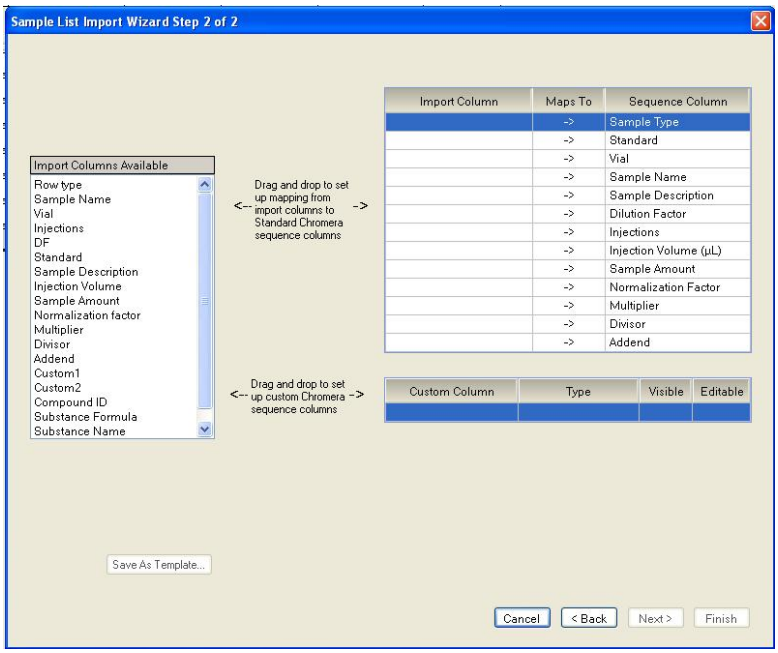
7. To **Select how columns from the worksheet will be mapped to the Chromera sequence columns**, you can select one of the following:
   - **I will set up my own mapping.**
     Using this selection, you will set up your own mapping in the Sample List Import Wizard Step 2 of 2.
   - **Use the mapping scheme from the specified template.**
     Using this selection, click the Browse button to display the **Select Import Template** dialog. The template name displays in the text field. You can create a template once you have initially defined a mapping scheme in the Sample List Import Wizard Step 2 of 2, so that you will never need to define that scheme again.



8. To **Select the Chromera method to be used in the sequence**, click the Browse button to display the **Data Selector - Single Method** dialog. Navigate to and select the method. Click the **Open** button to display the method in the **Method Name / Group Name** text field.

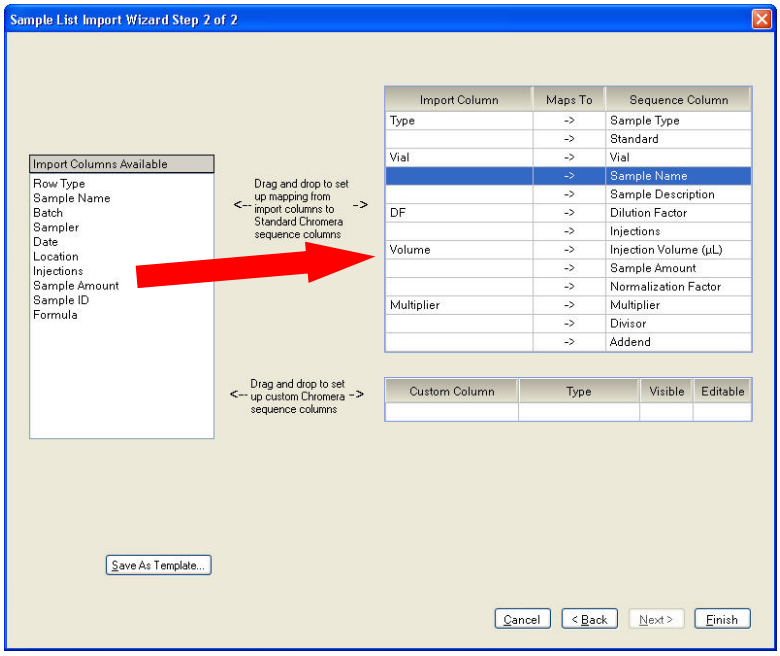
9. Click **Next**.

The **Sample List Import Wizard Step 2 of 2** dialog displays.

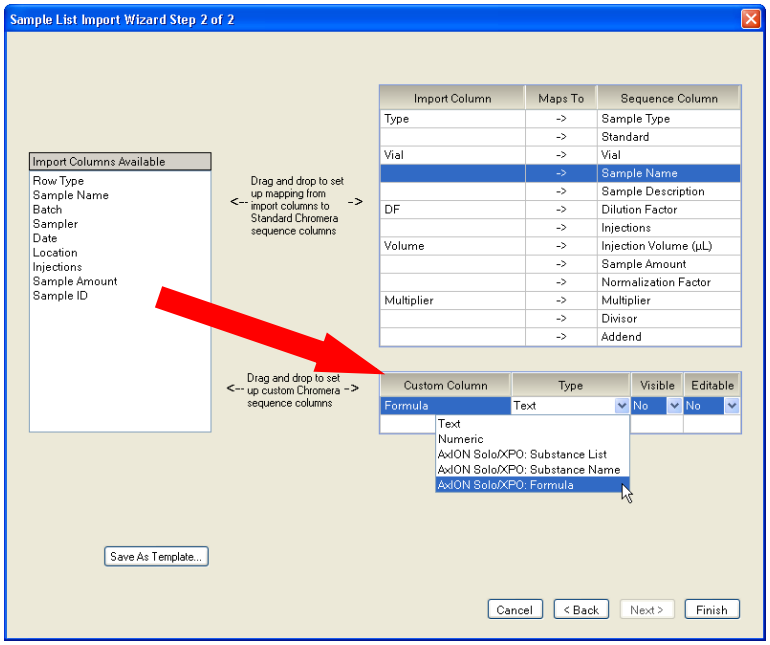


If you selected **I will set up my own mapping** on the previous screen (Step 1 of 2), then the dialog displays a list of the .csv source information headers in **Input Columns Available** and the **Import Column** is blank.

10. To define the mapping scheme for standard Chromera sequence columns, drag items from the **Import Columns Available** list and drop them in an **Import Column** cell adjacent to the appropriate Chromera **Sequence Column** item.



To define custom Chromera sequence fields, drag items from the **Import Columns Available** list to a **Custom Column** cell in the lower table. When an item is dropped in this table the data **Type**, **Visible**, and **Editable** drop-down lists will be activated.

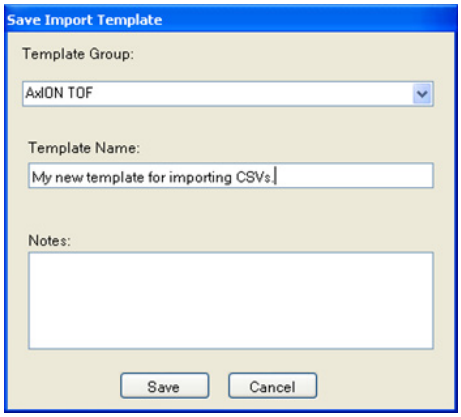


NOTE: *In the following example table AxION Solo/Xpo is shown as an example but not functional.*

Control	Description	Options
Type	A drop-down list to define the nature of custom field	Text Number AxION Solo/Xpo: Substance List AxION Solo/Xpo: Substance Name AxION Solo/Xpo: Formula
Visible	A drop-down list to indicate if the custom field will be visible in the Chromera Sequence Editor	Text /Number fields: Yes, No AxION Solo/Xpo fields: n/a (disabled)
Editable	A drop-down list to indicate if the custom field can be edited in the Chromera Sequence Editor	Text /Number fields: Yes, No AxION Solo/Xpo fields: n/a (disabled)

11. Once a mapping scheme has been defined, save this as a template by clicking the **Save As Template** button and entering identification for the template.

    The template preserves only the user fields that are mapped to Chromera sequence fields or defined as custom fields. Entries for the **Template Group** and **Template Name** fields are mandatory and the **Save** button will only be enabled when entries exist for both items. An entry in the **Notes** field is optional.



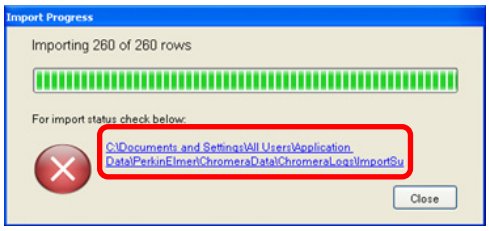
This template can then be specified for use in subsequent import sessions by selecting the template in **Use the mapping scheme from the specified template** on the initial page of the Wizard.

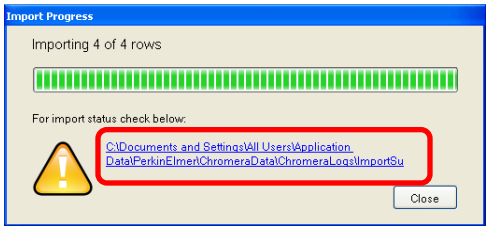
NOTE: *Since the settings on the first page of the Wizard are saved from session to session, if the import operation is a routine in which a file of the same name and structure is imported every day, all you have to do is select Import Sample List from the File menu, review the contents of the first wizard page and click Finish. The created sequence can then be reviewed and customized (e.g. with reports or other decision points) as required.*

12. When done click the **Save** button.

13. Click the **Finish** to import the sample list.

    The **Import Progress** dialog displays.





- A green circle with a ✓ indicates that all data imported successfully.
- A red circle with an **X** indicates that all rows did not import successfully.
- A yellow triangle with an **!** indicates that all rows imported successfully but there may be a problem with the fields.

14. If you want to check the import status, click the summary log link next to the green ✓, red **X**, or yellow **!**. The log indicates the success or failure for importing each data row in the import file.

15. Click the **Close** button. If no sequence is currently open one is created for the current instrument.

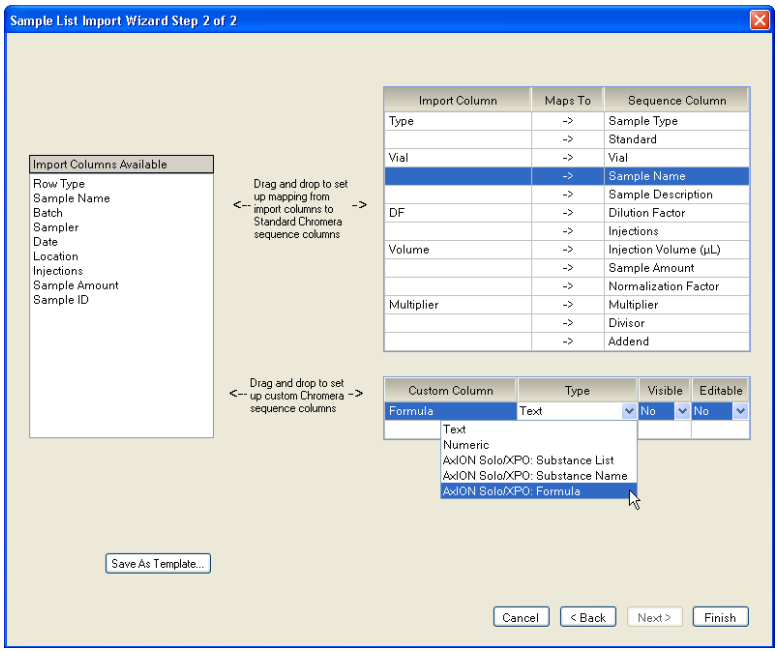
    The sample table is imported (appended to any existing rows) and displayed.

16. When done remember to name and save the sequence.

Importing Substance Data

Users importing sample list data from a .csv file are able to define a custom field as being a **Substance List** field. Chromera will interpret this as a string to be imported and saved in the custom field flagged as a substance list. This identification of the field is crucial to the correct passing of this information to the substance list source software when the sequence row is executed.

As an alternative to being able to define the name of a pre-existing substance list, you are able to indicate that fields being imported from the .csv file represent elements of a single target file. By definition, this is a file that does not yet exist and must be created during the import process.



If a column is headed **Formula** in the user's .csv file it has been defined as a **Formula**. This means that for every row imported an XML single target file will be created with the Formula element of the XML populated with the contents of the Formula field from the .csv file. If a user's data does not define the other element in the single target file (Substance Name), this element will not appear in the created single target file.

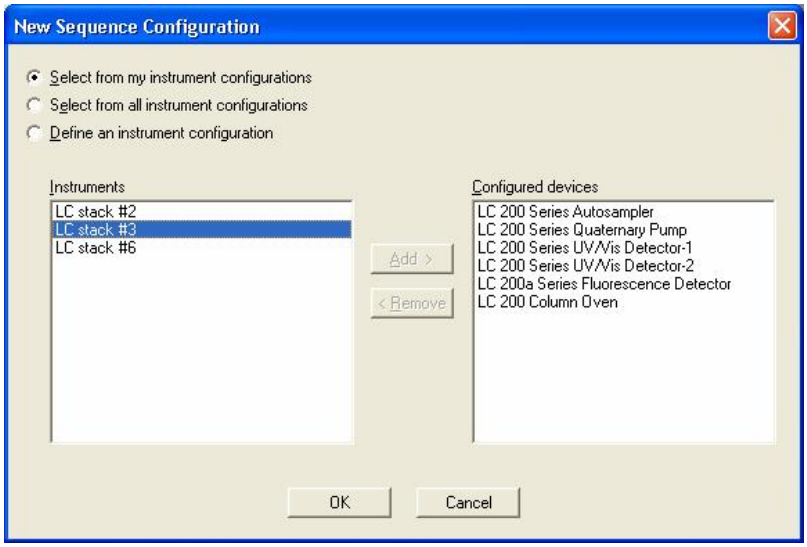
Note that for the substance list and the other elements in the custom fields grid shown above the **Visible** and **Editable** fields will be disabled and will show n/a. This is because these elements will have been written to the substance list XML file and will therefore not be available individually. The Chromera sequence will only retain the file name of the substance list or single target file and not the individual elements. By contrast, in the example above, the user could elect to display the Compound ID field in the Chromera sequence, since this is a simple text field and not part of a substance list or single target file.

NOTES:

1. If the user maps a .csv field to **Substance List** it will not be possible to map another field to any single target element (Name, Formula). The converse is also true – once an import field has been defined as a single target element it will not be possible to define another field as a Substance List.
2. Only **one instance** of any element can appear in the custom table. That is, it is not possible to define two substance lists, two Formula elements, or two Name elements.

New Sequence Configuration Dialog (Offline)

When you are in an **offline** instance of Chromera, the New Sequence Configuration dialog is displayed when you select the **New Sequence** command from the **File** menu. It enables you to define the instrument configuration to be used for the new sequence. Then you can select an existing configuration or define an instrument structure specifically for use with the new sequence.



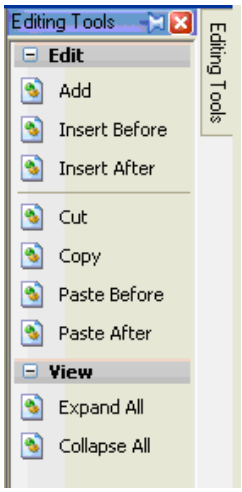
Control	Description
Select from my instrument configurations	Select to indicate an instrument configuration assigned to the current user will be selected for the new sequence.
Select from all instrument configurations	Select to indicate an instrument configuration from the full system collection will be selected for the new sequence.
Define an instrument configuration	Select to indicate that you will construct an instrument configuration for the new sequence.
Instruments	Displays a list of existing instrument configurations for you to define the new sequence structure. This list displays all of your existing defined configurations or all of the instrument configurations defined on the system (depending on the radio button selection).
Available Devices	Displays a list of devices comprising the instrument for the new sequence. It is only displayed when the third radio button option is selected. The list displays all instrument devices supported by the Chromera software.

Configured Devices	Displays a list of the **Configured Devices** making up the instrument. If an existing instrument is selected from the **Instruments** list then the devices assigned to that instrument are displayed in this list. If an instrument configuration is being defined then the list contains those devices assigned to the instrument so far.
	When a device name is selected in the **Available Devices** list, clicking this button copies the device name to the **Configured Devices** list. If a device of that type already exists then a numeric identifier will be appended, so that each occurrence of the device can be distinguished. When items from the **Available Devices** list are added to the **Configured Devices** list, the selected items in the **Available Devices** list are deselected/unhighlighted.
	Selecting one or more device names in the **Configured Devices** list and clicking this button, removes the device name(s) from the list. All items are then deselected in the **Configured Devices** list.

NOTES: *An instrument configuration is required in association with a sequence for two reasons: (1) An ICP-MS instrument sequence uses a different set of Sample types from 'pure' LC instruments. (2) If a Series LC 200 autosampler is to be used when the sequence is run, a tray type must be defined. This also serves to set the allowed range for vial number within the sequence.*

Sequence Editing Tools

The sequence Editing Tools are available as a tab on the right side of the sequence. To display them, move the cursor over the Editing Tools tab. To display those tools permanently, click on the thumbtack icon on top of the Editing Tools window. Use the editing tool to modify your sequence.



To **delete** a Sequence item (row) highlight the row and press the Delete key on your keyboard. To select multiple rows, hold down the Ctrl key and select the rows you want to delete, then press the **Delete** key.

You can also fill a column with the same information by right-clicking in a cell and select **Fill Down** or **Fill All**. To clear an entry in the cell select **Clear**.

Control	Description
Edit Controls	
Add	Displays the Add Item to Sequence dialog which will add samples to the end of the Sequence.
Insert Before	Displays the Insert Item in Sequence dialog. It places a new item before the selected row.
Insert After	Displays the Insert Item in Sequence dialog. It places a new item after the selected row.
Cut	Deletes selected rows from the sequence but retains them on a clipboard (not the Windows clipboard). Any existing rows on the clipboard will be replaced by the new Cut action.
Copy	Copies selected rows from the sequence and retains them on a clipboard. Any existing rows on the clipboard will be replaced by the new Cut action.

Paste Before	Inserts rows held on the clipboard into the sequence above the currently selected row. The clipboard rows are pasted as a contiguous block even if they were not adjacent when originally copied to the clipboard. If there is more than one selected row when the Paste command is given, the rows will be pasted above the first row number selected. If there is no selected row, an error message will be displayed.
Paste After	Inserts rows held on the clipboard into the sequence below the selected row. The clipboard rows are pasted as a contiguous block even if they were not adjacent when originally copied to the clipboard. If there is more than one selected row when the Paste command is given, the rows will be pasted below the last row number selected. If there is no selected row, an error message will be displayed.
View Controls	
Expand All	Displays all decision point rows for every line of the sequence.
Collapse All	Hides all rows of a sequence except for the identification row (first row).

Popup Menu

Right-clicking in a cell displays the sequence editor context menu to assist you in editing the sequence. It contains the following commands.

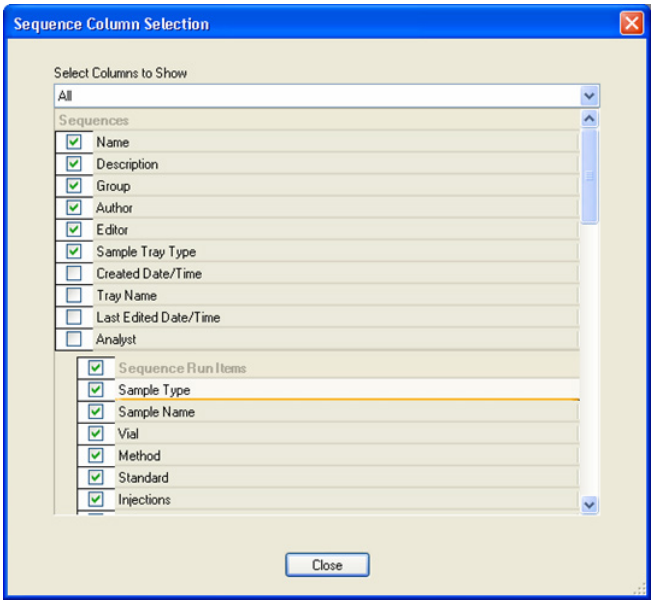
Command	Description
Fill Selected	Copies the value in the first cell of the selection to the remaining cells in the selection. See NOTE below.
Fill Down	Copies the value in the current cell to the corresponding cell in all rows below (where applicable). See NOTE below.
Fill All	Copies the value in the current cell to the corresponding cell in all rows (where applicable). See NOTE below.
Select Columns…	Displays the Sequence Column Selection dialog
Expand All	Fully expands the results grid to show all bands.
Collapse All	Hides all peaks bands in the grid leaving only the sample/injection bands shown.

NOTE: *The **Fill Selected, Fill Down, and Fill All** commands are disabled for Decision Point rows, since they make little sense in that context. The **Fill Down** and **Fill All commands** have slightly different behavior on the **Vial** cell. In this case the value is incremented for each applicable cell. The **Fill Selected** command copies the value from the first cell in the selection to the remaining cells.*

Sequence Column Ordering

The feature of column ordering by drag-and-drop is used in the sequence parameter display. This allows you to display the selected columns in each band in the preferred order. The order of the columns will be retained at the end of the session and used the next time that you open the sequence editor.

The Sequence Column Selection dialog (displays when you right click in the sequence) lists the columns available for display in all sections of the sequence in a hierarchical format.



Control	Description
Select columns to show	Select from the drop-down list the parameters to display in the list beneath, they are: **All** – Shows all parameters for all sections of the sequence. **Decision Points** – Shows only the decision point parameters. **Per Sample Report** – Shows only the columns for the Per Sample Report global parameters. **Sample Naming Template** – Shows only the columns for the Sample Naming Template global parameters. **Sequence Row Items** – Shows only the columns available for Sequence Row Items sequence sample rows. **Sequence** – Shows only the global sequence identification items.
Sequence	A section of the check list that shows the sequence identification parameters, they are: Author, Created Date/Time, Description, Editor, Group, Last Modified Date/Time, Name, and Sample Tray Type.

Sequence Row Items	A section of the check list that shows the parameters for each sample row, they are: Addend, Dilution Factor, Divisor, Injection Volume, Injections, Method, Multiplier, Normalization Factor, Sample Amount, Sample Description, Sample Name, Sample Type, Standard, and Vial. **NOTE**: The Details and Internal Standard Amounts columns contain buttons and therefore cannot be hidden by this mechanism.
Decision Points	A section of the check list that shows the decision point parameters, they are: Decision Mode, Decision Point Type, File Type, Input Name, Output Target, and Target Name.
Per Sample Report	A section of the check list that shows the Per Sample Report global parameters, they are: File Type, Frequency, Output Name, Output Target, Report Template, and Sample Type.
Sample Naming Template	A section of the check list that shows the Sample Naming Template global parameters, they are: Apply To, Number, Prefix, Suffix, Vial Increment, and Vial Start.

About Decision Points

Decision Points can be used in a running sequence or a reprocessing sequence. One type pauses the operation of the system and the other type performs an action.

- The **Peak Detection Review, Calibration Review,** and **Data Review** Decision Points pause the operation of the system thereby enabling you to examine the results, and take appropriate action. If executed during real time, the sequence pauses. You must be present to interact with Chromera since the system will remain in the paused state until a decision is made.

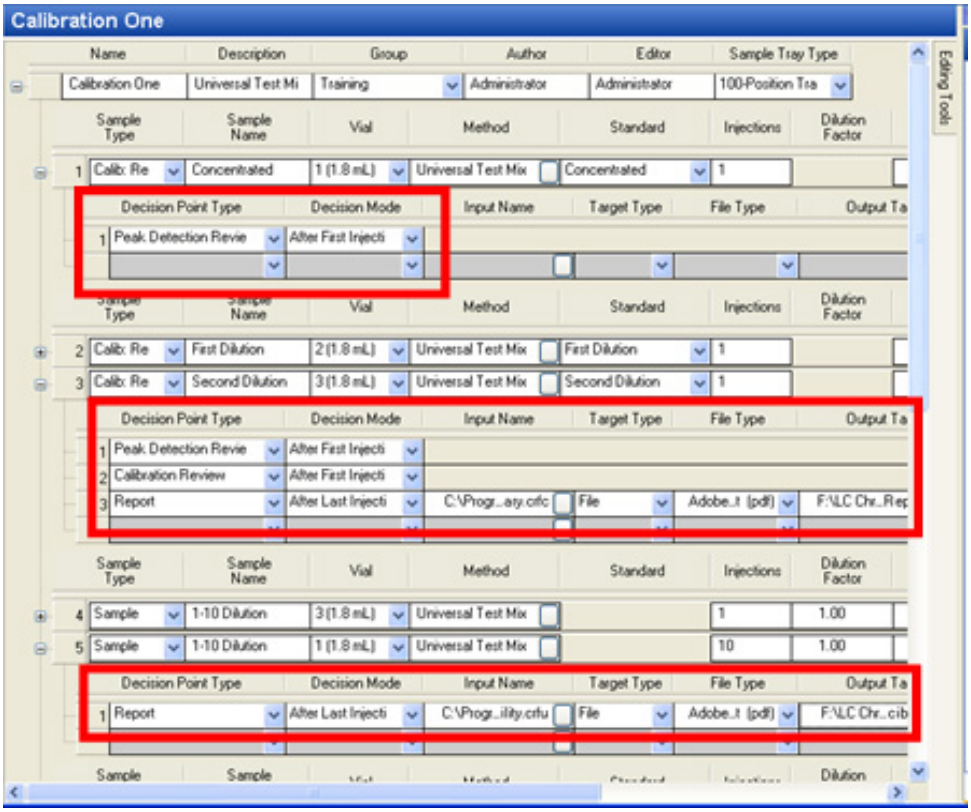
  For example: After running all the standards you can set a decision point to determine if the calibration curve is acceptable. If the calibration curve is not acceptable you can make changes (e.g., excluding a data point) and the adjusted calibration curve will be applied to all the subsequent runs.

- You do not need to be present for the **Report** and **Shutdown** Decision Points as they perform an action and the sequence continues.

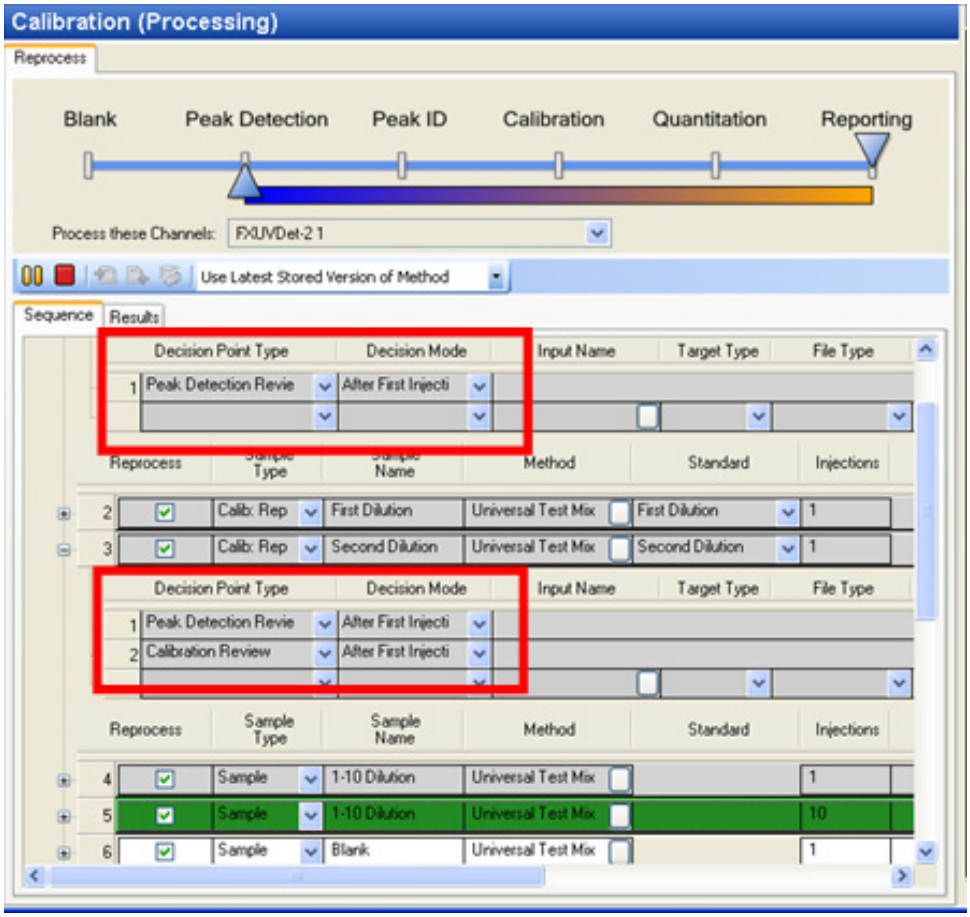
  For example: If you select Report, a report is printed or saved at that point in the sequence.

Decision points may be executed after the first injection, after the last injection, or after each injection. There is no limit to the number of decision points.

Example of Decision Points in a Sequence



Example of Decision Points in Reprocess



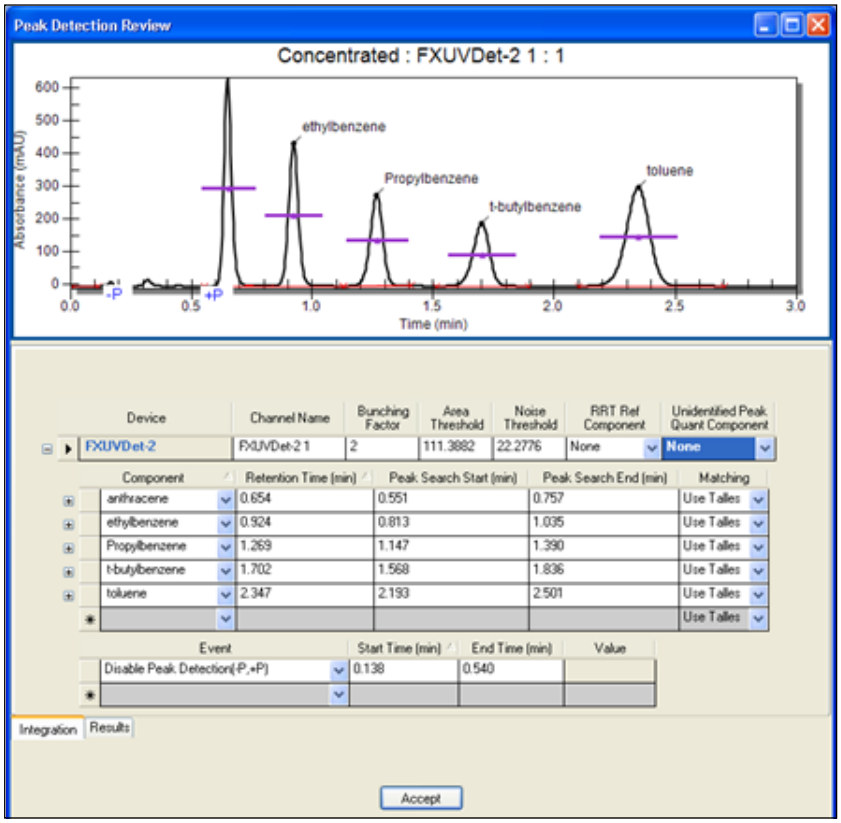
How to Set Decision Points

The following Decision Points can be set in a Sequence.

Setting a Peak Detection Review Decision Point

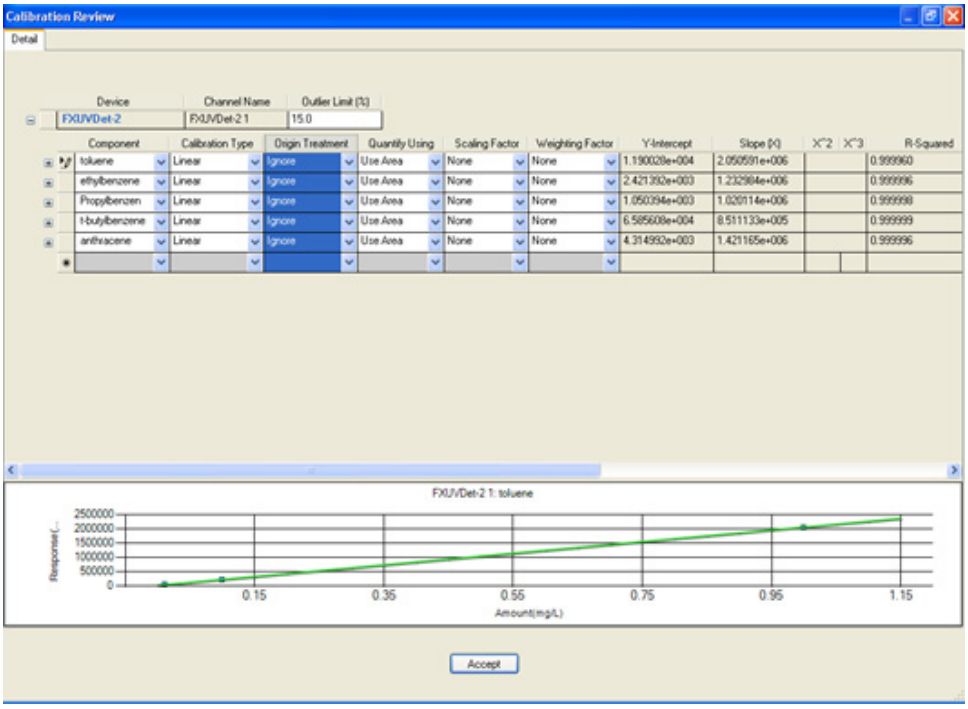
Selecting the **Peak Detection Review** decision point essentially pauses the sequence at this point and opens the Graphic Method Editor. Information that can be modified includes: Component Identification (Names, Retention times, and Search Windows), Data Handling Optimization (Bunching Factor, Noise and Area Threshold, and Baseline Timed Event).

After making modifications click **Accept**. The changes are saved to the method and the sequence progresses.

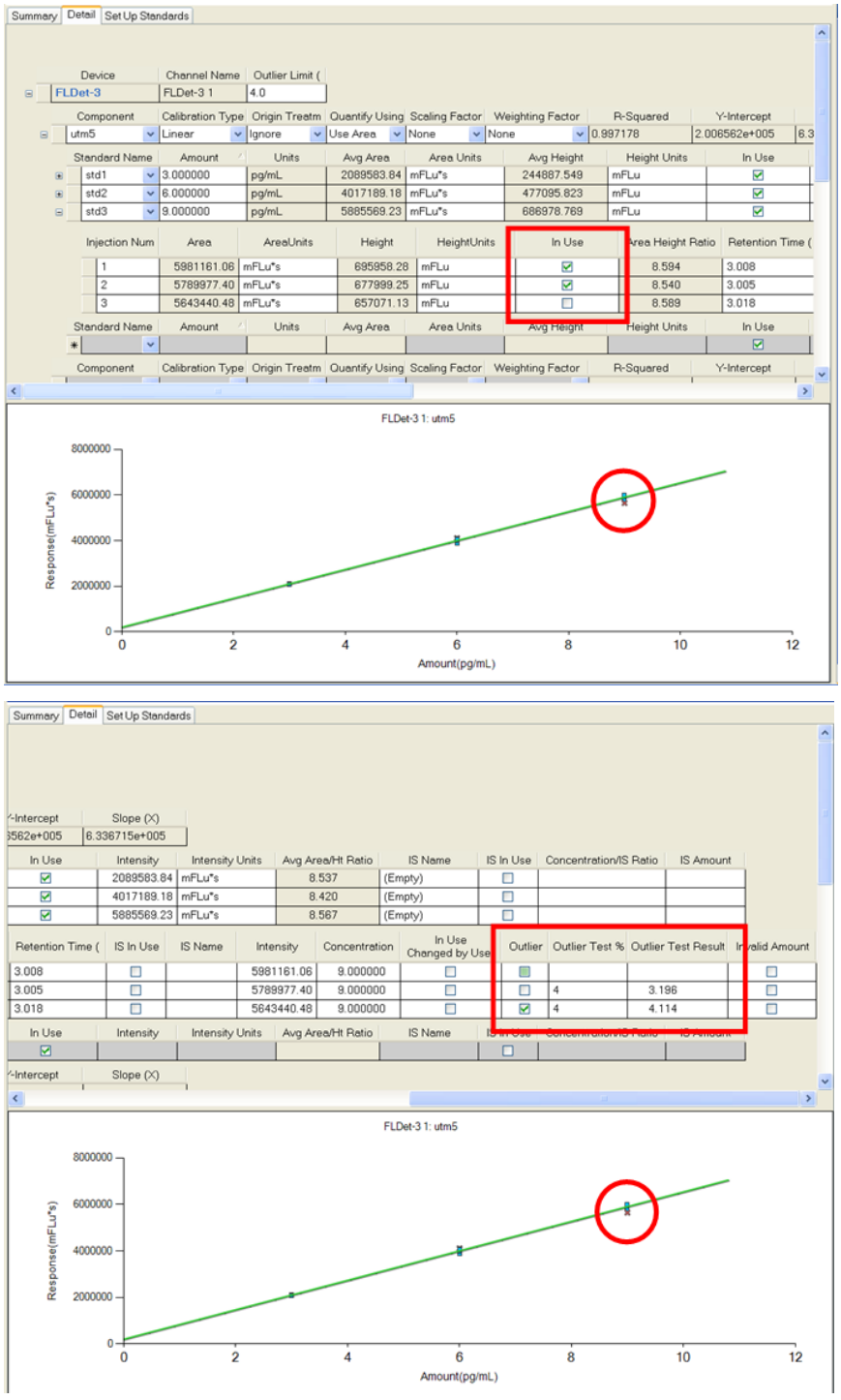


Setting a Calibration Review Decision Point

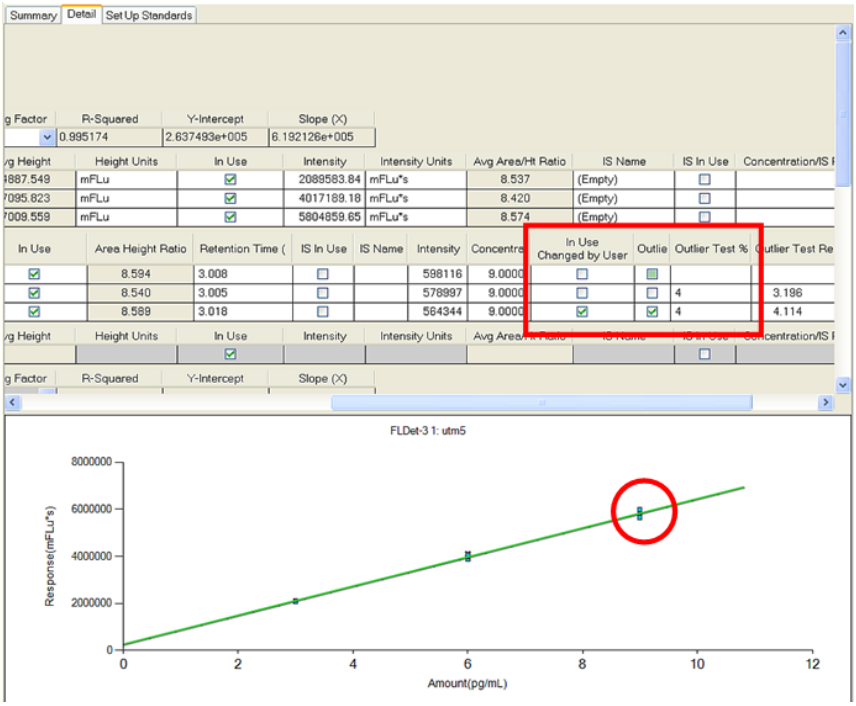
Selecting the **Calibration Review** decision point essentially pauses the sequence at this point and displays the curve information, data, and the calibration curve. Information you can modify includes: Type of Calibration, Data points used, and Treatment of the Origin (Ignore, Include, or Force).



Outlier points are calculated by Chromera based on the entered Outlier Limit value. When a point is calculated as an outlier the Outlier box is checked and the point is represented as an X on the graph. Also, the In Use box is not checked. In this example, Injection 3 is determined to be an outlier. The actual information on the screen is wider than your display so you need to use the scroll bar on the bottom of the screen to view all of the displayed information.



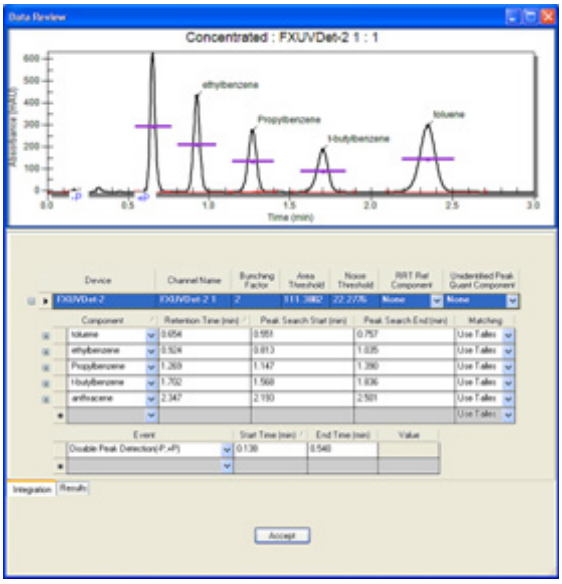
To include the Outlier in the calculations of the calibration curve check the **In Use** box. This is indicated by a check mark in the **In Use Changed by User** column.

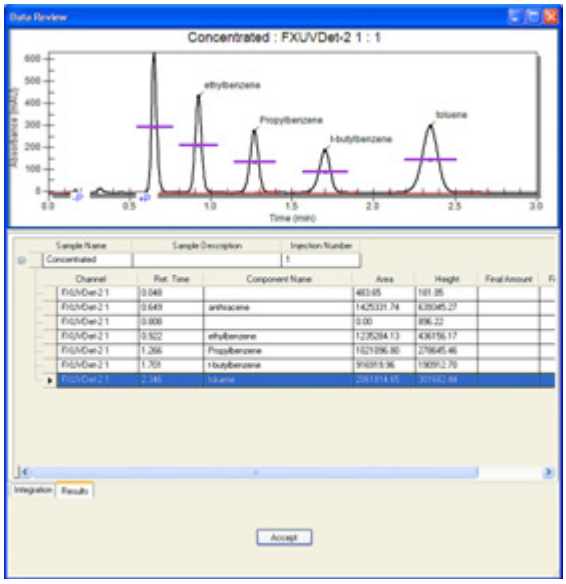


After making modifications, click the **Accept** button. This immediately updates the results.

Setting a Data Review Decision Point

Selecting the **Data Review** decision point essentially pauses the sequence at this point to review integrated chromatograms and results. The Results cannot be edited. Data Review has two views: Integration (Retention Times and Baseline Timed Events) and Results (Area, Height, Concentration, etc).

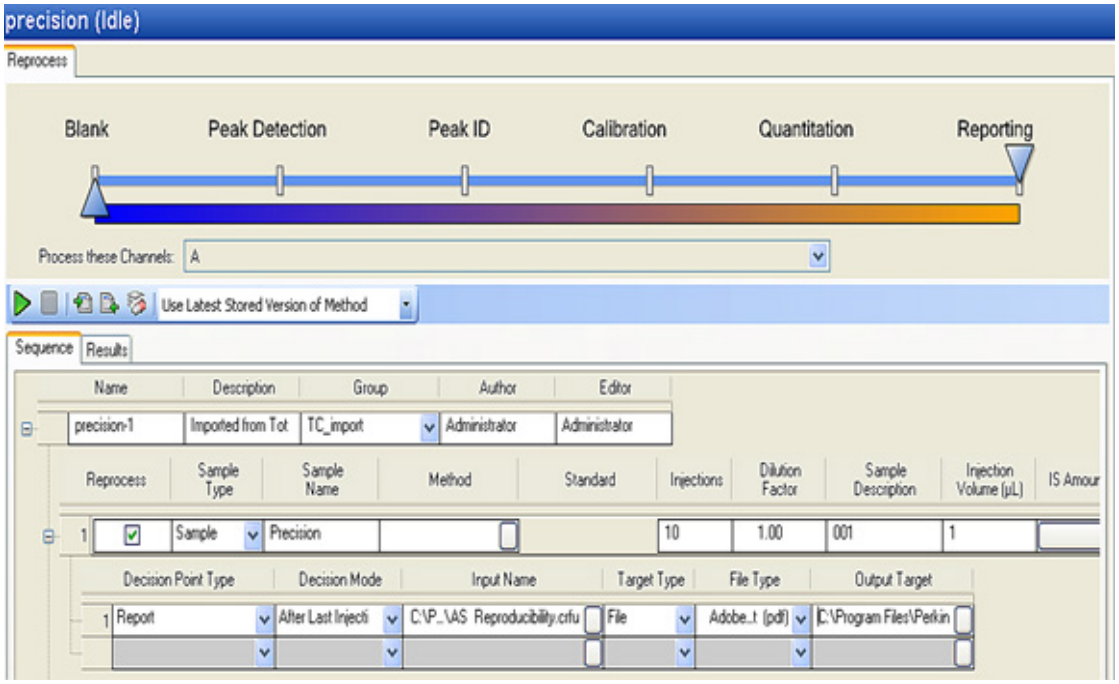




After reviewing click **Accept** and the sequence progresses.

Setting a Report Decision Point

Selecting a **Report** decision point enables the printing or saving of a specified type of report (e.g., Calibration, Method, Summary) at that point in the sequence. There is no user interaction with this Decision Point. Reports generated in this way are in addition to those specified as Global Reports in the sequence, or Per Sample Reports. Multi-sample Summary reports can ONLY be generated through a Report type Decision Point.



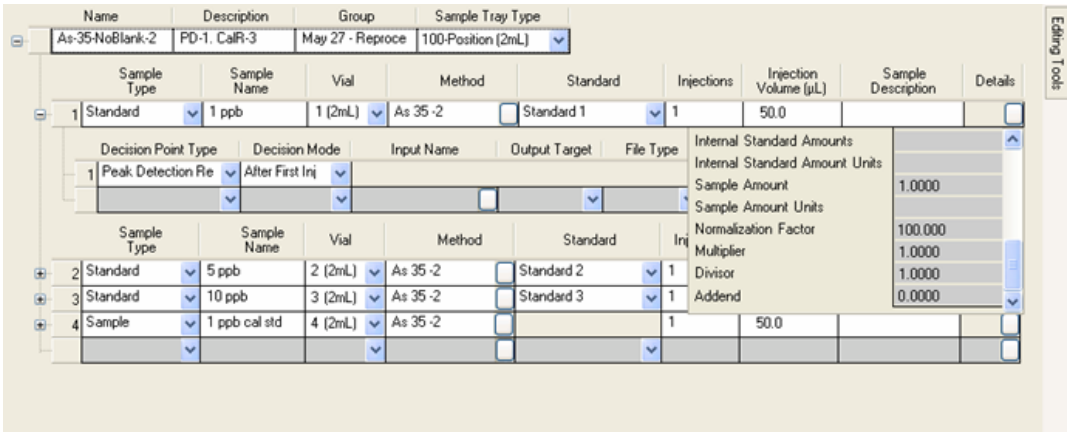
Setting a Shutdown Decision Point

The **Shutdown** decision point is ONLY available with a **Wash** sample type. In an LC application the pump stops and the detector lamp turn off. For LC/ICP-MS, the pump stops, the plasma turns off and peristaltic pump stops, the detector lamp turns off, and the oven temperature drops to 25 °C

This is executed after the **Wash** row in the sequence has been run.

Sequence Details View

Clicking the **Details** button on any row displays a popup window showing the parameters for the selected sample row that are not currently displayed.



The parameter values are editable within the popup window and the popup menu commands Fill Down and Fill All are available to propagate values from the current sample row to other sample rows. You are able to order the parameters within the View using drag-and-drop.

Vial Entry Validation

The samples vials defined within a sequence must be consistent with the type of sample tray selected for use with the sequence. There will be three distinct instances of validation, relating to different user actions. See also the discussion regarding tray and vial validation under Run Time.

NOTES: *Not all trays include vial positions that are a single contiguous range. The valid range or ranges of vial numbers for each tray type are defined in the Instrument Descriptor file for each autosampler model.*

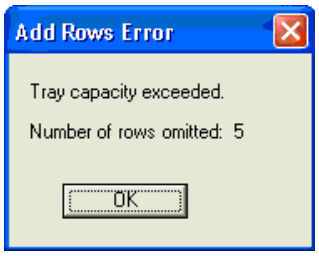
The only check is to ensure that every vial defined within the sequence actually exists in the specified tray.

Direct Vial Entry

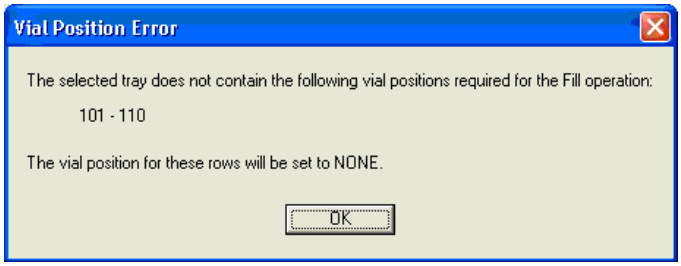
When you make an entry in the Vial field of a sequence row, what is actually occurring is a look-up in the list of available vials contained in the drop down list. If you press a character key that results in an entry not contained in the list, that character will not be displayed. For example, if the selected tray is the default 100-position tray and you press the key sequence 1 2 3, the final 3 will not appear since it creates an invalid vial number; leaving a vial entry of 12. It should therefore be impossible for you to enter an invalid vial number directly.

Add Rows or Fill Down

When you specify a range of sequence rows to be assigned a series of incrementing (not necessarily by 1) numbers, the software will check to ensure no vial numbers that do not exist for that tray are included in the generated series. If the Add procedure cannot be completed because it requires more vials than are available in the specified tray then an error message displays



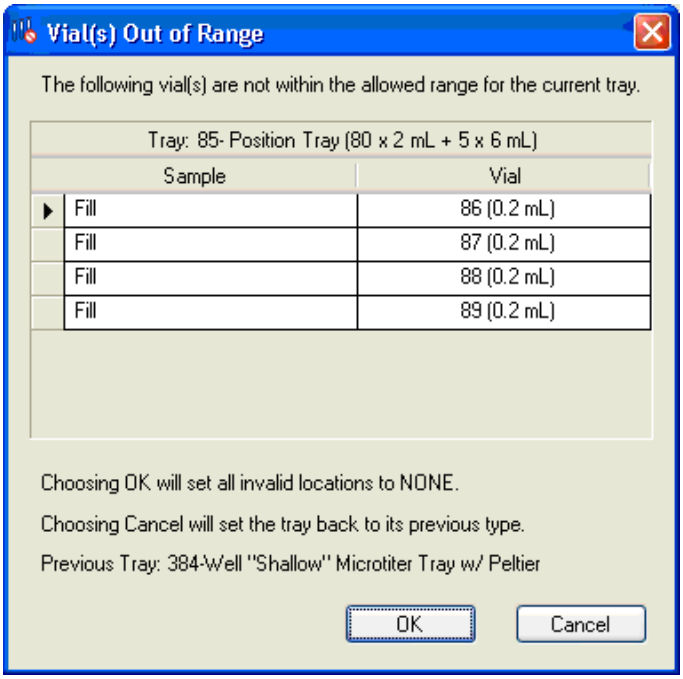
If the Fill Down cannot be completed because it requires more vials than are available in the specified tray then the Vial position Error message displays.



Clicking **OK** sets the Vial field for the indicated rows to NONE. This allows you to locate those rows easily once the error dialog has been closed, and to make the decision whether to delete the rows or assign valid vial numbers.

Tray Type change

If you change the tray type selection for an existing sequence, the software will check to see if all the vials specified in the sequence are available in the new tray. If any are not available then the Vial(s) Out of Range error message displays, indicating the vial positions not available in the new tray.

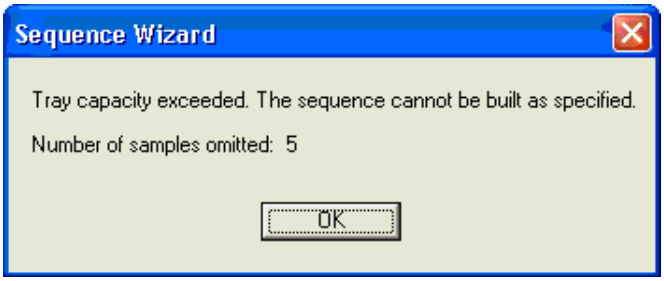


If you click **OK** the vial positions that are not available in the new tray type will be changed to NONE. This allows you to locate those rows easily once the error dialog has been closed, and to make the decision whether to delete the rows or assign valid vial numbers.

If you click **Cancel**, the tray type setting will revert to the prior selection.

Sequence Wizard Parameters

The validation action of the Sequence Wizard is to ensure that it never creates a sequence that references an invalid vial. If the specified tray includes discontiguous ranges, the wizard will take this into account and jump the gaps as necessary when building the sequence. If the building procedure cannot be completed because it requires more vials than are available in the specified tray then Sequence Wizard Tray capacity exceeded error message displays.



How to Build a Sequence using Sequence Wizard

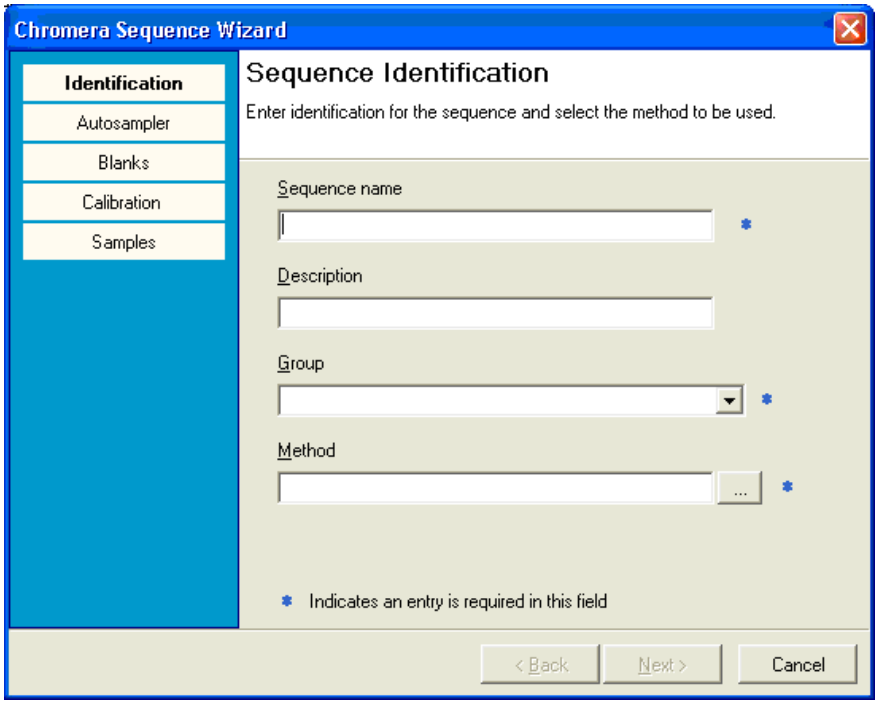
The Sequence Wizard provides a quick and simple way to build a sequence for routine sample analysis. It provides dialogs to enter information in a logical and structured way. The dialogs also contain next and back buttons to help you step through a sequence. The Wizard creates a sequence containing intermixed blanks, calibration standards, and samples, with the following constraints:

- The same method is used for all rows in the sequence
- If calibrations are specified, the first rows of the sequence (and hence first vials in the autosampler) is a calibration set
- Each calibration set will consist of one row per standard defined in the method
- If repeat calibrations are required they will appear at regular intervals (i.e. with a constant number of samples between them)
- Blanks can be inserted but only at regular intervals (i.e. with a constant number of samples between them)

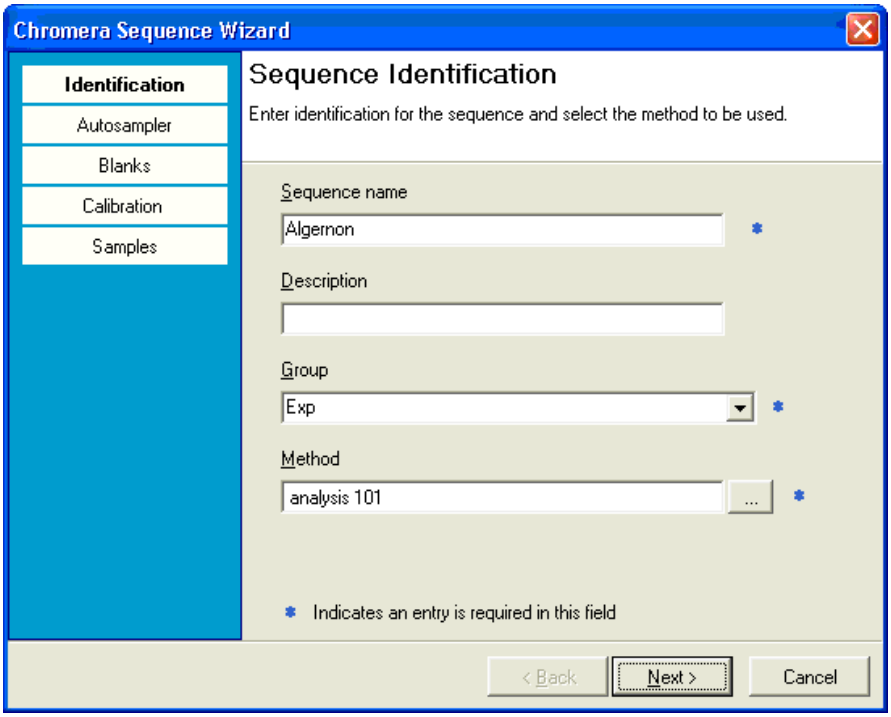
A sequence created using the Wizard can be edited in any way desired, using the standard Sequence Editor.

Sequence Identification Page

Use the Sequence Wizard initial screen to enter information to identify the sequence and select the method to be used. Required fields are indicated with an asterisk.



The Next button is enabled after entering a **Sequence name** and selecting a **Method**.



If the selected method is capable of being calibrated, that is if it contains at least one component with a valid calibration scheme (e.g. Linear through origin with one standard level, Linear with two standard levels, etc.), then the **Calibration** button will appear in the list.

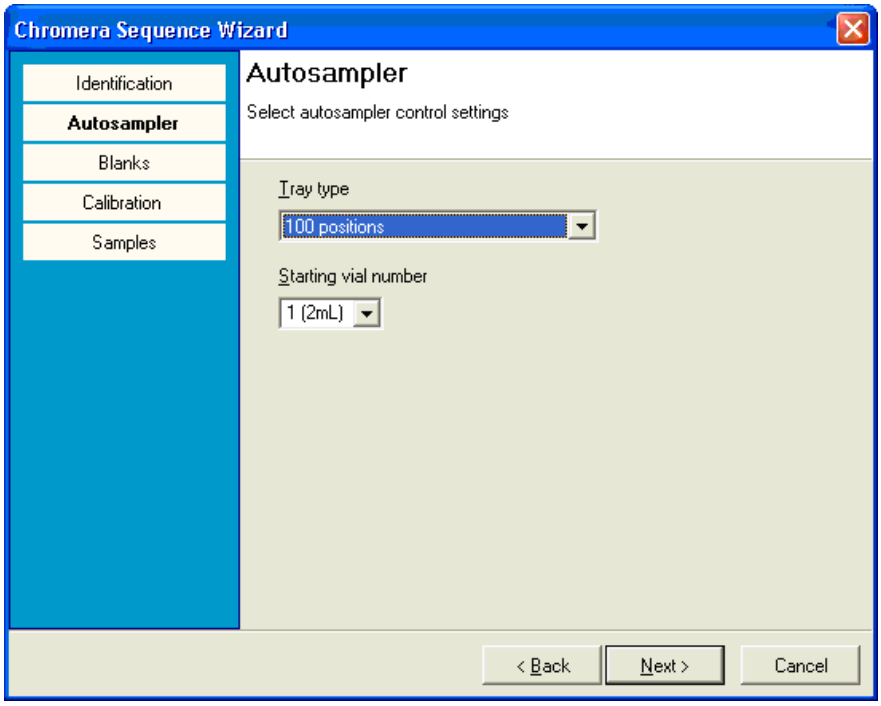
Enter the following parameters:

Control	**Description**
Sequence name	Type a name that defines the name by which to identify the sequence.
Description	Type a description about the sequence.
Group	Type a virtual folder name that can be used to assist in locating sequences in the database. You can also select an existing group name from the drop-down list.
Method	Select the method name to use in acquiring and processing data in the sequence. The method name is selected by clicking on the browse button.
...	Click the browse button to display the data selector in method mode, to select a method from the database.

The next page defines the **Autosampler** settings for the sequence.

Autosampler Page

The Autosampler page of the Wizard will only appear if an autosampler is configured on the instrument. This is where you select the autosampler control settings.



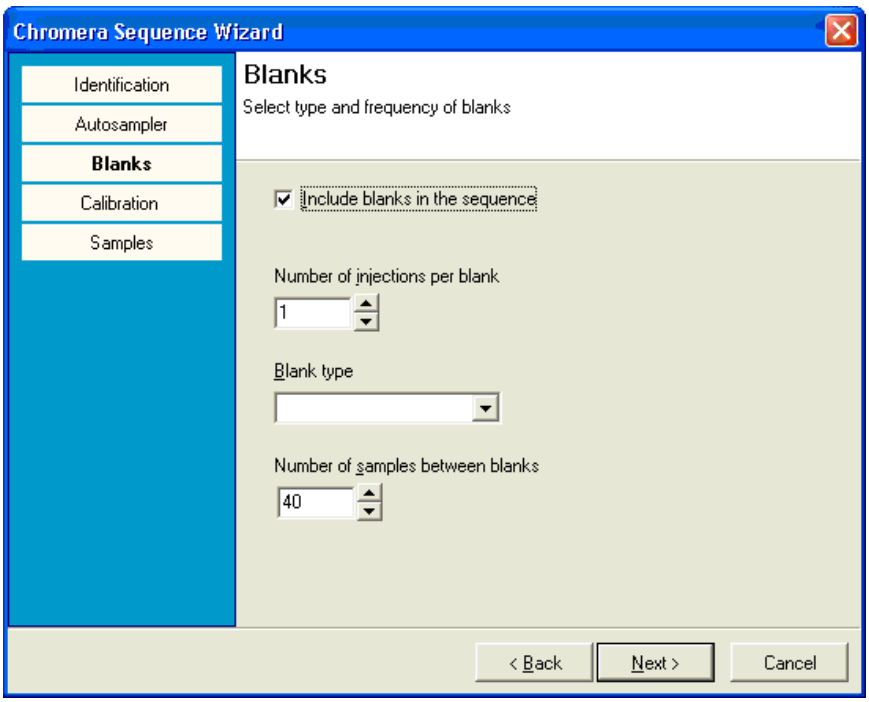
The page shown here is specific to the Series 200, Series 225, Series 275, and Flexar autosamplers, which are the models supported. Enter the following parameters:

Control	Description
Tray type	Select the tray to be used for injections made from this sequence. The list contains all available tray types for the current instrument.
Starting vial number	Set the vial position from which the current sample will be taken. Allowed range depends on the selection for Tray Type.

The next page defines the **Blanks** settings for the sequence.

Blanks Page

The Blanks page is where you set the type and frequency of blanks to be subtracted from sample analyses.



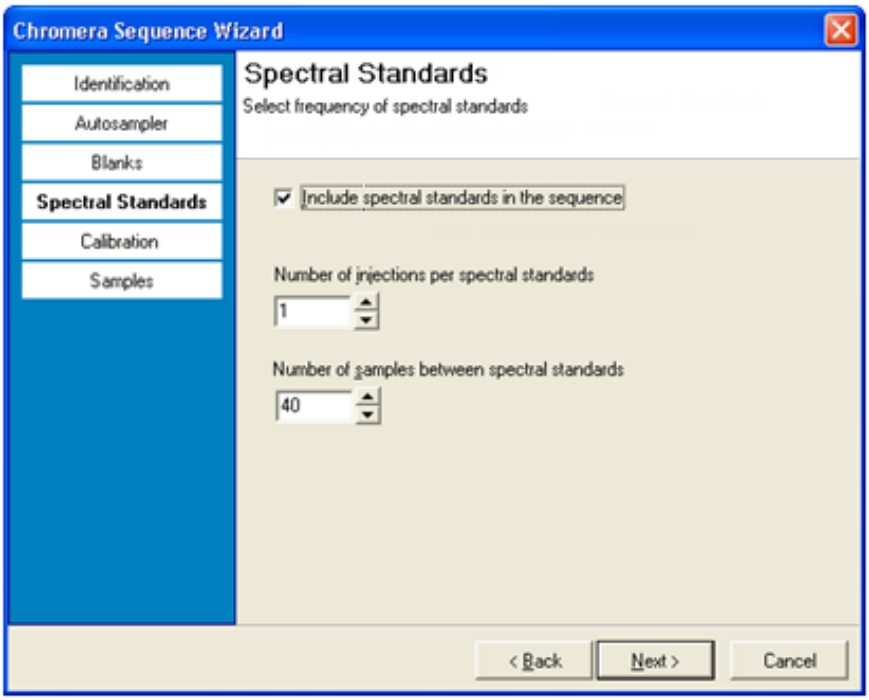
Enter the following parameters:

Control	**Description**
Include Blanks in the sequence	A check box to indicate that blanks will be run as part of the sequence.
Number of injections per blank	Set the number of times (from 1 to 9) each blank will be run. You can type in a value or use the buttons to set it.
Blank type	Select one of the applicable blank types for the current instrument. **For Speciation:** Blank (pt by pt), Reagent Blank, Standard Blank, CIS Blank **For General LC:** Matrix, Background
Number of samples between blanks	Set a value when an additional blank will appear in the sequence. You can type in a value (from 1 to a maximum number of vials available in the specified tray) or use the buttons to set it.

The next page defines the **Calibration** settings for the sequence.

Spectral Standards Page

The Spectral Standards page is where you set the type and frequency of blanks to be subtracted from sample analyses.

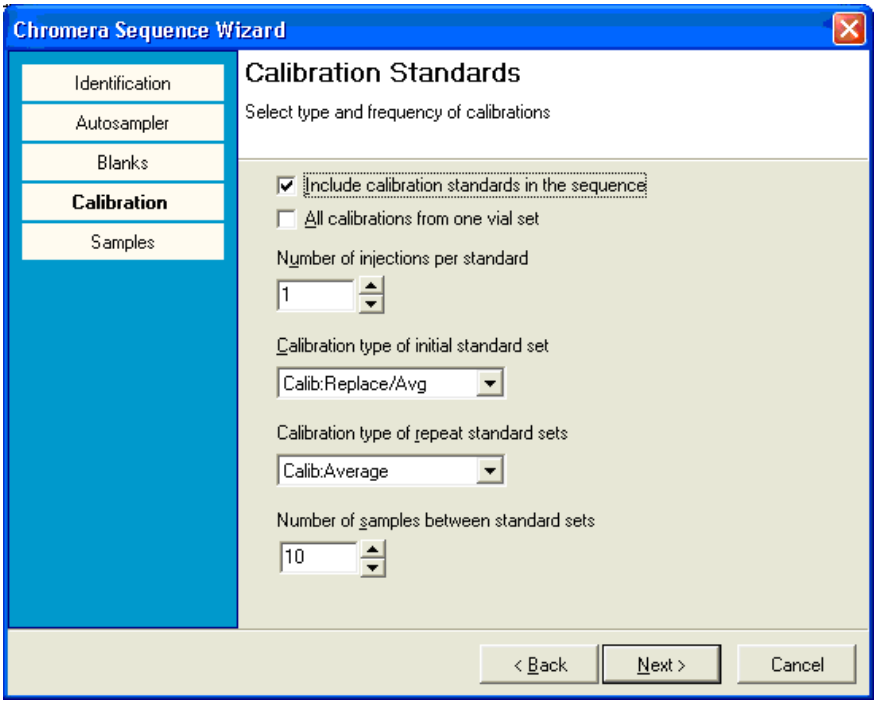


Enter the following parameters:

Control	Description
Include spectral standards in the sequence	A check box to indicate that spectral standards will be run as part of the sequence.
Number of injections per spectral standards	Set the number of times (from 1 to 9) each spectral standard will be run. You can type in a value or use the buttons to set it.
Number of samples between spectral standards	Set a value when an additional spectral standard will appear in the sequence. You can type in a value (from 1 to a maximum number of vials available in the specified tray) or use the buttons to set it.

Calibration Page

The Calibration page defines the interval at which calibration sets are inserted into the sequence, as well as the calibration types for the initial calibration set (first n rows of the sequence, where n is the number of standards defined in the method) and all subsequent sets.



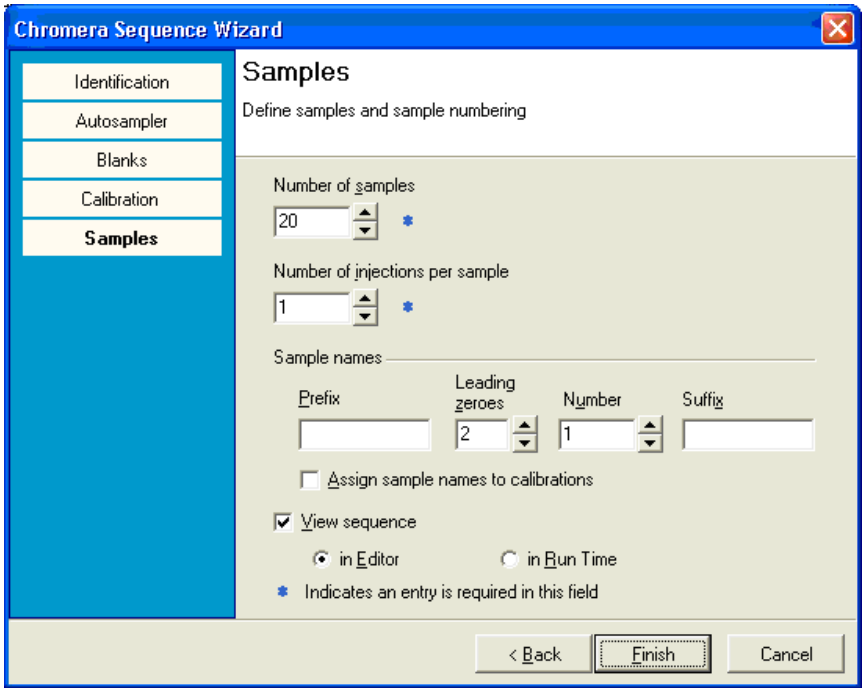
Enter the following parameters:

Control	Description
Include calibration standards in the sequence	A check box to indicate that calibration standards will be run as part of the sequence.
All calibrations from one vial set	A check box to indicate that only one set of calibration vials is present and that this set of vial numbers will be applied each time a calibration set is entered into the sequence.
Number of injections per standard	Set the number of injections (from 1 to 9) to be made of each calibration standard. You can type in a value or use the buttons to set it.
Calibration type for initial standard set	Select from the drop-down list the calibration type for the first set of calibration standards (the first rows) in the sequence. • for LC: Calib: Replace, Calib: Average • for ICP-MS: Standard
Calibration type for repeat standard sets	Select from the drop-down list the calibration type for additional sets of calibration standards appearing later in the sequence. • for LC: Calib: Replace, Calib: Average • for ICP-MS: Standard
Number of samples between standard sets	Set when an additional standard set will appear in the sequence. You can type in a value (from 1 to a maximum number of vials available in the specified tray) or use the buttons to set it.

The next page defines the **Sample** set for the sequence.

Samples Page

The Samples page enables you to define samples and sample numbering.



Enter the following parameters:

Control	Description
Number of samples	Set the number of injections (from 1 to a maximum number of vials available in the specified tray) to be made of each calibration standard. You can type in a value or use the buttons to set it.
Number of injections per sample	Set the number of injections to be made of each sample. You can type in a value from 1 to 9 or select it from the list.
Prefix	Set the start of each Sample Name for the added rows. The Sample Name consists of the **Prefix**, **Number** (incremented by one for each sample) and **Suffix** concatenated, without intervening spaces. You can use up to 25 characters.
Leading zeroes	Set the number of leading zeroes (from 0 to 4) appearing before the incrementing number within the sample name.
Number	Set the initial value of the incrementing number appearing in the Sample ID. The range is 0 to 9999 with as many digits as entered (e.g. 0000 is a valid entry).
Suffix	Set the end of each Sample ID for the added rows. You can use up to 25 characters.

Assign sample names to calibrations	A check box to set if the sample names constructed from Prefix/Number/Suffix should be entered for calibration standards as well as samples. The selections are Yes or No. This setting is enabled if calibration standards are included in the sequence.
View sequence	A check box to load the sequence into an editor for viewing after being generated.
In Editor In Run Time	Set the editor in which the generated sequence will be loaded.

NOTE: *The **Assign sample names to calibrations** check box on the page determines whether the sample names automatically generated from the 'Prefix', 'Number' and 'Suffix' fields is applied only for samples or also to calibration standards.*

When you click the **Finish** button, the sequence is built according to the settings within the Wizard and (optionally) displayed in the specified environment.

About Active Sequence

Any part of the sequence that has not yet been used, or is in the process of being used, can be changed. This means that the active row and all completed rows are read-only.

If you change the value of an editable sequence parameter and indicates the entry is complete (for example, by pressing the Tab key or selecting a different field) then the active sequence is updated immediately and the new value will replace the previous value as soon as that parameter is used.

All such changes to the active sequence will generate a log entry associated with the acquired data, indicating:

- The Row number and Sample Name of the changed row
- The change to parameter value or setting that was made
- The date and time of the change
- The user who made the change (the Full Name defined in the OS if available, otherwise the user account name)

For example - Row 5: Sample: "My test sample" Sample Amount: From: 1.0000 To: 2.5678 At: 02:34:55 PM August 30, 2008 By: Deb Peterson

The modified sequence row stored with the results data set will of course reflect the parameters that were actually used.

However, the sequence itself will remain unchanged in the database, unless the user chooses to save it. File/Save replaces the existing copy of the sequence with the modified version, as usual. The **Save As** command is available during active sequence editing but it will not affect the name of the active sequence or the name of the stored batch.

About Sequence Run Control

Starting/Ending Rows

By default a sequence is executed from the first row to the last, however it is possible for the user to specify starting and ending rows prior to giving the *Start Sequence* command.

Processing/Reporting

Options are available prior to starting (or resuming) a sequence to suppress all processing (peak detection to reporting) or just to suppress reporting. The latter (suppress reporting) setting over-rides both individual row decision point reporting as well as any global report setting.

Pause/Resume

During execution of a sequence it can be paused. This will have no effect on the currently running analysis (if any) but the next analysis will not take place while the sequence is paused. When you select Resume, by default the sequence will continue with the analysis immediately following the last completed one, however you may specify new a starting (resuming) row and/or ending row prior to giving the resume command.

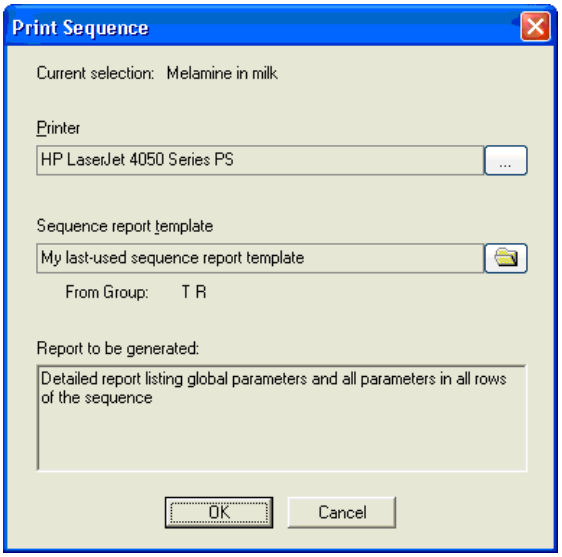
Also, a paused sequence may be stopped in which case the Resume command is no longer applicable.

Error Conditions

If for any reason the autosampler cannot make an injection from the specified vial (for example, there is no vial in the specified tray position), the software logs this event and continues sequence execution with the next row.

Print Sequence/Print Preview Sequence Dialog

The Print Sequence dialog is displayed when the **Print Sequence...** or **Print Preview Sequence...** command is chosen from the **File** menu within the Sequence environment. The dialog allows you to define on which printer the report will be printed and the report template to be used.



Set the following in this dialog:

Control	**Description**
Current selection:	Displays the name of the current sequence.
Printer	Displays the name of the printer to be used to output the sequence report. You can select from all the printers configured under Windows for the logged on (Windows) user.
...	Click the button to display the standard Windows select printer dialog. The name of the printer selected in the standard Windows dialog will be entered into the Printer field.
Sequence report template	Displays the name of the template to be used for generating the sequence printout.
	Click this button to select any of the sequence report templates available. Clicking this button displays the data selector with the filter set to **sequence report template** mode.

From Group:	Displays the name of the Group the currently selected report template is assigned to.
Report to be generated:	This field displays information about the selected template.

When this dialog is displayed, the default report template shown is the most recently used sequence report template used in this dialog. If no most recently used template exists for this user/instrument, then the field is blank. In this latter case the **From Group** text and **Report to be generated:** field will also be blank.

NOTE: *Although report templates are actually stored as files, the template name will be shown here without the file extension.*

When the folder button is clicked the standard report template selection dialog is displayed. However, the templates available for selection are filtered, so that only sequence templates will be shown.

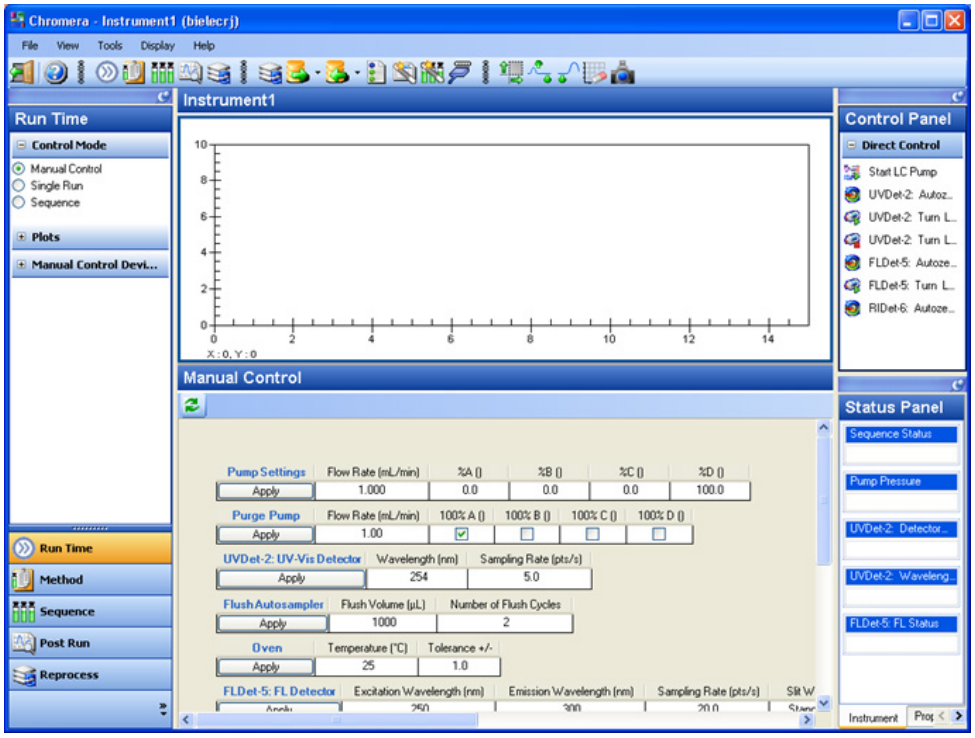
When the report template field is populated, the software examines the template to determine its basic characteristics. These are then displayed in the **Report to be generated:** field, as defined below.

Run Time

About the Run Time Environment

In the Run Time environment you can interactively control an instrument. For example, using the manual control of a specific device, data acquisition using a method, and sample analysis using a sequence are all controlled from here.

Run Time is accessed by clicking the **Run Time** button in the Navigation Pane (or selecting the **Run Time** command from the **View** menu or clicking the **Run Time** toolbar button). The Run Time environment is designed to bring together all elements required to monitor and control instrument operation. The intent here is that this environment should enable you to accomplish your primary instrument-related workflows in an intuitive and efficient manner.



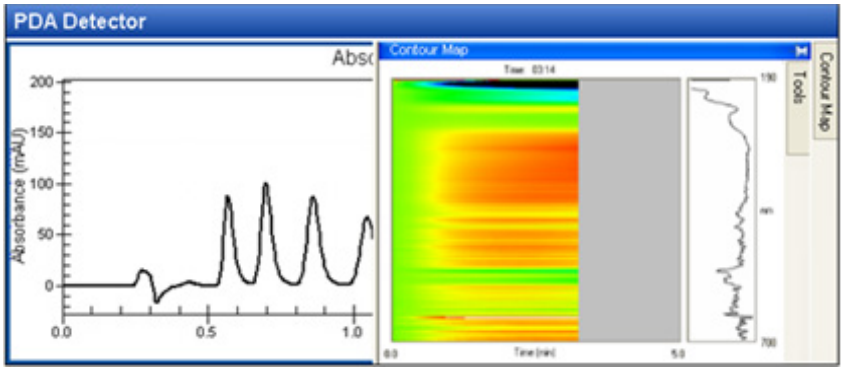
The workflows considered in the development of Run Time analysis are:

- Start up an instrument from shutdown or standby
- Routine analysis
- Instrument method development

NOTE: *In an online instance of Chromera, or an offline instance with an assigned instrument configuration, the title bar of the main display area always displays the name of the instrument. When data acquisition is in progress it also displays the name of the sample being analyzed.*

By default the main area of the screen contains two frames: a graphics frame to display the chromatograms and an instrument/sequence frame to display the settings. The basic contents of the instrument/sequence frame will depend on the **Control Mode** selection made in the upper section of the navigation pane.

If you are configured with a PDA, the graphics frame includes a real-time **Contour Map** monitor that flies-out from the right-hand side. The contour map also contains a **Tools** tab for you to control and scale the contour map.



- When **Manual Control** is selected, the controls enabling a user to make the various devices of the instrument ready for analysis is displayed.

The Single Run and Sequence modes have a toolbar below the title/status bar.

- When **Single Run** is selected, the frame will contain various run parameters or the method selected for control, depending on a toolbar selection.
- When **Sequence** is selected, the frame displays the sequence selected for control.

Navigation Pane

In the **Run Time** environment the Navigation Pane area above the buttons contains three sections:

- **Control Mode**

Control Mode Selection	Description
Manual Control	Disabled when a single run is in progress (for an automated injection this means from the time the Injection command is issued) or when a sequence is running.
Single Run	Disabled when a sequence is running.
Sequence	Disabled when a single run is in progress (for an automated injection this means from the time the Inject command is issued to the autosampler).

- **Plots**
- **Reference Plots -** Select channels to compare against the displayed graphics.
- **Manual Control Devices** - (when Manual Control is selected) or **Method** (when Single Run or Sequence is selected).

The display area of each of these sections can be toggled closed or open by clicking on the plus or minus button in the title bar.

NOTE: *The relative sizes of the two frames can be changed by dragging the splitter bar between them; or, double-clicking in the title bar of one of the frames will toggle between a maximized and restored view.*

Run Time Main Menu Commands - Manual Control

The menu commands displayed in the Run Time environment will depend on the run mode selected. All modes will have File, View, Display, Tools, and Help menus but the contents of the File menu differ for **Manual Control**, **Single Run**, and **Sequence** modes.

NOTE: *The Actions menu does not appear in Manual Control mode (all actions are controlled by buttons in that mode) and its contents are different in Single Run and Sequence modes.*

The table below displays the commands available in **Manual Control** mode. These commands are in addition to those described in **Common Menus**.

Menu	Command	Description
File	Lock	Lock is only displayed when user authentication/tracking is active. It minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	Closes the Chromera instance.
Display	Rescale	Displays the Rescale Axes dialog.
	Auto Rescale	Redraws the contents of the active plot frame at default scaling.
	Auto Offset	Applies to real-time plots only. Auto Offset redraws the contents of the active plot frame to reset the Y axis minimum value so that the current signal level is displayed at approximately 5% of full scale. The Y axis maximum is also adjusted so that the full scale span remains the same.
	Plot Styles	Displays the Plot Styles dialog.
	Clear Graphics	Clears the existing display of all real-time plots and starts plotting again for each selected channel. Also restores any data displayed for previous run or reference chromatograms.
	Snapshot	Displays the active plot in a full screen window.

Run Time Main Menu Commands - Single Run Mode

The table below displays the commands available in the **Single Run** mode. These commands are in addition to those described in Common Menus.

Menu	Command	Description
File	New Method	If the current method has been modified then you will first be asked if it should be saved. It creates a default method for the current instrument configuration and displays it. New Method is disabled when a run is in progress.
	Open Method…	If the current method has been modified then you will first be asked if it should be saved. It displays the data selector in select method mode. When you select a method it is displayed. Open Method is enabled at all times.
	Save Method	Saves the displayed method, replacing the existing version. Save Method is enabled when a method is displayed.
	Print Preview Method ...	Displays the Print Method dialog. After you have selected the required options in the Print Method dialog the method will be printed. Print Preview Method is enabled at all times.
	Print Method …	Displays the Print Method dialog (see Print Method). The method prints after selecting the required options in the Print Method dialog. Print Method is enabled at all times.
	Lock	Only displayed when user authentication/tracking is active. Minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	If a single run is in progress or a sequence is running you are prompted that the instrument must be in an idle state before the application can be closed (see Exit dialog). If the instrument is not active then if any unsaved data exists in any environment the software will ask the user if it is to be saved before exit.
Actions	Run Options	Displays the Run Options dialog appropriate to the current mode.

	Inject	Displayed only when an autosampler is configured on the instrument. Initiates an injection on the autosampler. Inject is disabled when a run is in progress (i.e. when injection cycle begins). It is re-enabled when the run ends. It is not required that the instrument be in Ready state before an injection can be initiated; just that it is not acquiring data.
	End Run ...	End Run is enabled when a run is in progress and displays the End Run dialog.
	Extend Run Time ...	Extend Run Time is enabled when a run is in progress on an LC-only instrument, it is always disabled for an ICP-MS instrument, and displays the Extend End Run dialog.

Run Time Main Menu Commands - Sequence Mode

The table below displays the commands available in Sequence mode. These commands are in addition to those described in Common Menus.

Menu	Command	Description
File	New Sequence	If the current sequence has been modified then you are first asked if it should be saved then it displays a new default sequence. The sequence configuration is that of the connected instrument. It is disabled in an when a sequence is running, otherwise it is enabled.
	Open Sequence …	Open Sequence is disabled when the sequence is running or paused. If the current sequence has been modified then you are first be asked if it should be saved. It displays the data selector in single sequence selection mode. Selecting a sequence displays it.
	Save Sequence	Save Sequence is enabled when a sequence is displayed. It saves the current sequence, replacing the existing version.
	Save Method	Save method is enabled when a method is displayed. It saves the current method, replacing the existing stored version.
	Print Preview ► Method ...	Enabled when a sequence is open. When a method is displayed that will be the method printed or previewed. When the sequence is displayed, the method printed or previewed will be that from the selected row or the first row, if no row is currently selected. Displays the Print Method dialog. The method prints after you have selected the required options in the Print Method dialog.

	Sequence ...	Enabled when a sequence is opened. It displays the Print Sequence dialog. The sequence prints after selecting the required options in the Print Sequence dialog.
	Print ► Method …	Enabled only when the Method is actually displayed in Sequence mode. Displays the Print Method dialog . The method prints after you have selected the required options in the Print Method dialog.
	Sequence ...	Enabled when a sequence is displayed. Displays the Print Sequence dialog. The sequence prints after selecting the required options in the Print Sequence dialog.
	Lock	Only displayed when user authentication/tracking is active. It minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	If a single run is in progress or a sequence is running then you are prompted that the instrument must be in an idle state before the application can be closed. If the instrument is not active then if any unsaved data exists in any environment the software will ask the user if it is to be saved before exit.
Display	Rescale	Displays the Rescale Axes dialog.
	Auto Rescale	Redraws the contents of the active plot frame at default scaling.
	Auto Offset	Applies to real-time plots only. It redraws the contents of the active plot frame to reset the Y axis minimum value so that the current signal level is displayed at approximately 5% of full scale. The Y axis maximum is also adjusted so that the full scale span remains the same.
	Plot Styles	Displays the Plot Styles dialog.
	Plot Layout	Displays the Run Time version of the Plot Layout dialog. Displayed in Sequence mode only. Displayed in Sequence mode only.
Actions	Run Options	Displays the Run Options dialog appropriate to the current mode (see Run options). It is disabled in Manual Control mode.
	Set as Start Row	Enabled when the sequence is idle (or Paused) and a row is selected in the sequence. Sets the selected row as the first row to be run when the sequence is started.

	Set as End Row	Enabled when the sequence is idle (or Paused) and a row is selected in the sequence. Sets the selected row as the last row to be run when the sequence is started.
	Start Sequence	Enabled when a sequence is open. Initiates the sequence run. The Start button also acts to restart the sequence from the Paused state.
	Pause Sequence	Enabled when a sequence is running. Inhibits the next injection when the current run (if any) is complete. Subsequent use of the **Start Sequence** command would (by default) cause the sequence to resume running with the next injection.
	Stop Sequence	Enabled when a sequence is running. Inhibits the next injection when the current run (if any) is complete. Subsequent use of the **Start Sequence** command would (by default) cause the sequence to begin running from the Start Row.
	End Run	Enabled when a run is in progress. It displays the End Run dialog.
	Extend Run Time	Enabled when a run is in progress on an LC-only instrument. It is always disabled for an ICP-MS instrument. It displays the Extend Run Time dialog.

Acquisition

About Channels

A channel is a digital data set received from an instrument, or derived from data received from an instrument. Some channels (those representing chromatograms) are processed by the software; others (such as solvent gradient profiles and pump pressure curves) are acquired and stored for subsequent display only. A channel may be a directly acquired signal from a device or it may be a derived stream (e.g. a chromatogram extracted from spectral data or a resultant chromatogram derived by subtracting a stored blank data stream from an acquired signal). The latter is used by the speciation application.

The supported detectors all provide data in the form of chromatograms. However, derived channels produced by the subtraction of one chromatogram from another will also be supported by the LC application.

There is no arbitrary limit to the number of channels that can be acquired, for example:

1. Simultaneously from a single detector (for example, absorbance output at different wavelengths).

2. Simultaneously from multiple detectors on a single instrument.

3. Simultaneously from multiple instruments.

Acquisition via a Method

In general, data acquisition is controlled by a sequence defining calibration standards and multiple samples. However, Chromera also supports a method only or single run mode of acquisition. This is described in the **Single Run Mode**.

Acquisition Via a Sequence

A sequence controls the acquisition and analysis of chromatographic data produced by a series of injections. Each row in the sequence contains, directly or by reference, all information relevant to the collection, processing and reporting of one sample. For further details refer to the **Sequence** section.

Acquired Data Sets

When data are acquired, a copy of the method used to control the acquisition is stored with the data. This copy will include all parts of the method; including peak detection and calibration replicate information for all channels, even if the data were acquired with the *Suppress Processing* flag set. That is, a copy of the complete method is stored with the batch entry even if no results were generated.

When data are acquired via a sequence, a copy of the sequence row used to control the acquisition is stored with the data. Since one sequence row may result in several sample data sets, resulting from multiple injections, the data will also include the injection number. This information is available for examination by the user whenever a data set is viewed in the Post Run/Graphic Editing windows.

When data are acquired using **Single Run** mode (using a method but no sequence), the subset of sequence parameters entered prior to the injection is stored with the data, in place of the complete sequence row.

Run Control

You can control a run by the following:

Extending an Analysis

A means is provided to extend the time of an analysis quickly and conveniently, separately from direct modification of the active method within the Method Editor. When you determine that a longer run is needed to allow expected peaks to elute you may not have time to examine all sections of the instrument method to find all those places where times need to be increased. When a run is extended the software performs the task of sending the necessary commands to all instrument devices that operate on a time basis. The devices are held at their final conditions (where programming occurs) for the additional time period.

Stopping an Analysis

When an analysis is in progress the following functions are available:

1. You can terminate data acquisition on all active channels and perform processing as specified. The raw data are stored as usual.

2. You can terminate data acquisition on all active channels and discard the raw data acquired up to that point.
   In the case of acquisition controlled by a sequence, you have a secondary option as to whether the next run should be a repeat of the current run (same sample, same injection number) or if execution should continue with the next injection in the sequence.

In both cases you are given two additional options:

- Whether the instrument devices should be commanded to reset immediately to initial conditions or whether they should be left to stop according to the current method conditions.
- Whether the next injection should be made as soon as the system comes ready or whether the sequence should pause until resumed by the user. (for instruments with a configured autosampler only)

Real-Time Plot

The term **real-time plot** is defined as the graphical display of the signal(s) from the channel(s) of an instrument during an analytical run. Each instance of Chromera associated with an instrument will include a view where the real-time plot(s) for that instrument can be viewed.

Function	Description
Presentation	Real-time plots are displayed using the Plot control described in the **Plot Control** section. If multiple channels are to be plotted the user may select whether they should be displayed within the same plot control (overlaid), or in separate plot controls (the layout of which is controlled by the user), or a combination of the two. Within a single plot control all plots are displayed on the same time scale, whereas for separate plot controls different time segments could be displayed in the different controls.
Signals Plotted	You can select to plot one or more of the data channels provided by the instrument. This includes detector output channels and channels from other devices, such as pump pressure or solvent gradients. The signals are all time-based and operating on the same basic time scale (although some channels may terminate before others).
Scaling	Any of the rescaling mechanisms provided by the plot control is available for a real-time plot. Real-time plot (and baseline monitor) also exhibit two additional scaling behaviors - one applied to the X axis and one to the Y-axis.
Scrolling	Scrolling (only during baseline monitor mode): The amount of time displayed across the plot remains the same and the percentage of scroll is fixed and not selectable by the user.
Auto Offset	This provides a simple mechanism to reset the Y axis minimum value so that the current signal level (assuming it is baseline, otherwise it's not a good idea to do this) is displayed at approximately 5% of full scale. The Y-axis maximum is also adjusted so that the full scale span remains the same.
Auto Zero	A command is available when the real-time plot window is displayed to auto zero the detector itself - for those detectors that support this feature.

Baseline Monitor

It is possible to monitor the detector signals from an instrument graphically, outside the context of an analytical run. This allows you to examine the stability of the detector in terms of noise, drift, ghost peaks, etc. The following sections define the intended functionality of the monitoring feature, however, the limitations of the device being monitored may impose constraints that will prevent the full functionality being implemented for some detector devices.

Baseline monitor differs from real-time plot in the following ways:

- The baseline can be monitored at any time (except during a run). Run Time plot depends on data acquisition activity.
- The time scale of baseline monitor is not constrained to the method run time (unless the instrument device imposes this)
- No data are stored permanently from a baseline monitoring session since it is clearly impracticable to save an infinite amount of data for this purpose. Data are only persisted for the purpose of rescaling.

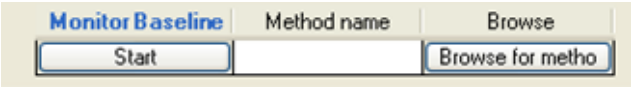
Baseline monitor will not specifically exclude the display of channels other than detector output signals but some other channels will not be of much value outside the context of an analytical run (e.g. solvent gradients).

All features of real-time plot described in **Signals Plotted to Auto Zero** are shared by baseline monitor; real-time integration is not available.

Access to Baseline Monitor

Access to the baseline monitor function is from the Run Time environment.

- For general LC, Baseline Monitor is an inherent feature of the Run Time environment and available in any run mode (Manual Control, Single Run, Sequence).
- For the ICP-MS application, Baseline Monitor displays in the Manual Control mode only (see ICP-MS Controls).



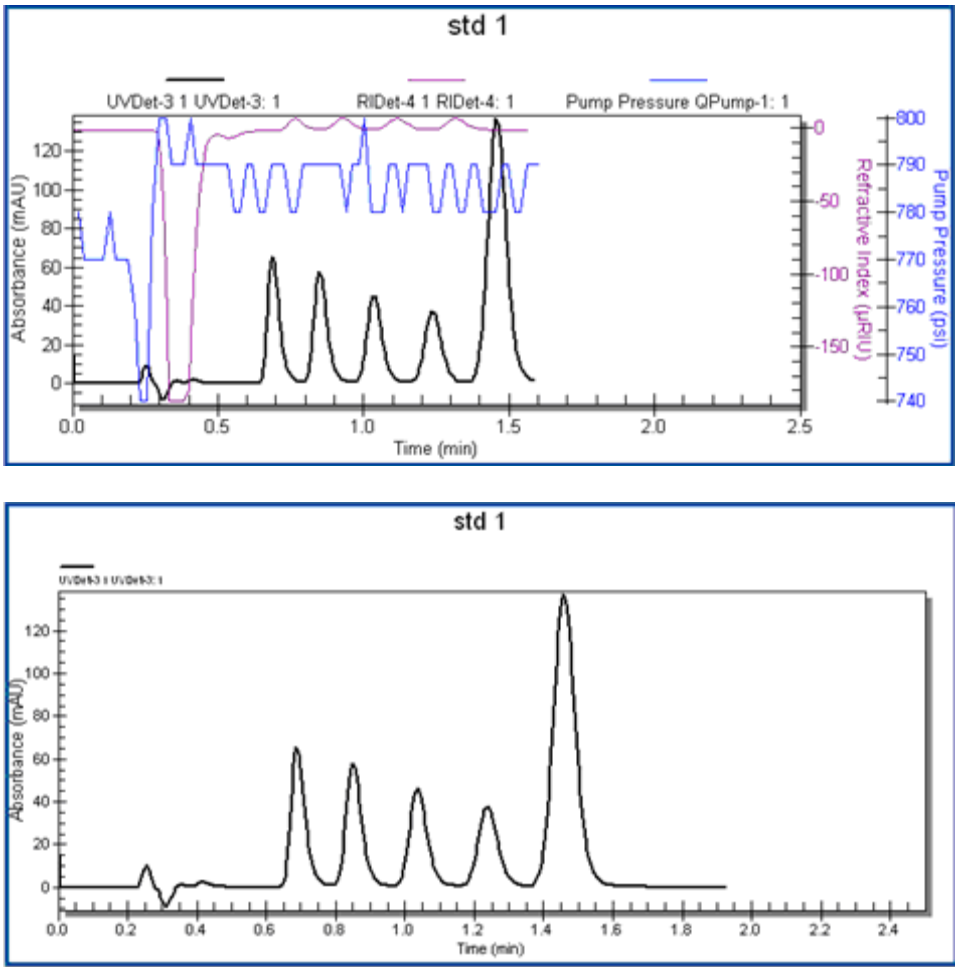
Control	Description
Method Name	Displays the Method selected in Browse for Method.
Browse for Method	Click this button to open the Data Selector and select a Method created in Chromera.
Start/Stop	Click Start to download the method displayed in the Method Name box to the Mass Spec. A check for the Source will occur here, and if the method is incompatible with the installed source, an error message is displayed. As the method runs, the Start button changes to Stop. When the method has finished or Stop has been clicked. The Start button appears again.

How to Use Run Time Graphics

This section describes the how real-time plotting works.

Run Time Plot Chart

Only a single chart is displayed within the graphics frame of the Run Time environment. All active channels will be plotting in overlay fashion on the single chart. Each curve will be plotted in a different color, based on the settings in Plot Styles.



Run Time plot with one active channel

The default behavior of curves for the Y axis is to be autoscaled on the chart. In Manual Control mode the default timescale will be 5 minutes; in Single Run or Sequence mode the default time scale will be the set run time. Baseline monitoring mode (in Manual Control or other modes outside a run) will also exhibit a scrolling time scale by default. When the plotted curves fill the time span across the screen, the time scale will be reset so the start and end times are both increased by 10%.

You are free to rescale the chart manually, using the Rescale Axes command. This action will disable the automatic rescaling of the chart, until the start of the next run.

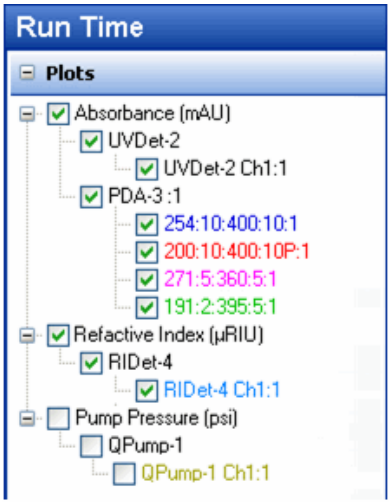
Plots Tree

The Plots tree will always contain a single top-level node (Plot 1) corresponding to the single chart. At the second level of the tree there is a node for each independent Y axis required, depending on the instrument configuration. The following table indicates the possible entries at this level of the tree.

Configured (and Active) Device	Y-Axis Node
UV/Vis Detector	Absorbance (mAU)
Refractive Index Detector	Refractive Index (µRIU)
Fluorescence Detector	Fluorescence (mFLU)
Pump	Pump Pressure (psi) [or other units as defined in Preferences]
Column Oven	Temperature (°C)
ICP-MS	Intensity (cps)

NOTE: *A device marked **not In Use** for the set up method is inactive and hence its channel(s) will not appear in the plot tree.*

Below each Y axis node, at the third level of the tree, will be each channel that is associated with that Y axis node.



This example shows three devices, each with one active channel having different Y axis units.

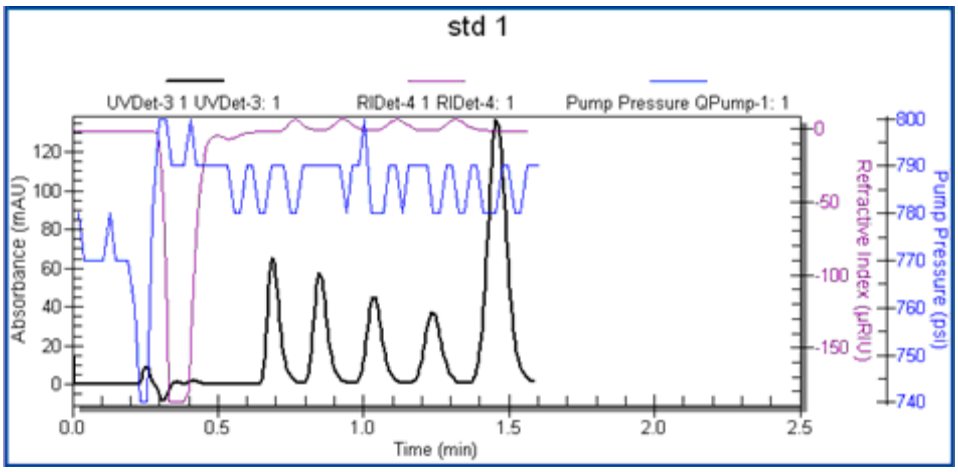
The UV/Vis detector (user name: Series 200UV) has an active channel named "UV-Vis1."

The Refractive Index detector (user name: Series 200aRI) has an active channel named "RI1."

The LC pump (user name: Quat) has an active pressure channel, which always has the name "Pump Pressure."

Detector channels will always appear first in the tree, followed by the subsidiary channels (pump pressure, oven temperature).

The top Y axis node is the primary axis, which appears at the left hand side of the chart. All other axes will appear on the right, in the order in which they appear in the tree, as shown below:

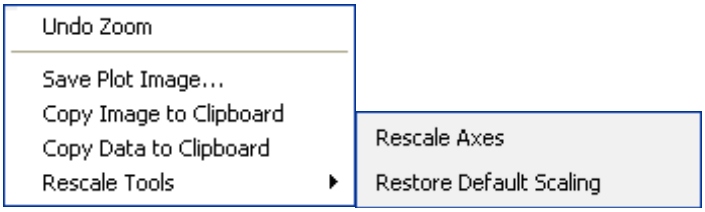


The chart from the plot tree shown above with Absorbance as the primary Y axis

You can change the order of items in the plot tree, to make the required curve occupy the primary axis, if this is not the default case.

Run Time Chart Popup Menu

The popup menu available on the chart within run time will be a subset of the menu available in Post Run.



Command	Description
Expand All	Fully expands the tree to show all channels under all Y axis nodes.
Collapse All	Collapses all lower levels so that only the 'Plot 1' node is shown
Move Up	Moves the selected (clicked on) Y axis node up one position in the plot tree. The chart will be redrawn to reflect the change in Y axis order. Disabled on any node other than a Y axis node.
Move Down	Moves the selected (clicked on) Y axis node down one position in the plot tree. The chart will be redrawn to reflect the change in Y axis order. Disabled on any node other than a Y axis node.

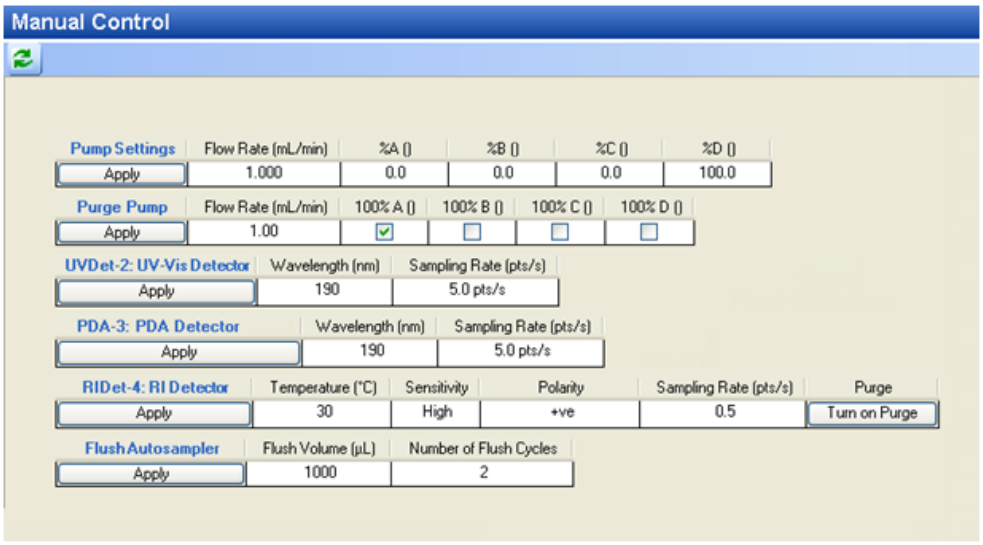
Manual Control

There are numerous possibilities for actions performed in this environment. It is designed to allow you a close and immediate degree of control over the instrument devices, for preparation or diagnostic purposes. A typical series of actions, for an instrument consisting of pump, detector(s), autosampler and column oven, is outlined here.

To prepare the instrument for use from shutdown:

- Set purge conditions and purge the pump
- Set analytical pump initial solvent composition and flow conditions, to begin system equilibration
- Set column oven temperature
- Set Peltier tray temperature (if required)
- Turn detector lamp on (if required)
- Set conditions and flush autosampler
- Monitor instrument status and detector signal(s) to assess instrument readiness (for the PDA: monitor instrument status and column equilibration via the PDA contour map to assess instrument readiness)

The content of the parameter frame in manual control mode depends on the currently connected devices configured on the instrument. For a pump module there are two bands: Pump Settings and Purge Pump. In general, each other device has a single band, but if the Autosampler has a Peltier Tray installed a separate band displays for this. Similarly the Peltier tray band displays for models of the Flexar or Series 225/275 Autosampler that are equipped with heating and/or cooling.



When the view is displayed each band is populated with the settings currently set in that device (with the exception of the Purge Pump settings, which is those last set for the current instrument). Any changes to the displayed values are only downloaded to the instrument device when the associated button is clicked.

When you leave the Manual Control environment, the instrument remains at the conditions last set in Manual Control until these are changed by means of a single run or sequence setup.

The parameter bands displayed for each device are summarized in the tables below.

1. Addition of a *Number of Flush Cycles* parameter to the *Flush Autosampler* band. This determines how many flush cycles are performed when the user clicks the Flush button.

2. For consistency with other devices, **Apply** is used for buttons associated with Autosampler, Oven, and Peltier tray. (The autosampler flush is a rather different action and hence merits a different command.)

There are manual controls for the ICP-MS. These provide access to the Baseline Monitor facility that previously occupied a separate window.

Pump Controls

The following pump controls are available:

Control	Description
Flow Rate (mL/min)	Flow rate of the LC mobile phase.
%A( ), %B( ), %C( ), %D( )	Percentage for solvents A, B, C and D in the mobile phase. Any assigned solvent name will appear in the parentheses. A, B, C and D for quaternary pump. A, B for binary pump. A (always 100%) for isocratic pump.
Apply	Start the pump (if not already running) and apply displayed settings.

NOTE: *The solvent names displayed by default in Manual Control will always be those from the instrument preferences. For your convenience it is also possible to edit the names from within Manual Control (via a context menu that displays the solvent dictionary).*

Purge Pump

Control	Description
Flow Rate (mL/min)	Flow rate of the LC mobile phase during purging.
%A( ), %B( ), %C( ), %D( )	Checked box indicates the solvent to use for purging. These check boxes actually act like radio buttons since only one can be selected. A, B, C and D for quaternary pump. A, B for binary pump. A (always 100%) for isocratic pump.
Apply	Start the pump (if not already running) and apply displayed settings.

Detector Controls

The following detector controls are available:

PDA Detector

Control	Description
Wavelength (nm)	Wavelength of the single (chromatographic) channel to be extracted from the spectra and displayed in real time. (Only a single pixel will be monitored and there is therefore no bandwidth or reference wavelength parameters required here.) (Range 190 – 700nm in 1nm steps. The system will select the nearest pixel to the selected wavelength.)
Sampling Rate (pts/s) or Peak Width (s)	The data acquisition rate for the detector (0.2, 0.5, 1, 2, 5, 10, 20, 50 samples/second). A user preference setting will determine which of these is displayed (see Method: PDA Detector).
Apply	Turns the lamp on (if not already on) and applies the displayed settings.

UV/Vis Detector

Control	Description
Wavelength (nm)	Wavelength to be monitored for signal output.
Sampling Rate (pts/s) or Peak Width (s)	The data acquisition rate for the detector. A user preference setting will determine which of these is displayed (see Method: Series 200 UV/Vis Detector).
Apply	Turns the lamp on (if not already on) and applies the displayed settings.

Fluorescence Detector

Control	Description
Excitation Wavelength (nm)	Excitation wavelength to be set.
Emission Wavelength (nm)	Wavelength to be monitored for signal output.
Slit Width	Emission Bandwidth value
Sensitivity	PMT Voltage parameter
Sampling Rate (pts/s	The data acquisition rate for the detector
Apply	Turns the lamp on (if not already on) and applies the displayed settings

Refractive Index Detector

Control	Description
Temperature (°C)	Temperature to be set for the RI cell.
Sensitivity (µRIU/V)	Detector sensitivity/dynamic range.

Polarity	Polarity of the signal.
Sampling Rate (pts/s)	The data acquisition rate for the detector.
Turn On Purge	Click to change to Turn on Purge.
Apply	Applies the displayed settings.

Autosampler Controls

The following autosampler controls are available:

Flush Autosampler

Control	Description
Flush Volume	Volume of solvent to be used to flush the autosampler.
Number of Flush Cycles	Number of times the flush operation is to be performed. (Range: 1 to 9)
Apply	Initiates the defined flush operation

Peltier Tray

Control	Description
Temperature	Sets the temperature for the Peltier tray
Apply	Updates the Peltier tray with the new temperature setting

Oven Controls

The following oven controls are available:

Control	Description
Temperature	Sets the temperature for the column oven
Apply	Updates the oven with the new temperature setting

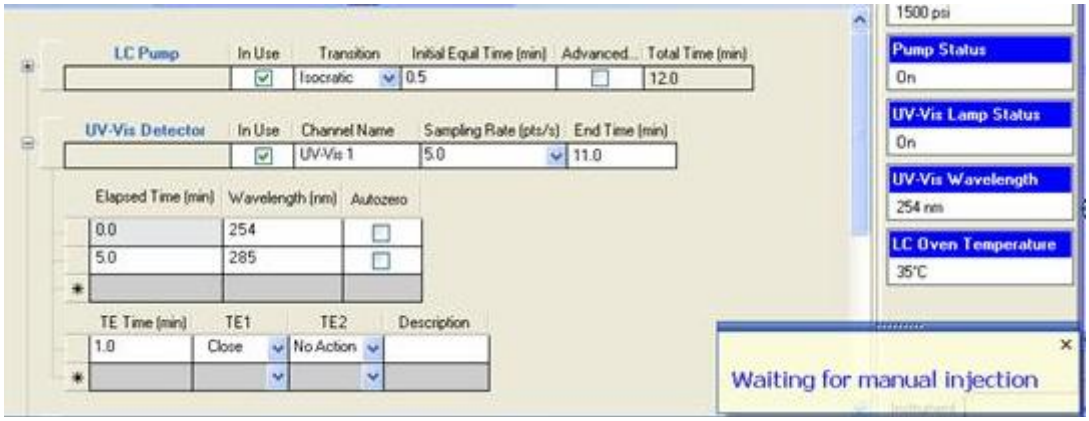
ICP-MS Controls

The following ICP-MS controls are available:

Control	Description
Method	Specifies the method to be used for control of the ICP-MS during baseline monitoring. The method can be selected by clicking on the button in the grid cell.
Start Baseline Monitoring	Initiates plotting of the ICP-MS signal in the run time graphics frame. When monitoring is in progress the button changes to Stop Baseline Monitoring.
Stop Baseline Monitoring	Terminates plotting of the ICP-MS signal and returns the ICP-MS to idle state.

Manual Injection

If no autosampler is configured on the instrument then the Inject toolbar button does not appear. Instead, when the instrument attains the Ready state, a notification is displayed at the bottom right of the screen.



The notification remains displayed until either:

- You explicitly close the notification window
- You make an injection and start the run. Once Chromera detects the run start the notification is removed.

NOTE: *The instrument does not have to be in the Ready state in order for a manual injection to be recognized. Once the software indicates that an Active method is present an injection can be made and acquisition will occur.*

The notification message display is enabled by default for a manual injection system but it may be disabled within User Preferences.

Run Time Manual Control Standard Toolbar

In this mode the toolbar contains only two buttons: Exit and Help Topics.

Or when user authentication active:

Icon	Command
	File/Lock (Only when user authentication active)
	File/Exit
	Help/Topics

About the Single Run Mode

The **Single Run** mode allows you to define the sample to be a run using single sequence row parameters (including sample name, vial number and method) and then initiate the single injection. Calibration cannot be performed automatically from this mode but if the specified method is already calibrated it is possible to obtain a quantitative report. This mode is intended primarily for instrument method development.

Parameters to identify the session are:

- Batch name
- Description
- Group

NOTE: *The Tray Type setting is also included with the above parameters, if an autosampler is configured.*

Parameters available to identify and control the sample run are:

- Method (this will not be present as a specific parameter field; the method itself is displayed)
- Sample Name
- Vial Number
- Sample Description
- Internal Standard Amounts
- Sample Amount
- Dilution Factor
- Normalization Factor

Decision Points available are:

- Peak Detection Review
- Calibration Review
- Data Review
- Report
- Shutdown (Wash sample type only – this is the only available decision point for Wash rows)

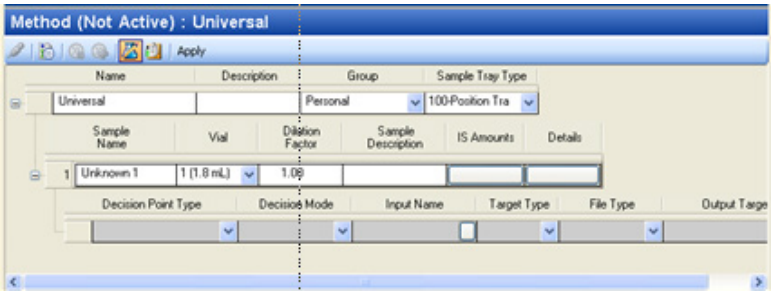
The options to suppress processing and/or reporting will also be available in this mode.

The data sets acquired during a single run mode session are associated together in a manner similar to that of a standard batch acquired via a sequence. The session is uniquely identified by the name you entered. The session is also identified with the instrument name and time and date of the most recent acquisition.

A single run mode session continues until a new session is started (by entry of a new batch name) or a sequence is set up on the instrument.

Run Time - Single Run

When selecting the Single Run mode the parameter display area displays either run parameters or the open method, depending on which is selected via the toolbar. When Chromera is run, by default, the last method open in this environment is opened but not activated (i.e. not downloaded to the instrument).



Single Run Mode - Run Parameters

The run parameters are a subset of the sequence parameters. The following indicates which parameters are displayed and in what order. Note that the Batch Parameters are exact equivalents for the same parameters at the top of a sequence. Data acquired in the Single Run mode session is stored under this batch. This information is recalled the next time you select Single Run mode on the instrument, so that data can be added to the batch if desired. This behavior is distinct from that of a standard batch.

Band 1 – Batch Parameters

Name
Description
Group
Sample Tray Type

Band 2 - Sample Parameters

The sample parameters will be handled in Single Run mode in exactly the same way as in Sequence. That is, the parameters to be displayed in columns can be selected by the user and the remaining parameters will be accessible via the drop-down **Details** field. The main difference is that in Single Run mode only a single row can be defined.

Band 4 - Decision Points

The Decision Points band will behave exactly as in the Sequence Editor.

NOTE: *The values of the run parameters are saved with the data set at the end of the run, prior to processing and decision points. This means that you can change any parameter value during the run and it will be saved with the data set.*

Single Run Mode - Method

The method display and editing facilities in the Run Time environment will not simply be a mirror of the Method Editor environment; it is possible for the two to contain different methods. It is possible to display the active method in the main Method Editor environment but it will not occur by default.

When a method is opened in the Run Time method frame it will not automatically become the active method; you must click the **Apply** button to have the method parameters downloaded to the devices and activated. Similarly if you edit the method in the Run Time window, those changes are not downloaded to the instrument until the Apply button is clicked. Since the displayed method may represent neither the method currently controlling the instrument nor the version of that method as it exists in the database, a clear indication of the method status is essential. For a complete discussion of Run Time method states see Run Time Method States. This status is shown in the title bar at the top of the grid frame, as described below.

Single Run Mode - Title Bar

Not only does the title bar at the top of the grid frame indicate that Single Run mode is selected but it also serves to convey status information concerning the method.

The basic format of the title bar contents are:

[**No**] **Method (**[<edit status> **-**] <download status>**) :** <method name>

- The initial **No** will appear only in the message **No Method**, when there is no method loaded. This will only occur when the system is first started after installation or when the last method opened in the previous session is no longer present in the database (or no longer matches the current instrument configuration).
- <edit status> indicates the current state of the method displayed relative to the database copy. Currently the only edit status defined is 'Modified'. The edit status (and the following hyphen) will not appear if the method displayed is the same as the version in the database.
- <download status> indicates whether or not the displayed method is being used to control the instrument devices. Values are Active and Not Active.
- <method name> is the name of the displayed method

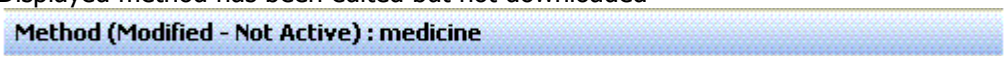
The following five examples illustrate the contents of the title bar under various conditions:

1. First access or last used method not found or not valid

2. Method open but not downloaded to instrument

3. Method downloaded and controlling instrument

4. Displayed method has been edited but not downloaded

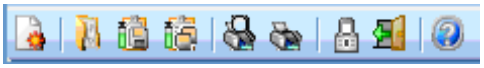


5. Method has been edited and downloaded to instrument

Run Time Single Run Mode Standard Toolbar

This toolbar displays in the Single Run mode:

Or when user authentication active:



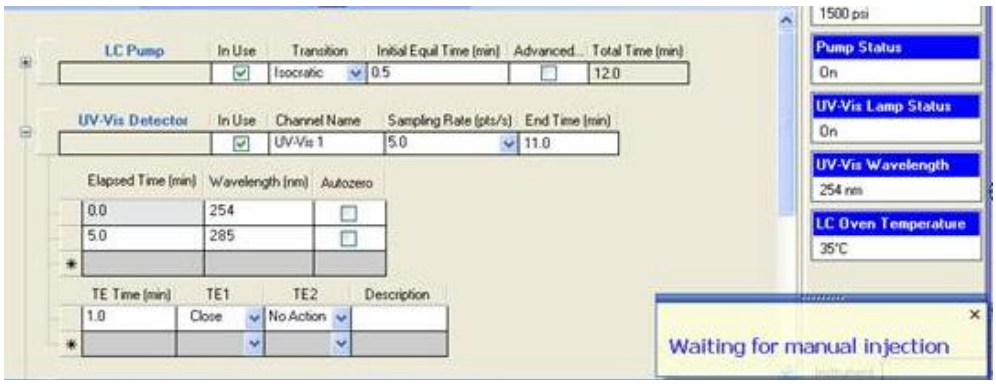
Icon	Command
	File/New Method
	File/Open Method
	File/Save Method
	File/Save Method As
	File/Print Preview Method
	File/Print Method
	File/Lock (Only when user authentication active)
	File/Exit
	Help/Topics

Single Run Mode - Toolbar

Icon	Command	Description
	Inject	Issues a command to the autosampler to inject the specified volume from the currently specified vial. Disabled when there is no active method or when the autosampler is not ready. **NOTE:** It is not required for the pump to be ready or the detector to be ready for this command to be enabled. **NOTE:** This button will not appear if there is no autosampler configured on the instrument. See Manual Injection below.
	Run Options	Displays a dialog enabling you to disable processing or disable generation of reports. See Run Options dialog for further details.
	End Run	Displays a dialog enabling you to confirm the run is to be stopped and providing options associated with stopping the run. See End Run dialog for further details. End Run is only enabled when a run is in progress.
	Extend Run	Displays a dialog enabling you to confirm the run is to be extended (or cancel the command) and to specify the length of time for which the run is to be extended. See Extend Run dialog for further details. Only enabled when a run is in progress on an LC-only instrument. It is always disabled for an ICP-MS instrument.
	Run Parameters	Displays the run parameters.
	Method	Displays the method parameters in the grid frame. Enabled when a method is open.
Apply	Activate Method	Downloads the method parameters to the instrument devices and activates them. This button is disabled when the displayed method is identical to the active method. (For example, immediately after the method has been downloaded.)
Restore Active	Restore Active Method	Replaces the displayed method with the method currently active in the instrument. This button will only be visible when the displayed method is different from the active method (either because another method has been opened or the method has been modified).

Manual Injection

If no autosampler is configured on the instrument then the Inject toolbar button does not appear. Instead, when the instrument attains the Ready state, a notification is displayed at the bottom right of the screen.



The notification remains displayed until either:

- You explicitly close the notification window
- You make an injection and starts the run. Once Chromera detects the run start the notification is removed.

NOTE: *It is not required that the instrument be in the Ready state in order for a manual injection to be recognized. Once the software indicates that an Active method is present an injection can be made and acquisition will occur.*

The notification message display is enabled by default for a manual injection system but it may be disabled within User Preferences.

Using the Single Run Mode

Prior to using **Single Run** mode first prepare the instrument using the Manual Control mode. To perform a typical single run analysis:

1. Select **Single Run** mode from the radio buttons in the upper panel of the navigation bar.
2. Choose the method to be run from the selector dialog displayed when the **Open Method** button is clicked.
3. Review the method as required by selecting devices or sections of interest from the method tree in the lower panel of the navigation bar.
4. Make changes to the method as desired (optionally saving the method back to the database).
5. Enter sample identification and other run parameters (including decision points) as required.
6. Click **Apply** to download the method to the instrument devices.
7. Review the status display and baseline monitor plot(s) to determine when the instrument is ready for the analysis.
8. Click the **Inject** button (to initiate an injection from the configured autosampler) or make a manual injection.

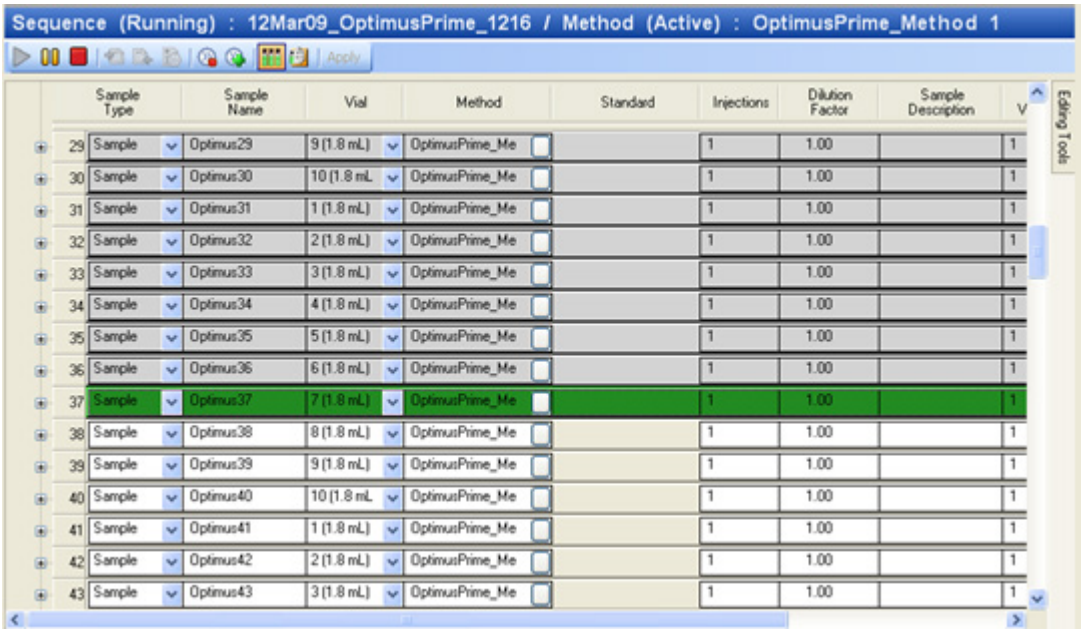
9. Review the chromatogram(s) displayed in the real-time plot frame.
10. Make adjustment to the run time (extend or stop early) if required.
11. At the end of the run, review data in **Peak Detection** decision point (if specified) or **Post Run** environment, as required.

About Run Time Sequence

The sequence in this environment is independent of that in the Sequence Editor. The only interaction is that a sequence open in Run Time cannot be saved under its existing name in the Sequence Editor (**Save As** is still available).

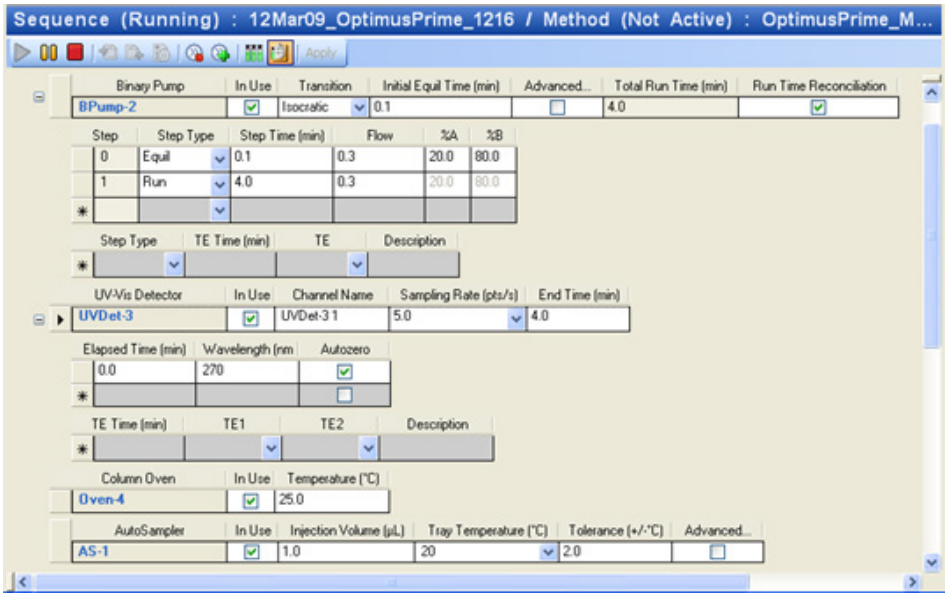
This frame is basically a mirror of the display in the Sequence Editor. Any changes made here are reflected in the main Sequence Editor window and vice versa. Similarly when the sequence is running the currently executing row is highlighted in both views, and rows that are set read-only (because they have been executed or are currently executing) cannot be edited in either view.

Selecting the **Sequence** mode displays either the sequence or the method (from currently selected row), depending on which is selected via the toolbar. When Chromera is run, by default the last sequence open in this environment is opened but not activated (i.e. no method parameters from the sequence are automatically downloaded to the instrument).



Sequence mode with sequence displayed

NOTE: *The Editing Tools fly-out tool panel appears in this environment just as it does in the main Sequence Editor.*



Sequence mode with method from selected row displayed

Run Time Main Menu Commands - Sequence Mode

The table below displays the commands available in Sequence mode. These commands are in addition to those described in Common Menus.

Menu	Command	Description
File	New Sequence	If the current sequence has been modified then you are first asked if it should be saved then it displays a new default sequence. The sequence configuration is that of the connected instrument. It is disabled in an when a sequence is running, otherwise it is enabled.
	Open Sequence …	Open Sequence is disabled when the sequence is running or paused. If the current sequence has been modified then you are first be asked if it should be saved. It displays the data selector in single sequence selection mode. Selecting a sequence displays it.
	Save Sequence	Save Sequence is enabled when a sequence is displayed. It saves the current sequence, replacing the existing version.
	Import Sample List ...	Opens the Import Sample List Wizard so you can import a sample list into a sequence.
	Save Method	Save method is enabled when a method is displayed. It saves the current method, replacing the existing stored version.

	Print Preview ► Method ...	Enabled when a sequence is open. When a method is displayed that will be the method printed or previewed. When the sequence is displayed, the method printed or previewed will be that from the selected row or the first row, if no row is currently selected. Displays the Print Method dialog . The method prints after you have selected the required options in the Print Method dialog.
	Sequence ...	Enabled when a sequence is opened. It displays the Print Sequence dialog. The sequence prints after selecting the required options in the Print Sequence dialog.
	Print ► Method …	Enabled only when the Method is actually displayed in Sequence mode. Displays the Print Method dialog . The method prints after you have selected the required options in the Print Method dialog.
	Sequence ...	Enabled when a sequence is displayed. Displays the Print Sequence dialog. The sequence prints after selecting the required options in the Print Sequence dialog.
	Lock	Only displayed when user authentication/tracking is active. It minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	If a single run is in progress or a sequence is running then you are prompted that the instrument must be in an idle state before the application can be closed. If the instrument is not active then if any unsaved data exists in any environment the software will ask the user if it is to be saved before exit.
Display	Rescale	Displays the Rescale Axes dialog.
	Auto Rescale	Redraws the contents of the active plot frame at default scaling.
	Auto Offset	Applies to real-time plots only. It redraws the contents of the active plot frame to reset the Y axis minimum value so that the current signal level is displayed at approximately 5% of full scale. The Y axis maximum is also adjusted so that the full scale span remains the same.
	Plot Styles	Displays the Plot Styles dialog.
	Plot Layout	Displays the Run Time version of the Plot Layout dialog. Displayed in Sequence mode only. Displayed in Sequence mode only.

Actions	Run Options	Displays the Run Options dialog appropriate to the current mode (see Run options). It is disabled in Manual Control mode.
	Set as Start Row	Enabled when the sequence is idle (or Paused) and a row is selected in the sequence. Sets the selected row as the first row to be run when the sequence is started.
	Set as End Row	Enabled when the sequence is idle (or Paused) and a row is selected in the sequence. Sets the selected row as the last row to be run when the sequence is started.
	Start Sequence	Enabled when a sequence is open. Initiates the sequence run. The Start button also acts to restart the sequence from the Paused state.
	Pause Sequence	Enabled when a sequence is running. Inhibits the next injection when the current run (if any) is complete. Subsequent use of the **Start Sequence** command would (by default) cause the sequence to resume running with the next injection.
	Stop Sequence	Enabled when a sequence is running. Inhibits the next injection when the current run (if any) is complete. Subsequent use of the **Start Sequence** command would (by default) cause the sequence to begin running from the Start Row.
	End Run	Enabled when a run is in progress. It displays the End Run dialog.
	Extend Run Time	Enabled when a run is in progress on an LC-only instrument. It is always disabled for an ICP-MS instrument. It displays the Extend Run Time dialog.

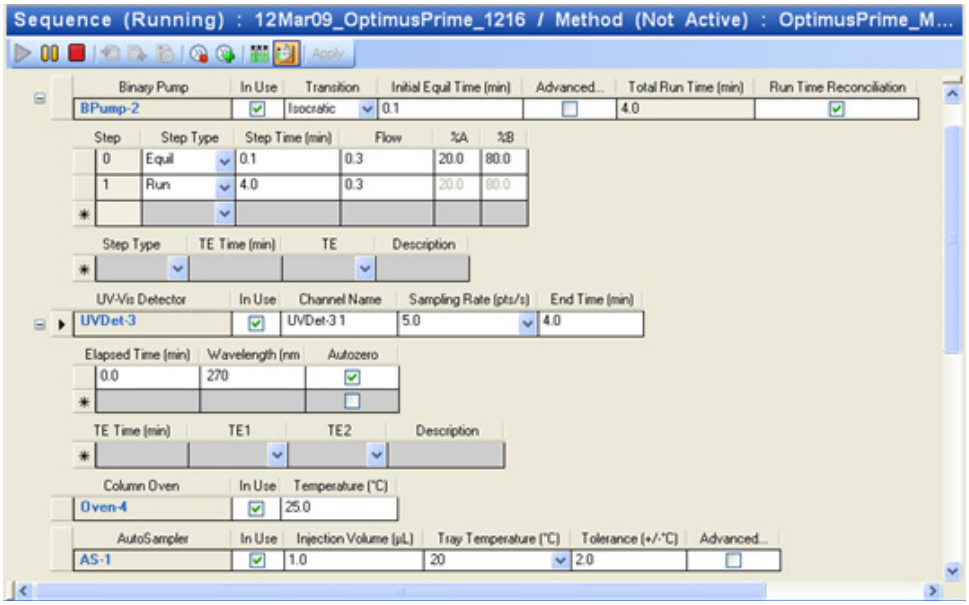
Sequence Mode - Validity Checks

When a sequence is opened, several checks must be performed. These include:

- That the sequence is compatible with the instrument. This means, for example, if the sequence specifies a **Mega Sample Tray** that the instrument has configured and it is not a PerkinElmer Series 200 Autosampler. If a different tray is installed a message displays indicating that the sequence cannot be run with a tray different from the one specified in the sequence.
- If the sequence includes any vial positions set to NONE (see Vial Entry validation) a message displays indicating that the sequence cannot be run until these rows are updated with valid vial positions or deleted.
- Method incompatibility will only be checked when the method is to be downloaded – so only the first method will be checked when the Sequence is run. If an incompatibility is found, that sequence row will be skipped and a message will be added to the error log.

Sequence Mode - Method

The method displayed is equivalent to the method display in Single Run mode but with the difference that a method can be selected for display only by selecting a row in the sequence. You can access a stored method by selecting an accessible row in the sequence, and then clicking the Method button, or the active method by selecting the currently executing row (i.e. when the sequence is running) and then clicking Method.



A modification to the active method will only take effect when you click the **Apply** button. This change will remain in effect for the run in progress (where applicable) and for all remaining runs from the currently executing row. For the change to be retained for subsequent rows (assuming they reference the same method) you must save the method.

A change to any method from a row yet to be executed will only take effect if you save the method, since the method is not read from the database until the row becomes the currently executing row.

NOTE: *You can only access and modify one active copy of the method and one stored copy. The active method is accessed by selecting the currently executing row; the stored method by selecting any other row (that references the same method).*

Sequence Mode - Title Bar

Not only does the title bar at the top of the display frame indicate that Sequence mode is selected but it also serves to convey status information concerning the sequence and the method from the current (or pending current) row. The basic format of the title bar contents depends on the current data set displayed.

When the sequence is displayed the format is:

[**No**] **Sequence (**[<edit status>]**) :** <sequence name>

- The initial **No** appears only in the message "No Sequence", when there is no sequence loaded. This will only occur when the system is first started after installation or when the last sequence opened in the previous session is no longer present in the database.

- <edit status> indicates the current state of the sequence displayed relative to the database copy. Currently the only edit status defined is 'Modified'. The edit status does not appear if the sequence displayed is the same as the version in the database.
- <sequence name> is the name of the displayed sequence

Note that the sequence run status is included in the title bar even though it is also indicated by the highlighting of the currently executing row (in green). This is because there is less space for display of the sequence in the Run Time environment and it is possible that the user may have scrolled the display, thereby concealing the currently executing row. Also, the currently executing row will not be visible when the Method display mode is selected.

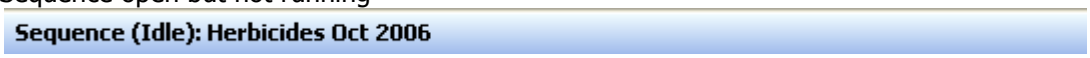
When the method is displayed the format is:

[**No**] **Sequence (**[<edit status> -]<run status>**) :** <sequence name> / **Method (**[<edit status> **-**]<download status>**) :** <method name>

The added method information is essentially the same as described for Single Run mode, except that the 'No Method' case cannot occur. When there is no sequence displayed the Method toolbar button is disabled so the method grid cannot be displayed.

The following examples illustrate the title bars under various conditions:

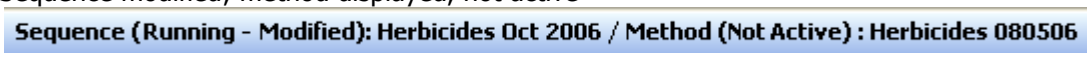
1. Sequence open but not running



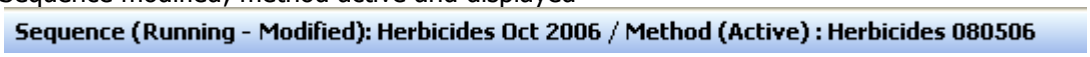
2. Sequence running; method active and displayed

3. Sequence paused; method active and displayed

4. Sequence modified; method displayed, not active

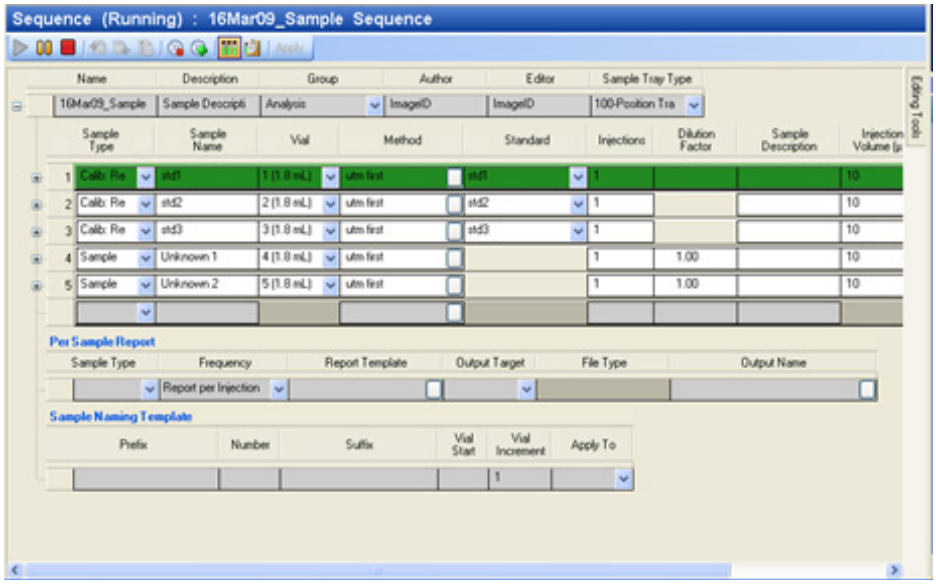


5. Sequence modified; method active and displayed

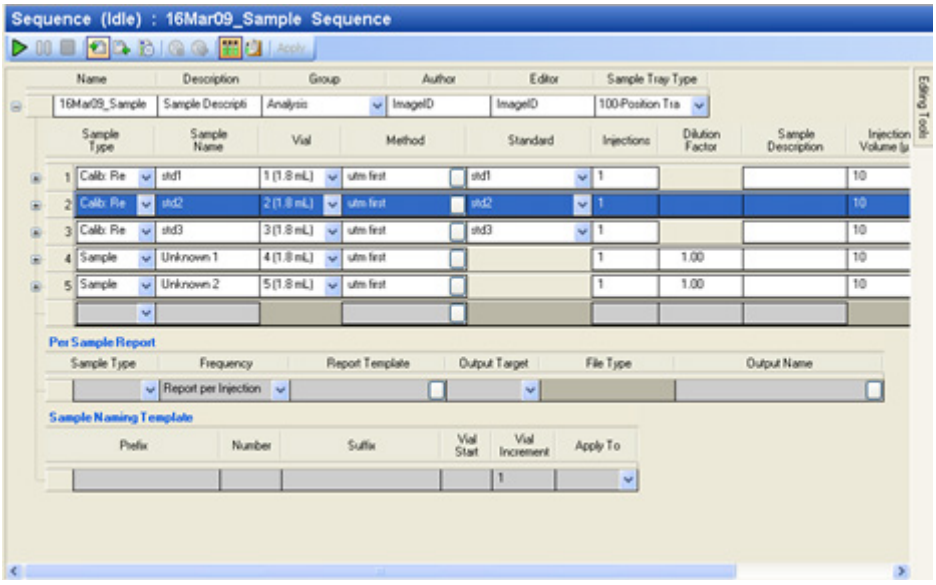


Sequence Mode - Parameter Display

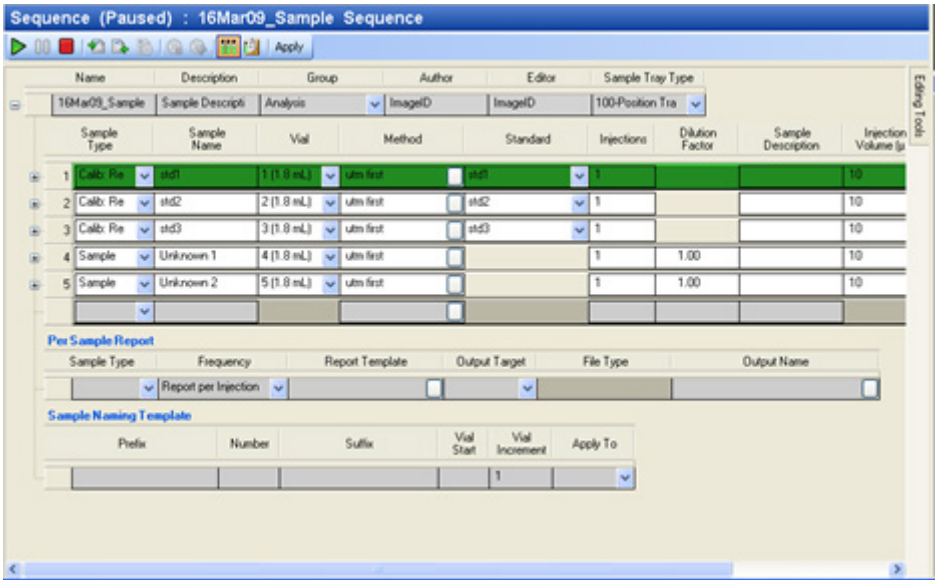
When the sequence is Idle you can select any row and it is highlighted in the standard way.



When the sequence is Running, the currently executing row is indicated by being highlighted by turning the background color for all cells in the row to green (note that the text color does not change to white as occurs with standard Windows highlighting).



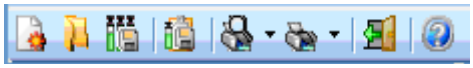
If the sequence is Paused, the Start Row is updated and hence all rows completed are displayed with a gray background (see Setting Start and Stop Rows for details of setting Start and Stop rows). The *resume point* in the sequence (by default) is the next row shown with a white background.



You can reset the Start Row to a different row if desired and this would update the display to indicate the new *resume point*.

Run Time Sequence Mode Standard Toolbar

In this mode the Standard toolbar contains buttons to access commands for both sequence and method actions.



Or when user authentication active:

Icon	Command
	File/New Sequence
	File/Open Sequence
	File/Save Sequence
	File/Save Method
	File/Print Preview ► Method Sequence
	File/Print ► Method Sequence
	File/Lock (Only when user authentication active)
	File/Exit
	Help/Topics

Sequence Mode - Toolbar

The toolbar in the Run Time sequence mode contains the following commands:

Icon	Command	Description
	Start Sequence	Begins execution of the sequence from the specified row. You can also use the Start button to restart the sequence from the Paused state.
	Pause Sequence	Does not begin the next run after the current run (if any) is completed. **NOTE:** If the sequence is paused the new default Start Row becomes the next row after the one completed before the pause takes effect (see Setting Start and Stop Rows).
	Stop Sequence	Terminates a running sequence when the current run (if any) is completed.
	Start Row	Sets the currently selected row as the staring row when the sequence is started (or restarted). Start Row is disabled when the sequence is running or when no row is selected. See Setting Start and Stop Rows.
	Stop Row	Sets the currently selected row as the ending row when the sequence is started (or restarted). Disabled when the sequence is running or when no row is selected. See Setting Start and Stop Rows.
	Run Options	Displays a dialog enabling you to select starting and ending rows for running (or resuming) the sequence, or to disable processing or disable generation of reports. See Run Options for further details. **NOTE:** Changing starting and ending rows will have no immediate effect if the sequence is running.
	End Run	Displays a dialog enabling the user to confirm the run is to be stopped and providing options associated with stopping the run. See End Run for further details.
	Extend Run	Displays a dialog enabling you to confirm the run is to be extended and to specify the length of time for which the run is to be extended. It is only enabled when a run is in progress on an LC-only instrument and always disabled for an ICP-MS instrument. See Extend Run for further details.
	Sequence	Displays the sequence.
	Method	Displays the method parameters.

Apply	Activate Method	Downloads the method parameters to the instrument devices and activates them. This button is only visible when the method page is selected (or always disabled when the sequence page is selected). This button is disabled when the displayed method is identical to the active method. (For example, immediately after the method has been downloaded.) Before the sequence is started the Apply button will be active when the starting row (Whether this is row 1 or another row) is selected. In this way you can download the initial method prior to starting the sequence, and monitor its stability at the set conditions. When the sequence is running the Apply button is disabled when the displayed method is not the active method (that is, when a method from a row other than the active row is being viewed).

Sequence Mode - Autosampler Tray Change

After changing an autosampler tray a validation is performed. Different actions will occur depending on whether the sequence is running or idle (not running or paused).

Sequence Idle

If a different tray from that specified in the sequence is replaced in the autosampler an error message will be displayed immediately warning that the sequence cannot be run with the installed tray. If you attempt to start the sequence (before fixing the problem) the same message will be displayed again and the sequence will remain idle.

If you do not install a new tray but leave the autosampler empty, any attempt to start the sequence will cause an error message to be displayed warning that the sequence cannot be run until the correct tray is installed.

Sequence Running

If a different tray from that specified in the sequence is replaced in the autosampler an error message will be displayed immediately warning that the sequence cannot be run with the installed tray. The sequence will be paused. If you attempt to restart the sequence (before rectifying the problem) the same message will be displayed again and the sequence will remain idle.

If you do not install a new tray but leave the autosampler empty, no error will appear until the system is ready to perform the next injection (since the user could replace the tray at any time prior to that, which should not trigger an error), at which time an error message will be displayed warning that the sequence cannot continue until the correct tray is installed. The sequence will be paused. If you attempt to restart the sequence (before rectifying the problem) the same message will be displayed again and the sequence will remain idle.

Setting Start and Stop Rows

The **Start Row** and **Stop Row** toolbar buttons are enabled when a row is selected in the sequence (and the sequence is not running).

Clicking on the **Start Row** button causes all rows in the sequence prior to that row to be shown as inactive; that is, with a cell background color of medium gray.

Similarly, if the **Stop Row** button is clicked then all rows following that row to the end of the sequence is marked as inactive.

Marking a row inactive does not prevent it being selected, so that the Start and Stop rows can easily be redefined. However, a Start Row cannot be set if it conflicts with an existing Stop Row, or vice versa. If you attempt to set a Stop Row earlier than the current Start Row then an error message is displayed (Stop Row must be later than the Start Row). Similarly, if you attempt to set a Start Row later than the current Stop Row then an error message is displayed (Start Row must be earlier than the Stop Row).

NOTE: *When a sequence is paused the Start Row is automatically updated to the row following the last one completed before the pause takes effect. In this way the Run command will, by default, resume the sequence from the next row. However, the user is able to change the default Start row to some other row and hence change execution of the sequence.*

Using the Sequence Mode

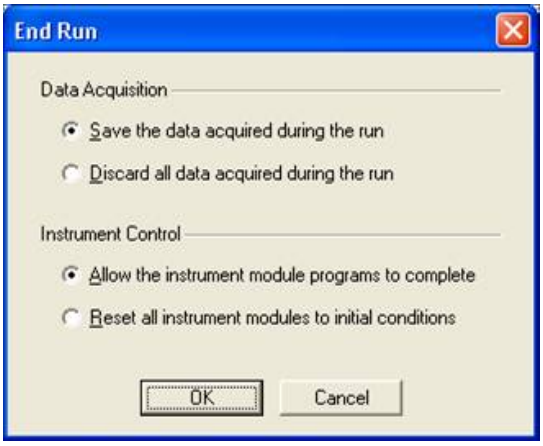
Prior to using Sequence mode first prepare the instrument as required using Manual Control mode. To analyze a set of samples in the run time Sequence mode:

1. Select **Sequence** mode from the radio buttons in the upper panel of the navigation bar.
2. Choose the sequence to be run from the selector dialog displayed when the **Open Sequence** button is clicked
3. Review the sequence and make any required changes (optionally saving the sequence back to the database)
4. Review the method(s) specified in the sequence, make any required changes and save the method(s)
5. If the sequence is not be run in its entirety, set starting and sending rows
6. Review the status display and baseline monitor plot(s) to determine when the instrument is ready to begin analysis
7. Click the **Start** button to initiate sequence execution (If manual injection, make the injection when prompted)
8. Review the chromatogram(s) displayed in the real-time plot frame
9. If Peak Detection or Calibration decision points are specified, review and take action as required

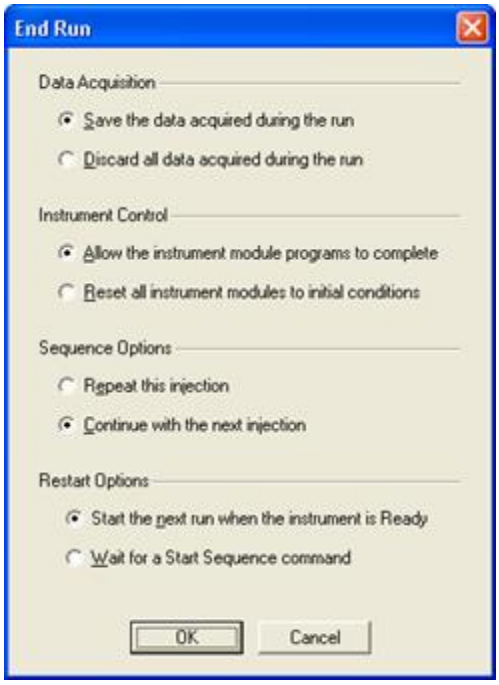
When the sequence is complete, review results in the **Post Run** environment, as required.

End Run Dialog

This dialog is displayed when the **End Run** command is chosen in **Single Run** or **Sequence mode**, via either the toolbar button or menu item. There are two versions of the dialog; one for Single Run mode and one for Sequence mode. The Single Run mode version is a simple subset of the Sequence version.



Single Run mode End Run dialog



Sequence mode End Run dialog

Controls	**Description**
Data Acquisition	Radio buttons to select whether the data acquired from the run in progress is **saved** or **discarded**. If the data are discarded then no batch entry will exist for the run.
Instrument Control	Radio buttons to select whether the other instrument modules (i.e. other than detectors) are left to complete the run or are immediately reset to initial conditions.
Sequence Options	Radio buttons to select whether the current run is repeated or if sequence execution will continue with the next logical run. If the current run is the final injection for a sample then the **Continue with next injection** option will cause the next sample to be injected.
Restart Options	Radio buttons to select whether the next run will begin automatically or whether the system will pause until you give a command.

Extend Run Dialog

The Extend Run Time dialog is displayed when the **Extend Run** command is chosen in Single Run or Sequence mode, via either the toolbar button or menu item. The dialog is identical in both modes.

Controls	Description
Extend run by (minutes)	Enter the amount by which the run time is extended (from 0 to 120.0 minutes). Note that this is not the new run time but the length by which the run is extended.
Do not extend data acquisition	A check box to set whether or not the data acquisition is extended as well as the instrument run time.
Current run time (minutes)	This display indicates the current run time. This is generally the pump run time.

The high-level mechanism (i.e., the way the active method is changed) for extending the run time for the various devices are:

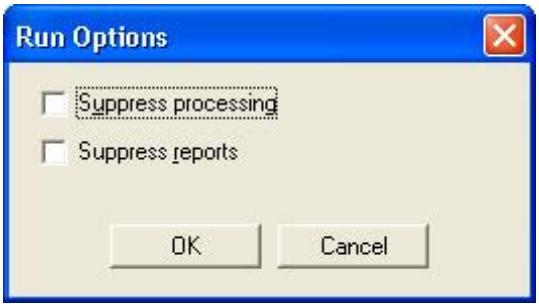
Pumps Extend the time of the final step (this has been determined to be possible for the PerkinElmer pumps).

LC Detectors Extend the time of the final step or add a new final step as required (and hence increase End Time) – unless acquisition not extended

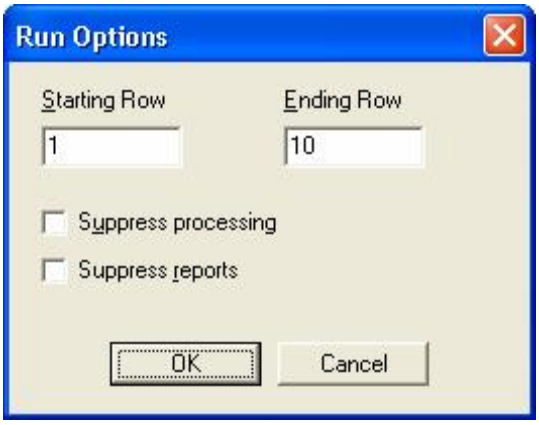
If the instrument includes a device for which extending the run is impossible then the Extend Run command is disabled.

Run Options Dialog

This dialog is displayed when the **Run Options** command is chosen in **Single Run** or **Sequence** mode, via either the toolbar button or menu item. There are two version of the dialog; one for Single Run mode and one for Sequence mode. The Single Run mode version is a simple subset of the Sequence version.



Run Options dialog in Single Run mode



Run Options dialog in Sequence mode

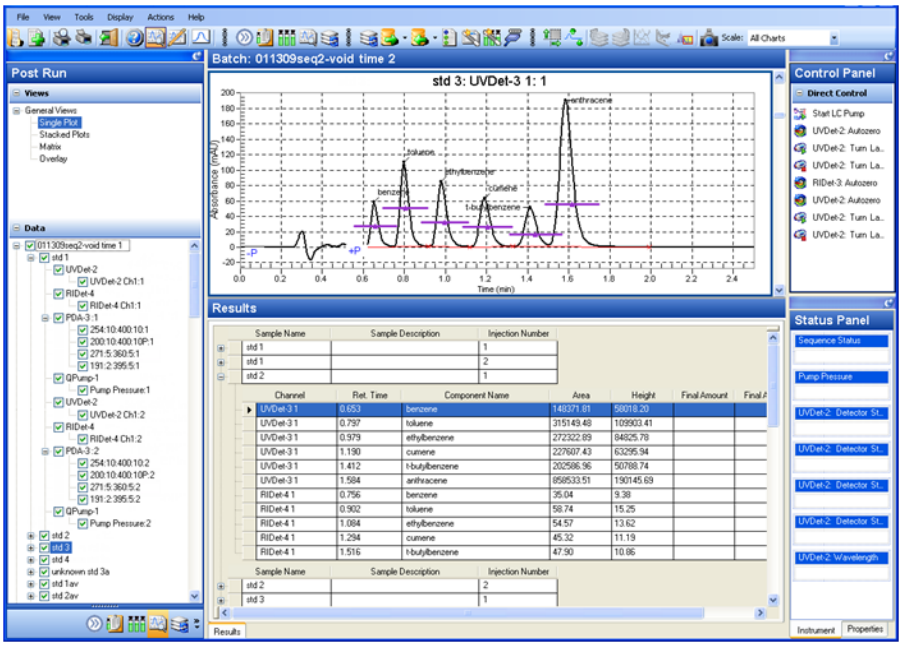
Controls	**Description**
Starting row	Enter a number to set the first row in the sequence The starting row must be less than the ending row. If it is not then the OK button is disabled. This setting does not appear in Single Run mode.
Ending row	Enter a number to set the last row in sequence. The ending row must be greater than the starting row. If it is not then the OK button is disabled. This setting does not appear in Single Run mode.
Suppress processing	A check box to set whether or not data acquired during the run is automatically processed at the end of the run. If **Suppress proceeding** is checked then **Suppress reports** is disabled, since the option is not available unless processing will occur.
Suppress reports	A check box to set whether or not any reports specified for the injection (via the global sequence parameter or via a Report decision point) is generated. This is disabled if **Suppress proceeding** is checked.

Post Run

About the Post Run Environment

The Post Run environment is where you review stored data. The examination of chromatograms, peak integration, and results are all performed here. You enter the Post Run environment by clicking the **Post Run** button in the Navigation Pane (or selecting the **Post Run** command from the **View** menu or clicking the **Post Run** toolbar button).

The Post Run environment is designed so that you can review all aspects of acquired data in a convenient and efficient manner. You are able to select between various views, each of which is designed to provide optimal display of a specific type of data set. In particular, you can configure the chromatogram display to accommodate the flexibility of instrument configurations supported by Chromera.



NOTE: *The relative sizes of two frames can be changed by dragging the splitter bar between them or double-clicking in the title bar of one of the frames toggles between a maximized and restored views.*

The basic concepts in matching the display environment to Chromera data are outlined in Display Data Sets.

The navigation pane is divided into two sections, a **Views** tree and a **Data** tree. By default the main area contains two frames: a graphics frame and a parameter frame. The basic format of the graphics frame depends on the selection made in the tree control located in the upper section of the navigation pane. The parameters frame displays results but the exact format will depend on user preferences.

The graphics frame in Post Run is configurable such that you can define a view port into a virtual display of columns and rows, with each cell containing a chromatogram. If the view port is only a subset of the full display then scroll bars are displayed in the frame as appropriate (i.e. vertical and/or horizontal.) To provide ease of use several predefined views are provided but you have a degree of flexibility to adjust these views to your needs.

Interactions between the various components of the Post Run environment are described below (see Interactions in the Post Run Environment).

Main Menu Commands

The commands shown in the table below are those for a **Post Run** display mode.

Menu	Command	Description
File	Open Data…	Replaces all existing data in the data tree with new data. Displays the data selector in **multiple batch/samples** selection mode. Selecting one or more data sets, populates the data tree with those items and selects the first data set and displays it as determined by the current View mode.
	Add Data…	Adds new data sets to the end of the data tree. Displays the data selector in **multiple batch/samples** selection mode. Selecting one or more data sets, adds the data at the end of the data tree but the current selection in the tree and display will not be changed.
	Close Batch	Closes the batch of which the currently selected plot is a part. All plots will be blanked but the view type will remain unchanged. This is enabled when the data tree is populated and an item in it is selected.
	Print Preview Report…	Displays the Print Report dialog. When you select the required options in the dialog (and click OK) a report will be generated from the data associated with the currently selected node in the data tree using the selected template and the selected print options. This report will be displayed in a standard Print Preview window.
	Print Report…	Displays the Print Report dialog. After selecting the required options in the dialog (and clicked OK) the data associated with the currently selected node in the data tree is reported using the selected template and the results are printed in accordance with the selected print options (see Print Results).
	Lock	Only displayed when user authentication/tracking is active. Minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	If a single run is in progress or a sequence is running then you are prompted that the instrument must be in an idle state before the application can be closed (see Exit dialog). If the instrument is not active then if any unsaved data exists in any environment the software will ask if it is to be saved before exit.

View	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Reprocess environment. The same action as clicking the Reprocessing button in the Navigation Pane.
	Reports	Opens the Report Viewer.
	Toolbars ► View	Toggles display of the View toolbar.
	Tools	Toggles display of the Tools toolbar.
	Graphics	Toggles display of the Graphics toolbar. It is disabled when the Graphics toolbar is not displayed.
	Show Small Icons Show Large Icons	Toggles the size of the icons in the Graphic toolbar.
Tools	Export ►	
	Chromera Results…	Displays the Database Export dialog.
	Methods…	Displays the Method Export dialog.
	Sequences…	Displays the Sequence export dialog.
	Report Templates...	Displays the Report Template Export dialog.
	Import ►	
	TotalChrom Data…	Displays the Import TotalChrom Data dialog.
	Chromera Results…	Displays the Import Results dialog.
	Chromera Methods…	Displays the Import Methods dialog.

	Chromera Sequences…	Displays the Import Sequences dialog.
	Chromera 2.0 Data	Displays the Import Chromera 2.0 Data dialog.
	Report Templates...	Displays the Report Template Import dialog.
	Preferences	Displays the Preferences dialog, showing the preferences associated with the current user (see Setting Preferences).
	Report Format Wizard	Displays the Report Format Wizard in New Report mode (see Report Format Wizard).
	Sequence Wizard	Displays the Sequence Wizard (see Sequence Wizard).
	Device Connections	Displays the Device Connections dialog.
	Error Log	Displays the Error Log dialog
	Dictionary Editor	Displays the Dictionary editor dialog
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector (see Batch Reprocess). It is disabled in an online instance when data acquisition is in progress, or a sequence is running.
Display	Instrument Method	Displays the Instrument parameters (only) stored with the selected sample injection, in a separate window. Enabled when the data tree is populated. **NOTE:** The parameters cannot be edited in this window.
	Peak Detection Parameters	Displays the Peaks section (only) of the method stored with the selected sample injection, in a separate window. Enabled when the data tree is populated. **NOTE:** The parameters cannot be edited in this window.
	Channels	Displays the Channels section of the method stored with the selected sample injection, in a separate window. Enabled when the data tree is populated. **NOTE:** The parameters cannot be edited in this window.
	Component (Species) Calibration	Displays the Calibration section (only) of the method stored with the selected sample injection, in a separate window. Enabled when the data tree is populated. **NOTE:** The parameters cannot be edited in this window.
	Rescale	Displays the Rescale Axes dialog for you to set the X-Axis and Y-Axis minimum and maximum values.

	Auto Rescale	Redraws the contents of the active plot frame at default scaling.
	Previous Layer	Enabled only in views that define layers (e.g. Stacked Plots or Matrix) and only when more than one layer of data exists. Displays the previous logical layer.
	Next layer	Enabled only in views that define layers (Stacked Plots or Matrix) and only when more than one layer of data exists. Displays the next logical layer.
	Overlay	Enabled in Stacked and Matrix views only (i.e. where layers may exist) but only where applicable for the current data set. Not enabled in graphic edit modes. **NOTE:** The Overlay display action depends on the view (see descriptions of each view mode.)
	3-D	Enabled in Overlay mode and the Overlay view only. Toggles 3-D mode on/off for all displayed plots.
	Radar Window	Toggles display of the radar window on and off. Only enabled in Single Plot views (this includes graphic editing modes).
	Plot Style	Displays the Plot Styles dialog (see Plot Styles).
	Plot Layout	Displays the appropriate version of the Plot Layout dialog for the selected view (see section Stacked Plots View). Plot Layout is enabled in standard Post Run mode only and in Stacked Plot and Matrix views.
	Snapshot	Displays the active plot in a full screen window. Use the slider at the top of the window to increase or decrease the transparency.
Actions	Standard Post Run Display	Displays the standard configuration of the Post Run environment (that is with a user configured graphics pane and an optional Results pane in the main display area. If unsaved data exist (from graphic editing) you are first be prompted if the current unsaved data should be saved.
	Peak Identification Review	Displays the graphic editing screen to optimize the results in the Post Run environment (that is with a single plot pane and a tabbed Results/Integration pane).

	Go to Graphic Method Editor	Switches to the Method Editor environment in graphic edit mode. The data from the data tree are carried over and the first data set is reprocessed with the current (stored in the database) version of its referenced method (see section Accessing Graphic Method Editing). **NOTE:** In order for this action to be performed the sample must have a method that exists in the Chromera database associated with it. If it does not (e.g. for imported data) the following error message will be displayed: ”The sample data was not processed with a Chromera method. Data must have been processed within Chromera before this command can be used.”
	Examine Spectra (PDA)	This will start and display the spectral processing in the Chrom/Spectra view mode. This selection is visible whenever the data tree contains a PDA node. If a PDA node is not present the selection is hidden. If execution of the Examine Spectra command fails to launch an error dialogue will be displayed with, if possible, the cause and solution to the problem. An entry into the Error Log will be made.
	Reprocess Current Chromatogram	Reprocesses the currently displayed chromatogram with the current (displayed) version of the method. This command is only used when automatic updating after each change to the method is disabled in user preferences (see Preferences).
Help	Topics	Opens the Chromera Help window.
	Consumables and Accessories	Opens the Consumable and Accessories Catalog
	About Chromera	Displays the copyright and version dialog.

Batch node selected	Report generated with default Summary template (most recently used in Results Print dialog), using all checked chromatograms
Sample node selected	If multiple injections per sample then report generated with default Replicate template (most recently used in Results Print dialog), using all checked chromatograms. If only single injections per sample then report generated with Sample template.
Chromatogram node selected	Report generated with default Sample template (most recently used i Results Print dialog). If the node is one of multi-channel data set the all channels are reported if the template accommodates them, otherwise just the selected channel is reported.

Post Run Standard Toolbar

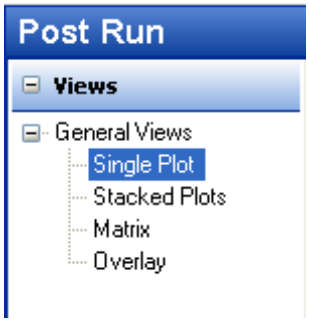
This toolbar displays in the standard Post Run mode:

Or when user authentication active the following toolbar displays:

Icon	Command
	File/Open Data
	File/Add Data
	File/Print Preview Report
	File/Print Report
	File/Lock (Only when user authentication active)
	File/Exit
	Actions/Standard Post Run Display
	Actions/Peak Identification Review
	Actions/Examine Spectra
	Help/Topics

Post Run - Views Tree

The Views tree contains a fixed set of items under a single main heading: **General Views**.

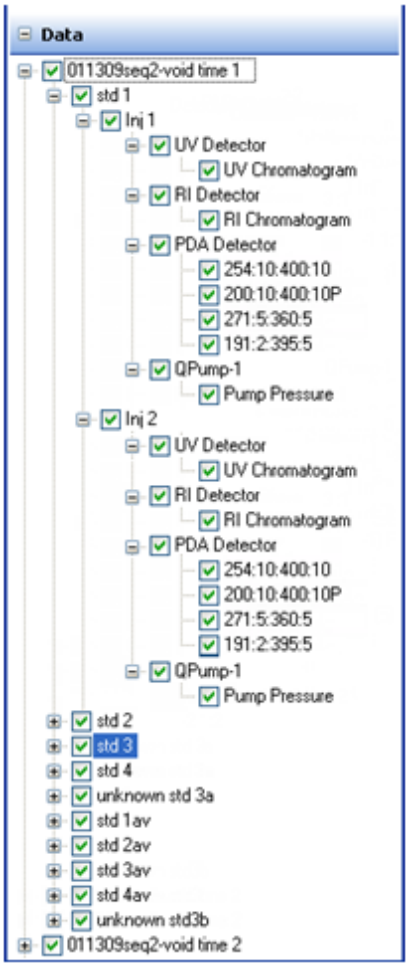


General Views contains the following sub items:

- Single Plot
- Stacked Plots
- Matrix
- Overlay

Post Run - Data Tree

The Data tree contains the levels: **Batch**, **Sample**, **Injection**, **Device**, and **Channel**. Each batch from which data have been selected for display is identified by a top level node consisting of the batch name. The data control tree has a check box associated with each node.



In this example, **Batch** (011309seq2-void time 1) , **Sample** (std 1), **Injection** (inj 1), **Device** (PDA Detector), and **Channel** (254:10:400:10).

The data tree may be populated:

- When you make selections in the data selection dialog, accessed from the **File>Open** menu command.
- Automatically as data are generated during data acquisition.

NOTE: *When an item in the tree is selected it is checked (and hence becomes the displayed plot). In other words, if an item is currently unchecked and it is selected by clicking on the text, the check box is automatically checked.*
When a data set is added to the tree, all chromatograms in the data set are checked by default and the first chromatogram in the data set becomes the selected plot. Which items in the data set are displayed will depend on the current view.

Check/Uncheck Data

When a top level node is checked or unchecked, all nodes under the top level are set to the same state.

Check/Uncheck nodes in the tree as follows:

Shift + Click	If the Shift key is held down while checking (or unchecking) an item in the data tree, then all items of that channel are checked (or unchecked).
Ctrl + Click	If the Ctrl key is held down while checking and/or unchecking items in the data tree, the graphics display is not updated until you release the Ctrl key.

Data Tree: Popup Menu

Right-clicking anywhere within the Data tree control will display a popup menu. The contents of the menu is always the same but which commands are enabled will depend on what specific part of the tree is clicked. If you click on a node in the tree, that node will first be selected before the command is implemented. If you click in empty space within the control

and not on a node then the only commands enabled are Expand All and/or Collapse All, as applicable.

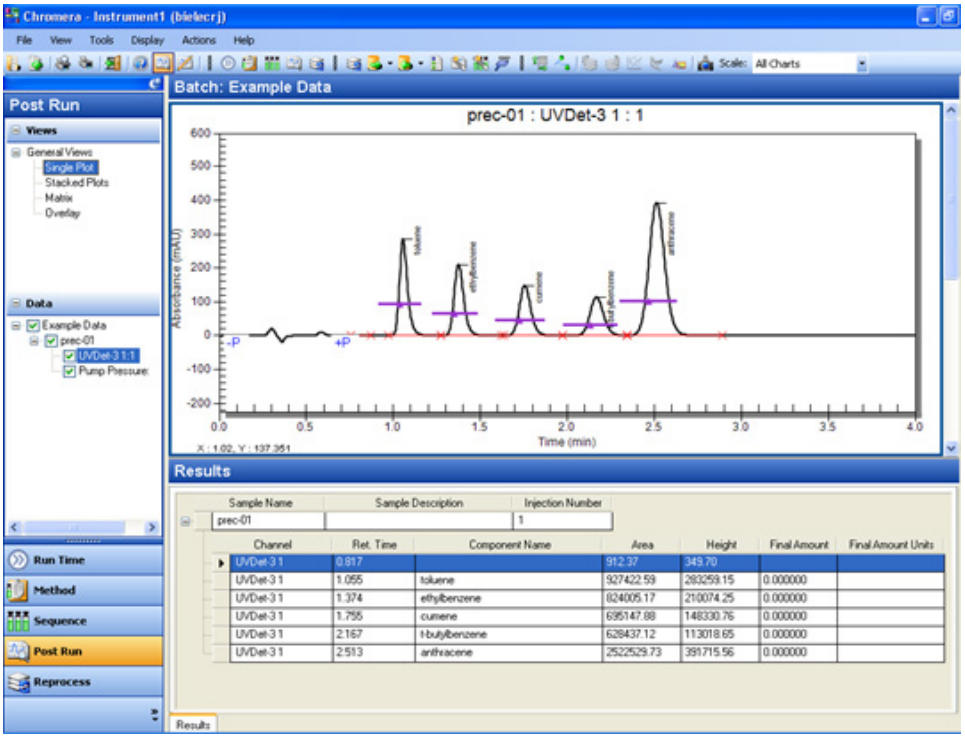
Command	Description
Expand	Displays the child nodes of the node clicked on.
Collapse	Hides the child nodes of the node clicked on.
Expand All Below Here	Fully expands the tree below this point to show all nodes.
Collapse All Below Here	Hides all nodes in the tree below this point other than the root node (the method name).
Select All in This Batch	Checks all items in the currently selected batch (i.e. the batch containing the selected node).
Select Only This Item	Removes the check from all items in the currently selected batch except for the currently selected node.
Close Batch	Closes the batch of which the currently selected plot is a part.

Post Run - Graphics

The graphics frame has two levels of configurability, enabling the presentation of data in the most appropriate manner. The first level of configuration is by selection of a basic layout from the General Views branch of the Views tree. The General Views are: Single Plot, Stacked Plots, and Matrix. The predefined views that include multiple plot panes also allow you to define the number of visible panes and how data are distributed in the panes, thereby providing a second level of configurability.

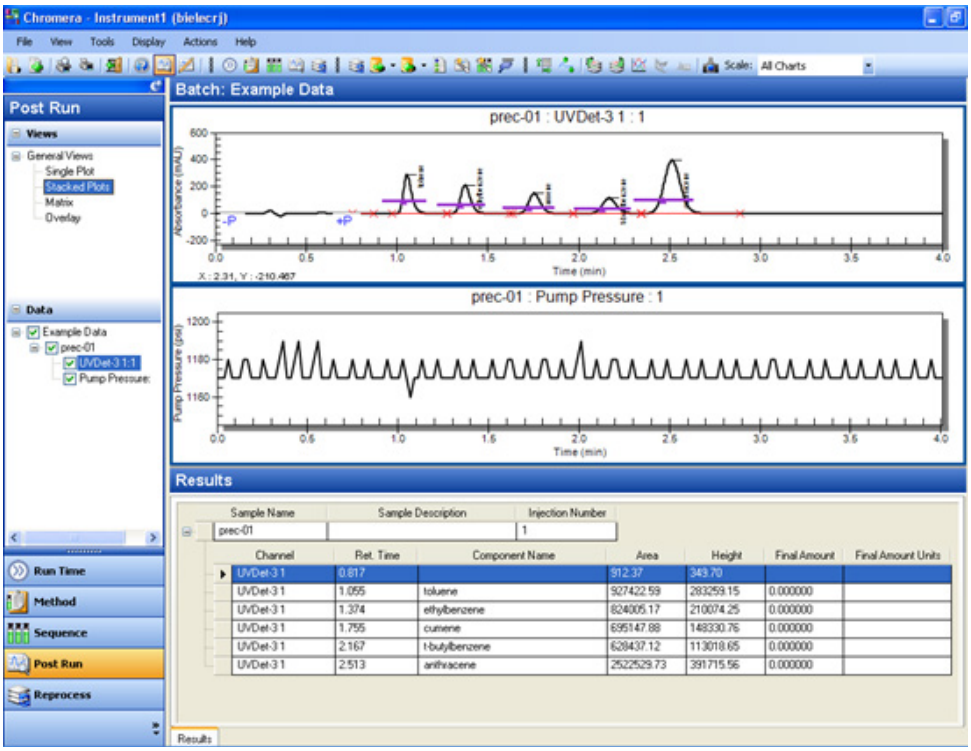
Single Plot View

This is the simplest view, where only one plot control (see Plot Control) is contained in the graphics frame. If more than one chromatogram is checked in the data tree then a vertical scroll bar is displayed. Operations on the scroll bar always result in display of a single, complete chromatogram. Clicking either of the arrows or clicking within the scroll bar displays the adjacent plot (previous or next as appropriate). Dragging and dropping the thumb will cause the plot nearest to the drop position to be displayed; it will not be possible to drag the thumb such that the display will show the bottom half of one plot and the top half of the next.



Stacked Plots View

In this view the plot frame is divided into a number of stacked plots. You can define the number of plots visible and saved as a user preference associated with this view. By default, the visible stack will contain one plot per channel (up to a maximum of four - if more channels exist it is necessary to scroll the display to see the additional channels). For example, you can have a Stacked View with data and pump pressure.



All **Display Mode** features of the plot control (as described in Plot Control) is exhibited by each plot in the stack. This includes:

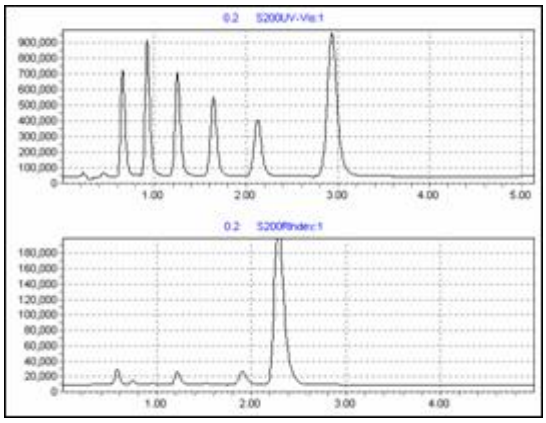
- Scaling changes via zoom box or by setting values in the Rescale dialog
- Display of baselines, peak labels, user labels

As before, manipulation of the scroll bar always results in the display of complete, not partial, plots. In this view the minor increment (arrow click) is one plot and the major increment (scroll bar click) is by the number of plots in the frame. For example, in the above example clicking in the scroll bar below the thumb would cause the two channels for the next sample to be displayed.

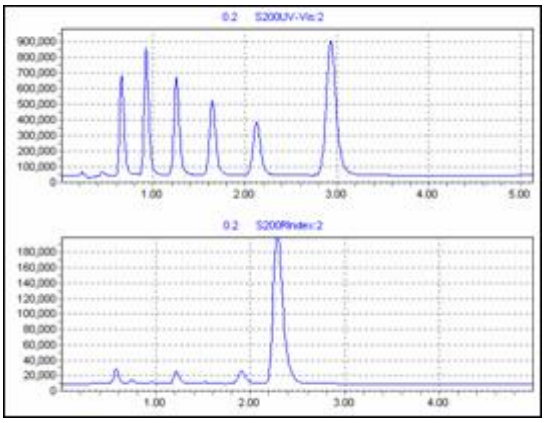
Layers

The Stacked Plots view introduces the concept of layers in the data organization to handle multiple injections of the same sample. In the default case the first injection of each sample from each channel is displayed initially and this is termed the primary layer. If there are replicate injections they are treated as invisible layers that can be made visible in place of the current display or by being overlaid on the current display. The **Next Layer** and **Previous Layer** commands allow you to move from injection to injection (in the default case) and the Overlay command will cause all injections to be displayed overlaid for the currently displayed sample/channels.

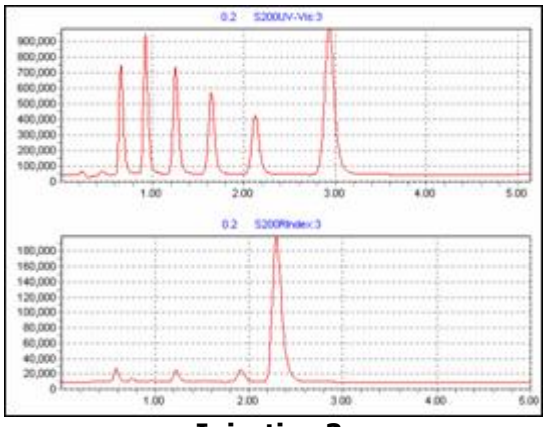
For example you can use the Next layer command to show the layers in succession (as three successive screen displays not a single screen).



Injection 1



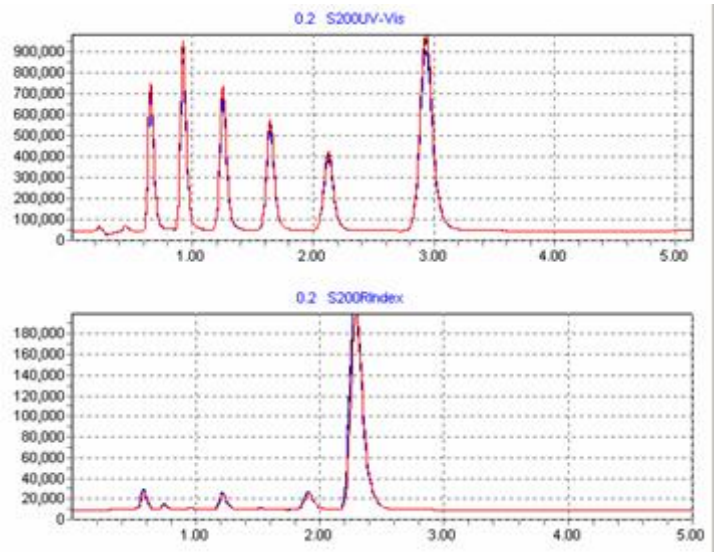
Injection2



Injection 3

If a chromatogram that is not currently checked is selected in the data tree then that chromatogram will be overlaid on the currently displayed plot associated with that sample/channel. For example, if the Injection 1 layer above was currently displayed and the unchecked item for injection 2 of the UV-Vis channel was selected in the data tree then that item would be automatically checked and the chromatogram overlaid on the chromatogram for injection 1 of the UV-Vis channel. The item selected in the data tree will always be displayed (and hence it will be checked).

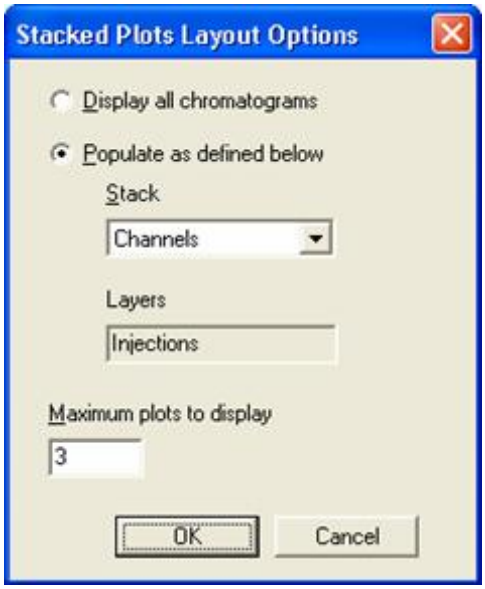
Also, all layers may be viewed simultaneously by choosing the **Overlay** command.



Note that the above description refers only to the default case; the user has other options available. For example, the user may select to display injections in the visible stack, in which case the channels will appear in layers. Or the user could elect to eliminate separate layers altogether and display all chromatograms in the primary layer. Details of configuration of the graphics frame in Stacked Plots view are described below.

Configuration of Stacked Plots View

When the **Stacked Plots** view is selected choosing the **Layout Options** command from the **Display** menu will display the **Stacked Plots Layout Options** dialog.



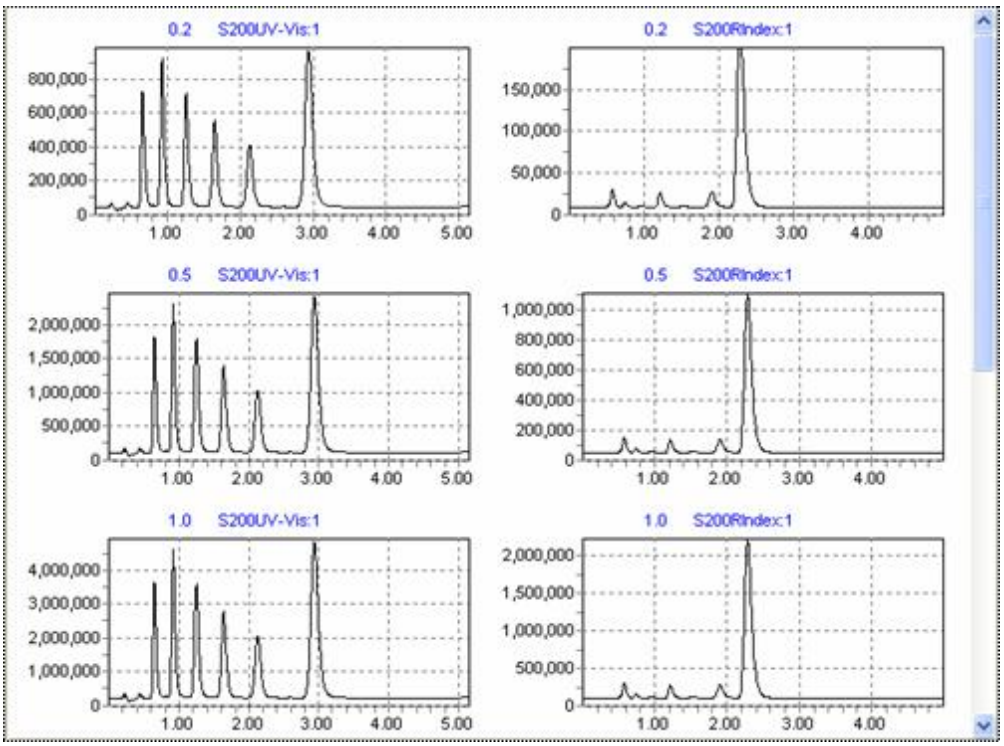
Control	Description
Display all chromatograms	A radio button to display (or not display) all chromatograms defined in the data tree in the primary layer (in the order shown in the data tree) and that the number of plots in the visible stack is as defined by the **Maximum plots to display** control.
Populate as defined below	A radio button to indicate that the Stack control will define the contents of the visible stack.
Stack	A drop-down list (containing **Channels or Injections**) to define the composition of the visible stack in the graphics frame.
Layers	A display indicating the source of data for secondary layers of the display. It displays **Injections** if Channels selected in the Stack control or **Channels** if Injections is selected in the Stack control.
Maximum plots to display	A text box defining the maximum number of plots (from 1 to 8) to be displayed in the visible stack within the graphics frame.

NOTE: *The **Maximum plots to display** control only defines the size of the visible stack when **Display all chromatograms** is set, or when the number of items defined by the Stack selection (e.g. channels) exceeds the value of **Maximum plots to display**.*

Matrix View

In the Matrix View you can change a two-dimensional view port into a virtual 3-D grid of x columns by y rows by z layers, with each grid cell containing a chromatogram. If the view port is only a subset of the full grid then scroll bars is displayed in the frame as appropriate (i.e. vertical and/or horizontal).

The following example shows a view port of three rows and two columns. The full data set includes more data in rows, as indicated by the vertical scroll bar. The organization of data in this example is that each sample in the batch occupies a row in the display with each data channel occupying a column. Replicate injections occupy layers that can be displayed one at a time, using the Next/Previous Layer commands, or simultaneously using the Overlay command (depending on the presentation required).



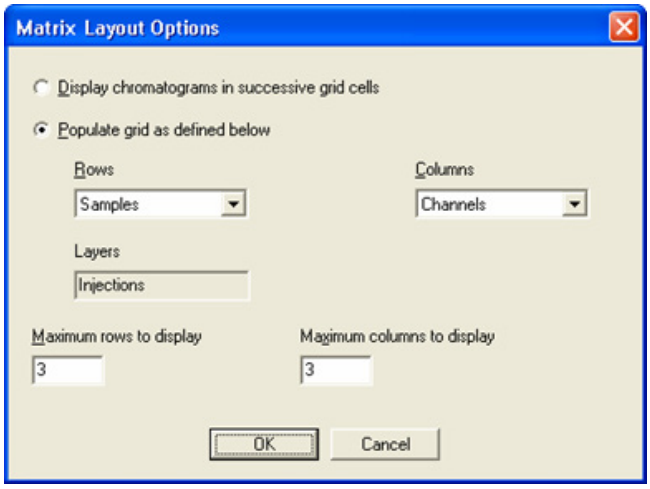
All **Display Mode** features of the Plot Control are exhibited by each plot in the grid. This includes:

- Scaling changes via zoom box or by setting values in the Rescale dialog
- Display of baselines, peak labels, user labels

The action of the scroll bar is similar in this view to that in the Stacked Plots view. That is, the minor increment is one row (scroll in the top sample and scroll in the next one in the above example) and the major increment is by the number of rows displayed (display the next three samples in the above example).

Configuration of the Matrix View

When the **Matrix** view is selected choosing the **Layout Options** command from the **Display** menu will display the **Matrix Layout Options** dialog.

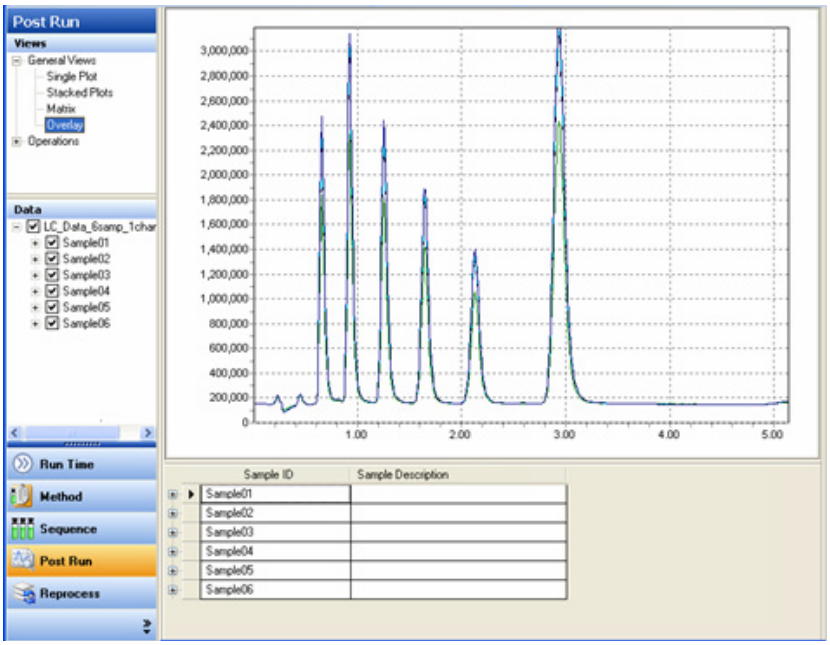


Control	Description
Display chromatograms in successive grid cells	Select this radio button to indicate that the display grid is filled sequentially, cell by cell, using all chromatograms defined in the data tree. The number of plots in the view port (visible in the frame) is as defined by the Maximum rows to display and Maximum columns to display settings.
Populate grid as defined below	Select this radio button to indicate that the Rows and Columns controls will define the contents of the grid.
Rows	Select from this drop-down list **(Samples, Channels,** and **Injections)** to define the contents of rows in the graphics frame.
Columns	Select from this drop-down list **(Samples, Channels,** and **Injections)** to define the contents of columns in the graphics frame.
Layers	Displays the source of data for secondary layers of the display. (The item of Samples, Channels, and Injections that has not been selected in the Rows and Columns controls.)
Maximum rows to display	Enter the maximum number of rows (from 2 to 8) to be displayed in the view port within the graphics frame.
Maximum columns to display	Enter the maximum number of plots (from 2 to 8) to be displayed in the view port within the graphics frame.

NOTES: *(1) The Maximum rows to display control only defines the number of rows displayed in the view port when Display all chromatograms in successive grid cells is set, or when the number of items defined by the Rows selection (e.g. samples) exceeds the value of Maximum rows to display. (2) The Maximum columns to display control only defines the number of columns displayed in the view port when Display all chromatograms in successive grid cells is set, or when the number of items defined by the Columns selection (e.g. channels) exceeds the value of Maximum columns to display.*

Overlay View

When **Overlay View** is selected (assuming some data is loaded into the Post Run environment), the data being inherited from the previous view will be examined to see how many items are checked. Firstly, the information about which items is retained for use if you switch back to a different view. If the number checked is less than or equal to the maximum number defined (shown here as 'N'), then the same items will remain checked in overlay view and all selected curves will be displayed on the chart. If the number checked is greater than N then checks will be removed from all but the first N items and only this first N curves will be displayed on the chart. In addition, a message box will be displayed saying "The number of items checked in the data tree exceeded the maximum (N) that can be overlaid. The first N checks have been retained and these curves are displayed in the overlay view. To display other curves you must first uncheck some of the items currently checked."



- If more data displays than you wish to view, you can clear the chart (and subsequently uncheck all of the Data tree nodes) by pressing and holding the CTRL key as you click Overlay in the Views pane. Once cleared you can check each item in the Data tree you want to display in the chart.

As you check and uncheck items in the data tree, the chart will update to show the currently selected curves (but always less than N+1). If you attempt to check a N+1 item it is prevented or a message displays informing you that the maximum number (N) of curves that can be overlaid are already selected. To display other curves you must first uncheck some of the items currently checked. The set of items checked in the data tree is maintained within overlay view, without affecting the set of items checked in the other views.

This view is customized for the display of multiple overlaid chromatograms. Its key features are:

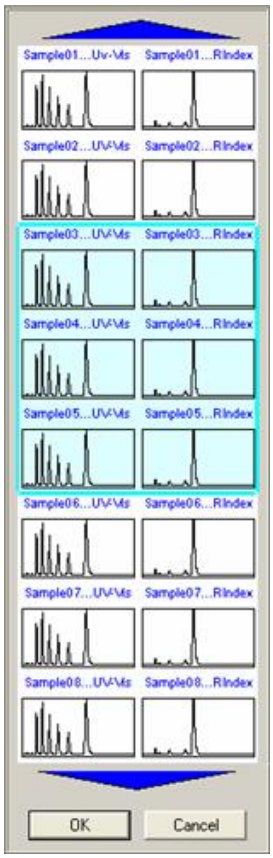
1. Only a single chart will be displayed, although this can include multiple Y axes.
2. There will never be a vertical scroll bar in the graphics pane.
3. Overlay view will display the same data tree contents as the other Post Run views but it will maintain a separate set of checkmarks. That is the plots that are selected for display in Overlay mode can be different from those selected in the other modes.
4. Up to 150 curves can be displayed on the chart at a time.

5. One of the curves displayed on the overlay chart can be selected as the active curve by clicking on it.
6. When data are opened in Overlay view, items will be unchecked by default.
7. The Display/Overlay menu command and the Overlay toolbar button will be disabled in Overlay view.
8. The Display/Plot Layout command will be disabled in Overlay view.
9. The Display/Previous Layer and Display/Next Layer commands will be disabled in Overlay view.

The Viewfinder

The Viewfinder provides you with the ability to adjust the position and/or size of the view port onto the data set. It is accessed by selecting the **Viewfinder** command from the popup menu displayed by right-clicking in the graphics frame of the Stacked Plots or Matrix View.

The principal purpose of the Viewfinder is to locate a particular chromatogram (or related chromatograms such as replicates or multiple channels from the same sample) within the batch data set. This is similar to the picture thumbnails in a digital camera. For this reason the chromatograms displayed within the Viewfinder should not be so small that the basic topography of the chromatograms cannot be discerned. To maximize the useful display area, chromatograms within the Viewfinder do not include Y axis labels since these are not of great significance in the pattern recognition process employed. However, they will have plot titles (displayed in the smallest legible font and shortened by the insertion of an ellipsis if necessary), otherwise distinguishing between similar samples would be impossible.



The view port does not change unless you change it. For this reason there are no scroll bars on the Viewfinder, since if you scrolled the display the viewport indicator would either get left behind and inaccessible or would have to move in some arbitrary manner.

The Viewfinder displays as much of the current data set at once (in the defined X / Y grid format) as can be accommodated on the screen, while maintaining a useful plot size. When the Viewfinder is first displayed the region of the data set where the view port is currently located will always be visible. In the example shown here the view port encompasses both channels of Sample03, Sample04 and Sample05.

The view port can be moved or sized by the usual drag-and-drop techniques (summarized in the table below) but it will resized as necessary at the drop point such that it always encompasses complete plots (i.e. rounded up or down to the nearest plot junction. Also, the minimum view port size is a single plot.

The buttons at the top and bottom of the dialog indicate that other samples exist in the dataset. Clicking on one of these buttons (when active) will scroll the display but this mechanism will only be required if the user wants a view port larger than the number of plots visible in the Viewfinder. If the first sample in the dataset is shown then the top arrow is grayed out (or invisible); similarly if the last sample is displayed the bottom arrow is grayed out (or invisible).

Dragging the view port (or the top or bottom boundary) to the top or bottom of the dialog then the plot thumbnails scroll appropriately. The scrolling speed must be set carefully to ensure this is a usable feature. Increasing the scrolling speed as long as the view port rectangle is kept at the top or bottom of the dialog would be a useful feature, as long as the rate of increase is not too great.

Clicking **OK** updates the main display to show the plots defined by the new view port size and position.

NOTE: *If the images used by the Viewfinder are not available for any reason, the Viewfinder will not be displayed. Instead a error message dialog will be displayed saying "There are no images to display in the Viewfinder." The Viewfinder Help topic should elaborate on this message since it will not be obvious to the user that the Viewfinder uses previously generated 'thumbnail' images rather than display regular plots.*

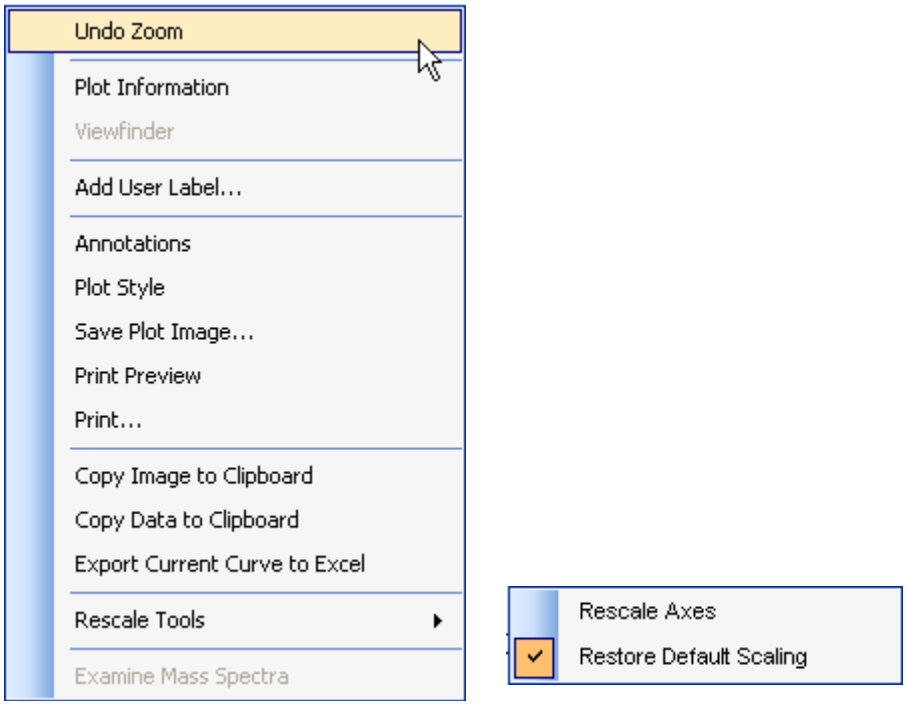
View Port Moving and Sizing

Icon	Action - Icon type	Position where cursor changes to icon and action on drag
	Move - four pointed arrow	Within view port and not within 'hit' range of an edge Moves view port to a new location in the data set
	Resize - arrow NW to SE	Upper left, lower right corners of view port Upper left - Change size of view port, leaving lower right corner fixed in place. Lower right - Change size of view port, leaving upper left corner fixed in place.
	Resize - arrow NE to SW	Upper right, lower left corners of view port Upper right - Change size of view port, leaving lower left corner fixed in place. Lower left - Change size of view port, leaving upper right corner fixed in place.
	Resize - vertical arrow	Upper, lower edges of view port Upper - Change height of view port, leaving lower edge fixed in place. Lower - Change height of view port, leaving upper edge fixed in place.
	Resize - horizontal arrow	Left, right edges of view port Left - Change width of view port, leaving right edge fixed in place. Right - Change width of view port, leaving left edge fixed in place.

NOTE: *The icons shown here are the Windows defaults; the actual icon displayed is defined in your Windows settings.*

Post Run - Graphics: Popup Menu

Post Run graphics plots inherits the basic plot popup menu items (Save Plot Image to Rescale Tools) and includes some additional ones to provide quick access to key plot properties. This menu is displayed whenever you click on an area of the plot other than an existing object, such as a user label or, when in graphic edit mode, a timed event.



The menu commands are described below.

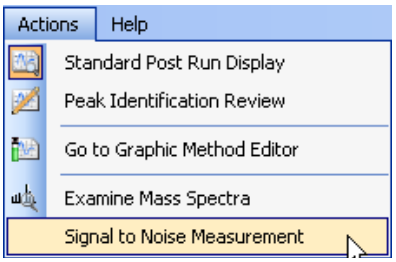
Command	Description
Zoom	Select zoom then hold the left mouse button to draw a zoom box by dragging the mouse across the plot area. Releasing the mouse button immediately rescales the plot. Depending on the relative X and Y dimensions of the box, the plot will be rescaled on the X axis, Y axis, or both.
Plot Information	Displays the Plot Information dialog for the selected plot.
Viewfinder	Displays the Viewfinder for moving or resizing the current viewport into the Grid View. It is displayed in Stacked and Matrix Views only.
Add User Label…	Displays the User Label dialog. The default text for a new label is the Y axis position (in minutes) at the point where the mouse was clicked.
Annotations	Displays the Annotations dialog.
Plot Style	Displays the Plot Style dialog.

Save Plot Image...	Displays the Save As dialog for you to name and save the image to a directory of your choice.
Print Preview	Opens a preview window and displays the chromatogram as it will be printed. You can select Portrait or Landscape orientation.
Print...	Prints the current chromatogram display to a selected printer. You can select Portrait or Landscape orientation.
Copy Image to Clipboard	Copies the current chromatogram display to the clipboard in bitmap format.
Copy Data to Clipboard	Copies the X and Y values for the current chromatogram to the clipboard in text format, with one pair of values per line. This command will only be available when a single chromatogram is displayed. Alternatively, it could just copy the values for the selected chromatogram (but that is not even always displayed in Chromera currently) or it could copy concatenate the sets of values for different plots.
Export Data to Excel	Exports to current data to Excel.
Rescale Tools ► Rescale Axes	Displays a dialog box to set the X- and Y- Axes.
Restore Default Scaling	Click to restore the X- and Y-axes to the default settings.

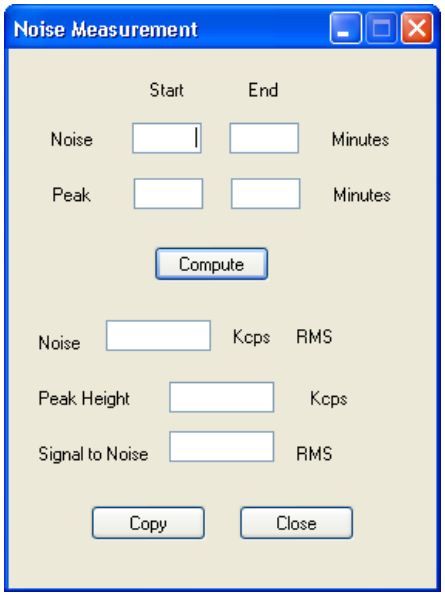
Signal to Noise Measurement

Signal to Noise measurement is available in **Post Run** and in the **Graphic Method Editor**.

1. Select **Signal to Noise Measurement** (S/N) from the **Actions** menu.



The **Noise Measurement dialog** appears.



2. When the Noise Measurement dialog displays, the focus is in the **Start** box for **Noise**.

3. Enter the appropriate values for determining the S/N, into the dialog. These values represent:
   - The start time and end time of a baseline segment you select as representative noise.
   - The start time and end time of an integrated peak whose signal to use for the calculation.

NOTE: *Values may be entered into the dialog either manually or graphically. If the values are entered manually, the **Compute** button must be clicked for the calculations to occur. If the values are selected graphically, the calculations are performed automatically. The following steps describe the graphical technique for selecting these values.*

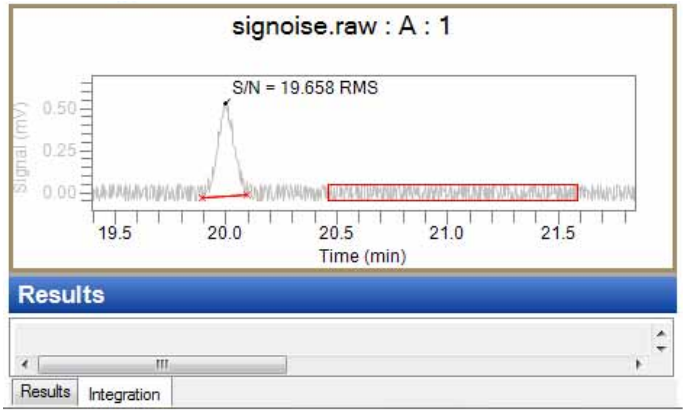
4. To select the Noise Start and End times, hold the CTRL key while dragging the mouse across a segment of baseline where the noise measurement is to be made. Within this window, the algorithm fits a straight line through that segment and determines the **RMS** noise for the selected baseline. This value is displayed in the **Noise** box in the lower half of the dialog.

NOTE: *(RMS = Root Mean Square. When comparing actual data with expected data the straight line, in this case, square the error at each data point add the squares together take the square root of that sum.)*

5. Then, the focus shifts to the **Peak Start** text box.

6. To select a peak whose signal will be used for the S/N calculation, either click on an integrated peak in the chromatogram, or hold the CTRL key and drag the mouse across the chromatogram to define a time window that includes a peak of interest. When a peak is selected by click-and-drag, it does not have to be an integrated peak. (See "How it Works" below.) The **Peak Height** of that peak is displayed in the lower half of the dialog.

7. Once both the noise segment and peak of interest have been selected, the calculated RMS Signal to Noise value will be displayed, along with the baseline Noise and Peak Height values used. A label containing the calculated S/N value will be added to the chromatogram, with an anchor line pointing to the peak selected.



Peak selection may be repeated as often as desired. If a different peak is selected, the new Signal to Noise value will be calculated automatically. To define a new noise window, simply click in one of the **Noise** text boxes in the dialog to move the focus back to that field, and then select or enter new values for the Start and End times. Again, the new Signal to Noise value will be calculated automatically.

How it Works

Here's what happens when you select a peak window, either by typing in Start and End time values or by CTRL-click dragging the mouse.

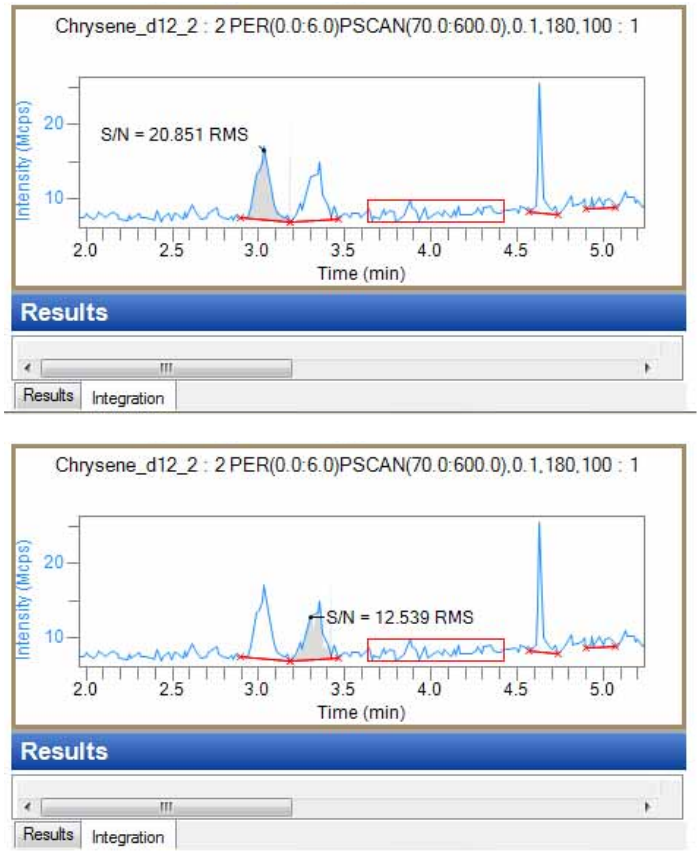
1. Within the selected window, the point of maximum amplitude is assigned to be the peak crest.

2. The points of **minimum** amplitude to the left and right of this crest are assigned as the ends of the peak's baseline.

3. The times in the **Peak Minutes** windows in the dialog are changed to show the actual peak window that was found in this way.

4. Peak height is determined as the vertical distance from the Crest point to the baseline that is drawn between the two points that were found in Step 2.

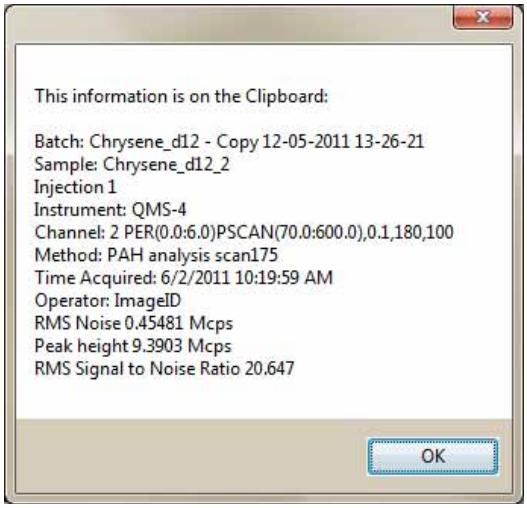
   If the peak you desire to use for the S/N calculation has already been detected and integrated, you do not need to define the peak window. All you need to do is to click within the peak on the chromatogram, and the existing baseline and peak-height values from the Results table will be used for the calculation.



Copying the Information

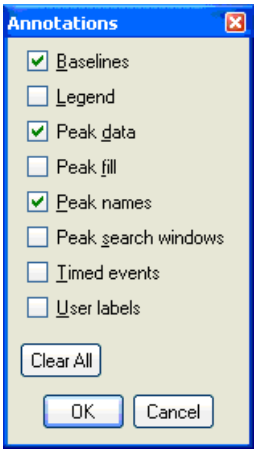
- Clicking the **Copy** button copies the measurement and identifying data for the channel to the Clipboard, and displays the information dialog shown below. From there, it may be pasted into any text editor.

NOTE: *There is no provision to print this information directly.*



Post Run Graphics Annotations Dialog

This dialog is displayed when the **Annotations** command is selected in the **Graphics Popup Menu**. It is provided so that you can make several changes to the annotations selection at one time. Unlike most dialogs this one is positioned at the point where the user clicked the popup menu, so that you do not have to move the mouse far to make the required changes.

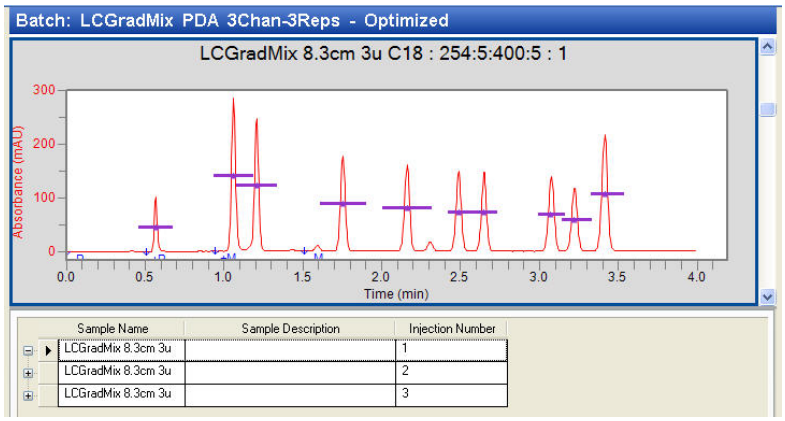


Command	Description
Baselines	A check box that indicates peak integration baselines will be displayed on all chromatograms.
Legend	A check box that indicates a legend relating curve color to sample name will be displayed above each chart.
Peak data	A check box that indicates the peak data items defined in Plot Options will be displayed for each integrated peak, in the orientation defined in plot options.
Peak fill	A check box that indicates integrated peaks will be filled in with the color defined in Plot Options.
Peak names	A check box that indicates names will be displayed for each peak that has been matched to a component
Peak search windows	A check box that indicates the peak search window gadget will be displayed for each component when graphic editing mode is active.
Timed events	A check box that indicates (instrument and) integration events from the processing method will be displayed on each chromatogram (where applicable).
User labels	A check box that indicates labels created by the user will be displayed on each chart for which labels have been created.
Clear All	A command button that will clear all check boxes in the dialog.

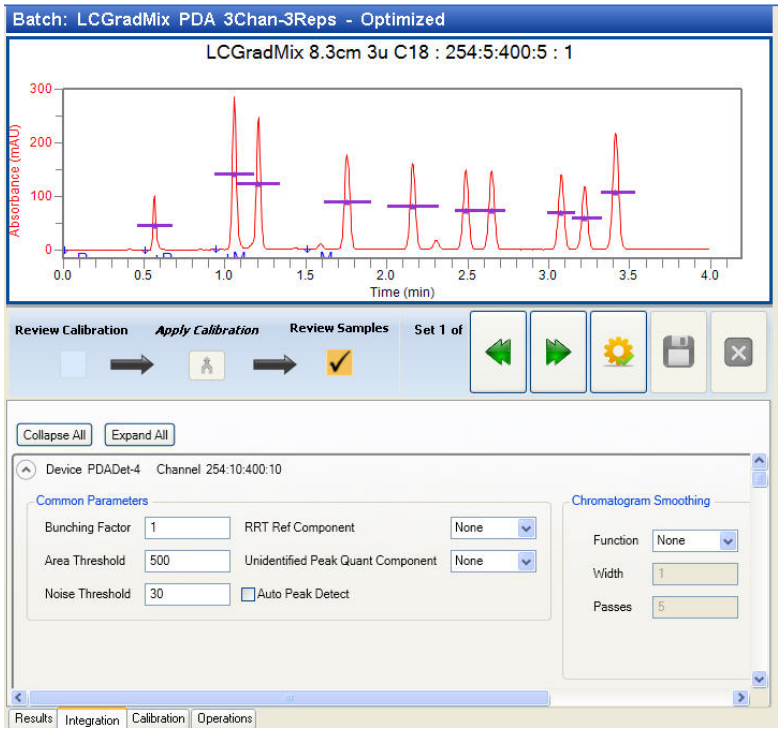
Clicking **OK** automatically applies the settings from the dialog to the current display and will sets in Plot Options. These are currently global settings and hence the Annotations dialog and Plot Options always show the same settings. Clicking **Cancel** in the Annotations dialog causes no changes to the display or Plot Options.

Peak Identification Review

Selecting **Peak Identification Review** from the **Actions** menu is an extension of the **Post Run** environment. The following shows how to switch from standard post run display to a graphic editing display to optimize results.



Standard Post Run Display

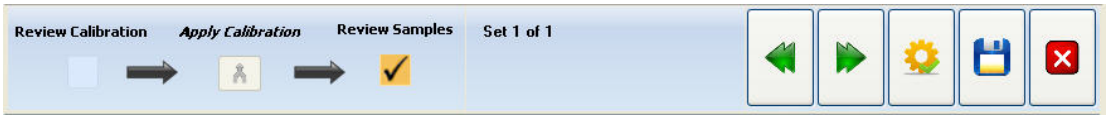


Peak Identification Display

At first this display looks very similar to that for graphic method editing, however there are several key differences. The most noticeable difference is that there is no method section tree. This is because there is no method in this case, or, more accurately there is no single entity called by a single method name that can be edited. A result may contain parameters from more than one method (instrument parameters from one method and processing parameters from a different method), and it is invalid to edit some of these parameters (for example the instrument parameters). In place of the Method tab from Graphic Method Editor the Graphic Results environment has an Integration tab. This provides convenient access to the parameters that you can edit.

The **Views** section in the Navigation Pane is eliminated in graphic results editing mode, since the plot frame only ever contains a single plot control displaying a single chromatogram.

Editing Toolbar

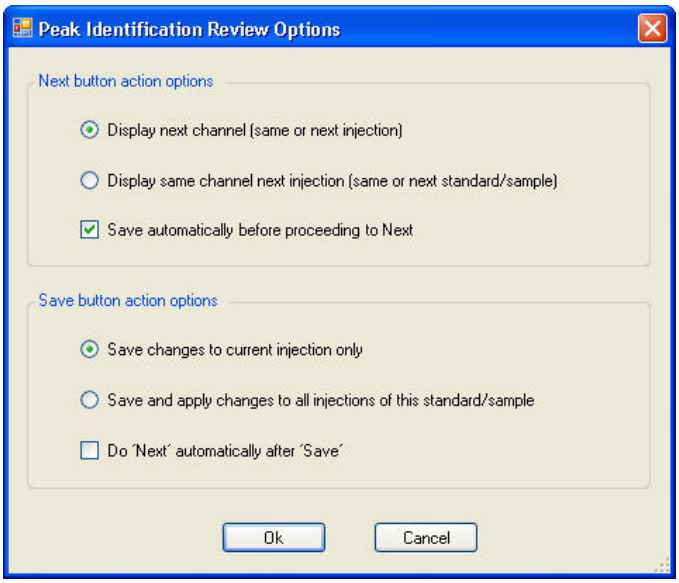


Control	Command/Caption	Description
(unselected) (selected)	**Review Calibration**	A button that indicates whether Review Calibration mode is selected. **NOTE:** It will be possible for both Review Calibration and Review Samples to be unselected at the same time.)
(unselected) (selected)	**Review Samples**	A button that indicates whether Review Samples mode is selected. **NOTE:** It will be possible for both Review Calibration and Review Samples to be unselected at the same time.)
(inactive) (active)	*Apply Calibration*	A button that updates the sample results using the current version of the 'composite calibration'. The button will be inactive (disabled) if no changes to calibration standards are currently pending. That is the button becomes active (enabled) when one or more calibration standards are edited and will be inactive again after it has been clicked to update the sample results. The terms 'inactive and 'active' are used (rather than simply 'disabled' and 'enabled') because for this control its state being active should indicate to the user that the command must be performed before proceeding to review samples.
	Set n of m	Displays the current 'set' number and the total number of 'sets' in the batch. NOTE: This will not actually be displayed if the batch consists of only one 'set' (as shown in the mockup example).

Control	Command/Caption	Description
	Prev (Previous)	A button that causes the previous chromatogram in the current 'set' (as defined in Option) to be displayed. If there are unsaved changes to the current chromatogram the user will be prompted whether to save the changes, discard them or cancel the 'Previous' command). This button will be disabled when Review Calibration is selected and the first channel of the first injection of the first calibration standard channel in the batch is displayed, or when Review Samples is selected and the first channel of the first injection of the first sample is displayed.
	Next	A button that causes the next chromatogram in the current 'set' (as defined in Options) to be displayed. If there are unsaved changes to the current chromatogram the user will be prompted whether to save the changes, discard them or cancel the 'Next' command) This button will be disabled when Review Calibration is selected and the last channel of the last injection of the last calibration standard channel in the batch is displayed, or when Review Samples is selected and the last channel of the last injection of the last sample is displayed.
	Options	A button that displays the Options dialog, which enables the user to customize elements of the user interaction.
	Save	A button that saves any integration or peak identification changes made to the current chromatogram. If the current chromatogram is a calibration standard then the composite calibration will be updated automatically. Optionally a 'Save' command will automatically execute a 'Next' command also. This button will only be enabled when unsaved changes exist to the current chromatogram.
	Cancel	A button that cancels any unsaved changes made to the current chromatogram and restores the original integration/peak identification – parameters and display.

Peak Identification Review Options

In addition, a number of options have been provided that enable users to customize certain elements of the process to better fit their specific needs.



Group/Field	Description
Next button action options	
Display next channel (same or next injection)	A radio button that sets the action of the 'Next' button to display the next detector channel, according to the data tree hierarchy.
Display same channel next injection (same or next standard/sample)	A radio button that sets the action of the 'Next' button to display the next instance of the current channel, if any, within the current review group (standards or samples).
Save button action options	
Save changes to current injection only	A radio button that sets the action of the Save button to save parameter and results changes to the current channel/injection only.
Save and apply changes to all injections of this standard/sample	A radio button that sets the action of the Save button to apply the parameter changes to all replicates of the current sample/channel, and to save the results.
Do 'Next' automatically after 'Save'	A check box that invokes the Next action (as defined herein automatically after the Save function. The Save option setting has no effect on which chromatogram will be displayed, since applying changes to all replicates does not change the current chromatogram.

Main Menu

Menu	Command	Description
File	The following is inserted into the File menu following the **Add Data** command.	
	Save Result	Saves the results for the selected sample run as a new version within the existing batch. Saves the results for all updated data sets as new versions within the existing batch.
	Save Method As…	Saves the displayed Integration, Identification and Calibration parameters, together with the Instrument section and Channels section from the method saved with the current data set, as a new method. Displays a dialog to enter a Name and a Group for the new method, which is then saved under a new identifier in the database.
Display	Rescale	Displays the **Rescale Axes** dialog (enabled when the data tree is populated).
	Auto Rescale	Redraws the contents of the active plot frame at default scaling (enabled when the data tree is populated).
	Radar Window	Toggles display of the radar window on and off. Only enabled according to options set in **Plot Styles** (not displayed/displayed in all single plot views/displayed in graphic edit only).
	Plot Styles	Displays the **Plot Styles** dialog. It is enabled at all times.
	Snapshot	Displays the active plot in a full screen window.
Actions	The following is appended to the Actions menu in graphic editing mode:	
	Reprocess Current Chromatogram	Reprocesses the currently displayed chromatogram with the current (displayed) version of the method. This command is only used when automatic updating after each change to the method is disabled in user preferences (see Preferences).

Standard Toolbar

This toolbar adds two buttons for Action menu items (Optimize Peak Detection and Reprocess Current Chromatogram) to those available in the standard Post Run environment:

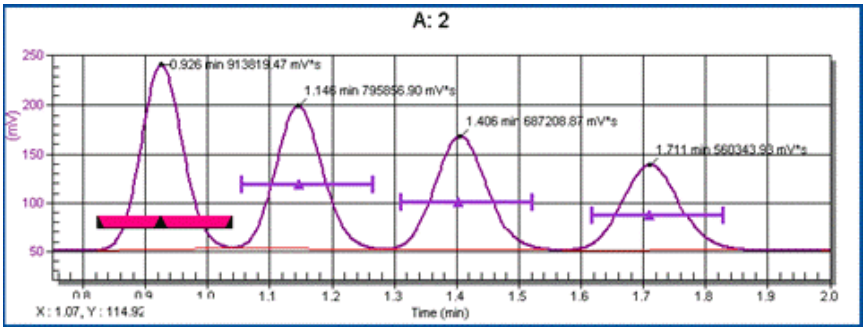
Or when user authentication is active:

Icon	Command
	File/Open Data
	File/Add Data
	File/Print Preview Report
	File/Print Report
	File/Lock (Only when user authentication active)
	File/Exit
	Actions/Standard Post Run Display
	Actions/Peak Identification review
	Actions/Reprocess Current Chromatogram
	Help/Topics

Chromatogram Control

Annotations on the chromatogram are determined by the **Plot Styles** settings. The plot exhibits the full range of interactive behavior described in Plot Control.

In graphic editing only one chromatogram is displayed at a time. That is, a single plot control (no stack) and no overlaid chromatograms. This is to make it clear as to which chromatogram the integration and component settings apply to.

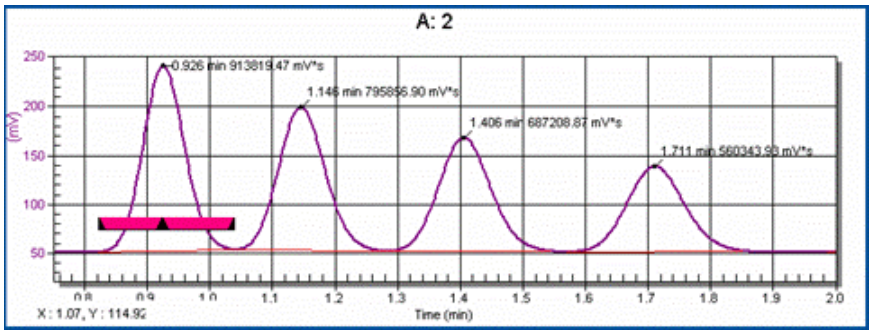


When a sample injection includes data from multiple channels, the chromatogram displayed depends on the current selection in the method tree.

Component Search Window Tool

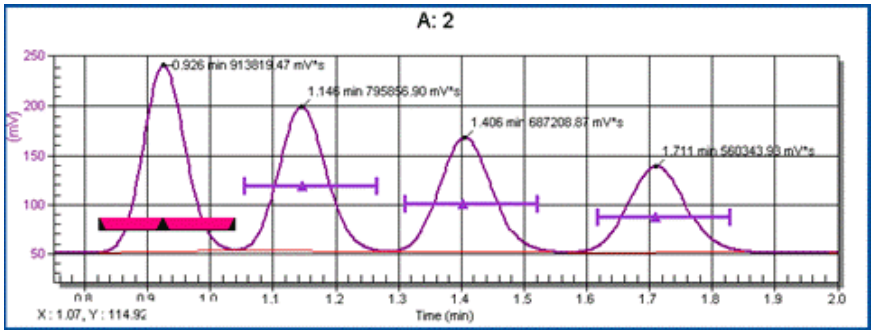
This is a graphic element that enables you to set the expected retention time and the search window for a component. The tool initially appears (assuming that Component Search Windows are selected as displayed Annotations) when you click on the chart to indicate the peak to be identified as the component. The selected form of the tool is similar to the Bunching Factor tool but it has an additional triangle marker indicating the expected retention time of the component within the search window. You can drag this central triangle to a new position within the search window, as well as repositioning the whole tool and

resizing the tool. Whenever you make a change to the position or size of any element of the tool, the Retention Time, Peak Search Start and Peak Search End fields will be updated in the parameters grid of the selected channel.



When the mouse pointer is positioned over any of the triangular handles the cursor changes to ↔ , indicating that the width of the object can be changed and the central marker can be moved.

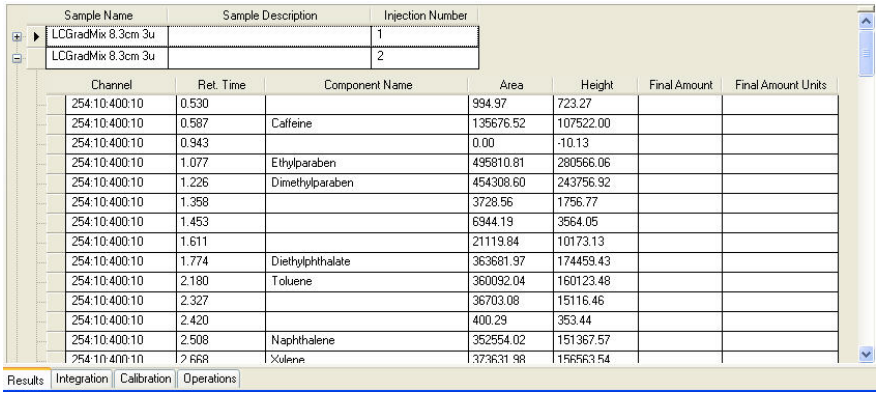
When multiple component tools are displayed, the single selected component is indicated by its different form as well its different color. The colors used for the body of the tool in the case of selected and non-selected components are user-selectable within Plot Options. The handles for resizing the gadget are always black.



Tab Control

Results Tab

The data displayed on this tab will depend on the selection in the data tree. If a sample node is selected then results from all channels for all injections is displayed in the peak table. If a channel node is selected then the results from just that channel is displayed.



Sample Name	Sample Description	Injection Number
LCGradMix 8.3cm 3u		1
LCGradMix 8.3cm 3u		2

Channel	Ret. Time	Component Name	Area	Height	Final Amount	Final Amount Units
254:10:400:10	0.530		994.97	723.27		
254:10:400:10	0.587	Caffeine	135676.52	107522.00		
254:10:400:10	0.943		0.00	-10.13		
254:10:400:10	1.077	Ethylparaben	495810.81	280566.06		
254:10:400:10	1.226	Dimethylparaben	454308.60	243756.92		
254:10:400:10	1.358		3728.56	1756.77		
254:10:400:10	1.453		6944.19	3564.05		
254:10:400:10	1.611		21119.84	10173.13		
254:10:400:10	1.774	Diethylphthalate	363681.97	174459.43		
254:10:400:10	2.180	Toluene	360092.04	160123.48		
254:10:400:10	2.327		36703.08	15116.46		
254:10:400:10	2.420		400.29	353.44		
254:10:400:10	2.508	Naphthalene	352554.02	151367.57		
254:10:400:10	2.668	Xylene	373631.98	156563.54		

Results | Integration | Calibration | Operations

This means that results may be displayed for a chromatogram that is not currently visible but the convenience outweighs the inconsistency (this is also true for Post Run). Note that the results displayed are those generated by processing the selected data set with the currently displayed version of the method parameters – even on the initial display. Results stored in the database for the selected data set are never displayed here, although it is possible that the results are the same if the method and the peak processing algorithms are unchanged.

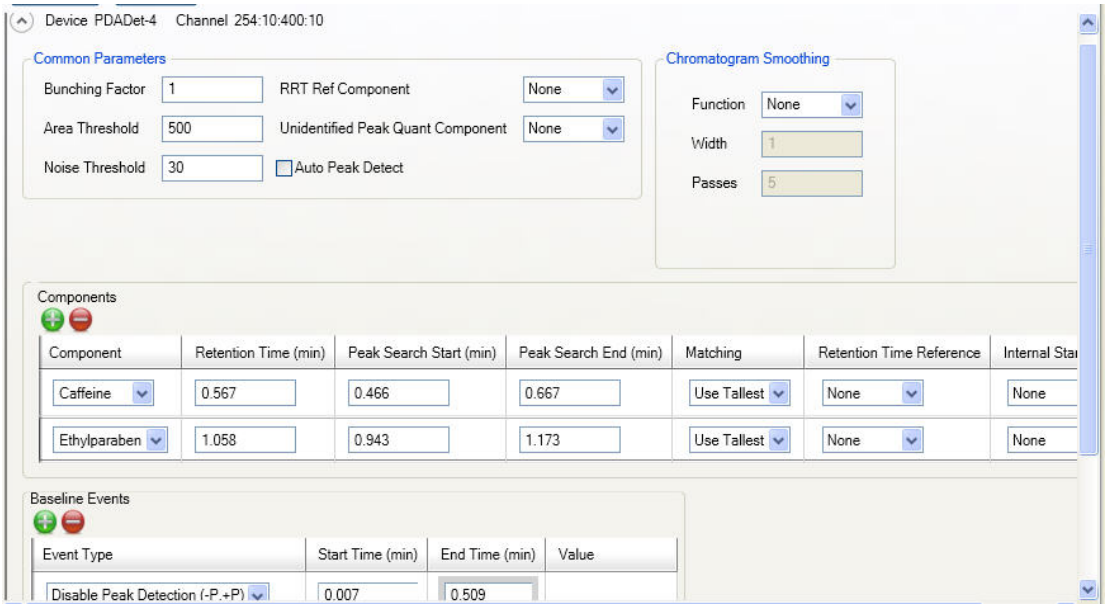
NOTE: *Although it can be disconcerting to see different results here from in the Post Run environment, it is an inevitable consequence of bringing in data from an earlier release that had different integration algorithms. To see old results unaffected by updated algorithms simply view the data in standard Post Run mode.*

The first band in the results grid will always display the Sample Name, Sample Description, and Injection Number for the selected data set. If the selected data set is a single injection of a sample (i.e. not the top level batch name node) then the Injection Number is displayed also (as in the above example). The format of the secondary band (i.e. the columns displayed) can be defined by the user in a *Properties* dialog, accessed via a right-click popup menu. The contents of the display cannot be edited on this tab.

Integration Tab

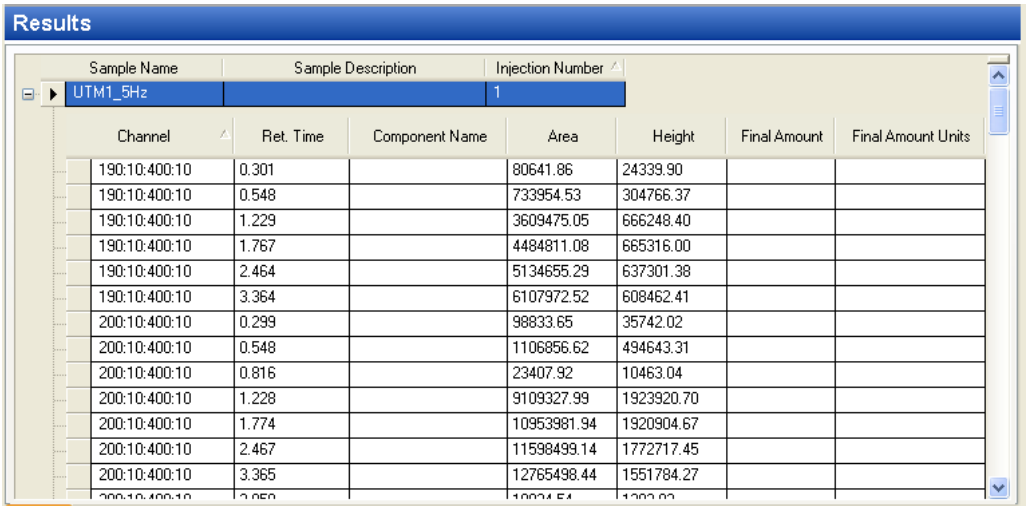
This tab displays the Peaks section of the method for the selected channel. The term Integration is used rather than Peaks to help reinforce the fact that this is not a free-standing method that is being edited.

NOTE: *The **In Use** field does not appear here since it is not applicable in the post run environment*



Post Run - Results

The basic layout of the Results pane in Post Run is a band for each injection, which in turn each have a secondary band containing a row per reported component/peak (this may be all peaks or identified peaks only). The top level bands can be expanded or collapsed in the usual way.



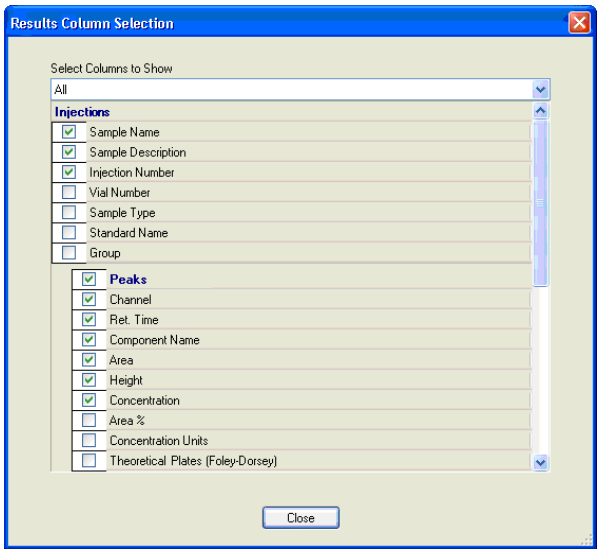
You can define the columns displayed in each band by right-clicking within the results area to display a popup menu. This displays a dialog to select the displayed columns.

Results Displayed

A selection in the data tree will determine which result node is visible (i.e. selected and brought into view) in the results display but all results are displayed at all times.

Results Column Selection Dialog

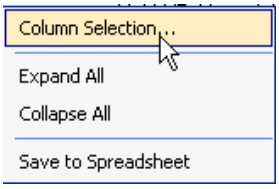
The Results Column Selection dialog lists the columns available for display in the injection band and the peak table in a hierarchical format.



Control	Description
Select columns to show	Select from the drop-down list the parameters to be displayed in the list. The selections are: **All** – Shows both sample/injection parameters and peak table columns. **Components –** Shows only the peak table columns. **Injections** – Shows only the sample/injection parameter columns.
Injections	A section of the check list that shows the sample/injection parameters. The initial default display includes: Sample Name, Sample Description and Injection Number. Any change you make will be remembered and the display will reflect the user's preferences the next time the results frame is displayed.
Components/Peaks	A section of the check list that shows the peak table columns. The initial default display includes: Components, Channel, Retention Time, Component Name, Area, Height, Final Amount and Final Amount Units. Any change you make will be remembered and the display will reflect the user's preferences the next time the results frame is displayed.
Close	Clicking this button closes the dialog and updates the results table display based on the current settings.

Post Run - Results Popup Menu

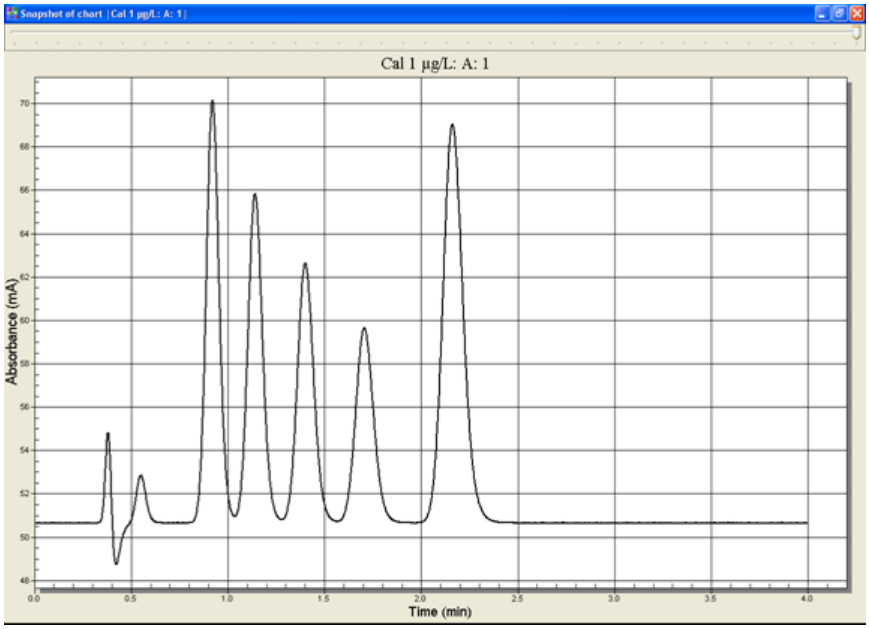
The Results popup menu appears whenever you right-click anywhere within the results area.



Command	Description
Column Selection...	Displays the Column Selection dialog.
Expand All	Fully expands the results grid to show all bands.
Collapse All	Hides all peaks bands in the grid leaving only the sample/injection bands shown.
Save to Spreadsheet	Displays a standard File Save dialog, enabling you to save the results in Excel (.xls) format. The entire batch will be saved with sample/injection information in header rows, followed by the results table, as a set of expanding rows below the header rows.

Post Run Snapshot Window

The Snapshot command displays the currently selected chromatogram (at default scaling) in a full-screen window, with exactly the same content of the display in the Post Run view. The only manipulations of the chart within the snapshot window are to zoom (via rubber-band box) and unzoom (via popup menu).



The slider control at the top of the window allows you to control the transparency of the Snapshot window. As transparency is increased (by moving the slider from right to left) the Chromera Post Run window below will become more and more visible, enabling you to review the contents of the view without closing the Snapshot window.

The Snapshot window has its own icon in the Windows taskbar, enabling you to switch to Chromera (or any other application) and back to the Snapshot as required. You can close the Snapshot window by clicking the standard Windows close button in the top right-hand corner.

Snapshot Window Popup Menu

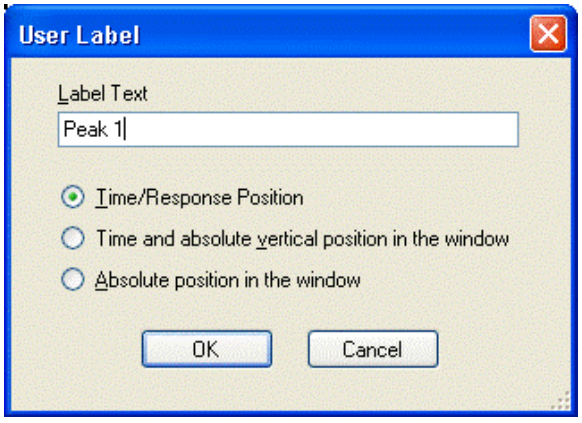
A popup menu containing an **Unzoom** command is available in the Snapshot window after you have zoomed in on the plot using the standard rubber-band box technique. This restores the Snapshot window to its previous scaling. No scaling changes made in the Snapshot window will be reflected in the main display when the Snapshot window is closed.

User Label Dialog

When the mouse pointer is on the plot, right-clicking displays a pop-up menu where one selection is **Add User Label**. This allows you to add custom text at the location designated by the mouse click. Note that this means the location of the initial mouse click that displayed the context menu, not the mouse position when the command was selected (which you have no control over).

NOTE: *Entering a new User Label to an existing chromatogram will take the current format settings. The existing User Labels will keep their original format settings and not change to the current format settings. You must re-enter the User Labels to get the updated format settings.*

In addition to specifying the text, you can set its text properties, and also specify whether the label is to be locked to X and/or Y data. If the label is locked to X data then it always appears at the same time on the plot, regardless of the scaling. If it is not locked to X data then it always appears at the same relative distance along the X-axis (i.e. as % of time span), and therefore moves relative to the chromatogram topography when the scaling changes. Similarly, if the label is locked to Y data then it always appears at the same response level, regardless of scaling. If it is not locked to Y data then it always appears at the same relative distance along the Y-axis (i.e. as % of full scale).



Control	Description
Label text	Enter text (up to 25 characters) to be displayed as a user label.
Time/Response Position	Select to display the label locked to the data. That is, at the data coordinates defined by the right mouse click. The label X/Y position is affected by plot scaling just as the chromatogram is affected.
Time and absolute vertical position in the window	Select to display the label locked to the time defined by the right mouse click but always at the same vertical position (i.e. % full scale) in the plot window. The X position of the label is affected by plot scaling but not the Y position.
Absolute position in the window	Select to display the label at the absolute position (% full scale of X and Y) in the plot window defined by the right mouse click. Neither X or Y position of the label in the plot window are affected by plot scaling.

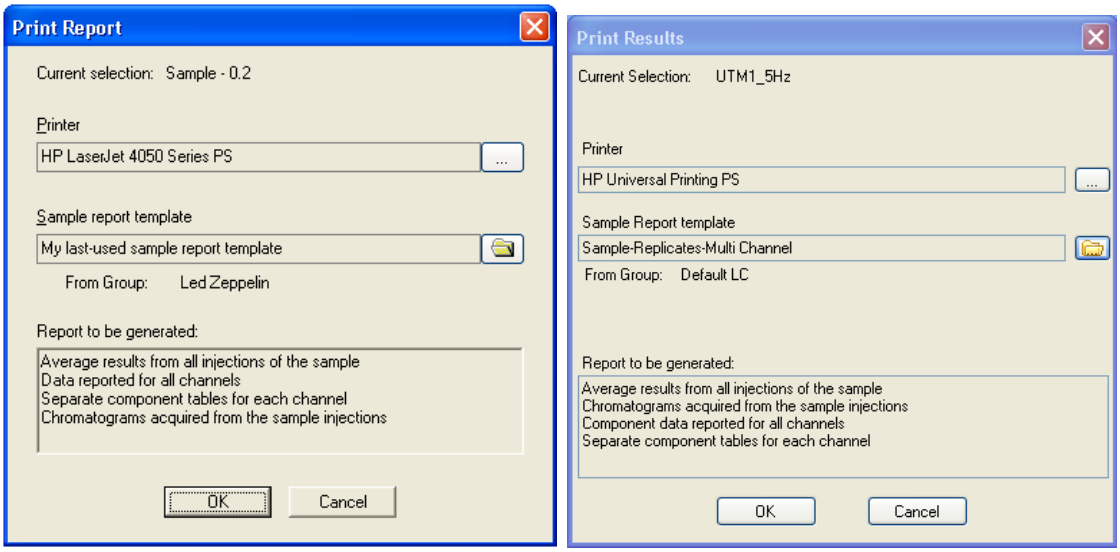
User Label Popup Menu

The popup menu appears whenever you right-click on a user label.

Command	Description
Change Label Font and Color	Displays standard Windows Font dialog, allowing the user to set Font Name, Style, Size and Color.
Change Label Background Color	Displays standard Windows color selection dialog, allowing the user to select the background color for the label.
Label Border	Toggles display of a black border around the label (one pixel thickness) on or off. The menu item is checked (or highlighted) when a border is displayed.
Delete This User Label	Deletes the selected user label (without confirmation). There is no undo.
Delete All User Labels	Deletes all user labels from the selected chart (without confirmation). There is no undo.

Print Report/Print Preview Report Dialog

The Print Report dialog displays when the **Print Report...** or **Print Preview Report...** command is chosen from the **File** menu within the Post Run environment. The dialog allows you to define what type of report will be printed (Summary report template or Sample report template depending on data selection), on which printer the report will be printed, and the report template to be used. Note that only one report can be generated at a time.



Set the following in this dialog:

Control	Description
Current Selection	Displays the identification for the item selected in the data tree. Batch node: Displays 'Batch – ' + batch name Sample node: Displays 'Sample – ' + sample name Injection/Channel node: Displays Sample:Channel:Injection – ' + sample name + ':' + channel name + ':' + injection number
Printer	Clicking the browse button  displays the standard Windows printer selection dialog to select a printer to be used to output the report.
Summary/Sample report template	Clicking the browse button  , displays a standard File Open dialog for you to select a report template file to be used for generating the results printout. The caption associated with the field indicates the type of report that can be generated from the current data selection.
From Group:	Displays the name of the Group the currently selected report template is assigned to.
Report to be generated:	Displays information about the selected template.

When the dialog is displayed, the default report template shown will the most recently used template associated with the current data selection type (i.e. Batch or Sample/Channel/Injection). If no most recently used template exists for this user/instrument, then the field is blank. In this latter case the **Report to be generated:** field will also be blank.

Clicking the Report Template Selection folder button, the standard report template selection dialog is displayed. However, the templates available for selection are filtered, so that only templates appropriate to the current data selection are shown. When a batch node is selected, only Summary report templates are listed. When a sample or channel/injection node is selected, only Sample report templates are listed.

When the report template field is populated, the software examines the template to determine its basic characteristics. These are displayed in the 'Report to be generated:' field.

Reprocess

About Reprocess

After you have acquired data and stored it, you can process the data repeatedly. This is called reprocessing the data. Reprocessing allows previously acquired data to be viewed and reprocessed under its original conditions or modifications can be made to the original conditions. For example, if reports were not generated when the original data were acquired, the original data file can be reprocessed so that reports are generated.

Reprocessing matches the processing performed during data acquisition; that is, each row (and each injection) is processed according to its designated sample type. For example, if a Blank/Matrix row appears then the data from that run is subtracted from standards and samples that follow (and use the same method). Similarly, the presence of standards will result in a change to the calibration of the method, if Calibration is included in the processing functions specified.

The term "batch data set" reflects the set of data initially generated by execution of a sequence, that is, data acquisition. A batch data set consists of the following:

- Identification for the batch derived from the sequence name, group and date/time at the start of sequence execution
- The sequence parameters from each row used to acquire the data
- The results data for each channel (peak RTs, areas, amounts, etc)
- The method parameters used to control the instrument and process each channel of data
- References to the raw data acquired (time/response values derived from the detector(s)
- References to auxiliary data from some non-detector devices (e.g. time/pump pressure values from a pump)

The functionality of batch reprocessing within Chromera are:

- The input data for reprocessing is any batch from the database, selected using the Chromera query and browsing tools
- It is possible to reprocess all channels from a sample data set or just a selected subset
- The reprocessing performs the actions required for the designated sample type (e.g. Calibration, Baseline Subtraction)
- You may elect to reprocess with the current version of the method specified in the batch row or the version stored with the current results
- It is possible to edit the sequence parameters prior to reprocessing

About the Data to be Reprocessed

Batch Selection	Basic selection of the batch data set to be reprocessed will be made using the standard Chromera data selection tools. At present you are restricted to selecting a single batch for reprocessing.
Sample Selection	Within the Reprocess environment you are able to elect not to reprocess certain samples but all of the batch will appear within the display.
Channel Selection	For any given sample it is possible to select to reprocess only specific channels of data within the data set. However, this option is not available if you change any sequence parameters. This is because sequence parameters are global to all channels and therefore reprocessing only one channel with modified parameters would result in inconsistent or invalid results.

About Channels

A channel is a digital data set received from an instrument, or derived from data received from an instrument. Some channels (those representing chromatograms) are processed by the software; others (such as solvent gradient profiles and pump pressure curves) are acquired and stored for subsequent display only. A channel may be a directly acquired signal from a device or it may be a derived stream (e.g. a chromatogram extracted from spectral data or a resultant chromatogram derived by subtracting a stored blank data stream from an acquired signal). The latter is used by the speciation application.

The supported detectors all provide data in the form of chromatograms. However, derived channels produced by the subtraction of one chromatogram from another will also be supported by the LC application.

There is no arbitrary limit to the number of channels that can be acquired, for example:

- Simultaneously from a single detector (for example, absorbance output at different wavelengths).
- Simultaneously from multiple detectors on a single instrument.
- Simultaneously from multiple instruments.

Processing Method

You have two global options for reprocessing the batch:

- To use the current version of the method specified in the batch row
- To use the version of the method stored with the current batch data set

These two options may provide a different set of available reprocessing functions.

You also have the option of editing the batch row to specify a completely different method. Of course if this option is selected the current version of that method will always be used for reprocessing.

It is not possible to use sections from two different methods simultaneously, or different versions of the same method. However, in some cases, this can be achieved by using graphical reprocessing followed by batch reprocessing. For example, adjusting the integration of a chromatogram and then reporting it using the calibration stored with the original results could be accomplished in this way.

Sequence Parameters

Most sequence row parameters will be editable when batch reprocessing is set up and any modified parameters will be used during reprocessing. Changes to sequence parameters will have implications for the reprocessing functions available and/or for the resulting data set(s).

Specific sequence row parameters that will not be editable are:

- Vial Number - this clearly cannot be changed after acquisition when an autosampler is used
- Injection Volume - this is currently a control parameter for acquisition and not used in calculations
- Number of Injections - this clearly cannot be changed after acquisition

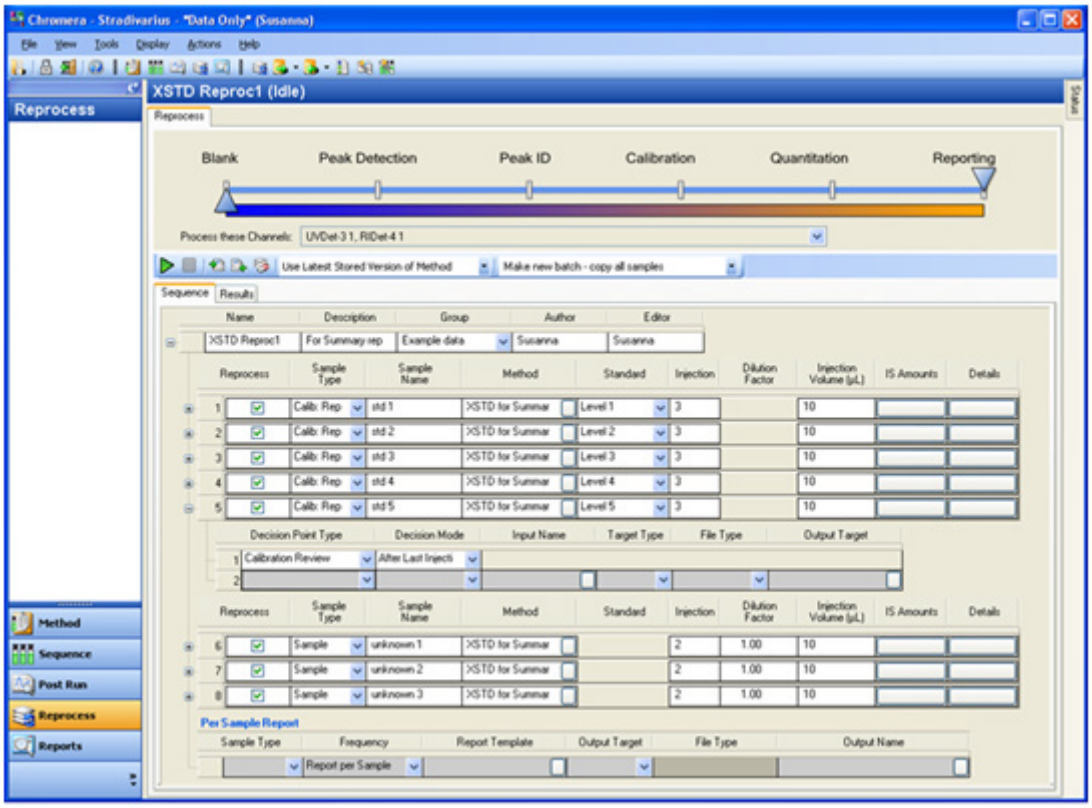
Resulting Data Sets

Reprocessing involving simply a modified (including recalibrated) method will generate a new version of results for that injection within the Batch dataset. These new results will become the default results displayed for that injection, although previous version will still be accessible via a database query.

If any sequence parameters (more accurately, batch identification or sample parameters) are modified then a completely new batch data set is generated. The extent to which data are copied from the original batch to the new one depends on several factors, including the nature of the parameter changes made.

Reprocess – Main Screen

The general appearance of the Batch Reprocess environment showing a single Batch open for reprocessing is shown below:



The main display area contains two frames: an options selection frame and a parameter control frame. The options frame will show controls for selection of the scope of batch reprocessing (start and end points and the channels to be reprocessed). The parameter area contains two tabs, one displaying the batch to be reprocessed in a format very similar to the sequence editor frame in Sequence or Run Time, and the other displaying Results identical to the one in Post Run.

The header of the main display shows the name of the batch currently being viewed in this environment, together with its status (see Title Bar).

Menus and Toolbars

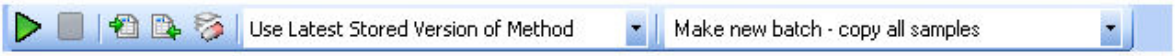
Main Menu Commands

Menu	Command	Description
File	Open Data…	Displays the data selector in single batch selection mode. When you select a data set the parameter display is populated with those items. Any existing batch will be replaced by the new batch.
	Lock	Only displayed when user authentication/tracking is active. Minimizes the Chromera window to the taskbar. Before the window can be restored the valid password for the associated user must be entered.
	Exit	Online instance: If data acquisition or a sequence is running then the user will be prompted that the instrument must be in an idle state before the application can be closed (see Exit Dialog). If the instrument is not active then the following procedure for an offline instance will apply. Off line instance: If unsaved data are present in any other environment then the user will be prompted to save each in turn. Closes the application window.
View	Run Time	Switches to the Run Time environment. The same action as clicking the Run Time button in the Navigation Pane.
	Method	Switches to the Method environment. The same action as clicking the Method button in the Navigation Pane.
	Sequence	Switches to the Sequence environment. The same action as clicking the Sequence button in the Navigation Pane.
	Post Run	Switches to the Post Run environment. The same action as clicking the Post Run button in the Navigation Pane.
	Reprocess	Switches to the Reprocess environment. The same action as clicking the Reprocessing button in the Navigation Pane.
	Reports	Displays the Report Viewer window to view reports generated in this environment.

	Toolbars ► View Tools Graphics	 Toggles display of the View toolbar. Toggles display of the Tools toolbar. Toggles the display of the Graphic toolbar.
Tools	Export ► Chromera Results… Methods… Sequences… Report Templates…	 Displays the Database export dialog. Displays the Method export dialog. Displays the Sequence export dialog. Displays the Report template Export dialog.
	Import ► TotalChrom Data… Chromera Results… Chromera Methods… Chromera Sequences… Chromera 2.0 Data... Report Templates…	 Displays the Import TotalChrom data dialog. Displays the Import Results dialog. Displays the Import Methods dialog. Displays the Import Sequences dialog. Displays the Import Chromera 2.0 data dialog. Displays the Report template Import dialog.
	Preferences	Displays the Preferences window, showing the preferences associated with the current user (see Setting Preferences).
	Report Format Wizard	Displays the Report Format Wizard in New Report mode (see Report Format Wizard).
	Sequence Wizard	Starts the Sequence Wizard (see Sequence Wizard).
	Device Connections	Displays the Device Connections dialog.
	Error Log	Displays the Error Log dialog.
	Dictionary Editor	Displays the Dictionary editor dialog.
	Reprocess	Initiates the Batch Reprocessing function by displaying the Data Selector (see Batch Reprocess). It is disabled in an online instance when data acquisition is in progress, or a sequence is running.
	Batch Builder	Starts Batch Builder.
Display	----------	----------
Actions	Run Options	Displays the Run Options dialog, to set the Start and End Rows for reprocessing.

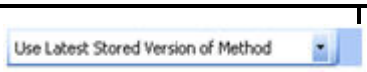
	Set as Start Row	Sets the selected row as the first row to be run when the batch reprocess is started. Enabled when the batch is idle (or Paused) and a row is selected.
	Set as End Row	Sets the selected row as the last row to be run when the batch reprocess is started. Enabled when the batch is idle (or Paused) and a row is selected.
	Start Reprocess	Initiates the batch reprocess. Enabled when a batch is open.
	Pause Reprocess	Inhibits reprocessing of the next row when the current row is complete. Enabled when a batch is being reprocessed. Subsequent use of the **Start Reprocess** command would (by default) cause the batch to resume reprocessing with the next row.
	Stop Reprocess	Inhibits reprocessing of the next row when the current row is complete. Enabled when a batch is being reprocessed. Subsequent use of the **Start Reprocess** command would (by default) cause the batch to be reprocessed from the Start Row.
	Clear Batch	Closes the displayed batch.
Help	Topics	Opens the Chromera Help window.
	Consumables and Accessories	Opens the Consumable and Accessories Catalog
	About Chromera	Displays the copyright and version dialog.

Batch Reprocess Toolbar



The function of each command is described below:

Icon	Command	Description
	Start Reprocess	Begins reprocessing of selected rows only, starting with the specified Start Row. The Start button is enabled when the batch is not being processed. Each icon is always enabled when it is displayed. The current state of the batch is indicated in the header in the main display and by highlighting of the currently executed row in the display.
	Pause Reprocess	Does not process the next sample after the current sample has been reprocessing is completed. The Pause button is enabled when the batch is being reprocessed.
	Stop Reprocess	Terminates reprocessing of the batch when the current sample (if any) has been processed. Enabled when the batch is being processed or when paused.
	Start Row	Sets the currently selected row as the staring row when the batch is started (or restarted). If the designated Start Row is not marked for reprocessing then the first marked row following that is the first row reprocessed. This button is disabled when the batch is being processed. **NOTE:** Start and Stop Rows are defined in Batch Reprocess in a similar manner to that used in the Run Time environment (see Setting Start and Stop Rows).
	Stop Row	Sets the currently selected row as the stop row when the batch is started (or restarted). This must be a later row than the Start Row. If the designated Stop Row is not marked for reprocessing then the last marked row prior to that is the last row reprocessed. This button is disabled when the batch is being processed.
	Clear Batch	Clears the batch display from the current view. This button is disabled when the batch is being processed.

	Method Options	Select the method version from the drop-down list to use for processing. Options available are: **Use Latest Stored Version of Method:** The current version of the method specified in the sample row is read from the method database and used for reprocessing. **Use Method Saved with Results:** The version of the method saved with the results are used for processing (processing must start with Quantitation or later – see below). **NOTE:** Rows in which a new method name is selected will always be processed with the current version of that method. This is disabled when the batch is being processed.
	Save/Copy Options	Select from the drop-down list whether a new batch should be created and what data should be copied to the new batch. **Make new batch – copy all samples:** Creates a new batch and copies all samples (and all injection versions) of the original batch to the new batch prior to reprocessing. **Make new batch – copy reprocessed samples only:** Creates a new batch and copies only the raw data of the samples selected for reprocessing, together with any injection version data required for reprocessing, to the new batch. **Update current batch – no new copy:** Updates the original batch with results of the reprocessing (as new injection versions). This is the default selection when a batch is opened. This is disabled when the batch is being processed.

Batch Reprocess Standard Toolbar

This toolbar displays in Reprocess: 

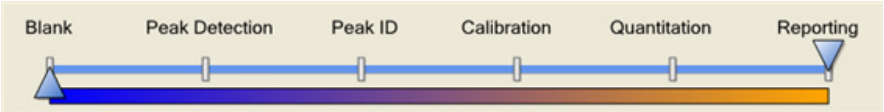
Or when user authentication active: 

Icon	Command
	File/Open Data
	File/Lock (Only when user authentication active)
	File/Exit
	Help/Topics

Processing Functions and Rules

Processing Functions

Using the Options Panel on the Reprocess screen, you may select to have the software reprocess the data completely (from blank/baseline subtraction to report generation) or to perform only certain functions. For example, you may select to re-quantify results without re-integrating or re-identifying the peaks. The process steps that may be selected are:



1. **Blank/Baseline Subtraction** - (where applicable)
2. **Peak Detection and Integration** – When peak areas, heights, and other peak properties are determined.
3. **Peak Identification** - When detected peaks are identified and matched with components (species) in the method, based upon retention time windows and reference peaks, etc.
4. **Calibration** – When identified peaks in a Calibration Standard Sample are used to update the component calibration section of the method, with the appropriate information from that standard.
5. **Quantitation** – When the peaks in an Unknown Sample are processed against the current calibration information in the method, and new results are generated from that processing.
6. **Reporting** – When new sample reports and/or chromatograms are generated, by simply reporting the current set of data for the current sample.

Processing Rules and Other Considerations

The following list summarizes the key rules concerning the scope of reprocessing:

- An analyst is not allowed to select an ending step that occurs before the starting step. For example, it is invalid to start processing with Quantitation and end processing with Peak Identification.
- Processing cannot skip steps. For example, it is invalid to perform Peak Identification and Reporting without performing Quantitation.
- Peak Detection and Integration are considered a single step for the purpose of reprocessing.
- It is possible to reprocess unknown samples only, without the need to include calibration standard samples, by using the current calibration state of the method for each row to re-quantitate that sample.
- Quantitated results will not be calculated for calibration standard samples. Until the calibration is complete the results of using the intermediate states of the method can be grossly misleading. If you wish to re-report calibration standards to treat them as samples, the batch must be reprocessed with the Sample Type changed from **Calib** to **Sample** (or **Standard** to sample for speciation applications).
- All reportable data rows should have full accessibility to all reports. It is possible for the customer to print a Sample report for a calibration standard, if desired.
- When reprocessing starts with the Reporting step no data will ever be changed; existing data are read and reported.

How Batch Reprocess Works

There are different ways to arrive in the Batch Reprocess environment with a batch displayed and ready to be processed. Before describing the Batch Reprocess environment itself, the various ways of launching it, and populating it with data, is described. All approaches to Batch Reprocess share the characteristic that they will load a single batch at a time.

NOTE: *Ensure that the data set is correct before starting reprocess, rather than making adjustments as part of the reprocessing interaction itself.*

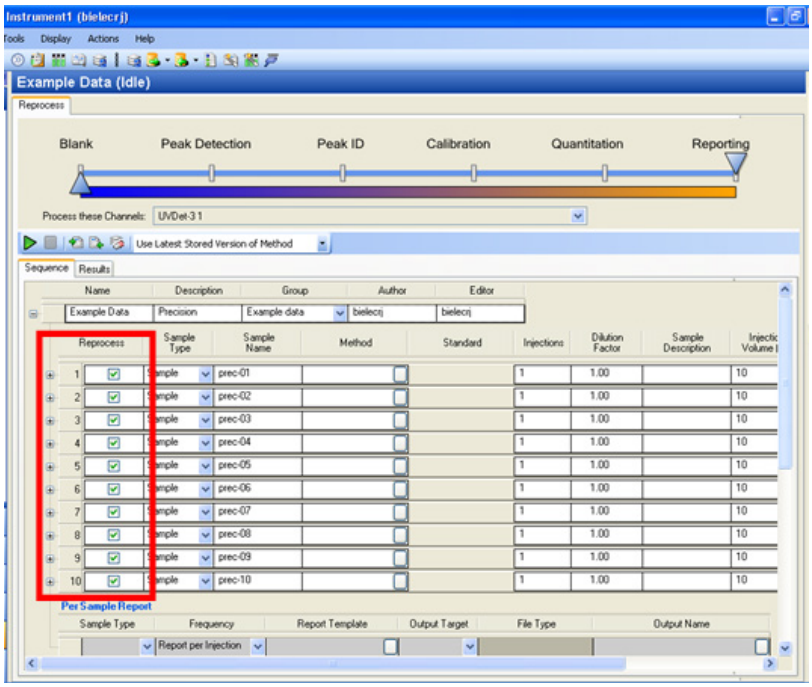
The following procedure is a overview on How Batch Reprocess Works.

1. Clicking the **Reprocess** button in the Navigation Pane (or selecting the **Reprocess** command from the **View** menu or clicking the **Reprocess** toolbar button) displays the Batch Reprocess environment in its last-viewed state which can be one of the following:
    - The data display pane is blank if no data has ever been reprocessed, or if the you cleared the view.
    - The data display pane may contain a data set, consisting of samples from a single batch.
2. To open data from within the Batch Reprocess environment the select **Open Data...** from the **File** menu. This displays the Data Selector, enabling you to locate and select the data (Batch) to be reprocessed.
    - If you choose the **Reprocess...** command from the **Tools** menu the Data Selector is displayed (in single batch selection mode). After selecting the required data set (and click the **Open** button) the Data Selector closes, the Batch Reprocess environment becomes the active view and the selected data set are displayed there replacing any existing data set.
3. After you select the Batch, click the **Open**. The selected Batch data populates the Reprocess window.
4. Select the samples to be reprocessed.
5. Select the processing method for this reprocessed batch.
6. Set the sequence parameters you wish to change. Some cannot be changed.
7. Set the processing functions and channels to be processed in the Options Panel.
8. In the Reprocess Toolbar select the method version to be used for processing from the drop-down menu.
9. In the Reprocess Toolbar select if a new batch should be created and what data should be copied to the new batch.
10. Click the Start Reprocess button  to start the processing. The sample row turns green as the row is being reprocessed
11. Click the Results tab to see the results of the reprocessing.

The Displayed Data

The display of the selected data in Batch Reprocess is very similar to that of a sequence in the Sequence or Run Time environments. The display shows the hierarchy of Batch/Sample/Decision Points and all the associated bands. The primary differences between the batch display and the sequence editor display are:

- The batch display includes a Reprocess check box cell for each sample row.



- Certain fields are set read-only in the Batch Reprocess environment, as defined below.
- The editing tools fly-out panel is not shown in the Batch Reprocess environment.
- Rows cannot be deleted from (or added to) the batch display.

Changing Parameter Values

Some sample parameters can be changed in the batch prior to reprocessing. In general, the following **instrument related parameters cannot be changed**: the Vial, Injections, and Injection Volume. If you make any change to batch or sample parameters then a new batch must be created, since changing this information in the original batch is not permitted. For this reason, if you make such a change when the current batch save selection is set to '**Update current batch – no new copy'** then a warning dialog displays, giving you the option of changing the **save/copy option** to one that creates a new batch or reverting to the original batch.

NOTE: *Any change to sample parameters (but not batch information) will also require that all channels be reprocessed, since prior results from any channel are potentially rendered invalid by the sample parameter change. Therefore the channels selector will be set to 'all' and will be disabled.*

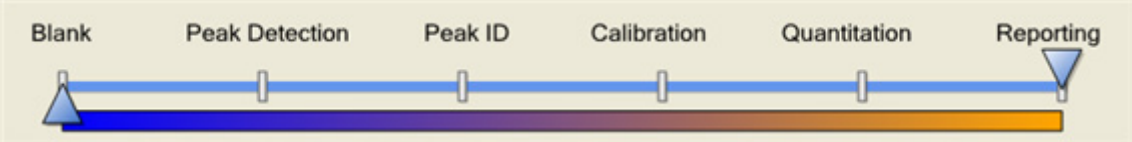
A new method can be specified if the method option is set to **Use Latest Stored Version of method**. If **Use Method Saved with Results** is selected the method column will be set read-only. If the method is changed the latest version of the method (i.e. in the method database) will always be used and the method option drop-down list will be set appropriately.

About the Options Panel

The controls in the Option Panel are used to set two key aspects of the scope of batch reprocessing: The start and stop points of the processing cycle and the data channels to be reprocessed.

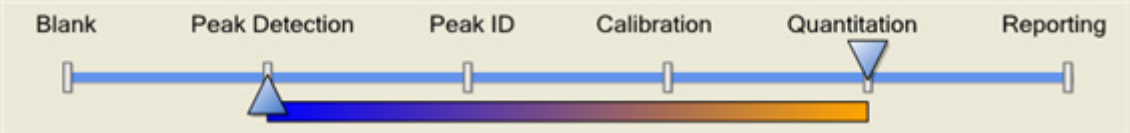
Processing Start/Stop Selector

Use this control to select the step with which reprocessing will start and the step after which it will stop.



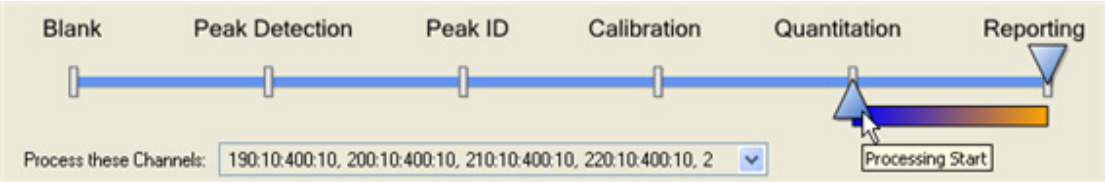
The scale has six divisions representing the six steps of processing (see Processing Functions). The terminology used in labeling the processing steps on this control employs verbs, since these are shorter than the (usually employed) nouns and better convey the sense of action.

The Start point marker (the left triangle) can be dragged to a new location, but only up to the current location of the Stop marker (the right triangle). Similarly the Stop marker can be dragged to a new location, but not before the Start marker. When a marker is dropped it is set to the nearest processing point. For example:



Note that the limits indicator (the blue to orange band) resizes with the marker positions to show the range of processing steps that would be executed.

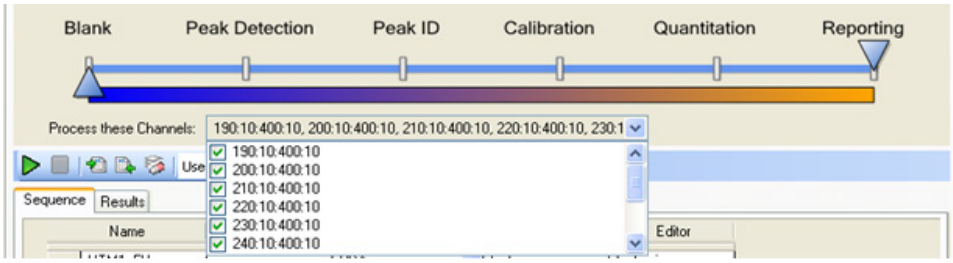
When you click the left mouse button on one of the markers to start a drag operation, the limits indicator is updated immediately to show the valid range in which the marker can be dragged. For example in the example above, if you press the left mouse button over the green triangle the display would change to:



When the Method option is set to **Use Method Saved with Results** the start point will be defaulted to **Quantitation** and it will not be possible to move it to an earlier stage.

Channels Selector

This control enables you to choose the data channels that are processed. It is a combination drop-down list and check list.



- The drop-down list will always contain all the channels for which data exists in the batch.
- If individual channel names are checked then when the control is closed it will display all the selected channel names, separated by commas (as shown in the example above). You are able to select and un-select all channels in one action via a popup menu that will appear when right-clicking on the drop-down list.
- If any sample parameter is changed then the channels selector is automatically set to *All Channels* and disabled (see section Changing Parameter Values).

Control and States

The control of Batch Reprocessing follows the same pattern as the control of the sequence in Run Time. Also, its status is indicated in a very similar way.

State	State Indicators	Possible Actions
Batch Idle	If Start/Stop Rows have been set the excluded rows are shown with a gray cell background (and Reprocess cell is unchecked).	Set Start/Stop Rows Run
Batch Processing	Currently processed row is highlighted with green cell background	Pause Stop
Batch Paused	Already processed rows (and excluded rows) are shown with a gray cell background, so the 'resume point' is the first row with white cell background.	Set Start/Stop Rows Run Stop

NOTE: *When reprocessing is paused the Start Row is automatically updated to the row following the last one completed before the pause takes effect. In this way the Run command will, by default, resume reprocessing from the next row. However, you are able to change the default Start row to some other row and hence change reprocessing execution. If this involves a repetition of processing of rows already reprocessed then the prior results is retained and a new version of results are generated for these repeated rows.*

Title Bar

The header above the main area displays the current batch name and state. This is similar to the behavior of the title bar above the frame in Sequence mode of Run Time.

Batch displayed but no activity is taking place

Batch is being reprocessed

Batch reprocessing has been paused

Results Display

Selecting the Results tab will display the results only after reprocessing is complete. The results are not displayed for the original batch when it is first opened. This is identical to the Results tab displayed in Post Run (see Post Run - Results)

Resulting Data Sets

Depending on the various selections and entries made, the results of batch reprocessing could be:

- Updating of the original batch with a new set of results.
- A new batch containing all the original data or a subset of it, together with the new results.
- One or more reports, with no change whatever to the original batch.

Updated Batch

If no change is made to any of the batch or sample values (e.g. if only parameters within the method have been changed), you can choose to have the original batch updated with the new results. The previous results are still retained within the database but in the current version of Chromera you only have access to the most recent set of results.

New Batch

You can always choose to have the results of reprocessing saved to a new batch. You can also select whether the new batch should contain all the original data, as well as the new reprocessed results, or if only the reprocessed samples and the latest results should be saved in the new batch.

If you make changes to any values in the displayed batch grid then a new batch must be created. This is because only one set of batch identification parameters and one set of parameters per sample are saved in the batch, and you are not permitted to overwrite the original values. When a new batch is created you have complete freedom to change most batch values (including Sample Type), however, you cannot change injection volume, number of injections, or vial number.

NOTE: *Any change to sample parameters will also require that all channels be reprocessed, since prior results from any channel are potentially rendered invalid by the sample parameter change.*

If the user has chosen to create a new batch but does not enter a new batch name then the software will append an identifier to the batch name to distinguish it from the original.

The batch creation date will be set to the time at which the new batch is created in the database, since this operation will be treated as creation of a new batch and not modification of an existing one.

New Batch Contents

All originally acquired raw data from all channels for all samples (or optionally only those samples selected for reprocessing) are copied to the new batch. The existing processed data copied to the new batch depends on the 'save' option selected and the processing step defined as the **Start** point for reprocessing. The new data saved with the batch depends on the processing step defined as the **End** point for reprocessing.

If you choose to copy all samples to the new batch, all prior versions of the results for all samples/injections will be copied to the new batch (in addition to the raw data). The exact nature of this data depends on the processing carried out in each case (see the table below).

If instead you choose to copy reprocessed samples only, then only the raw data and any data required to perform the requested processing will be copied to the new batch. For example, by selecting to reprocess from **Blank** to **Reports** then only the raw data will be copied, since no other input is required. However, if you select to start reprocessing at **Peak ID** then the peak data from the original batch (most recent injection version) would be copied to the new batch to act as the input for the **Peak Identification** stage.

The table below defines the processed data saved with the batch for the injection version created by reprocessing.

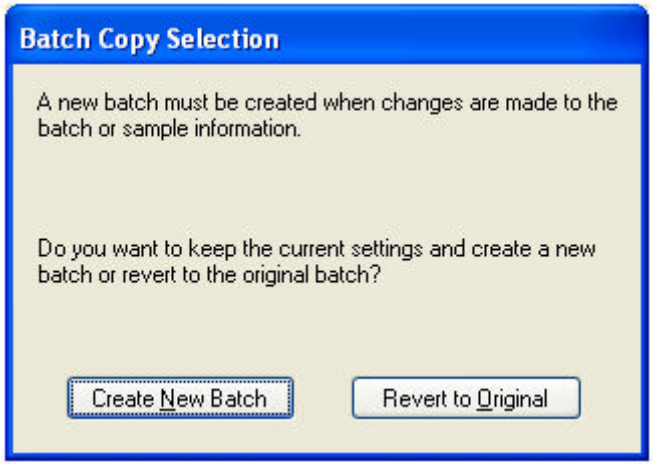
End Analysis	**New processed data saved to new batch**
Blank Subtraction	Derived raw data from subtraction of blank from sample raw data.
Peak Detection and Integration	Derived raw data (from blank subtraction – if any) Peak data (RT, area, height, system suitability values)
Peak Identification	Acquired raw data, derived raw data (from blank subtraction – if any) Peak data (RT, area, height, system suitability values) Component identification information
Calibration	Acquired raw data, derived raw data (from blank subtraction – if any) Peak data (RT, area, height, system suitability values) Component identification information Component calibration results
Quantitation	Acquired raw data, derived raw data (from blank subtraction – if any) Peak data (RT, area, height, system suitability values) Component identification information and quantitation values
Reporting	No new batch will be created in this case

Reports Only

When reprocessing starts (and, by definition also ends) with Reporting no new batch will be created nor will any changes be made to the original batch. Since reporting only involves reading the batch there is no new (saved) data involved.

Batch Copy Selection

This dialog is displayed under the following circumstances:



- When the current batch save/copy selection is **Update existing batch – no new copy** and you make change to any value in the batch (to batch or sample parameters).
- When you have already made a change to a value in the batch and then change the save/copy selection from either of the '**Make new batch**' options to '**Update existing batch – no new copy**.'

You are warned that making changes to the batch or sample information is incompatible with updating the existing batch. You are given the option to undo the changes made or to make a new batch.

If you choose to **Create New Batch**, the save/copy drop-down list changes to show the **Make new batch – copy all samples** selection. You can change this to the **Make new batch – copy reprocessed samples only** option without triggering any additional warning.

If your choose to **Revert to Original** then the original batch is reloaded, thereby undoing changes made to the batch or sample parameters.

Batch Builder

The Batch Builder utility enables you to create a custom batch containing the data from selected injections of selected samples copied from existing batches. The resultant batch is flagged internally as a 'synthesized' batch but Chromera will treat it like any other batch, that is, it can be displayed, edited graphically or reprocessed and the results reported.

Selecting **Batch Builder** from the **Tools** menu opens the Batch Builder dialog. The dialog contains a toolbar, two tree controls side-by-side and below them are a custom data table and a thumbnail graphics pane, like that in Data Selector. Initially the two tree controls will be empty (and the custom data table and thumbnail graphics will not be displayed).

Batch Builder Toolbar

The toolbar contains the following Batch Builder functions:

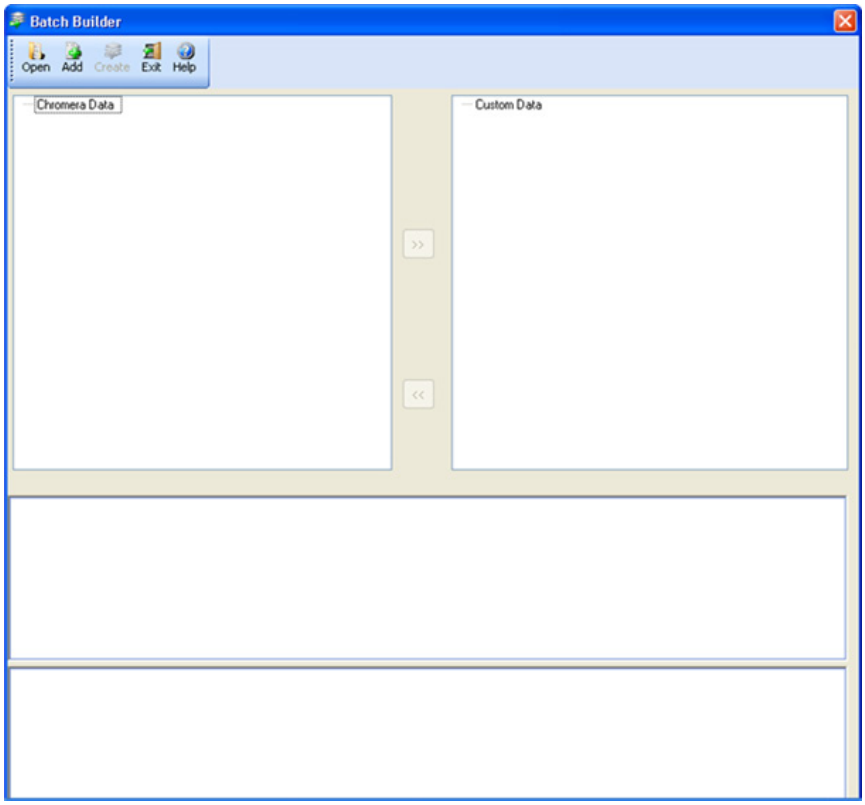
Icon	Command
Open	Click to open the Data Selector in multi-batch select mode. If one or more batches are selected then these **replace** any batches previously displayed in the Chromera data tree. This loads one or more Chromera batches into the data tree.
Add	Click to open the Data Selector in multi-batch select mode. If one or more batches are selected then these are **appended** to any existing batches displayed in the Chromera data tree. This Appends one or more Chromera batches to the data tree
Create	Click to start the process of creating the custom batch defined in the custom data tree. This creates the custom batch and save it in the Chromera database.
Exit	Click to close the Batch Builder dialog.
Help	Click to open a Help window with the How to Build a Batch topic selected.

How to Build a Batch

The following procedure describes how to build a batch:

1. Select **Batch Builder** from the **Tools** menu.

   The Batch Builder dialog opens. The dialog contains a toolbar, two tree controls side-by-side and below them are a grid control and a thumbnail graphics pane, like that in Data Selector.

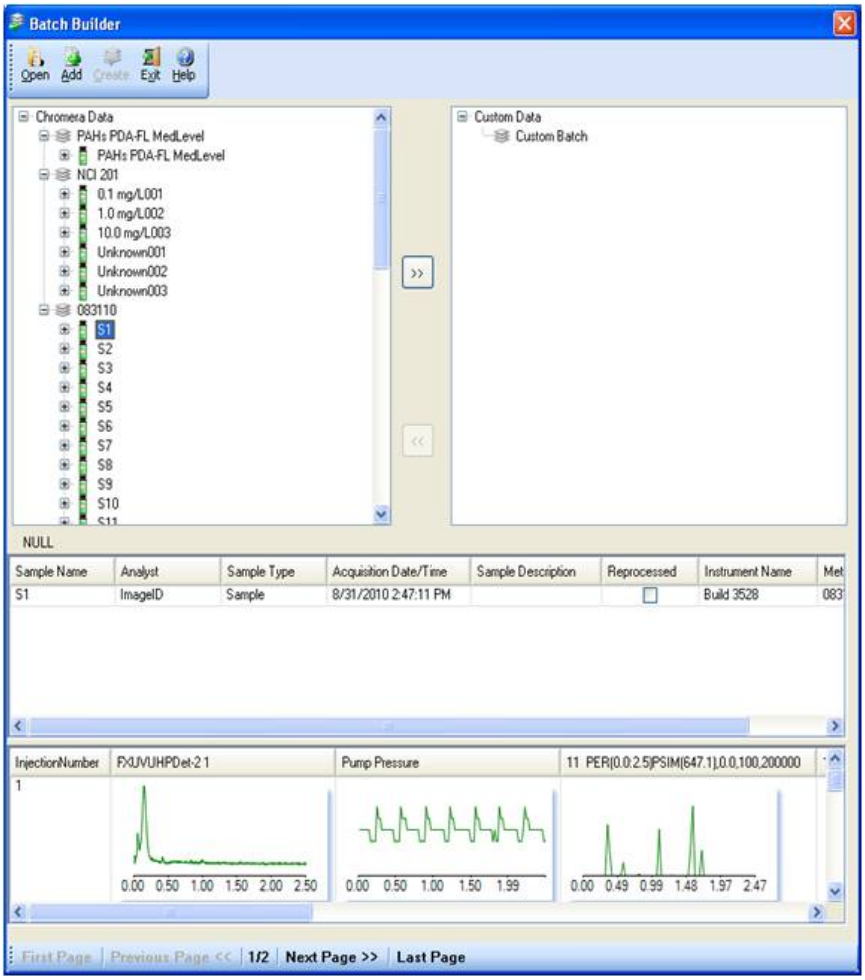


2. Click the **Open Data** or **Add Data** button.

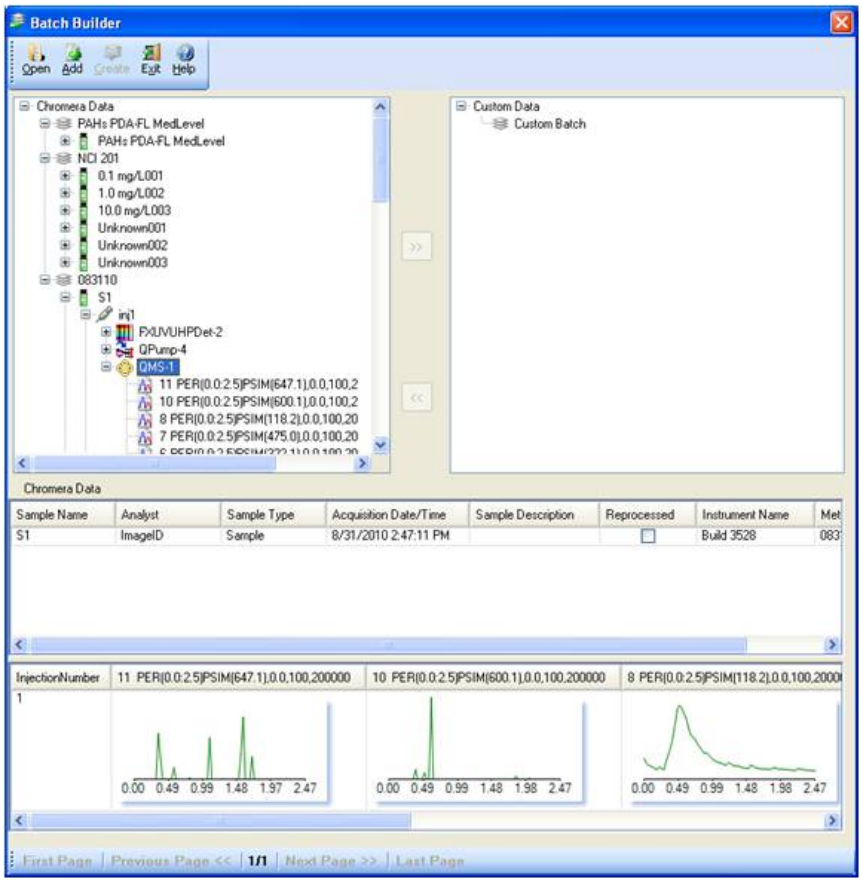
   The Data Selector opens in the multi-batch selection mode. When one or more batches have been selected and the 'Open' button in the Data Selector has been clicked, the selected batches will be loaded into the Chromera Data tree. As in Post Run, Open Data will replace any existing batches in the tree with the newly selected batches, whereas Add Data retains existing batches and appends the newly selected batches at the end of the tree.

3. Review the Chromera data.

   When one or more batches are loaded into the Chromera Data tree, selecting nodes in the tree will display associated information in the table and graphic thumbnail pane.



If more specific nodes are selected (i.e. injection, device, channel) the sample table display will remain unchanged but the thumbnail pane will be filtered to include only the applicable plots. That is, with an injection node selected, plots for all channels of all devices from that injection will be displayed; with a device node selected, plots for all channels of that device only (from that injection) will be displayed; with a channel node selected, only the plot for that channel (from that injection) will be displayed. The following example shows the display when a device node is selected (LC-MS in this case). Note that the UV and pump channel plots (shown in the prior example) are no longer displayed.



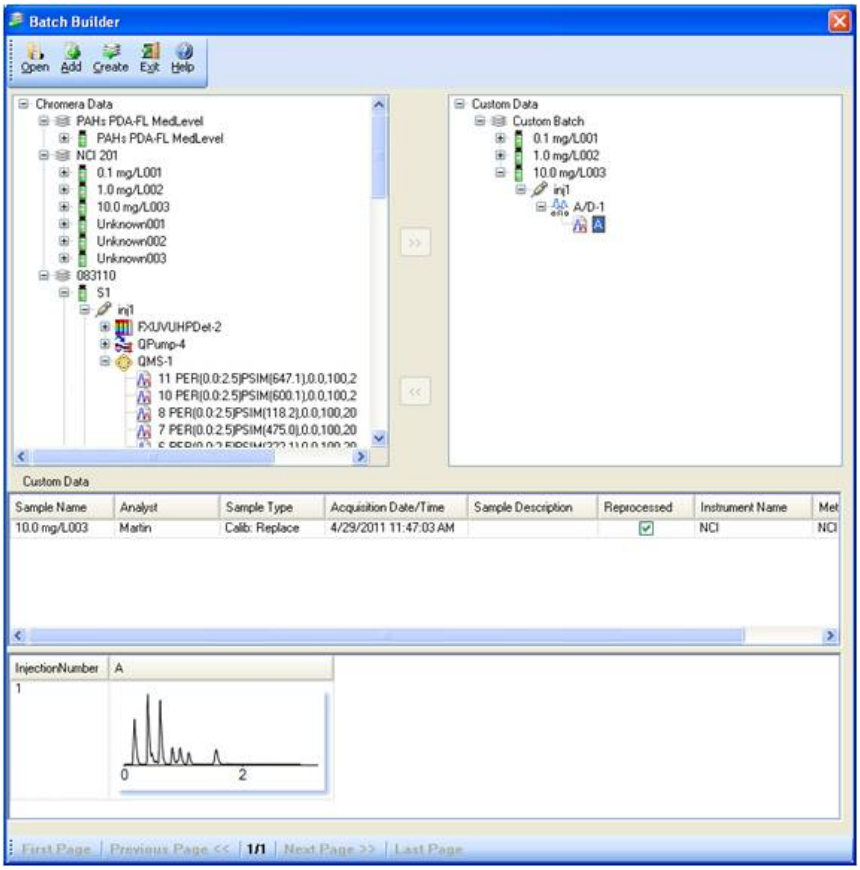
4. Build a **Custom Batch** by selecting items to copy to the new batch. This can be accomplished by doing any of the following:

   - Dragging one or more selected sample and/or injection nodes from the Chromera Data tree and dropping them on the Custom Data tree.

   - Selecting one or more sample and/or injection nodes from the Chromera Data tree and then clicking the >> button.

   - Double-clicking a node.

   The following example shows the custom batch tree after a number of samples have been copied from the Chromera Data tree.



Note that with a node in the Custom Data tree selected, the Custom Data table and the thumbnail pane will display the data associated with that node. In the above example the table displays the sample information for the sample '10.0 mg/L003' and the thumbnail pane displays the NCI channel plots for injection 1. The label above the table displays 'Custom Data' in this case (as oppose to 'Chromera Data', which is shown in all the prior examples) to confirm which tree the information is coming from.

Note also that with data in the Custom Data tree the Create button will become enabled.

5. You can remove nodes from the **Custom Data** tree by basically reversing the action of adding them:

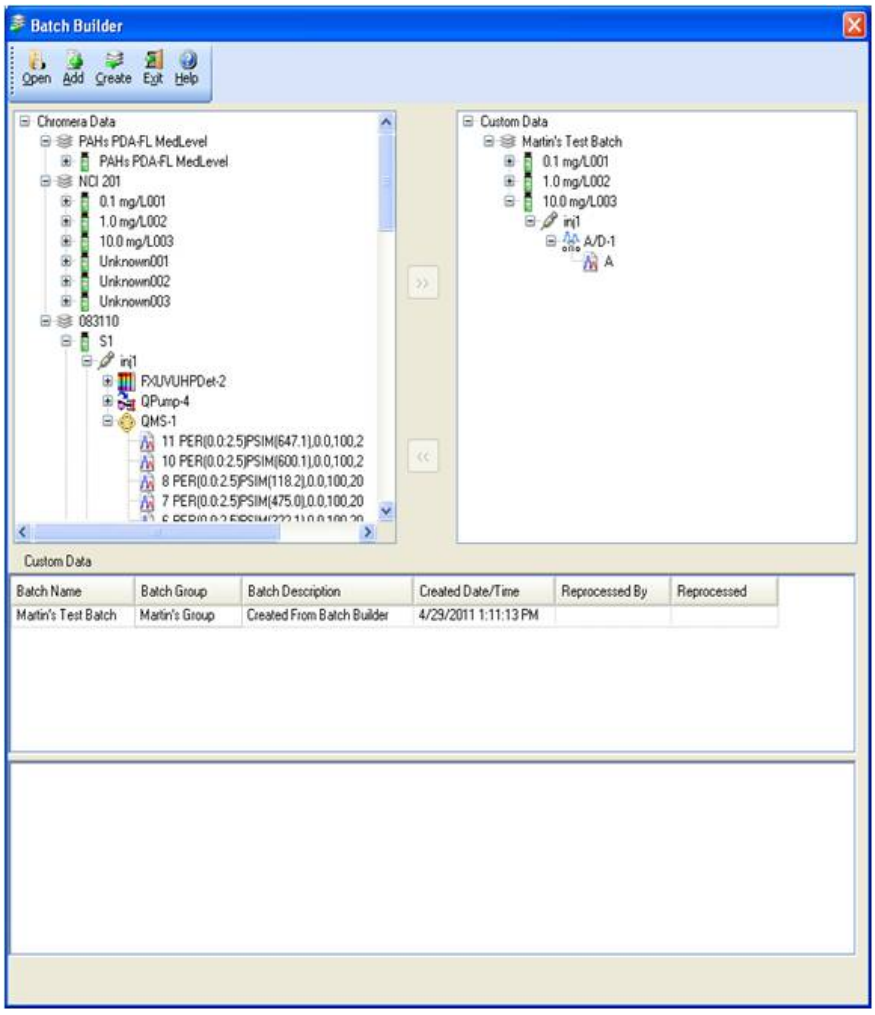
   - Dragging one or more selected sample and/or injection nodes from the Custom Data tree and dropping them on the Chromera Data tree.

   - Selecting one or more sample and/or injection nodes from the Custom Data tree and then clicking the << button.

   - Double-clicking a node.

6. Identify the Custom Batch.

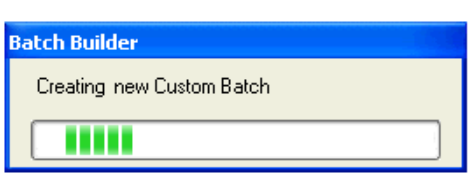
   The default batch name **Custom Batch**) can be modified by selecting the batch node in the Custom data tree and editing the batch name in the table. The **Group** name for the batch and the **Batch Description**' ('Created from Batch Builder' is the default) can also be edited in the table. The following example shows the display after editing the custom batch name and the batch group name. Note that the new batch name appears in the Custom Data tree as well as in the table.

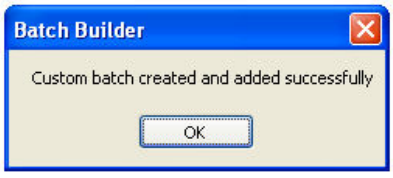


The **Created Date/Time** field will initially show the time the first sample was added to the Custom Data tree but this value will be updated when the batch is actually written to the database (see next section). By definition the 'Reprocessed By' and 'Reprocessed' fields will always be blank for the custom batch. The **Created Date/Time**, **Reprocessed By** and **Reprocessed** fields will not be editable.

7. Save the Custom Batch.

   After adding all the required data to the Custom Data tree the batch can be generated and saved to the Chromera database by clicking the **Create Custom Batch** toolbar button. While the custom batch is being constructed in the database a message box is displayed, indicating that activity is in progress.



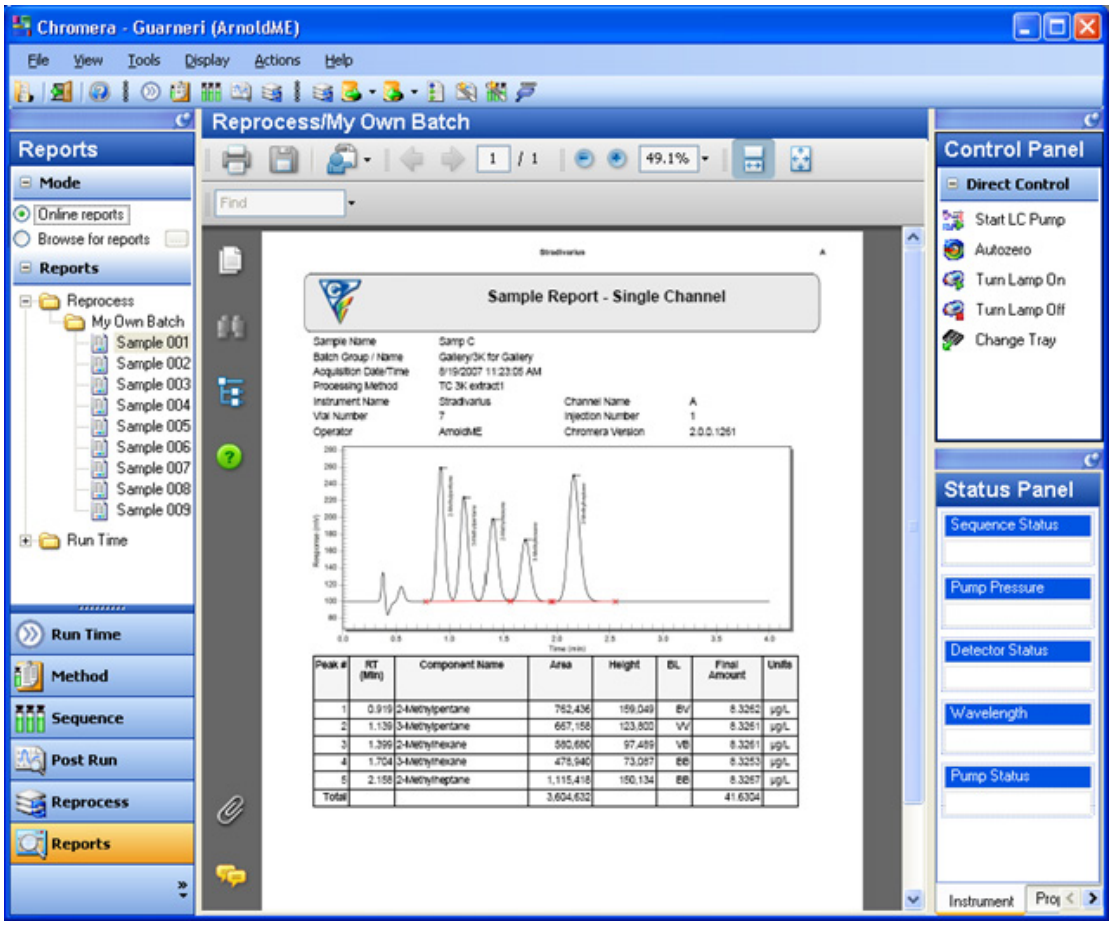


8. Upon successful completion, click **OK** to clear the message and return to the Batch Builder dialog, the Custom Data tree will be blank, as it was when the dialog was first opened. However, the Chromera Data tree contents will be unchanged (in case you wish to construct another batch from these data).

Reports (Report Viewer)

About Report Viewer

The main area of Report Viewer is a PDF viewer. It provides the basic requirements of page selection, variable magnification display, and printing. In addition it provides some search capabilities.



The Report Viewer will display, by default, the reports from both Run Time and Reprocess environments. The reports are presented to you in the Navigation pane via a tree structure. When the Report Viewer environment is selected the tree structure is updated to show all the reports present in the directory structure for that instrument instance.

The viewer lists all batches (as secondary nodes) and reports (PDFs, as extremity nodes under the batch) found in the Run Time directory in a tree control, under a top level node with the base name Run Time, and all reports found in the Reprocess directory in a tree control, under a top level node with the base name Reprocess. There is only a maximum of one batch node for each environment, as previous batch nodes (and report files) are deleted when a new batch is acquired and/or processed. When you select a report in the tree, that report is displayed in the viewer pane.

You can also set the Report Viewer into an alternate mode that allows you to browse for PDF files anywhere on the system and display them. This functionality is intended for the viewing of Chromera reports saved in PDF format but it will allow display of any PDF file.

Mode Control

The mode control basically consists of two radio buttons:

Online Reports	Displays the reports generated from the last acquisition and reprocess actions performed for this instrument instance (or the last reprocess action only in the case of a Data-Only instance).
Browse for Reports	Allows you to select a directory and view the PDF files within that directory branch.
	Displays a Browse for Folder dialog which enables you to choose the directory branch displayed within the tree control.

Plot Control

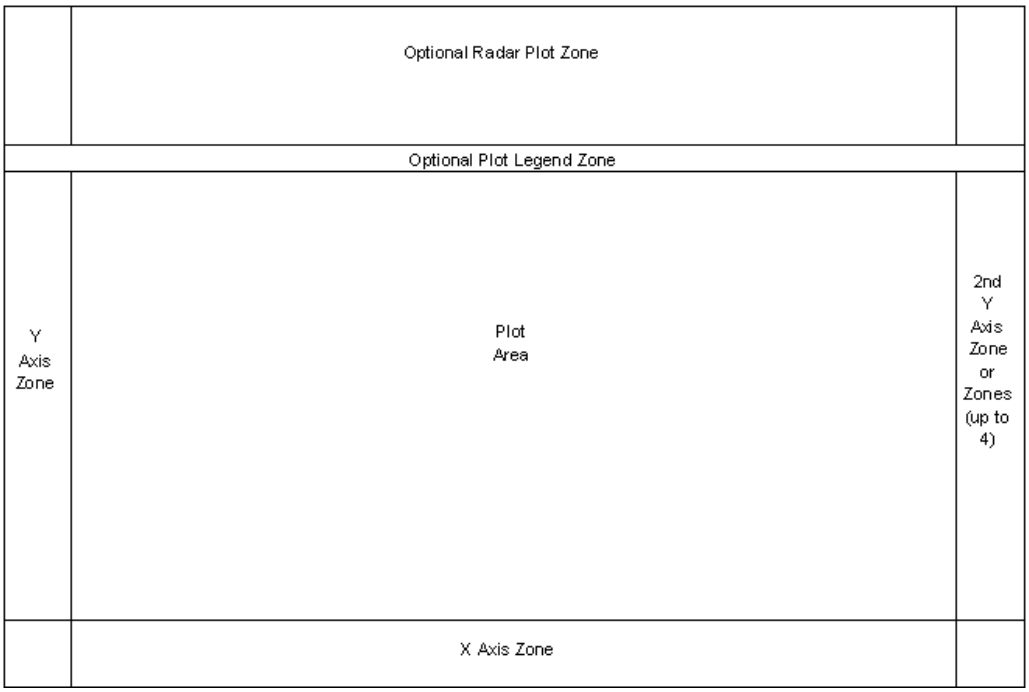
About the Plot

The Plot is a display of a chromatogram, or several overlaid chromatograms, annotated with any or all of the following: baselines, timed events, peak names, retention times, user labels, etc. The Plot may also include instrument data profiles overlaid on the chromatogram(s).

Plot Control is how you view and edit the displayed chromatograms and other run-related signals, such as solvent gradient profiles. This section describes some of its basic characteristics and general behavior. Plot Control is used in two distinct modes - **Display** and **Edit**. The **Display** mode allows you to examine any available aspect of the plotted data but without changing the results of data processing (obviously plot scaling and annotations displayed can be changed). In **Edit** mode you are able to alter parameters and interact with the data in other ways that affect the results.

Plot Area

In simple terms a plot is a set of axes and an area where the data is displayed. The Plot Area is the central subdivision of the whole plot in which the actual data are plotted. Since the general use of the control is to display chromatograms, we will refer to chromatograms most of the time and only reference other data when there is distinct difference of behavior.



The radar plot always displays the full chromatogram with the part highlighted that is currently displayed in the main plot area. It exhibits an extremely limited set of capabilities that are closely tied to its associated plot. The radar window is only available when a single plot is displayed.

When up to four overlaid chromatograms are shown, additional Y-axes will appear on the right-hand side of the plot. The labels on these axes use the same color as the associated chromatogram. The Y-axis displayed on the left-hand side is that of the currently active chromatogram. When more than four chromatograms are overlaid, no additional Y-axes are displayed (only that of the active curve).

The following terms describe the parts of the plot:

Chromatogram

In its simplest form the chromatogram is a curve drawn (plot) as a visual representation of the raw data collected of the data acquired from a chromatographic detector. Provided associated results data exist, the chromatogram may optionally be annotated with peak names, retention times, baselines and timed events. If results data exist, a peak may be selected by clicking on it (within the area enclosed by the signal and the baseline).

Profile

A curve displaying instrument signals other than detector output. For example solvent gradients, pressure or temperature profiles.

Peak

A peak is a sub-element of a chromatogram, providing that results data exist (i.e. the peak was detected and integrated).

Peak Name (Components/Species)

Peak names are an example of system labels. The text is taken from the result data (or the method depending on the environment). You may choose to have the peak names displayed horizontally or vertically above the peak.

If the peak apex is off scale then the name will be displayed superimposed on the peak (i.e. the label is moved down so that it is visible). A 'tie line' is used to connect the label to the peak if repositioning causes any ambiguity as to which peak the label is associated with.

Peak Data Label

You may select up to three data values as part of the peak annotation. Any three of the following items may be selected: RT, relative retention, area, height, area %, final amount, peak width (@ 5%, 10% 50% or base), resolution, plate number, alpha, k', tailing factor. If the peak apex is off scale then the label is moved as described above for peak names. A 'tie line' is used to connect the label to the peak if this repositioning causes any ambiguity as to which peak the label is associated with.

Peak data labels are displayed for unidentified peaks as well as identified peaks. In the case of identified peaks the peak name appears as an additional first item in the annotation.

Component Search Window

There are two forms of the component search window – one purely for display (the unselected form) and one that allows modification of the search window and expected retention time (the selected form).

The unselected form is a line displayed on the chromatogram to indicate the search window for all components. The triangle within the bounds of the window indicates the expected RT of the component (which does not have to be the center of the window).

In **Edit mode** when a search window is selected, the selected form is displayed.

This form has three handles (the black triangles), one at each end, indicating the overall search window region, and one in between that indicates the expected retention time of the component. Each of these handles may be dragged to a new position. Moving one of the two end handles will adjust the time period of the search window. Moving the handle within the window will update the expected RT of the component. The middle handle cannot be dragged outside the window, however the whole window can be moved to a new position by dragging in the region between the handles.

Baseline

You can optionally select to have baseline segments displayed on the plot, provided results data exist for the chromatogram. The term *baseline segments* includes drop lines drawn between unresolved peaks and skim lines (exponential or tangential). You may select the color and line type to be used to draw baselines.

Legend

This identifies each chromatogram displayed in the plot area, by displaying a sample of the line color together with the sample name. As occurs on the plot itself, the line for the selected chromatogram is double thickness.

Axes

Each axis zone consists of three elements: a title, divisions (tick marks) indicating the scale, and labels associated with some of these tick marks (major divisions).

The title for each axis indicates the quantity displayed on that axis and the units. The title is displayed horizontally on X axis and vertically on the Y axis.

Timed Event

A timed event is indicated by its standard Chromera abbreviation. A timed event label is generally located above the signal level at the time at which it occurs.

In Edit mode, an event is moved simply by dragging it. Moving the timed event horizontally causes the time at which the event is to occur to be updated. Except in the case of UF events (to be described in detail in graphic editing), movement of the event in the vertical direction has no effect and the event will be relocated at the correct height when the event is dropped.

The color and font attributes (including text and background colors) of timed events is a user preference (on a global basis).

Annotation

Additional information displayed superimposed on the chromatogram or plot area. This can be in the form of lines (as in baselines), text, numbers or pictures.

Labels

Annotations consisting of text. These may be system labels or user labels.

System labels

Labels whose content is supplied by the system rather than by the user. System labels will generally be locked to the data (as for peak names) but certain types may be at a fixed position in the window (as for header information).

User labels

This consists of free form text that may be positioned anywhere on the plot. When you enter the label text you can also define (for each label) whether the label is linked to the chromatogram (in the manner of peak names positioned at the peak apex), displayed at a data axis location or shown at a fixed position in the window (regardless of rescaling). The color and font attributes (including text and background colors) of user labels is set as user preferences (on a global basis). A user label can be edited after it has been added to the plot.

User labels added to a plot are associated with the raw data set; so that when that raw data set is displayed in the future the previous view of the data can be restored. Labels added when plots are overlaid are associated with the currently focused curve.

Zoom box

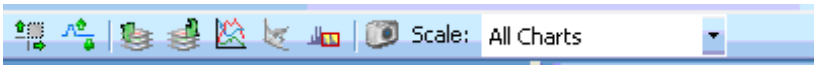
A rectangular object drawn on the plot by the user by performing a drag operation with the mouse, for the purpose of immediately rescaling the plot. To obtain an immediate rescaling of the plot you can draw a zoom box by dragging with the mouse across the plot area, when the default mouse pointer is displayed. Depending on the relative X- and Y-dimensions of the box, the plot is rescaled on the X-axis, Y-axis, or both.

Coordinates Display

As you move the mouse pointer around in the plot area, the coordinates of the hot spot are displayed (in the same units as the axes labels) below the plot. Note that when multiple plots are displayed only one set of coordinates is displayed.

Graphics Toolbar

This toolbar is displayed whenever a plot control is displayed on the screen. Not all buttons are enabled in all environments where a plot control appears. The commands available in each environment are described in detail in the associated section below.



Icon	Command	Description
	Rescale	Displays the Rescale Axes dialog, enabling you to enter minimum and maximum settings for X and Y axes. The action of this command is affected by the Scaling Scope setting (see below).
	AutoScale	Sets the plot to default scaling settings. The action of this command is affected by the Scaling Scope setting (see below).
	AutoOffset	Sets the Y axis minimum value so that the current signal level is displayed at approximately 5% of full scale. The Y axis maximum is also adjusted so that the full scale span remains the same. Displayed in Run Time environment only.
	Previous Layer	Displays the previous layer in a three dimensional view consisting of a visible layer and hidden layers (for details see Stacked Plots View). Only enabled when a multi-layer view is displayed in Post Run.
	Next Layer	Displays the next layer in a three dimensional view consisting of a visible layer and hidden layers (for details see Stacked Plots View). Only enabled when a multi-layer view is displayed in Post Run.
	Overlay	Overlays multiple chromatograms. Only enabled in Post Run when a suitable data set is displayed. See Post Run Environment.
	3-D View	Displays overlaid plots in 3-D Mode. Only enabled when overlaid plots are displayed.
	Radar Window	Toggles display of radar window.
	Clear Graphics	Clears the current chart and starts plotting again from the X minimum. Displayed in Run Time environment only.
	Snapshot	Displays the active plot in a full screen window.
Scale : All Plots	Scaling Scope	Determines the scope of scaling changes made using Rescale command, AutoScale command or zoom box (for example, all plots or single plot). Options available depend on the environment and the view.

About Plot Styles

The global (at least global within each environment) setting of plot properties is referred to as **Plot Styles**. This is the command used to access these values and the title of the dialog used to set them.

The plot properties/options are divided into three categories:

Appearance general properties affecting the appearance of the plot window but not its content

Annotations settings that determine the additional information to be displayed on the chromatogram and its appearance

Curve Colors a list of colors to be used within curve sets and options defining their use

The majority of plot properties are global. That is they will be used for all plots in all given environments for a given user/instrument. For example, changing the peak name text color to blue within Post Run would set all peak names displayed on all plots in that environment to blue but it would not change the color of peak names displayed in Graphic Results Editing or Run time.

The key exceptions to the above rule is curve colors and user labels. Each chromatogram can be assigned a different color (if required) and user labels can specify individual lock properties as well as having individual text properties.

Appearance

The Appearance settings are:

Title Set the optional title to appear at the top of the plot. You can define up to three title segments: left justified, centered, and right justified over the plot. Each segment contains a data item selectable from a list or text that you can enter. You can also specify the name, size, color, and style (regular, bold, italic, bold italic) of the font used for the title display.

Plot style Set whether chromatograms are displayed as lines or as individual data points or as a combination of line and bunched data points (averaged response).

Legend Set (check box) to display the legend relating chromatograms colors and identifiers (sample name + channel + injection).

Grid lines Select to display the horizontal and vertical lines across the chart (from minimum to maximum) at each major division (i.e. labeled divisions) of the X- and Y-axes. The grid lines, when shown, are closely spaced dashed lines in black.

Axis titles and units Set to label the X- and Y-axes with the quantities plotted. For the X-axis this label (when shown) is always Time (min). The Y-axis label depends on the detector that generated the chromatogram. For example, chromatograms from a UV/Vis detector would show Absorbance (mAU).
Note that this option does not affect display of the numeric marking of axis divisions – these are always displayed.

Annotations

You are able to select the combination of annotations that should appear on chromatograms (this selection is global). Each annotation type also has a set of properties that can be set. Many of the annotations are text objects for which the following properties can be set:

- Font name
- Font size
- Text color
- Text style (regular, bold, italic, bold italic)
- Background color
- Orientation (horizontal or vertical)
- Border (a thin, black, rectangular line surrounding the text)

The annotations that can be selected for display are listed below:

Baselines	Lines indicating the integration of peaks. This includes both lines drawn under the peak and drop lines drawn separating unresolved peaks. You can define the color and style (solid, dashed, dotted) of the lines.
Component Windows	Markers indicating the search window used for matching peaks to components defined in the method. You can define the color of the markers.
Timed Events	Text abbreviations indicating the position of timed events affecting peak detection and integration, as well as those associated with instrument devices (i.e. contact closures). You can specify the text properties as defined above.
Legend	A key that matches the curve colors to the sample identifiers. Although the legend is not strictly an annotation, since it appears above the chart, it will be treated as one.
Peak Data	Data values associated with a peak. You can select up to three items to be displayed. The text properties of Peak Data annotations are the same as those for Peak Names.
Peak Names	Names associated with peaks that have been matched to components defined in the method. You can specify the text properties as defined above.
User Labels	Text that you enter for display on the plot. You can specify the text properties as defined above.

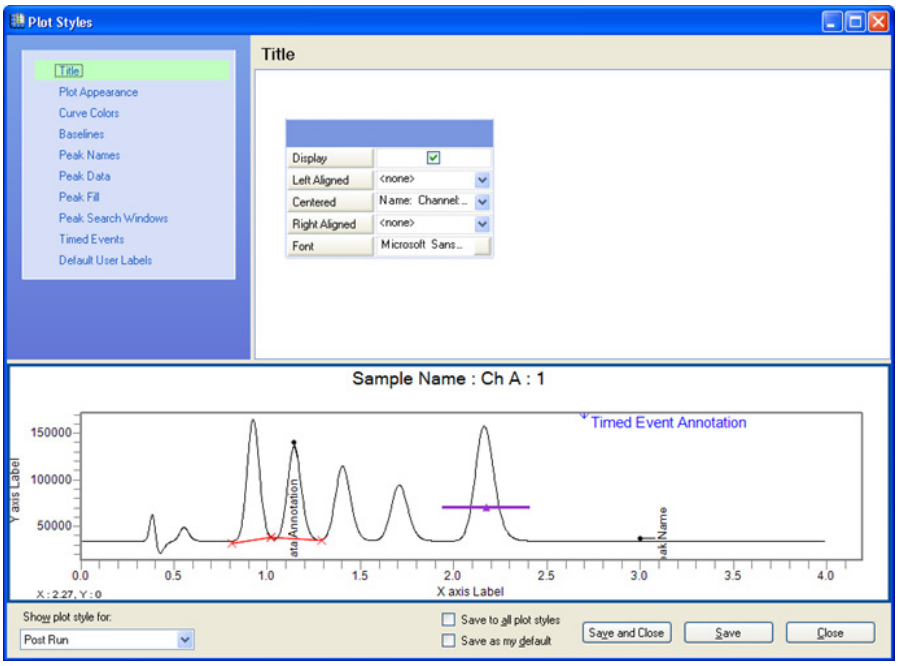
Curve Colors

It is important to specify different colors for chromatograms displayed in overlay mode. However, the various views available within the Post Run environment allow for different arrangements of data sets that affect what combination of chromatograms is overlaid. The definition of curve colors therefore allows you to determine the context in which the colors are used.

Curve colors definition will consist of a user-defined set of colors to be used in turn for chromatograms (of a specific type). Ideally this set should be unlimited in number but if a limit is required to simplify the implementation that number should be 20.

Setting Plot Styles

The Plot Styles dialog displays when you choose **Plot Styles** from the **Display** menu or from the popup menu on a chart in Post Run, Run Time, or Graphic Method Editor. The Plot Styles dialog contains two main sections: (1) at the top is a parameter section, displaying the various user-settable parameters associated with charts, and (2) at the bottom is an example chromatogram, showing examples of many of the plot styles. The example display provides you with an immediate impression of what the selections will look like on the plot.



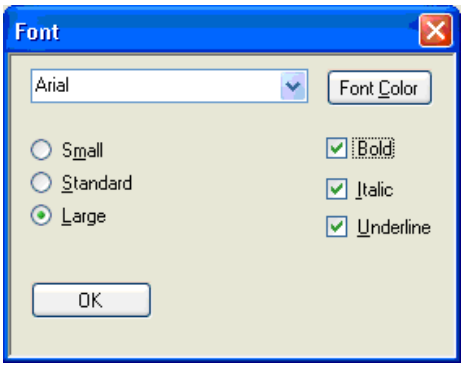
When setting Plot Styles, it interacts with the rest of the Chromera software as follows:

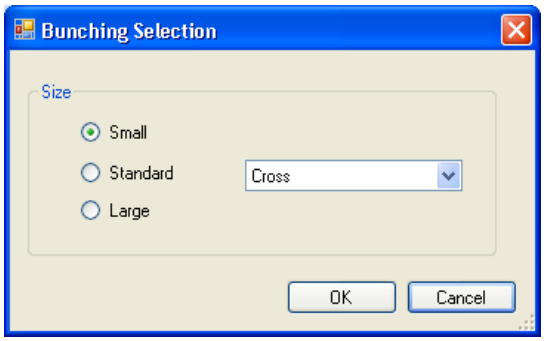
1. Changes made (and saved) within the Plot Styles dialog are reflected immediately (as applicable) within the Run Time, Post Run, and Graphic Edit environments.

2. The Display settings in Plot Styles and the check boxes in the Annotations dialog are kept synchronized at all times, so that changes in either location are reflected in the other the next time it is accessed.

Set the following Plot Styles:

Title	**Description**
Display	A check box to set whether a title line will be display at the top of the chart.
Left Justified	Select from the drop-down list the sample identification value to be displayed left-justified on the title line. Select one of the following: **<none>, Name:Channel:Injection (the standard identifier), Acquisition Date/Time, Channel Name, Injection Number, Instrument Name, Analyst, Sample Description, Sample Name, Vial Number**

Centered	Select from the drop-down list the sample identification value to be displayed centered on the title line. Same list as for Left Justified
Right Justified	Select from the drop-down list the sample identification value to be displayed right-justified on the title line. Same list as for Left Justified
Font	Set the font to be used for the title. Set this value by clicking on the button in the cell and selecting the required properties in the displayed dialog. The font characteristics that can be set are: typeface (font name), size (small, standard, large), and style (regular, bold, italic, underline). 

Plot Appearance	**Description**
Bunched Points symbol	Define the symbol and size to be displayed on the chromatogram for bunched points. Set this value by clicking on the button in the cell and selecting symbol properties (size and type) from the displayed dialog. 
Plot Style	Select from the drop-down list the type of data display (Line, Raw Points, or Bunched Points) to be used for chromatograms.
Axis Title and Units	A check box to set whether the X and Y axes on each chart will be labeled with a title and the unit values.
Legend	A check box to set whether a color legend will be displayed above the plot area. This shows the color used to plot each displayed curve on the chart.
Grid Lines	Select from the drop-down list whether grid lines (None, Vertical, Horizontal, or Both) will be displayed on each chart (at major axis divisions).

Grid Line Style	Select from the drop-down list the type of line to display (Thin, Dashed, Dotted, or Thick).
Grid Line Color	Select the color using a popup color chart, which is displayed when the down arrow in the field is clicked.
Background Color	Set the color to be used as a background on each chart. Select the color using a popup color chart, which is displayed when the down arrow in the field is clicked.
Tooltip	A check box to set whether plot information will be displayed in a tooltip when the mouse pointer hovers over the chart.

Curve Colors	**Description**
Curve 1 through Curve 30	Select a color from the drop-down list for each curve (1 - 30).

Baselines	**Description**
Display	A check box to set whether baselines will be displayed on integrated chromatograms.
Color	Set the color to be used for baselines. Select the color using a popup color chart, which is displayed when the down arrow in the field is clicked.
Line Style	Select from the drop-down list the line style (Solid Line, Dashed Line, or Dotted Line) to be used for drawing baselines on the plot.

Peak Names	**Description**
Display	A check box to set whether peak names will be displayed for identified peaks.
Font	Set the font to be used for the peak names. Set this value by clicking on the button in the cell and selecting the required properties in the displayed dialog. The font characteristics that can be set are: typeface (font name), size (small, standard, large), and style (regular, bold, italic, underline).
Orientation	Select from the drop-down list the direction (Horizontal or Vertical) peak names (and peak data labels) will be displayed on the chart.

Peak Data	**Description**
Display	A check box field to indicate whether peak data labels will be displayed.
Data Item #1	A drop-down list to indicate the first data item to be displayed for each integrated peak. **<none>, Alpha, Area, Area %, Capacity Factor, Final Amount, Height, Peak Width (5%), Peak Width (10%), Peak Width (50%), Peak Width (Base) Resolution, Retention Time, Tailing Factor**

Data Item #2	A drop-down list to indicate the second data item to be displayed for each integrated peak.
Data Item #3	A drop-down list to indicate the third data item to be displayed for each integrated peak.

Peak Fill	**Description**
Display	A check box set whether integrated peaks will be filled with color.
Color	A color field to set the color used to fill integrated peaks. Select the color using a popup color chart, which is displayed when the down arrow in the field is clicked.

Peak Search Windows	**Description**
Display	A check box to set whether peak search windows will be displayed.
Selected Color	Set the color to be used for the currently selected peak search window. The color is selected using a popup color chart, displayed when the down arrow in the field is clicked.
Unselected Color	Set the color to be used for all unselected peak search windows. The color is selected using a popup color chart, displayed when the down arrow in the field is clicked.

Timed Events	**Description**
Display	A check box to set whether timed event annotations will be displayed.
Font	Set the font to be used for the timed event annotations. Set this value by clicking on the button in the cell and selecting the required properties in the displayed dialog. The font characteristics that can be set are: typeface (font name), size (small, standard, large), and style (regular, bold, italic, underline).

Default User Labels	**Description**
Display	A check box to set whether user labels will be displayed.
Font	Set the font to be used for the user labels. Set this value by clicking on the button in the cell and selecting the required properties in the displayed dialog. The font characteristics that can be set are: typeface (font name), size (small, standard, large), and style (regular, bold, italic, underline). Note that, unlike other plot labels, each user label can have its properties (font, background color and border) customized after it has been created.
Background	Set the color to be used as background on each chart. Select the color

Color	using a popup color chart, which is displayed when the down arrow in the field is clicked.
Border	A check box to set whether user labels will be surrounded by a border.

Show plot style for:	You can set different plot styles for different environments of Chromera. Select from the drop-down list (**Graphic Method Editor, Post Run, Reporting, or Run Time**).
Save to all plot styles	A check box to set whether or not you want to save the settings to all plot styles.
Save as my default	A check box to set whether or not you want to save the settings as your default plot styles.
Save and Close, Save, or Close	Click the button for the action you want to perform.

About Plot Information

When you want to see more information about a specific chromatogram you can view a summary optionally in any of three formats:

- As a popup tooltip when the mouse hovers over the chromatogram
- In a modeless dialog
- In the Status panel

Plot Information Content

The information displayed via all three mechanisms is the same, although the format (and behavior) will not be identical. The data items displayed are:

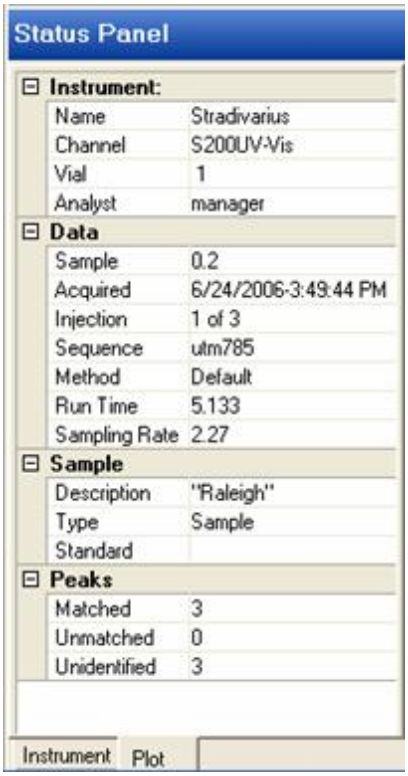
1. Instrument name
2. Channel name
3. Vial number
4. Operator (the name of the user who set up the instrument for acquisition)
5. Sample name
6. Time and date acquired
7. Injection number (and total number of injections)
8. Sequence name (or Session name if acquired via Single Run mode)
9. Method name
10. Run time (i.e. the data acquisition time)
11. Time Adjustment (min) (from Channel section of acquisition method)
12. Sampling rate
13. Sample description
14. Sample type
15. Standard name (if any)
16. Number of identified (named) peaks
17. Number of components not found
18. Number of unidentified peaks

Selection vs. Current

There is an important distinction between the behavior of the tooltip display and the other two forms. The tooltip is displayed when the mouse pointer hovers over a plot regardless of whether that is the selected plot or not. The other two forms specifically display the plot information from the selected plot (if any). When there is no selected plot, rather than display nothing in the Status Panel and/or Plot Information dialog, summary information about the Batch is displayed instead.

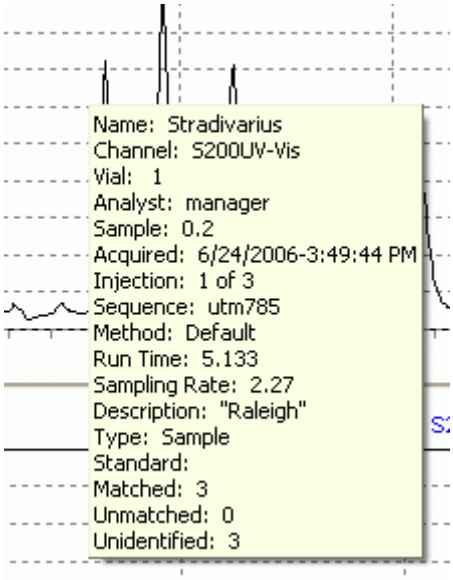
Status Panel Display

When the plot information is displayed in the Status Panel, it is formatted in the manner of a properties tree. The information is divided into categories to make it easier to view. For example, Instrument, Data, Sample, Peaks.



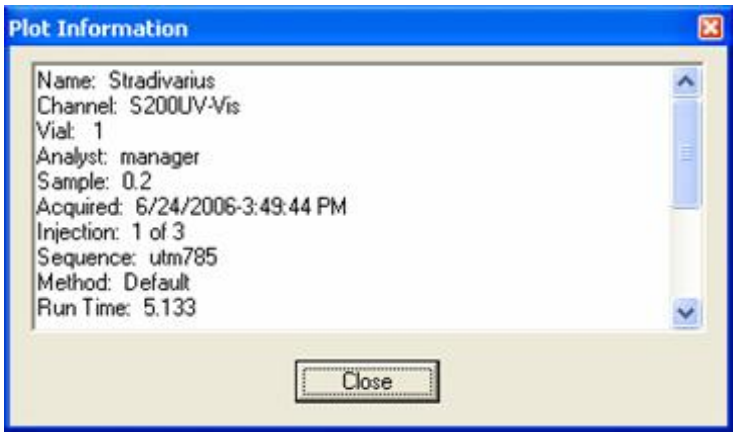
Tooltip Display

The tooltip appears when the mouse hovers over the actual plot area within the axes and not in the blank area between plots.



Dialog Display

The Plot Information dialog displays so that it is immediately adjacent to, but does not cover up, the plot it references. As long as the dialog is displayed, selection of a different plot (via the data tree or by clicking on a plot) will update the information in the dialog.



It is possible to select some or all of the text in the list box and copy the information to the clipboard. This could be achieved either by use of a popup menu and/or using hot keys (Ctrl+A = Select All, Ctrl+C = Copy). Cutting and pasting of text is not possible.

Batch Information Display

In the Post Run environment, when there is no selected plot, rather than have the plot status tab and the plot information dialog be blank, some summary information about the batch is displayed. The following lists the information and indicates the basic format:

Batch: <batch name>

Created Date/Time: <created date/time>

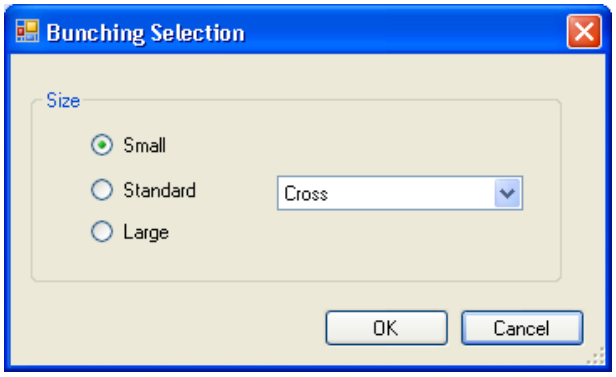
Instrument: <instrument name>

Number of samples: <number of samples>

Total number of runs: <Total number runs (injections)>

Bunched Points Symbol Dialog

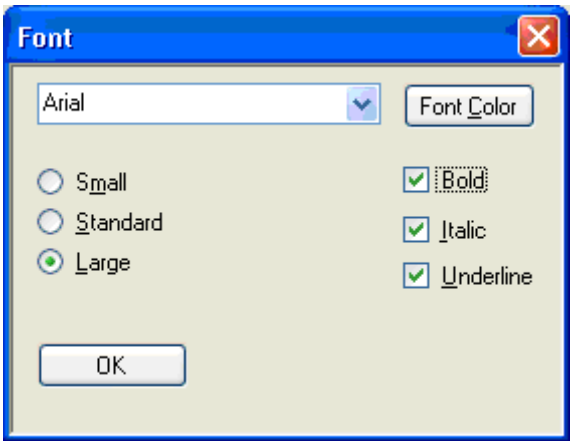
This dialog displays when the button within the Bunched Points Symbol field of the Plot Appearance band is clicked.



Control	Description
Size	A set of radio buttons that indicate the size of the symbol. The choices are: Small, Standard, or Large.
Drop-down list	Select a symbol from the drop-down list . The choices are: Plus, Cross, Circle, Solid Circle, Square, Solid Square, Diamond, Solid Diamond, Upward Triangle, Solid Upward Triangle, Downward Triangle, or Solid Downward Triangle.

Font Dialog

This dialog displays when the button is clicked in the Font field in the Title or Peak Names band.



Control	Description
<font name>	Select a font from the drop-down list to be used for plot titles. The list contains all available fonts on the computer.
Font Color	Click this button to display the standard Windows color selection dialog. This control is only displayed when the dialog is opened from the Peak Names band.
<size>	Set the size (Small, Standard, or Large) of the Title font.
Bold	A check box to set the title in boldface type.
Italic	A check box to set the title in italic type.
Underline	A check box to underline the title.

Other Commands

The following commands are available in Plot Control.

Save Plot Image	Saves the current chromatogram display to a file.
Copy Image to Clipboard	Copies the current chromatogram display to the clipboard in bitmap format.
Copy Data to Clipboard	Copies the X, Y values for the current chromatogram to the clipboard in text format, with one pair of values per line. This command will only be available when a single chromatogram is displayed. Alternatively, it could just copy the values for the selected chromatogram (but that is not even always displayed in Chromera currently) or it could copy concatenate the sets of values for different plots (but that implies some additional lines of identification would be required.)
Print	Prints the current chromatogram display to a selected printer.
Print Preview	Opens a preview window and displays the chromatogram as it is printed.
Rescale Axes	Enables the user to set minimum and maximum values for the X and Y axes.
Restore Default Scaling	Restores the initial display scaling.
Export Data to Excel	Displays a **Save As** dialog (with File Type of .xls). When you enter a file name, the raw data (as time, intensity pairs) together with header information, for the active curve will be saved to an Excel file (Excel 2003 .xls format).

Interaction Modes and Mouse pointers

Interaction Modes and Mouse Pointers

The software indicates when it is in one of the special interactive modes by displaying a different mouse pointer. The following pointers are used for the indicated mode or sub-mode.

Mouse Pointer Icon	**When Displayed**
	Default pointer for plot is used for drawing a zoom box.
	When over an object that can be selected or moved.
	When over a selected component search window use the handles for resizing the search window or adjusting the expected RT in graphic edit.
	When over a region of a selected component and the search window is between handles, it is used for moving the window to a new location (in graphic edit).
	When in *add component* or*add event with single time value* mode, it indicates a single time value (in graphic edit).
	When in *add event with start and end time values* it is used to draw a box indicating start and end times (in graphic edit).
	When mouse is moved beyond the left hand axis of a zoomed plot it is used to scroll the plot to an earlier time region.
	When mouse is moved beyond the right hand axis of a zoomed plot it is used to scroll the plot to a later time region.
	When mouse is moved beyond the top axis of a zoomed plot it is used to scroll the plot to display a higher intensity region.
	When mouse is moved beyond the bottom axis of a zoomed plot it is used to scroll the plot to display a lower intensity region.

How to Interact with the Plot - Display or Edit Mode

In the Display or Edit mode you can interact with the plot in the following ways.

Scaling the Chromatogram via a Zoom Box

When the default mouse pointer is displayed and your drag the mouse across the plot area, a zoom box is displayed. When you end the drag, the plot is rescaled. Usual behavior is that the plot is rescaled such that the area of the plot enclosed by the zoom box occupies the full plot area.

Scrolling a Zoomed Plot

When the mouse pointer is moved just outside the confines of the plot area (i.e. onto the border surrounding the curve) on a zoomed plot, the mouse pointer changes to one of the shapes shown in the table. Clicking when such a pointer is displayed will cause the plot to scroll in accordance with Windows conventions. For example, clicking with a down-pointing arrow displayed causes the document (the plot) to move upward and display a region of lower intensity, and clicking with a right-pointing arrow displayed causes the document to move leftward to display a later time region.

A single mouse click will scroll the plot 10% of the displayed region. If the Shift key is held down while the moue is clicked the plot will scroll 50% of the displayed region.

Displaying System Annotations

The primary system annotations are baselines, component names, component search windows, peak data labels and event mnemonics. The default display for each type of system annotation is set as part of the general plot properties.

Each type of system annotation also has its own set of the following graphic properties:

- For baselines, you can select the color and the line type.
- For timed events you can select some of the text properties.
- For component names you can select the display orientation and the text properties.
- For component search windows you can select the colors for the selected and unselected windows.
- For peak data labels you can select display orientation, text properties and the data items to be displayed.

Adding a User Label

When the mouse pointer is not over any other object on the plot, one of the items in the pop-up menu is **Add User Label**. This allows you to add custom text at the location designated by the mouse click. Note that this means the location of the initial mouse click that displayed the context menu, not the mouse position when the command was selected (which you have no control over).

NOTE: *Entering a new User Label to an existing chromatogram will take the current format settings. The existing User Labels will keep their original format settings and not change to the current format settings. You must re-enter the User Labels to get the updated format settings.*

In addition to specifying the text, you can set its orientation (vertical or horizontal), its text properties, and also specify whether the label is to be locked to X and/or Y data. If the label is locked to X data then it always appears at the same time on the plot, regardless of the scaling. If it is not locked to X data then it always appears at the same relative distance along the X-axis (i.e. as % of time span), and therefore moves relative to the chromatogram topography when the scaling changes. Similarly, if the label is locked to Y data then it always appears at the same response level, regardless of scaling. If it is not locked to Y data then it always appears at the same relative distance along the Y-axis (i.e. as % of full scale).

Moving a User Label

Labels can be repositioned by being dragged to a new location. If the label was defined to be locked to the data or to a specific location within the plot area then the lock is updated to the new position.

Deleting a User Label

Right-clicking on an existing user label will display a context menu, providing to commands to delete the label or delete all labels.

How to Interact with the Plot - Edit Mode

The following additional interactions apply in edit mode. By default, a change to any graphic object related to peak integration or identification (e.g. adding an integration event or resizing a component search window) will automatically cause the data to be reprocessed. You are able to disable automatic processing in Preferences, in which case a Reprocess command will become available, so that you can update results at a convenient time.

Setting Initial Bunching Factor

Refer to Chromatogram Control - Bunching Factor Tool

Setting Initial Area and Noise Thresholds

Refer to Chromatogram Control - Thresholds Tool

Adding a Timed Event

You can add a timed event...

- From the Peak Detection Method, or
- From the Toolbar

From the Peak Detection Method

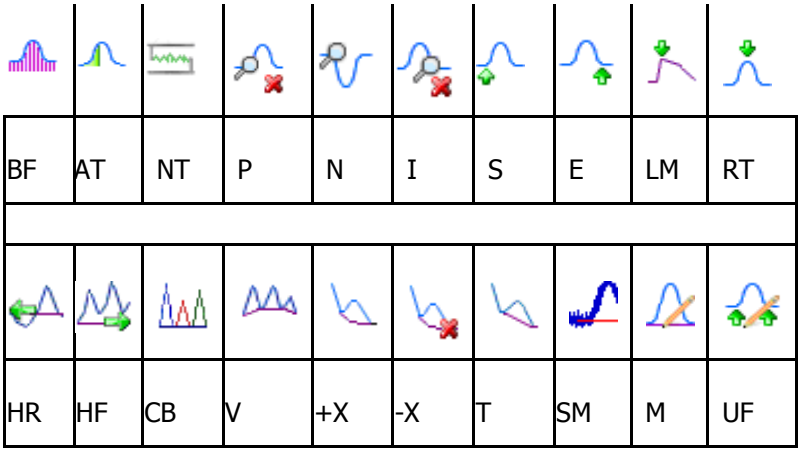
Selecting an event type from the drop-down list on the blank line of the events list within the peak detection method window sets the plot into add event mode, and the mouse pointer changes appropriately. At least two mouse pointers appear; one indicating a single-point event and another for time zone events. For the former, only a single click is required to define the event. For the latter, a drag is required to indicate the start and end points. If you perform the wrong action for the event type the following will occur:

- A single point event is positioned at the point of the button up action. (If the you click the event it will be at the click point; if you drag to create a rectangle, the event will be at the edge of the rectangle at completion of the drag.).
- The start of a time zone event is positioned at the start and end of the rectangle drawn. If you click instead of dragging, the event will not be added to the plot; instead the software will remain in the **adding event** mode, with the Start field empty but selected.

Ideally the mouse pointer would change to a prohibited icon (see below) if you move it into a zone where the current selected event would be invalid.

From the Toolbar

Clicking one of the integration event toolbar buttons (shown below) sets the plot into the add event mode, and the mouse pointer changes appropriately. The behavior described above also applies here; the only difference being that here the event does not appear in the event list of the peak detection method view until your indicate the position on the plot.



BF	AT	NT	P	N	I	S	E	LM	RT
HR	HF	CB	V	+X	-X	T	SM	M	UF

Moving a timed event

If you drag the timed event to a new location on the chromatogram, the time of that event will be updated in the events list. The event label being dragged is highlighted to indicate that you are dragging the right (or wrong) event, when two events are very close together.

The mouse pointer changes to a prohibition sign  if you drag it into a zone where the event is not permitted, based on the presence of other events.

Deleting a timed event

A timed event can be deleted by selecting the event in the method parameters display and pressing the Delete key.

Manual Integration

There are two kinds of manual integration features. The first is Manual Baselines, which is simply the addition of +M and -M timed events. The second is Manual Peak Integration, which is a special graphical approach to the addition of User Forced integration (UF) events.

The major difference between these two functions is that Manual Peak Integration covers both peak detection and baseline drawing. While UF events are in effect the standard integration algorithms are not active and other baseline timed events are ignored. Manual Baselines, on the other hand, determines only where the baseline is drawn; peak detection is carried out as normal (modified by timed events if present).

You cannot nest or overlap +M/-M and UF events.

Manual Baselines

Adding a new manual baseline segment

To specify a manual baseline, select the 'Manual Baseline' event and drag from the required peak start to the peak end. As the mouse is dragged a 'rubber-band' box is drawn from the start point to the current mouse position. When you release the mouse button a +M event is added at the start time and a -M event at the end time. The Y-axis location and size of the box is of no consequence, since +M/-M events always use the signal level at the specified time. The chromatogram is automatically reprocessed so that the display and report information reflect the new manual baseline.

No overlap of any kind is allowed between manual baseline segments. If you draw a segment that wholly contains, or is contained, by an existing segment the software will assume that the new segment is to replace the existing one. No confirmation is required. If a new segment partially overlaps an existing one, the existing segment is truncated immediately before the start (or after the end as appropriate) of the new segment so that no overlap occurs.

Editing a Manual Baseline

Manual baselines may be edited simply by dragging the start or end event to a new location. The same limitations on the new location described above still apply.

Adding a component

To define a new component in the selected data channel follow this procedure.

1. Select the Name field in the new component row within the Peaks section of the method for the selected channel. A name for the component can be defined either by selecting an existing value from the component dictionary contained in the drop-down list, or by typing in a new name (which will then be added to the dictionary).
2. When a name has been defined, the retention time field will be automatically selected in the grid and the mouse pointer on the chart will change to an upward pointing arrow, indicating that the expected retention time of the component can be set by clicking on the chart.
3. When you click on the chart a value will be entered into the RT field for the component and the search window will be set to default size (defined by Start and End values entered into the appropriate fields in the grid. The value set for the RT depends on where you click and on the current state of the chromatogram. If the chromatogram is integrated and you click within a peak envelope (between start and end times) then the calculated crest of that peak (based on curve fit, where applicable) will be entered as the component expected RT. If any other conditions apply then the time at which you clicked on the chart will be entered as the component expected RT.
4. The component search window gadget is displayed on the chart as soon as you define the component RT and the default search window start and end times have been calculated.
5. Once you click on the chart to define the component RT, the chart cursor reverts to the standard mouse pointer and the chart is once again in zooming mode.
6. You can adjust the size of the component search window as required by drag-and-drop using the start and end handles of the component search window gadget. Updated values for these times will be entered into the grid upon each drop.

Radar Window

The radar window is a frame displayed at the top of a graphics pane, with the working plot below. The radar window displays the full chromatogram and highlights the portion of the chromatogram displayed in the working plot frame below. The radar plot uses the curve color and background color set for the working chromatogram, and the region of the radar plot showing a selection (i.e. the portion displayed in the working plot) is displayed with a contrasting background color.

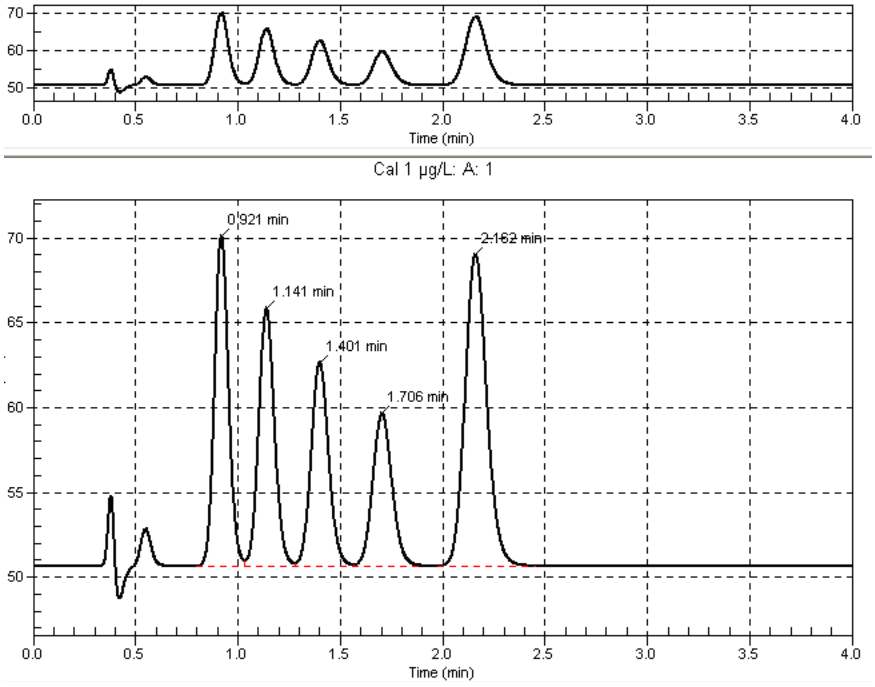
The relative sizes of the radar window and working plot frames within the graphics pane is directly adjustable by dragging a dividing bar. The limits on the size of the radar window will be about 5% to 95% of the size of the plot window as a whole, however it is not possible to hide either window completely. A command is available to toggle display of the radar plot on or off.

Annotations such as baselines, retention times, component names, labels and events will never be displayed on the radar plot. Nor will the peak or peaks currently selected in the working chromatogram be shown as selected on the radar window.

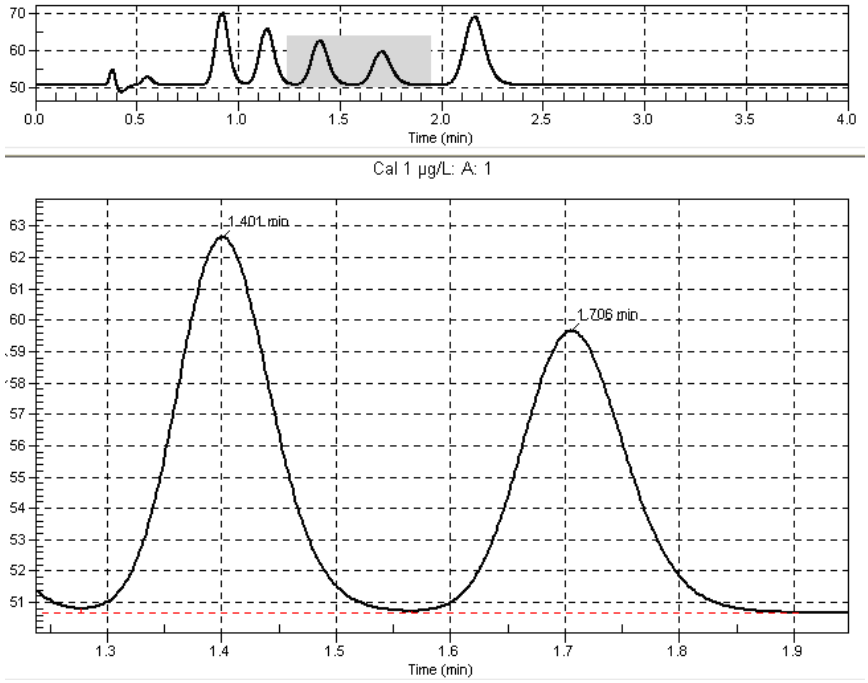
It is possible to scale the working plot by drawing a zoom box or selection box on the radar window. This is a means to zoom in on a portion of the working plot that is currently not even displayed in the working plot at its current scaling.

Radar plot behavior

When a data file is loaded (under default conditions) both the working plot and the radar plot show the full chromatogram.



If you draw a zoom box on the working plot and expand it, the display changes to something like the following:



The highlighting within the radar chromatogram shows the portion displayed in the working area.

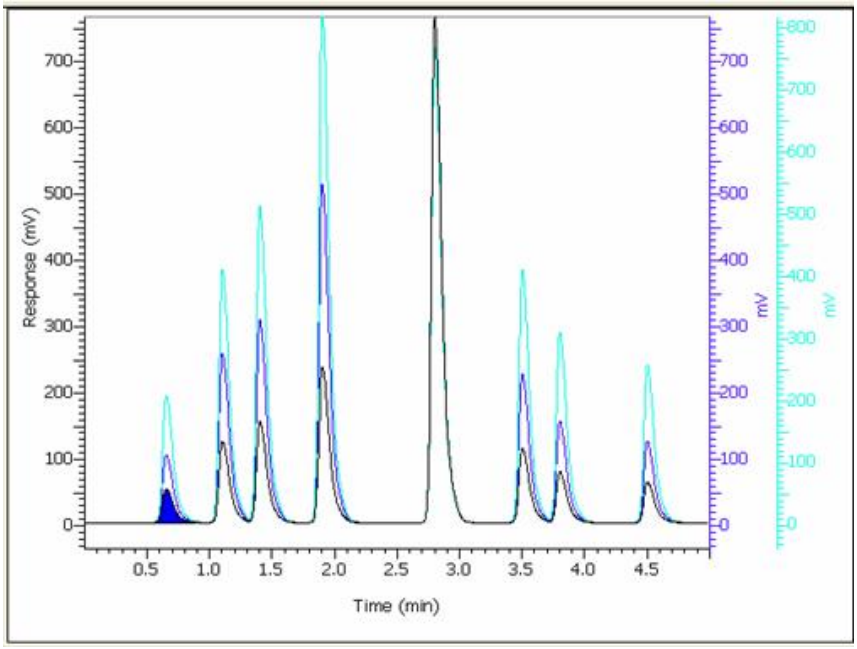
Manipulating the Radar Window

The selection rectangle marking the portion of the full chromatogram displayed in the working frame is movable. Dragging it to a different part of the radar plot causes the working chromatogram display to be updated to display the newly highlighted region.

It is also possible to draw a new rectangle within the radar window area. When this is done the region outlined on the radar window is highlighted and the working chromatogram is rescaled to display just that region.

Multi-Chromatogram Overlay

The plot control can display more than one chromatogram at a time in an overlay format.



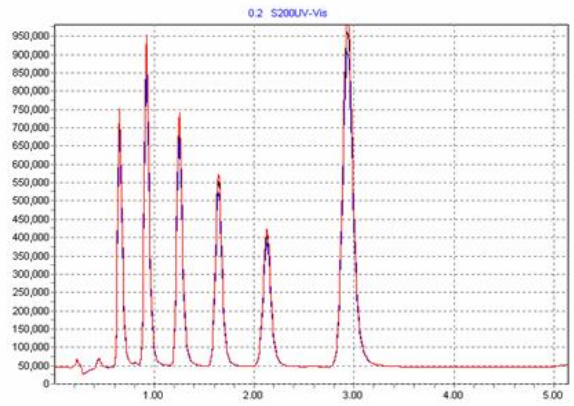
Overlayed Chromatograms

NOTE: *This example shows only the basic plot control and does not show the associated data tree and/or legend that would be present to identify the chromatograms.*

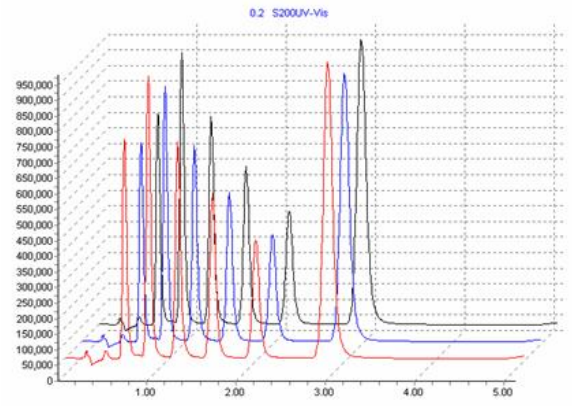
As described, the Y axis on the left is associated with the primary (selected) chromatogram and the additional Y axes on the right indicate the different scales of the overlaid plots. An optional legend at the top of the plot, occupying multiple rows if necessary, indicates the mapping of color to the chromatogram (identified by sample ID: channel: injection number).

3-D Mode

You can also display an overlay plot in a 3-D format. When the 3-D command is issued for an overlaid plot the presentation changes to show the plots spaced out along a Z axis.

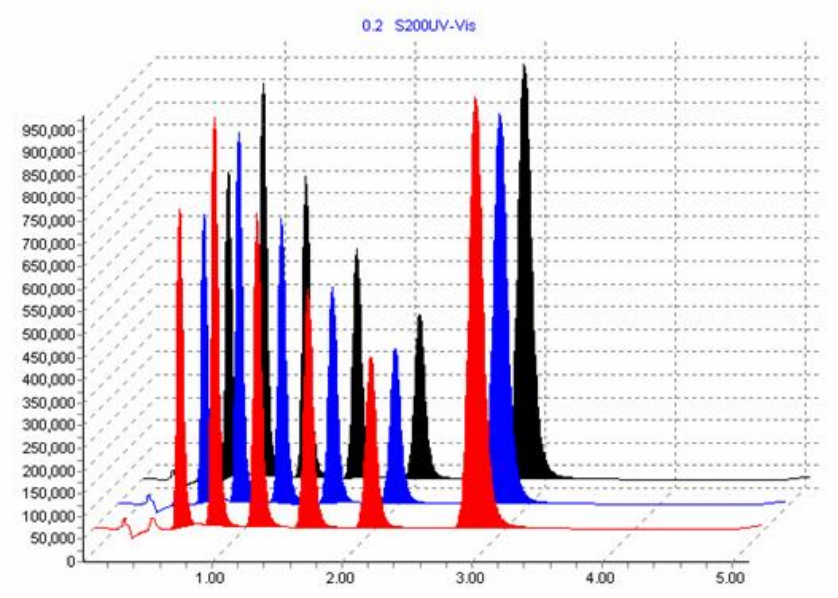


Standard Overlay Mode



3-D Overlay Mode

If the area under each curve is filled in and if the order in which the plots are drawn are set from back to front then this would effectively provide a hidden line display, as shown in the following example. This makes it much easier to track the peaks in each chromatogram.



Processing

Processing

The processing of detector channel output is the complete integration of the digital signal stream. A separate set of peak detection parameters (including an integration events list) is available for each detector channel configured on the instrument.

By default acquired data are processed according to the specified method but there is an option to suppress processing (and/or report generation) when a sequence is run.

What is Data Analysis?

Data analysis is the process by which Chromera interprets the data obtained from an instrument and stored in the database. The outcome of this interpretation is stored as results within the database.

Chromera analyzes data at three different points:

- Immediately after the software collects the data from an instrument and stores it in the database. This is the original data analysis.
- When you reprocess a batch of raw data using the Reprocessing function.
- When you reprocess an individual raw data set using the Graphic Method Editor or Optimize Results function.

Data Analysis Parameters

Chromera acquires and analyzes data based on the values (parameters) you enter in a method. A method consists of four sections — instrument, channels, peaks, and calibration — each of which has its own set of parameters that guide their respective parts of the analysis.

The instrument and channels sections are essential to all methods. Peaks and Calibration parameters are essential to identifying peaks and calculating component amounts. A separate but related file, the report format, defines the contents and layout of the printed report containing the results of the analysis.

How the Method Controls Data Analysis

For every injection, you define the method parameters you want to use to analyze the data. For a series of injections, this is done through the sequence file, where you can use different methods to define the instrument, processing, and calibration parameters. This sequence file, which you create using the Sequence Editor, defines how you want Chromera to acquire and analyze the data from a particular series of injections.

When you initialize and set up an instrument to acquire data from these injections, you specify the sequence name. Chromera then acquires and analyzes the data based on the information in this sequence.

For more information on how to create and modify a sequence file, please refer Sequence.

Overview of the Steps in Data Analysis

There are distinct steps in the data analysis process. Some of the steps in data analysis are optional — their occurrence depends on the information you provide in the method and sequence. For example, you can omit from the method instructions for adding component names to peaks, generating a report and plot, and running a user program during analysis.

Also, the calibration step is relevant only when Chromera analyzes the data from a standard sample.

When you reprocess data, you can choose which steps you want to perform as part of the reprocessing operation. For example, you can start with component identification or quantitation and end with report generation. As analysis proceeds from one step to the next, the icon at the bottom of the screen changes to reflect the step currently under way.

The following is a brief description of each step of data analysis. More detailed information about each step (except report generation) is discussed in the appropriate section.

Channel Extraction — When the chromatographic channels defined in the method are extracted from the spectral array.

Blank Subtraction — Subtracts the points in a baseline data set, obtained by running an instrument or a sample blank, from those in the current raw data set in order to correct the baseline. The results of this are stored in a modified raw data set (in addition to the raw data set).

Peak Detection and Integration — Scans the data points in a raw data set (or modified raw data set) to find peaks. This produces a peak list that consists solely of peak starting and ending points. To edit peak detection parameters, refer to Peak Detection Parameters. Then peaks are grouped into clusters and the area under each peak is calculated. This adds final values to the results for peak starting and ending points, retention times, areas and heights. To edit peak integration parameters, refer to, "Developing Processing Parameters in the Method."

Component/Peak Identification (optional) — Determines the peak identities in the results by comparing their retention times with a pre-defined list of expected components and retention times specified in the Peaks section of the method. This step adds component names to peaks in the results.

Spectral Processing — Performed solely by the Spectral Processing window either when requested by Chromera (post-acquisition calculations or commands to display spectral operations annotations or results) or by the user, from within the Spectral Processing window. In either case the results will be passed back to Chromera for storage and display. Only one set of spectral operation parameters will be available for each injection since the spectral data is common to all derived chromatographic channels from that injection. Thus the identification obtained using a spectral library search of the chromatographic peak at 2.37 mins in derived chromatogram A will be exactly the same as the identification of the chromatographic peak at 2.37 mins in derived chromatogram B (it is after all searching on exactly the same spectrum).

Calibration — Updates amount and response values for components during the analysis of calibration standards. Chromera stores calibration data, including the results of the analysis of standard samples, in the calibration section of the method. The calibration step in data

analysis also produces a report containing the current calibration data for each component. Refer to Calibration Parameters.

Quantitation — Calculates the amounts of components represented by peaks in the result data set. These calculations are based on the peak areas or heights and on the calibration data for the corresponding components. This step adds component amounts to the results.

Report Generation (optional) — Produces one or more reports containing the results of the analysis. You specify the reports in the Global Settings of the sequence controlling the analysis, or in Decision Points associated with individual sequence rows.

Peak Detection

During the peak detection step in data analysis, Chromera scans the data points in a raw data file (or modified raw data file) to find peaks. It saves the point data and point index data it collects in a file. A point index is a number indicating the position of a data point in the series of data points within the raw data file.

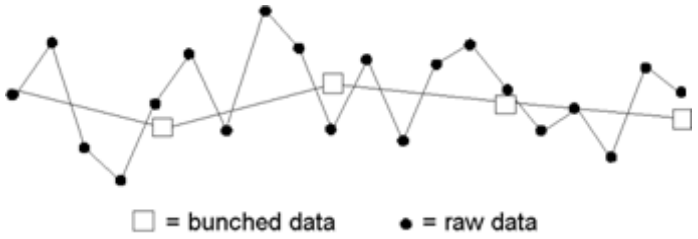
Peak Detection Parameters

The peak detection parameters include bunching factor, noise threshold, and area threshold.

Bunching Factor

The bunching factor specifies how many sequential data points in a raw data file are grouped in a bunch. During peak detection, Chromera counts bunches and then averages the voltage values of the points in each bunch. The resulting averages are assigned to bunched points.

The following diagram shows how bunching smoothes out raw data, which helps prevent Chromera from identifying baseline noise as peaks. Small dots represent the raw data, and squares represent bunched points. In the following figure, one bunched point is shown for every five raw points; thus, the bunching factor is 5. Each bunched point is located at the same time position as the last raw point in a bunch.

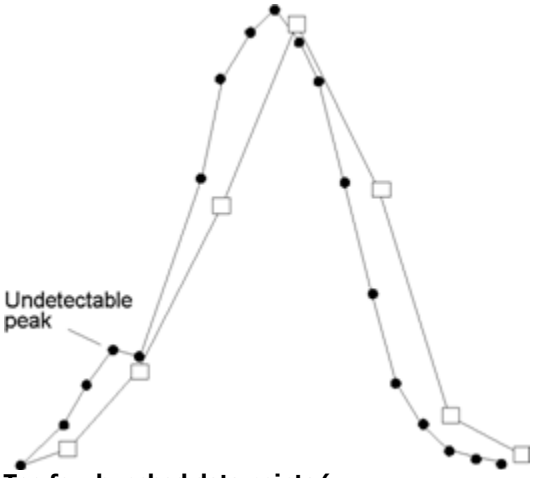


A bunching factor also compensates for over-sampling: that is, collecting more points than are necessary in a peak. Ideally, peaks will have about 20 points from start to end. This provides the best balance between acceptable processing time and correct peak detection and integration.

If the sampling rate remains constant throughout a run, you might not be able to avoid over-sampling because peak widths can vary, perhaps broadening later in the run. The broader a peak, the more points it is likely to have; thus, it can be over-sampled.

In this situation, where both narrow and broad peaks occur in the same run, you cannot lower the sampling rate. However, you can increase the bunching factor one or more times by using a timed event. You specify the event (increasing the bunching factor) and the time in the run when the event will take place.

Setting the bunching factor too high can lead to small, unresolved peaks being smoothed out completely, so they are undetected. The goal in setting a bunching factor by timed event is to maintain the number of points within the peaks reasonably close to the 20-point optimum.



Too few bunched data points (☐) can smooth small peaks, mak

Because bunched points are positioned at the time of the latest point in the bunch, a peak plotted from bunched data tends to shift to the right (refer to the above figure). This shift has no adverse effect on peak detection and integration because the bunched data are used only to identify the potential start, top, and end of a peak. After finding the potential peak values, the software reverts back to the bunched data points to pinpoint the actual peak start, top, and end. Therefore, there is no loss of resolution in calculating baseline positioning or peak integration.

Stages in Peak Detection

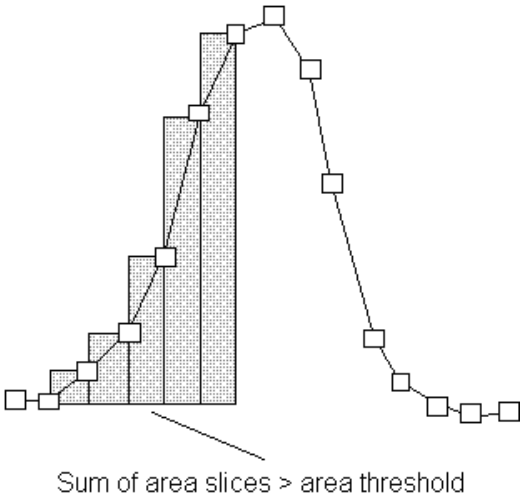
In order to use timed events and peak detection and integration parameters correctly, you must be familiar with the stages in the peak detection process. A simplified discussion of each stage in peak detection follows.

Finding the Potential Peak Start

To find the potential start of a peak, Chromera examines the difference in value between each bunched data point and the preceding one. If the difference exceeds the current noise threshold value, then a potential peak start point has been found.

Confirming the Peak Start

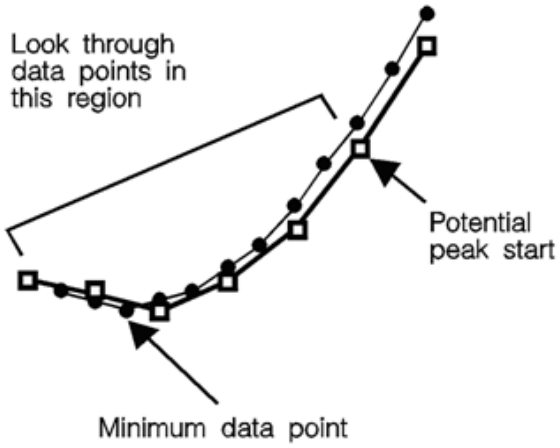
After a potential peak start is found, Chromera begins to sum the differences between each bunched point and the last baseline point. Because each bunched point represents an area slice, the sum is the accumulated area for the potential peak, as shown in the figure that follows. The differences between bunched points must also continue to exceed the noise threshold, or the peak start will be canceled. If the accumulated area exceeds the area threshold before a bunched point fails the noise threshold test, then the peak is confirmed.



A peak is confirmed when it passes the area threshold test.

After a peak is confirmed, the peak detection software scans backward from the potential peak start looking for the lowest raw data point. It scans backward through five bunches of raw data to find this minimum, but will stop the search if it reaches the end of the preceding peak. The lowest raw data point found in this process becomes the actual peak start.

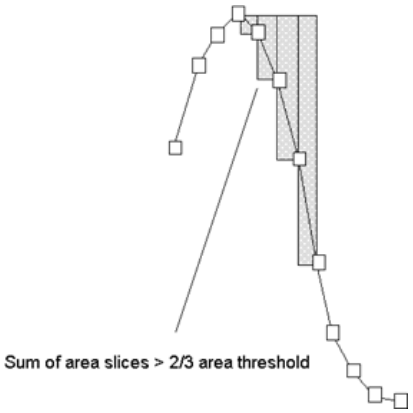
The following figure shows how the software finds the actual start point. In this example, the bunching factor is set to 2, so there are only two raw data points for each bunched point. Therefore, the bunched points do not rise much faster than the raw data. The potential peak start was found at a point well up the side of the peak because previous consecutive pairs of bunched points failed the noise threshold test. It is evident in this figure that, without the backward search, a high noise threshold would drastically alter the peak start value. The same principle applies to peak end values.



Finding the Peak Top

To find the top of the peak, the software first tries to identify a local maximum bunched point value. When a bunched point is lower than the previous one, the previous point is considered to be the potential peak top. To avoid finding a false peak top because of noise,

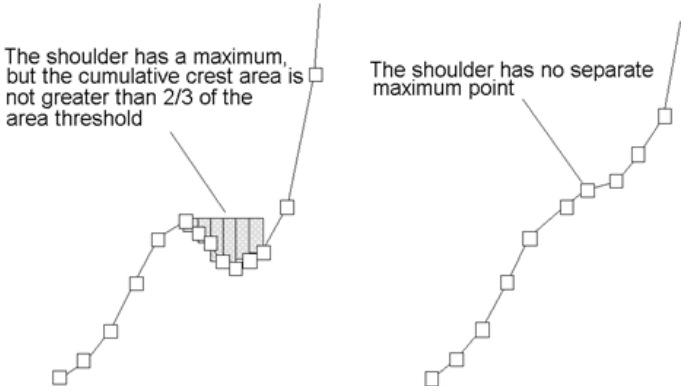
Chromera performs a confirmation test by summing the differences between the potential top and subsequent bunched points. If the sum exceeds two-thirds of the area threshold value, the potential peak top is confirmed. However, if a higher bunched point is found before the area test is passed, a new potential top is identified and the area test is restarted.



If the shaded area exceeds two-third of the area threshold, the maximum point becomes the peak top.

Note: *The reported retention time for a peak is not simply the time of the point identified as the peak top. The reported retention time is determined from a quadratic fit based on the five highest bunched data points.*

Because of this top-of-peak test, the choice of an area threshold value affects both peak confirmation and how shoulders are detected on the leading edge of a larger peak. Shoulders on the leading edge of a larger peak are not detected as peaks unless they have a discernible maximum point and a crest area that is greater than two-thirds of the area threshold.



The shoulder in the first figure could be detected as a peak if the area threshold were lower. However, the shoulder in the second figure could not be detected automatically regardless of the peak detection parameter values that are used because it lacks a maximum. To separate this shoulder as a distinct peak, you must use the timed event S (split peak).

Finding the Peak End

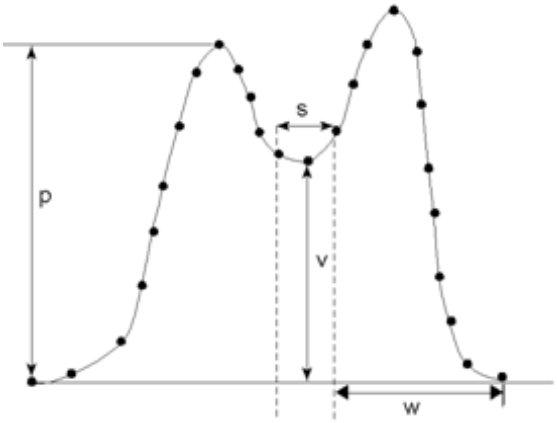
There are two indicators of a peak end:

- Two consecutive bunched point differences are less than half the noise threshold.
  or
- The start of another peak is detected.

Peak Separation Criteria

Width Ratio and Valley-To-Peak Ratio are two peak separation criteria that are considered overlapped or separated. This determination will affect how the baseline is drawn beneath the peaks. In peak detection, a peak is defined as overlapped with its neighbor on the right if the pair meet two criteria: the valley-to-peak ratio is greater than 0.01, and the separation is less than 0.2w, where w is the width of the first peak in the pair. (You can change these values in Chromera.) Peaks defined as overlapped are assigned an overlap flag of 1; those not defined as overlapped receive an overlap flag of 0. A set of overlapped peaks is called a cluster and, by default, shares a common baseline. Peaks that are separated each have an individual segment of the baseline.

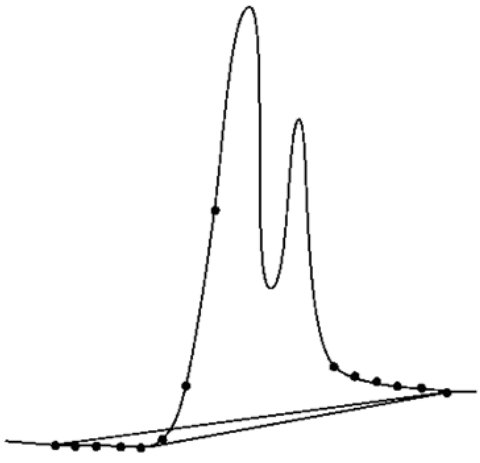
The Width Ratio is the ratio of the distance(s) between the end of the first peak and the start of the second peak to the width of the second peak at its base (w). If this ratio is greater than the set value, the peaks are considered to be separated. Otherwise, they are marked as overlapped.



Criteria for overlapping peaks

The Valley-to-Peak Ratio is the ratio of the height of the valley between peaks (v) to the height of the smaller peak (p). If this ratio is less than or equal to the set value, the peaks are considered to be separated.

Baseline Penetration at the Start or End of a Cluster



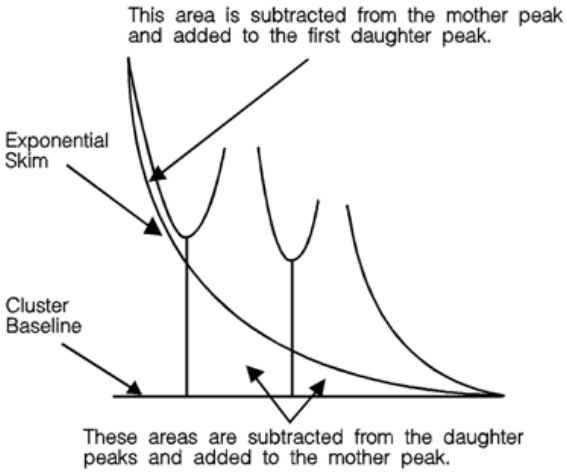
In addition to checking each valley point, the software examines all the points of each cluster for baseline penetration.

If any point penetrates the baseline, the start point of the first peak in the cluster is set to the point following the penetrator. If any of the final points penetrates the baseline, the end point of the last peak in the cluster is set to the point preceding the penetrator. A new cluster baseline is calculated, and the software continues testing for penetration. The software corrects baseline penetration at the start of a cluster.

Area Adjustment

For some peaks, additional processing beyond dropline integration is required to obtain a suitable peak area. The system offers two methods to adjust peak areas: exponential skimming and tangential skimming. The necessity for an exponential skim is determined automatically by the software; however, you can also impose an exponential skim by means of a timed event. A tangential skim is never implemented automatically; it can only be imposed by a timed event.

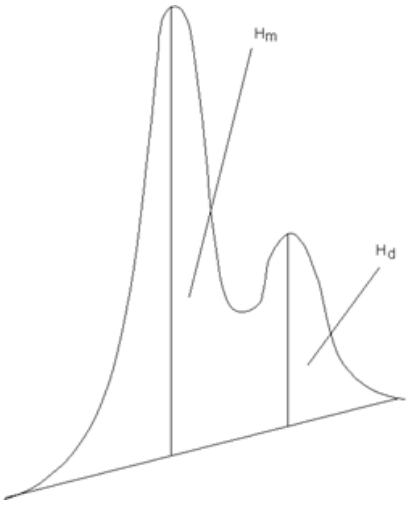
Exponential Skims



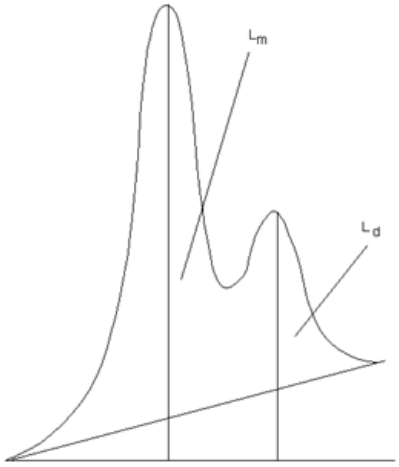
An exponential skim is a curve drawn by using an exponential equation to approximate the trailing edge of a parent peak. The skim passes under one or more peaks that follow the parent. These are called child peaks. The area underneath the skim is subtracted from the child peaks and given to the parent peak. A small area above the skim is subtracted from the parent peak and given to the first child peak. All droplines, beginning at the end of the first child, are adjusted to drop only to the skim.

Exponential Skim Criteria

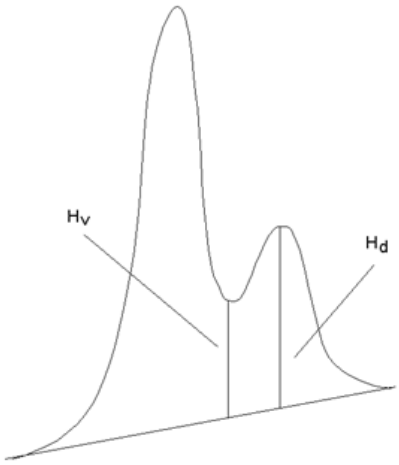
Peak Height Ratio, Adjusted Height Ratio, and Valley Height Ratio are exponential skim criteria that determine whether an exponential skim line will be used to calculate the area of a child peak eluting on the trailing edge of a parent peak. Chromera will not use these parameters if a +X timed event (which always forces an exponential skim) or a *X timed event (which prevents an exponential skim) is in effect.



Peak Height Ratio is the ratio of the baseline-corrected height of the parent peak (Hm) to the baseline corrected height of the child peak (Hd). This ratio must be greater than the set value for the child peak to be skimmed. To disable exponential skimming throughout a run, you can set this parameter to its maximum value (1.0e+06). Hm divided by Hd must exceed the set value for peak height ratio.



Adjusted Height Ratio is the ratio of the height of the parent above its start point (Lm) to the height of the child above the same point (Ld). This ratio must be greater than the set value for the child peak to be skimmed. Lm divided by Ld must exceed the set value for adjusted height ratio.

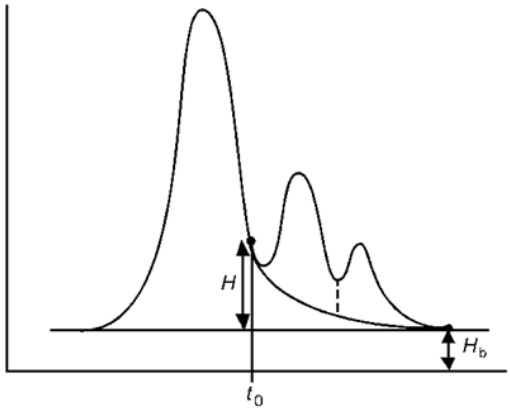


Valley Height Ratio is the ratio of the baseline corrected height of the child peak (Hd) to the height of the valley between the parent and child peaks above the baseline (Hv). This ratio must be less than the set value for the child peak to be skimmed. Hd divided by Hv must be less than the set value for valley height ratio.

Calculation of Exponential Skims

Following is the equation you use to calculate an exponential skim:

$$Y = H_b + H_e - G^{(t-t_0)}$$



where:

Y is the height of the exponential skim at time t

H is the height (above the cluster baseline) of the start of the exponential skim

H_b is the height of the cluster baseline at the end of the exponential skim

G is the decay factor of the exponential

t_0 is the time corresponding to the start of the exponential skim

Integration Timed Events

Integration (baseline) timed events are commands defined in the processing section of the method that affect peak detection and/or integration at specific times during a run. Chromera implements compatibility rules for the list of timed events to prevent invalid combinations of events. For details see **Compatibility Rules.**

Supported Events

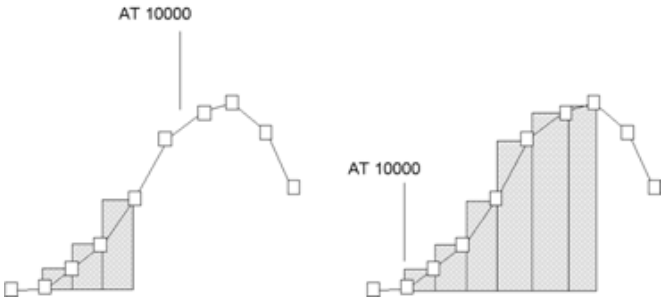
The following table provides an overview of the supported events.

Control	Description
Set Bunching Factor	A parameter in the processing section of the method that specifies how many sequential data points in the raw data file is grouped in a bunch. It can range from 1 to 99. Bunching factors should be based on the narrowest peak you want the system to detect.
Set Noise Threshold	A value that helps determines the difference between baseline noise and the start of a peak.
Set Area Threshold	A value that helps discriminates between noise spikes and peaks. As a general rule, set the area threshold to approximately five times the noise threshold. However, the optimal ratio depends on the actual peak size and signal-to-noise ratio.
Disable Peak Detection	Stops peak detection during a run. At end time peak detection is enabled again.
Inhibit End of Peak Detection	When this event is in effect, the software does not attempt to determine the ending point of a peak. This may be used to include the area of a shoulder within in a peak when it might otherwise be interpreted as baseline.
Enable Negative Peak Detection	When this event is in effect the software will attempt to detect negative peaks as well as positive ones. Positive peak detection (+P) must be in effect before negative peak detection (+N) can be enabled.
Force Start of New Peak	Starts a new peak when the event occurs, regardless of whether a peak would normally be detected and confirmed at this position
Force End of Peak	The E event establishes a peak end at a specific point in time.
Force Baseline	Forces the baseline to the start of the current peak. (The current peak is the one on which the event occurs, or the one that follows the event if it occurs between peaks.) As a result, the preceding peak is treated as though it is resolved from the current peak.
Force Common Baseline	Causes the software to treat all peaks as though they were clustered even if they do not meet the criteria for being overlapped.

Force Horizontal Forward Baseline	Projects a horizontal baseline from the time of the event to the end of the chromatogram or until the end of the event time.
Force Horizontal Backward Baseline	Projects a horizontal baseline from the time of the event back to the start of the run.
Enable Valley-to-Valley Baselines	Defines segment in which a baseline is drawn directly from each peak start to peak end (i.e. o common baselines or drop-lines from valley points)
Force Exponential Skim	A curve drawn by using an exponential equation to approximate the trailing edge of a parent peak. The skim passes under one or more child peaks. The area underneath the skim is subtracted from the child peak(s) and given to the parent peak.
Prevent Exponential Skim	Projects a horizontal baseline backward from the time the event is scheduled to the start of the chromatogram. The baseline is set at the voltage level and time value of the raw data point nearest the event placement. The software does not adjust the baseline to prevent penetration by valleys; thus the baseline remains horizontal.
Enable Tangential Skim	Use of a straight baseline segment, rather that an exponential curve, when skimming minor (child) peaks off the trailing edge of a tailing major (parent) peak. A tangential skim is never created automatically by the system. It must be accomplished by a this timed event placed between the peak's start and end points.
Locate Retention Time at Maximum	Locates the peak retention time at the maximum data point that falls within the peak rather than attempting to fit a quadratic to the peak crest.
Force Retention Time	Forces the retention time of the current peak to be the event time.
Enable Smooth Peak Ends	Performs a Savitsky-Golay smooth of the raw data at the Start and End of peaks when determining the position of baseline. The value (representing the Savitsky-Golay smoothing interval) can be any odd value between 5 and 25, inclusive.
Start Manual Baseline	Defines the start and end of manual baseline for peak integration.

About Bunching Factor, Noise Threshold, and Area Threshold Events

Using the bunching factor (BF), noise threshold (NT)), and area threshold (AT) timed events allows you to override previously set values. These events take effect at the time they are scheduled: they do not work retroactively. For example, in the first figure that follows, the current area threshold is low, which enables Chromera to confirm the peak before the AT event occurs. The new area threshold value does not affect the confirmation of the peak.



In the second figure, the AT event is scheduled during peak confirmation, so Chromera uses the new, larger area threshold to determine where the peak actually starts.

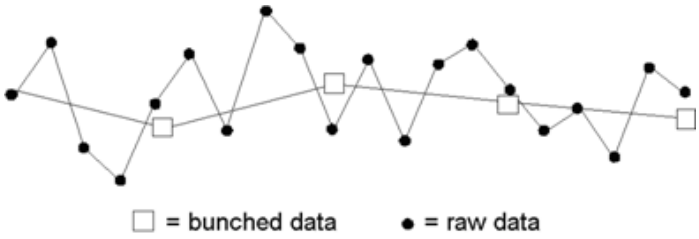
For best results, schedule the BF, NT, or AT timed events on the baseline as far away from peaks as possible. If you schedule these events at times too close to a transition from one peak detection stage to another, detection might differ significantly from chromatogram to chromatogram because of small shifts in retention time or random noise. For example, in the previous figure, a slight shift by the peak to an earlier retention time would move the AT timed event past the Confirm stage.

Bunching Factor (BF)

The BF event helps compensate for changes in peak width over the course of a run. Peaks tend to broaden as the run progresses. In these cases, scheduling this event at a higher value later in the chromatogram helps maintain the ideal 20-30 bunched points per peak.

The bunching factor specifies how many sequential data points in a raw data file are grouped in a bunch. During peak detection, Chromera counts bunches and then averages the voltage values of the points in each bunch. The resulting averages are assigned to bunched points.

Bunching smoothes out raw data, which helps prevent Chromera from identifying baseline noise as peaks. Small dots represent the raw data, and squares represent bunched points. One bunched point is shown for every five raw points; thus, the bunching factor is 5. Each bunched point is located at the same time position as the last raw point in a bunch.



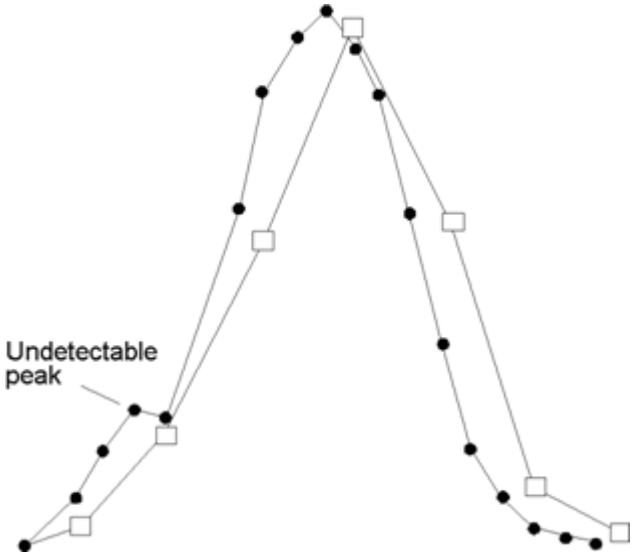
A bunching factor also compensates for over-sampling: that is, collecting more points than are necessary in a peak. Ideally, peaks will have about 20 points from start to end. This provides the best balance between acceptable processing time and correct peak detection and integration.

If the sampling rate remains constant throughout a run, you might not be able to avoid over-sampling because peak widths can vary, perhaps broadening later in the run. The broader a peak, the more points it is likely to have; thus, it can be over-sampled.

In this situation, where both narrow and broad peaks occur in the same run, you cannot lower the sampling rate. However, you can increase the bunching factor one or more times

by using a timed event. You specify the event (increasing the bunching factor) and the time in the run when the event will take place.

Setting the bunching factor too high can lead to small, unresolved peaks being smoothed out completely, so they are undetected. The goal in setting a bunching factor by timed event is to maintain the number of points within the peaks reasonably close to the 20-point optimum.



Too few bunched data points (*) can smooth small peaks, making them invisible to peak detection.

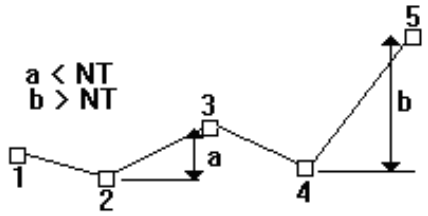
Because bunched points are positioned at the time of the latest point in the bunch, a peak plotted from bunched data tends to shift to the right (refer to the above figure). This shift has no adverse effect on peak detection and integration because the bunched data are used only to identify the potential start, top, and end of a peak. After finding the potential peak values, the software reverts back to the bunched data points to pinpoint the actual peak start, top, and end. Therefore, there is no loss of resolution in calculating baseline positioning or peak integration.

Noise Threshold (NT)

The NT event helps compensate for changes in baseline noise. You can increase the noise threshold to decrease peak detection sensitivity or decrease the noise threshold to increase sensitivity. For example, suppose the beginning of a chromatogram is noisy because of fast-eluting contaminants in the solvent, and this noise diminishes later in the run.

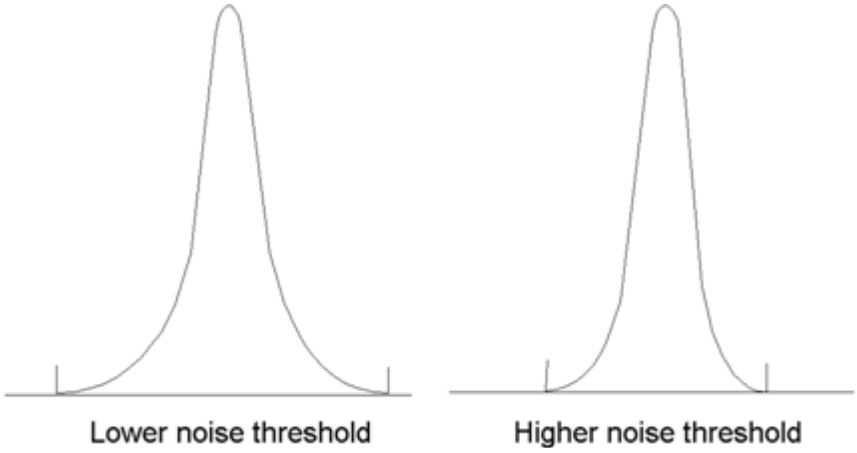
To optimize peak detection, you would set the default noise threshold high enough to screen out the initial noise and schedule the NT event to decrease the noise threshold at the time when the noise diminishes.

Expressed in units of microvolts, the noise threshold (NT) is the parameter that enables Chromera to discriminate between baseline noise and peaks. If the vertical difference between two consecutive bunched data points is greater than the noise threshold, Chromera recognizes the potential start of a peak. For example, point 4 would be detected as a potential peak start because the distance (b) between points 4 and 5 exceeds the noise threshold. Point 2 would not be detected as a peak start because distance (a) is too small.



The lower the noise threshold, the more sensitive peak detection will be. Conversely, raising the noise threshold decreases sensitivity. If the threshold is too high, however, Chromera will not be able to detect wanted peaks.

Increasing the noise threshold affects peak detection. The lower the noise threshold, the earlier the peak start is detected, and the later the peak end. The data values at the end taper off to a point that is below the noise threshold.



When the noise threshold is higher, peaks start and end more abruptly.

A higher noise threshold requires a more abrupt rise between data values before a peak start can be detected. By the same token, the peak end is found sooner as differences between consecutive data values quickly reach the threshold.

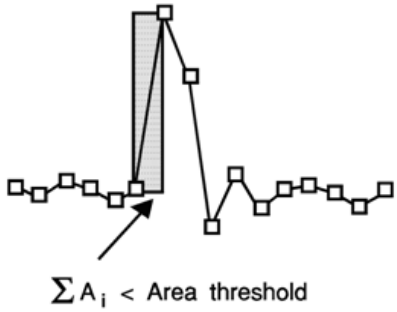
Area Threshold (AT)

In addition to the noise threshold (NT) event, the area threshold (AT) event also affects peak detection sensitivity. If you are scheduling an NT event to change the noise threshold, schedule an AT event at the same time to change the area threshold. For example, if you are lowering the noise threshold to gain sensitivity, lower the area threshold as well. Otherwise, the system might not confirm the smaller potential peaks that the lower noise threshold allows it to detect. *A general guideline is to set the area threshold to* ***five times*** *the value of the noise threshold.*

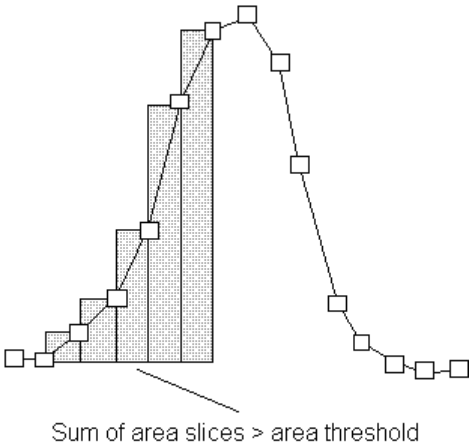
Area threshold is used to discriminate between noise spikes and peaks. Expressed in microvolts, this parameter is used after the noise threshold to confirm the potential start of peaks that pass the noise threshold test.

After passing the noise threshold test, pairs of bunched data points must continue to exceed the noise threshold, and the cumulative sum of the bunched data points on the leading edge must eventually exceed the area threshold for the peak to be confirmed.

For example, You can have a noise spike whose first two points pass the noise threshold test, but subsequent pairs of consecutive bunched points fail the area threshold test. Consequently, the spike does not sustain a leading edge that accumulates enough area under it to exceed the area threshold. The spike is not detected as a peak.



Another example illustrates a peak that passes the area threshold test. The sum of the bunched area slices on the leading edge exceeds the area threshold, so the peak is detected. There is no direct relationship between area threshold and the smallest peak that can appear on a report. To exclude all peaks smaller than 100 *V-sec, use the Area Reject setting in the Report Format Options dialog box.



A peak is confirmed when it passes the area threshold test.

Higher area threshold values make it harder to confirm a peak, and Chromera might not detect smaller peaks at all, especially those that appear as shoulders on the leading or trailing edges of larger peaks.

A good policy when starting out is to use a low noise threshold and a high area threshold. This maintains a high degree of sensitivity in detecting peak starting and ending points, but still screens out noise spikes. You can visually optimize noise and area threshold settings/values for your data in the Graphic Method Editor.

In addition to confirming a peak start, area threshold also helps determine a peak top. Refer to the Stages in Peak Detection.

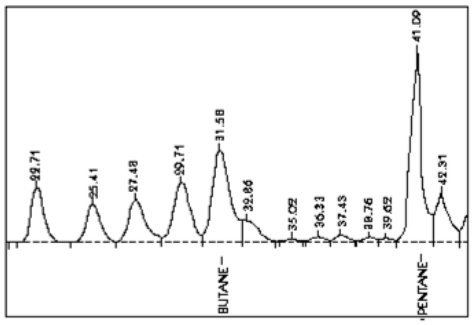
Disable/Enable Peak Detection (-P/+P)

The pair of timed events -P and +P turn peak detection off and on, respectively, allowing you to disable and re-enable peak detection during a run. For example, if valve switching causes

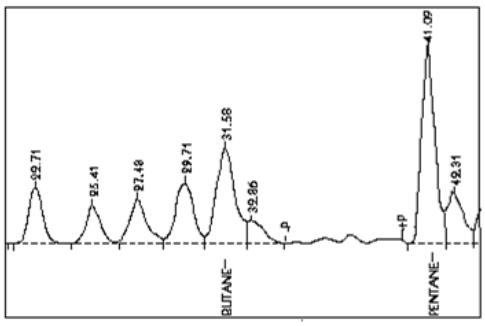
noise spikes in the middle of a chromatogram, you can schedule a P event before the affected region and a +P event after it to avoid the detection of false peaks.

If the software is at the point where it is searching for a peak ending point (for example, after the peak crest) when the P event occurs, the peak will end at exactly that point and all peak detection will stop. If the system is at any other point in peak detection, the process will stop and the current peak will not be detected.

The +P event has no effect unless peak detection has been disabled by a -P event. Likewise, a -P event has no effect if peak detection is currently disabled.



Without –P and +P timed events insignificant peaks are detected.



With –P and +P timed events, peak detection is disabled in this region.

Enable/Disable Negative Peak Detection (+N/-N)

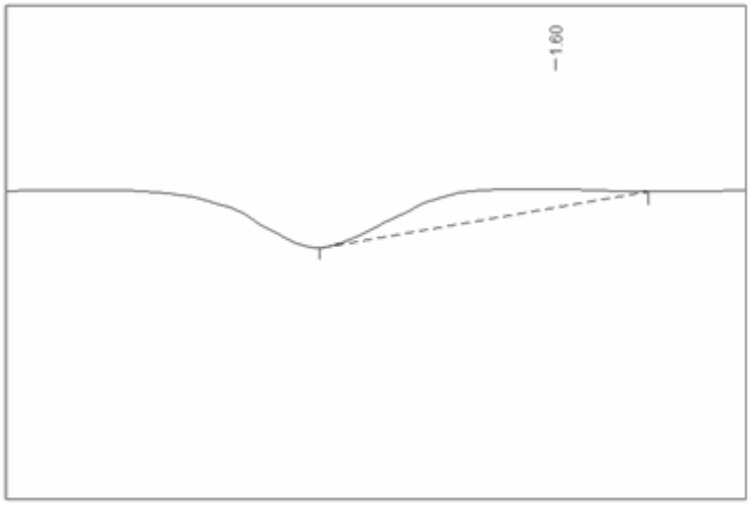
NOTE: *Certain events are needed as pairs. For example, it will not be possible to include a +N event without also including a –N event (even if it is at the end of the run). Therefore, the name of this event within Chromera is simply* Enable Negative Peak Detection *(the disable is inherent when the enable expires).*

The pair of timed events +N and -N turn negative peak detection on and off, thereby enabling and disabling the detection of negative peaks during a run. However, positive peak detection (+P) must be in effect before negative peak detection (+N) can be enabled. By default, Chromera detects only positive signals, so a +N event must be included to have it detect negative peaks.

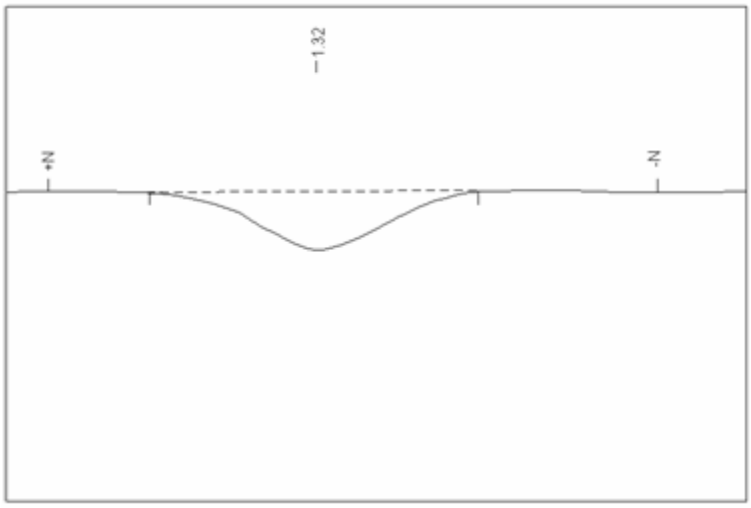
When negative peak detection is in effect, the tests normally performed when the signal increases are also performed on a signal decrease. Thus, the software will detect a potential negative peak if a bunched data point value decreases from the previous bunched point value by more than the noise threshold. The negative peak is confirmed if the area accumulated between the horizontally projected baseline and the decreasing signal exceeds the area threshold.

The following examples show the effect of the +N and -N timed events.

- Without the +N and -N events, the negative peak is not detected, and a false positive peak is found.



- With the +N and -N events, the negative peak is detected, and then negative peak detection is disabled.



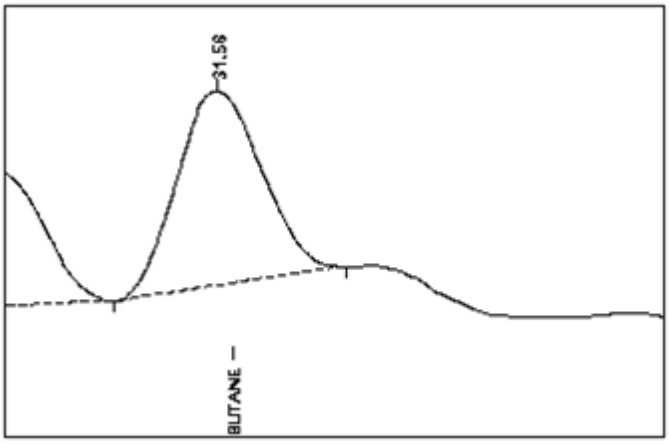
The -N event takes effect immediately if peak detection is in the Begin or Confirm stages. Otherwise, it is delayed until the end of the current peak is found.

If a +N event is added when negative peak detection is already enabled, the event has no effect. Likewise, nothing happens if a -N event occurs when negative peak detection is disabled.

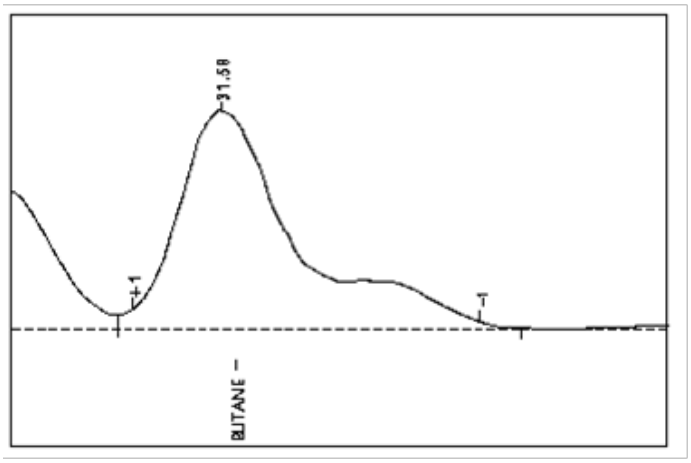
Inhibit/Allow End of Peak Detection (+I/-I)

The pair of timed events +I and -I inhibit and allow the stage in peak detection when the peak end is detected. When +I is in effect, Chromera does not attempt to determine the ending point of a peak. The -I event re-establishes peak detection for ending points.

It is appropriate to use these events when isomers of a compound elute as shoulders on the trailing side of the main peak, and you want all isomers to be treated as part of the main peak. In this case, you can schedule the +I event to prevent Chromera from finding the end of the main peak, and schedule the -I event to allow peak end detection again after the last isomer. For example, without the +I and –I events, a peak at 31.58 minutes ends naturally, but the shoulder on its trailing edge goes undetected.



When you use the +I and –I events, the shoulder is included in the peak.



The +I event forces the software to remain in the Find End stage. The result is that it will neither find a peak end nor detect the start of the next peak until the event is switched off. All data from the time of a +I event to the time of a *I event is perceived as part of the same peak.

The +I event takes effect immediately, but has no impact on processing until peak detection naturally enters the Find End stage. The *I event takes effect immediately, and allows Chromera to find the first peak end it encounters, based on the current noise threshold.

You should never use +I and -I events to group together distinct resolved peaks whose area you want to report as a single area. This is because the baseline that be would drawn under such a composite peak may be penetrated in places by the chromatographic signal. If Chromera detects that this has occurred between the start of a peak and the crest (or the crest and the end point), it will adjust the start (or end) point to eliminate the penetration. (See Baseline Penetration at the Start or End of a Cluster) If this process occurs during a composite peak, you will get results you did not intend.

Instead of +I/-I events, use the timed group component capability of Chromera to group distinct resolved peaks into a single component for quantitation and reporting.

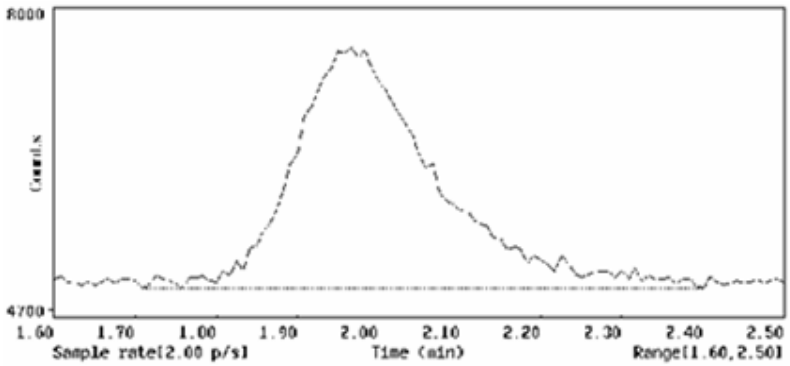
Force Retention Time (RT)

The RT event forces the retention time of the current peak to be the event time.

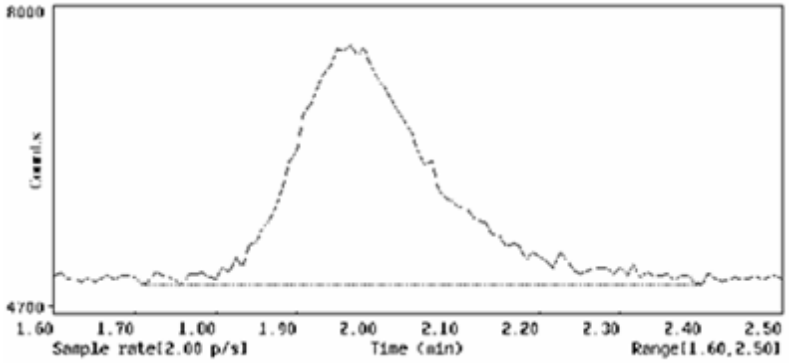
The RT event can be used in combination with the User Forced Peak event (UFn). If it is used within a user forced events cluster with daughter peaks, the optional peak number n specifies to which peak the RT event is applied.

Enable Smooth Peak Ends (+SM/-SM)

The SM event smoothes the beginning and ending of all peaks that start or end on a baseline. To illustrate this we can compare a peak before the SM event is added to a peak after the SM event was added.



This event applies a Savitsky-Golay smoothing algorithm to a group of **n** data points at both ends of a peak, where **n** can be any odd value between 5 and 25, inclusive. The algorithm adjusts the **start** and **end** data levels used for the baseline calculation to average baseline **values** (start and end times are not affected). The same peak after the SM event has been included shows the start and end point for each peak are put in the center of the noise band.



The SM event is useful when a high noise level, regardless of the signal strength, or a poor signal-to-noise ratio makes baseline resolution a problem. It is especially useful where accurate, reproducible integration is critical.

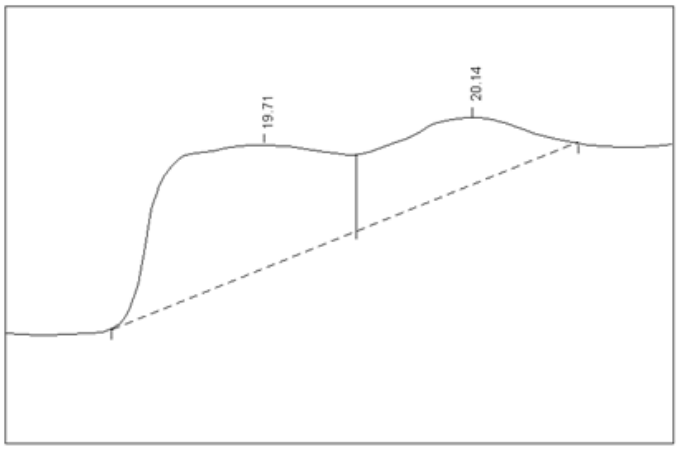
Force Baseline to Point (BL)

The BL event forces the baseline to the start of the current peak. (The current peak is the one on which the event occurs, or the one that follows the event if it occurs between peaks.)

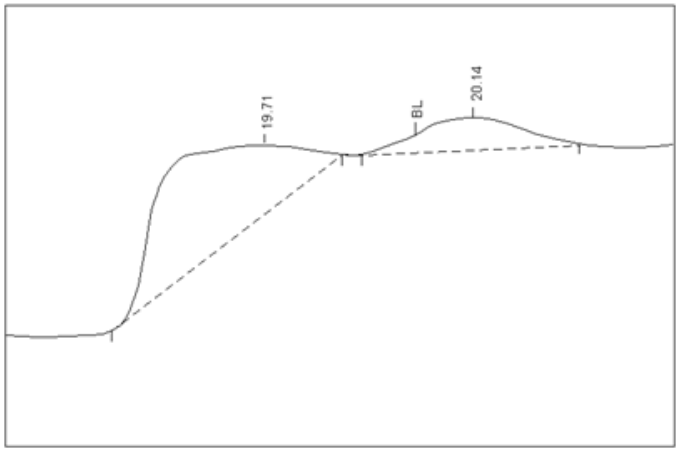
BL causes Chromera to assign an overlap flag of 0 to the peak preceding the current peak. As a result, the preceding peak is treated as though it is resolved from the current peak. The software terminates the peak cluster at the end of the preceding peak by ending the cluster baseline there. Because the baseline is forced into this position, the baseline for the current peak is also forced to start at or near the peak start.

If this event occurs when peak detection is in the Begin or Confirm stages, the baseline of the current peak is forced to start at the bunched point representing the data bunch at the event's time. If the event occurs when peak detection is in the Find Top or Find End stages, the baseline is forced to start at the starting point of the current peak.

When no BL event has been scheduled, a common baseline has been drawn for the two peaks because they meet the overlap criteria. Without the BL event, the two peaks are clustered and integrated by using drop lines.



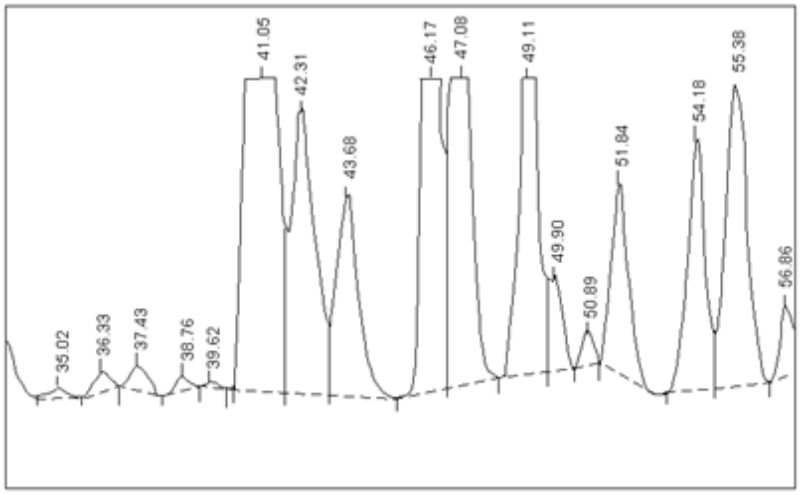
With the BL event when it occurs during the Find Top or Find End stages. The preceding peak is assigned a 0 overlap flag and thus has a valley-to-valley baseline. As a result, the baseline of the current peak is forced to begin at the peak starting point. With the BL event, a baseline is forced to begin at the peak start.



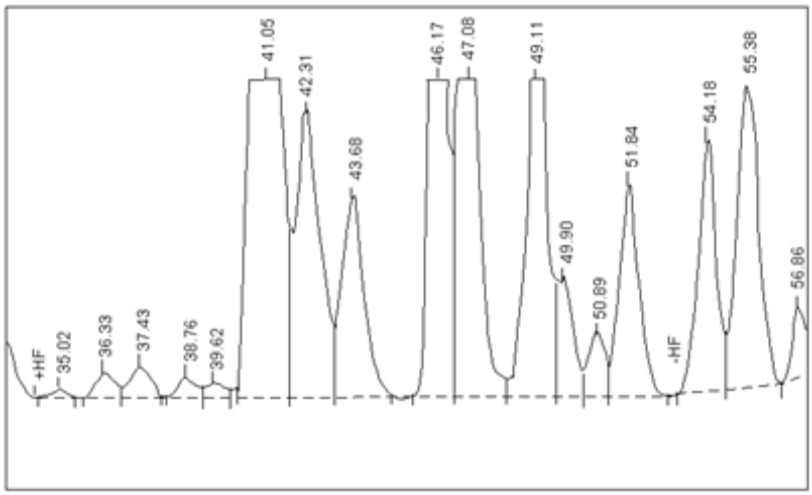
You can use the BL event to reposition the theoretical baseline used for negative peak detection when a negative peak and a positive peak are contiguous. If the BL event is placed within the negative or positive peak, the theoretical baseline is moved to the level of the peak start. If the BL event is outside either peak, the theoretical baseline is set at the level of the point within whose range the event is scheduled.

Force/Discontinue Horizontal Baseline Forward (+HF/-HF)

The +HF event projects a horizontal baseline from the time of the event to the end of the chromatogram or until the event is turned off by a *HF event. When the +HF event is not in use, Chromera adjusts the baseline to avoid penetration by valleys. Without the +HF event, baseline placement is determined by peak separation criteria.

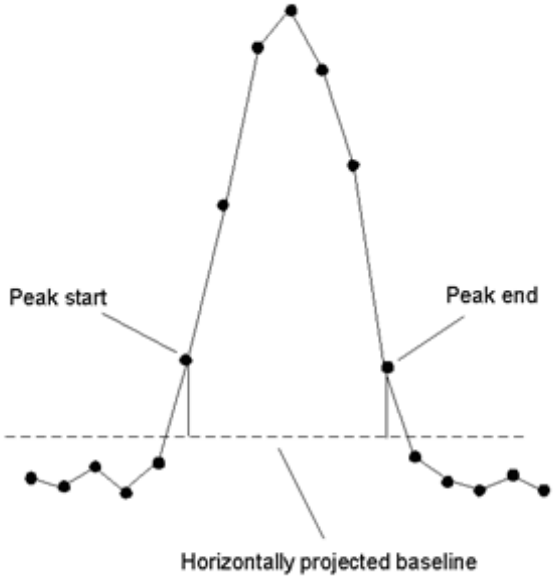


Conversely, the baseline established by the +HF event always remains horizontal. Peaks within the +HF event are treated as a single cluster and share a common, horizontal baseline. The –HF event restores normal baseline treatment.

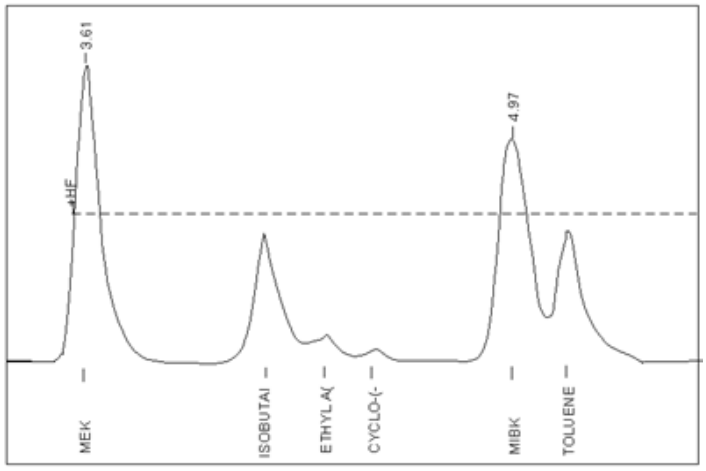


The +HF event takes effect at the time it is scheduled. The baseline is set at the voltage level and time value of the raw data point nearest the event placement.

In sections of the chromatogram where the +HF event is in effect, peaks cannot have starting or ending points below the baseline. If the baseline intersects a peak, it is not likely to intersect exactly at a raw data point, so the peak starting and ending points usually lie some distance above the projected baseline. As a result, the area between the projected baseline and peak starting and ending points are excluded from the peak area. The peak starting and ending points usually lie above the horizontally projected baseline.



You must be careful where you place the +HF event. If the projected baseline is set too high, large areas of peaks will be submerged below the baseline and, therefore, will not be integrated. Some peaks might be submerged entirely and go undetected. To avoid this problem, do not schedule a +HF event on a peak: place it on the baseline just before the start of a peak.

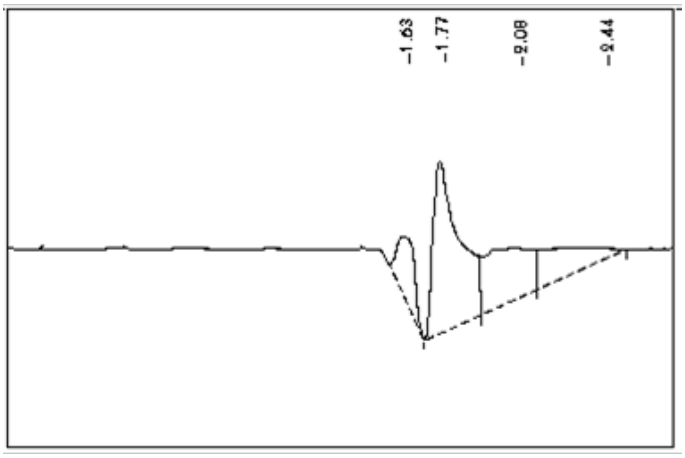


The -HF event discontinues the +HF event. Chromera then resumes evaluating each peak against the overlap criteria and assigns overlap flags of 0 or 1 as warranted. If Chromera is searching for a peak ending point when either event is encountered, the current peak will end at the event. Otherwise, the current peak (if any) will be discarded.

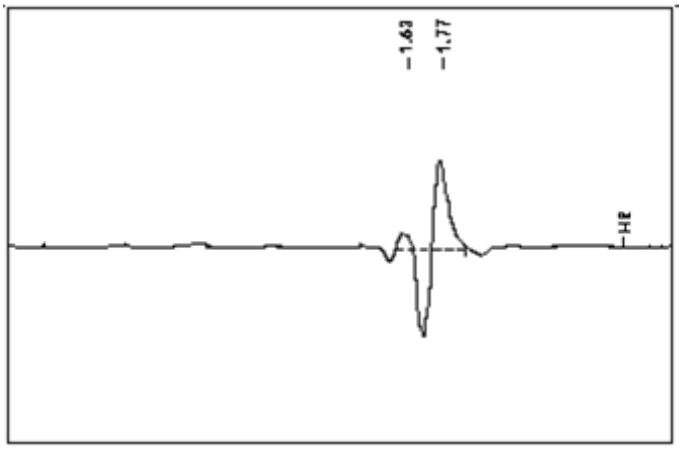
You can schedule any number of +HF/-HF event pairs in a chromatogram, but they must not be overlapped. If two +HF events are scheduled in a row, without an intervening -HF event, the second event will be ignored. The lack of an intervening -HF event might even prevent Chromera from drawing a baseline.

Force Horizontal Baseline Backward (HR)

The HR timed event projects a horizontal baseline backward from the time the event is scheduled to the start of the chromatogram. The baseline event is set at the voltage level and time value of the raw data point nearest the event placement. Chromera does not adjust the baseline to prevent penetration by valleys; thus the baseline remains horizontal. Without an HR event, peaks can have separate baselines.



Where the HR event is in effect, peaks cannot have starting or ending points below the baseline. If the baseline intersects a peak, the intersection is not likely to occur exactly at a raw data point. Therefore, the peak starting and ending points will usually lie some distance above the projected baseline. As a result, the area between the projected baseline and the peak starting and ending points will be excluded from the peak area. This result is because of the algorithm that requires peak boundaries to occur at raw data points, not between them. With the HR event, a common horizontal baseline projects backward to the start of the chromatogram.

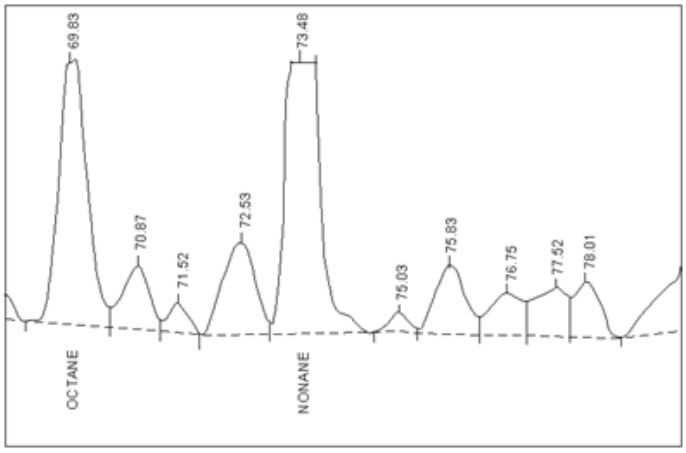


To achieve the best result, do not schedule the HR event on a peak, but place it on the baseline between peaks or after any peak. This helps avoid baselines that are too high or too low.

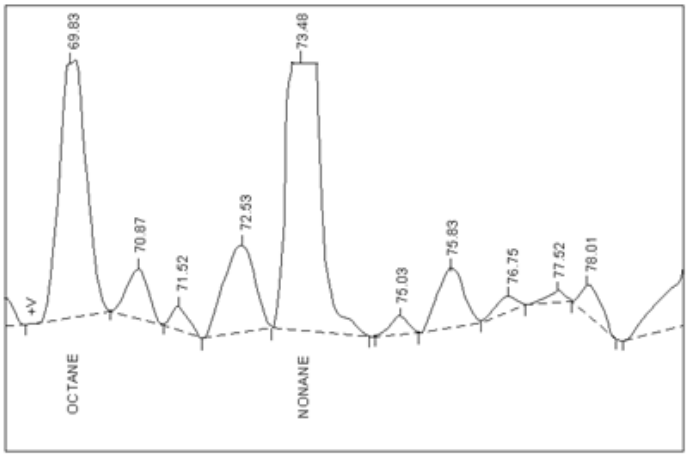
Force/Discontinue Valley-to-Valley Baselines (+V/−V)

The +V event assigns an overlap flag of 0 to all peaks that occur while the event is in effect. As a result, during integration, all peaks are treated as though they are resolved, even if they

meet the criteria for being overlapped. Chromera draws an individual baseline for each peak extending from valley to valley. No peaks are clustered. Without the +V event, some peaks are clustered, having a common baseline.



With the +V event, each peak has an individual baseline regardless of possible overlapping.

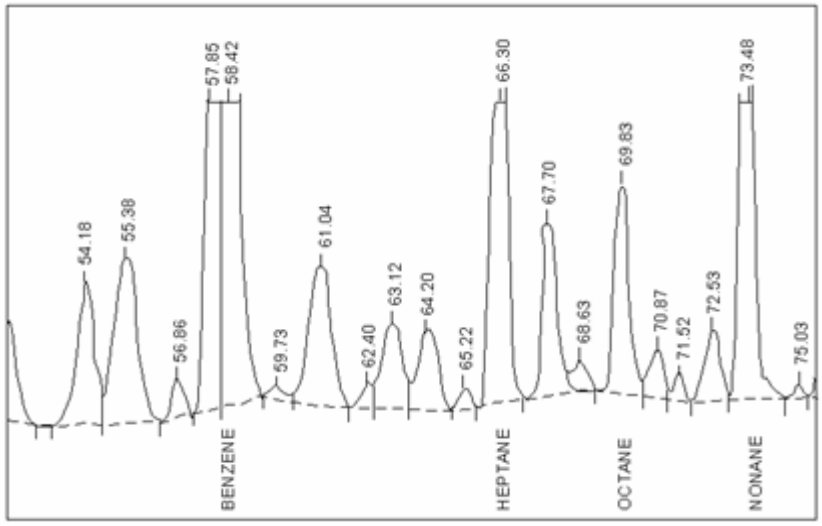


The +V event takes effect at the time it is scheduled and applies to the current peak. It also applies to subsequent peaks.

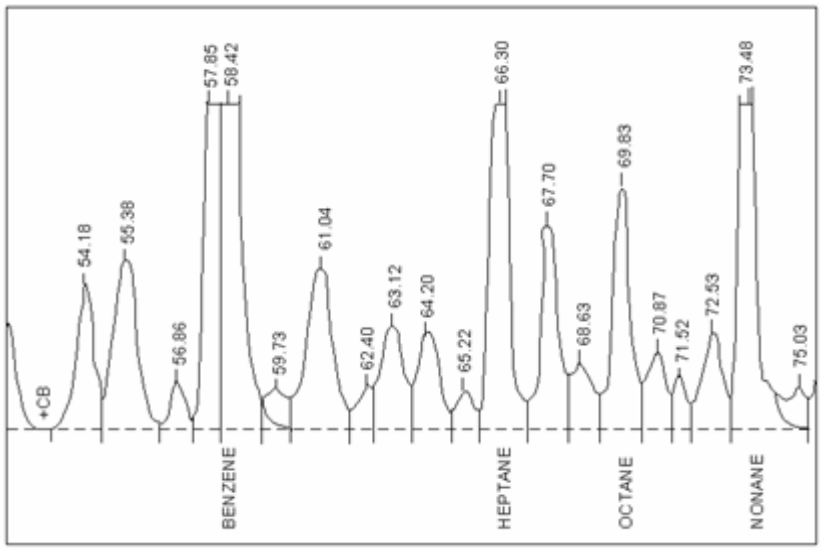
The -V event discontinues the +V event. Chromera resumes evaluating each peak against the overlap criteria and assigns overlap flags of 0 or 1 as warranted. This event takes effect immediately and applies to the current peak and subsequent peaks.

Force Common Baseline (+CB/-CB)

The +CB event causes the software to treat all peaks as though they were clustered, even if they do not meet the criteria for being overlapped. A common baseline is drawn for these peaks. Without the +CB event, baseline placement is determined by the peak separation criteria.



With the +CB event, all peaks share a common baseline.



NOTE: *Even when the +CB event is in effect, the software checks the artificial cluster for valleys that penetrate the forced baseline. If such valleys are present, it redefines the baseline, where necessary, to eliminate penetration.*

The +CB event takes effect at the time it is scheduled and applies to the current peak — the peak on which the event is located, if any. It also applies to subsequent peaks. The -CB event takes effect immediately and applies to the current peak and subsequent peaks.

Force Exponential Skim (+X)

To ensure that Chromera creates an exponential skim, schedule the +X event anywhere within the parent peak on which you want the skim to start. The +X will have no effect unless you place it between the starting and ending points of a peak: it will have no effect if you place it on the baseline outside a peak.

If you schedule more than one +X event on the same peak, only one event will be recognized. If you place +X events on two adjacent peaks in the same cluster, the software will ignore the second one because it cannot decipher multiple skims when they converge.

If you place +X events on non-adjacent peaks in the same cluster, the software draws exponential skims. However, what is drawn from the first peak will not extend past the start of the next peak with a +X event.

See Area Adjustment for more information.

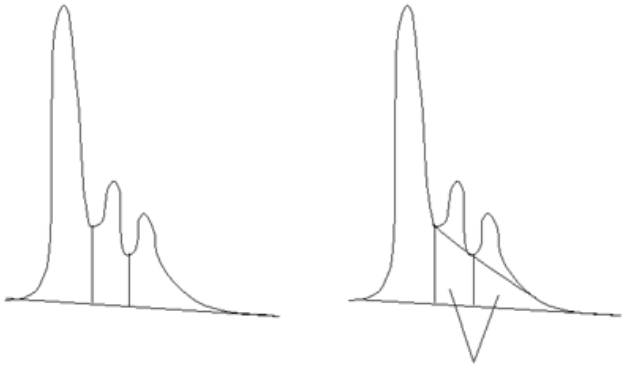
Prevent Exponential Skim (–X)

Scheduling a -X event anywhere on a peak ensures that Chromera will not create an exponential skim that originates on that peak. This event does not prevent the software from drawing a skim beneath the peak if the skim originated on a preceding peak.

The -X event will have no effect unless placed between a peak's starting and ending points: it will have no effect if you place it on the baseline outside a peak.

Tangential Skim (T)

To create a tangential skim, Chromera draws a straight baseline from the starting point to the ending point of each child peak of a designated parent peak, as shown in the following figure. Such baselines are drawn for each child peak that occurs before the end of the cluster. The software then checks to see that the new baselines are not penetrated by the child peaks. Finally, new areas for the child peaks are calculated, and the difference between the old and new areas (the area between the tangent baselines and the cluster baseline) is added to the parent peak.



Unlike an exponential skim, Chromera never creates a tangential skim automatically. If you want to adjust peak areas by using a tangential skim, you must use a T-timed event. The T event must be placed between a peak's starting and ending points; it will have no effect if you place it on the baseline outside a peak.

If you schedule more than one T event on the same peak, the effect will be the same as having one T event. Avoid scheduling T events on more than one peak in a cluster. If you place T events on two adjacent peaks in the same cluster, the second one will be ignored.

Start/End Manual Integration (+M/–M)

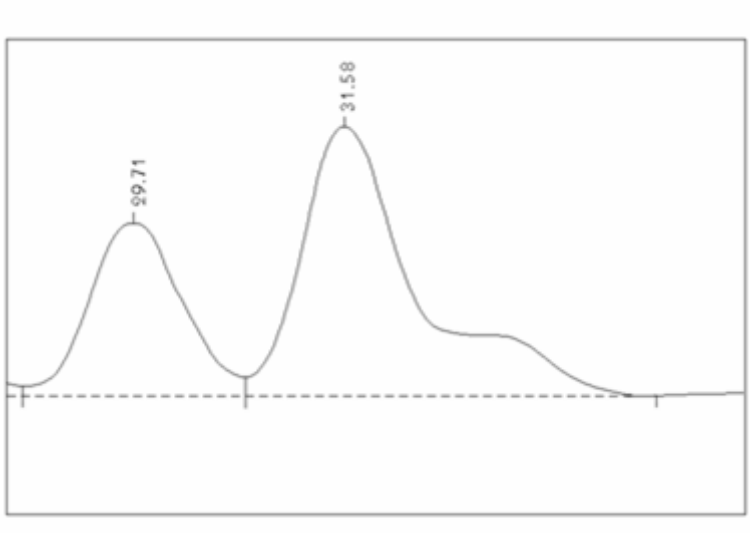
The timed events +M and -M allow you to manually integrate peaks. You place the +M event where you want a peak's baseline to start and the -M event where you want it to end. If necessary Chromera will interpolate within a data point to start and/or end manual integration at exactly the time you specify.

Locate Retention Time at Maximum (LM)

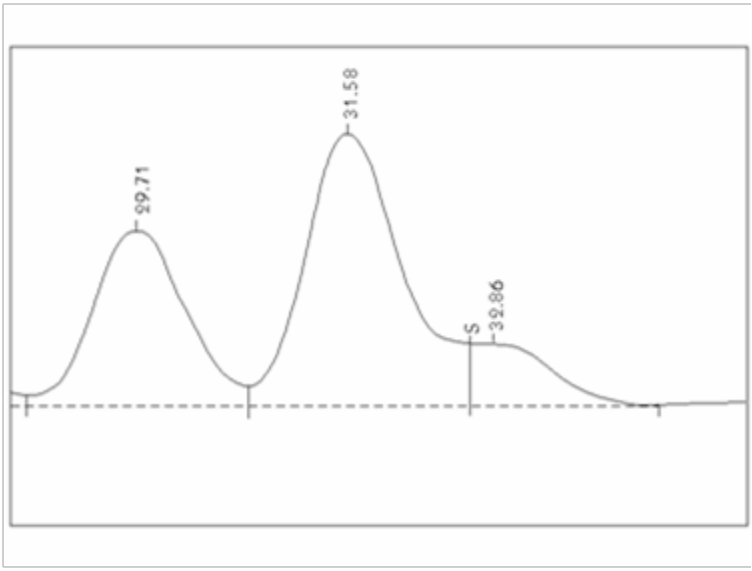
The LM event locates the peak retention time at the maximum data point that falls within the peak rather than attempting to fit a quadratic to the peak crest.

Force Start of New Peak (S)

The S event starts a new peak when the event occurs, regardless of whether a peak would normally be detected and confirmed at this position. Typically, you use this event to force a split between a shoulder of a parent peak. Without the S event, the shoulder on the peak at 31.58 minutes is not detected as a separate peak.



With the S event, the shoulder is integrated as a separate peak.

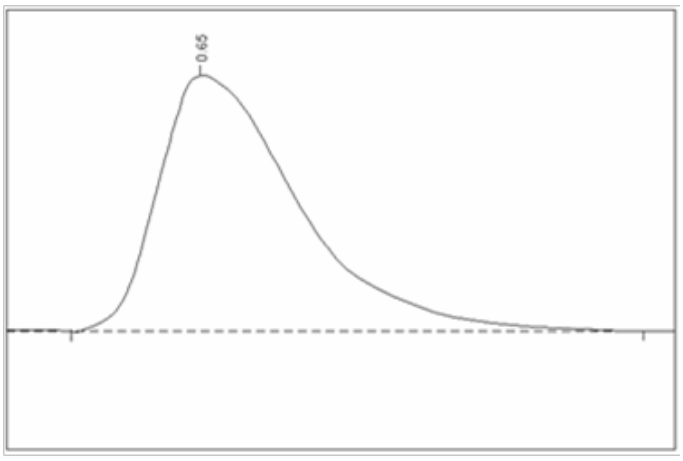


If there is no confirmed peak eluting at the time the S event occurs, the last raw data point of the current bunch (located at the time the event is scheduled) becomes the peak starting point. Also, Chromera enters into the Find Top stage.

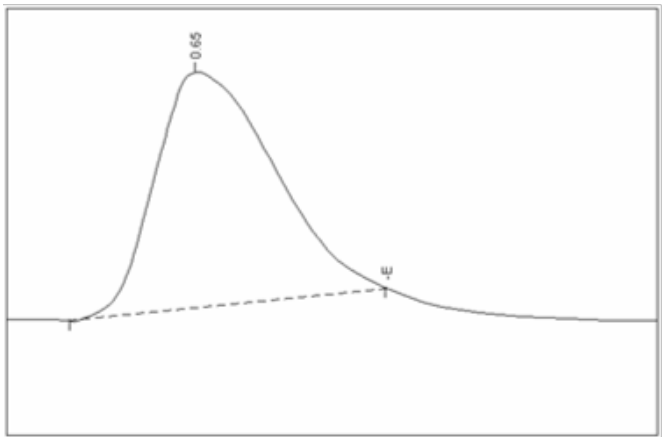
If there is a confirmed peak eluting at the time the S event is scheduled, the software forces the present peak to end on the last point of the current bunch, and a new peak is forced to begin.

Force End of Peak (E)

The E event establishes a peak end at a specific point in time. If there is no confirmed peak eluting at the time the E event occurs, the event will have no effect. Otherwise, the last point of the current bunch becomes the ending point. After this event, the software re-starts peak detection. Without the E event, the peak end is found automatically.



With the E event, the peak end is set at the specified time.



About Calibration Processing

This is an overview of the way that calibration processing is handled in the Chromera software. Specifics of the calibration curve types and the calculations associated with each are described in **Calibration**.

Calibration processing uses the **Replace/Average** mechanism where calibration standards are processed in the order in which they appear in the sequence or batch, with new standards either replacing the existing calibration information for the identified calibration level in the method with values from the current calibration row, or averaging the new response values with those already existing in the method. The quantitation of unknown samples is then performed using this state of calibration for all subsequent sample injections in the sequence or batch, until new calibration injections are made and the calibration state changes again. At which point, any subsequent samples are processed using the new state of calibration (i.e. the current calibration factor or curve).

As each calibration standard is processed, the peak response information from that standard is appropriately updated in the calibration section of the original or source method, as well as being stored with the batch records of the samples for which that calibration applies. In this way, the current version of the method file is always complete. That is, it always contains all of the constituent data from all the injections and replicates of its calibration, for each component and calibration level, since each level was last replaced. What this mechanism enables is the ability to acquire and process new data from unknown samples using the fully calibrated state of the current method, without making any new calibration injections, or without specifying new calibration standards in a sequence or batch.

This mechanism requires two new sample types to be available in the sequence:

Calib: Replace	A calibration standard for which the first injection replaces any existing calibration data for that standard (level) and any replicate injections are averaged with the first injection results.
Calib: Average	A calibration standard for which results from all injections are averaged with the existing calibration for that standard (level).

How Calibration Works

The following steps outline how calibration works in the saving of the calibration information generated during data processing.

1. The method always contains the entire table of calibration data history relevant to that method since each calibration level in the method was last replaced.

2. It is possible to reprocess and re-quantitate a set of samples from a sequence or batch without the need to have calibration standards in the sequence. The calibration information to be used for quantitating those samples comes from the current state of calibration in the method specified for each of the rows in the sequence or batch.

3. When a new calibration standard is analyzed using the *Calib: Replace/Avg* option for a calibration level of a method specified in the sequence, the peak responses (i.e., peak areas and heights) will replace those values currently existing in that method, for each of the identified compounds detected for that level in that calibration standard. The calibration factor or calibration curve is updated accordingly.

4. When a new calibration standard is analyzed using the *Calib: Average* option for a calibration level of a method specified in the sequence, the peak responses (i.e. peak areas and heights) will replace those values currently existing in that method, for each of the identified compounds detected for that level in that calibration standard. The calibration factor or calibration curve is updated accordingly.

5. The complete record of each calibration replicate, not just the cumulative averages, is saved in the method along with a reference to the associated raw data, so that the method contains all of the discrete processed calibration values, as well as the means to identify the original raw calibration data. The purpose for maintaining the discrete values within the method is to provide the ability to exclude individual calibration points and/or replicates, if desired, from quantitation of samples using that method.

6. The complete set of calibration information used to quantitate a sample, including the replicate values, is saved with that batch record for that sample.

7. Calibration standards will also be processed as any other sample. That is, quantitated amounts are calculated for them so sample reports can be printed for them, if so desired.

Calculations

Calibration

Average Calibration Factor

The calibration factor for a component is the ratio of response (area or height) to (known) amount in a standard for external standard calibration, or the ratio of response-ratio to amount-ratio for internal standard calibration. This is effectively a single point linear fit forced through the origin.

To obtain the average calibration factor (ACF), the calibration factors for all replicates are averaged using the equation:

$$ACF = \frac{\sum_{i=1}^{n} \frac{Rsp_i}{Amt_i}}{n}$$

where:

ACF	is the average calibration factor
Rsp_i	is the response (response ratio in the case of internal standard calibration) for the i th replicate of the standard
Amt_i	is the amount (amount ratio in the case of internal standard calibration) for the i th replicate of the standard
n	is the number of replicates for the standard

For components using average calibration factor, the ACF value replaces the Slope value currently displayed for components using linear fit. This should be clearly indicated as Average Calibration Factor. No r^2 value is calculated for ACF components.

Scaling and Weighting

Amount Scaling

The software will offer alternatives to simply plotting response as a function of amount or response ratio as a function of amount ratio. For example, you can choose to plot response as a function of the log of the component amount. Or, in the case of internal standard calibration, you could plot response ratio as a function of the ratio:

$$\log \frac{x_{comp}}{x_{istd}}$$

where:

x_{comp}	is the component amount
x_{istd}	is the internal standard amount

.

The following is a list of the available functions for amount scaling:

1/x	Response is a function of the reciprocal of the amount.
1/x2	Response is a function of the reciprocal of the square of the amount.
log(x)	Response is a function of the base-10 log of the amount.
1/log(x)	Response is a function of the reciprocal of the log of the amount.

Use of these scaling alternatives rules out some values for amount that otherwise would be allowed. Specifically, the amount 0.0 cannot be used with 1/x or 1/x2 scaling. Also, amounts less than 1.0 cannot be used with a logarithmic option; otherwise, an invalid operation will result.

Weighting

As shown in determination of least squares fit in Quadratic and cubic curve fits, different weighting can be applied to the various data points (levels) in the calibration curve.

The following is a list of the available functions for weighting:

1/X	The reciprocal of the point's amount value
1/Y	The reciprocal of the point's response value
$1/X^2$	The reciprocal of the square of the point's amount value
$1/Y^2$	The reciprocal of the square of the point's response value

Exclusion of Replicates

It is possible to eliminate any replicate data point from the calibration, on a component-by-component basis. When a point is excluded the calibration results (curve equation or average calibration factor) is automatically updated to reflect the change. The excluded point is retained in the method so that it can be restored for use in the calibration if necessary.

Excluded points should be shown on the calibration curve in the calibration review window but using a distinct color and/or symbol to indicate it is an excluded point.

Internal Standard Calibration

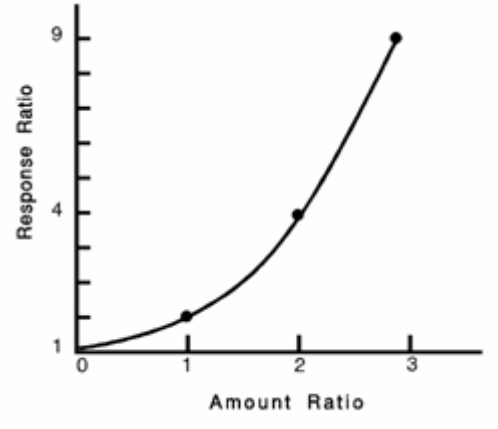
Internal standards are compounds introduced in known amounts into chromatography samples * both samples to be analyzed and standard samples. One or more internal standards can be added to a sample.

When the internal standard method is selected, the system relates amount ratios with response ratios to compute the calibration curve. The amount ratio is the amount of component in a standard sample divided by the amount of an internal standard component in the same sample. The response ratio is the area or height of the component divided by the response of an internal standard in the same sample.

The amount and response ratios at each calibration level contribute a data point to a component's calibration curve. The amount ratio is the x coordinate (the independent variable), and the response ratio is the y coordinate (the dependent variable). For example, suppose a component has the following calibration data:

Component		**Internal Standard**	
Amount	**Response**	**Amount**	**Response**
10	10,000	10	10,000
20	40,000	10	10,000
30	90,000	10	10,000

Using a quadratic curve fit, the system would generate the calibration curve.



Method

Internal standards will be treated in the method much like other sample components. That is, when a method for samples that contain internal standards is created, component information will be entered for the internal standards, as well as for the components to be quantified. This information includes calibration level data. Like other components, internal standards can have multiple calibration levels.

Frequently, the amount of internal standard added will be the same for all calibration levels (and often in unknown samples as well), but, as long as the response for both components is linear, this will not be required to be the case. In some instances it is not practical to maintain exactly the same amount of internal standard in every sample, which is why the calibration curve plots response ratio against amount ratio, to eliminate any such variation. This is also why Chromera will support entry of individual amounts for multiple internal standards in the sample row of the sequence.

Computation of the Calibration Curve

After a calibration replicate for a component is added to the method, the software will compute or recompute the component's calibration curve (except in the case of Average Calibration Factor calibration, when the ratio of response to amount is calculated for each replicate in all of the levels and averaged together to give the average calibration factor (ACF), as described in detail elsewhere).

The data used to create a calibration curve depend upon the calibration method specified: internal standard or external standard.

If the external standard method has been specified, the curve will show the relation between amount and response values. Response can be either the peak area or the peak height, whichever is specified for a given component in the method.

If the internal standard method has been specified, the curve will show the relation between amount ratios and response ratios.

Calibration Quality Metrics

Outlier Test

This test checks the response for each component from a calibration run against the current expected response. That is, the current average calibration factor or the response given by the current calibration curve, depending on the calibration type specified. If the response falls outside the percentage deviation specified by the user the result is excluded from the calibration calculations but the result is retained within the calibration information for the method. In other words this test acts in the manner of a user reviewing calibration results and marking the replicate to be excluded from the calculation of calibration curve or ACF. When the user does review the calibration results the replicate is shown as excluded, so that he/she can include again if required.

NOTE: *The outlier test is not performed if there is no existing calibration to compare the current result against (e.g. if the current standard is the first data at that level). For internal standard analyses the outlier test is performed using the response ratio and not the raw response. One implication of this is that the outlier test is not performed on internal standard peaks themselves, only on the components associated with internal standards. Thus all internal standard replicate data are always reported and never excluded as will occur when a target component replicate fails the outlier test.*

Confidence Limits Test

The following is an outline of the test.

For any normal distribution, the confidence limits of the distribution can be expressed as

$$\mu = \bar{x} \pm t \times \left(\frac{s}{\sqrt{n}} \right)$$

where:

μ	is the true mean
x-bar	is the average of the replicates
t	is the coefficient for the confidence interval at (n-1) degrees of freedom
s	is the standard deviation of the replicates
n	is the number of replicates

To find if a given value is within the confidence interval, the right side of the equation is calculated compared to the value of interest. Chromera tests the hypothesis that..

$$\left| x_i - \bar{x} \right| \leq t \times \left(\frac{s}{\sqrt{n}} \right)$$

or rearranged

$$\left| x_i - \bar{x} \right| \times \left(\frac{\sqrt{n}}{s} \right) \leq t$$

The first pass calculates the limits for the distribution without the current replicate and compares them for the 95% and the 99.9%. The second pass calculates the limits for the distribution including the current replicate at the same points.

The above equations were derived from the discussion of confidence limits in J.C. Miller and J.N. Miller, "Statistics For Analytical Chemistry," 2nd Ed., John Wiley and Sons, 1988, pp. 41ff.

The values for t were taken from a table in G.E.P. Box, W.G. Hunter, and J.S. Hunter, "Statistics for Experimenters," John Wiley and Sons, 1978, p. 631.

TotalChrom reports confidence limits ranges and such an approach is probably adequate for Chromera also. The ranges reported by Chromera are:

1. Insufficient data for outlier test (<3 replicates)
2. Significant outlier (failed at 99.9% 2nd pass)
3. Probable outlier (failed at 95% 1st pass, failed at 95% 2nd pass)
4. Probable outlier (failed at 95% 1st pass, failed at 99.9% 1st pass)
5. Probably not outlier (failed at 95% 1st pass, passed at 99.9% 1st pass)
6. Not outlier (passed at 95% 1st pass)

NOTE: *The Confidence Limits test does not exclude any values from the calibration; it simply reports the confidence level for each replicate within the calibration (for each component).*

Component Amount

Using Average Calibration Factor

For external standard calibrations, the preliminary unknown amount is calculated as:

$$Amt_c = \frac{Rsp_c}{ACF_c}$$

where:

Amt_c	is the amount for the component
Rsp_c	is the response for the component
ACF_c	is the average calibration factor for the component

For internal standard calibrations, the preliminary unknown amount is calculated as:

$$Amt_c = \frac{Rsp_c \times Amt_{is}}{Rsp_{is} \times ACF_c}$$

where:

Amt_c	is the amount for the component
Rsp_c	is the response for the component
Rsp_{is}	is the response for the internal standard
Amt_{is}	is the amount of the internal standard
ACF_c	is the average calibration factor for the component

Final Result

Calculation of the reported amount (concentration) of a component in an analyzed sample in Chromera is basically a three stage process:

1. Determination of the preliminary amount (or amount ratio) by solving the component's calibration curve equation or by applying a calibration factor.

2. Conversion of the amount ratio to amount by multiplying by the associated internal standard amount in the sample, where applicable (i.e. components using curve fit and internal standard calibration).

$$A = A_{ratio} \times IS$$

where:

A	is the corrected component amount
A_{ratio}	is the initial component amount ratio
IS	is the internal standard amount (taken from the sequence)

3. Calculation of the final adjusted component amount using the following equation:

$$A_{adj} = \frac{A_{init} \times DF \times m}{S \times d} + a$$

where:

A_{init}	is the initial component amount (after ISTD correction where appropriate)
DF	is the Dilution Factor value from the sequence
m	is the Multiplier from the sequence
d	is the Divisor from the sequence
S	is the Sample Amount value from the sequence
a	is the Addend from the sequence

Relative Retention Time (RRT)

If a RRT reference peak has been defined in the method, this value is calculated for each peak in the run, using the equation:

$$RRT = \frac{(RT_{obs} - \text{void time})}{(RT_{ref} - \text{void time})}$$

where:

RT_{obs}	is the observed retention time of the peak
RT_{ref}	is the observed retention time of the RRT reference peak
void time	is the observed retention time of an unretained peak

This value is calculated for reporting purpose only and is not utilized in the peak identification process.

System Suitability

System Suitability

System Suitability values are calculated for all identified and unidentified peaks (where sufficient information exits) for all sample types. The values calculated are defined below. All calculated values are stored in the results data and will therefore be available for reporting.

Peak Width

Peak width at base is determined by performing a linear fit on the points between 60 - 80% of the baseline-corrected peak height (for both leading and trailing edges of the peak) and then finding the intersection of these lines with the baseline drawn under the peak. The base width is the time between the two intersections. If the peak is highly unresolved then this process may not be possible (e.g. the signal changes from +ve slope to –ve slope, or vice versa within the 60-80% region) and hence no value is available for peak width at base.

Peak width at a specified percentage (e.g. 5%, 10%, 50%) of peak height is calculated in the following way. Starting at the peak crest (which determines the corrected peak height) the software scans the preceding data (and then the data following), recalculating the corrected peak height for each data point, until the data points encompassing the desired percentage of peak height are located. An interpolation is then performed to determine the exact times at which the desired fractional peak height occurs. The peak width is the difference between these two times.

Theoretical plates by the tangential method

For ASTM and USP compliance, system efficiency N_{sys} is calculated using this equation:

$$N_{sys} = 16\left(\frac{T_r}{W}\right)^2$$

where:

T_r	is the peak retention time
W	is the calculated peak width at base (see Peak Width for the calculation)

For BP compliance, the width of the peak is measured at half its height, and system efficiency N_{sys} is calculated using the equation:

$$N_{sys} = 5.54\left(\frac{T_r}{W_{h/2}}\right)^2$$

where:

T_r	is the peak retention time
$W_{h/2}$	is the peak width at half height (in the same time units as T_r) (see Peak Width for the calculation)

NOTE: *BP 2005 shows the multiplier to two decimal places only (5.54), although the traditional version of this equation (e.g. "Basic Relationships of Gas Chromatography" L.S. Ettre 1977) uses the factor 5.545.*

NOTE: *USP 29 states that the above equation may be convenient when an electronic integrator is used, but that "in the event of a dispute, only equations based on peak width at baseline are to be used."*

Theoretical plates by the Foley-Dorsey approximation

The Foley-Dorsey method provides a significantly more accurate calculation of theoretical plates than the tangential method. Foley-Dorsey assumes an exponentially modified Gaussian distribution as the skewed peak model. The equation for system efficiency N_{sys} is:

$$N_{sys} = \frac{41.7\left(\frac{T_r}{W_{0.1}}\right)^2}{B/A + 1.25}$$

where:

T_r	is the peak retention time
$W_{0.1}$	is the peak width at 10% of peak height. Again the value is in the same time units as T_r. (see Peak Width for calculation)
B/A	is an empirical asymmetry ratio (A is the width of the front half of the peak at 10% height and B is the width of the back half - so that A + B = $W_{0.1}$

Tailing factor (Symmetry Factor - BP)

The tailing factor T is calculated by the equation:

$$T = \frac{W_{\%}}{2f}$$

where:

$W_{\%}$	is the peak width at the specified % peak height (5 or 10 - see Peak Width for the calculation)
f	is the width (time) between the peak maximum and the front edge of the peak at the specified % peak height (5 or 10)

NOTE: *USP 29 and BP 2005 only reference 5% of peak height for W and f measurement.*

Alpha

The alpha value reported for a given peak is the relative retention of that peak to the previous named peak.
Alpha (relative retention) is calculated by the following equation:

$$\alpha_{p2p1} = \frac{T_{p2} - T_v}{T_{p1} - T_v}$$

where:

α_{p2p1}	is the relative retention of peak 2
T_{p1}	is the retention time of peak 1
T_{p2}	is the retention time of peak 2
T_v	is the void time (retention time of an unretained peak)

Capacity factor (k')

The capacity factor is calculated using the following equation:

$$k' = (T_r / T_v) - 1$$

where:

T_r	is the peak retention time
T_v	is the void time (retention time of an unretained peak)

Resolution

The resolution reported for a given peak is the value calculated between itself and the previous named peak. For USP compliance, resolution is calculated by the following equation:

$$R_{p2} = \frac{2(t_{p2} - t_{p1})}{(W_{p1} + W_{p2})}$$

where:

R_{p2}	is the resolution of peak 2
T_p1	is the retention time of peak 1
T_{p2}	is the retention time of peak 2
W_{p1}	is the width at the base of peak 1 (in the same time units as the RT measurements)
W_{p2}	is the width at the base of peak 2

$$R_{p2} = \frac{2(t_{p2} - t_{p1})}{1.70(W_{p1,h/2} + W_{p2,h/2})}$$

where:

R_{p2}	is the resolution of peak 2
T_{p1}	is the retention time of peak 1
T_{p2}	is the retention time of peak 2
$W_{p1, h/2}$	is the width at half-height of peak 1
$W_{p2, h/2}$	is the width at half-height of peak 2

For BP compliance, resolution is calculated by the following equation:

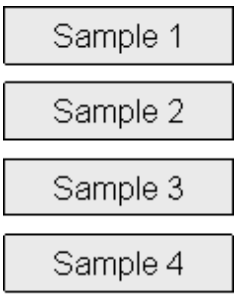
$$R_{p2} = \frac{1.18(T_{p2} - T_{p1})}{(W_{h1/2} + W_{h2/2})}$$

where:

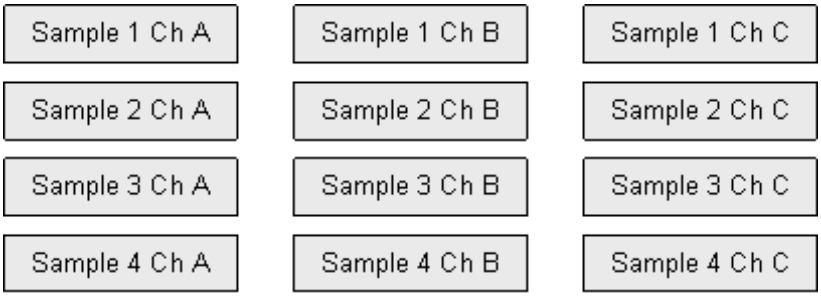
R_{p2}	is the resolution of peak 2
T_{p1}	is the retention time of peak 1
T_{p2}	is the retention time of peak 2
$W_{h1/2}$	is the width of peak 1 at half height (in the same time units as the RT measurements)
$W_{h2/2}$	is the width of peak 2 at half height

Display Data Sets

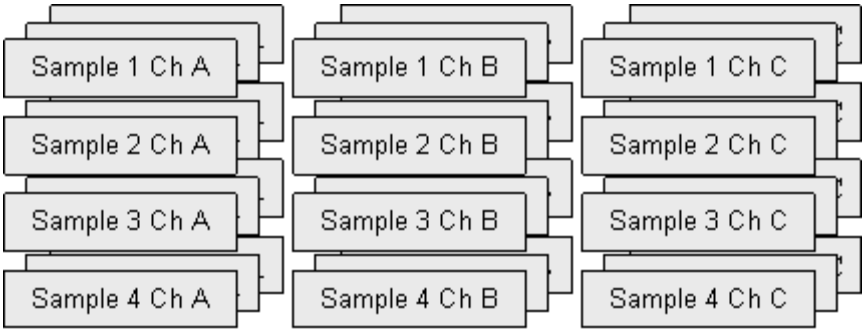
A Batch can contain data from many Samples. For example:



Each Sample run can include chromatograms from several channels. For example:



Each Sample can be injected multiple times (replicates). For example:



The default view of the Post Run graphics display reflects the above structure: samples displayed in rows, channels in columns and replicates in layers, in a 2-D format so that any single layer can be displayed or all layers can be displayed at once (overlaid). You can change these default settings; for example the channels could be displayed in rows, the replicates in columns and the samples in layers.

Since not all data sets to be displayed will contain multiples of each element (sample, channel, replicate), Chromera also provides other predefined views that are suitable for displaying simpler data sets.

Reports (Report Format Wizard)

About Reports

Chromera provides you with complete control over the data content of reports within a set of basic report formats.

Most of the printed output is handled through the reporting system, with the exception of some direct printing from other controls (such as the Plot Control). Reports include: the output of sample results (both single injection and replicate injections), summaries of multiple samples, calibration results, method parameters and sequence information. The options of format and content available differ considerably for each of these categories of reports; from none for method printouts (other than section selection) to extensive, for sample and summary reports.

A key benefit is that graphics are generated at the time the report is rendered and each is optimized for the space allocated for it in the report template; thereby providing a consistently high quality of plot regardless of the size.

About Report Templates

You can create report templates through the Report Format Wizard for your needs. In essence these templates provide a set of structured containers into which you can insert the specific data items to report.

Once a template has been generated using the Wizard it can be modified using essentially the same user interface. See Report Format Wizard for complete details. These report templates are stored in the database and may be referenced in Report Decision Points or as sequence-wide reports (Per Sample or Per Injection).

There are five types of reports:

Sample	Single or multiple injections of one sample
Summary	Results from multiple samples
Calibration	Calibration curves, statistics and standard results
Sequence	Information from each defined row
Method	Instrument, processing and component parameters

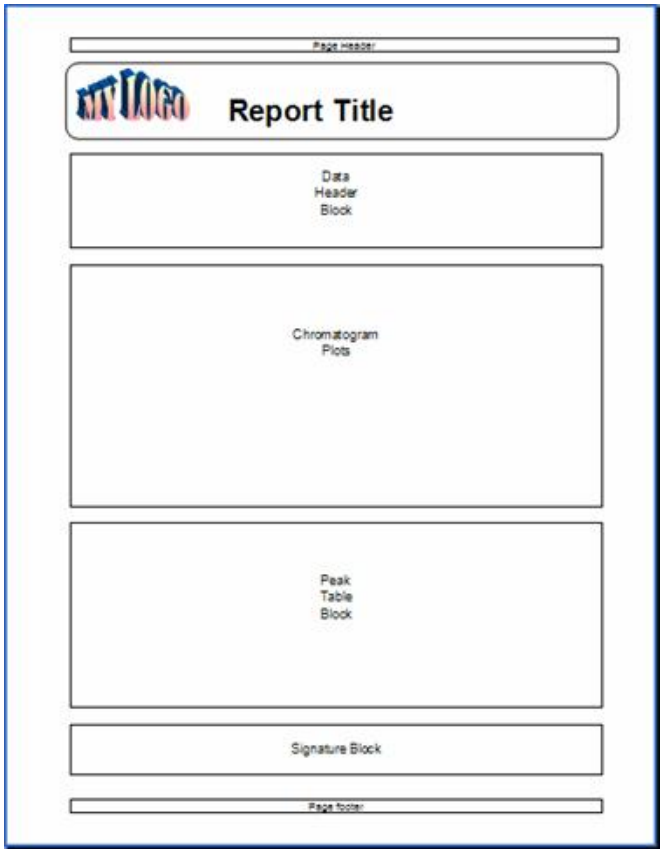
Some of the report types are available in more than one basic format.

Page Setup

The template definition includes the orientation of the page (portrait/landscape) and the size of the page margins (top/bottom/left/right) but not the paper size, as this is determined when the report is generated. If the report is printed, it is adapted to fit (as closely as possible) the default paper size contained in the printer. If the report is to be saved to a file, then paper size (where applicable) is the default paper size in the default printer.

Sample Reports

Sample Reports show results from a single sample injection and those showing average results from multiple injections (replicate reports). There are several variations of the Sample Report but all are derived from a single basic structure.

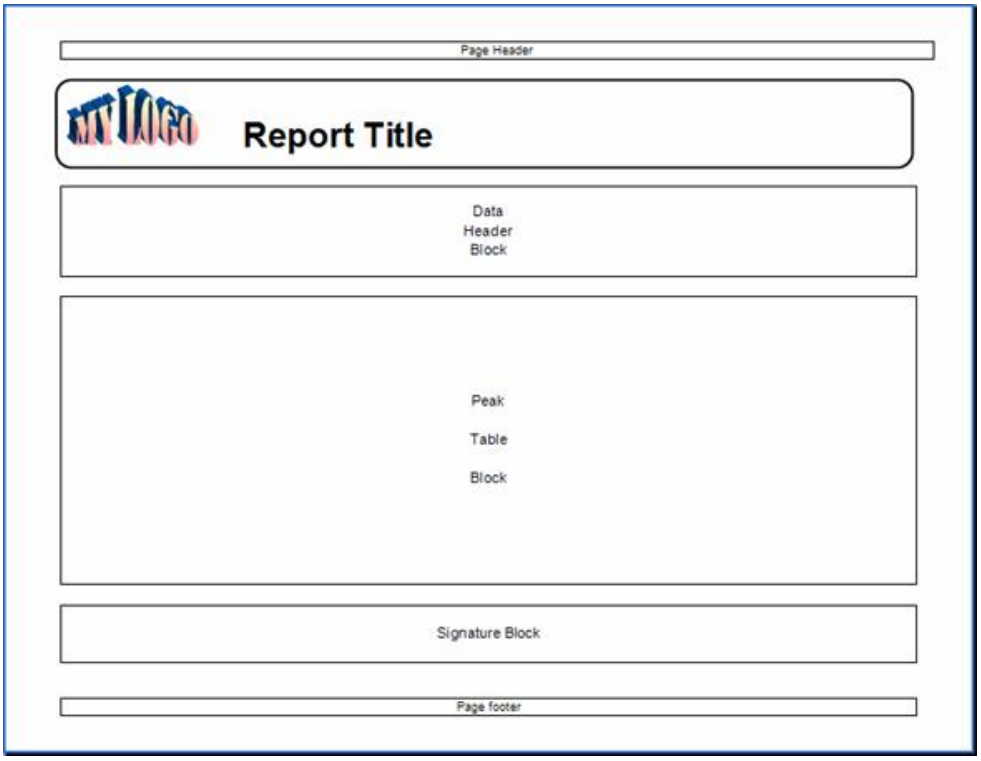


A Sample Report consists of the following elements:

Page Header	A single line of information identifying the report that appears on every page (except, optionally, the first page).
Title	A text title (up to 50 characters) and optional logo graphic that appears at the top (but below the page header) of the first page (and optionally on every page).
Data Header	Information identifying the sample and/or other identifying information. Multiple lines of data can be defined.
Chromatogram Plot(s)	The chromatogram from the sample injection (or chromatograms in the case of a multi-channel or replicate report). The chromatogram plots block may contain one chromatogram (single injection sample report with one data channel) or several (multi-channel sample or replicate report). Multiple chromatograms may be overlaid on the same plot or displayed in separate plots.
Peak Table	The results for components/peaks found in the run.
Signature Block	Up to two lines providing space for signatures indicating report review and/or approval.
Page Footer	A single line of information identifying the report that appears on every page (except, optionally, the first page).

Summary Reports

Summary Reports shows data for identified peaks (only) from multiple sample injections. This can be multiple injections from one sample or single injections from multiple samples, or any combination. The components results are presented in a tabular form where there is one row per injection and a set of repeating columns for each component. There are three variants of the Summary Report but all of them are derived from a single basic structure:



A Summary Report is generally shown in landscape orientation, but this is a user defined option.

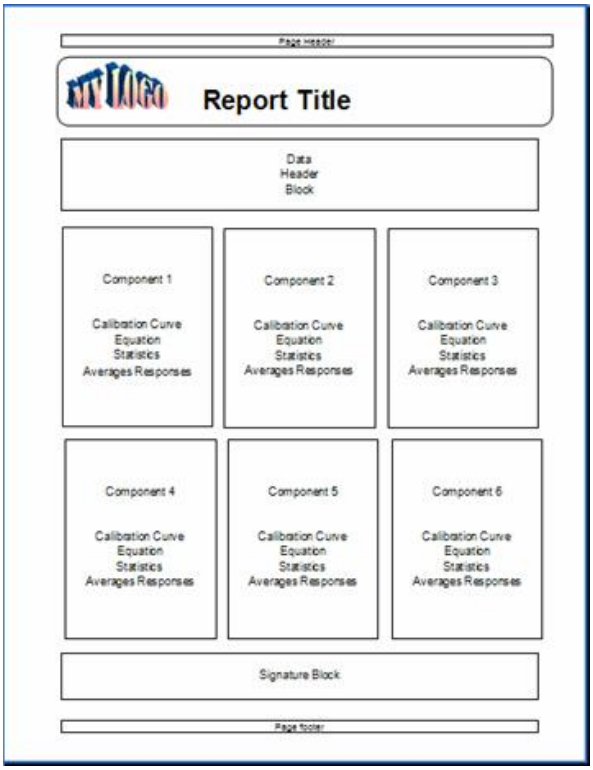
A Summary Report consists of six elements:

Page Header	A single line of information identifying the report that appears on every page (except, optionally, the first page).
Title	A text title (up to 50 characters) and optional logo graphic that appears at the top (but below the page header) of the first page (and optionally on every page).
Data Header	Information identifying the samples and/or other identifying information. Multiple lines of data can be defined
Peak Table	The results for components found in the runs
Signature Lines	Up to two lines providing space for signatures indicating report review and/or approval.
Page Footer	A single line of information identifying the report that appears on every page (except, optionally, the first page)

Calibration Reports

Calibration Reports show the results of calibrating a method containing a list of components with a set of standards. There are two basic forms of a Calibration Report:

Detailed Calibration Report Structure



Condensed Summary Calibration Report Structure

1. A detailed report, which includes not only the calibration curve, equation and statistics for each component but also the full results from each standard run.

2. A summary (or condensed) report that includes the calibration curve, equation, statistics and average results for each standard level in a much more compressed format.

The two formats share the five common report elements. Any element can be omitted from the basic structure. The space on the page occupied by each block depends on the contents and properties defined for it. Omitted blocks occupy no space on the page:

Page Header	A single line of information identifying the report that appears on every page (except, optionally, the first page).
Title	A text title (up to 50 characters) and optional logo graphic that appears at the top (but below the page header) of the first page (and optionally on every page).
Data Header	Information identifying the samples and/or other identifying information. Multiple lines of data can be defined.
Signature Lines	Up to two lines providing space for signatures indicating report review and/or approval.
Page Footer	A single line of information identifying the report that appears on every page (except, optionally, the first page).

The detailed report also contains the following elements (which are repeated for each component in the method):

Calibration Curve	A graph plotting the response obtained from each injection of each standard
Equation Data	A display of curve type, equation, intercept, slope and goodness of fit coefficient (r^2)
Results Table	A table listing selected results from each injection of a particular standard level (repeated for each level)

In addition to the common elements the condensed report contains an element that is a hybrid of some of the above:

Calibration Data	Includes the calibration curve, the equation data and a table listing the average response and %RSD at each standard level

Equation Data

The equation data block is a simple listing of values related to the calibration equation:

Calibration Type	The order of the equation used to fit the curve to the data (from the method). [Linear, Average Calibration Factor]
Equation	The equation derived from the calibration data [or Average Calibration Factor if that type applies]
Curve Statistics	Intercept, Slope and r^2 [Intercept and Slope only appear for Linear] (Note that the term Correlation Coefficient is not used since it is only applicable to linear fits) [For Average Calibration this line is replaced with one that shows the %RSD for the results]

NOTE: *If an equation does not exist for a component for any reason then the Equation field and all related fields (Intercept, Slope, r^2) will be blank.*

Calibration Data

This element (used within the condensed summary calibration report) contains several of the elements described previously but compressed into a smaller space.
The summary results information is preceded by display of the number of standards (levels) defined. The table will always contain the following columns and is not configurable:

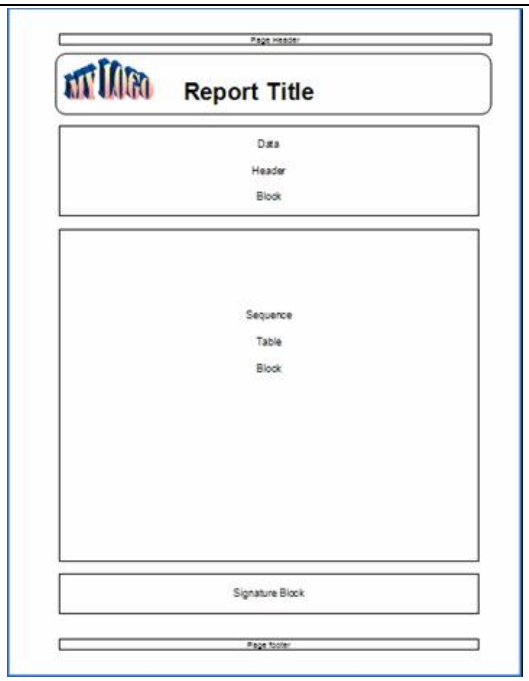
- Standard [standard/level name from the method]
- %RSD calculated from all injection at the current level
- Avg. Response Average response (area or height) from all injections of the current standard

NOTE: *Whether the %RSD and Average Response values are calculated from area or height depends on which was used for the calibration curve, which in term is determined by the* ***Quantify Using*** *parameter from the calibration section of the method.*

Sequence Report

A Sequence Report shows information from a specific sequence. There are two basic forms of a Sequence Report:

Sequence Detailed Report



Sequence Summary Report

1. A detailed report, which includes all data items from each sequence row, including full details of all decision points.

2. A summary includes only values from columns of the sequence row selected by the user. This report does not include decision points.

The two formats share the following five common report blocks. Any element can be omitted from the basic structure. The space on the page occupied by each block will depend on the contents and properties defined for it. Omitted blocks occupy no space on the page:

Page Header	A single line of information identifying the report that appears on every page (except, optionally, the first page).
Title	A text title (up to 50 characters) and optional logo graphic that appears at the top (but below the page header) of the first page (and optionally on every page).
Data Header	Information identifying the samples and/or other identifying information. Multiple lines of data can be defined.
Signature Lines	Up to two lines providing space for signatures indicating report review and/or approval.
Page Footer	A single line of information identifying the report that appears on every page (except, optionally, the first page).

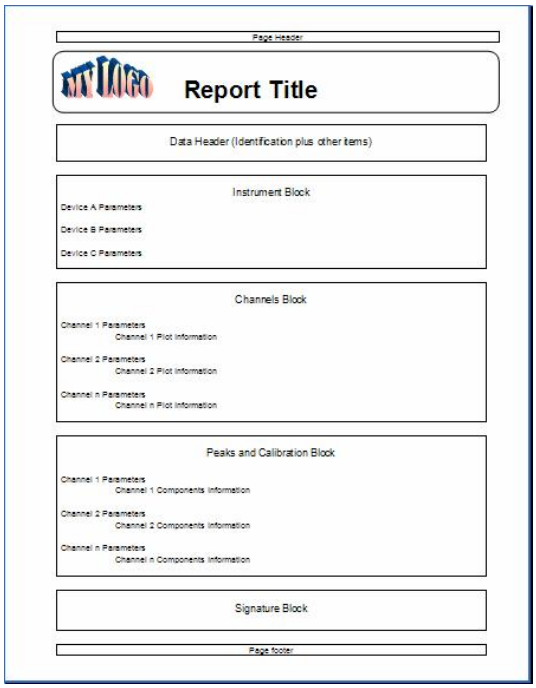
The detailed report also contains the following elements:

Global Settings Block	A structured listing of all the reports defined and the sample naming template. There can be two sections to the Global Settings Block: The first titled **Per Sample Reports** will only appear if you have defined at least one report to be generated on a per-sample-type. Each per sample report sub block always contains entries for Sample Type, Frequency, Report Template, Output Target and Output Name. In the case of a report where the Output Target is file then there will also be an entry for File Type. The second section to the Global Settings Block is titled **Sample Naming Template** and this will always be printed. Only one sample naming template can be defined. The fields will be Prefix, Vial Start, Number, Vial Increment, Suffix and Applies To.
Sequence Data Block	A structured listing of all the basic parameters in a sequence row. One block is reported for each row in the sequence.
Decision Point Block	A structured listing of decision point data (one block per decision point)
Sequence Row Table	A table showing selected data items from each row of the sequence.

Method Report

A Method Report shows instrument and processing parameters from a method. There is only one basic template for this report but it is a complex one since it presents a variety of data sets of both fixed and variable length.

A very simplified form of the basic template is shown below.



The method report includes the following five common report blocks (any of which can be omitted):

Page Header	A single line of information identifying the report that appears on every page (except, optionally, the first page).
Title	A text title (up to 50 characters) and optional logo graphic that appears at the top (but below the page header) of the first page (and optionally on every page).
Data Header	Information identifying the samples and/or other identifying information. Multiple lines of data can be defined
Instrument Block	Within the Instrument block is a section for each device configured in the instrument.
Channels Block	Each channel section begins with channel identification, followed by a set of fixed parameters, which is followed in turn by a block of parameters (in two-column format) for each defined plot.
Peaks and Calibration Block	Within the Peaks and Calibration block is a section for each channel defined in the Instrument section.
Signature Lines	Up to two lines providing space for signatures indicating report review and/or approval.
Page Footer	A single line of information identifying the report that appears on every page (except, optionally, the first page).

Report Format Wizard

The **Report Format Wizard** enables you to tailor the basic report templates provided with the system to more closely match your requirements. The basic approach to reporting has been described in Reports along with the elements of each report type (sample, summary, calibration, sequence and method). The report format wizard provides a user interface that allows users to define the data items included in each report element and also to specify some basic formatting.

After completing entries into the wizard you are able to save the tailored report format and then can specify that format subsequently when defining report output (via a sequence or directly from one of the main Chromera environments, where applicable).

Running the Report Format Wizard

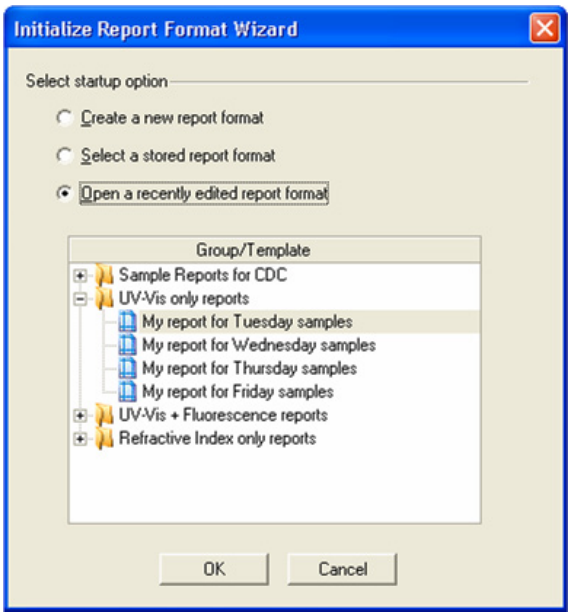
There are two entry points into the wizard:

One when you have chosen to create a new report format and one when you have chosen to edit an existing, saved format. Note that the terms new and saved are from the user's viewpoint. In the first case what you are really be doing is specifying a set of preferences to be applied to one of the basic PerkinElmer-supplied templates, and in the second case you are modifying a previously defined and saved set of preferences. There is no case where you are expected to create a report format from a blank sheet of paper.

The difference in the wizard's form and behavior between the *create new format* and *edit existing format* commands is that in the latter case the page on which you select the report type (sample, summary, calibration, sequence or method) and basic content will not appear.

Report Format Wizard Startup Dialog

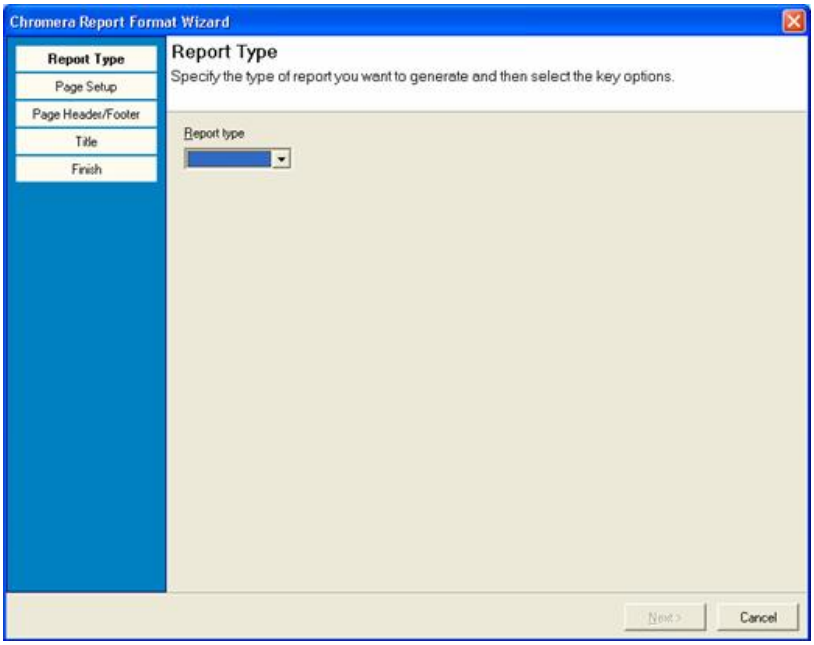
The Initialize Report Format Wizard dialog is displayed when the **Report Format Wizard** command is chosen from the **Tools** menu. Select the option within this dialog to determine the mode in which the Report Format Wizard will be run.



Control	Description
Create a new report format	Select this button to run the Report Format Wizard in *create new* mode.
Select a stored report format	Select this button to run the Report Format Wizard in edit existing mode, operating on a report format to be selected from the database/
Open a recently edited report format	Select this button to run the Report Format Wizard in edit mode, operating on a report format to be selected from the displayed recently used list.
Group/Report Format Name	A tree control that displays the names of report formats, along with their associated group name, in a hierarchical structure when either **Select a stored report format** or **Open a recently edited report format** is selected. In the former case, all stored report formats are displayed. In the latter case the most recently used (MRU) list is displayed. The group names are displayed in alphabetical order and the report formats are displayed in alphabetical order within each group. The MRU list can contain up to 10 names and will operate as a FIFO list. When ten report format names exist, editing a new report format will displace the oldest name from the list.
OK	This button closes the dialog and initiates action based on your selection: **Create a new report format** – Runs the Report Format Wizard in 'create new' mode. **Select a stored report format** – Opens the report format selected in the stored formats list in the Report Format Wizard, running in edit mode **Open a recently edited report format** – Opens the report format selected in the recently used list within the Report Format Wizard running in edit mode.
Cancel	This button closes the dialog and cancels the Report Format Wizard command.

Report Format Wizard – Overview

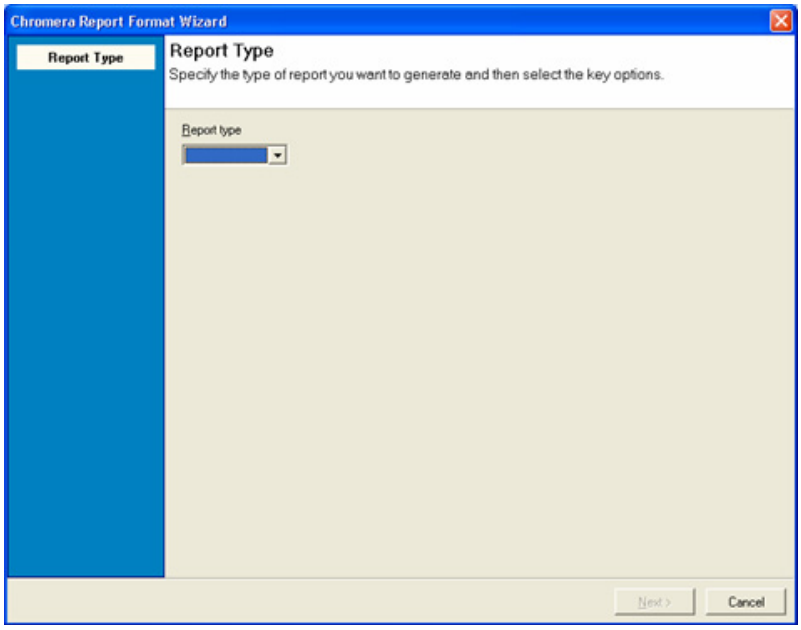
The title bar identifies the window as the **Chromera Report Format Wizard**. The window is designed to display a panel at the left indicating the steps (pages) in the wizard, a title and subtitle for the current page and a main area displaying the controls for the current page.



The buttons provided at the bottom of the window are a **Cancel** button always present, together with **Back** (except on the first page) and **Next** (replaced by **Finish** on the final page) buttons. On pages where at least one entry or selection is mandatory (e.g. the first page shown above), the **Next** button is disabled until the required entries/selections have been made.

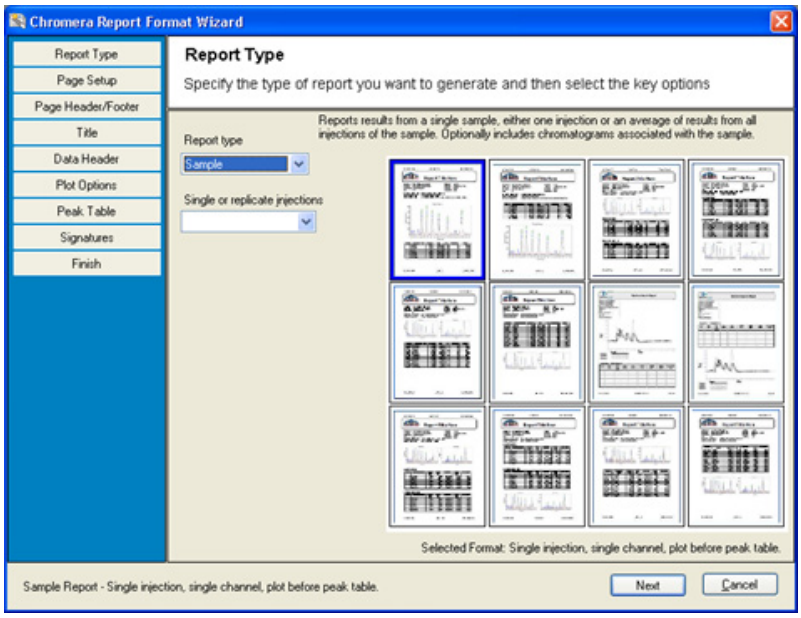
Report Format Wizard – Report Type

This is the first page displayed when you choose to generate a new report format.



The **Next** button is disabled until you make a selection in the **Report type** drop-down list. Only the Report Type button is displayed in the panel at the left side of the dialog until a selection has been made for the report type.

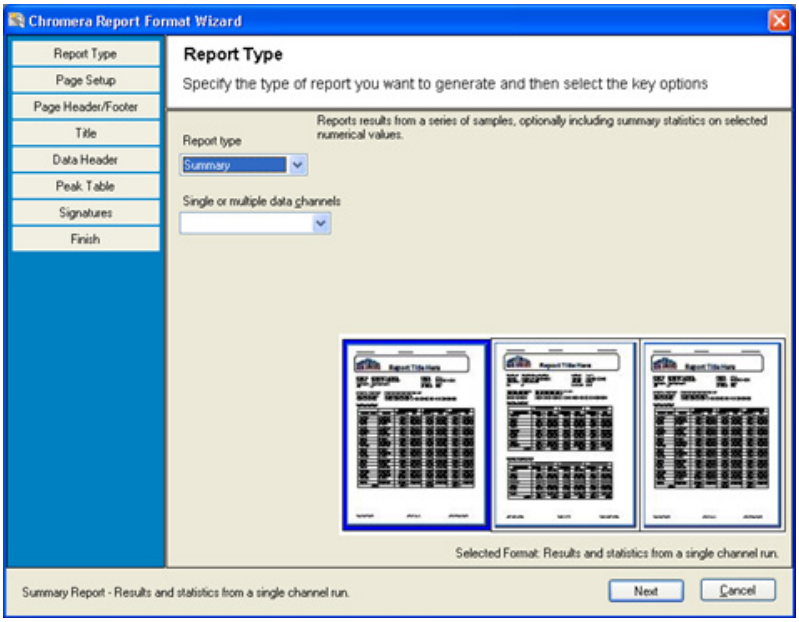
The selection of report type affects both the buttons displayed in the left hand panel and the contents of the main display area, as shown in the examples below.



Sample selected:

- Report Type, Page Setup, Page Header/Footer, Title, Data Header, Plot Options, Peak Table, Signatures, and Finish buttons shown.
- Thumbnail depictions of all basic sample report formats are displayed in a grid format (Default selection: Col 1 Row 1).
- Description of the sample report type and its variants is displayed at the top of the page.
- Description of the format currently selected in the thumbnail grid is displayed below the thumbnails.
- An additional drop-down list (Single or multiple injections) is displayed. Note that there is no default selection in the drop-down list.
- A message displays in the bottom panel of the dialog indicating the selected report type and variant (this appears on all pages).

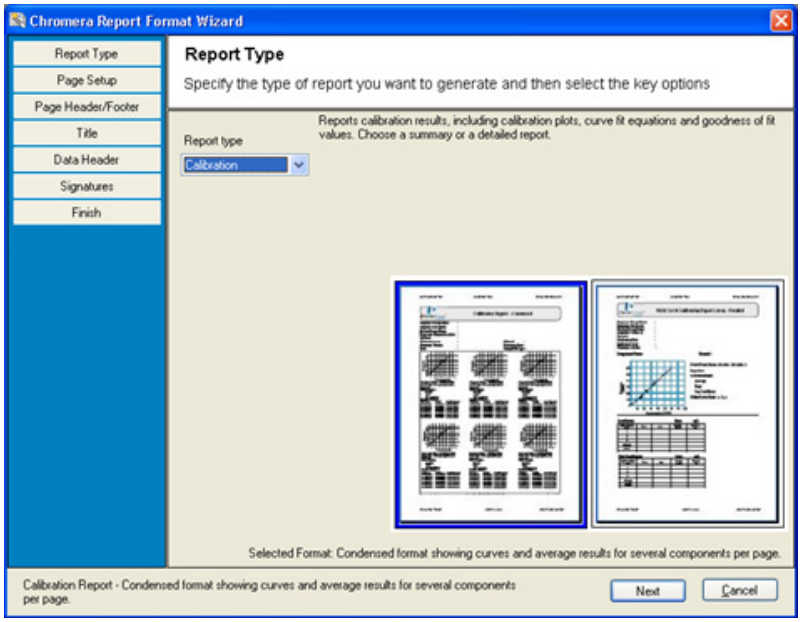
The progressively specific selections for defining the format of the sample report is described once the basic layout of the report type page has been shown for each report type.



Summary selected:

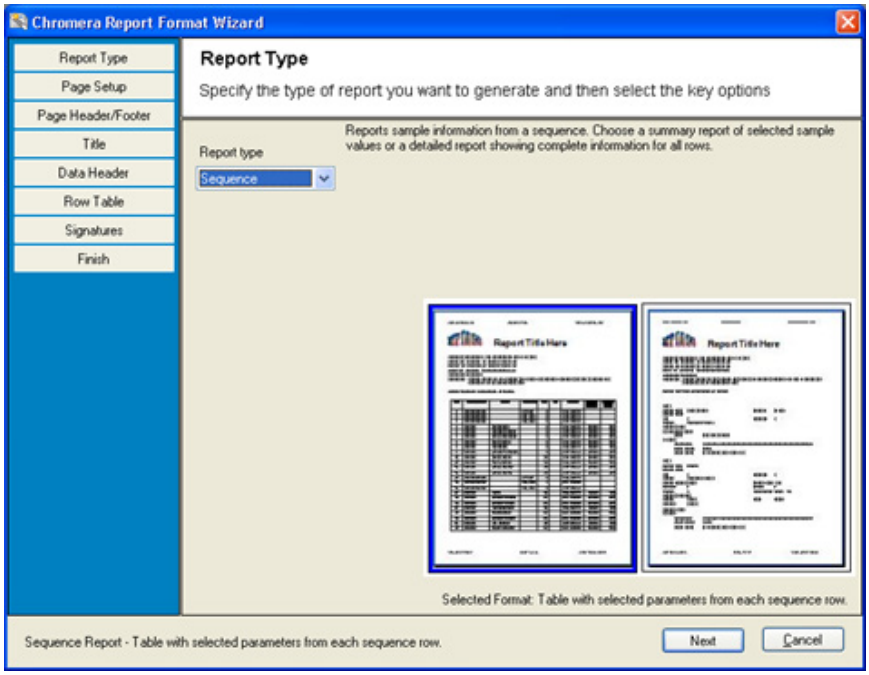
- Report Type, Page Setup, Page Header/Footer, Title, Data Header, Peak Table, Signatures, and Finish buttons shown
- Thumbnail depictions of all basic summary report formats are displayed
- Description of the summary report type and its variants is displayed at the top of the page
- Description of the format currently selected in the thumbnail grid is displayed below the thumbnails
- An additional drop-down list (Single or multiple data channels) is displayed. Note that there is no default selection in the drop-down list.
- A message displays in the bottom panel of the dialog indicating the selected report type and variant (this appears on all pages).

The secondary selection for defining the format of the summary report is described once the basic layout of the report type page has been shown for each report type.



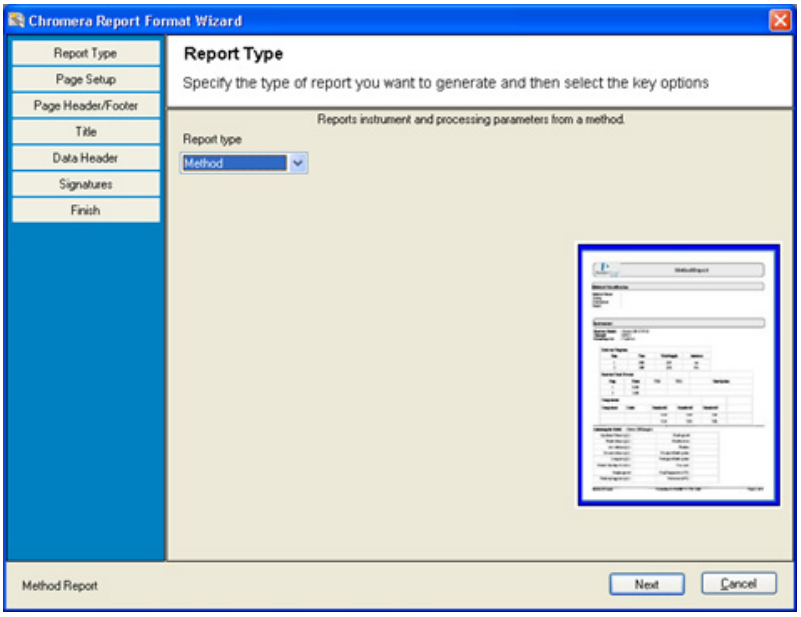
Calibration selected:

- Report Type, Page Setup, Page Header/Footer, Title, Data Header, Signature, and Finish buttons shown.
- Thumbnail depictions of both basic calibration report formats are displayed.
- Description of the calibration report type and its variants is displayed at the top of the page.
- Description of the format currently selected in the thumbnail grid is displayed below the thumbnails.
- A message displays in the bottom panel of the dialog indicating the selected report type and variant (this appears on all pages).



Sequence selected:

- Report Type, Page Setup, Page Header/Footer, Title, Data Header, Row Table, Signatures, and Finish buttons shown.
- Thumbnail depictions of both basic sequence report formats are displayed.
- Description of the sequence report type and its variants is displayed at the top of the page.
- Description of the format currently selected in the thumbnail grid is displayed below the thumbnails.
- A message displays in the bottom panel of the dialog indicating the selected report type and variant (this appears on all pages).



Method selected:

- Report Type, Page Setup, Page Header/Footer, Title, Data Header, Signatures, and Finish buttons shown.
- Thumbnail depictions of basic method report format is displayed.
- A message displays in the bottom panel of the dialog indicating the selected report type and variant (this appears on all pages).

Sample Format Selection

Sample Format Selection

As shown there are twelve basic formats available for **Sample type** reports. Select one of these before continuing with the next page of the wizard. You can select a specific format in one of two ways:

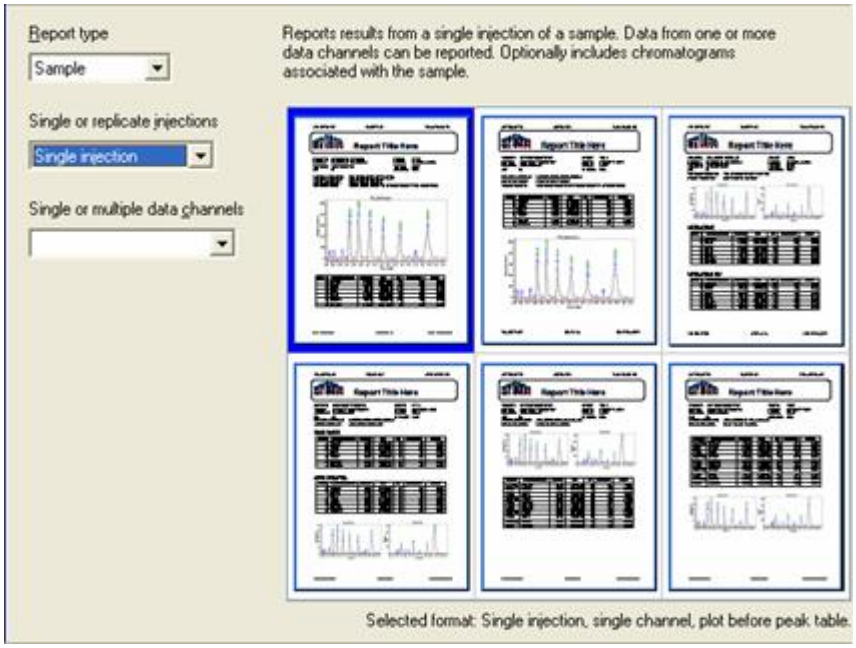
- By selecting a specific thumbnail in the example grid.
- By selecting options from the progressively displayed set of drop-down lists (and then one of the two remaining thumbnails).

The basic characteristics that must be defined and their effect on the report (and the report format wizard) are indicated in the following table:

Setting	Description
Number of injections	Defines whether the report will include results from a single injection of the sample or average results from multiple injections.
Number of channels	Defines whether the report will include results from a single data channel or multiple data channels.
Peak Table format	Defines whether results from multiple data channels are reported in separate tables or merged in a single table. (Only applicable to multi-channel results)
Plot position	Defines whether the plot(s) (if any) in the report will appear before or after the peak table. (Selected via thumbnail display only)

As shown above, selection of the Sample report type causes the **Single or replicate injections** drop down list to be displayed. A selection in this control will cause another drop-down list (Single or multiple data channels) to appear. Selection of the **Multiple channel**s option in this control will cause another drop-down list 'Separate or merged peak tables) to appear. Selection of **Merge peak data from all channels** will cause another drop-down list (Sorted by) to be displayed. As each selection is made the grid of report format thumbnails is updated to show only those that still apply. For example, the following show the effect of selecting Single Injection, then Multiple Channels and finally Separate Peak Tables.

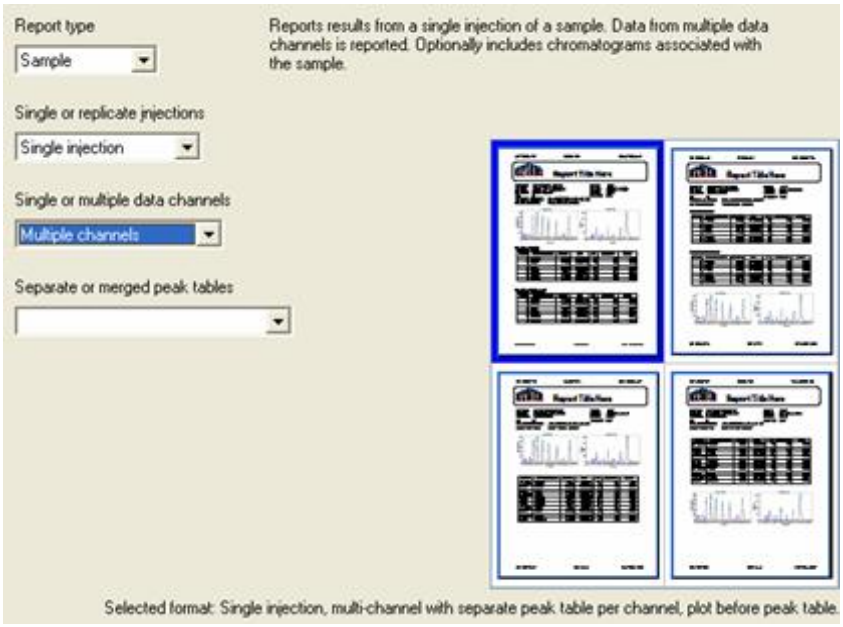
Single injection selected:



Examples of replicate reports are eliminated from the thumbnail grid.

The **Single or multiple data channels** drop-down list is displayed. Note that there is no default selection in the drop-down list.

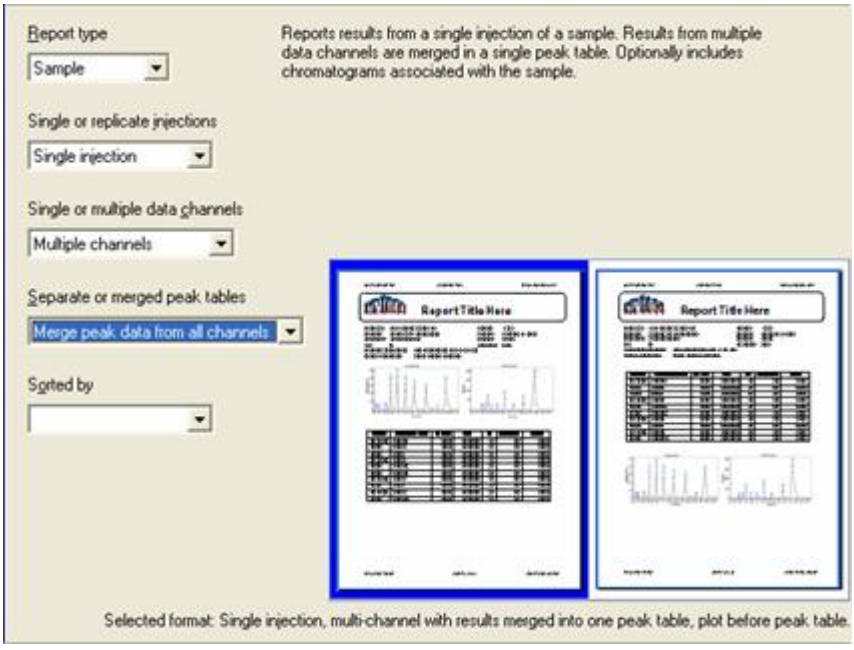
Multiple channels selected:



The **Separate or merged peak tables** drop-down list is displayed.

The descriptive text at the top of the page is updated to reflect the user's latest selection.

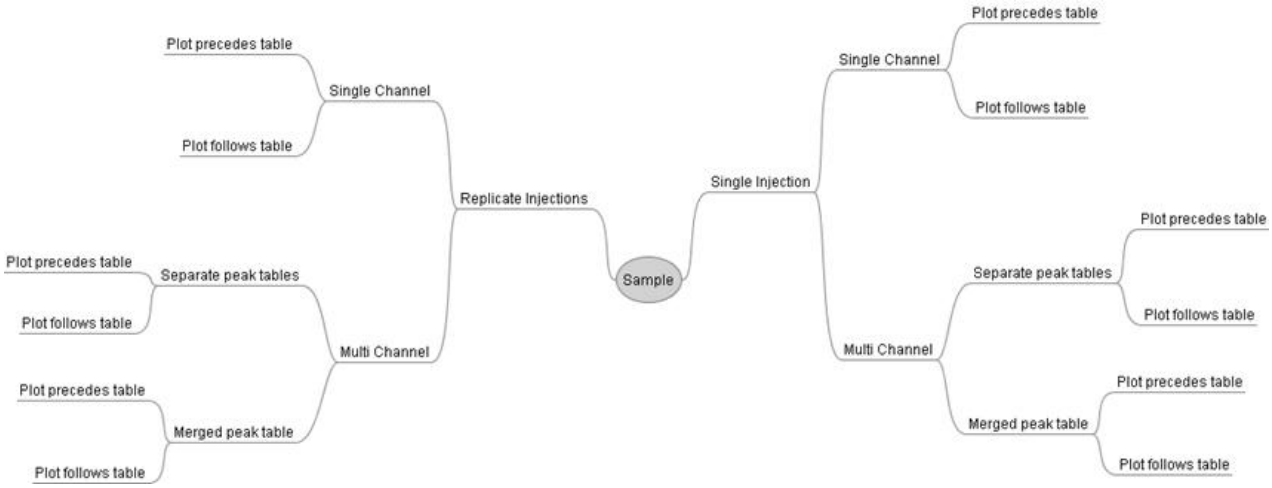
Merge peak data from all channels selected:



The descriptive text at the top of the page is updated to reflect your latest selection.

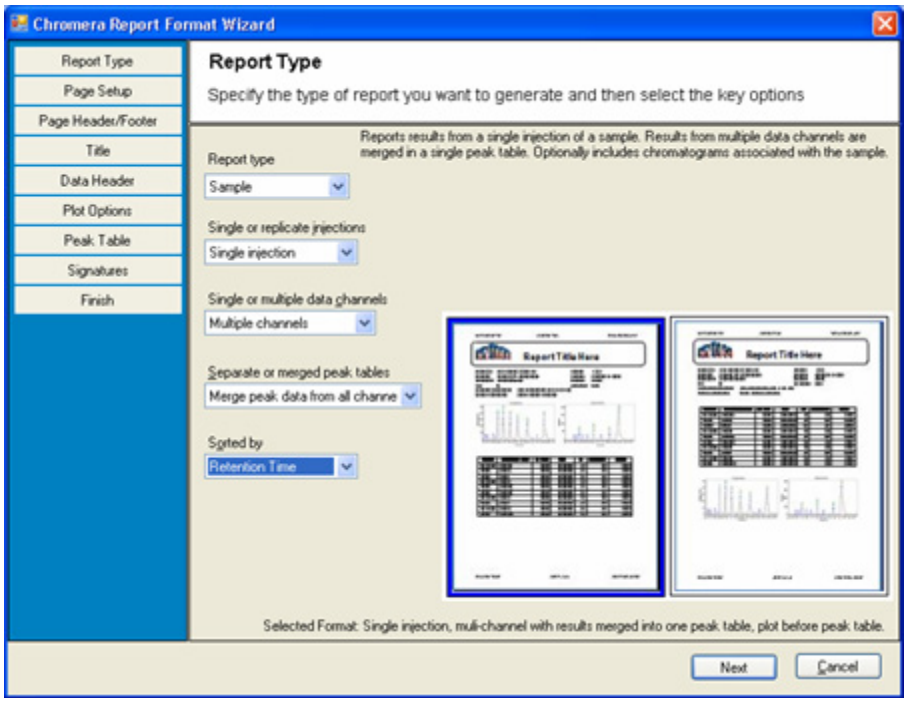
The **Sorted by** drop-down list is displayed, allowing you to choose the order in which peaks appear in the merged table (default setting is by Retention Time);

By making selections in the drop-down lists you are following a decision tree, with the final selection at the end of each branch made by selecting one of the final two thumbnails remaining.



Sample Report Type

A summary of the form and behavior of the drop-down list controls on the **Report type** page for a Sample report appears below.



Control	Description
Report type	Select from the drop-down list (**Sample, Summary, Calibration, Sequence, Method)** the type of data set to be reported.

For the section of the table below the assumption is that the **Report type** selection is Sample.

Control	Description
Single or Replicate injections	Select from the drop-down list whether the report includes data from a single injection (run) or replicate injections of the same sample.
Single or Multiple data channels	Select from the drop-down list whether the report includes data from a single data channel or more than one channel.
Separate or Merged peak tables	Select from the drop-down list whether the report will contain a separate peak table for each data channel or a single peak table with results merged from all data channels.
Sorted by	Select from the drop-down list (**Component name or Retention time)** the ordering of peaks in the merged peak list.

The descriptive text to appear at the top of the page for each combination of settings in the drop-down lists is shown in Report Type Descriptive Text.

Summary Format Selections

As shown, there are three basic formats available for **Summary type reports**. You must select one of these before continuing with the next page of the wizard. The selection of a specific format can be made in one of two ways:

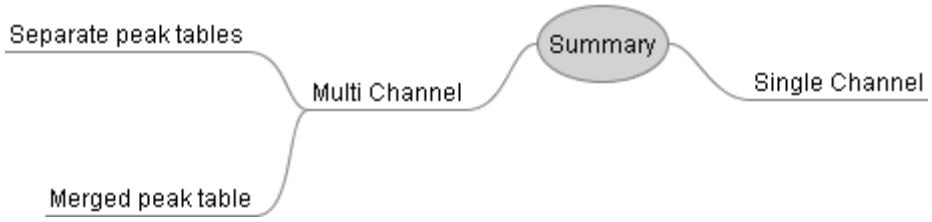
- By selecting a specific thumbnail in the example set.
- By selecting an option from the displayed drop-down list (and then one of the two remaining thumbnails).

The basic characteristics to be defined and their effect on the report (and the report format wizard) are:

Setting	Description	Effects on Report Element(s)
Number of channels	Whether the report will include results from a single data channel or multiple data channels.	Determines format options available for peak table.
Peak Table format	Whether results from multiple data channels are reported in separate tables or merged in a single table. (Only applicable to multi-channel results) (Selected via thumbnail display only)	Determines the number of peak tables in the report.

Selection of the Sample report type causes the Single or multiple data channels drop down list to be displayed. A selection in this control causes the thumbnails display to be reduced to one (for single channel) or two (for multiple channels). In the latter case the final selection of peak table format can be made by selecting one of the thumbnails.

The decision tree for the Summary report type is shown below. In this case the selection between separate peak tables or a merged peak table for multiple channel data is made by selecting the appropriate thumbnail.



Report Type - Summary

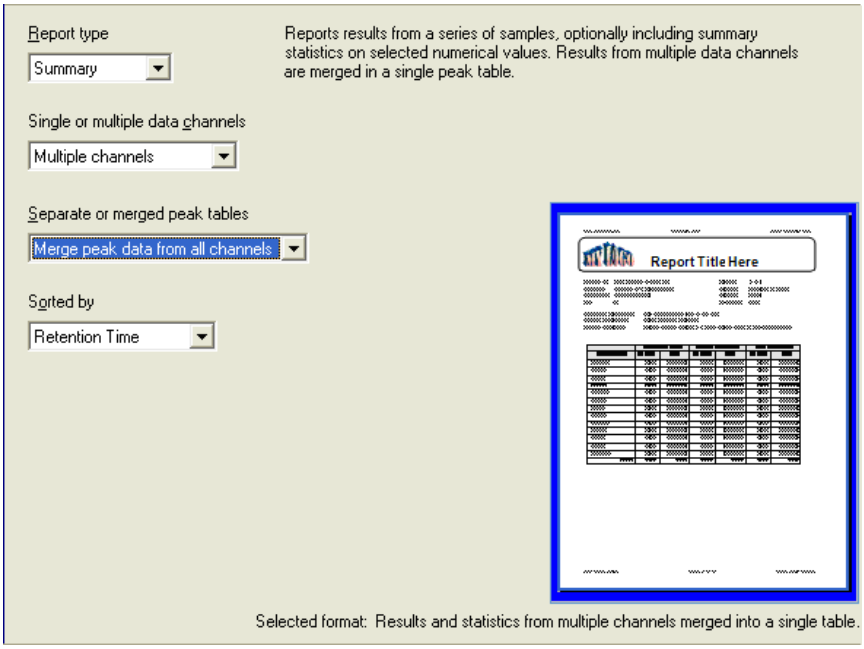
A summary of the form and behavior of the drop-down list controls on the Report Type page for a Summary report appears below.

Control	Description
Report type	Select one of the following **Sample**, **Summary**, **Calibration**, **Sequence**, or **Method** from a drop-down list that determines the type of data set to be reported.

For the section of the table below the assumption is that the Report type selection is Summary.

Control	Description
Single or multiple channels	Select **Single channel** or **Multiple channels** from a drop-down list to indicate whether the report includes data from a single data channel or more than one channel. The control and its associated label are not displayed until the Summary selection has been made in the Report type control.
Separate or merged peak tables	Select **Separate peak table per channel** or **Merge peak data from all channels** from a drop-down list to indicate whether the report will contain a separate peak table for each data channel or a single peak table with results merged from all data channels. The control and its associated label are only displayed if and when Multiple channels is selected in the Single or multiple channels control.
Sorted by	Select **Component name** or **Retention time** from a drop-down list defining the ordering of peaks in the merged peak list. The control and its associated label are only displayed if and when Multiple channels is selected in the Single or multiple channels control and Merge peak data from all channels is selected in the Separate or merged peak tables control.

The following example screen shows the Report Type page with all Summary report type selections displayed. Note the spacing of the controls; there is no 'gap' even though the Single or multiple injections option for the Sample report type does not appear.



Calibration Format Selection

As shown above there are two basic formats for Calibration reports. For this reason no further controls are necessary to reduce selections and the choice between formats can be made from the thumbnail depictions. The options are:

- A condensed report, fitting basic results (curve, equation, average results) from several components on each page.
- A detailed report showing results for all injections of each standard level, in addition to the curve and equation. The data for each component occupies as much space as required.

Sequence Format Selection

As shown above there are two basic formats for Sequence reports. For this reason no further controls are necessary to reduce selections and the choice between formats can be made from the thumbnail depictions. The options are:

- A tabular summary report showing data items selected by the user (on the Row Table page). There is one row in the table for each row in the sequence.
- A detailed report showing every data item and decision point for each row in the sequence. The definition of each row occupies as much space as required.

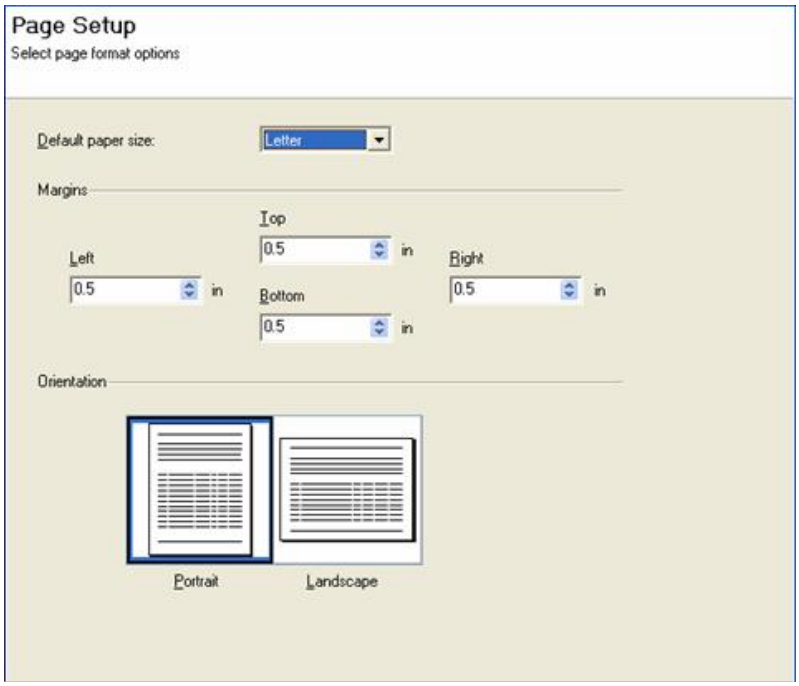
Method Format Selection

There is only a single basic format for Method reports. There are therefore no further controls for selection and only a single thumbnail is displayed.

The Method report type option exists only to allow you to customize page header/footer, title and data header (Identification items plus general items) for the report, for consistency with the other report types.

Report Format Wizard – Page Setup

This page displays when you click the **Next** button (or click **Page Setup**) after making a selection for the basic report format on the Report Type page (when a new format is being generated). This is the first page displayed when an existing report format is being edited.

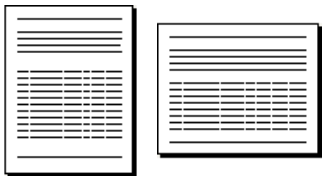


Main Display Area of the Page Setup Page

As mentioned previously, the *Default paper size* setting on this page is simply to define (along with the other settings) the page area available for data. This information is used later in the wizard to guide the user with regard to the amount of information that can be fitted on the page. Although the margin and orientation settings are stored with the resulting report template, the paper size is determined at the time the report is generated.

Since default values always exist for these settings, the Next button will always be enabled on this page. The Back button will also be visible and enabled.

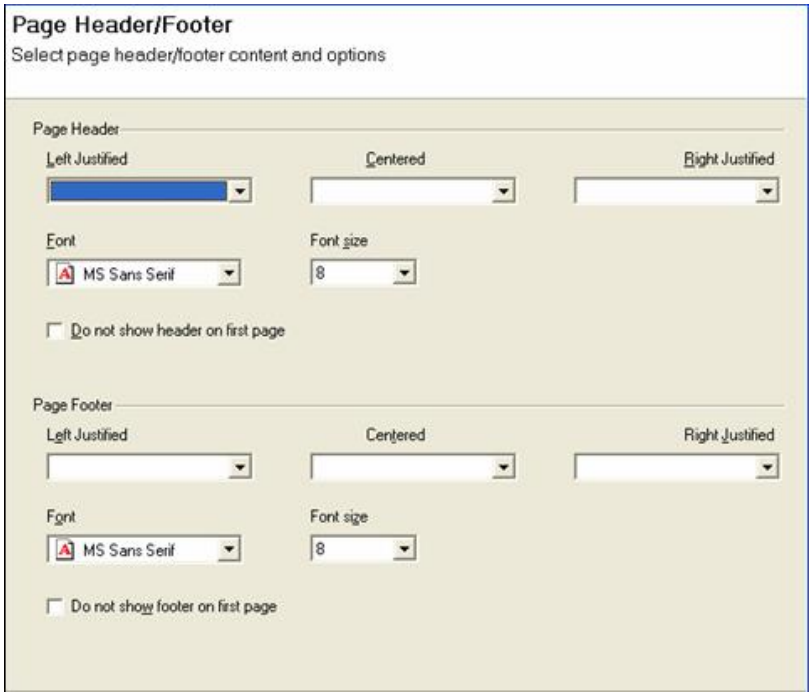
Control (in tab order)	Description
Default paper size	Select **A4**, **Legal**, or **Letter** from a drop-down list to indicate the paper size the report is designed for. If computer Time Zone is GMT -2 or higher (i.e. higher negative number) = Letter size otherwise the paper size is A4

Top	Use the up/down arrow buttons to select the size of the page top margin. A units label appearing after the control should match the paper size (**Letter** or **Legal** shows **in** and **A4** shows **cm)** **Range:** 0.0 to 1.5 in - for **Letter** or **Legal** paper size 0.0 to 4.0 cm – for **A4** paper size
Left	Use the up/down arrow buttons to select the size of the page left margin. A units label appearing after the control should match the paper size (**Letter** or **Legal** shows **in** and **A4** shows **cm)** **Range:** 0.0 to 1.5 in - for **Letter** or **Legal** paper size 0.0 to 4.0 cm – for **A4** paper size
Right	Use the up/down arrow buttons to select the size of the page right margin. A units label appearing after the control should match the paper size (**Letter** or **Legal** shows **in** and **A4** shows **cm)** **Range:** 0.0 to 1.5 in - for **Letter** or **Legal** paper size 0.0 to 4.0 cm – for **A4** paper size
Bottom	Use the up/down arrow buttons to select the size of the page bottom margin. A units label appearing after the control should match the paper size (**Letter** or **Legal** shows **in** and **A4** shows **cm)** **Range:** 0.0 to 1.5 in - for **Letter** or **Legal** paper size 0.0 to 4.0 cm – for **A4** paper size
	Select **Portrait** or **Landscape** from a graphic selection control indicating the page orientation for the report.

NOTES: *(1) If you change the setting for paper size from Letter or Legal to A4 (or vice versa) the software will make direct conversions of any current settings for plot sizes and table column widths. (2) If you make some changes that affect the printable page width (e.g. changing margins or changing paper size) and the change results in an existing table being too wide for the page you are given the choice of clearing the table contents or canceling the change(s).*

Report Format Wizard – Page Header/Footer

This page is displayed when you click the **Next** button from the **Page Setup** page (or click **Page Header/Footer**).



Main Display Area of the Page Header/Footer Page

Since default values always exist for these settings (blank header/footer lines are quite valid), the Next button is always enabled on this page. The Back button is also visible and enabled.

Page Header Controls (in tab order)	**Description**
Left Justified	A drop-down list indicating the data item (if any) to be shown at the left side of the header line.
Centered	A drop-down list indicating the data item (if any) to be shown at the left side of the header line.
Right Justified	A drop-down list indicating the data item (if any) to be shown at the left side of the header line.
Font	A drop-down list indicating the font (typeface) to be used for the header line.
Font size	A drop-down list indicating the size of the font (in pts) to be used for the header line.
Do not show header on first page	A check box that indicates if the header line should be suppressed on the first page of the report.
Page Footer Controls (in tab order)	**Description**
Left Justified	A drop-down list indicating the data item (if any) to be shown at the left side of the footer line.

Center	A drop-down list indicating the data item (if any) to be shown at the left side of the footer line.
Right Justified	A drop-down list indicating the data item (if any) to be shown at the left side of the footer line.
Font	A drop-down list indicating the font (typeface) to be used for the footer line.
Font size	A drop-down list indicating the size of the font (in pts) to be used for the footer line.
Do not show header on first page	A check box that indicates if the header line should be suppressed on the first page of the report.

Data Items available for Page Header/Footer

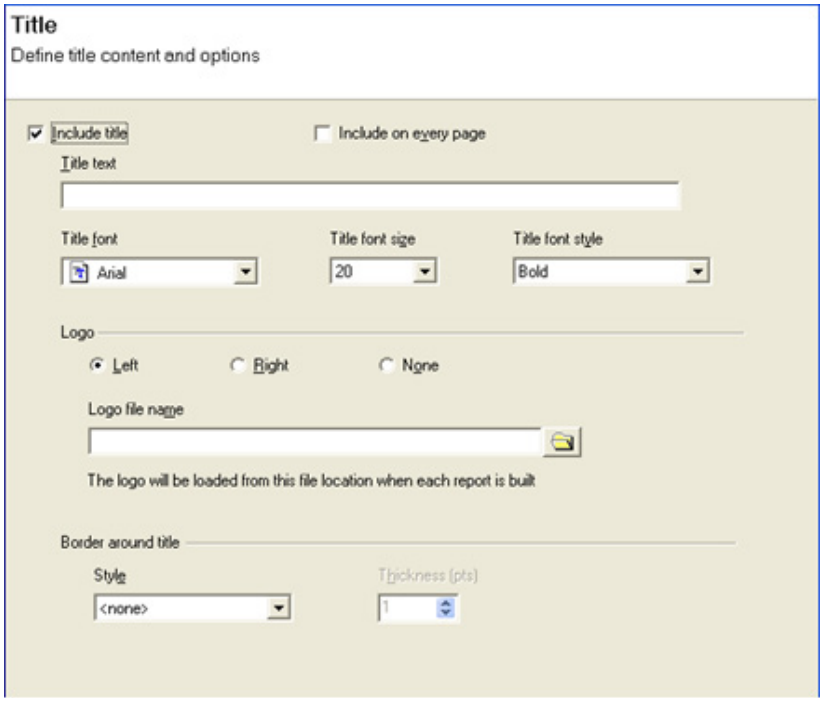
The data items that may be selected for the page header and footer lines depend on the report type (and format in some cases). For any given report type the contents of the six drop-down lists (Left Justified/Centered/Right Justified for Header and Footer) is identical.

The items available for any given report type/format are taken from the following:

Acquisition Method	The name of the method from which the instrument parameters used during acquisition were taken.
Batch Name	This is the sequence used to acquire the data or a name entered in Reprocess.
Channel Name	The name of the data channel from which the results were acquired.
Group Name	The database group associated with the sequence or method.
Instrument Name	The name of the instrument from which the results were acquired.
Method Name	The name of the method being reported.
Page n of m	Where n = current page, m = total pages in the report.
Processing Method	The name of the method from which the parameters used for processing the data were taken.
Report Date	The date of report generation (in Long format from Regional Settings).
Report Date/Time	The date and time of report generation (using Long date format from Regional Settings).
Sample Name	The name associated with the sample (from Sequence row).
Sequence Name	The name of the sequence. In the case of a Sample/Summary report this is the sequence (or Single Run batch) used to acquire the data.

Report Format Wizard – Title

This page displays when you click the **Next** button from the Page Header/Footer page (or click **Title**).



Main Display Area of the Title Page

Since default values always exist for these settings (a blank title line is quite valid), the Next button is always enabled on this page. The Back button is also visible and enabled.

Controls (in tab order)	**Description**
Include title	A check box that indicates if the title block define on this page will appear in the report.
Include on every page	A check box that indicates if the title block will appear on just the first page or on every page of the report.
Title text	An edit box to enter the text to appear in the title block.
Title font	A drop-down list indicating the font (typeface) to be used for the title text.
Title font size	A drop-down list indicating the size of the font (in pts) to be used for the title text.
Title font style	A drop-down list indicating the text style (**Regular, Bold, Italic, or Bold Italic)** to be applied to the title text.

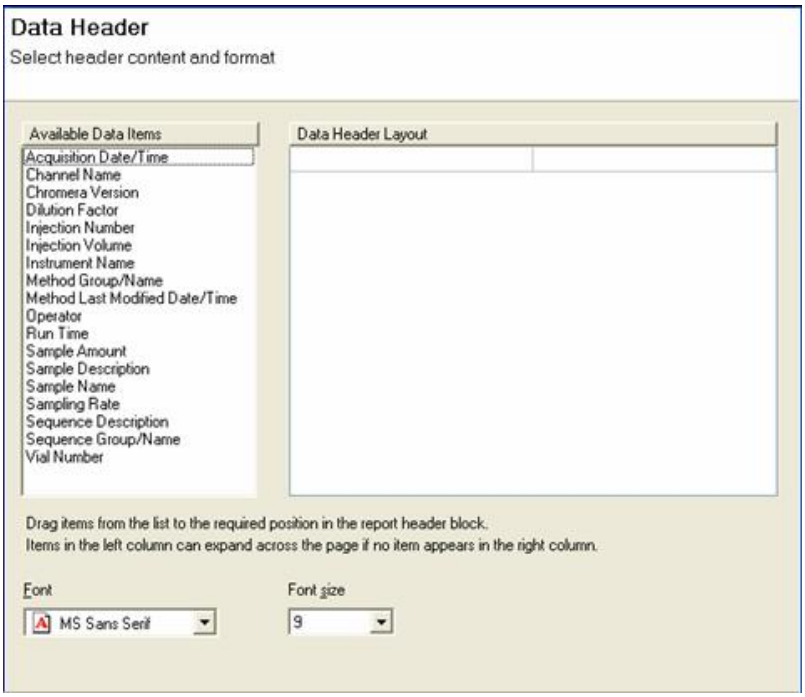
Logo (position)	A set of radio buttons defining the position of the logo (if any). If **None** is selected then no logo will appear even if a logo file name is specified. **Left** the logo will appear to the left of the title text. **Right** the logo will appear to the right of the title text. **None** no logo will appear
Logo file name	An edit box that defines the file to be used for the logo.
	A button that displays a **File Open** dialog, enabling you to browse for and select the logo file.
Scale (%)	A spin button control to set the scale to be applied to the logo graphic defined by the specified file when it is included in the report.
Style	A drop-down list that defines the type of border to appear around the title block.
Thickness (pts)	A spin button that determines the thickness of the border around the title block.

Report Format Wizard – Data Header

This page displays when you click the **Next** button from the Title page (or click **Data Header**).

In the actual format of the data header in the final report each data item value is preceded by the name of the data item. Thus **Acquisition Date/Time** in the final report appears something like:

Acquisition Date/Time: Wednesday, February 11, 2009, 3:14:10 PM



Since default values always exist for these settings (a blank/non-existent data header is valid), the Next button is always enabled on this page. The Back button is also visible and enabled.

The contents of the Available Data Items list depends on the currently selected report type/format, as described later in this section.

Controls **(in tab order)**	**Description**
Available Data Items	A list box showing the data items available to be added to the data header block. An item can be added to the header only once. When it has been added to the header it is removed from the list box. Conversely, when the item is deleted from the data header it is returned to the list box. The items are sorted alphabetically.
Data Header Layout	A two-column grid showing the current contents of the data header block. The procedure by which the user builds a data header is described in section Building the Data Header. A single blank row is shown when the page is first displayed. When a data item is added to either cell in the blank row a new blank row will appear at the bottom of the header grid.
Font	A drop-down list indicating the font (typeface) to be used for the data header block. These include fonts available on the system, excluding non-alphanumeric fonts.
Font size	A drop-down list indicating the size of the font (in pts) to be used for the data header block.

Data Header Layout Popup Menu

This popup menu appears when you right click on a row in the Data Header Layout .

Command	**Description**
Add Row	Adds a new blank row to the end of the grid
Insert Row	Inserts a blank row above the one under the mouse pointer when the right button was clicked.
Delete Row	Deletes the row under the mouse pointer when the right button was clicked. Returns any data items on the deleted row to the list box.

Building the Data Header

Drag-and-Drop

To add a data item to a specific position drag it from the list and drop it in the required position. Whether you populate both columns in a row or just the left hand column will depend on the expected length of the item. For example, it would be appropriate to position Sequence Group/Name on a row by itself but Vial Number and Injection Number (always shown as 'n of m') would certainly fit on the same line.

- When an item has been added to the header it is removed from the list box.
- If you drop an item onto the bottom row of the header block then a new blank row is appended to the header.
- If you drop an item onto a previously occupied cell, the new data item displaces the previous one, which is returned to the list box.
- To reposition an item drag it from one cell in the header block and drop it on another cell.
- You can remove an item from the header by dragging it from the header block and dropping it on the list box (or by deleting the row, which also returns the item to the list box).

Double-Click Operation

If you double-click on an item in the list box, that item is added to the left hand cell in the blank row at the bottom of the header grid, and a new blank row is appended. This is a rapid way to add several items that are to occupy complete rows. Of course an additional item could be added to the right hand column of any of these rows subsequently via a drag-and-drop operation.

Keyboard Operation

If you select an item in the list box and then presses the right arrow key the item is added to left hand cell in the blank row at the bottom of the header grid. If this key operation is repeated then next item is added to the right hand column of the same row. A third keystroke would add the next item to the left hand cell in the new blank row at the bottom of the header grid, and so on.

If you select an item in the list box and then presses the space bar, this acts in the same way as a double-click operation. That is, the item is added to the left hand cell in the blank row at the bottom of the header grid, and a new blank row is appended.

Data Items available for Data Header

The data items that may be selected for the data header block depend on the report type (and format in some cases).

The items available for any given report type/format are taken from the following:

Acquisition Date	The date of acquisition of the first injection of a replicate series.
Acquisition Date/Time	The date and time of data acquisition (using Long date format from Regional Settings)
Acquisition Method	Group name and Method name for the method used to acquire the data
Acquisition Method Last Modified	The date and time the acquisition method was last modified (using Long date format from Regional Settings) prior to the acquisition. In the case of replicate injections this refers to acquisition of the final injection.
Author	The full name of the user logged in when the method or sequence was created
Batch Description	The description from the acquisition sequence or the description entered for the batch in Reprocess
Batch Group/Name	The group and name inherited from the acquisition sequence or entered in Reprocess
Calibration Date/Time	The date and time when the calibration was performed (or completed)

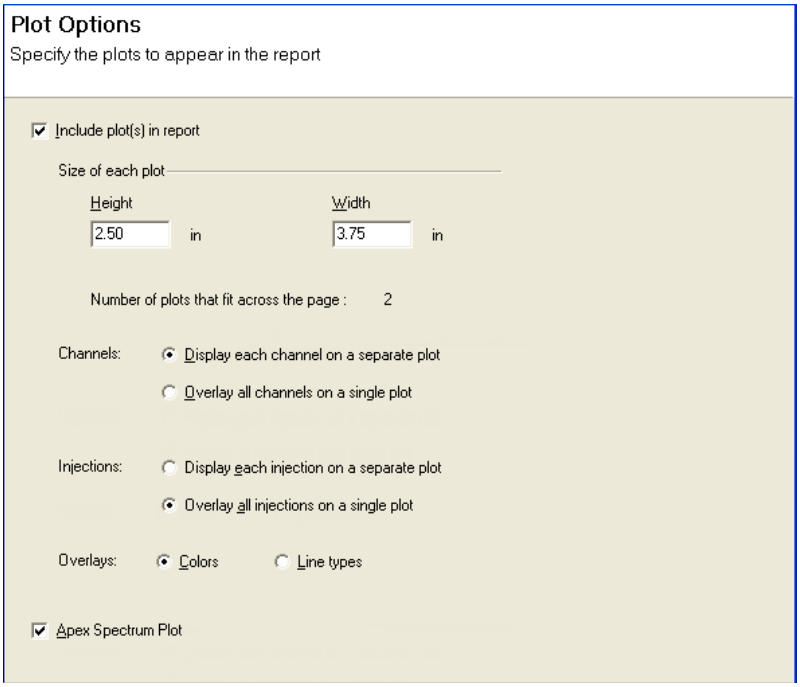
Channel Name	The name of the data channel from which the results were acquired
Chromera Version	The version of the Chromera software generating the report
Created On	Date/Time when method or sequence was created
Dilution Factor	The dilution factor associated with the sample (from Sequence row)
Edited On	Date/Time when method or sequence last edited
Editor	The full name of the user logged in when the method or sequence was last edited
Injection Number	The replicate number for the sample run and the total number of injections made on the sample (n of m)
Injection Volume	The volume of sample injected (from Sequence row)
Instrument Name	The name of the instrument from which the results were acquired
Method Description	The descriptive text associated with the method
Method Group/Name	The name of the method – applies to Calibration and Method reports only
Method Notes	Additional descriptive text associated with the method
Operator	The full name of the user logged in when the data were acquired
OS Version	The version of the operating system at the time of report generation
Processing Method	Group name and Method name for the method process the data and generate current results
Processing Method Last Modified	The date and time the acquisition method was last modified (using Long date format from Regional Settings) prior to processing the data. In the case of replicate injections this refers to processing of the final injection.
Report Date/Time	The date and time of report generation (using Long date format from Regional Settings)
Run Time	The actual run time for acquisition (longest of all data channels plus pump method)
Sample Amount	Value from the sequence row for the sample
Sample Description	Value from the sequence row for the sample
Sample Name	The name associated with the sample (from Sequence row)
Sampling Rate	The rate of data acquisition
Sequence Description	The descriptive text associated with the sequence
Sequence Group/Name	The group and name of the sequence. In the case of a Sample/Summary report this is the sequence (or Single Run batch) used to acquire the data.
Vial Number	The vial from which the sample was taken

Report Format Wizard – Plot Options

This page is displayed when you click the **Next** button from the Data Header page (or click **Plot Options**). This page is only displayed for Sample report types.

The contents of the page depends on the current report type and format. The example below shows the fully populated page, which is displayed only for a replicate sample report for multiple data channels.

NOTE: *The Apex Spectrum Plot will only be valid if a PDA has been included in the current instrument configuration.*



Main Display Area of the Plot Options Page

Controls (in tab order)	**Description**
Include plot(s) in report	Click in the check box to include plots in the report.
Height	Type a value in the text box to set the height of each plot to be included in the report. If there are multiple plots in the report they will all be the same height. The units in which the height is set should match the current paper size (**Letter** or **Legal = in, A4 = cm)**
Width	A text box to set the width of each plot to be included in the report. If there are multiple plots in the report they will all be the same width. The units in which the width is set should match the current paper size (**Letter** or **Legal = in, A4 = cm)**
Number of plots that fit across the page:	A display that indicates how many of the plots defined by the dimensions in the previous controls would fit across the page defined on the Page Setup page. This value is updated whenever a change is made to any of the controls that determine its value.

Channels:	Radio buttons to set how the chromatograms from multiple data channels are handled. These controls only appear for Sample reports that have been defined to include multiple data channels.
Injections:	Radio buttons to set how the chromatograms from multiple replicate injections are handled. These controls only appear for Sample reports that have been defined to include multiple injections.
Overlays:	Radio buttons to set how overlaid chromatograms are distinguished from one another. These controls only appear if either Channels or Injections has the option to overlay chromatograms set.
Apex Spectrum Plot (PDA only)	**NOTE:** The Apex Spectra Plot is created by the Spectral Processing window. It can cover multiple pages depending on the number of spectra associated with the selected chromatogram. Click in the check box to specify if the standard chromatogram plot should be supplemented by Peak Apex Spectrum image provided by the Spectral Processing window. The Apex Spectra Plot will follow, starting on a new page, whatever template pages are selected in the Report Format Wizard. This option will only be valid if a PDA has been included in the current instrument configuration.

Report Format Wizard - Tables

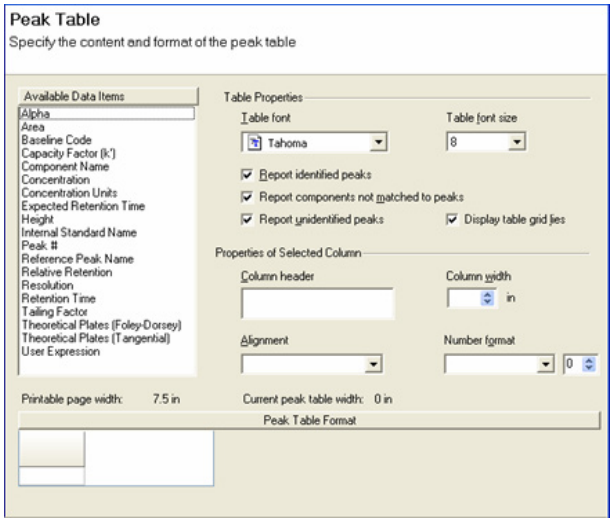
The title and contents of this page depend on the report type and format:

- For Sample and Summary reports the title is **Peak Table**,
- For Calibration the title is **Data Table,** and
- For Sequence the title is **Row Table**.

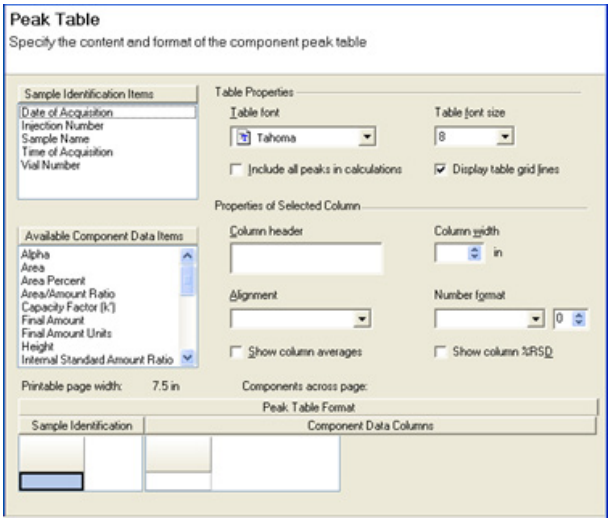
NOTE: *The page does not appear for the Sequence/Detailed report format.*

There are examples of the four major variants of the Table page. The **Sample/Single Injection** and **Sample/Multiple Injections** variants have the same page layout but differ in the data items available for reporting.

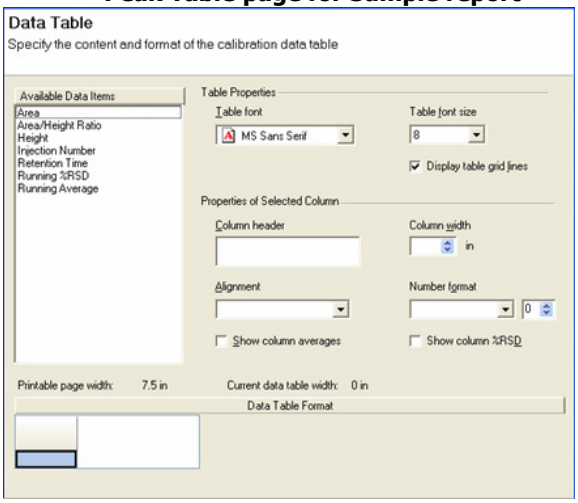
NOTE: *The examples shown here indicate the general layout of the controls on the page.*



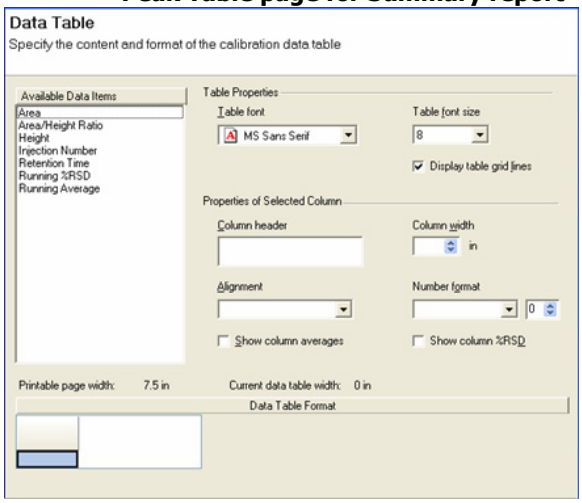
Peak Table page for Sample report



Peak Table page for Summary report



Data Table page for Calibration/Detailed report



Row Table page for Sequence/Table report

Sample/Single Injection Peak Table

Sample/Single Injection Peak Table

The layout of the Peak Table page when creating a new template is shown above.

Control	Description
<Page title>	Peak Table
<Page subtitle/description>	Specify the content and format of the peak table.
Available Data Items	Select the data items from this list box that can be included as columns in the peak table. You define the peak table by moving items from this list to the Peak Table Format grid.

Table font	Select the font (typeface) from this drop-down list to be used for the peak table.
Table font size	Select the size of the font (in pts) from this drop-down list to be used for the peak table.
Report Identified peak	Click in the check box to indicate that the report will include peaks found in the run that were matched to components defined in the processing method.
Report component not matched to peaks	Click in this check box to indicate that the report will include components from the processing method that were not matched with peaks found in the run. Note that unmatched components are not included in the Peak # count.
Report unidentified peaks	Click in this check box to indicate that the report will include peaks found in the run that were not matched to components defined in the processing method.
Display table grid lines	Click in this check box to indicate if the peak table will include borders around each cell.
Column header	A text control which determines the heading (two lines of up to 20 characters each) to appear at the top of the selected column in the peak table.
Column width	Set the width of the selected column in the peak table. The units in which the width is set should match the current paper size (**Letter** or **Legal = in, A4 = cm)**
Alignment	Select from the drop-down to define the alignment of values in the selected column.
Number format	Select from the drop-down to define whether values in the selected column are formatted by number of significant figures or number of decimal places. Disabled (together with caption) when selected data item type is text or integer.
<number>	Select from the drop-down list (or a spin control) to indicate the number of decimal places when Number format = Decimal Places or the number of significant figures when Number format = Significant Figures. Disabled (together with caption) when selected data item type is text or integer.
Printable page width:	A read-only display that indicates the usable space based on the defined page setup settings.
Current peak table width:	A read-only display that indicates the width of the currently defined peak table. The units in which the width is set should match the current paper size (**Letter** or **Legal = in, A4 = cm)**

Peak Table Format	A two-row grid that displays the currently defined peak table. The Column Header text is displayed in the top row for each column and a simplified representation of the data type is displayed in the second row. Nothing is editable directly in the grid.

Peak Table Format Popup Menu

This menu appears when you right click on a cell in the Peak Table Format. The cell clicked on also becomes the selected cell and the Properties of Selected Column controls are updated accordingly.

Command	Description
Add Column	Adds a new blank column to the end of the grid.
Insert Column	Inserts a blank column to the left of the one under the mouse pointer when the right button was clicked.
Delete Column	Deletes the column under the mouse pointer when the right button was clicked. Returns any data items on the deleted column to the list box.

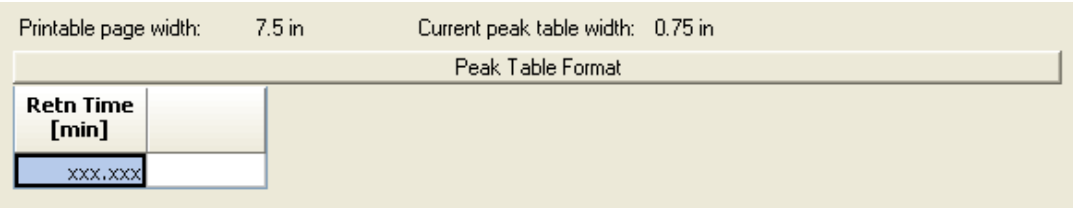
NOTE: *The blank column at the right hand side of the peak table is purely for the purpose of allowing items to be added to the table. When the report is generated that column will not appear. However, a blank column within the peak table is valid and it is rendered when the report is generated (i.e. a blank column may be used to add space between other columns).*

Building the Peak Table

Drag-and-Drop

To add a data item to a specific column in the grid drag it from the list of available items and drop it in the required position. The result is the same regardless of whether you drop the item on the header row or the second row. The header row displays the default column header text for that item and the second row displays a representation of the current item format.

For example, if the Retention Time is dropped onto the empty column (initially there is only one, blank column) the Peak Table Format grid would show:



The 'xxx.xxx' display indicates that the default format for retention time includes three decimal places. Since the column into which the item is dropped becomes the selected column then the controls in the *Properties of Selected Column* group display the default properties for the data item (see below for more details).

- When an item has been added to the peak table it is removed from the list box.
- If you drop an item onto the blank column at the end of the table then a new blank column is appended to the right hand side of the table.
- If you drop an item onto a previously occupied column, the new data item displaces the previous one, which is returned to the list box.
- To reposition an item drag it from one column in the peak table and drop it on another column.
- Remove an item from the peak table by dragging it from the grid and dropping it on the list box (or by deleting the column, which also returns the item to the list box).

Double-Click Operation

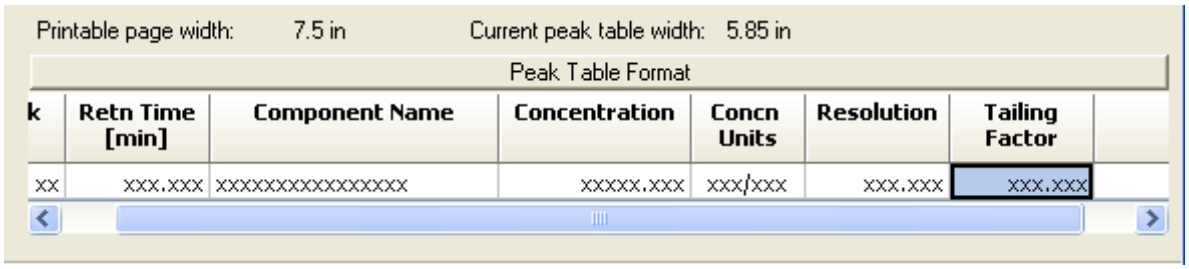
If you double-click on an item in the list box, that item is added to the empty column at the right hand side of the peak table, and a new blank column is added to the table. This is a rapid way to add columns to the table.

Keyboard Operation

If you select an item in the list box and then press the space bar, this acts in the same way as a double-click operation. That is, the item is added to the empty column at the right hand side of the peak table, and a new blank column is added to the table

Peak Table Scrolling

If the number of columns assigned to the peak table exceeds the width allotted to the Peak Table Format area, the grid can scroll below the header bar as necessary. For example:



When an item is added by double-clicking the grid is scrolled automatically, if necessary, to show the new column.

Maximum Table Size

As items are added to the peak table the accumulated column width is maintained and displayed in the 'Current peak table width' field. If adding a column at its default width would make the total peak table width exceed the printable page width then one of two possibilities exists:

1. If there is at least 0.5 in (or 1.27 cm) available (i.e. the minimum allowed column width could be accommodated) then the item is added with that width instead of the normal default width.

2. If there is less than 0.5 in (or 1.27 cm) available then an error message is displayed ('Insufficient space is available for this column') and the item will not be added to the peak table.

Sorting of Peak Tables

All peak tables containing data from a single channel are sorted by retention times and peak or component numbers (as appropriate for the report type) will be assigned on this basis. This includes all single channel reports and multiple channel reports where separate peak tables are used for each channel.

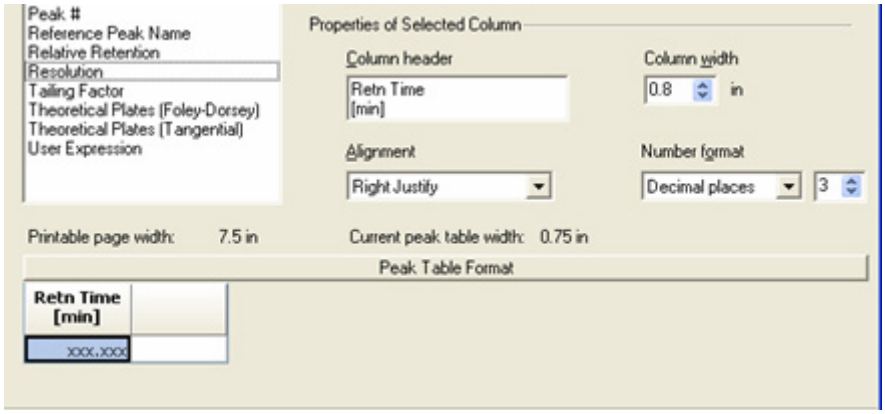
In the case of merged peak tables you can select (within the report template) whether rows are sorted by retention time or component name. This includes both single injection and replicate reports.

Thus the complete definition of sorting for sample reports is:

Report Type	**Sort By**
Single Injection, Single Channel (Plot before or after table)	Retention Time
Single Injection, Multiple Channel, Merged Peak Table (Plot before or after table)	User selected value (RT or component name)
Single Injection, Multiple Channel, Separate Peak Table (Plot before or after table)	Retention Time
Replicate Injection, Single Channel, (Plot before or after table)	Retention Time
Replicate Injection, Multiple Channel, Merged Peak Table (Plot before or after table)	User selected value (RT or component name)
Replicate Injection, Multiple Channel, Separate Peak Table (Plot before or after table)	Retention Time

Properties of Selected Column

The controls within the **Properties of Selected Column** group display the settings for the currently selected column. When no columns exist they are blank. When a data item is added to the peak table these controls will display the default settings for that item. For example, if the Retention Time item is added the display would be



The display of the Retention Time column header will reflect the text in the **Column header** control, and the representation of the RT in the second row will reflect the settings of the **Alignment** and **Number format** controls. The actual width of the column in the Peak Table Format display reflects the value set in the **Column Width** control. The default values for all available data items are defined below.

Where column properties are not applicable to the current data item then those controls (and the associated caption) are disabled. For example, when Component Name or Peak # is the selected column, the **Number format** controls are disabled.

The Peak Table Format display also reflects the settings for **Table font** and **Table font size**. The column header is also always shown in **Bold** style and is centered in the header cell, regardless of the alignment set for the data item itself.

NOTE: *The screen display will not match precisely the printed (or stored) output. The intent here is to enable you to see a close approximation to the final output.*

Modifying Column Properties

You can modify any of the displayed properties for the selected column. Changes to all controls (Column header, Column Width, Alignment and Number format – both drop-down list and spin control) will be reflected in the grids display immediately

The modified properties remain in effect only while the data item is included in the peak table. If a data item is deleted from the peak table (returned to the list box) and then subsequently re-added to the peak table; it is restored will its default settings.

Data Items Available for Single Injection Peak Table

The data items available for inclusion as columns in the peak table for a single injection Sample report are described below.

Data Item	Description
Absorbance Ratio	The ratio of the absorbance at two user defined wavelengths. Can be used for component confirmation.
Alpha	Relative retention of the peak to the previous named peak (System Suitability value).
Area	Area measured for the peak.

Area Percent	Area of the peak expressed as percentage of total area for all peaks in the run (channel).
Area/Amount Ratio	Ratio of the peak area to its Final Amount.
Baseline Type	The codes indicating baseline treatment at the start and end of the peak.
Capacity Factor (k')	Corrected RT ratioed to unretained peak time (System Suitability value).
Calibration +/- Range	Flag to show if peak are is above or below range covered by calibration standards.
Channel Name	The name of the data channel in which a peak was found (or Analyte associated with Species for ICP-MS).
Component Name	The name of the component the peak has been matched with.
Expected Retention Time	The retention time defined in the method at which the component peak was expected to elute.
Final Amount	The amount calculated for the component, including dilution factor, multiplier, normalization factor, etc.
Final Amount Units	The units for the final amount as entered in the method.
Height	Height measured for the peak.
Internal Standard Amount Ratio	The ratio of the component amount to the related internal standard amount. This data type applies only to peaks that have been identified and quantified based on the internal standard method. The amount ratio is always based on raw amounts, never on adjusted amounts.
Internal Standard Name	The name of the component specified as the internal standard for the current component.
Internal Standard Response Ratio	The ratio of a peak's response to the response of the related internal standard component. This data type applies only to peaks that have been identified and quantified based on the internal standard method.
Normalized Area Percent	The peak's percentage contribution to the total area for all peaks (for which results are reported). These percentages add up to the normalization factor specified in the sequence file.
Normalized Amount	The adjusted amount of the peak expressed as a percentage of the total reported amounts. These percentages add up to the normalization factor specified in the sequence.
Peak Library Search	The component name plus a hit value resulting from a search in a user specified spectral library on each peak apex spectrum in the chromatogram.
Peak Purity	An estimate of the probability that the peak is contaminated with a co-eluting component as determined using the spectral information across

	the peak.
Peak #	Number of the current peak within the set being reported.
Peak Width @5% Height	The width of the peak at 5% of its height (System Suitability value).
Peak Width @10% Height	The width of the peak at 10% of its height (System Suitability value).
Peak Width @50% Height	The width of the peak at 50% of its height (System Suitability value).
Peak Width @Base	The width of the peak at its base System Suitability value).
Raw Amount	The amount calculated for the component directly from the calibration curve, before the adjustments made for dilution factor, multiplier, normalization factor, etc.
Reference Peak Name	The name of the component specified as the time reference peak for the current component.
Relative Retention	The ratio of the peak retention time to the component specified as the 'RRT Component' in the method.
Resolution	Resolution between current peak and previous peak (System Suitability value).
Retention Time	The time into the run at which the peak eluted.
Spectral Library Confirmation	The hit value plus a Pass/Fail flag for the comparison between a peak apex spectrum of an identified peak in the sample to the spectrum with the same name in a user specified spectral library
Spectral Standard Confirmation	The Absorbance Index plus a Pass/Fail flag for the comparison between the peak apex spectrum of an identified peak in the sample to the peak apex spectrum of the peak with the same name in a user defined standard chromatogram.
Tailing Factor	Measure of peak asymmetry (System Suitability value).
Theoretical Plates (Foley-Dorsey)	Measure of column efficiency for this peak (System Suitability value).
Theoretical Plates (Tangential)	Measure of column efficiency for this peak (System Suitability value).
User Expression	A value calculated according to a user defined equation.
Wavelength Maximum	The wavelength of the maximum absorbance in the peak apex spectrum. Can be used for both component confirmation and for developing a wavelength program.

User Expressions

In addition to the standard data items available for reporting, users can create their own expressions for reporting calculated values. A user expression can be reported in a column of the peak table in the same way as any standard numeric item. The user expression is evaluated for each reported peak in the table. By definition a user expression is a numeric value and has the properties of a numeric value, including **Number format**.

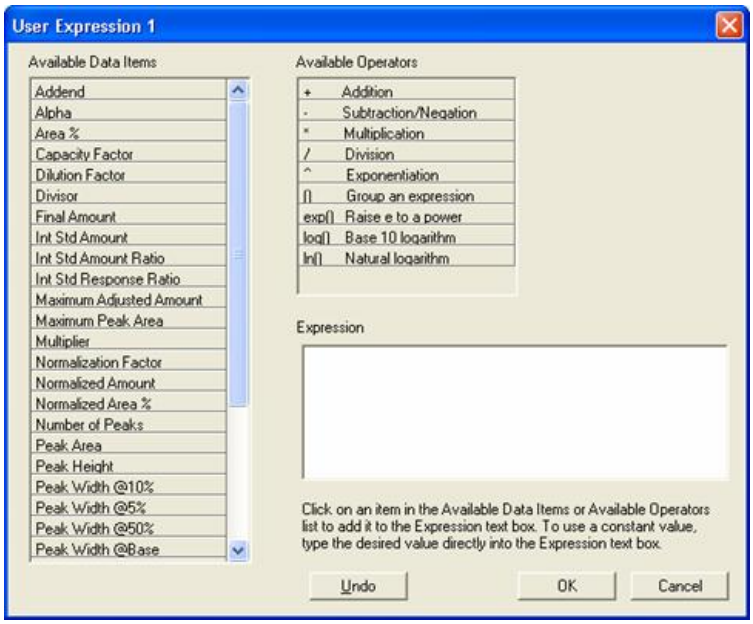
- Any of the standard data items listed in the table above. Only data items associated with the current peak is accessible.
- Any of the following operators: Addition, Subtraction, Multiplication, Division, Exponentiation, Raise e to a Power, Base 10 Logarithm, Base e Logarithm and Brackets (to group sub-expressions).
- Constant numeric values
- Any of the additional data values defined in the table below.

Data Item	**Description**
Addend	From the sequence row associated with the run (i.e. the same value for all peaks)
Dilution Factor	From the sequence row associated with the run (i.e. the same value for all peaks)
Divisor	From the sequence row associated with the run (i.e. the same value for all peaks)
Maximum Final Amount	The largest Final Amount value calculated for all peaks located in the channel/run
Maximum Peak Area	The largest Peak Area value for all peaks located in the channel/run
Maximum Peak Height	The largest Peak Height value for all peaks located in the channel/run
Multiplier	From the sequence row associated with the run (i.e. the same value for all peaks)
Normalization Factor	From the sequence row associated with the run (i.e. the same value for all peaks)
Number of Peaks	The total number of peaks found in the channel/run
Sample Amount	From the sequence row associated with the run (i.e. the same value for all peaks)
Total Final Amount	The total of all Final Amount values calculated for all peaks in the channel/run
Total Peak Area	The total of all Peak Area values calculated for all peaks in the channel/run
Unretained Peak Time	The Unretained Peak Time from the method for the channel

NOTES: ***(1)*** *For reports where multiple channels are reported in separate tables, the Maximum and Total values are those from the channel. Where channels are merged into a single peak table, the Maximum and Total values are those from the merged peak set.* ***(2)*** *For reports where multiple channels are reported in separate tables, the Unretained Peak Time is that from the appropriate channel. Where channels are merged into a single peak table, the Unretained Peak Time is that from the (first) channel in the method having a 'Time Offset' value equal to zero.*

User Expression Editor Dialog

When you add a User Expression to the peak table, the User Expression Editor dialog is displayed.



User Expression Editor dialog for a new expression

Note that the title bar of the dialog displays the index number associated with the user expression.

Control	Description
Available Data Items	A button list that displays all the data items available for inclusion in a user expression.
Available Operators	A button list that displays all the operators available for inclusion in a user expression.
	+ Addition - Subtraction/Negation * Multiplication / Division ^ Exponentiation () Group an expression exp() Raise e to a power log() Base 10 logarithm ln() Natural logarithm

Expression	A edit box that displays the user expression. When this field has focus a text insertion point cursor is displayed (by default at the end of the current contents of the edit box).
Undo	A command button that reverses the last action you made.

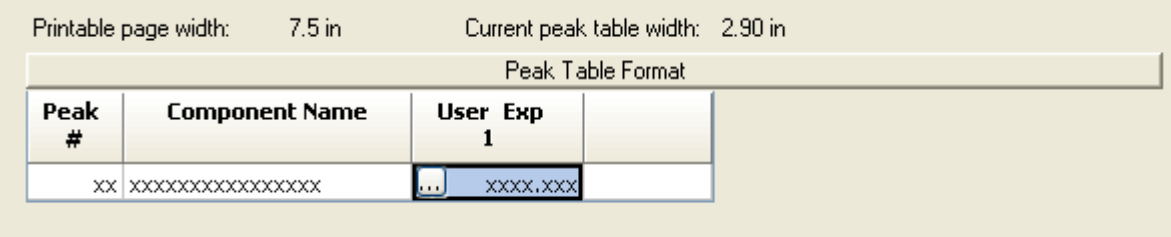
User Expression Editor Dialog

Control	Description
Available Data Items	Clicking on an item in the list will add it to the Expression edit box within square brackets, at the current position of the insertion point. For example: [Peak Width@10%] The insertion point will then be positioned following the closing bracket of the data item term. The list remains unchanged, since any data item can be included multiple times in an expression.
Available Operators	Clicking on an item in the list will add it to the Expression edit box at the current position of the insertion point. If the operator includes brackets ( ) then the insertion point is positioned within the brackets after the operator has been added to the Expression box, otherwise the insertion point is positioned at the end of the expression text.
Expression	The user can type freely in this edit box, although anything other than a numeric constant entry is liable to cause the expression to be invalidated. It is distinctly preferable that user entry of operators (and even data items) by typing in the appropriate characters (with complete accuracy) be accepted by the syntax check (see below). However, it would be acceptable if a third-party control is utilized for this function and it does not support such a feature. The user can move the insertion point within the edit box using the left and right arrows (and up and down arrows if this can be supported).
Undo	For example, undo would remove a data item just added to the expression or a constant value just typed into the Expression box.

OK	When this button is clicked the expression is parsed for validity. If its syntax is correct then the dialog will close and the defined user expression is associated with the current peak table column. If the syntax is not correct then an error message is displayed and the insertion point is positioned at the point where the problem was found. The error message should provide as clear an indication of the error as practicable. That is, a single error message "Syntax error" (or similar) is inadequate. If the text between square brackets is found not to match one of the defined data items then the message "Invalid data item" (or similar) is displayed and the insertion point should be located within the brackets. This same message would be displayed if the user accidentally deleted an ending square bracket. If a ending square bracket is immediately followed by an opening square bracket, or a numeric constant, then the message "Missing operator" (or similar) is displayed. If two operators appear in succession then the error message "Operator follows operator" (or similar) is displayed. If any extraneous non-numeric characters (i.e. that do not represent a data item and operator or a constant value) appear in the expression then the error message "Invalid characters in expression" (or similar) is displayed.
Cancel	When this button is clicked the dialog is closed. If the dialog was displayed for a newly added expression then the expression is voided and the column in the peak table will remain blank. If the dialog was displayed for editing an existing expression then the expression will remain unchanged.

User Expression Column

When a User Expression has been successfully added to the peak table grid it appears as follows:



The default column header shows **User Expr** followed by the index number of the expression. The user can, of course, change this to any valid header text.

The default example representation of the expression value is **xxx.xxx** (as shown in the table in section Data items Available for Page Header/Footer) and is shown right justified in the cell.

The cell also contains a button labeled with an ellipsis. The button is shown on the left hand side of the cell in the example above but it could be on the right hand side as long as it does not interfere with the example representation of the expression value.

When the button is clicked, the User Expression Editor dialog is displayed, with the expression itself (i.e. the text string making up the expression) shown in the Expression edit box. The user may then edit the expression as desired.

Sample/Multiple Injection Peak Table

The Peak Table page for the Sample/Multiple Injection report formats is identical to that of the Sample/Single Injection page, with the exception of the contents of the Available Data Items list. There are two basic kinds of value available:

- Average values from all injections for numeric quantities such as Retention Time, Area, etc.
- Percent relative standard deviation values for numeric quantities such as Retention Time, Area, etc. The user documentation should warn the user that these results only have statistical validity when the number of replicate injections exceeds nine.

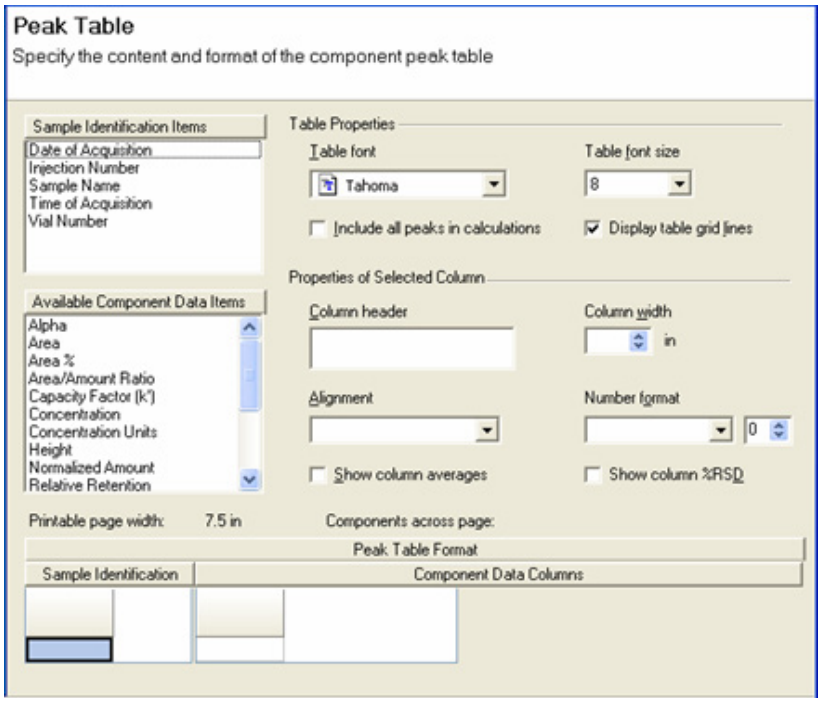
NOTE: *When data from only a single injection are available, average and RSD values will (obviously) not be shown and N/A will be displayed instead, to indicate a problem with the data.*

Summary Peak Table

Although the table in a summary report is still termed a peak table it differs from a sample report peak table in three important ways:

- A row in a sample report reports the results for a single peak (either a single injection or the average of several injections). A row in a summary report reports results for a single sample.
- A summary report includes results from identified peaks only.
- The summary report includes a set of columns that are repeated for each identified peak in the run. This frequently results in the table wrapping (as described in Summary Reports).

The layout of the Peak Table page when creating a new template is shown below.



The key difference between this page and the Sample Peak Table page is that the Summary version has a peak table consisting of two sections: Columns that identify the sample on the left and a set of columns of peak data, which are repeated for each identified peak in the channel or run, on the right.

Although some of the controls on this page are identical in form and behavior to controls on the Sample Peak Table page, the entire page is described in the table below for completeness.

Control	**Description**
<Page title>	Peak Table
<Page subtitle/description>	Specify the content and format of the component peak table.
Sample Identification Items	Select the data items in this list box that can be included as columns in the sample identification section of the table. You can define the section by moving items from this list to the Sample Identification grid in the Peak Table Format area.
Available Component Data Items	Select the data items in this list box that can be included as columns in the component section peak table. You can define the section by moving items from this list to the Component Data Columns grid in the Peak Table Format area.
Table font	Select the font (typeface) from this drop-down list to be used for the peak table.
Table font size	Select the size of the font (in pts) from this drop-down list to be used for the peak table.

Include all peak in calculations	Click in this check box to indicate that the values calculated for data items will include results from all peaks found in the run not just from the reported identified peaks.
Display table grid lines	Click in this check box to indicate if the peak table will include borders around each cell.
Column header	A text control which determines the heading (two lines of up to 20 characters each) to appear at the top of the selected column in the peak table.
Column width	Set the width of the selected column in the peak table. The units in which the width is set should match the current paper size (**Letter** or **Legal = in, A4 = cm)**
Alignment	Select from the drop-down list to define the alignment of values (**Left Justify, Center, Right Justify)** in the selected column.
Number format	Select from the drop-down to define whether values in the selected column are formatted by number of significant figures or number of decimal places. Disabled (together with caption) when selected data item type is text or integer.
<number>	Select from the drop-down list (or a spin control) to indicate the number of decimal places when Number format = Decimal Places or the number of significant figures when Number format = Significant Figures. Disabled (together with caption) when selected data item type is text or integer.
Show column averages	Click in this check box to indicate if average results are displayed at the foot of each column, in an Averages row. When average values are calculated they are printed with the same numeric format as defined for the column they are derived from.
Show column %RSD	Click in this check box to indicate if the %RSD results are displayed at the foot of each column, in an Averages row. %RSD values will be printed to 3 decimal places.
Printable page width:	A read-only display that indicates the usable space based on the defined page setup settings.
Components across page:	A read-only display that indicates the number of component column sets (which are always kept together as a set) will fit across the page. It depends upon the page setup settings, the sample identification column settings and the component column settings.

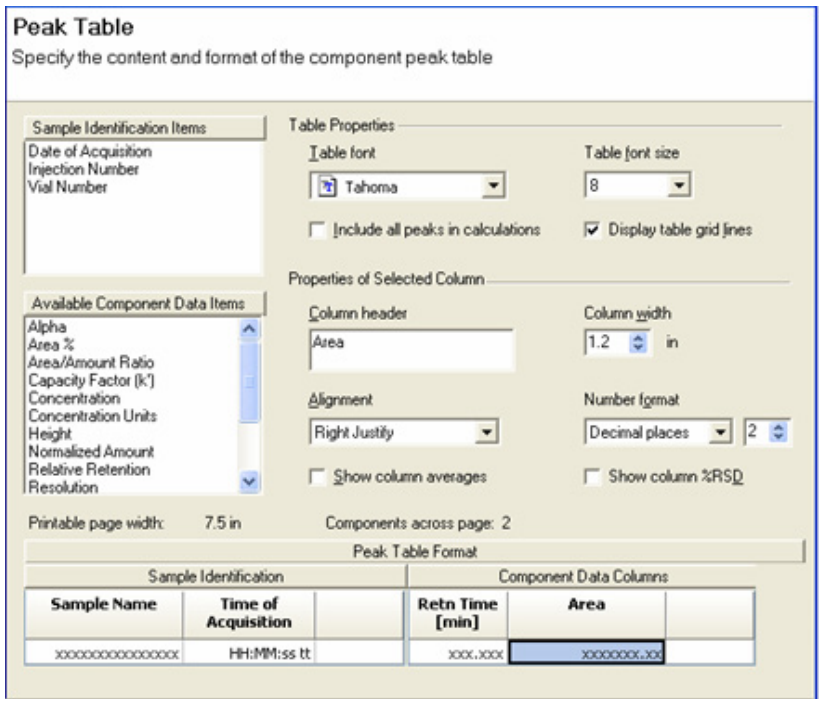
Peak Table Format – Sample Identification	A two-row grid that displays the currently defined sample identification section of the peak table. The Column Header text is displayed in the top row for each column and a simplified representation of the data type is displayed in the second row. Nothing is editable directly in the grid.
Peak Table Format – Component Data Columns	A two-row grid that displays the currently defined component data section of the peak table. The Column Header text is displayed in the top row for each column and a simplified representation of the data type is displayed in the second row. Nothing is editable directly in the grid.

Building the Summary Peak Table

The basic approach to building the **Summary Peak Table** is the same as for the Sample Peak Table. The drag-and-drop, double-click, and keystroke techniques described for the Sample Peak Table page are all applicable here. The key difference is that data items from the Sample Identification list can only be dropped on the **Sample Identification** grid and items from the Available Component Data Items list can only be dropped on the **Component Data Columns** grids. Similarly, the double click and keystroke actions on each list adds the data item to the appropriate grid.

NOTE: *If you does not select at least one data item from the Sample Identification list and also at least one item from the Component Data list then no table will be generated when the report is rendered.*

The Components across page readout is updated whenever a column is added to or removed from either grid, or when a column width is modified.



User Expressions are handled exactly the same way on the Summary Peak Table as for Sample reports.

Peak Table Scrolling

When the space occupied by the defined columns exceeds the available screen width, both grids are scrolled together, along with their individual headers, as shown below.

Data Items Available for Summary Peak Table

The data items available for inclusion as columns in the Sample Identification section of the Summary report peak table are:

Data Item	Description
Date of Acquisition	Date the injection reported on the current row was acquired.
Injection Number	The original injection number of the sample.
Sample Name	The name associated with the sample (from the sequence row).
Time of Acquisition	Time the injection reported on the current row was acquired.

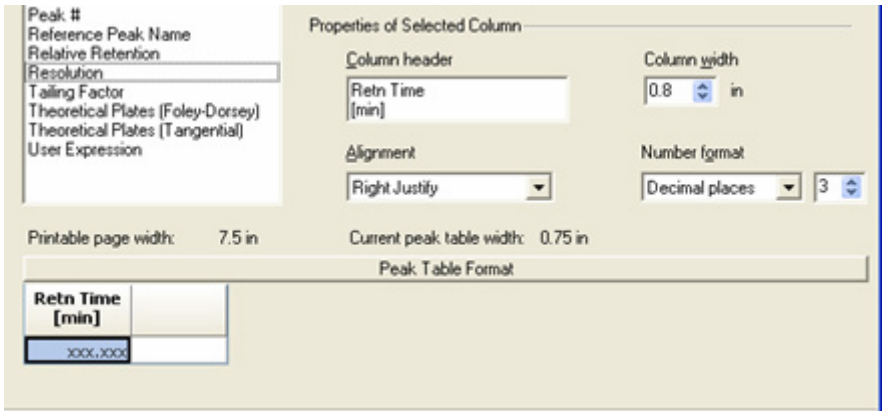
Vial Number The number of the vial from which the sample was injected.

The data items available for inclusion as columns in the Component Data section of the peak table for a Summary report are a subset of the items available in Sample reports.

NOTE: *When data from only a single injection are available for calculation, average and RSD values will not be shown and N/A will be displayed instead.*

Properties of Selected Column

The controls within the **Properties of Selected Column** group display the settings for the currently selected column. When no columns exist they are blank. When a data item is added to the peak table these controls will display the default settings for that item. For example, if the Retention Time item is added the display would be



The display of the Retention Time column header will reflect the text in the **Column header** control, and the representation of the RT in the second row will reflect the settings of the **Alignment** and **Number format** controls. The actual width of the column in the Peak Table Format display reflects the value set in the **Column Width** control. The default values for all available data items are defined below.

Where column properties are not applicable to the current data item then those controls (and the associated caption) are disabled. For example, when Component Name or Peak # is the selected column, the **Number format** controls are disabled.

The Peak Table Format display also reflects the settings for **Table font** and **Table font size**. The column header is also always shown in **Bold** style and is centered in the header cell, regardless of the alignment set for the data item itself.

NOTE: *The screen display will not match precisely the printed (or stored) output. The intent here is to enable you to see a close approximation to the final output.*

Modifying Column Properties

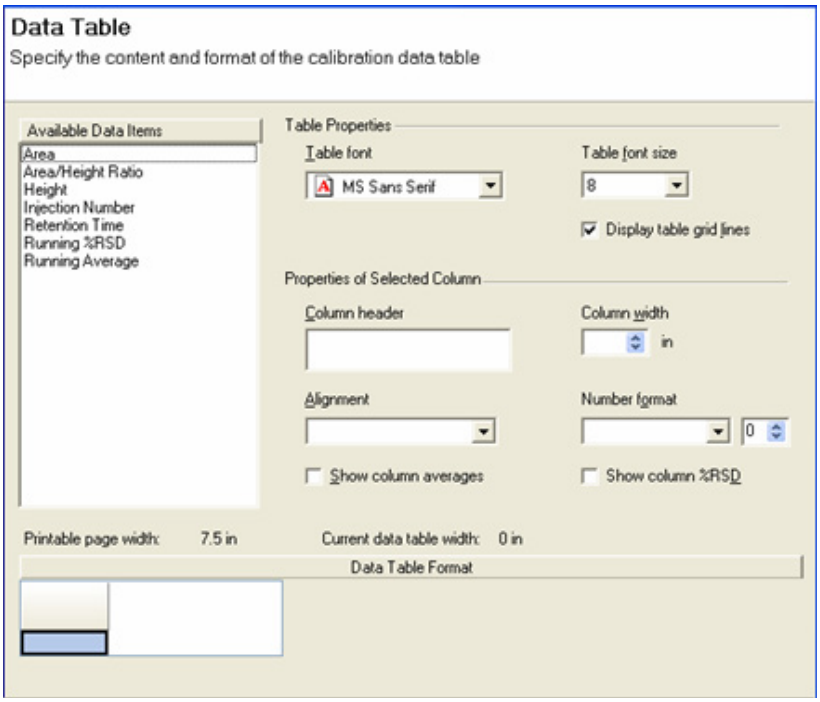
You can modify any of the displayed properties for the selected column. Changes to all controls (Column header, Column Width, Alignment and Number format – both drop-down list and spin control) will be reflected in the grids display immediately

The modified properties remain in effect only while the data item is included in the peak table. If a data item is deleted from the peak table (returned to the list box) and then subsequently re-added to the peak table; it is restored will its default settings.

Calibration Data Table

Calibration Data Table

On this page the table is reporting calibration data (one table for each standard level of each calibrated component) and hence is not termed a **Peak Table** but a **Data Table**. The page title and contents are labeled accordingly:



In form the Calibration peak table page is a combination of the Sample (single Available Data Items list and Table Format grid subset of Table Properties) and Summary (Properties of Selected Column) controls.

Control	**Description**
<Page title>	Data Table
<Page subtitle/description>	Specify the content and format of the calibration data table.
Available Data Items	Select the data items from this list box that can be included as columns in the data. You can define the peak table by moving items from this list to the Data Table Format grid.
Table font	Select the font (typeface)from this drop-down to be used for the calibration data table.
Table font size	Select the size of the font (in pts) from this drop-down list to be used for the calibration data table.
Display table grid lines	Click in this check box to indicate if the peak table will include borders around each cell.

Column header	A text control which determines the heading to appear at the top of the selected column in the peak table. It can be two lines of up to 20 characters each.
Column width	Set the width of the selected column in the peak table. The units in which the width is set should match the current paper size (**Letter** or **Legal = in** (from 0.5 to 6.0 in)**, A4 = cm** (from 1.0 to 15.0 cm)
Alignment	Select from the drop-down list the alignment **(Left Justify, Center**, **Right Justify)** of values in the selected column.
Number format	A drop-down list that determines whether values in the selected column is formatted by number of significant figures or number of decimal places. Disabled (together with caption) when selected data item type is text or integer.
<number>	Select from the drop-down list (or a spin control) to indicate the number of decimal places when Number format = Decimal Places or the number of significant figures when Number format = Significant Figures. Disabled (together with caption) when selected data item type is text or integer.
Printable page width:	A read-only display that indicates the usable space based on the defined page setup settings.
Current data table width:	A read-only display that indicates the width of the currently defined data table. The units in which the width is set should match the current paper size (**Letter** or **Legal = in, A4 = cm)**
Data Table Format	A two-row grid that displays the currently defined data table. The Column Header text is displayed in the top row for each column and a simplified representation of the data type is displayed in the second row. Nothing is editable directly in the grid.

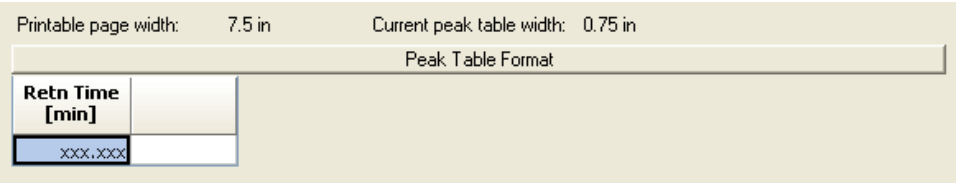
NOTE: *The display in the Data Table Format area has an associated popup menu identical to that described in Peak Table Format Popup Menu*

Building the Data Table

Drag-and-Drop

To add a data item to a specific column drag it from the list of available items and drop it in the required position. The result is the same regardless of whether you drop the item on the header row or the second row. The header row displays the default column header text for that item and the second row displays a representation of the current item format.

For example, if the Retention Time is dropped onto the empty column (initially there is only one, blank column) the Table Format would show:



The 'xxx.xxx' display indicates that the default format for retention time includes three decimal places. Since the column into which the item is dropped becomes the selected column then the controls in the *Properties of Selected Column* group display the default properties for the data item (see below for more details).

- When an item has been added to the table it is removed from the list box.
- If you drop an item onto the blank column at the end of the table then a new blank column is appended to the right hand side of the table.
- If you drop an item onto a previously occupied column, the new data item displaces the previous one, which is returned to the list box.
- To reposition an item drag it from one column in the peak table and drop it on another column.
- Remove an item from the peak table by dragging it from the grid and dropping it on the list box (or by deleting the column, which also returns the item to the list box).

Double-Click Operation

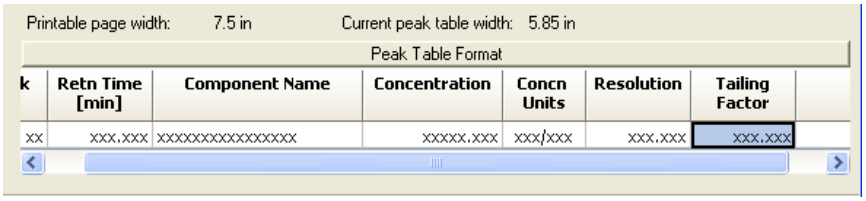
If you double-click on an item in the list box, that item is added to the empty column at the right hand side of the table, and a new blank column is added to the table. This is a rapid way to add columns to the table.

Keyboard Operation

If you select an item in the list box and then press the space bar, this acts in the same way as a double-click operation. That is, the item is added to the empty column at the right hand side of the peak table, and a new blank column is added to the table

Peak Table Scrolling

If the number of columns assigned to the peak table exceeds the width allotted to the Table Format area, you can scroll below the header bar as necessary. For example:



When an item is added by double-clicking the grid is scrolled automatically, if necessary, to show the new column.

Maximum Table Size

As items are added to the table the accumulated column width is maintained and displayed in the 'Current peak table width' field. If adding a column at its default width would make the total peak table width exceed the printable page width then one of two possibilities exists:

1. If there is at least 0.5 in (or 1 cm) available (i.e. the minimum allowed column width could be accommodated) then the item is added with that width instead of the normal default width.

2. If there is less than 0.5 in (or 1 cm) available then an error message is displayed ('Insufficient space is available for this column') and the item will not be added to the table.

Data Items Available for Data Table

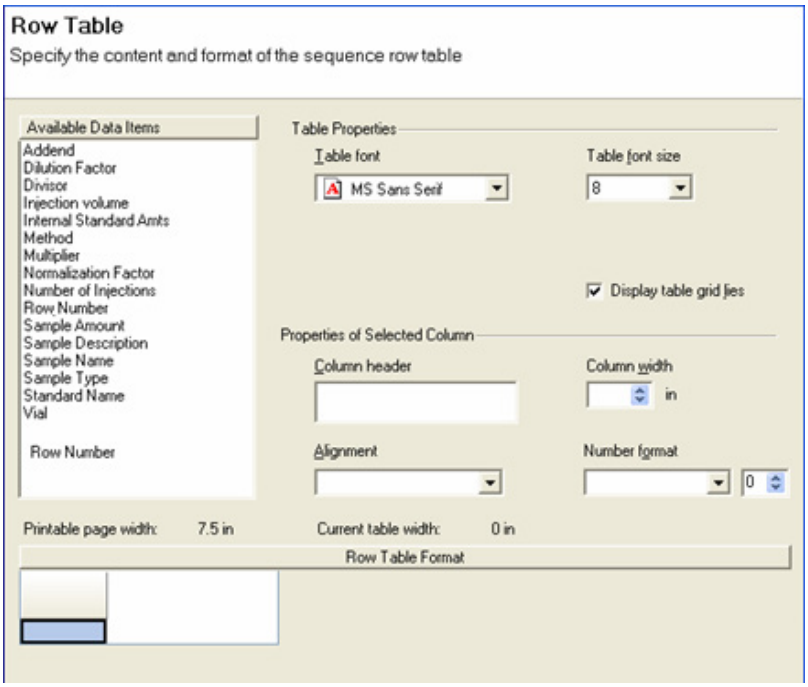
The data items available for inclusion as columns in the data table for a detailed Calibration report are listed in the table below:

Data Item	Description
Area	Area measured for the current component peak.
Area/Height Ratio	Ratio of area measured for the current component peak to its height.
Acquisition Date	Date the current calibration standard injection was acquired.
Height	Height measured for the current component peak
Injection Number	An index for each injection made of a particular standard. This is not necessarily the injection number as originally reported since any calibration level could contain injections from more than one series of calibrations (i.e. resulting from more than one sequence row).
Internal Standard Area	The area measured for the associated internal standard peak.
Internal Standard Area Ratio	The ratio of a peak's area to the area of the associated internal standard peak.
Internal Standard Concentration Ratio	The ratio of the component amount to the related internal standard amount at the current calibration level.
Internal Standard Height	The area measured for the associated internal standard peak.
Internal Standard Height Ratio	The ratio of a peak's height to the height of the associated internal standard peak.
Internal Standard Name	The name of the component specified as the internal standard for the current component peak.
Retention Time	The time into the run at which the current component peak eluted.
Running Average (Area)	The average area for all rows up to the current row, for the current component peak.
Running Average (Height)	The average height for all rows up to the current row, for the current component peak.
Sample Name	The name associated with the sample (from Sequence row)
Time of Acquisition	Time the current calibration standard injection was acquired.

NOTES: *(1) Average and %RSD values are only calculated for Retention Time, Area, Height and Area/Height Ratio data. The check boxes, Show column averages and Show column %RSD are disabled (and unchecked) when any other data column is selected in the table. (2) When data from only a single injection are available for calculation, average and RSD values are not shown and N/A is displayed instead.*

Sequence Row Data Table

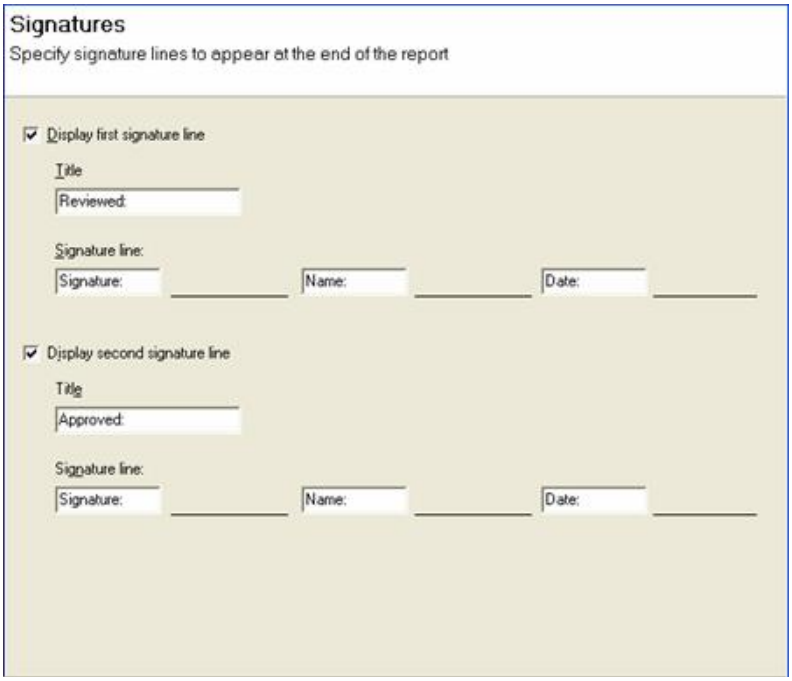
On this page the table is reporting sequence row information and hence is not termed a **Peak Table** but a **Row Table**. The page title and contents is labeled accordingly:



This page is a simple subset of the Sample Peak Table page and the controls exhibit all the same behaviors. Only the available data items are unique to this page.

Report Format Wizard – Signatures

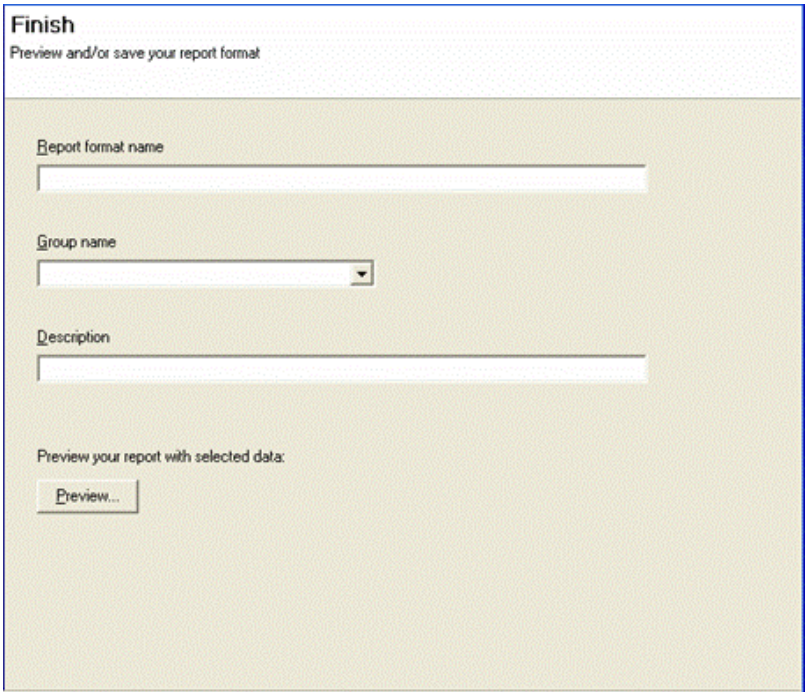
This page is displayed for all report types, to provide up to two signature lines for report review and/or approval.



Control	Description
Display first signature line	Click in the check box to indicate if the first signature line is included in the report.
Title	Enter the title to appear above the first signature line. You can use up to 25 characters.
Signature line:<first field>	Enter the text to appear preceding the first entry space on the signature line. You can use up to 15 characters.
Signature line:<second field>	Enter the text to appear preceding the second entry space on the signature line. You can use up to 15 characters.
Signature line:<third field>	Enter the text to appear preceding the third entry space on the signature line. You can use up to 15 characters.
Display second signature line	Click in the check box to indicate if the second signature line is included in the report.
Title	Enter the title to appear above the second signature line. You can use up to 25 characters.
Signature line:<first field>	Enter the text to appear preceding the second entry space on the signature line. You can use up to 15 characters.
Signature line: <second field>	Enter the text to appear preceding the second entry space on the signature line. You can use up to 15 characters.
Signature line: <third field>	Enter the text to appear preceding the third entry space on the signature line. You can use up to 15 characters.

Report Format Wizard – Finish

This page is always displayed as the final page of every report type.



Here you can preview the report, if desired, and save the generated format to the Chromera database for subsequent use.

Control	Description
Report format name	Enter the name (up to 25 characters) associated with the report format.
Group name	Enter (or select) the report format (up to 25 characters) that you want to assign to an existing group or to a new group. To use an existing group type in the group name or select it from the drop-down list. To create a new group and assign the method to it the user types in a group name that does not already exist.
Description	Enter a description of the report format (up to 50 characters).
Preview…	Click this button to display a preview of the report showing example data.
Finish	Click this button to save the report format to the database and close the Report Format Wizard.

Data Selector

About Data Selector

The Data Selector enables you to locate the required data set(s) based on your current environment (Method, Sequence, Post Run, Reprocess, etc.) either by browsing the database or by searching for specific values in key data fields. These are not really separate modes; rather the search capabilities allow you to filter the list of data sets being browsed. The ability to sort and group the displayed list of data sets further assists you in finding the required data set(s). The Data Selector displays whenever you select one or more data sets. Commands that display the Data Selector include:

- Open **Method** from the **File** menu
- Open **Sequence** from the **File** menu
- Open **Data** from the **File** menu
- **Reprocess** from the **Tools** menu
- **View Current Session Logs** from the **Tools** menu
- **View Retrieved Archived Logs** from the **Tools** menu

When browsing, the default sort order (i.e. the order when the Data Selector is displayed) with the most recently edited (or created in the case of data) items appears at the top of the list.

When selecting data, batches are sorted in inverse order of Batch Created Date/Time (i.e. newest first) and within each batch the samples are sorted in order of Acquisition Date/Time (i.e. oldest first).

About Methods and Sequences

The browse pane consists of a list of data sets of the type to be selected. However, the data displayed are not a simple list but include grouping ability, in addition to simple sorting.

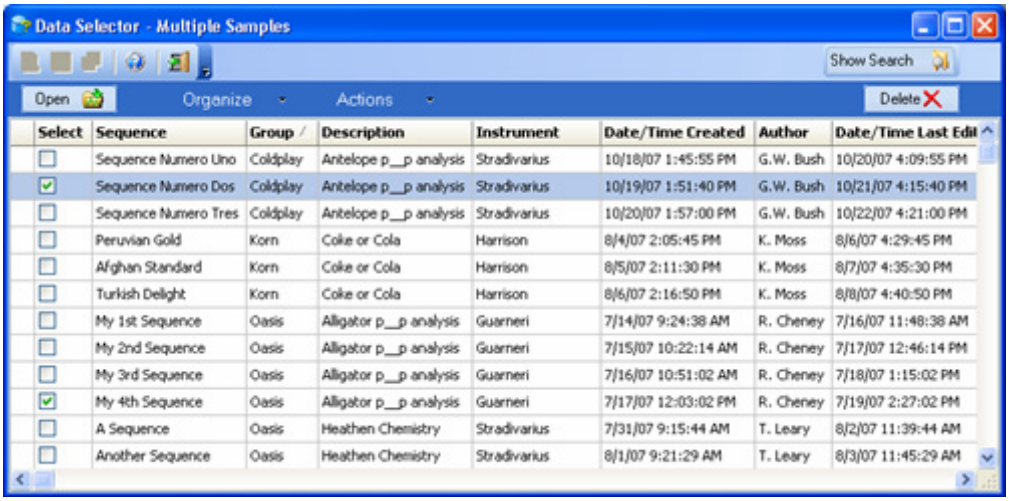
Browsing and Sorting

Methods and sequences can be stored under a **Group** name, so the initial level of browsing is using a group list, where each group can be expanded to show the methods or sequences assigned to it. It is also possible to group methods or sequences by Author or Editor name.

It is also possible to select a display that eliminates the Groups and shows all methods or sequences in a continuous list. It is possible to sort the displayed list of methods or sequences, in ascending or descending order, on any field shown. The displayed fields are: Name, Group , Description, Created Date/Time, Last Edited Date/Time, Author, and Editor.

The list can also be grouped by **Author** or **Editor**. Only a single level of grouping is supported.

By default Sequences and Methods are listed by group; however, you can use the **Organize** menu to turn off grouping and display all Sequences and Methods as a continuous list, as shown in the next example screen.



Select	Sequence	Group	Description	Instrument	Date/Time Created	Author	Date/Time Last Edit
☐	Sequence Numero Uno	Coldplay	Antelope p__p analysis	Stradivarius	10/18/07 1:45:55 PM	G.W. Bush	10/20/07 4:09:55 PM
☑	Sequence Numero Dos	Coldplay	Antelope p__p analysis	Stradivarius	10/19/07 1:51:40 PM	G.W. Bush	10/21/07 4:15:40 PM
☐	Sequence Numero Tres	Coldplay	Antelope p__p analysis	Stradivarius	10/20/07 1:57:00 PM	G.W. Bush	10/22/07 4:21:00 PM
☐	Peruvian Gold	Korn	Coke or Cola	Harrison	8/4/07 2:05:45 PM	K. Moss	8/6/07 4:29:45 PM
☐	Afghan Standard	Korn	Coke or Cola	Harrison	8/5/07 2:11:30 PM	K. Moss	8/7/07 4:35:30 PM
☐	Turkish Delight	Korn	Coke or Cola	Harrison	8/6/07 2:16:50 PM	K. Moss	8/8/07 4:40:50 PM
☐	My 1st Sequence	Oasis	Alligator p__p analysis	Guarneri	7/14/07 9:24:38 AM	R. Cheney	7/16/07 11:48:38 AM
☐	My 2nd Sequence	Oasis	Alligator p__p analysis	Guarneri	7/15/07 10:22:14 AM	R. Cheney	7/17/07 12:46:14 PM
☐	My 3rd Sequence	Oasis	Alligator p__p analysis	Guarneri	7/16/07 10:51:02 AM	R. Cheney	7/18/07 1:15:02 PM
☑	My 4th Sequence	Oasis	Alligator p__p analysis	Guarneri	7/17/07 12:03:02 PM	R. Cheney	7/19/07 2:27:02 PM
☐	A Sequence	Oasis	Heathen Chemistry	Stradivarius	7/31/07 9:15:44 AM	T. Leary	8/2/07 11:39:44 AM
☐	Another Sequence	Oasis	Heathen Chemistry	Stradivarius	8/1/07 9:21:29 AM	T. Leary	8/3/07 11:45:29 AM

You can use the **Organize** menu to select which columns are available for display. From this screen you can also perform the following tasks:

- Use the Organize menu to select which columns are to be displayed, and to specify sorting.
- Use the Organize menu to select all (or no) data sets or display the Search pane.
- Adjust column widths by dragging on the divider line between column headers
- Select a column and drag it to a new position.
- Right-click on the columns to display a dialog to select the columns to be displayed.
- Click a column heading to sort the displayed items in ascending and descending order of that field.
- Delete a sequence/method (or more than one if it is a multi-select environment)

Querying

Method queries can include the following data items: Name, Group, Created Date/Time, Last Edited Date/Time, Author, Editor.

Sequence queries can include the following data items: Name, Group, Created Date/Time, Last Edited Date/Time, Author, Editor.

The results of a query are displayed in the same basic format as the continuous list display (i.e. without grouping) and can be sorted in exactly the same way. Secondary queries can be applied to this collection as required.

How to Open a Method

To open a method:

1. Select the Method environment by clicking the **Method** button in the Navigation Pane.

2. From the **File** menu select **Open Method**. The Data Selector dialog opens.

3. Expand the Method group by clicking the plus sign  .

4. Select the method you want to open by clicking the check box at the beginning of the row.

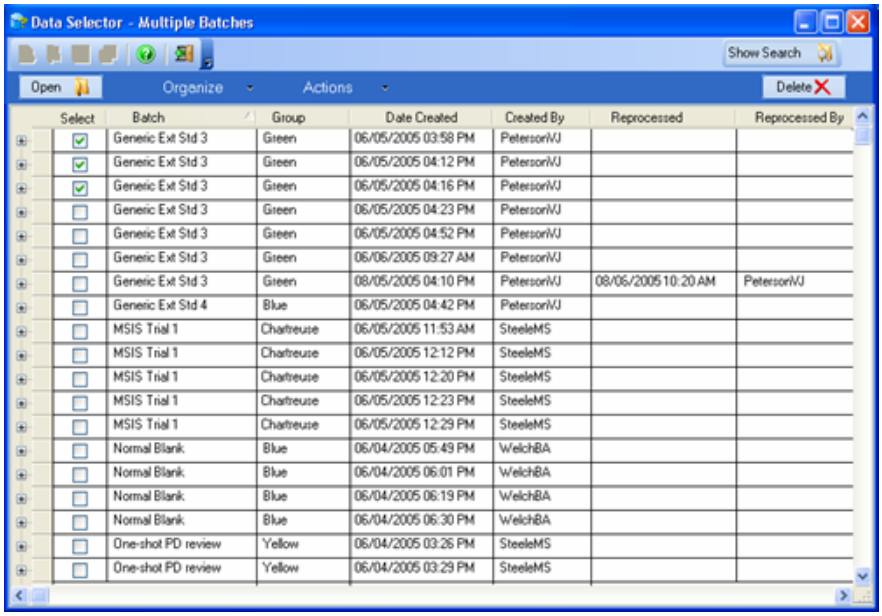
5. Click the **Open** button, the first page of the method displays.

About Sample Data

Browsing and Sorting

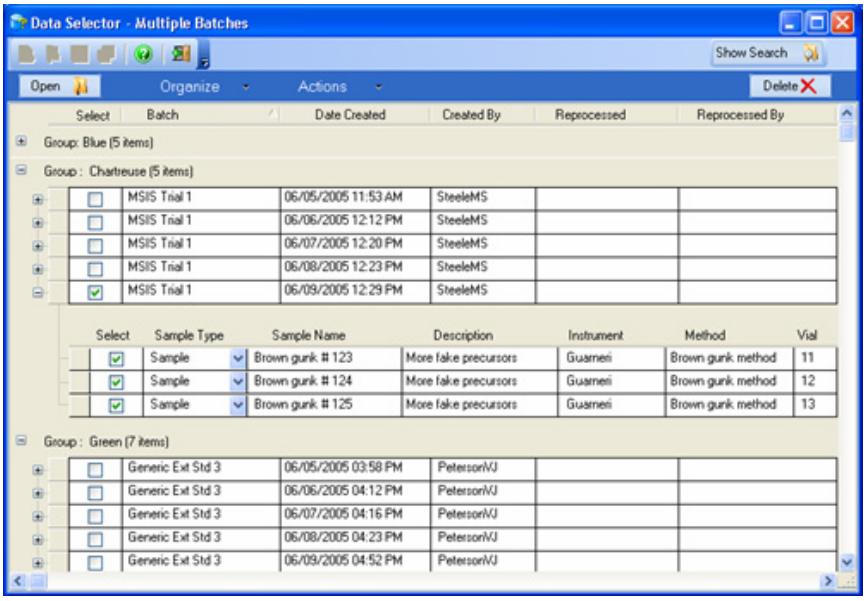
Sample data are normally displayed under the batch name; that is the name of the sequence from which the data were acquired (or the batch from which the reprocessed batch was derived). You can expand each batch to display the samples contained within that batch.

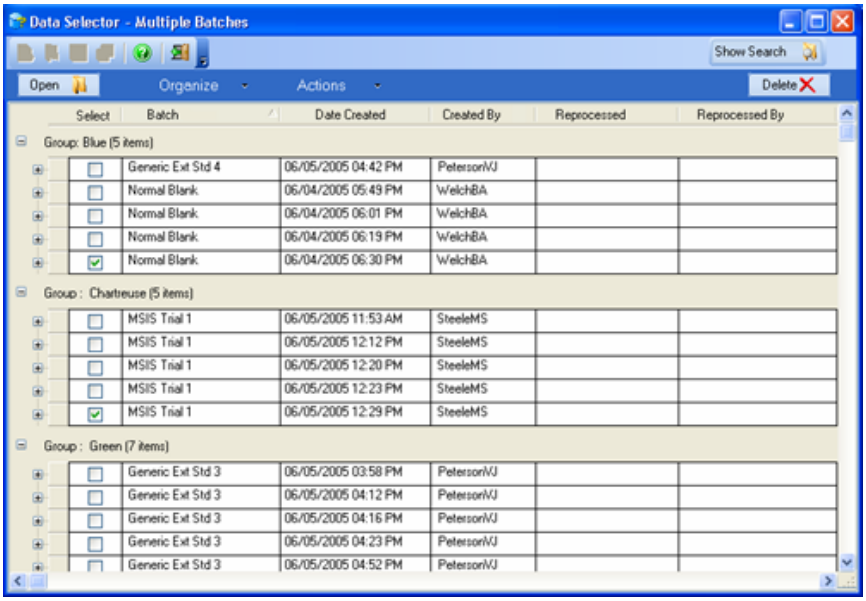
You can sort the displayed batch list, in ascending or descending order, on any field shown in the display. The displayed fields are: Batch Name, Batch Group, Description, Created Date/Time, Editor (the user who reprocessed the batch), and Reprocessed Date/Time. Analyst is not available at this level because it is at the sample level in the sequence/batch database table. One type of Batch is a continuous list.



Select	Batch	Group	Date Created	Created By	Reprocessed	Reprocessed By
☑	Generic Ext Std 3	Green	06/05/2005 03:58 PM	PetersonVJ		
☑	Generic Ext Std 3	Green	06/05/2005 04:12 PM	PetersonVJ		
☑	Generic Ext Std 3	Green	06/05/2005 04:16 PM	PetersonVJ		
☐	Generic Ext Std 3	Green	06/05/2005 04:23 PM	PetersonVJ		
☐	Generic Ext Std 3	Green	06/05/2005 04:52 PM	PetersonVJ		
☐	Generic Ext Std 3	Green	06/06/2005 09:27 AM	PetersonVJ		
☐	Generic Ext Std 3	Green	08/05/2005 04:10 PM	PetersonVJ	08/06/2005 10:20 AM	PetersonVJ
☐	Generic Ext Std 4	Blue	06/05/2005 04:42 PM	PetersonVJ		
☐	MSIS Trial 1	Chartreuse	06/05/2005 11:53 AM	SteeleMS		
☐	MSIS Trial 1	Chartreuse	06/05/2005 12:12 PM	SteeleMS		
☐	MSIS Trial 1	Chartreuse	06/05/2005 12:20 PM	SteeleMS		
☐	MSIS Trial 1	Chartreuse	06/05/2005 12:23 PM	SteeleMS		
☐	MSIS Trial 1	Chartreuse	06/05/2005 12:29 PM	SteeleMS		
☐	Normal Blank	Blue	06/04/2005 05:49 PM	WelchBA		
☐	Normal Blank	Blue	06/04/2005 06:01 PM	WelchBA		
☐	Normal Blank	Blue	06/04/2005 06:19 PM	WelchBA		
☐	Normal Blank	Blue	06/04/2005 06:30 PM	WelchBA		
☐	One-shot PD review	Yellow	06/04/2005 03:26 PM	SteeleMS		
☐	One-shot PD review	Yellow	06/04/2005 03:29 PM	SteeleMS		

Batches can also be grouped by group name and editor (user reprocessed by). Only a single level of grouping is supported. Any batch can be expanded to display the sample contained within the batch.





Querying

Two levels of query are available for sample data:

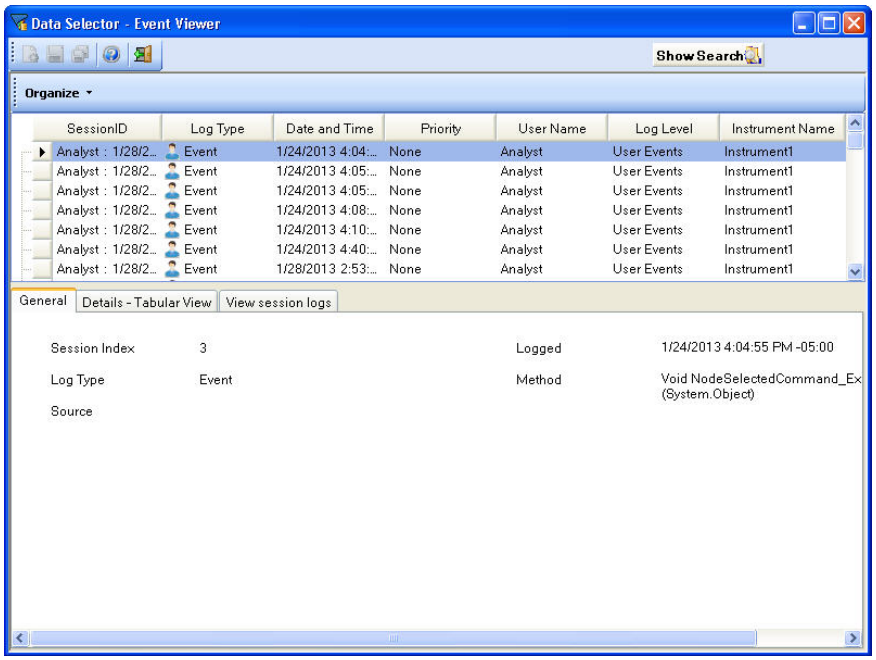
- Querying to locate a Batch with certain characteristics
- Querying to locate sample data sets (injections) with certain characteristics, across batches

The two query types have the same search items available but the results of the query are quite different. For example, a batch query equivalent to "where sample name contains 'cocaine' and date created is May 2006" would return a list of batches, whereas a sample query with the same criteria would return a list of samples.

Sample data queries can include the following items: Batch Name, Group , Description, Acquisition Date/Time, Analyst, Sample Type, Sample Name, Sample Description, Instrument, and Method Name.

About the Data Selector – Event Viewer

Event logging records information about "what happens" in the system. Events are changes in the status of the system that can affect its operation or the data being generated. Some status changes are informational, and others record errors. The contents of the active Event Log Database can be reviewed by selecting the View Current Session Logs command from the Chromera Tools menu. This command is available at all times. Activating the Event Log Viewer launches a special mode of the Data Selector, which displays a Microsoft Windows Event Viewer styled listing of the current events recorded in the database. The upper frame of the selector displays a sortable list of all the events recorded for the current Chromera session, identified by the user name and timestamp of when the session began, and the lower frame displays a selection of tabs providing various options for viewing a selected event from the list, or for generating a report of all logged events from the database.



Events fall into three categories in Chromera:

User Events – Specific actions that occur within Chromera as a consequence of normal user interaction, such as launching Chromera, or saving a method. These are selectable by the end-user and can be enabled as desired.

System Events – Actions that may occur within or outside of normal operations, which are not specifically user initiated, but are common and are anticipated, such as a missing vial in an autosampler.

How to Interact with the Event Viewer

There is only one, single Event Log Database for all of Chromera. It is stored in the folder - **C:\Program Files\Microsoft SQL Server\MSSQL10_50.SQLEXPRESS2008\MSSQL\DATA**, along with most other Chromera databases.

- Each time Chromera Manager is launched, a new **SessionID** is created and the events that occur during that session are recorded in the Event Log Database under that SessionID.

- Each time a Chromera Instrument instance or a Data Only instance is launched, a new **SessionID** is created and the events that occur during that session are recorded in the Event Log Database under that SessionID.

NOTE: *User and System Events cannot be configured/modified while a Chromera instance is already running. The Configure User Events option will be grayed-out. Close any open Chromera instances to enable the configuration option in the Chromera Manager Actions menu.*

Preparing to Capture Events in a Log (Chromera Manager)

To create a new Event Log database, follow this procedure:

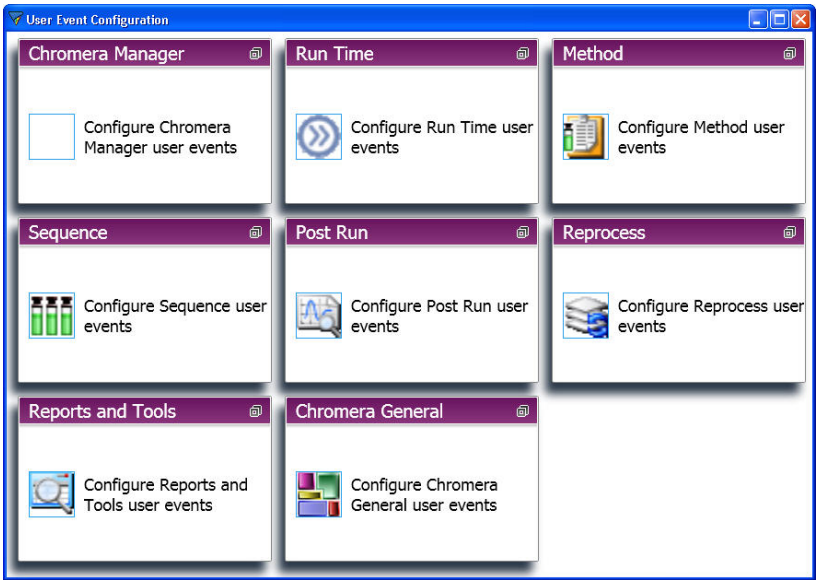
1. Delete the existing Event Log Database through the Event Logs Database Management section of the Chromera Manager.

2. Select Yes to the prompt to Archive the existing database if you need to preserve previous event logs. Otherwise, the existing Event Log Database and all events will be deleted.

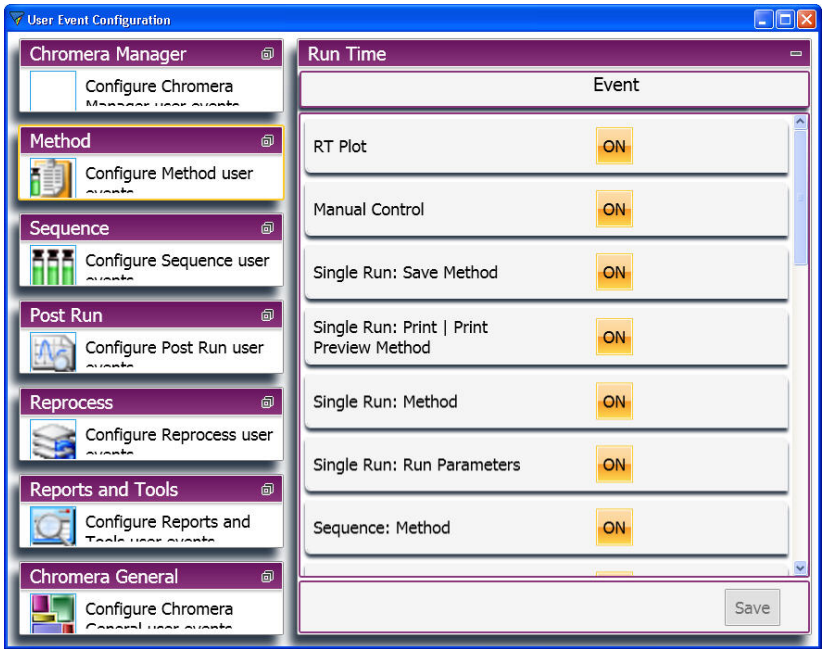
3. Create a new Event Log Database through the Event Logs Database Management section of the Chromera Manager.

To ensure that the User and System events are enabled (they should all be ON by default) follow this procedure:

1. From the **Chromera Manager Actions** menu, select **Configure User Events**.



2. With the **User Event Configuration** window open, click through each of the Chromera environment sections to verify all events for that environment are set to ON.



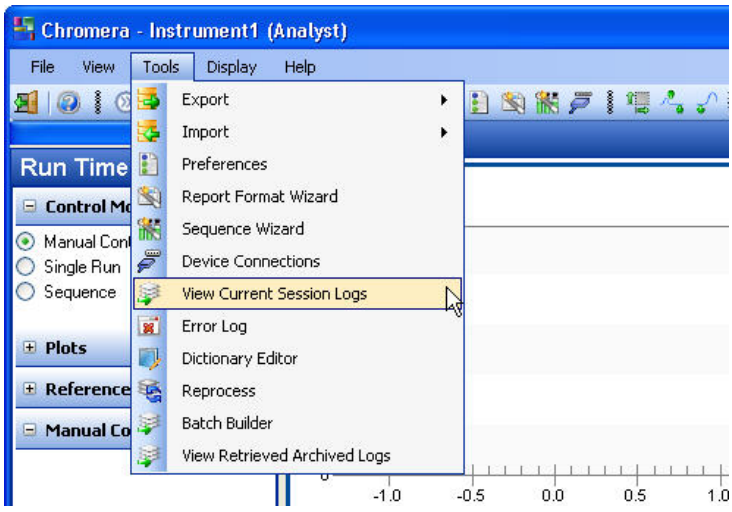
3. Save any changes and close **User Event Configuration**.

4. Launch Chromera.

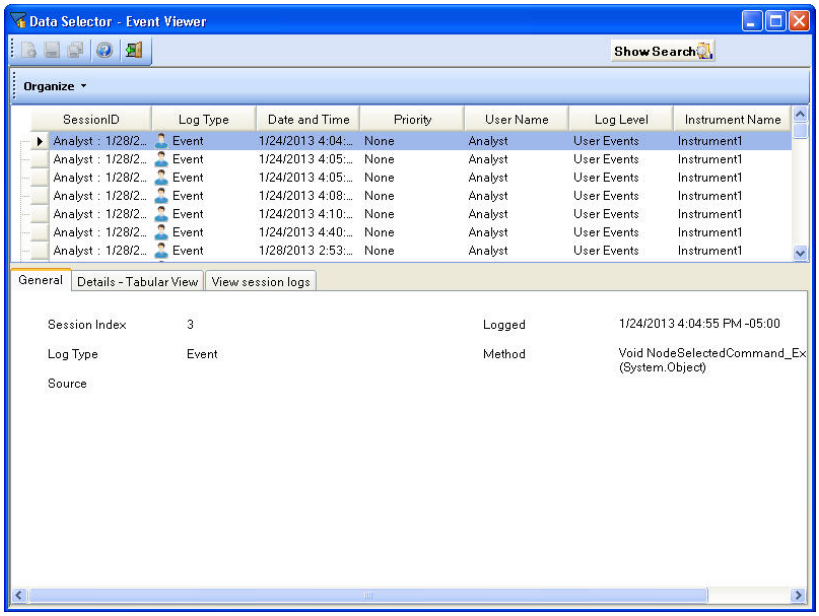
Viewing and Outputting the Event Log through the Data Selector

To view the Event Log through the Data Selector, follow this procedure:

1. Select View Current Session Logs from the Chromera Tools menu. (Or from the Actions menu in Chromera Manager.)

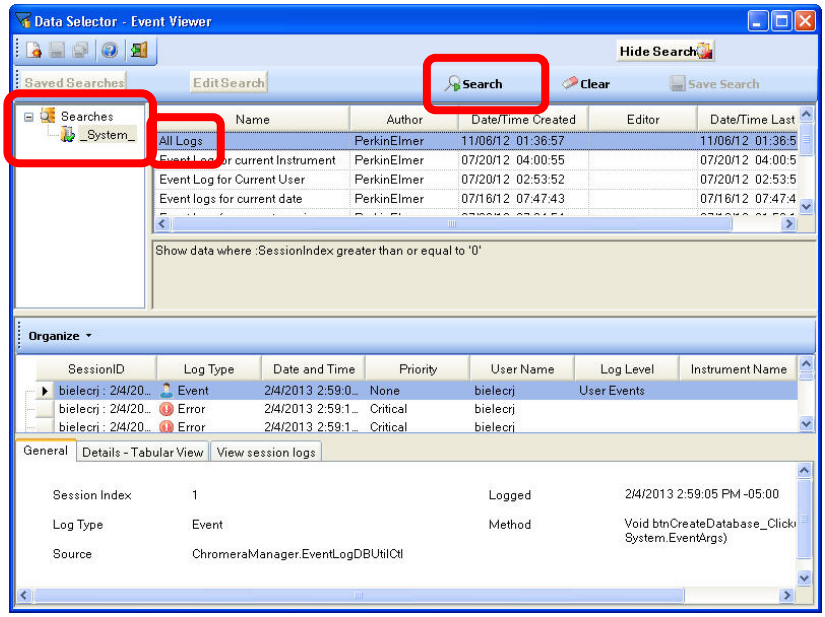


The **Data Selector - Event Viewer** screen appears.



The default is to display only the events from the current **SessionID**. Therefore, by default, only the events that have occurred since the current instance of Chromera was launched will be visible.

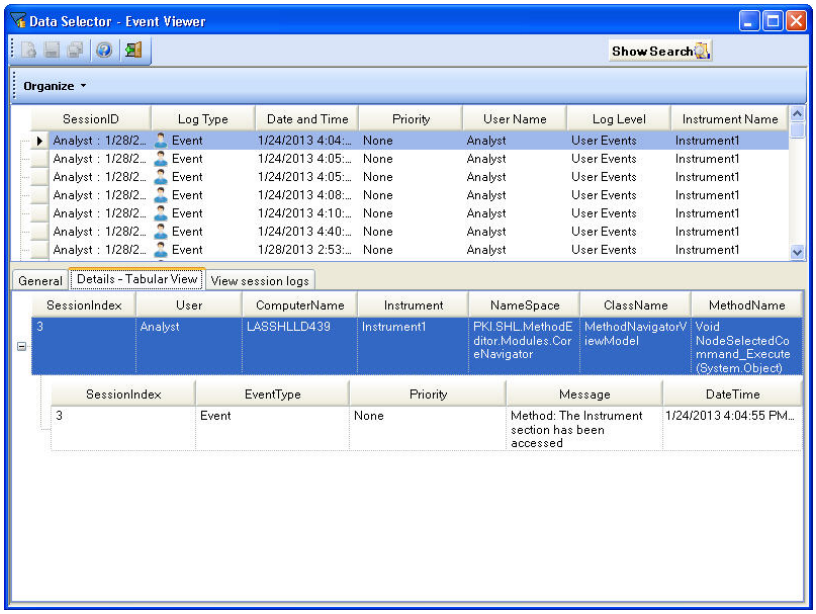
2. Click the Show Search button and select _System_ from the Searches directory to choose the desired collection of events to display, and then click the Search button to return all of the items for that selection.



3. Select **All Logs**; then clicking the **Search** button displays all of the events for all of the SessionIDs in the database.

   The other searches return the events that their **Names** imply.

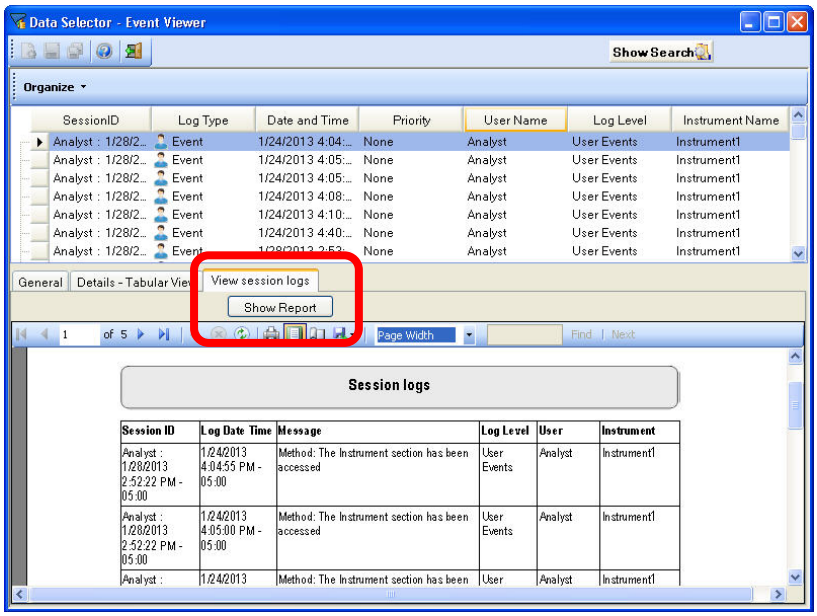
4. Select the **Details - Tabular View** tab to display the contents of individual events while scrolling through the list.



The **Message** field contains the relevant information about the selected event. Move through the list using the Up and Down arrow keys or scroll bar

To output the Event log, follow this procedure:

1. Select the **View Session Logs** tab, and then click the **Show Report** button to print the complete list of events from the selected search.



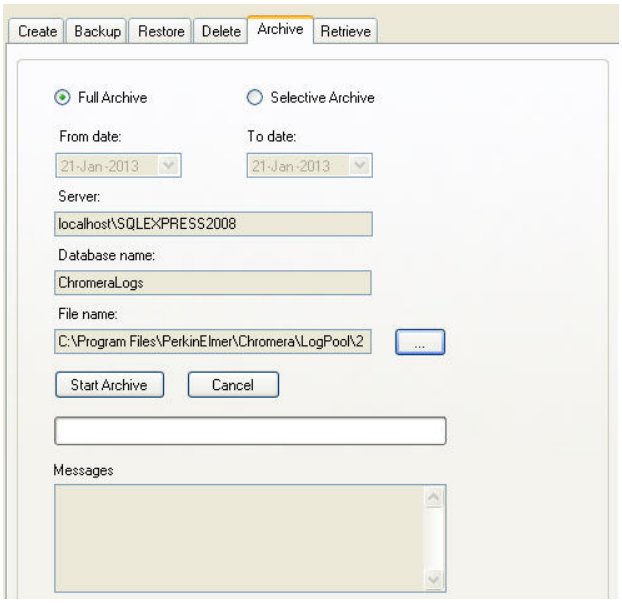
2. Click the **Export** button on the Toolbar, and select **Excel**, **PDF**, or **Word** as the file type to export.

   Selecting an Excel file type allows the greatest flexibility for viewing, sorting, and filtering the exported data.

Using Event Log Archives (Chromera Manager)

To create an archive of the Event Log for future viewing, follow this procedure:

1. From the Chromera Manager, select the **Archive** tab from the **Event Logs Database Management** section.



2. Choose the option for **Full Archive** to copy the complete database, with all events, or just a **Selective Archive**, to copy only the events from a specified date range.

NOTE: *Both of these options will remove the associated events from the active Event Log database.*

3. Select a name and a location for the .archive file. The default is to create a file name from the date/timestamp of the archive range, and to save into the folder C:\Program Files\PerkinElmer\Chromera\LogPool.

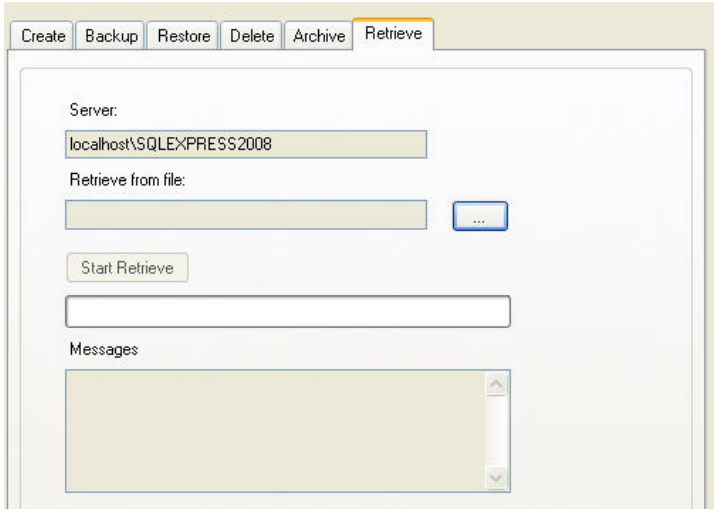
4. Click the **Save** button, and then **Start Archive**.

   This will copy the selected records to the .archive file, and then delete them from the current Event Log Database. The .archive file then can be copied to another computer for subsequent review.

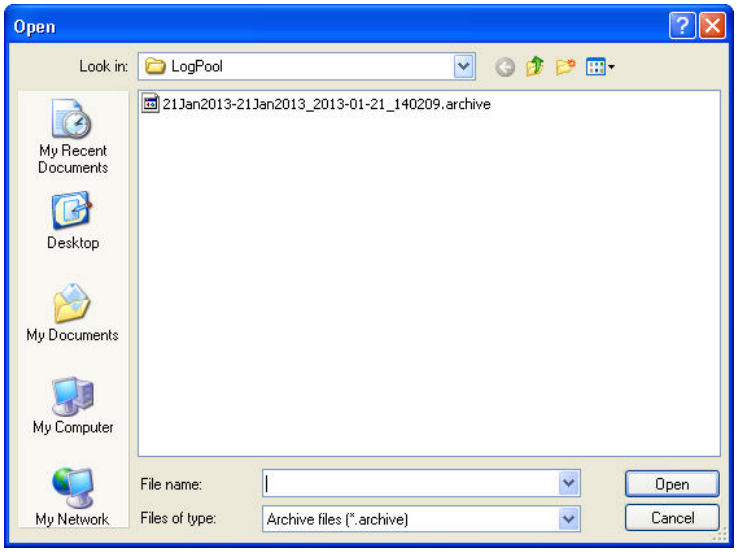
To view an Archive Event Log file, follow this procedure:

1. Copy the .archive file to the **LogPool** folder (the default location) if it is not already there.

2. From the Chromera Manager, select the **Retrieve** tab from the **Event Logs Database Management** section.

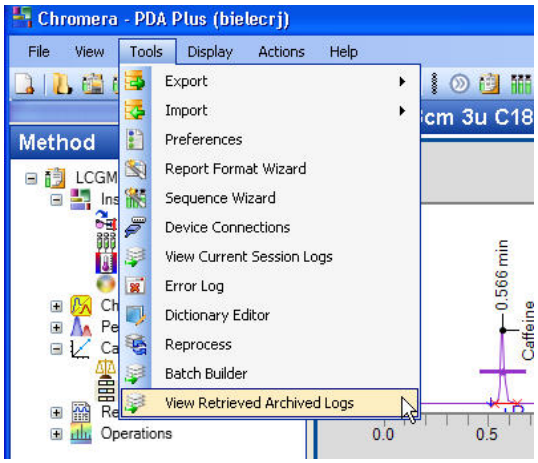


3. Select the appropriate .archive file from the **LogPool** folder and click the **Open** button.

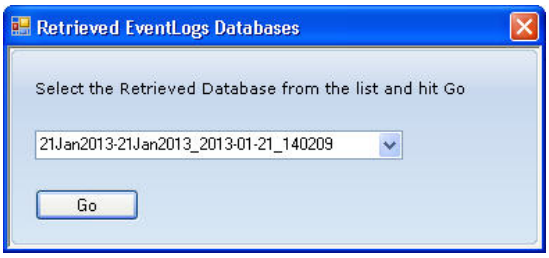


This will convert the .archive file back into a database formatted file. It WILL NOT copy the archived records into the active Event Log Database.

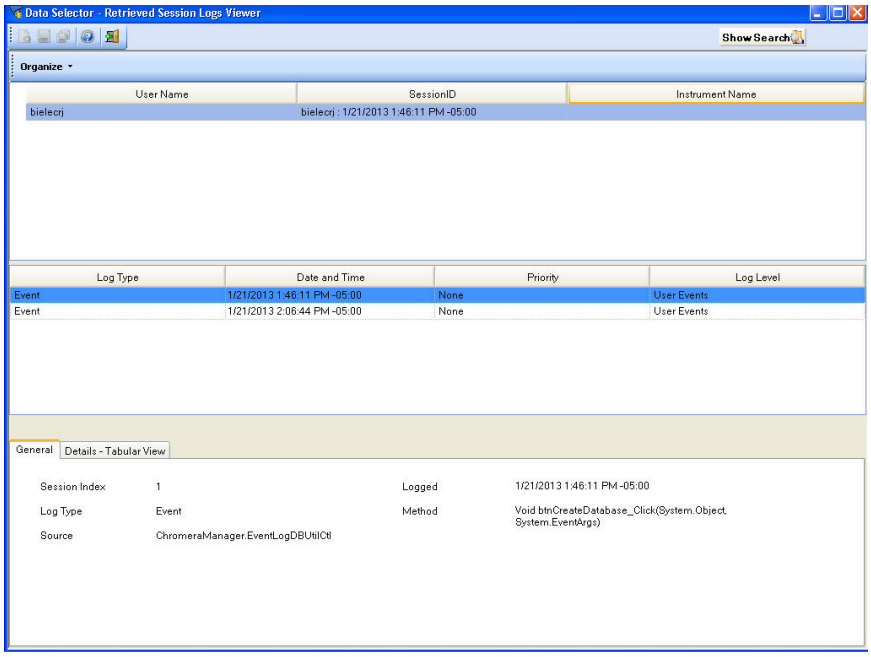
4. Launch Chromera and select **View Retrieved Archive Logs** from the **Tools** menu.



5. Select the desired database from the drop-down list and click the **Go** button.



This allows browsing of the events from the Retrieved database in exactly the same manner as browsing the active Event Log Database.



Selecting Data

Each row in the data list (representing a data set) includes a **Select** check box. Whenever at least one item is checked the **Open** button is enabled.

Rows can be checked or unchecked in the following ways:

1. Clicking in the **Select** box will toggle the state of the check for that row. If the row is a batch row then changing its state also sets all sample rows within that batch to the same state.

2. Choosing the **Select All** command from the **Organize** menu will cause all items displayed in the Browse grid to be checked.

3. Choosing the **Select None** command from the **Organize** menu will cause all items displayed in the Browse grid to be unchecked.

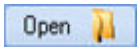
4. Making an extended selection using Shift + Click. If the Shift key is held down while a row is checked or unchecked and then a second row is clicked, all rows from the first up to and including the second row will assume the state of the first clicked row. Note that an extended selection can only include rows at the same level. An extended selection of sample rows cannot extend from one batch to another.

NOTE: *When the data selector is opened in any mode, double-clicking on an item will open that item (as it would in a standard Windows File Open dialog). Thus double-clicking an item is a shortcut for checking the item and then clicking the Open button.*

When the **Open** button is clicked the Data Selector closes and the selected data set(s) return to the function that initiated the data selector.

Button/Menu Commands

The Data Selector uses the following button and menu commands.

Menu	Command	Description
	Open	A command button that opens your selection. This duplicates the Actions/Open command
Organize	Select All	Selects all of the results displayed on the screen. Enabled when the Data Selector is in multi-select mode and items are available for browsing.
	Select None	Selects none of the results displayed on the screen.
	List	Displays all items in a continuous list.

	Group by ► Analyst Batch Group Reprocessed By Expand all groups Collapse all groups	Displays a submenu containing items the list can be grouped by.
	Search pane	Toggles display of the Search pane.
	Preview	Toggles display of the Preview pane (see Overview of the Search Pane).
	Select Columns	Displays the Select Columns dialog for customizing the list columns.
Actions	Open	Opens the selected data items and closes the data selector window.
	Delete	Displays a confirmation dialog **Are you sure you want to delete the selected item(s)?**. Clicking the **Yes** button deletes the selected items and the updates the displayed list of data items. Clicking **No**, then no action is taken.
	Delete	A simple command button that duplicates the action of the Actions/Delete command described above.

Group by submenu contents:

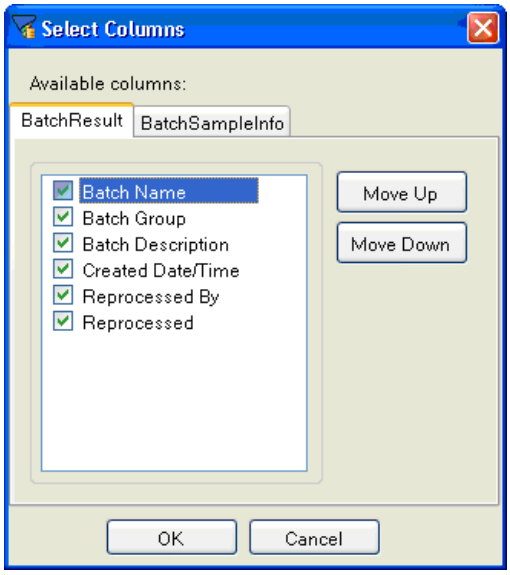
Method	Author, Editor, Method Group
Sequence	Author, Editor, Sequence Group
Batch/Samples	Batch Group, Reprocessed By

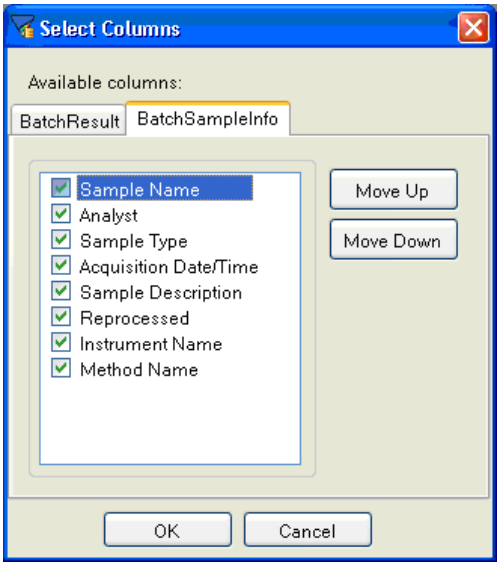
How to Customize the Browse Data List

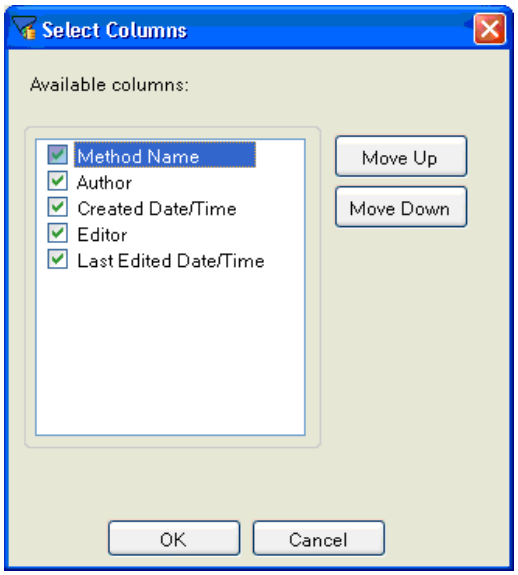
The basic approach to customizing the data list display is the same for all data types; however, the two-level display of sample data necessitates the ability to customize the display columns for both the batches and the samples.

To add or remove columns in the data list:

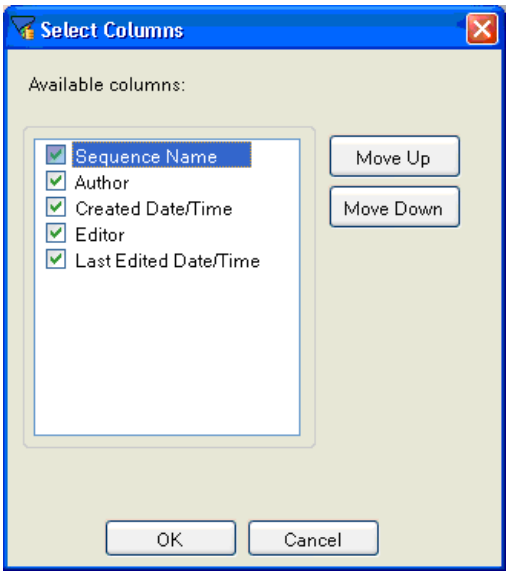
1. Click the **Organize** button to display the menu items, then click **Select Columns**. The form of this dialog will depend on the type of data being displayed. For example:







Select Columns dialog for display of Methods



Select Columns dialog for display of Sequences

2. From the **Select Columns** dialog you can either add a column or remove a column. To add a column, check the check box next to a column name in the available columns list. To remove a column, uncheck the check box next to a column.

3. You can also use the **Move Up** and **Move Down** buttons to change the order in which columns are displayed.

To move a column up or down select that column name in the list, and then use the Move Up and Move Down buttons to move it up or down the list.
(The order of columns can also be modified by direct drag-and-drop within the grid display.)

To sort the items in the data list:

1. Click on the column heading of the data item to be sorted.

2. A drop down arrow appears next to the text on the column to indicate whether the column is sorted in ascending or descending order. An arrow facing upwards indicates the column is sorted in ascending order. An arrow facing downwards indicates the column is sorted in descending order. The order toggles each time the column heading is clicked.

Toolbar Commands

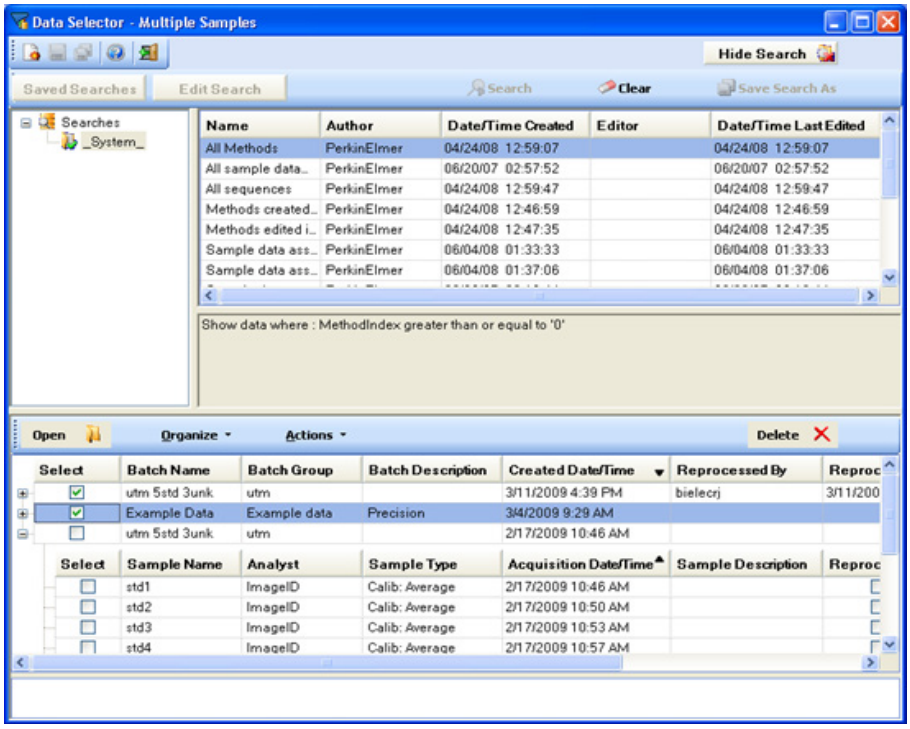
The Data Selector contains the following icon buttons for standard interactions that are typically performed frequently.

Toolbar Button	Associated Command
	New search definition
	Save search (enabled only when an existing search is being edited and it is a valid search)
	Save search as (enabled only when a search has been defined)
	Display Help file
	Exit - Close the Data Selector

Overview of the Search Pane

To set up a search definition, click on the **Show Search** button and the Search pane appears in the upper part of the window. Initially the Search pane is set to display Saved Searches. These are saved under a user defined folder hierarchy. This is a Windows Explorer-like display with the folder structure displayed in a tree control on the left and the searches contained within the selected folder displayed in a list view on the right. When a saved search is selected in the list view, the search definition is displayed in the lower pane on the right. This display is the text **Show data where:** followed by the search definition. Where the search definition includes bracketed expressions (see below) then these are include in the statement. For example: Show data where: Acquisition date/time is between 6/5/07 and 7/7/07 and (Sample name contains 'iso' or Sample name contains 'propanol')

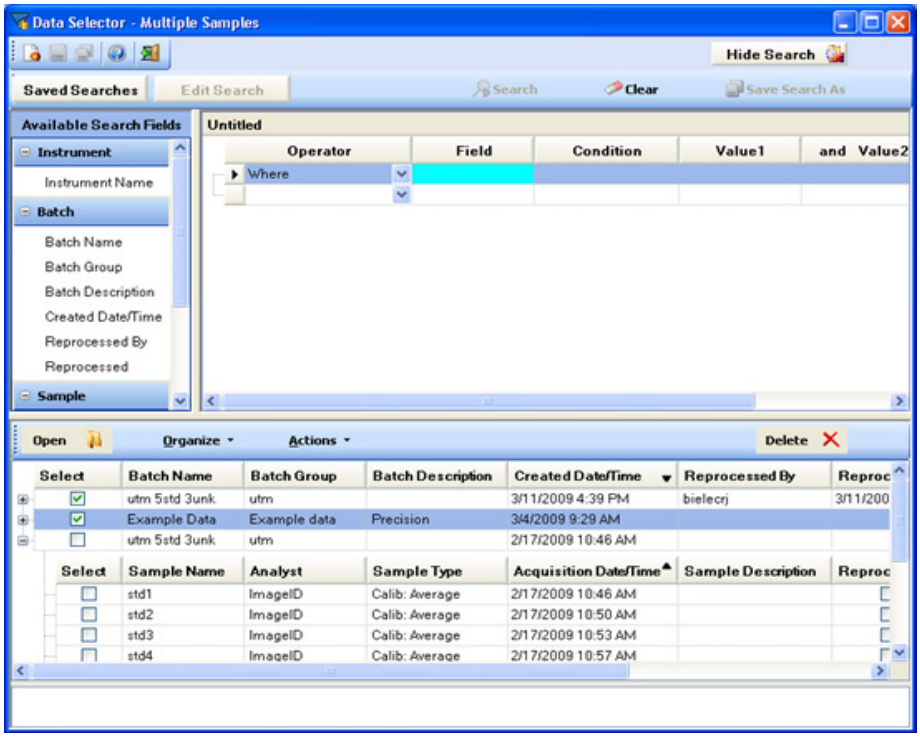
Note that literal strings are shown enclosed in quotes.



Data Selector showing Search pane with display of saved searches

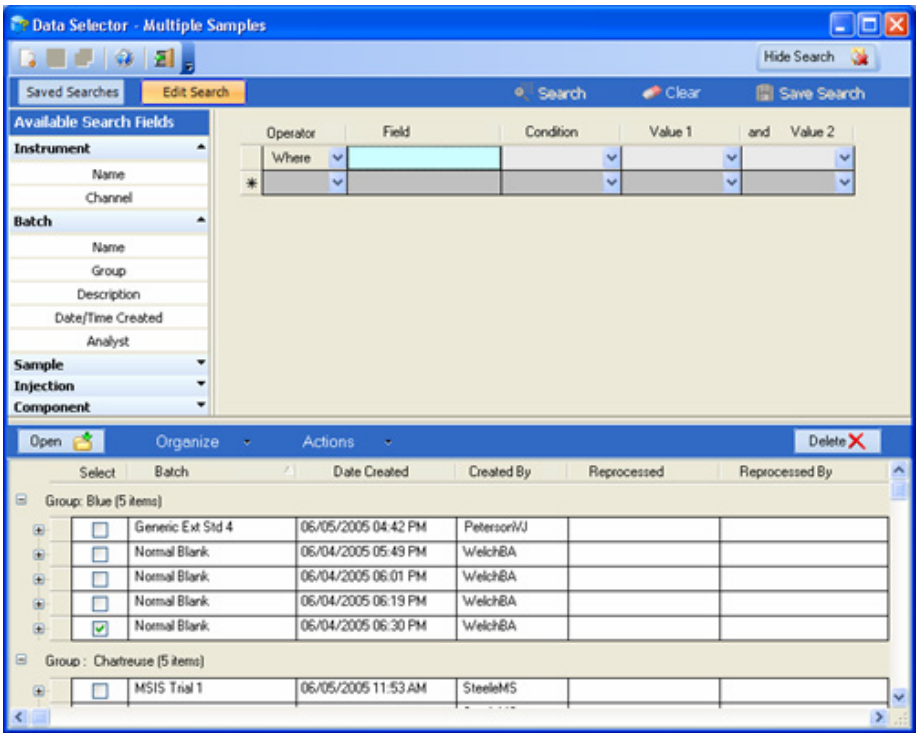
The relative size of all three panes within the Search pane is adjustable using the splitter bars. The sizes of these panes are retained within user preferences and restored when that user opens the window.

Clicking the **New Search** toolbar button or the **Edit Search** button (when an editable saved search is selected in the tree) will display the Search pane in edit mode:



Data Selector showing Search pane set to edit mode

NOTE: *Entering the edit mode by the latter route displays the currently selected search ready for editing. Clicking the* ***New Search*** *toolbar button displays the edit mode with a blank search definition.*

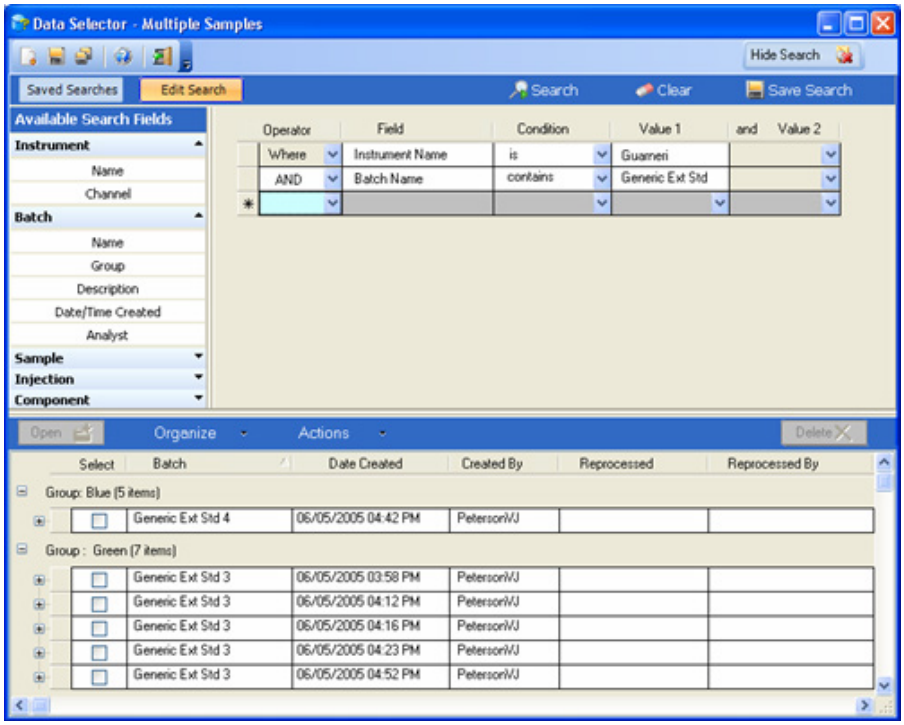


Data Selector showing Search pane after New Search command

NOTE: *Although not shown in the above examples, when the edit mode of Search is displayed the name of the currently shown search definition (or Untitled if not yet saved) is shown in the title bar.*

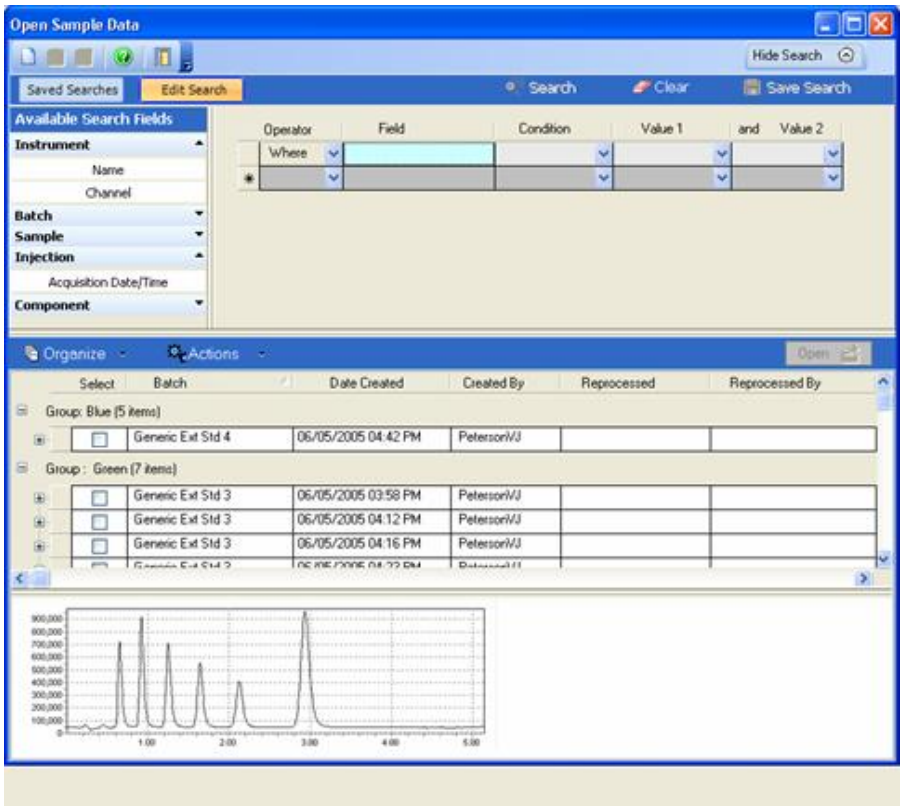
When a valid search is shown (see below for procedure for defining a search) the Search button is enabled. Clicking this button will cause the search to be executed and the results are displayed in the browse pane at the bottom of the screen.

NOTE: *A search can also be run directly from the Saved Searches view by clicking the Search button when the desired saved search is selected.*



Data Selector showing results of Search

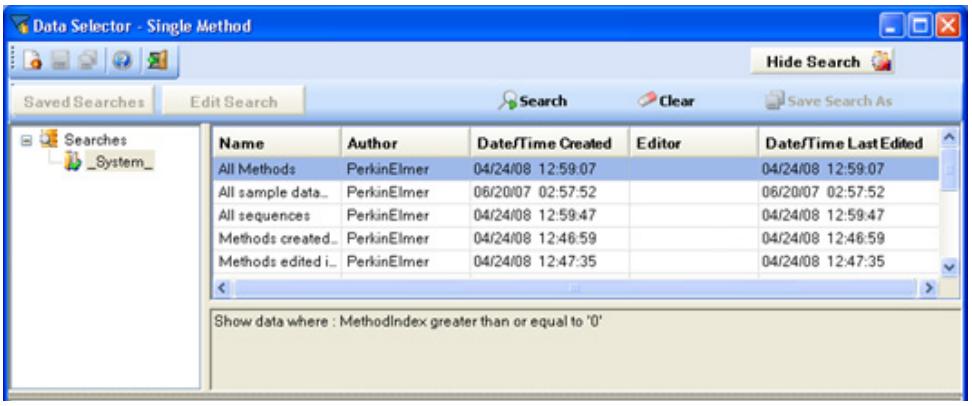
When the optional Preview pane is displayed, the screen will appears as follows:



The preview pane displays the chromatograms associated with the sample row selected in the data grid. This would include all channels for all replicate injections (scrolled as required).

Data Selector: Saved Searches

Selecting Saved Searches displays the folder structure in which searches are saved. Functionally this operates in an identical way to the directory tree within Windows Explorer. Folders are displayed in ascending alphabetical order.



The **Searches** root is always displayed. It is possible to store searches in the root folder and so when this node is selected the display in the upper right pane will list any such searches. However, the icon used for the root will not change when it is selected.

When you right-click on the tree, the popup menu described below is displayed. If you click on a node in the tree then that node first becomes the selected node, and hence the command will relate to that node.

Command	**Description**
New Folder	Creates a new folder as a sub folder to the currently selected folder (as in Windows Explorer). If you did not click on a node then the new folder is at the first level (i.e. in the root folder).
Delete Folder	Deletes the selected folder, provided it contains no saved searches. If it is not empty then a message is displayed: **The selected folder cannot be deleted as it is not empty.**
Expand	Expands the selected folder to show all its subfolders.
Expand All	Expands all folder (recursively) to show all subfolders
Collapse	Closes the selected folder, hiding its subfolder
Collapse All	Closes all subfolders so that only the folders at the top level are displayed.

Data Selector: Saved Searches List

This is a list view showing all the searches saved within the folder selected in the tree. The list view contains the following columns:

Name	The name you give to the search
Author	The name of the person who created (saved) the search
Created Date/Time	The date and time (in format defined by regional settings) that the search was first saved
Editor	The name of the person who last edited (and saved!) the search. This is blank if the search has not been modified since its original creation.
Edited Date/Time Last	The date and time (in format defined by regional settings) that the search was last edited.

You can change the order of these columns using drag-and-drop. The searches will initially be listed in ascending alphabetical order where you should be able to sort on any column by clicking on the header (toggling between ascending and descending) in the usual way.

When you right-click on the list view, the popup menu described below is displayed. If you click on a row in the list then that row first becomes the selected row, and hence the command will relate to that node.

Command	Description
Delete Search	Deletes the selected search. There is no confirmation dialog.
Run this Search	Executes the selected search and displays the results in the lower part of the screen.

Search Definition

This pane displays the selected search in pseudo-sentence form. However, for clarity, when parentheses are used in the search definition these are shown in the sentence. All literal strings entered by the user as search criteria (e.g. sample names, instrument names, etc) are displayed in quotes.

Data Selector: Edit Search View

Available Search Fields List

This displays the search fields that can be used within a search for the data set type being searched for. Each category heading is a button that can be clicked to display or hide the list of fields within that category.

Clicking on any item within a section (when the Field part of the search definition is selected will cause that field to be added to the search definition.

The fields displayed in the list depend on the data set type, as defined below:

Method Search

Category	Field	Comment
Identification	Method Name	Displayed as Method Name in the search definition.
	Method Group	Displayed as Method Group in the search definition.
	Author	The person who created the method
	Created Date/Time	The date and time when the method was first saved
	Editor	The person who last modified the method (blank if the method has not been modified since it was created).
	Last Edited Date/Time	The date and time when the method was last changed.

Sequence Search

Category	Field	Comment
Identification	Sequence Name	Displayed as Sequence Name in the search definition.
	Sequence Group	Displayed as Sequence Group in the search definition.
	Author	The user who created the sequence
	Created Date/Time	The date and time when the sequence was first saved

	Editor	The user who last modified the sequence (blank if the sequence has not been modified since it was created).
	Last Edited Date/Time	The date and time when the sequence was last changed.

Data Search

Category	Field	Comment
Instrument	Instrument Name	The instrument on which the data were acquired. Displayed as Instrument Name in the search definition.
Batch	Batch Name	
	Batch Group	Displayed as Batch Group in the search definition.
	Batch Description	Displayed as Batch Description in the search definition.
	Created Date/Time	The date and time when the batch was created (acquisition was started).
	Reprocessed By	The user who last reprocessed data in the batch. This is blank if the data has not been reprocessed since original; post-run processing.
	Reprocessed	The date and time when the batch (or any channel in it) was last reprocessed.
Sample	Acquisition Date/Time	Date/Time of injection of first sample injection.
	Sample Name	Displayed as Sample Name in the search definition.
	Sample Type	Displayed as Sample Type in the search definition.
	Sample Description	Displayed as Sample Description in the search definition.
	Method Name	The name of a method associated with this sample (acquisition or processing method)
	Analyst	The user who acquired the data.

Search Definition

The basic fields within the display are described below.

Control	Description
Operator	Select from the drop-down list the operator (**AND**, **OR**, **AND(**, **OR(**) to be applied between clauses of the search definition. For the first row of the search definition this is a read-only field which displays the term **Where**. If the selection is **AND(** or **OR(** then a secondary band is displayed below the current row.
Field	Displays the name of the field selected from the Available Search Fields list.
Condition	Use the drop down list to select the nature of the search that applies to a criteria field. The drop down list contents depend on the type of field selected to search on. Text fields: **is**, **contains**, **does not contain**, **starts with** Numeric fields: **is equal to**, **is not null**, **less than or equal to**, **greater than or equal to**, **is between** Date fields: **after**, **before**, **between**, **on**
Value 1	The nature of this field will depend on the Field selection. Ideally the appearance of the cell is distinct for each Field type but if this cannot be achieved with the control then a text field with an associated button should be used. The button would display the date selector for a date field but be inactive for text and numeric fields. Text fields: **Any text string up to 50 characters** Numeric fields: **Any valid number** Data fields: **A date selected from the popup date selector.**
Value 2	The nature of this field will depend on the Field selection. This field will only be enabled when the selected Condition is '**is between**' (numeric) or '**between**' (date). Text fields: **Any text string up to 50 characters** Numeric fields: **Any valid number** Data fields: **A date selected from the popup date selector.**
<close parenthesis>	Select from the drop-down list the end of a sub-expression. This field ONLY appears on secondary bands. Selection of '**)**' will cause the blank secondary band row below the current one to be hidden and the Operator field of the blank entry band at the next higher level is selected.

Search Definition Popup Menu

The popup menu defined below is displayed when you right-click on the search definition. The right-click action also first selects the cell beneath the mouse pointer. (If the you click on a header or on blank space then the popup menu will not be displayed.)

Command	Description
Clear Entry	Clears the entry in the selected cell. This command is only enabled on cells in which the user types an entry, not on drop-down list cells or date cells.
Delete Row	Deletes the selected row and any child rows associated with it. If the deleted row is a child row containing the closing parenthesis then either: (i) If a preceding child row exists then the closing parenthesis is added to that row. (ii) If there is no other child row then a blank child row is displayed below the parent row and the query is invalid until a child row has been added or the parent row deleted. Enabled whenever the selected row is not a blank (entry) row. **NOTE:** A row can also be deleted by selecting that row and then pressing the Delete key.
Insert Row Above	Inserts a new row above the currently selected one. This will always be at the same level as the selected row. Thus if the first secondary row of a sub clause is selected the new row is an addition at the secondary level. If the selected row is the very first row (containing the read-only 'Where' operator) then the new row will show 'Where' as its operator and the selected row's Operator field will become enabled and set to 'AND' by default. Enabled whenever the selected row is not a blank (entry) row.

Data Selector: System Searches

A number of default search definitions are provided with the software. These searches are unique in the following ways:

- They cannot be edited
- They cannot be deleted
- They contain dynamic criteria. That is, the search parameters are not fixed values but depend on the environment in which the search is executed.

The proposed default searches are:

1. Sample data associated with any instrument acquired or reprocessed in the past 24 hours
2. Sample data associated with the current instrument acquired or reprocessed in the past 24 hours
3. Sample data associated with any instrument acquired or reprocessed in the past 7 days
4. Sample data associated with the current instrument acquired or reprocessed in the past 7 days

5. All sample data associated with any instrument. This is the default case and therefore does not require an explicit saved search.
6. All sample data associated with the current instrument
7. Sequences created in the past 7 days
8. Sequences edited in the past 7 days
9. All sequences. This is the default case and therefore does not require an explicit saved search.
10. Methods created in the past 7 days
11. Methods edited in the past 7 days
12. All methods. This is the default case and therefore does not require an explicit saved search.

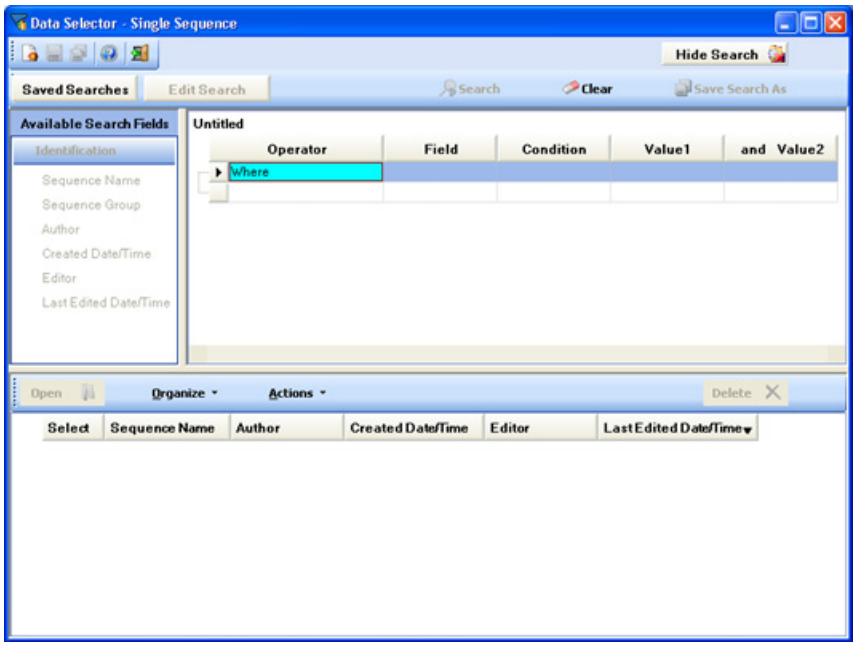
In all cases the date range for the search is determined when the query is run. For searches 2 and 4 the instrument associated with the current instance of Chromera will act as an additional filter (if no instrument is associated with the instance then these searches will return no results).

The default searches are displayed in the 'Saved Searches' tree within the folder 'System'.

Searching for Methods, Sequences or Sample Data

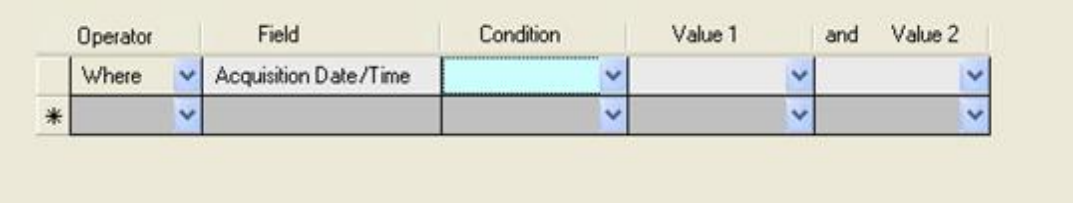
By selecting available search fields and setting search criteria for those fields, it is possible to generate a very specific search of the database.

1. From the browse pane of the Data Selector click **Show Search**.
   The Search pane appears, with a blank search definition and the Field cell selected:

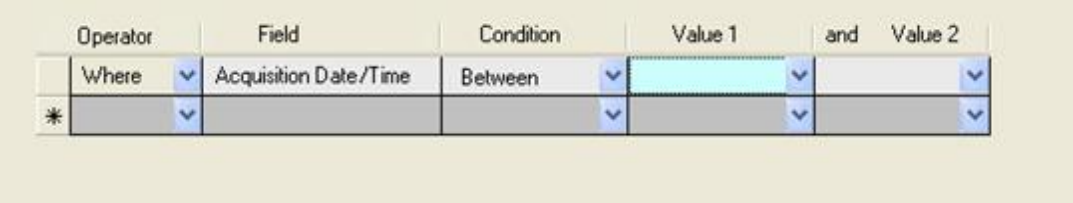


2. To choose a field to search on click on an item in the Available Search Fields list (expanding and/or collapsing sections as required). When a field has been clicked it is displayed in the field cell and the Condition cell is selected.

NOTE: *The name that appears in the Field cell is sometimes a composite of the category and field name (for example Instrument Name not just Name).*

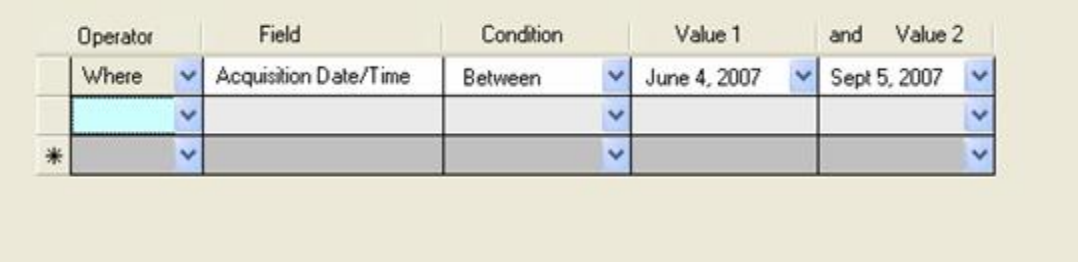


3. From the drop-down list in the **Condition** cell, select the desired search criterion/condition. The available conditions depend on the field selected as defined above. When a Condition has been selected, the Value 1 cell is selected.



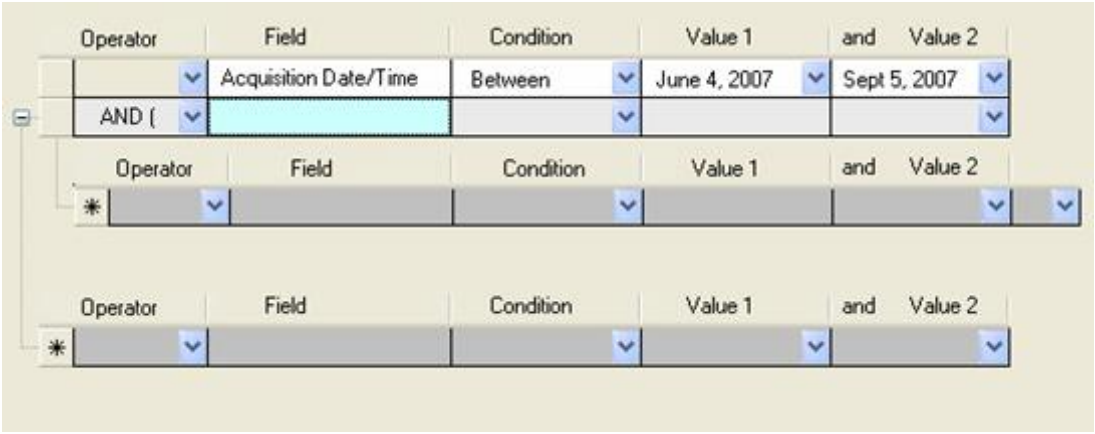
4. Depending on the Field type either enter a value (text or numeric) into the Value 1 cell or choose a date from the popup calendar displayed when the button associated with the cell is clicked. If the Condition selected requires two values then the Value 2 cell is selected (immediately for date selection, or when user presses Tab or Enter for text or numeric entry), otherwise the Operator field in the next row is selected. This is a row at the same level for the first search clause.

5. If the Condition selected requires two values then enter or choose a value as for Value 1. When you leave that field the Operator field in the next row is selected and a new blank line will appear at the bottom of the grid.

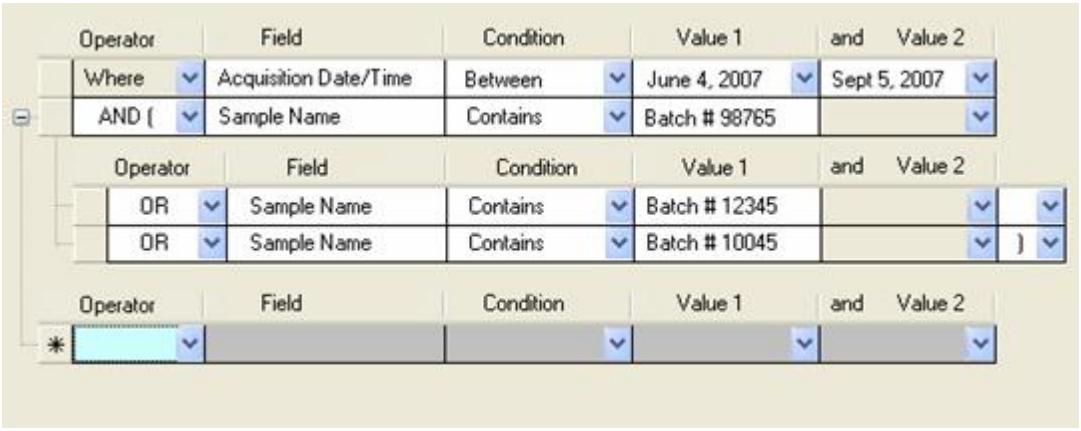


6. At this point the search is valid and the Save Search and Search buttons are enabled.

7. To add additional search criteria select an Operator from the drop-down list. If 'AND' or 'OR' is selected then a new blank row at the same level is displayed below the current entry row. If 'AND(' or 'OR(' is selected then a blank secondary band entry row is displayed.



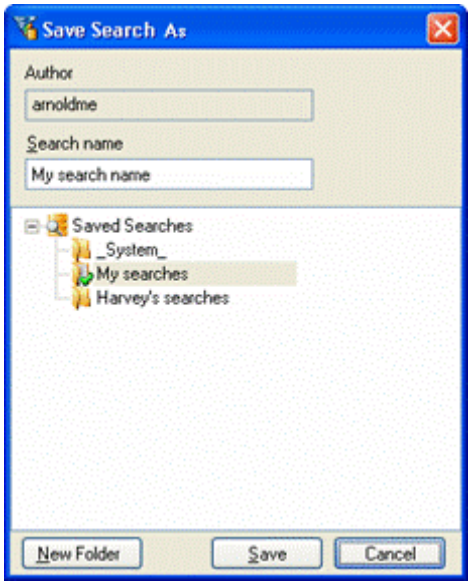
8. Steps 2 - 6 may be repeated to add additional search criteria. The procedure for search criteria grouped within parentheses is basically the same as described above. The user signals the end of the grouped search criteria by selecting '**)**' from the drop-down list in the final cell of the secondary entry row. Only a single level of nesting of bracketed criteria is allowed.



9. Once you complete the search definition the search can be activated by clicking **Search**.
   The search begins and the mouse pointer will change to an hour glass to indicate that a search is in progress. When the search has completed the mouse pointer will change back to the pointer icon and the search results will appear in the Browse grid below the Search pane.

To save a search definition

Click **Save**. If the search has not previously been saved then the Save Search As dialog will appear. This dialog allows the user to select the folder the search is saved in and to enter the name under which the search is saved.



Control	Description
Author	Displays the name of the logged-on user.
Search name	Enter the name under which the search will be saved.
<search folders tree>	A tree control displaying the folders in which searches can be saved
New Folder	Click this button to create a new folder in the search folders tree where you can enter a name for the folder. The name entered must be unique in the tree.
Save	Click this button to save the search under the specified name within the selected folder. This command also closes the dialog.
Cancel	Click this button to cancel the save operation and close the dialog.

Selecting a Data Set

To select a set of samples (for example, in the Post Run environment):

1. Choose **Open Data** from the **File** menu in Post Run to display the **Data Selector**.
2. Click the **New Search** button (assuming the required data is not contained in the list displayed when the Data Selector opens).
3. Set search parameters, based on information known about the required samples.
4. Click the **Search** button to retrieve sample data meeting the entered criteria.
5. Review the displayed list of Batches. You can group the batches and/or expand one or more batches to review the constituent sample information.
6. Select a complete batch (or batches) by checking the **Select** box on the batch row(s), or select specific samples by clicking on the Select box on sample rows.
7. Click the **Open** button to open the selected data set in the Post run environment.

Search Messages

The following search messages are displayed depending on the circumstance.

Unsaved Search Definition

If you attempt to close the Data Selector window or go to open the selected items without saving the current search definition, then the following message appears:

> Do you want to save changes to this search before closing?
>
> Yes No Cancel

- Clicking Yes, displays the Save Search dialog from where you can save the search.
- Clicking Cancel, returns you to the Data Selector.
- Clicking No, closes the Data Selector window.

Search Name Exists

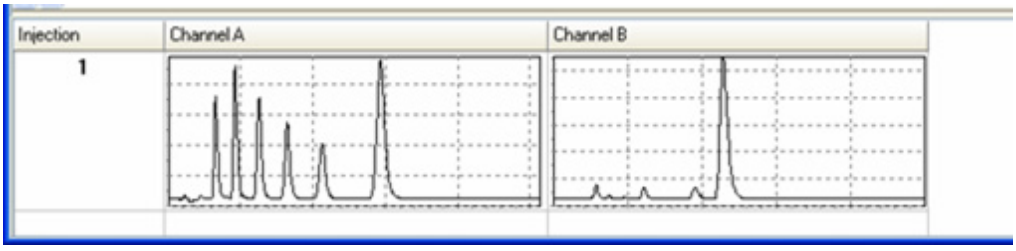
If you try to save a search with a name that already exists, then you are prompted with the following message:

> The search "name" already exists. Do you want to replace it?
>
> OK Cancel

- Clicking Cancel, returns you to the Data Selector.
- Clicking OK, overwrites the existing search with the new definition.

Preview Pane

The Preview pane displays the chromatograms associated with the sample row highlighted in the data area. This includes all channels for all replicate injections. A row appears in the display for each injection associated with the selected sample. A column appears for each data channel, as well as a column indicating the injection number. The display of the chromatograms show the data stored with each raw data set. If no thumbnail is available for any given cell then that cell will be blank. The entire pane is blank when a batch is selected. Two different sizes of thumbnail are available. Select the size to display in the Preview pane via a popup menu.



Selection of Single Injection

When the Data Selector is opened in single injection selection mode the Preview pane displays automatically. In this case there is also a Select column as the first column in the

display. Only a single injection can be selected; checking any injection row will cause all other rows to be unchecked.

Preview Pane Popup Menu

When you right-click on the Preview pane the following menu is displayed:

Command	**Description**
Enable Magnify	When this item is checked, hovering the mouse pointer over a thumbnail chromatogram will cause a larger version of the chromatogram to be displayed in a tooltip-like window.
Show Large Image	When this item is checked the larger of the two thumbnail images will be displayed in the Preview pane for each channel of each injection.
Show Small Image	When this item is checked the smaller of the two thumbnail images will be displayed in the Preview pane for each channel of each injection.

Data Transfer (Import/Export)

Import

The Import functions are accessed from the **Import** submenu in the **Tools** menu. The distinct import functions are:

- Import TotalChrom data (index files, raw data, or component names)
- Import Chromera Results (from a previously exported Chromera 3.x database file)
- Import Chromera Methods (from a previously exported Chromera 3.x database file)
- Import Chromera Sequences (from a previously exported Chromera 3.x database file)
- Migrate (Import) Chromera 2.0 Data
- Report Templates

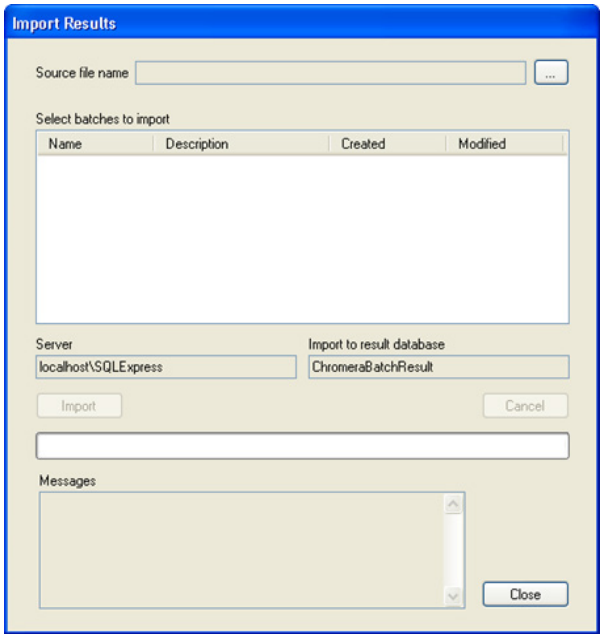
Importing Chromera Data

NOTE: *This function retrieves data from the database file format used by the Chromera 2.x or the 3.x Export function. The migration of Chromera version 1.2 results is handled by a separate utility in the Database Utilities of Chromera Manager.*

This function is similar to that of the Retrieve operation in the Database Utilities with one significant difference. Whereas Retrieve uses the Data Selector to enable you to browse or search the archive database and displays only the selected batches in the retrieve list, this dialog displays all the batches contained in the export file directly in the list. You then select which batches are to be imported by selecting them in the list. This difference in behavior is a reflection of the fact that an export database, by definition, likely to be much smaller than an archive database, and therefore querying for the required batches would not be required.

To import Chromera results:

1. From the **Tools** menu selecting **Chromera Results** from the **Import** submenu, displays the following dialog:



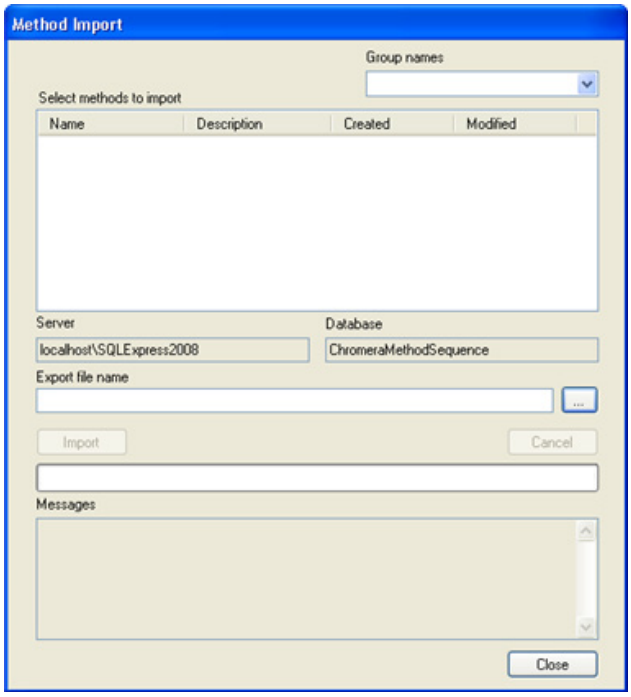
2. Enter the following information to import your results files.

Field	Description
Source file name	Displays the name of the database from which the data will be imported. Select this file by clicking the **Browse** button.
 <Browse button>	The **Browse** button displays a standard **Open** dialog to select a directory containing an exported results file that you want to import. After selecting a directory and file (and if necessary, entering a File name) click the **Open** button.
Select batches to import <displayed in a table of data selected for import>	A table that displays the **Name, Description, Creation Data/Time**, and **Last Modified Data/Time** for each batch contained in the export file. If the selected file was not a valid results export database file then an error message (Selected export file does not contain Chromera data batches) will be displayed in the Messages box and the table will remain empty. Click on the desired displayed file.
Server	Displays the name of the server configured for the selected instrument. The field is populated automatically from the configuration of the current instrument.
Import to results database	Displays the name of the results database configured for the selected instrument. The field is populated automatically from the configuration of the current instrument.
Import	Click this button to start the import process. When this button is clicked the software attempts to import the selected batches in the export database into the main results database defined in the fields above. As the operation proceeds the progress bar underneath the Import and Cancel buttons is progressively filled in. The result of the import operation is indicated in the **Messages** box. This button is only enabled when one or more batches in the table are selected.
Cancel	This button that can be used to cancel a import operation while it is in progress.
Messages	A box that displays messages associated with the Import Results function. If the selected file is not a valid Chromera results export database file then a message will be displayed here and the selection list will remain empty. Any existing messages (from a previous operation) will be cleared when a new export file is selected, the Refresh Items button is clicked or the Import button is clicked. If the Import operation is successful a message indicating this is displayed.
Close	This button closes the Import Results dialog Use of this button will not cancel an import operation in progress; the dialog closes when the import is complete.

Import Chromera Methods

To import Chromera methods:

1. From the **Tools** menu selecting **Chromera Methods** from the **Import** submenu, displays the following dialog:



2. Enter the following information to import your method files.

Field	Description
Group names	You can type in the group name or select it from the drop-down list.
Select methods to import <displayed in a table of data selected for import>	A table that displays the **Name, Description, Creation Data/Time**, and **Last Modified Data/Time** for each method contained in the export file. If the selected export file does not contain Chromera methods, then a error (Selected export file does not contain Chromera methods) is displayed in the Messages box and the table will remain empty.
Server	Displays the name of the server configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.
Database	Displays the name of the methods database configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.
Export file name	Select this file by clicking the **Browse** button.

 <Browse button>	The **Browse** button displays a standard **Open** dialog for you to select a directory containing an exported method file that you want to import. After selecting a directory and file (and if necessary, entering a File name) click the **Open** button.
Import	This button starts the import process. When this button is clicked the software attempts to import the selected batches in the export database into the main results database defined in the fields above. As the operation proceeds the progress bar underneath the Import and Cancel buttons is progressively filled in. The result of the import operation is indicated in the Messages box. This button is only enabled when one or more batches in the table are selected.
Cancel	This button that can be used to cancel a import operation while it is in progress.
Messages	A box that displays messages associated with the Import Results function. If the selected file is not a valid Chromera results export database file then a message will be displayed here and the selection list will remain empty. Any existing messages (from a previous operation) will be cleared when a new export file is selected, the Refresh Items button is clicked or the Import button is clicked. If the Import operation is successful a message indicating this is displayed.
Close	This button closes the Method Import dialog. Use of this button will not cancel an import operation in progress; the dialog closes when the import is complete.

Unique Naming of Methods

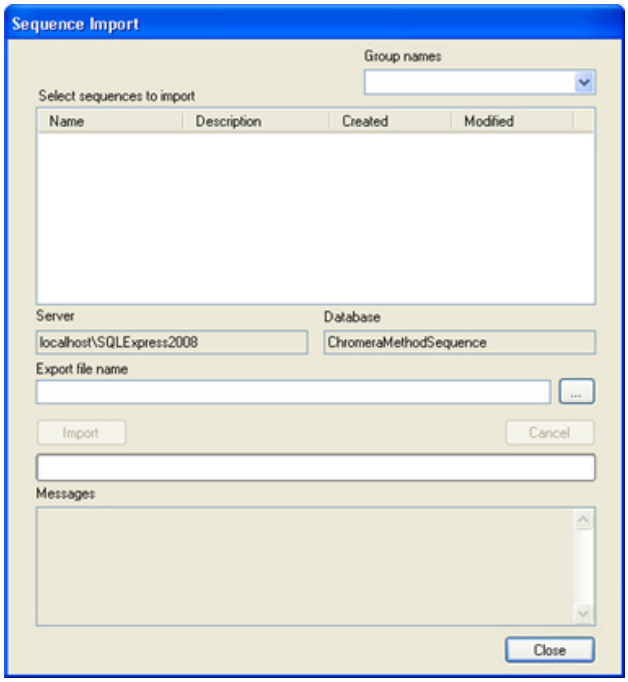
Each method must have a unique name within the group to which it is assigned. The complications arising when methods and sequences are imported require some additional actions, since many methods can be imported in one operation and separately renaming in the case of each 'collision' would be impractical and annoying.

When one or more methods are to be imported the software will determine in advance whether one or more 'name collisions' will occur. If so, you will be presented with a list of the methods involved and will be asked to create a new group (this must therefore be a new and unique name). You will be given the option of assigning only those methods for which a name collision occurs to the new group or all of the methods being imported. The imported methods will be updated with the new group name as required.

Import Chromera Sequences

To import Chromera sequences:

1. From the **Tools** menu selecting **Chromera Sequences** from the **Import** submenu, displays the following dialog:



2. Enter the following information to import your sequence files.

Field	Description
Group names	You can type in the group name or select it from the drop-down list.
Select sequences to import <displayed in a table of data selected for import>	A table that displays the **Name, Description, Creation Data/Time**, and **Last Modified Data/Time** for each sequence contained in the export file. If the selected export file does not contain Chromera sequences then a error (Selected export file does not contain Chromera sequences) is displayed in the Messages box and the table will remain empty.
Server	Displays the name of the server configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.
Database	Displays the name of the methods database configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.
Export file name	Select this file by clicking the **Browse** button.
 <Browse button>	The **Browse** button displays a standard **Open** dialog for you to select a directory containing an exported sequence file that you want to import. After selecting a directory and file (and if necessary, entering a File name) click the **Open**

	button.
Import	This button starts the import process. When this button is clicked the software attempts to import the selected batches in the export database into the main results database defined in the fields above. As the operation proceeds the progress bar underneath the Import and Cancel buttons is progressively filled in. The result of the import operation is indicated in the Messages box. This button is only enabled when one or more batches in the table are selected.
Cancel	This button that can be used to cancel a import operation while it is in progress.
Messages	A box that displays messages associated with the Import Results function. If the selected file is not a valid Chromera results export database file then a message will be displayed here and the selection list will remain empty. Any existing messages (from a previous operation) will be cleared when a new export file is selected, the Refresh Items button is clicked or the Import button is clicked. If the Import operation is successful a message indicating this is displayed.
Close	This button closes the Sequence Import dialog. Use of this button will not cancel an import operation in progress; the dialog closes when the import is complete.

Unique Naming of Sequences

When one or more sequences are to be imported the software will determine in advance whether one or more name collisions will occur for both the sequences and the associated methods. Collisions of sequence names and methods names are handled separately from each other but each will be treated in a similar way to that described in Unique Naming of Methods in the section Import Chromera Methods. You will be given the option of assigning all sequences to the new group or only those for which a name collision occurs. The imported sequences will be updated with the new group name as required.

If methods referenced in any sequence have to be assigned to a new group because of a name collision then you will have the option of assigning all methods to the new group or only those which a collision occurs. The sequences will be updated as necessary with the revised method references/indices, and the methods will be updated with the new group name as required

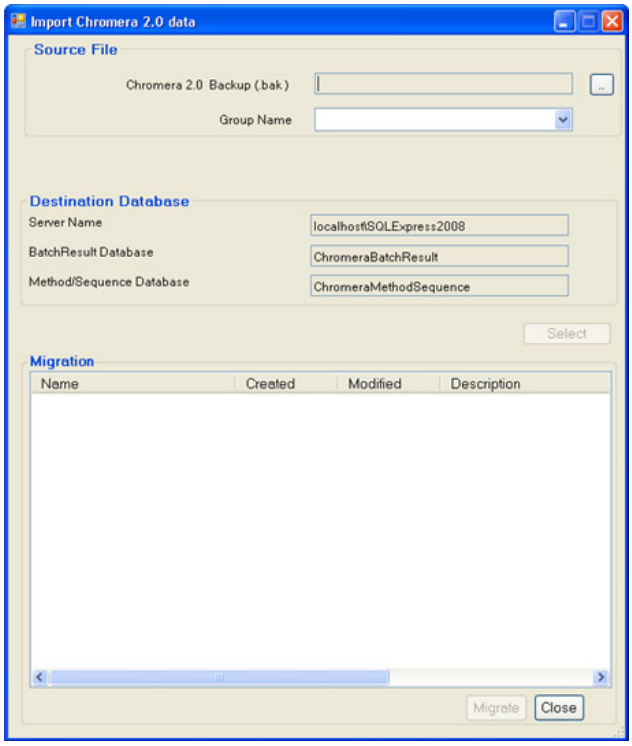
Import Chromera 2.0 Data

Data acquired in Chromera 2.0 can be imported (migrated) into Chromera 3.0. Although Methods created in Chromera 2.0 cannot be imported directly, they can be extracted from the data after it has been imported. Sequences created in Chromera 2.0 cannot be imported into Chromera 3.0.

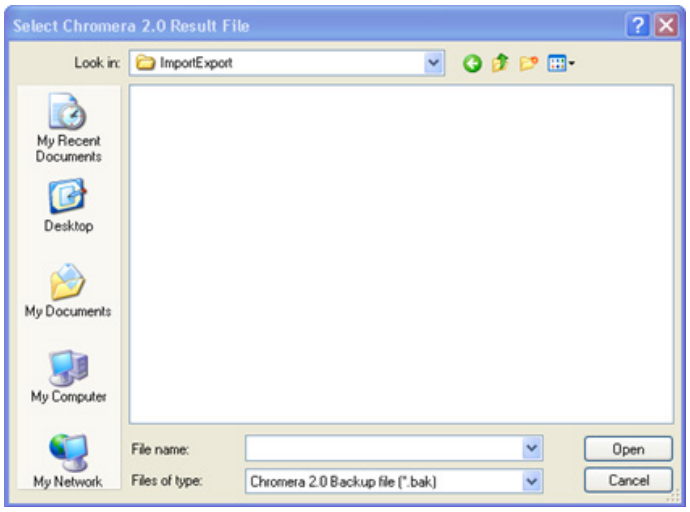
The following procedure shows how to import (migrate) data acquired in Chromera 2.0 into Chromera 3.0 and how to extract the Methods. To import Chromera 2.0 data , follow this procedure:

3. Select Chromera 2.0 Data from Import in the Tools menu.

   The Database Settings dialog appears:



4. Click the browse button  to display the **Select Chromera 2.0 Result File** dialog.



5. Locate your desired Source Files by navigating to the directory that contains your Chromera 2.0 data.

6. Select the .BAK file then click **Open**.
   This file then appears to the right of **Chromera 2.0 Backup (.BAK).**

7. Select a **Group Name** from the drop-down list or type a new Group Name.

8. Select the type you want to import: **Result**, **Method**, or **Sequence**.

9. The Destination Database displays the Server Name, Batch Result Database, and Method/Sequence Database.

10. Click the **Select** button.
    The files populate the table under **Migration**.

11. Select individual data or select all batches by clicking in the **Select all Batches** checkbox.

12. Click the **Migrate** button to save these batches to your database.
    A status message appears in the lower left corner of this dialog (for example, **Status: Data Imported successfully)**.

13. When done click the **Close** button.
    The data is now in the Chromera v3.0 format.

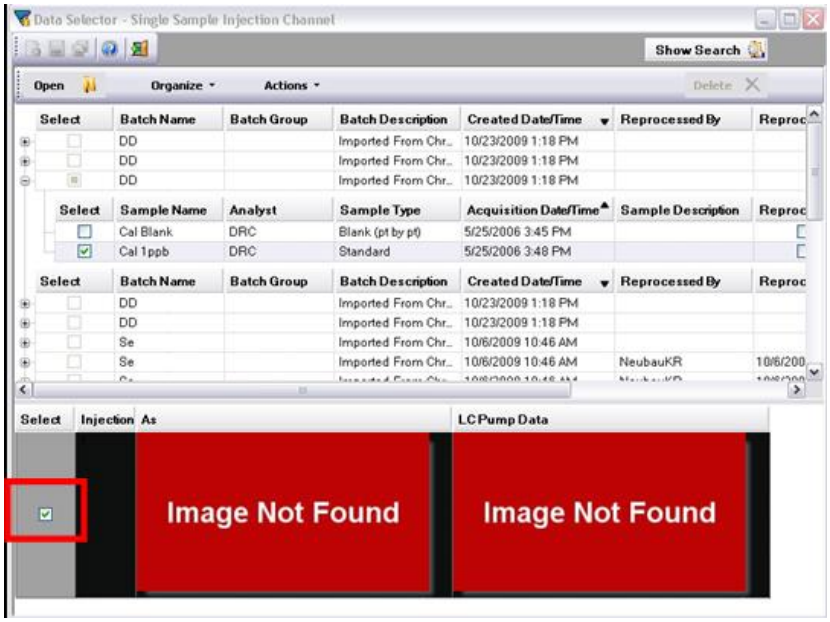
To view the data:

14. Click on the **Post Run** button to enter the Post Run environment, and select **Open Data** from the **File** menu.
    In the Data Viewer, the Batches which were imported are present. The **Batch Description** for the Imported Data displays Imported from Chromera 2.0. Data previews are not available for Chromera 2.0 data.

15. Check the check box next to the data you want to open, and click the **Open** button. The data will not appear in the Post Run window.

16. To view the Method associated with the 2.0 data, click the **Method** button to enter the Method environment.

17. Select Extract Method from Results from the File menu.
    The **Data Selector** opens.



18. Click on the  next to the desired batch, click in the check box of the appropriate sample, then on the bottom of the screen, click in the check box of the appropriate injection where the chromatogram preview would be. Finally, click the **Open** button.

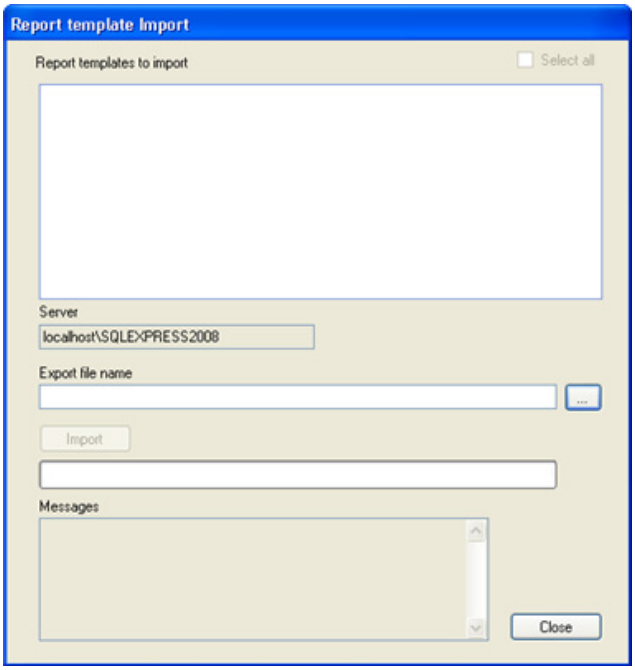
19. The **Save As** dialog will open. Enter an appropriate **Name** and **Group**, then click **Ok**.

20. The Method is now saved into the Chromera 3 database and can be treated as any method which was created in Chromera 3.

Import Report Templates

To import Chromera report templates:

1. From the **Tools** menu selecting **Report Templates...** from the **Import** submenu, displays the following dialog.



2. Enter the following information to import your template files.

Field	Description
Select templates to import <displayed in a table of data selected for import>	A table that displays the **Name, Description, Creation Data/Time**, and **Last Modified Data/Time** for each template contained in the export file. If the selected file was not a valid template file then an error message (Selected export file does not contain Chromera templates) will be displayed in the Messages box and the table will remain empty.
Server	Displays the name of the server configured for the selected instrument. The field is populated automatically from the configuration of the current instrument.
Export file name	Displays the name of the file from which the data will be imported. Select this file by clicking the **Browse** button.
 <Browse button>	The **Browse** button displays a standard **Open** dialog for you to select a directory containing an exported template file that you want to import. After selecting a directory and file (and if necessary, entering a File name) click the **Open** button.
Import	This button starts the import process. When this button is clicked the software attempts to import the selected batches in the export database into the main results database defined in the fields above. As the operation proceeds the progress bar underneath the Import and Cancel buttons is progressively filled in. The result of the import operation is indicated in the Messages box. This button is only enabled when one or more batches in the table are selected.

Cancel	This button that can be used to cancel a import operation while it is in progress.
Messages	A box that displays messages associated with the Import Results function. If the selected file is not a valid Chromera results export database file then a message will be displayed here and the selection list will remain empty. Any existing messages (from a previous operation) will be cleared when a new export file is selected, the Refresh Items button is clicked or the Import button is clicked. If the Import operation is successful a message indicating this is displayed.
Close	This button closes the Import Results dialog Use of this button will not cancel an import operation in progress; the dialog closes when the import is complete.

Importing TotalChrom Data

Chromera supports importing raw data and some parts of methods from TotalChrom versions that utilize the latest file raw and method file versions only. For each imported file in which items that could not be imported, a log is created that contains a record indicating the parameter and the nature of the problem. At the end of the import operation you are informed of files that generated such logs. Observe the following points about importing TotalChrom data:

- It is not necessary for TotalChrom to be installed on the Chromera workstation on which the import is performed. Chromera is able to access and read the TotalChrom files directly, without the need for TotalChrom or TCAccess.
- Only the latest version of each TotalChrom file type (Raw and Method) is imported. Earlier file versions will have to be updated using TotalChrom before they can be imported.
- A Chromera method will not be automatically created based on information contained in a TotalChrom RAW data file. Only the absolute minimum of information required to make sense of the raw data (i.e. sampling rate and end time) will be saved in the Chromera batch with the raw data; no instrument or other parameters will be extracted from the TotalChrom instrument method in the raw file. However, it will be possible to construct a valid (if minimal) Chromera method by extracting the method from the batch result. For this purpose a TotalChrom device will be utilized by Chromera to construct each data channel.

Importing TotalChrom Raw Data

When multiple files are imported the raw data files will be assigned to batches given the same name as the sequence file associated with the TotalChrom RAW file.

The information necessary to construct the batch records are determined from the contents of the various RAW file header sections as described in a separate document. In particular, sample identification, sample weights and values, and Instrument Method parameters can be

used to map the appropriate fields. Some of the fields present in the TotalChrom file headers will not be used in the Chromera environment.

1. A single TotalChrom RAW file represents one sample row in a Chromera Batch. The header information and Sequence Cycle record stored within that RAW file will allow the construction of that Batch record.

2. Since a TotalChrom sequence does not have a **Number of Injections** field (e.g. 3 injections of the same sample requires 3 rows in a TotalChrom sequence), that field in Chromera will always be determined by gathering all raw files that share the same Sample Name, Sample Number and Vial Number as replicates.

3. It must be considered that TotalChrom Sequence Cycle Numbers for RAW files to be imported are not necessarily exactly sequential. Cycles in a TotalChrom sequence may represent non-data rows, such as User Programs or Cleanup-Shutdown rows.

4. The Instrument Method information from the RAW file will only be used to populate the Instrument Method section of the Chromera batch record to the minimum extent required to make the data usable within Chromera. This means the channel identifier (used to create a Chromera channel of the same name) and the data sampling rate. Only references to the identification of the TotalChrom Process and Calibration methods will be found in the RAW file header. Therefore, the Peak and Calibration sections of that batch record will be left blank (or default values). Descriptive fields from the TotalChrom method (Method Description and Header text) should be included in the Chromera Method Description and Notes fields respectively.

5. There is no peak information or result data associated with the Batch record created form the RAW file. These fields are left blank. This is the same situation that would result within Sirius by acquiring data with processing being suppressed. That, too, would cause the creation of a batch record containing no peak information or results.

GC Raw Data

There is no fundamental difference in the structure of a RAW file from GC vs. HPLC, but there are a few considerations that must be made.

1. Data from a GC (Clarus or AutoSystem, or Agilent 5890/6890) may be dual channel in nature. That is, the data may come from two different detectors on the same instrument for the same sample.
   The acquisition sequence for that sample will have both the A and B channels fully populated. This is reflected in the header information of the raw data files. One file will represent the A channel data and the other will represent the B channel data. The sample information and Instrument Method is common for both the A and B channels. The Process and Calibration methods may be different for the two channels (but these are irrelevant since none of the parameters are imported).

2. The raw data may still be single channel in nature, but may come from either Channel A or Channel B of the Instrument Method. Therefore, the Instrument Method for GC data may indicate Channel A, or Channel B, or Dual.

3. The only relevant Instrument Method information that can be imported from a TotalChrom GC raw data file are:
   Description (Free text)
   Header Text (Instrument description)
   Data Channel and source (Detector A, Detector B, etc.)
   Sampling Rate (in points per second)

A/D Converter Raw Data

The ability to import raw data generated by an NCI or 900 Series A/D interface is supported in this release of Sirius.

There is no fundamental difference in the structure of a RAW file from GC vs. HPLC, but there are a few considerations that must be made.

1. Data from an NCI or 900 Series interface may be dual channel in nature. That is, the data may come from two different detectors on the same instrument for the same sample.
   The acquisition sequence for that sample will have both the A and B channels fully populated. This is reflected in the header information of the raw data files. One file will represent the A channel data and the other will represent the B channel data. The sample information and Instrument Method is common for both the A and B channels. The Process and Calibration methods is different for the two channels (but these are irrelevant since none of the parameters are imported).

2. The raw data may still be single channel in nature, but may come from either Channel A or Channel B of the instrument. Therefore, the Instrument method may indicate Channel A, or Channel B, or Dual. These are indicated in the method by the following codes:
   41 = Channel A
   42 = Channel B

3. The only relevant Instrument Method information that can be imported from a TotalChrom A/D raw data file are:
   Description (Free text)
   Header Text (Instrument description)
   Data Channel (Channel A, or Channel B)
   Sampling Rate (in points per second)

Importing TotalChrom Methods

TotalChrom Methods are not imported directly or in their entirety; instead the following two functions are provided.

Import of Component Information

It is possible to import component (and optionally calibration and baseline events) information into an existing Chromera method. This obviates the need to handle the generation of Chromera detector devices and data channels from TotalChrom parameters. Specific information to be imported will be component retention times, names, identification windows, Use Tallest flag, peak type (analyte, reference peak, internal standard), associated reference peak and/or internal standard (if any). Optionally baseline events, calibration standard names, and amounts can also be imported.

Import of Component Names

A separate function is the importation into the Chromera component dictionary of all unique component names from TotalChrom method files located within a specified directory tree. The basic process is as follows:

1. If you have specified a particular set of methods then only these will be examined for component names, otherwise all valid methods found within the specified directory tree (starting nodes and all subdirectories beneath that node) will be examined.

2. A composite list of all unique component names within the examined methods will be generated. Duplicate names, based on case-insensitive comparison, will be eliminated from the list.

3. The list of component names from TotalChrom will be merged with the Chromera component name dictionary. As before, duplicate names, based on case-insensitive comparison, will be eliminated. **NOTE:** In such cases, the original name from the dictionary will be retained and the version originating from TotalChrom will be eliminated.

Import TotalChrom Data Wizard

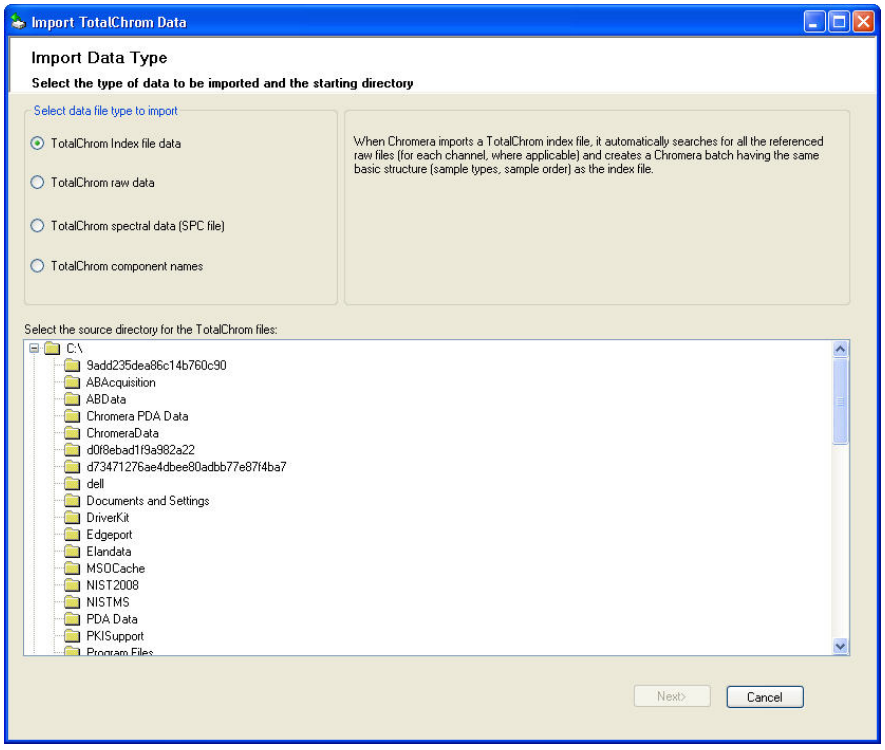
Selecting the **TotalChrom Data** option from the **Import** submenu in the **Tools** menu, displays a dialog that guides you through the import process. The purpose of the wizard is to guide you through importation of either TotalChrom raw data or component names from TotalChrom methods (into the Chromera dictionary).

Import Data Type

This initial page displays for you to select the type of TotalChrom data to be imported (TotalChrom Index Files Data, TotalChrom raw data, TotalChrom Spectral data (SPC file), or TotalChrom component names) and select the source directory containing the TotalChrom files.

After selecting the source directory click the **Next** button.

NOTE: *Next button will not be enabled until you select a directory containing files corresponding to the selected data type. For example, if you select* ***TotalChrom Index Files Data****, the directory must contain IDX files.*



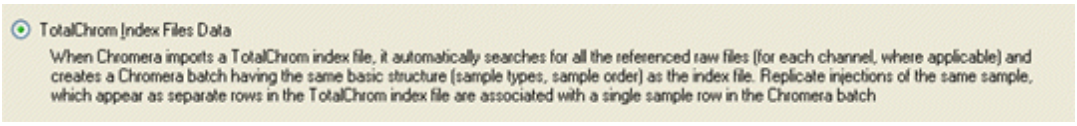
About the Settings on this Page

Control	Description
TotalChrom Index Files Data	Selecting **TotalChrom Index Files Data** indicates data will be imported from TotalChrom Index files.

TotalChrom raw data	Selecting **TotalChrom raw data** indicates data will be imported from TotalChrom raw files. When Chromera imports TotalChrom raw data it examines the header information to determine which files within the specified directory form part of the same data set. File referencing the same sequence file name will be gathered into a single Chromera Batch. Files that share the same sample name, sample number and vial number will designated as replicate injections of the same sample. Separate data channel designations will be identified in the case of dual channel data.
TotalChrom spectral data (SPC file)	Selecting **TotalChrom spectral data (SPC file)** indicates data will be imported from TotalChrom spectral files. When Chromera imports a TotalChrom spectral file, it uses the name if the file you have selected to find its location. Spectral data from a single sequence will be gathered into a Chromera Batch and a Chromera Batch structure created.
TotalChrom component names	Selecting **TotalChrom component names** indicates component names from one or more TotalChrom methods will be imported into the Chromera component name dictionary. This function merges the component names found in TotalChrom method files into the Chromera component name dictionary. You have the option of importing names from specific methods or from all methods found within a specified directory tree.
Select the source directory for the TotalChrom files:	A browse control that allows you to select the directory containing the required TotalChrom files.
Search subdirectories for methods	Select this check box to indicate that methods from all subdirectories found in the tree of which the selected directory is the root will be examined and all components from all of these methods will be extracted. This check box is only displayed when the **TotalChrom component names** option is selected.
Back	Click this button to display the previous page in the wizard.
Next	Click this button to display the next page in the wizard.
Cancel	Click this button to close the Import Wizard window without any data being imported.

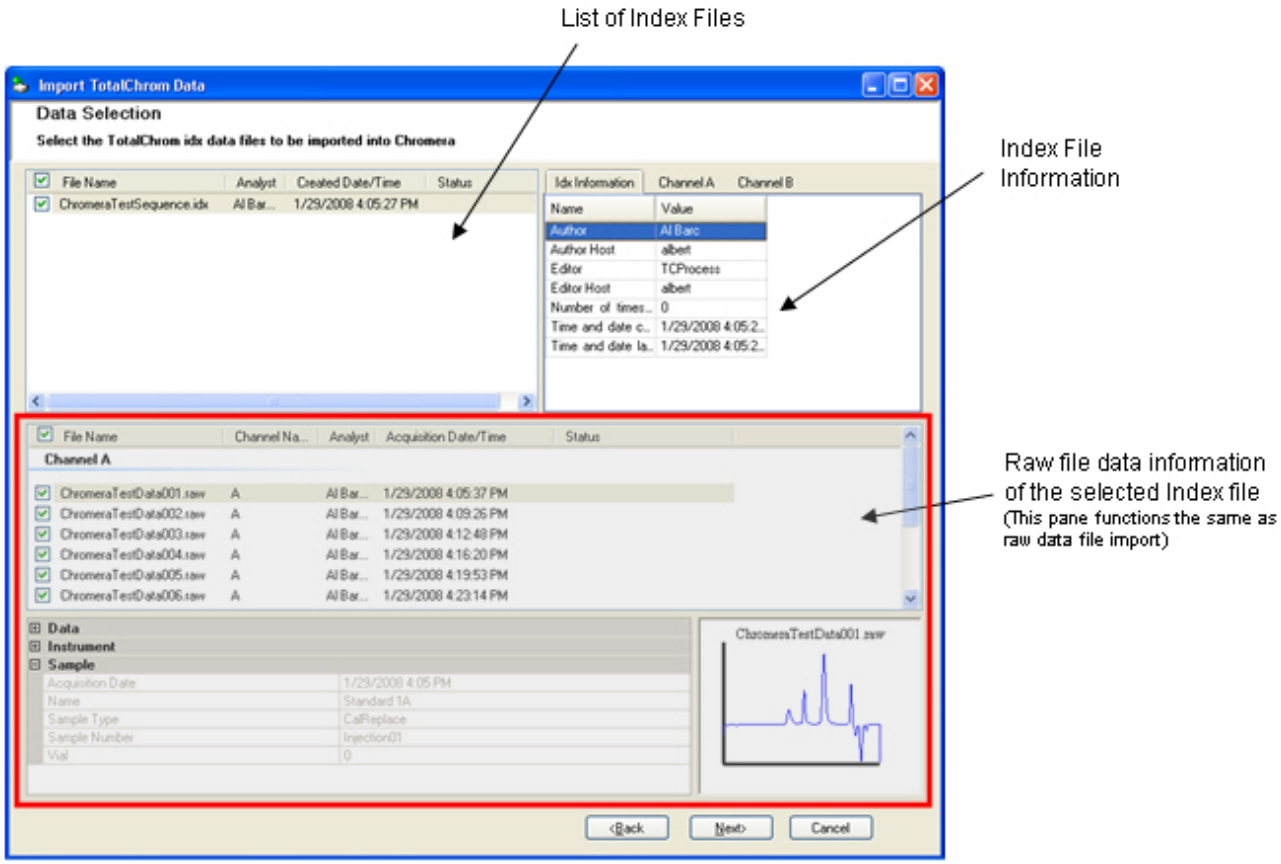
TotalChrom Index Files Data Selection

This **Data Selection** screen displays if you selected **TotalChrom Index Files Data** on the first wizard page, chose a directory that contains the index file data, and clicked the **Next** button.



This option enables you to import a group of TotalChrom RAW data files by selecting the Index (IDX) file that points to them. This creates a corresponding Chromera Batch as a consequence.

The software reads each file in the selected directory with a **.idx** extension and determines if it is a valid TotalChrom index file. For the purposes of import into Chromera, valid means that its format is the latest TotalChrom index file revision. If the file is valid, an entry for it will be included in the upper pane display list. Also, a list of raw files for each index file is displayed in the lower pane (the view is same as seen in raw file import). The first row is selected and highlighted in the IDX file pane, and the first raw file is highlighted with its information and chromatogram displayed in the bottom two panes.



This screen contains the following information.

Control	Description
Check box next to **File Name**	You can select all / deselect all option by clicking in this check box. This selects / deselects all valid IDX and all its corresponding valid raw files. Also, selecting any raw file will automatically select its corresponding IDX file.
List of Index Files	All valid index and valid raw files are checked and ready to import. The first row is selected and highlighted in index file pane. This displays the associated raw files and highlights the first raw file. This raw file information and chromatogram displays in the bottom two panes.
The **Status** column in the Idx file pane	Contains the following information: • No raw files found (color code : red) If a search fails those IDX files are marked in red and also its corresponding raw (one/more/all) will be marked in red. If a missing file is found in other location, you can paste it in the IDX path or IDX folder then click next to refresh the raw file information. This selection preserves all states of check/uncheck for raw and IDX still present in that directory. • Few raw files found (color code : blue) • Invalid IDX file (color code : red) • Blank IDX file - Created during setup but data not acquired (color code : red)
Idx Information	Information about the selected index file.
Raw Files	Associated with the selected index file. You can select individual raw files from total available raw files by clicking in each check box.
The **Status** column in RAW file pane	Contains the following information: • A File missing at acquired path or a File missing in IDX folder (a tool tip indicates the path where the raw file is expected to be) • Invalid raw file (color code : red)
Back	Click this button to display the previous page in the wizard.
Next	Click this button to display the next page (Review Batch Data) in the wizard. This button is disabled unless at least one file is checked in the list.
Cancel	Click this button to close the Import Wizard window without any data being imported. Enabled at all times.

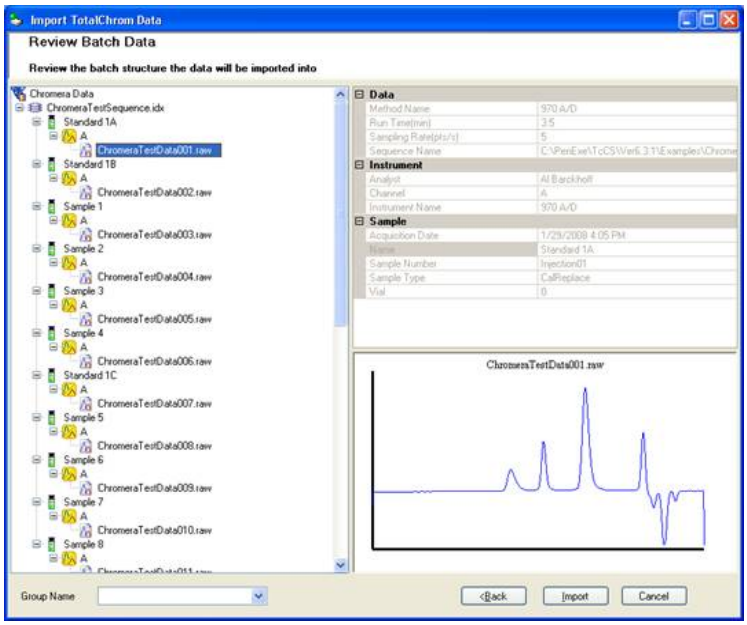
Index Files Review Batch Data

1. After clicking **Next** on the previous Data Selection screen the following screen appears.

2. When reviewing batch data from an index file, you can rename the batch name to the right of  by right-clicking on it, selecting **Rename Node**, and typing a new name.

3. Clicking **Next** on the index files data selection page displays the **Review Batch Data** page for you to review the batch structure the data will be imported into.

4. Clicking on a raw file displays the information and chromatogram associated with the raw file in the top right and lower right boxes.



Right-clicking on any node other than the batch name displays a popup menu containing the commands **Collapse All** or **Expand All**.

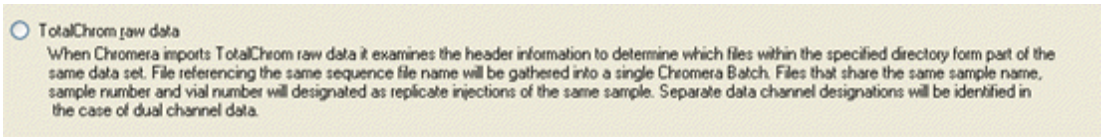
Control	Description
Display of the data tree	A tree view that displays the Index File data that will be imported into Chromera. Clicking on a node displays the information for that node in the upper box on the right and lower box on the right. You can you can rename the batch name by right-clicking on it, selecting **Rename Node**, and typing a new name.
Properties Display <upper box on the right>	Displays the properties of the selected file. Properties are shown when a file is selected (highlighted) in the list.
Chromatogram Display <lower box on the right>	Displays the chromatogram from the selected file. The chromatogram will be shown when a file is selected (highlighted) in the list.

Group Name	Select a name from the drop-down list or enter the name of the Chromera group to which the imported data will be assigned. All imported batches are assigned to this group. All groups currently defined in Chromera are displayed in the drop-down list. If you enter a group name that does not yet exist in Chromera, that group will be created.
Back	Click this button to display the previous page (file selection page) in the wizard.
Import	Click this button to begin the actual import process.
Cancel	Click this button to close the Import Wizard window without any data being imported.

5. Select or enter a **Group Name** and click **Import** to import the TotalChrom index files into Chromera.

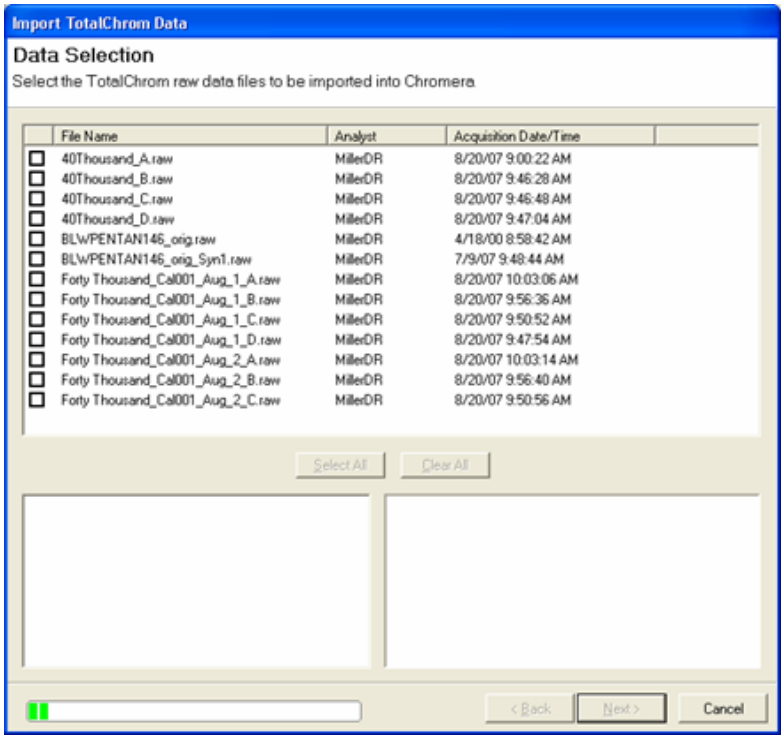
TotalChrom Raw Data Selection

This **Data Selection** screen displays if you selected **TotalChrom raw data** on the first wizard page, chose a directory that contained the raw data, and clicked the **Next** button.



This option enables you to import a group of TotalChrom RAW data files by selecting them directly from a folder. A corresponding Chromera Batch will be created as a consequence.

The software reads each file in the selected directory with a **.raw** extension and determines if it is a valid TotalChrom raw file. For the purposes of import into Chromera, "valid" means that its format is the latest TotalChrom raw file revision. If the file is valid, an entry for it will be included in the display list.



A progress bar on the bottom of the screen displays while files are examined. The progress is updated based on the percentage of raw files contained in the directory that have been examined. The **Select All**, **Clear All** and **Back** buttons are disabled during this process.

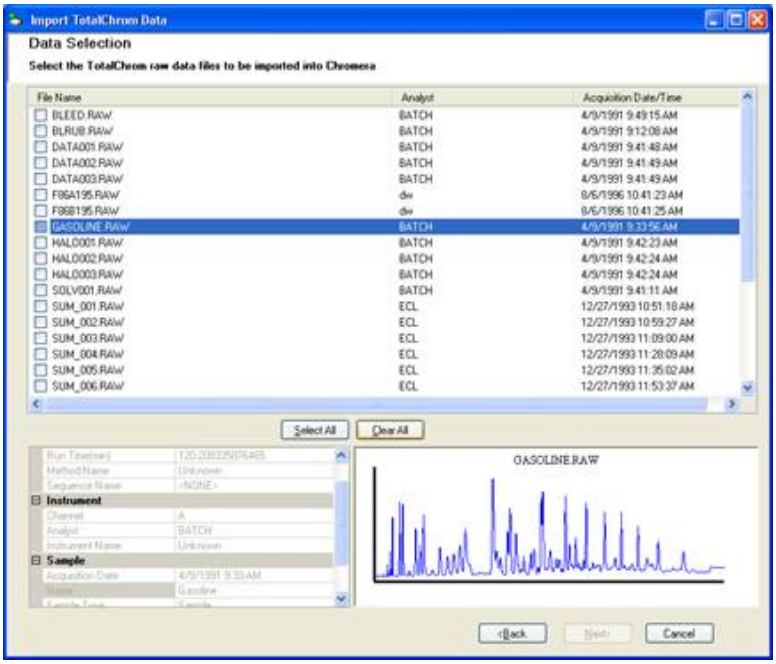
The displayed **File Name** list contains the valid TotalChrom raw files (only) from the directory selected on the first page of the wizard.

Control	**Description**
Display of the files	Displays the valid TotalChrom raw files from the selected directory. The files will be displayed in alphabetical order. This is a single selection list: Only one line can be selected (highlighted) at a time. Of course any number can be checked. Clicking on the file name selects (highlights) the file but does not check it. Clicking on the check box both selects the file and toggles the state of the check box.
File Name	Displays the file name, including extension.
Analyst	Displays the user who acquired the data.
Acquisition Date/Time	Displays the date and time the analysis was started.
Select All	Clicking this button causes all files in the list to be checked. This button is labeled **Select All** but what it really does is **Check All**.
Clear All	Click this button to clear the checks from all files in the list.
<left-hand box>	Displays the properties of the selected file. Properties will be shown when a file is selected (highlighted) in the list.
<right-hand box>	Displays the chromatogram from the selected file. The chromatogram will be shown when a file is selected (highlighted) in the list.

Back	Click this button to display the previous page in the wizard.
Next	Click this button to display the next page in the wizard. This button is disabled unless at least one file is checked in the list.
Cancel	Click this button to close the Import Wizard window without any data being imported. Enabled at all times.

Display of File Details

Details of a selected file are in the boxes at the bottom of the page. A typical details display is shown below:



Click on the **File Name** to highlight it and display its **Properties** in the lower left box and **Chromatogram** in the lower right box. Clicking on the file name highlights the file and display its information but does not select it. Clicking in the check box both selects the file and toggles the state of the check box.

Properties Display

The file details are displayed in a typical properties-style expanding list with several sections. The sections and the items in each section are defined below (not in the picture).

Section	**Items**
Batch Info	Batch Date
	Batch Name
	Data Modified
	File Description

Section	**Items**
Data	Method Name
	Run Time (min)
	Sampling Rate (pts/s)
	Sequence Name

Instrument	Analyst
	Channel
	Instrument Name
Sample	Acquisition Date/Time
	Name
	Sample Number
	Sample Type
	Vial

NOTE: *The above properties include Analyst and Acquisition Date/Time even though these values are included in the file list. This is mainly for consistency with the properties display on the next wizard page, which does not include the file list.*

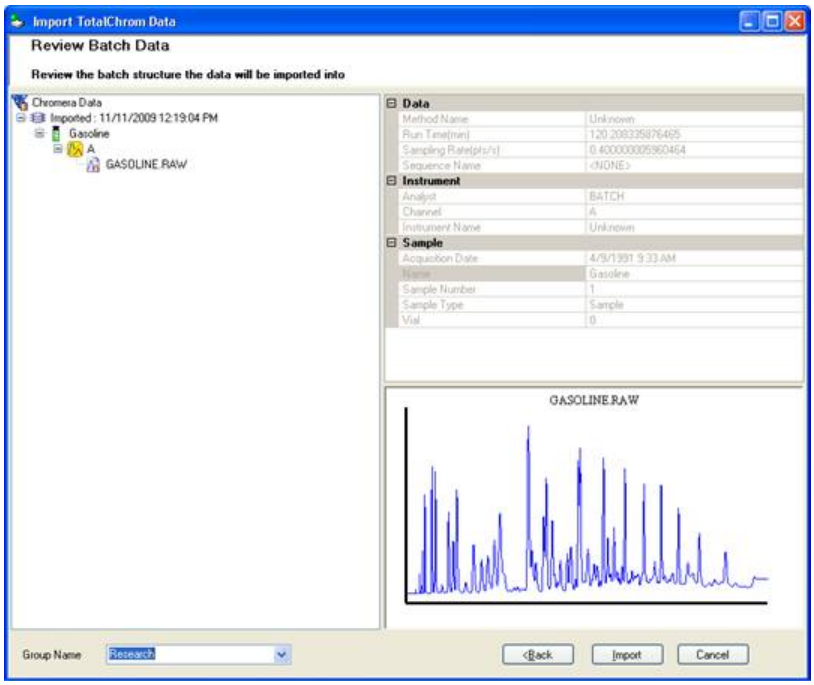
Chromatogram Display

The chromatogram from the raw file is displayed at default scaling (i.e. with the maximum data point set to full scale). Only the curve will be displayed, without axes, labels or annotations of any kind. This is also the plot that will be stored, along with the raw data itself, as a thumbnail representation of the chromatogram, for use elsewhere in Chromera.

Review Batch Data

Clicking **Next** on the raw data selection page displays the **Review Batch Data** page for you to review the batch structure the data will be imported into.

Right-clicking on any node other than the root (Chromera Data) of the data tree displays a popup menu containing the command **Remove Node**. Selecting this command will cause the node clicked on, and all its child nodes, to be removed from the tree. The data associated with deleted nodes will not be imported when you click **Import**.



You can set the following in this dialog.

Control	Description
Display of the data tree	A tree view that displays the raw data that will be imported into Chromera.
Properties Display <upper box on the right>	Displays the properties of the selected file. Properties are shown when a file is selected (highlighted) in the list. See Display of File Details.
Chromatogram Display <lower box on the right>	Displays the chromatogram from the selected file. The chromatogram will be shown when a file is selected (highlighted) in the list. See Display of File Details.
Group Name	Select a name from the drop-down list or enter the name of the Chromera group to which the imported data will be assigned. All imported batches are assigned to this group. All groups currently defined in Chromera are displayed in the drop-down list. If you enter a group name that does not yet exist in Chromera, that group will be created.
Back	Click this button to display the previous page (file selection page) in the wizard.
Import	Click this button to begin the actual import process.
Cancel	Click this button to close the Import Wizard window without any data being imported.

- Select or enter a **Group Name** and click **Import** to import the TotalChrom raw data into Chromera.

About the Batch Data Tree

The batch tree indicates the data structures that will be created in the Chromera database to accommodate the imported data. The structure will be based on data found within the raw file headers. There are five levels in the tree:

Level	Icon	Description
1		The root node of the tree which indicates that this will be the structure of the set of selected raw data once it is imported into Chromera. Only one node of this type will be present.
2		Represents a Chromera batch. One of these nodes will be present in the tree for each unique sequence name references within the selected TotalChrom index files or raw data files.
3		Represents a sample contained within the parent batch. All TotalChrom files that reference a given sequence name will be included as children of that batch node. A sample node will be created for each unique combination of Sample Name + Sample Number + Vial Number found in the raw data file headers. It is this combination (referred to as the 'Sample ID' here) that is used as the label for the sample node.
4		Represents a channel of data associated with the parent sample. A channel node will be created for each unique TotalChrom channel designator found within the set of raw data files associated with each sample. The maximum number of channels from TotalChrom data will be

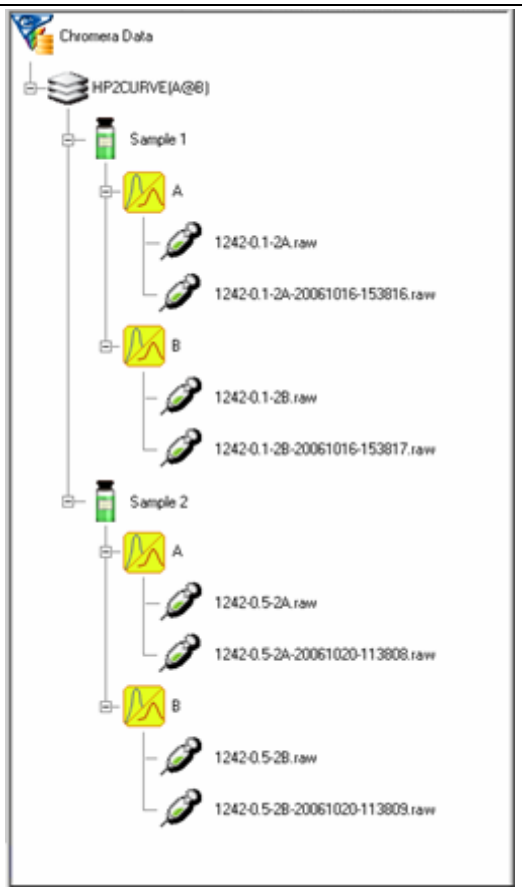
		two. The text version of the channel designator ('A' or 'B') will be used as the label for the node.
5		Represents an chromatogram (i.e. the raw data) associated with the parent channel. An injection node will be created for each raw file found having the identical combination of: sequence name + Sample ID + channel name. The node label will be the raw file name.

Example of the Review Batch Data Tree

The following example describes the structure of the tree. This example shows a set of data files ready to be imported. The tree indicates the data consist of:

Two samples: Identified as **Sample 1** and **Sample 2**

Two data channels in each sample: Identified as **A** and **B**

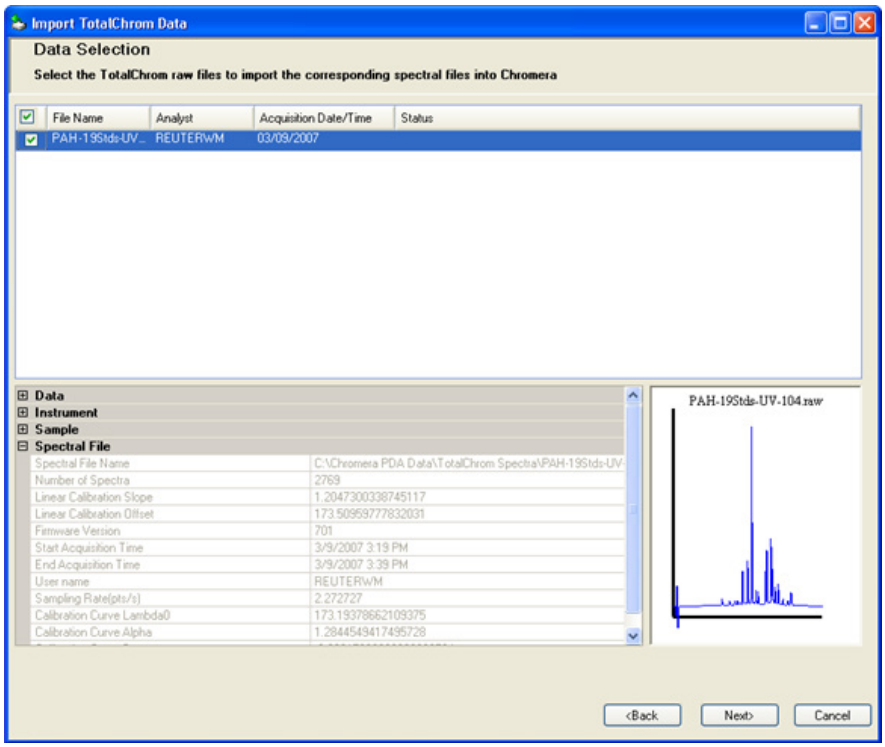


Two injections of each sample:
Sample 1 Injection 1 Channel A: 'A1242-01-2A.raw'
Sample 1 Injection 1 Channel B: 'A1242-01-2B.raw'
Sample 1 Injection 2 Channel A: 'A1242-01-2A-020061016-153816.raw'
Sample 1 Injection 2 Channel B: 'A1242-01-2B-020061016-153817.raw'
Sample 2 Injection 1 Channel A: 'A1242-05-2A.raw'
Sample 2 Injection 1 Channel B: 'A1242-05-2B. raw'
Sample 2 Injection 2 Channel A: 'A1242-05-2A-020061020-113808.raw'
Sample 2 Injection 2 Channel B: 'A1242-05-2B-020061020-113809.raw'

TotalChrom spectral data (SPC file) Selection

Use the **Data Selection** screen to import **TotalChrom spectral data (SPC file).** It uses the name of the raw file you have selected to find its location. Spectral data from a single sequence will be gathered into a Chromera Batch and a Chromera Batch structure created. The selection of a data file that has an associated spectra file, imports the spectral into the appropriate spectral format and any necessary links between the chromatogram and spectra file being established. This conversion occurs independent of the current instrument configuration; i.e., a PDA need not be configured into the current instrument for this conversion to occur.

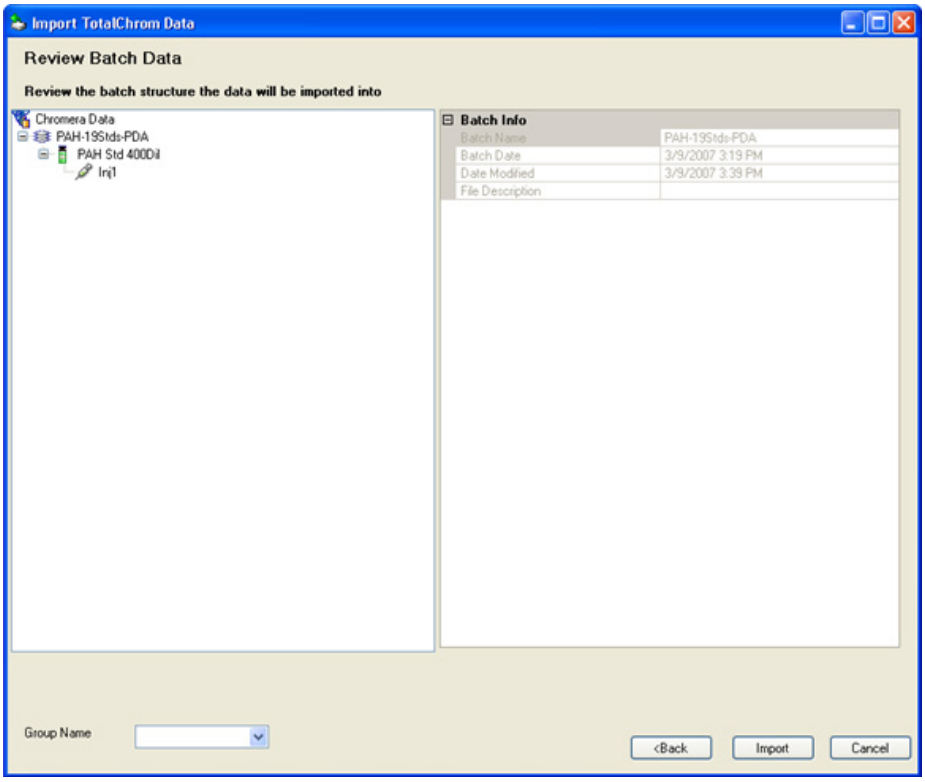
This screen displays the spectral files found with their details.



Column headers displayed in the **File** selection list will be identical to those shown for TotalChrom raw files. Only raw files with associated spectra will be included in this list.

Review Batch Data (SPC)

The Review Batch page displays when you click Next on the raw Data Selection page (This follows the behavior of the Chromera Platform). Raw file names are displayed when importing via the .raw files. Result file names are displayed when importing via the .rst files.



1. Click Import to import spectral files using the raw file as the identifier of the chromatogram. This file will be imported as a batch (with only injections) and will not have any associated channel or method.

2. After importing the spectral file, you will need to manually reprocess the file to extract the channels.

3. Once it is reprocessed, the behavior will be similar to any other batch in Chromera.

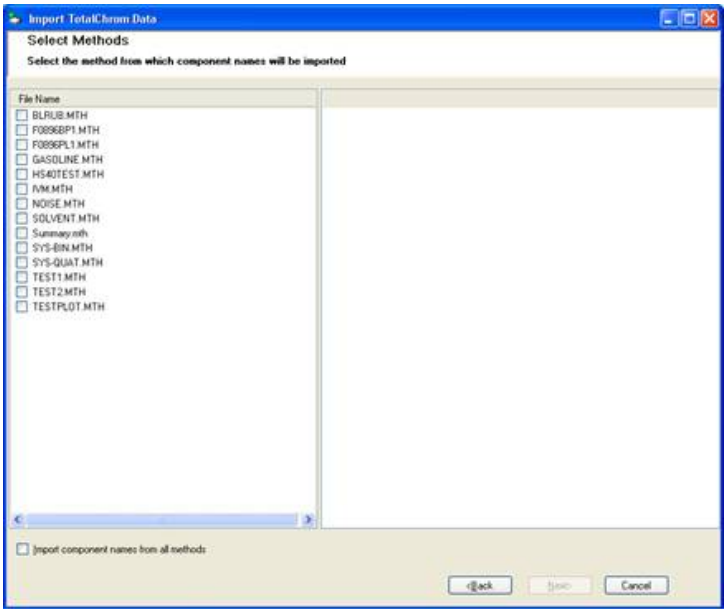
TotalChrom Component Names Selection

This page is displayed if you selected **TotalChrom component names** on the first wizard page, chose a directory, and clicked the **Next** button.

This option enables you to populate the Chromera Component Dictionary with the complete list of component names read from all the TotalChrom methods found in a selected folder and subfolders.

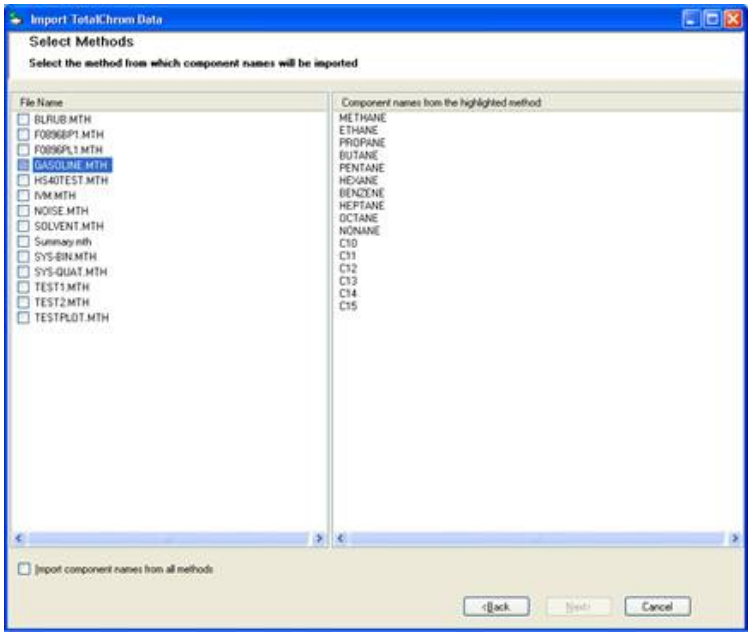
NOTE: *The **Search subdirectories for methods:** check box indicates that methods from all subdirectories found in the tree of which the selected directory is the root will be examined and all components from all of these methods will be extracted.*

The software reads each file in the selected directory with a .mth extension and determines if it is a valid TotalChrom method file. A progress bar is displayed while files are examined. For the purposes of import into Chromera, valid here means that its format is the latest TotalChrom method file revision. If the file is valid it will be included in the check list box.



Control	Description
Right-hand box displays component names	Displays the component names from the highlighted file.
Import component names from all methods	Click on this check box to indicate component names will be extracted from all valid method files found in the designated path(s).
Back	Click on this button to display the previous page (data type selection page) in the wizard.
Next	Click on this button to display the next page in the wizard. This button is disabled if the 'Import component names from selected methods only' option is selected and no methods are checked in the list.
Cancel	Click on this button to close the Import Wizard window without any data being imported.

4. Click on the method name to highlight it and display the method's components in the right pane.



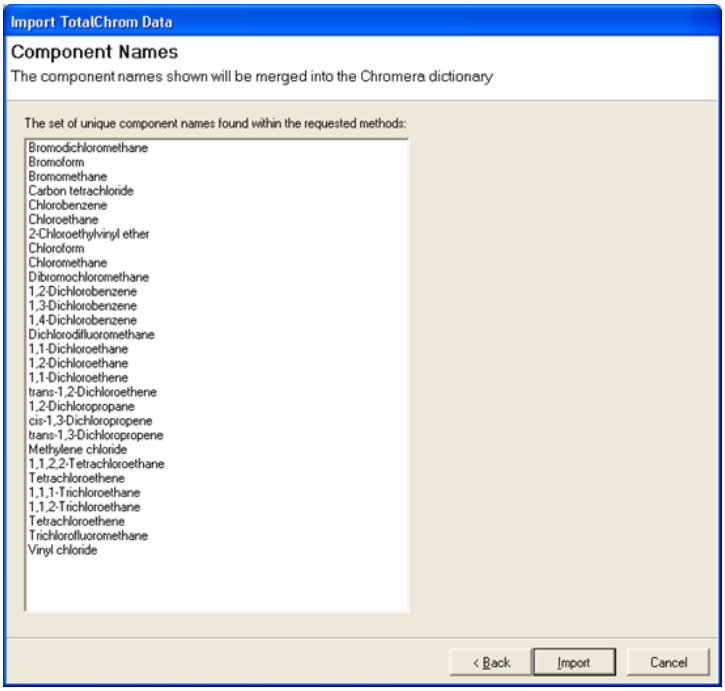
5. Highlight each method to see if it contains the components you want. If the method contains your desired components, click in the check box to select the method. You can continue to highlight methods to see components and select methods that contain the components you want.

6. Click in a check box to select a method.

7. After selecting the methods that contain your desired components, click **Next**.
   A list of components shown will be merged into the Chromera directory.

Component Names Final Page

Clicking **Next** on the **Select Method** page displays this page:



The list contains the complete set of component names from all of the selected methods to be merged into the Chromera component name dictionary. This list will be compiled as described in Import of Component Names.

5. Click **Import** to import the component names into the Chromera Component Dictionary.

Control	**Description**
The set of unique component names found within the requested methods:	Displays the list of component names that will be merged into the Chromera component name dictionary.
Back	Click this button to display the previous page (Select Method page) in the wizard.
Import	Click this button to initiate the merging of the displayed component names into the Chromera component name dictionary.
Cancel	Click this button to close the Import Wizard window without any data being imported.

Method and Sequence Formats

Method Formats:

- Chromera 2.x database structure (to provide 100% lossless transfer of data between Chromera systems)
- XML
- CSV

Each of the above formats will contain all data from the method, including calibration replicate information.

Sequences Formats:

- Chromera 2.x database structure (to provide 100% lossless transfer of data between Chromera systems)
- XML
- CSV

Each of the above formats will contain all data from the sequence.

How to Copy Components from TotalChrom

This dialog displays when you select the **From TotalChrom Method...** command from the **Copy Components** submenu of the **Actions** menu within the Method Editor.

Control	Description
Copy from TotalChrom method	Displays the source of the components to be copied. The field is populated by selecting a file from a standard File Open dialog displayed when the browse button is clicked.
	A browse button that displays files with .mth extension in a standard Windows File Open dialog, enabling you to select a TotalChrom method file.
Copy to channel	A checkbox list to set the data channel(s) the components will be copied to. The list is populated with all data channels from the method. You can copy the components to more than one channel by selecting the required channels within this control.
Include integration events	Check this box to copy the integration events from the TotalChrom method to the destination channel.
Include calibration standards	Check this box to copy the calibration standard amounts with the other component information.
<component list>	A display listing the components found in the TotalChrom method, together with their retention times.

NOTE: *The **OK** button is disabled until you select a TotalChrom method and at least one destination channel (as required).*

When the **OK** button is clicked the components from the TotalChrom method are copied to the destination channel(s). In general, the copied components are merged with the existing components and will not replace them. However, if a component with exactly the same retention time exists in the destination channel then that component will not be copied. All components will be copied; any selection made in the component list has no impact on the component copied.

Data Mapping – TotalChrom Raw File to Chromera

The raw data values from TotalChrom are in counts but Chromera will convert them to either mAU (for LC UV-Visible data) or mV (for GC or A/D data). The appropriate conversion functions are:

$$\text{Data Value in mV} = \frac{\text{Counts}}{\text{Sampling Rate Factor} \times \text{Voltage Conversion Factor} \times 1000}$$

Where: Counts is the raw data point value from TotalChrom

Sampling Rate Factor is an adjustment factor for sampling rate used only for AutoSystem and Clarus GC data. In the TotalChrom raw file it is stored as d_ps_conversion in the INST_CONFIG structure.

Voltage Conversion Factor represents the number of counts per microvolt. In the TotalChrom raw file it is stored as d_volt_conversion in the INST_CONFIG structure.

$$\text{Data Value in mAU} = \frac{(\text{Counts} - \text{Offset}) \times 1000}{\text{Conversion Factor}}$$

Where: Counts is the raw data point value from TotalChrom

Offset is a zero adjustment. In the TotalChrom raw file it is stored d_plot_offset in the INST_CONFIG structure.

Conversion Factor represents the number of counts per Absorbance unit. In the TotalChrom raw file it is stored as d_plot_conversion in the INST_CONFIG structure.

Import Messages

Duplicate Batch Name

When importing multiple files you have the option of associating all the data sets in a single batch or importing each as its own batch. By default, the Batch is given the same name as the sequence file associated with the TotalChrom RAW file. If a Batch by that name already exists, you are notified, and a dialog is presented to specify how to proceed. You may enter a new name for the Batch, or add the new data to the existing batch. The ability to associate separate TotalChrom raw data files into a single dual channel Chromera sample run would be of significant value. If this can be achieved without extensive effort it is supported.

If you try to import a batch with a name that already exists, then the following message is displayed:

The batch '<batchname>' already exists. How would you like to proceed? [Enter a new name for the batch] [Add new data to existing batch] [Cancel]

- Click **Cancel**, to return to the Import dialog.

- Click **Enter a new name for the batch**, then a dialog appears to specify a new name for the batch.

- Click **Add new data to existing batch**, then the new data is added to the existing batch.

Import Errors

For each imported file in which items that could not be imported were encountered a log is created that contains a record indicating the parameter and the nature of the problem. At the end of the import operation you are informed of files that generated such logs .

You can view any errors associated with importing a data item, by clicking on a data item in the Data Conversion Tree that has an icon with a red border and viewing the error message in the Details pane.

Below is a list of the error messages that you may encounter:

- No Error
- File Read Error
- File Write Error
- Header Checksum Error
- File Checksum Error
- File or Path not Found
- Invalid TotalChrom Signature
- Invalid TotalChrom RAW File
- Invalid TotalChrom Method File
- Invalid TotalChrom File Type
- Invalid TotalChrom Revision (Must be v6.2.0)
- Invalid TotalChrom technique
- Database Write Error

Export

About Export

You can export batches, methods and sequences in Chromera database format. The procedure for each of the data types will be basically the same: You will use the data Selector to select the data sets to be exported and specify a file name and path for the export file.

Exporting Data

Export of methods, sequences and data (raw/results) to various formats are supported. No information is changed in the source database during this process.

NOTE: *You cannot export all three items at the same time.*

Chromera 3.x

Only export of complete batches is supported. The export format is itself be a database export, which allows all relationships to be maintained intact. This allows the batch to be imported into another Chromera system as an entity.

Excel

It will be possible to export the results from a set of samples displayed in the Post Run environment to an Excel spreadsheet. This will be a manual function in this release, that will save the results for all samples in the batch to a single Excel spreadsheet. It will also be possible to save the raw data from a data channel (from a single injection) to an Excel spreadsheet, as a manual operation.

Methods

Chromera 3.x database structure provides 100% transfer without loss of data between Chromera systems.

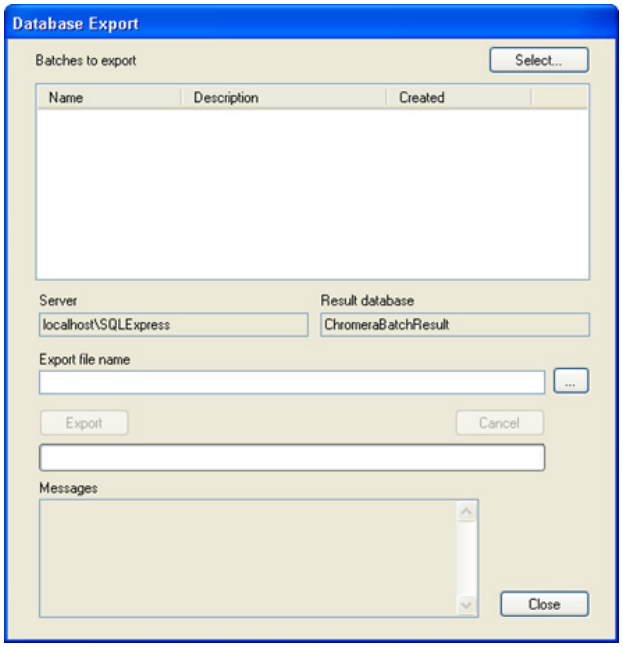
Sequences

Chromera 3.x database structure provides 100% transfer without loss of data between Chromera systems.

Export Chromera Results

To Export Chromera results:

1. From the **Tools** menu select **Chromera Results** from the **Export** submenu. The following, dialog displays.



Enter the following information to export your batches.

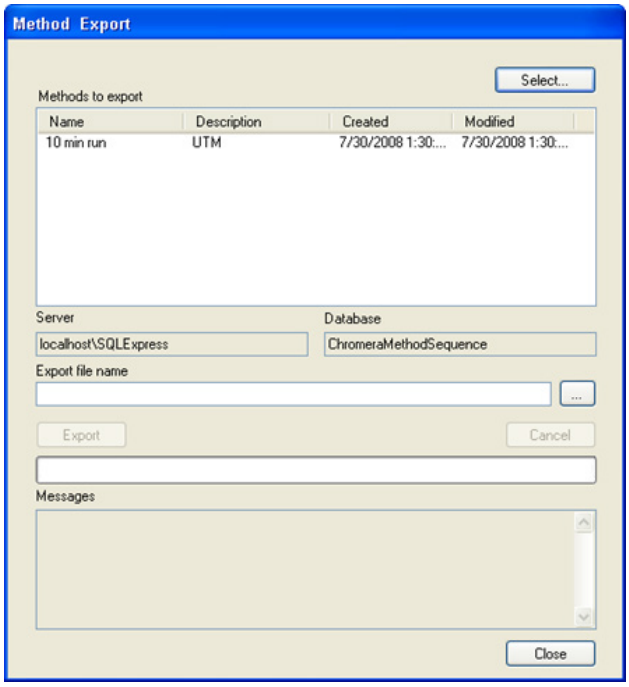
Field	Description
Select ...	Clicking this button displays the Chromera **Data Selector** in the **Multiple Batches** mode so you can locate and select the results to be exported. When you select one or more batches and click the **Open** button the Data Selector closes and information about the selected batches appears in the **Batches to export** table.
Batches to export <displayed in a table of data selected for import>	A table that displays the **Name, Description, Creation Data/Time**, and **Last Modified Data/Time** for each batch contained in the export file. If the selected file was not a valid results export database file then an error message (Selected export file does not contain Chromera data batches) is displayed in the Messages box and the table remains empty.
Server	Displays the name of the server configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.
Results database	Displays the name of the results database configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.

Export file name	Select a file by clicking the **Browse** button or you can enter a fully-qualified file name into the field.
 <Browse button>	The **Browse** button displays a standard **Save As** dialog for you to select a directory for your exported file. After selecting a directory (and if necessary, entering a File name) click the **Save** button.
Export	This button starts the export process and the software will attempt to export the selected batches in the Chromera results database into the specified file. As the operation proceeds the progress bar underneath the Export and Cancel buttons is progressively filled in. The result of the export operation is indicated in the Messages box.
Cancel	This button that can be used to cancel a export operation while it is in progress.
Messages	A box that displays messages associated with the Export Results function. If the Export operation is successful a message indicating this is displayed.
Close	This button closes the Database Export dialog. Use of this button will not cancel an export operation in progress; the dialog closes when the export is complete.

Export Chromera Methods

To Export Chromera methods:

1. From the **Tools** menu selecting **Methods** from the **Export** submenu, displays the following dialog.



2. Enter the following information to export your method files.

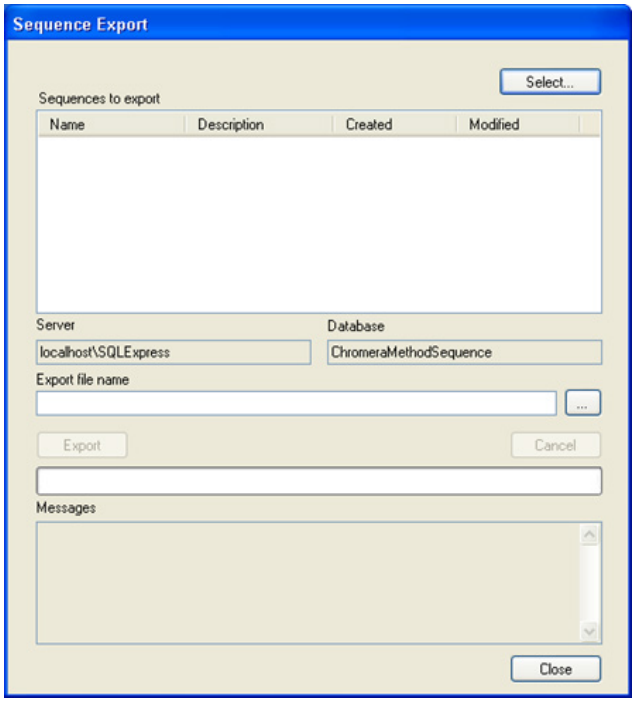
Field	Description
Select ...	Clicking this button displays the Chromera **Data Selector** in the **Multiple Methods** mode, to select the methods to be exported. When you select one or more methods and click the Open button the Data Selector closes and information about the selected batches appears in the **Methods to export** table.
Methods to export <displayed in a table of data selected for import>	A table that displays the **Name, Description, Creation Data/Time**, and **Last Modified Data/Time** for each method contained in the export file. If the selected file was not a valid method export database file then an error message (Selected export file does not contain Chromera methods) is displayed in the Messages box and the table remains empty.
Server	Displays the name of the server configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.
Database	Displays the name of the database configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.

Export file name	Select a file by clicking the **Browse** button or you can enter a fully-qualified file name into the field.
 <Browse button>	The **Browse** button displays a standard **Save As** dialog to select a directory for you exported file. After selecting a directory (and if necessary, entering a File name) click the **Save** button.
Export	This button starts the export process and the software will attempt to export the selected methods in the Chromera database into the specified file. As the operation proceeds the progress bar underneath the Export and Cancel buttons is progressively filled in. The result of the export operation is indicated in the Messages box.
Cancel	This button that can be used to cancel a export operation while it is in progress.
Messages	A box that displays messages associated with the Export Methods function. If the Export operation is successful a message indicating this is displayed.
Close	This button closes the Method Export dialog. Use of this button will not cancel an export operation in progress; the dialog closes when the export is complete.

Export Chromera Sequences

To Export Chromera sequences:

1. From the **Tools** menu selecting **Sequences** from the **Export** submenu, displays the following dialog.



2. Enter the following information to export your sequence files.

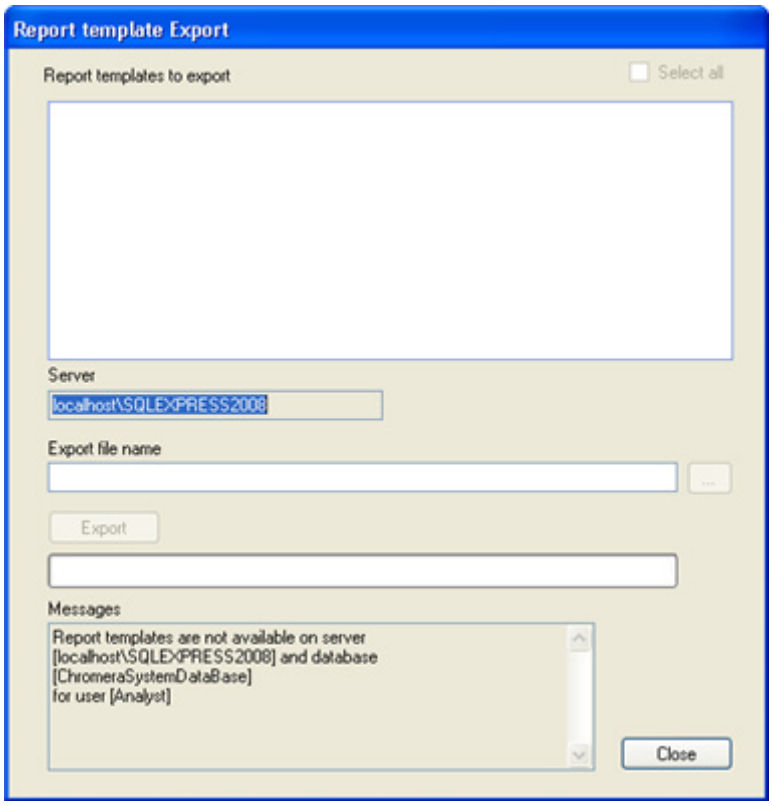
Field	Description
Select ...	Clicking this button displays the Chromera **Data Selector** in the **Multiple Sequences** mode, to select the sequences to be exported. When you select one or more sequences and click the **Open** button the Data Selector closes and information about the selected batches appears in the **Sequences to export** table.
Sequences to export <displayed in a table of data selected for import>	A table that displays the **Name, Description, Creation Data/Time,** and **Last Modified Data/Time** for each sequence contained in the export file. If the selected file was not a valid sequence export database file then an error message (Selected export file does not contain Chromera sequences) is displayed in the Messages box and the table remains empty.
Server	Displays the name of the server configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.

Database	Displays the name of the database configured for the selected instrument. This field is populated automatically from the configuration of the current instrument.
Export file name	Select a file by clicking the **Browse** button or you can enter a fully-qualified file name into the field.
 <Browse button>	The **Browse** button displays a standard **Save As** dialog to select a directory for your exported file. After selecting a directory (and if necessary, entering a File name) click the **Save** button.
Export	This button starts the export process and the software will attempt to export the selected sequences in the Chromera database into the specified file. As the operation proceeds the progress bar underneath the Export and Cancel buttons is progressively filled in. The result of the export operation is indicated in the Messages box.
Cancel	This button that can be used to cancel a export operation while it is in progress.
Messages	A box that displays messages associated with the Export Sequences function. If the Export operation is successful a message indicating this is displayed.
Close	This button closes the Sequence Export dialog. Use of this button will not cancel an export operation in progress; the dialog closes when the export is complete.

Export Report Templates

To export Chromera report templates:

1. From the **Tools** menu selecting **Report Templates...** from the **Export** submenu, displays the following dialog.



2. Enter the following information to import your files.

Field	Description
Select templates to export <displayed in a table of data selected for import>	A table that displays the Name, Description, Creation Data/Time, and Last Modified Data/Time for each template contained in the export file. If the selected file was not a valid template file then an error message (Selected export file does not contain Chromera templates) will be displayed in the Messages box and the table will remain empty.
Server	Displays the name of the server configured for the selected instrument. The field is populated automatically from the configuration of the current instrument.
Export file name	Displays the name of the file from which the data will be imported. Select this file by clicking the browse button. Using the Open dialog, navigate to the directory containing the file, select the name of the stored template file then click the Open button.

 <Browse button>	The Browse button displays a standard file selector dialog in single selection mode, enabling you to select a previously exported results database. After selecting an export file and closing the Open dialog, information about the batches in the export file will appear in the table.
Export	This button starts the Export process. When this button is clicked the software attempts to export the selected templates into the main results database defined in the fields above. As the operation proceeds the progress bar underneath the Export and Cancel buttons is progressively filled in. The result of the export operation is indicated in the Messages box. This button is only enabled when one or more batches in the table are selected.
Cancel	This button that can be used to cancel an export operation while it is in progress.
Messages	A box that displays messages associated with the Import Results function. If the selected file is not a valid Chromera results export database file then a message will be displayed here and the selection list will remain empty. Any existing messages (from a previous operation) will be cleared when a new export file is selected, the Refresh Items button is clicked or the Import button is clicked. If the Import operation is successful a message indicating this is displayed.
Close	This button closes the Import Results dialog Use of this button will not cancel an import operation in progress; the dialog closes when the import is complete.

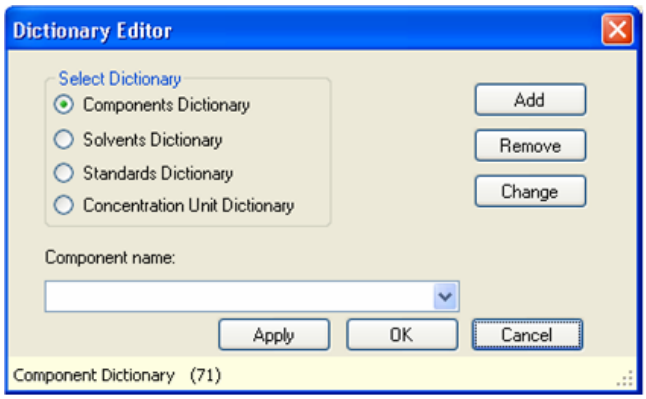
Dictionaries

About Dictionaries

Chromera maintains several dictionaries to facilitate the consistent entry various names into method and other user-entry fields. The supported dictionaries are for component names, solvent descriptors, concentration units, and calibration standard names. The functionality of these dictionaries will be identical.

Dictionary Editor

This dialog displays when you choose the **Dictionary Editor** command from the **Tools** menu. The same dialog provide access for editing all system dictionaries.



Control	Description
Select Dictionary	A set of radio buttons to select the dictionary to be edited.
<combo box>	Select an existing or enter a new dictionary item to be modified or deleted. The drop-down list display the current contents of the selected dictionary.
Add	Click the button to add the name in the combo box to the selected dictionary.
Remove	Click the button to delete the item displayed in the combo box from the selected dictionary.
Change	Click the button to display a dialog enabling to change the item displayed in the combo box within the selected dictionary.
Apply	Click the button to save all changes made within the editing session to the database. The Cancel button will therefore not function to undo these changes. The dialog will remain open.
OK	Click the button to save all changes made within the editing session to the database and closes the dialog.
Cancel	Click the button to undo all changes made since the last click of the apply button. The dialog closes.

<status bar>	Displays messages relating to operation of the Dictionary editor. When a dictionary is selected using the radio buttons the name of the selected dictionary is displayed, along with a count of its contents. For example: Components Dictionary (124) When a command is issued, the status panel will display a message indicating the result. For example: The item was successfully added to the Components Dictionary or The Solvents Dictionary was successfully updated

NOTE: *If the Add, Remove or Change button is clicked when the combo box is empty (or does not contain an existing dictionary entry) then an error message will be displayed. This approach has been chosen over the alternative, of disabling the buttons when there is no entry, so that the error message can be used to provide guidance on how to perform the function. The specific error message for each button will be:*

- *Add - Please enter the item you want to add to the selected dictionary before clicking the Add button.*
- *Remove - Please enter (or select) the item you want to remove from the selected dictionary before clicking the Remove button.*
- *Change - Please enter (or select) the item you want to change before clicking the Change button.*

Choosing Names

When entering one of the supported identifier types into the method, sequence, etc. you will be able to easily select a name from the associated dictionary. This is an auto-complete style of interaction where you are offered choices as you type in the first few characters of a name.

NOTE: *The dictionary will NOT be available in controls where you are not entering a name but selecting an existing one from the method. For example, in the Relative Reference Component field of the Channel parameters, the Reference Peak field of the Peak Detection parameters or the Internal Standard Component field of the Peak Detection parameters.*

Adding Names

When you type into one of the dictionary-supported fields a name that does not already exist in the associated dictionary, that name will be added to the dictionary.

Saving Names

When you enter one of the supported names types into the method or sequence a copy of the complete name will be stored in the data set (and not just a pointer to the dictionary entry).

This is a practical necessity when considering the import and export of methods (and sequences). It is also not necessarily desirable that a change to the spelling of a name in the

dictionary should affect stored methods (as would occur if indexes to the dictionary were stored in the method).

Editing Names

Since typing in a misspelled name would result in a new entry to the dictionary (as described in Adding Names), a dictionary editor is provided.

Note that changes to the dictionary will not affect names already stored in methods (as explained in section Saving Names).

Typical LC Workflows

About LC Workflows

The following are examples of some typical LC workflows. In some cases they describe the actions you need to perform in order to carry out a specific task, whether or not a data system were to be used. In other cases the workflow is only performed because it is necessary in the context of a data system and hence use of some kind of software tool is assumed. However, even in the latter case the description is as generic as possible, so as to minimize the assumptions made about the software behavior.

Starting Conditions

Instrument Method Development

The following is an overview of the steps for method development.

Starting Conditions

Instrument operational

Set Run Conditions

Modify active method to set up trial instrument conditions.

Set Up Run

Enter parameters required to set up and identify sample run, according to preference. This will likely include vial for injection and some kind of sample or run identification.

Run Sample

Inject sample. Examine chromatogram produced. Optionally integrate the chromatogram for accurate determination of relative peak size to get information about peak separation order.

Evaluation

Compare latest chromatogram with those obtained earlier in the session.

Iteration

Repeat Set Run Conditions through Evaluation until satisfied with the separation achieved.

Instrument Startup from a Shutdown State

Starting Conditions

The instrument is in standby (pump operating at reduced flow, detector lamp may be on or off) or shutdown (pump off, detector lamp off) state, following previous day's analyses.

Instrument Startup from Shutdown

(This step is not performed if the instrument in standby state - see step *Startup Instrument from Standby*

1. Replenish solvents (if required)
2. Purge the pump
3. Set the analytical pump flow conditions
4. Turn the detector lamp on

5. Purge the autosampler
6. *Go to step Preparation Activities*

Startup Instrument from Standby

7. If solvent replenishment required, turn off pump and replenish the solvents
8. Set analytical pump flow conditions
9. Turn detector lamp on (if off)
10. Purge autosampler

Preparation Activities

Following steps are likely performed in parallel

Monitor Instrument

Evaluate noise and drift of detector signal.

Evaluate pump pressure stability.

Review Prior Data

(See separate workflow)

If previous day's results indicate remedial action is required on the instrument this workflow will terminate.

Prepare Samples and Sequence

Prepare samples for injection and place in autosampler.

Create/edit the sequence to be used for analysis of the samples.

Run validation sample

Provided the general instrument indicators are acceptable a sample is run to confirm chromatographic performance is acceptable for sample analysis. (If noise/drift/pressure stability indicate remedial action is required on the instrument this workflow will terminate.)

This validation sample may be run as an isolated analysis or as the first row of the sequence, depending on SOP and/or confidence level in the instrument system.

If the results from the validation sample (which could be suitability values, absolute peaks areas and/or calibration results) are not acceptable remedial action is required on the instrument and this workflow will terminate.

Run/Continue Sequence

Chromera has this under control.

Starting Conditions

The instrument is in standby (pump operating at reduced flow, detector lamp may be on or off) or shutdown (pump off, detector lamp off) state, following previous day's analyses.

Instrument Startup from Shutdown

(This step is not performed if the instrument in standby state - see step *Startup Instrument from Standby*

1. **Replenish solvents (if required)**
2. Purge the pump
3. Set the analytical pump flow conditions
4. Turn the detector lamp on
5. Purge the autosampler
6. *Go to step Preparation Activities*

Startup Instrument from Standby

7. If solvent replenishment required, turn off pump and replenish the solvents
8. Set analytical pump flow conditions
9. Turn detector lamp on (if off)
10. Purge autosampler

Preparation Activities

Following steps are likely performed in parallel

Monitor Instrument

Evaluate noise and drift of detector signal.

Evaluate pump pressure stability.

Review Prior Data

(See separate workflow)

If previous day's results indicate remedial action is required on the instrument this workflow will terminate.

Prepare Samples and Sequence

Prepare samples for injection and place in autosampler.

Create/edit the sequence to be used for analysis of the samples.

Run validation sample

Provided the general instrument indicators are acceptable a sample is run to confirm chromatographic performance is acceptable for sample analysis. (If noise/drift/pressure stability indicate remedial action is required on the instrument this workflow will terminate.)

This validation sample may be run as an isolated analysis or as the first row of the sequence, depending on SOP and/or confidence level in the instrument system.

If the results from the validation sample (which could be suitability values, absolute peaks areas and/or calibration results) are not acceptable remedial action is required on the instrument and this workflow will terminate.

Run/Continue Sequence

Chromera has this under control.

Instrument Method Development

The following is an overview of the steps for method development.

Starting Conditions

Instrument operational

Set Run Conditions

Modify active method to set up trial instrument conditions.

Set Up Run

Enter parameters required to set up and identify sample run, according to preference. This will likely include vial for injection and some kind of sample or run identification.

Run Sample

Inject sample. Examine chromatogram produced. Optionally integrate the chromatogram for accurate determination of relative peak size to get information about peak separation order.

Evaluation

Compare latest chromatogram with those obtained earlier in the session.

Iteration

Repeat Set Run Conditions through Evaluation until satisfied with the separation achieved.

Errors

About Errors

Errors are divided into three classes:

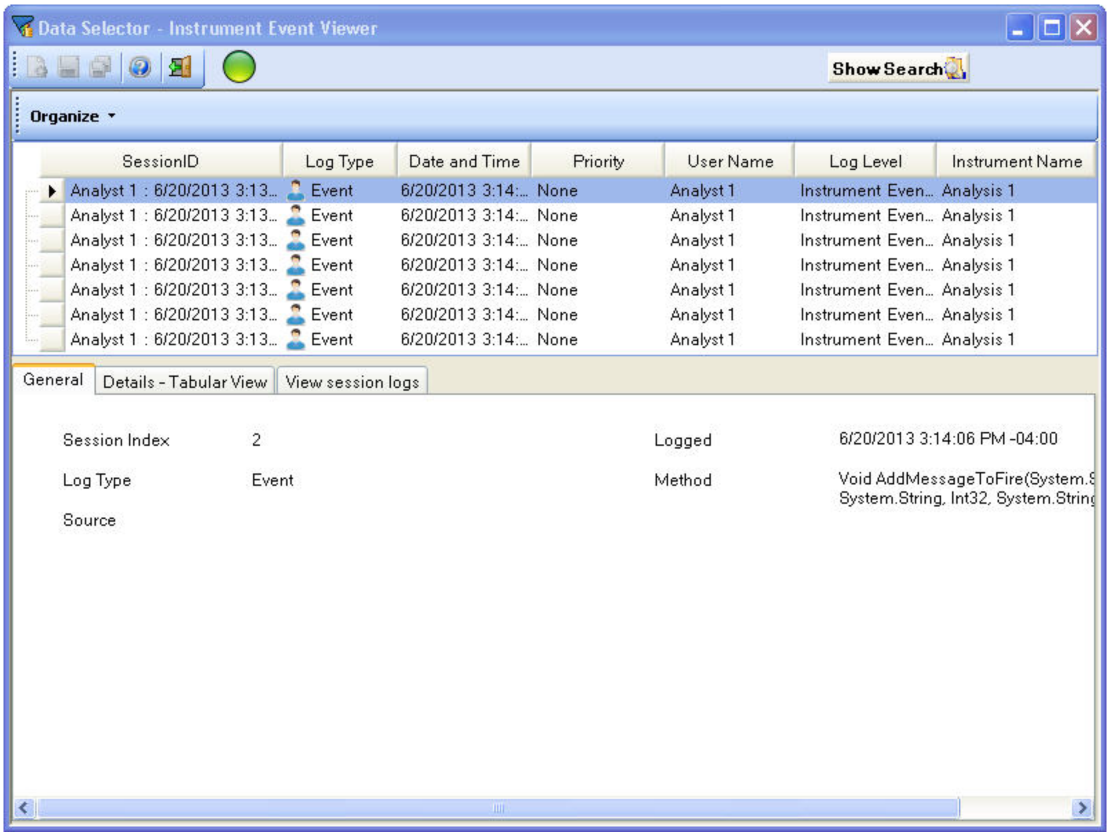
1. Errors relating to instrument operation. These include both errors reported by the device itself and any errors occurring in the communication between a device and the software.

2. Errors occurring during background operations such as end-of-run processing or batch reprocessing.

3. Errors occurring in interactive sessions where the user can be assumed to be present.

Error Logs

The **Instrument Event Viewer** displays when Instrument Error Log command is selected from the **Tools** menu. The **Instrument Event Viewer** displays all errors associated with connection to, and communication with, the devices in the current instance for the current instrument configuration. In addition it displays errors relating to processing and other system operations.



Closing an Instance

If a sequence is running, or data acquisition is in progress under method setup (single run) mode, on an instrument then are not allowed to close that online instance of Chromera. Any attempt to do so results in an error message (You cannot shut down Chromera while the instrument is active) and the Chromera window will remain open.

Method Changed

The current version of this method in the database contains changes made within the Run Time or Reprocess environment since you opened it with the Method Editor.

These changes could involve one or more of the following:

- Modifications you made to the method and saved from Run Time
- Updating of the calibration information within the method as a result of running a calibration sample
- Calculation of suitable Area and Noise Threshold values during automatic processing to replace blank values in the method.

It is recommended **not to overwrite** the updated method with the version displayed here, unless you are certain you wish to do so. You can save the displayed method under a new name by choosing **No** within the message box and then choosing the **Save as** command from the File menu.

Appendix 1 PDA Spectral Processing

Overview of Spectral Processing

In Spectral Processing you can manipulate, process, and display spectral data associated with chromatograms obtained using the PerkinElmer photo diode-array (PDA) detectors.

With spectral processing software you can:

- View chromatographic data as a chromatogram, as a contour map, or as a three-dimensional plot.
- Gain access to all of a chromatogram's associated spectra, and perform various calculations using those spectra.
- Conduct on-screen spectral comparisons, or use the software to perform more complex operations such as adding, subtracting, dividing, and obtaining derivatives of spectra.
- Identify peaks and calculate peak purity.
- Confirm the identity of chromatographic peaks by comparing them with other peaks or standards
- Annotate chromatograms with spectral positions, retention times, component names, or calculated values such as concentrations or purity indices.
- You can select positions on any of the displayed chromatograms and call up their spectra in a separate window.
- Chromatograms and Spectra can be normalized, offset-normalized, or displayed full scale to help you compare them visually.
- The software also allows you to create and browse through spectral libraries, to search those libraries for spectral matches to an unknown spectrum, and to confirm peak identities by matching their spectra to those of known peaks in the libraries.
- Use a two-dimensional contour map to find chromatographic peaks, which may not be showing at the wavelength used for the chromatogram. You can use the contour map to obtain a spectrum and chromatogram at any point on the display. The time, absorbance, and wavelength display axes can be scaled independently.
- View chromatographic data on a three-dimensional plot that provides a scaleable perspective on the complete data set.
- Print chromatograms, spectra, and other data using a color printer. You can annotate and export screen displays to other Windows programs to generate presentation graphics or documentation. You can also export numerical data to programs such as Microsoft Excel for additional study.

About the Spectral Processing Window

Chromera Spectral Processing is designed so that you can easily process, manipulate, and display spectral data associated with chromatograms from one main window. Each instance of Chromera will use one or multiple separate instances of Spectral Processing and each instance will not be shared between different instances of Chromera.

The following topics provide a high-level look at the user interface.

Tell me about the Menu bar

The **Menu Bar**, located along the top of the program window, contains the menu commands that enable you to process, manipulate, and display spectral data associated with chromatograms, as described below:

NOTE: *The symbol ✓ in front of an item indicates that this is an on/off toggle command. The check mark appears in front of the item when the function is active or selected. The default for these commands is switched on.*

Menu	Command	Description
File	Remove	Removes the currently selected spectrum from the Data tree.
	Remove all spectra	Removes all spectra from the Data tree.
	Print	Displays a Print dialog from where you specify which details you want to print from the current view and which printer you want to use. Enabled at all times.
	Apex Spectrum Plot Setup	Displays the Apex Spectrum Plot Setup...dialog. This option is available at all times and in all environments in the Spectra Processing window but will display an error dialog if no channel is selected in the data tree. When not in Graphics Method Editor (GME) the command will also yield an information dialogue that the Apex Spectra Plot report parameters can only be set in the Method environment of the Spectral Processing window.
	Exit	Closes this application.
Edit	Copy Object	Copies the selected object to the clipboard. If the cursor is in either a chromatogram or spectrum region then the numerical values of that trace will be placed on the clipboard. This allows export of data to spreadsheet programs such as Excel.
	Copy Screen As ►Bitmap ►Metafile	**Bitmap** - Copies the entire screen as a bitmap image to the clipboard. **Metafile** - Copies the entire screen as a Windows metafile to the clipboard.
View	✓Chromatogram Baselines	Displays or hides baselines on all chromatograms. Baselines are displayed as a solid red line.

	✓Baseline Spectra	Adds the baseline spectra for the selected spectra to the Data Tree. The baseline spectra are identified by the retention time of the spectrum (or, if a range of spectra are being used, the start and end times of the range) followed by the word **base**; and they appear immediately after the parent spectrum in the tree. This command is enabled when a spectrum is selected in the data tree. The command does not work if a baseline spectrum is selected.
	✓Baseline Corrected Spectra	On the Main and Chrom/Spectra view the command will toggle the displayed spectra to be baseline corrected or uncorrected. On the Contour Map and 3D Plot the command will toggle the ghost spectra to be baseline corrected or uncorrected but has no affect on previously captured spectra. This command has no affect in any of the Spectral Operations views which are all controlled by the Baseline Corrected Spectra in the parameter list for that operation.
	Overlay Chromatograms	Displays the contents of all chromatogram windows with all chromatograms being displayed on the one set of axes. When selected the menu item changes to Stack Chromatograms, and selecting the option returns all chromatograms to being displayed on separate axes.
	Stack Spectra	Displays the contents of all spectral windows split onto separate axes. When selected the menu item changes to Overlay Spectra, and selecting the option again will return to Stack Spectra.
	✓ Cursor	Toggles the cross-hair cursor On or Off. Enabled when a chromatogram, spectrum or contour map is selected on a view (not an operation).
	✓Toolbars	Toggles whether or not the toolbars are displayed. When Toolbars is checked, all three tool bars are displayed.
	✓View tree	Toggles whether or not the View tree is displayed.
	✓Data tree	Toggles whether or not the Data Tree is displayed.
	✓Parameters	Toggles whether or not the Parameters pane for Operations is displayed. Enabled only on Operations. ***NOTE:*** *The Parameters pane always includes the Display or Hits list.*
	Panes ► Chromatogram Spectrum Contour Map 3D Plot	This command allows you to select whether or not a pane, selected in the menu, is displayed on the page. When you hide a pane, a Custom View is formed. Shown panes are checked, hidden panes are unchecked. Enabled for all View pages. This command is disabled on Operation pages. ***NOTE:*** *Hiding a pane does NOT unload the chromatogram or extracted spectra. This command only hides/shows the pane. The contents do not change.*

	View Template ► Save As Delete Export Import	**Save As...** - Displays the Save View As dialog. **Delete** - Deletes the selected custom view. Enabled only when a custom view is selected. **Export** - Displays a standard file selector enabling the selected custom view to be exported to disk. Enabled only when a custom view is selected. **Import** - Displays a standard file selector enabling a custom view exported to disk to be imported into this view tree.
	Log	Displays the log file.
Actions	Zoom control ► ✓X axis ✓Y axis ✓Z axis	Sets the control over the zoom slider. All three are independent toggles enabling any combination to be switched on at the same time to create a multi-directional zoom. X and Y are enabled when a 2D graph is selected, all three when a 3D plot is selected.
	Autoscale ► X and Y Y only	Rescales the selected graph to the maximum range of the data displayed, in either just the Y direction or both, depending on the command selected. Enabled when a 2D graph is selected and the full range is not currently shown.
	Normalize ► X and Y Y only Offset Point	Rescales the selected graph in line with the command selected. **X and Y** and **Y only** will operate based on the full graph. **Offset** will normalize to the maximum point to the right of the X-axis cursor. **Point** on a spectrum or chromatogram will normalize at the current X-axis cursor position, while on a contour map this command will assign all absorbance values above the current X-axis cursor position to the top contour.
	Zero	This command only applies to contour maps. When a Contour Map pane is selected, this command assigns all absorbance values below the mid-point of the cursor to the bottom of the contour. Enabled at all times.
	Previous scale	Returns the selected graph to the previous scaling, stepping back one at a time through previous scale changes. Enabled only when the selected 2D or 3D graph has been scaled, normalized or zoomed.
	3D Rotation Controls ► ✓X only ✓Y only	**Rotate X** - Sets rotation slider to rotate around the X axis. **Rotate Y** - Sets rotation slider to rotate around the Y axis.

	Label Chromatograms	Displays the Label Chromatograms dialog. Enabled any time a chromatogram is selected on a View (not an Operation).
	Annotations ► Add Edit Delete Delete All	**Add** - Opens an empty Edit Annotations dialog. Enabled when a graph is selected. **Edit** - Opens the Edit Annotations dialog with the details of the selected annotation available to edit. Enabled when an annotation is selected. **Delete** - Removes the selected annotation. Enabled when an annotation is selected. **Delete All** - Removes all annotations from the selected graph. Enabled when a graph is selected that has at least one annotation.
	Range	This command is used to display all the spectra within a given time range. When this command is selected a green range box is displayed on the chromatogram: • Positioning the mouse pointer in the range box and clicking actives the range box. When activated: Handles are displayed on the left and right edges of the box. Positioning the mouse pointer over the left or right handles of the box changes it to a horizontal two-headed arrow and click and hold enables the edge to be stretched. Upon releasing the mouse the range box is deactivated. Positioning the mouse pointer over the left or right edge of the box (except for the position of the handles) changes it to a four-headed arrow and click and hold enables the complete box to be moved. Upon releasing the mouse the range box is deactivated. • Positioning the mouse pointer within the box and double-clicking loads all the spectra enclosed by the box into the Data Tree and sets them as selected. The range box is cleared. Clicking on the chromatogram but outside of the box clears the range box. ***NOTE:*** *Range is also an option in the Context menu for a stacked chromatogram.*

	Add All to View	Used both for adding spectra that have come from library searches, mathematical operations, etc, to the Data Tree so they can be viewed on other pages and for permanently displaying spectra temporarily displayed on a View. Enabled in Views at any time a spectrum is temporarily viewed or in Operations when a spectral graph is selected that includes spectra that are not currently part of the Data Tree. When the user selects the **Add All to View** command in any Operation, a Response dialog appears that informs you that spectra have been added to the Data Tree. In any Operations, all the displayed/checked hit spectra will be added to the Data tree under the appropriate chromatogram. Derivative Spectra will be named with the time of the source spectrum plus the label "Derivative" and the derivative order. Spectra from Math operations will be labeled "MATH #" where # will increment to provide a unique name. Information on the math spectrum will yield the source chromatograms and spectra and all necessary parameter values. They will be shown in the non-operation views. If the spectrum already exists in the Data tree, the spectrum will not be duplicated. Individual spectra may be added by context menus in the specific operation.
Tools	View Library	Displays a file select to select the library to be displayed followed by the View Library dialog.
	Build Library ► Create Add Apexes Edit	Displays a file selector either to select the library to be edited or to name the new one, followed by the Edit Library dialog. **Create** - Displays a file selector titled New Library. From the New Library dialog you specify a name and location for the new library. Once you specify a new library, the Create Library dialog appears. **Add Apexes** - Displays a file selector titled New Library. From this dialog you specify a name and location for the new library. Once you specify a library, the Create Library dialog appears. This in turn will be followed by the Edit Library dialog. The Edit Library dialog shows all the named peaks from the selected chromatogram in the Library list by component name. Enabled only if a single chromatogram is selected and it has named peaks in it. **Edit** - Displays a file selector titled Open Library Once you select a library to open, the Edit Library dialog appears. Enabled at all times.
	Chromera ► Reprocess Graphic Method Edit	Opens the selected application with the selected chromatogram passed to the application. Enabled at all times a single chromatogram is selected.
	Format Graphs	Displays the Format Graphs dialog and either goes to the correct tab for the currently active graph type or the Chromatogram tab if no graph is active. Changes made here effect the defaults, to change the current graph only use the context menu for the graph.

	Spectral Baseline correction	Displays the Spectral Baseline correction dialog. Enabled only if a single chromatogram is selected in the Data tree. ***NOTE:*** *This command is also available as context menu for chromatograms only.*
	Override save dialog	Displays the dialog that enables you to set whether the you want to turn off the automatic save dialog .
	Edit Default Method	Displays the Method Editor dialog for the default method, enabling it to be edited.
Help	Contents and Index	Displays the opening page of the HTML Help.
	✓Display Tooltips	Toggles the tool tips on and off. Enabled at all times. Default is on.
	PerkinElmer on the Web	Goes to www.perkinelmer.com. Enabled at all times, if there is a web browser installed and connected to an ISP.
	About	Displays the About dialog.

Tell me about the Tool bars

There are three default tool bars located below the menu bar. The tool bars contain icons for standard interactions that will be performed frequently. The default tool bars are as follows:

Main Tool Bar

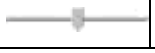
The **Main Tool bar** contains the following commands:

Icon	**Associated menu command**	**Description**
	Remove	Removes the currently selected items in the Data tree.
	Information…	Enables you to review the information associated with a file.
	Print	Prints details from the current view or operation.
	Exit	Closes the application.
	Copy Object	Copies the selected object to the Windows clipboard.
	Baseline Corrected Spectra	Determines whether spectra are shown in their baseline corrected state or not.
	Cursor	Displays or hides the cursor.
	Label Chromatograms…	Enables you to choose the labels to be displayed on chromatograms.

	Add/Edit Annotation	Enables you to add or edit text on a graph.
	View Tree	Switches on and off the View Tree.
	Data Tree	Switches on and off the Data Tree.
	Parameters	Switches on and off the Parameters pane.
	Range	Displays a range box enabling you to add all the spectra within the box to be added to the Data tree.
	Add All to View…	Enables you to add spectra from Operations to the Data Tree.
	Format Graph	Enables you to change the formatting of the selected graph.
	Help	Displays the Help File.
	Display Tool tips	Determines whether tool tips are displayed or not.
	PerkinElmer on the web	Links to www.perkinelmer.com.

2D Graph Tool Bar

This tool bar contains commands that allow you to modify how chromatograms and spectra are displayed.

Icon	Associated Menu Command	Description
	Stack/Overlay	Switches the currently selected pane between a stacked display and an overlaid display.
	Autoscale X and Y	Rescales the graph to the maximum and minimum of all data in the X and Y directions.
	Autoscale Y only	Rescales the graph to the maximum and minimum of all data in the Y direction only.
	Normalize X and Y	Normalizes the graph so all plots are full scale.
	Normalize Y only	Normalizes the graph so all plots are full scale without changing the X axis.
	Offset Normalize	Normalizes all plots to the highest point to the right of the cursor position.
	Normalize Point	Normalizes all plots at the cursor position.
	Zero	Sets the point at the cursor position to zero.
	Previous scale	Steps back through previous scale changes.
	Zoom X	Sets whether the zoom slider works on the X axis.
	Zoom Y	Sets whether the zoom slider works on the Y axis.
	Zoom Z	Sets whether the zoom slider works on the Z axis.
	Zoom slider	Zooms in on the graph as set by the zoom tools.

3D Graph Tool Bar

This toolbar contains commands for rotating a 3D Plot.

Icon	Associated Menu Command	Description
	Rotate X	Sets rotation slider to rotate around the X axis.
	Rotate Y	Sets rotation slider to rotate around the Y axis.
	Rotation slider	Rotate around the set 3D rotation control axis.

Tell me about the Views Tree

The **Views Tree** appears in the upper left hand pane of the window, and it is your means of navigation. The Views Tree will change depending on the environment from which it was opened (Method/GME or Post-Run/GRE). From the Views Tree you select what you want displayed in the right-hand portion of the screen.

When you select an item on the Views Tree, the right-hand portion of the window displays the selected view. If you select a header item, such as Operations or Custom View, the right-hand side of the window displays empty panes.

Tell me about the Views and Operations that are listed on the Views Tree

When in Spectral Processing, you work with **Views** and **Operations**. Basically, **Views** allow you to view chromatograms and spectra on the right-hand side of the window. Meanwhile, **Operations**, which are also displayed on the right hand side of the window, are used to obtain important information on chromatograms and to help you analyze spectra. All of the Views and Operations that can be displayed on the window are listed on the Views Tree.

Views

The Main View is displayed by default. This view is divided into four panes: a **Chromatogram pane**, a **Spectra pane**, a **Contour Map pane**, and a **3D Plot pane**.

In addition to the Main View, you have four other default views that you can select from the Views Tree. The four other default views are labeled: Chrom/Spectra View, Contour Map View, Spectra 3D View, and Compare View.

You can also create your own Custom View by modifying an existing view. You can also create your own Custom View page by modifying an existing view.

Operations

Operations that you can perform on chromatograms and spectra are listed under the **Operations** node of the Views Tree. The operations listed on the Views tree provide you with a number of options for identifying, storing, and performing calculations on spectra; and for obtaining important information about your chromatograms, such as verifying the purity of chromatographic peaks, or building your own libraries of stored spectra that can be used in a search to identify an unknown spectrum.

When you click on an Operation, such as **Wavelength Maximum**, the right-hand side of the window displays panes for displaying the required Chromatogram or Spectrum (which you select from the Data Tree), a Parameters pane, which is used to set and investigate the various parameters used to determine the results of a particular operation, a Results pane where the result of an operation is displayed, and a Display List that contains a list of items you can select to display on the Results pane.

Tell me about the Data Tree

The Data Tree serves two functions. On the **Main View**, **Chrom/Spectra View**, and **Compare View**, the Data Tree displays a list of currently loaded chromatograms and spectra that are grouped by the parent chromatogram. On these Views, you use the Data Tree to select

the chromatograms and spectra you want to see in the relevant panes (chromatograms are displayed in the Chromatogram pane, Spectra are displayed in the Spectra pane). Items that appear checked on the Data Tree are displayed in the relevant panes; while unchecked items on the Data Tree are not displayed.

When specialized Views are displayed, such as the **3D Spectra View** or the **Contour Map View**, the Data Tree displays a list of open chromatograms. From the Data Tree, you select the chromatogram you want to display. Only one chromatogram can be selected at a time.

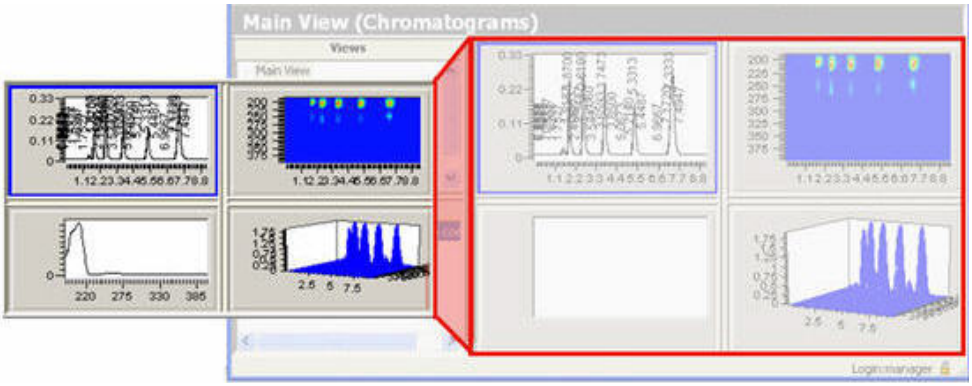
NOTE: *When the 3D Spectra View or the Contour Map View is displayed, the spectra that appeared on the Data Tree prior to accessing either view, are not shown on the Data Tree. Only opened chromatograms are displayed on the Data Tree for the 3D Spectra View and the Contour Map view. The spectra are not lost and will reappear when you change the view.*

It should also be noted that when you extract spectra from a chromatogram displayed on a 3D Plot or Contour Map, the spectra are not displayed in the Data Tree until you select another view such as the Main View or Chrom/Spectra View. In addition, chromatograms that are extracted on these views are temporary and will be cleared from the chromatogram pane when you select a different view.

Tell me about the right-hand side of the window

The right-hand side of the window can consist of multiple panes for displaying chromatograms and spectra. The type of panes displayed on the right-hand side of the window depends on whether you are looking at a View or an Operation.

The screen below shows the panes that are displayed when the Main View is selected on the Views Tree. In the Main View you can display chromatograms, spectra, contour maps, and 3D plots.



- The Main View, Chrom/Spectra View, Contour Map View, Spectra 3D View, and Compare View, have a predefined set of panes for displaying chromatographic data. You cannot add panes to these predefined Views. However, you can hide a pane that is associated with the view in order to create a Custom View.

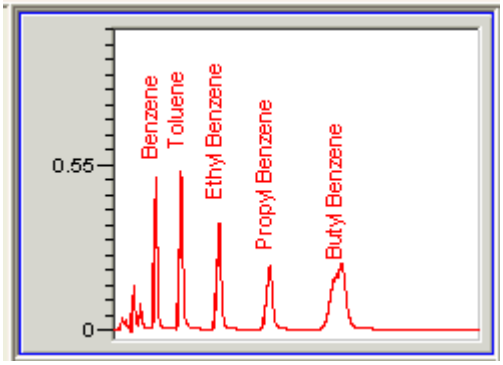
- If an **Operation** is selected on the Views Tree, the panes that appear in the right-hand portion of the window consist of: panes for displaying the required **Chromatogram** or **Spectrum** (which you select from the Data Tree), a **Parameters** pane, which is used to set and investigate the various parameters used to determine the results of a particular operation, a **Results** pane where the result of an operation is displayed, and a **Display List** that contains a list of items you can select to display on the Results pane.

Viewing Chromatograms

About the Chromatogram Pane

The Chromatogram pane shows one more chromatograms, which have been selected on the Data Tree. The chromatograms displayed on this pane are color coded to match the chromatogram names listed on the Data Tree.

You can display chromatograms either stacked or overlaid, as determined by the **Stack/Overlay Chromatograms** command.



This section shows you how to work with chromatograms displayed on the Chromatogram pane.

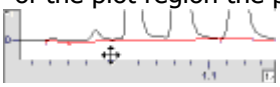
- To work with the Chromatogram pane, you must first select the pane by clicking anywhere inside the plot region of the pane. When the Chromatogram Pane is selected, the border of the pane turns blue.

How do I work with the Chromatogram Pane?

When the Chromatogram Pane is selected, the border of the pane turns blue. Once you have selected the Chromatogram pane you can do the following:

- If the **Cursor** command appears checked on the **View** menu, you can click anywhere on the displayed chromatogram and cross hairs appear at the point in the chromatogram region where you clicked the cursor. The exact positions of the X and Y axes are also displayed as text boxes on the axes.

- On any Chromatogram pane, when you move the mouse pointer close to any edge of the plot region the pointer changes into a four headed arrow.



A drag operation, when the mouse pointer is in this form, drags the graph around the plot region.

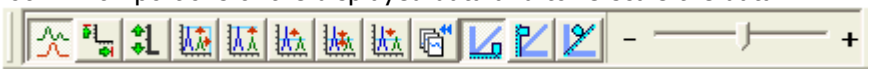
- If the Chromatogram pane is stacked, as indicated when the stacked icon appears to be pressed down  , a separate scale is shown for each curve.

- If overlaid, as indicated when the stack/overlay icon appears  , a single Y scale is shown, unless the Y values for the selected spectra are too dissimilar, in which case, the Y axis has no labels and the curves are artificially overlaid.

- On the **MainView**, **Chrom/Spectra**, and **Compare** Views, the Stack/Overlay command can be used to display chromatograms as either stacked or overlaid. If the Chromatogram pane is stacked, as indicated when the stacked icon appears to be pressed down  , a separate scale is shown for each curve.

If overlaid, as indicated when the stack/overlay icon appears  , a single Y scale is shown, unless the Y values for the selected spectra are

- The tool bar buttons along the upper right-hand side of the window allow you to zoom in on portions of the displayed data and to re-scale the data.



For more information on scaling chromatograms, see Scaling Chromatograms.

What happens when I open a chromatogram?

For information on opening a chromatogram, see the topic titled Opening Chromatograms.

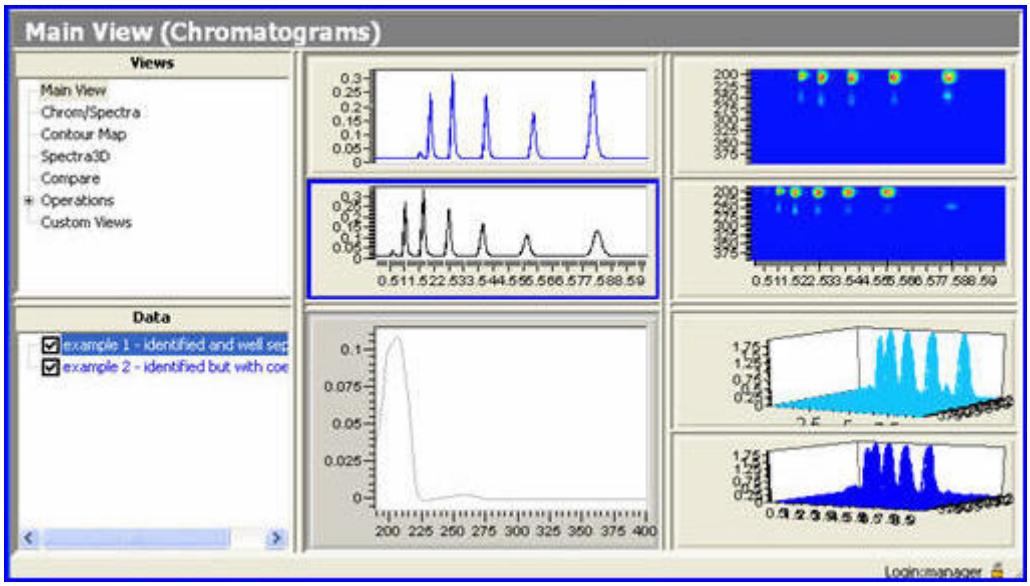
Each time you open a new chromatogram the following occurs:

The file name of the chromatogram you just opened is listed at the bottom of the Data Tree.

Depending upon the View you have selected you can specify whether or not a chromatogram is displayed on the view by doing the following:

- To have the chromatogram displayed on the current view, check the chromatogram.
- Or, if the current view only allows one chromatogram to be displayed on the View, you can select which chromatogram you want displayed on the view by selecting it on the Data Tree.

For example, on the Main View each chromatogram that appears checked on the Data Tree is displayed on the Chromatogram pane, on the Contour Map pane, and the 3D Plot pane.



NOTE: *In order for you to easily identify the newly opened chromatogram, please note that the chromatogram graph is color coded to match the chromatogram name listed on the Data Tree.*

If you are looking at a view where multiple chromatograms can be displayed, and the Chromatogram pane is set to Stacked mode, the newly opened chromatogram appears at the top of the Chromatogram pane; this is the reverse order of how chromatograms are listed on the Data Tree. The Contour Map and 3D plot for the newly opened chromatogram are displayed in the same position as shown on the Chromatogram pane.

Tell me about the context menu that appears when I right mouse click on the Chromatogram Pane.

When you right click on the Chromatogram pane a context menu appears that contains the following commands:

NOTE: *When the Chromatogram Pane is in Stacked mode, each of the chromatograms has its own context menu. When the Chromatogram Pane is in Overlay mode, the context menu items apply to all chromatograms.*

Command	**Description**
Print Pane…	Prints the current pane.
✓ Chromatogram Baselines	Displays or hides baselines on all chromatograms as a red line.
Overlay Chromatograms/Stack Chromatograms	This command toggles between Stack Chromatograms and Overlay Chromatograms, and determines how the contents of the pane are displayed. This option is not available when the Chromatogram pane is displayed on an Operation. • When **Overlay Chromatograms** is selected all chromatograms are displayed on one set of axes and the menu command changes to Stack Chromatograms. • When **Stack Chromatograms** is selected, all chromatograms are displayed on separate exes and the menu command changes to Overlay Chromatograms.
✓Cursor	Toggles the cross-hair cursor on and off.
Hide Pane	Hides the chromatogram pane forming a custom view. This option is not available when the Chromatogram pane is displayed on an Operation.
Label Chromatograms…	Displays the Label Chromatograms dialog. This option is not available when the Chromatogram pane is displayed on an Operation.
Range	Displays a green range box on the chromatogram. This option is not available when the Chromatogram pane is displayed on an Operation.
Add to View…	Adds the spectrum temporarily displayed from a selected point on the chromatogram to the Data Tree and selects it. This option is not available when the Chromatogram pane is displayed on an Operation. ***NOTE:*** *To use this option you must have the Chromatogram pane in Stacked mode and the Spectra pane in Overlay mode.*

Format Graphs	Displays the Format Graphs dialog at the Chromatogram tab. Changes made here effect the current graph only.
Spectral Baseline Correction	Displays the Spectral Baseline Correction dialog.

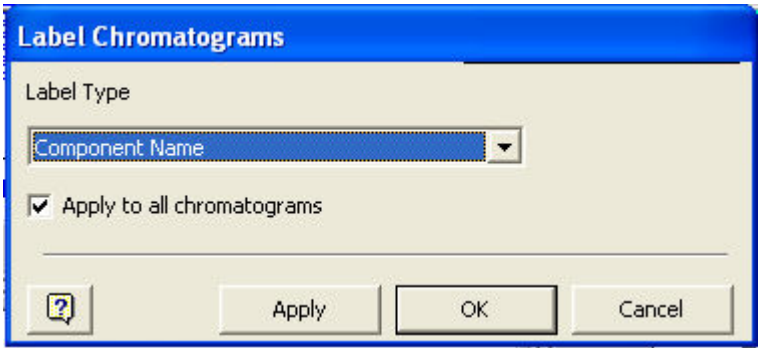
How do I label Chromatograms?

NOTE: *Labels can only be applied to chromatograms that are displayed on a View. You can not select a label for a chromatogram that is displayed on an Operation.*

You can apply labels to all chromatograms that appear in a Chromatogram pane, or, if the current view displays chromatograms as stacked, you can apply a label to a single chromatogram.

To apply a label to all currently loaded chromatograms:

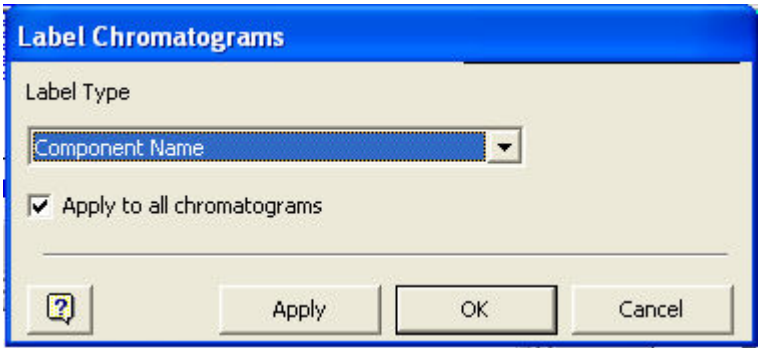
1. From any of the Views, select **Actions > Label Chromatograms**.
   The Label Chromatograms dialog appears.



2. From the Label drop down, select a label you wish to apply.
3. Check **Apply** to all chromatograms.
4. Click **OK** to close the Label Chromatograms dialog.

To apply a label to a single chromatogram:

1. From any of the Views, click on the Chromatogram pane that contains the chromatogram you wish to label.
2. A blue border appears around the selected Chromatogram pane.
3. From the Actions menu select **Actions > Label Chromatograms**.
4. The Label Chromatograms dialog appears.



5. From the Label drop down list, select a label you wish to apply to the chromatogram.
6. Uncheck the Apply to all chromatograms check box.
7. Click **OK**.
   The selected label is applied to the chromatogram.

How do I extract and/or display spectra from a chromatogram?

To view spectra associated with a particular chromatogram, as well as extract spectra from a chromatogram, the Chromatogram pane use a Stacked display, and the Spectrum pane must use an Overlaid display.

- You can set the display modes for both panes by selecting **Stack Chromatograms** and **Overlay Spectra** from the **View** menu.

To extract spectra:

1. Click on any area in the white space of the Chromatogram pane.
2. Set the selected Chromatogram pane to Stacked mode by clicking on the icon so that the icon appears to be pushed down
3. Float your mouse pointer over a point on the chromatogram to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the chromatogram, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.
4. If you see a spectrum that you want extract, just double click on the spot in the chromatogram that contains the spectrum you want to extract.
   The spectrum at the wavelength and time you clicked appears in the Spectra pane.

Viewing Chromatograms

Now that you have a general understanding of the user interface, this section will introduce you to the most basic element, the chromatogram. Chromera Spectral Processing is designed to answer the question **"What chromatographic information can I get from the spectra associated with this chromatogram?"** — or, perhaps more clearly, **"What information about the peaks in this chromatogram and the components in the sample can I get from the spectra associated with this chromatogram?"** It's not surprising, then, that the basic element is the chromatogram. Most operations begin with chromatograms.

Scaling Chromatograms

There are a variety of options to scale a chromatogram. You can:

- Visually specify an area of the display to zoom into using the **Box Zoom** function.
- Scale chromatograms by specifying the axis range from the **Format Graphs** dialog.
- **Autoscale** only the absorbance axis, to fit the chromatogram between 10% and 90% of the display, or you can scale both axes to display the full retention time range and re-scale the absorbance to fit between 10% and 90% of the display.
- **Zoom** in a continuous fashion.
- **Normalize** an entire chromatogram using the **Normalize X and Y** command  (i.e. all points in the displayed chromatogram), normalize along the Y axis only using the **Normalize Y** command  , normalize at the current cursor position using the **Normalize Point** command  , or you can normalize to the maximum absorbance value to the right of the current cursor position using the **Offset Normalize** command  .

Following is an overview of the scaling options that are available to you when you are working with a Chromatogram pane:

How do I visually specify an area of the display to zoom into using the Box Zoom function?

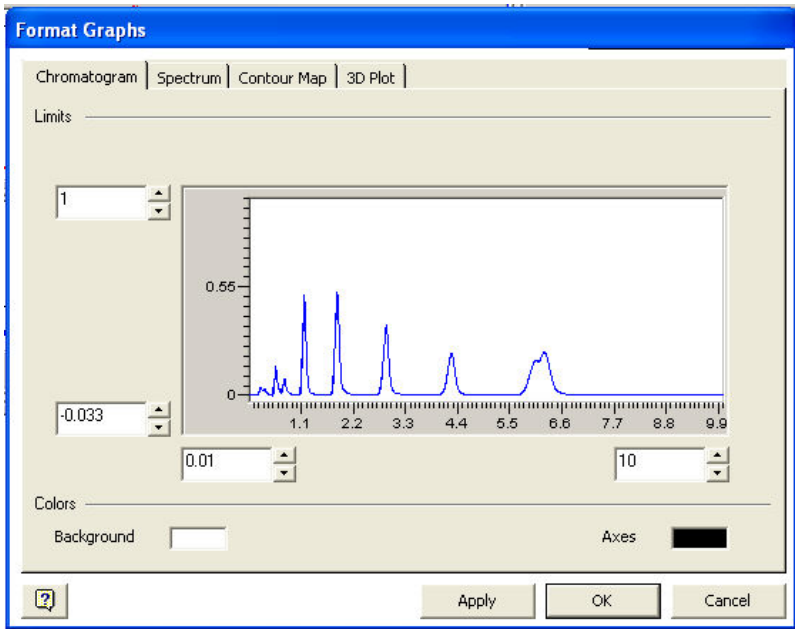
To zoom into a visually defined region:

1. Move the mouse cursor inside the plot region and position it at a corner of the area you want to zoom.
2. Hold down the left mouse button and drag the mouse to form a box around the area to be zoomed.
3. Release the mouse button.
4. The region you wish to zoom into will be outlined with a box containing eight control points. The box can be resized by clicking and dragging on any of the control points.
5. Move the mouse pointer into the zoom region and click the left mouse button.
6. The zoomed area now fills the trace display region.
7. You can abort the zoom before step 4 by clicking the left mouse button outside the zoom box.
8. To return to a view of the entire trace, select **Actions > Autoscale > X and Y.**

How do I view and change the axis range for a particular chromatogram?

1. You can view and change the axis range for particular chromatogram by right mouse clicking on a chromatogram and selecting **Format Graphs** from the context menu that appears.

2. The Format Graphs window appears with the Chromatogram tab selected. This window allows you to set the minimum and maximum values for the selected chromatogram on both the X and Y axes.



3. From the Chromatogram tab page specify the Maximum and Minimum values for the X and Y axes.

4. Click OK to apply changes and close the Format Graphs dialog.

How do I use the Autoscale tools?

The autoscale commands allow you to rescale the data in the chromatogram pane so that all of the data are visible. There are two commands which you can use to scale a chromatogram to fit the display region. **Autoscale X and Y** scales the chromatogram to between 10% and 90% of the absorbance axis as well as displaying the full retention time range of the chromatogram. **Autoscale Y** only scales only the absorbance axis without changing the retention time range.

To autoscale the absorbance only:

1. Click inside the Chromatogram pane that contains the chromatogram/s that you want to autoscale in the Y direction.

2. Select Actions > Autoscale > Y only.

   OR

   Click on the Autoscale Y icon .

   The absorbance axis of the selected chromatogram is scaled to fill 80% of the display.

To autoscale both the retention time and absorbance:

1. Click inside the Chromatogram pane that contains the chromatogram/s you want to autoscale in the X and Y directions.

2. Select Actions > Autoscale > X and Y.
   OR
   Click on the Autoscale X and Y icon .
   The retention time axis of the selected chromatogram is scaled to fill the region. All other chromatograms will be re-scalled to this retention time range. The absorbance axis is scaled to fill 80% of the display.

How do I zoom in a continuous fashion?

You can zoom a chromatogram in a continuous fashion using the slider zoom feature in conjunction with the **Zoom X** and **Zoom Y** icons. The slider zoom enables you to expand or contract the chromatogram in a continuous manner. The **Zoom X** and **Zoom Y** commands determine what is zoomed when you are interacting with the zoom slider.

To continuously zoom a chromatogram:

1. Before performing the zoom, place the cursor at the position that you want to zoom, since the zoom is around the position of the cross-hair cursor.

2. Click on the ZoomX axis icon and or the **Zoom Y** axis icon on the tool bar to set the required zoom mode.
   You can select both the X and Y axis icons to create an X and Y zoom.
   The buttons appear depressed when active.

3. Push the tool bar slider  to the right or left.

How do I use the use the Normalization commands to scale a chromatogram?

The Normalization commands are useful when multiple, overlaid chromatograms are displayed, as these commands are used to find subtle differences in the plot. Basically, the Normalize commands help you compare the exact shapes of peaks by converting all of the displayed peaks to the same maximum height.

NOTE: *For a detailed description of how the Normalization commands can be used, refer to Viewing Spectra - Detailed Description of Normalization commands.*

The Normalization commands are as follows:

Icon	Associated menu command	Description
	Normalize X and Y	Normalizes the graph so all plots are full scale.
	Normalize Y only	Normalizes the graph so all plots are full scale without changing the X axis.

	Offset Normalize	Normalizes all plots to the highest point to the right of the cursor position.
	Normalize Point	Normalizes all plots at the cursor position.

NOTE: *The Normalize Y command is useful if you have zoomed in on a specific region of the chromatogram and want to normalize without resetting the X axis, since Normalize All evaluates all points in the chromatogram, and therefore, resets the X axis.*

To normalize both the retention time and absorbance:

1. Click anywhere inside the chromatogram pane that contains the chromatogram/s you want to scale.

2. Click on the **Normalize X and Y** icon  .
   OR
   Select **Actions > Normalize > X and Y** from the menu bar.
   The retention time axis of the selected chromatogram is scaled to fill the region. All other chromatograms will be re-scaled to this retention time range. The absorbance axis is scaled to fill 80% of the display.

To normalize along the Y axis only:

1. Click the region below the point where you wish normalization to occur.

2. Click on the Normalize Y icon  .
   OR
   Select **Actions > Normalize > Y** only from the menu bar.
   The chromatogram will be normalized at the maximum point in the displayed region without changing or resetting the X axis value.

To normalize to the highest point to the right of the cursor:

1. Click the region below the point where you wish normalization to occur.

2. Click on the Offset Normalize  icon.
   OR
   Select **Actions > Normalize > Offset** from the menu bar
   The chromatogram is normalized to the maximum point above the cursor position.

To normalize at the position of the cursor:

1. Click the region at the point where you wish normalization to occur.

2. Click on the Normalize Point icon  .
   OR
   Select **Actions > Normalize > Point** from the menu bar.
   The chromatogram is normalized at the cursor position.

Labeling Chromatograms

You can label individual chromatographic peaks. There are 14 different labels available for annotating the chromatograms, however, only one type of label can displayed at a time. You may change the type of labeling on one or all of the chromatograms any time you are viewing chromatograms. You cannot apply a label to a chromatogram from an Operation.

How do I apply a label to chromatograms?

NOTE: *Labels can only be applied to chromatograms that are displayed on a View. You can not select a label for a chromatogram that is displayed on an Operation.*

You can apply labels to all chromatograms that appear in a Chromatogram pane, or, if you are on a View where the Chromatogram panes are stacked, you can apply a label to a single chromatogram.

To apply a label to all currently loaded chromatograms:

1. From any of the Views, select Actions > Label Chromatograms.
   The Label Chromatograms dialog appears.
2. From the Label drop down, select a label you wish to apply.
3. Check Apply to all chromatograms.
4. Click OK to close the Label Chromatograms dialog.

To apply a label to a single chromatogram:

1. From any of the View pages click on the Chromatogram pane that contains the chromatogram you wish to label.
   A blue border appears on the selected Chromatogram pane.
2. From the Actions menu select **Actions > Label Chromatograms**.
   The Label Chromatograms dialog appears.
3. From the **Label** drop down list, select a label you wish to apply to the chromatogram.
4. Uncheck the Apply to all chromatograms check box.
5. Click **OK**.
   The selected label is applied to the chromatogram.

Temporarily Displaying Extracted Chromatograms

Chromatograms can be temporarily captured and displayed on the 3D Spectra View, and the Contour Map View. The chromatograms you capture and display on these views are not added to the Data Tree and are cleared when you select another View.

To temporarily display extracted chromatograms:

1. Click on an area inside the white space of the pane that you wish to extracting from.
   A blue border appears around the selected pane.

2. Set the selected pane to stacked mode, as indicated when the stacked icon appears to be pressed down  .
   You used a stacked display whenever you want to extract data from a pane.

3. Click on any area in the white space of the **Chromatogram** pane.
   A blue border appears around the selected pane.

4. Set the Chromatogram to Overlay mode, as indicated when the stack/overlay icon appears  .
   The pane that you are extracting data into must be in Overlay mode.

5. You can now "float" the mouse pointer on the pane you are extracting from and have the associated chromatogram displayed on the Chromatogram pane.
   OR
   Double click on a specific area of the pane to temporarily extract a chromatogram at the selected wavelength.

Extracting and Displaying Spectra from a Chromatogram pane

To view spectra associated with a particular chromatogram, as well as extract spectra from a chromatogram, the **Chromatogram** pane must use a **Stacked** display, and the **Spectrum** pane must use an **Overlaid** display.

- You can set the display modes for both panes by selecting **Stack Chromatograms** and **Overlay Spectra** from the **View** menu.

To extract spectra:

1. Click on any area in the white space of the Chromatogram pane.
2. Set the selected Chromatogram pane to Stacked mode by clicking on the  icon so that the icon appears to be pushed down 
3. Float your mouse pointer over a point on the chromatogram to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the chromatogram, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.
4. If you see a spectrum that you want extract, just double click on the spot in the chromatogram that contains the spectrum you want to extract.
   The spectrum at the wavelength and time you clicked appears in the Spectra pane.

Viewing Spectra

About Viewing Spectra

Now that you've opened a chromatogram, you can look at its associated spectra. You can use the Main View and Chrom/Spectra View to open, extract, and view spectra from a chromatogram.

In this Help topic we first describe how spectra are collected for viewing. You will also learn how to examine spectra on the various Views as well as learn how to modify the spectrum display so that you can more easily compare spectra.

Spectral Formats

Spectra are stored in three different formats: chromatographic, independent and library spectra.

Chromatographic Spectra - These are the spectra associated with a chromatogram. These spectra can be accessed from the chromatogram, when the chromatogram is selected on the Data Tree and displayed on the current View.

To see spectra associated with a chromatogram, simply float your mouse pointer over the chromatogram; and the spectrum from that point is temporarily displayed on the Spectra pane on the current view. We recommend you use the Main View, Chrom/Spectra View, Contour Map View, or Spectra 3D View to perform this task.

You can also capture spectra by double clicking your mouse over an area on the chromatogram, and the spectrum at the wavelength and time you clicked appears in the Spectra pane for the current view. The spectrum is also added to the Data Tree under the chromatogram from which the spectrum was extracted.

NOTE: *A "captured" spectrum is a spectrum extracted from its source and displayed permanently in the Spectra Pane. A captured spectrum can be hidden and/or removed.*

- **Individual Spectra** -These are the files that result when you select a chromatographic spectrum that you have extracted, and then select the spectrum on the Data Tree.

- **Library Spectra** - You can use the Add Apexes command to automatically create a spectral library using the apex spectra from named peaks in a chromatogram. You can also add a previously stored, individual spectrum to a library; to do this you must first open this spectrum, and then use the **Build Library** commands, located under the **Tools** menu. In addition, spectra extracted from a chromatogram can be added to a spectral library by using the **Build Library** commands, located under the **Tools** menu.

For more information on set up a library of stored spectra, refer to Spectral Libraries.

Viewing Chromatographic Spectra

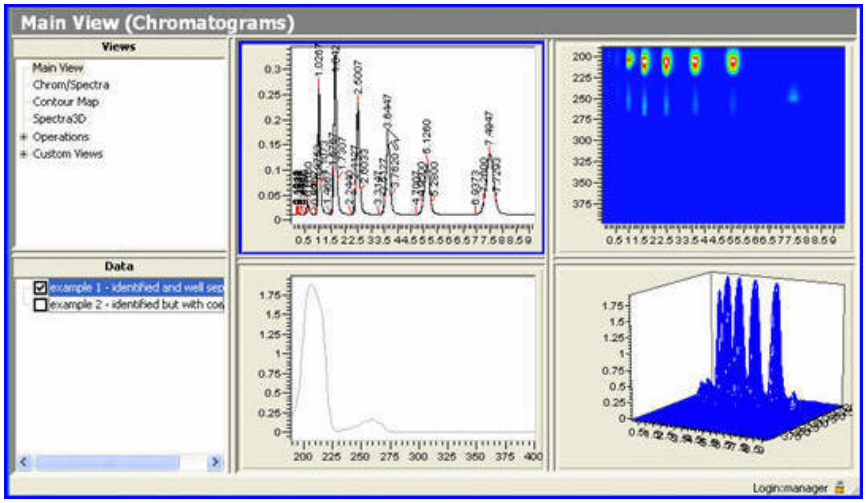
This Help topic shows you how to capture and display a spectrum from a chromatogram in a chromatogram pane, contour map, or 3D plot pane using the **Main View**. For more information on extracting spectra using the other Views, refer to the individual Help on each of the default views.

NOTE: *A "captured" spectrum is a spectrum extracted from its source and displayed permanently in the Spectra Pane. A captured spectrum can be hidden and/or removed.*

How do I see spectra associated with a chromatogram?

- You can see spectra associated with a chromatogram without capturing the spectra permanently in the Spectra pane. If you do not want permanently capture a spectrum from a chromatogram, simply float your mouse pointer over the chromatogram, 3D plot or contour map, and the spectrum from that point is temporarily displayed on the Spectra pane of the current View.

NOTE: *To preview spectra associated with a particular chromatogram, as well as extract spectra from a chromatogram, the pane you are extracting from must be set to a* ***Stacked*** *display, and the Spectra pane must be set to an* ***Overlaid*** *display.*



How do I capture a spectrum from a Chromatogram pane?

To view spectra associated with a particular chromatogram, as well as extract spectra from a chromatogram, the **Chromatogram pane** must use a **Stacked** display, and the **Spectrum pane** must use an **Overlaid** display.

- You can set the display modes for both panes by selecting **Stack Chromatograms** and **Overlay Spectra** from the **View** menu.

To extract spectra:

1. Click on any area in the white space of the Chromatogram pane.

2. Set the selected Chromatogram pane to Stacked mode by clicking on the  icon so that the icon appears to be pushed down 

3. Float your mouse pointer over a point on the chromatogram to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the chromatogram, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.

4. If you see a spectrum that you want extract, just double click on the spot in the chromatogram that contains the spectrum you want to extract.

   The spectrum at the wavelength and time you clicked appears in the Spectra pane.

How do I capture a spectrum from a Contour Map?

To extract and display a spectrum from a point on the Contour Map:

1. On the Main View, check the chromatogram on the Data Tree that you want displayed as a contour map.

   The chromatogram is displayed on the Chromatogram pane, Contour Map pane, and 3D Plot pane.

2. Float your mouse pointer over a point on the contour map plot to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the contour map, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.

3. If you see a spectrum that you want extract, just double click on that area in the contour map; and the spectrum at the wavelength and time you clicked appears in the Spectra pane and is now listed on the Data Tree.

NOTE: *Unlike the Main View, where extracted spectra are displayed on the Data Tree, when you extract a spectrum from the* Contour Map View*, the spectrum is added to the Data Tree; however, you will not see the extracted spectrum on the Data Tree until you exit the Contour Map View. The reason for this is because on the Contour Map View, the Data Tree is used only for selecting the chromatogram that you want displayed. You will be able to see all of the spectra you have extracted once you select a different view such as the* Main View *or the* Chrom/Spectra View*.*

How do I capture a spectrum from a 3D Plot?

To extract and display a spectrum from a point on the Contour Map:

1. On the MainView, check the chromatogram on the Data Tree that you want displayed as the 3D Plot.

   The chromatogram is displayed on the Chromatogram pane, Contour Map pane, and 3D Plot pane.

2. Float your mouse pointer over a point on the 3D Plot to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the 3D Plot, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.

3. If you see a spectrum that you want extract, just double click on that area in the 3D Plot; and the spectrum at the wavelength and time you clicked appears in the Spectra pane.

NOTE: *Unlike the Main View, where extracted spectra are displayed on the Data Tree, when you extract a spectrum from the* Spectra 3D View*, the spectrum is added to the Data Tree; however, you will not see the extracted spectrum on the Data Tree until you exit the Spectra 3D View. The reason for this is because on the Spectra 3D View, the Data Tree is used only for selecting the chromatogram that you want displayed. You will be able to see all of the spectra you have extracted once you select a different View such as the* Main View *or the* Chrom/Spectra View*.*

What Views should I use to capture spectra?

Although you can use the Main View to capture spectra from a chromatogram that is displayed on a Chromatogram pane, Contour Map, and/or 3D Plot, we recommend you use the following Views to capture spectra:

- If you want to capture spectra from a chromatogram use the Chrom/Spectra View, or the Main View.
- If you want to capture spectra from a point on a contour map, use the Contour Map View.
- If you want to, capture spectra from a point on a 3D plot use the 3D Spectra View.

NOTE: *Unlike the* Main View*, where extracted spectra are displayed on the Data Tree, when you extract a spectrum from the* Contour Map View *or* 3D Spectra View*, you will not see the extracted spectrum on the Data Tree until you exit the Contour Map View or 3D Spectra View. The reason for this is because while you are viewing the Contour Map View or 3D Spectra View, the Data Tree is used only for selecting the chromatogram that you want displayed on the view. You will be able to see all of the spectra you have extracted once you select a different View such as the* Main View *or the* Chrom/Spectra View.

How do I use the Range Box to extract spectra?

NOTE: *The Range command is enabled only on Views, not Operations, when the View contains a Chromatogram pane with a single chromatogram displayed. This command is also available from the context menu for a stacked chromatogram.*

This Range command  , located on the tool bar, is used to display all the spectra within a given time range. When the Range icon  is selected a green range box is displayed on the chromatogram. To activate the range box, simply position your mouse pointer in the range box and click.

When the range box is activated:

- Handles are displayed on the left and right edges of the green range box.
- Positioning your mouse pointer over the left or right handles of the box changes it to a horizontal two-headed arrow; and if you click and hold you can stretch the edge of the range box. When you release the mouse, the range box is deactivated.

- Positioning your mouse pointer over the left or right edge of the box (except for the position of the handles) changes the handles to a four-headed arrow. When the four-headed arrow appears you can move the entire range box by clicking and holding. Upon releasing the mouse the range box is deactivated.

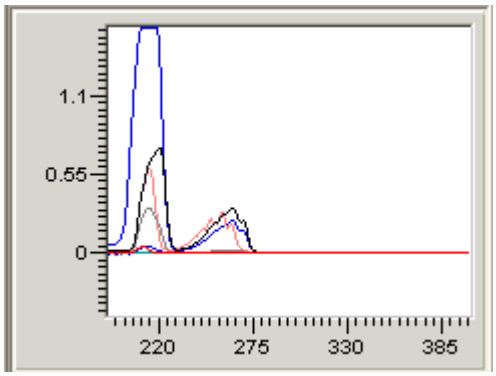
- Positioning the mouse pointer in the middle of the range box changes it to a four-headed arrow and click and hold enables the box to be moved.

- Positioning the mouse pointer within the box and double-clicking loads all the spectra enclosed by the box into the Data Tree and sets them as selected. When you release the mouse, the range box is cleared.

- Clicking on the chromatogram, in an area outside of the box, clears the range box.

About the Spectra Pane

Previously stored, individual spectrums can be opened and viewed on the Main View, Chrom/Spectra View, and the Compare View. Meanwhile, spectra associated with a chromatogram are accessed from the chromatogram. You can view the spectra in a chromatogram from the Main View, Chrom/Spectra View, Spectra 3D View, Contour Map View, and Compare View.



You can see spectra associated with a chromatogram without capturing the spectra permanently in the Spectra pane. If you do not want permanently capture a spectrum from a chromatogram, simply float your mouse pointer over the chromatogram, 3D plot or contour map, and the spectrum from that point is temporarily displayed on the Spectra pane of the current View.

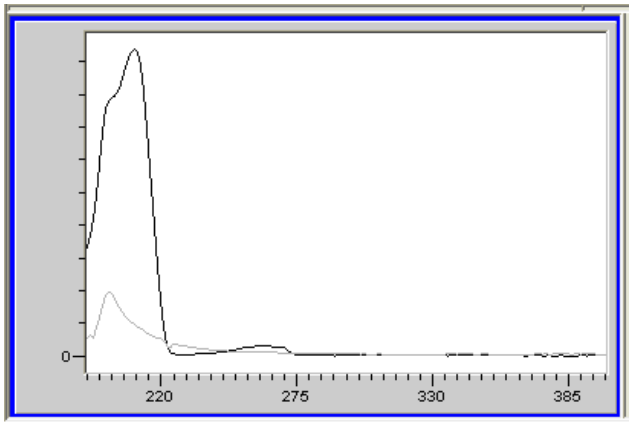
On the various Views referenced above, spectra are displayed on the Spectra pane. On this pane spectra are color coded to match the spectra that are checked on the Data Tree; however, if you have extracted spectra on the Spectra 3D View or the Contour Map View, the spectra you extracted will appear on the Spectra pane, but they will not be displayed on the Data Tree until you select a different view such as the Chrom/Spectra View.

The Spectra pane can display spectra as either stacked or overlaid, as determined by **Stack Spectra**/**Overlay Spectra**, which is available from the **View** menu. If a stacked display is selected, a separate Y scale is shown for each curve. If an overlaid display is selected, a single Y scale is shown, unless the Y values for the selected spectra are too dissimilar in which case the Y axis has no labels and the curves are artificially overlaid.

How do I work with the Spectra Pane?

- To work with the Spectra pane, you must first select the pane by clicking on the white space of the pane.

The pane appears selected and the border of the pane turns blue.



- If a **Stacked** display is being used on the **Chromatogram** pane and an **Overlaid** display is being used on the **Spectra** pane, you can float the cursor over a point on the Chromatogram pane and have the associated spectrum displayed.

- When the mouse pointer is moved close to any edge of the plot region it changes into a four headed arrow.

A drag operation when the mouse pointer is in this form, drags the graph around the plot region.

- You can also scale spectra with a large selection of tools. For more information on scaling spectra, see Scaling Spectra.

Tell me about the context menu that appears when I right click on a Spectra Pane.

When you right click on the Spectra Pane a context menu appears that contains the following commands:

Command	**Description**
Print Pane…	Prints just the current pane.
✓Baseline Spectra	Adds the baseline spectrum for the selected spectrum to the Data Tree and displays the baseline spectrum on the Spectra pane. The baseline spectrum is identified by the retention time of the spectrum (or if a range of spectra are being used, the start and end times of the range) followed by the word **base**.
Stack Spectra/Overlay Spectra	This command toggles between **Stack Spectra** and **Overlay Spectra** and determines how the contents of the pane are displayed. The menu command displays **Overlaid** Spectra when the current display is set to Stacked. If an overlaid display is selected and all plots are plotted on the same Y scale a single Y scale is shown. If each plot is plotted with its own Y scale then the Y axis has no labels. When **Stack Spectra** is selected, all spectra are split onto separate axes and the menu command changes to Overlay Chromatograms.
✓Cursor	Toggles the cross-hair cursor.
Hide Pane	Closes the selected pane forming a custom view.
Format Graphs	Displays the Format Graphics dialog at the Spectrum tab. Changes made here effect the current graph only.

Scaling Spectra

The default display protocol for spectra displayed is an X/Y autoscale mode that automatically scales the display so that every spectrum is completely visible in both the X and Y axis directions. If you add a new spectrum with a wider range, the display's range is adjusted to accommodate it.

In addition you are provided with a number of features that let you modify the spectrum display so that you can more easily compare spectra. This section begins with a brief description of the scaling commands available to you.

Options for scaling spectra

This section describes the available scaling commands used to modify the spectrum display. You will find that the information in this section is useful when multiple, overlaid spectra are displayed.

Please note that before you use any of the scaling commands to modify how spectra are displayed, you must first select the Spectra pane by clicking on the white space of the pane. When the Spectra pane is selected a blue border appears around the pane. Now that you have the Spectra pane selected you can use the scaling commands described below to more easily compare spectra.

Tell me about Autoscaling Spectra.

The autoscale commands allow you to rescale the data in the Spectra pane so that all of the data are visible. As noted earlier, the autoscale commands are particularly useful when multiple, overlaid spectra are displayed.

There are two commands which you can use to scale spectra to fit the display region:

- The **Autoscale X and Y** command  rescales spectra on the current view in both the X and Y directions, so that all of the data are visible. This command is particularly useful when multiple overlaid spectra are displayed, because clicking on this command rescales the data so that all points for the largest data set, in both the X and Y axis directions, fit in the window.

NOTE: *Most LC solvents absorb strongly in the low UV and so allow very little energy to reach the pixels of the array at those wavelengths, making any data suspect. Scaling on the Y-axis is always done above the pixel threshold. Thus, if the first valid pixel is 210 nm, the scale will be determined by the minimum and maximum absorbance values found in the region (210–700 nm for the Series 200 DAD). With strong end absorbance, this may mean that the spectrum below 210 nm is off-scale.*

- The **Autoscale Y Only** command  works like the Autoscale X and Y command described above, except that it leaves the X axis unchanged and scales only the Y axis.

Tell me about Normalizing Spectra.

The Normalization commands are useful when multiple, overlaid spectra are displayed and you want to find subtle differences between spectra. Basically, the Normalize commands

help you compare the exact shapes of peaks by converting all of the displayed peaks to the same maximum height.

The Normalization commands are as follows:

NOTE: *If you are trying to scale a single chromatogram or spectrum, only* ***Offset Normalize*** *and* ***Normalize Point*** *commands are useful commands.*

- The **Normalize X and Y** command  is useful when multiple, overlaid spectra are displayed. This command scales the maximum Y value in the active window to 90% for each spectrum and sets the X axis scale so that the entire largest spectrum is displayed.

- The **Normalize Y Only** command  , works like "Normalize All," except it rescales only the Y axis.

- The **Offset Normalize** command  is useful when multiple, overlaid spectra are displayed, or when a single spectrum is displayed. This command scales the data point with the maximum Y value to the right of the current cursor position to 90% in the region for each spectrum.

- The **Normalize Point** command  is useful when multiple, overlaid spectra are displayed, or when a single spectrum is displayed. This command scales the Y value at the current cursor point to 90%. The new (90%) scaling is applied to every spectrum.

In addition to the Autoscale and Normalize commands, the following additional options are available for scaling spectra:

- Use the **Box Zoom** function to visually specify an area of the display you want to zoom into.

- Use the **Format Graphs** dialog to scale spectra by specifying the axis range.

- **Zoom** in a continuous fashion.

How do I visually specify an area of the display to zoom into using the Zoom Box function?

It is possible to zoom into a visually defined region of a spectrum using the Box Zoom. To scale individual spectra set the Spectra pane to Stacked mode.

To zoom into a visually defined region:

1. Move the mouse cursor inside the plot region and position it at a corner of the area you want to zoom.

2. Hold down the left mouse button and drag the mouse to form a box around the area to be zoomed.

3. Release the mouse button.

The region you wish to zoom into will be outlined with a box containing eight control points. The box can be resized by clicking and dragging on any of the control points.

4. Move the mouse pointer into the zoom region and click the left mouse button.

NOTE: *You can individually scale the absorbance of each spectrum. All spectra, however, share the same wavelength axis, so zooming into the wavelength range on one spectrum zooms into that wavelength range for all spectra.*

5. You can abort the zoom before step 4 by clicking the left mouse button outside the zoom box.

6. To return to a view of the entire trace, select **Actions> Autoscale > X and Y**.
   OR
   Use the **Previous Scale** command  , which is also located under the Actions menu.

How do I view and change the axis range for spectra?

1. You can view and change the axis range for spectra by right mouse clicking on a spectra pane and selecting Format Graphs from the context menu that appears.
   The Format Graphs window appears with the Spectra tab selected. This window allows you to set the minimum and maximum values on both the X and Y axes..

2. From the **Spectra** tab page specify the Maximum and Minimum values for the X and Y axes.

3. Click **OK** to apply changes and close the Format Graphs dialog.

NOTE: *Changes in the wavelength range always affect all the spectra.*

How do I Zoom in a continuous fashion?

You can zoom spectra in a continuous fashion using the slider zoom feature in conjunction with the **ZoomX** and **ZoomY** icons. The slider zoom enables you to expand or spectra in a continuous manner about the cursor position. The Zoom X and Zoom Y commands determine what is zoomed when you are interacting with the zoom slider.

To zoom a spectrum:

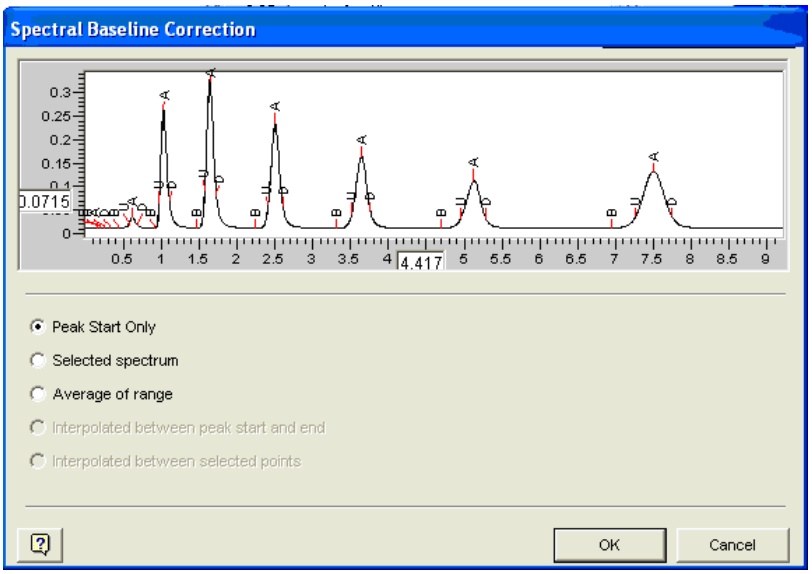
1. Click on the Spectra pane that you wish to zoom.

2. Click on the Zoom X axis icon and or the Zoom Y axis icon on the Toolbar to set the required zoom mode.
   You can select both the X and Y axis icons to zoom the X and Y axes simultaneously.
   The buttons appear depressed when active.

3. Push the Toolbar slider  to the right or left.

Defining How You are Going to Perform the Baseline Correction of the Spectra

The Spectra Baseline Correction dialog allows you to define how you are going to perform the baseline correction of the spectra. Baseline correction is achieved by subtracting the appropriate absorbance baseline spectrum from the absorbance spectrum.



On the Spectral Baseline Correction dialog you can select from three options available to you for calculating/selecting the baseline spectrum. You can correct baseline spectra within a chromatogram using the dialog. Baseline correction is achieved by subtracting the appropriate absorbance baseline spectrum from the absorbance spectrum.

Your options are:

- **Peak Start Only** – This option selects the spectrum at the Baseline Start position before the current chromatographic peak as the baseline spectrum. (If all peaks are baseline separated, each peak in the chromatogram would have a different baseline spectrum.) This spectrum will be used for baseline correction until the next Baseline Start position.

  For spectra before the first start of the first peak in the chromatogram the first baseline spectrum will be used for all spectra before the first peak.

  For spectra after the first end of the last peak in the chromatogram the last baseline spectrum will be used for all spectra after the last peak.

- **Selected Spectrum** – This option uses a spectrum at a point you select on the chromatogram, currently displayed on this dialog, as the baseline spectrum.

  When you select this option, a vertical marker is displayed on the graph (at the start of the chromatogram). You then drag the marker to the required position. The baseline spectrum will now be the spectrum from this point. This marker will be re-displayed whenever Spectrum Type is selected as the Peak Label.

- **Average of range** - This option creates the baseline spectrum by averaging the spectra in a range that you select on the chromatogram that is currently displayed on the Spectral Baseline Correction dialog.

  When this option is enabled a square is displayed on the graph that you drag to the required position and resize to select a range of spectra.

Removing Spectra

Removing Spectra from the Data Tree

- If you want to remove all of the spectra from the Data Tree select **File > Remove All Spectra**.

  All of the spectra are removed from the Data Tree.

- If you want to remove a spectrum from the Data Tree, right mouse click on the spectrum you want to remove and select **Close** from the context menu that appears.

Hiding Spectra from Being Displayed

- If you do not want to display a spectrum on the Main View or Chrom/Spectra View you can hide it by unchecking the spectrum on the Data Tree.

Viewing the Data

About Viewing the Data

You examine chromatograms and spectra from the five default **Views**: Main View, Chrom/Spectra View, Contour Map View, Spectra 3D View, and Compare. By default, the Main View is displayed. However, you can select any one of the five default views, which are listed on the Views Tree, to display the selected view on the right hand portion of the spectral processing window. The menu bar and tool bars always appear on the window.

You can also create your own Custom Views by modifying an existing view. You can modify existing views by selecting to hide or show a particular pane. To hide or show a pane on a view, select **View > Panes** from the menu bar. The Panes sub menu allows you to select whether or not a **Chromatogram** pane, **Spectrum** pane, **Contour Map** pane, or **3D Plot** pane is displayed on the View. Once you have modified an existing view you can save it as a Custom View by selecting **View > View Template > Save As...** from the menu bar. Custom Views appear under the Custom node on the Views tree.

The data that is displayed on a View is controlled by the Data Tree. From the Data Tree you select the chromatogram/s and or spectra that you wish to display.

This chapter describes the five default views as well as provides you with information on creating Custom Views.

Main View

Launching Spectral Processing displays the Main View. This view allows you to look at chromatograms and their associated spectra, as well as the contour map and 3D plot for a chromatogram.

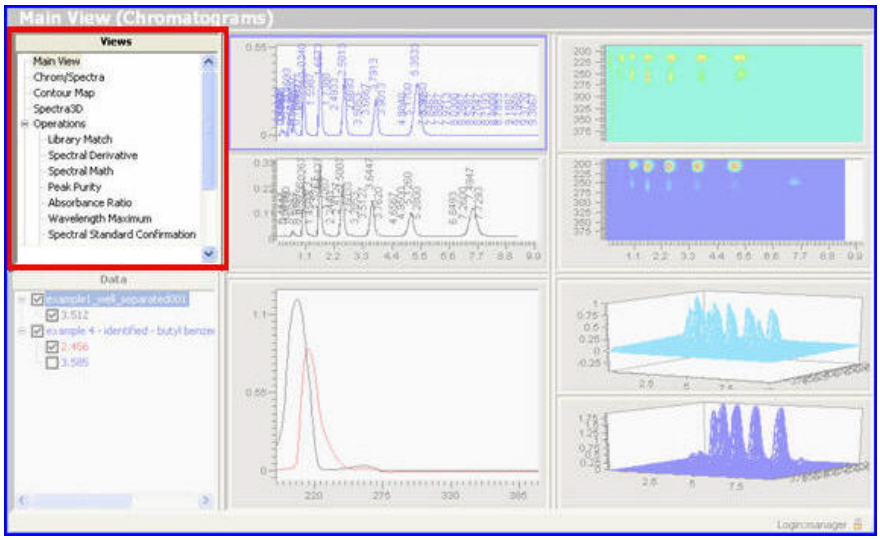
NOTE: *You can scale each of the four panes that are displayed on the Main View with a wide selection of tools. For more information on scaling chromatograms, see* Scaling Chromatograms*. For more information on scaling spectra, see* Scaling Spectra*. For more information on scaling the Contour Map, see* Scaling a Contour Map*. For more information on scaling a 3D Plot, see* Scaling a 3D Plot Pane

Tell me about the Main View

The Main view consists of the following areas:

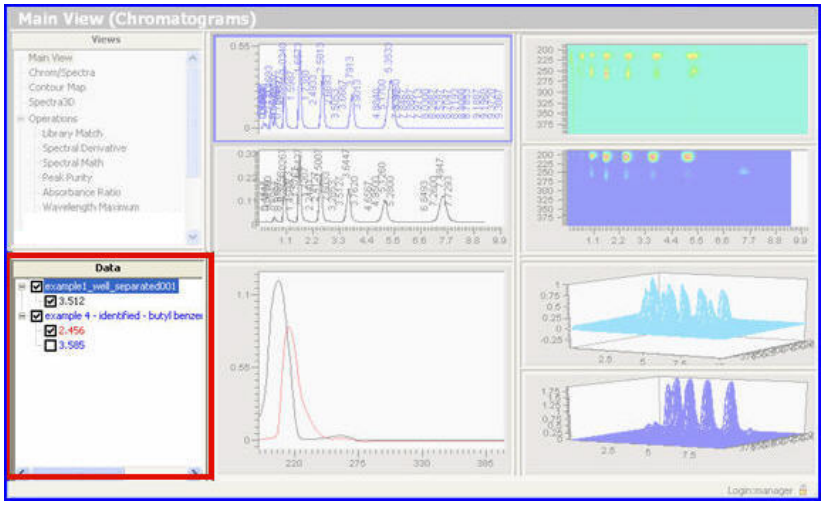
NOTE: *The area to the right of the Views Tree and Data Tree is referred to as the Main Pane, and in the Main View consists of four pane.*

- **Views Tree** - The Views Tree provides you access to the Main View. The Main View is displayed by default.

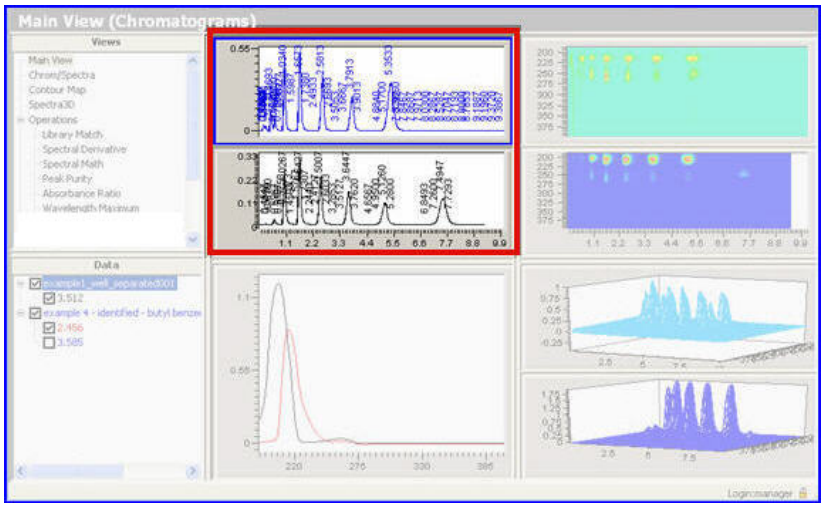


- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms and spectra. You use the Data Tree to select the chromatograms and spectra that you want to see in the relevant panes (chromatograms are displayed in the Chromatogram pane, Contour Map pane, and 3D Spectra pane, while spectra are displayed in the Spectra pane). Items that appear checked on the Data Tree are displayed; while unchecked items on the Data Tree are not displayed.

NOTE: *When you extract spectra from a chromatogram, the extracted spectra are listed on the Data Tree under the parent chromatogram. Meanwhile, previously stored individual spectrum files, that you have opened by selecting* ***File > Open > Spectrum…****, appear as individual branches on the Data Tree.*

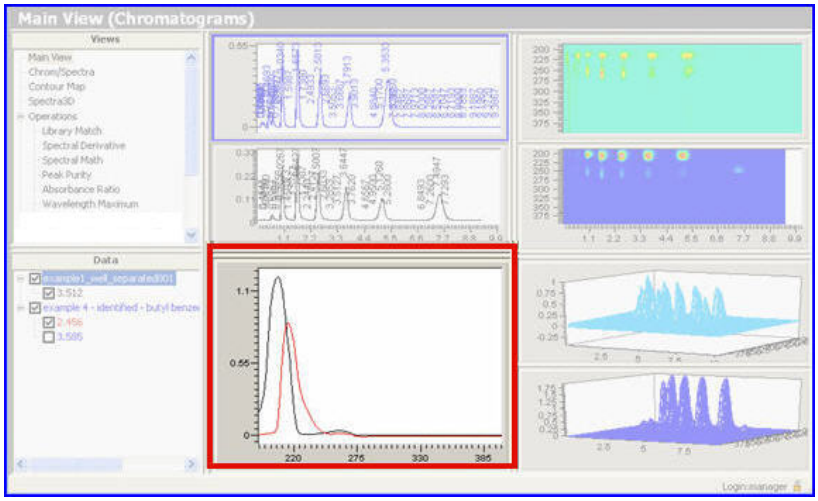


- **Chromatogram Pane -** The Chromatogram pane displays the chromatograms that are checked on the Data Tree.

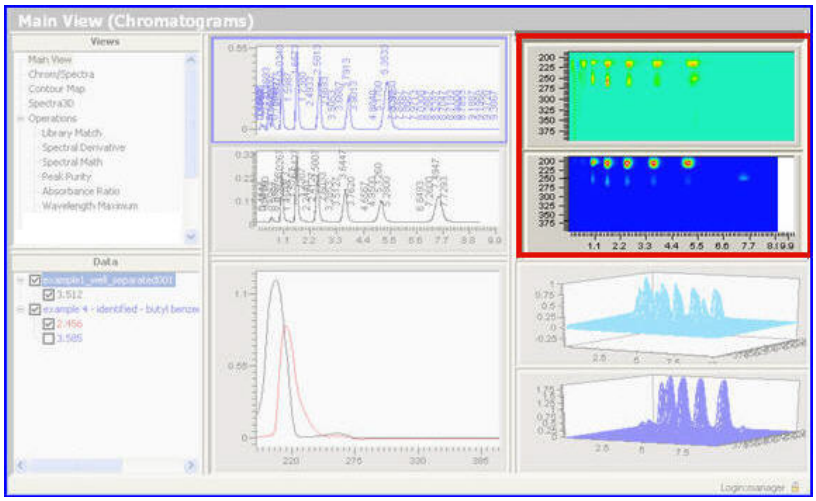


When this pane is in Stacked mode, as indicated when the stacked icon appears to be pressed down  , and the Spectra pane is set to Overlay mode, as indicated when the stack/overlay icon appears  , you can float your cursor over a point on the Chromatogram pane and have the associated spectrum temporarily displayed in the Spectrum pane. You can also extract a spectrum by double clicking on the area in the Chromatogram pane that contains the spectrum you wish to extract. The extracted spectrum is added to the Data Tree and displayed in the Spectrum pane..

- **Spectra Pane -** The Spectra pane displays the spectra that are checked on the Data Tree.



- **Contour Map -** This pane displays contour maps for all chromatograms that appear checked on the Data Tree. When multiple chromatograms are checked on the Data Tree, this pane displays stacked contour maps. The stacked contour maps appear in the same order as the chromatograms displayed on the Chromatogram pane.



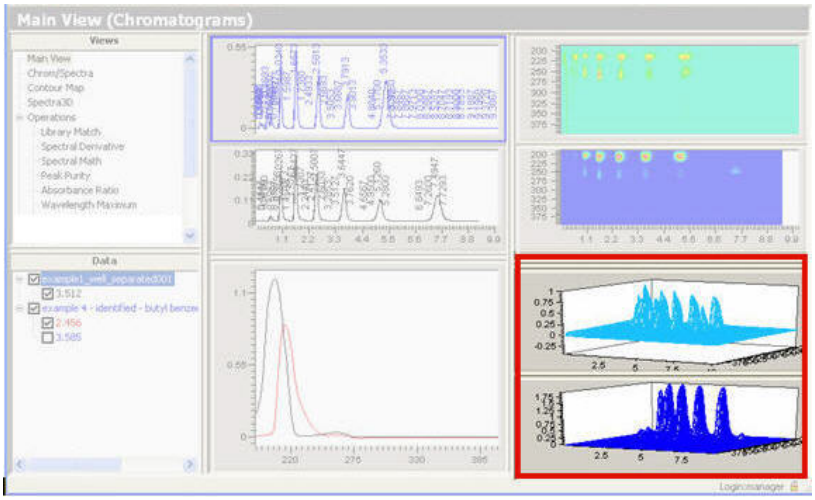
You can float your mouse pointer over the Contour Map to temporarily view a single spectrum and chromatogram from a point on the Contour Map. Or, if you want to extract spectra from this pane: click on any area inside the white space of the Spectra pane and set the Spectra pane to Overlay mode, as indicated when the stack/overlay icon for the Spectra pane appears as follows:  , and then double click on the area in the Contour Map that contains the spectrum you want to extract.

You can also temporarily view a chromatogram from a point on the contour map. To display a chromatogram from a point on a contour map, first make sure the Chromatogram pane is set to an Overlaid display. Then, simply float your mouse pointer over the contour map, and the chromatogram from that point is temporarily displayed on the Chromatogram pane in the Main View.

If you want to temporarily capture a chromatogram from the Contour Map, first make sure the Chromatogram pane is set to an Overlaid display. Then, just double click on an area in the Contour Map; and the chromatogram at the wavelength and time you clicked appears in the Chromatogram pane.

NOTE: *Chromatograms that are captured from a contour map are only temporary and will be cleared when you leave the Main View.*

- **3D Plot Pane -** This pane displays a three-dimensional perspective plot for each chromatogram that is checked on the Data Tree. When multiple chromatograms are checked on the Data Tree, this pane displays stacked 3D plots . The stacked plots appear in the same order as the chromatograms displayed on the Chromatograms pane.



You can float your mouse pointer over the 3D Plot to temporarily view a single spectrum and chromatogram from a point on the 3D Plot. *(**NOTE:** The Chromatogram and Spectra panes must be set to an Overlaid Display)*

If you want to extract spectra from the 3D plot: click on any area inside the white space of the Spectra pane, set the Spectra pane to an Overlaid display, and then double click on the area in the 3D plot that contains the spectrum you want to extract.

If you want to temporarily capture a chromatogram from the 3D plot, just double click on an area in the 3D plot; and the chromatogram at the wavelength and time you clicked appears in the Chromatogram pane in the Main View.

NOTE: *Chromatograms that are captured from a 3D plot are only temporary and will be cleared when you leave the Main View.*

How do I adjust the pane width and height?

You can adjust the height and widths of any of these panes to make them smaller or larger.

To adjust panes:

1. Place the pointer over the edge of the pane that you want to adjust.

   The pointer turns into a line with arrows on each end ↕ ↔.

2. Press the left mouse button and drag up, down, left or right. The pane is resized after you release the mouse button.

   The program maintains these settings until you adjust the panes again.

How do I open and display chromatograms?

- You can open chromatograms by clicking in the check box corresponding to your chromatogram in the Data Tree.

  The chromatogram is displayed in the chromatogram pane. If you do not want the chromatogram displayed, uncheck the chromatogram in the Data Tree.

NOTE: *You can find information on a chromatogram - the number of spectra and how they were collected - before it is opened by selecting the* ***Information*** *command in the* ***File*** *menu and then selecting the chromatogram you wish to view more information on from the Open Chromatogram dialog that appears.*

How do I capture a spectrum from a Chromatogram pane?

To view spectra associated with a particular chromatogram, as well as extract spectra from a chromatogram, the **Chromatogram pane** must use a **Stacked** display, and the **Spectrum pane** must use an **Overlaid** display.

- You can set the display modes for both panes by selecting **Stack Chromatograms** and **Overlay Spectra** from the **View** menu.

To extract spectra:

1. Click on any area in the white space of the Chromatogram pane.

2. Set the selected Chromatogram pane to Stacked mode by clicking on the  icon so that the icon appears to be pushed down 

3. Float your mouse pointer over a point on the chromatogram to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the chromatogram, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.

4. If you see a spectrum that you want extract, just double click on the spot in the chromatogram that contains the spectrum you want to extract.

   The spectrum at the wavelength and time you clicked appears in the Spectra pane.

How do I capture a spectrum from a Contour Map?

To extract and display a spectrum from a point on the Contour Map:

1. On the MainView, check the chromatogram on the Data Tree that you want displayed as a contour map.

   The chromatogram is displayed on the Chromatogram pane, Contour Map pane, and 3D Plot pane.

2. Float your mouse pointer over a point on the contour map plot to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the contour map, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.

3. If you see a spectrum that you want extract, just double click on that area in the contour map; and the spectrum at the wavelength and time you clicked appears in the Spectra pane and is now listed on the Data Tree.

NOTE: *Unlike the Main View, where extracted spectra are displayed on the Data Tree, when you extract a spectrum from the* Contour Map View*, the spectrum is added to the Data Tree; however, you will not see the extracted spectrum on the Data Tree until you exit the Contour Map View. The reason for this is because on the Contour Map View, the Data Tree is used only for selecting the chromatogram that you want displayed. You will be able to see all of the spectra you have extracted once you select a different view such as the* Main View *or the* Chrom/Spectra View*.*

How do I capture a spectrum from a 3D Plot?

To extract and display a spectrum from a point on the Contour Map:

1. On the MainView, check the chromatogram on the Data Tree that you want displayed as the 3D Plot.

   The chromatogram is displayed on the Chromatogram pane, Contour Map pane, and 3D Plot pane.

2. Float your mouse pointer over a point on the 3D Plot to temporarily view, in the Spectra pane, the spectrum from that point. As you continue to float your mouse pointer over the 3D Plot, the Spectra pane automatically updates, so that the spectrum from the current location of your mouse pointer is displayed in the appropriate pane.

3. If you see a spectrum that you want extract, just double click on that area in the 3D Plot; and the spectrum at the wavelength and time you clicked appears in the Spectra pane.

NOTE: *Unlike the Main View, where extracted spectra are displayed on the Data Tree, when you extract a spectrum from the* Spectra 3D View*, the spectrum is added to the Data Tree; however, you will not see the extracted spectrum on the Data Tree until you exit the Spectra 3D View. The reason for this is because on the Spectra 3D View, the Data Tree is used only for selecting the chromatogram that you want displayed. You will be able to see all of the spectra you have extracted once you select a different View such as the* Main View *or the* Chrom/Spectra View*.*

How do I temporarily capture a chromatogram from a Spectra pane?

On the Main View, you can temporarily preview and capture chromatograms from a spectrum. However, it is important to note that any chromatograms you capture from the Spectra pane are temporary and will be cleared from the display when you select a different view.

To temporarily preview and capture a chromatogram from a spectrum displayed on the Spectra pane, the **Chromatogrampane** must use an **Overlaid** display, and the **Spectrumpane** must use an **Stacked** display.

- You can set the display modes for both panes by selecting **Overlay Chromatograms** and **Stack Spectra** from the **View** menu.

To temporarily capture a chromatogram:

1. Click on any area in the white space of the Chromatogram pane.

2. Set the selected Chromatogram pane to an Overlaid display by selecting OverlayChromatograms from the View menu.

3. Click on any area in the white space of the Spectra pane and set the pane to a Stacked display by selecting Stack Spectra from the View menu.

4. Float your mouse pointer over a point on a displayed spectrum to temporarily view, in the Chromatogram pane, the chromatogram from that point. As you continue to float your mouse pointer over the spectrum, the Chromatogram pane automatically updates, so that the chromatogram from the current location of your mouse pointer is displayed in the appropriate pane.

5. If you see a chromatogram that you want to temporarily capture, just double click on the spot in the spectrum that contains the chromatogram you want to extract.

   The chromatogram at the point you clicked appears in the Chromatogram pane.

NOTE: *Chromatograms that are captured from a Spectra pane are only temporary and will be cleared when you select a different view.*

How do I temporarily capture a chromatogram from a Contour Map?

On the Main View, you can temporarily capture chromatograms from a Contour Map. However, it is important to note that any chromatograms you capture from the Contour Map pane are temporary and will be cleared from the display when you select a different view.

To temporarily capture a chromatogram from a Contour Map pane, the **Chromatogram pane** must use an **Overlaid** display, and the **Contour Map pane** must use a **Stacked** display.

To temporarily extract and display a chromatogram from a point on the Contour Map:

1. On the **Main View**, check the chromatogram on the Data Tree that you want displayed as a contour map.

   The chromatogram is displayed on the Chromatogram pane, Contour Map pane, and 3D Plot pane.

2. Make sure that the Chromatogram pane is set to an Overlaid display.

3. Float your mouse pointer over a point on the contour map plot to temporarily view, in the Chromatogram pane, the chromatogram from that point. As you continue to float your mouse pointer over the contour map, the Chromatogram pane automatically updates, so that the chromatogram from the current location of your mouse pointer is displayed in the appropriate pane.

4. If you see a chromatogram that you want to temporarily capture, just double click on that area in the contour map; and the chromatogram from the location you clicked appears in the Chromatogram pane.

NOTE: *Chromatograms that are captured from a Contour Map are only temporary and will be cleared when you select a different view.*

How do I temporarily capture a chromatogram from a 3D Plot?

On the Main View, you can temporarily capture chromatograms from a 3D Plot. However, it is important to note that any chromatograms you capture from the 3D Plot pane are temporary and will be cleared from the display when you select a different view.

To temporarily capture a chromatogram from a 3D Plot pane, the **Chromatogrampane** must use an **Overlaid** display.

To temporarily extract and display a chromatogram from a point on the 3D Plot:

1. On the Main View, check the chromatogram on the Data Tree that you want displayed as the 3D Plot.

   The chromatogram is displayed on the Chromatogram pane, Contour Map pane, and 3D Plot pane.

2. Make sure that the Chromatogram pane uses an Overlaid display.

3. Float your mouse pointer over a point on the 3D Plot to temporarily view, in the Chromatogram pane, the chromatogram from that point. As you continue to float your mouse pointer over the 3D Plot, the Chromatogram pane automatically updates, so that the chromatogram from the current location of your mouse pointer is displayed in the appropriate pane.

4. If you see a chromatogram that you want to temporarily extract, just double click on that area in the 3D Plot; and the chromatogram at the at the point you clicked appears in the Chromatogram pane.

NOTE: *Chromatograms that are captured from a 3D Plot are only temporary and will be cleared when you select a different view.*

Chrom/Spectra View

The Chrom/Spectra View functions similar to the Main View; both views are designed for viewing unlimited chromatograms and spectra at one time. However, unlike the Main View page, which shows you chromatograms, spectra, contour maps, and 3D plots, the Chrom/Spectra page just shows you the chromatograms and spectra that are checked on the Data Tree. For more information on how to use the Chrom/Spectra View you can refer to the Main View Help topic.

Contour Map View

The Contour Map View is intended to help you explore complex chromatographic data. This view gives you a wide array of tools for visual investigation of chromatographic and spectroscopic features.

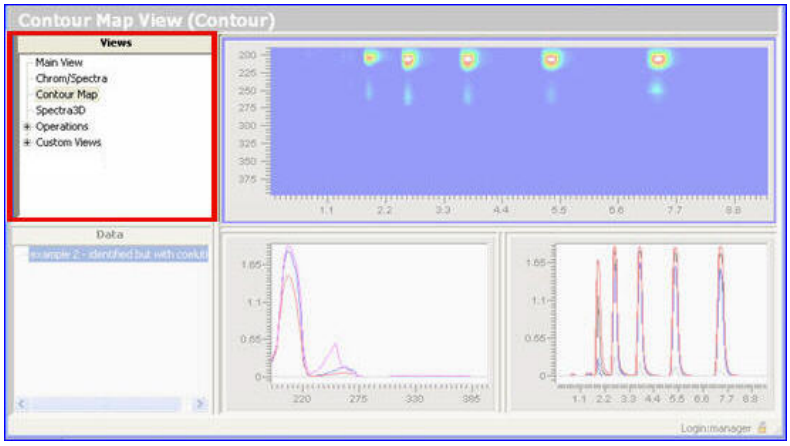
NOTE: *Although this page allows you view spectral and chromatographic data during analysis, the data cannot be stored for use in other areas of the program.*

More specifically, the Contour Map view allows you to obtain a plot of spectral data that resembles a geographical contour map, except that the lines represent equal absorbance rather than equal altitude. The axes for the display are Retention Time and Wavelength. The contour levels show the absorbances. There are 100 colored levels on the Contour Map. The color at each level is fixed, starting with blue at the bottom and finishing with white at the top. The absorbance range covered can be set using the various scaling commands on the view, for more information see the topic titled Scaling a Contour Map.

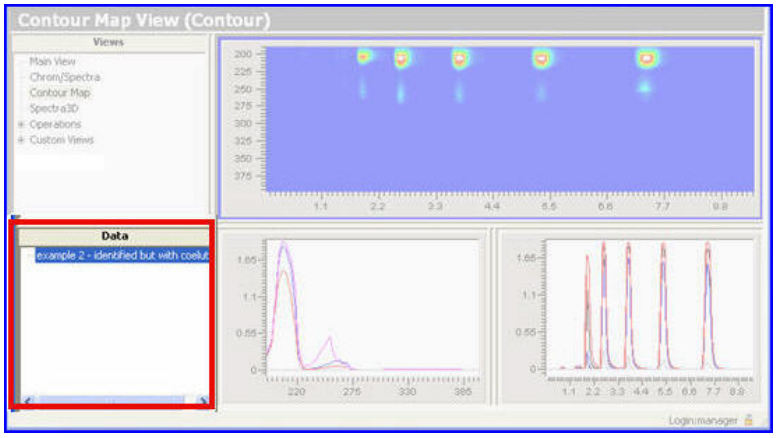
Tell me about the Contour Map.

The Contour Map view consists of the following areas:

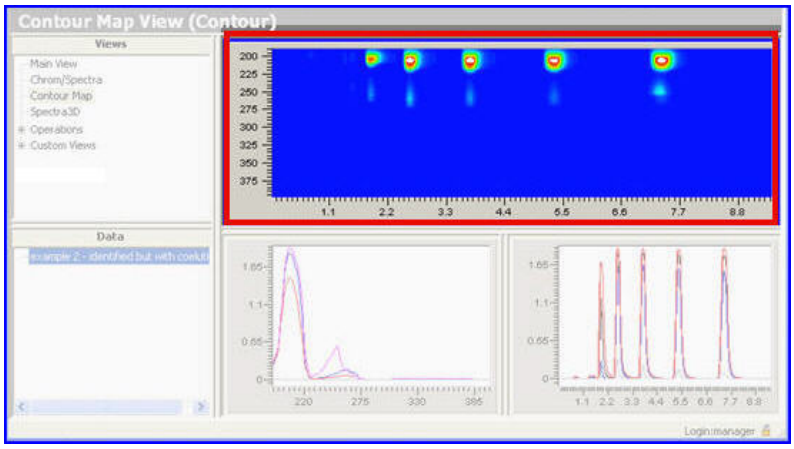
- **Views Tree** - The Views Tree provides you access to the Contour Map View.



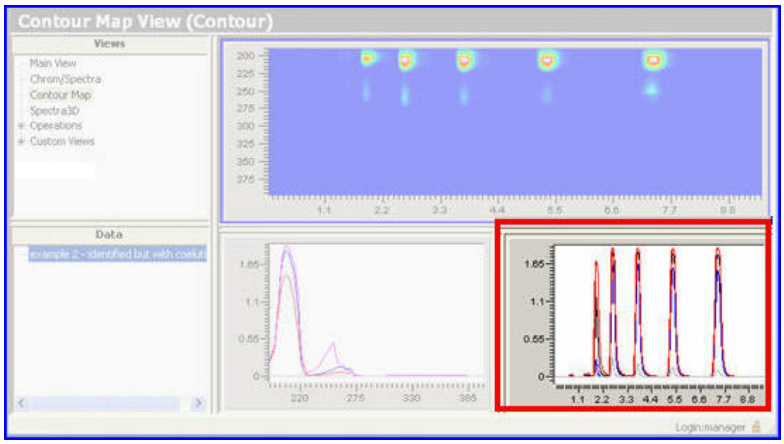
- **Data Tree -** The Data Tree displays a list of opened chromatograms. From the Data Tree you select the required chromatogram for the contour map. Only one chromatogram can be selected and displayed on this page at a time.



- **Contour Map Pane -** This pane displays the contour map of a chromatogram that is selected on the Data Tree. The display includes a crosshair cursor which is used to select a point on either the retention time axis or the wavelength axis and the relevant chromatogram and spectrum will be shown in the corresponding panes below. The contour levels show the absorbances.



- **Chromatogram Pane** -The Chromatogram pane displays the chromatogram from the wavelength axis point selected on the Contour Map pane.



To display a chromatogram on this pane, simply float your mouse pointer over the Contour Map, and the chromatogram from that point is temporarily displayed on the Chromatogram pane.

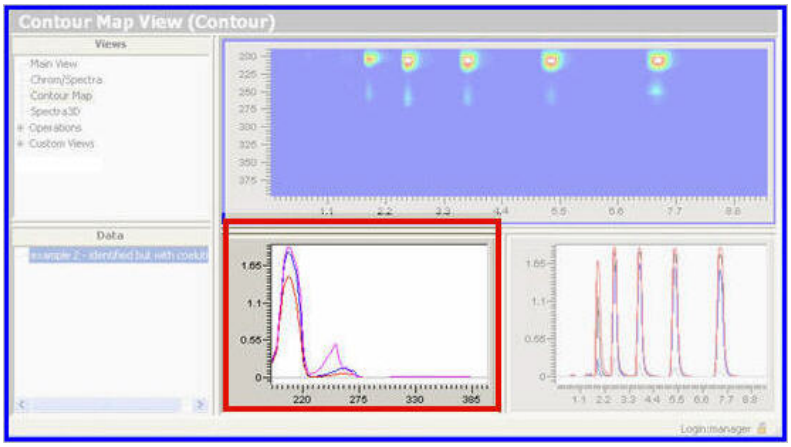
If you want to temporarily extract a chromatogram from the Contour Map, just double click on an area in the Contour Map; and the chromatogram at the wavelength and time you clicked appears in the chromatogram pane.

NOTE: *Chromatograms that are captured from the Contour Map pane are only temporary and will be cleared when you leave the Contour Map View.*

- **Spectra Pane -** This pane displays the spectrum from the point selected on the Contour Map. To display a spectrum on this pane, simply float your mouse pointer over the Contour Map, and the spectrum from that point is temporarily displayed on the Spectra pane.

  If you want to extract a spectrum from the Contour Map, just double click on an area in the Contour Map; and the spectrum at the wavelength and time you clicked appears in the Spectra pane.

NOTE: *When you extract a spectrum from the Contour Map View, the spectrum is added to the Data Tree; however, you will not see the extracted spectrum listed on the Data Tree until you exit the Contour Map View. The reason for this is because while you are viewing the Contour Map View, the Data Tree is used only for selecting the chromatogram that you want displayed on the view. You will be able to see the extracted spectra once you select a different view, such as the Main View or the Chrom/Spectra View.*



How do I adjust the pane width and height?

You can adjust the height and widths of any of these panes to make them smaller or larger.

To adjust panes:

1. Place the pointer over the edge of the pane that you want to adjust.

   The pointer turns into a line with arrows on each end ↕ ↔.

2. Press the left mouse button and drag up, down, left or right. The pane is resized after you release the mouse button.

   The program maintains these settings until you adjust the panes again.

How do I view chromatograms and spectra using the Contour Map?

In this section you will learn how to capture and display spectra and chromatograms from a contour map.

NOTE: *A "captured" spectrum is a spectrum extracted from its source and displayed permanently in the Spectra pane and is listed on the Data Tree. A captured spectrum can be hidden and/or removed.*

To extract and display a chromatogram and spectrum from a point on the Contour Map:

1. From the Views tree select ContourMap View.

   The Contour Map View displays.

2. From the Data Tree, click on the chromatogram you want displayed as the contour map.

3. Float your mouse pointer over a point on the Contour Map plot to temporarily view, in their respective panes, the chromatogram and spectrum from that point. As you continue to float your mouse pointer over the Contour Map, the Chromatogram and Spectra panes automatically update, so that the chromatogram and spectrum from the current location of your mouse pointer are displayed in the appropriate panes.

4. If you see a chromatogram/spectrum that you want extract, then double click on the Contour Map at the wavelength of the chromatogram you want to see.

   The chromatogram and spectrum, at the wavelength and time you clicked, are displayed in their respective panes.

   Please note that chromatograms are only temporarily displayed and will be cleared when you change the view. On the other hand, the spectra that are displayed on the page are extracted and therefore added to the Data Tree. However, you won't see the extracted spectra listed on the Data Tree until you select another view, such as the Main View or Chrom/Spectra View.

Spectra 3D View

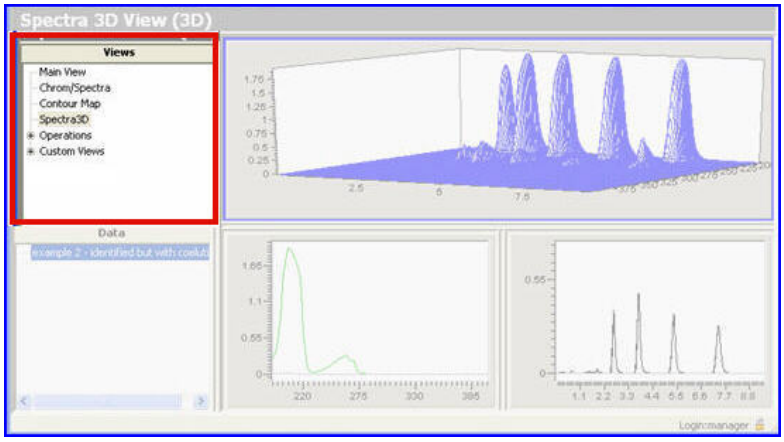
The Spectra 3D View is intended to help you explore complex chromatographic data. This view gives you a wide array of tools for visual investigation of chromatographic and spectroscopic features. Although it does let you view spectral and chromatographic data during analysis, the data cannot be stored for use in other areas of the program.

When a chromatogram is selected on the Data Tree, and the Spectra 3D View is selected, the spectrum and chromatogram positions come from the projection of the mouse cursor position down onto the plane of the perspective outline box. Once you click on the 3D display at the point of interest, the cursor position is identified by a cross-hair marker and is defined in the information bar at the bottom of the Spectra 3D window on the X (time) axis, the Y (absorbance) axis, and the Z (wavelength) axis.

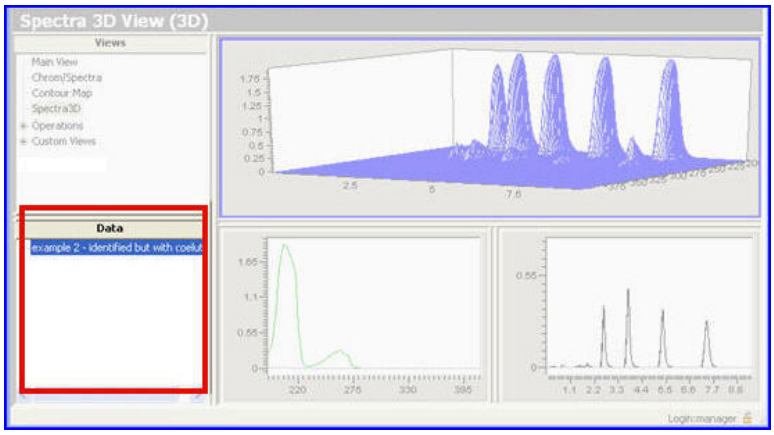
Tell me about the Spectra 3D View.

The Spectra3D page is comprised of the following areas:

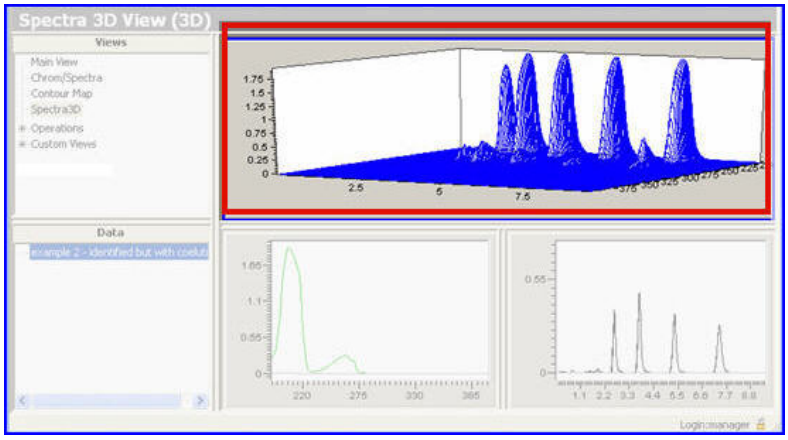
- **ViewsTree** - The Views Tree provides you access to the Spectra 3D View.



- **Data Tree** - The Data Tree displays a list of opened chromatograms. From the Data Tree you select the required chromatogram for 3D plot. Only one chromatogram can be selected and displayed on this page at a time.



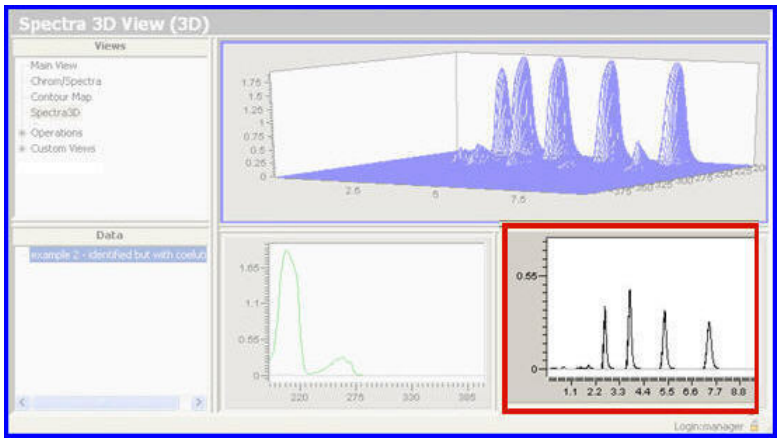
- **3D Plot Pane** – This pane displays the Spectra 3D View from the chromatogram selected on the Data Tree. The display includes a crosshair cursor which is used to select a point on either the retention time axis or the wavelength axis and the relevant chromatogram or spectrum will be shown in the corresponding panes below.



- **Chromatogram Pane** - The Chromatogram pane displays the chromatogram from the wavelength axis point selected on the 3D Plot pane. To display a chromatogram on this pane, simply float your mouse pointer over the 3D Plot, and the chromatogram from that point is temporarily displayed on the Chromatogram pane.

  If you want to temporarily extract a chromatogram from the 3D Plot, just double click on an area in the 3D Plot; and the chromatogram at the wavelength and time you clicked appears in the Chromatogram pane.

NOTE: *Chromatograms that are displayed on this pane are only temporary and will be cleared when you leave the Spectra 3D View.*

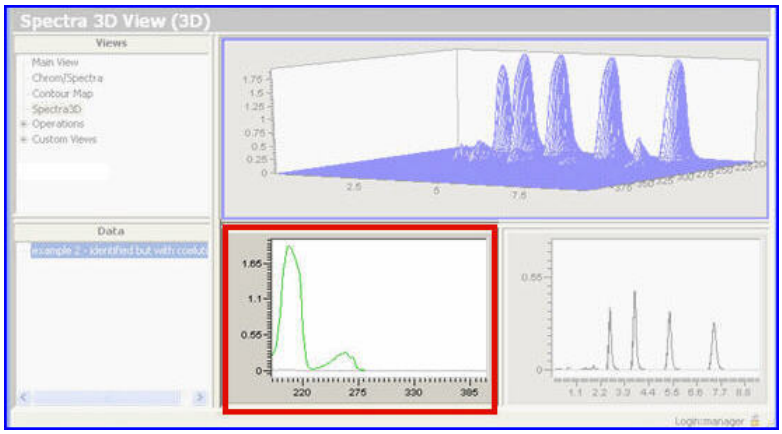


- **Spectra Pane** - This pane displays the spectrum from the retention axis point selected on the 3D Plot pane. To display a spectrum on this pane, simply float your mouse pointer over the 3D Plot, and the spectrum from that point is temporarily displayed on the Spectra pane.

  If you want to extract a spectrum from the 3D Plot, just double click on an area in the 3D Plot; and the spectrum at the wavelength and time you clicked appears in the Spectra pane.

NOTE: *When you extract a spectrum from the Spectra 3D View, the spectrum is added to the Data Tree; however, you will not see the extracted spectrum listed on the Data Tree until you exit the Spectra 3D View. The reason for this is because while you are viewing the 3D Spectra View, the Data Tree is used only for selecting the chromatogram that you want displayed on the view. You will be able to see the extracted spectra once you select a different view such as the Main View page or the Chrom/Spectra View.*

- The **Add to View** command from the context menu will add the Spectrum to the Data Tree, or drag-drop of the label can be used



How do I adjust the pane width and height?

You can adjust the height and widths of any of these panes to make them smaller or larger.

To adjust panes:

1. Place the pointer over the edge of the pane that you want to adjust.

   The pointer turns into a line with arrows on each end ↕↔.

2. Press the left mouse button and drag up, down, left or right. The pane is resized after you release the mouse button.

   The program maintains these settings until you adjust the panes again.

How do I display and extract a chromatogram and spectrum from the 3D Plot?

In this section you will learn how to capture and display spectra and chromatograms from the 3D Plot on the Spectra 3D View.

NOTE: *A "captured" spectrum is a spectrum extracted from its source and displayed permanently in the Spectra pane and is listed on the Data Tree. A captured spectrum can be hidden and/or removed.*

To extract and display a chromatogram and spectrum from a point on the 3D Plot:

1. From the **Views Tree** select **Spectra 3D View**.

   The 3D Spectra View page displays.

2. From the **Data Tree**, click on the chromatogram you want displayed as a 3D plot.

3. Float your mouse pointer over a point on the 3D plot to temporarily view, in their respective panes, the chromatogram and spectrum from that point. As you continue to float your mouse pointer over the 3D plot, the Chromatogram and Spectra panes automatically update, so that the chromatogram and spectrum from the current location of your mouse pointer are displayed in the relevant panes.

NOTE: *The horizontal cursor on the 3D Plot indicates exactly where you are viewing the chromatogram.*

4. If you see a chromatogram/spectrum that you want extract, while you are floating your mouse pointer over the 3D plot, just double click.

   The chromatogram and spectrum at the wavelength and time you clicked are displayed in their respective panes.

   Please note that chromatograms are only temporarily displayed and will be cleared when you change the view. On the other hand, the spectra that are displayed on the view are extracted and therefore added to the Data Tree. However, you won't see the extracted spectra listed on the Data Tree until you select another View, such as the Main View or Chrom/Spectra View.

How do I rotate the display?

You can rotate the 3D Plot around one axis at a time to identify chromatographic or spectral characteristics more accurately. To rotate the 3D plot, you must first select the axis that you wish to rotate the 3D plot around; you can then use the Rotation slider to rotate the 3D Plot a set number of degrees around the selected axis.

To rotate the 3D Display:

1. First select the rotation controls for rotating the 3D plot.

2. Click on the **Rotate X**  icon if you want to rotate the 3D plot around the X axis.

   OR

   Click on the **Rotate Y**  if you want to rotate the 3D plot around the Y axis.
   The selected icon appears to be pressed down to indicate that the 3D plot will be roated around the selected axis.

3. Use the Rotation slider  to rotate the 3D Plot a set number of degrees around the axis you selected in step 1.

   Move the slide to the right to rotate the plot to the right, or the "up" direction.

   Move the slide to the left to move the plot in the reverse direction.

4. Release the slider to return it to the center position.

Creating Custom Views

You can create your own Custom views by modifying an existing view page. You can modify existing views by selecting to hide or show a particular pane.

- To hide or show a pane on a view page select **View > Panes** from the menu bar. The Panes sub menu allows you to select whether or not a C**hromatogram pane, Spectrum pane, Contour Map pane**, or **3D Plot pane** is displayed on the View.

  OR

  Right mouse click on the pane you wish to hide, and select Hide Pane from the context menu that appears.

- Once you have modified an existing view you can save it as a Custom View by selecting **View > View Template > Save As...** from the menu bar.
  The name of the view you saved now appears under the Custom node on the Views tree.

Scaling Tools

Scaling Spectra

The default display protocol for spectra displayed is an X/Y autoscale mode that automatically scales the display so that every spectrum is completely visible in both the X and Y axis directions. If you add a new spectrum with a wider range, the display's range is adjusted to accommodate it.

In addition you are provided with a number of features that let you modify the spectrum display so that you can more easily compare spectra. This section begins with a brief description of the scaling commands available to you.

Options for scaling spectra

This section describes the available scaling commands used to modify the spectrum display. You will find that the information in this section is useful when multiple, overlaid spectra are displayed.

Please note that before you use any of the scaling commands to modify how spectra are displayed, you must first select the Spectra pane by clicking on the white space of the pane. When the Spectra pane is selected a blue border appears around the pane. Now that you have the Spectra pane selected you can use the scaling commands described below to more easily compare spectra.

Tell me about Autoscaling Spectra.

The autoscale commands allow you to rescale the data in the Spectra pane so that all of the data are visible. As noted earlier, the autoscale commands are particularly useful when multiple, overlaid spectra are displayed.

There are two commands which you can use to scale spectra to fit the display region:

- The **Autoscale X and Y** command  rescales spectra on the current view in both the X and Y directions, so that all of the data are visible. This command is particularly useful when multiple overlaid spectra are displayed, because clicking on this command rescales the data so that all points for the largest data set, in both the X and Y axis directions, fit in the window.

NOTE: *Most LC solvents absorb strongly in the low UV and so allow very little energy to reach the pixels of the array at those wavelengths, making any data suspect. Scaling on the Y-axis is always done above the pixel threshold. Thus, if the first valid pixel is 210 nm, the scale will be determined by the minimum and maximum absorbance values found in the region (210–700 nm for the Series 200 DAD). With strong end absorbance, this may mean that the spectrum below 210 nm is off-scale.*

- The **Autoscale Y Only** command  works like the Autoscale X and Y command described above, except that it leaves the X axis unchanged and scales only the Y axis.

Tell me about Normalizing Spectra.

The Normalization commands are useful when multiple, overlaid spectra are displayed and you want to find subtle differences between spectra. Basically, the Normalize commands

help you compare the exact shapes of peaks by converting all of the displayed peaks to the same maximum height.

The Normalization commands are as follows:

NOTE: *If you are trying to scale a single chromatogram or spectrum, only* ***OffsetNormalize*** *and* ***NormalizePoint*** *commands are useful commands.*

- The **Normalize X and Y** command  is useful when multiple, overlaid spectra are displayed. This command scales the maximum Y value in the active window to 90% for each spectrum and sets the X axis scale so that the entire largest spectrum is displayed.

- The **NormalizeYOnly** command  , works like "Normalize All," except it rescales only the Y axis.

- The **OffsetNormalize** command  is useful when multiple, overlaid spectra are displayed, or when a single spectrum is displayed. This command scales the data point with the maximum Y value to the right of the current cursor position to 90% in the region for each spectrum.

- The **NormalizePoint** command  is useful when multiple, overlaid spectra are displayed, or when a single spectrum is displayed. This command scales the Y value at the current cursor point to 90%. The new (90%) scaling is applied to every spectrum.

In addition to the Autoscale and Normalize commands, the following additional options are available for scaling spectra:

- Use the **Box Zoom** function to visually specify an area of the display you want to zoom into.

- Use the **Format Graphs** dialog to scale spectra by specifying the axis range.

- **Zoom** in a continuous fashion.

How do I visually specify an area of the display to zoom into using the Zoom Box function?

It is possible to zoom into a visually defined region of a spectrum using the Box Zoom. To scale individual spectra set the Spectra pane to Stacked mode.

To zoom into a visually defined region:

1. Move the mouse cursor inside the plot region and position it at a corner of the area you want to zoom.

2. Hold down the left mouse button and drag the mouse to form a box around the area to be zoomed.

3. Release the mouse button.

   The region you wish to zoom into will be outlined with a box containing eight control points. The box can be resized by clicking and dragging on any of the control points.

4. Move the mouse pointer into the zoom region and click the left mouse button.

NOTE: *You can individually scale the absorbance of each spectrum. All spectra, however, share the same wavelength axis, so zooming into the wavelength range on one spectrum zooms into that wavelength range for all spectra.*

5. You can abort the zoom before step 4 by clicking the left mouse button outside the zoom box.

6. To return to a view of the entire trace, select Actions> Autoscale > X and Y.
   OR
   Use the Previous Scale command  , which is also located under the Actions menu.

How do I view and change the axis range for spectra?

1. You can view and change the axis range for spectra by right mouse clicking on a spectra pane and selecting Format Graphs from the context menu that appears.
   The Format Graphs window appears with the Spectra tab selected. This window allows you to set the minimum and maximum values on both the X and Y axes..

2. From the **Spectra** tab page specify the Maximum and Minimum values for the X and Y axes.

3. Click **OK** to apply changes and close the Format Graphs dialog.

NOTE: *Changes in the wavelength range always affect all the spectra.*

How do I Zoom in a continuous fashion?

You can zoom spectra in a continuous fashion using the slider zoom feature in conjunction with the **Zoom X** and **Zoom Y** icons. The slider zoom enables you to expand or spectra in a continuous manner about the cursor position. The Zoom X and Zoom Y commands determine what is zoomed when you are interacting with the zoom slider.

To zoom a spectrum:

1. Click on the Spectra pane that you wish to zoom.

2. Click on the **Zoom X axis** icon and or the Zoom Y axis icon on the Toolbar to set the required zoom mode.
   You can select both the X and Y axis icons to zoom the X and Y axes simultaneously. The buttons appear depressed when active.

3. Push the Toolbar slider  to the right or left.

Scaling Chromatograms

There are a variety of options to scale a chromatogram. You can:

- Visually specify an area of the display to zoom into using the **Box Zoom** function.
- Scale chromatograms by specifying the axis range from the **Format Graphs** dialog.
- **Autoscale** only the absorbance axis, to fit the chromatogram between 10% and 90% of the display, or you can scale both axes to display the full retention time range and re-scale the absorbance to fit between 10% and 90% of the display.
- **Zoom** in a continuous fashion.
- **Normalize** an entire chromatogram using the **Normalize X and Y** command  (i.e. all points in the displayed chromatogram), normalize along the Y axis only using the **Normalize Y** command  , normalize at the current cursor position using the **Normalize Point** command  , or you can normalize to the maximum absorbance value to the right of the current cursor position using the **Offset Normalize** command  .

Following is an overview of the scaling options that are available to you when you are working with a Chromatogram pane:

How do I visually specify an area of the display to zoom into using the Box Zoom function?

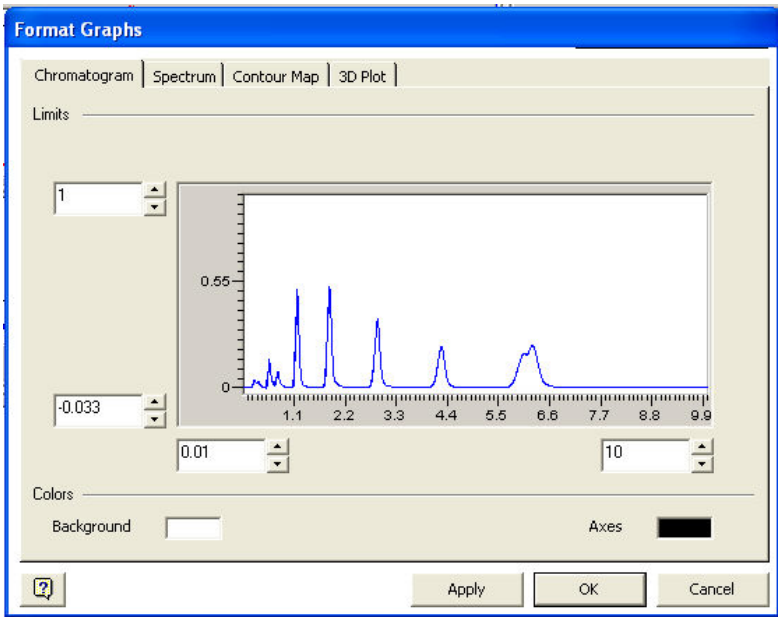
To zoom into a visually defined region:

1. Move the mouse cursor inside the plot region and position it at a corner of the area you want to zoom.
2. Hold down the left mouse button and drag the mouse to form a box around the area to be zoomed.
3. Release the mouse button.
   The region you wish to zoom into will be outlined with a box containing eight control points. The box can be resized by clicking and dragging on any of the control points.
4. Move the mouse pointer into the zoom region and click the left mouse button.
   The zoomed area now fills the trace display region.
5. You can abort the zoom before step 4 by clicking the left mouse button outside the zoom box.
6. To return to a view of the entire trace, select **Actions > Autoscale > X and Y.**

How do I view and change the axis range for a particular chromatogram?

1. You can view and change the axis range for particular chromatogram by right mouse clicking on a chromatogram and selecting Format Graphs from the context menu that appears.

The Format Graphs window appears with the Chromatogram tab selected. This window allows you to set the minimum and maximum values for the selected chromatogram on both the X and Y axes.



2. From the **Chromatogram** tab page specify the Maximum and Minimum values for the X and Y axes.

3. Click **OK** to apply changes and close the Format Graphs dialog.

How do I use the Autoscale tools?

The autoscale commands allow you to rescale the data in the chromatogram pane so that all of the data are visible. There are two commands which you can use to scale a chromatogram to fit the display region. **Autoscale X and Y** scales the chromatogram to between 10% and 90% of the absorbance axis as well as displaying the full retention time range of the chromatogram. **Autoscale Y** only scales only the absorbance axis without changing the retention time range.

To autoscale the absorbance only:

1. Click inside the **Chromatogram** pane that contains the chromatogram/s that you want to autoscale in the Y direction.

2. Select Actions > Autoscale > Y only.
   OR
   Click on the Autoscale Y icon .
   The absorbance axis of the selected chromatogram is scaled to fill 80% of the display.

To autoscale both the retention time and absorbance:

1. Click inside the Chromatogram pane that contains the chromatogram/s you want to autoscale in the X and Y directions.

2. Select **Actions > Autoscale > X and Y**.
   OR
   Click on the Autoscale X and Y icon .
   The retention time axis of the selected chromatogram is scaled to fill the region. All other chromatograms will be re-scalled to this retention time range. The absorbance axis is scaled to fill 80% of the display.

How do I zoom in a continuous fashion?

You can zoom a chromatogram in a continuous fashion using the slider zoom feature in conjunction with the **Zoom X** and **Zoom Y** icons. The slider zoom enables you to expand or contract the chromatogram in a continuous manner. The **Zoom X** and **Zoom Y** commands determine what is zoomed when you are interacting with the zoom slider.

To continuously zoom a chromatogram:

1. Before performing the zoom, place the cursor at the position that you want to zoom, since the zoom is around the position of the cross-hair cursor.

2. Click on the **Zoom X** axis icon and or the **Zoom Y** axis icon on the tool bar to set the required zoom mode.
   You can select both the X and Y axis icons to create an X and Y zoom.
   The buttons appear depressed when active.

3. Push the tool bar slider to the right or left.

How do I use the use the Normalization commands to scale a chromatogram?

The Normalization commands are useful when multiple, overlaid chromatograms are displayed, as these commands are used to find subtle differences in the plot. Basically, the Normalize commands help you compare the exact shapes of peaks by converting all of the displayed peaks to the same maximum height.

NOTE: *For a detailed description of how the Normalization commands can be used, refer to Viewing Spectra - Detailed Description of Normalization commands.*

The Normalization commands are as follows:

Icon	Associated menu command	Description
	Normalize X and Y	Normalizes the graph so all plots are full scale.
	Normalize Y only	Normalizes the graph so all plots are full scale without changing the X axis.
	Offset Normalize	Normalizes all plots to the highest point to the right of the cursor position.
	Normalize Point	Normalizes all plots at the cursor position.

NOTE: *The Normalize Y command is useful if you have zoomed in on a specific region of the chromatogram and want to normalize without resetting the X axis, since Normalize All evaluates all points in the chromatogram, and therefore, resets the X axis.*

To normalize both the retention time and absorbance:

1. Click anywhere inside the chromatogram pane that contains the chromatogram/s you want to scale.

2. Click on the **Normalize X and Y** icon  .
   OR
   Select **Actions > Normalize > X and Y** from the menu bar.
   The retention time axis of the selected chromatogram is scaled to fill the region. All other chromatograms will be re-scaled to this retention time range. The absorbance axis is scaled to fill 80% of the display.

To normalize along the Y axis only:

1. Click the region below the point where you wish normalization to occur.

2. Click on the **Normalize Y** icon  .
   OR
   Select **Actions > Normalize > Y** only from the menu bar.
   The chromatogram will be normalized at the maximum point in the displayed region without changing or resetting the X axis value.

To normalize to the highest point to the right of the cursor:

1. Click the region below the point where you wish normalization to occur.

2. Click on the **Offset Normalize**  icon.
   OR
   Select **Actions > Normalize > Offset** from the menu bar
   The chromatogram is normalized to the maximum point above the cursor position.

To normalize at the position of the cursor:

1. Click the region at the point where you wish normalization to occur.

2. Click on the **Normalize Point** icon  .
   OR
   Select **Actions > Normalize > Point** from the menu bar.
   The chromatogram is normalized at the cursor position.

Scaling a Contour Map

The most useful scaling commands for a Contour Map view are the **Normalize Point** and **Zero** commands. Both commands adjust the scale based on the current position of the cross-hair cursor. However, you are not limited to using the Normalize Point and Zero commands to scale a Contour Map. In fact, you can scale a Contour Map performing any one of the following options:

- Use the **Normalize** and **Zero** commands to set the absorbance range covered by the map.
- Zoom into a visually defined wavelength and/or time region on the contour map using the **Box Zoom**.
- Use the **Autoscale** commands to return the map to its default scaling.
- Scale all three axes on the Contour Map page by specifying Limits on the **Format Graphs** dialog.

How do I scale the Contour Map using the Normalize Point and Zero commands?

As stated earlier, probably the two most useful scaling commands on the contour map are the **Normalize Point** and **Zero** commands in the Tool bar.

On a Contour Map the **Normalize Point** command adjusts the scale so that the absorbance at the cursor position is represented by the top color in the scale. If the absorbance at the cursor position was originally less than the highest absorbance, this has the effect of stretching the scale and revealing more detail in the map.

The **Zero** command does the opposite action and assigns a specified absorbance to the bottom contour. All data below this level is displayed in the bottom color (blue) and thus is hidden.

By combining the use of the two commands you can set any absorbance range on the map to examine specific areas of interest.

To assign an absorbance to the top contour:

1. Click in the map at the point to be assigned to the top contour.
2. Click on the **Normalize Point** icon.
   All absorbance values equal to or above the absorbance at this point will be assigned to the top contour (white). All absorbance values between the value at the zero level and the new top level will be assigned to the remaining fourteen contours.

To assign an absorbance to the bottom contour:

1. Click in the map at the point to be assigned to the bottom contour.
2. Click on the Zero icon.

All absorbance values equal to or below the absorbance at this point will be assigned to the bottom contour (black). All absorbance values between the value at the top level and the new bottom level will be assigned to the remaining fourteen contours.

How do I use the Box Zoom on the Contour Map?

It is possible to zoom into a visually defined wavelength and/or time region on the contour map using the box zoom.

To zoom into a visually defined wavelength and/or time region:

1. Move the mouse cursor inside the plot region and position it at a corner of the area you want to zoom.

2. Hold down the left mouse button and drag the mouse to form a box around the area to be zoomed.

3. Release the mouse button.
   The region you wish to zoom into will be outlined with a box containing eight control points. The box can be resized by clicking and dragging on any of the control points.

4. Move the mouse pointer into the zoom region and click the left mouse button.
   The zoomed area now fills the trace display region.

5. You can abort the zoom before step 4 by clicking the left mouse button outside the zoom box.

6. To return to a view of the entire trace, select **Actions > Autoscale > X and Y.**

How do I use the Autoscale tools on the Contour Map?

Both the Autoscale Y and Autoscale X and Y commands are useful tools in scaling the Contour Map.

To autoscale the absorbance without changing the wavelength or time axes:

Use this command to scale the absorbance range after a zoom on the wavelength and/or time axis.

1. Click anywhere on the map.

2. Click on the **Autoscale Y** icon  .
   The absorbance range is scaled such that the lowest absorbance in the displayed data is set to the bottom contour and the top absorbance is set to the top contour.

To return to the full display of the map:

Use this command to return to the full display of the map.

1. Click anywhere on the map.

2. Click on the **Autoscale X and Y** icon  .

The wavelength and time axes are returned to their full range. The absorbance range is scaled such that the lowest absorbance in the displayed data is set to the bottom contour and the top absorbance is set to the top contour.

How do I scale the Time, Absorbance, and Wavelength axes on the Contour Map?

To scale the Time, Absorbance, and Wavelength axes on the Contour Map:

3. You can scale all three axes on a Contour Map by right mouse clicking on the Contour Map, and selecting **Format Graphs** from the context menu that appears.

   The Format Graphs window appears with the Contour Map tab selected. From this tab page you can scale each of the three regions independently by specifying the limits for the **Time**, **Wavelength**, and **Absorbance** axes; and you can preview the effect of the values you specify for the axes directly on the screen. The display shows you how the plot will appear. You can hover your mouse over the controls on the tab page to view information about the control.

4. After you have specified the limits for all 3 axes click **OK**.

   The Format Graphs dialog closes and the new limits are applied.

Scaling a 3D Plot

Most of the panning and zooming tools that can be used on other panes are not particularly useful or do not operation on 3D panes. Scaling a 3-D Plot view is best done using the Format Graphs command, which is located under the **Tools** menu bar. From the Format Graphs dialog you can scale all three axes: wavelength, retention time, and absorbance, on the plot as well as select to reverse the wavelength axis to "look behind" large peaks. In addition to using the Format Graphs dialog to scale a 3-D plot you can also use the **Autoscale** commands to bring all data into view.

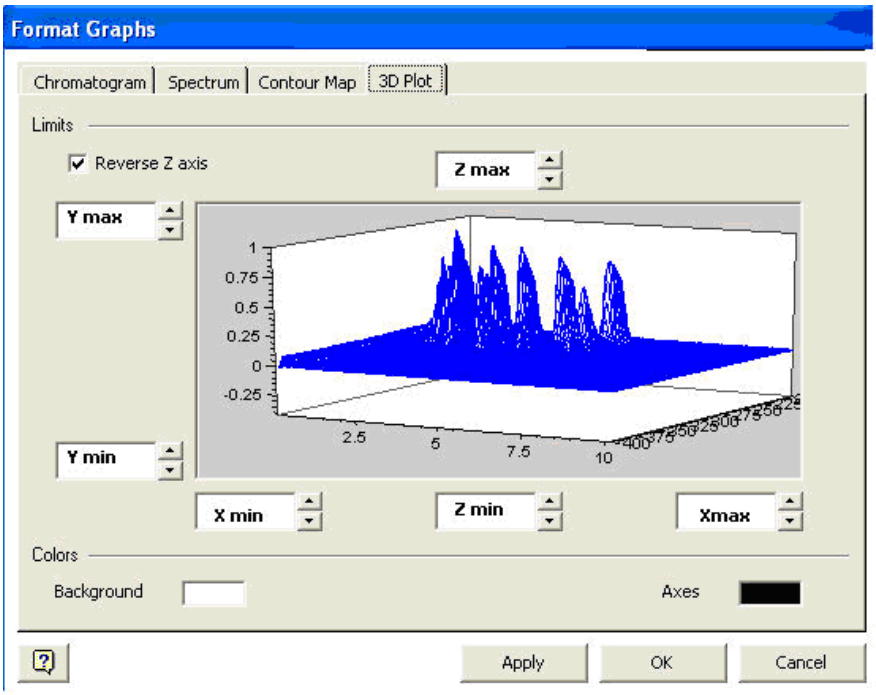
How do I specify limits for a 3D plot and reverse the Wavelength Axis?

The **3D Plot** tab on the Format graphs dialog allows you specify limits for all three axes as well as reverse the wavelength axis (Z axis).

To specify limits for a 3D plot:

1. On the **3D Plot** tab of the Format Graphs dialog specify the minimum and maximum values for the X, Y, and Z axes.

   The six spin boxes on this page represent the minimum and maximum values for the X, Y, and Z axes. When you specify values for the three axes on this dialog, you can preview the effect directly on the Format Graphs dialog.



2. When you are finished setting the numerical limits for the plot click **OK**.

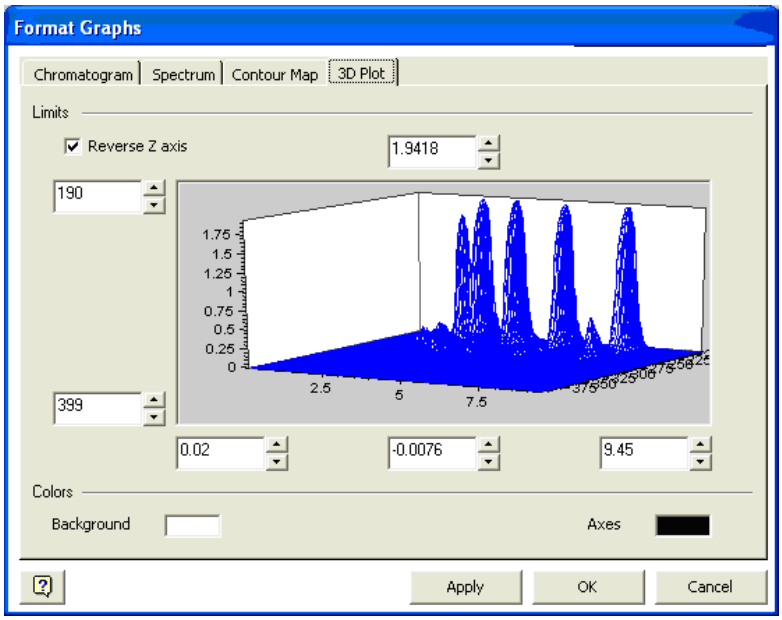
   The Format Graphs dialog closes and the selected plot is scaled according to the limits you specified.

How do I reverse the wavelength axis of a 3-D Plot?

To reverse the Wavelength Axis on a 3D plot:

The 3D Plot tab page on the Format Graphs dialog contains a switch for reversing the wavelength axis. Most UV spectra have very high absorbance in the low UV. Therefore, the 3-D plot is normally displayed reversed,, i.e. with the low UV at the back of the display so it does not obscure the information in the mid-UV range, 220 - 300 nm. The switch is useful if you want to display the low UV at the front, or if you want to look behind one of the peaks in the display.

1. On the 3D Plot tab page of the Format Graphs dialog click on the **Reverse Z Axis** check box to preview the effect.



The check mark is cleared or returned depending on its previous state.

When this option appears checked the 3D plot is displayed reversed.

2. Click **OK**.

The Format Graphs dialog closes and the plot is redrawn with wavelength axis reversed from its previous state.

How do I use the Autoscale commands to scale a 3D Plot?

The **Autoscale X and Y** and **Autoscale Yonly** commands can be used on any plot region to bring all the data into view. **Autoscale Y only** can be used after a change on the wavelength and/or time axis to re-scale the absorbance range only. **Autoscale X and Y** can be used to return to the full display of the plot. However, since the initial scaling for the 3D-Plots and Contour Maps is based on the scaling in the previous view the Autoscale commands will not necessarily return them to their initial states.

To autoscale the absorbance without changing the wavelength or time axes:

Use this command to scale the absorbance range after a change on the wavelength and/or time axis.

1. Click anywhere on the region to be re-scalled.

2. Click on the **AutoscaleY** icon  .
   The Absorbance axis is automatically scaled to fill 80% of the display region.

To return to the full display of the plot:

Use this command to return to the full display of the plot.

1. Click anywhere on the region to be re-scaled.

2. Click on the **Autoscale X and Y** icon  .
   The wavelength and/or time axis will be returned to their full range. The Absorbance axis is automatically scaled to fill 80% of the display region.

Spectral Libraries

About Spectral Libraries

You can identify a spectrum by searching one or multiple libraries for a match to an unknown spectrum, to build your own libraries of stored spectra, build a library directly from the peak apex spectra in a chromatogram, as well as browse through the individual spectra in a library, and if necessary, edit that library.

Note: *When copying spectral libraries used by the Spectral Processing window within Chromera, three files need to be copied [Spectral Library].alb, [Spectral Library].idx and [Spectral Library].aud. Unless all three are copied to the new location the library will not work the next time it is used.*

This section provides you with information on the following topics:

- Automatically Creating a Library from Named Peaks
- Manually Creating a Library
- Viewing a Library
- Editing a Library
- Identifying a Spectrum using the Library Match operation

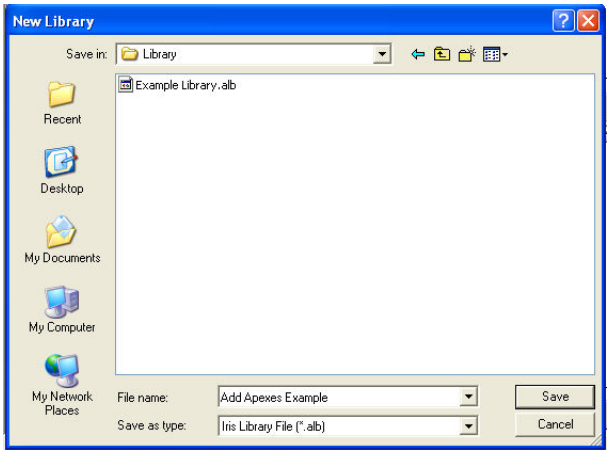
Automatically Creating a Library from Named Peaks

The **Add Apexes** command is accessed by selecting **Tools > Build Library > Add Apexes**. This command allows you to automatically create a spectral library using the apex spectra from named peaks in a chromatogram. This feature is particularly useful when you wish to create a library for use with the Spectral Library Confirmation function, since this function requires that the names of the peaks in the chromatogram are identical to the component names in the library.

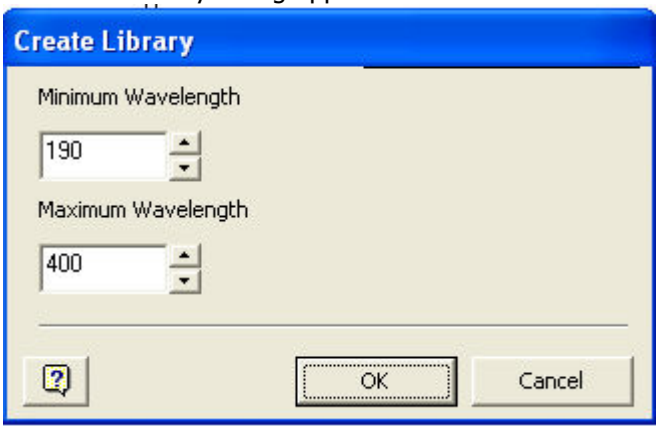
NOTE: *If the chromatogram you have selected contains no identified peaks, an error is displayed that tells you that you cannot add the apex spectra.*

How do I create a spectral library using the apex spectra from named peaks in a chromatogram?

1. From the **DataTree**, select a chromatogram that has named peaks.
2. Select **Tools > Build Library > Add Apexes**.
   The New Library dialog appears.
3. Select location to save the new library and enter a name for the library in the **File name** field.



4. Click **Save**.
   The Create Library dialog appears.



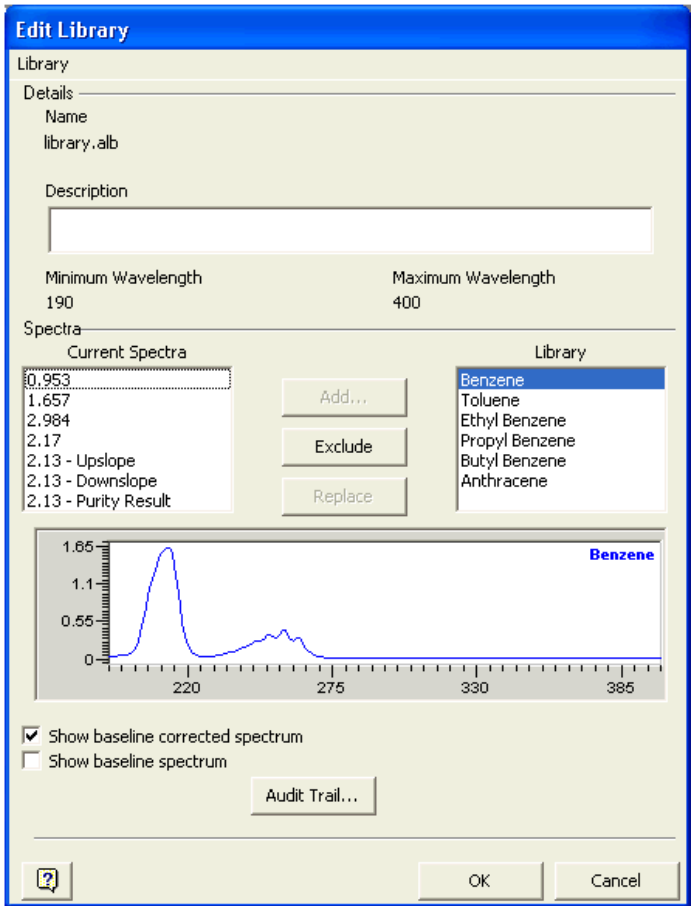
5. From the Create Library dialog, use either the default values for the Library **Minimum** and **Maximum Wavelengths**.

   OR

   If you are working in a different range, enter new Minimum and Maximum Wavelength values that cover the range of spectra collected.

   For example, specify a range of 190-400 for UV only data, or specify 400-700 for visible only data, or 190-700 for UV and visible data

6. Click **OK** to close the Create Library dialog.



7. Click **OK** to exit the Edit Library dialog.

Manually Creating a Library

The Create Library Command, found under the Tools menu by selecting **Tools > Build Library > Create**, allows you to create new libraries. You may find this function useful for creating a library for each general class of compound that you work with. This reduces the number of spectra that must be searched when you are trying to match an unknown, (you can match an unknown spectra by accessing the Library Match Operation). It also decreases the risk of false matches, because the search can be restricted to the most likely class of compounds. (If no matches are found, it is always possible to widen the search by adding other libraries to the search list.)

You can also create a spectral library automatically with the apex spectra from named peaks in a chromatogram using the Add Apexes command.

There are three steps involved in creating a new library.

1. Select **Tools > Build Library > Create**.

   The New Library Dialog appears and from this dialog you specify a name for the library as well as select a location where the library will be saved.

2. Once you have specified a library name and location where the library will be saved, click Save.

   The Create Library dialog appears. This dialog is used to specify the Library Minimum and Maximum Wavelengths.

3. From the Create Library dialog make sure that the Library **Minimum** and **Maximum Wavelengths** values cover the range of the spectra collected.

   For example, specify a range of 190-400 for UV only data, or specify 400-700 for visible only data, or 190-700 for UV and visible data.

   Once you have specified the Library Minimum and Maximum Wavelengths the Edit Library dialog appears. At this point, the new library has been created, although it does not contain spectra. Spectra may be added to the library at any time. Therefore, you may exit at this point, and add the spectra later, or you may continue and add any displayed spectra that you want to include in the library.

The following procedures describe how to create a new library.

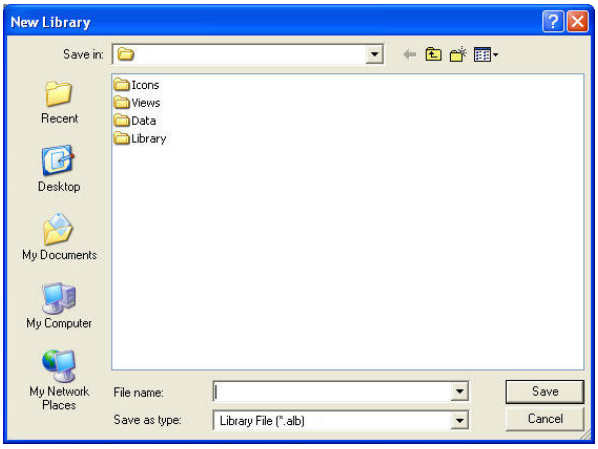
The following procedure describes how to create a new library. *For information on adding, deleting, or replacing spectra in the library as well as how to browse the library once spectra have been added, refer to the topic titled Edit Library.*

How do I create a new library?

To create a new library:

1. From the Tools menu select **Build Library > Create**.

   The New Library dialog appears.

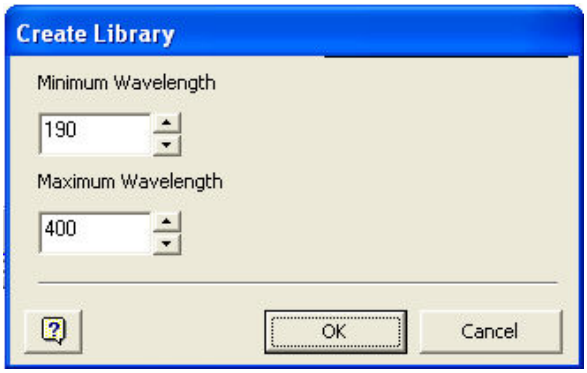


2. From the **Save in** drop down list select the folder where you wish to save the Library you are creating.

3. Enter a name for the Library in the **Filename** field.

4. Click **Save**.

   The Create Library dialog appears.



5. From the Create Library dialog, use either the default values for the Library **Minimum** and **MaximumWavelengths**.

   OR

   If you are working in a different range, enter new Minimum and Maximum Wavelength values that cover the range of spectra collected.

   For example, specify a range of 190-400 for UV only data, or specify 400-700 for visible only data, or 190-700 for UV and visible data.

6. Click **OK** to close the Create Library dialog.

   At this point, the new library has been created, although it contains no spectra. Spectra may be added to the library at any time.

   Refer to the topic titled Edit Library for information on using the Edit Library dialog.

Viewing a Library

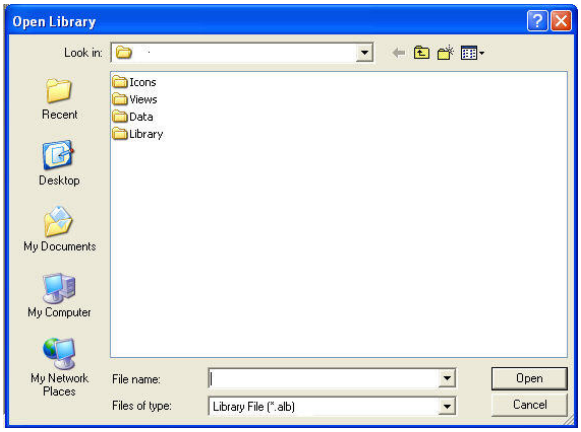
The **View Library** dialog allows you to view information about a selected library, such as the compounds in the library and their spectra.

How do I access the View Library dialog?

To access the view Library dialog:

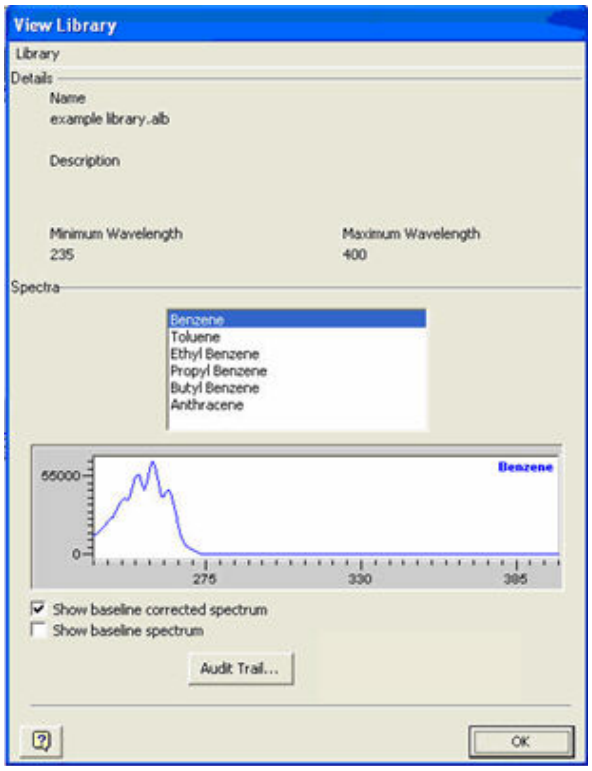
1. From the **Tools** menu select **View Library**.

   The Open Library dialog appears.



2. Select the Library you wish to view and click **Open**.

   The View Library dialog appears.



Tell me about the View Library dialog.

The View Library dialog displays the following information:

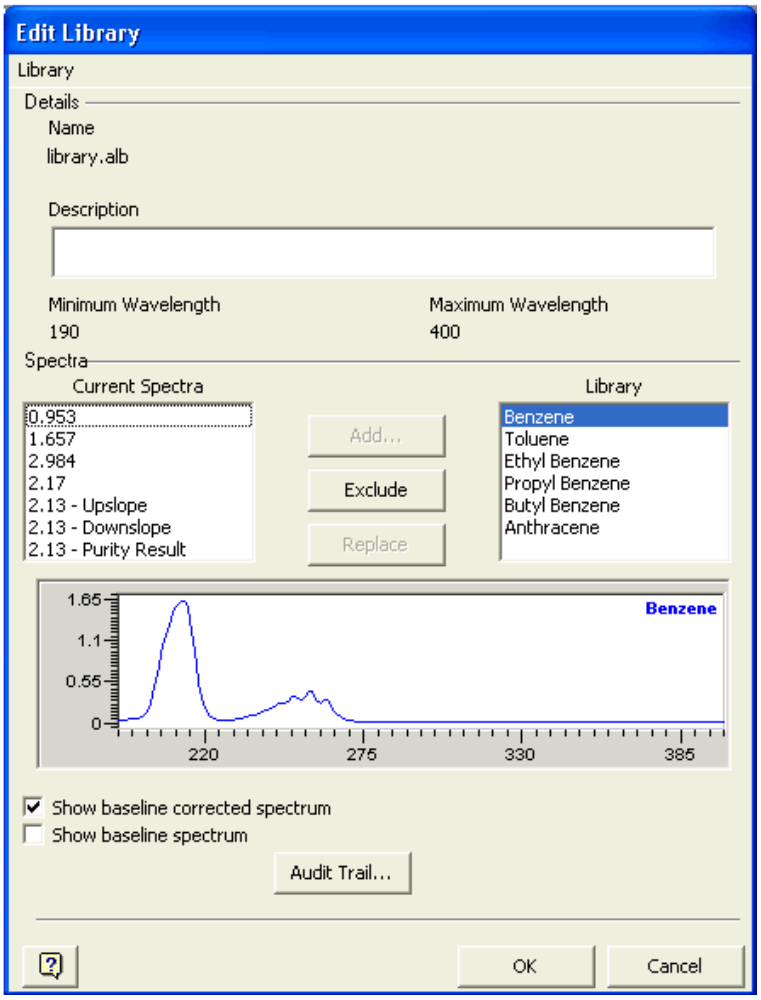
Control	Description
Name	The name of the library file you opened appears here.
Description	A description of the library appears here. ***NOTE:*** *You can specify a description for the library when you are editing/creating a library and the Edit Library dialog is displayed. For more information on entering a description for the library, see Editing a Library.*
Minimum Wavelength	The library's minimum wavelength value appears here.
Maximum Wavelength	The library's maximum wavelength value appears here.
Spectra List	The Spectra List shows all the spectra currently in the Library by component name. You can click on a spectrum from this list to have the selected spectrum displayed the graph below. If you right mouse click, on a select spectrum and select **Properties**, a dialog appears that displays the source, the chromatogram and retention, the baseline, and who added the spectrum and when.
Baseline corrected	This check box allows you to select whether or not the spectra displayed on this dialog have been baseline corrected. ***NOTE:*** *This checkbox only determines what is displayed on the View Library dialog; it does not determine whether or not baseline corrected spectra are used in library operations.*
Show Baseline	This checkbox allows you to select whether or not the baseline spectrum is also displayed on the graph.

Editing a Library

The Edit Library dialog is used to add, replace, or delete spectra; in addition, this dialog can be used to browse through the spectra in a library. The Edit Library dialog automatically appears after you have created a new library. However, this dialog can also be accessed by selecting **Tools > Build Library > Edit...**, and then selecting the library you wish to edit from the Library File Selector dialog box.

Tell me about the Edit Library Dialog.

Click here to see a screen shot of the Edit Library dialog.



The Edit Library dialog consists of the following controls:

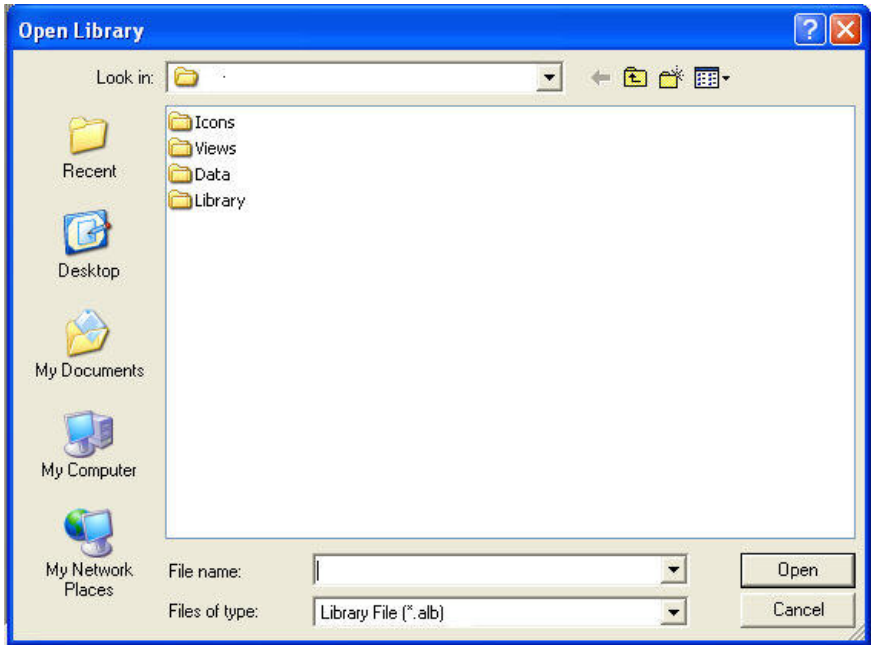
Control	Description
Name	The file name of the library appears here.
Description	You can enter or edit a description for the library in this text box. The description you enter here will help you identify the library later on when you are performing Operations that require you to select a list of libraries to be searched.

Minimum Wavelength	The minimum wavelength of the spectra in the library is displayed here.
Maximum Wavelength	The maximum wavelength of the spectra in the library is displayed here.
Current spectra	This list box shows all the spectra currently listed on the Data Tree, branched by chromatogram. • You can select a spectrum from this list and then use the **Add** command to add the Spectra to a Library. When you add a selected spectrum you are prompted to enter the component name of the spectra before it is added to the library. **OR** • You can select a spectrum from this list that you wish to use to replace a Library component. To replace a component you must first select the component from the Library list box, then select the replacement spectrum from the Current Spectra list box, and finally click the **Replace** button.
Add	This command button allows you to add a spectrum that is currently selected on the Current Spectra list to the Library. When you select a spectrum from the **Current Spectra** list box and click **Add**, the Add to Library dialog box appears. From this dialog box you enter the component name for the spectrum. ***NOTE:*** *Make sure that you enter the component name correctly, on the Add to Library dialog box, before you click OK. The component name cannot be edited once it has been entered into the library.*
Exclude	This command button is enabled when a spectrum, that has not already been excluded, is selected from the Library list box. T**his command permanently excludes a selected spectrum, so it is not used for operations.** When a spectrum is excluded it remains in the Library list box and you can still click on the spectrum and view a graph of the selected spectrum. However, when an excluded spectrum is selected the Add, Exclude, and Replace command buttons are disabled.
Replace	This command button deletes the spectrum that is selected on the **Library list** box and replaces it with the spectrum that is currently selected in the **Current Spectra** list box. This command is only enabled when a spectrum is selected from both lists.
Library	This list box shows all the spectra currently listed in the library, alphabetically by component name. You can select a spectrum from this list to view a graphical display of the spectrum in the pane directly below the Current Spectra and Library list boxes. • You can view additional information about a component by right mouse clicking on a **Library spectrum** and selecting **Properties** from the context menu that appears. A dialog appears that shows the source, the chromatogram and retention, the baseline, and who added the spectrum and when.
Baseline corrected	This check box allows you to select whether or not the spectra displayed on this dialog have been baseline corrected. ***NOTE:*** *This checkbox only determines what is displayed on the Edit Library dialog; it does not determine whether or not baseline corrected spectra are used in library operations.*
Show Baseline	This checkbox allows you to select whether or not the baseline spectrum is also displayed on the graph.

How do I access the Edit Library dialog?

To access the Edit Library dialog:

1. From the Tools menu select **Build Library > Edit**.
   The Open Library dialog appears.

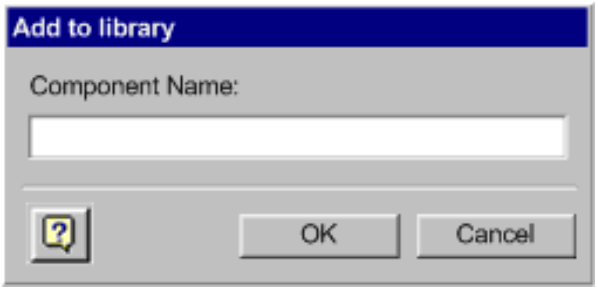


2. Select the Library you wish to view and click **Open**.
   The Edit Library dialog appears.

How do I add extracted spectra into a library?

1. From the Edit Library dialog select the spectrum you want to add from the **Current Spectra** list.

2. Click the **Add** button.
   The Add to Library dialog box appears and prompts you to enter a **Component Name**.



3. Enter the name of the component carefully.

NOTE: *Make sure that you enter the component name correctly before you click OK. The component name cannot be edited once it has been entered into the library.*

4. Click **OK**.

   The dialog closes and you are returned to the Edit Library dialog, from where the newly added spectrum is displayed in the Library list.

How do I exclude a spectrum in the Library List?

NOTE: *The exclude command permanently excludes a selected spectrum; you cannot undo this action.*

To permanently exclude a library spectrum:

1. Select the spectrum you wish to exclude from the **LibraryList**.

   The Exclude button is enabled.

2. Click **Exclude**.

   A dialog appears that prompts you to select whether or not you want to permanently exclude the selected spectrum.

3. Click **Yes** to permanently exclude the spectrum.

   The spectrum still appears in the Library List; however, if you click on the spectrum the Add, Exclude, and Replace buttons are disabled, and the Spectrum Display shows that the selected spectrum is excluded.

How do I replace a spectrum in the Library List with one of the Current Spectra?

The **Replace** command button deletes the spectrum you have selected from the Library list box and replaces it with the spectrum you select from the Current spectra list box.

To replace a Library spectrum with a spectrum listed in the Current Spectra list box:

1. Click on the spectrum in the **Library List** that you want to delete.

2. Click on a replacement spectrum in the **Current Spectra** list.
   The Replace button is enabled.

3. Click **Replace**.

   A dialog appears that informs you that the spectrum selected on the Library list box will be replaced with the spectrum selected on the Current Spectra list box.

4. Click **Yes**.

   The Library List spectrum is replaced with the spectrum selected on the Current Spectra list.

Library Match

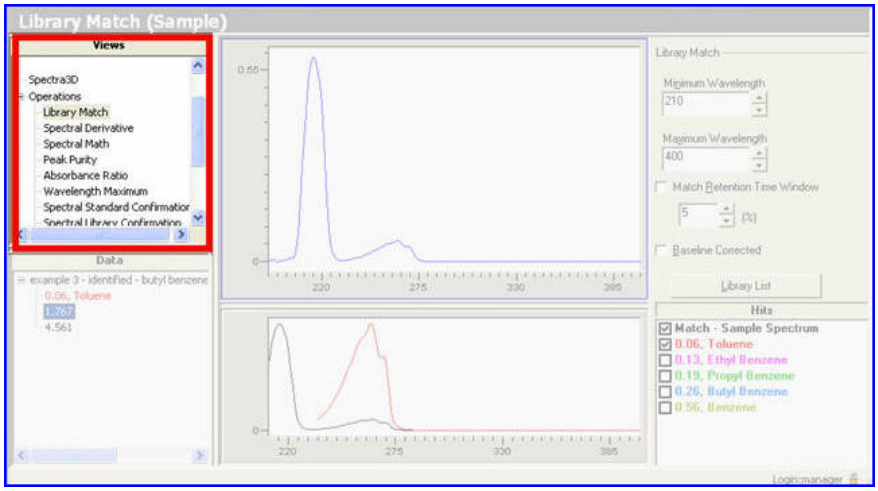
You use the **Library Match** operation to match a selected spectrum against the spectra in a list of libraries. The results of this match allow you to identify the sample spectrum. You can access the Library Match Operation by selecting **Operations > Library Match** from the Views tree.

NOTE: *Prior to accessing the Library Match operation, the spectrum you wish to identify must already be extracted and displayed on the Data Tree.*

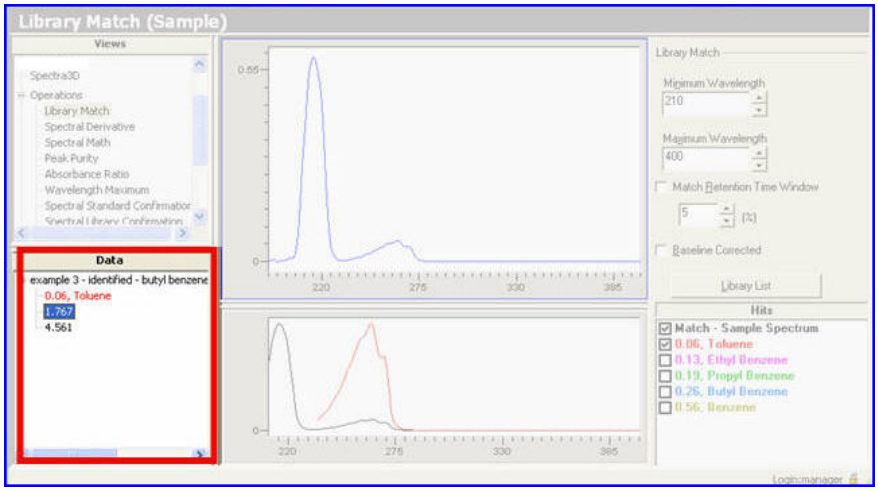
Tell me about the Library Match view.

The Library Match operation is comprised of the following areas:

- **Views Tree** - From the Views tree you select **Operations > Library Match** to access the Library Match operation.

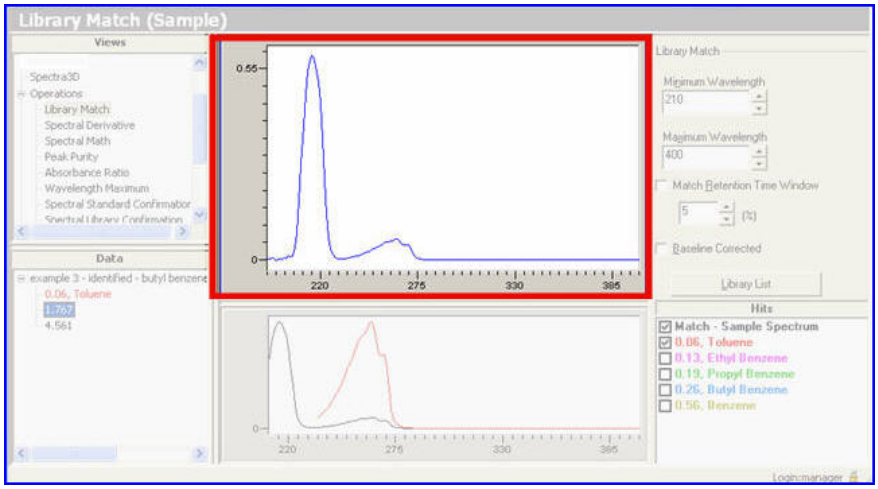


- **Data Tree** - The Data Tree displays a list of extracted spectra that are grouped by their parent chromatogram. From the Data Tree you select the spectrum you wish to identify. When you click on a spectrum listed in this tree, a graphical representation of the spectrum appears in the Spectra pane. The spectrum selected on the Data Tree is highlighted in blue.

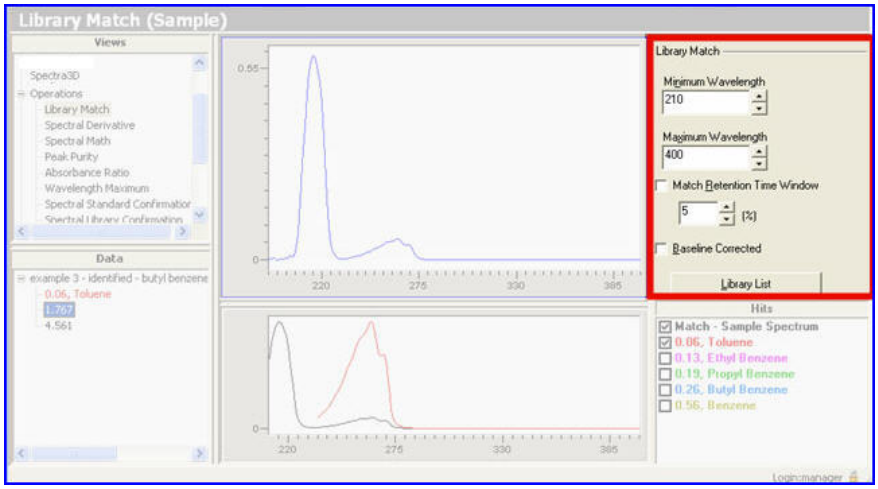


NOTE: *Whenever you select a spectrum from the Data Tree, the software automatically runs a search, using the parameters specified on this page, and updates the Hits List and Hits Pane.*

- **Spectrum Pane -** This pane displays the spectrum that you selected from the Data Tree.

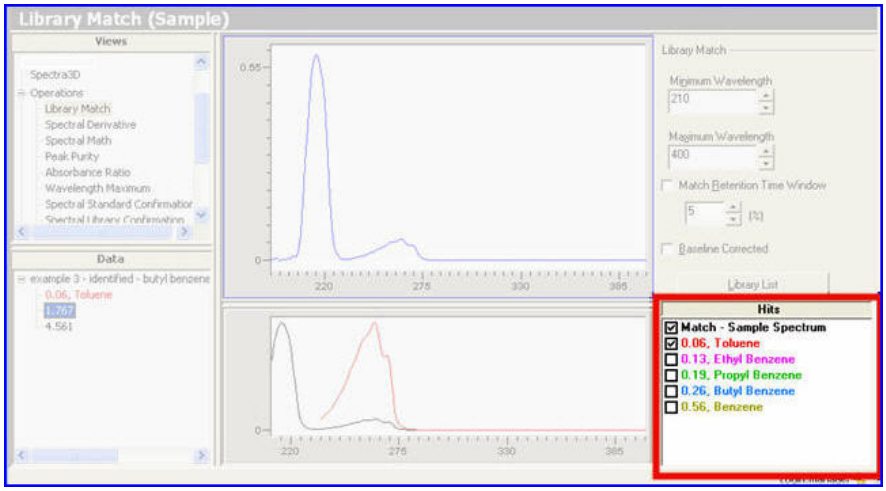


- **Parameters** - The Parameters pane is where you set and investigate the various parameters (including the list of libraries that will be searched) that control the matching process. Whenever you change a parameter displayed on this page, and have a spectrum selected on the Data Tree, the software automatically re-runs a search and updates the Hits List and Hits pane.



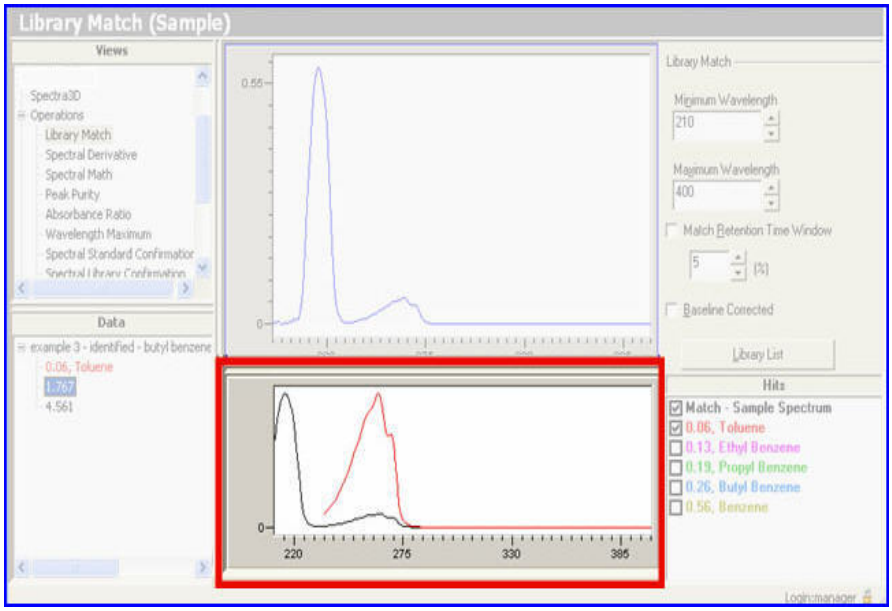
- **Hits List** - The Hits List contains a list of the spectra from the library(s) that best match the sample spectrum, in hit order. These matching spectra are listed by their "hit quality" and name. The hit quality is displayed as a numeric value; a hit quality of 0 indicates a perfect match.

  The spectra that appear checked on this list are displayed in the Hits Pane. Therefore, it should also be noted that the first spectrum that is always listed on the Hits List is the Sample Spectrum. You can check the Sample Spectrum, listed here, to compare it to matching spectra that are also checked on this list.



NOTE: *Spectra that appear checked on this list can be added to the Data Tree by selecting* ***Actions > Add All to View****.*

- **Hits Pane** - The Hits pane provides a graphical display of the spectra that are checked on the Hits List. From this pane you can compare the sample spectrum with selected Hits; the spectra that appear on this graph are color coded to match the Hits List.



How do I identify a spectrum by matching it to a spectral library or libraries?

To match a spectrum to one or multiple libraries:

NOTE: *Before you perform the Library Match Operation, the spectrum you wish to identify must already be extracted from a chromatogram and displayed on the Data Tree. From the Views Tree, select Operations> Library Match.*

If you have already selected a spectrum on the Data Tree, and a list of libraries has been built, prior to you selecting the Library Match view, then, as soon as the Library Match view is displayed, Chromera matches the selected spectrum and displays the results on the page.

OR
If you have not already selected a list of libraries to search, the Libraries List dialog appears.

1. From the Libraries List dialog select the libraries you wish to search.

NOTE: *From the Library Match page you can open the Libraries List dialog by clicking on the Library list button.*

2. Once you have selected the libraries you wish to search, click **OK** to close the Libraries List dialog and return to the Library Match page.

3. Select the spectrum in the Data Tree that you want to identify.

   The match is immediately performed and the results are automatically displayed on the Hits List.

   The original, selected spectrum appears first in the Hits List. The subsequent hits are displayed in the order of the best match. Each hit is listed first by its Hit Quality value, the numeric value representing how close a match the library spectrum is to the currently selected sample spectrum, and then by its library component name.

NOTE: *The Hit Quality is a measure of the similarity of the sample spectrum and the library spectrum. Lower numbers are better matches; 0 means a perfect match.*

4. If you want to compare the sample spectrum to specific matches, check the **Sample Spectrum** check box and check any additional spectra listed on the Hits List that you want displayed on the Hits Pane.

NOTE: *At any time you, can select a different spectrum from the Data Tree that you want to identify.*

To modify the wavelength range used to perform the Library Match:

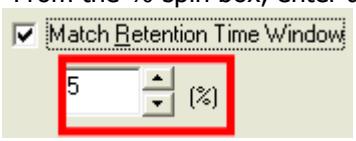
- Enter a new **Minimum Wavelength** and **Maximum Wavelength** in the corresponding spin boxes.

To restrict the search to spectra from peaks with similar retention times:

1. Check the **Match retention time** check box.

   A % spin box is enabled.

2. From the % spin box, enter a percentage window for the retention time match.



To examine the effect of using baseline corrected spectra on the Library Match:

- Check the **Baseline corrected** check box to use baseline corrected spectra for the match.

How do I add spectra from the Hit List to the Data Tree?

1. Check the items on the **Hits List** that you wish to add to the Data Tree.

2. Select **Actions > Add All to View**.

   A dialogue box is displayed with the message "The data was added successfully". The selected spectra now appear in the Data Tree and are labelled with the Hit Quality and component name.

How do I build or modify the list of libraries to be searched?

NOTE: *The list you build is remembered by the system, and will be used on subsequent searches, unless you modify the list of selected libraries to be searched.*

1. From the Library Match view click on the **Library List...** button.

   The Libraries List dialog appears.

2. Specify the directory path where the libraries you wish to search are located by typing the directory path in the **Look in** text box, or by clicking on the **Browse...** button and selecting a directory path from the Browse for Directory dialog box.

   Once you have specified a directory path, a list of libraries available for selection appears on the Libraries list dialog.

3. From the **Available libraries list**, click on the libraries you wish to search and then click on the **Add** button.

You can either click on a single library to add it to the Libraries list, or you can select multiple libraries to add to the Libraries List by using CTRL+click or SHIFT+click.

The selected libraries now appear in the Libraries List.

4. To remove a library from the **Libraries list** click on the library and then click **Delete**.

   The selected library is removed from the Libraries list and reappears in the Available Libraries list.

5. You can view a description of a library by clicking on the library, from either list. The **Description** of the selected library appears below the list boxes.

6. Click **OK**.

   You are returned to the Library Match view, and the results your search are displayed in the Hits pane.

How do I specify search parameters?

The Parameters Pane on the Library Match page contains the following parameters, which you can modify so that the software re-runs the match and updates the Hits List and Hits Pane:

Control	**Description**
Minimum Wavelength	This spin box is where you set the minimum wavelength to be used for the match.
Maximum Wavelength	This spin box is where you set the maximum wavelength to be used for the match.
Match retention time window	Check this field to select the retention time of the spectrum as one of the search criteria. When this field is checked a % spin box appears from where you specify the search window, as ± percent of retention time
Baseline corrected	Check this field so that the sample spectrum and the spectra in the libraries being searched are used with baseline correction. If this field is not checked, baseline corrected spectra will not be used for the library match. ***NOTE:*** *Checking this option affects the results of the operation; however, this option does not change the sample spectrum displayed in the top pane. To baseline correct the sample spectrum, displayed in the top pane, select* ***View > Baseline Corrected Spectra****.*
Library List	Click on this command button to display the Library List dialog from where you select the libraries that will be searched.

NOTE: *If* ***Match retention time window*** *is checked, the search first creates a list of all spectra in the library that fall within the window; it then does a spectrum-by-spectrum comparison. If Match retention time window is not checked, the comparison is made with all spectra in the library.*

Spectral Operations

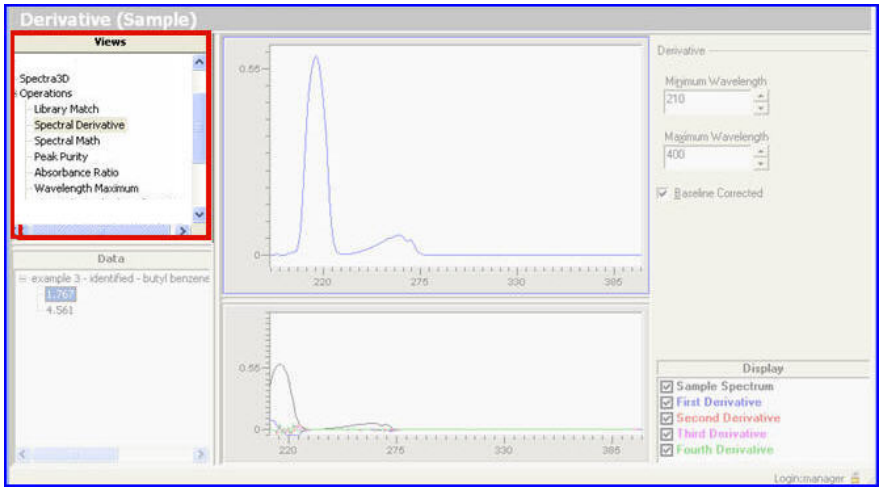
Spectral Derivative

The Spectral Derivative operation allows you to you calculate the first, second, third, or forth derivative for a selected spectrum. You can access this operation from the **Views** tree by selecting **Operations > Spectral Derivative.** In order to calculate spectral derivatives you must select a spectrum from the Data Tree.

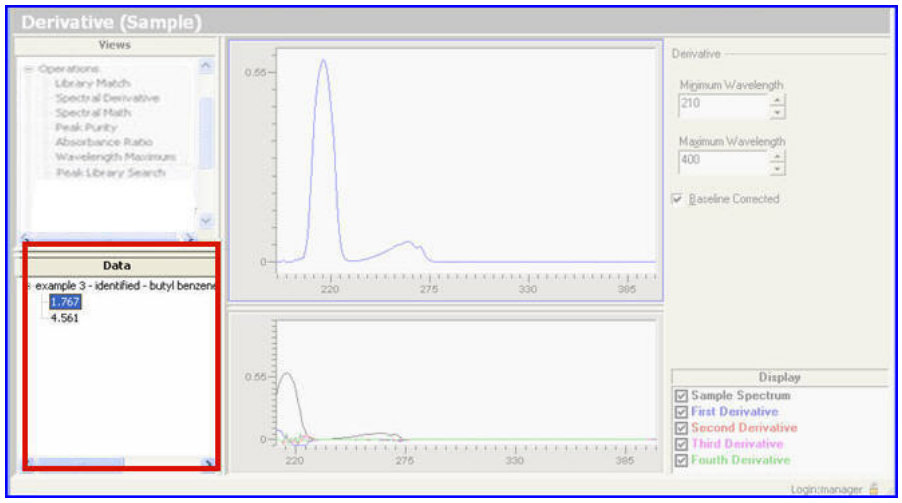
Tell me about the Spectral Derivative view.

The Spectral Derivatives view consists of the following areas:

- **Views Tree -** This pane provides you access to the Spectral Derivatives view.

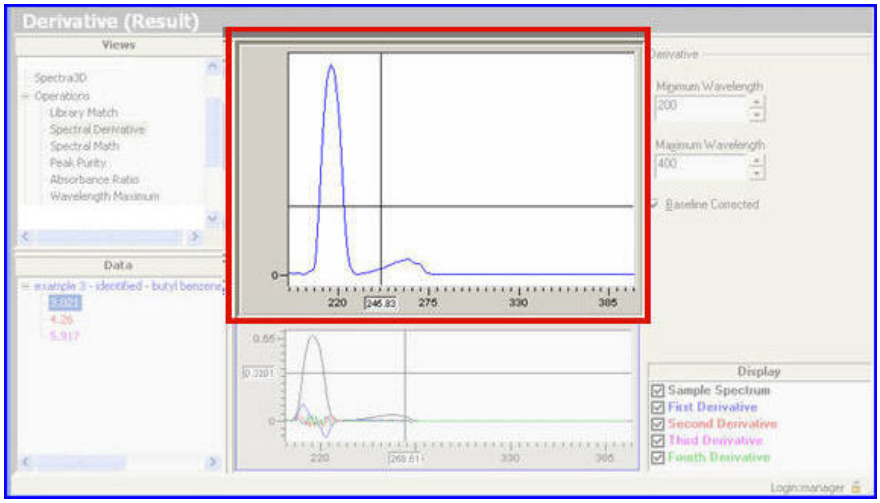


- **Data Tree -** The Data Tree displays a list of extracted spectra that are grouped by their parent chromatogram. From the Data Tree, you select a spectrum for calculating spectral derivatives. Only one spectrum can be selected at a time; and the selected spectrum is displayed in the Spectrum Pane. As different spectra are selected the software re-calculates the derivatives and updates the Derivatives graph.

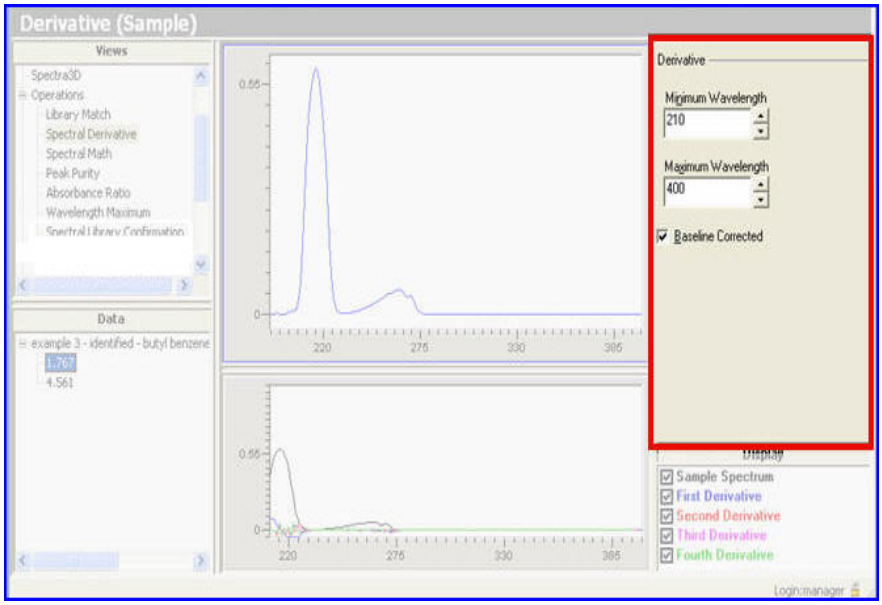


NOTE: *If you will be performing the Spectral Derivative operation on spectra extracted from a chromatogram, the extracted spectra must be displayed on the Data Tree prior to accessing the Spectral Derivative view.*

- **Spectrum Pane** - This pane displays the original sample spectrum that you have selected from the Data Tree.



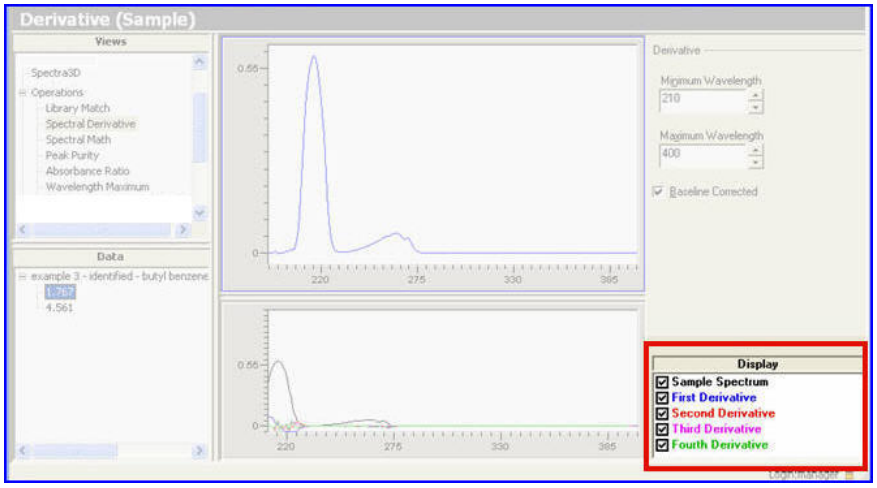
- **Parameters** - The Parameters Pane allows you to set the **minimum** and **maximum wavelengths** used for calculating spectral derivatives as well as select whether or not you wish to use the **baseline corrected spectrum** for the derivative.



- **Display List -** You use the Display List to select which spectra you want to see on the Derivatives Pane. Items that appear checked are displayed on the Derivatives Pane, while unchecked items are not displayed.

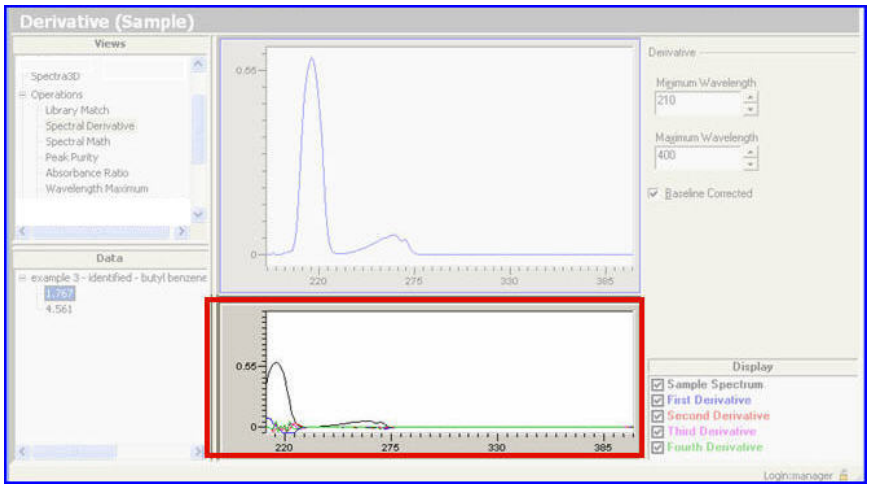
  You can select to display the **Sample Spectrum**, **First Derivative**, **Second Derivative**, **Third Derivative**, and/or the **Fourth Derivative**. When you select or

deselect any one of the check boxes in the Display list, the Derivatives Pane is automatically updated.



NOTE: *The check box options from the Display List and the curves on the Derivatives Pane have matching color coding.*

- **Derivatives Pane** - This pane provides a graphical display of the derivative spectra that appear checked on the Display List. It should also be noted that whenever you select a new sample spectrum, change the parameters for the calculation, or select/deselect an item on the Display List, the Derivatives Pane is automatically updated.



How do I calculate spectral derivatives?

To calculate spectral derivatives:

NOTE: *If you want to calculate spectral derivatives on a spectrum extracted from a chromatogram, the spectrum must be extracted and displayed on the Data Tree prior to accessing the Spectral Derivative operation.*

1. From the Views Tree select **Operations > Spectral Derivative**.

   The Spectral Derivatives view is displayed.

2. Click on a spectrum listed on the Data Tree.

   The selected sample spectrum is displayed in the Spectrum Pane. The spectral derivatives are automatically calculated using the Parameters specified on this page. The Derivatives Pane displays the spectral derivatives that appear checked on the Display List.

3. If you want to change the parameters used to calculate spectral derivatives for the sample spectrum, you may do so by modifying the following parameters:

   - Select the lower limit of the spectrum range to be included in the calculation from the **Minimum Wavelength** spin box.

   - Select the upper limit of the spectrum range to be included in the calculation from the **Maximum Wavelength** spin box.

   - Check the **Baseline corrected** check box to use the baseline corrected spectrum for the derivative.

4. From the **Display List**, check the spectral derives you wish to be displayed on the Derivatives Graph. You can select to display the **Sample spectrum**, the **First Derivative**, **Second Derivative**, **Third Derivative**, and **Fourth Derivative**.

   The Derivatives Graph updates to display what is checked on the Display List.

How do I add result spectrum to the Data Tree?

- From the menu bar select **Actions > Add All to View** to add all the derivative spectra that appear checked in the Display List to the Data Tree.

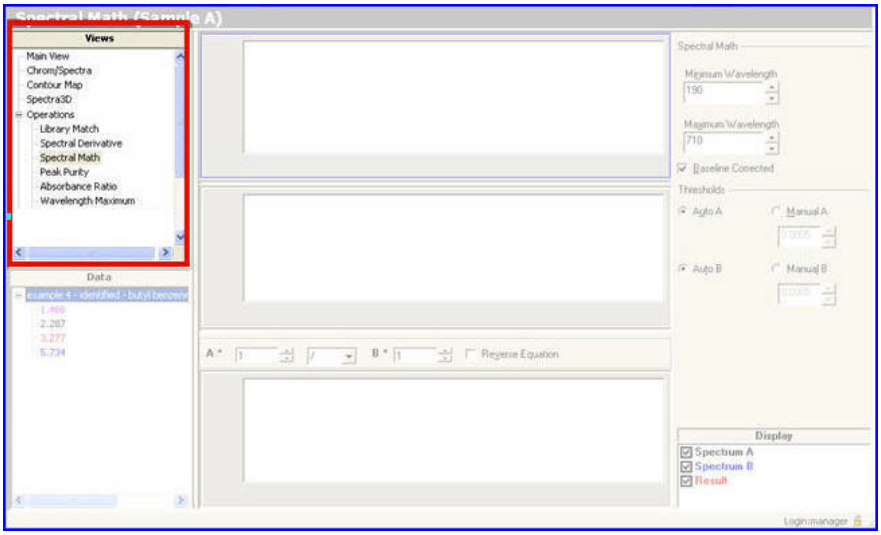
Spectral Math

The Spectral Math operation allows you to perform mathematical operations (add, subtract, and divide) on spectra and view the results. On the Spectral Math view, you select two spectrums that you wish to add, subtract, or divide, as well as specify the wavelength range of the operation, and the Absorbance Threshold if necessary. The results of the calculation are displayed in the lower portion of this screen, and can be added to the Data Tree using the Add to View command.

Tell me about the Spectral Math view.

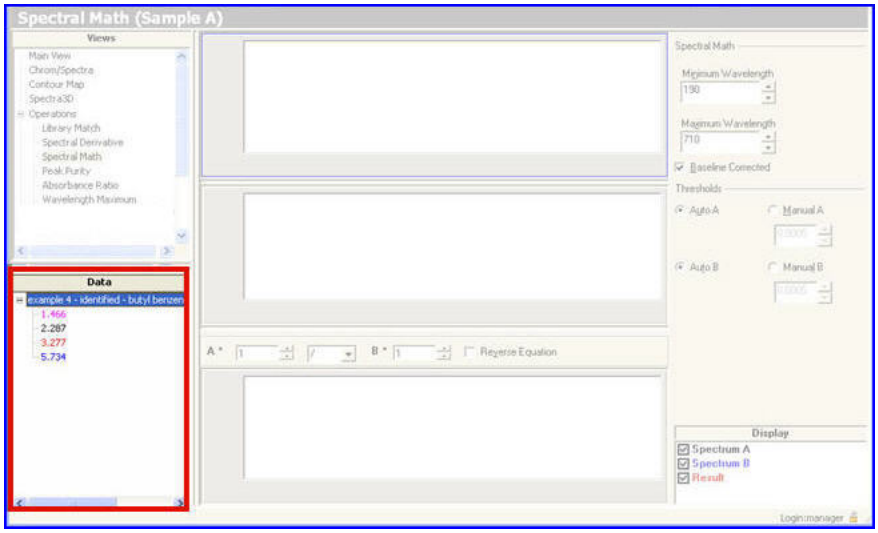
The Spectral Math view consists of the following areas:

- **Views Tree -** This pane provides you access to the Spectral Math operation. You can access the Spectral Math view by selecting **Operations > Spectral Math** from the Views Tree.



- **Data Tree**: The Data Tree displays a list of extracted spectra that are grouped by their parent chromatogram. From the Data Tree you select spectra that will be used to perform mathematical operations on this page. You must select two spectra from the Data Tree in order to perform operations on this view.

NOTE: *If you will be performing the Spectral Math operation on spectra extracted from a chromatogram, the extracted spectra must be displayed on the Data Tree prior to accessing the Spectral Math view.*

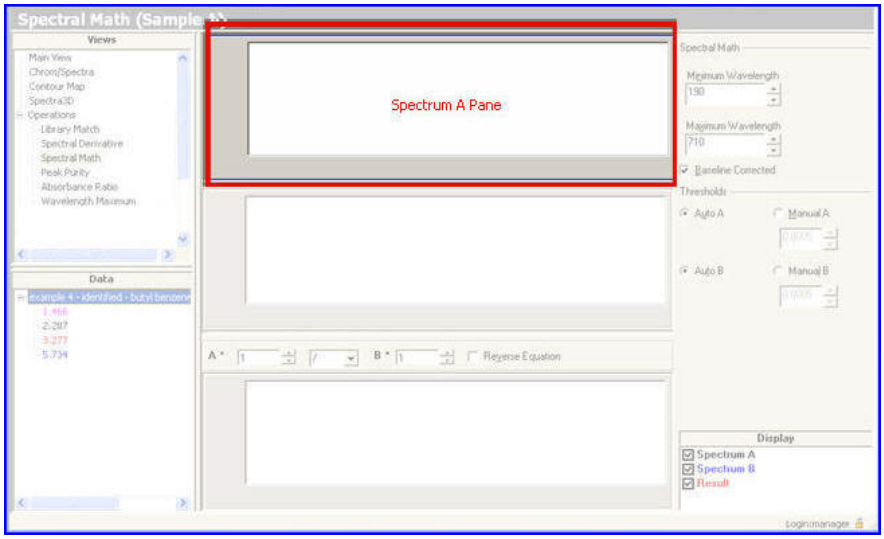


NOTE: *The Spectrum Panes remain blank until a spectrum is selected for the pane, and the Result pane is blank until both spectrum have been selected.*

- **Spectrum A Pane** - This pane displays the spectrum you selected on the Data Tree to represent Spectrum A for mathematical operations performed on this page.

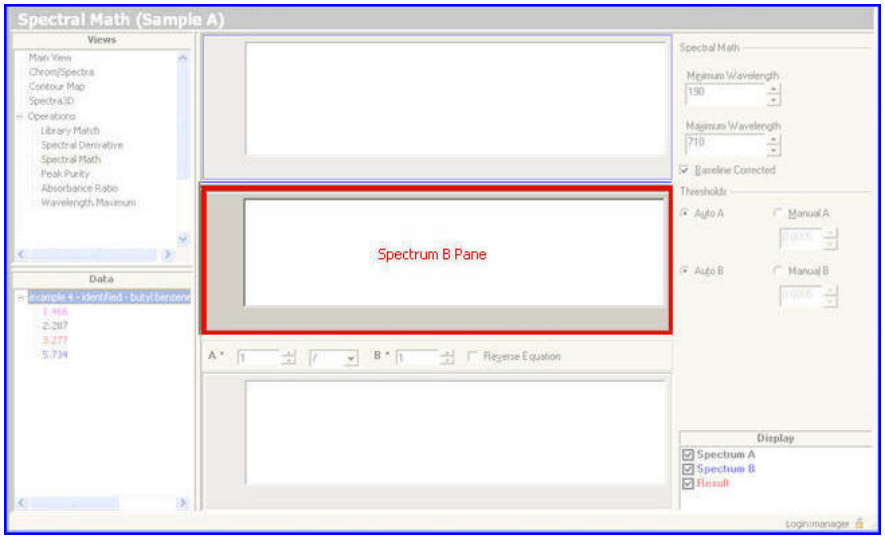
  If you do not have a spectrum already selected on the Data Tree, when you first access the Spectral Math page, the Spectrum A Pane appears blank. To display a spectrum in this pane, you must click on the Spectrum A Pane, and then select a spectrum that is listed on the Data Tree.

  On the other hand, if a spectrum is already selected on the Data Tree, before you access this page, the selected spectrum is automatically displayed in this pane. If the spectrum currently displayed on this pane is not the spectrum you wish to use for the operation, simply click on an area inside the white space of the pane, and then select a different spectrum from the Data Tree. The new spectrum is now displayed on the Spectrum A Pane.

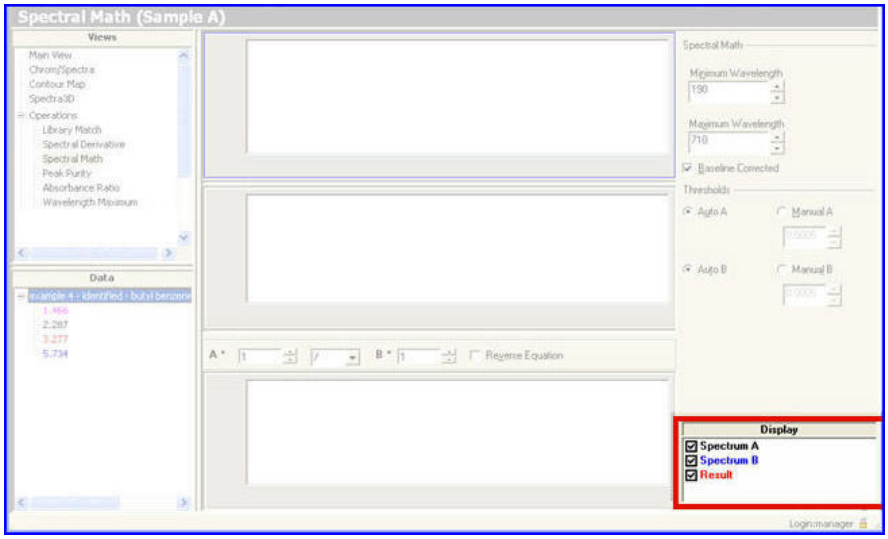


- **Spectrum B Pane** - This pane displays the spectrum you selected on the Data Tree to represent Spectrum B for mathematical operations performed on this page.

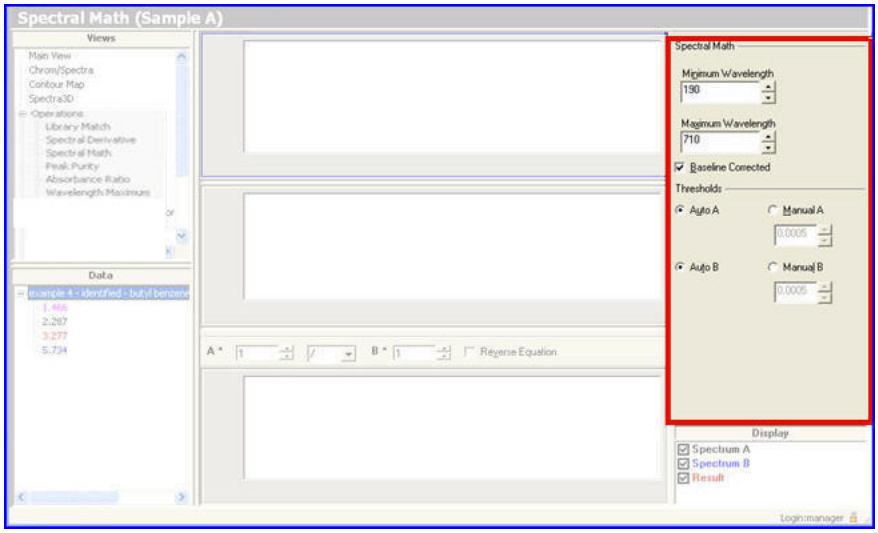
When you first access the Spectral Math page this pane appears blank. To add a spectrum to this pane you must first click on an area inside the whites pace of the pane, and then click on a spectrum listed on the Data Tree. The selected spectrum now appears on this pane.



- **Display List** - You use the Display List to select the information you want displayed on the Results Pane. When items appear checked on this list, they are displayed in the Results Pane. Meanwhile, unchecked items are not displayed.



- **Parameters Pane -** The Parameters pane displays the wavelength range for the calculation, whether or not baseline corrected spectra are used for the spectral math, as well as the Threshold controls. You can modify these parameters and the Results pane automatically updates.



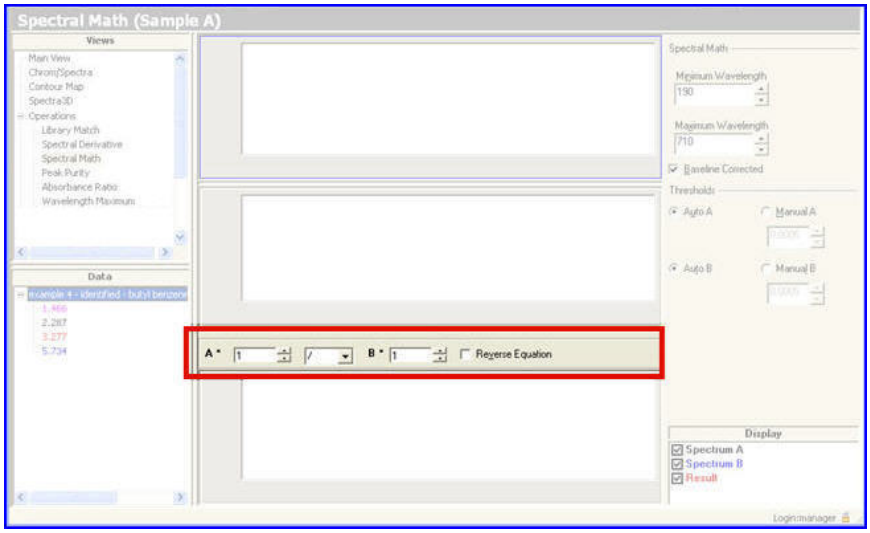
More specifically, the Parameters Pane allows you to specify the following parameters:

- **Minimum Wavelength** - Sets the lower limit of the spectrum range to be included in a calculation.
- **Maximum Wavelength** - Lets you set the upper limit of the spectrum range to be included in the calculation.
- **Baseline Corrected** - Check this option to use baseline corrected spectra for the spectral math.

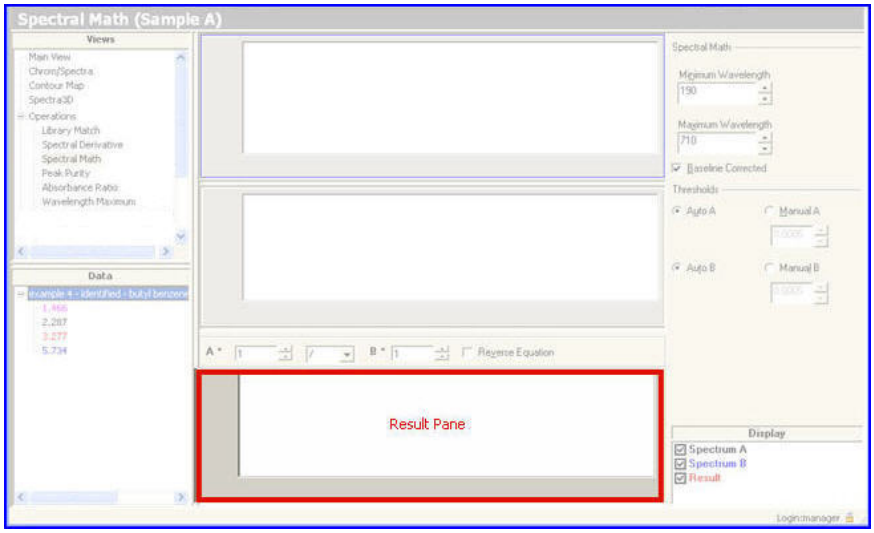
NOTE: *Checking this option affects the results of the operation; however, this option does not change the sample spectrum displayed in the top pane. To baseline correct the sample spectrum, displayed in the top pane, select* ***View > Baseline Corrected Spectra****.*

- **Auto A** - Select this option to automatically calculate the minimum absorbance value that can be used in the calculation for Spectrum A. If the value at a specific wavelength falls below this threshold, the calculated result for that wavelength will be set to 0.
- **Auto B** - Select this option to automatically calculate the minimum absorbance value that can be used in the calculation for Spectrum B.
- **Manual A** - Select this option to manually set the minimum absorbance value for Spectrum A.
- **Manual B** - Select this option to manually set the minimum absorbance value for Spectrum B.

Calculation Parameters - This area is where you define the mathematical operation to be performed (add, subtract, or divide) as well as specify multiplication factors for Spectrum A and B, and whether or not you wish to switch Spectrum A and B and their multiplication factors, in the function.



- **Result Pane -** This pane displays a graphical representation of the items that are checked on the Display List. You can view Spectrum A, Spectrum B, and the Result Spectrum on this pane.



How do I select spectra for performing mathematical operations?

You must have two spectrums selected in order to perform mathematical operations on spectra. Once you select two spectrum the software automatically calculates and displays the result of the mathematical operation that is currently defined on this page. You can modify any of the parameters on this page to recalculate and display a different result.

To select spectra:

1. From the Spectral Math page click on the top Spectrum Pane, this is the spectrum pane that represents Spectrum A for any mathematical operations you perform on this page.

   A blue border appears around the pane to indicate that the pane is selected.

2. From the **Data Tree** click on the spectrum that you wish to use as Spectrum A for performing mathematical operations.

   The selected spectrum appears in the top Spectrum pane and will be considered Spectrum A in the mathematical operations you perform on this page.

   You must now select a second spectrum to be used in the calculation, this spectrum will be considered Spectrum B in the mathematical operations you perform on this page.

3. To select the second spectrum click on the lower Spectrum pane (Spectrum B), this pane is below the Spectrum A pane.

   A blue border appears around the pane to indicate that the pane is selected.

4. From the **Data Tree** click on the spectrum that you wish to use as Spectrum B for performing mathematical operations on this page.

   The selected spectrum appears in the Spectrum B pane; and the program automatically calculates and displays the result of the currently defined mathematical operation, using the two selected spectrum and the parameters defined on this page. The results of the currently defined operation are displayed on the Result Pane of this page.

   You can change any of the parameters used in the calculation, as well as modify the calculation you wish to perform; and Chromera will automatically recalculate the result and display it in the Result Pane.

How do I perform mathematical operations on spectra?

The summary below illustrates how to use the Spectral Math page to perform mathematical operations on any two spectra that are listed on the Data Tree. Please note that if you are working with spectra extracted from chromatograms, the two spectra must be present in the Data Tree before you access the Spectral Math page. Once you have selected the two spectra for the operation, Chromera automatically calculates and displays the result of the operation using the parameters currently displayed on the page. You can modify any of the parameters on this page and the new result is automatically displayed.

To select spectra:

1. If you are working with spectra extracted from chromatograms, the two spectra must be present in the Data Tree prior to displaying the Spectral Math view.

2. From the **Views** tree, expand the **Operations** node and click on **Spectral Math**.

   The Spectral Math view displays.

3. From the Spectral Math view, click anywhere inside the white space of the top Spectrum pane, this is the spectrum pane that represents Spectrum A for any mathematical operations you perform on this view.

   A blue border appears around the pane to indicate that the pane is selected.

4. From the **Data Tree**, click on the spectrum that you wish to use as **Spectrum A** for performing mathematical operations.

   The selected spectrum appears in the top Spectrum pane and will be considered Spectrum A in the mathematical operations you perform on this view.

You must now select a second spectrum to be used in the calculation, this spectrum will be considered **Spectrum B** in the mathematical operations you perform on this page.

5. To select the second spectrum click anywhere inside the white space of the lower Spectrum pane (Spectrum B), this pane is below the Spectrum A pane.
   A blue border appears around the pane to indicate that the pane is selected.

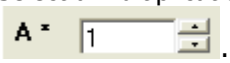
6. From the **Data Tree** click on the spectrum that you wish to use as Spectrum B for performing mathematical operations on this page.
   The selected spectrum appears in the Spectrum B pane; and Chromera automatically calculates and displays the result of the currently defined mathematical operation, using the two selected spectra and the parameters currently displayed on this page.

NOTE: *You can change any of the parameters used in the calculation, as well as modify the calculation you wish to perform; and Chromera will automatically recalculate the result and display it in the Results Pane.*

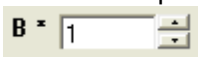
To perform a mathematical operation on two spectra using a different set of parameters:

Once you have selected Spectrum A and Spectrum B, Chromera automatically performs the calculation, using the current parameter values displayed on your screen. You can then modify any of the parameters displayed on the page; for example, you can specify new multiplication factors for Spectrum A and B, or select a different mathematical operation to perform, and with each parameter you modify, the results are automatically recalculated and displayed on the page.

To select new multiplication factors for Spectrum A and B:

1. Select a multiplication factor for Spectrum A using the spin box next to **A***
   

   .

2. Select a multiplication factor for Spectrum B using the spin box next to **B***
   

   .

To select a different operator:

- From the drop down list, located between A* and B* select an operator  for the calculation.
  You can select to add spectra, subtract spectra, or divide spectra.

To reverse the order of a Division or Subtraction operation:

- Check the **Reverse Equation** check box to switch Spectrum A and B and their multiplication factors in the function. For example, a function that read A*1-B*2, becomes B*2-A*1 when Reverse equation is checked. This option is only enabled when the operator is / or -.

How do I add the Result Spectrum to the Data Tree?

- From the menu bar select **Actions > Add all to View**.
  The Result Spectrum is added to the Data Tree.

Chromatogram Operations

Performing Operations on Chromatograms

There are a number of built-in functions that allow you to obtain important information about your chromatograms. These functions include Peak Purity (which checks the homogeneity of each peak in the chromatogram), Peak Library Search (which identifies each peak in the chromatogram by comparing its spectrum to a spectral library), and Spectral Standard Confirmation (which confirms the identity of each peak in the chromatogram by comparing its spectrum to the spectrum from the same named peak in a reference chromatogram).

This section shows you how to perform operations, such as Peak Purity, and Peak Library Search to investigate a chromatogram.

More specifically, you will learn how to:

- Use the Peak Purity operation to check the purity of chromatographic peaks.
- Use the Absorbance Ratio operation to calculate the Absorbance Ratio of chromatographic peaks.
- Use the Wavelength Maximum operation to determine the Wavelength Maximum of chromatographic peaks.
- Use the Spectral Library Confirmation operation to verify the identity of chromatographic peaks by comparing to a spectral library.
- Use the Peak Library Search operation to identify chromatographic peaks.
- Use the Preview Chromatograms operation to output new channel definitions that will be added to the controlling method.
- Use the Apex Optimized Chromatogram operation to create a chromatogram with the optimum wavelength set for each peak
- Save the results of the operations you performed on a chromatogram, so that the corresponding file is updated with this information and the corresponding spectral method files are updated with the parameter values used to calculate the results.

Peak Purity

The purity of a chromatographic peak can be checked by comparing the spectra on the upslope and down slope of the peak. If the two spectra are not the same, then two or more components with different spectra must be present in the peak envelope. Use the Peak Purity operation to calculate the peak purity for all peaks in a specific chromatogram.

- To access the Peak Purity operation, expand the **Operations** node on the **Views** tree and select **Peak Purity**.

Calculating the Purity of a Chromatographic Peak

In order to perform the Peak Purity operation, you must first select a chromatogram from the Data Tree. The selected chromatogram is displayed on the page in the upper graph (Chromatogram pane) and each peak in the chromatogram is annotated with a Purity Index and a pass fail rating. The purity indices and corresponding pass fail ratings are calculated according to the wavelength range, pass threshold, data point threshold, and absorbance threshold values that are currently displayed on the page. You can modify any of these parameters; and the software automatically recalculates and displays the new purity indices for each peak in the chromatogram.

The Purity Index is calculated by dividing the peak upslope and down-slope spectra at each wavelength; and then dividing the maximum value in the resulting plot by the minimum value. If the two spectra were identical then the division of the two spectra would result in a straight line and dividing the maximum value by the minimum value in this plot would give a value of 1.00. A Peak Index of 1.00 would therefore mean that the upslope and downslope spectra matched exactly and thus the peak was spectrally homogeneous, most likely consisting of a single component. A value above 1.00 implies that the two spectra are different. Because of noise and other variables a peak with a value of 1.00 to 1.50 is usually considered pure.

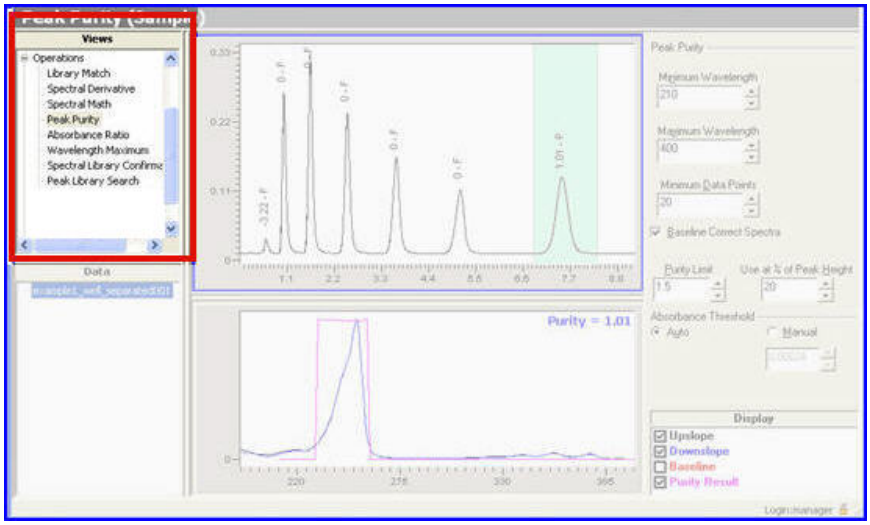
If you are interested in seeing how the peak purity was evaluated for a specific peak, simply click on that peak. The Results pane, located beneath the chromatogram display, provides you with a graphical representation of the upslope, down slope, and baseline spectra for the selected peak, as well as a graphical representation of the purity difference, which is a plot resulting from the division of the upslope and down slope spectra. For more information on how the software calculates Peak Purity, refer to Calculation Algorithms.

NOTE: *Use the Display List to specify what is displayed on the Results pane. Items that appear checked on the Display List are plotted on the Results pane.*

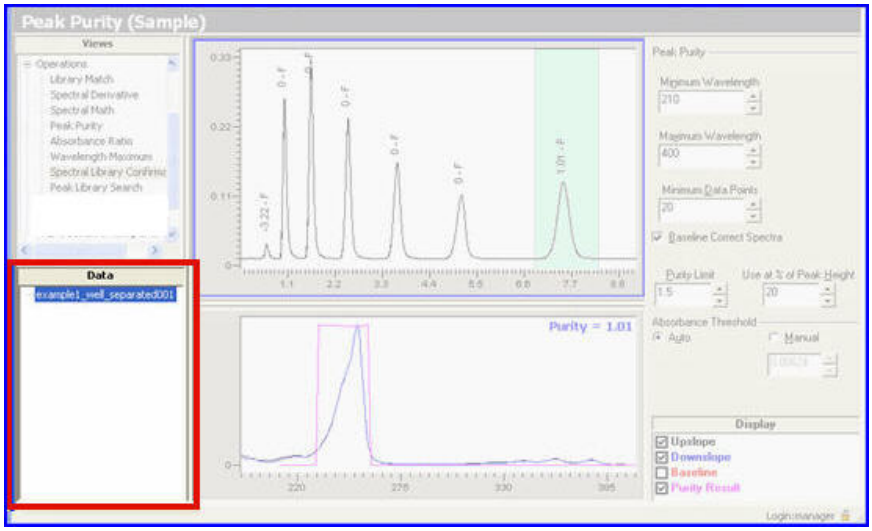
Tell me about the Peak Purity operation.

The Peak Purity operation consists of the following areas:

- **Views Tree** - The Views Tree provides you access to the Peak Purity operation.



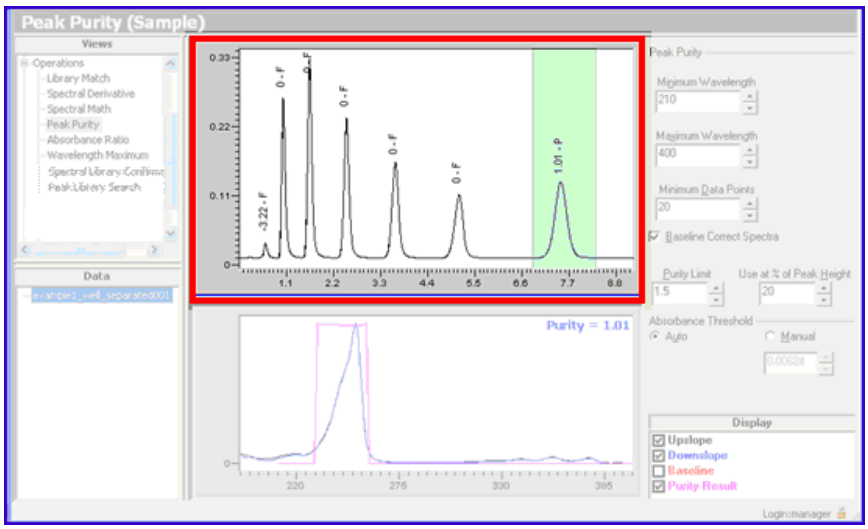
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.



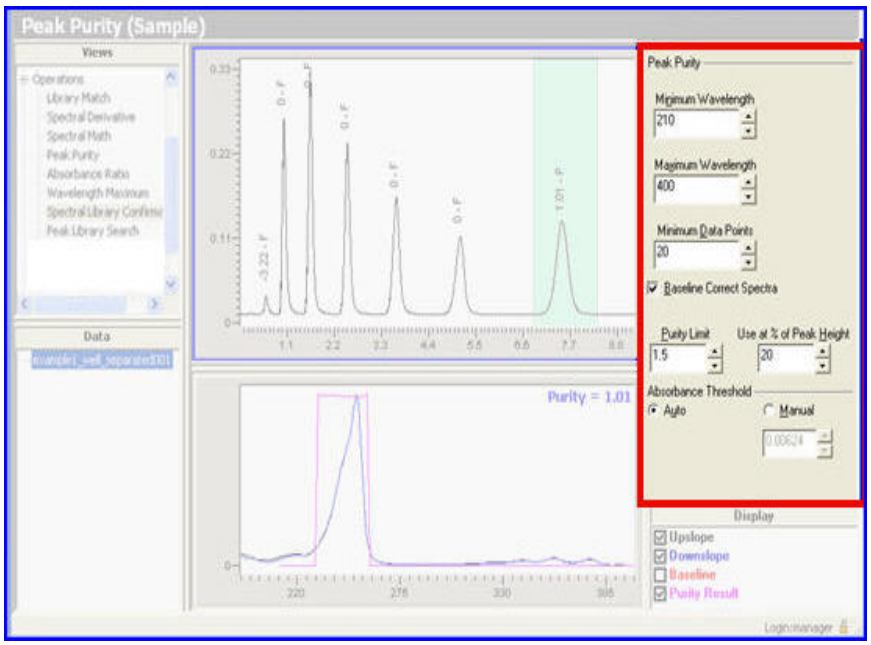
- **Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree.

  Each peak on the displayed chromatogram is labelled with its purity index and an associated Pass/Fail Rating. If the purity index for a peak exceeds the Purity Limit value currently displayed on the Parameters pane, then the peak is considered impure and an **F** appears next to the purity value. If the purity index for a peak is less than or equal to the Purity Limit currently defined on the Parameters pane, then the peak is considered pure and a **P** is displayed next to the purity value.

NOTE: *A purity index of 1.00 means that a peak is homogeneous, most likely consisting of a single component. A high value means that the upslope and downslope spectra are quite different, and the peak is highly impure. Typically, a peak with a purity value of 1.00 to 1.50 is considered pure. Chromera uses an initial default value of 1.5 for the Purity Limit; however, you can specify a different maximum value at which the upslope and downslope spectra are considered to match, using the Purity Limit parameter that is displayed on this view.*



- **Parameters Pane** - This pane is where you set and investigate the various parameters used to calculate Peak Purity. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, the Display List and Results pane are automatically updated with the modified results.



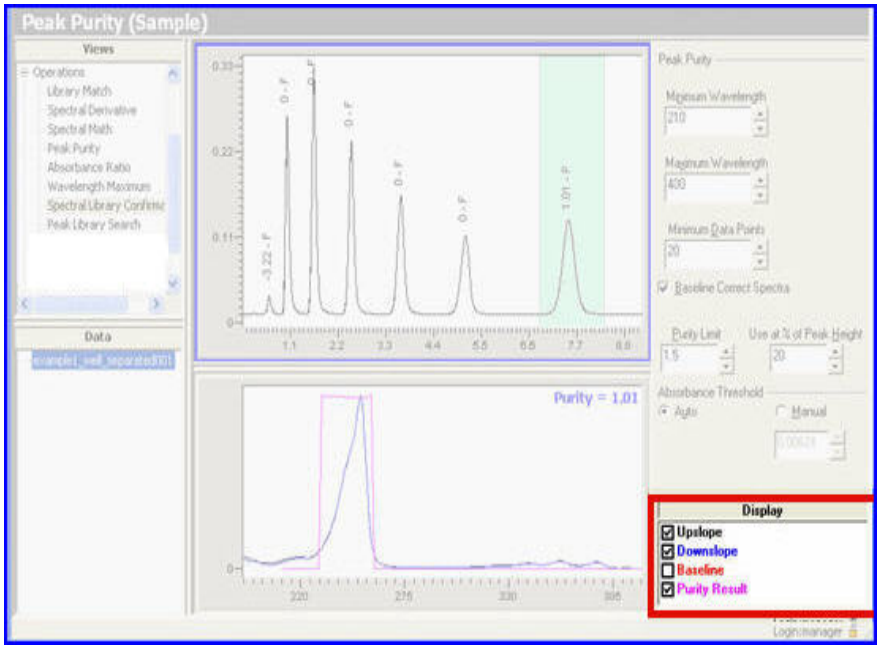
- **Display List** - You use the Display List to select the information you want displayed on the Results pane (including the spectra used in the calculation of the purity and the purity result). Items that appear checked are displayed on the Results pane, while unchecked items are not displayed.

The following items are available for selection on the Display List:
Upslope spectrum - Check **Upslope spectrum** to display the upslope spectrum from the current peak.

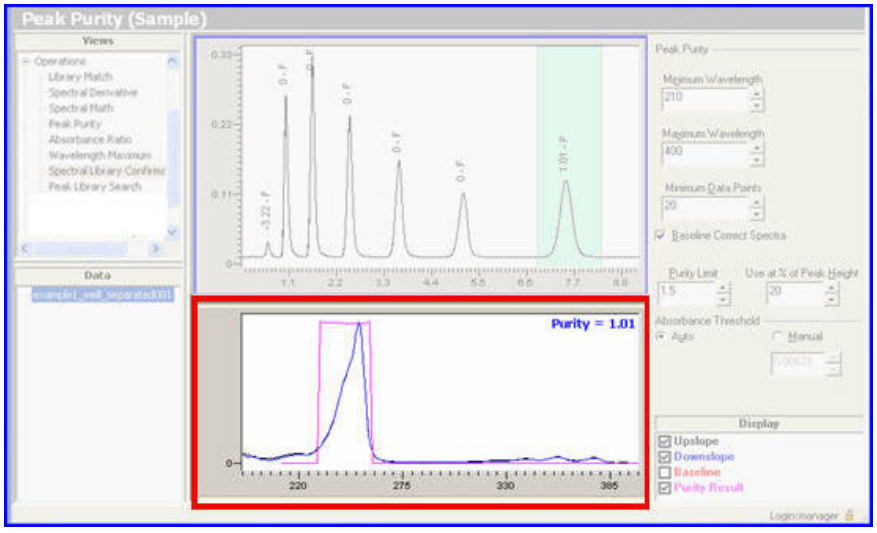
NOTE: *You can define at what position of the peak height the upslope and downslope spectra are obtained. The position is defined on the Parameters pane as a percentage of the chromatographic peak height. The lower the percentage value that is entered for the Use at*

% of Peak Height parameter, the closer to the baseline are the spectra taken. When looking for possible impurities, spectral differences will be increased as you move further out on the peak, I.e. closer to the baseline, but the signal-to-nosie will decrease and therefore increase the risk of false positives.

- **Downslope spectrum** – Check **Downslope spectrum** to display the downslope spectrum from the current peak.
- **Baseline** - Check **Baseline** to display the baseline spectrum from the current peak.
- **Purity Result** - Check Purity Result to display the plot resulting from the division of the upslope and down slope spectra at each wavelength.



- **Results Pane** - This pane displays a graphical representation of the items that are checked on the Display List. In addition, in the right hand corner of this pane, the Purity Index for currently selected peak is displayed. If the peak's purity value exceeds the Purity Limit currently defined on the Parameters pane, then the Purity Index is shown in red. If the peak's purity value is less than or equal to the currently defined Purity Limit, then the Purity Index is shown in blue.



NOTE: *Whenever you select a different peak, change the parameters for the operation, or select/deselect an item on the Display List, the Result pane is automatically updated to reflect the change.*

How do I modify the parameters that are used to calculate Peak Purity?

Peak Purity Parameters

The Parameters pane is where you set and investigate the various parameters for calculating Peak Purity. At any time you can optimize/adjust the parameters that are listed on the Peak Purity operation; and the Peak Purity value labels are updated for every peak in the chromatogram.

Below is a list of the Peak Purity parameters you can modify:

Parameter	**Description**
Minimum Wavelength	This parameter allows you to set the lower limit of the spectrum range to be included in calculation.
Maximum Wavelength	This parameter allows you to set the upper limit of the spectrum range to be included in the calculation.
Minimum Data Points	The absorbance threshold value will eliminate certain data points from the calculation of the result. If too many data points are eliminated, the result becomes questionable. Therefore, the Minimum Data Points parameter is used to set the minimum number of data points that must be present for a valid result. The default value is twenty. If the number of data points falls below this number, the result is reported as 0 and the test is considered as a fail.
Baseline Correct Spectra	Check this box use baseline corrected spectra in the calculation.
Purity Limit	This parameter allows you to set the maximum value at which the two spectra are considered to match. If the two spectra match exactly then the value will be 1. To allow for noise and other errors in the system, this value is normally set at 1.5. You can reduce or increase this value to fit your own criteria. Values that exceed this number are labeled in red on the Results pane.

Use at % of Peak Height	This parameter allows you to change the position from where the upslope and downslope spectra are extracted. The position is defined as a percentage of the chromatographic peak height – the lower the percentage, the closer to the baseline are the spectra taken. This parameter is useful if you are looking for possible impurities. When you specify a lower percentage value and thereby move further out on the peak, i.e. closer to the baseline, you will notice that spectral differences are increased. However, it should be noted that by setting a lower percentage value for this parameter, the signal-to-noise will decrease, thereby increasing the risk of false positives.
Absorbance Threshold	The result of dividing two noisy small numbers can be very unstable; therefore, you can use the Threshold controls to set the minimum absorbance values above the system noise and therefore obtain a more reproducible result. When **Auto** is selected, the absorbance threshold values are calculated automatically by the system and are set to either 0.0005 A.U. or 2% of the maximum absorbance in the spectrum, whichever is greater. The entry field is grayed when Auto is selected. When **Manual** is selected, enter the required value in the entry field, by either typing directly or using the spin buttons. When Manual is first selected the minimum absorbance value calculated by the Auto function is displayed in the Manual threshold field.

Summary of Operation: Peak Purity

The summary below illustrates how to calculate the purity of peaks in a chromatogram, and then save the new results and parameters used in the calculation.

How do I review and recalculate Peak Purity values for the peaks in a chromatogram?

1. From the **Views Tree** expand the **Operations** node and click on **Peak Purity**.

   The Peak Purity operation displays.

2. Select a chromatogram from the **Data** Tree.

   The selected chromatogram is displayed. The first peak is selected.

   The Results pane, located directly below the chromatogram, shows, for the selected peak, its Purity Index, its upslope and downslope spectra, the plot resulting from the division of the upslope and downslope spectra at each wavelength, and optionally its baseline spectrum.

How do I view the Peak Purity value for a different peak?

- Select the required peak by clicking on it in the chromatogram.

  The Results pane is updated to show the Purity Index, the upslope and downslope spectra for the current peak, and the baseline spectrum for the selected peak. The Purity Result for the current peak is also displayed (The Purity Result is the plot

resulting from the division of the upslope and downslope spectra at each wavelength).

How do I examine the Purity Values at different wavelengths?

- Enter a new value for the **Minimum Wavelength** and/or **Maximum Wavelength**.
  The Peak Purity value labels are updated for every peak in the chromatogram.

How do I examine the effect of using baseline corrected spectra on the Peak Purity values?

- Check **Baseline Correct Spectra**.
  The Peak Purity value labels are updated for every peak in the chromatogram.

How do I change the minimum absorbance value that is used in the calculation of the Peak Purity values and set the Minimum Data Points?

The Peak Purity operation is very sensitive, and two spectra are rarely absolutely identical. We have found in practice that a value between 1.00 and 1.50 will usually indicate that the spectra are the same and that the peak is pure. However, two practical factors have to be considered when actually performing the Peak Purity operation. The first is that a result of dividing two noisy numbers close to zero is very unstable and could easily affect the minimum or maximum value in the final calculation. To avoid such errors, you can set an absorbance threshold above the system noise. Absorbances in the spectra that fall below this threshold are not used in the calculation.

The second factor results from the threshold just described. Clearly, the threshold removes some data points from the calculation. It is possible for low- absorbance spectra, especially if the minimum and maximum wavelength has to be set to a narrow range, to have insufficient points for a valid calculation to be performed. A second threshold, labeled **Minimum Data Points**, is therefore applied, which determines if the calculation can be performed. This value defaults to 20, but it can be changed.

1. Select the **Manual** radio button.
   The **Manual** spin box is enabled
2. Use the spin box to specify a new minimum absorbance value.
   The Peak Purity value labels are updated for every peak in the chromatogram.

How do I return to automatically calculating the minimum absorbance value?

- Select the **Auto** radio button if you wish to have IRIS automatically calculate the minimum absorbance value that can be used in the calculation.
  The Peak Purity value labels are updated for every peak in the chromatogram.

How do I add the displayed spectra in the results pane to the Data Tree?

- Select **Actions > Add All to View**.

A dialogue box is be displayed with the message "The data was added successfully". On re-displaying the Main or Chrom/Spectra views the added spectra will appear in the Data Tree.

How do I save the results and parameters used in the calculation?

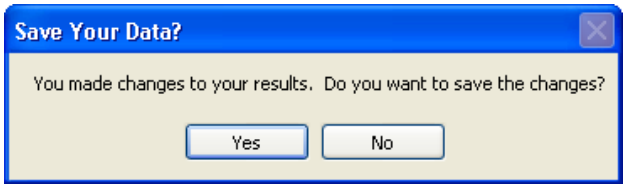
Once you are satisfied with the purity results, you can save the updated purity values and the parameters used in the calculation, by selecting **File > Save Results** in the main Chromera application. Once you have saved the updated purity values, they can be included in a report.

1. In the main Chromera application, select **File > Save Results**

   A save dialog appears that asks you to confirm whether or not you want to save the new values.

   Or

   If you select another item in the Spectral Processing Window the following dialog appears:



2. Click **Yes** to save the results and calculation parameters.

   The saved results can now be displayed as peak labels when the chromatogram is displayed in the Main View or Chrom/Spectra
   View. In addition, when you select this chromatogram again on the Peak Purity page, the saved results and the parameters used are displayed on the page.

How do I display the Peak Purity results in the Main View or Chrom/Spectra View?

1. Select **Actions > Label Chromatograms...**

2. Select **Purity** from the list of available **Label Types**.

How do I review for any chromatogram previously calculated Peak Purity values and the parameters used?

- Select this chromatogram again on the Peak Purity operation.

  The saved results and the calculation parameters are automatically displayed.

Absorbance Ratio

The Absorbance Ratio of a compound is defined as the ratio of the absorbance at two specified wavelengths in its peak apex spectrum. This value is constant for any given component, and its value can therefore be used to confirm identification and purity.

You use the **Absorbance Ratio** operation to optimize/adjust the absorbance ratio for each peak in an open and visually selected chromatogram.

- To access this operation, expand the **Operations** node on the **Views** tree and select **Absorbance Ratio**.

About the Absorbance Ratio Operation

In order to use this page you must first select a chromatogram that is listed on the Data Tree. The selected chromatogram is displayed on the page in the upper graph (Chromatogram pane) and each peak in the chromatogram is labeled with the Absorbance Ratio value. As noted in the previous section, the Absorbance Ratio of a compound is defined as the ratio of the absorbance at two specified wavelengths in its peak apex spectrum. The value is independent of concentration, characteristic for the compound but not necessarily unique.

Chromera calculates the absorbance ratio values using the parameters that are currently displayed on the page. At any time you can recalculate the absorbance ratio values by modifying the two wavelength values used in the calculation, specifying a different absorbance threshold, as well as by selecting whether or not baseline corrected spectra are used in the calculation. Chromera automatically updates the Absorbance Ratio page to display the recalculated results.

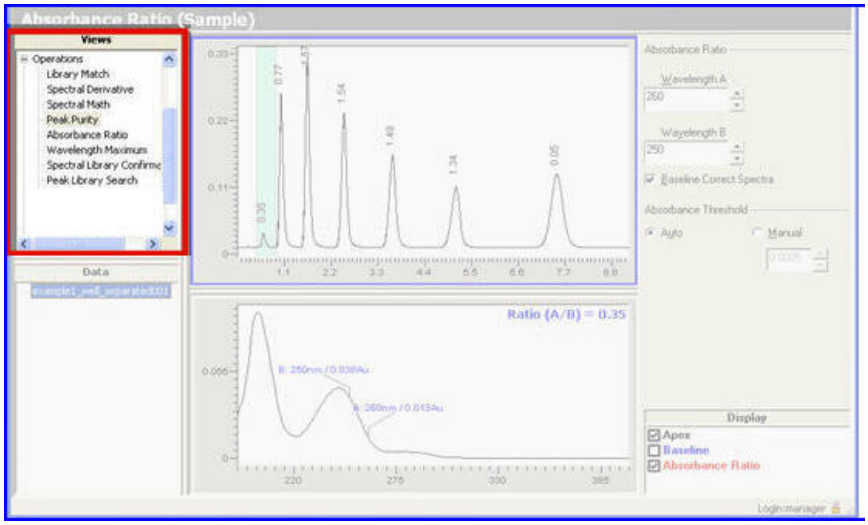
If you are interested in the absorbance ratio for a specific peak, click on the peak; and, in the bottom graph (Results pane), the apex and baseline spectra are displayed for the peak as well as the Absorbance Ratio value. The apex spectra are annotated with the positions of the two wavelengths used and the absorbances at each wavelength.

NOTE: *Use the Display List to determine what is displayed on the Results Pane. Items that appear checked on the Display List are plotted on the Results Pane.*

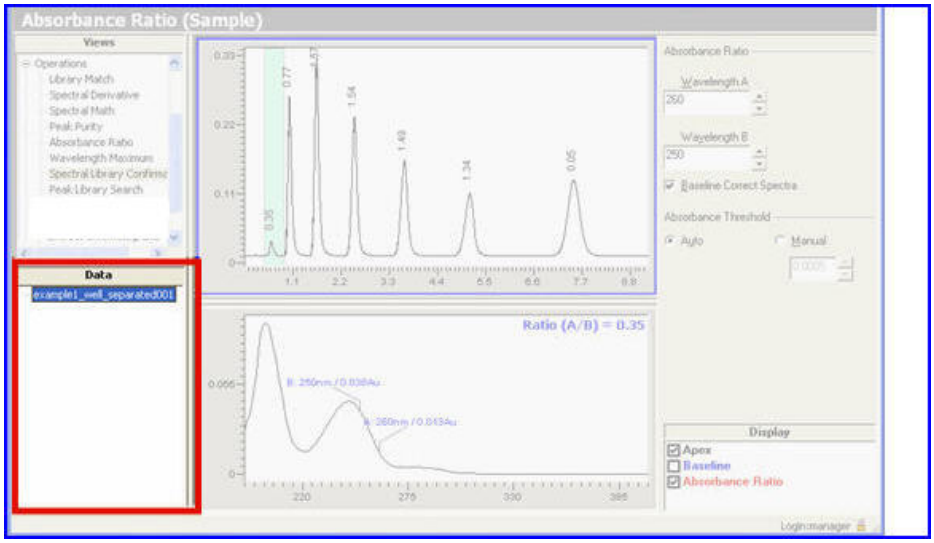
Tell me about this window

The Absorbance Ratio page consists of the following areas:

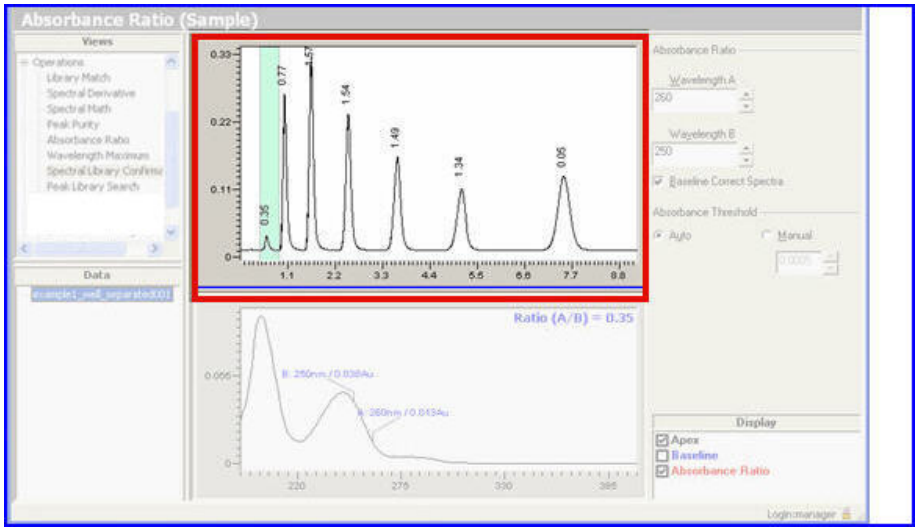
- **Views Tree** - The Views Tree provides you access to the Absorbance Ratio operation. You can access this operation by selecting **Operations >Absorbance Ratio** from the Views Tree.



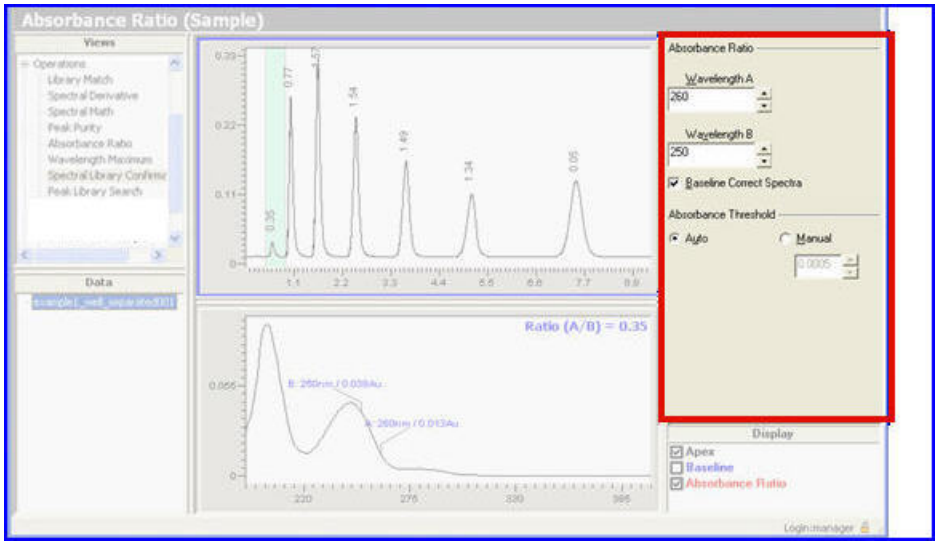
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree will is displayed in the upper pane (Chromatogram pane) for the Operation.



- **Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree.



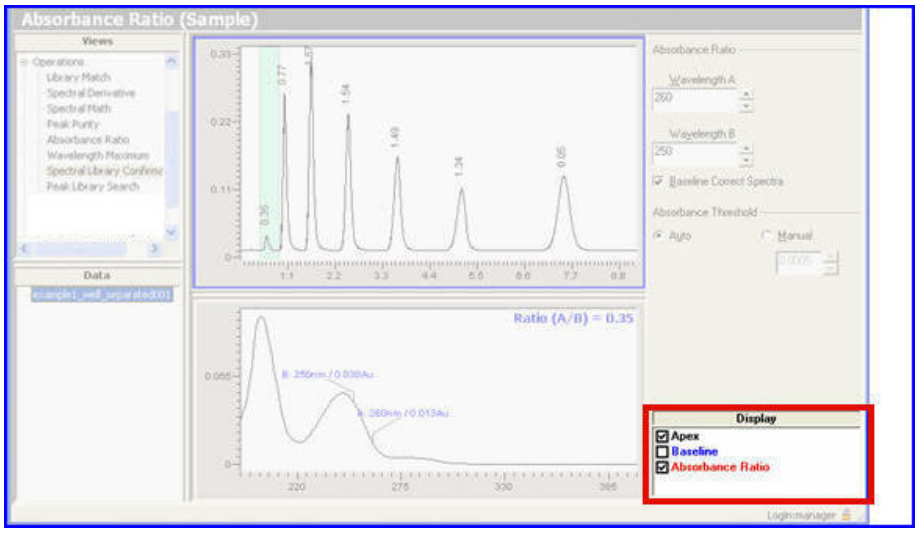
- **Parameters Pane** - This pane is where you set and investigate the various parameters used to calculate the Absorbance Ratio values. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, the Display List and Results pane are automatically updated with the modified results.



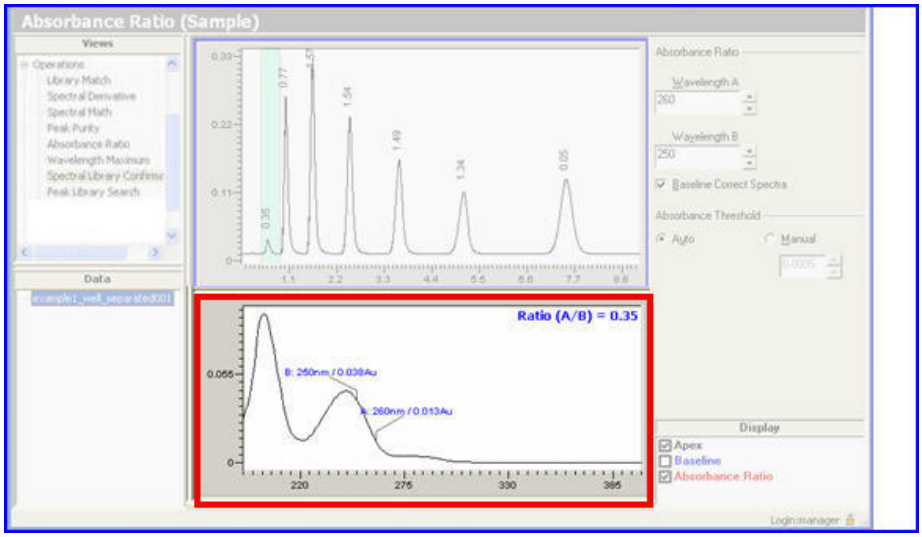
- **Display List** - You use the Display List to select the information you want displayed on the Results Pane. When items appear checked on this list, they are displayed in the Results Pane. Meanwhile, unchecked items are not displayed.

  The following items are available for selection on the Display List:
  - **Apex** - Check this option to display the apex spectrum from the currently selected peak on the Results pane.
  - **Baseline** - Check this option to display baseline spectrum associated with the currently selected peak on the Results pane.
  - **Absorbance Ratio** - This option is only available when Apex is checked. When Absorbance Ratio is checked, the apex spectrum, currently displayed in the Results pane, is annotated with the position of the two wavelengths and the absorbances used for calculating the Absorbance Ratio.

NOTE: *You can select* ***Actions > Add All to View*** *to add all selected spectra in the Display list to the Data Tree.*



- **Results Pane** - This pane displays a graphical representation of the items that are checked on the Display List. In addition, when a peak is selected on the chromatogram, the Results pane displays the Absorbance Ratio for the selected peak. The Absorbance Ratio is labeled as: **Ratio (A/B) =**.



NOTE: *Whenever you select a different peak, change the parameters for the calculation, or select/deselect an item on the Display List, the Results pane is automatically updated to reflect the change.*

How do I modify the parameters that are used to determine the Absorbance Ratio values?

Absorbance Ratio Parameters

The Parameters pane displays the values that are currently used in the operation. At any time you can optimize/adjust the parameters that are listed on the Absorbance Ratio operation; the display updates with the new results.

Below is a list of the parameters you can modify:

Parameter	Description
Wavelength A and Wavelength B	These spin boxes show the two wavelengths used for the ratio, which you can modify. The ratio is always calculated A/B. Wavelength A may be less than, equal to or greater than Wavelength B.
Baseline correct spectra	Check this option to use the baseline corrected spectra in the calculation.
Absorbance Threshold	To ensure that the Absorbance Ratios are calculated for small peaks, but the baseline noise is not interpreted as peaks, you can use this parameter to set the minimum absorbance values above the system noise, and therefore, obtain more reproducible results. When **Auto** is selected, the absorbance threshold values are calculated automatically by the system and are set to either 0.0005 A.U. or 2% of the maximum absorbance in the spectrum, whichever is greater. The entry field is grayed when Auto is selected. When **Manual** is selected, enter the required value in the entry field, by either typing directly or using the spin buttons. When Manual is first selected the minimum absorbance value calculated by the Auto function is displayed in the Manual threshold field.

Summary of Operation: Absorbance Ratio

The summary below illustrates how to optimize/adjust the Absorbance Ratios for a chromatogram and then save the new results and parameters used in the calculation.

How do I review and recalculate Absorbance Ratios for a chromatogram?

1. From the **Views Tree** expand the **Operations** node and click on **Absorbance Ratio**.

   The Absorbance Ratio operation displays.

2. Select a chromatogram from the **Data** tree.

   The selected chromatogram is displayed. The first peak is selected.

   The results pane, directly below the chromatogram, shows, for the selected peak, its peak apex spectrum annotated with the position of the two wavelengths and absorbances, its Absorbance Ratio value, in the top right corner of the pane, and optionally its baseline spectrum.

How do I view the Absorbance Ratio for a different peak?

- Select the required peak by clicking on it in the chromatogram.

  The results pane is updated to show the peak apex spectrum, the Absorbance Ratio value, and the baseline spectrum for the selected peak.

  How do I examine the Absorbance Ratios at different wavelengths?

- Enter a new value for **Wavelength A** or **Wavelength B**.
  The Absorbance Ratio value labels are updated for every peak in the chromatogram.

How do I examine the effect of using baseline corrected spectra on the Absorbance Ratio values?.

- Check **Baseline Correct Spectra**.
  The Absorbance Ratio value labels are updated for every peak in the chromatogram.

How do I change the minimum absorbance value that is used in the calculation of the Absorbance Ratios?

1. Select the **Manual** radio button.

   The minimum absorbance spin box is enabled.

2. Use the spin box to specify a new minimum absorbance value.

   The Absorbance Ratio value labels are updated for every peak in the chromatogram.

How do I return to automatically calculating the minimum absorbance value?

- Select the **Auto** radio button if you wish to automatically calculate the minimum absorbance value that can be used in the calculation.

  The Absorbance Ratio value labels are updated for every peak in the chromatogram.

How do I add the displayed spectra in the results pane to the Data Tree?

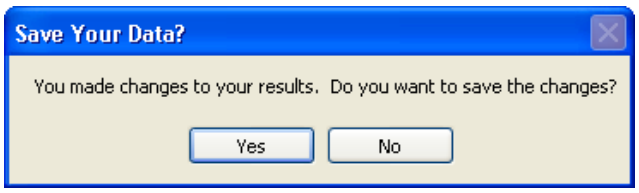
- Select **Actions > Add All to View**.
  A dialogue box is be displayed with the message "The data was added successfully". On re-displaying the Main or Chrom/Spectra views the added spectra will appear in the Data Tree.

How do I save the results and parameters used in the calculation?

Once you are satisfied with the results, you can save the updated values and the parameters used in the calculation, by selecting **File > Save Results** in the main Chromera application. Once you have saved the updated values, they can be included in a report.

1. In the main Chromera application, select **File > Save Results**

   A save dialog appears that asks you to confirm whether or not you want to save the new values.
   Or
   If you select another item in the Spectral Processing Window the following dialog appears:



2. Click **Yes** to save the results and calculation parameters.

   The saved results can now be displayed as peak labels when the chromatogram is displayed in the Main View or Chrom/Spectra View. In addition, when you select this chromatogram again, the saved results and the parameters used are displayed on the page.

Wavelength Maximum

The Wavelength Maximum operation is used to determine the wavelength maximum of the apex spectrum of each peak in a chromatogram. The Wavelength Maximum of a spectrum is defined as the wavelength of the highest absorbance peak in the spectrum above a specifiable minimum wavelength.

- To access this operation expand the **Operations** node on the **Views** tree and select **Wavelength Maximum**.

Determining the wavelength maximum of chromatographic peaks

In order to use this page you must first select a chromatogram that is listed on the Data Tree. The selected chromatogram is displayed on the view in the upper graph (Chromatogram pane) and each peak in the chromatogram is labeled with a Wavelength Maximum value. Chromera determines the wavelength maximum values using the parameter values that are currently displayed on the page. You can modify the calculation parameters at any time and Chromera automatically recalculates and displays the new values on the page.

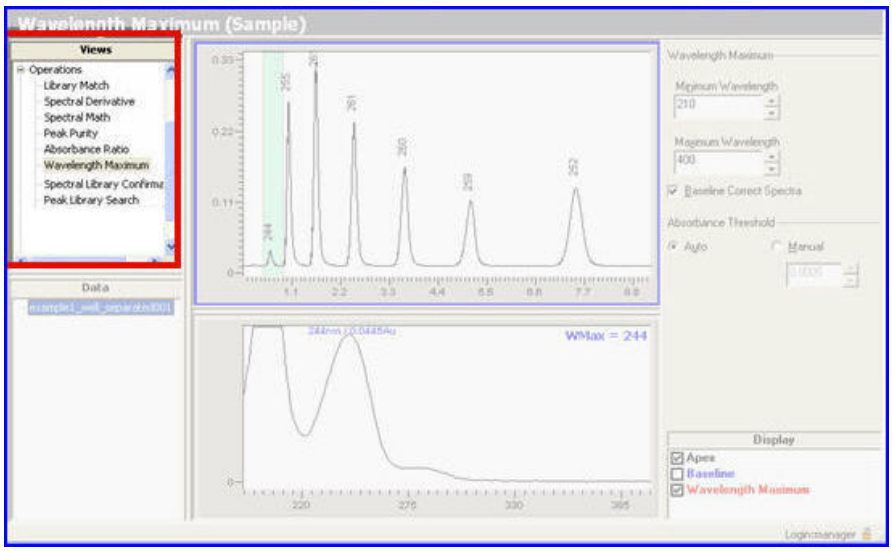
If you are interested in the wavelength maximum of a specific peak, click on the peak; and, in the bottom graph (Results pane), the apex and baseline spectra for the peak are displayed. The apex spectra are annotated with the position and the absorbance of the wavelength maximum. In addition, in the right hand corner of the Results pane the Wavelength Maximum value for the selected peak is displayed.

NOTE: *Use the Display List to specify what is displayed on the Results pane. Items that appear checked on the Display List are plotted on the Results pane.*

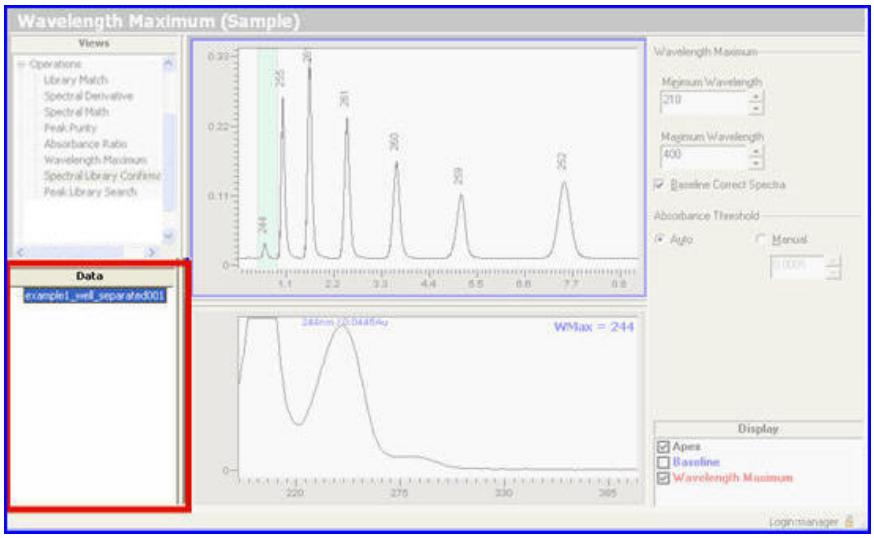
Tell me about the Wavelength Maximum operation.

The Wavelength Maximum operation is comprised of the following areas:

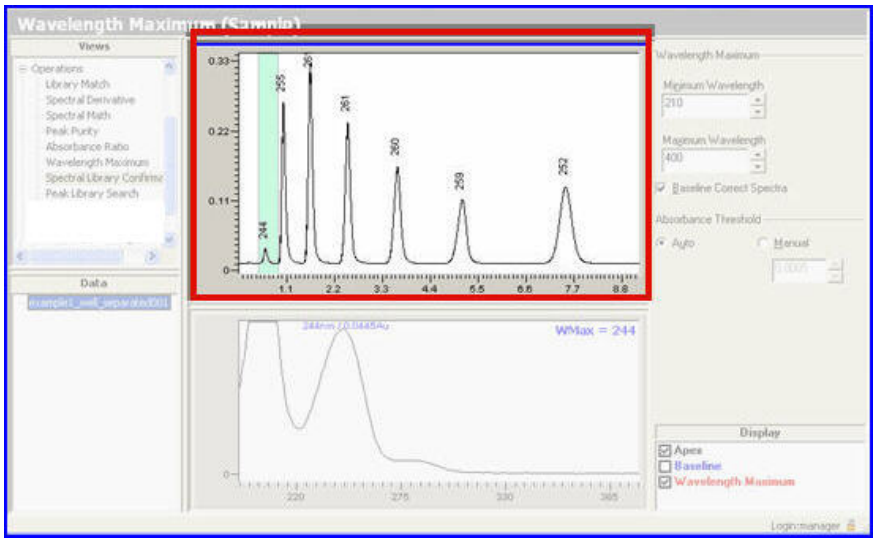
- **Views Tree** - The Views Tree provides you access to the Wavelength Maximum operation. You can access this operation by selecting **Operations >Wavelength Maximum** from the Views Tree.



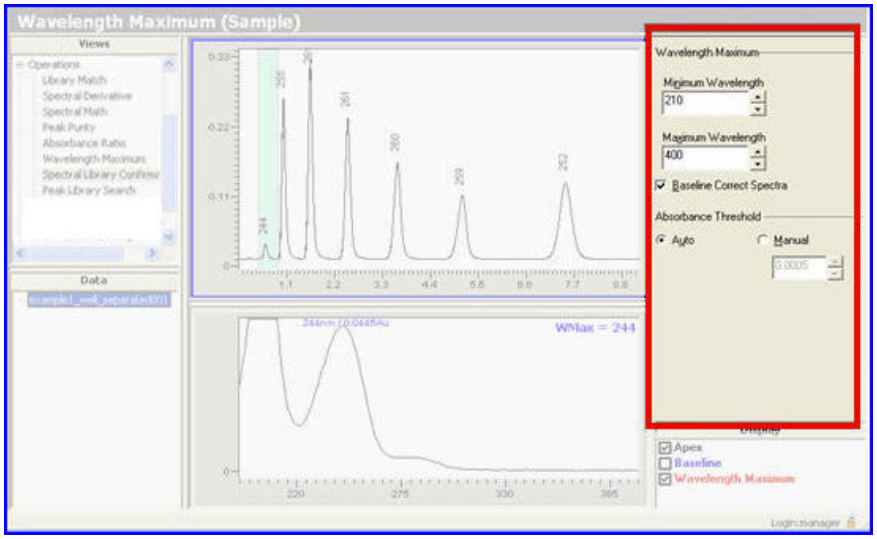
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation



- **Chromatogram Pane** - This pane displays the chromatogram that is currently selected on the Data Tree. Each peak in the displayed chromatogram is labeled with the wavelength maxima.



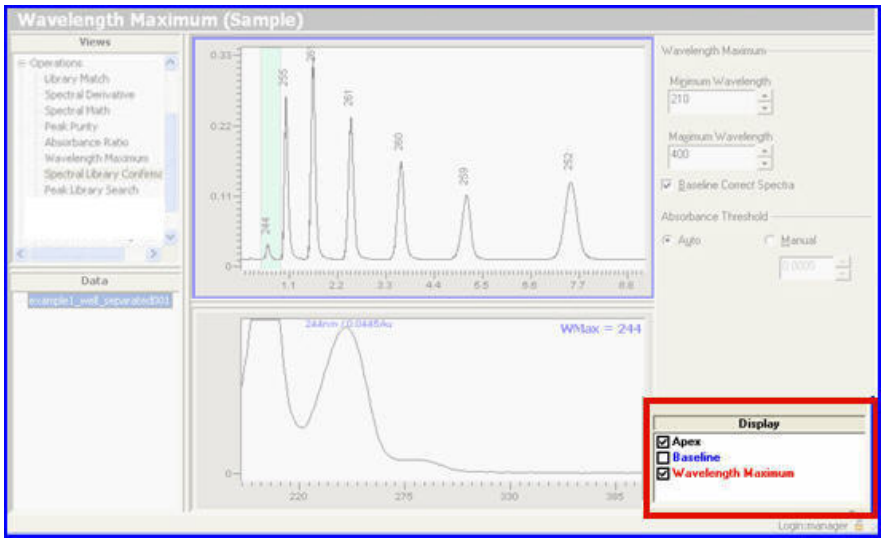
- **Parameters Pane** - This pane is where you set and investigate the various parameters used to determine the Wavelength Maximum values. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, Chromera automatically updates the Display List and Results pane with the modified results.



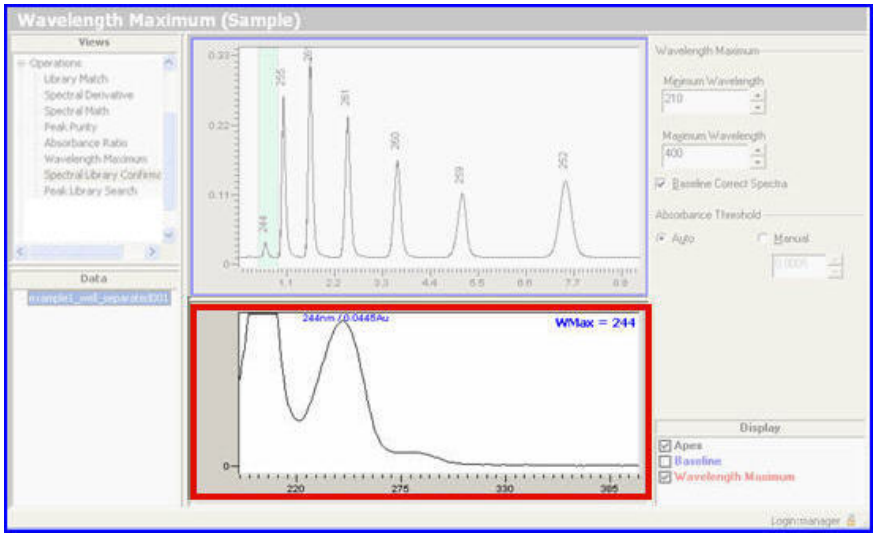
- **Display List** - You use the Display List to select the information you want displayed on the Results pane. When items appear checked on this list, they are displayed in the Results Pane. Meanwhile, unchecked items are not displayed.

  The following items are available for selection on the Display List:

  - **Apex -** Check this option to display the apex spectrum for the selected peak.
  - **Wavelength Maximum**- When Apex is checked you can check Wavelength Maximum to have the Apex spectrum annotated with the position and absorbance of the wavelength maximum.
  - **Baseline** - Check this option to display the baseline spectrum associated with the peak that is currently selected.



- **Results Pane** - This pane displays a graphical representation of the items that are checked on the Display List. In addition, when a peak is selected on the chromatogram, this pane displays the Wavelength Maximum value for the selected peak in the upper right hand corner as **WMAX =**.



NOTE: *Whenever you select a different peak, change the parameters for the operation, or select/deselect an item on the Display List, the Results pane is automatically updated to reflect the change.*

How do I modify the parameters used that are used to determine the Wavelength Maximum values?

Wavelength Maximum Parameters

The Parameters pane displays the values that are currently used in the operation. At any time you can optimize/adjust the parameters that are listed on the Wavelength Maximum operation; and the display is automatically updated with the new results.

Below is a list of the parameters you can modify:

Parameter	**Description**
Minimum Wavelength	You can use the spin box to specify the minimum wavelength for the determination of the Wavelength Maximum.
Maximum Wavelength	You can use the spin box to set the upper limit of the spectrum range to be included in the calculation.
Baseline correct spectra	Check this option to use the baseline corrected spectra in the calculation.

Absorbance Threshold	To ensure that Chromera determines the Wavelength Maximum values for small peaks, but does not interpret baseline noise as peaks, you can use this parameter to set the minimum absorbance values above the system noise, and therefore, obtain more reproducible results. When **Auto** is selected, the absorbance threshold values are calculated automatically by the system and are set to either 0.0005 A.U. or 2% of the maximum absorbance in the spectrum, whichever is greater. The entry field is grayed when **Auto** is selected. When **Manual** is selected, enter the required value in the entry field, by either typing directly or using the spin buttons. When **Manual** is first selected the minimum absorbance value calculated by the **Auto** function is displayed in the Manual threshold field.

Summary of Operation: Wavelength Maximum

The summary below illustrates how to determine the Wavelength Maximum for all the peaks in a chromatogram and then save the new results and parameters used in the calculation.

How do I determine the Wavelength Maximum of all the peaks in a chromatogram?

1. From the **Views** tree expand the **Operations** node and click on **Wavelength Maximum**.

   The Wavelength Maximum operation displays.

2. Select a chromatogram from the Data tree.

   The selected chromatogram is displayed. The first peak is selected.

   The results pane, located directly below the chromatogram, shows, for the selected peak, its peak apex spectrum annotated with the position and absorbance of the wavelength maximum, and optionally its baseline spectrum. In the top right corner of the pane, the Wavelength Maximum value is displayed.

How do I view the Wavelength Maximum for a different peak?

- Select the required peak by clicking on it in the chromatogram.

  The results pane is updated to show the peak apex spectrum, the Wavelength Maximum value, and optionally the baseline spectrum for the selected peak.

How do I examine the Wavelength Maximum values at different wavelengths?

- Enter a new value for **Wavelength A** or **Wavelength B**.

  The Wavelength Maximum value labels are updated for every peak in the chromatogram.

How do I examine the effect of using baseline corrected spectra on the Wavelength Maximum values?

- Check **Baseline Correct Spectra**.

  The Wavelength Maximum value labels are updated for every peak in the chromatogram.

How do I change the minimum absorbance value that is used to determine the Wavelength Maximum values?

1. Select the **Manual** radio button.

   The minimum absorbance spin box is enabled

2. Use the spin box to specify a new minimum absorbance value.

   The Wavelength Maximum value labels are updated for every peak in the chromatogram

How do I return to automatically calculating the minimum absorbance value?

- Select the **Auto** radio button if you wish to have IRIS automatically calculate the minimum absorbance value that can be used in the calculation.

  The Wavelength Maximum value labels are updated for every peak in the chromatogram.

How do I add the displayed spectra in the results pane to the Data tree?

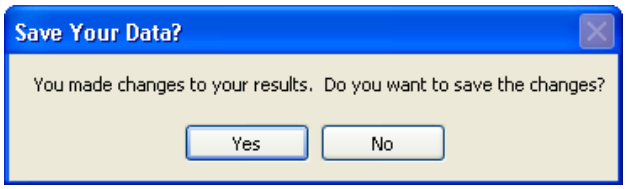
- Select **Actions > Add All to View**.

  A dialogue box is be displayed with the message "The data was added successfully". On re-displaying the Main or Chrom/Spectra views the added spectra will appear in the Data Tree.

How do I save the results and the parameters used in the calculation?

1. In the main Chromera application, select **File > Save Results**

   A save dialog appears that asks you to confirm whether or not you want to save the new values.
   Or
   If you select another item in the Spectral Processing Window the following dialog appears:



2. Click **Yes** to save the results and calculation parameters.

   The saved results can now be displayed as peak labels when the chromatogram is displayed in the Main View or Chrom/Spectra
   View.

How do I display the Wavelength Maximum results in the Main View or Chrom/Spectra View?

1. Select **Actions > Label Chromatograms...**

2. Select **WavelengthMaximum** from the list of available labels.

How do I review for any chromatogram previously determined Wavelength Maximum values and the parameters used?

- Select this chromatogram again on the Wavelength Maximum operation.

  The saved results and the calculation parameters are automatically displayed.

Spectral Library Confirmation

This **Spectral Library Confirmation** operation is used to check the identity of named peaks in a chromatogram by comparing the apex spectrum of each named peak to a spectrum of the same name in a library. The comparison of the spectra is made using a Euclidean distance algorithm, the numerical result of which is called the Hit Quality.

Chromera uses a Euclidean distance algorithm to evaluate the comparison between a selected spectrum and a library spectrum.

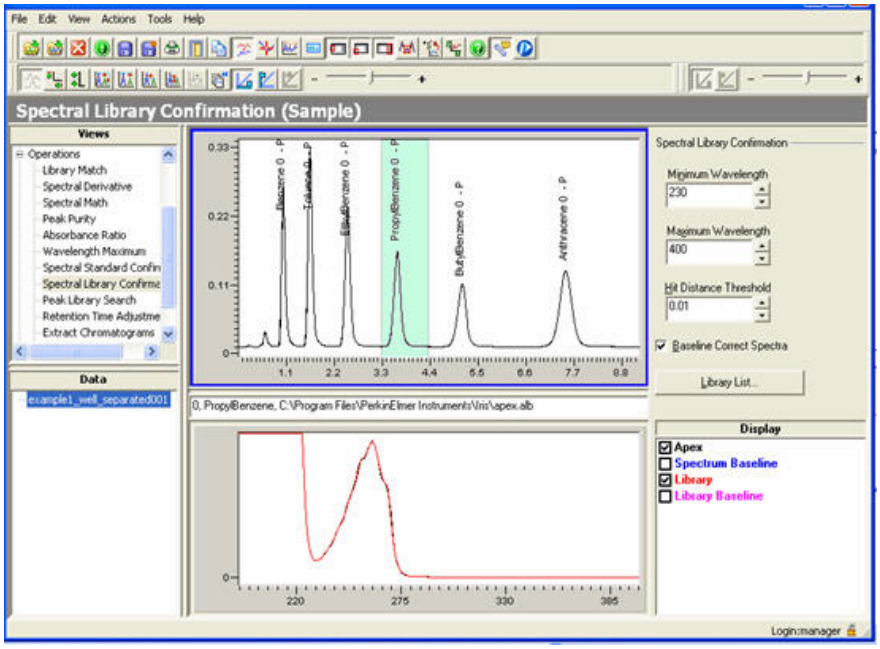
Each normalized spectrum is defined as a point in N-dimensional space (where the number of dimensions is equal to the number of points in the spectrum). The Hit Quality is simply the distance between the sample and library spectra. Since the space is normalized, the maximum possible distance between any two spectra is root 2. The distance between the spectra therefore varies between 0 and 1.4 - the lower the number, the closer the spectra and the better the match.

For verification purposes in the Spectral Library Confirmation task and Peak Library Search task, a Hit Distance Threshold of 0.05 is used to confirm that the peak in the sample chromatogram is the same component as that in the spectral library. A value higher than 0.05 suggests either that the peak in the sample is impure or that it is not the same component as in the spectral library.
For identification purposes in the Spectral Library Search task a lower Hit Distance Threshold of 0.01 is used in order to prevent false matches.

The Hit Quality threshold can be modified, up or down, on the Parameters pane for each of these tasks. . You can set a maximum hit threshold value to prevent unlikely matches.

- To access this page expand the **Operations** node on the **Views** tree and select **Spectral Library Confirmation**.

  The Spectral Library Confirmation operation displays.



Verifying the Identity of Chromatographic Peaks by Comparing to a Spectral Library

To perform this operation you must select a chromatogram that is listed on the Data Tree. Next, you must specify the spectral libraries to be searched. Chromera then matches each named peak in the chromatogram to a library spectrum that has the same component name.

As a result of the search, each named peak, on the chromatogram, that could be matched to a library spectrum with the same component name is labeled with the **Component Name**, a **Hit Quality Value** (a numerical value that indicates how close the apex spectrum matches to the library component of the same name), and a **P**ass/**F**ail value that indicates whether or not the component from the sample chromatogram matches the library component based on the Hit Threshold you specify.

NOTE: *If the chromatogram that you selected on the Data Tree has no identified peaks, Chromera informs you of the error and you will not be able to perform this operation. Only identified peaks can be matched.*

If you are interested in the Spectral Library Confirmation for a specific peak, simply click on the peak you wish to evaluate. Below the chromatogram plot, an overlaid plot of the apex spectrum for the currently selected peak is displayed along with the library spectrum with the same component name.

How does Chromera perform Spectral Library Confirmation?

As stated earlier, the Spectral Library Confirmation operation verifies the identity of named peaks in a sample chromatogram by comparing the apex spectrum of each named peak in the sample to an identically named spectrum in a spectral library. The comparison of the spectra is made by calculating the Euclidean distance algorithm.

Chromera uses a Euclidean distance algorithm to evaluate the comparison between a selected spectrum and a library spectrum.

Each normalized spectrum is defined as a point in N-dimensional space (where the number of dimensions is equal to the number of points in the spectrum). The Hit Quality is simply the distance between the sample and library spectra. Since the space is normalized, the maximum possible distance between any two spectra is root 2. The distance between the spectra therefore varies between 0 and 1.4 - the lower the number, the closer the spectra and the better the match.

For verification purposes in the Spectral Library Confirmation task and Peak Library Search task, a Hit Distance Threshold of 0.05 is used to confirm that the peak in the sample chromatogram is the same component as that in the spectral library. A value higher than 0.05 suggests either that the peak in the sample is impure or that it is not the same component as in the spectral library.
For identification purposes in the Spectral Library Search task a lower Hit Distance Threshold of 0.01 is used in order to prevent false matches.

The Hit Quality threshold can be modified, up or down, on the Parameters pane for each of these tasks. The numerical result of this comparison is called the Hit Quality; and this value can vary between 0 and 1.4. The lower the Hit Quality number, the closer the spectra and the better the match.

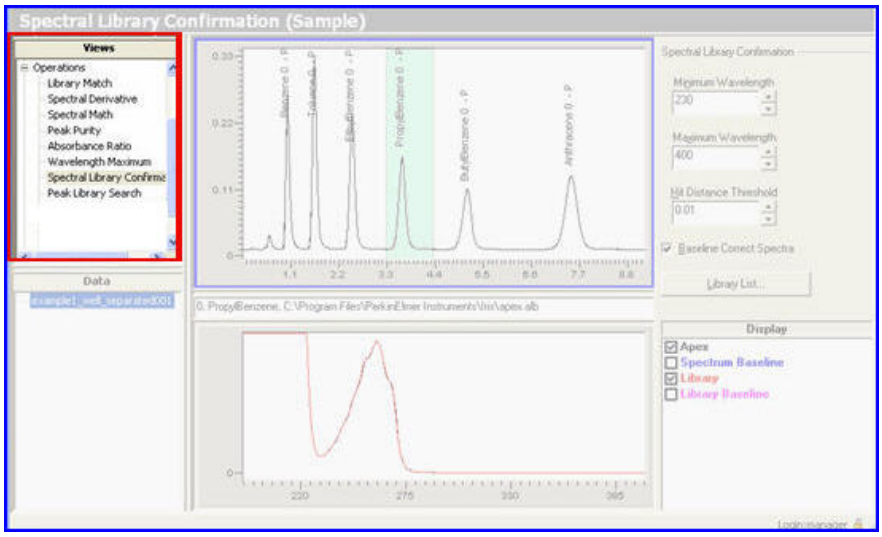
For identification purposes in the Spectral Library Confirmation, it is recommended that a Hit Quality threshold of 0.01 be used in order to confirm that the component in the sample is the

same component as that in the spectral library. This low value of 0.01 for the Hit Quality Threshold is used to prevent false matches. However, it should be noted that you can determine the value that you want to use for the Hit Quality threshold and are not required to use the recommended Hit Quality threshold of 0.01.

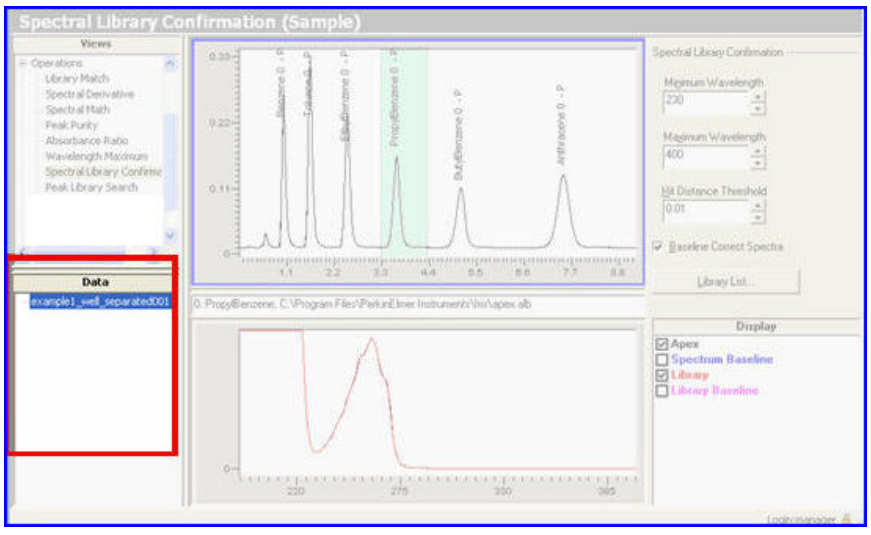
Tell me about the Main Window.

The Spectral Library Confirmation operation is comprised of the following areas:

- **Views Tree** - The Views Tree provides you access to the Spectral Library Confirmation operation. To access this operation, expand the **Operations** node and click on **Spectral** Library **Confirmation**.

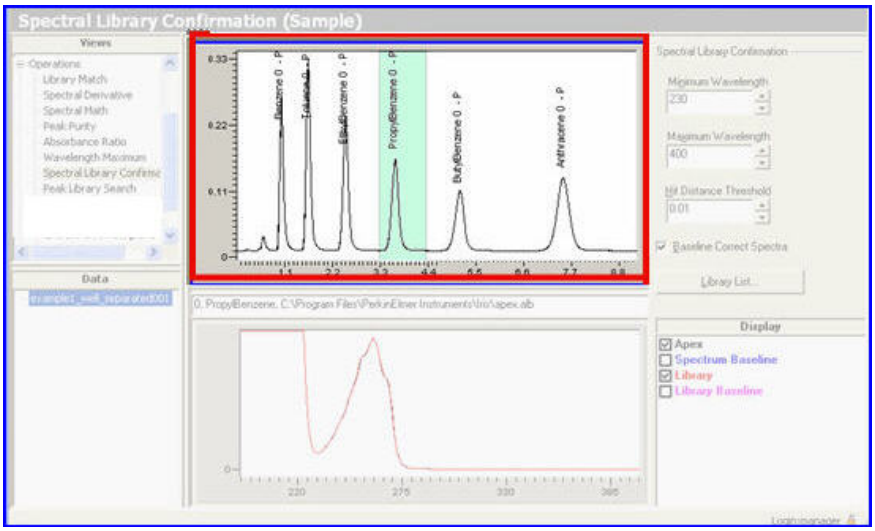


- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.

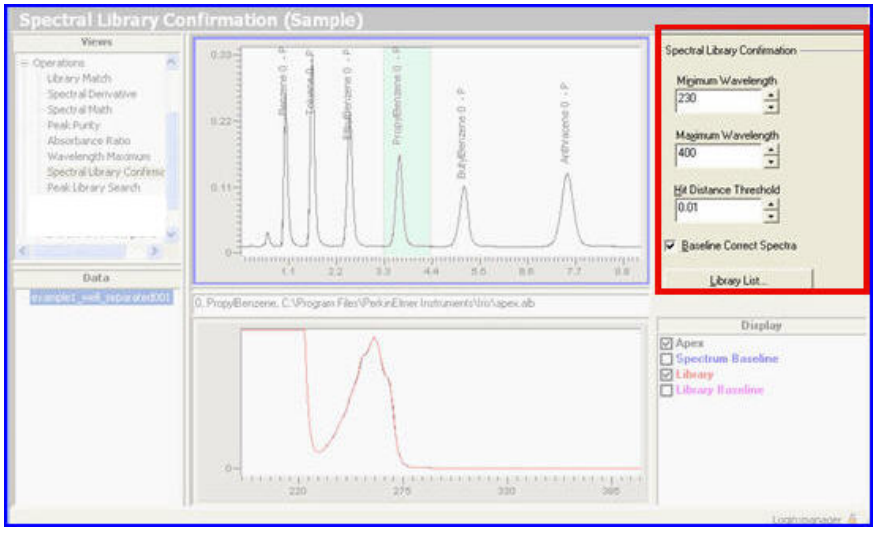


- **Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree. Named peaks in the displayed chromatogram are labeled with

their **Component Name**, the **Hit Quality Value**, and either a **P** or an **F** to indicate a Pass or Fail status, which is dependent on the current value of the Hit Distance Threshold.



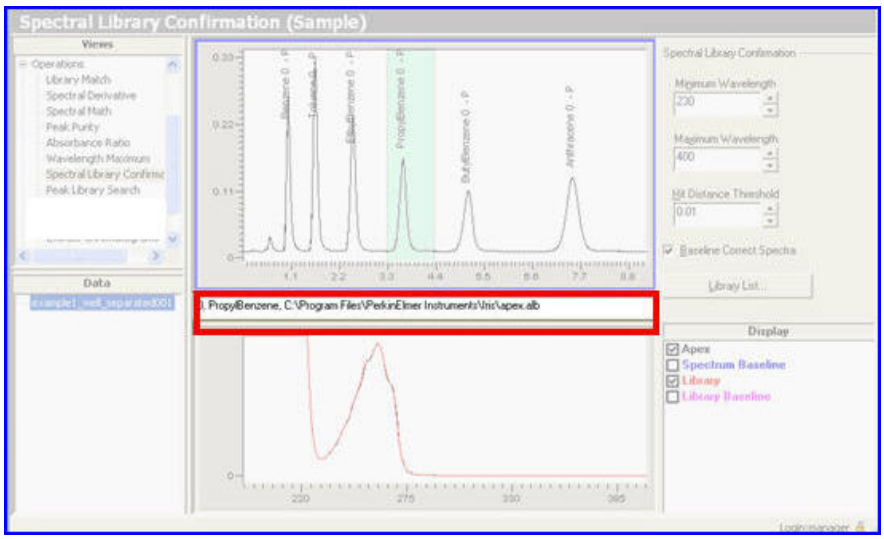
- **Parameters Pane** - This pane is where you set and investigate the various parameters used to perform the Spectral Library Confirmation operation. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, Chromera automatically updates the Display List and Results pane with the modified result.



- **Hit Details** - The file name and path for the library spectrum, that has the same name as the peak selected on the chromatogram, is displayed here.

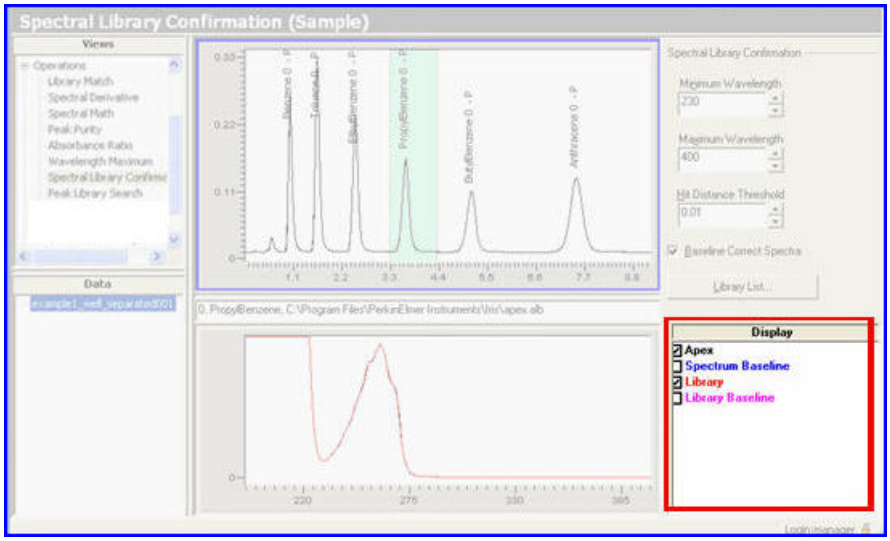
  You can right mouse click on this section and select Properties from the context menu that appears to view detailed information on the library spectrum, such as the source, the chromatogram and retention, the baseline, and who added the spectrum and when.

.

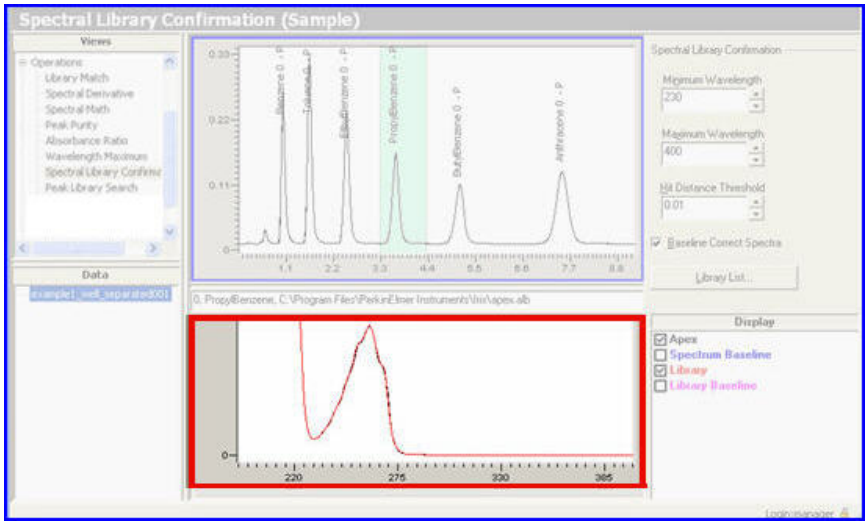


- **Display List** - You use the Display List to select the information you want displayed on the Results pane. When items appear checked on this list, they are displayed in the Results Pane. Meanwhile, unchecked items are not displayed.

  The following items are available for selection on the Display List:
  - **Apex** - Check this item to display the apex spectrum from the chromatogram on the Results pane.
  - **Library** - Check this item to display the matching named spectrum from the library on the Result pane.
  - **Spectrum baseline** - Check this item to display the baseline spectrum used for the apex spectrum on the Results pane.
  - **Library baseline** - Check this item to display the baseline spectrum used for the library spectrum on the Results pane.



- **Results Pane** - The Results pane displays the items that are checked on the Display List.



How do I modify the parameters used in the search?

Spectral Library Confirmation Parameters

The Parameters pane is where you set and investigate the various parameters for perform the Spectral Library Confirmation on a chromatogram that is currently selected on the Data Tree. Once you have a chromatogram selected on the Data Tree, you can optimize/adjust the parameters that are displayed on the screen at any time; and Chromera automatically updates the display with the new results.

Below is a list of the parameters you can optimize/adjust:

Parameter	Description
Minimum Wavelength	Use the Minimum Wavelength spin box to set the lower limit of the spectrum range to be used in the calculation.
Maximum Wavelength	Use the Maximum Wavelength spin box to set upper limit of the spectrum range to be used in the calculation.
Hit distance threshold	Use the Hit Distance Threshold spin box to specify the Hit Quality above which two spectra will not be considered a match. The results of the match are displayed as labels for each peak on the chromatogram. If the match for a peak on the chromatogram meets the Hit Distance Threshold specified, then the peak is labeled with a **P**, to indicate the test passed. If the match for a peak on the chromatogram does not fall within the Hit Distance Threshold, then the peak is labeled with an **F**, indicating that the test failed.
Baseline corrected	Check this option to use the baseline corrected spectra in the operation.
Library list	Select this command button to display the Libraries List dialog.

Summary of Operation: Spectral Library Confirmation

The summary below illustrates how to optimize/adjust the Spectral Library Confirmation results for a chromatogram and then save the new results and parameters used in the operation.

How do I review and adjust the Spectral Library Confirmation values for a chromatogram?

1. From the **Views** tree, expand the **Operations** node and click on **Spectral Library Confirmation**.

   The Spectral Library Confirmation operation displays.

2. If necessary open the required chromatogram using **File > Open > Chromatogram...**

3. Select a chromatogram from the Data Tree.

   The selected chromatogram is displayed. The first peak is selected.

   The results pane, directly below the chromatogram, shows, for the selected peak, its peak apex spectrum, the matching library spectrum, and optionally the baseline spectrum for the peak selected on the chromatogram and the baseline spectrum for the matching library spectrum.

How do I view the Spectral Library Confirmation values for a different peak?

- Select the required peak by clicking on it in the chromatogram.
  The results pane is updated to show the peak apex spectrum, the matching library spectrum, and optionally the baseline spectrum for the selected peak and the matching library spectrum.

How do I examine the Spectral Library Confirmation values at different wavelengths?

- Enter a new value for **Wavelength A** or **Wavelength B**.
  The Spectral Library Confirmation value labels are updated for every peak in the chromatogram.

How do I examine the effect of using baseline corrected spectra on the Spectral Library Confirmation values?

- Check **Baseline Correct Spectra**.
  The Spectral Library Confirmation value labels are updated for every peak in the chromatogram.

How do I change the spectral library/libraries that are used in the match?

Select the Library List... button.

The Libraries List dialog appears.

1. Specify the directory path where the libraries you wish to search are located by typing the directory path in the Look in text box, or by clicking on the **Browse...** button and selecting a directory path from the Browse for Directory dialog box.

Once you have specified a directory path, a list of libraries available for selection appears on the Libraries list dialog.

2. From the **Available libraries** list, click on the spectral library/libraries you want to use and then click on the **Add** button.

   You can either click on a single library to add it to the Libraries list, or you can select multiple libraries to add to the Libraries List by using CTRL+click or SHIFT+click.

   The selected libraries now appear in the Libraries List.

3. To remove a library from the Libraries list click on the library and then click **Delete**.

   The selected library is removed from the Libraries list and reappears in the Available Libraries list.

4. Click **OK**.

   You are returned to the Spectral Library Confirmation operation, and each peak that Chromera was able to match is labeled with the identically named spectrum from the spectral library, along with the Hit Quality of the match, and a Pass/Fail flag that indicates whether or not the test passed or failed.

How do I add the spectra that are displayed in the Results pane to the Data Tree?

- Select **Actions > Add All to View**.
  A dialogue box is displayed with the message "The data was added successfully". On re-displaying the Main View or Chrom/Spectra View the added spectra will appear in the Data Tree.

How do I save the results and parameters used in the calculation?

1. In the main Chromera application, select **File > Save Results.**

   A save dialog appears that asks you to confirm whether or not you want to save the new values.

   Or

   If you select another item in the Spectral Processing Window the following dialog appears:

2. Click **Yes** to save the results and calculation parameters.

3. The saved results can now be displayed as peak labels when the chromatogram is displayed in the Main View or Chrom/Spectra View.

How do I display the Spectral Library Confirmation results in the Main View or Chrom/Spectra View?

1. Select Actions > Label Chromatograms...

2. Select **Spectral Library Confirmation** from the list of available labels

How do I review for any chromatogram previously calculated Spectral Library Confirmation values and the parameters used?

- Select this chromatogram again on the **Spectral Library Confirmation** operation.

Peak Library Search

The Peak Library Search operation allows you to easily identify all the peaks in a chromatogram by comparing the apex spectrum of each peak in a chromatogram to spectra contained in libraries that you specify.

- To access this operation, expand the **Operations** node on the **Views** tree and select **Peak Library Search**

Identifying Chromatographic Peaks

In order to perform a Peak Library Search you must first select a chromatogram from the Data Tree. At this point you may be prompted to select a list of libraries to search, if a search has not been previously performed on the chromatogram. If you have already performed a search on the chromatogram, Chromera uses the previously defined set of libraries for the search. At any time, however, you can modify the list of libraries that are used in the search by clicking on the Library List button, which is located in the right-hand portion of the Peak Library Search operation.

Once you have selected a chromatogram and built a list of libraries to be searched, Chromera automatically searches the specified spectral libraries for a match to the apex spectrum of each peak in the chromatogram; and the results are immediately displayed on the screen. Notice that each identified peak on the displayed chromatogram is labeled with the **Component Name** of the best match as well as the **Hit Quality Value** (a numerical value that indicates how close a match the currently displayed peak is to the component name from the matching library spectrum, the lower the Hit Quality Value, the closer the match). If a match for a peak could not be found, the unmatched peak is labeled with "Not Found."

If you want to evaluate how the best match for a specific peak was calculated, then click on that peak. In the pane located below the chromatogram plot, the apex spectra for the currently selected peak is displayed along with the library spectrum considered to be the best match.

In addition, in the lower right hand corner of the Peak Library Search operation, a list of library spectra check boxes is displayed. The Library spectra checkboxes are listed by their Hit Quality, then by component name; the spectra with the lowest Hit Quality number is considered the best match. To visually compare how close the apex spectrum of the currently selected peak matches to additional library spectra, check the Sample check box, and then check any number of library spectra listed on the Hits List that you wish to compare. The selected spectra are displayed in the Results pane, which is located directly below the Chromatogram pane.

How does is a Peak Library Search performed?

As stated earlier, the Peak Library Search operation searches spectral libraries for a match to the apex spectrum of each peak in the chromatogram. The comparison of the spectra is made by calculating the Euclidean distance algorithm; the numerical result of which is called the Hit Quality. The Hit Quality Value, or distance between the two spectra, can very between 0 and 1.4. The lower the Hit Quality number, the closer the spectra and the better the match.

When the Peak Library Search operation is performed, and a peak is selected on the chromatogram you will notice that a list of all the library spectra that are considered a match

to the peak apex spectrum of the currently selected peak are displayed. The library spectra displayed here are considered a match, because the distance between these library spectra and the peak apex spectrum (for the currently selected peak) fall within a user-specified Hit Distance Threshold.

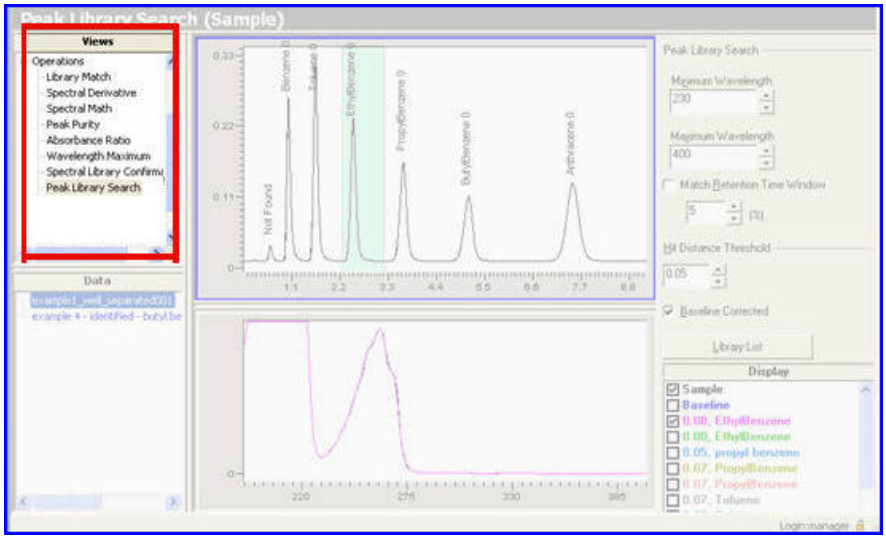
For verification purposes, Chromera uses a default Hit Distance Threshold of 0.05 to confirm which peak apex spectrum is the same component as that in the spectral library. Therefore, when the distance between a sample spectrum and a library spectrum is less than 0.05, the sample peak is labeled with the component name of the best matching library spectrum. It should be noted that, a Hit Quality value higher than 0.05 suggests either the peak in the sample is impure or that it is not the same component as in the spectral library.

You can modify the Hit Distance Threshold value, to include or exclude library spectra as a match to a spectrum at the peak apex in the chromatogram. For instance, to prevent unlikely matches you may wish to set a lower maximum hit threshold.

Tell me about the Peak Library Search Operation.

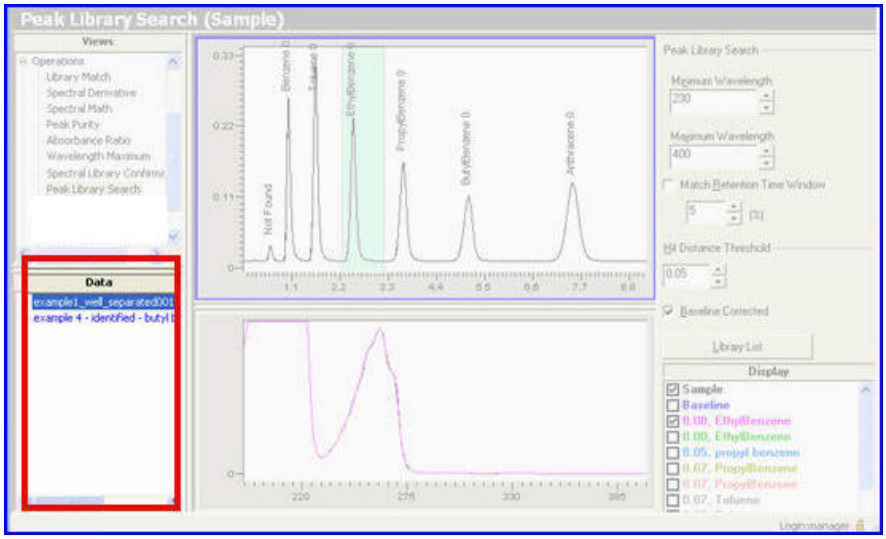
The Peak Library Search operation is comprised of the following areas:

- **Views Tree** - The Views Tree provides you access to the Peak Library Search operation. You can access this operation by expanding the **Operations** node and selecting **Peak Library Search**.

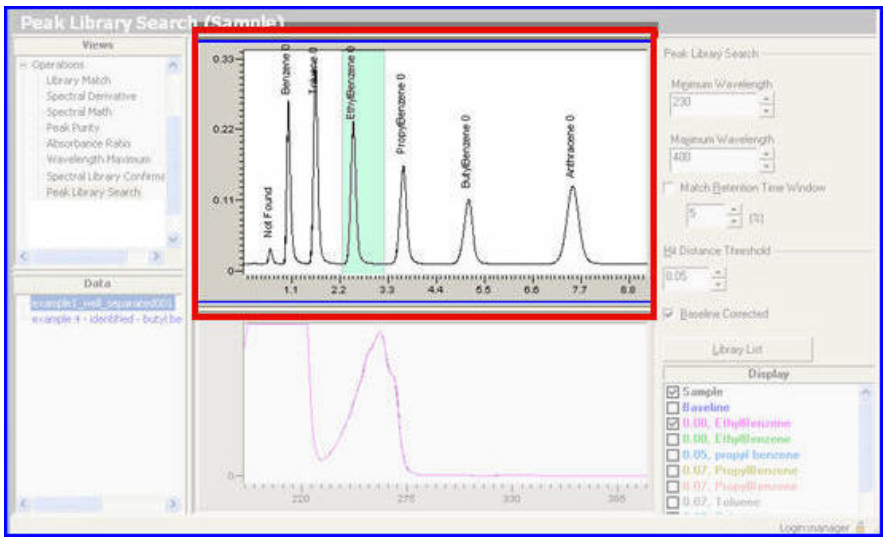


- **Data Tree** - The Data tree here shows only a list of chromatograms and is used to select the required chromatogram from the ones available. The selected chromatogram is displayed in the top graph (Chromatogram pane).

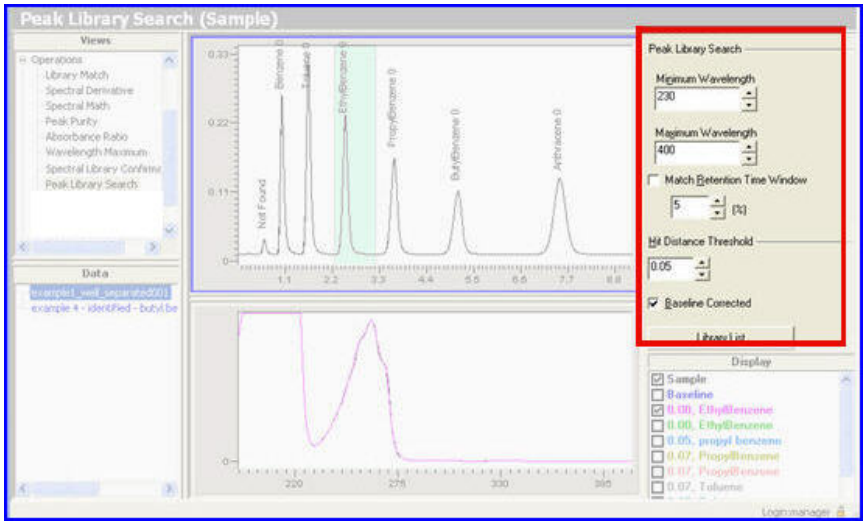
  As different chromatograms are selected the nearest peak is selected, the other two graphs are updated and chromatogram labels are updated.



- **Chromatogram Pane** - When a chromatogram is selected on the Data Tree, the chromatogram is displayed on this pane and the peak maxima are labelled with the top library hit name, as calculated by the current settings or with "Not found" when unable to find a match for the peak. The labels are automatically updated with every change in parameters. When a chromatogram is loaded or changed these labels are calculated and displayed as soon as possible.



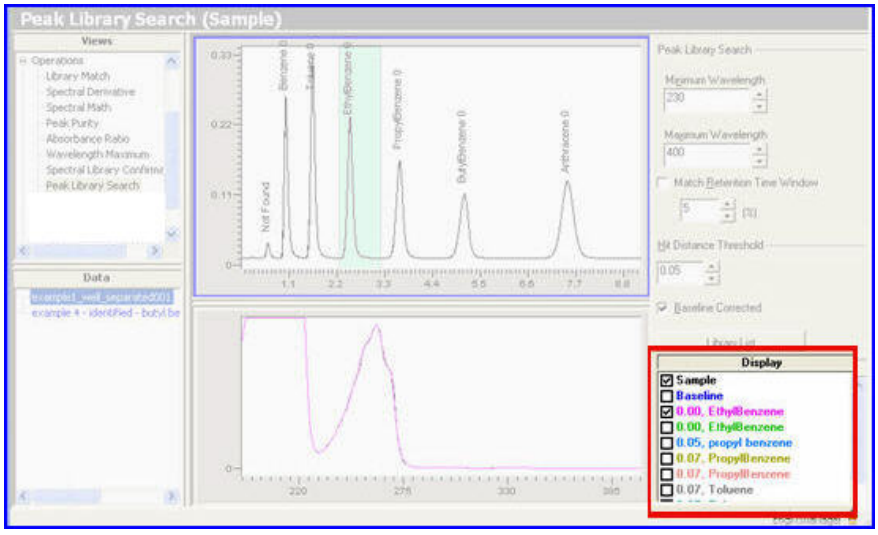
- **Parameters Pane** - From this pane you can change the minimum and maximum wavelengths to be used for the search; you can also change the Hit Threshold value, which defines whether a valid match has been found, and you can specify whether retention time is to be used to restrict the search, as well as whether or not you wish to use baseline corrected spectra for the library match.



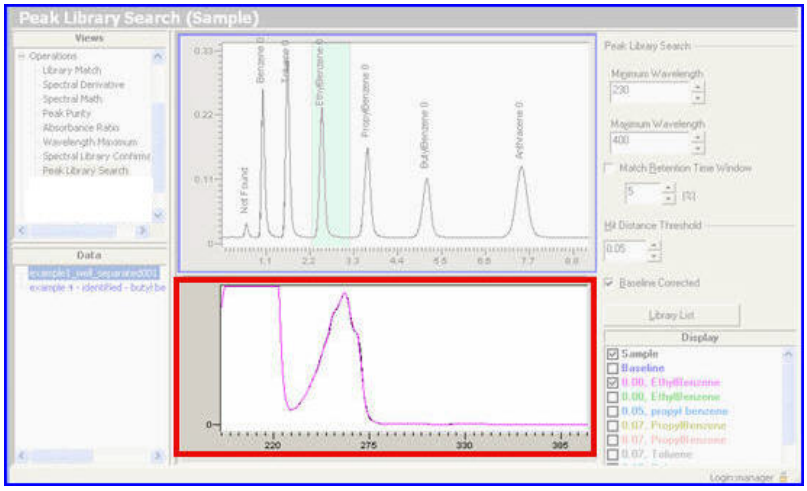
- **Hits List** - The Hits List contains a list of check boxes for selecting what is displayed on the Results Pane. Items that appear checked on this list are displayed in the Results pane, unchecked items are not displayed in the Results pane.

  The first item in the list, labeled Sample, is the apex spectrum of the currently selected peak. You can check this item to compare how close the apex spectrum of the currently selected peak matches any of the library spectra that are listed here as hits.

  The remaining items, that appear on this list, are the hits from the library match, listed in hit order, with the best hit first. Each hit is listed by the Hit Quality value, and component name. You can right mouse click on a hit and select Properties from the context menu that appears to display additional information about the spectrum.



- **Results Pane** - The Results pane displays the items that are checked on the Hits List. You can use this pane to compare how close the apex spectrum of the currently selected peak matches to the hits returned from the search.



How do I modify the parameters used in the search?

Peak Library Search Parameters

The Parameters pane displays the values that are currently used in the search. At any time you can modify the parameters that are listed on the Peak Library Search Page; and the display automatically updates with the recalculated results.

Below is a list of the parameters you can modify:

Parameter	**Description**
Minimum Wavelength	Use the Minimum Wavelength spin box to set the lower limit of the spectrum range to be used for the match.
Maximum Wavelength	Use the Maximum Wavelength spin box to set upper limit of the spectrum range to be used for the match.
Match retention time window	Check this option if you wish to use the retention time of the spectrum as one of the search criteria. When this option is checked the % spin box is enabled.
%	This spin box specifies the search window, as ± percent of retention time, if you have checked the Retention Time Search option. If you use retention time as a search criterion, the search first creates a list of all spectra in the library that fall within the window; it then does a spectrum-by-spectrum comparison. If you don't use the retention time option, the comparison is made with all spectra in the library.
Hit distance threshold	Use the Hit Distance Threshold spin box to specify the Hit Quality above which two spectra will not be considered a match.
Baseline corrected	Check this option if you wish to use baseline corrected spectra for the library match.
Library List	Click this button to display the Libraries List dialog.
Update Component List	Click this button to update the component list in the Chromera method with the new component names and retention times. The new identified peaks and their retention times will simply be added to the current component list.

Summary of Operation: Peak Library Search

The summary below illustrates how to perform the Peak Library Search Operation for a chromatogram and then label the peaks in that chromatogram with the results.

How do I identify the peaks in a chromatogram using a library/s of standard spectra?

1. From the Views tree expand the **Operations** node and click on **Peak Library Search**.

   The Peak Library Search operation displays.

2. Select a chromatogram from the Data Tree.

NOTE: *If the Peak Library Search operation has not been previously performed on the chromatogram that you just selected, the Libraries List dialog is displayed from where you must select the spectral libraries you want to search. This dialog is not displayed if the Peak Library Search operation has already been performed on the chromatogram and the results were saved via the File > Save Results command. Instead, the Peak Library Search operation is displayed and the previously selected library/s are automatically selected for the operation.*

The selected chromatogram is displayed and the first peak is selected.

Note that each identified peak on the chromatogram is labeled with the **Component Name** of the best match as well as the **Hit Quality Value** (a numerical value that indicates how close the peak apex spectrum matches the component name from the matching library spectrum, the lower the Hit Quality Value, the closer the match). If a match for a peak is not found, the unmatched peak is labeled with "Not Found."

In addition, the Results pane, directly below the chromatogram, shows, for the selected peak the apex spectrum of the peak, and optionally its baseline spectrum. The spectrum from the library with the lowest Hit Quality is also displayed in the Results pane.

How do I view the Peak Library Search values for a different peak?

- Select the required peak by clicking on it in the chromatogram.
  The Results pane is updated to show the peak apex spectrum and the spectrum from the library with the lowest Hit Quality.

How do I visually compare how close the apex spectrum of the currently selected peak matches to additional library spectra?

1. Check **Sample** on the Hits List.

   The peak apex spectrum from the currently selected peak is displayed.

2. Check any number of library spectra on the **Hits List** that you want to compare.

   The selected spectra are displayed in the Results pane.

How do I examine the Peak Library Search values at different wavelengths?

- Enter a new value for **Wavelength A** or **Wavelength B**.

The Peak Library Search value labels are updated for every peak in the chromatogram.

How do I examine the effect of using baseline corrected spectra on the Peak Library Search values?

- Check **Baseline Correct Spectra**.
  The Peak Library Search value labels are updated for every peak in the chromatogram.

How do I add the displayed spectra in the results pane to the Data Tree?

- Select **Actions > Add All to View**.
  A dialogue box is be displayed with the message "The data was added successfully". On re-displaying the Main View or Chrom/Spectra View the added spectra will appear in the Data Tree.

How do I save the results and parameters used in the calculation?

NOTE: *When you save the results of this operation, any existing component lists are deleted and a new component list is created.*

1. In the Chromera main application, select F**ile > Save Results**

   A save dialog appears that asks you to confirm whether or not you want to replace the component list in the chromatogram's file and asks you to confirm whether or not you wan to save the new values to the chromatogram's file, and save the parameters that were used calculate the new values to the associated spectral method files.

2. Click **Yes**.

   A dialog appears that asks you to confirm that you are sure you want to replace the component list in the chromatogram's result file.

3. Click **Yes**.

   Peak Library Search values added to the result file.

How do I display the Peak Library Search results in the Main View or Chrom/Spectra View?

1. Select Actions > Label Chromatograms...

2. Select **Peak Library Search** from the list of available labels.

How do I review for any chromatogram previously calculated Peak Library Search values and the parameters used?

- Select this chromatogram again on the Peak Library Search operation.
  The saved results and the search parameters are automatically displayed.

Preview Chromatograms

The purpose of this function is to automatically generate the optimum wavelength program. No chromatogram will actually be created by the Spectral Processing window (other than that used to preview within the Spectral Processing window the resulting chromatogram), instead a wavelength program channel description will be generated which will be added to the method being edited in GME. Preview Chromatograms has no controlling parameters and is used simply to preview possible extracted chromatograms. It is not stored with the method but will revert to default values each time it is called, even within the same session. **Preview Chromatograms is only accessible when you are in Graphics Method Editor (GME).**

Note: *The result from the Spectral Processing window function Preview Chromatograms is not included in this list since the output from this function is not a numerical value but a chromatogram which is not of course a result in the terms used here. A user may add the definition of the parameters required for the generation of this chromatogram (Analytical and Reference wavelengths and bandwidths) to the PDA channels section of the method if they so wish when saving the results from the Spectral Processing window back into Chromera.*

The **Preview Chromatogram** operation allows you to preview chromatograms from a spectral file and automatically process them.

- To access this page expand the **Operations** node on the **Views** tree and select **Preview Chromatograms**.

Previewing Chromatograms at Different Wavelengths

To preview chromatograms at different wavelengths you must first select a **Channel** in the **Data Tree**. The selected chromatogram is displayed in the top graph of this page. You can then use the bottom graph to preview a chromatograms. To preview chromatograms all you need to do is enter an analytical **Wavelength** for the chromatogram you want to preview. This operation also provides you with the ability to specify the following additional parameters for each channel you want to preview: Bandwidth, Reference Wavelength, and Reference Bandwidth.

After you have finished establishing the preview parameters click the **Add to Method** button. Once the parameters are saved, you can then use Reprocess to reprocess the batch.

For example...

In the following example a chromatogram named **Example 2 - identified but with coelution** is selected for the operation and the following parameters are specified to produce 1 chromatogram:

AnalyticalWavelength of **260**

Bandwidth of **1**

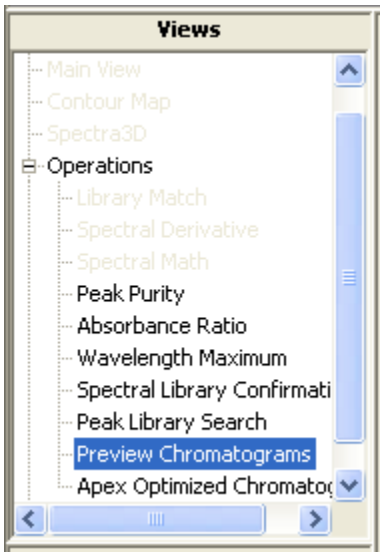
Reference Wavelength of **400**

ReferenceBandwidth of 1

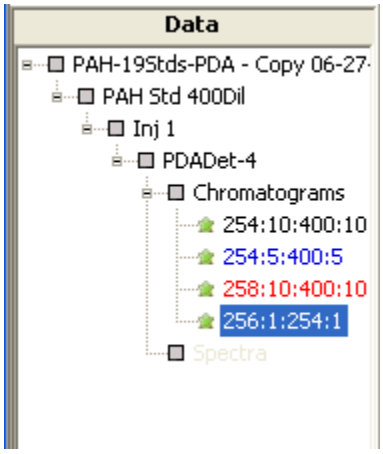
*Tell me about the Preview C**hrom**atograms operation.*

The Preview Chromatograms operation is comprised of the following areas:

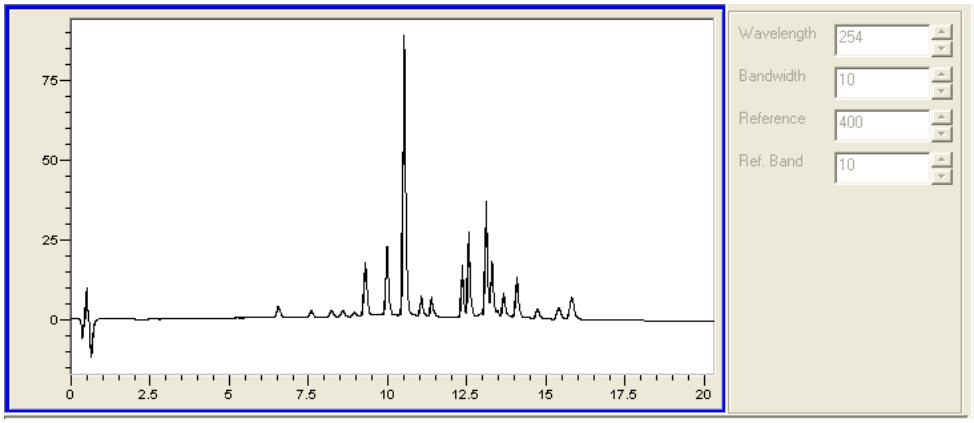
- **Views Tree** - The Views Tree provides you access to the Extract Chromatograms page. You can access this page by expanding the **Operations** node and selecting **Extract Chromatograms**.



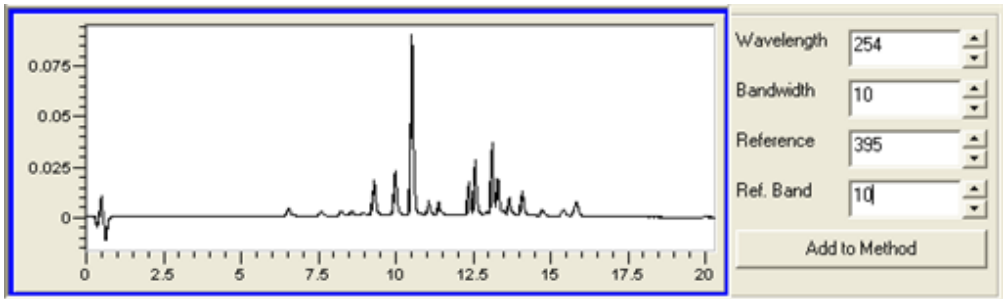
- **Data Tree** -The Data Tree displays a list of the current batches open in Chromera. You use the Data Tree to select the channel that you want to use in the operation. The channel you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.



- **Chromatogram pane** - When you select a channel from the Data Tree, the selected channel is displayed here. To the right of the displayed channel are the parameters under which the channel was originally extracted.



- **Preview Chromatograms pane** – Once you have selected a channel from the Data Tree, you can use the Preview Chromatograms pane to view the sample chromatogram at different wavelengths.



Preview Chromatograms Parameters

You can specify the following parameters for the chromatogram you want to preview:

Control	Description
Wavelength	Enter the analytical wavelength for the extracted chromatogram in this text box. The available wavelength range for this text box depends upon the range available from the original spectrum.
Bandwidth	Enter the analytical bandwidth for the extracted chromatogram in this text box. The bandwidth is the total width of the wavelengths used. The Analytical or Reference Wavelength is centered in this range, thus an Analytical Wavelength of 250 nm and a Bandwidth of 10nm would use data in the range of 245-255nm. ***Note:*** *The Bandwidth cannot be set such that it would result in data stretching past either end of the current wavelength range. Thus, if you are working in the UV range, 190-400 nm, and set an analytical wavelength of 195 nm, the maximum bandwidth you can set is 10 nm, +/- 5nm.* *Setting a bandwidth too wide or too close to the end of the range for the current bandwidth will result in an error message that provides you with assistance in setting valid values.*
Reference	Enter the reference wavelength to be used in this text box.
Reference bandwidth	*A text box for entering the reference bandwidth to be used.* ***Note:*** *To turn off the Reference Channel enter 0 as the value for Reference bandwidth. A message will appear when you enter 0 that informs you that when you enter 0 the Reference Channel is turned off, to re-enable the Reference Channel.*
Add to Method	This command adds the channel description created by the Preview Chromatogram operation to the channels section of the method. This operation is therefore always synchronized to the Channels section of the Chromera method.

Summary of Operation: Preview Chromatograms

The following procedure shows you how to preview a chromatogram at conditions other than the original conditions.

1. From the **Views Tree** expand the **Operations** node and select **Preview Chromatograms**.

2. The Preview Chromatograms operation displays.

3. Select a **channel** from the **Data Tree**.

4. The selected chromatogram is displayed in the top pane of this page.

5. In the bottom pane, specify the **Analytical Wavelength**, **Bandwidth**, **Reference Wavelength**, and **Reference Bandwidth** for the chromatogram you wish to extract.

6. As soon as you enter a valid Analytical Wavelength, the chromatogram is displayed.

7. After entering the parameters, click the **Add to Method** button to add this new channel to the method.
   You can use this method with new channel information to acquire or when you reprocess data.

About Calculation Algorithms

The following two major calculation algorithms are used:

- Peak Purity Algorithm
- Euclidean Distance Algorithm

Peak Purity Algorithm

If a chromatographic peak is homogeneous, then the spectra on the leading edge of the peak should be identical to those on the trailing edge. If, however, two or more components are eluting in a single peak envelope, then as long as the two components do not have the identical retention time, the leading edge of the peak will have a higher concentration of the faster eluting component. Spectra from the leading edge will differ from those on the trailing edge.

You therefore can check the homogeneity or purity of a peak by comparing the spectra from its front and back edge. Spectra taken close to the peak start will obviously have a higher percentage of the earlier-eluting component than those taken at the peak apex. Similarly, spectra taken close to the end of the peak will have a higher percentage of the later-eluting component. In practice, of course, the closer to the baseline the spectra are taken, the lower the signal-to-noise will be in the spectrum.

For all spectra collected from the Series 200 DAD, spectra are collected continuously across the peak. Within the spectral processing software, you can therefore select where on the peak you want to take the two spectra.

To check the peak purity, the software first divides the two spectra. If they are identical except for concentration, the result of this division will be a straight line, parallel to the wavelength axis. If, however, the two spectra are different, then deviations from the straight line will occur. The greater the difference between the two spectra, in terms of both the shape of the spectral peak and its wavelength maximum, the greater will be this deviation. The deviation is quantitated simply by dividing the maximum in the resulting plot by the minimum.

If the spectra are identical, the resulting plot is a straight line, and the Purity Index will be 1.00. Note that this result is independent of the concentration of the components in the two spectra.

If the two spectra are not identical, then the plot deviates from the straight line and the Absorbance or Purity Index will always be greater than 1.00.

The test is very sensitive, and two spectra are rarely absolutely identical. We have found in practice that a value between 1.00 and 1.50 will usually indicate that the spectra are the same and that the peak is pure.

Two practical factors have to be considered when actually performing the test. The first is that a result of dividing two noisy numbers close to zero is very unstable and could easily affect the minimum or maximum value in the final calculation. To avoid such errors, an absorbance threshold is set in the calculation. Absorbances in the spectra that fall below this threshold are not used in the calculation. The default value for this absorbance threshold is 0.0005 AU, or 2% of the maximum absorbance in the spectrum, whichever is the greater. This threshold value can be changed within the application.

The second factor results from the threshold just described. Clearly, the threshold removes some data points from the calculation. It is possible for low- absorbance spectra, especially if the minimum and maximum wavelength has to be set to a narrow range, to have insufficient points for a valid calculation to be performed. A second threshold, the Minimum Data Points threshold, is therefore applied, which determines if the calculation can be performed. This value defaults to 20, but it can be changed.

Euclidean Distance Algorithm

The Euclidean Distance is basically a vector product correlation calculation. If S is an array of absorbances for the "unknown" sample, and L is an array of a spectrum in a library, the HQI (Hit Quality Index) for the Euclidean Distance method is calculated by:

HQI = sqrt(2) * sqrt(1-(sum(S * L)/[(sum(S * S)) * (sum(L * L))])

The "sum(S * S)", etc. items indicate a dot product operation. The dot product operation results in a single value that is the sum of the multiplication of two vectors (arrays). Once additional note is that both the sample S and library L spectra are normalized to have a minimum value of zero (0) and a maximum value of one (1) before the HQI calculation. As you can see, if the S and L vectors are identical, the algebra works out to give an HQI of zero (0) indicating a perfect match. If the S and L vectors are exact "antispectra," then the HQI will return the maximum allowed value of sqrt(2) which is ~1.414. In practice, neither of these cases ever happens. Basically, the smaller the HQI, the better the match.

One thing to remember about searching is that, although you will always get a hit list, this does not mean that the hit with the lowest HQI in the list is the actual compound you measured. It only means it is the most similar one in the library. Unfortunately, there is no statistical significance or cutoff level that can be assigned to the HQI to determine whether you actually have the same compound as the library spectrum. In other words, it is a fairly relative measure. What is more useful, in many cases, is to look at the whole hit list for compounds with similar chemical makeup to elucidate the class of the "unknown" compound.

Printing and Presentations

Printing and Presentations

You can easily create high-quality presentation output.

- You can add your own annotations to the views
- You can copy chromatograms and spectra other programs
- You can print chromatograms and spectra at high resolution and in color

Annotations

You can create freehand labels in any view page, and then print the annotated view at high resolution. The annotations are free-floating text and are not associated with a specific position on a chromatogram or spectrum. If you annotate a specific point on a chromatogram and then change the scaling, the annotation will not move its position on the screen.

NOTE: *It should be noted that annotations are associated with a view; annotations on a chromatogram are not associated with that chromatogram. For example, annotations on a chromatogram in the Chrom/Spectra view will not be displayed in the any other view page. They will, however, stay with the Chrom/Spectra view and will be restored when the view is re-displayed.*

How do I add an annotation?

To create freehand labels in any view:

1. Click on the pane where you want to add an annotation.
2. From the menu bar select **Actions > Annotations >Add**.
   The Edit Annotations dialog appears. From this dialog you enter the text of the annotation and specify the text color, justification, and orientation, as well as the font, size, and style.
3. Make your choices and click **OK**
   The text appears in a box that is the same size as the final annotation.
4. Move the box to the exact location you want.
5. Click outside the box
   The text is placed on the screen.
6. The text can be selected and moved at any time by clicking on it and dragging.

How do I modify an annotation?

You can modify the font and style of any annotation you have placed on the screen as well as several of the automatic text labels (the chromatogram and spectrum labels fall into this category) by selecting **Actions > Annotations > Edit** from the menu bar.

To modify an annotation:

The Edit Annotation command operates only on a selected annotation.

1. Select the annotation to be changed by clicking on it.
   The cursor turns into a four-way cursor, a box appears around the label and the text color is changed to white.
2. Select **Actions > Annotations > Edit** from the menu bar.
   The Edit Annotations dialog appears and from this dialog you can change the annotation or its font, size, and style.

3. Click on the **Annotation tab** to modify the **Text** and **Orientation**.

4. Click on the **Font tab** to select a **Font**, **Font Size**, **Style**, **Color**, and to select whether or not the label is **underlined**.
   This tab page provides you with a preview that shows you the name of the selected font formatted as set on the two tabs, and is automatically updated for each selection made on the dialog.

5. Once you have finished making your changes click **OK**.
   The dialogue box is closed and the selected label with the changes appears.

6. Move the box to the exact location you want.

7. Click outside the box
   The text is placed on the screen.

How do I delete annotations?

- You can delete any annotation you have placed on the screen as well as several of the automatic text labels by selecting the annotation you wish to delete and then selecting A**ctions > Annotations > Delete** from the menu bar.

- If you wish to delete all annotations that appear on a page select **Actions > Annotations > Delete All**.

Copying Images to other Locations

You can copy complete screen images and paste them into applications, such as, Microsoft Word for Windows.

How do I copy the pixels of the screen display as a bitmap image?

To copy the view as a bitmap image:

- From the menu bar select **Edit > Copy Screen As > Bitmap**.
  The entire screen is copied to the clipboard. You can now paste this image into any Windows application that supports bitmap images. If you wish to edit the image you can past the image into an application such as Microsoft Windows Paintbrush.

How do I export the numerical values of a trace for a displayed chromatogram or spectra to spreadsheet programs such as Microsoft Excel?

To export the numerical values of a trace:

You can export data displayed on a chromatogram or spectra pane to spreadsheet programs such as Microsoft Excel by using the Edit > Copy Object command. This command copies the numerical values of the trace to the clipboard.

1. Click on the chromatogram or spectra pane that you wish to copy.

NOTE: *You can only copy contents from a stacked chromatogram or spectra pane.*

2. From the menu bar select **Edit > Copy Object**.
   The numerical values of the trace are copied to the clipboard. You can paste these values into a spreadsheet program such as Microsoft Excel.
   Spectra data are copied to the clipboard in wavelength/absorbance pairs in 1nm resolution from 195nm to 365nm. Chromatographic data are copied to the clipboard in retention time/absorbance pairs. The resolution of the data is at the original data collection rate.

Printing Chromatograms and Spectra

The Print dialog allows you to specify the format for printing chromatograms and spectra that are currently displayed.

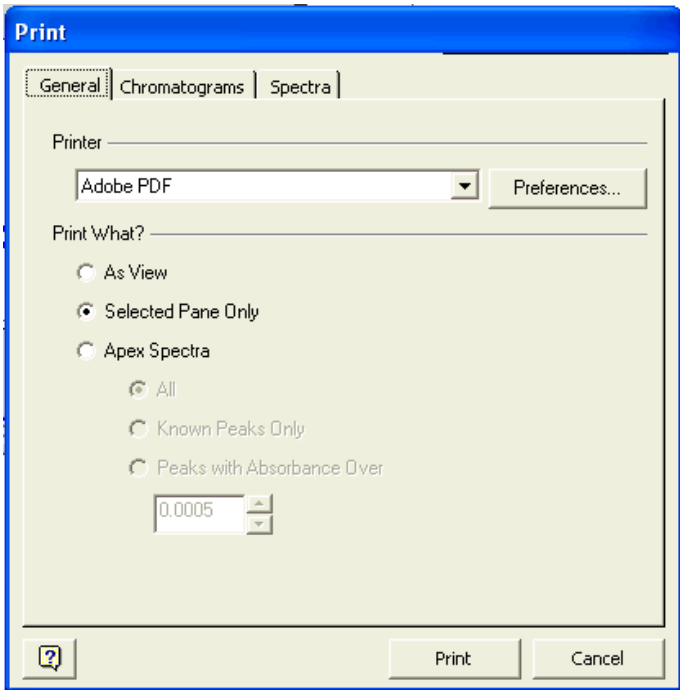
- To print chromatograms and spectra from a view select **File > Print...** from the menu bar.

NOTE: *Operations can only be printed as the view displayed on your screen. You can, however, select a printer and set the printer preferences.*

Tell me about the Print dialog.

The print dialog consists of three tab pages:

- **General tab page** - This tab page is always enabled and allows you to select where and what you would like to print.



The following options are available to on the General tab page:

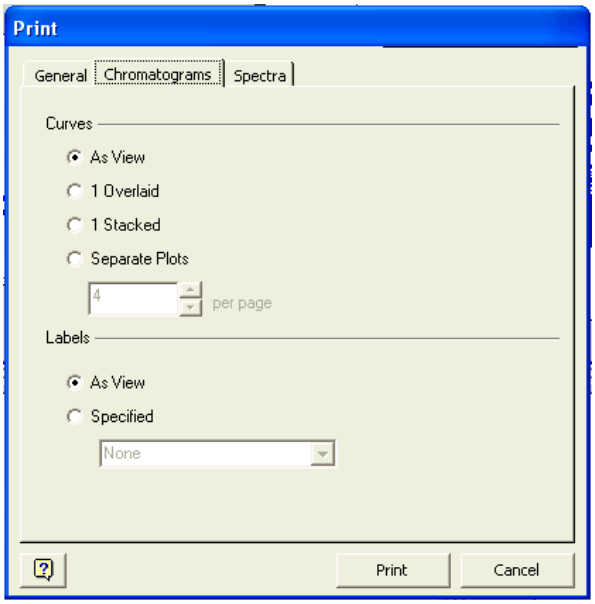
- **Printer** - Select a printer from the drop down list.
- **Preferences** - Click on the Preferences button to define the settings for the printer.
- **Print What** - Select one of the following radio buttons:

  - **All Panes in View** - Prints the current workspace.
  - **Selected Pane Only** - Prints only the contents of the pane that is currently selected. When this option is selected the Chromatograms and Spectra tab pages are enabled.

  - **Apex Spectra** - Allows you to print the parent chromatogram and associated apex spectra. When this radio button is selected you must also select whether or not you wish to print All of the apex spectra, or just the apex spectra from known peaks, or to print the apex spectra for peaks that are above the set absorbance limit.

- **Chromatogram tab page** - This tab is enabled when a chromatogram pane is selected on a View page and the Selected Pane only radio button is selected on the General tab of the Print dialog.

From the Chromatograms tab page you can select define whether or not you want the chromatograms to be printed as overlaid on one plot, stacked on one plot, or printed on separate plots. You can also specify which label will be displayed on the printed chromatograms.



The following options are available to you on the Chromatograms tab page:

Curves - From this section you select how chromatograms will be displayed on the print-out.

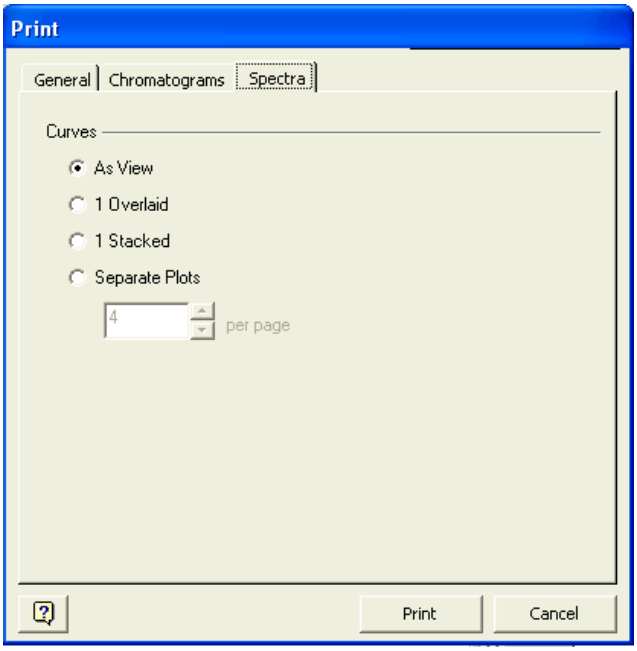
- **As View** - Prints an image of the chromatogram pane that is currently selected.
- **1 Overlaid** - Prints the currently displayed chromatograms overlaid on one plot.
- **1 Stacked** - Prints the currently displayed chromatograms stacked on one plot.
- **Separate Plots** - Prints each chromatogram on a separate plot. When this option is selected you the **per page** spin box is enabled; and from this spin box you must specify the number of plots that will be printed on each page.

Labels - From this section you specify which label will be displayed on the printout.

- **As View** - Prints the label currently displayed on the workspace for the selected chromatogram pane.
- **Specified** - Enables a drop down menu from where you select a specific label for the printout.

Spectra tab page - This tab page is enabled when a spectra pane is selected on the View page and the Selected Pane only radio button is selected on the General tab of the Print dialog.

From the Spectra tab page you can define the print layout for the spectra displayed in the currently selected pane. You can select to print the currently displayed spectra overlaid on one plot, or stacked on one plot, or you can select to print separate plots for each currently displayed spectrum.



The following options are available to you on the Spectra tab page:

- **All Panes in View** - Prints an image of the spectra pane that is currently selected.
- **1 Overlaid** - Prints the currently displayed spectra overlaid on one plot.
- **1 Stacked** - Prints the currently displayed spectra stacked on one plot.
- **Separate Plots** - Prints each spectrum on a separate plot. When this option is selected you the per page spin box is enabled; and from this spin box you must specify the number of plots that will be printed on each page. on a separate plot. When this option is selected you the **per page** spin box is enabled; and from this spin box you must specify the number of plots that will be printed on each page.

What information is included on the printouts?

The first page of each print out provides you with the following information:

- A title for the document being printed.
  This title is set by the software.

- The complete source identification information for all displayed objects in the report.
  The first page(s) of the printout color coded list all of the chromatograms/spectra printed on the report, so that you can easily identify the source. Please note that this information may be printed on more than one page.

- A date and time stamp of the printing in the time zone where the printing occurred.

- The User name and full name of the person generating the report, that is the person logged in to the software.

- The page number in the form of Page X of N

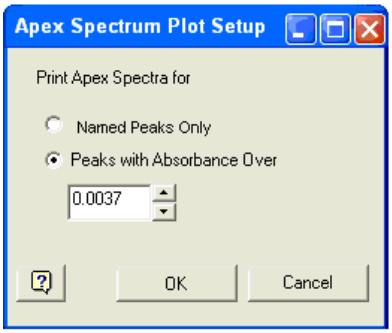
All subsequent pages include the title of the document being printed, the date and time stamp, the user name and full name of the person generating the report, and the page number in the form of Page X of N.

Apex Spectrum Plot Setup

The **Apex Spectrum Plot** is a special report type for use with UV spectral data acquired from a Photo Diode Array (PDA) detector. This report, which must be based upon a Chromera **Single Injection, Multi-Channel** report format, will generate a chromatographic plot of the current injection, as well as a separate plot of the spectrum at the apex of each of the desired peaks from that chromatogram. These reports can be produced either manually, from the **File Print** command within **Post Run** or **Graphic Method Edit**, or automatically from a sequence in **Run Time** or **Batch Reprocess**.

The selection to generate an Apex Spectrum Plot is made though the **Plot Options** of the Chromera **Report Format Wizard**, and the settings for which spectra to print are made through the **Apex Spectrum Plot Setup** dialog of the **Spectral Processing Window** (**SPW**), shown below. These settings are then saved in the **Spectral Operations** section of the associated Chromera method.

1. From the menu bar select **File > Apex Spectrum Plot Setup...**
   The **Apex Spectrum Plot Setup** dialog appears.



2. Select one of the following options to indicate for which peaks **Apex Spectra** are to be printed:
   - **Named Peaks Only** - Select this option to print the apex spectra only for identified peaks.
   - **Peaks with Absorbance Over** - Select this option to print the apex spectra only for peaks that are above the absorbance limit that you enter in the box. By setting this threshold value to zero, the report will produce an apex spectrum plot for every detected peak in the chromatogram.

3. Click **OK** when are finished.

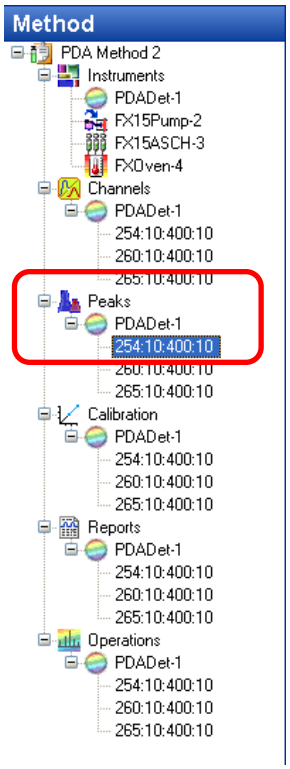
How to Prepare for Printing an Apex Spectrum Plot Report

NOTE: *The following steps must be performed in the order described before preparing chromatograms to be annotated with apex spectra through the Plot Options of the Chromera Report Format Wizard's. This procedure presumes a PDA method has already been created to acquire or reprocess representative spectral data.*

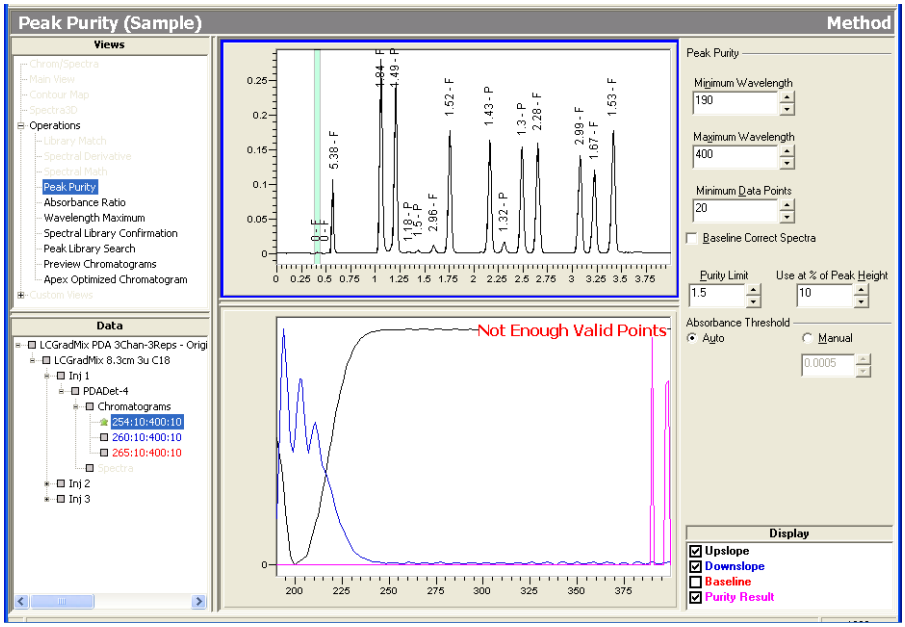
1. First, prepare a method that will be used for automated printing of apex spectra. You can open an existing PDA method with representative data in the Chromera Graphic Method Editor (GME), or open previously acquired PDA spectral data in the Graphic Results Editor (GRE).

NOTE: *A PDA method and associated data must be opened in either GME or GRE, and not through the basic Method Editor, in order to have access to the Spectral Processing Window, which is required to complete the method setup described below.*

2. Select a data Channel (e.g., 254:10:400:10) from the **Peaks** section of the **Method**.

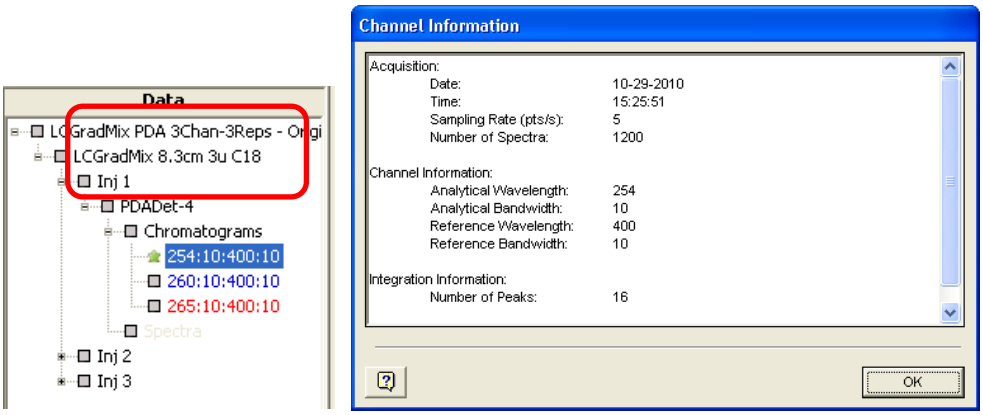


3. Select the option to **Edit Spectral Operation Graphically** from the **Actions** menu. (This command will be visible only from the Peaks or Operations section of the Method. It will be hidden for all other method sections.)



This opens the **Spectral Processing window** in the Peak Purity Spectral Operation view, displaying the same chromatogram that is open in the Graphic Method Edit environment within Chromera, with the Peak Purity parameters being those shown in the Operations section of the method in GME.

4. To view detailed information associated with the selected channel, right mouse click on the chromatogram in the **Data** tree and select **Information** from the popup menu that appears.

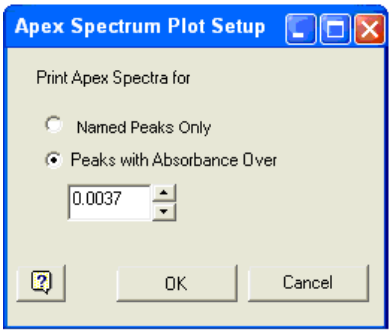


An information dialog appears that shows Channel Information associated with the selected chromatogram. When done viewing the information, click OK to close the dialog box.

5. Make sure that the chromatogram that you just selected is still selected highlighted in the Data tree.

6. From the menu bar select the item **File > Apex Spectrum Plot Setup...**

   The **Apex Spectrum Plot Setup** dialog appears.



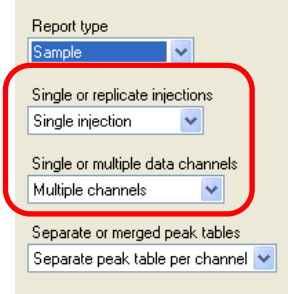
7. From the dialog, select one of the following options to indicate for which peaks **Apex Spectra** are to be printed:

   - **Named Peaks Only** - Select this option to print the apex spectra only for identified peaks.
   - **Peaks with Absorbance Over** - Select this option to print the apex spectra only for peaks that are above the absorbance limit that you enter in the box. By setting this threshold value to zero, the report will produce an apex spectrum plot for every detected peak in the chromatogram.

8. Click **OK** when finished, and these settings will be transferred back to the Spectral Operations Section of the method open in GME, or they will be inserted into the embedded method for the chromatogram open in GRE.

Once the appropriate plot settings have been made through the Spectral Operations section, return to the Chromera application to complete the setup of the report to include the Apex Spectrum plots.

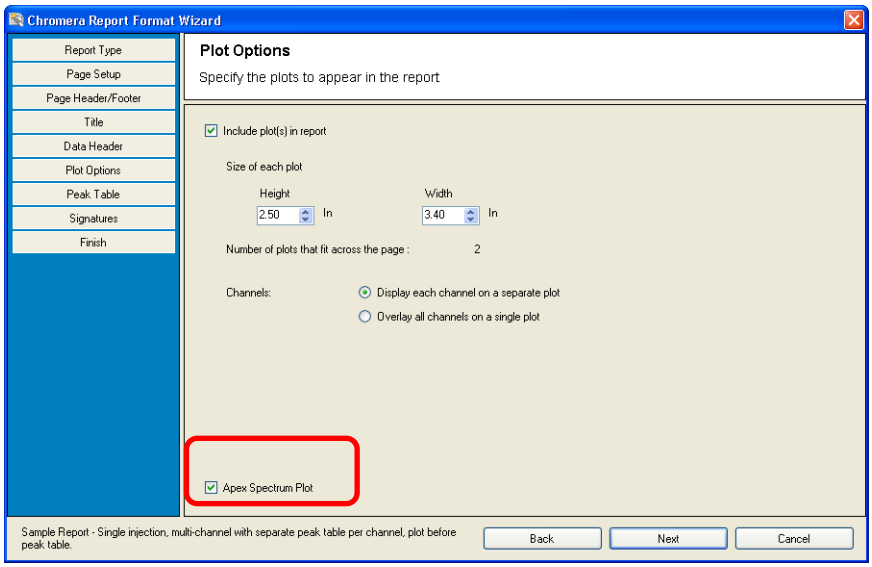
9. In the main Chromera application, select **Report Format Wizard** from the Tools menu to create a new report format or modify an existing one to select the option of generating Apex Spectrum Plots. For this type of report, it is important to select **Single Injection** and **Multiple channels** For the report type.



10. Continue in the Report Format Wizard to adjust the report layout as appropriate.

11. In the Plot Options dialog, select the Apex Spectrum Plot check box.

    This is the only report option necessary for generating these plots. The actual spectra printed will depend upon the peak selection option set in the Spectral Operations section of the Chromera method.



12. Complete the Report Format Wizard as desired, then move to the Finish dialog. Enter a Report Name and Group Name for the template, then click the Finish button.

    The modified report will now be available for producing Apex Spectrum Plots either manually, or from Run Time or Reprocess.

Index

S

T

U

V

PerkinElmer
710 Bridgeport Avenue
Shelton, CT 06484-4794, U.S.A.

Internet: http://www.perkinelmer.com
email: info@perkinelmer.com