IRIS – HPLC Spectral Processing Software

User Manual

PerkinElmer®
precisely.

Iris - HPLC Spectral Processing Software

Release History

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Chapter 1. Introduction

Introduction

IRIS is a spectral application program designed for use with TotalChrom 6.3.1 (or higher) and the Series 200 HPLC System equipped with a Series 200 PDA Detector (Photo Diode Array). With IRIS you can process, manipulate, and display spectral data associated with chromatograms obtained using the Series 200 PDA. In conjunction with TotalChrom, IRIS also contains features and functions to assist laboratories in meeting FDA regulations, such as 21 CFR Part 11, by providing electronic records controls and electronic signature support for all data modified or created by the IRIS application.

This chapter introduces you to the IRIS application and describes how to access the IRIS application. In addition, this chapter also provides you with an overview of the IRIS user interface and information on concepts you should be familiar with before using IRIS, such as how spectral methods are used in IRIS.

About IRIS Spectral Processing Software

IRIS Spectral Processing software provides an easy navigation through the wealth of spectral information provided by Photo Diode Array detection. With IRIS, you can access, display and manipulate spectral data with speed and flexibility. In addition, IRIS provides you with the ability to perform various chromatographic calculations such as confirming and performing peak identification via spectral library matches, calculating and reporting peak purity and wavelength maxima for each component. You can also use the IRIS software to extract and reprocess actual chromatograms at any wavelength you desire - directly from spectral data files.

One of the benefits you will discover, when using IRIS, is that the integration between the IRIS Spectral Processing software and TotalChrom allows you to:

- Easily pass chromatograms between the two applications.

  For example, if you're looking at a chromatogram in TotalChrom's Reprocess, and you wish to examine the same chromatogram in IRIS, all you need to do is select **Spectra** from the **Other** menu and the chromatogram is displayed in IRIS. On the other hand, if you are looking at a chromatogram in IRIS, you can move that chromatogram to one of two TotalChrom environments: **Reprocess** or **Graphic Method Edit**. To pass a chromatogram from IRIS to TotalChrom all you need to do is right mouse click on the chromatogram in the IRIS Data Tree and select **TotalChrom > Reprocess** or **Graphic Method Edit** from the context menu that appears.

- Save the results of any arithmetic operations and/or chromatographic extractions that were performed in IRIS, back in the TotalChrom result file. These results can then be included in a printed TotalChrom report.

- Run the AutoCalc user program, which is shipped with your IRIS spectral processing software, during a TotalChrom sequence to automatically perform any combination of the arithmetic operations provided by IRIS, such as Peak Purity and Spectral Standard Confirmation. As part of either a real-time or reprocessed sequence, the results of these automated operations are automatically saved to the TotalChrom Result file (.RST) and can automatically be included in a TotalChrom report.

  Note: This program also allows the automated extraction of up to 8 chromatograms and printing of chromatograms annotated with apex spectra.

Features of IRIS

With the IRIS 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

- Using IRIS, you can annotate chromatograms with spectral positions, retention times, component names, or calculated values such as concentrations or purity indices.

- To help you visually compare data, chromatograms and spectra can be normalized, offset-normalized, or displayed full scale.

- 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.

- IRIS also provides you with the ability to view chromatographic data on a three-dimensional plot that provides a scaleable perspective on the complete data set.

- Extract and reprocess actual chromatograms at any wavelength you desire - directly from spectral data files.

- Chromatograms, spectra, and other data can be printed using a **color printer**, such as the HP Deskjet 5650. 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.

Starting IRIS

Before accessing the IRIS application, make sure you have a valid **TotalChrom User Name** and **Password**, since access to IRIS is controlled by TotalChrom. If your user account and password have not been set up in TotalChrom, contact your System Administrator who is responsible for maintaining the TotalChrom application, and is the contact person for all technical support issues. *For more information on setting up user accounts, see the section titled **User Management** on page **44**.*

Once you have a valid TotalChrom User Name and Password, you can start IRIS a number of different ways. This section introduces you to the various ways you can start IRIS.

Starting IRIS from Windows

You can start IRIS directly from Windows from the Windows Start menu, or if you have added the IRIS application as a shortcut to your Windows Desktop, you can double click on

the IRIS icon .

➢ If TotalChrom is not running, you will be prompted with the TotalChrom log on screen. From the log on screen, enter your TotalChrom **User Name** and **Password**.
   OR

➢ If you have already logged into the TotalChrom application, you will not need to log on to TotalChrom again to start IRIS. Instead, IRIS will open immediately when you start IRIS from Windows.

To start IRIS directly from Windows:

1. From the Windows Start menu select **All Programs > PerkinElmer > IRIS**.
   The IRIS menu displays.

2. From the IRIS menu click on **IRIS**.
   If you have not already logged into TotalChrom, the TotalChrom log on dialog appears. From the TotalChrom log on dialog, enter your TotalChrom **User Name** and **Password** can click **OK**.
   The IRIS application launches.
   OR
   If you have already logged into TotalChrom, the IRIS application launches.

Starting IRIS from TotalChrom

There are two ways to start IRIS from TotalChrom: from the IRIS icon on the **Navigator screen**, or from a menu command in **Reprocess**.

Starting IRIS from the TotalChrom Navigator:

➢ You can start IRIS directly from the TotalChrom Navigator by clicking on the **Spectra**
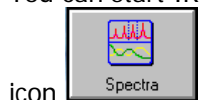
icon .

Note: *The Spectra icon is active whenever an instrument containing a Series 200 DAD has been selected.*

Starting IRIS from Reprocess:

Note: *Before you can start IRIS from Reprocess you must first save the result file. In addition, you must save the result file after you make any change in Reprocess and upon entry into Reprocess, since TotalChrom's Reprocess always reprocesses the data upon entry and the results obtained may differ from the results in the saved *.rst file.*

Any chromatogram you can view in TotalChrom can be opened quickly and easily in IRIS with no need to select the chromatogram again within IRIS. The file you were viewing in TotalChrom appears in the chromatogram region of the IRIS Main View.

➢ If you're looking at a chromatogram in TotalChrom's Reprocess, and you wish to examine the same chromatogram in IRIS, select **Spectra** from the **Other** menu.

Note: *The* **Spectra** *command is active only if there are spectra associated with the chromatogram you are currently viewing.*

You are moved directly into IRIS and the chromatogram is selected on the IRIS Data Tree and displayed on the Main View.

Overview of the IRIS User Interface

IRIS is designed so that you can easily process, manipulate, and display spectral data associated with chromatograms from one main window. Following is a high level look at the IRIS user interface.
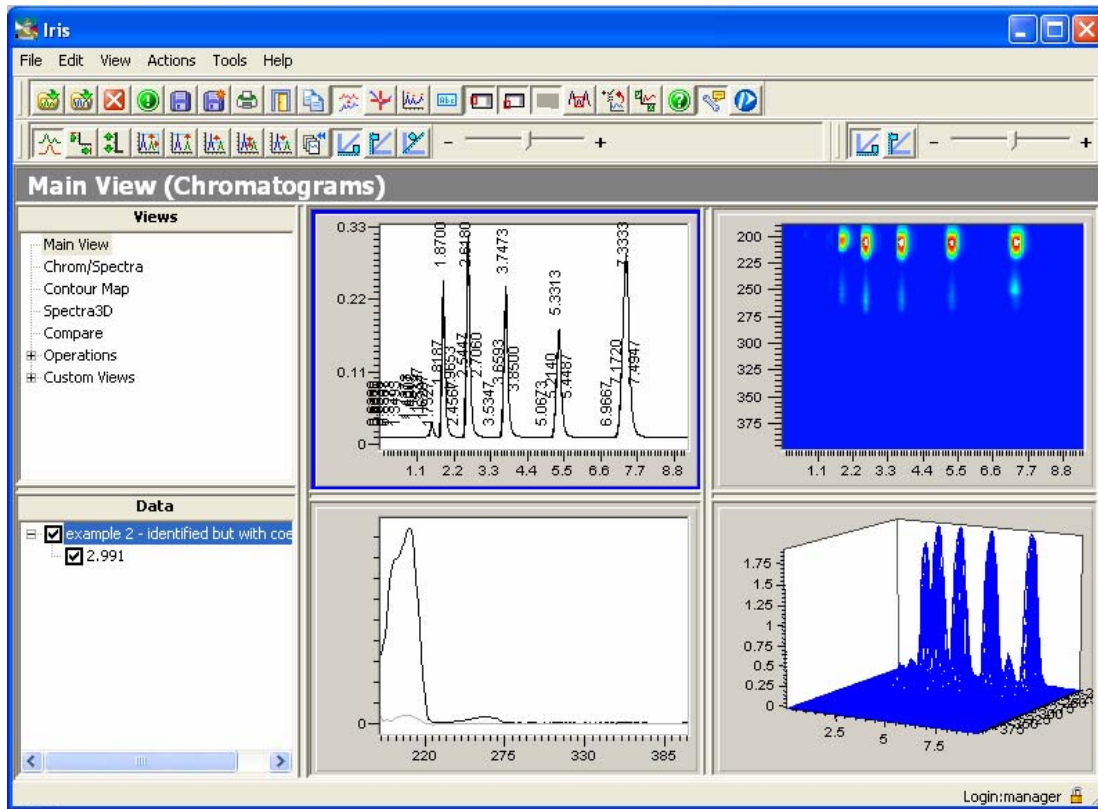
Figure 1-1 The Main View is displayed when the IRIS application is started

The Views Tree

The Views Tree appears in the upper left hand pane of the IRIS window, and is your means of navigating though IRIS. From the Views Tree you select what you want displayed in the right-hand portion of the screen.
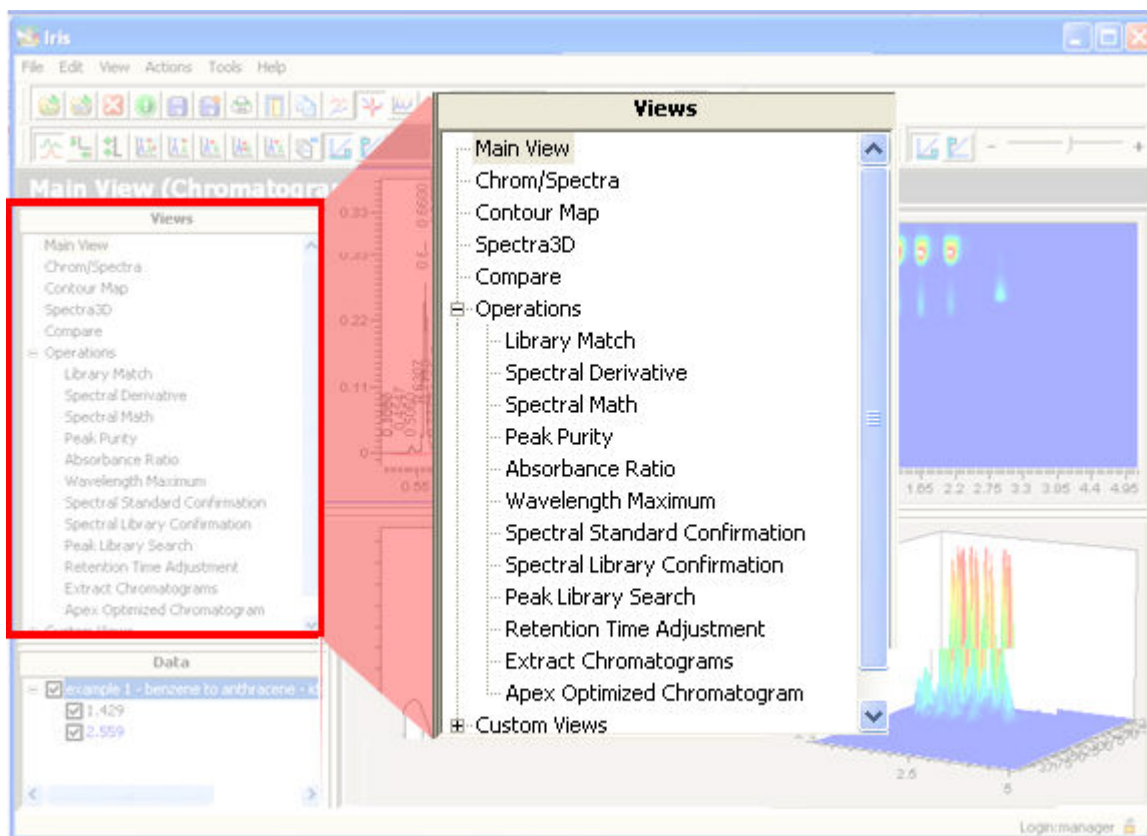
Figure 1-2 The Views Tree

When you select an item on the Views Tree, the right-hand portion of the IRIS 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.

In IRIS you work with Views and **Operations**. Basically, Views allow you to view chromatograms and spectra on the right hand side of the IRIS window. Meanwhile, Operations, which are also displayed on the right hand side of the IRIS 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 IRIS window are listed on the Views Tree.

Views

When IRIS launches, the **Main View** is displayed by default. This view is divided into four panes: a **Chromatogram pane**, a **Spectra pane**, a **Contour Map**, and a **3D Plot pane**.

In addition to the Main View, IRIS also provides you with four other default views that you can select from the Views Tree. The four other default views are labeled: **Chrom/Spectra**, **Contour Map View**, **Spectra 3D View**, and **Compare**.

You can also create your own **Custom View** by modifying an existing view. *For more information on Views in IRIS, refer to the chapter titled **Chapter 5. Viewing the Data** on page **105**.*

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 IRIS 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 that 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. It should be noted that the parameter values that are displayed on an operation come from the parameter values stored in a spectral method. *For more information about spectral methods, refer to* **About Spectral Methods** *on page* **28**.

*For more information on Operations, refer to the chapter titled **Chapter 7. Performing Operations on Spectra** on page **178**, and the chapter titled **Chapter 8. Performing Operations on Chromatograms** on page **199**.*

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.
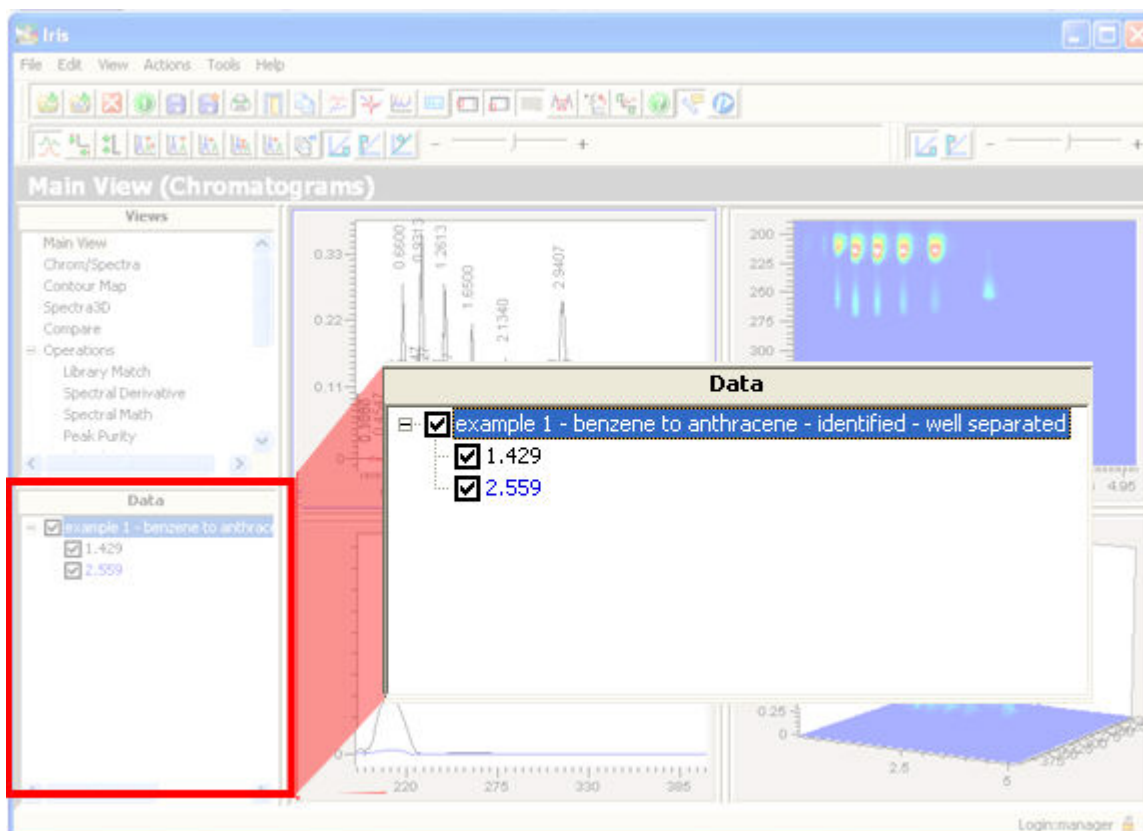
Figure 1-3 The Data Tree

How are Chromatograms and Spectra listed on the Data Tree?

When you open a chromatogram or spectrum, via the **File > Open > Chromatogram/Spectrum...** command, the chromatogram or spectrum appears at the bottom of the list on the Data Tree and the newly opened item is selected on the Data Tree and displayed in the appropriate pane (unless a View or Operation is selected that does not allow you to display the newly opened chromatogram or spectrum).

Spectra that you have extracted from a chromatogram are listed in ascending order on the Data Tree under the parent chromatogram. *For information on extracting spectra, see page 78.*

The Data Tree Context Menu

A right-click on a chromatogram or spectrum in the Data tree displays the context menu that contains a list of commands you can select. The table shown below lists all of the context menu commands. Please note that certain menu commands are enabled or disabled depending upon whether you have a chromatogram or spectrum selected.

Note: *The command selected only applies to the item right clicked on.*

Command	Description
Information...	Displays the relevant information for the item right clicked on.
Audit trail...	Displays the Audit Trail dialog for the item right clicked on.
Close	Deletes the chromatogram or spectrum right clicked on from the Data tree. Closing the chromatogram will remove the Chromatogram from the data tree and close all its associated files such as Contour Map and 3D Plot. Closing the chromatogram will close all its associated spectra.
Save Results...	This command allows you to save the results and the parameters used in an operation performed on a chromatogram. *Refer to the section titled **Saving the Results of a Calculation** on page Error! Bookmark not defined. for more information.*
Save As...	If a **spectrum** is right-clicked on, this command allows you to name and save the spectrum as **.uv** file. If a **chromatogram** is right-clicked on, this command allows you to name and save the chromatogram with a **.RAW** extension
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.
✓ Match color	Toggles all the spectra belonging to the chromatogram right clicked on to the same color as the chromatogram, all other spectra are then turned grey. This option is enabled only when a chromatogram is right clicked on.
✓Baseline Spectra	Adds/removes the baseline spectrum for the spectrum right clicked on in the Data Tree. Baseline spectra are identified by the retention time of the spectrum followed by the word **base**). This command is enabled if a spectrum is right clicked on; and disabled for a baseline spectrum.
Spectral baseline correction...	Displays the Spectral Baseline correction dialog. This option is enabled if a chromatogram is right-clicked on.

Command	Description
S<u>h</u>ow scale	Displays the Y axis scale for the spectrum or chromatogram right clicked on. This command is enabled if the current graph is displayed as overlaid and normalized; and so by default the scale is blank.
View <u>M</u>ethod....	Opens the Method Editor dialog as read-only and displays the parameter values in the chromatogram's spectral method. The chromatogram's spectral method has the same name as the chromatogram's result file; however, the file extension is .tsm. *Fore more information on spectral methods, see page* **Error! Bookmark not defined.**.
TotalChrom ▶ Reprocess... Graphic Method Edit...	Opens the TotalChrom Reprocess or Graphic Method Edit application with the selected chromatogram passed to the application. Enabled if a chromatogram is right-clicked on.

Panes for Displaying Chromatograms, Spectra, Contour Maps, and 3D Plots

The right hand side of the IRIS window can consist of multiple panes for displaying chromatograms and spectra. The type of panes displayed on the right-hand side of the IRIS 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.
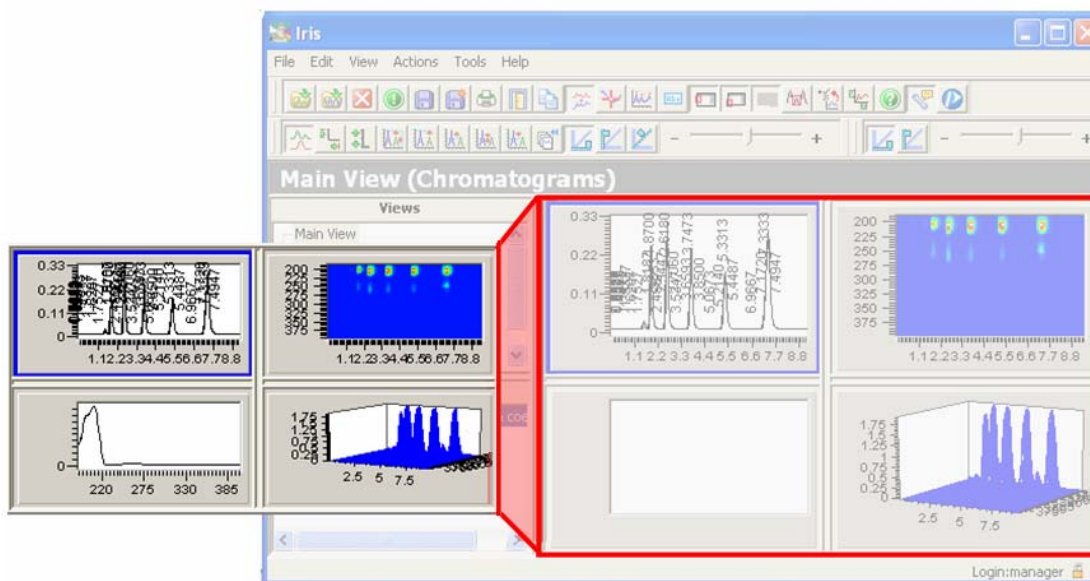
Figure 1-4 The Main View consists of four panes: a Chromatogram pane, Spectra pane, Contour Map pane, and a 3D Plot pane

➢ 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. Detailed information on each of the predefined Views can be found in the chapter titled *Chapter 5. Viewing the Data*.

➢ If an **operation** is selected on the Views Tree, the panes that appear in the right-hand portion of the IRIS 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. Detailed information on each operation can be found in the chapters titled *Chapter 6. Spectral Libraries* and *Chapter 8. Performing Operations on Chromatograms*.

The IRIS Menu Bar

The IRIS 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 actual availability of menu items is determined by their privileges, which are set in TotalChrom.*

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.*

Menu	Command	Description
File	Open ►	Displays a standard Windows file selector set to show the correct type of file (.rst for chromatograms, .uv for spectra, and .tsm for methods).
	Chromatogram...	When you open .rst and .uv files, they are added to the Data Tree and appear selected, so that the opened items are immediately displayed in the relevant panes in addition to the data that is already displayed in the panes. When you open a .tsm file, a read-only version of the Method Editor dialog box appears. From this dialog box you can view the parameter values in the method.
	Spectrum...	
	Method...	

Note about opening files:
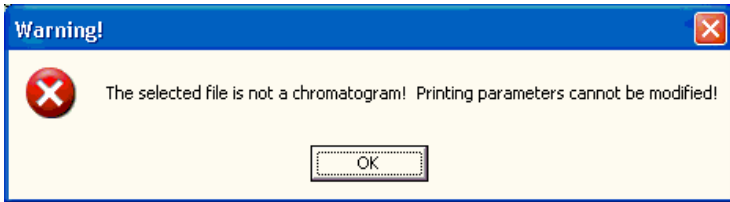
- *If the spectral energy of the file being opened is low, the Warning Low Energy Spectra dialog appears.*

- *If the spectral energy of the file being opened is inconsistent with the associated method, the "Inconsistent Wavelength Range" dialog appears and shows the method's wavelength range and the energy determined wavelength range.*
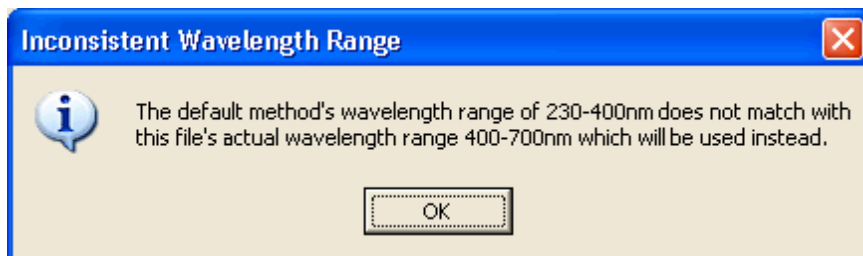
*If you click **OK** on this dialog box, the associated method is updated with the actual range shown on the dialog.*

If the checksums are not correct, or not present for the TotalChrom .raw, .rst files, or the checksum is not correct for the raw spectra file .spc a warning is displayed that states: "Invalid spectral file (checksum failed), file cannot be opened and the file is not opened." If the checksum is not present for the raw spectra file .spc, a warning is displayed that states: "This file was collected using an earlier version of PerkinElmer's spectral software." The file is opened but all data created that is related to this spectral file will indicate that the checksum was missing.

Menu	Command	Description
	Close	Removes the currently selected spectra and chromatograms from the Data tree. If the selection includes an extracted chromatogram or calculated spectrum that has not yet been saved a message will be displayed that states: "*The items selected for closing include data that has not been saved. Do you want to continue?*" The command buttons are **Yes** and **No**.
	Close all spectra	Removes all spectra from the Data tree. If this includes calculated spectra that have not yet been saved a message will be displayed that prompts you whether or not you wish to close the spectra without saving the calculated data.

Menu	Command	Description
	Information ► Chromatogram... Spectrum...	This command allows you to view information about a specific chromatogram or a spectrum without actually opening the chromatogram's .rst file or the spectrum's .uv file.
		For more information on viewing information on a chromatogram, see **Obtaining Information** *about a Chromatogram on page **63**.*
		For more information on viewing information on previously stored individual spectrum file, see **Obtaining Information on** *a Stored Spectrum on page **87**.*

Menu	Command	Description
	Save Results	This command allows you to save the results and the parameters used in an operation performed on a chromatogram.
		Refer to the section titled **Saving the Results of a Calculation** *on page Error! Bookmark not defined. for more information.*
	Save As	If a **spectrum** is selected on the Data Tree and you select **File > Save As…** from the menu bar, a standard Windows file selector appears. From this dialog box you can save the spectrum as a .uv file. An audit trail entry is created for this newly created file that identifies the source of the chromatogram, the user's full name and logon name, as well as a date and time stamp.
		If a **chromatogram** is selected on the Data Tree and you select **File > Save As…** from the menu bar, a standard Windows file selector is displayed. From the dialog box that appears you can name and save the chromatogram with a .RAW extension.

Menu	Command	Description
	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.

Menu	Command	Description
	AutoCalc Print Setup	If you plan on using AutoCalc to automatically print out the Apex spectra display for each sample in a running sequence, then you can use this command to specify details of that output, including the printer to be used.

For more information on using this dialog, refer to the chapter titled **AutoCalc: Automating Chromatographic Tasks***.*

Note: *This command is enabled at all times; however, in order to access the AutoCalc Print Setup dialog you must first select a chromatogram on the Data Tree that has been processed by the same TotalChrom method that will be used in the sequence. If a chromatogram is not selected on the Data Tree when you select the AutoCalc Print Setup command, the following warning message displays:*

Menu	Command	Description
	E<u>x</u>it	Closes the IRIS application.
		Enabled at all times.
<u>E</u>dit	Copy <u>O</u>bject	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.
		Enabled at all times.
	Copy <u>S</u>creen As ► <u>B</u>itmap <u>M</u>etafile	**Bitmap** - Copies the entire screen as a bitmap image to the clipboard. Enabled at all times.
<u>V</u>iew	✓<u>C</u>hromatogram Baselines	Displays or hides baselines on all chromatograms. Baselines are displayed as a solid red line. Enabled at all times.
	✓Baseline <u>S</u>pectra	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.

Menu	Command	Description
	✓<u>B</u>aseline Corrected Spectra	Sets whether or not spectra are displayed as baseline corrected. When this command is checked, spectra are displayed as baseline corrected
		Note: *On individual operations, you can check **Baseline Corrected** to perform the operation using baseline corrected spectra. On the other hand, the **Baseline Corrected Spectra** menu command only affects the display of the spectra.*
		Enabled at all times.
	------------------ ---	
	Overlay C<u>h</u>romatograms	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.
		Enabled at all times.
	Stac<u>k</u> 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 all spectra being displayed on the one set of axes.
		Enabled at all times.
	------------------ ---	
	✓ C<u>u</u>rsor	Toggles the cross-hair cursor.
		Enabled on any of the IRIS views (this command is not enabled on operations).
	------------------ ---	
	✓<u>T</u>oolbars	Toggles whether or not the toolbars are displayed. When Toolbars is checked, all three tool bars are displayed.
		Enabled at all times.
	------------------ ---	
	✓<u>V</u>iew tree	Toggles whether or not the View tree is displayed.
		Enabled at all times.
	✓<u>D</u>ata tree	Toggles whether or not the Data Tree is displayed.
		Enabled at all times.
	✓<u>P</u>arameters	Toggles whether or not the Parameters pane for Operations is displayed.
		Note: *The Parameters pane always includes the Display or Hits list.*

Menu	Command	Description
		Enabled only on Operations.
	----------------- ---	
	Panes ▶ Chromato**g**ram Sp**e**ctrum Contour **M**ap	This command allows you to select whether or not a pane, selected in the menu, is displayed on the view. When you hide a pane, a Custom View is formed.
		For more information on Custom Views, refer to the section titled **Custom View** *on page* **150**.
	3**D** Plot	Shown panes are checked, hidden panes are unchecked.
		Enabled for all Views. This command is disabled on Operations.
		Note: *Hiding a pane does NOT unload the chromatogram or extracted spectra. This command only hides/shows the pane. The contents do not change.*
	Vie**w** Template ▶ **S**ave As **D**elete **E**xport **I**mport	**Save As...** - Displays the Save View As dialog. Always enabled
		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. Enabled at all times.
<u>A</u>ctions	Zoo**m** 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.
	<u>A</u>utoscale ▶ <u>X</u> and Y <u>Y</u> 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.

Menu	Command	Description
	Normalize ►	Rescales the selected graph in line with the command selected.
	X and Y	**X and Y** and **Y only** will operate based on the full graph.
	Y only	**Offset** will normalize to the maximum point to the right of the X-axis
	Offset	cursor.
	Point	**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 X-axis 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 graph tools ►	**Rotate X** - Sets rotation slider to rotate around the X axis
		Rotate Y - Sets rotation slider to rotate around the Y axis
	✓X only	
	✓Y only	
	-------------------- ---	
	Label Chromatograms	Displays the Label Chromatograms dialog. Enabled any time a chromatogram is selected on a View (not an Operation).
	Annotations ►	Add - Opens an empty Edit Annotations dialog. Enabled when a graph is selected.
	Add	
	Edit	**Edit** - Opens the Edit Annotations dialog with the details of the selected annotation available to edit. Enabled when an annotation is selected.
	Delete	**Delete** - Removes the selected annotation. Enabled when an annotation is selected.
	Delete All	**Delete All** - Removes all annotations from the selected graph. Enabled when a graph is selected that has at least one annotation.
	-------------------- ---	

Menu	**Command**	**Description**

<u>R</u>ange — 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.*

Menu	Command	Description
	Add All to <u>V</u>iew	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 views; 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.

Menu	Command	Description
<u>T</u>ools	<u>V</u>iew Library	Displays a file select to select the library to be displayed followed by the View Library dialog.
		Enabled at all times.
	<u>B</u>uild Library ► <u>C</u>reate <u>A</u>dd Apexes <u>E</u>dit	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.

Menu	Command	Description
	<u>T</u>otalChrom ► <u>R</u>eprocess <u>G</u>raphic Method	Opens the selected TotalChrom application with the selected chromatogram passed to the application.
		Enabled at all times a single chromatogram is selected.

Menu	Command	Description
	Edit	

	Audit trail	Displays the Audit trail dialog.
		Enabled only when a single chromatogram or spectrum 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.
		Enabled at all times.
	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 .
		Enabled at all times.

	Edit Default Method	Displays the Method Editor dialog for the default method, enabling it to be edited.
		Enabled at all times.
Help	Contents and Index	Displays the opening page of the HTML Help system iris.chm.
		Enabled at all times.
	✓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.

Menu	Command	Description
	<u>A</u>bout	Displays the About dialog.
		Enabled at all times.

IRIS Tool Bars

In IRIS 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
	Open Chromatogram	Enables you to open a chromatogram and add it to the Data Tree.
	Open Spectrum	Enables you to open a spectrum and add it to the Data Tree.
	Close	Closes the currently selected items in the Data tree.
	Information...	Enables you to review the information associated with a file.
	Save	Saves current changes to a file.
	Save As...	Saves new spectrum or extracted chromatogram files.
	Print	Prints details from the current view or operation.
	Exit	Closes the IRIS software.
	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.

["

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.

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

About Spectral Methods

In *Chapter 8. Performing Operations on Chromatograms* you will learn about the chromatographic operations in IRIS, which allow you to obtain important information about your chromatograms. These operations 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). However, before you begin performing these operations it is important that you learn about how these operations are calculated. In IRIS all of the parameter values used to calculate the results of an operation are stored in spectral method files.

There are three types of spectral methods in IRIS: **Default Method**, **Chromatogram's Spectral Method**, and a **Process Spectral Method**. Following is s description of each method and how it is used by IRIS.

Default Method

IRIS provides you with a method named **Default.TSM** that contains the default set of parameters for all operations that can be performed in IRIS. The parameters specified in this default method are used to set the initial parameter values for new spectral methods created by IRIS.   In fact, whenever you open a chromatogram in IRIS for the first time, and the chromatogram has no associated Process Spectral Method, then both the Chromatogram's Spectral Method and the Process Spectral Method are created as a copy of the Default.tsm method.

You can view and edit the parameter values in the default method by selecting **Tools > Edit Default** method from the IRIS menu bar. The Method Editor Default.tsm dialog that appears provides you with a convenient way to set the default/initial conditions for new spectral methods that will be created by IRIS. *For more information, see page* *39*.

Figure 1- 5 The Default Method.

Chromatogram's Spectral Method

This is the spectral method associated with a chromatogram. When a chromatogram is opened in IRIS for the first time, IRIS automatically creates and saves this method, which we refer to as the Chromatogram's Spectral Method. IRIS names this method with the same name as the chromatogram's result file; however the file extension is .tsm instead of .mth. This method contains the parameter values that are responsible for the results you can see when a chromatogram is displayed on an operation in IRIS, and the results that are stored with the TotalChrom result file.

IRIS initially creates this method by either copying the parameter values in the Default.tsm method. Or, if you open a chromatogram in IRIS that has been processed by the same TotalChrom Method, as a chromatogram previously opened in IRIS, then IRIS creates the new Chromatogram's Spectral Method by copying the parameters values from its associated Process Spectral Method (the Process Spectral Method is the method associated with all chromatograms that have been processed by the same TotalChrom method; and is used by AutoCalc).

The example below shows the spectral method that was created the first time the following example chromatogram: **example 1 – identified and well separated.rst** was opened in IRIS. You can access the dialog shown below by right mouse clicking on a chromatogram from the IRIS Data Tree, and selecting **View Method** from the context menu that appears.

Note: *The example chromatogram, referenced above, is located in the IRIS Data directory, which is installed as part of the IRIS Installation process. The .tsm spectral method file is not installed with IRIS, but rather it is created the first time you open the chromatogram in IRIS.*
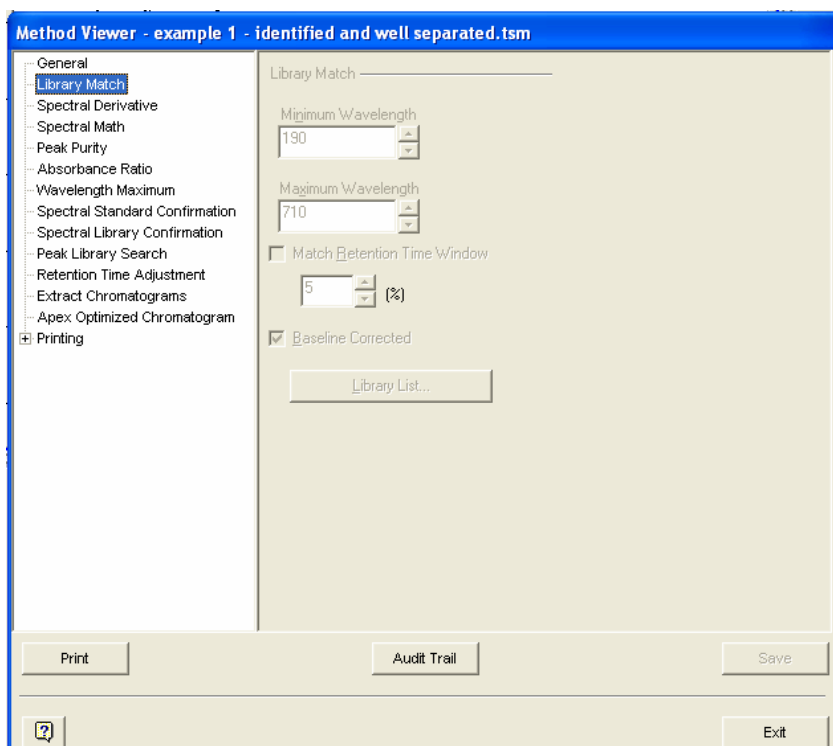
Figure 1- 6 A Chromatogram's Spectral Method controls what you see when you select a chromatogram on an operation in IRIS, it also contains a record of the parameters used to calculate any results you have saved back to the TotalChrom Result file.

Process Spectral Method

The Process Spectral Method is also created when a chromatogram is first opened in IRIS. This spectral method is given the same name and stored in the same location as the TotalChrom Method that was used to create the result file that you opened in IRIS; however the file extension is .tsm instead of .mth.

The screen shot below shows the Chromatogram Information dialog for the example chromatogram named **example 1 – identified and well separated.rst**. This dialog shows that the chromatogram was processed by the TotalChrom method named **ex1 identified.mth.**
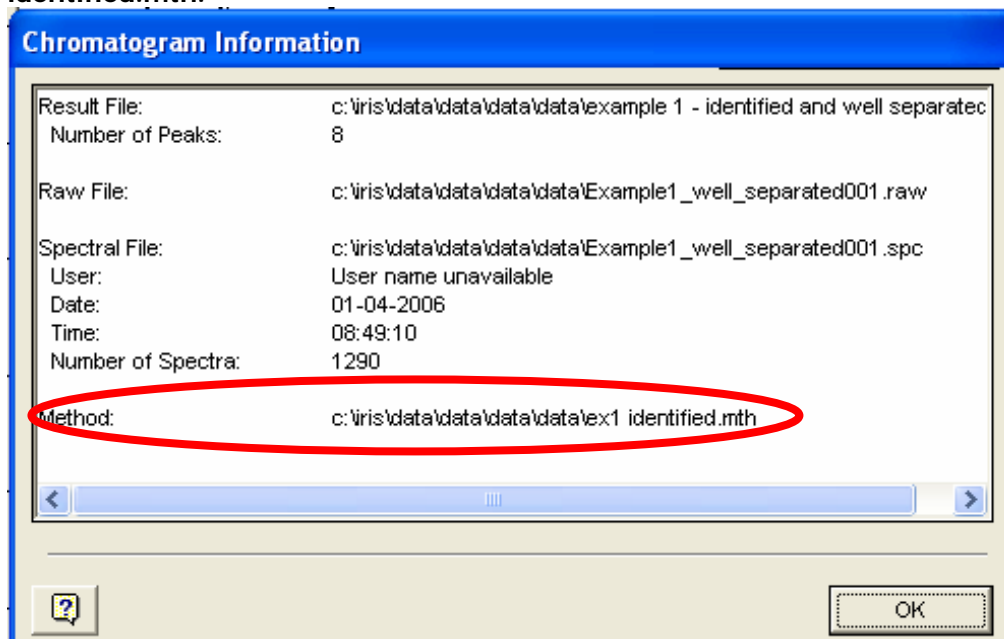
Figure 1-7 The Chromatogram Information dialog can be accessed by right mouse clicking on a Chromatogram in the IRIS Data Tree and selecting Information… from the context menu that appears.

Now that we know the TotalChrom method used to process the chromatogram, we can use the **File > Open Method..** command to locate and view the chromatogram's Process Spectral Method, which should be named **ex 1 identified.tsm**. The screen shot below shows the properties for this Process Spectral Method.
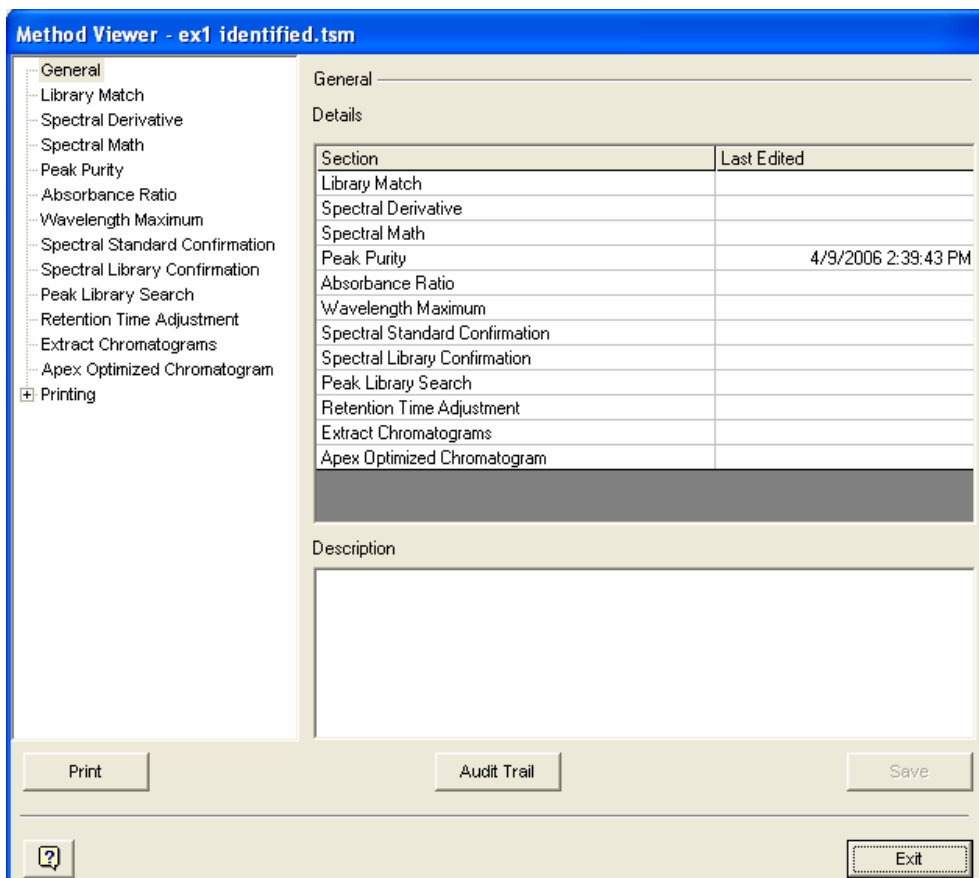
Figure 1- 8 The Process Spectral Method for example 1 – identified and well separated.rst has the same name as the TotalChrom method used to process the result. This method can be used by all chromatograms that are processed by the same TotalChrom Method.

When a Process Spectral Method is first created, the parameter values for the method are copied from the parameter values in the Default Method. Then, whenever you perform an operation on a chromatogram in IRIS and save the results, IRIS not only updates the Chromatogram's Spectral Method, IRIS also updates the associated Process Spectral Method with the new parameters.

Note: *For more information on saving the results of a chromatographic operation, refer to page* **309**.

It is important to note that all chromatograms that are opened in IRIS, which have been processed by the same TotalChrom method, share the same Process Spectral Method. Therefore, once this method is initially created, its parameter values are then used to set the parameter values in the Chromatogram's Spectral Method for new chromatogram you open in IRIS, which has been processed by the same TotalChrom Method.

 In addition, the parameter values stored in a Process Spectral Method can be used by AutoCalc, during a TotalChrom sequence, to automatically perform any of the arithmetic operations and chromatographic extractions provided by IRIS. *For more information on AutoCalc, see page* **314**.

Viewing a Spectral Method

The **View Method** dialog allows you to view and print, in its entirety the parameter values in a spectral method associated with a chromatogram (Chromatogram's Spectral Method) or a Process Spectral Method. In addition, the View Method dialog allows you to view the Audit Trail for a spectral method.

Below is an example of the View Method dialog.
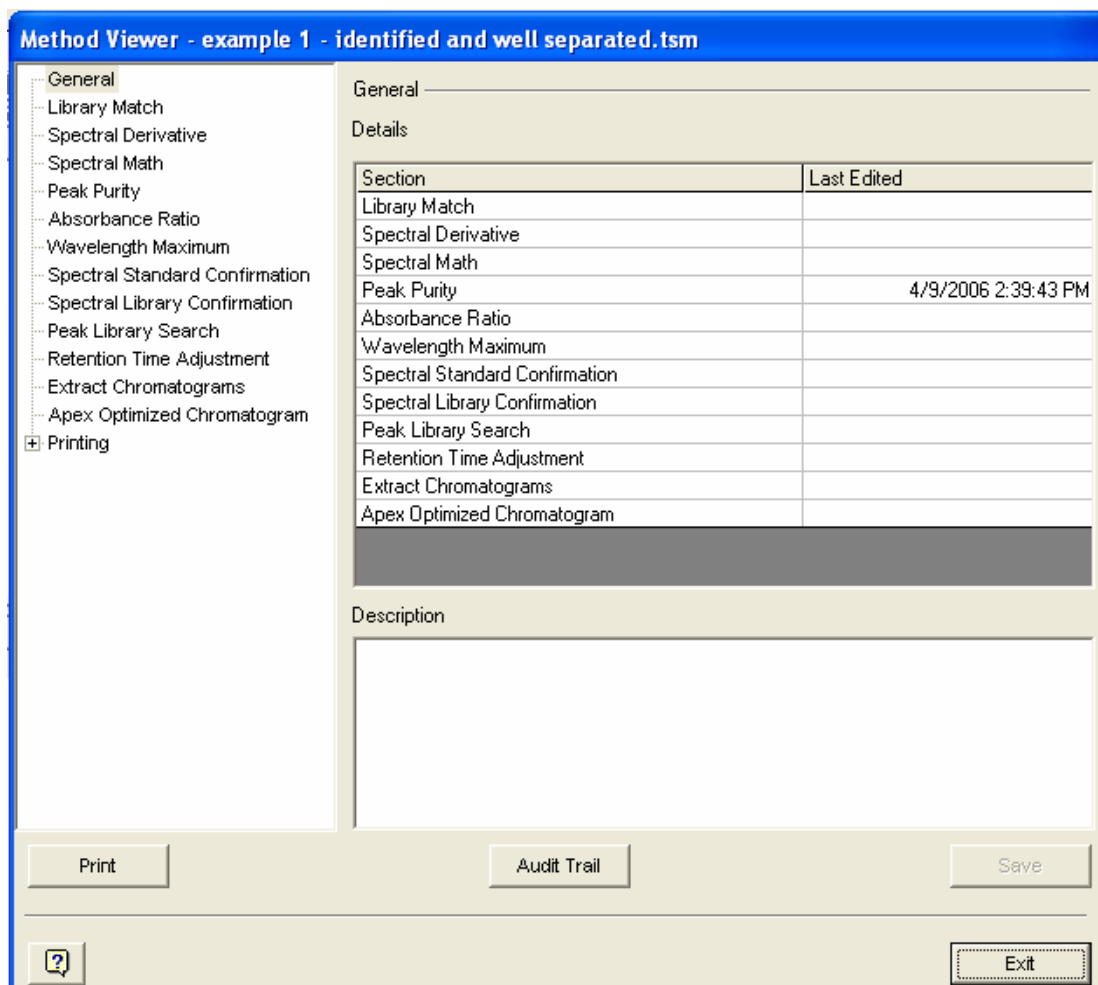
Note: *If you have the appropriate user permissions you can edit the default method by selecting* **Tools > Edit Default Method...** *from the IRIS menu bar. For more information on editing the default method see the topic titled Method Editor.*

How do I view the parameter values in a spectral method?

To view the parameters in a Chromatogram's Spectral Method:

1. From the IRIS workspace select **File > Open > Method**.
   The Select Method dialog box appears.

2. Select a spectral method to view and click **Open**.
   The View Method dialog displays.
   OR
   If you have opened a chromatogram in IRIS, right mouse click on the chromatogram listed on the Data Tree and select **View Method** from the context menu that appears.
   The View Method dialog is displayed.

To view the parameters in a Process Spectral Method:

1. From the IRIS workspace select **File > Open > Method**.
   The Select Method dialog box appears.

2. Select a spectral method to view and click **Open**.
   The View Method dialog displays.

Tell me about the View Method dialog.

The View Method dialog is comprised three main areas:

- A **list box** that lists the components of the spectral method file.

  Click on one of the items listed here, and the right hand portion of the dialog displays, in read-only format, the parameters for the selected item.
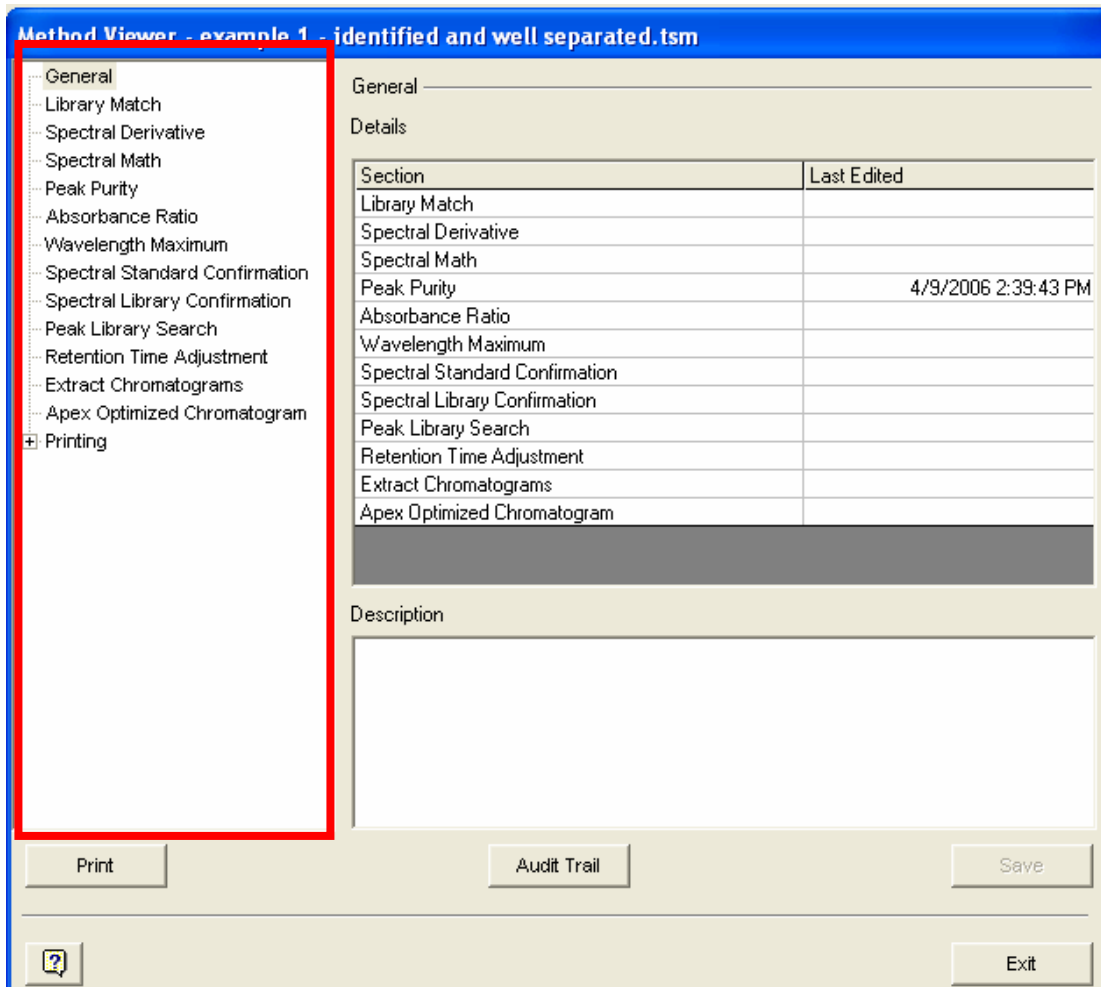
- The **Parameters display** shows you the parameters and their values for the operation have selected.
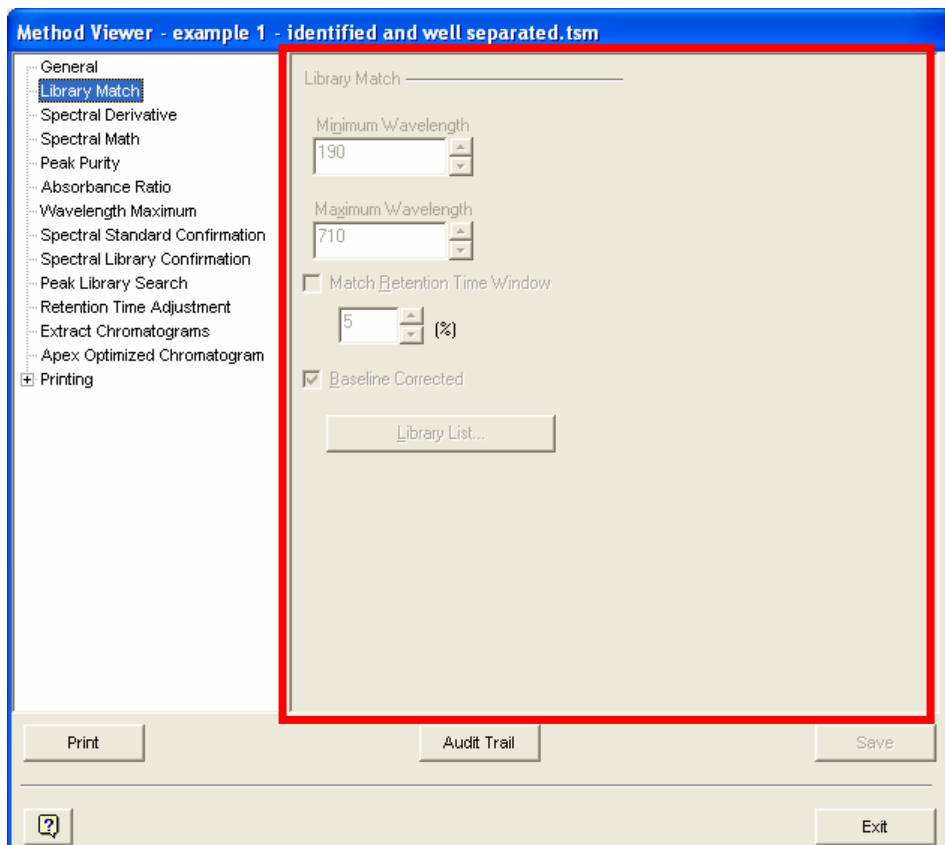
- If you select **General** from the list box, the right-hand portion of the dialog lists the date and time when each section of the spectral method was last edited.

Note: *If the date/time stamp is empty for an operation, then this section of the method has never been used. When you first use this operation, the parameter values will automatically be filled in using the default values that rare stored in the IRIS default method.*

Note Carefully: *AutoCalc cannot perform an operation if the corresponding section in the method is empty.*

- If you select an **operation**, such as Absorbance Ratio, from the list box, the right-hand portion of the dialog displays the parameter values that are used to calculate the results for that operation.
- If you select any of the items listed under the **Printing** node, the right hand portion of the dialog displays the default print settings for the spectral method.

- A **Print** button that you can select to print a report of the entire spectral method details.

- An **Audit Trail** button that you can select to view the audit trail for the spectral method.

  The Audit Trail can also be printed.

- An **Exit** button that you select to close the dialog.

How do I modify the parameters values stored in a Chromatogram's Spectral Method and a Process Spectral Method?

Unless you are viewing the **Default.TSM** method (This method contains the default/initial conditions for all new methods that are created by IRIS, when you open a chromatogram in IRIS for the first time) , you cannot edit any of the fields that are displayed on the View Method dialog. The View Method dialog only allows you to view and print the parameter values for a spectral method.

All other IRIS spectral methods are updated only when you save the results of a chromatographic operation.

To modify a Chromatogram's Spectral Method:

1. Open the chromatogram in IRIS.

2. Select the operation from the Views tree that you wish to modify.

3. Select the chromatogram on the Data Tree.
   The Chromatogram is displayed on the operation you have selected.

4. Modify the parameters that are listed on the right-hand side of the operation.

5. When you are satisfied with the results select **File > Save Results...** from the menu bar.
   The results of any of the operations you perform on a chromatogram in IRIS can be saved to the chromatogram's result file (.RST), and the parameters that were used calculate the results are saved the associated spectral method files (.TSM). The saved results can to be included in a printed TotalChrom report.
   You can View the new parameter values from the View Method dialog.

To modify a Process Spectral Method:

When you perform an operation on a chromatogram in IRIS and save the results, IRIS not only updates the chromatogram's spectral method, IRIS also saves the parameter values to a Process Spectral Method that will be used by other chromatograms that are processed by the same TotalChrom method, i.e. all chromatograms in this family.

1. In IRIS, open a chromatogram that has been processed with the TotalChrom method that has the same name as the Process Spectral Method you want to modify.

Note: *You can verify the name of the chromatogram's associated TotalChrom method by right mouse clicking on the chromatogram in the IRIS Data Tree and selecting* **Information** *from the context menu that appears. A Chromatogram Information dialog appears that shows the TotalChrom method associated with the selected chromatogram. If the TotalChrom method has the same name as the Process Spectral Method you want to update, then you can proceed to the next step. If the TotalChrom method does not have the same name as the Process Spectral Method you want to update, then open a different chromatogram and repeat the steps you just performed.*

2. If the TotalChrom method has the same name as the Process Spectral Method you want to update, you can modify the Process Spectral Method by doing the following:

   - For the chromatogram opened in Step 1, select any of the desired operations that are listed under Operations in the IRIS Views tree.

   - Make any required changes to the parameters listed for the operation you selected.

   - Select the next operation that you want to make changes to. When you select a different operation, a dialog appears that prompts you to select if you want to save the changes. From this dialog select the desired save mode and click **Yes**.

3. After completing the changes for the last operation, save the results via **File > Save Results.**

   The Process Spectral Method and the Chromatogram's Spectral Method are then updated with all the modified parameter values. Moreover, for **AutoCalc** , the Process Spectral Method parameters can now be used by any chromatograms processed by the TotalChrom Method having the same name.

Editing the Default Method to set the default/initial conditions of a new method

Note: *If you have the appropriate user permissions you can edit the default method by selecting* **Tools > Edit Default Method...** *from the IRIS menu bar.*

The Default Method contains the default set of parameter values for all operations that can be performed in IRIS. When you open a chromatogram in IRIS for the first time, and the chromatogram has no associated Process Spectral Method, then both the Chromatogram's Spectral Method and the Process Spectral Method are created as a copy of the Default.tsm method. The Edit Default Method dialog provides you with a convenient way to specify the default/initial conditions that IRIS will use to create a new method.

➢ To access the Default Method select **Tools > Edit Default Method...** from the IRIS menu bar.

Tell me about the Edit Method dialog.

The Edit Method dialog is comprised three main areas:

• A **list box** that lists the components of the spectral method file.

Click on one of the items listed here, and the right hand portion of the dialog displays the parameters for the selected item.
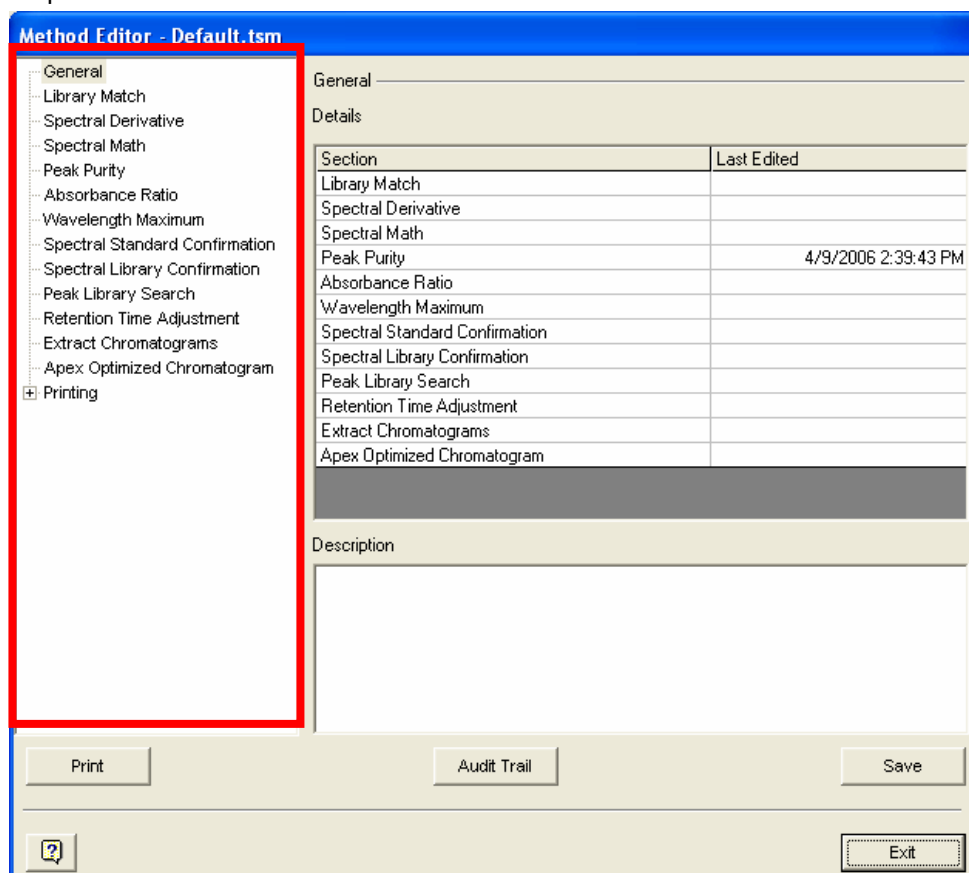
You can modify any of the displayed parameter values and select **Save** to update the default method.

- The **Parameters display** shows the parameters and their values for the operation you have selected.
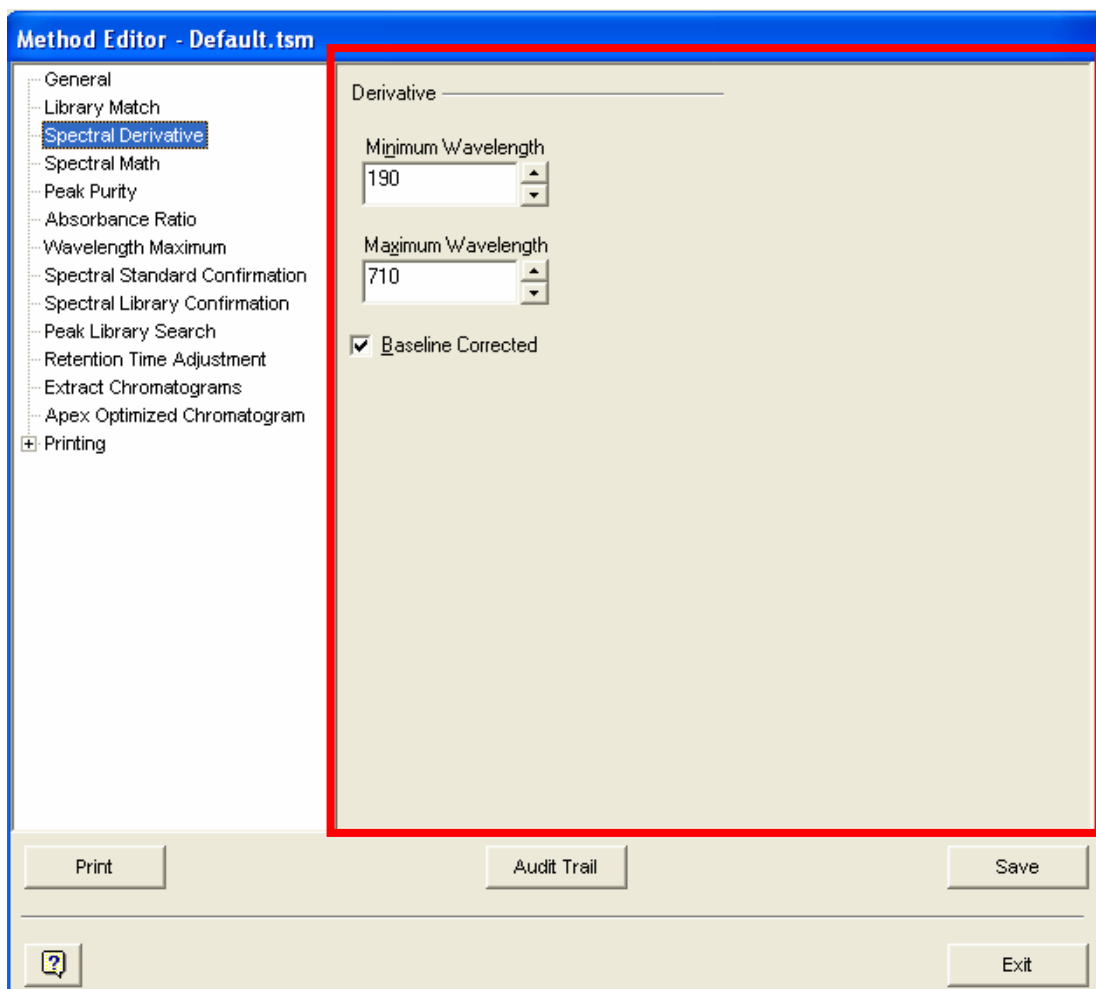
- If you select **General** from the list box, the right-hand portion of the dialog lists the date and time when each section of the spectral method was last edited.
- If you select an **operation**, such as Absorbance Ratio, from the list box, the right-hand portion of the dialog displays the parameter values that are used to calculate the results for that operation.
- If you select any of the items listed under the **Printing** node, the right hand portion of the dialog displays the default print settings for the spectral method.

- A **Print** button that you can select to print a report of the entire spectral method details.

- An **Audit Trail** button that you can select to view the audit trail for the spectral method.
  The Audit Trail can also be printed.

- An **Exit** button that you select to close the dialog.

How do I edit the default method?

To edit the Default Method:

1. From the IRIS menu bar select **Tools > Edit Default Method...**
   The Edit Method dialog appears.

2. From the list box select the operation that you wish to modify.

3. The parameters for the selected operation are displayed on the right-hand side of the dialog.

4. When you have finished modifying parameters click **Save**.

Chapter. 2 Enhanced Security

Enhanced Security

In response to Title 21 (Food and Drug Administration) of the United States Code of Federal Regulations (CFR), Chapter 1, Part 11, as well as the increasing data security requirements in other industries, PerkinElmer has developed IRIS to work in conjunction with TotalChrom in order to provide you with the technical compliance tools needed to meet these regulations. For example, in IRIS enhanced security features such as electronic records controls and electronic signatures are supported for all data modified or created by the IRIS application.

More specifically, the integration between IRIS and TotalChrom provides you with the following enhanced security features:

- **User Level Management and Security**

   IRIS uses the secure login process provided by TotalChrom in order to control access to the application. More specifically, IRIS provides for three security levels that can be set in the TotalChrom SysConfig utility to control access to the application. This means that users who are granted one type of access level may perform all IRIS functions, while other users, who are granted a more restrictive access level, can use the software to calculate and save chromatographic results, but are not able to perform functions such changing methods or libraries.

- **Audit Trails**

   If Audit Trailing is turned on in TotalChrom for a specific chromatogram then IRIS will create secure, computer generated, time-stamped audit trails for all IRIS files linked to that chromatogram. In addition, Spectral Libraries are always audit trailed. All IRIS audit trails can be viewed on screen, printed, and exported.

- **Electronic Signatures**

   In IRIS, electronic signatures work in conjunction with audit trailing. Electronic Signatures can be enabled in TotalChrom, only on files for which audit trail has been enabled. The Electronic Signature feature forces a user who saves a file to enter the TotalChrom password that corresponds to the logged on user.

This chapter covers User Management, Audit Trailing, and Electronic Signatures.

User Management

The IRIS Spectral Processing software is designed to be used by several different users who are granted one of three access levels to the application. This means that users who are granted one type of access level may perform all IRIS functions, while other users, who are granted a more restrictive access level, can use the software to calculate and save chromatographic results, but are not able to perform functions such changing methods or libraries.

The level of access available to users the IRIS software depends on the permission level you have been assigned to in TotalChrom. The TotalChrom Administrator or Lab Manager is responsible for setting up users, groups, and permissions through **the TotalChrom SysConfig** utility.

User Permissions

IRIS provides for various security levels that can be set in the TotalChrom SysConfig utility. Therefore, depending upon the permission level you have been assigned to in TotalChrom, you are granted one of the following access levels to IRIS:

- **Manager permission** – This permission grants a user full access to IRIS.

- **User permission** – This permission provides a user with the ability to calculate and save chromatographic results. However, users with this permission cannot change methods or libraries.

Note: *For more information on methods, refer to the chapter titled **IRIS Spectral Methods**.*

- **Read Only permission** – This permission provides a user with the ability to perform calculations as well as change any parameter values that are used in the calculation. However, users with this permission cannot save anything.

Audit Trails

IRIS provides you with an Audit Trail feature that tracks and records all changes made within the IRIS software. All IRIS audit trails can be viewed on screen, printed, and exported.

It should be noted that audit trailing of Spectral Libraries is always enabled in IRIS. On the other hand, audit trailing of a chromatogram and its associated spectral methods is activated only when Audit Trailing is turned on in TotalChrom for a specific chromatogram. In other words, when Audit Trailing is turned on in TotalChrom for a specific chromatogram, IRIS will create secure, computer generated, time-stamped audit trails for all IRIS files linked to that chromatogram; meanwhile, all changes, made in IRIS, to the actual chromatogram are audit trailed by TotalChrom.

Note: *Because the TotalChrom chromatographic raw data and the Diode Array spectral file cannot be modified, this data is not continuously audit trailed. However, you can view the name of the user who created the data as well as the creation date by right mouse clicking on a chromatogram or spectral file from the IRIS Data Tree and then selecting **Audit Trail** from the context menu that appears.*

What information is captured in an Audit Trail?

The following information is captured in the Audit Trail for any file that can be audit trailed:

- The name of the modified parameter.

- The original value.

- The new value.

- The unambiguous time/date stamp of the modification, including the Time Zone where the modification occurred.

- The User Name of the person making the change..

- If necessary, a selection from the appropriate TotalChrom Reasons List and free text. The enforcement of the reason will be based upon settings in the TotalChrom System Configuration.

New files created in IRIS contain the following audit trail information:

- The unambiguous time stamp of the file creation.

- An indication of the "source" of the new file.

- "File filename created as a new file"

- "File filename2 created as a Save As from filename1"

- The User Name of the person creating the file, recorded automatically.

- The "reason" will be automatically recorded as "File creation".

Note: *Any file with a corrupted audit trail can only be accessed in read-only mode to allow for investigation of the failure and cannot be used for any IRIS or TotalChrom processing or data generation.*

Entering Audit Trail Information

When audit trailing has been enabled in TotalChrom for a specific chromatogram and a user attempts to make any changes in IRIS to the chromatogram and its associated spectral methods, the user is prompted with the Audit Trail dialog. In addition, any time a user attempts to modify or create a spectral library, an Audit Trail dialog displays. The Audit Trail dialog displays the user name of the individual who is currently logged in and the date and time of the event. In addition, your application manager may have set up the system to require you to select a reason for the event and you also may be required to or have the option to enter a comment about the event.

To enter audit trail information when the Audit Trail dialog is displayed:

Note: *Your application manager may have set up the system so that you must select a Reason from a list that is displayed when the Audit Trail is triggered. Or, you may be able to enter your own reason for the event that triggered the Audit Trail dialog.*

1. Select or enter a reason for the change(s) in the **Reason** list.

2. Enter any information about the change(s) you made to the file in the **Comment** text box.

3. Click **OK**.

   If Electronic Signatures is enabled the Electronic Signature dialog appears.

4. Enter your TotalChrom **User Name** and **Password**.

5. Click **OK**.

Viewing an Audit Trail

All audit trailed files in IRIS can be viewed from an Audit Trail reader. You can view the Audit Trail for:

- A Chromatogram's Spectral Method (.tsm) and Spectral file (.spc).

- The default method for IRIS (Default.tsm), or a Process spectral method (.tsm method associated with all chromatograms that are processed by the same TotalChrom method)

- A spectral library.

Note: *For more information on spectral methods, refer to page* **Error! Bookmark not defined.**.,

The following section describes how to access the audit trail for a particular file as well as describes the components of the Audit Trail reader.
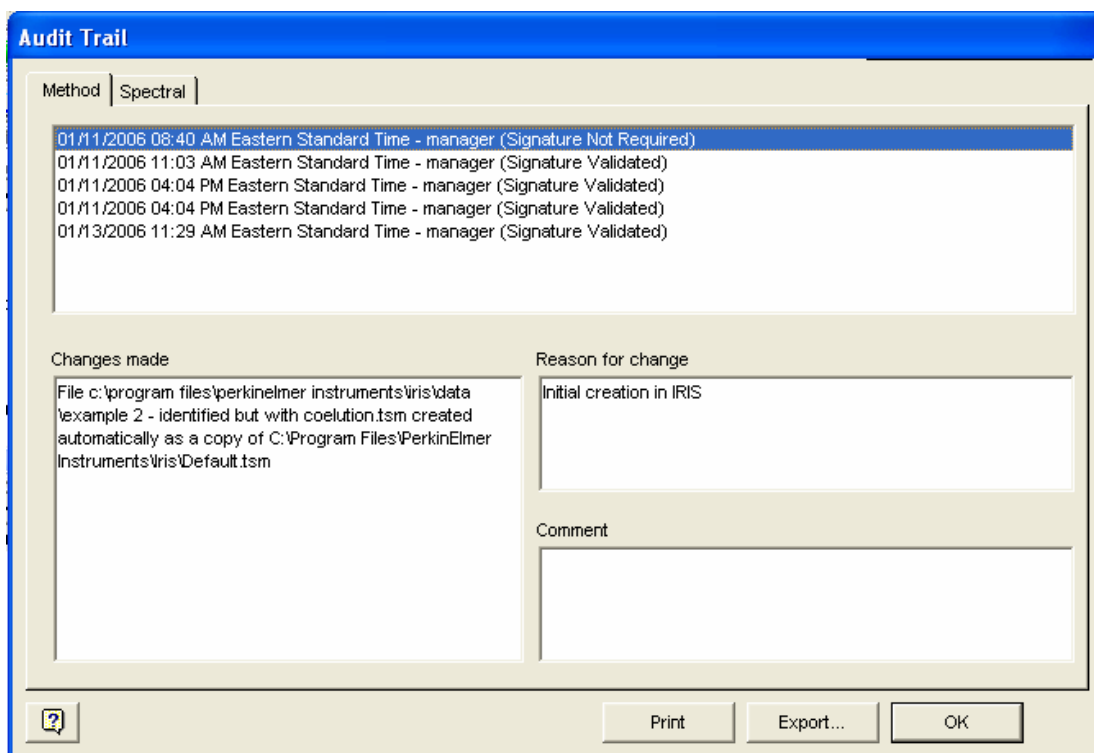
Figure 2-1 The Audit Trail Reader

Tell me about the Audit Trail Reader

The Audit Trail dialog displays the audit trial for a specific object and consists of the following areas:

Tab pages

The Audit Trail dialog displays tab pages that correspond to the type of Audit Trail you are viewing:

- When called from the Audit trail button on the Edit Library and View Library dialogs a **Library** tab page is displayed.
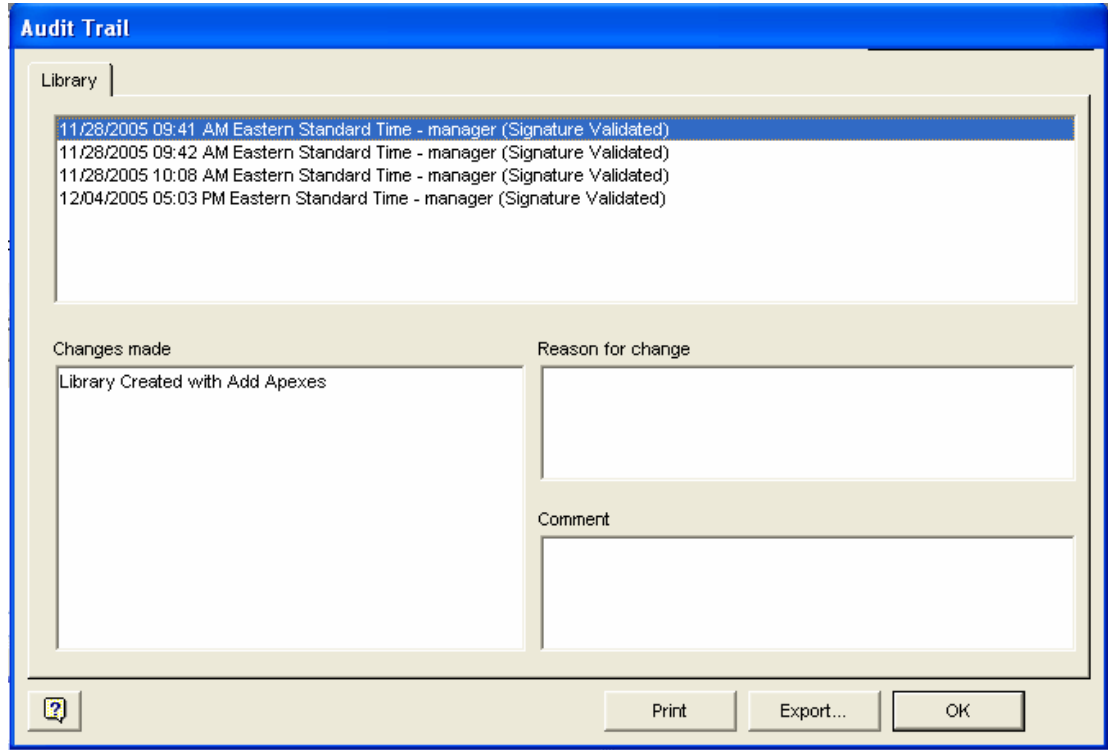
Figure 2-2 The Audit Trail Reader for a Spectral Library

- When called from the Audit trail button on the **Method Editor** dialog, a **Method** tab page appears. More specifically, this dialog appears when you open a Chromatogram's Spectral Method or Process Spectral Method via the **File > Open Method Command**; then when the Method Editor dialog is displayed, click on the **Audit Trail** button to view the Audit Trail for the Process Spectral Method or Chromatogram's Spectral Method that you selected.

Figure 2-3 The Audit Trail Reader for a Spectral Method

- When you right-click on a chromatogram in the Data Tree and select Audit Trail... from the Context menu, the Audit Trail dialog is displayed; and consists of two tab pages: a **Method** tab and **Spectral** tab. The **Method** tab page displays the Audit Trail of the Chromatogram's Spectral Method (.TSM having the same name as the TotalChrom Result file that was selected). The **Spectra** tab page displays the Audit Trail of the source .SPC file.
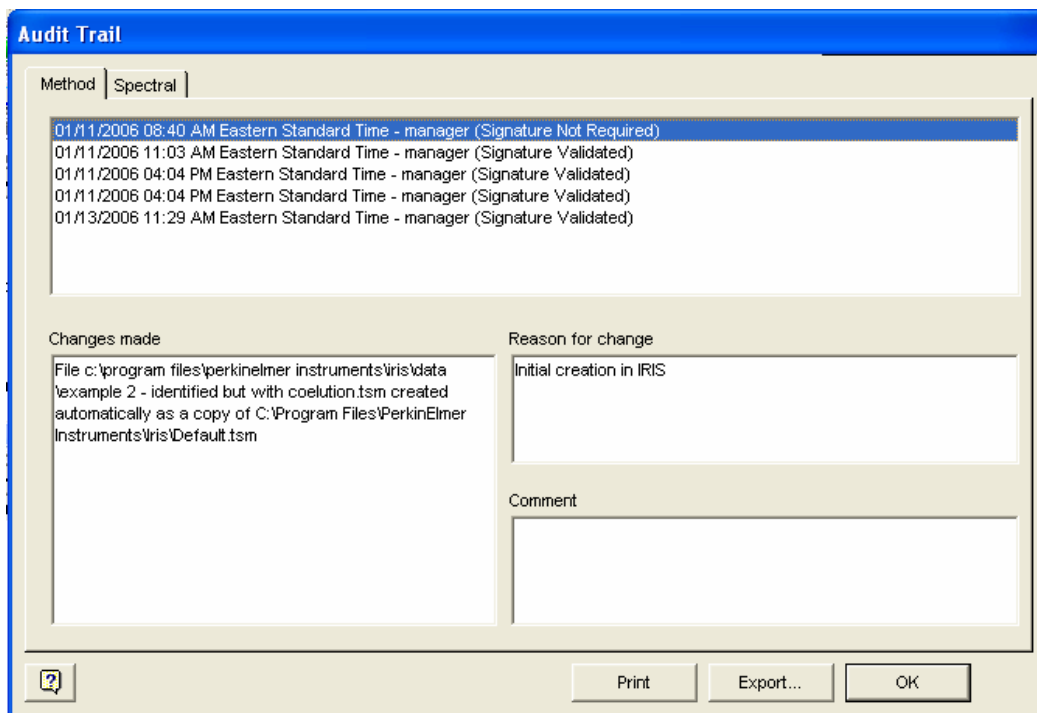
Figure 2-4 The Method tab page on the Audit Trail Reader for a chromatogram
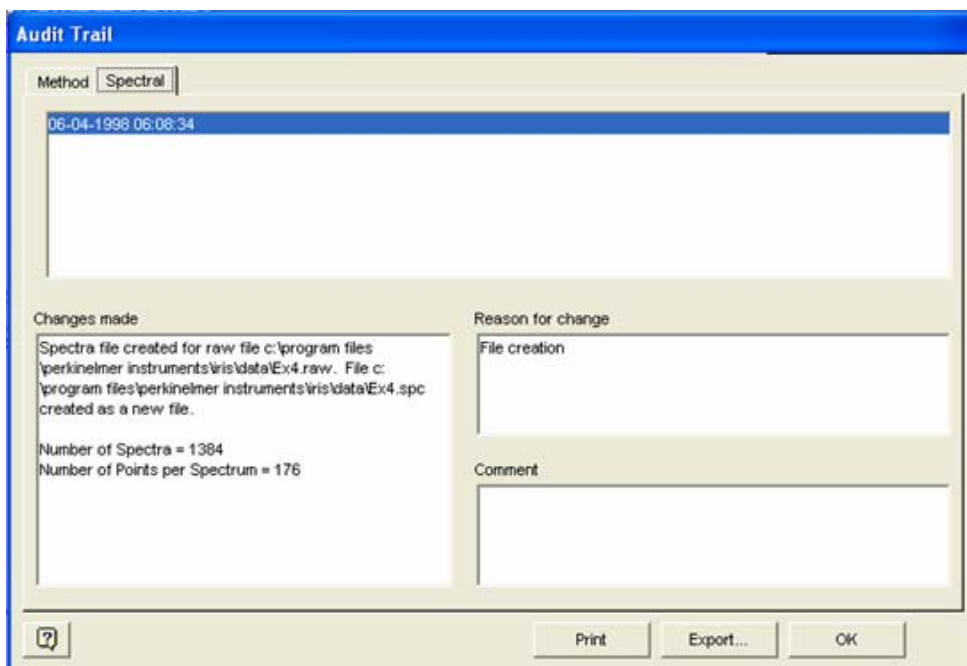
Figure 2-5 The Spectral tab page on the Audit Trail Reader for a chromatogram

Event Table

The Event List displays a list of the events when changes were made to the object. The events are listed by date and time and the user who made the change. When you click on an item listed here, the **Changes made** section is populated with a list of changes made to the selected object.
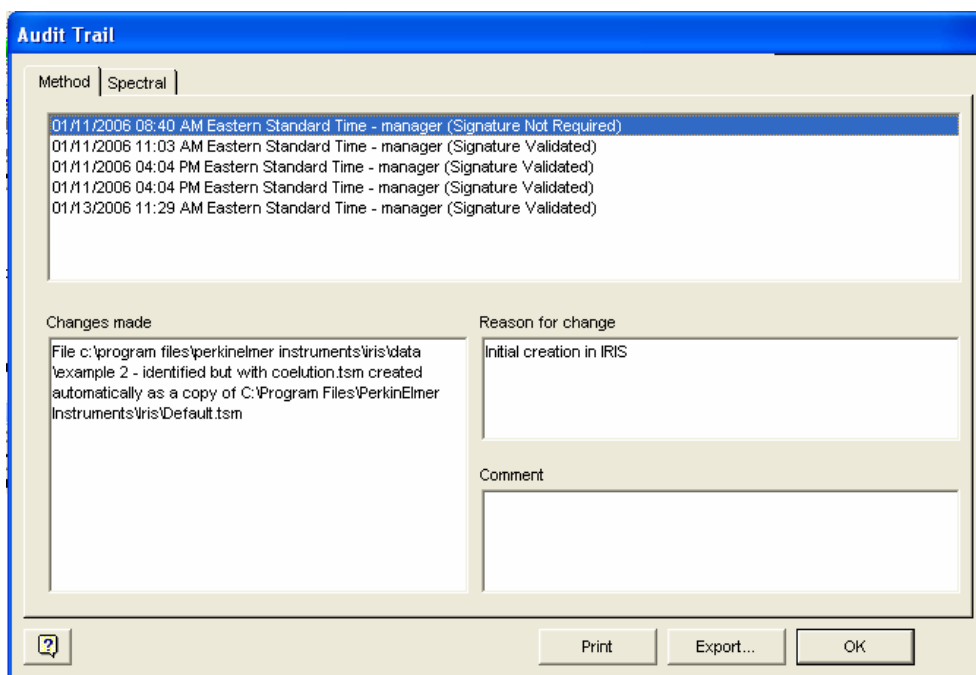
Figure 2-6 The Events List for a chromatogram

Changes Made
When you select an event on the **Event List**, the Changes Made text box displays the changes that were made for the selected date and time.

➢ You can select any of the changes listed here to view the **Reason for** the **change** and a **Comment** associated with the change in the corresponding text boxes.

Note: *If you select a change from the **Changes made** list, the **Reason for change** and **Comment** text boxes may not display any information, if the user was not required to select a reason or enter a comment.*

Reason for Changes
This text box displays the reason that was selected when a specific change was made to an audit trail item.

Comment
This text box displays the comments that were entered at the time the selected change was made.

Print Button
This command button enables you to print the audit trail.

Export Button

This command button displays a standard Microsoft file selector for you to select where to export and save the audit trail as a text (.txt) file.

Electronic Signatures

An electronic signature as defined by 21 CFR Part 11 means a computer data compilation of any symbol or series of symbols executed, adopted, or authorized by an individual to be the legally binding equivalent of the individual's handwritten signature.

Electronic Signature Support in IRIS

Note: *Electronic Signature support in IRIS is controlled by TotalChrom. For more information on enabling electronic signatures, refer to the TotalChrom Help.*

The electronic signature feature forces a user who saves a file to enter the TotalChrom password that corresponds to the logged on user. The electronic signature feature of IRIS is provided to assist in satisfying the requirements of FDA Final Rule in 21 CFR 11.

Electronic signature works in conjunction with audit trail; you can enable electronic signature, in TotalChrom, only on files for which audit trail has been enabled. Once electronic signature has been enabled for a file, it cannot be turned off. Electronic signature for a result file is automatically enabled if any of the methods used to create it have electronic signature enabled.

IRIS records the following information about electronic signatures in the audit trails:

- When electronic signature is enabled for a file, an entry is made in the audit trail to record the fact that signature requirement has been started.

- When a user enters the correct password for a file that has electronic signature enabled, the audit trail entry for the transaction will show that the electronic signature was verified.

Chapter 3. Opening and Viewing Chromatograms

Introduction

Now that you have a general understanding of the IRIS user interface, this section will introduce you to the most basic element in IRIS, the chromatogram. IRIS was designed 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 most of the tasks you will perform in IRIS begin with chromatograms.

This chapter shows you how to open a chromatogram to begin your investigation. You will also learn how to obtain information about a chromatogram before you open it.

Opening a Chromatogram

You can open a chromatogram at any time while using IRIS by following the steps listed in this section. However, to help you get started, this chapter will show you how to open and work with chromatograms on the Main View.

To open a new chromatogram:

1. From the IRIS menu bar select **File > Open > Chromatogram**.

   The Open Chromatogram dialog appears.

2. Select the result file (.RST) that you want to open and click **Open**.

   The Open dialog closes and the chromatogram you just opened is now checked on the Data Tree and displayed on the current view (if the view contains a pane for displaying chromatograms).

   For this example, the newly opened chromatogram appears checked on the Data Tree and is displayed on the Main View in the following panes: Chromatogram pane, Contour Map pane, and 3D Plot pane. To hide the chromatogram from being displayed on the Main View, simply uncheck the chromatogram on the Data Tree.
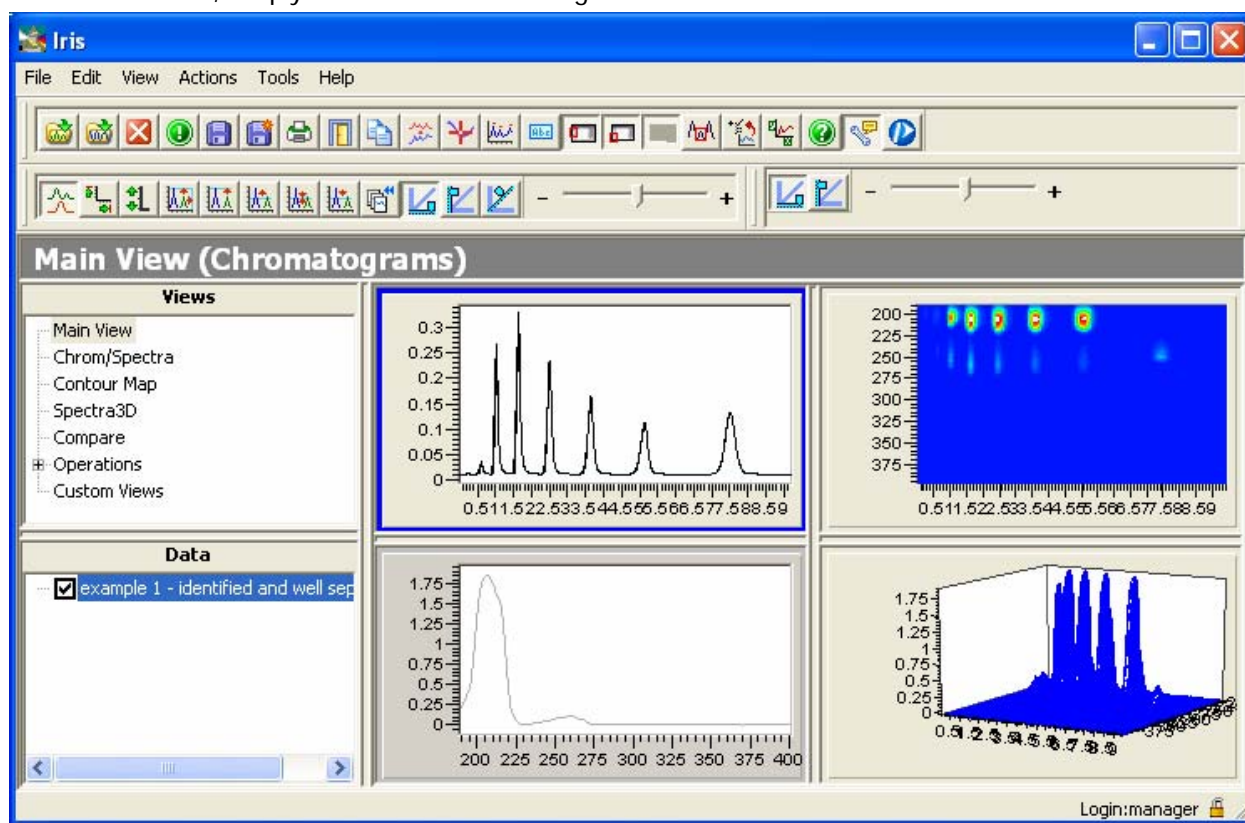
Figure 3-1 Main View after opening a chromatogram

3. Repeat steps **1 and 2** for each additional chromatogram you want to open.

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.*

What happens when I open a chromatogram in IRIS?

When you open a chromatogram, IRIS automatically determines and specifies, on the chromatogram, the times at which the baseline, upslope, apex, and down slope spectra occur.

- The upslope and down slope spectrum positions (times) are determined as a percentage of the peak height.

- By default, the TotalChrom peak-start position (time) is used as the origin of the baseline spectrum; however, you can change how spectra are baseline corrected within a chromatogram from the Spectral Baseline Correction dialog. On this dialog you can select from three options to select/calculate the baseline spectrum. *For more information on the defining how you are going to perform the baseline correction of the spectra, refer to* **103**.

- The TotalChrom peak-retention time is used as the origin of the apex spectrum.

What happens when I open multiple 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 on the Data Tree.

   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.
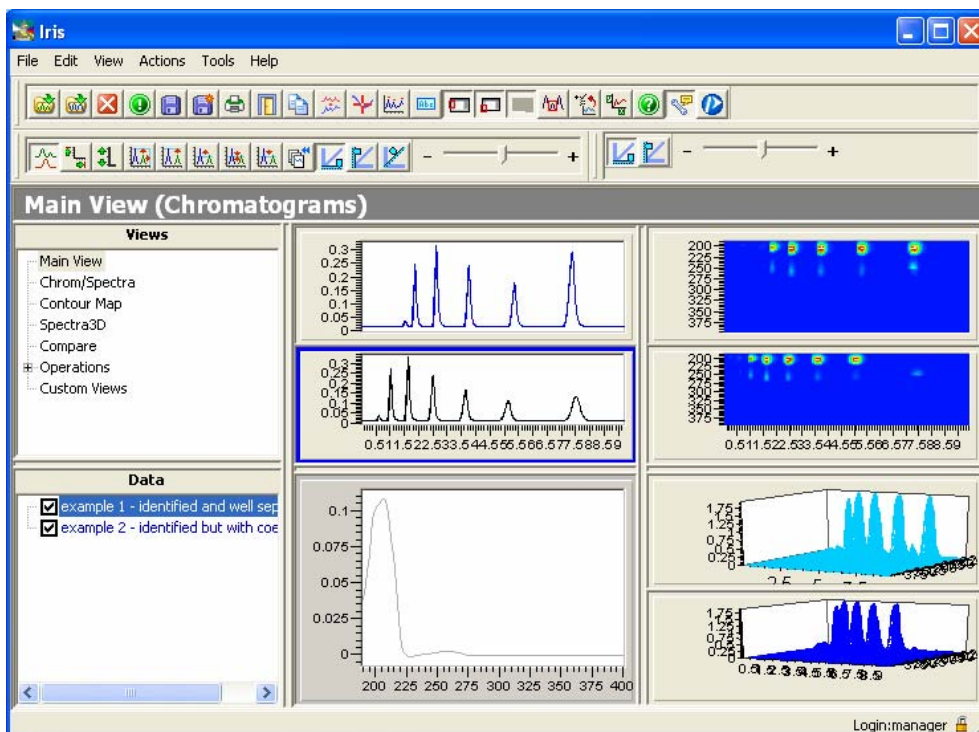
Figure 3-2 The Main View with multiple chromatograms displayed

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 a Stacked display, 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.

Viewing Chromatograms

In the previous section you learned how to open chromatograms and select which chromatograms on the Data Tree you want displayed in the current View. The following section shows you how to work with chromatograms that are displayed on the Chromatogram pane. This section does not cover viewing chromatograms on a Contour Map or 3D Plot. For more information on working with Contour Maps and 3D plots, refer to ***The Contour Map View*** on page ***122*** and The ***Spectra 3D View*** on page ***132***.

Working with 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.
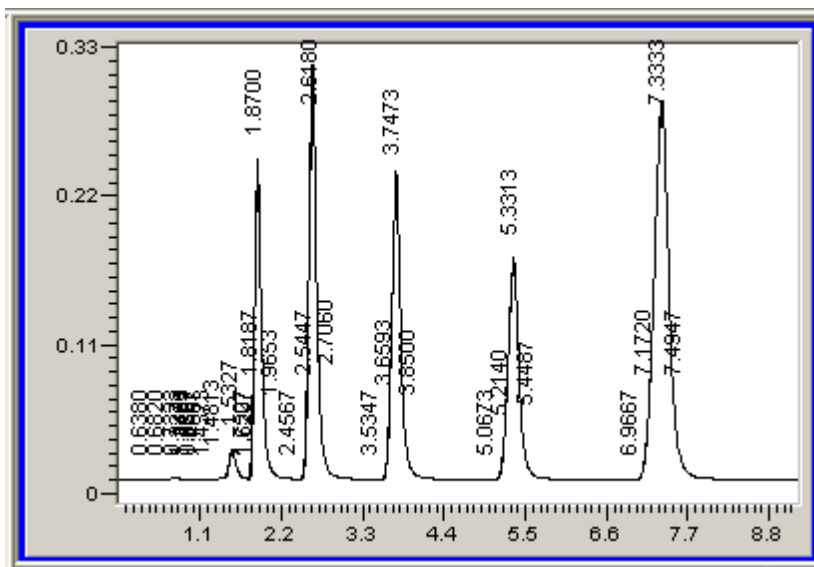
Figure 3-3 A blue border appears around the Chromatogram pane to indicate that it is selected

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.
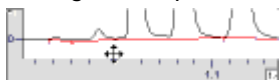
  A drag operation, when the mouse pointer is in this form, drags the graph around the plot region.

- On the Main/View, Chrom/Spectra, and Compare Views, the Stack/Ov**erlay** 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** display is selected, as indicated when the stack/overlay icon appears , and all plots are plotted on the same Y scale, then a single Y scale is shown. If each plot is plotted with its own Y scale, then the Y axis has no labels.

- The toolbar 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** on page **66**.*

The Context Menu for the Chromatogram pane

When you right click on a 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
P̲rint Pane...	Prints the current pane.

✓ Chromatogram B̲aselines	Displays or hides baselines on all chromatograms as a red line.
Overlay C̲hromatograms/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 Operations page. • 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.
✓C̲ursor	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 Operations page.

Label Chromatograms...	Displays the Label Chromatograms dialog. This option is not available when the Chromatogram Pane is displayed on an Operations page.

Command	Description
Range	Displays a green range box on the chromatogram. This option is not available when the Chromatogram Pane is displayed on an Operations page.
Add to <u>V</u>iew...	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 Operations page. **Note:** *To use this option you must have the Chromatogram pane in Stacked mode and the Spectra pane in Overlay mode.*

<u>F</u>ormat 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.

Obtaining Information about a Chromatogram

There are two ways you can obtain information about a stored chromatogram. You can use the **Information** command, located in the **File** menu, to obtain additional information on a stored chromatogram that is not open. Or, you can also view information about a chromatogram that is listed on the Data Tree by right mouse clicking on the chromatogram, and selecting **Information** from the context menu that appears.

To view information about a chromatogram without opening the chromatogram:

1. From the **File** menu select **Information > Chromatogram…**

   The Open Chromatogram dialog appears.
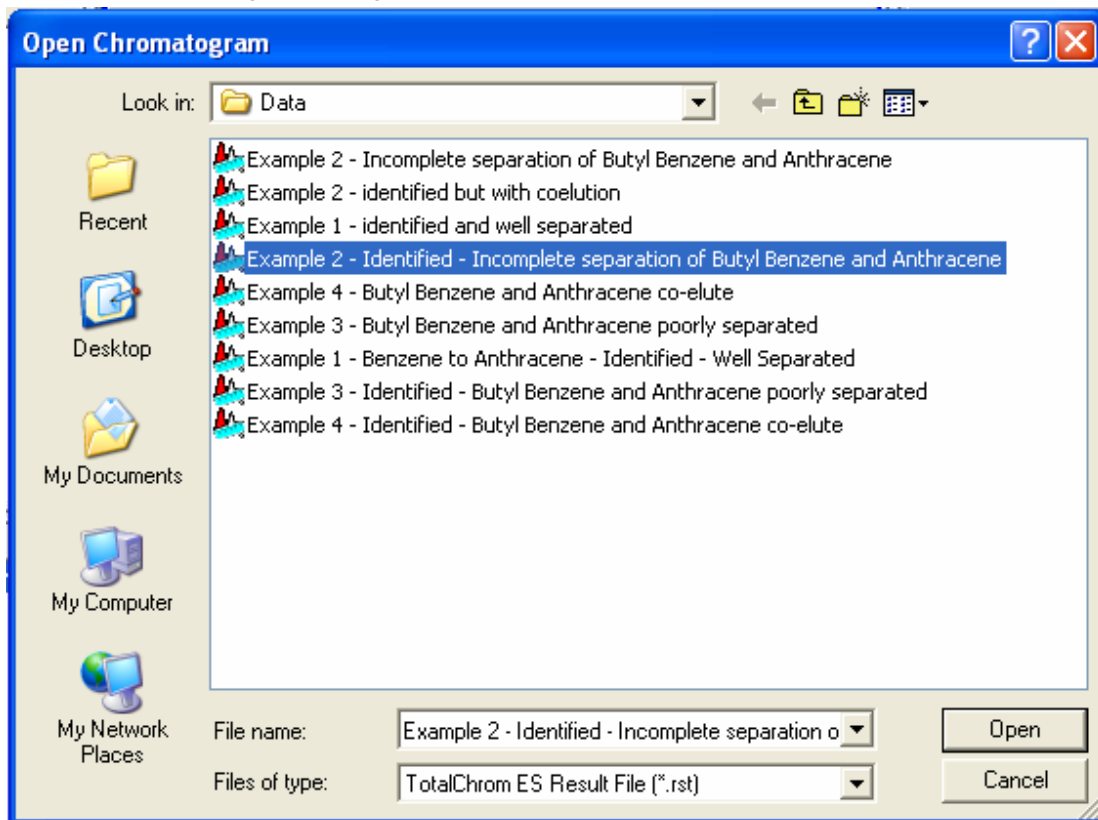
2. From the Open Chromatogram dialog select the result file (.RST) that you want to view information on, and then click **Open**.

   The Information dialog box for the selected chromatogram appears.

To obtain information about a chromatogram that is listed in the Data Tree:

➢ From the Data Tree, right mouse click on a chromatogram, and select **Information** from the context menu that appears.

The Information dialog box for the selected chromatogram appears.
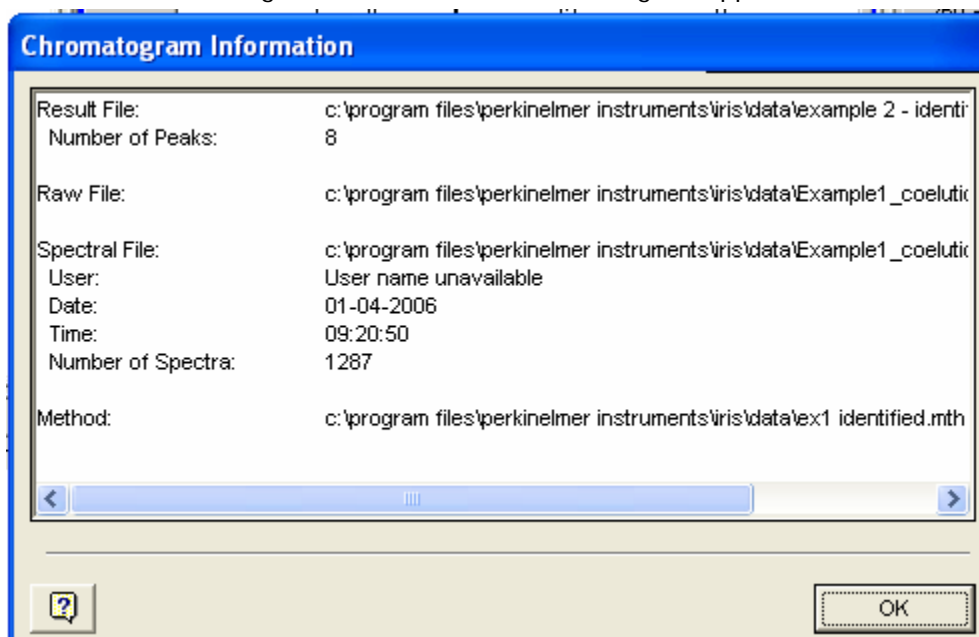
Figure 3-4 Chromatogram Information

Labeling Chromatograms

IRIS provides you with the ability to label individual chromatographic peaks. There are 14 different labels available for annotating the chromatograms. 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 Operations page.

How do I apply a label to chromatograms?

Note: *Labels can only be applied to chromatograms that are displayed on a View. When you are viewing an Operation, you cannot select a label for a chromatogram.*

You can apply labels to all chromatograms that appear in a Chromatogram pane. Or, if you are looking at 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. Select a view from the Views Tree.

2. Select **Actions > Label Chromatograms**.
   The Label Chromatograms dialog appears.

3. From the **Label** drop down, select a label you wish to apply.

4. Check Apply to all chromatograms.

5. Click **OK** to close the Label Chromatograms dialog.

To apply a label to a single chromatogram:

1. Select a view from the Views Tree.

2. Click on any area in the white space of a stacked chromatogram display that contains the chromatogram you wish to label.
   A blue border appears around the selected stacked display.

3. From the Actions menu select **Actions > Label Chromatograms**.
   The Label Chromatograms dialog appears.

4. From the **Label** drop down list, select a label you wish to apply to the chromatogram.

5. Uncheck **Apply to all chromatograms**.

6. Click **OK**.
   The label you selected is applied to the chromatogram.

Scaling Chromatograms

IRIS offers you 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.
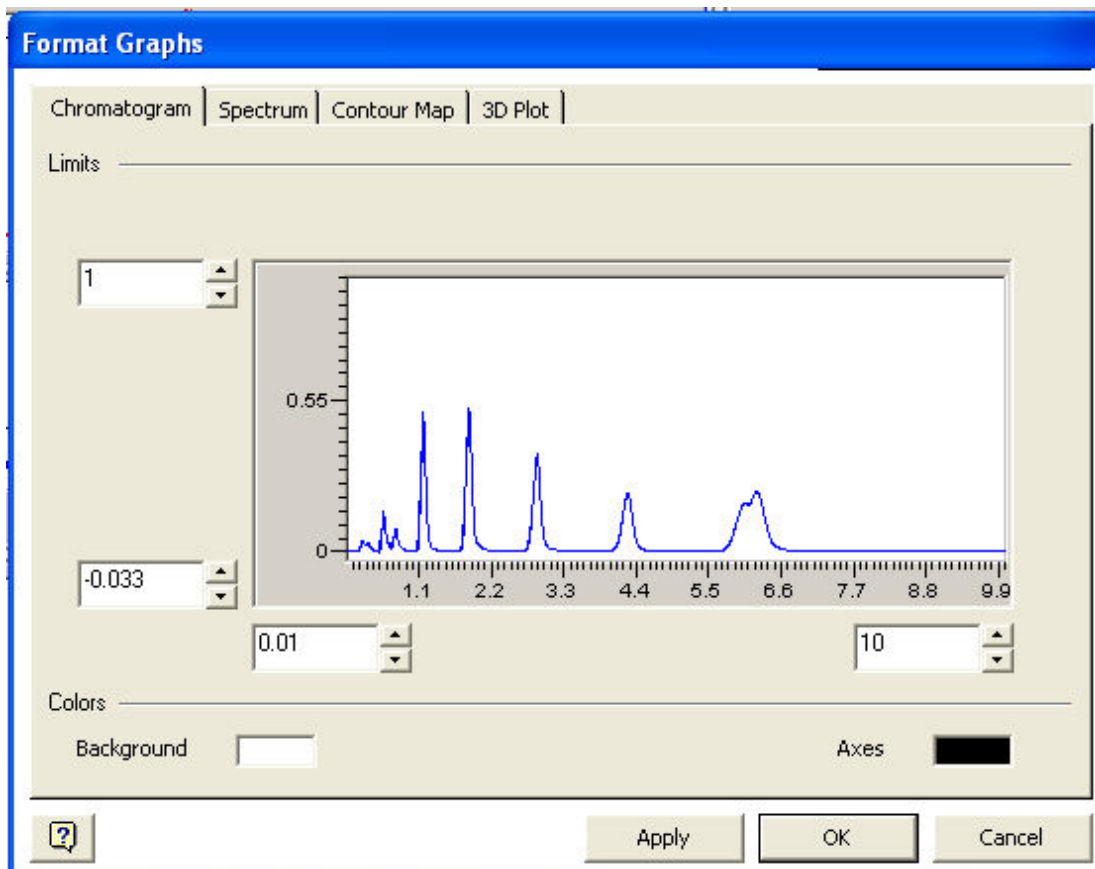
Figure 3-5 The Chromatogram tab page allows you to scale the X and Y axes for a chromatogram

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 on the Chromatogram pane 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 on the Chromatogram you wish 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?
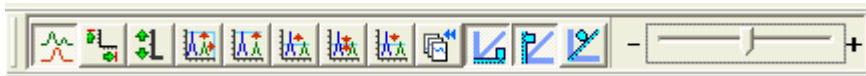
You can zoom a chromatogram in a continuous fashion using the slider zoom feature in conjunction with the **Zoom X** and **Zoom Y** icons. The slider zoom enables you to expand or contract the chromatogram in a continuous manner. The Zoom X and Zoom Y commands determine what is zoomed when you are interacting 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 Toolbar 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 Toolbar slider to the right or left.

How do I use the use the Normalization commands to find subtle differences between chromatographic peaks?

The Normalization commands are most useful when multiple, overlaid chromatograms are displayed and you want 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.

One example, in which the normalize commands may prove useful, is if you have multiple overlaid chromatograms displayed on your screen and you want to confirm the presence of closely co-eluting components. You can select pane containing the multiple overlaid chromatograms and then use the normalize commands described below to better qualitatively compare the exact shapes of the peaks.

For a detailed description of how the Normalization commands can be used, refer to the section titled **Detailed Description of Normalization commands** *in* **Chapter 4. Viewing Spectra**.

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 on the chromatogram you want to normalize.

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-scalled 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.

How do I close a chromatogram?

> ➢ You can close a chromatogram by selecting the chromatogram from the Data Tree and then selecting **File > Close**.
>
> When you close a chromatogram, the chromatogram is removed from the Data Tree and all of the chromatogram's associated displays, such as the Contour Map and 3D Plot are no longer shown. In addition, all of the chromatogram's associated spectra are removed from the Data Tree and are no longer displayed.

Note: *If you do not want to remove a chromatogram from the Data Tree, you can hide it from being displayed by unchecking the chromatogram on the Data Tree when you are looking at the Main View, Chrom/Spectra View, and Compare View. On all other Views, you can hide the chromatogram from being displayed by simply selecting a different chromatogram on the Data Tree. .*

Initializing a TotalChrom Environment with an IRIS Chromatogram

This topic shows you how to pass a chromatogram that is open in IRIS back to TotalChrom. It is possible to move a chromatogram you are viewing in IRIS to one of two TotalChrom environments: **Reprocess** or **Graphic Method Edit**.

➢ Right mouse click on the chromatogram in the Data Tree and select **TotalChrom > Reprocess** or **Graphic Method Edit** from the context menu that appears.

The selected chromatogram is displayed in the requested environment.

Chapter 4. Viewing Spectra

Introduction

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.

In this chapter we first describe how spectra are collected for viewing in IRIS. In this chapter you will also learn how to examine spectra on the various IRIS Views as well as learn how to modify the spectrum display so that you can more easily compare spectra.

Spectral Formats

IRIS stores spectra in three different formats: chromatographic, independent and library spectra.

- **Chromatographic Spectra** - These are the spectra associated with a chromatogram. Spectra are collected by the Series 200 Diode Array Detector in equal-time increments across the entire chromatogram (TotalChrom lets you define the time range and the collection increment, i.e., the spectral collection rate).

  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 3D Spectra View to perform this task.

  You can also capture spectra by double clicking your mouse over an area on the chraomtogram, 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.*

Note: *You cannot use the* **File > Open > Spectrum** *command to access a spectrum associated with a chromatogram.*

- **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 and then use the Save As... command. (*Note that you must first extract and display the spectrum; you cannot save spectra directly from a chromatogram to a file.*) Only this type of spectrum can be opened using the Open > Spectrum option under the File menu.

- **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 in IRIS, 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 Libr**ary commands, located under the **Tools** menu.

For more information on setting up a library of stored spectra, refer to the Spectral Libraries chapter on page **151***.*

Viewing a Spectrum

This section shows you how to open and view chromatographic spectra and individual spectrums that have been previously extracted and saved using the **File > Save As…** command in IRIS.

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**.

Viewing Chromatographic Spectra

In this section you will learn 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 View pages in IRIS, refer to the chapter titled **Chapter 5. Viewing the Data**..*

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?

IRIS also allows you to 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.*
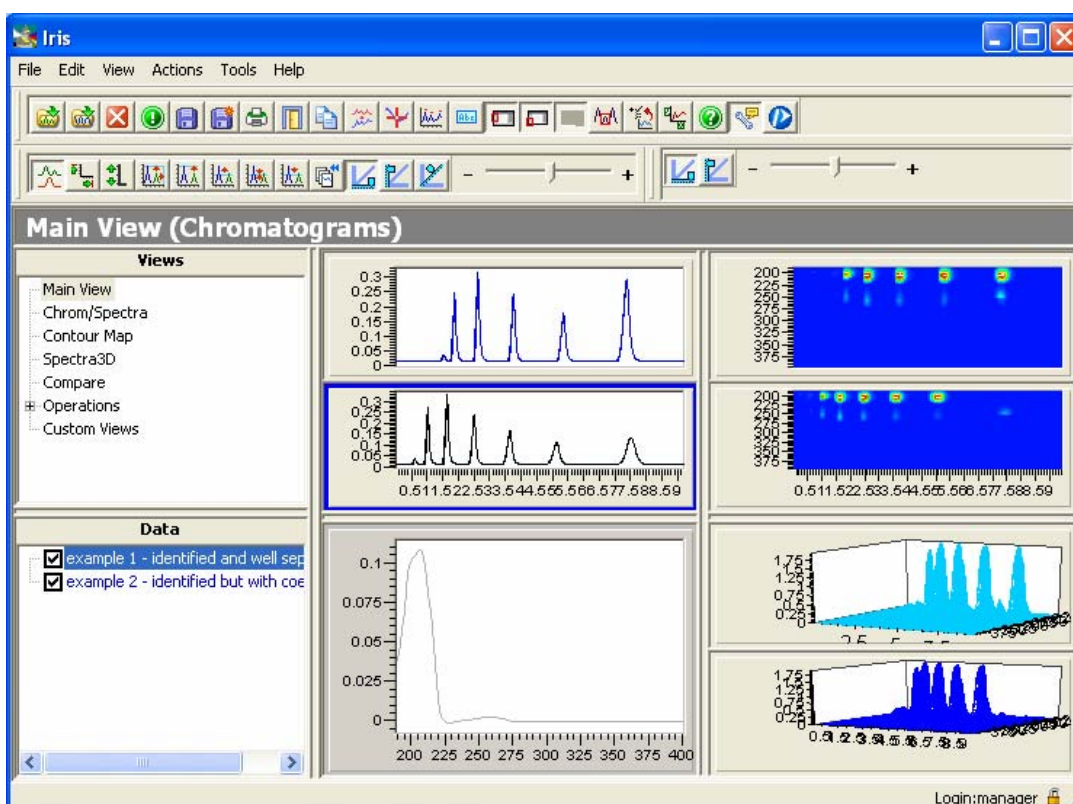
Figure 4-1 On a stacked chromatogram display you can hover your mouse over the chromatogram and the spectrum from that point on the chromatogram is displayed in the Spectra pane

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.
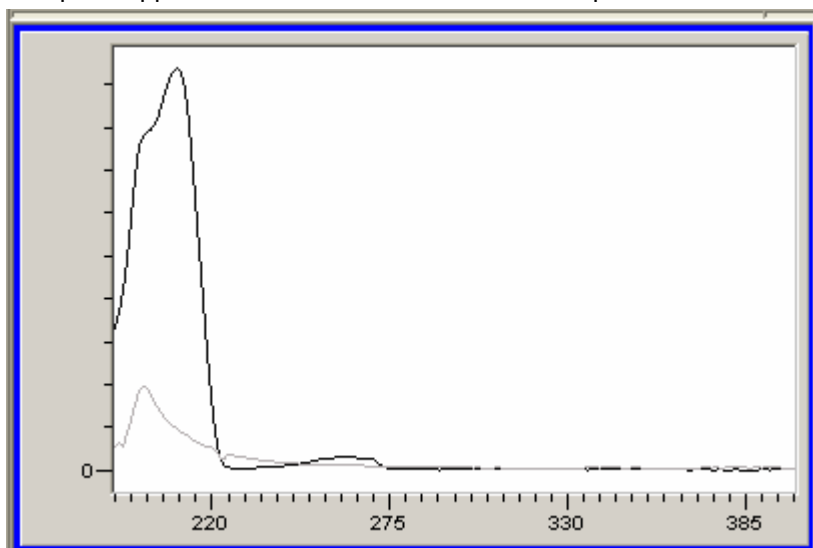
- If a Stacked display is being used on the Chromatogram pane and an Overlaid display is being used on the Spectra pane, you can float the cursor over a point on the Chromatogram pane and have the associated spectrum displayed.

- When the mouse pointer is moved close to any edge of the plot region it changes into a four headed arrow.
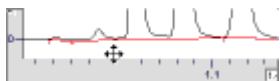
  A drag operation when the mouse pointer is in this form, drags the graph around the plot region.

- You can also scale spectra with a large selection of tools.

  For more information on scaling spectra, see the section titled **Visually Comparing Spectra** *on page* **89**.

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 **Overlay 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 Graphs dialog at the Spectrum tab. Changes made here effect the current graph only.

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:

5. 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.

6. 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.

7. 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 **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. 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.
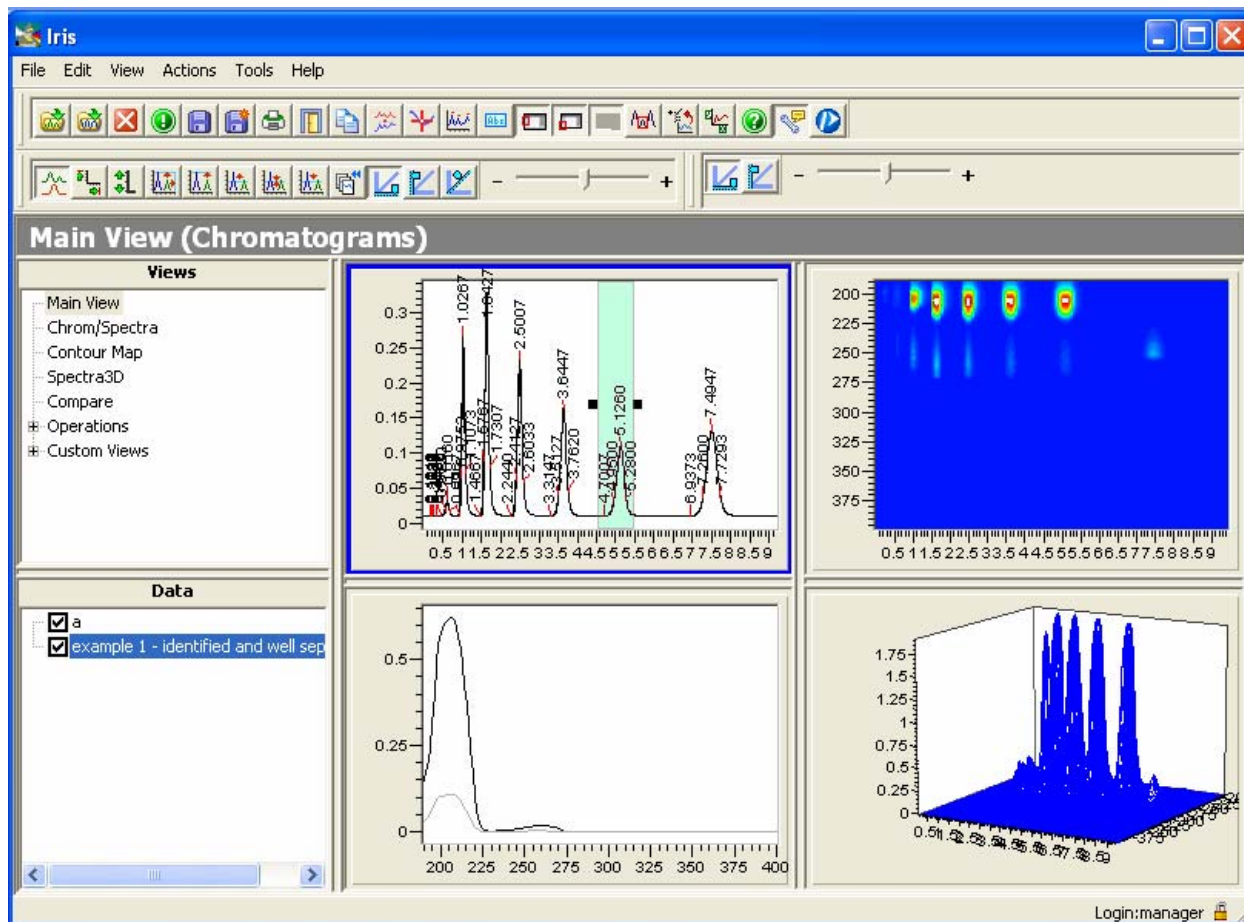
Figure 4-2 Click on the Range icon to activate the range box

When the range box is activated:

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 checked. When you release the mouse, the range box is cleared.

Note: *If more than 70 spectra are included in the range, you will be informed of the number of spectra you have requested and are warned that the operation may take some time.*

- Clicking on the chromatogram, in an area outside of the box, clears the range box.

Viewing Individual, Previously Stored Spectra

You can save individual spectrums, plus its baseline spectrum for comparison purposes by first extracting the spectrum from a chromatogram. Once the spectrum is extracted and appears on the Data Tree, you select the spectrum on the Data Tree, right mouse click on the spectrum, and select Save As... A Save Spectrum As dialog appears. On the Save Spectrum dialog you specify a file name and location for the spectrum.

How do I view a previously stored individual spectrum?

1. Once a spectrum is saved, using the procedure described above, you can view the spectrum again by selecting **File > Open > Spectrum**....

   The Open Spectrum dialog appears.

2. Select the spectrum you want to open and click **Open**.

   The selected spectrum is opened and now appears as its own node on the Data Tree. On the Main View and Chrom/Spectra View, and Compare View you can display this spectrum by checking it on the Data Tree. The spectrum is displayed on the Spectra pane.

Removing Spectra and Hiding Spectra from the Data Tree

Removing Spectra from the Data Tree

➢ If you want to remove all of the spectra from the Data Tree select **File > Close 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.

Obtaining Information on a Stored Spectrum

There are two ways you can obtain information about a spectrum. You can use the **Information** command, located in the **File** menu, to obtain additional information on a stored spectrum that is not open. Or, you can also view information about a spectrum that is listed on the Data Tree by right mouse clicking on the spectrum, and selecting **Information** from the context menu that appears.

Note: *If you are viewing Information on spectra created via a mathematical operation in IRIS, the details of the operation and the source of the spectra will be included on the Information dialog. If you are viewing Information on spectra extracted from chromatograms, the source of the spectra is shown on the Information dialog along with the baseline source and type (single point, peak start, interpolated, etc.).***To view information about a spectrum without opening the spectral file:**

1. From the **File** menu select **Information > Spectrum**.

   The Open Spectrum dialog appears.

2. From this Open dialog, select the spectral file (.UV) that contains the spectrum that you wish to view more information on and click **Open**.

   The Information dialog box for the selected spectrum appears.
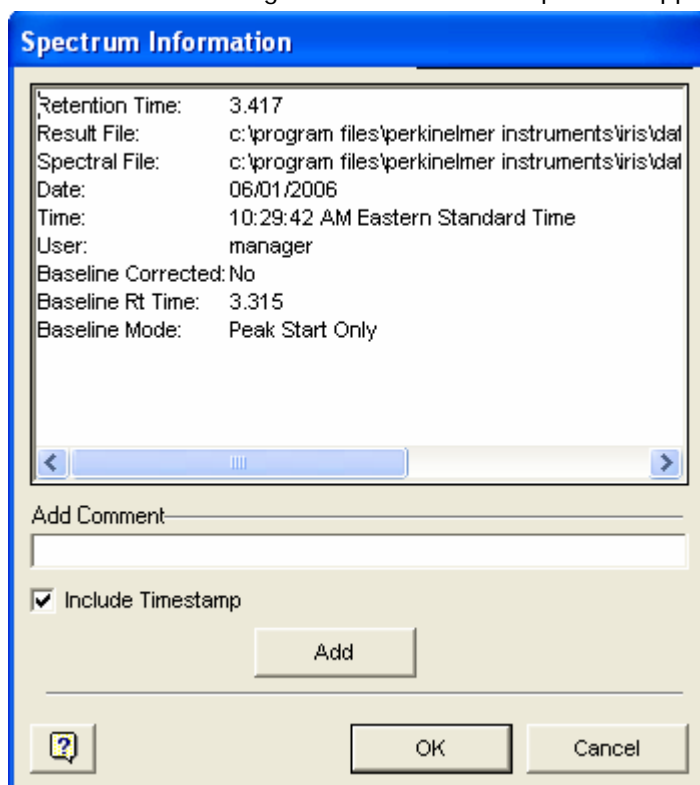
Figure 4-3 Information dialog for a previously saved spectrum

To obtain information about a chromatogram that is listed in the Data Tree:

➢ From the Data Tree, right mouse click on a spectrum, and select **Information** from the context menu that appears.

The Information dialog box for the selected spectrum appears.

Spectrum Information

Retention Time:	3.417
Result File:	c:\program files\perkinelmer instruments\iris\dat
Spectral File:	c:\program files\perkinelmer instruments\iris\dat
Date:	06/01/2006
Time:	10:29:42 AM Eastern Standard Time
User:	manager
Baseline Corrected:	No
Baseline Rt Time:	3.315
Baseline Mode:	Peak Start Only

Add Comment

☑ Include Timestamp

Add

OK   Cancel
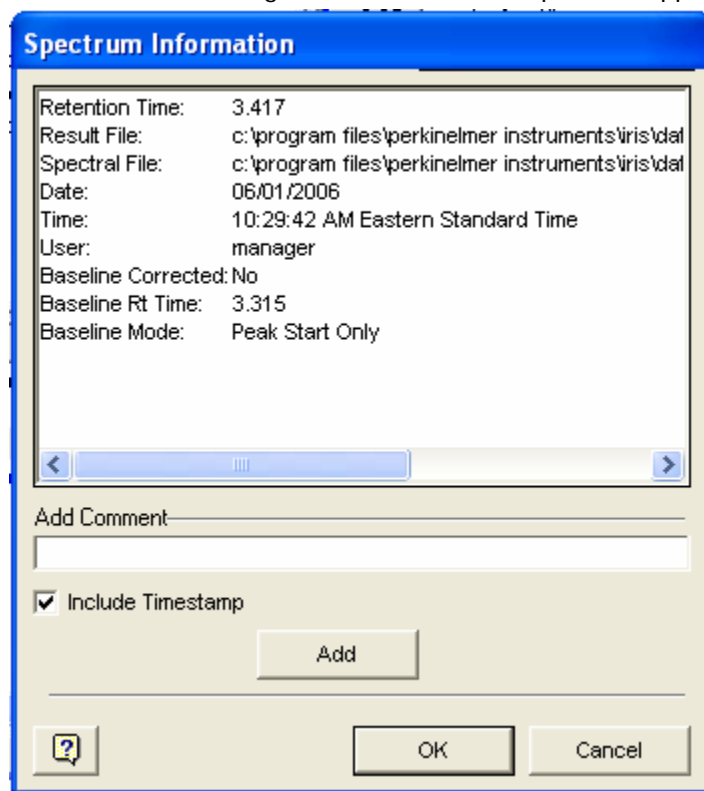
Figure 4-4 Information dialog for a spectrum that was selected on the Data Tree.

Visually Comparing Spectra

The default display protocol for spectra displayed in IRIS 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.

IRIS does, however, provide you 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, and then describes in detail the various forms of spectrum normalization.

Options for Scaling Spectra

This section describes the scaling commands you can use to in order to modify the spectrum display. You will find that the information in this section is useful when multiple, overlaid spectra are displayed.

Note: *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.*

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.

- 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

Normalizing Spectra

The Normalization commands are **most** useful when multiple, overlaid spectra are displayed.

The Normalization commands are as follows:

Note: *If you are trying to scale a single chromatogram or spectrum, only Offset Normalize and Point Normalize 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.

Detailed Descriptions of Normalize Buttons

Normalize All

The **Normalize X and Y** command ![icon] applies to chromatogram displays, as well as to spectral displays containing multiple, overlaid spectra. This command scales the maximum Y value in the active window (i.e. the Chromatogram or Spectra pane that is currently selected) to 90% for each spectrum or chromatogram and sets the X axis scale so that all of the largest spectrum or chromatogram is displayed.   All chromatograms or spectra in the selected pane are affected by this command.

Figure 4-5, below, shows a range of spectra displayed over the last peak. Because the spectra, in the display below, are of varying sizes it is not clear if the chromatographic peak is homogeneous, nor is it clear whether or not all the spectra are identical, or if the spectra vary across the peak. To better compare the peaks, you can use the Normalize commands, since these commands will convert all of the peaks to the same maximum height.
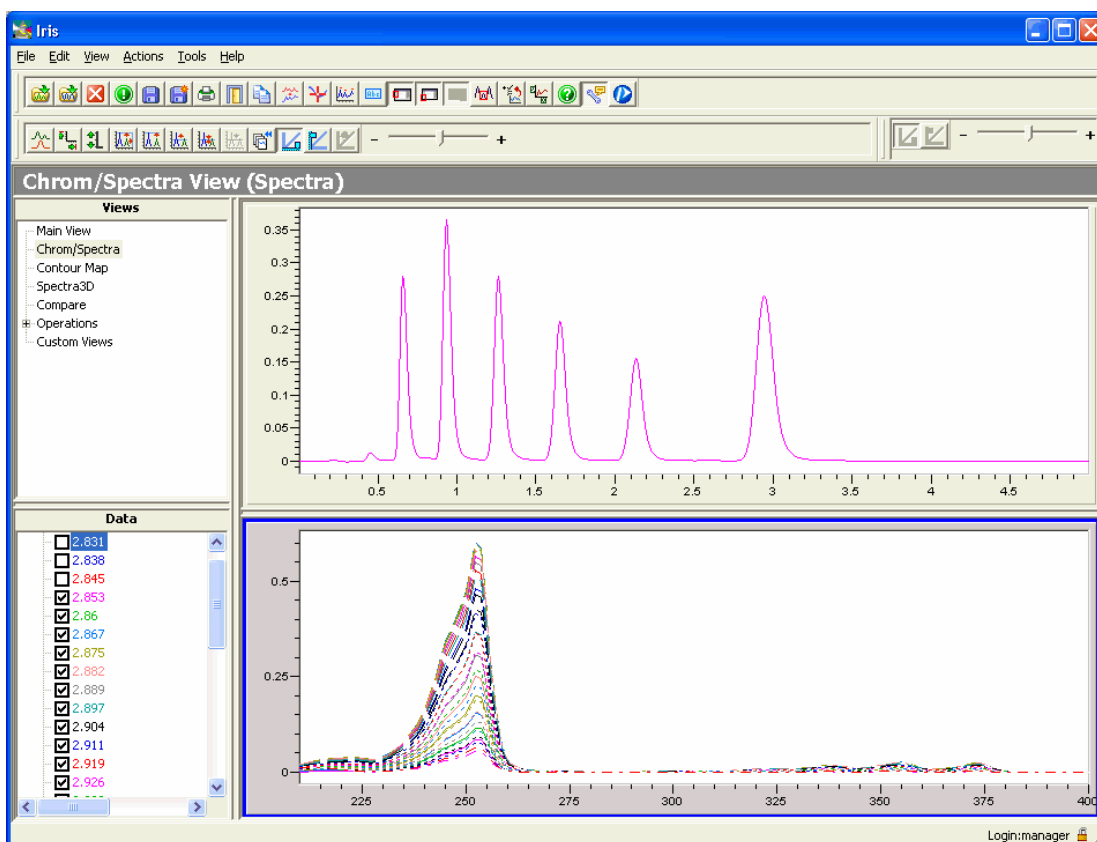
Figure 4-5 Range Spectra display over the last peak

If the **Normalize X and Y** command is applied to **Figure 4-5**, it is evident that all the spectra do appear to be identical; and therefore, the chromatographic peak is homogeneous.
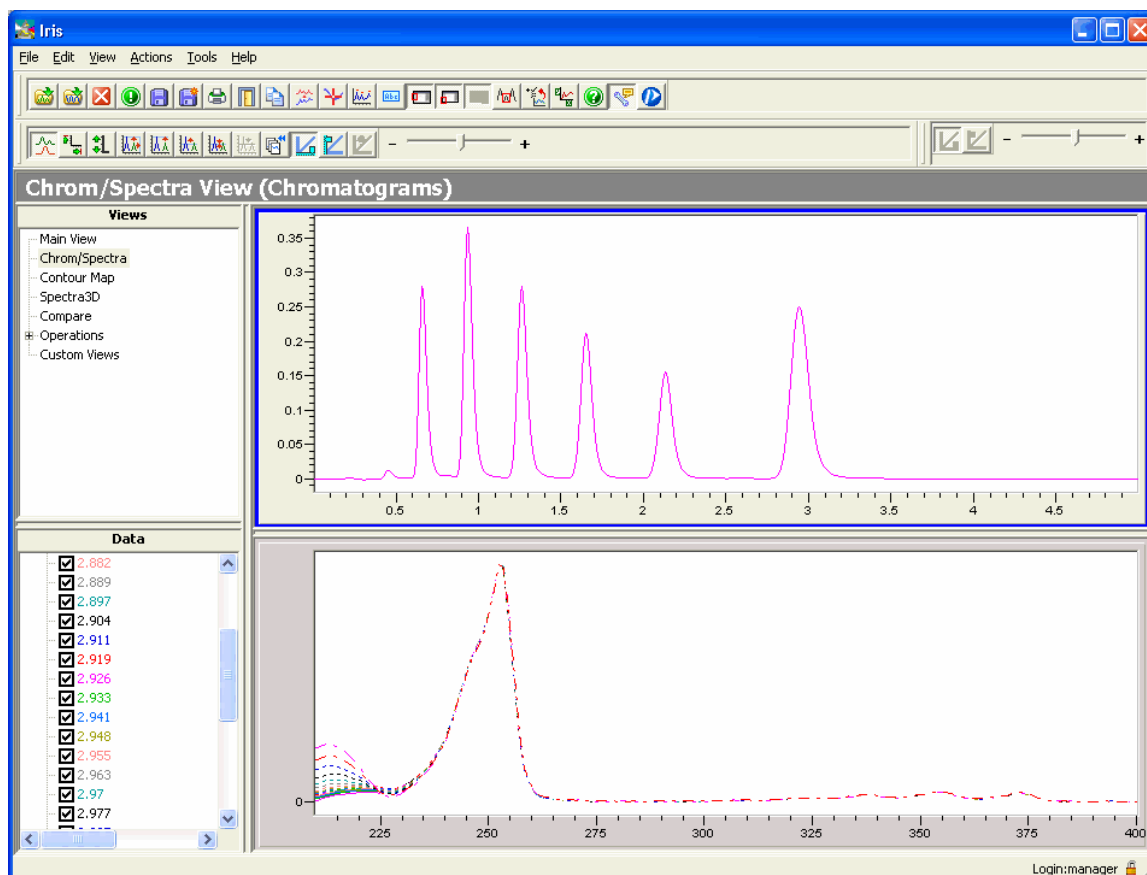
Figure 4-6 Range Spectra display after the Normalize X and Y command has been applied

Normalize Y Only

The **Normalize Y Only** command ![icon] works like the **Normalize X and Y** command, except that it leaves the X axis unchanged and rescales only the Y axis.

In the example below, four spectra were captured from four different peaks: Toluene, Ethyl Benzene, Propyl Benzene, and Butyl Benzene. Because of the high absorbance in the low UV of the spectra shown in the figure below, it is very difficult to see any subtle differences in the higher part of the UV spectrum.
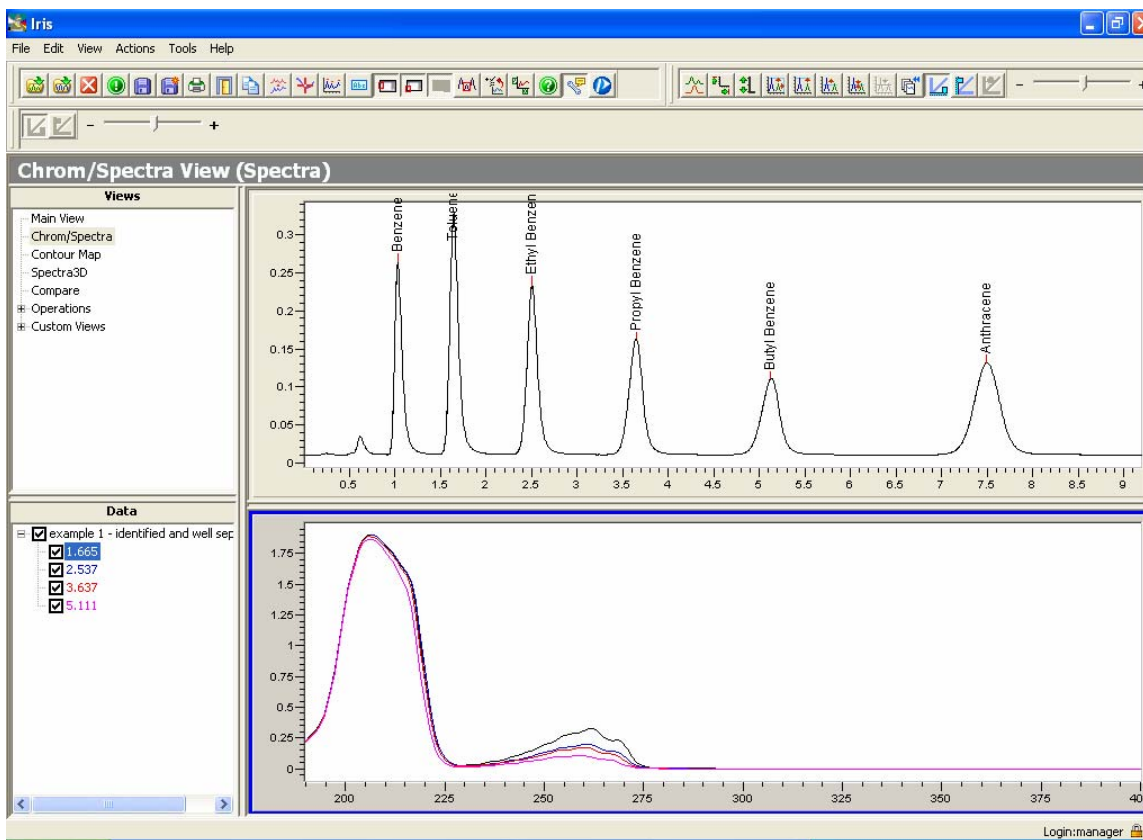
Figure 4-7 Before applying the Normalize Y only command, it is difficult to see any subtle differences in the higher part of the UV spectrum

To better compare the spectra, we can zoom into this region and then apply the **Normalize Y Only** command.
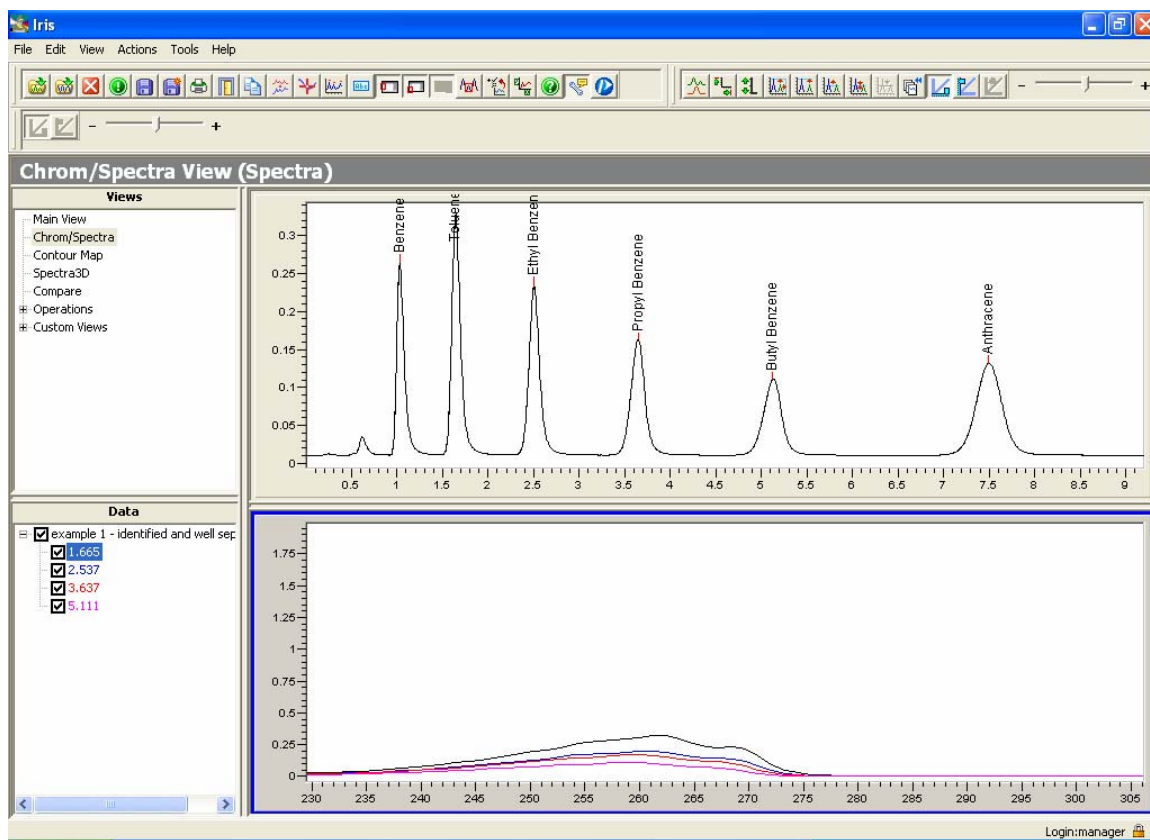
Figure 4-8 Figure showing the results after zooming into the region of interest
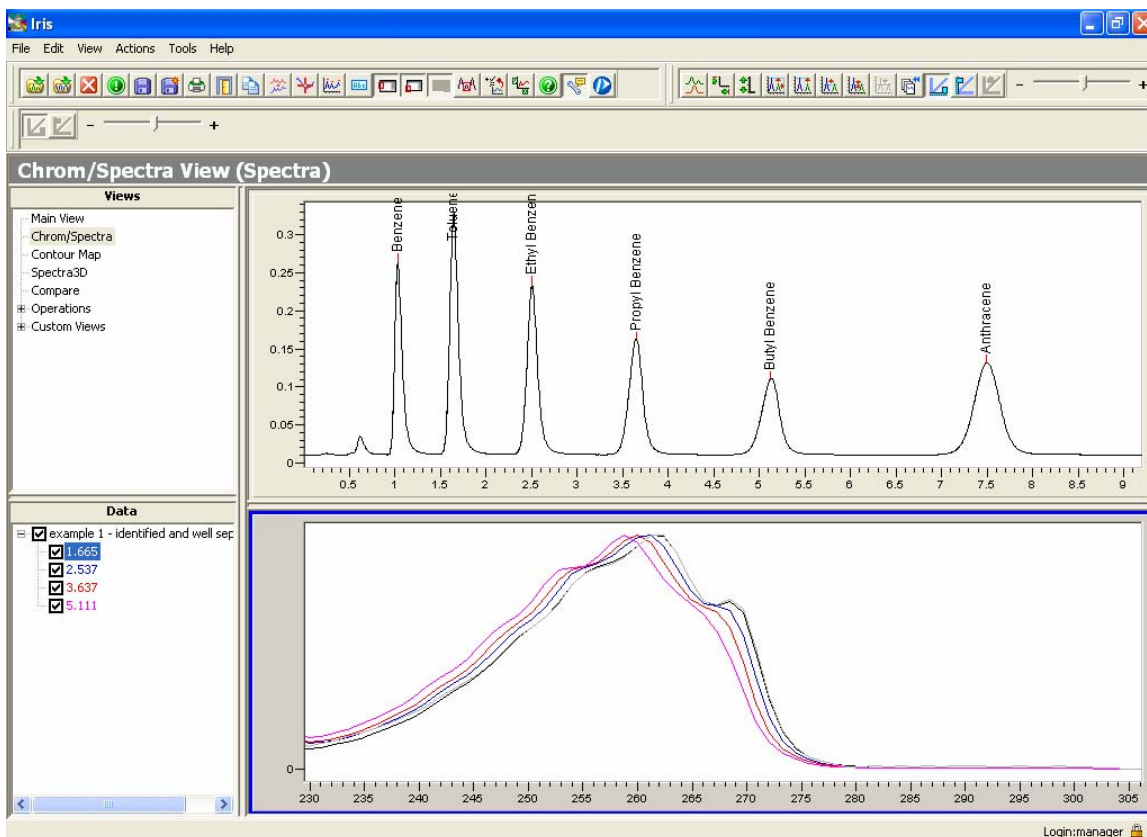
Figure 4-9 Figure after the Normalize Y command has been applied

As shown in the figure above, the spectra are not identical. Since the spectra were all captured from different peaks, the results shown in the figure above are expected; however, by using the **Normalize Y** command we were able to confirm the expectation that the four spectra were not identical.

Offset Normalize

The **Offset Normalize** command ⬚ applies to chromatogram displays, as well as to spectral displays containing multiple, overlaid spectra. 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.

In the **Figure 4-7**, when examining a set of spectra to observe their differences, we expanded the scale to observe only the wavelength range of interest, and then we used the Normalize Y command. Since there was no higher absorbance to the right of the region of interest, we could have more easily achieved our goal by using the Offset Normalize command, as shown in the figure below. In this case, we positioned the cursor at

approximately 230 nm, and then selected **Offset Normalize** ⬚.
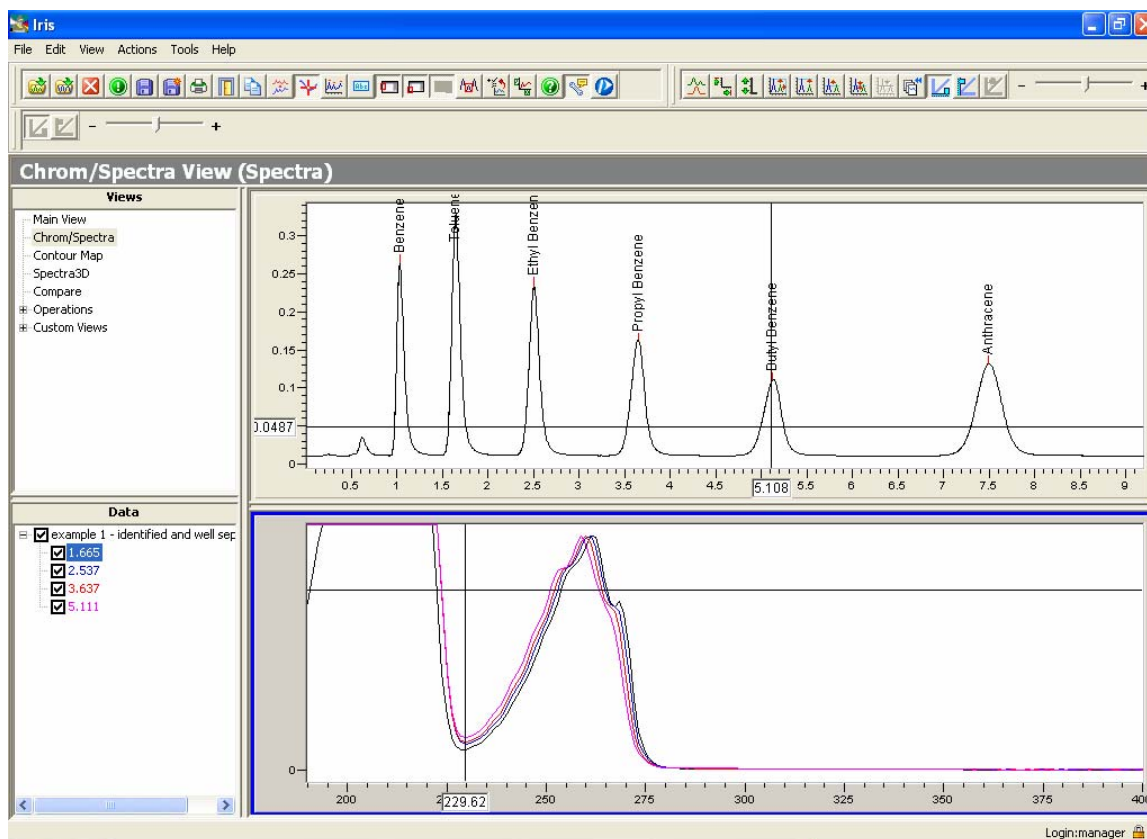
Figure 4-10 Using Offset Normalize

Normalize Point

The **Normalize Point** command ![icon] applies to chromatograms, contour maps, and spectra. For chromatograms and spectra, this command scales the Y value at the current cursor point for every chromatogram or spectrum to 90%.

Note: *The Normalize Point button is valuable in the Contour Map view, as discussed in the section titled* ***Scaling a Contour Map*** *on page* ***129****.*

The figure below shows detail from spectra extracted at different points on an Anthracene peak. To amplify any possible differences, we can use the Normalize Point command ![icon] to force all the curves to an identical absorbance at a specific point on the peak.
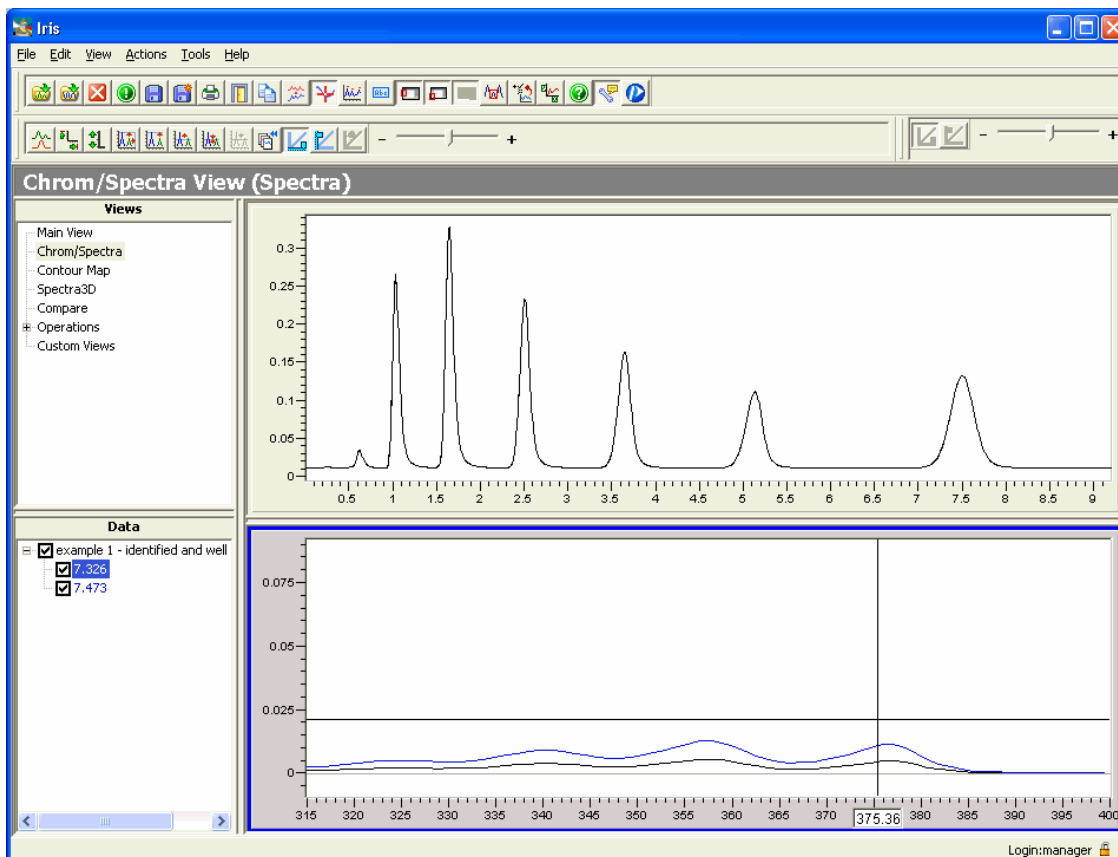
Figure 4-11 Spectra Detail
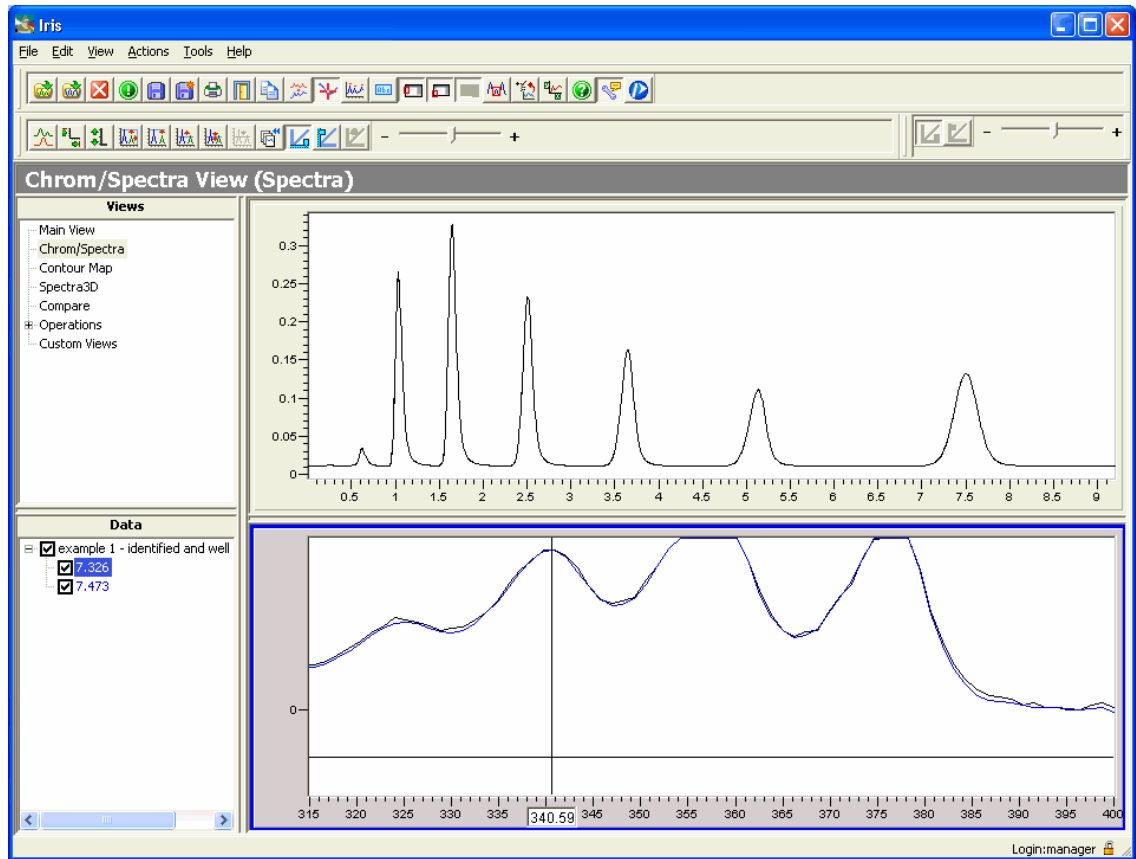
Figure 4-12 Detailed examination using Normalize Point

Additional Options for Scaling Spectra

In addition to the Autoscale and Normalize commands, IRIS offers the following additional options 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.*

Saving an Individual Spectrum

You can use the **File > Save As...** command to save an individual spectrum, plus its baseline spectrum for comparison purposes. The spectrum is saved as an .UV file. You must have the spectrum selected on the Data Tree in order to save it. Once you have saved the spectrum it can be reopened by selecting **File > Open > Spectrum...** from the IRIS menu bar.

To save a spectrum that you have extracted from a chromatogram:

1. From the Data Tree click on the spectrum you wish to save.

2. From the menu bar select **File > Save As...**
   The Save Spectrum As dialog appears.

3. From the Save As dialog enter a **File name** for the .UV file and click **Save**.
   The spectrum is saved and a information header is added to the .UV file that identifies the source of the chromatogram, the user's full name, user id, and the date and time the spectrum was saved.

Defining How You are Going to Perform the Baseline Correction of the Spectra

The Spectral 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.
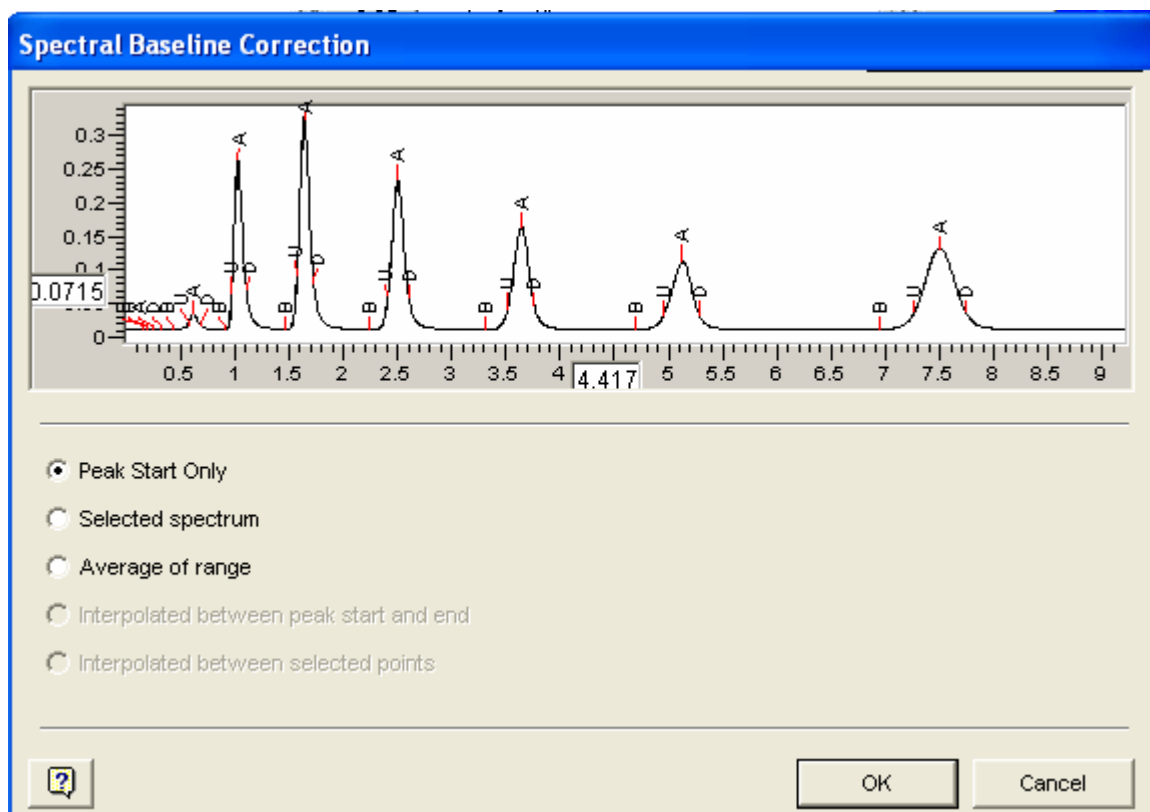
Figure 4-13 The Spectral Baseline Correction dialog allows you to define how you are going to perform the baseline correction of the spectra

On the Spectral Baseline Correction dialog you can select from the following three options that are available to you for calculating/selecting the baseline spectrum:

- **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.

Chapter 5. Viewing the Data

IRIS Views

You examine chromatograms and spectra from Views. IRIS provides you with five default Views: **Main View**, **Chrom/Spectra View**, **Contour Map View**, **Spectra 3D View**, and **Compare**. By default, when IRIS is started 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 IRIS window. The menu bar and tool bars always appear on the IRIS 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 page 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, or 3D Plot 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 Page 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

When you first launch IRIS the Main View is displayed on the IRIS window. 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 page **66**. For more information on **scaling spectra**, see page **89**. For more information **on scaling the Contour Map**, see page **129** . For more information on scaling a **3D Plot**, see page **141**.*

Tell me about the Main View.
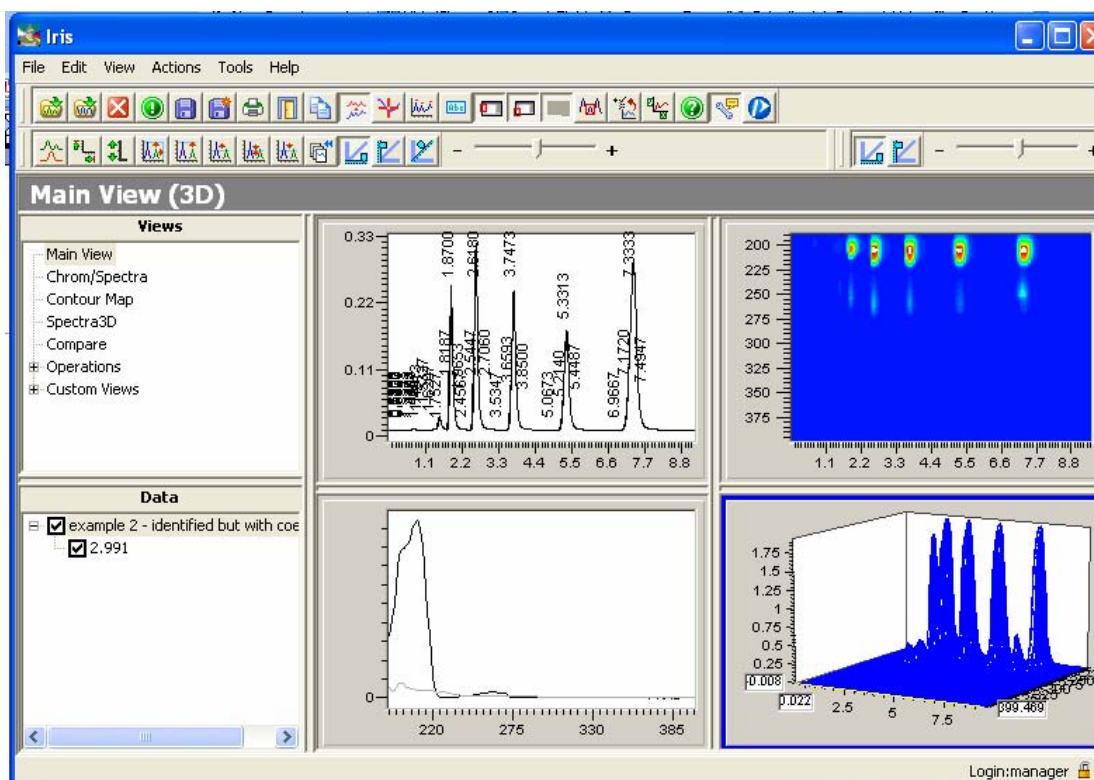
Figure 5-1 The Main View is used to display chromatograms, spectra, contour maps, and 3D plots

The Main View consists of the following areas:

- **Views Tree** - The Views Tree provides you access to the Main View. The Main View is selected by default when IRIS is launched.

- **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 ![icon], and the Spectra pane is set to **Overlay** mode, as indicated when the stack/overlay icon appears ![icon], 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.
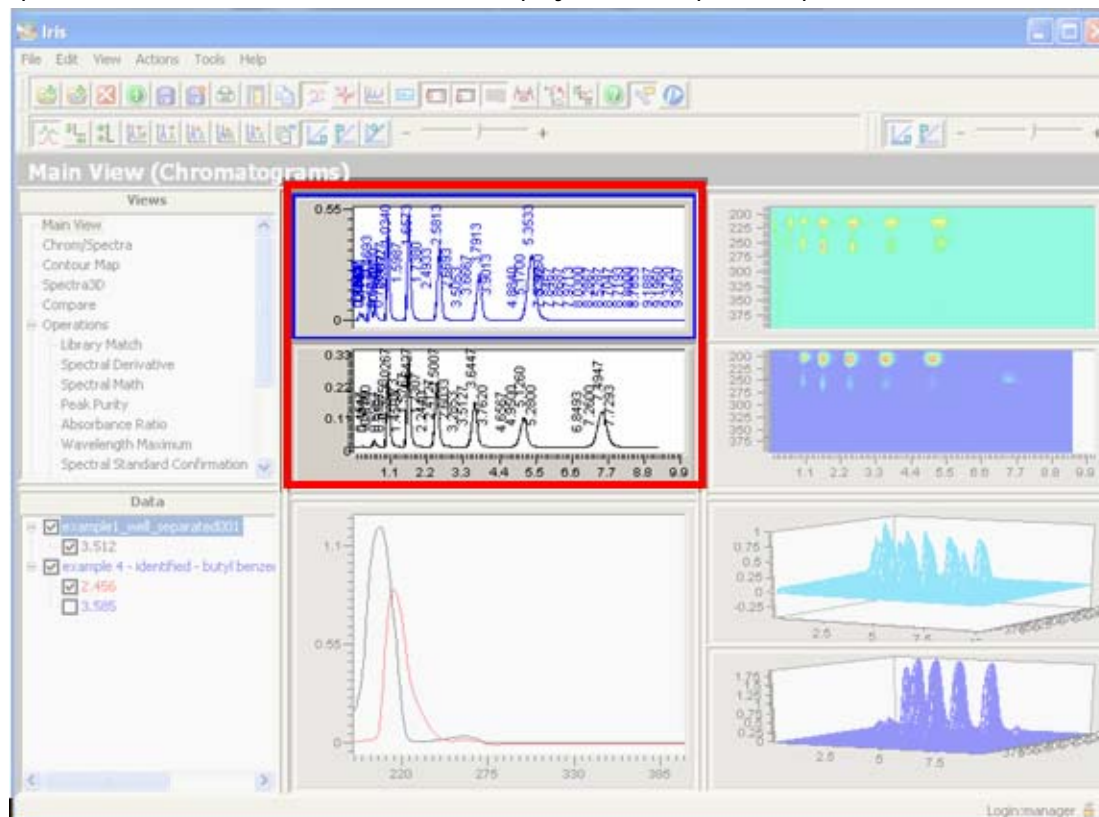
- **Spectra Pane** - The Spectra pane displays the spectra that are checked on the Data Tree.
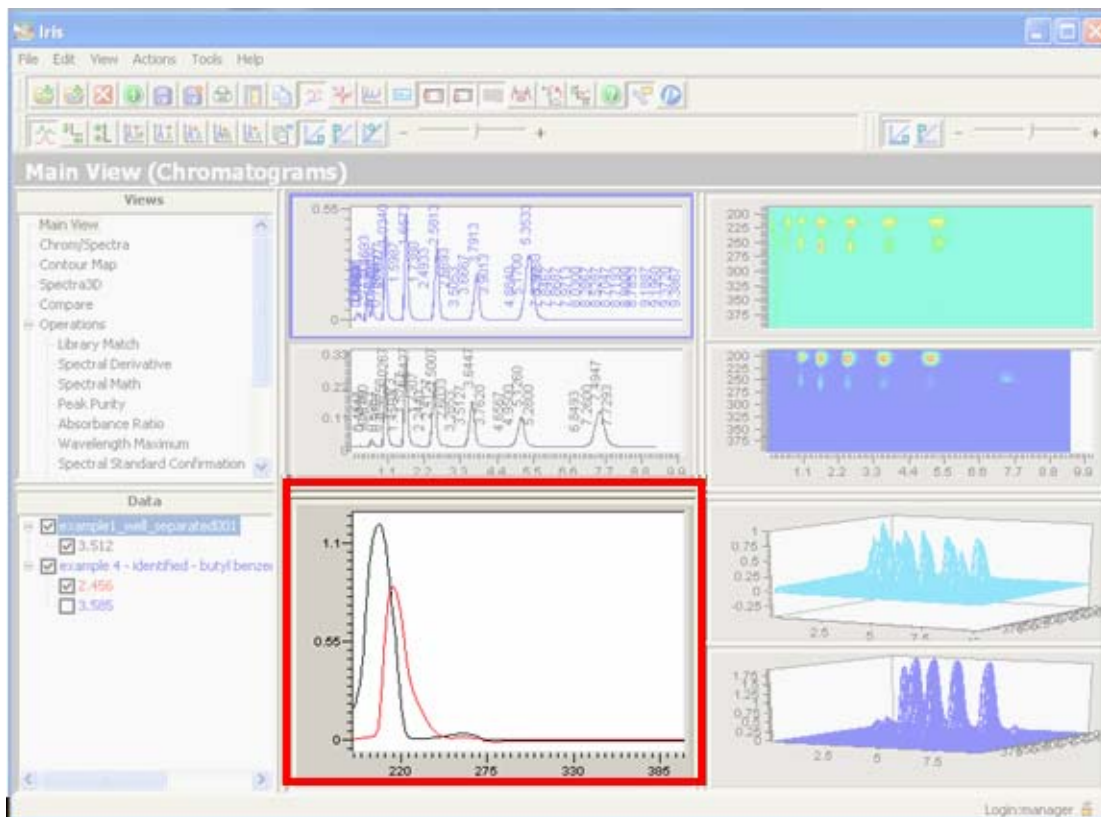
- **Contour Map** - This pane displays stacked contour maps for all chromatograms that appear checked on the Data Tree. The stacked contour maps appear in the same order as the chromatograms displayed on the Chromatogram pane.

  You can float your mouse pointer over this pane 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 an overlaid display, 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 this pane, first make sure the Chromatogram pane is set to an overlaid display. Then, double click on an area in the Contour Map pane; 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.*

*For information on capturing chromatograms and spectra from the Contour Map View, see page **122**.*
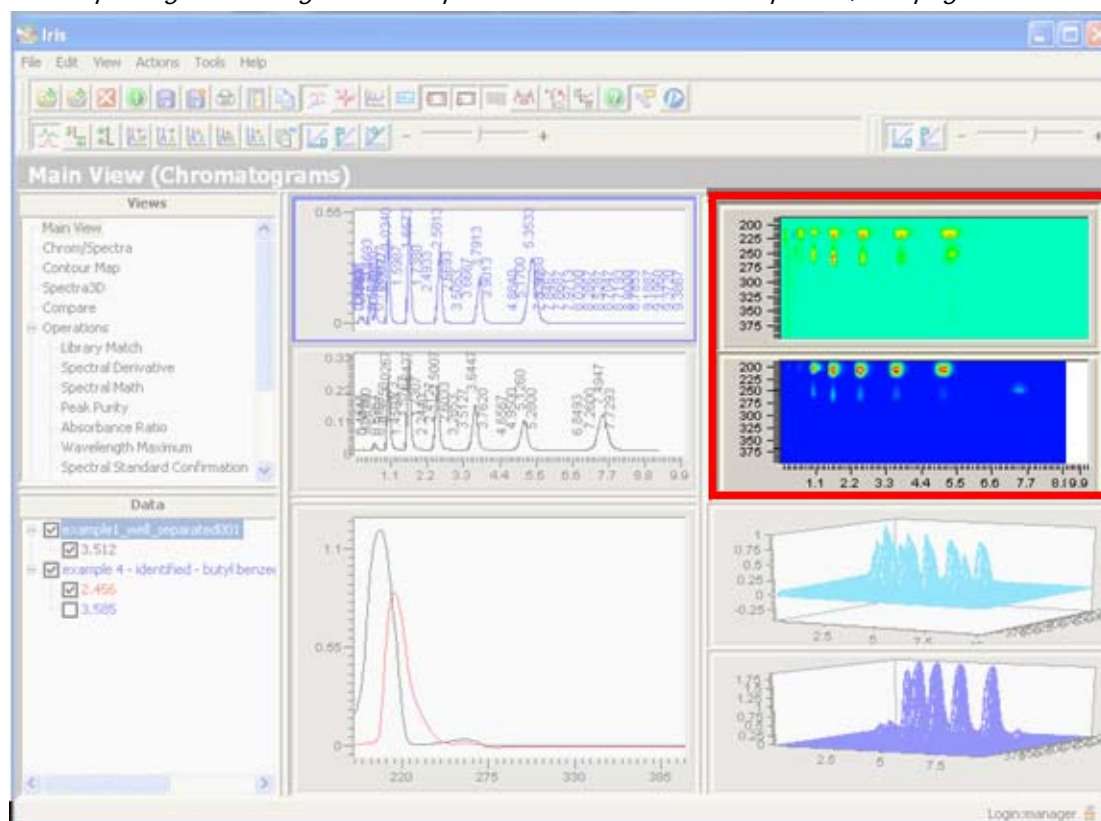
- **3D Plot Pane** - This pane displays stacked three-dimensional perspective plots for each chromatogram that is checked on the Data Tree. The stacked plots appear in the same order as the chromatograms displayed on the Chromatograms pane.

  You can float your mouse pointer over the 3D Plot pane to temporarily view a single spectrum and chromatogram from a point on the 3D Plot pane. *(**Note:** the Chromatogram and Spectra panes must be set to an Overlaid Display)*

  If you want to extract spectra from this pane: 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 pane; 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.*

For information on using the 3D Spectra View to capture chromatograms and spectra, see page **132***.*
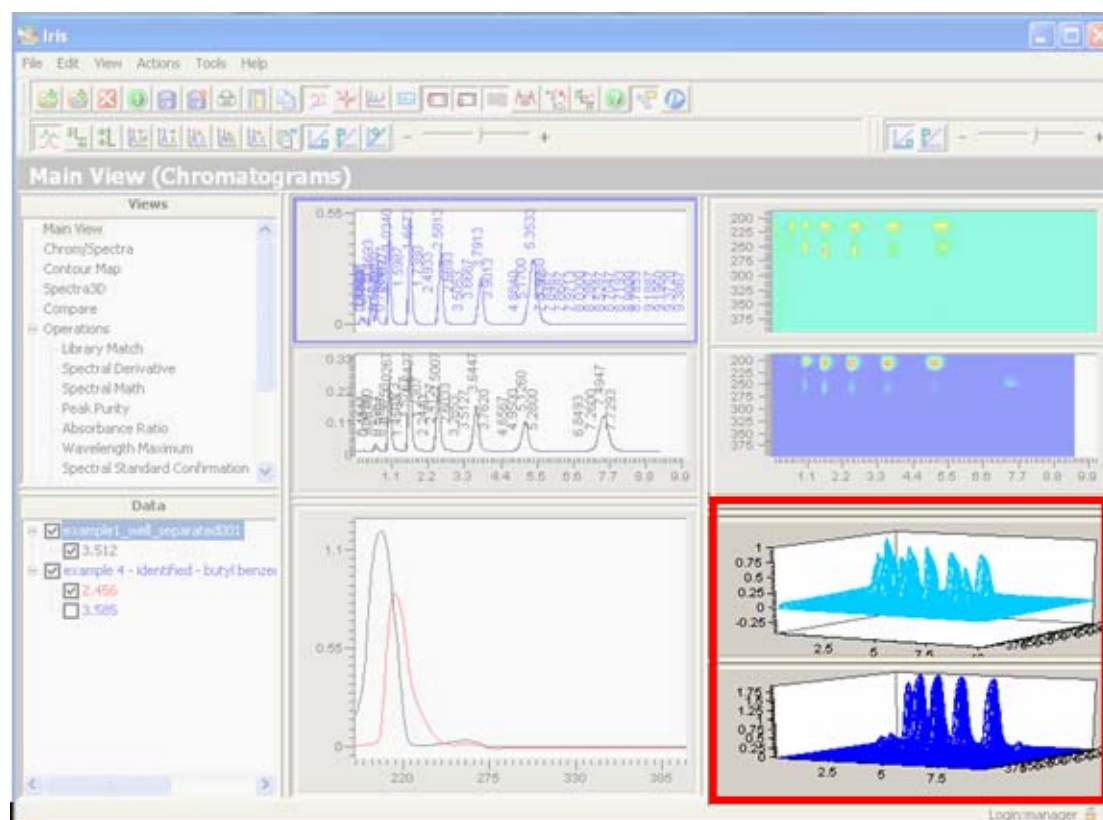
How do I adjust the pane width and height?

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 using the **File > Open > Chromatogram** command on the menu bar. To display chromatograms on the Main View, check the chromatograms you wish to be displayed from the Data Tree.

To open a chromatogram:

1. From the menu bar select **File > Open > Chromatogram**.
    The Open Chromatogram dialog appears.

2. Select the result file (.RST) that you want to look at and click **Open**.
    The Open dialog closes and the name of the chromatogram is added to the Data Tree and the chromatogram is displayed in the Chromatogram pane. The chromatogram can be hidden by unchecking it from the Data Tree.

Note: *You can find information on a chromatogram, 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 open a previously stored, individual spectrum ?

You can open previously stored, individual spectrum files using the **File > Open > Spectrum** command. You can also select the spectra that you wish to be displayed on this window by checking the spectra you want to display on the Data Tree.

To open previously stored, individual spectrum files:

1. From the menu bar select **File > Open > Spectrum**.
   The Open Spectrum dialog appears.

2. Select the spectral file (.UV) that you want to open and click **Open**.
   The Open dialog closes and the spectrum appears checked in the Data Tree.

How do I capture a spectrum from the 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 a **stacked** display by clicking on the
   Stack/Overlay 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 the Contour Map pane?

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 the 3D Plot pane?

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 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 the 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 **Chromatogram pane** must use an **overlaid** display, and the **Spectrum pane** must use a **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 **Overlay Chromatograms** 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 the Contour Map pane?

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 the 3D Plot pane?

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.

Note: *To temporarily capture a chromatogram from a 3D Plot pane, the **Chromatogram pane** 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.*

How do I close displayed chromatograms?

➢   You can close a chromatogram by selecting the chromatogram from the data tree and then selecting **File > Close**.

    When you close a chromatogram, the chromatogram is removed from the Data Tree and all of the chromatogram's associated displays, such as the chromatogram, Contour Map, and 3D Plot, as well as all of the associated spectra are no longer shown.

    If you attempt to close an extracted chromatogram or calculated spectrum that has not yet been saved a message appears and states: "The items selected for closing include data that has not been saved. Do you want to continue?". Click the **Yes** button on the message dialog to save the Data.

How do I close all displayed spectra?

➢ You can close all of the spectra that appear on the Data Tree by selecting **File > Close All Spectra**.

When you select this command all spectra from the Data Tree are removed.

If the Data Tree displays calculated spectra that have not yet been saved a message appears that states: "The items selected for closing include data that has not been saved. Do you want to continue? Click the **Yes** button on the message dialog to save the Data.

Chrom/Spectra View

The Chrom/Spectra View page functions similar to the Main View; both views are designed for viewing unlimited chromatograms and spectra at one time. However, unlike the Main View, which shows you chromatograms, spectra, contour maps, and 3D plots, the Chrom/Spectra View 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, refer to the information found in the section titled* **Tell me about the Main View.** *on page* **107**.
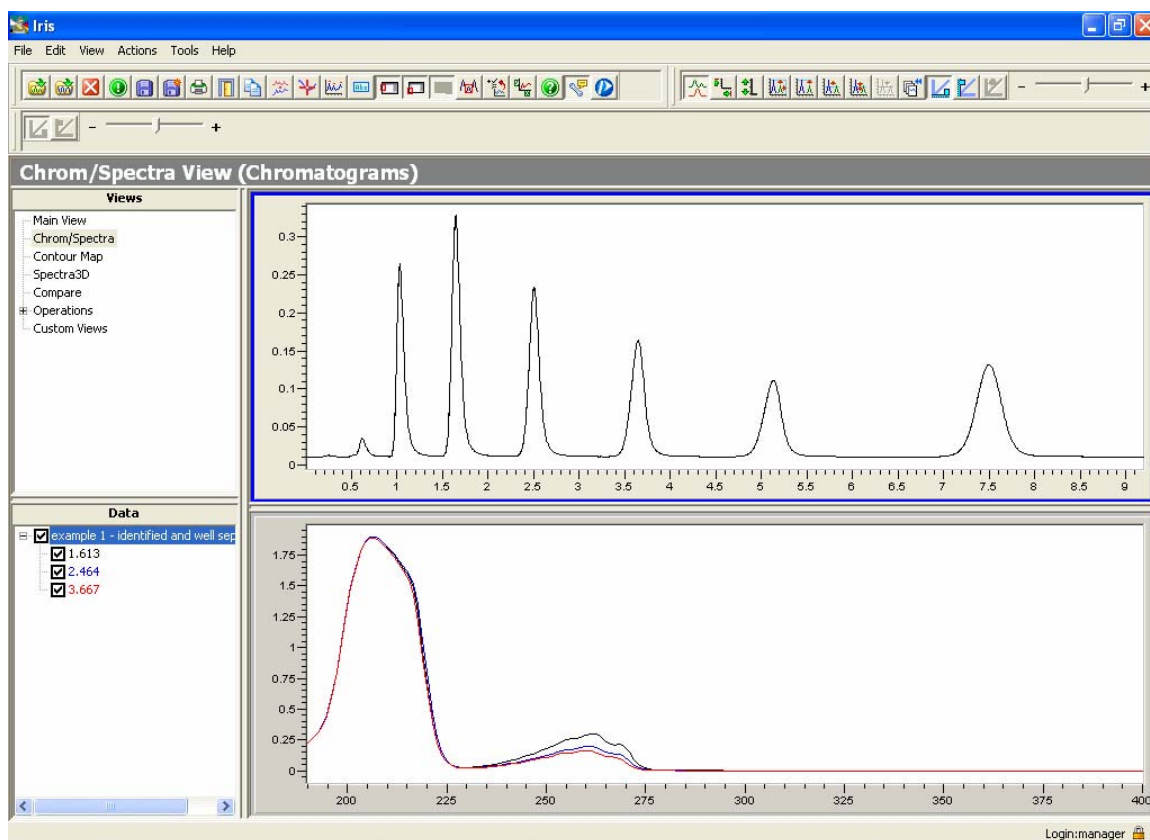
Figure 5-2 The Chrom/Spectra View

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 IRIS 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*** on page ***129***.

Tell me about the Contour Map View.
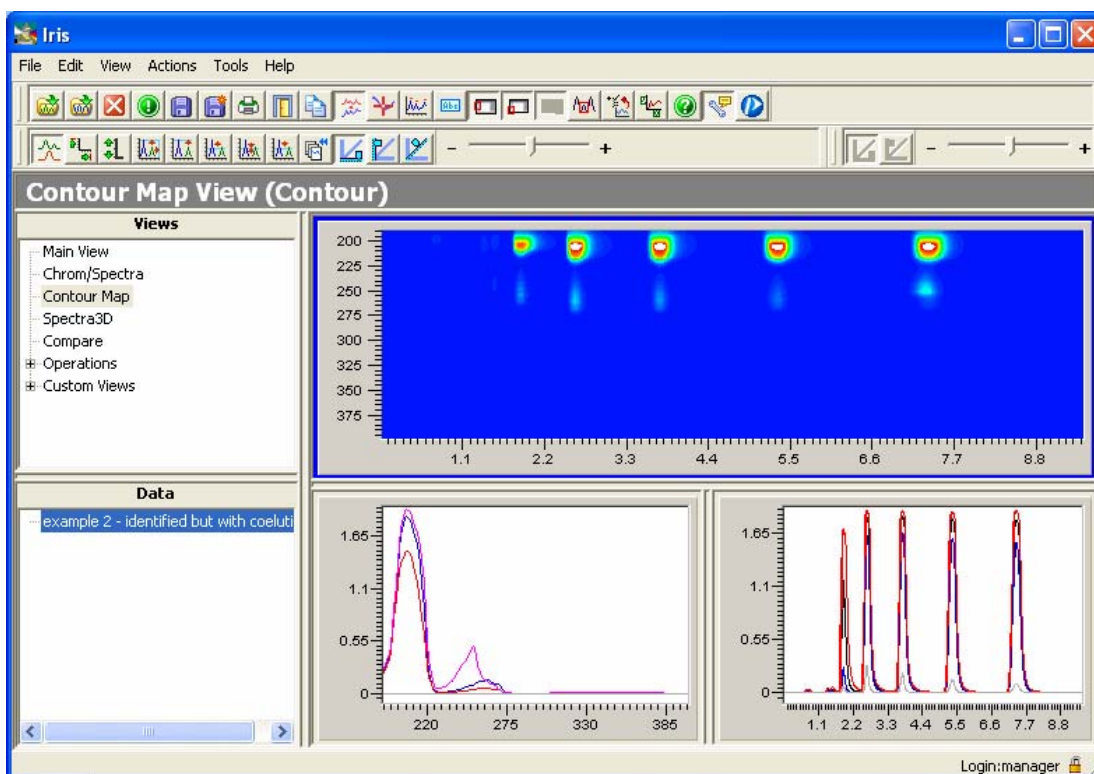
Figure 5-3 The Contour Map View

The Contour Map View consists of the following areas:

- **Views Tree** - The Views Tree provides you access to the Contour Map View.
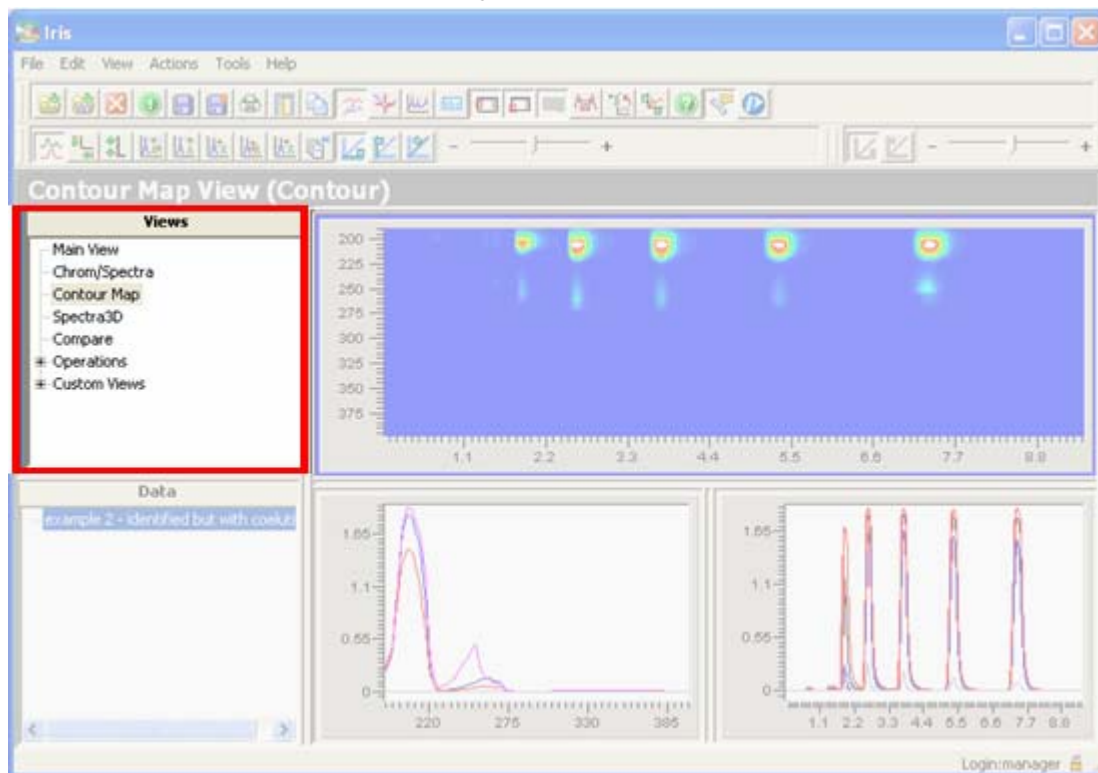
- **Data Tree** - The Data Tree displays a list of opened chromatograms. From the Data Tree you select the required chromatogram for the contour map. Only one chromatogram can be selected and displayed on this view at a time.
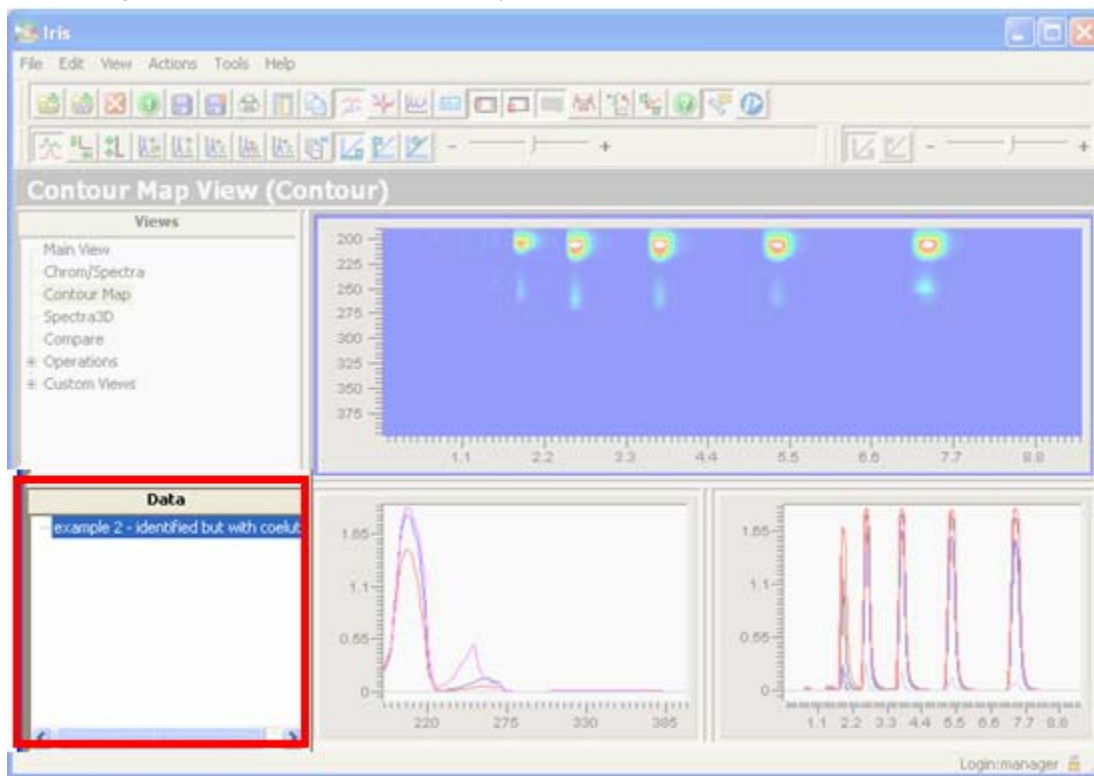
- **Contour Map Pane** - This pane displays the contour map of a chromatogram that is selected on the Data Tree. The display includes a crosshair cursor which is used to select a point on either the retention time axis or the wavelength axis and the relevant chromatogram and spectrum will be shown in the corresponding panes below. The contour levels show the absorbances.
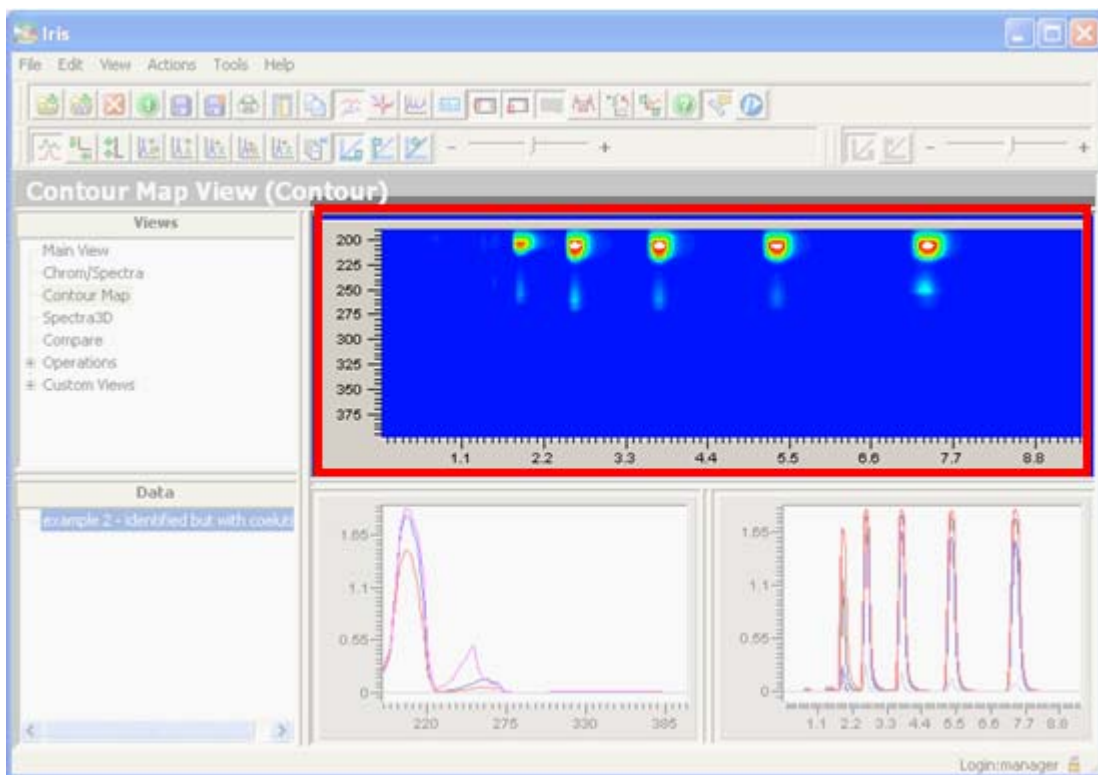
- **Chromatogram Pane** - The Chromatogram pane displays the chromatogram from the wavelength axis point selected on the Contour Map pane.   To display a chromatogram on this pane, simply float your mouse pointer over the Contour Map, and the chromatogram from that point is temporarily displayed on the 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 displayed on this pane are only temporary and will be cleared when you leave the Contour Map View.*
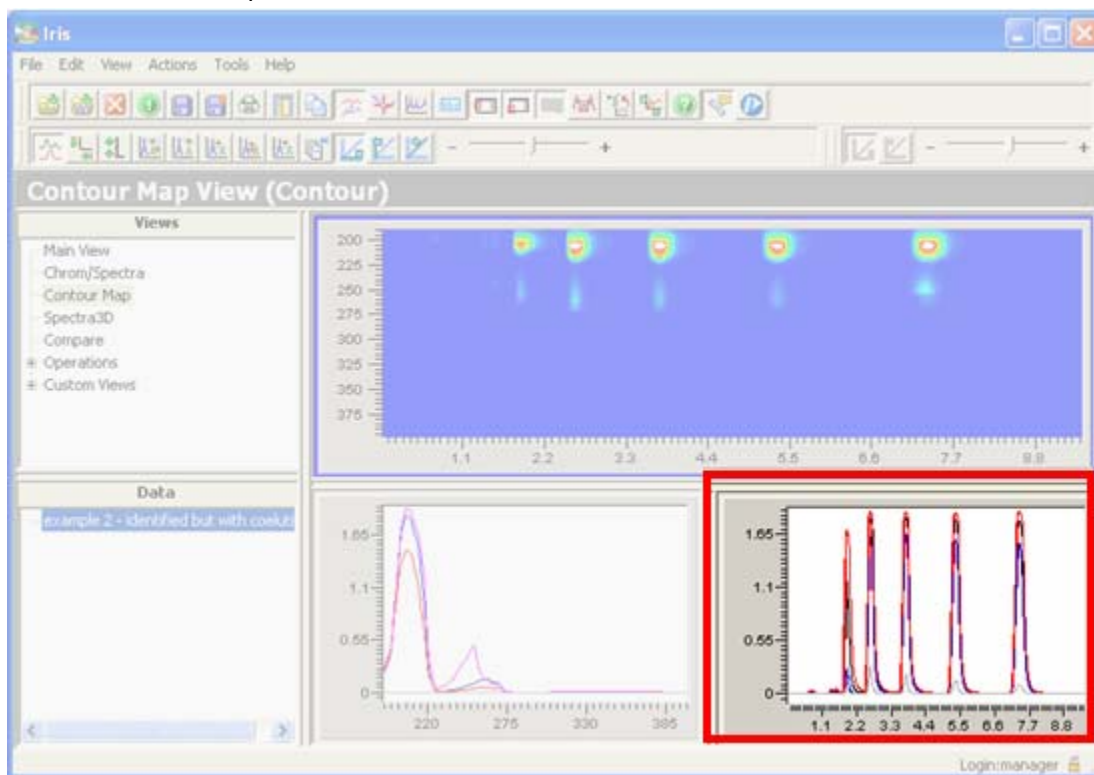
- **Spectra Pane** - This pane displays the spectrum from the point selected on the Contour Map. To display a spectrum on this pane, simply float your mouse pointer over the Contour Map, and the spectrum from that point is temporarily displayed on the Spectra pane.

  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, 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 on the Contour Map View?

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 **Contour Map 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 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.*

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:

1. 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.

2. After you have specified the limits for all 3 axes click **OK**.

    The Format Graphs dialog closes and the new limits are applied.

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 IRIS 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
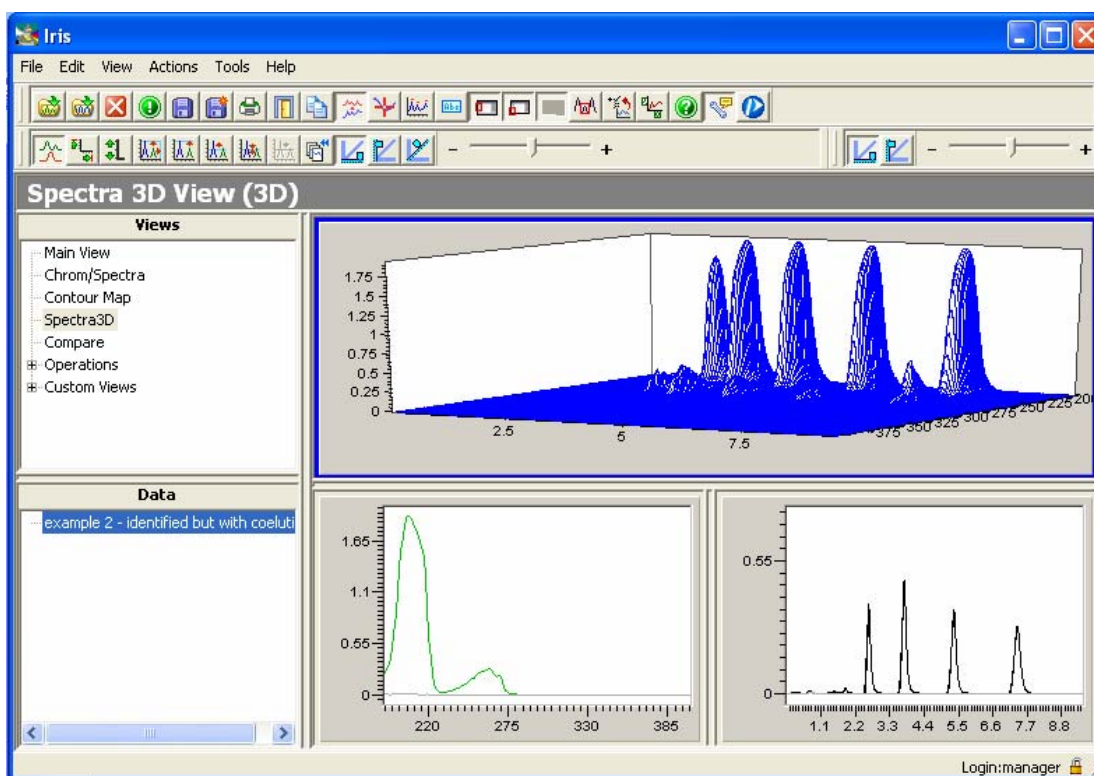
Figure 5-4 The Spectra 3D View

The Spectra 3D View is comprised of the following areas:

- **Views Tree** - The Views Tree provides you access to the Spectra 3D View.
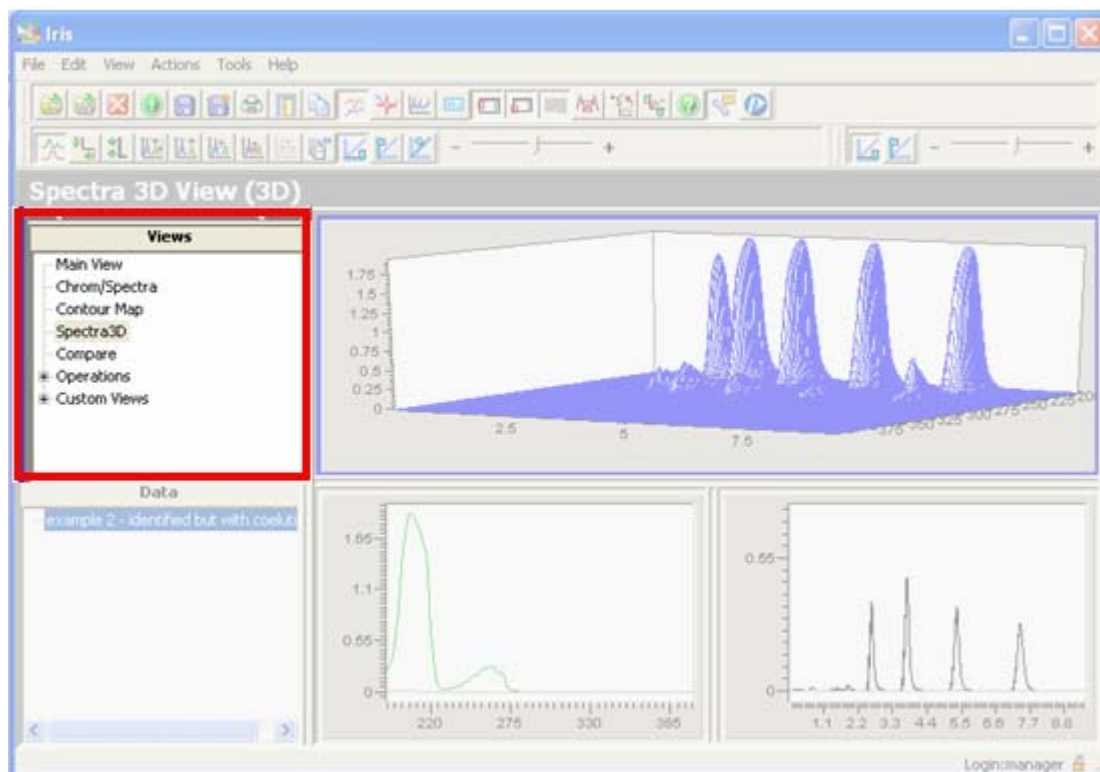
- **Data Tree** - The Data Tree displays a list of opened chromatograms. From the Data Tree you select the required chromatogram for 3D plot. Only one chromatogram can be selected and displayed on this view at a time.
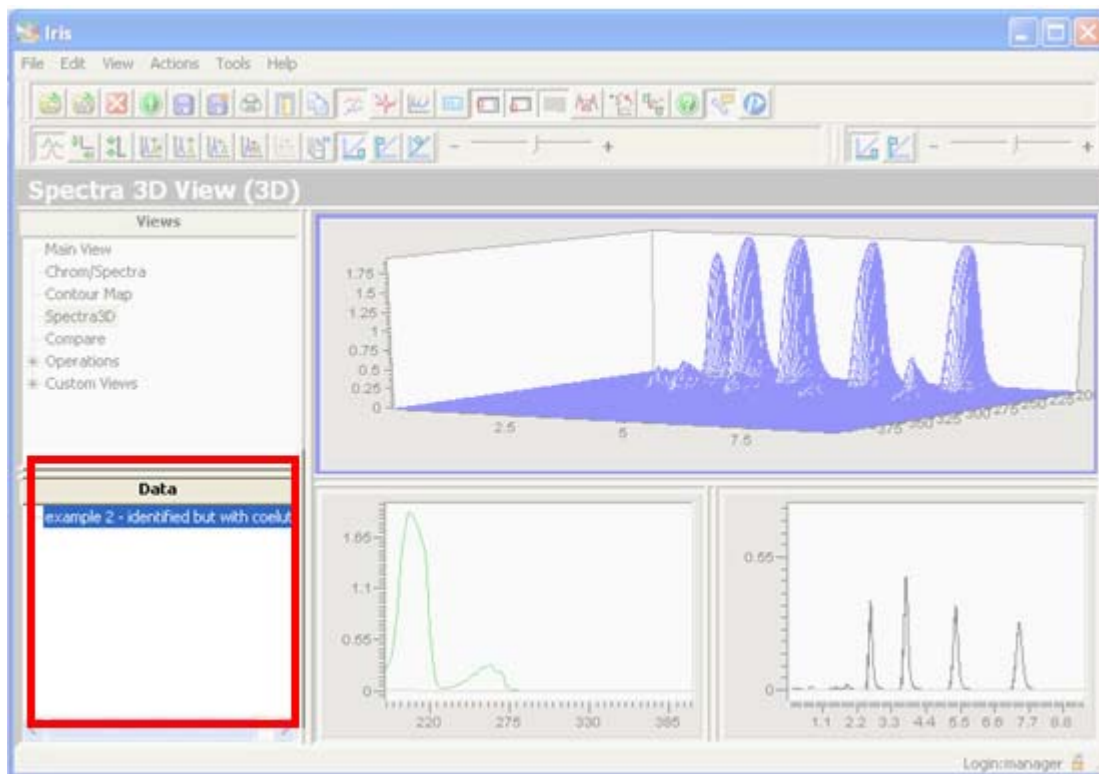
- **3D Plot Pane** – This pane displays the Spectra 3D View from the chromatogram selected on the Data Tree. The display includes a crosshair cursor which is used to select a point on either the retention time axis or the wavelength axis and the relevant chromatogram or spectrum will be shown in the corresponding panes below.
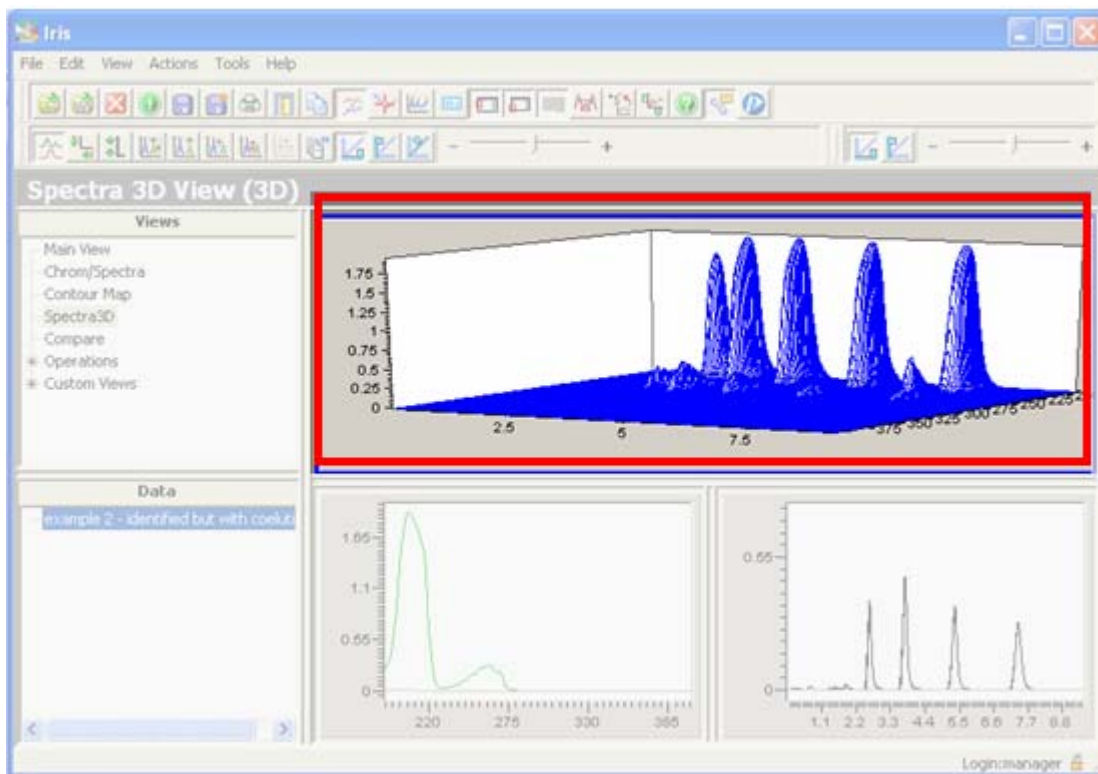
- **Chromatogram Pane** - The Chromatogram pane displays the chromatogram from the wavelength axis point selected on the 3D Plot pane. To display a chromatogram on this pane, simply float your mouse pointer over the 3D Plot, and the chromatogram from that point is temporarily displayed on the Chromatogram 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.*
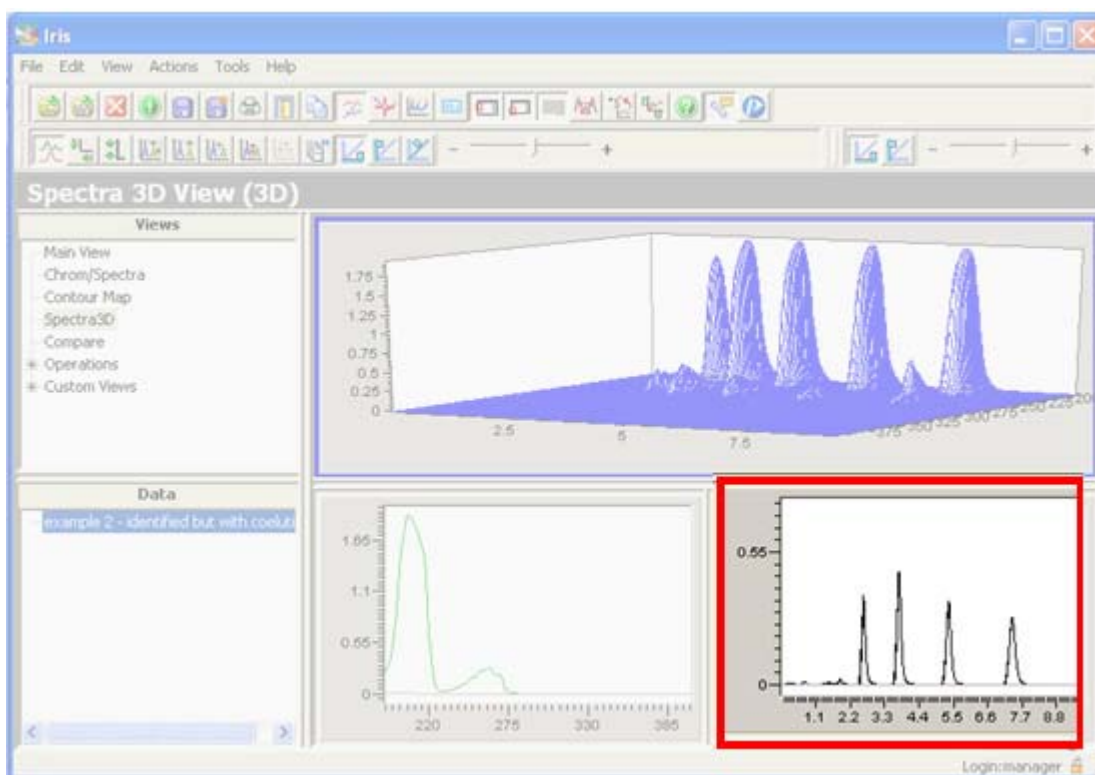
- **Spectra Pane** - This pane displays the spectrum from the retention axis point selected on the 3D Plot pane. To display a spectrum on this pane, simply float your mouse pointer over the 3D Plot, and the spectrum from that point is temporarily displayed on the Spectra pane.

   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.
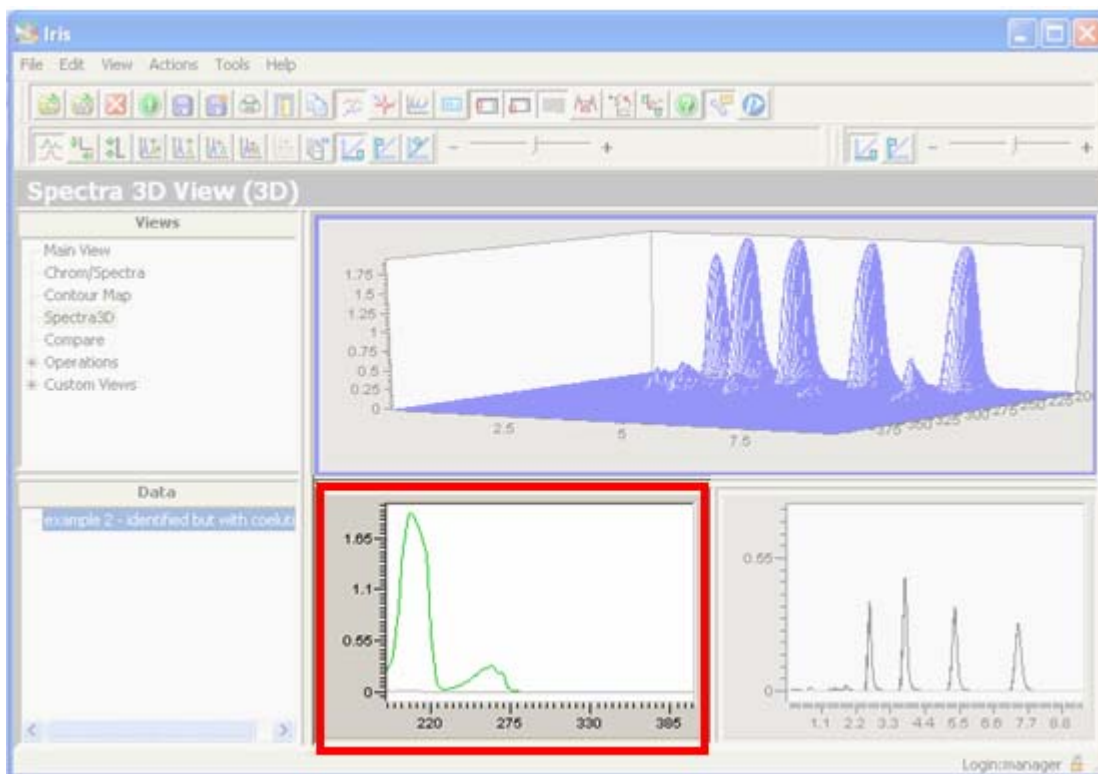
How do I adjust the pane width and height?

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 view chromatograms and spectra on the Spectra 3D View?

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 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.

    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.

2. 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.

3. Release the slider to return it to the center position.

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.
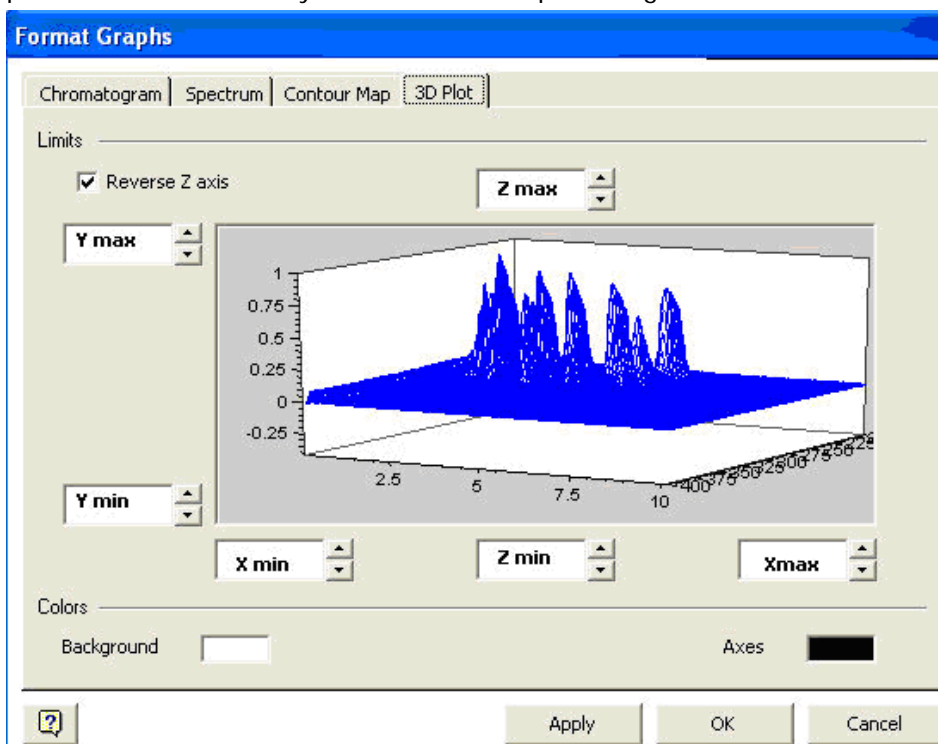
2. When you are finished setting the numerical limits for the plot click **OK**.

    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.
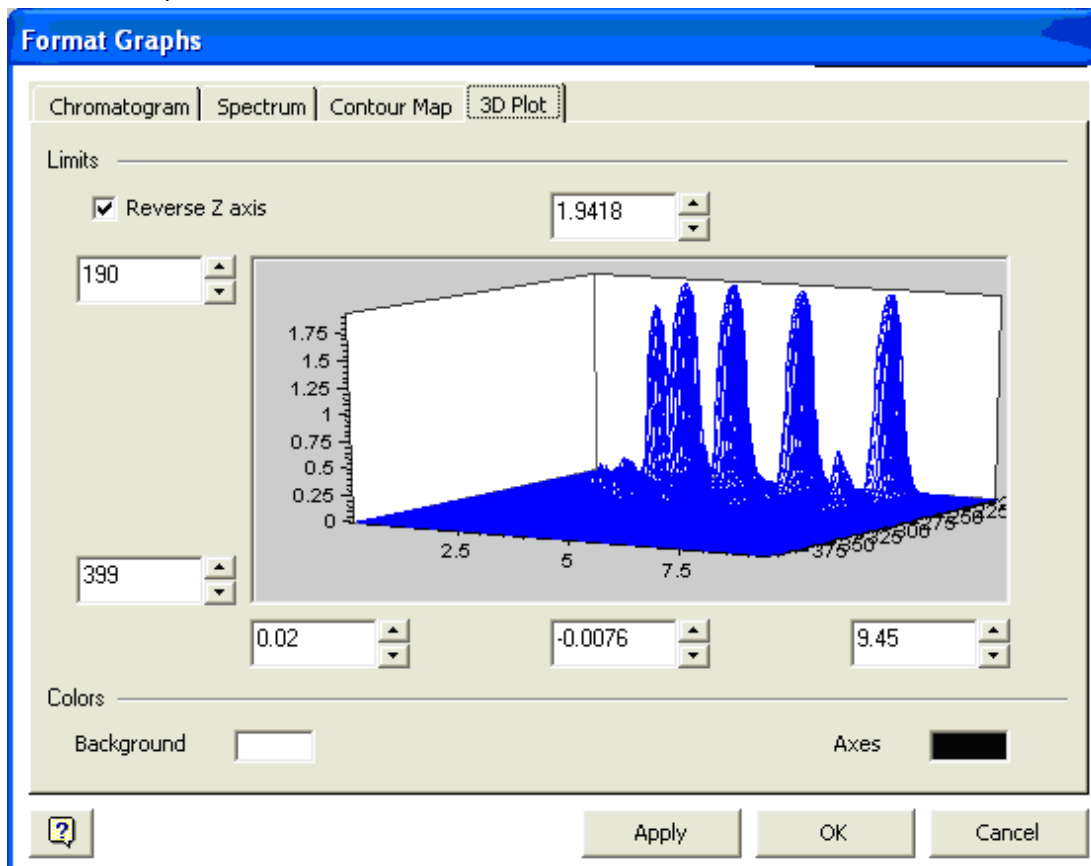
The check mark is cleared or returned depending on its previous state.

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 Y only** 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-scaled.

2. Click on the **Autoscale Y** 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.

Compare View

The Compare View allows you to compare two chromatogram or spectra side-by side for easy comparison.

Tell me about the Compare View
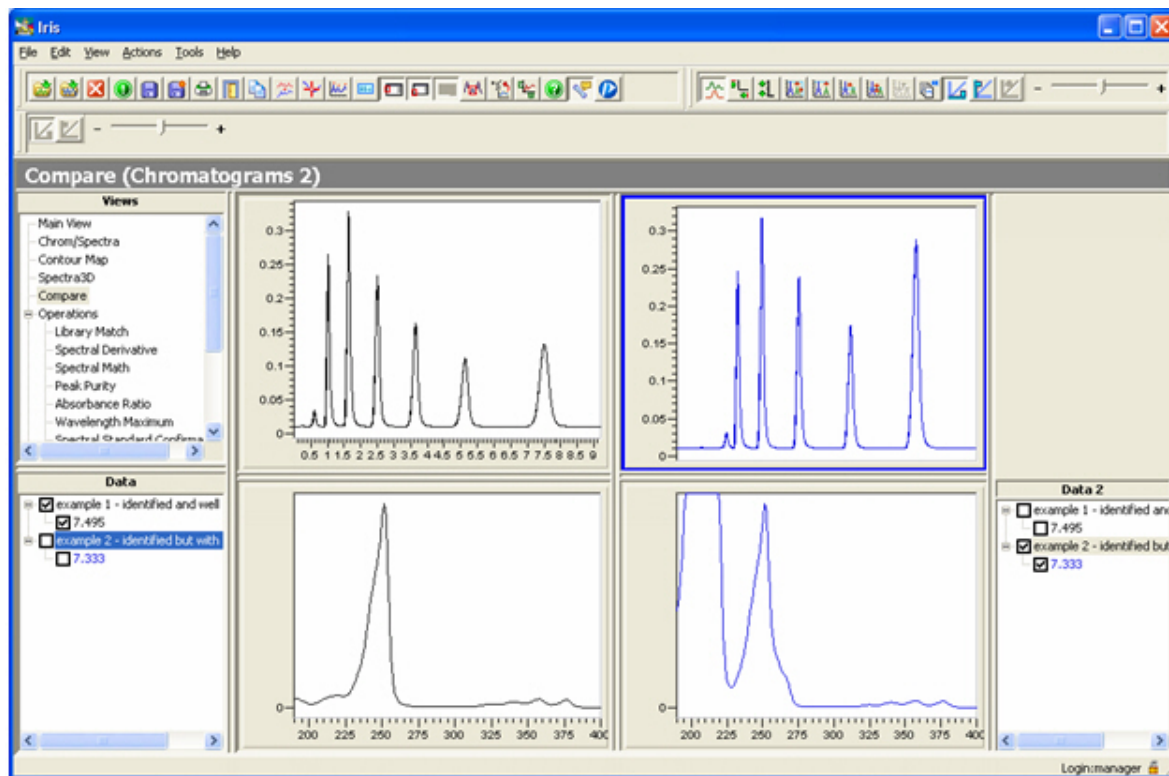
Figure 5-5 The Compare View

The Compare View page is comprised of the following areas:

- **Views Tree** - The Views Tree provides you access to the Compare View.
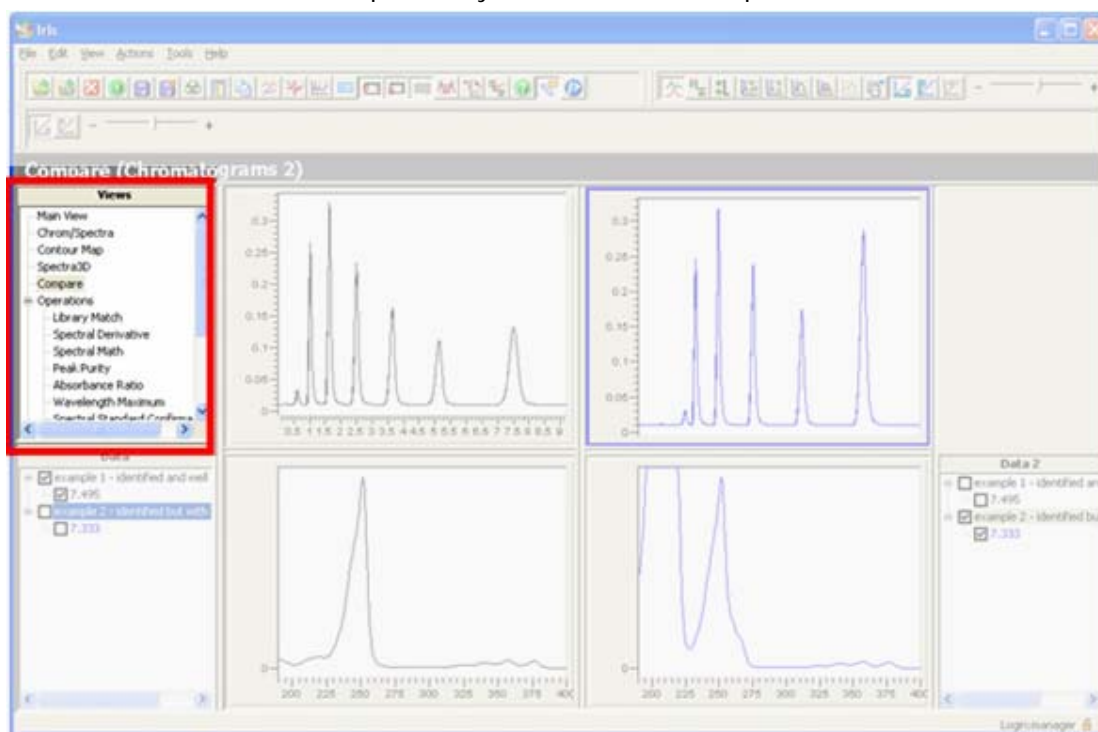
- **Data Trees** - Both the Data Trees display a list of currently loaded chromatograms and spectra. You use the Data Trees to select the chromatograms and spectra that you want to compare side by side in the relevant panes. Items that appear checked on the Data Trees are displayed; while unchecked items on the Data Trees are not displayed.

  Use Data Tree on the left to check the items that you want displayed in the left-hand Chromatogram and Spectra panes. Use the Data Tree on the right to check the items that you want displayed in the right-hand Chromatogram and Spectra panes.
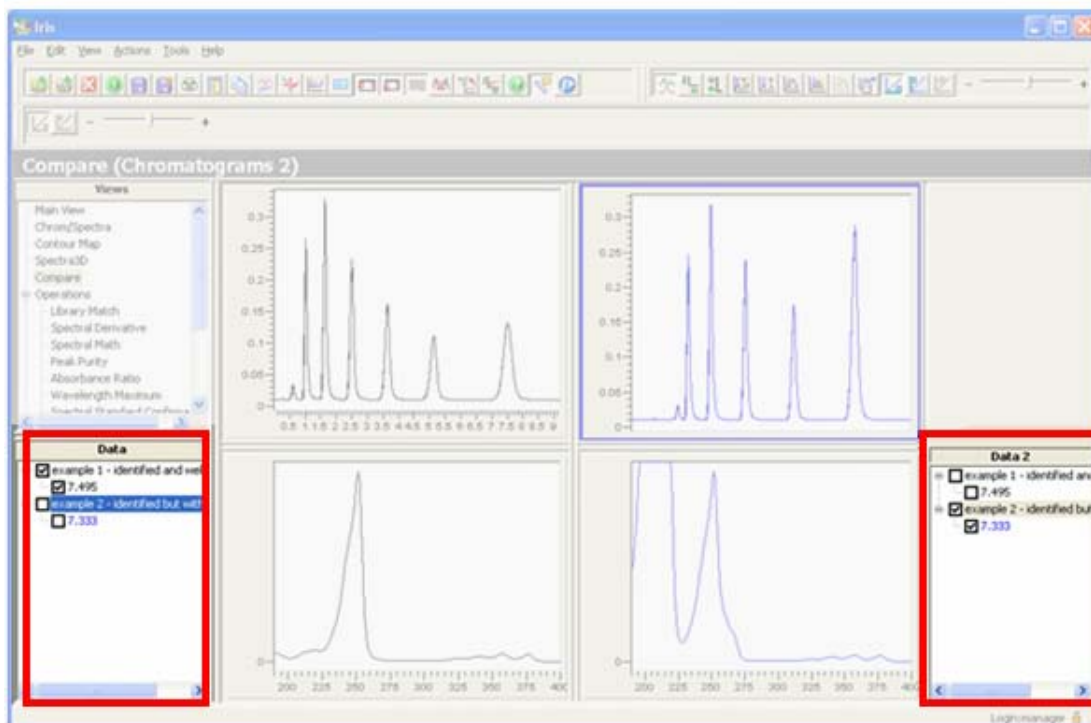
- **Chromatogram Panes** - The Chromatogram pane on the right-hand side of the window displays the chromatograms that are checked on the Data Tree that is located on the right-hand side of the window. Conversely, the Chromatogram pane on the left, displays the chromatograms that are checked on the Data Tree that is located on the left-hand side of the window.
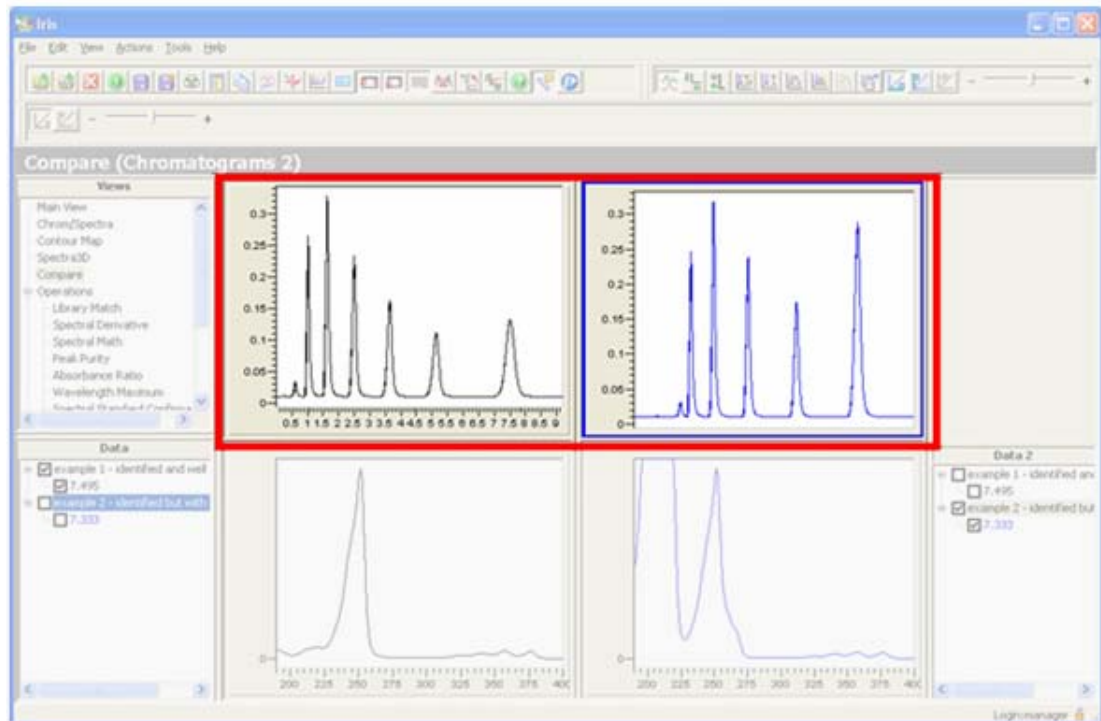
- **Spectra Panes** - The Spectrum pane on the right-hand side of the window displays the spectra that are checked on Data Tree that is located on the right-hand side of the window. The Spectrum pane on the left, displays the spectra that are checked on the Data Tree that is located on the left hand side of the window.

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.

Custom Views

You can create your own Custom views by modifying an existing view. You can modify existing views by selecting to hide or show a particular pane. When a default view is modified a custom view is automatically created.

➢ 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 **Chromatogram 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.
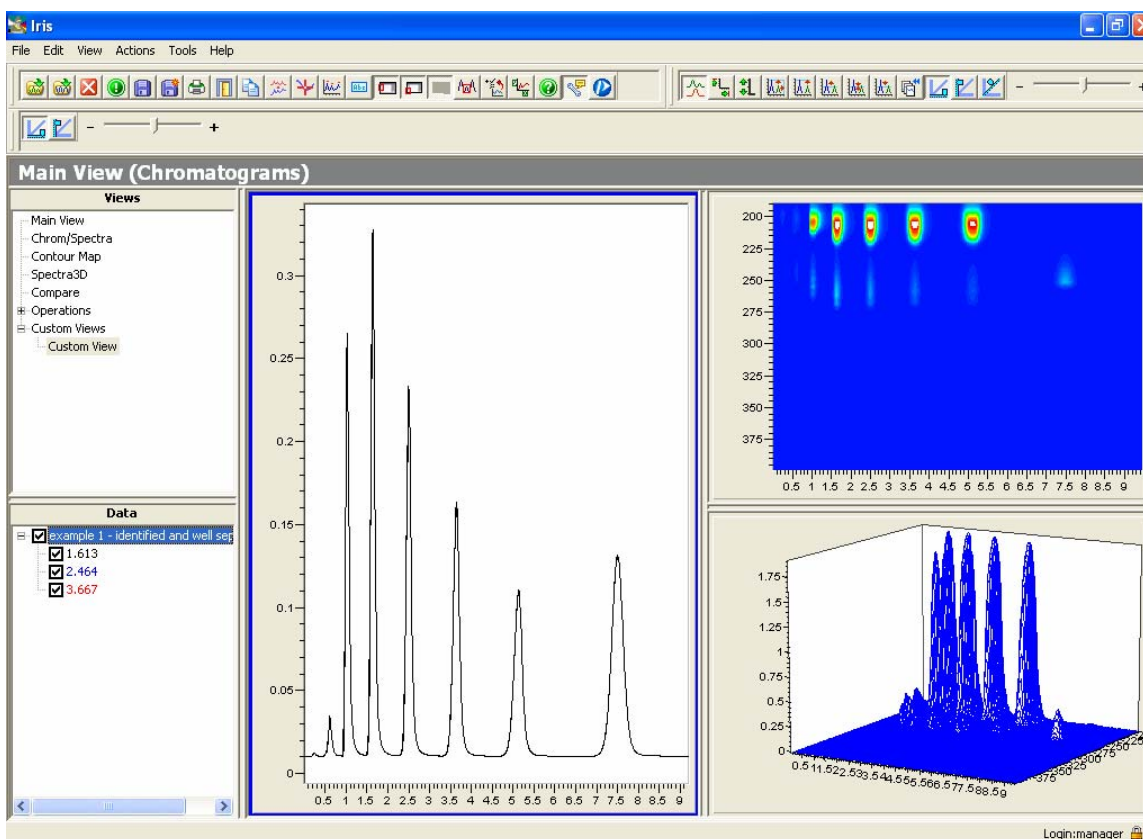
Figure 5-6 An example of a Custom View

Chapter 6. Spectral Libraries

About Spectral Libraries

IRIS provides you with the ability to 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.

This chapter 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 Data Tree, 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.

    The Audit Trail dialog appears.

7. Depending upon how your TotalChrom Administrator has configured Audit Trailing you may be required to enter a **Comment** about the library you are creating. If your TotalChrom Administrator has created a "Reasons list" in TotalChrom, you may also be required to specify a **Reason** on the Audit Trail dialog*. .*

8. Click **OK** when you have finished entering the required information on the Audit Trail dialog.

    The Electronic Signature dialog appears.

9. From the Electronic Signature dialog, enter your **User Name** and **Password** and click **OK**.

    The Electronic Signature dialog closes and the Edit Library dialog appears. On the Edit Library dialog all the named peaks from the selected chromatogram are displayed in the Library list.

Note: *The components that appear in the Library list are named from the component names in the result file.*
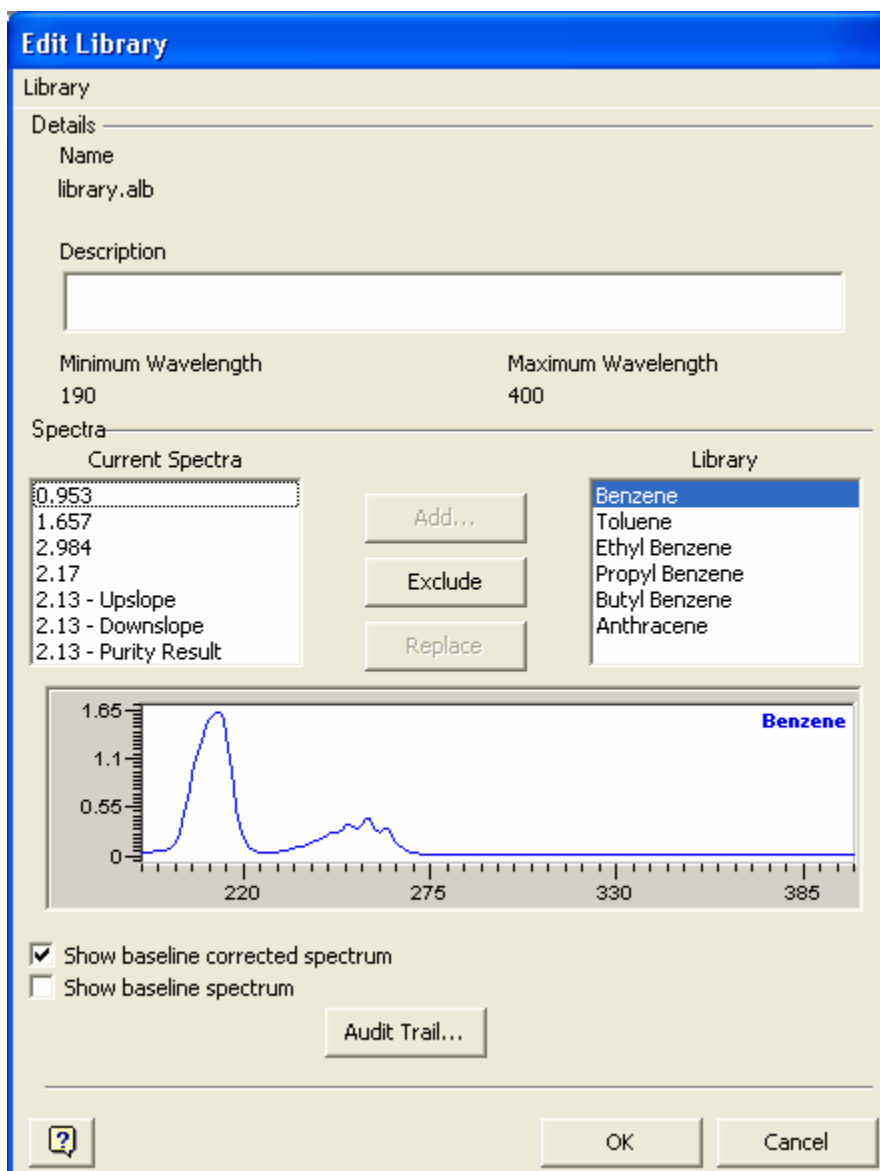
10. For more information on using the Edit Library dialog, see ***Editing a Library*** on page ***162***.

    OR

    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 page). 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**. *For more information on using the Add Apexes command, see page* **153**.

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.

For information on adding, deleting, or replacing spectra in the library, and for information on how to browse the library once spectra have been added, see page **162**.

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.
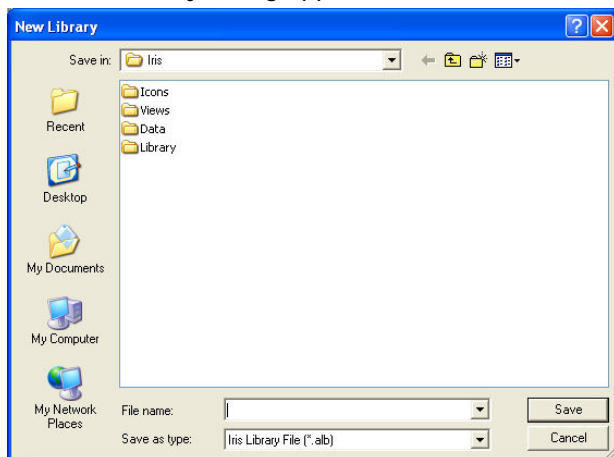
2. From the **Save in** drop down list select the folder where you wish to save the Library you are creating.

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 **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.

The Audit Trail dialog appears.

7.   Depending upon how your TotalChrom Administrator has configured audit trailing you may be required to enter a **Comment** about the library you are creating on the Audit Trail dialog. In addition, if your TotalChrom Administrator has created a "Reasons list" in TotalChrom, you may also be required to specify a **Reason** on the Audit Trail dialog.

8.   Click **OK** when you have finished entering the required information on the Audit Trail dialog.

The Electronic Signature dialog appears.

9.   From the Electronic Signature dialog, enter your **User Name** and **Password** and click **OK**.

The Electronic Signature dialog closes; and the Edit Library dialog appears. At this point, the new library has been created, although it contains no spectra. Spectra may be added to the library at any time. *For more information on adding spectra, see* **How do I add extracted spectra into a library?** *on page* **166**.

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.
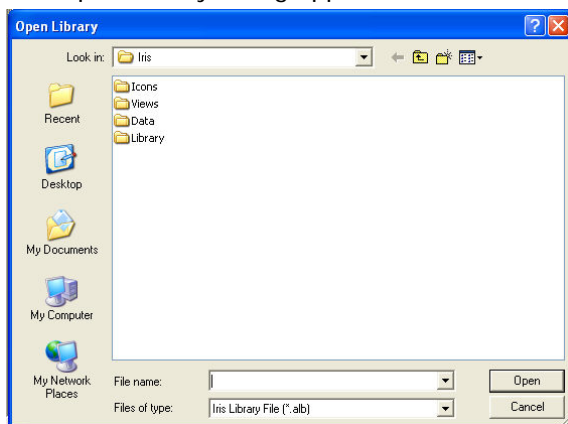
2. Select the Library you wish to view and click **Open**.

   The View Library dialog appears.
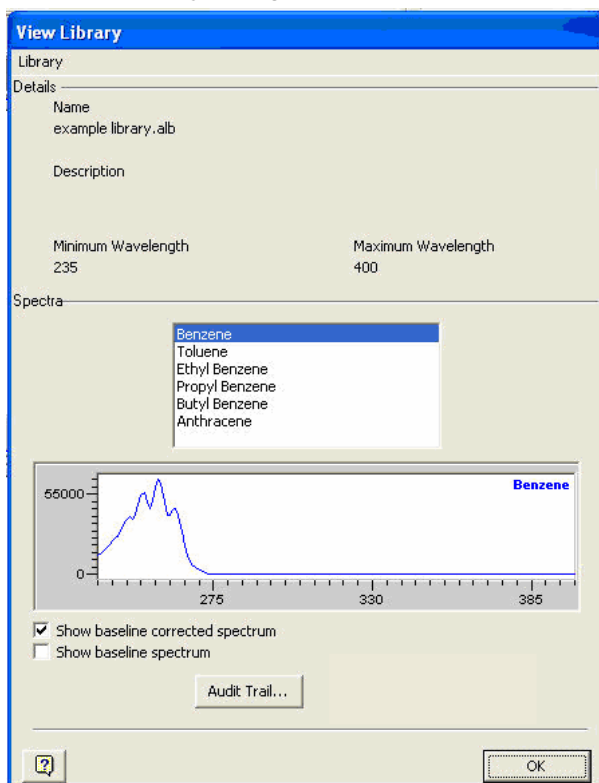
Tell me about the View Library dialog.

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** *on page* **162**.
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.
Audit Trail...	Click on this button to display the audit trail for the library.

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.
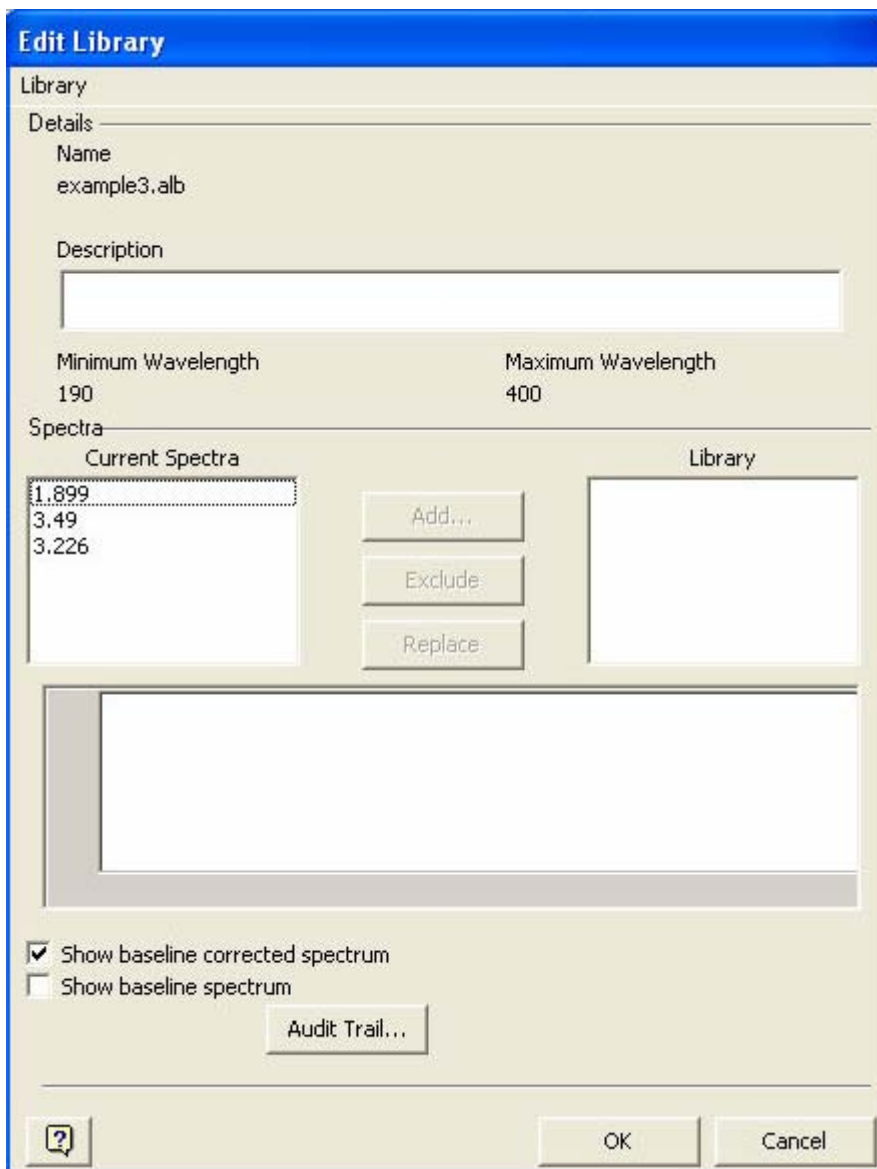
Figure 6-1. The Edit Library dialog.

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 in IRIS 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. **This 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.

Control	Description
<u>R</u>eplace	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.
<u>L</u>ibrary	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.
<u>B</u>aseline 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.*
<u>S</u>how Baseline	This checkbox allows you to select whether or not the baseline spectrum is also displayed on the graph.
Audit <u>T</u>rail...	This command button displays the audit trail for the current library.

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.
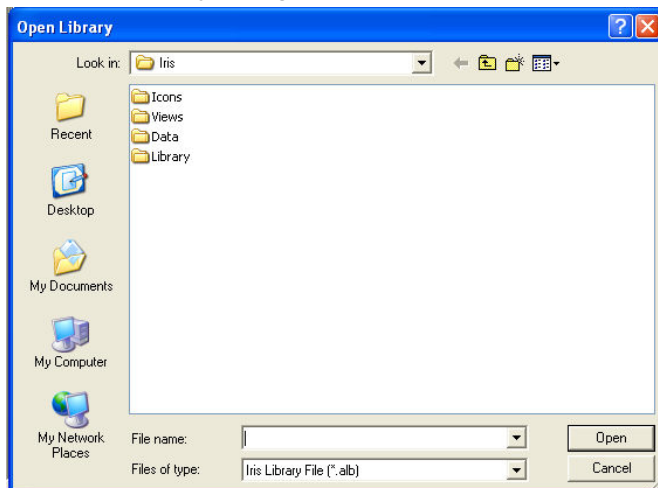
2. Select the Library you wish to view and click **Open**.
   The Edit Library dialog appears.
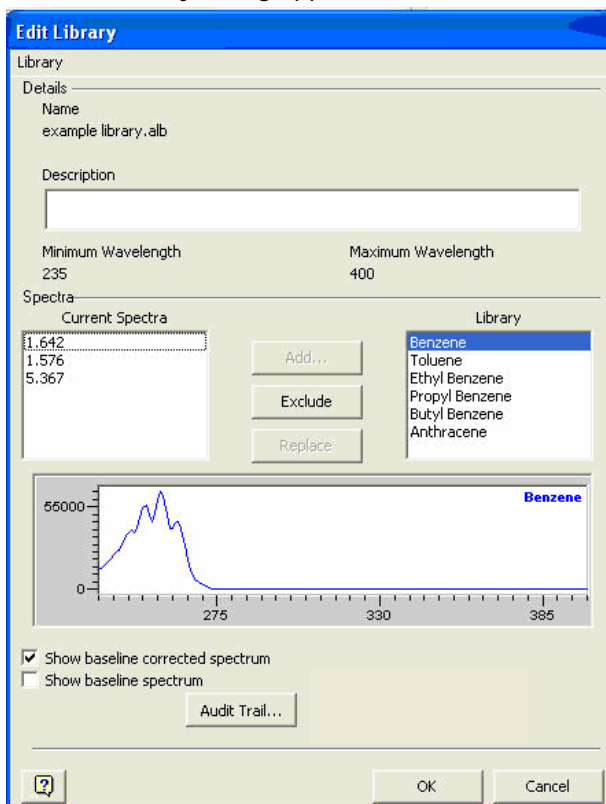
How do I add extracted spectra into a library?

1. From the Edit Library dialog select the spectrum you wish to add from the **Current Spectra** list box.

2. Click the **Add** button.

    The Add to Library dialog box appears and prompts you to enter a **Component Name**.
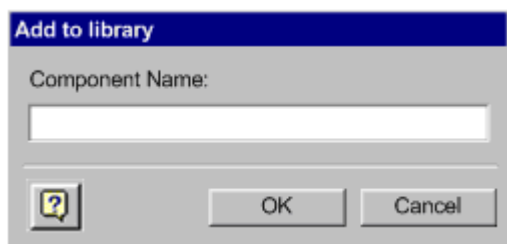
3. Enter the name of the component carefully.

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 **Library** list box.
    The Exclude button is enabled.

2. Click **Exclude**.

    A dialog appears that prompts you to select whether or not you wish 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 box that you wish to delete.

2. Click on a replacement spectrum in the **Current Spectra** list box.
   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 spectrum is replaced with the spectrum selected on the Current Spectra list box.

Identifying a Spectrum-using 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 operation.

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.
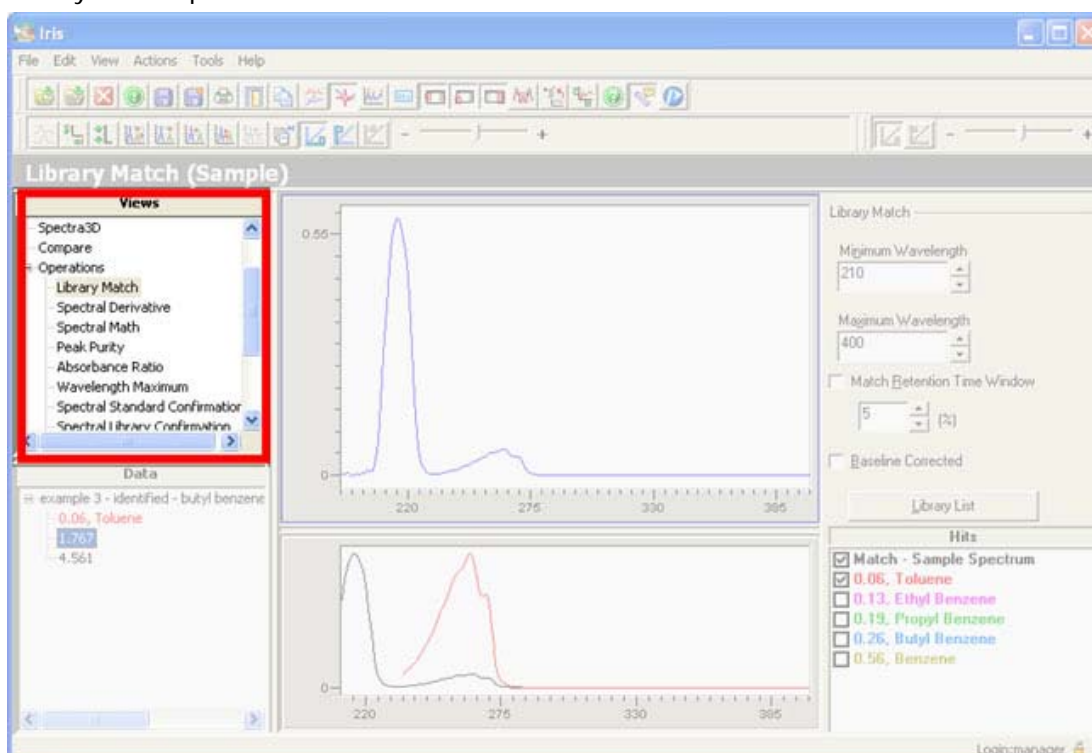
- **Data Tree** - The Data Tree displays a list of extracted spectra that are grouped by their parent chromatogram. From the Data Tree you select the spectrum you wish to identify. When you click on a spectrum listed in this tree, a graphical representation of the spectrum appears in the Spectrum Pane. The spectrum selected on the Data Tree is highlighted in blue.
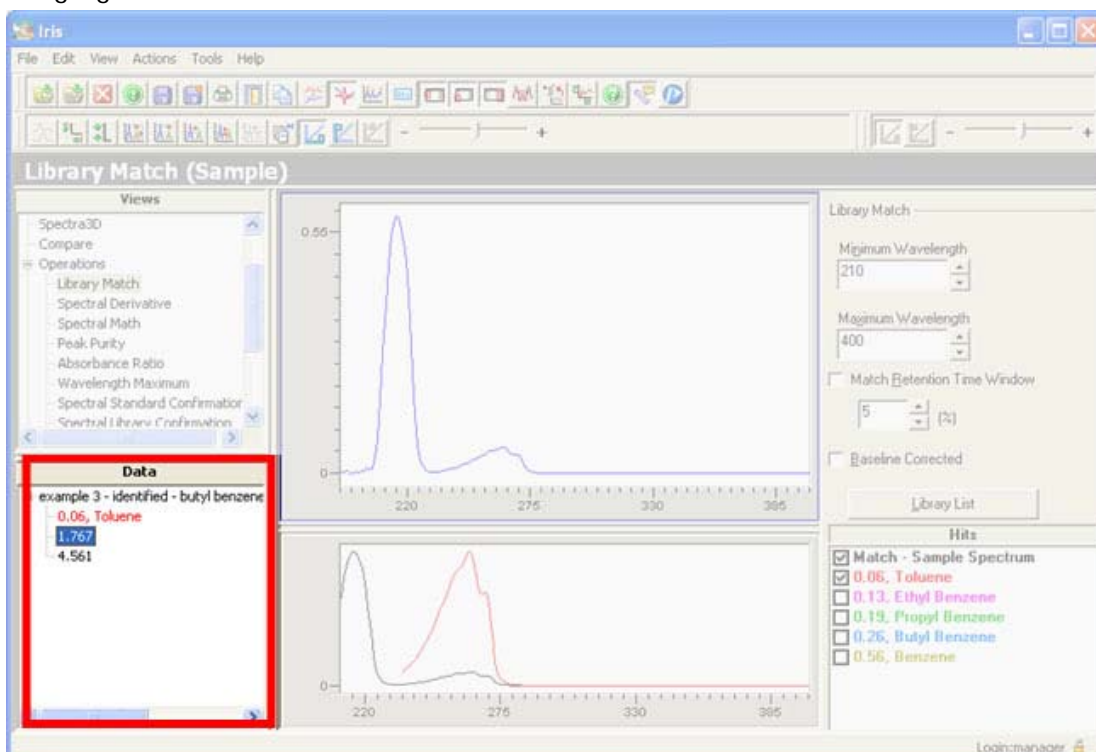
Note: *Whenever you select a spectrum from the Data Tree, the software automatically runs a search, using the parameters specified on this operation, and updates the Hits List and Hits Pane.*

- **Spectrum Pane** - This pane displays the spectrum that you selected from the Data Tree.
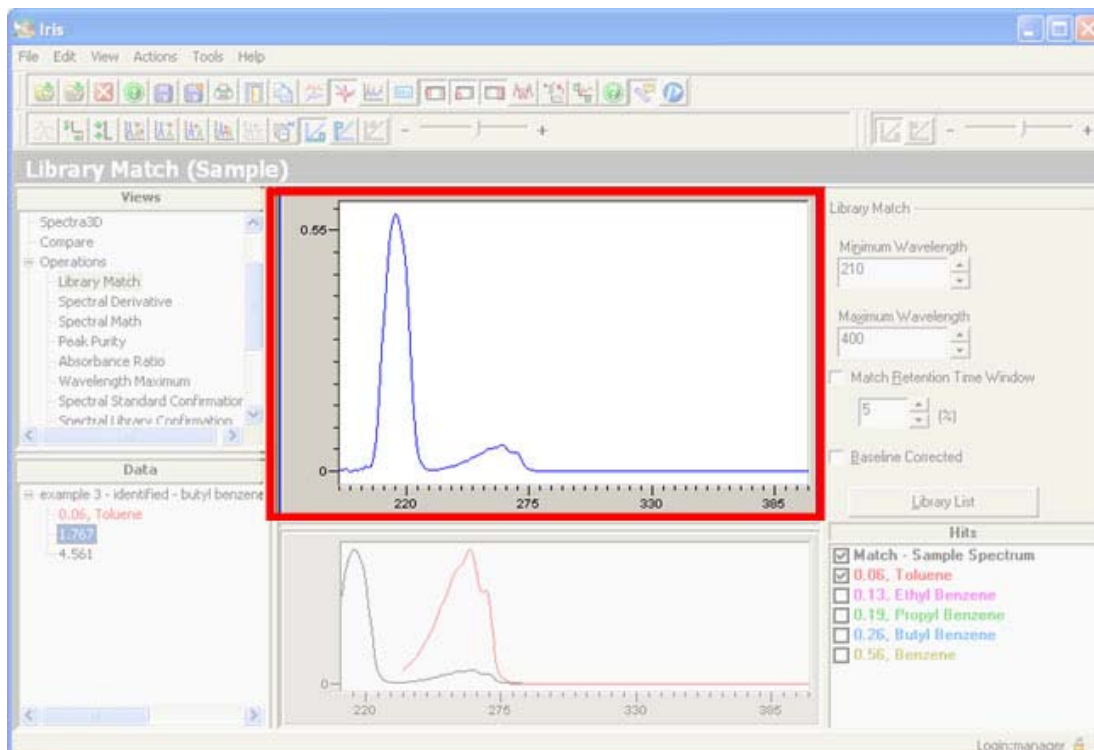
- **Parameters Pane** – This pane is where you set and investigate the various parameters (including the list of libraries that will be searched) that control the matching process. Whenever you change a parameter displayed on this operation, and have a spectrum selected on the Data Tree, Iris automatically re-runs a search and updates the Hits List and Hits Pane.
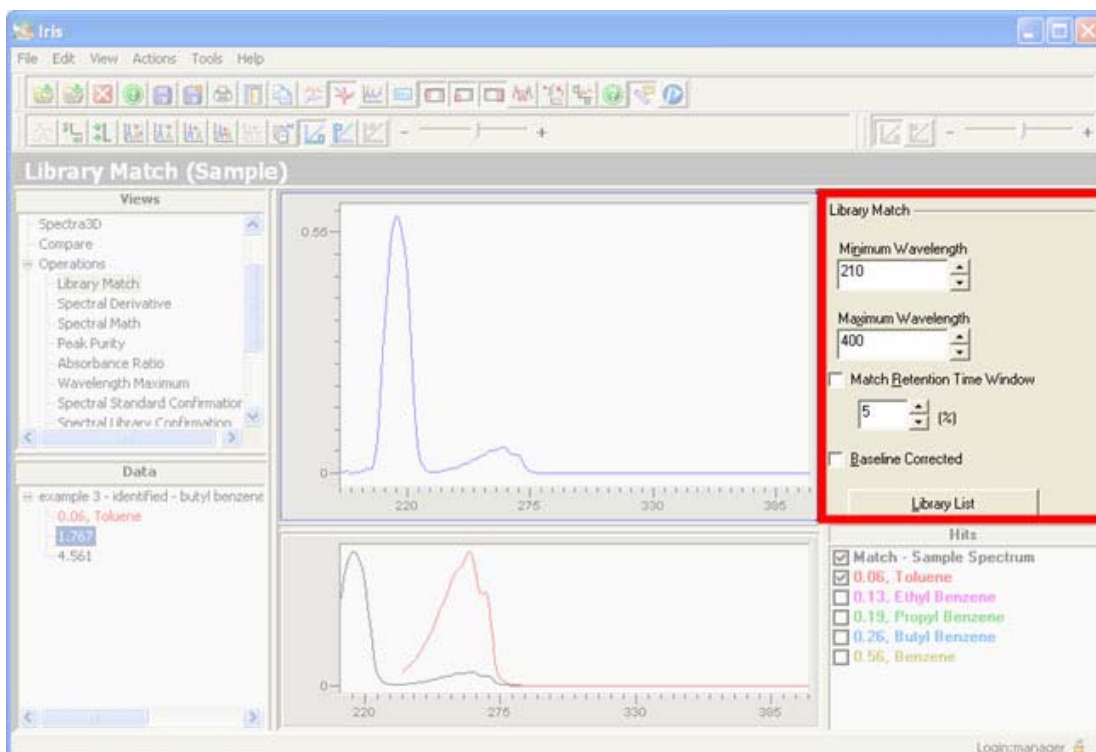
- **Hits List** – The Hits List contains a list of the spectra from the library(s) that best match the sample spectrum, in hit order. These matching spectra are listed by their "hit quality" and name. The hit quality is displayed as a numeric value; a hit quality of 0 indicates a perfect match.

  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.
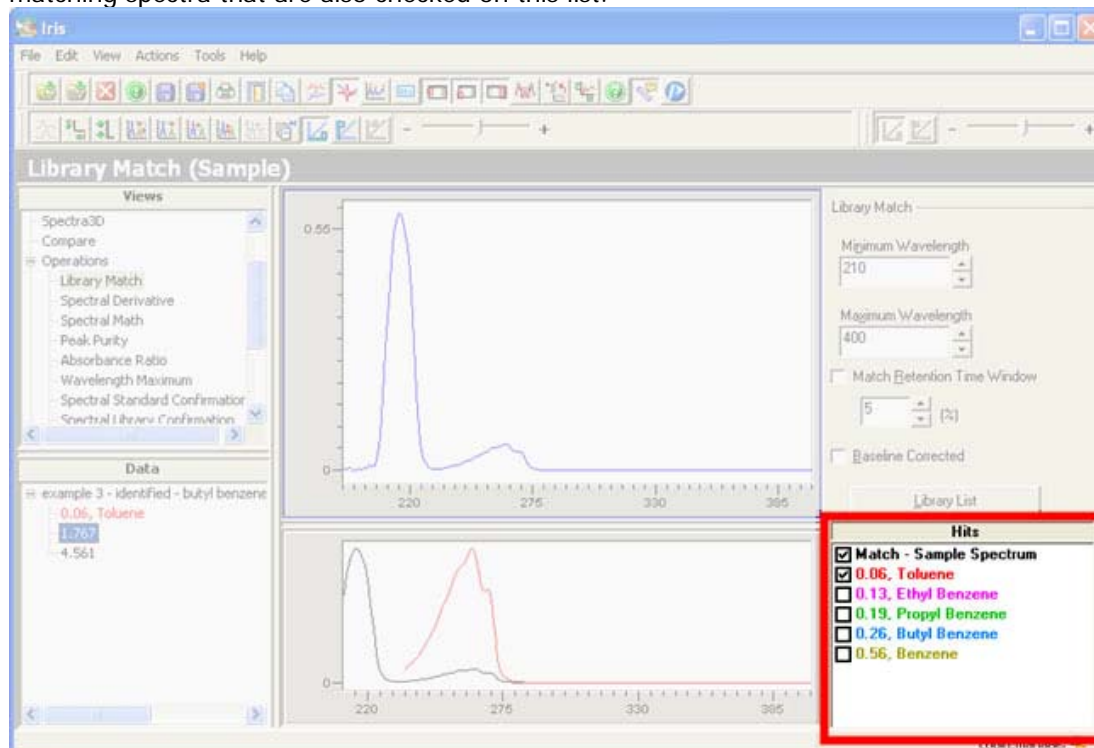
Note: *Spectra that appear checked on this list can be added to the Data Tree by selecting* **Actions > Add All to View**.

- **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.
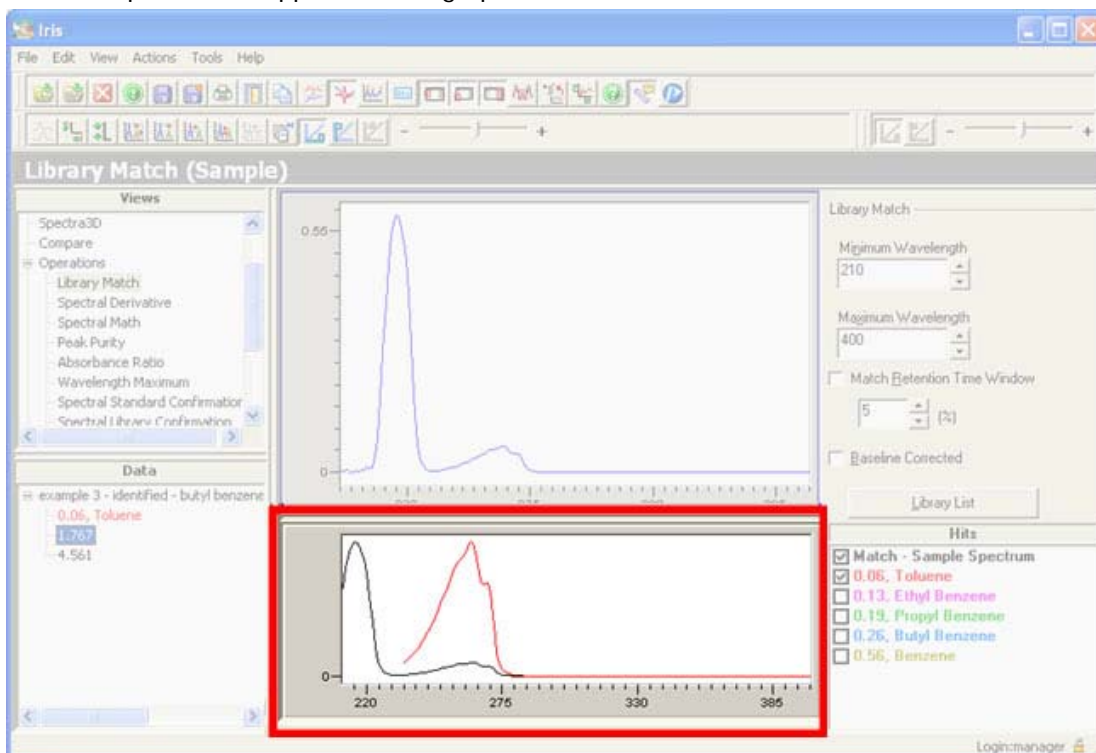
Summary of Operation: Library Match

The summary below illustrates how 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. For more information on extracting spectra, see page **78.***

1. 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 page, then, as soon as the Library Match operations is displayed, IRIS matches the selected spectrum and displays the results on the screen. *If this is the case, you can skip to step 5.*

   OR

   If you have not already selected a list of libraries to search, the Libraries List dialog appears.

2. From the Libraries List dialog select the libraries you wish to search.

Note: *From the Library Match operation you can open the Libraries List dialog by clicking on the* **Library list** *button.*

3. Once you have selected the libraries you wish to search, click **OK** to close the Libraries List dialog and return to the Library Match operation.

4. 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.*

5. 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.
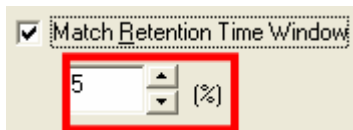
To examine the effect of using baseline corrected spectra on the Library Match:

➢ Check the **Baseline corrected** check box to use baseline corrected spectra for the match.

To add the displayed spectra in the Hits pane 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.

Note: *Whenever you have modified the Parameters or selected a different spectrum on the Data Tree the match is re-run and the Hit List and Hits Pane are updated.*

To 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 operation 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 operation, and the results your search are displayed in the Hits Pane.

How do I specify search parameters?

The Parameters Pane on the Library Match operations contains the following parameters, which you can modify so that Iris 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.*

Chapter 7. Performing Operations on Spectra

Spectral Derivative

The Spectral Derivative operation is where you calculate the first, second, third, or forth derivative for a selected spectrum. This operation provides you with the ability to enhance subtle differences of the shape of the spectrum and therefore provide you with more accurate comparisons. In fact, second order derivative spectra are very useful when the "native" UV-Vis spectrum is not very indicative and does not show sharp maximum of minimum.

Note: *The spectrum you select from the operation should be intense and noise free. Taking the derivative of a noisy spectrum multiplies variance due to the noise and can result in inaccurate data.*

In this section you will learn how to use the Spectral Derivative operation to provide more specific details of the spectra.

➢ 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.
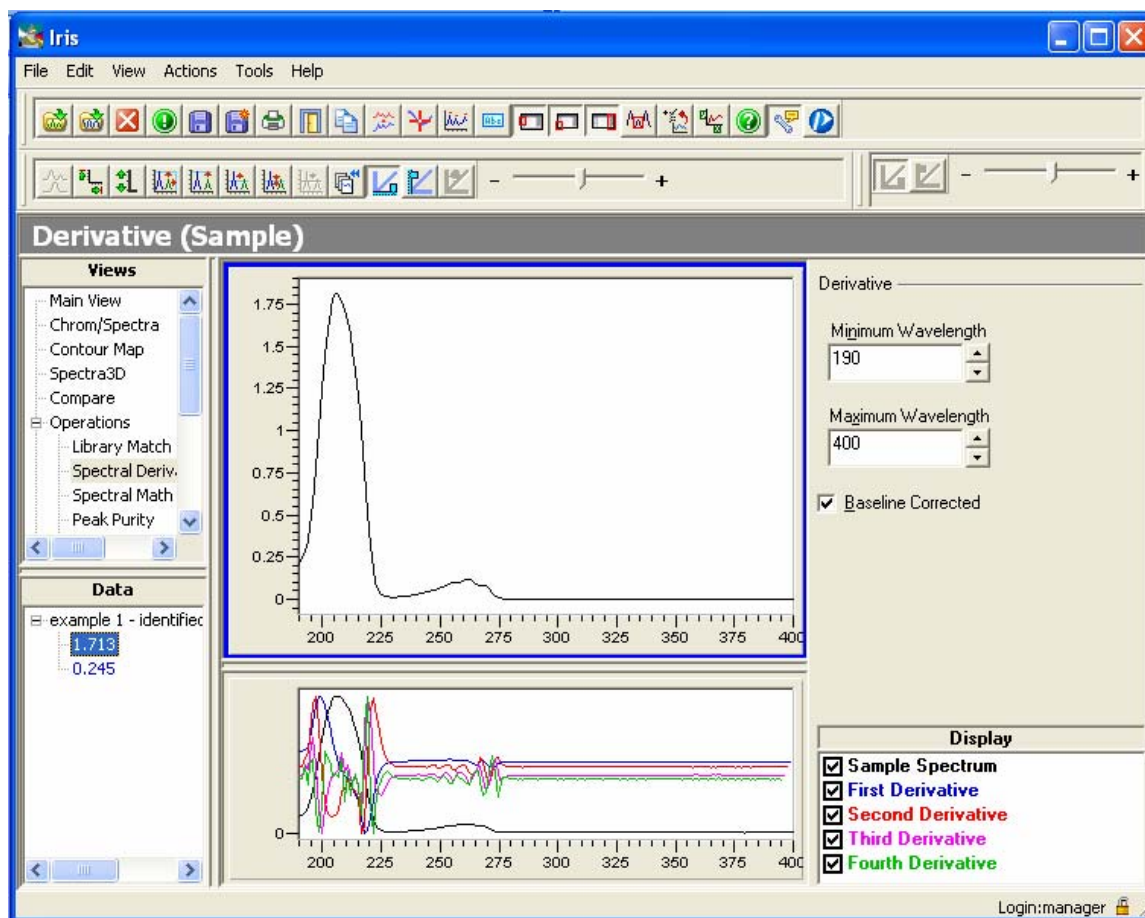
Figure 7-1 The Spectral Derivative operation

Tell me about the Spectral Derivatives operation.

The Spectral Derivatives operation consists of the following areas:

- **Views Tree** - This pane provides you access to the Spectral Derivatives operation.
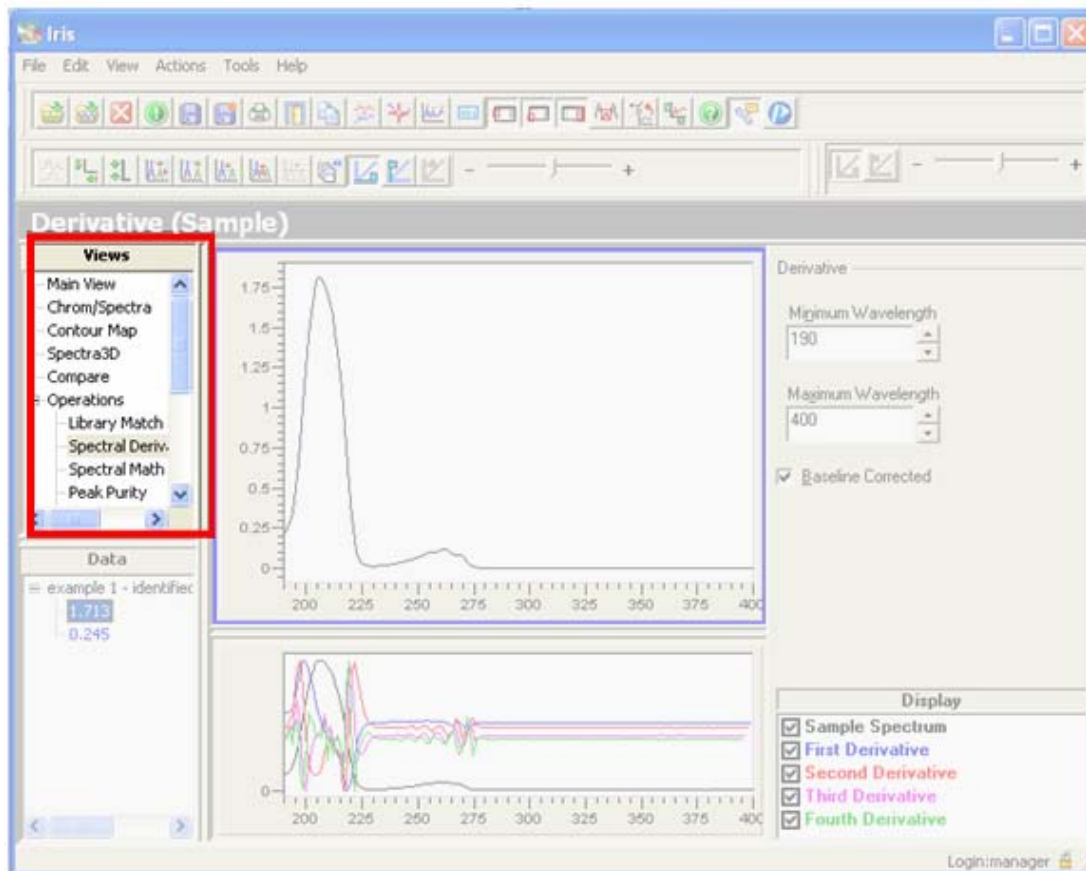
- **Data Tree** - The Data Tree displays a list of extracted spectra that are grouped by their parent chromatogram. From the Data Tree, you select a spectrum for calculating spectral derivatives. Only one spectrum can be selected at a time; and the selected spectrum is displayed in the Spectrum Pane. 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 Derivate operation.*
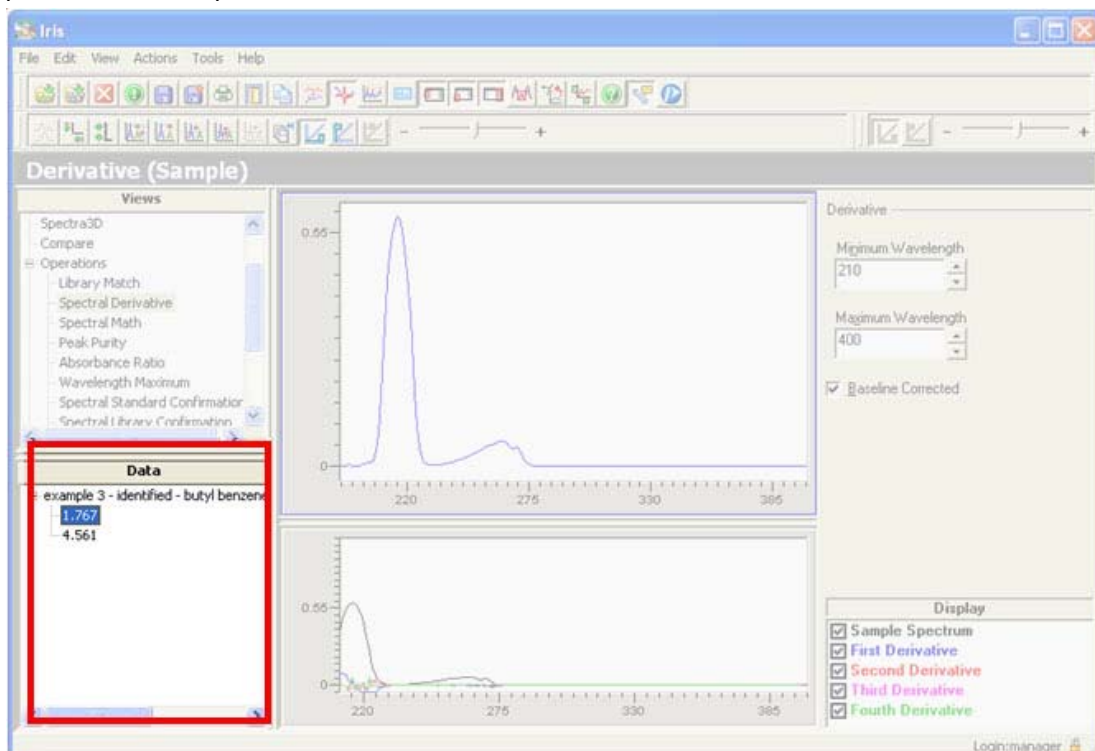
- **Spectrum Pane** - This pane displays the original sample spectrum that you have selected from the Data Tree.
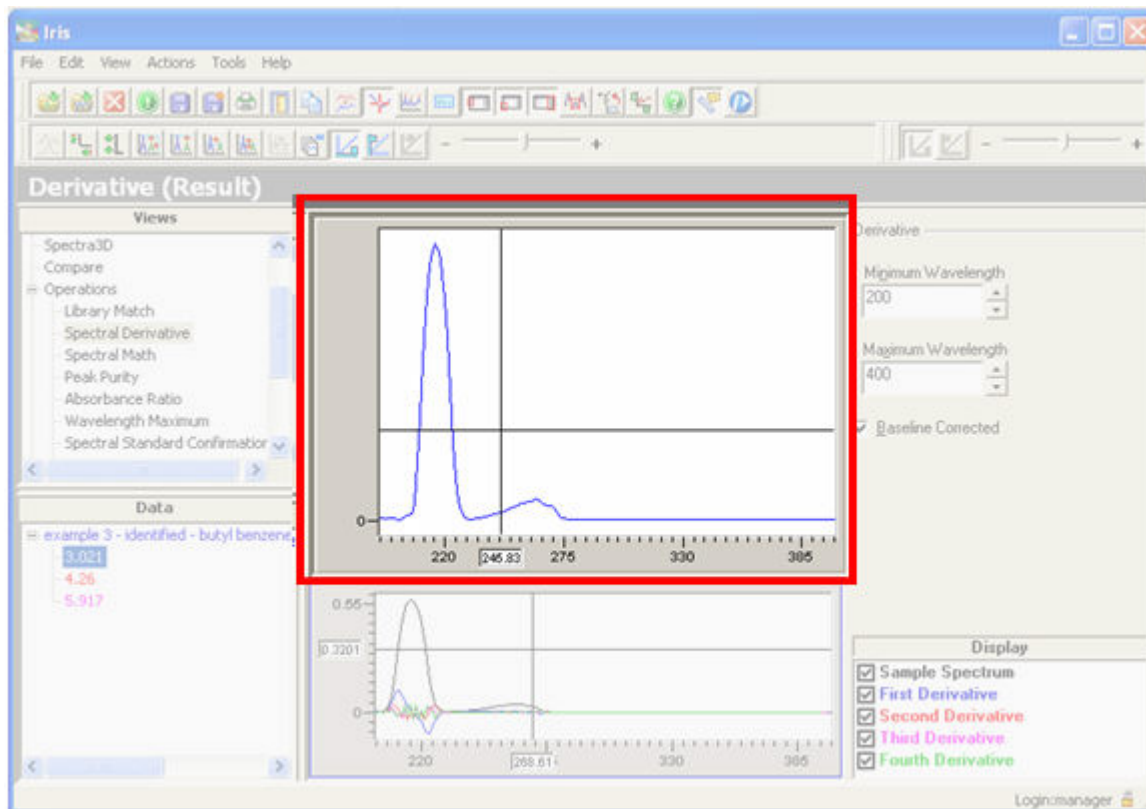
- **Parameters Pane** – This pane allows you to set the minimum and maximum wavelengths used for calculating spectral derivatives as well as select whether or not you wish to use the baseline corrected spectrum for the derivative.
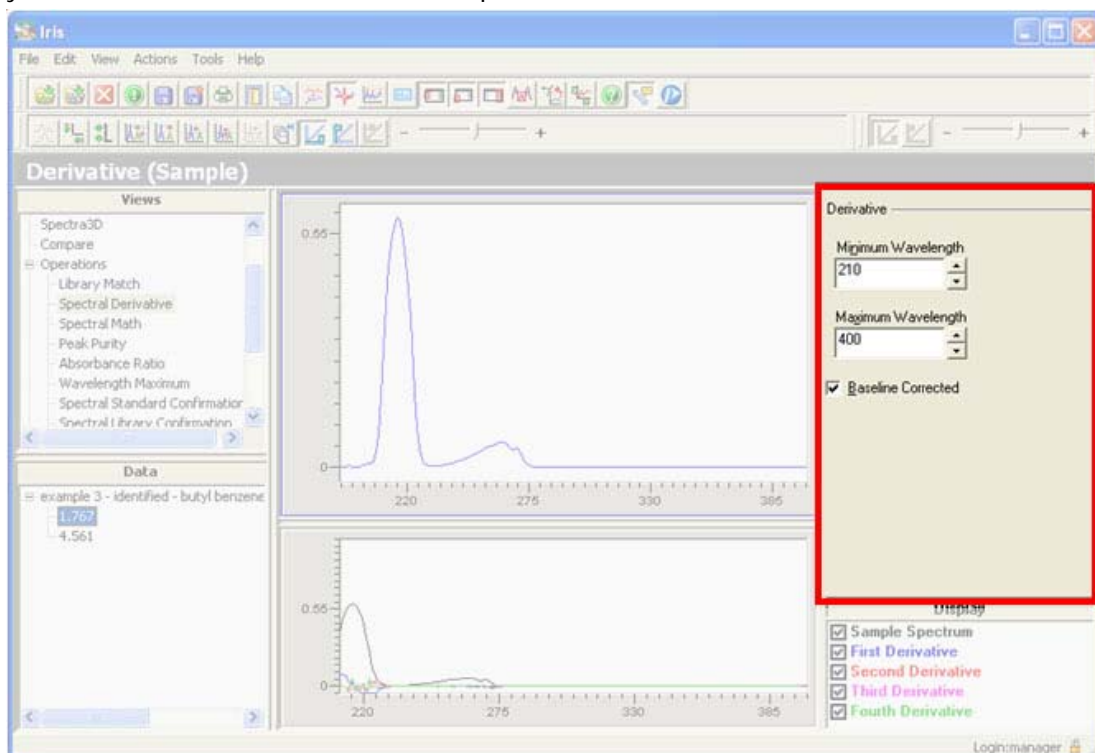
- **Display List** – You use the Display List to select which spectra you want to see on the Derivatives Pane. Items that appear checked are displayed on the Derivates Pane, while unchecked items are not displayed.

  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.
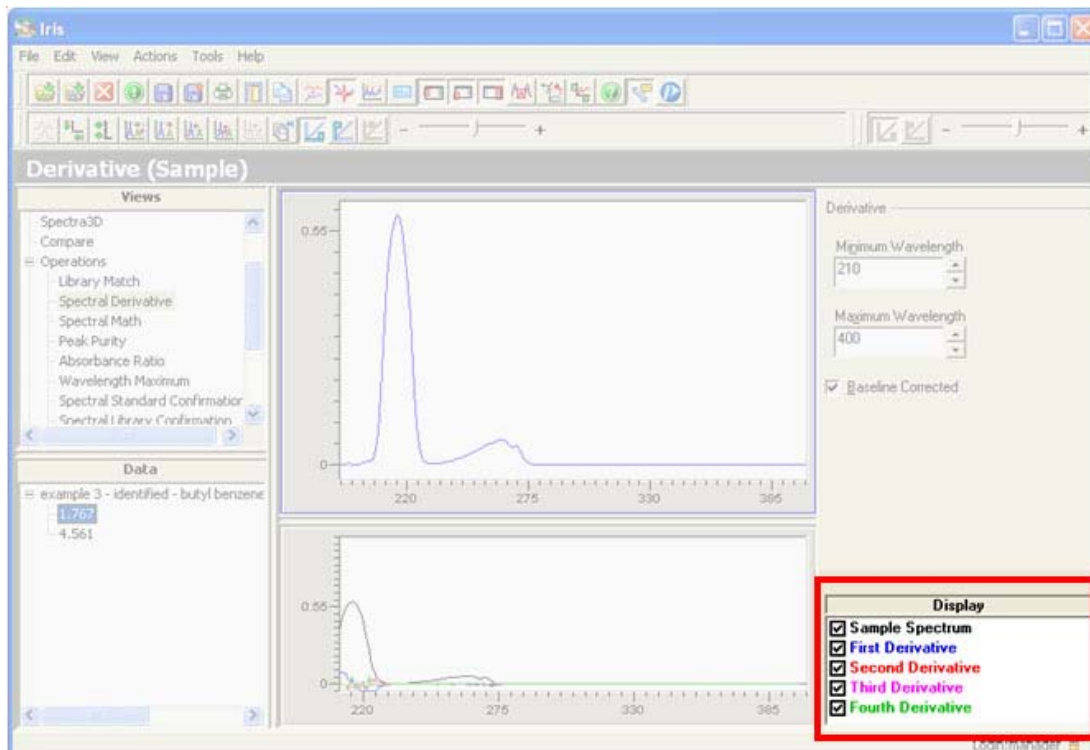
Note: *The check box options from the Display List and the curves on the Derivatives Pane have matching color coding.*

- **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.
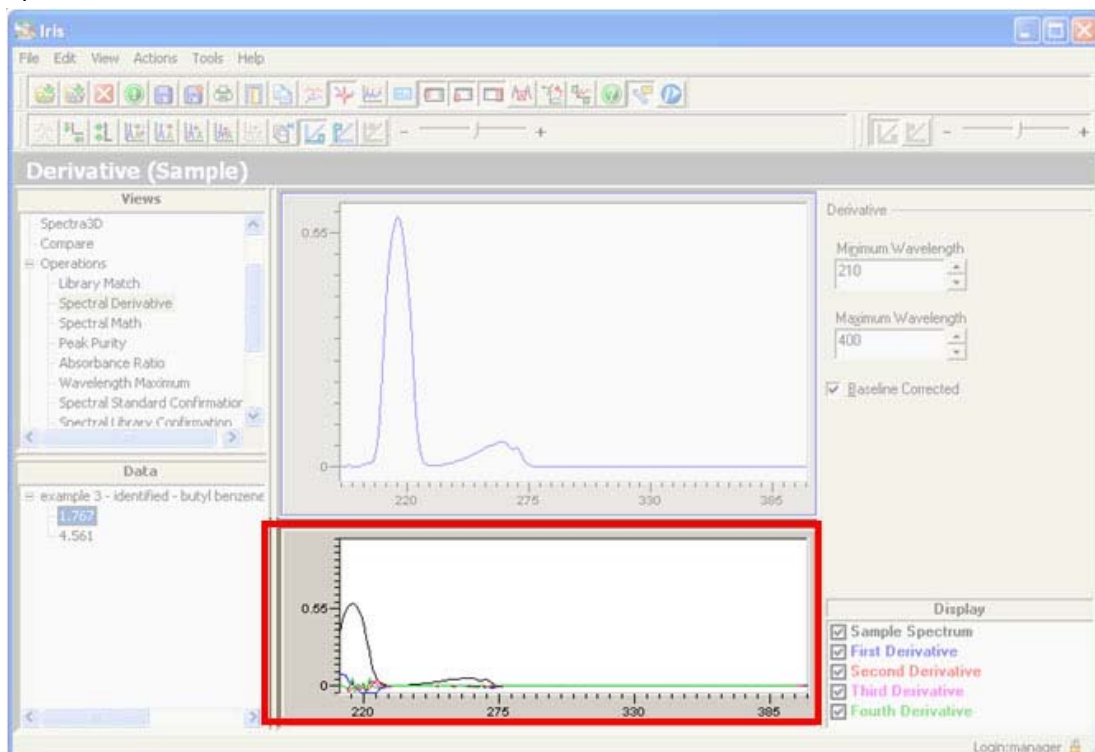
Summary of Operation: Spectral Derivitives

The summary below illustrates how calculate spectral derivatives for a spectrum.

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 page.*

7. From the Views Tree select **Operations > Spectral Derivative**.

    The Spectral Derivatives operation is displayed.

8. 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 operation. The Derivatives Pane displays the spectral derivatives that appear checked on the Display List.

9. If you wish 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.

10. 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.

To 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.

To save a result spectrum to a .uv file:

11. From the Data Tree, select the spectrum you wish to save.

12. From the **File** menu select **Save As…**
    The Save Spectrum As dialog appears.

13. Specify a **filename** and **location** for the spectral file.

Spectral Math

The Spectral Math operation is where you perform mathematical operations (add, subtract, and divide) on spectra and view the results. From this operation, 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 operation.
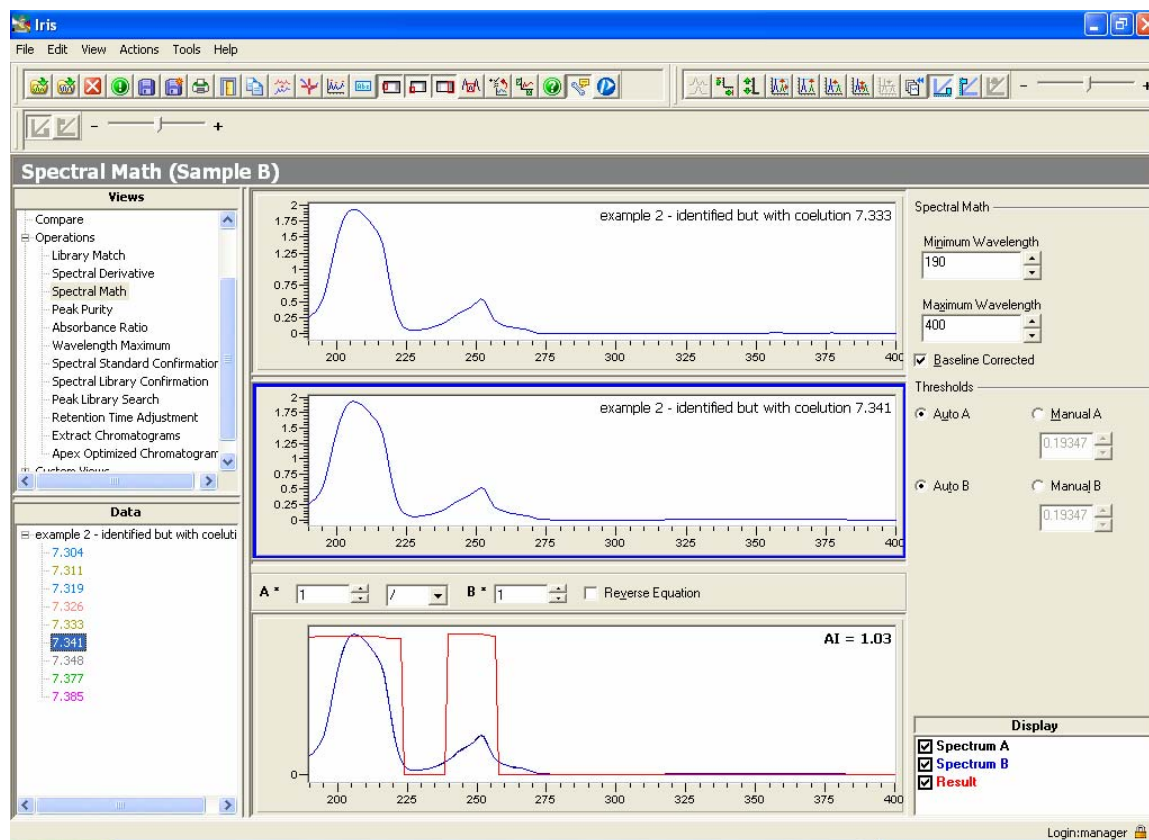
Figure 7-2 The Spectral Math Operation

The Spectral Math operation consists of the following areas:

- **Views Tree** - This pane provides you access to the Spectral Math operation. You can access the Spectral Math operation by selecting Operations > Spectral M**ath** from the Views Tree.
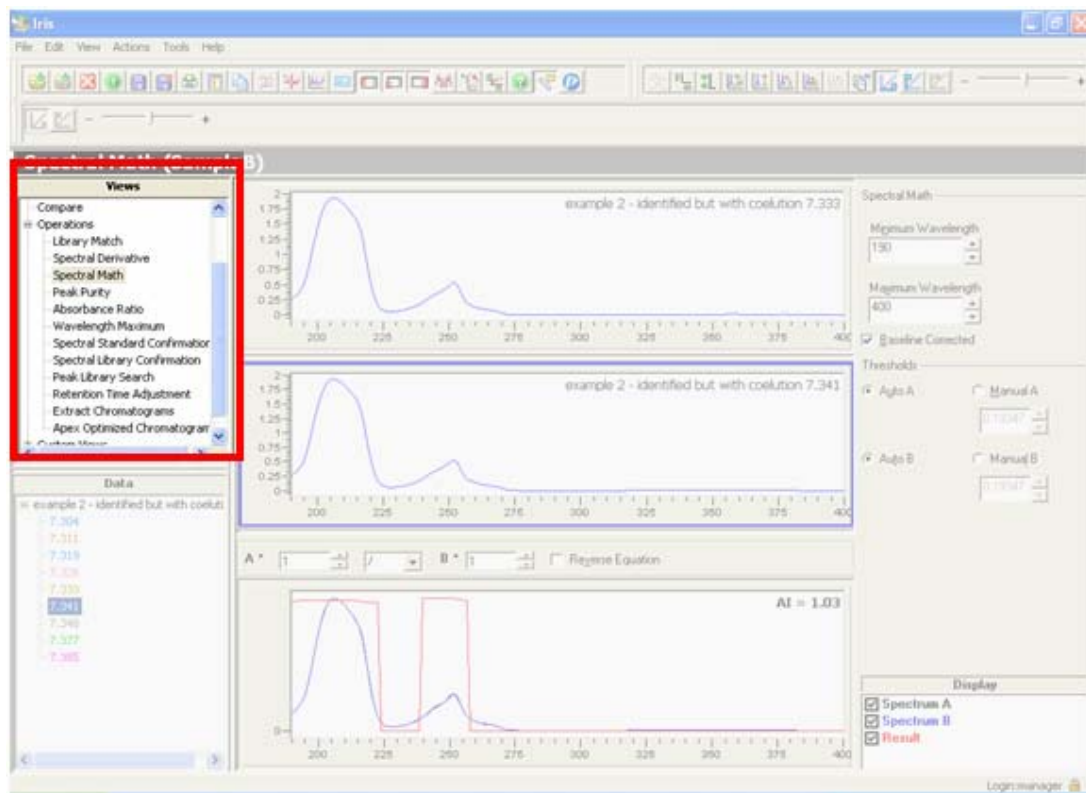
- **Data Tree -** The Data Tree displays a list of extracted spectra that are grouped by their parent chromatogram. From the Data Tree you select spectra that will be used to perform mathematical operations on this page. You must select two spectra from the Data Tree in order to perform operations on this page. *For information on selecting spectra, see* **To select spectra:** *on page* **197***.*

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 operation.*
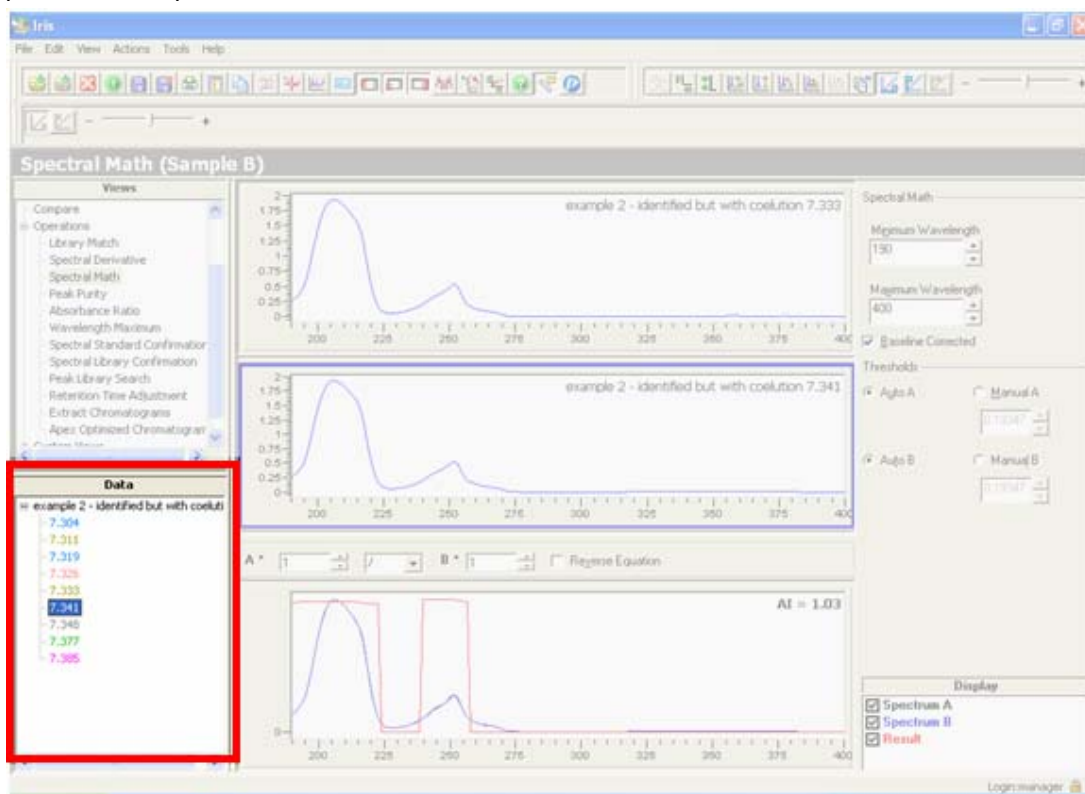
Note: *The Spectrum panes remain blank until a spectrum is selected for the pane, and the Result pane is blank until both spectrums have been selected.*

- **Spectrum A Pane** - This pane displays the spectrum you selected on the Data Tree as Spectrum A for mathematical operations performed on this view.

  If you do not have a spectrum already selected on the Data Tree, when you first access the Spectral Math opeartion, 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 as Spectrum A, simply click on an area inside the whitespace of the pane, and then select a different spectrum from the Data Tree. The new spectrum is now displayed on the Spectrum A Pane.
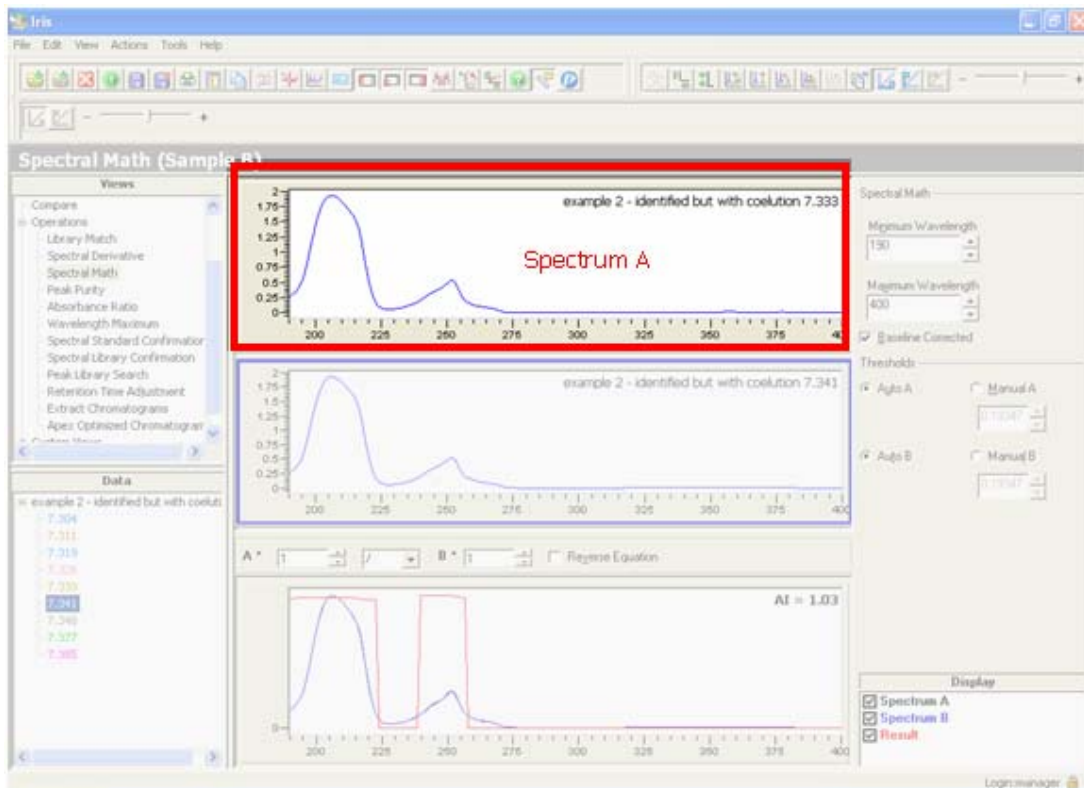
- **Spectrum B Pane** -- This pane displays the spectrum you selected on the Data Tree as Spectrum B for mathematical operations performed on this view.

  When you first access the Spectral Math operation this pane appears blank. To add a spectrum to this pane you must first click on an area inside the whitespace of the pane, and then click on a spectrum listed on the Data Tree. The selected spectrum now appears on this pane.
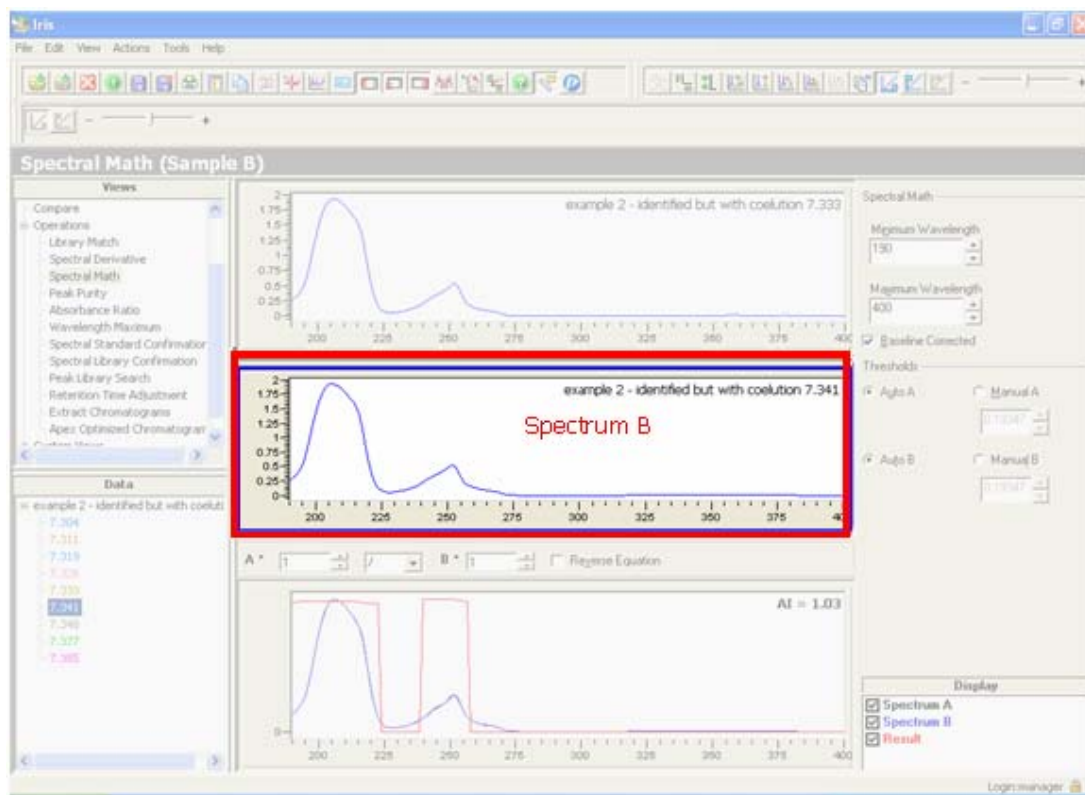
- **Display List** – You use the Display List to select the information you want displayed on the Results Pane. When items appear checked on this list, they are displayed in the Results Pane. Meanwhile, unchecked items are not displayed.
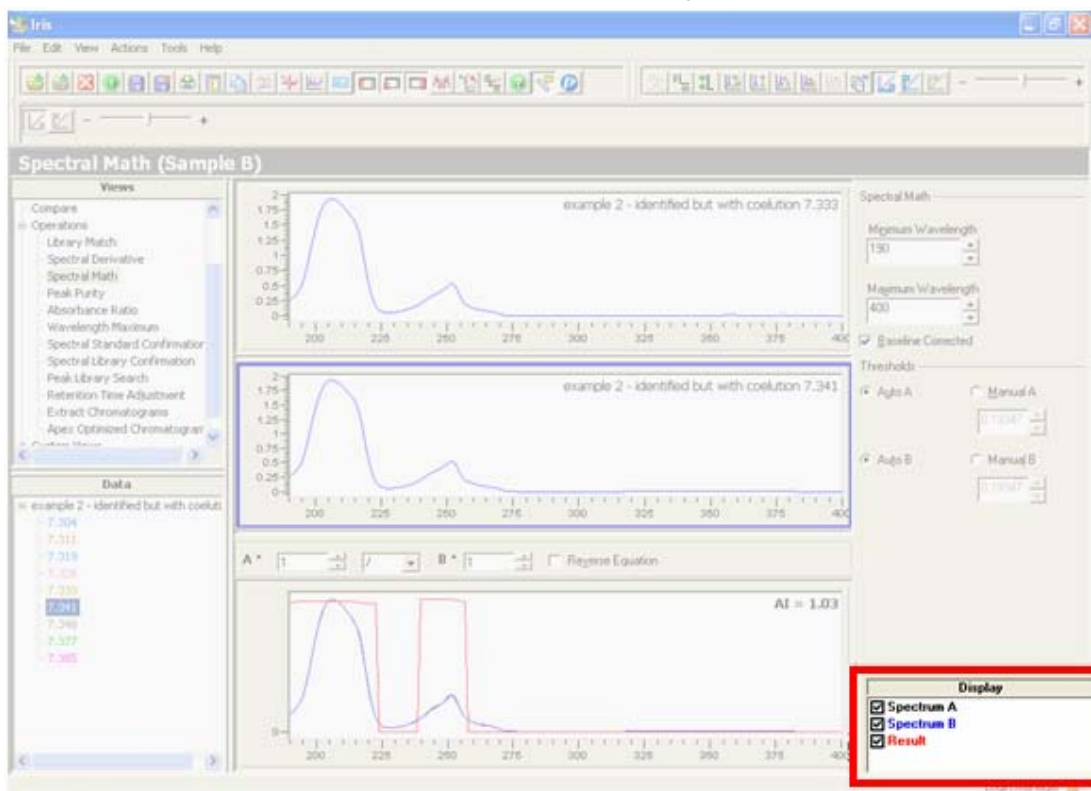
- **Parameters Pane** - The Parameters Pane displays the wavelength range for the calculation, whether or not baseline corrected spectra are used for the spectral math, as well as the Threshold controls, which you can manually set to a value above the system noise. You can modify these parameters and IRIS automatically updates the Results pane.

Note: *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.*
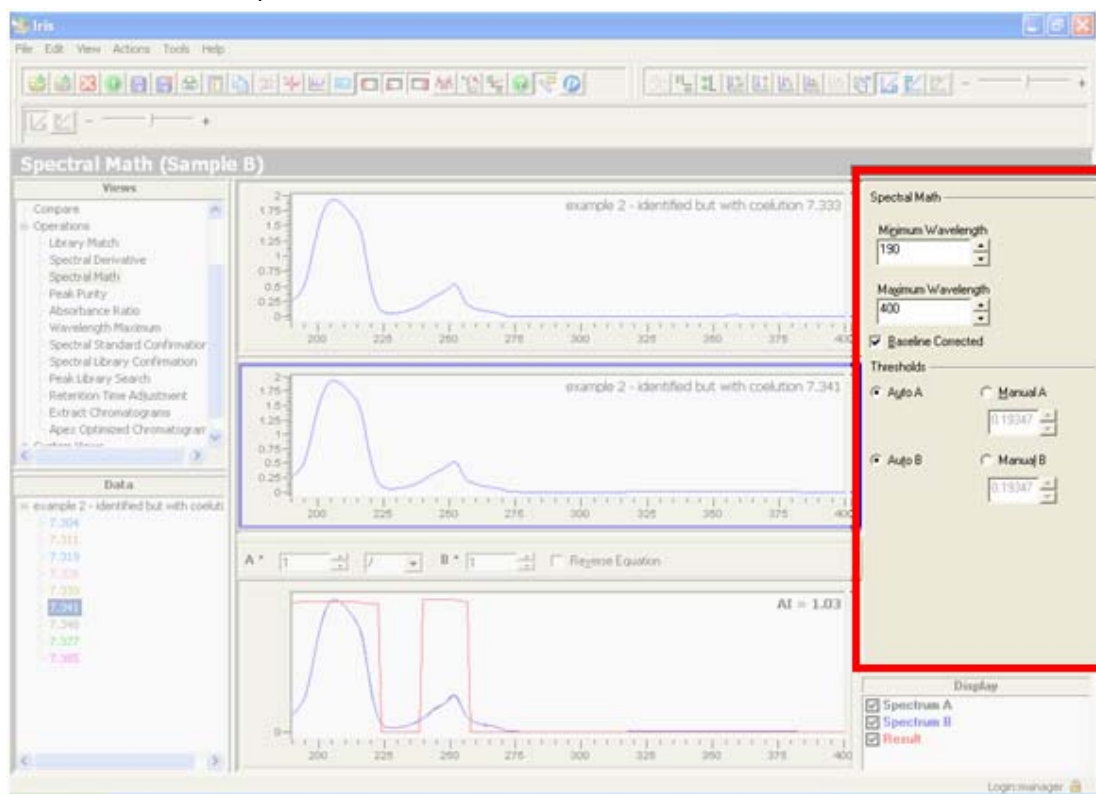
More specifically, the Parameters Pane allows you to specify the following parameters:

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 have IRIS 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 have IRIS 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.
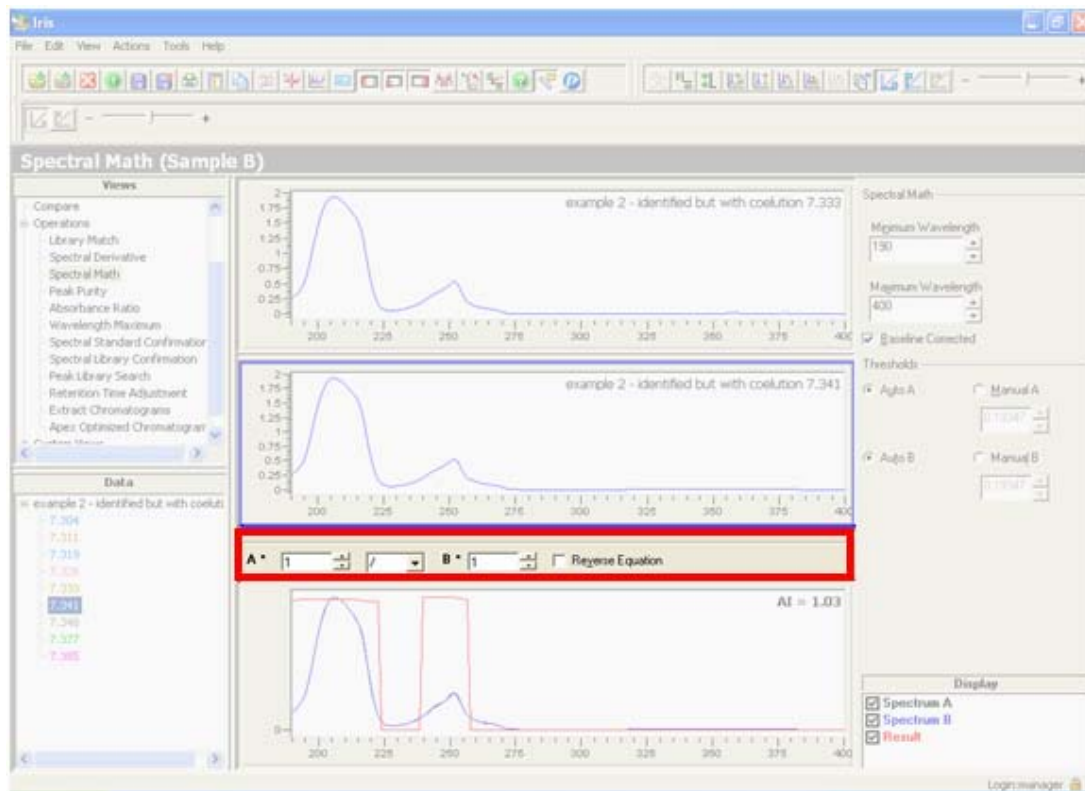
- **Results Pane** - This pane displays a graphical representation of the items that are checked on the Display List. You can view Spectrum A, Spectrum B, and the Result Spectrum on this pane.
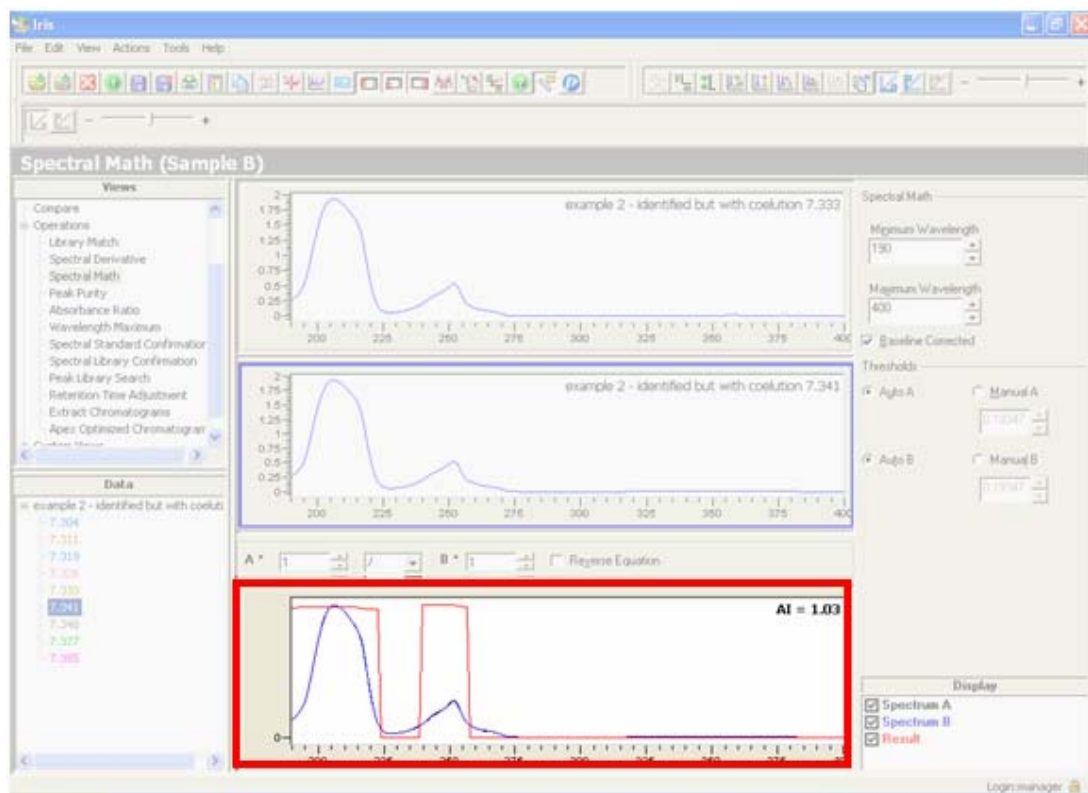
Summary of Operation: Spectral Math

The summary below illustrates how to use the Spectral Math operation 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 <u>must</u> be present in the Data Tree before you access the Spectral Math page.*** Once you have selected the two spectra for the operation, IRIS automatically calculates and displays the result of the operation using the parameters currently displayed on the operation. You can modify any of the parameters on this page and IRIS will automatically display the new result.

To select spectra:

1. If you are working with spectra extracted from chromatograms, the two spectra <u>must</u> be present in the Data Tree prior to displaying the Spectral Math operation.

2. From the Views tree, expand the **Operations** node and click on **Spectral Math**.
    The Spectral Math opeartion displays.

3. If you are not working with spectra extracted from chromatograms, open one spectrum file at a time using **File > Open > Spectrum…**
    The opened spectra appear in the Data Tree.

4. From the Spectral Math operation, 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 operation.
    A blue border appears around the pane to indicate that the pane is selected.

5. 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 want to perform.
    You must now select a second spectrum to be used in the calculation, this spectrum will be considered Spectrum B in the mathematical you wan tto perform.

6. 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.

7. 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 IRIS automatically calculates and displays the result of the currently defined mathematical operation, using the two selected spectra and the parameters currently displayed on this operation.
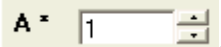
Note: *You can change any of the parameters used in the calculation, as well as modify the calculation you wish to perform; and IRIS will automatically recalculate the result and display it in the Results Pane.*

To perform a mathematical operation on two spectra using a different set of parameters:

Once you have selected Spectrum A and Spectrum B, IRIS 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 operation.

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* A * [1   ] .

2. Select a multiplication factor for Spectrum B using the spin box next to
   B* B * [1   ] .

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 -.

To 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.

To save a result spectrum to a .uv file:

1. From the Data Tree select the spectrum you wish to save.

2. From the **File** menu select **Save As...**
   The Save Spectrum As dialog appears.

3. Specify a **filename** and **location** for the spectral file.

Chapter 8. Performing Operations on Chromatograms

Performing Chromatogram Operations

IRIS has 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 chapter shows you how to use IRIS to perform operations, such as peak Purity, and Peak Library Search to investigate a chromatogram.

More specifically, in this chapter 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 Standard Confirmation operation to verify the identity of chromatographic peaks by comparing to a standard chromatogram.

- Use the Spectral Library C**onfirmation** 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 Retention Time Adjustment operation to properly assign the component names to a peak when TotalChrom misses the identification of peaks because they are outside the peak tolerance window or they are misassigned.

- Use the Extract Chromatograms operation to extract up to 8 chromatograms from a spectral file and automatically process them.

- 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 TotalChrom Result file is updated with this information and the corresponding spectral method files are updated with the parameter values used to calculate the results.

Note: *Any of the arithmetic operations and chromatographic extractions listed above can be automatically performed during a TotalChrom sequence, by means of the **AutoCalc** user program, which is installed with your IRIS Spectral Processing software. The results of the automated operations are automatically saved to the TotalChrom Result file (.RST), and can be automatically included in a TotalChrom Report.*

*For more information, on automating arithmetic operations and chromatographic extractions, refer to the chapter titled **AutoCalc: Automating Arithmetic Operations, Extractions and Apex Spectra Printing.***

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. In IRIS you 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**.
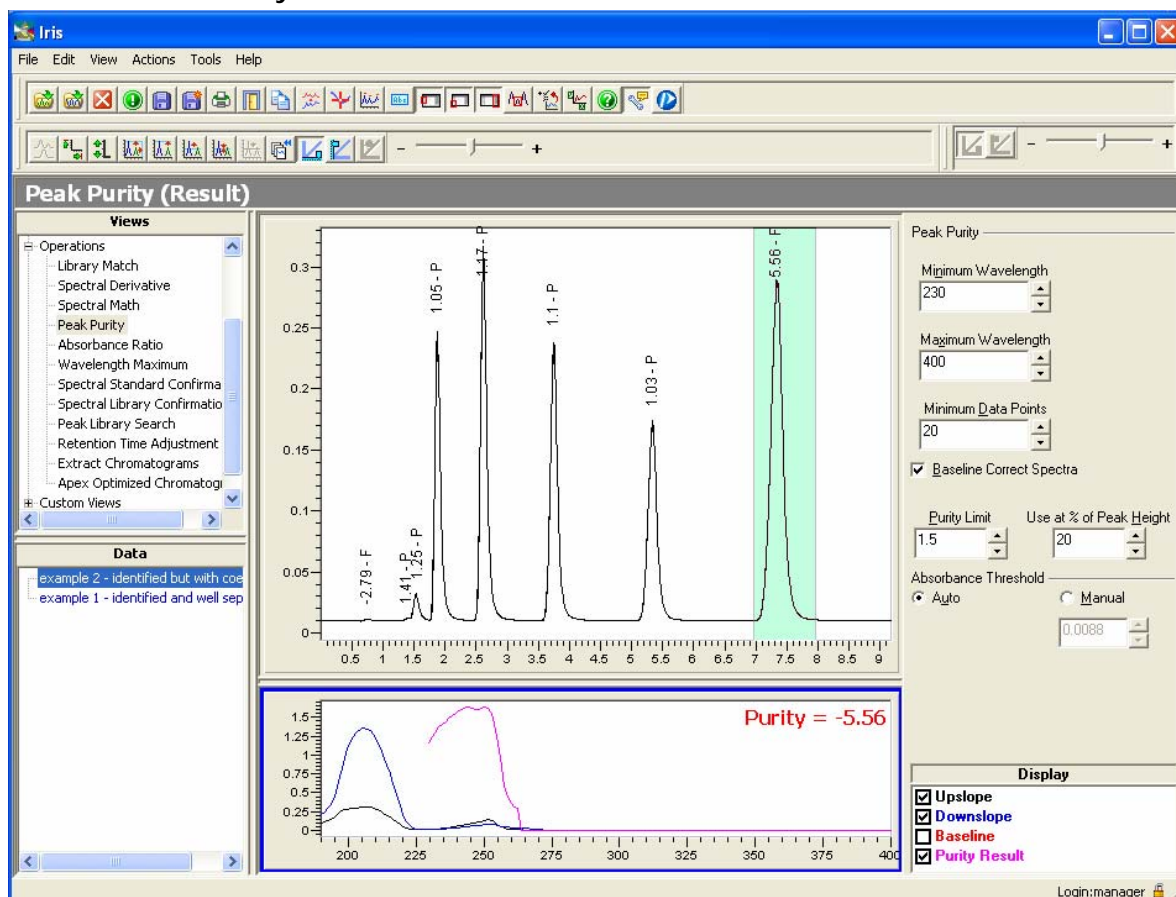
Figure 8-1The Peak Purity operation

Checking the Purity of Chromatographic Peaks

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 IRIS automatically recalculates and displays the new purity indices for each peak in the chromatogram.

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 IRIS calculates Peak Purity, refer to* **Appendix 1** *on page* **349***.*

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.
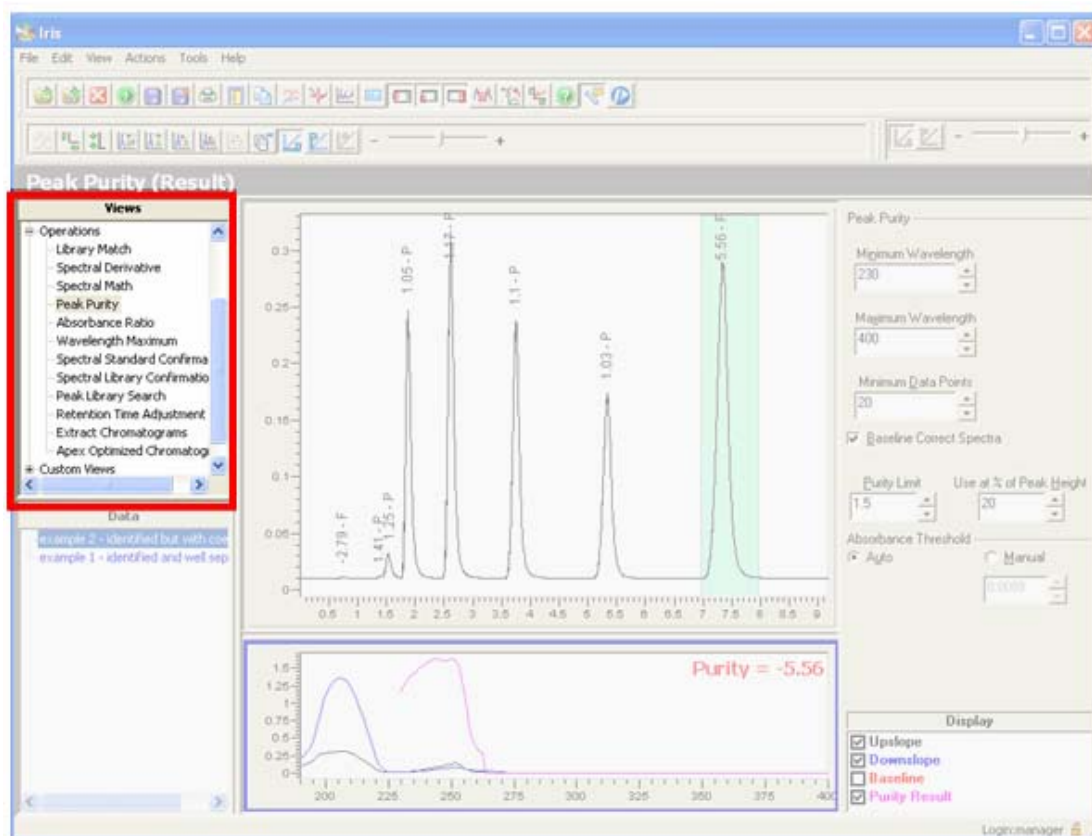
- **Data Tree** – The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.
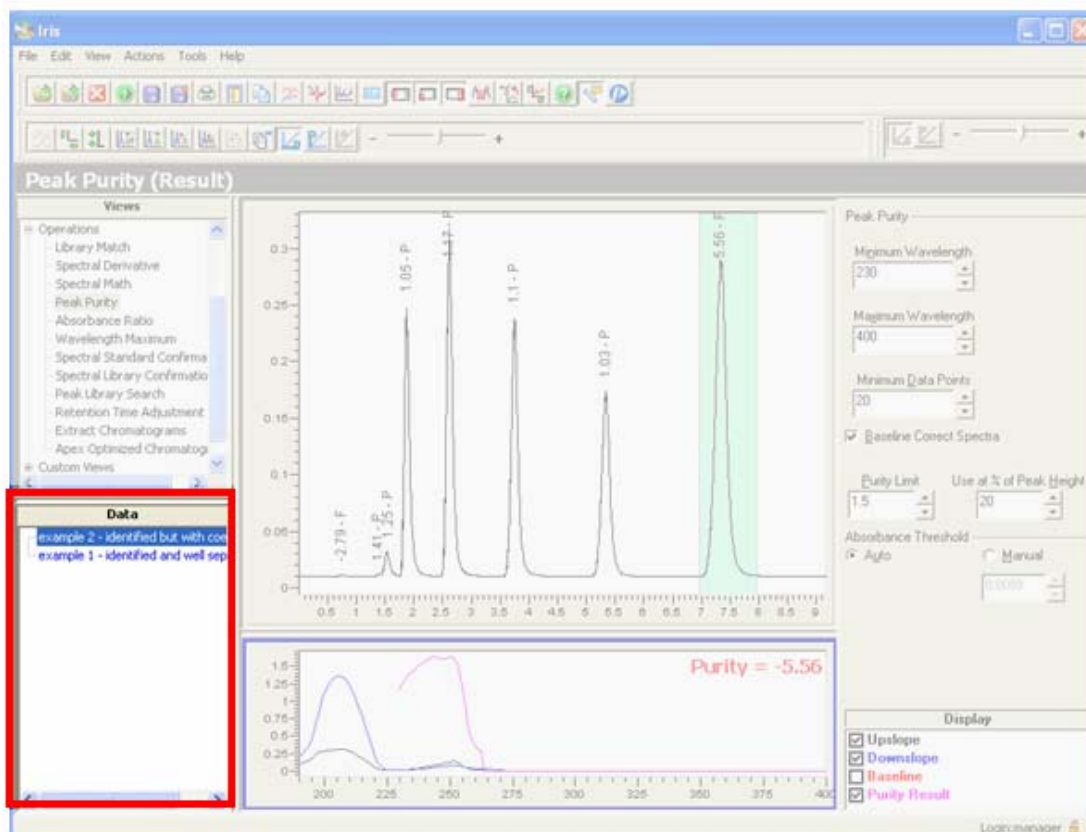
- **Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree.

    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 isconsidered 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. IRIS 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.*
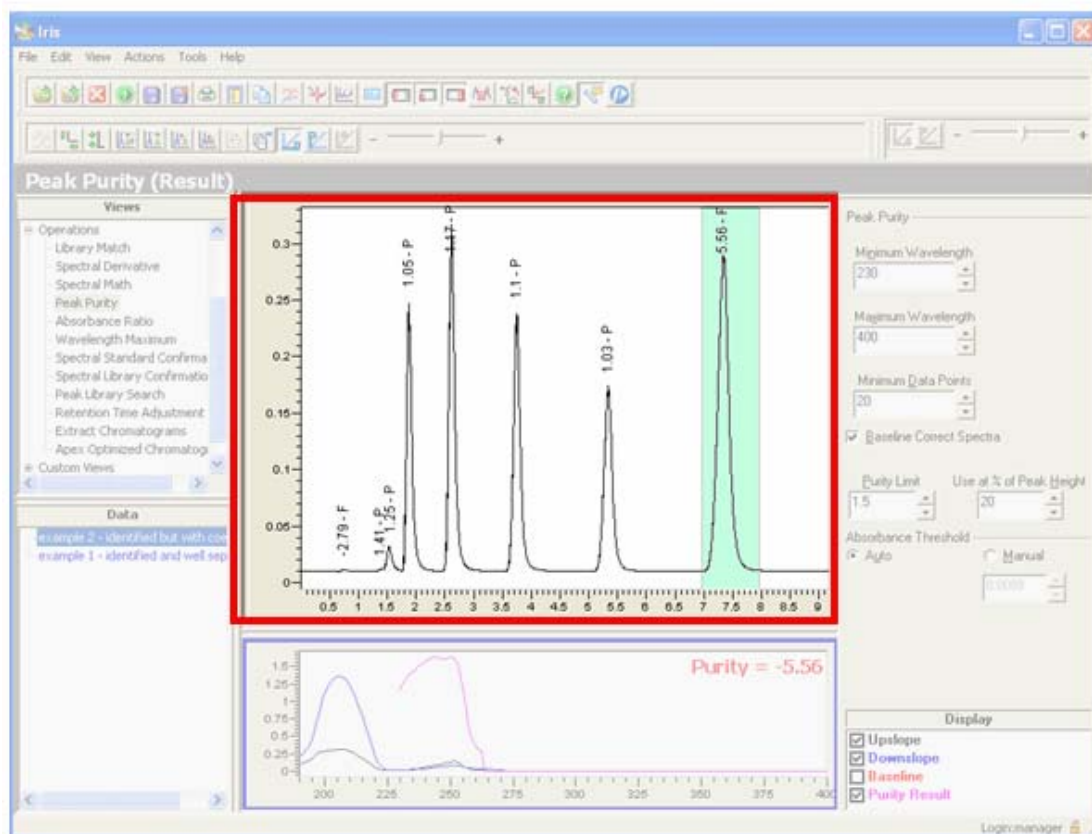
- **Parameters Pane** – This pane is where you set and investigate the various parameters used to calculate Peak Purity. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, IRIS automatically updates the Display List and Results pane with the modified results.

  *For more information on the Peak Purity parameters, see **How do I** on page **209**.*
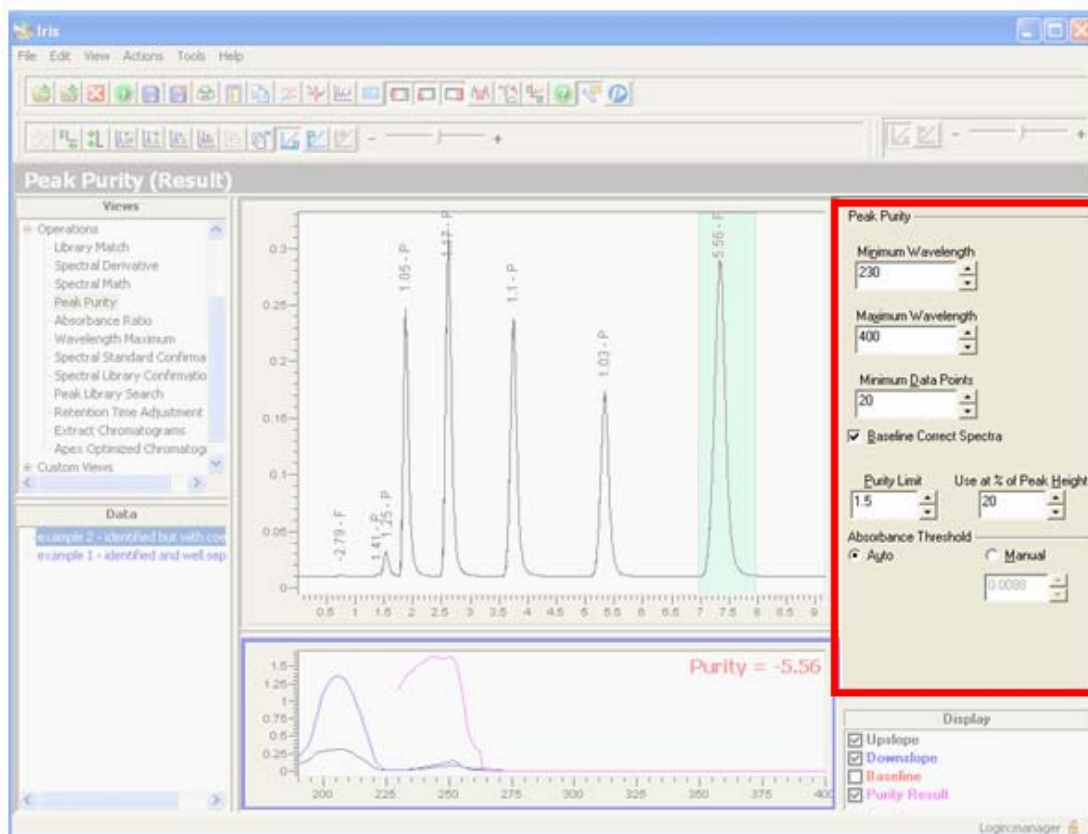
- **Display List** - You use the Display List to select the information you want displayed on the Results pane (including the spectra used in the calculation of the purity and the purity result). Items that appear checked are displayed on the Results pane, while unchecked items are not displayed.
The 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-noise 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.
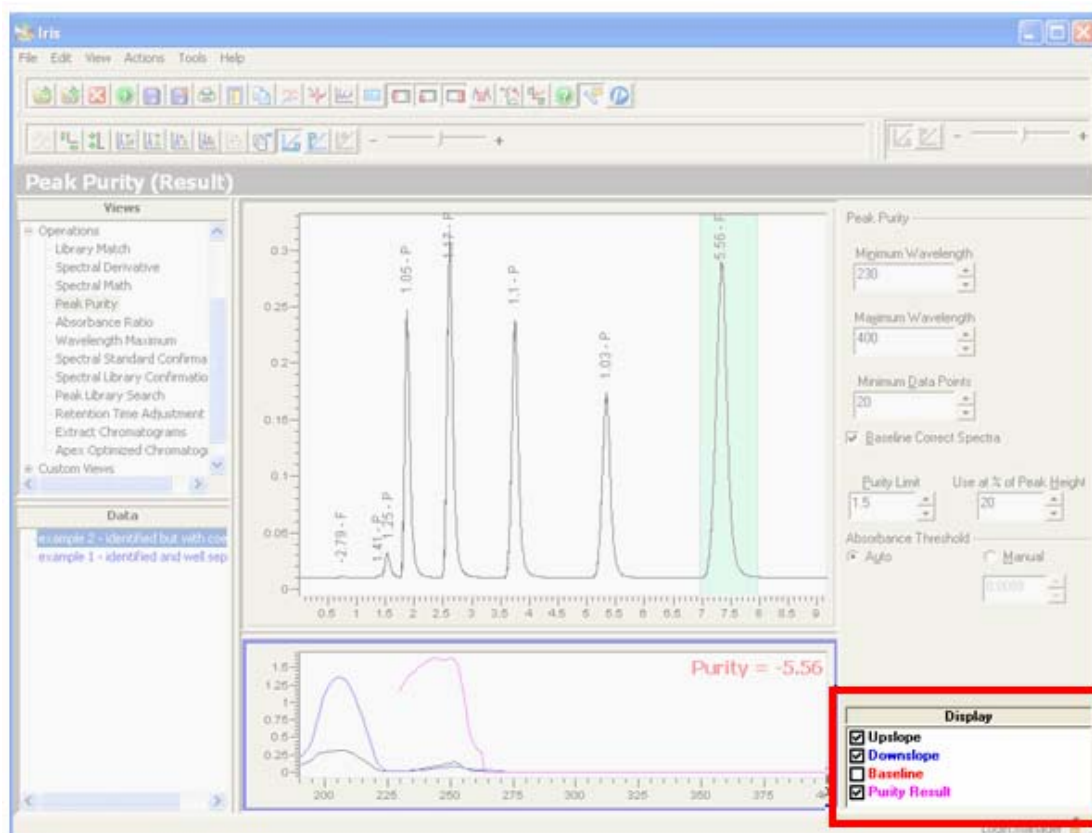
- **Results Pane** - This pane displays a graphical representation of the items that are checked on the Display List. In addition, in the right hand corner of this pane, the Purity Index for currently selected peak is displayed. If the peak's purity value exceeds the Purity Limit currently defined on 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.
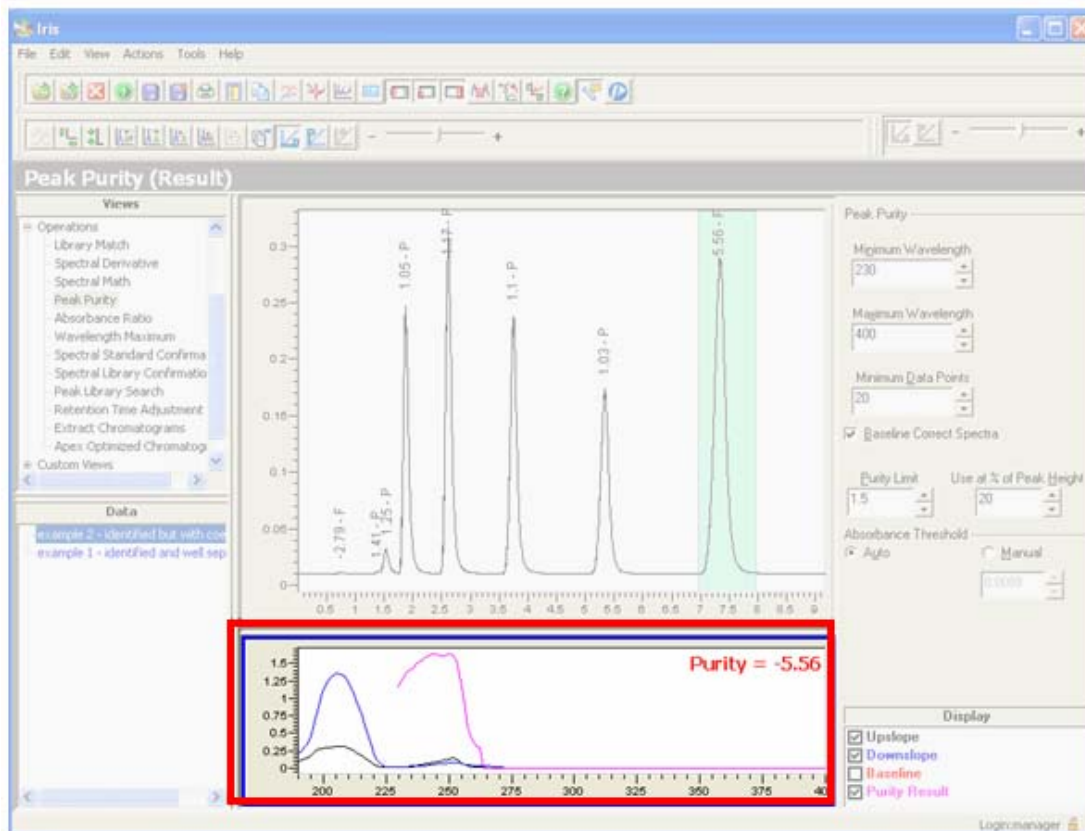
Note: *Whenever you select a different peak, change the parameters for the operation, or select/deselect an item on the Display List, the Result pane is automatically updated to reflect the change.*

How do I modify the parameters that are used to calculate Peak Purity?

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 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.

Parameter	Description
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.

To 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. 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, 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.

To 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)*.

To 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.

To 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.

To adjust the Minimum Absorbance value and the Minimum Data Points that are used in the calculation of Peak Purity:

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 the **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, the **Minimum Data Points** threshold, is therefore applied, which determines if the calculation can be performed. This value defaults to 20 within IRIS, 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.
   If you set the **Absorbance Threshold** above the system noise, then you may want to modify the Minimum Data Points parameter to ensure that there are sufficient points for a valid calculation to be performed.

3. Use the spin box for the **Maximum Data Points** parameter to specify the minimum number of data points to be found for the result to be valid.

To 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.

To 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 apear in the Data Tree.

To 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**. Once you have saved the updated purity values, they can be included in a TotalChrom report.

1. Select **File > Save Results...**

   A save dialog appears that asks you to confirm whether or not you want to save the new values to the chromatogram's result file (.RST), and save the parameters that were used calculate the results to the associated spectral method file (.TSM).

2. To only save results for peaks that are currently identified (named) in TotalChrom select **Currently identified peaks**.

   OR

   To save results for all peaks, select **Include unidentified peaks**.

   Any unidentified peaks for which results have been calculated are added to the TotalChrom result file as Unknown 1, Unknown 2, etc. (Any unidentified peaks which do not have results are not added to the file.)

3. Click **Yes** to save the results and calculation parameters.

   Peak Purity values added to the result file for all identified peaks.

   The saved results can now be displayed as peak labels when the chromatogram is displayed in IRIS in the Main or Chrom/Spectra View. In addition, when you select this chromatogram again on the Peak Purity operation, the saved results and the parameters used are displayed on the screen.

To display the Peak Purity results in the Main or Chrom/Spectra View:

1. Select **Actions > Label Chromatograms...**

2. Select **Peak Purity** from the list of available labels

To 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.

In IRIS 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**.
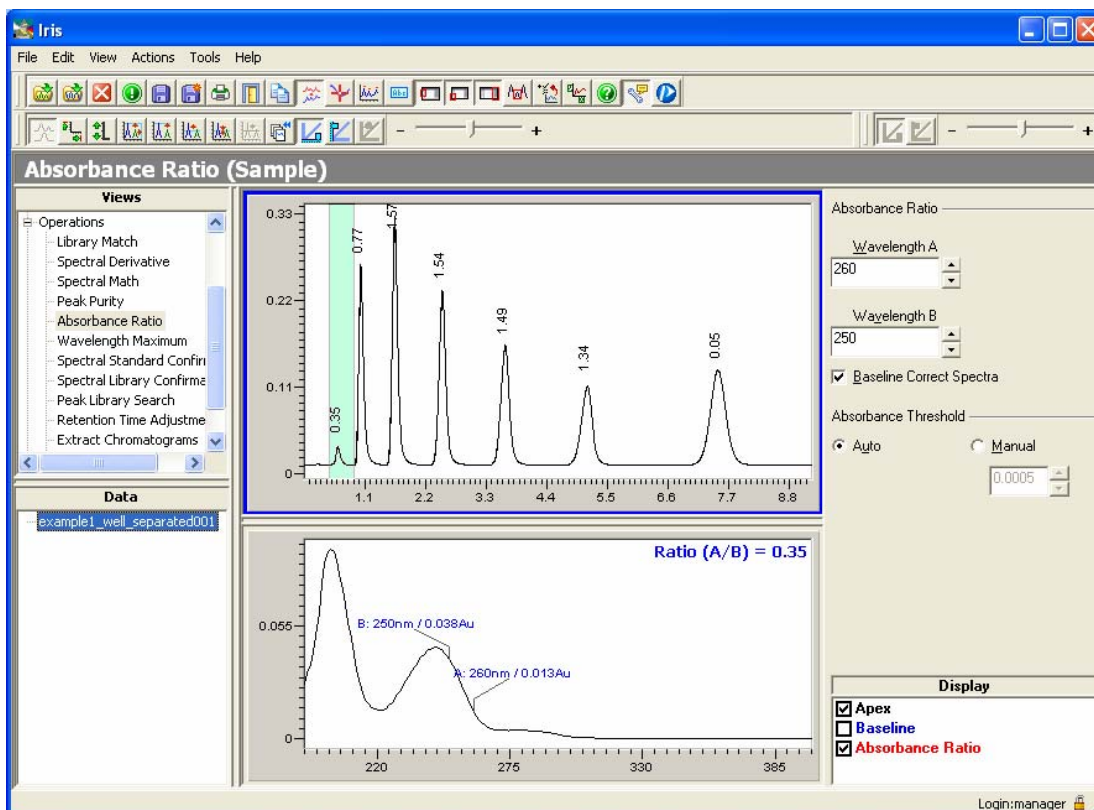
Figure 8-2 The Absorbance Ratio operation

Determining the Absorbance Ratio for each peak in a chromatogram

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.

IRIS 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. IRIS 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), IRIS displays the apex and baseline spectra 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 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 Absorbance Ratio Operation.

The Absorbance Ratio operation 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.
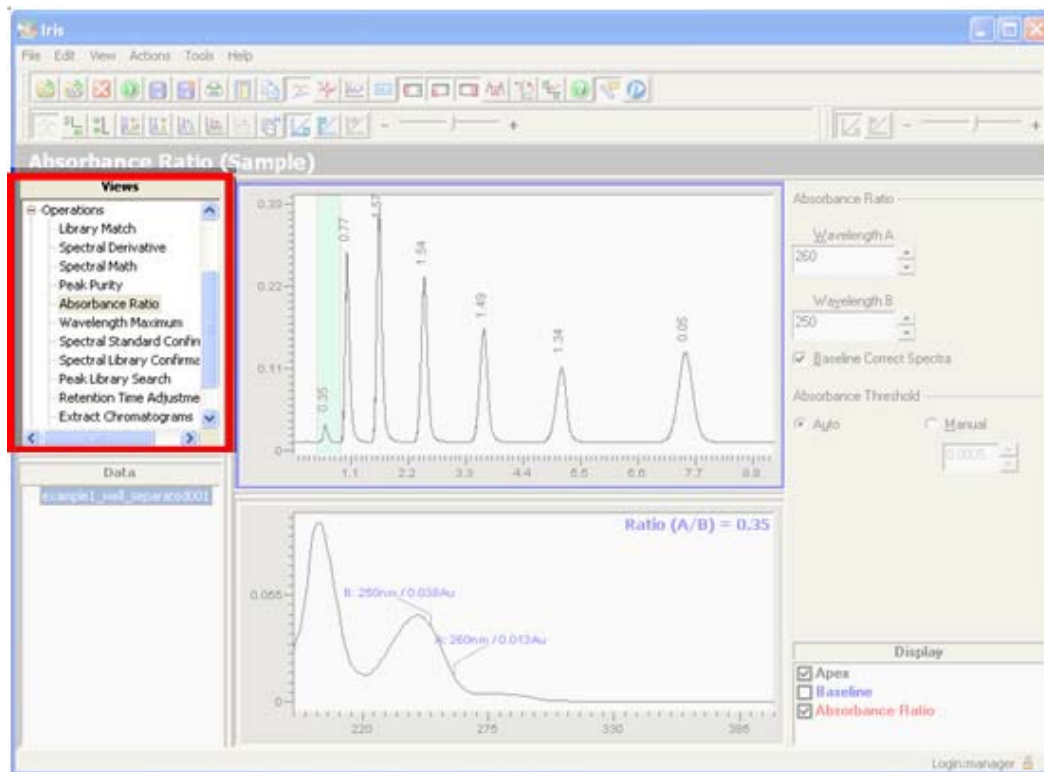
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree will is displayed in the upper pane (Chromatogram pane) for the Operation.
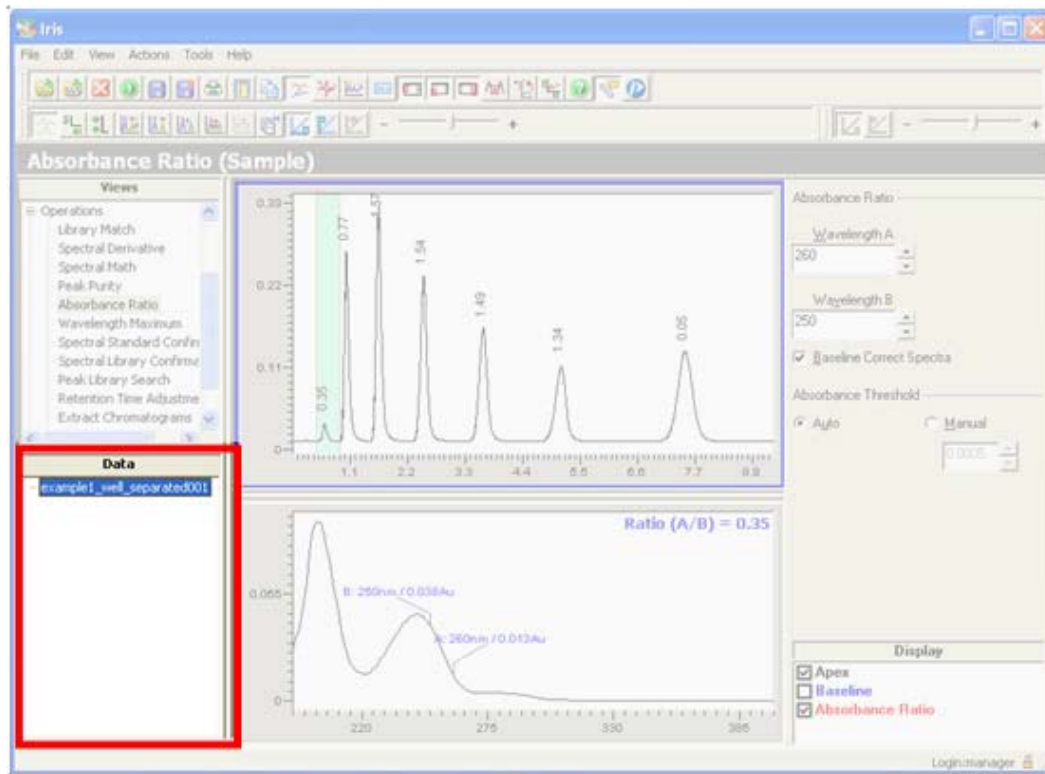
- **Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree.
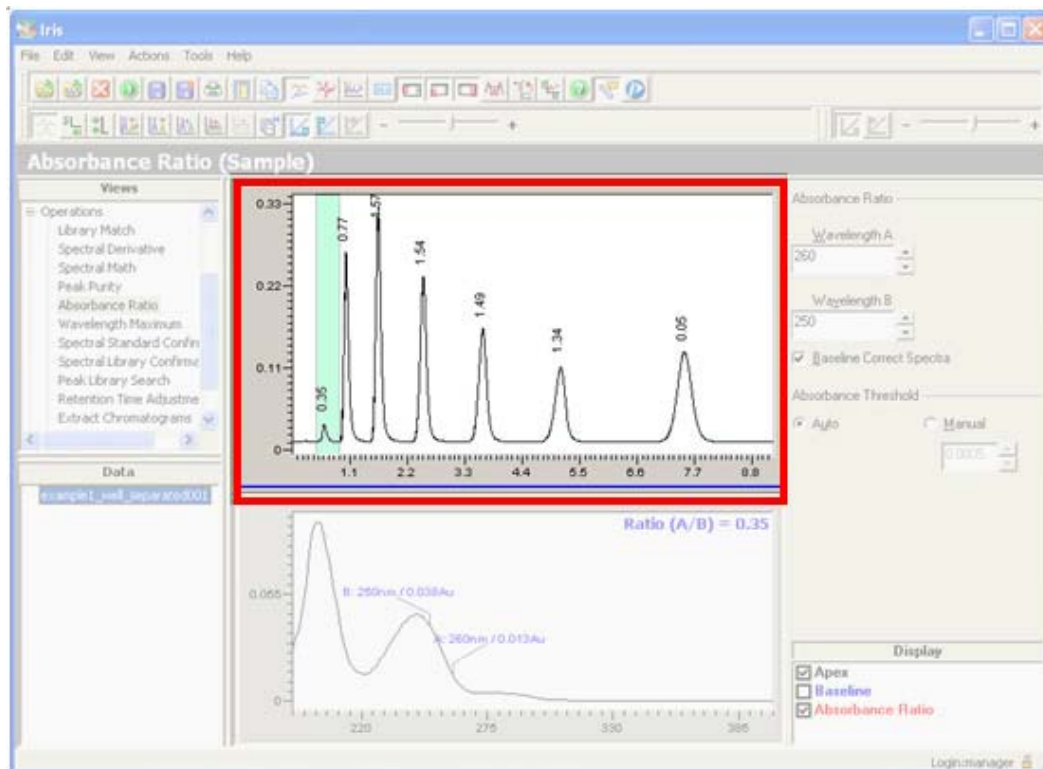
- **Parameters Pane**– This pane is where you set and investigate the various parameters used to calculate the Absorbance Ratio values. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, IRIS automatically updates the Display List and Results 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 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.
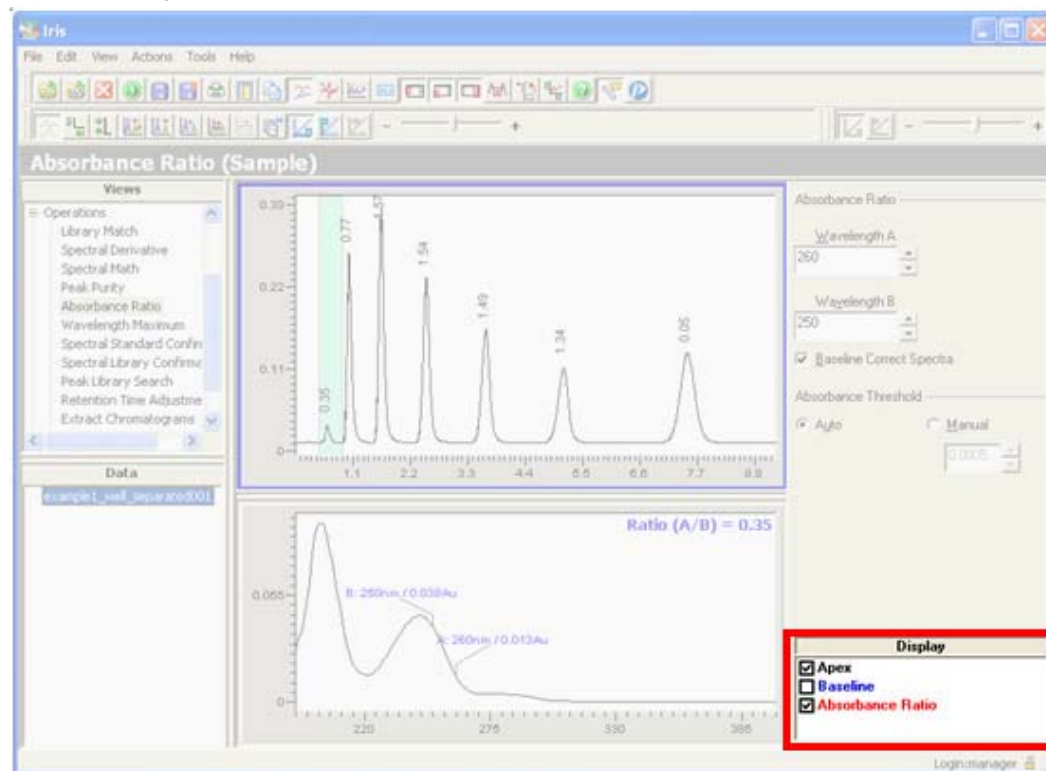
Note: You can select **Actions > Add All to View** to add all selected spectra in the Display list to the Data Tree.

- **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 labelled as: Ratio (A/B) =.
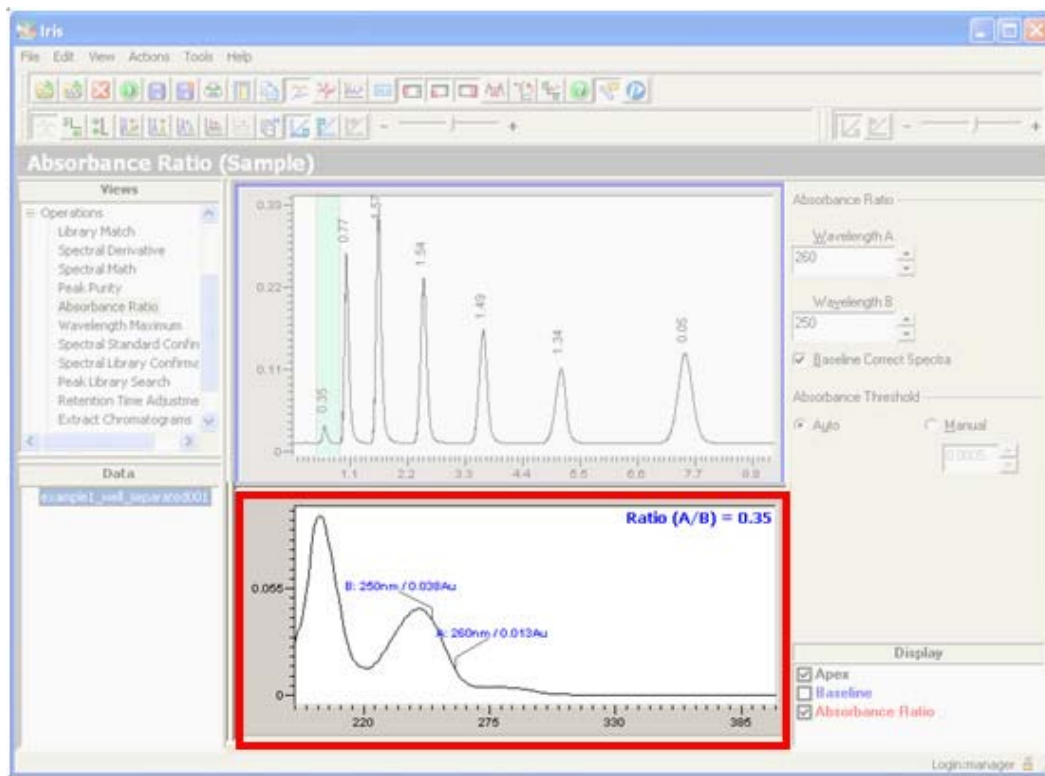
Note: *Whenever you select a different peak, change the parameters for the operation, or select/deselect an item on the Display List, the Result pane is automatically updated to reflect the change.*

How do I modify the parameters that are used to determine the Absorbance Ratio values?

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; and IRIS automatically updates the display 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 IRIS calculates the Absorbance Ratios 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 greyed 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.

To 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. 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 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.

To 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.

To 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.

To 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.

To 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.

To 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 Absorbance Ratio value labels are updated for every peak in the chromatogram.

To 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 apear in the Data Tree.

To save the results and parameters used in the calculation:

1. Select **File > Save Results...**

    A save dialog appears that asks you to confirm whether or not you want to save the new values to the chromatogram's result file (.RST), and save the parameters that were used calculate the results to the associated spectral method file (.TSM).

2. To only save results for peaks that are currently identified (named) in TotalChrom select **Currently identified peaks**.
    OR
    To save results for all peaks, select **Include unidentified peaks**.

    Any unidentified peaks for which results have been calculated are added to the TotalChrom result file as Unknown 1, Unknown 2, etc. (Any unidentified peaks which do not have results are not added to the file.)

3. Click **Yes** to save the results and calculation parameters.

    Absorbance Ratio values added to the result file for all identified peaks.

To display the Absorbance Ratio results in the Main or Chrom/Spectra view:

1. Select **Actions > Label Chromatograms...**

2. Select **Absorbance Ratio** from the list of available labels

To review for any chromatogram previously calculated Absorbance Ratio values and the parameters used:

➢ Select this chromatogram again on the **Absorbance Ratio** operation.

The saved results and the calculation parameters are automatically displayed.

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**.
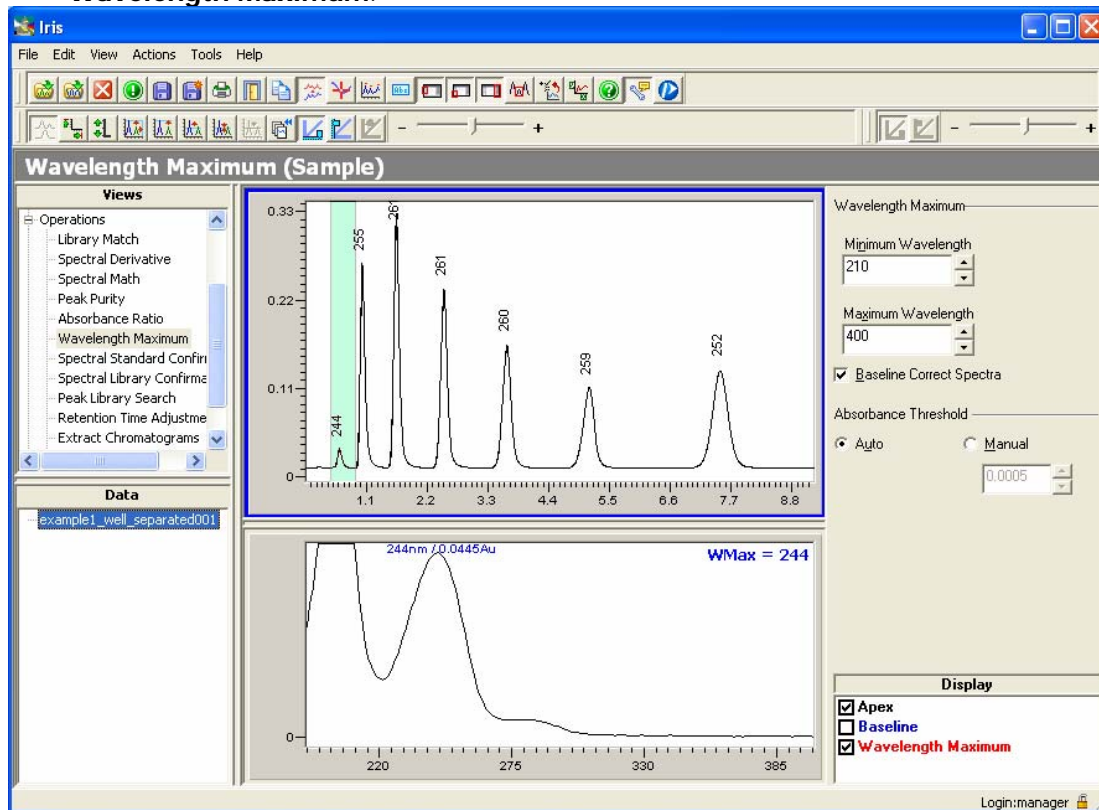
Figure 8-3 The Wavelength Maximum operation

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. IRIS 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 IRIS 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. To access this operation, expand the Operations node and click on Wavelength Maximum.

- **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.
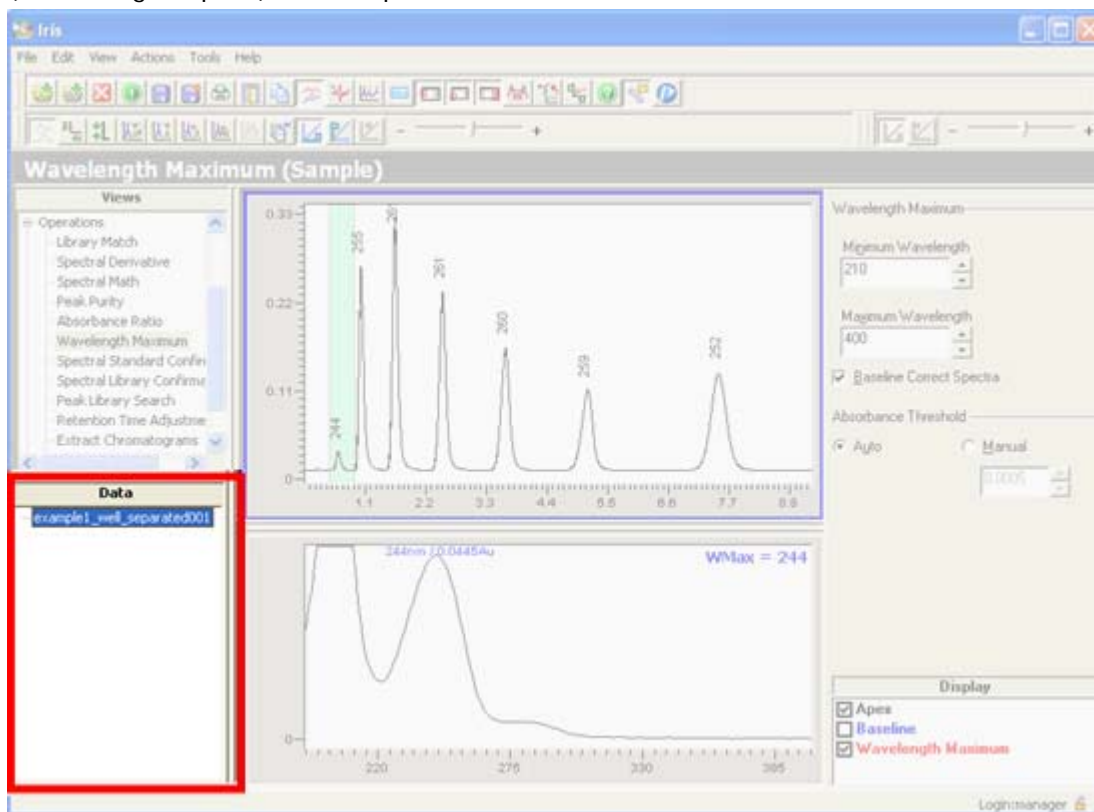
- **Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree.
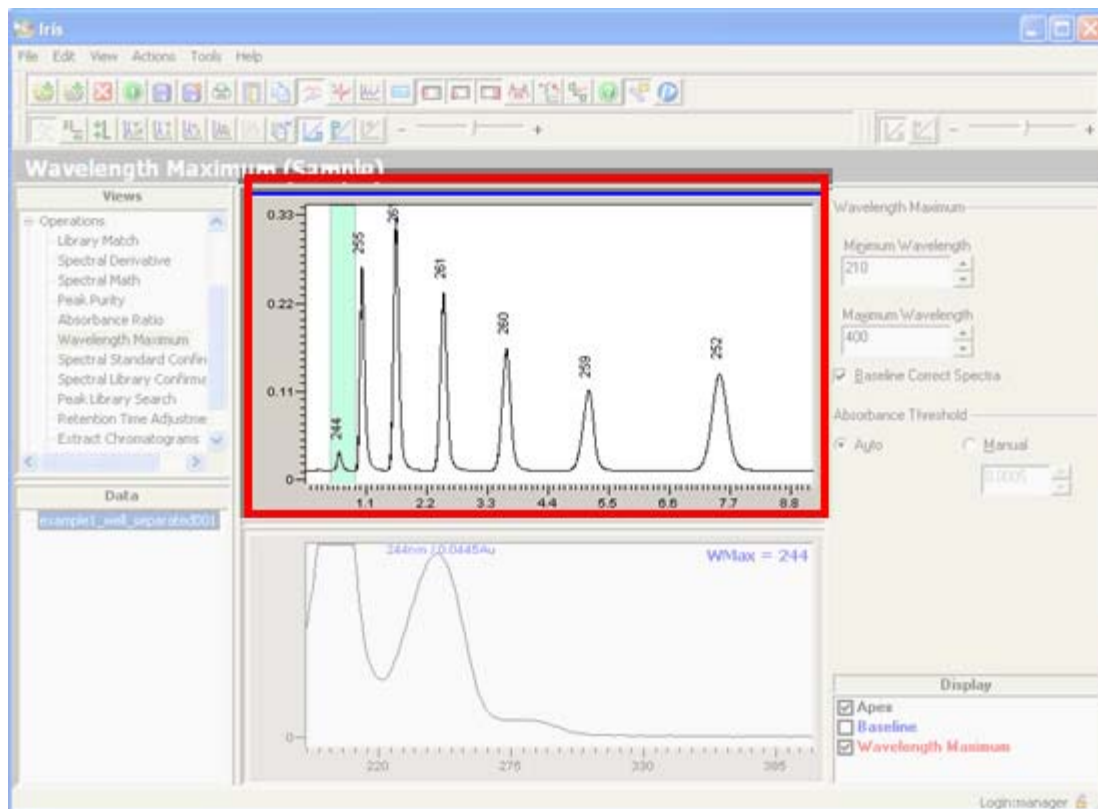
- **Parameters Pane** – This pane is where you set and investigate the various parameters used to determine the Wavelength Maximum values. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, IRIS automatically updates the Display List and Results 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:
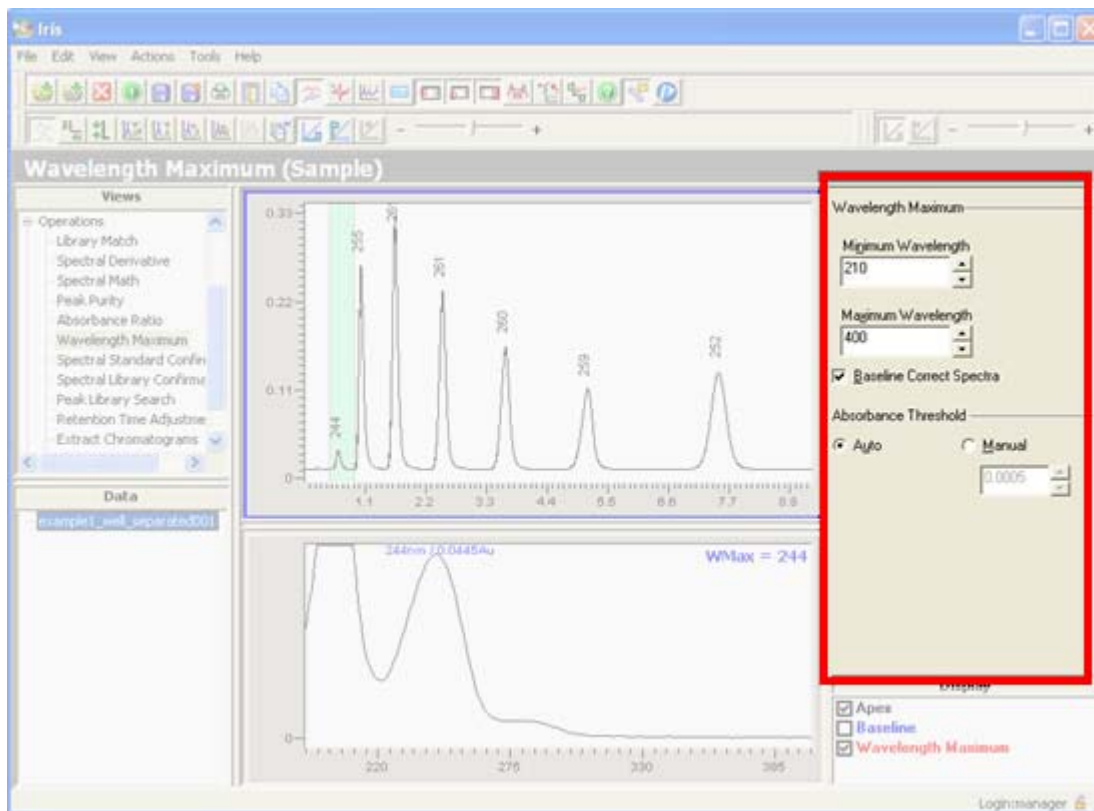
  Apex - Check this option to display the apex spectrum for the selected peak.

  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.
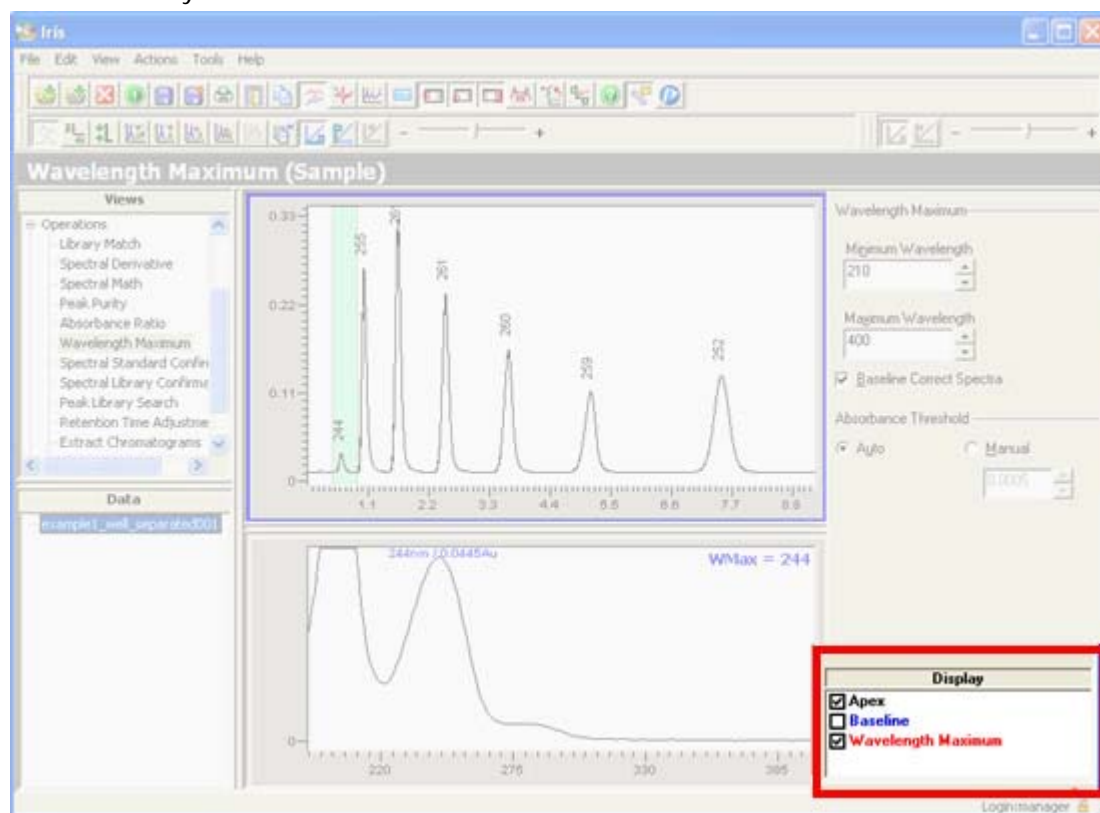
Note: *You can select **Actions > Add All to View** to add all selected spectra in the Display List to the Data Tree.*

- **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, this pane displays the Wavelength Maximum value for the selected peak in the upper right hand corner as **WMAX =**.
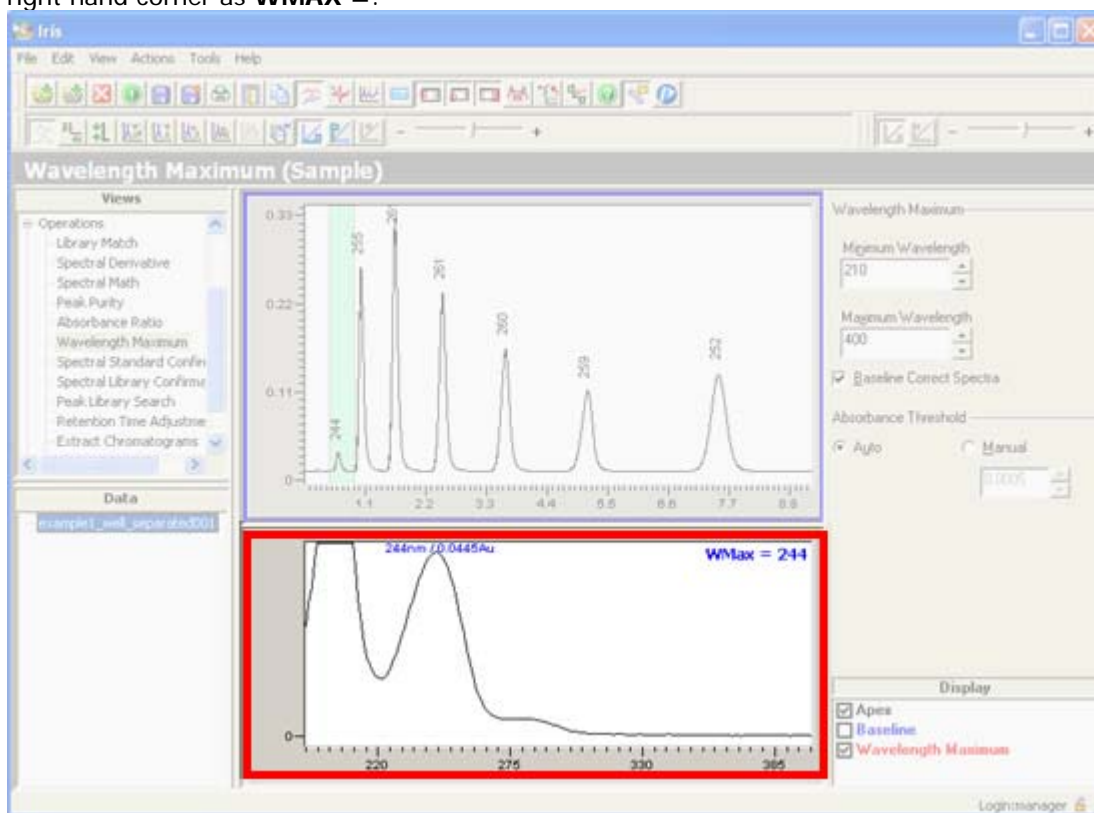
Note: *Whenever you select a different peak, change the parameters for the operation, or select/deselect an item on the Display List, the Results pane is automatically updated to reflect the change.*

How do I modify the parameters used that are used to determine the Wavelength Maximum values?

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 IRIS automatically updates the display 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 IRIS 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 greyed 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 operation.

To 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. 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, 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 Wavlength Maximum value is displayed.

To 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 Wavlength Maximum value, and optionally the baseline spectrum for the selected peak.

To examine the Wavelength Maximum values at different wavelengths:

➢ Enter a new value for the **Minimum Wavelength** and/or **Maximum Wavelength**.
    The Wavelength Maximum value labels are updated for every peak in the chromatogram.

To 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.

To 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.

To 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.

To 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 apear in the Data Tree.

To save the results and parameters used in the calculation:

1. Select **File > Save Results…**

   A save dialog appears that asks you to confirm whether or not you want to save the new values to the chromatogram's result file (.RST), and save the parameters that were used calculate the results to the associated spectral method file (.TSM).

2. To only save results for peaks that are currently identified (named) in TotalChrom select **Currently identified peaks**.

   OR

   To save results for all peaks, select **Include unidentified peaks**.

   Any unidentified peaks for which results have been calculated are added to the TotalChrom result file as Unknown 1, Unknown 2, etc. (Any unidentified peaks which do not have results are not added to the file.)

3. Click **Yes** to save the results and calculation parameters.

   The Wavlength Maximum values are added to the result file for all identified peaks.

To display the Wavelength Maximum results in the Main or Chrom/Spectra view:

1. Select **Actions > Label Chromatograms…**

2. Select **Wavelength Maximum** from the list of available labels.

To 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 Standard Confirmation

The **Spectral Standard Confirmation** operation allows you to verify the identity of named peaks in a sample chromatogram by comparing the apex spectrum of each peak in the sample to the apex spectrum of an identically named peak in a standard chromatogram. The comparison is made using the **Absorbance Index test**.

The Absorbance Index is a numerical measure of the similarity of two spectra. The value is calculated by dividing the two spectra and then dividing the maximum value in the resulting plot by the minimum value. If the two spectra are identical this quotient, the Absorbance Index, is 1.00. An AI value above 1.00 implies that the two spectra are different, however, because of noise and other variables, two spectra are usually considered to match well if the AI value is between 1.00 to 1.50; this same test is used in the Peak Purity calculation.

➢ To access this operation, expand the **Operations** node on the **Views** tree and select **Spectral Standard Confirmation**.

To perform this operation you must have a sample chromatogram selected from the Data Tree as well as have specified a standard chromatogram. If this is the first time you are performing Spectral Standard Confirmation on a chromatogram, IRIS automatically prompts you to select a Standard Chromatogram upon accessing this operation. The sample chromatogram is displayed in the upper pane, and standard chromatogram is displayed directly below the sample chromatogram.
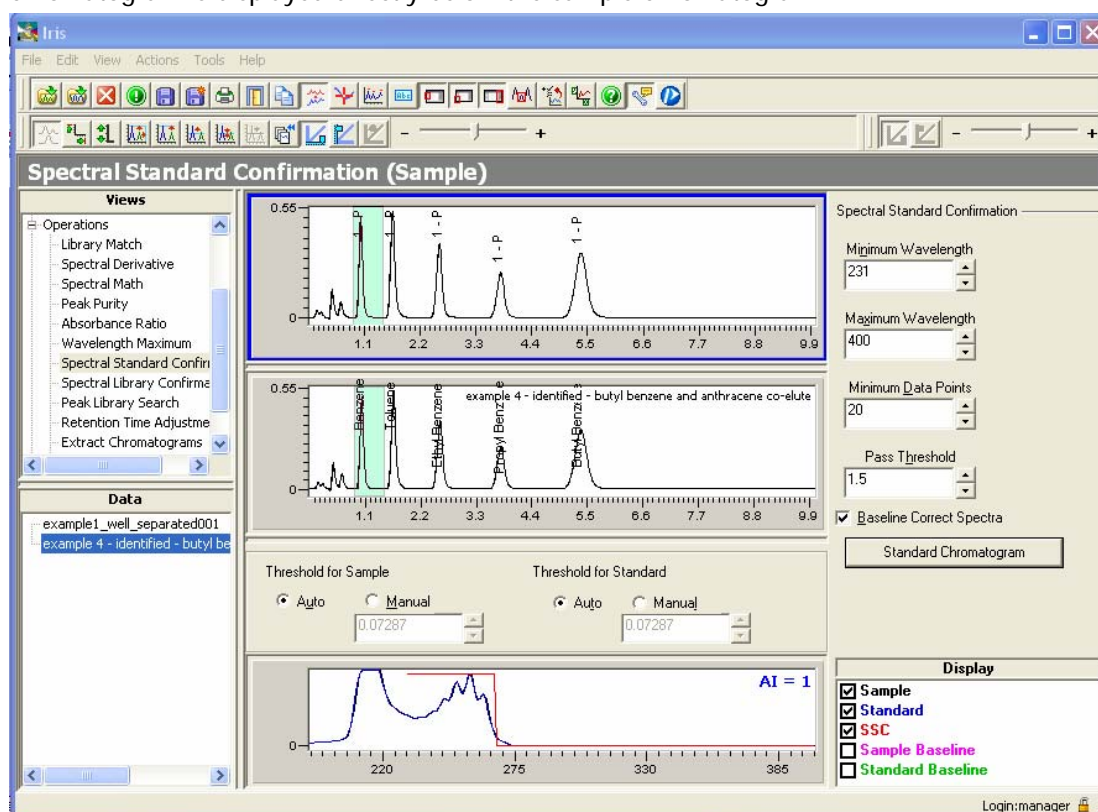
Figure 8-4 The Spectral Standard Confirmation

Verifying the Identity of Chromatographic Peaks by Comparing to a Standard Chromatogram

Because Spectral Standard Confirmation identifies the corresponding peak in the standard chromatograms by its component name, this function applies only to named peaks. If the chromatograms were run using different TotalChrom methods, peak names may not exactly match. IRIS warns you when this is the case.

If you apply the Spectral Standard Confirmation function to a chromatogram with no identified peaks, an error message is displayed, and you will not be able to confirm the identity of any peaks, until you select a new chromatogram that has identified peaks.

It should also be noted that the Spectral Standard Confirmation operation ignores any unnamed peaks in the chromatogram; as well as ignores named peaks in the sample chromatogram that are not present in the standard chromatogram.

Tell me about the Spectral Standard Confirmation operation.

The Spectral Standard Confirmation operation is comprised of the following areas:

- **Views Tree** - The Views Tree provides you access to the Spectral Standard Confirmation operation. To access this operation, expand the **Operations** node and click on **Spectral Standard Confirmation**.
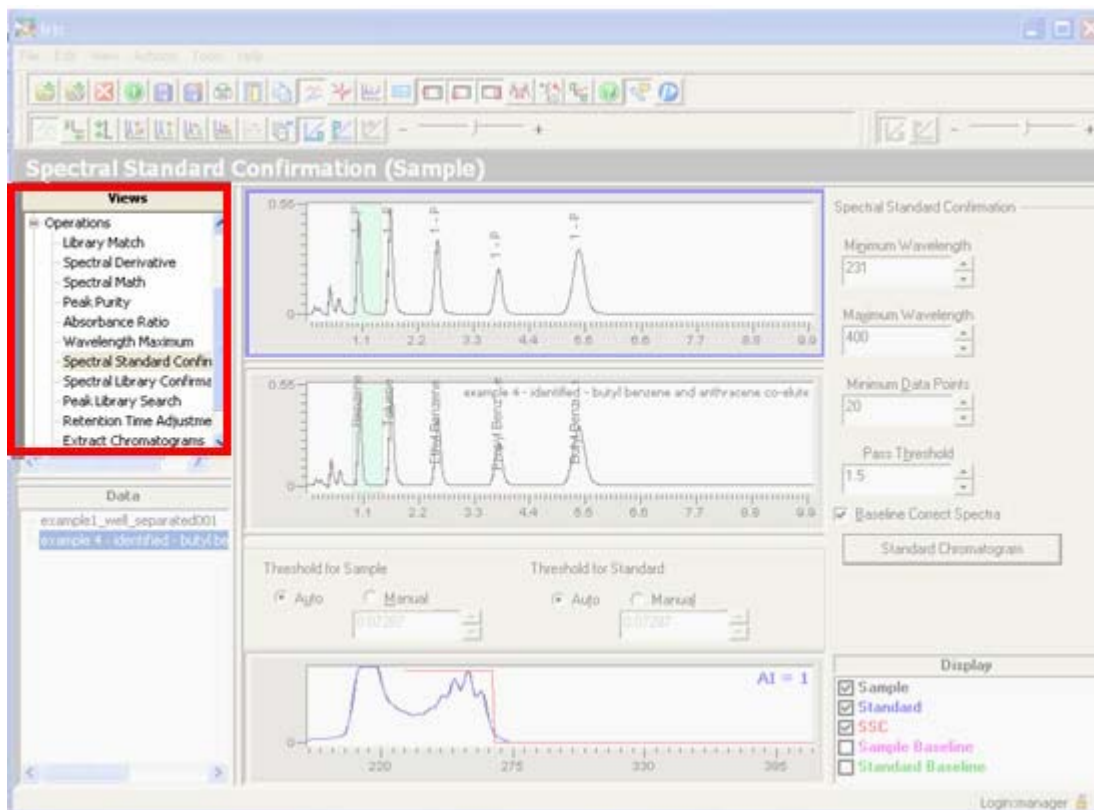
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.
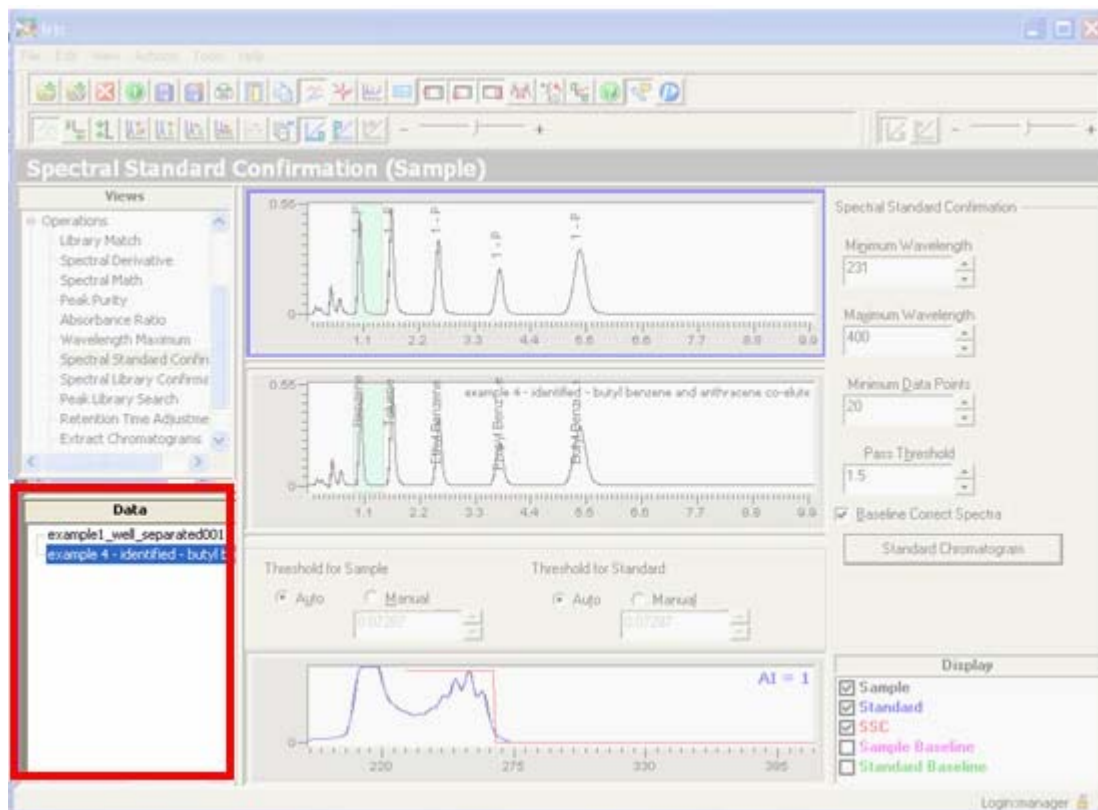
Note: *If the selected chromatogram has no named peaks, then a message is displayed on the Chromatogram pane that states:* "**Selected chromatogram has no named peaks"** *and the Standard Chromatogram pane and Results pane are blank.*

- **Sample Chromatogram Pane** – This pane displays the chromatogram that you selected from the Data Tree. When a chromatogram is first displayed on this pane, the first peak is selected and appears highlighted in pale green.

  You can select any named peak on the chromatogram by clicking on the peak. When you select a named peak on this pane, the peak with the same name is selected on the **Standard Chromatogram** pane and vice-versa. If the selected peak in the sample chromatogram has no equivalent in the standard chromatogram the message **No corresponding component in standard** is displayed on the Result pane.
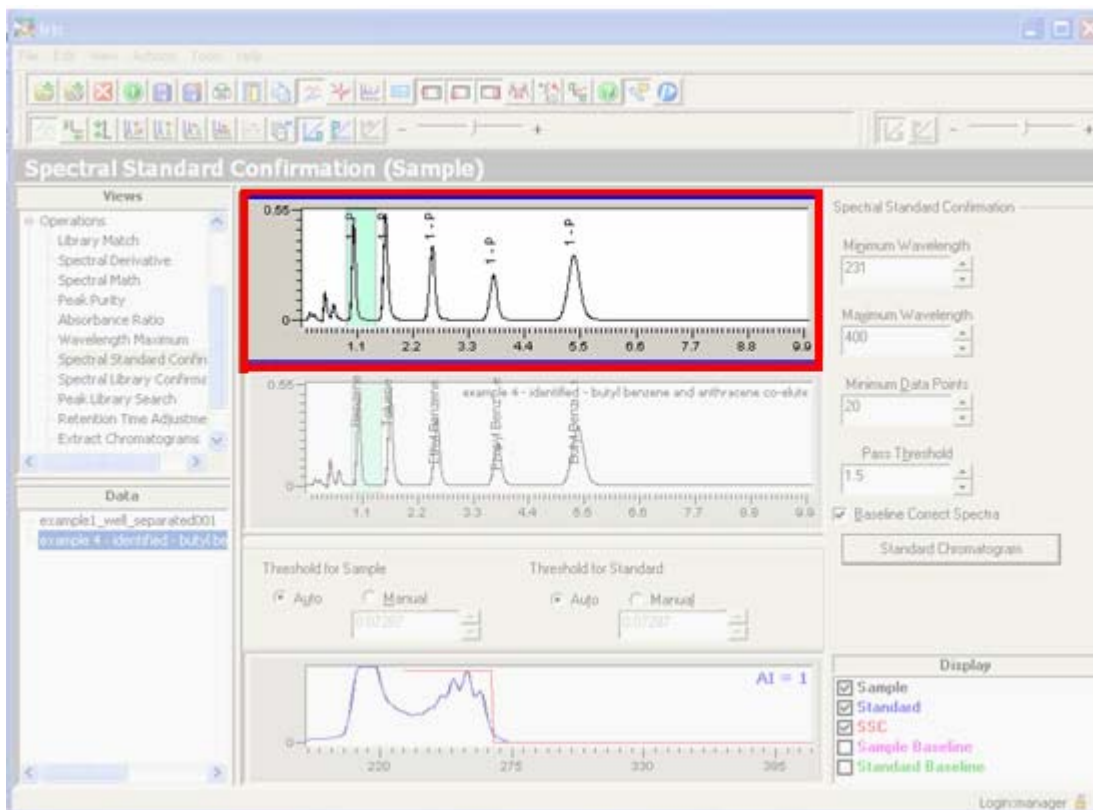
- **Standard Chromatogram Pane** - This pane displays the standard chromatogram that you have selected. When the standard chromatogram is first displayed, the peak matching the peak selected in the sample chromatogram is automatically selected. You can select any other identified peak in the standard by clicking on it. The corresponding peak in the Sample is then automatically selected.

  If you want to change the chromatogram that is displayed on this pane, you can click on the **Standard Chromatogram** button, which is located on the Parameters pane for this operation, and select a different standard chromatogram from the dialog box that appears.

  If you open a standard chromatogram with a method that does not match the sample chromatogram, a warning message is displayed that states "*Sample and Standard chromatogram methods do not match.*" Meanwhile, if the selected peak in the standard chromatogram has no equivalent in the sample chromatogram the message is displayed: *"No corresponding component in sample"* on the Results pane.
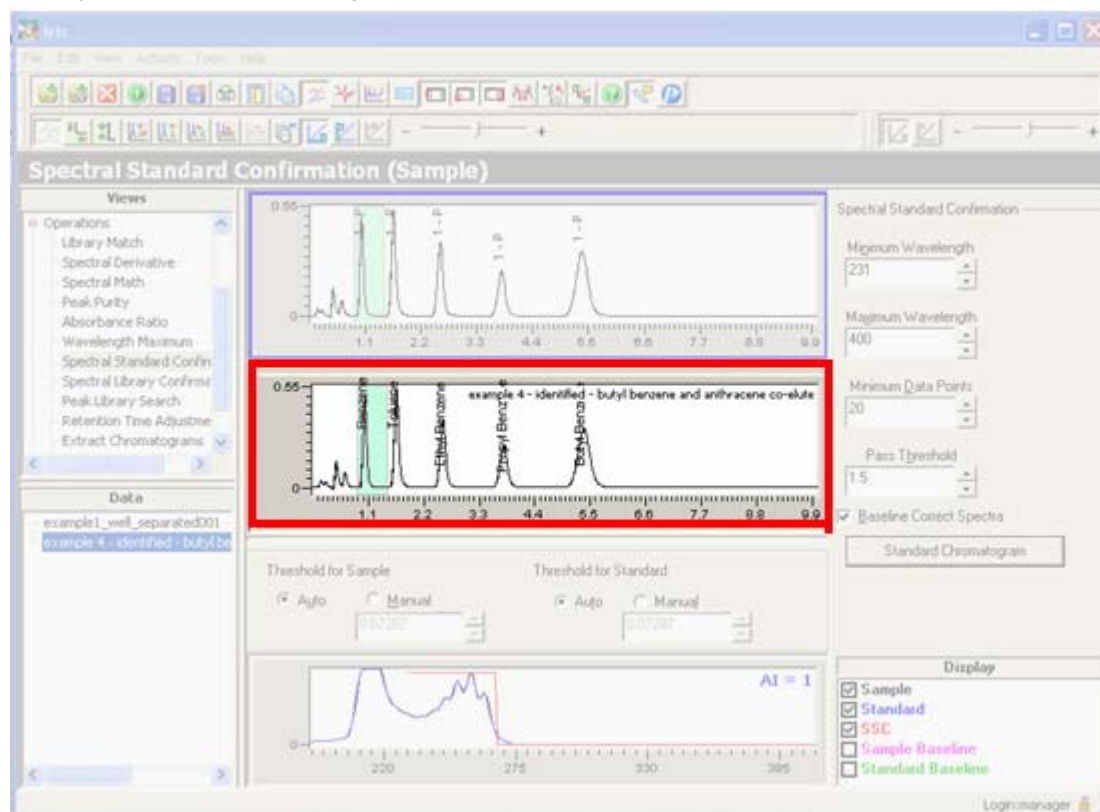
- **Parameters Pane** – This pane is where you set and investigate the various parameters used to perform the Spectral Standard Confirmation operation. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, IRIS automatically updates the Display List and Results pane with the modified results.
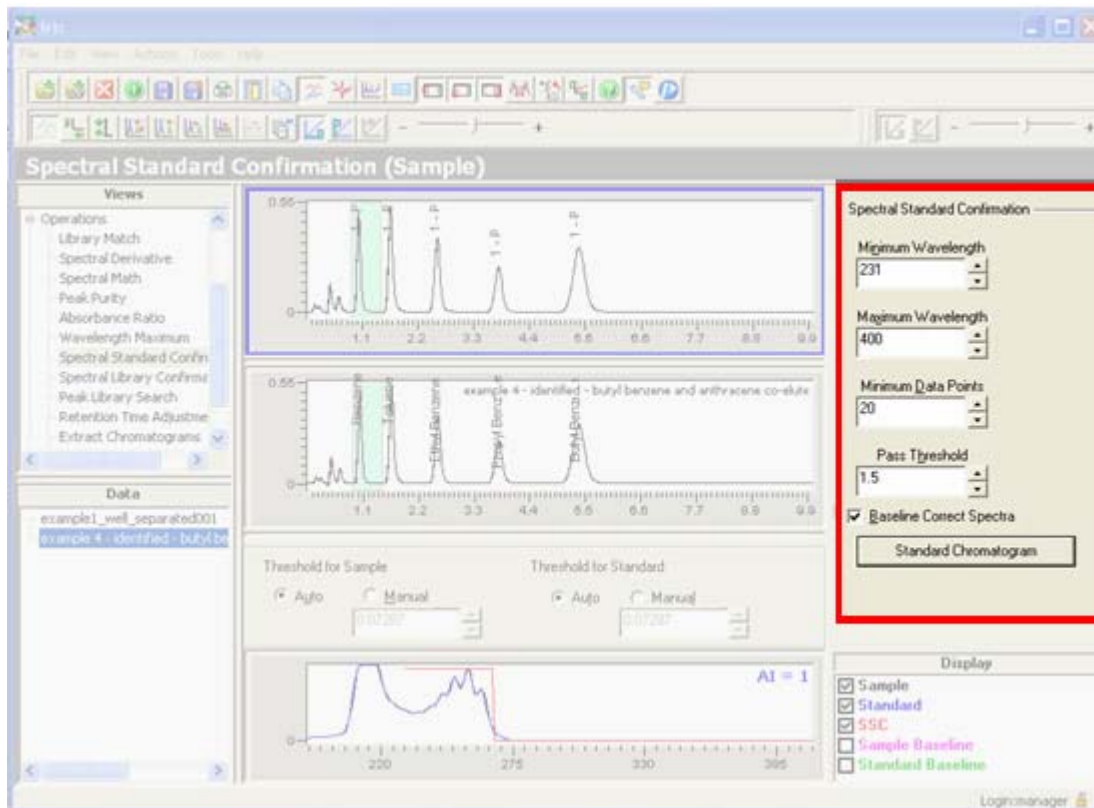
- **Display List** - You use the Display List to select the information you want displayed on the Results pane. When items appear checked on this list, they are displayed in the Results Pane. Meanwhile, unchecked items are not displayed.

  The following items are available for selection on the Display List:

  Sample spectrum - Check this item to display the spectrum from the selected peak on the sample chromatogram.

  Standard Spectrum – Check this item to display the spectrum from the selected peak on the standard chromatogram.

  Sample Baseline/Standard Baseline – Check either of these items to display the baseline spectrum associated with each chromatographic peak.

  SCC - Check this item to display the result plot, which is the line calculated by dividing the sample and standard spectra.
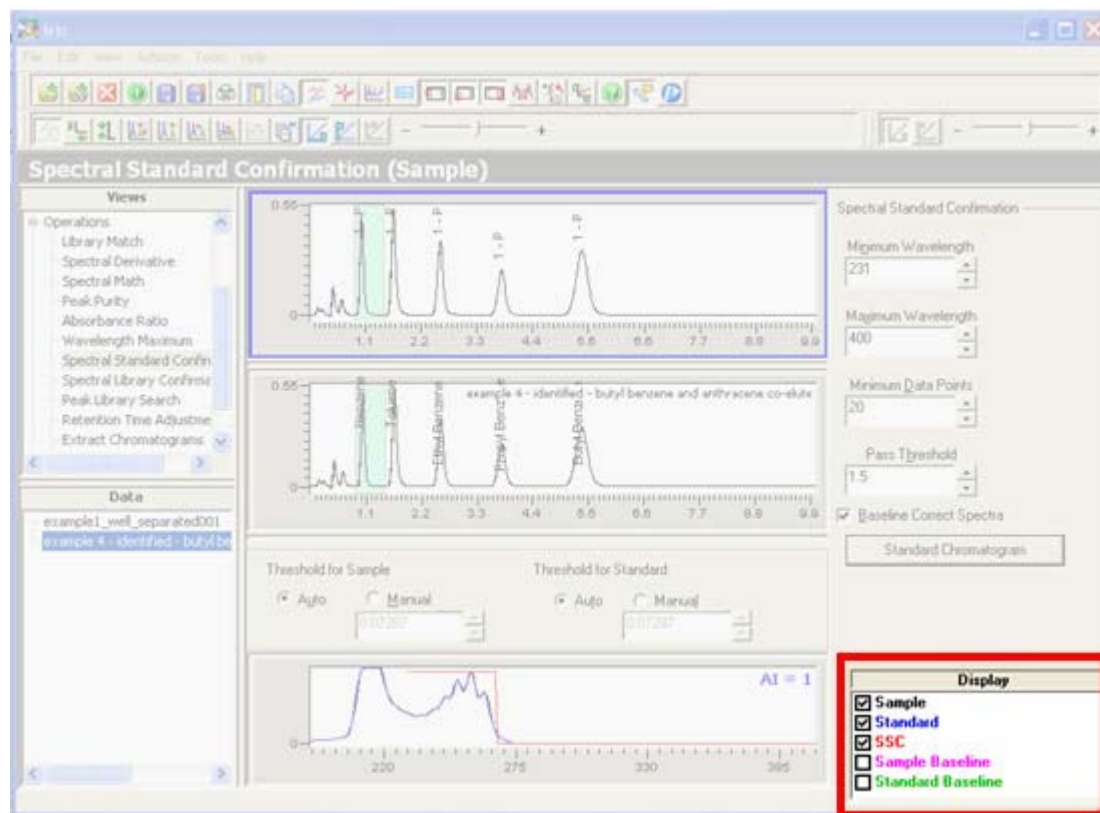
Note: You can select **Actions > Add All to View** to add all selected spectra in the Display list to the Data Tree.

- **Results Pane** - This pane displays a graphical representation of the items that are checked on the Display List. If the selected peak on the sample chromatogram matches the selected peak on the standard chromatogram, then the Results pane also displays the Absorbance Index. By default, the Absorbance Index is displayed in the upper right hand corner of the pane as **AI =**.
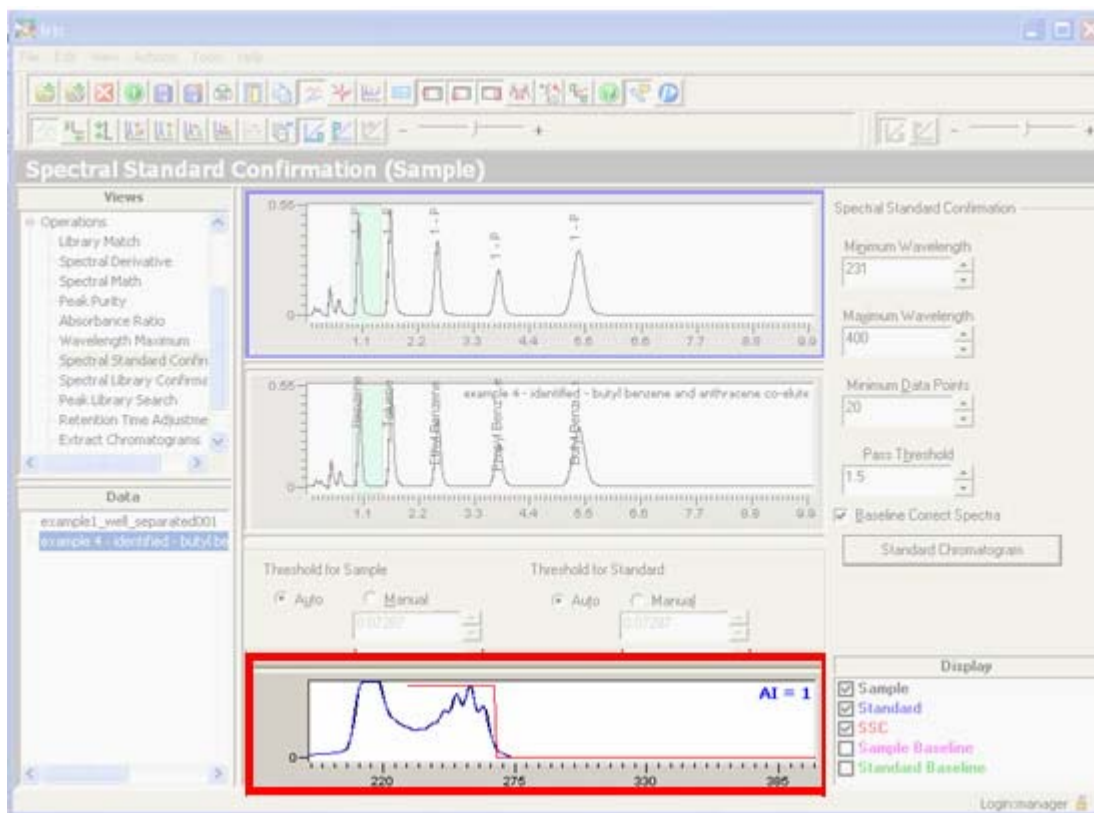
Note: *Whenever you select a different peak, change the parameters for the operation, or select/deselect an item on the Display List, the Results pane is automatically updated to reflect the change.*

How do I modify the parameters that are used to perform the Spectral Standard Confirmation operation?

The Parameters pane displays the values that are currently used in the operation. At any time you can modify the parameters that are listed on the Spectral Standard Confirmation operation; and IRIS automatically updates the display with the new results.

Below is a list of the parameters you can modify:

Parameter	Description
Minimum Wavelength	Specify the minimum wavelength to be used.
Maximum Wavelength	Specify the maximum wavelength to be used.
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, use the **Minimum Data Points** spin box to set the minimum number of data points that must be present for the result to be valid. If the number of data points calculated is less than the minimum set, then the results are considered invalid and the absorbance index result is shown as 0.
Pass Threshold	Use this spin box 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. If the calculated absorbance index result is greater than the threshold set here then the test is failed and the AI result is shown in bold red.
Baseline correct spectra	Check this option to use the baseline corrected spectra in the calculation.
Standard Chromatogram	Click on this button to change the Standard Chromatogram that you want to use for the operation.

Parameter	Description
Absorbance Threshold	To ensure that IRIS determines performs Spectral Standard Confirmation on 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 greyed 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: Spectral Standard Confirmation

The summary below shows you how to perform the Spectral Standard Confirmation operation on a chromatogram and then save the results.

To confirm the identity of peaks by comparing to a standard chromatogram:

1. From the Views tree select **Operations > Spectral Standard Confirmation**.

   The Spectral Standard Confirmation operation displays.

2. From the Data Tree click on the chromatogram that contains the named peaks that you want to verify against a standard chromatogram.

   The selected chromatogram appears on the Chromatogram pane with a vertical cursor that snaps to the nearest named peak.

Note: *If the selected Chromatogram has no named peaks, a message is displayed on the chromatogram graph stating: "**Selected chromatogram has no named peaks**", and the Standard Chromatogram pane and Result pane are blank.*

3. Select a standard chromatogram from the **Select a Standard Chromatogram** dialog box that automatically appears if you have not already performed spectral standard confirmation on the selected sample.

   OR

   If this dialog does not automatically appear you can click on the **Standard Chromatogram** button, located on the Parameters pane, to select the standard chromatogram.

   Once you have selected a standard chromatogram, you are returned to the Spectral Standard Confirmation operation and the standard chromatogram appears on the Standard Chromatogram pane with a vertical cursor that snaps to the nearest peak that corresponds to the peak selected on the Sample Chromatogram pane.

   The results of the operation are displayed in the lower Results pane. In addition, the peaks in the sample chromatogram are labeled accordingly: peaks in the sample that correspond to a peak in the standard are labeled with the Absorbance Index value, while peaks in the sample chromatogram that do not have a corresponding peak in the standard are labeled with "**Not found**".

To view the Spectral Standard Confirmation plot for a different peak:

➢ Select the required peak by clicking on it in the sample chromatogram.

   The Results pane is updated to show the peak apex spectrum for the peak selected on the sample chromatogram and the peak apex spectrum for the peak selected on the standard chromatogram, the Spectral Standard Confirmation plot, the Absorbance Index value, and optionally the baseline spectrum for the selected peaks on the sample chromatogram and standard chromatogram.

To examine the Spectral Standard Confirmation values at different wavelengths:

➢ Enter a new value for **Minimum Wavelength** and/or **Maximum Wavelength**.

The Spectral Standard Confirmation value labels are updated for every peak in the chromatogram.

To examine the effect of using baseline corrected spectra on the Spectral Standard Confirmation values:

➢ Check **Baseline Correct Spectra**.

The Spectral Standard Confirmation value labels are updated for every peak in the chromatogram.

To change the minimum absorbance values that are used in the operation:

1. Select the **Manual** radio button under **Threshold for Sample**.

The Minimum Absorbance spin box is enabled.

2. Use the spin box to specify a new minimum absorbance value.

The Spectral Standard Confirmation value labels are updated for every peak in the chromatogram.

3. Select the **Manual** radio button under **Threshold for Standard**.

The Minimum Absorbance spin box is enabled.

4. Use the spin box to specify a new minimum absorbance value.

The Spectral Standard Confirmation value labels are updated for every peak in the chromatogram.

To return to automatically calculating the minimum absorbance value:

➢ Select the **Auto** radio button, under **Threshold for Sample** and/or **Threshold for Standard** if you want to have IRIS automatically calculate the minimum absorbance value that can be used in the calculation.

The Spectral Standard confirmation value labels are updated for every peak in the chromatogram.

To 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 apear in the Data Tree.

To save the results and parameters used in the calculation:

1. Select **File > Save Results...**
    A save dialog appears that asks you to confirm whether or not you want to save the new values to the chromatogram's result file (.RST), and save the parameters that were used calculate the results to the associated spectral method file (.TSM).

2. Click **Yes** to save the results and calculation parameters.
    Spectral Standard Confirmation values added to the result file.

To display the Spectral Standard Confirmation results in the Main View or Chrom/Spectra View:

1. Select **Actions > Label Chromatograms...**

2. Select **Spectral Standard Confirmation** from the list of available labels

To review for any chromatogram previously calculated Absorbance Ratio values and the parameters used:

➢   Select this chromatogram again on the Spectral Standard Confirmation operation.
    The saved results and the calculation parameters are automatically displayed.

Spectral Library Confirmation

The **Spectral Library Confirmation** operation allows you to verify the identity of named peaks in a sample chromatogram by automatically comparing the apex spectrum of each named peak in the sample to an identically named spectrum from a spectral library that you specify.

➢ To access this operation expand the **Operations** node on the **Views** tree and select **Spectral Library Confirmation**.
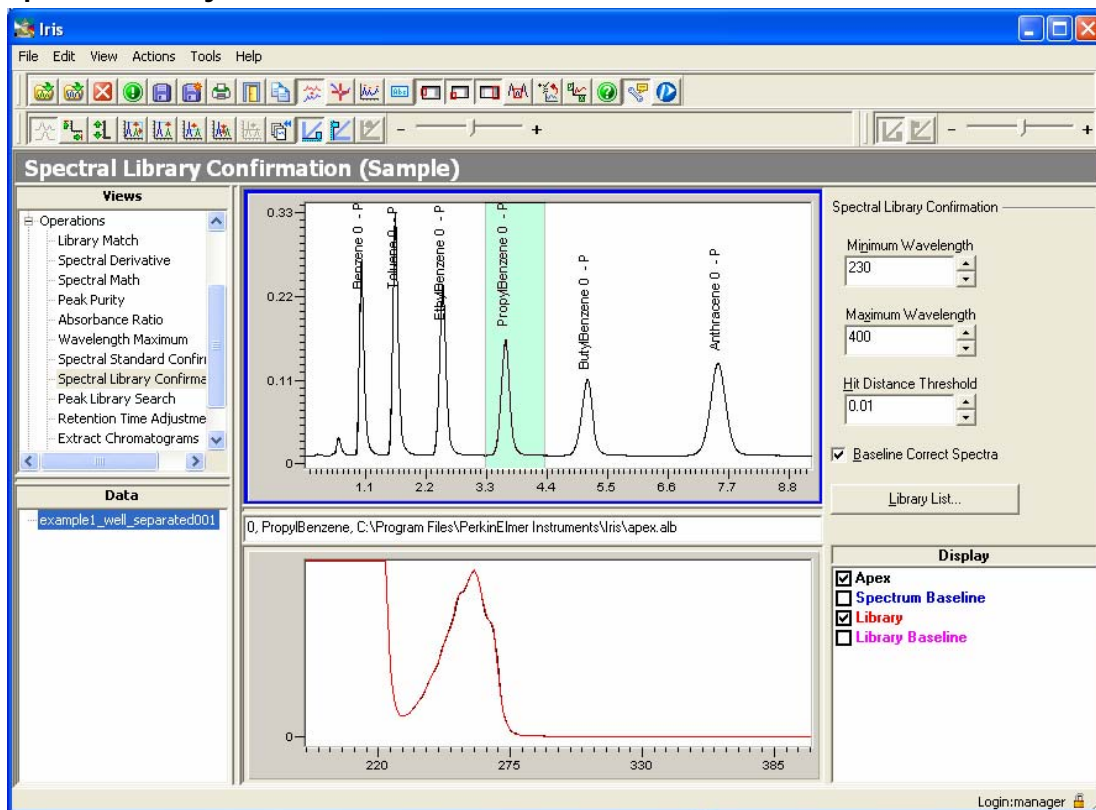
Figure 8-5 The Spectral Library Confirmation operation

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. IRIS 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, IRIS 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 IRIS 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. *For more information on the Euclidean distance algorithm, refer to* **Appendix 1**. 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. *For information on specifying a Hit Quality Threshold for the operation, refer to* **How do I modify the parameters that are used in** the search? *on page* **259**.

Tell me about the Spectral Library Confirmation operation.

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.
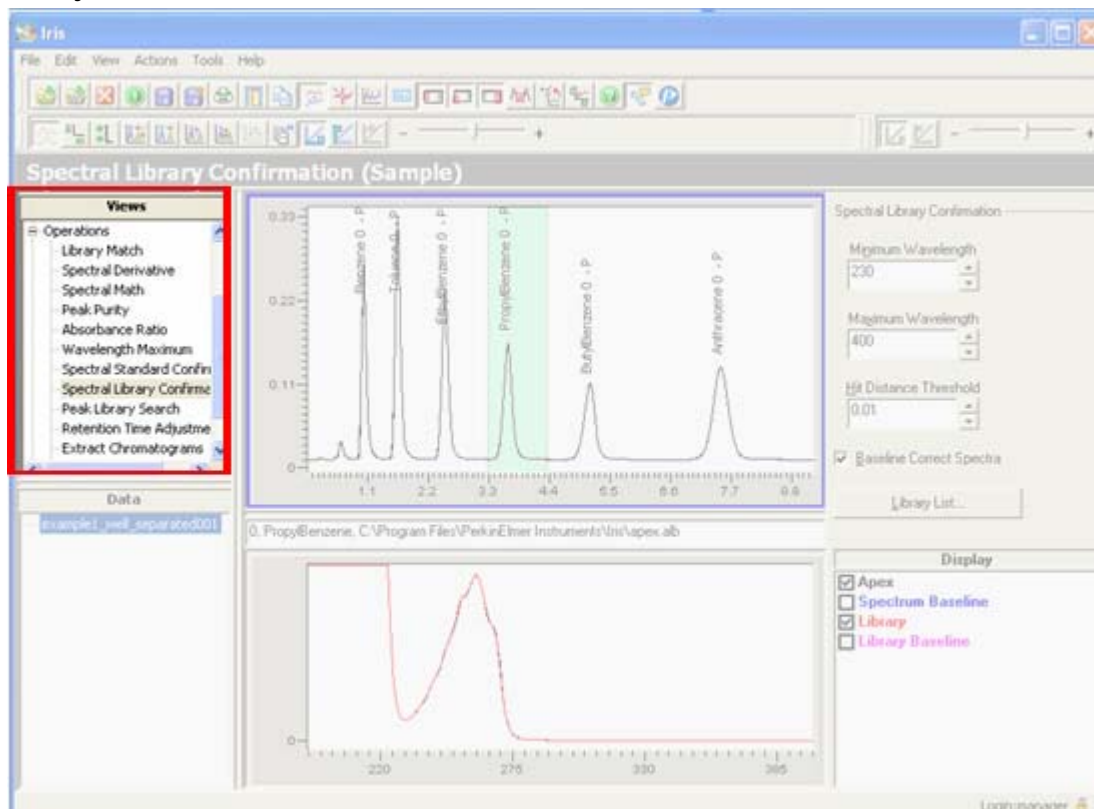
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.
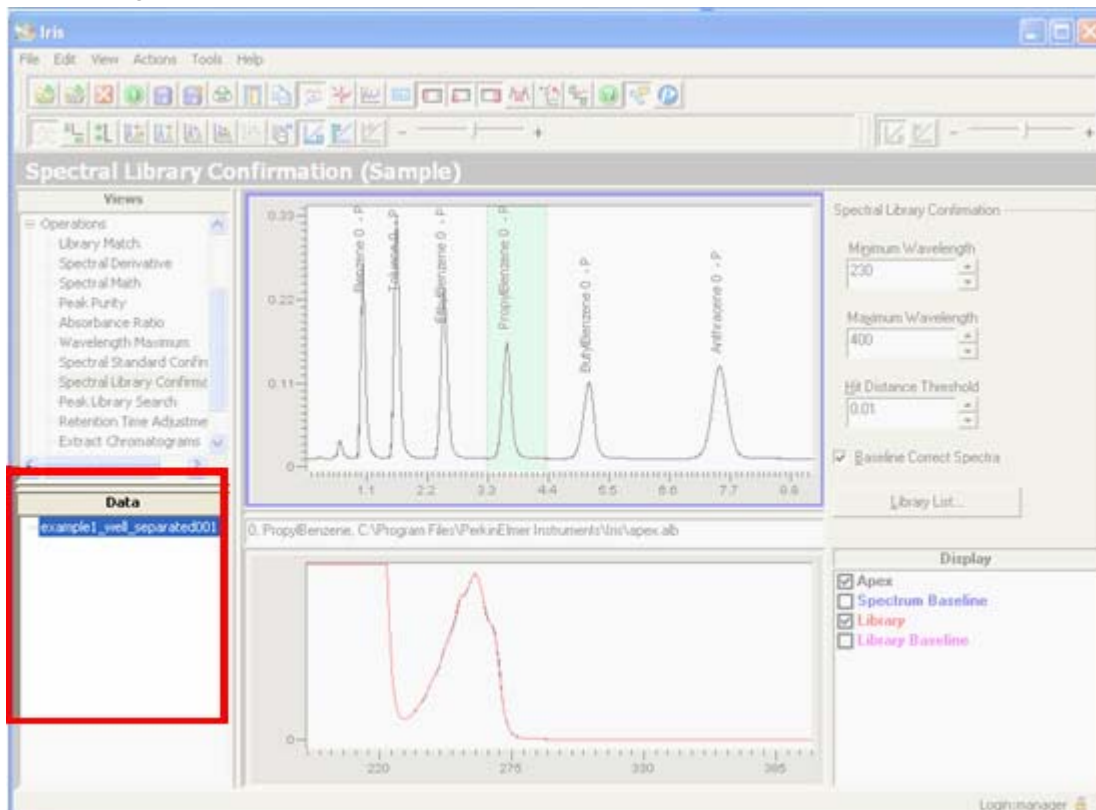
- **Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree. Named peaks in the displayed chromatogram are labelled with their Component Name, the **Hit Quality Value**, and either a P or an F to indicate a Pass or Fail status, which is dependent on the current value of the Hit Distance Threshold.
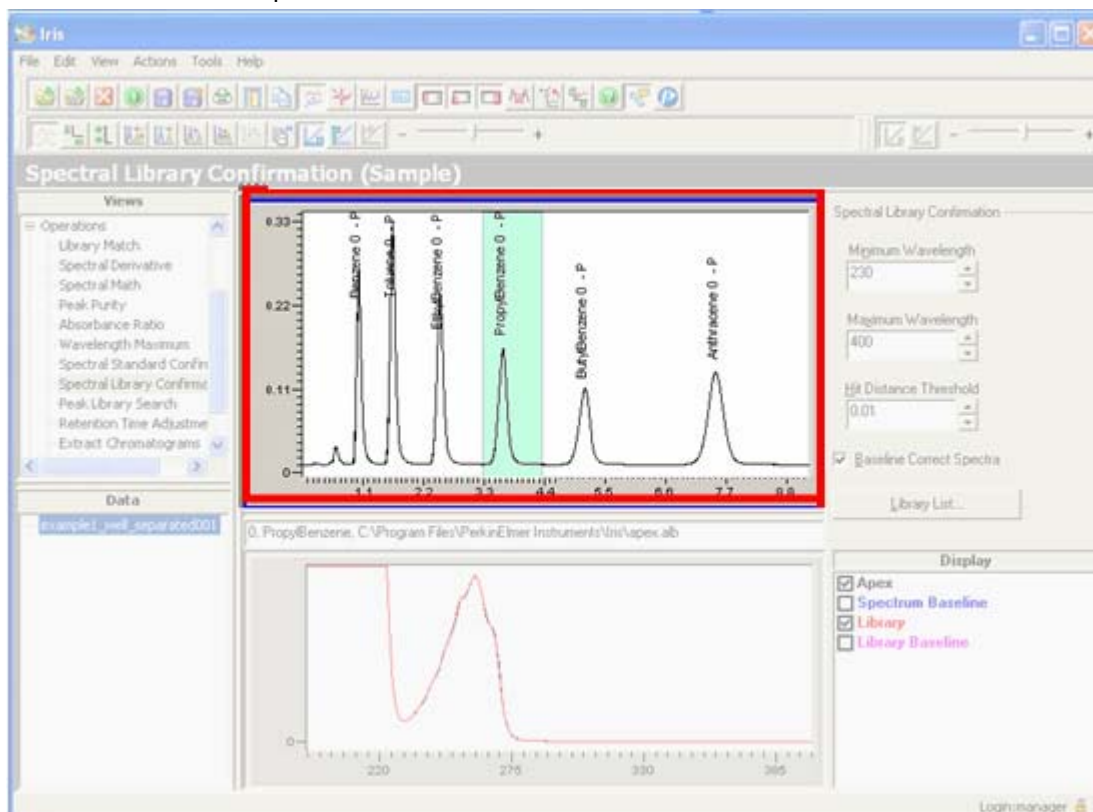
- **Parameters Pane** – This pane is where you set and investigate the various parameters used to perform the Spectral Library Confirmation operation. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, IRIS automatically updates the Display List and Results pane with the modified results.
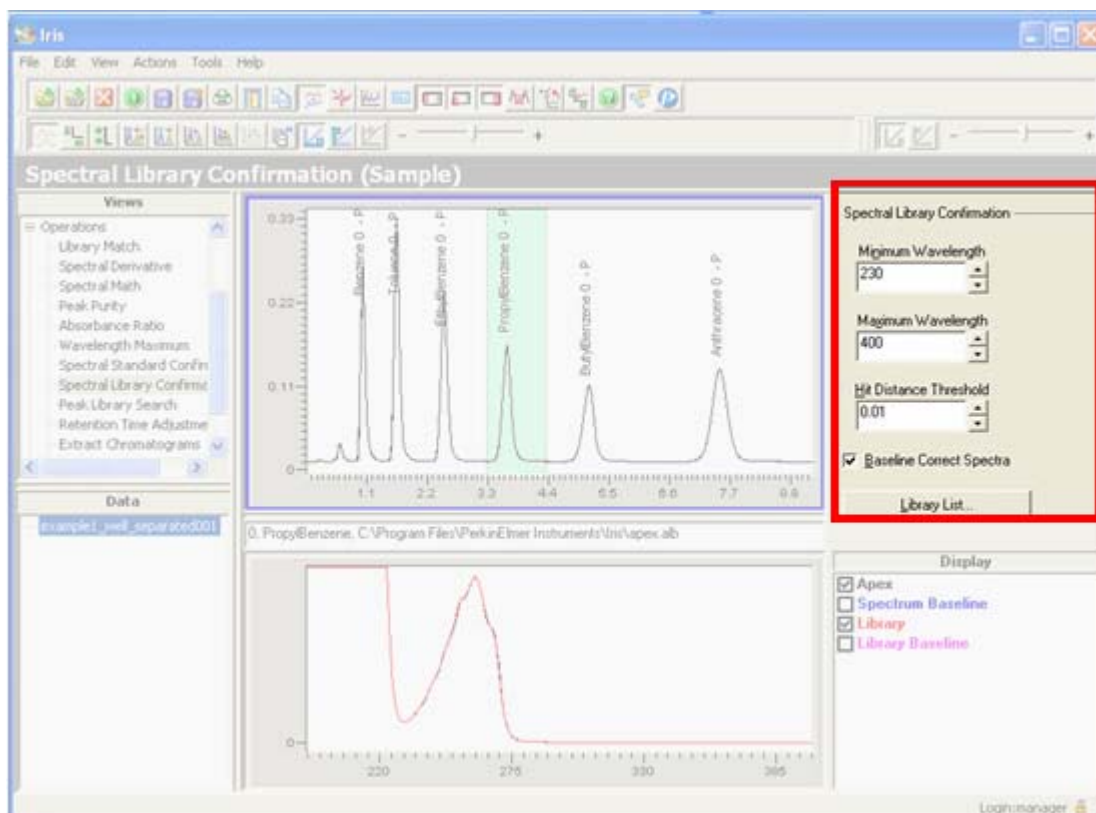
- **Hit Details** - The file name and path for the library spectrum that has the same name as the peak selected on the chromatogram, is displayed here.

  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:
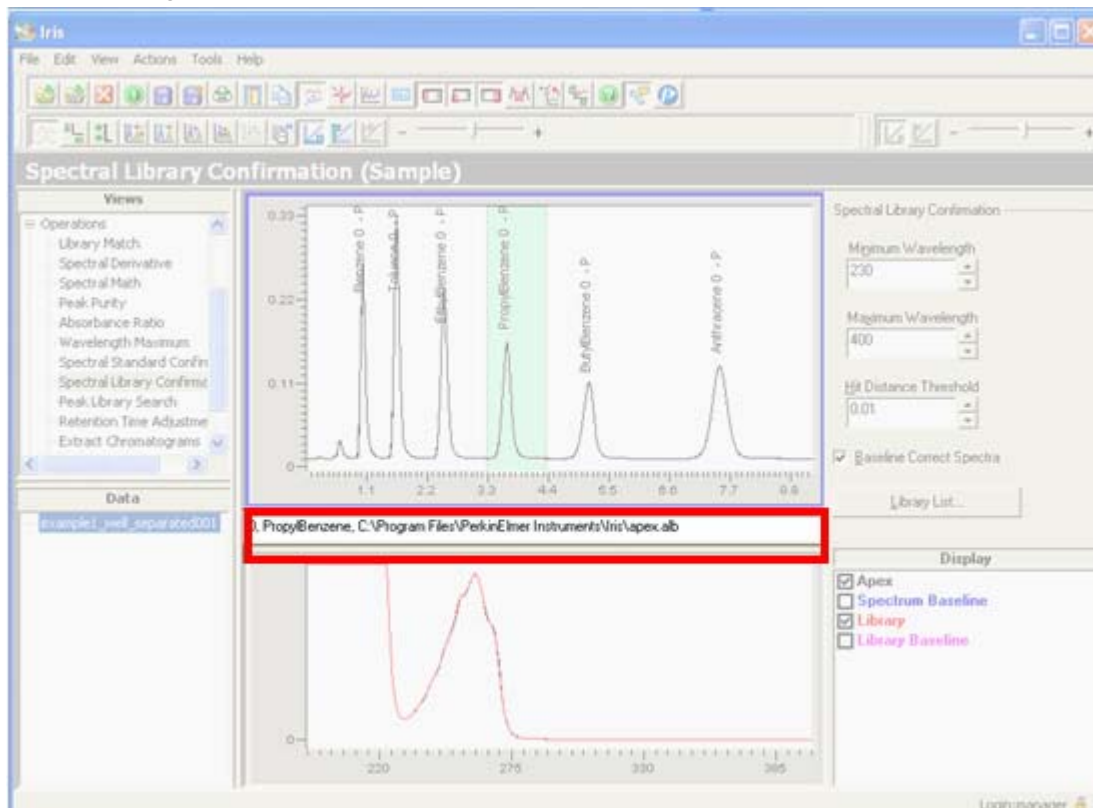
  Apex - Check this item to display the apex spectrum from the chromatogram on the Results pane.

  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.
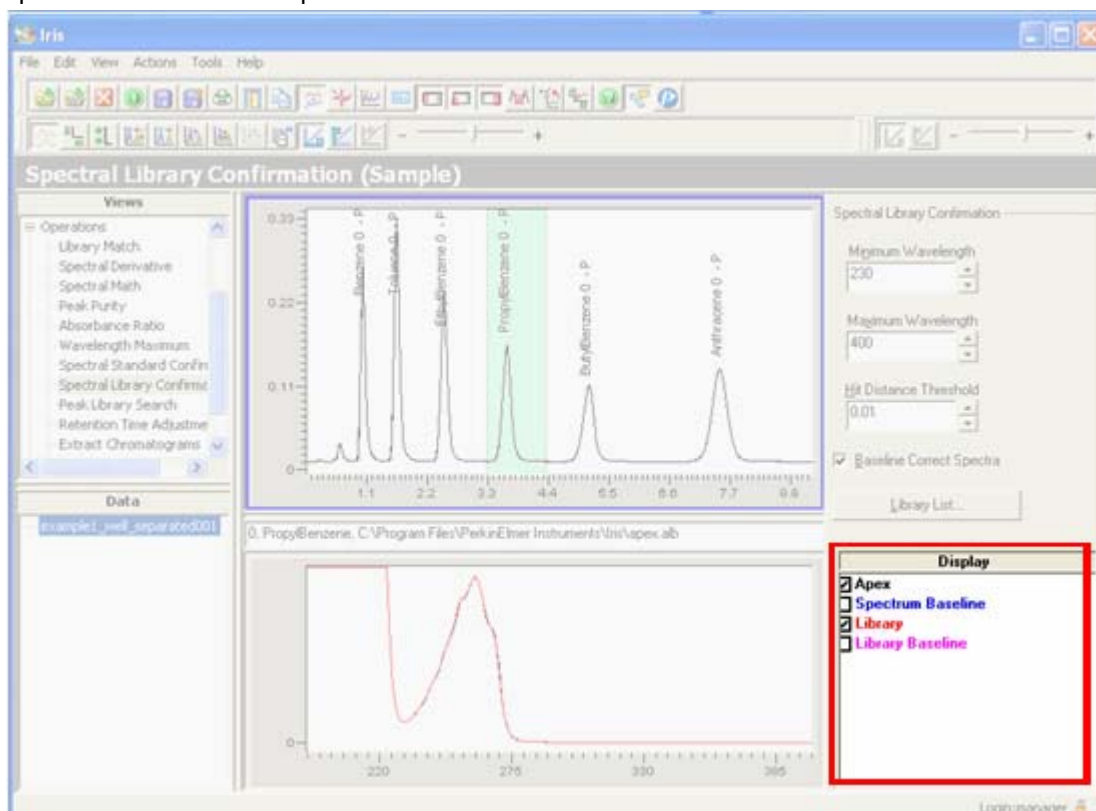
- **Results Pane** - This pane displays a graphical representation of the items that are checked on the Display List.
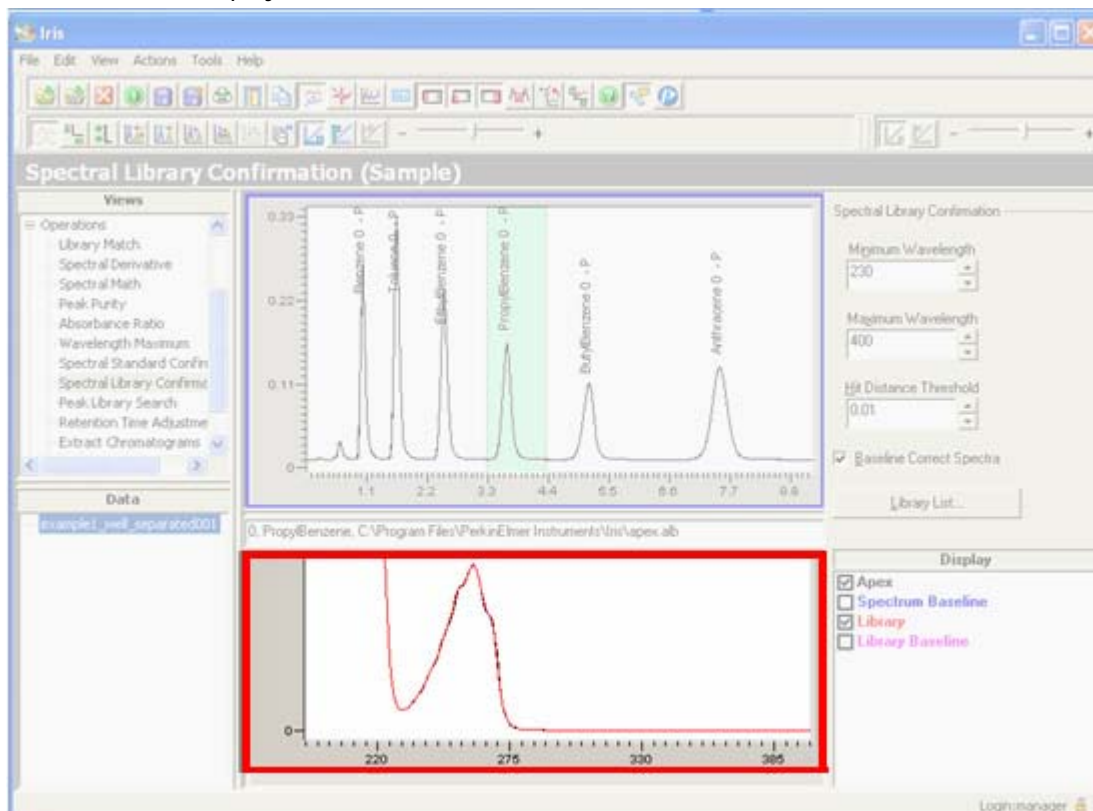
How do I modify the parameters that are used in the search?

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 IRIS automatically updates the display with the new results.

Below is a list of the parameters you can optimize/adjust:

Parameter	Description
Mi<u>n</u>imum Wavelength	Use the Minimum Wavelength spin box to set the lower limit of the spectrum range to be used in the calculation.
Ma<u>x</u>imum Wavelength	Use the Maximum Wavelength spin box to set upper limit of the spectrum range to be used in the calculation.
Hi<u>t</u> 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.
<u>B</u>aseline corrected	Check this option to use the baseline corrected spectra in the operation.
<u>L</u>ibrary list	Select this command button to display the Libraries List dialog from where you select the spectral libraries to search.

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.

To 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.

To 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.

To examine the Spectral Library Confirmation values at different wavelengths:

➢ Enter a new value for **Minimum Wavelength** and/or **Maximum Wavelength**.

   The Spectral Library Confirmation value labels are updated for every peak in the chromatogram.

To 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.

To change the spectral library/libraries that are used in the match:

1. Select 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 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.

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. Click **OK**.

6. You are returned to the Spectral Library Confirmation operation, and each peak that IRIS was able to match is labelled 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.

To 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.

To save the results and parameters used in the calculation:

1. Select **File > Save Results…**
    A save dialog appears that asks you to confirm whether or not you want to save the new values to the chromatogram's result file (.RST), and save the parameters that were used calculate the results to the associated spectral method file (.TSM).

2. Click **Yes** to save the results and calculation parameters.
    Spectral Library Confirmation values added to the result file and the parameter values are added to the chromatogram's process spectral method file and the chromatogram's spectral method file.

To 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

To 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 page expand the **Operations** node on the **Views** tree and select **Peak Library Search**.
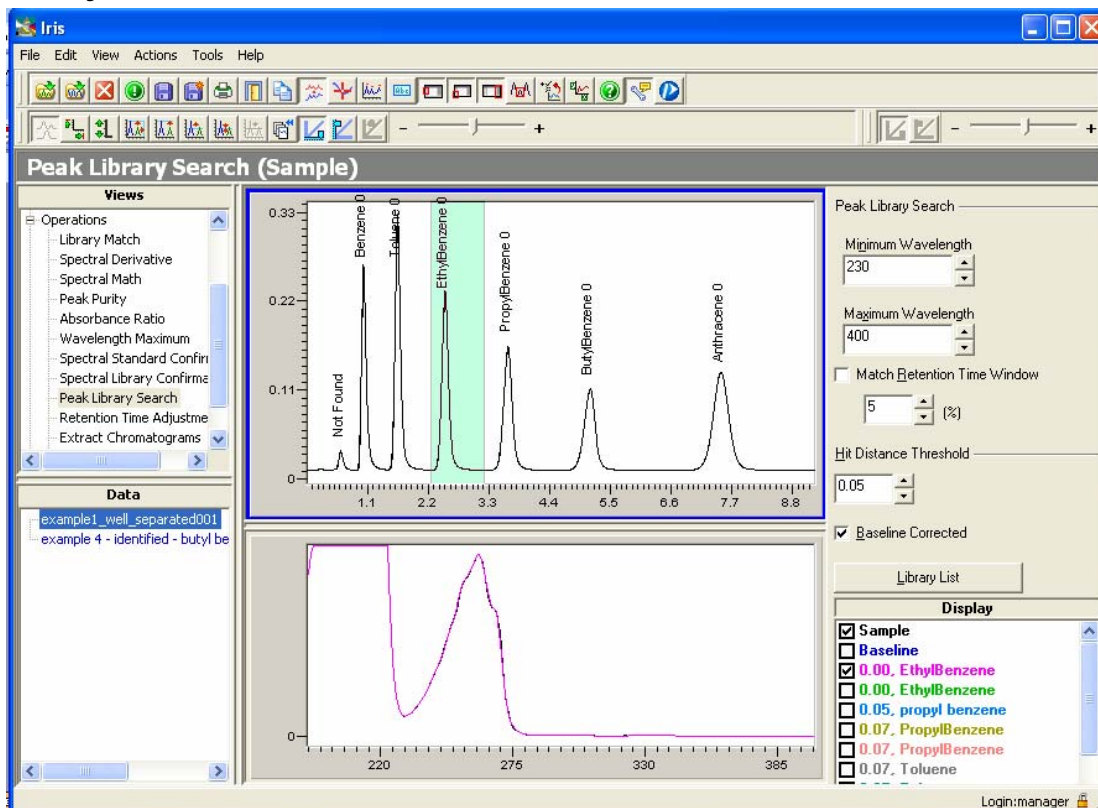
Figure 8-6 The Peak Library Search operation

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, IRIS 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, IRIS 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 IRIS could not find a match for a peak, 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 IRIS perform a Peak Library Search?

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.

Note: *For more information on the Euclidean Distance algorithm, refer to Appendix 1.*

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, IRIS 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, IRIS labels the sample peak 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 page by expanding the **Operations** node and selecting **Peak Library Search**.
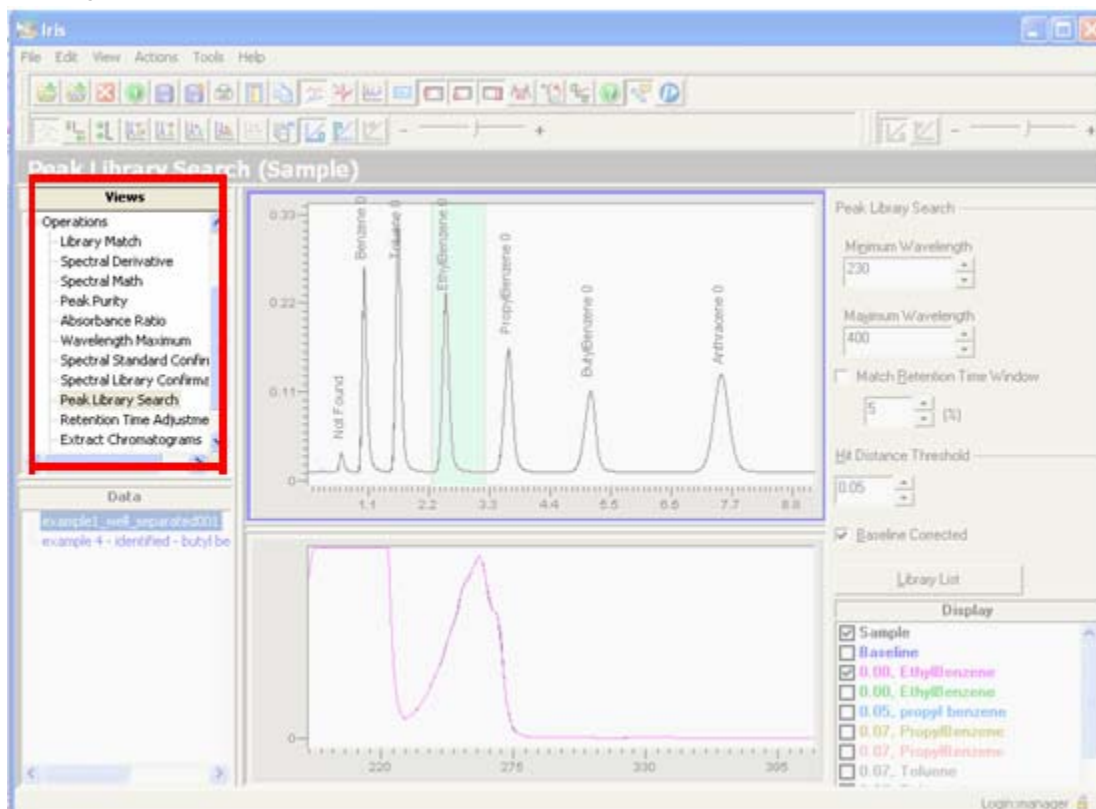
- **Data Tree** - The Data tree here shows only a list of chromatograms and is used to select the required chromatogram from the ones available. The selected chromatogram is displayed in the top graph (Chromatogram pane).

  As different chromatograms are selected the nearest peak is selected, the other two graphs are updated and chromatogram labels are updated.
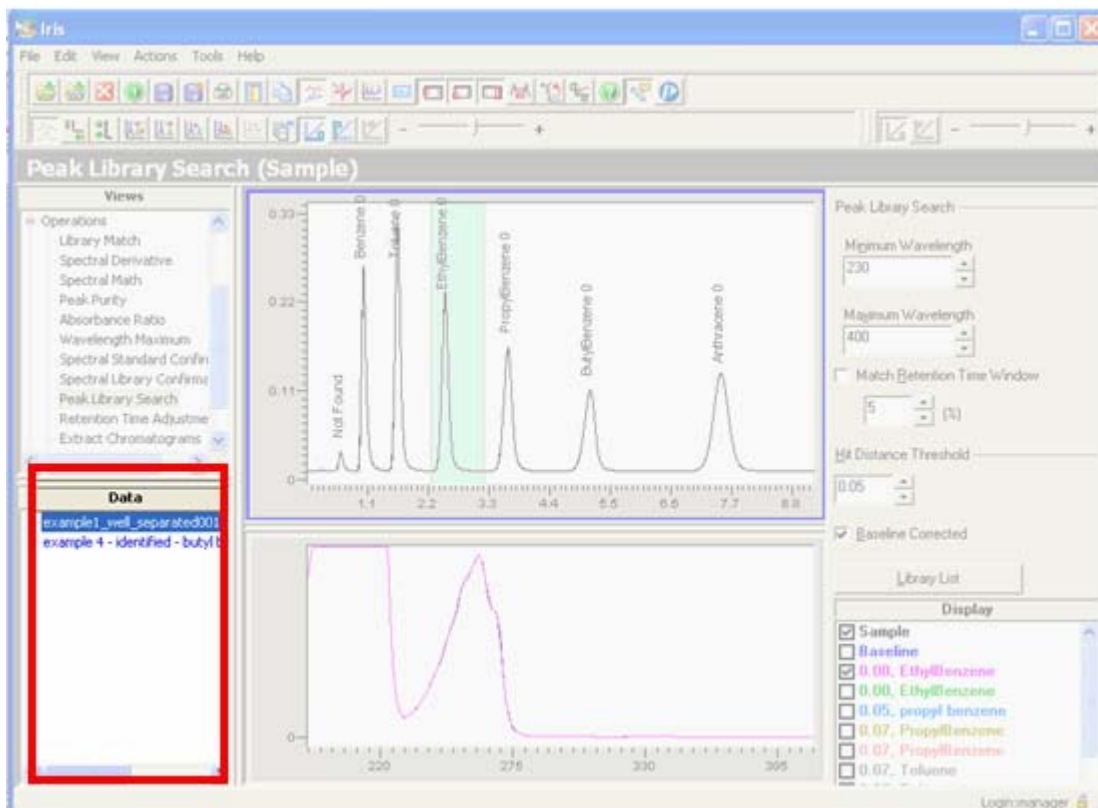
- **Chromatogram Pane** - When a chromatogram is selected on the Data Tree, the chromatogram is displayed on this pane and the peak maxima are labelled with the top library hit name, as calculated by the current settings or with "*Not found*" when IRIS was unable to find a match for the peak. The labels 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.
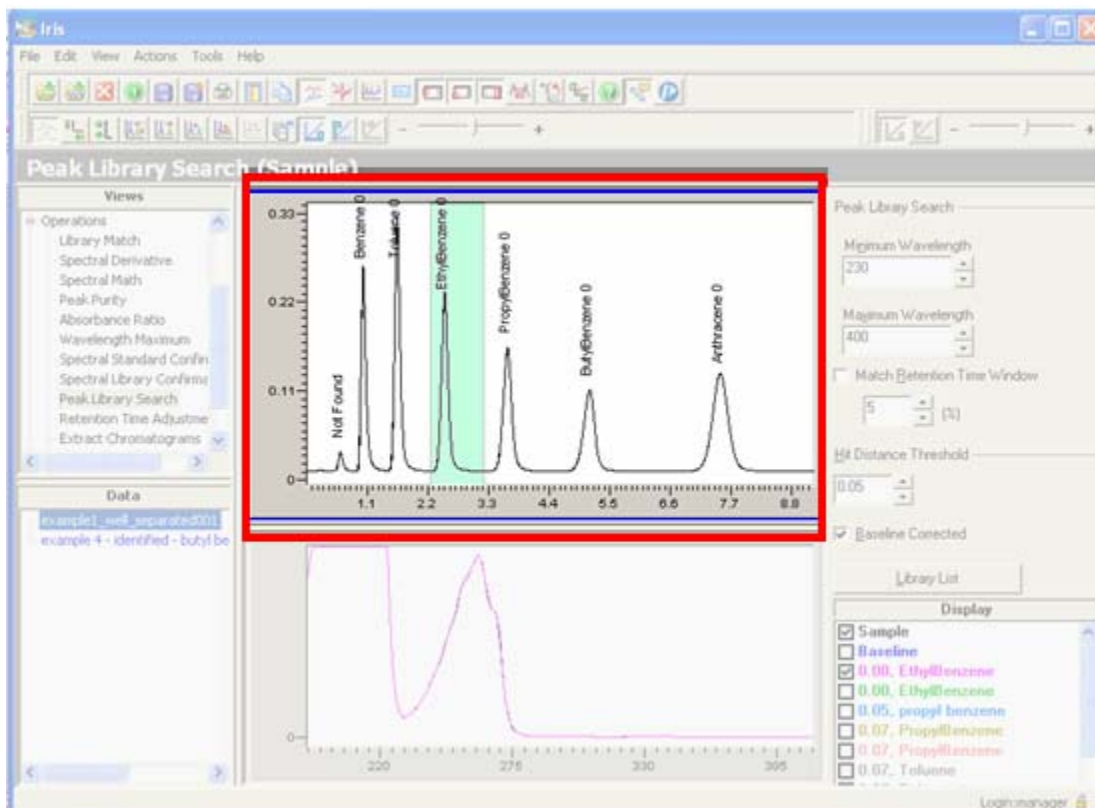
- **Parameters Pane** - From this pane you can change the minimum and maximum wavelengths to be used for the search; you can also change the Hit Threshold value, which defines whether a valid match has been found, and you can specify whether retention time is to be used to restrict the search, as well as whether or not you wish to use baseline corrected spectra for the library match.
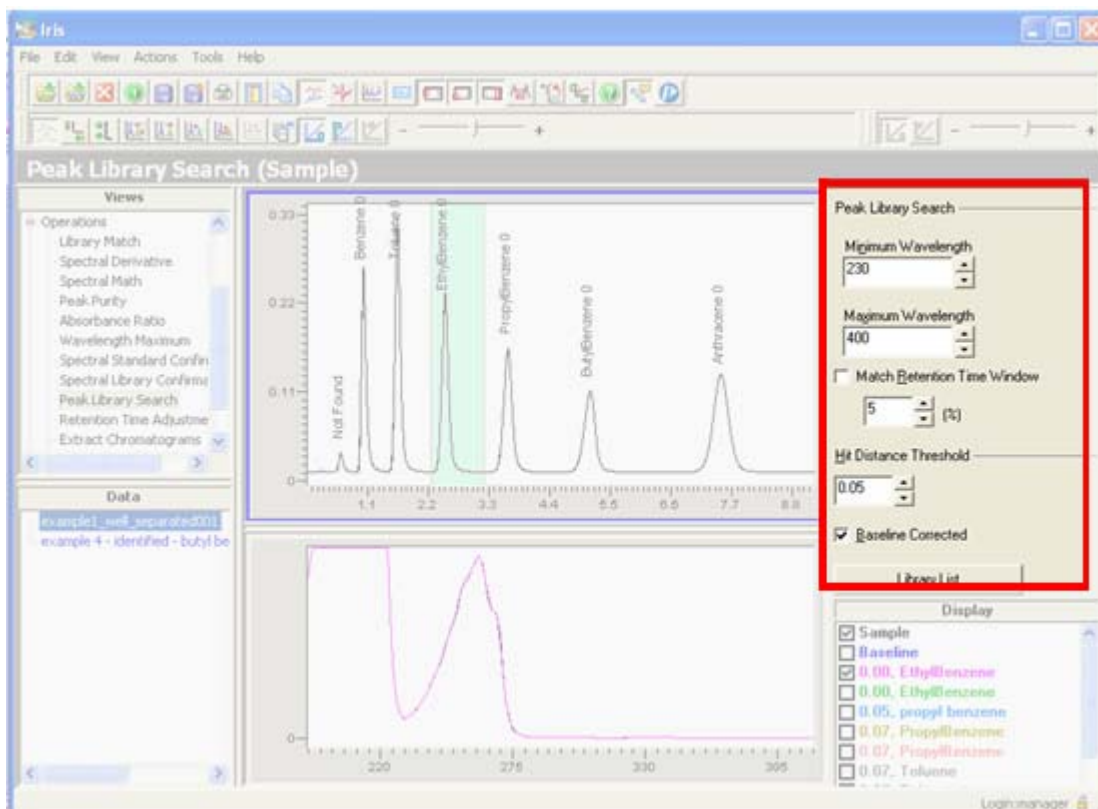
- **Hits List** - The Hits List contains a list of check boxes for selecting what is displayed on the Results Pane. Items that appear checked on this list are displayed in the Results pane; unchecked items are not displayed in the Results pane.

  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.
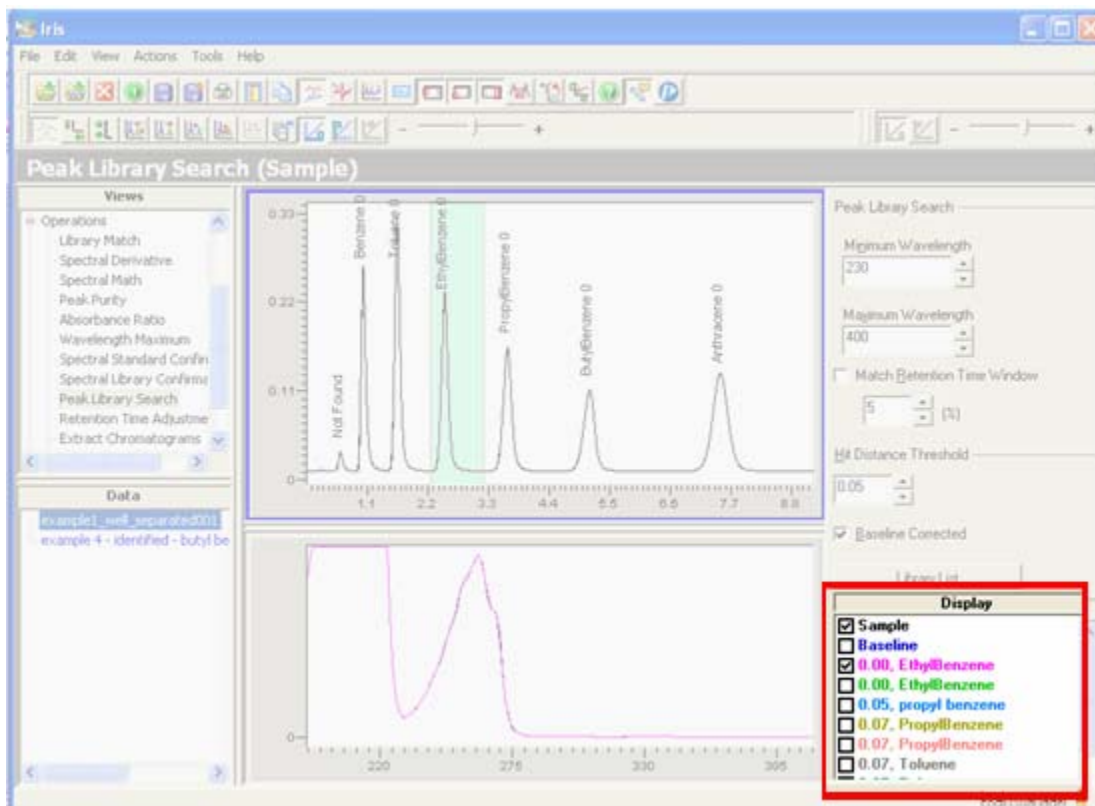
- **Results Pane** - The Results pane displays the items that are checked on the Hits List. You can use this pane to compare how close the apex spectrum of the currently selected peak matches to the hits returned from the search.
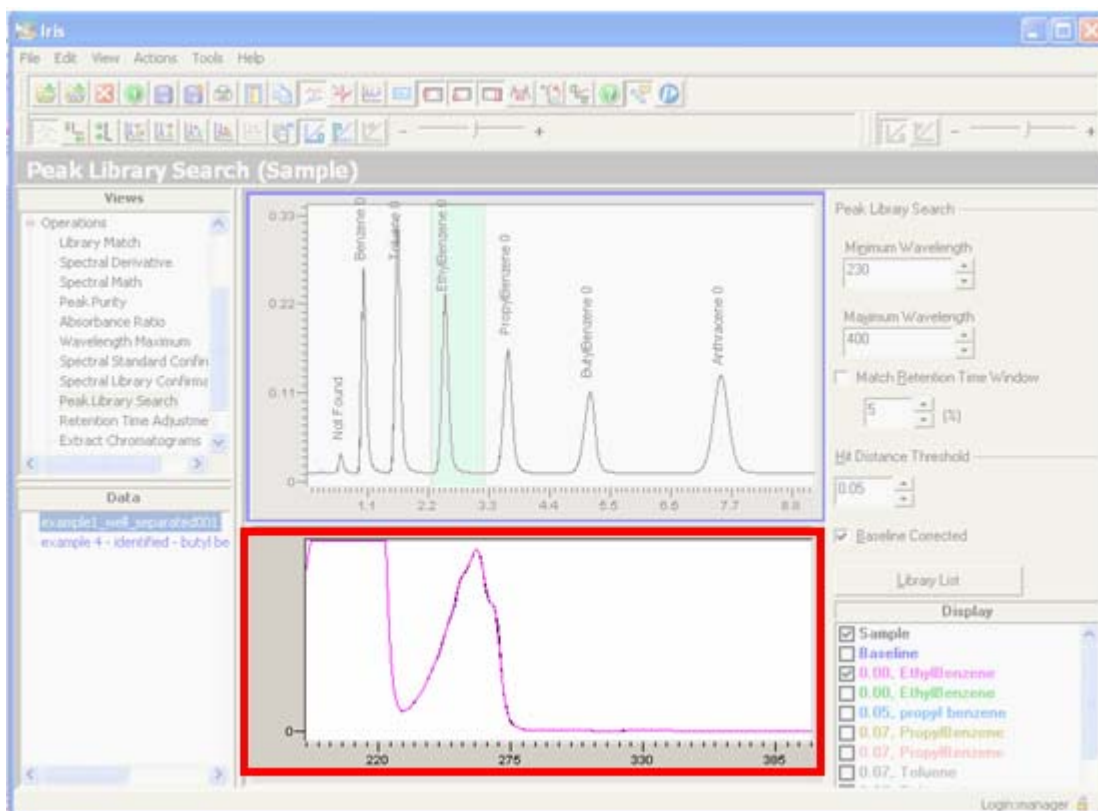
How do I modify the parameters used in the search?

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 IRIS automatically updates the display 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	Select this command button to display the Libraries List dialog.

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.

To 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. If necessary open the required chromatogram using **File** > **Open** > **Chromatogram**…

3. 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/ies 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 IRIS could not find a match for a peak, 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.

To 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.

To 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.

To examine the Peak Library Search values at different wavelengths:

➢ Enter a new value for **Minimum Wavelength** and/or **Maximum Wavelength**.

    The Peak Library Search value labels are updated for every peak in the chromatogram.

To 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.

To 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.

To 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. Select **File > 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 result file and asks you to confirm whether or not you want to save the new values to the chromatogram's result file (.RST), and save the parameters that were used calculate the new values to the associated spectral method files (.TSM).

2. Click **Yes**.

    A dialog apepars 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.

To 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.

To 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.

Retention Time Adjustment

Within TotalChrom peaks are identified on the basis of their retention time. If a peak moves outside a preassigned retention time window, that peak can be either misidentified or be left unidentified. The **Retention Time Adjustment** operation allows you to readjust the retention time window, so that TotalChrom can then successfully identify the peak. It accomplishes this by matching the peak apex spectrum in the chromatogram to library spectra, and then adjusting/correcting the retention times in a chromatogram's result file (*.rst). It should be noted, however, that if there are matching results in the elution times of the component being switched, IRIS cannot update these components, and the existing retention time will not be modified. If the scenario just described is true, IRIS will notify you of this situation.

➢ To access this operation expand the **Operations** node on the **Views** tree and select **Retention Time Adjustment**.
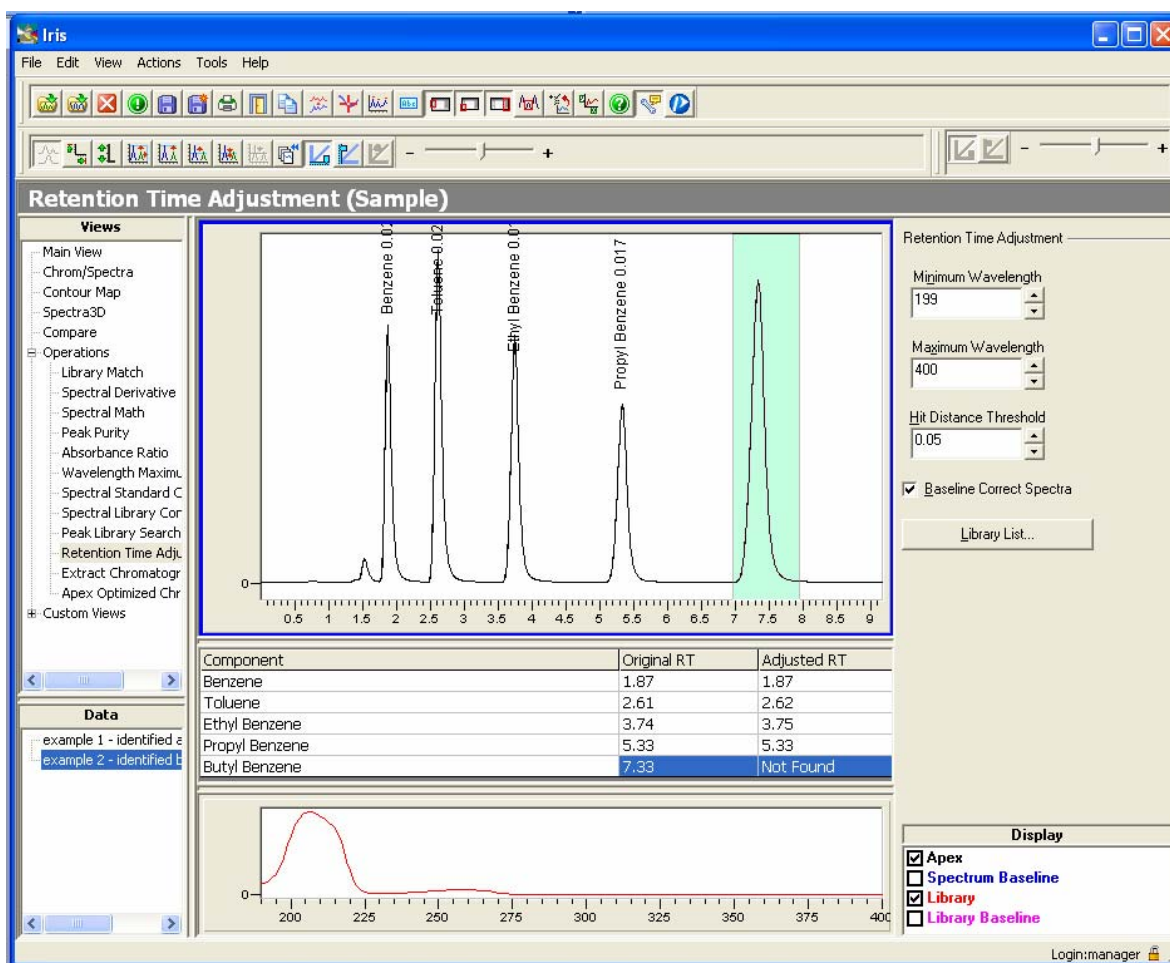
Figure 8-7 The Retention Time Adjustment operation

When should I use this operation?

This operation is useful because TotalChrom identifies a peak because it falls within a retention time window; and if the peak moves outside that window, then TotalChrom can no longer identify it. Therefore, this operation allows you to identify the peaks even when they move outside the retention time window. In addition, you may want to perform this operation if the chromatography and/or column have changed when applying an existing method. In addition, this operation can be particularly useful during the method development or method robustness phases in any applications lab. This operation can also be helpful when performing method validation on older columns.

Assigning a Component Name to a Peak and Correcting the Retention Time in the Chromatogram's Result File.

For this operation, IRIS uses the component list (the list of components that you are looking for in the chromatogram) present in the result file. For each component the operation finds the spectrum in the library matching the component name; and then finds which peak in the chromatogram best matches that (named) spectrum. If you so choose, the retention times in the component list can then be updated with the new retention times and the chromatogram re-identified by TotalChrom (**File > Save Results...**).

Note: *If in reassigning the retention times, two peaks have changed their elution order, this update process cannot be completed and an error message will appear informing you of this situation.*

Tell me about the Retention Time Adjustment operation.

The Retention Time Adjustment operation is comprised of the following areas:

- **Views Tree** – The Views Tree provides you access to the Retention Time Adjustment operation. You can access this operation by expanding the Operations node and selecting Retention Time Adjustment.
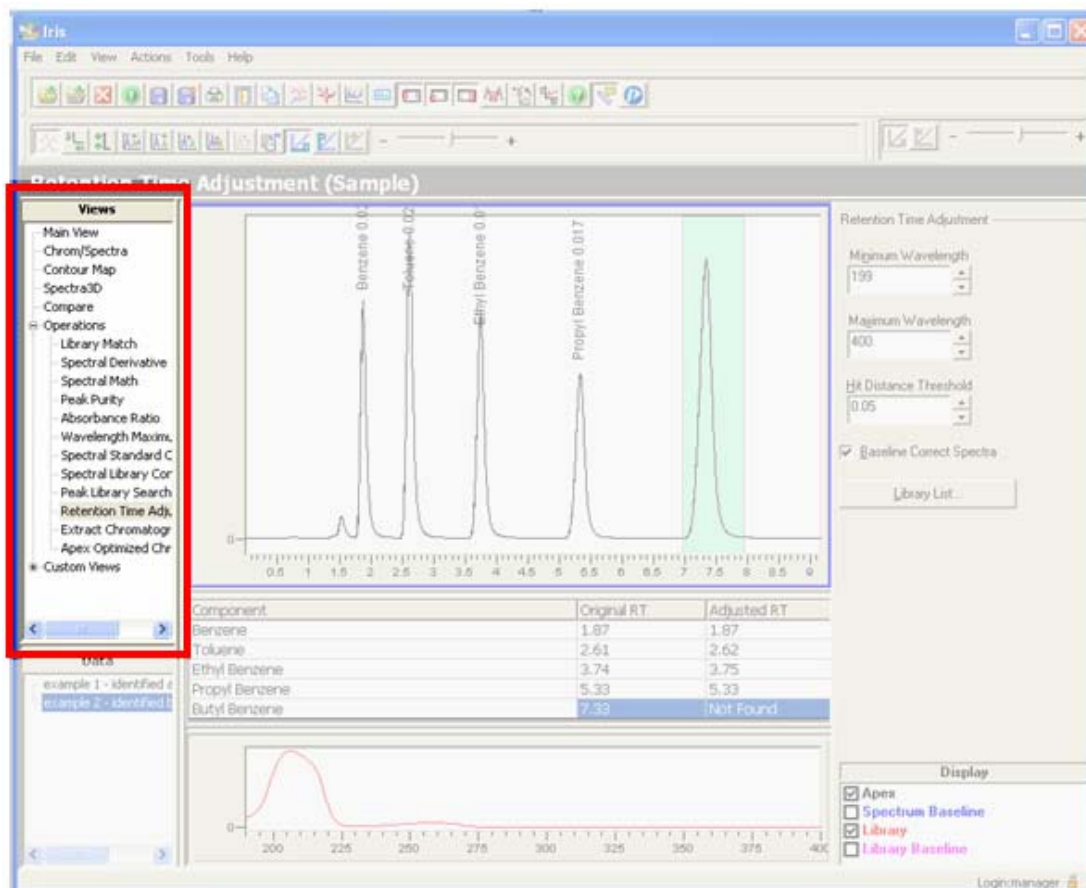
- **Data Tree** - The Data Tree shows a list of opened chromatograms, and is used to select the required chromatogram from the ones available. The selected chromatogram is displayed in the top graph (Chromatogram pane).
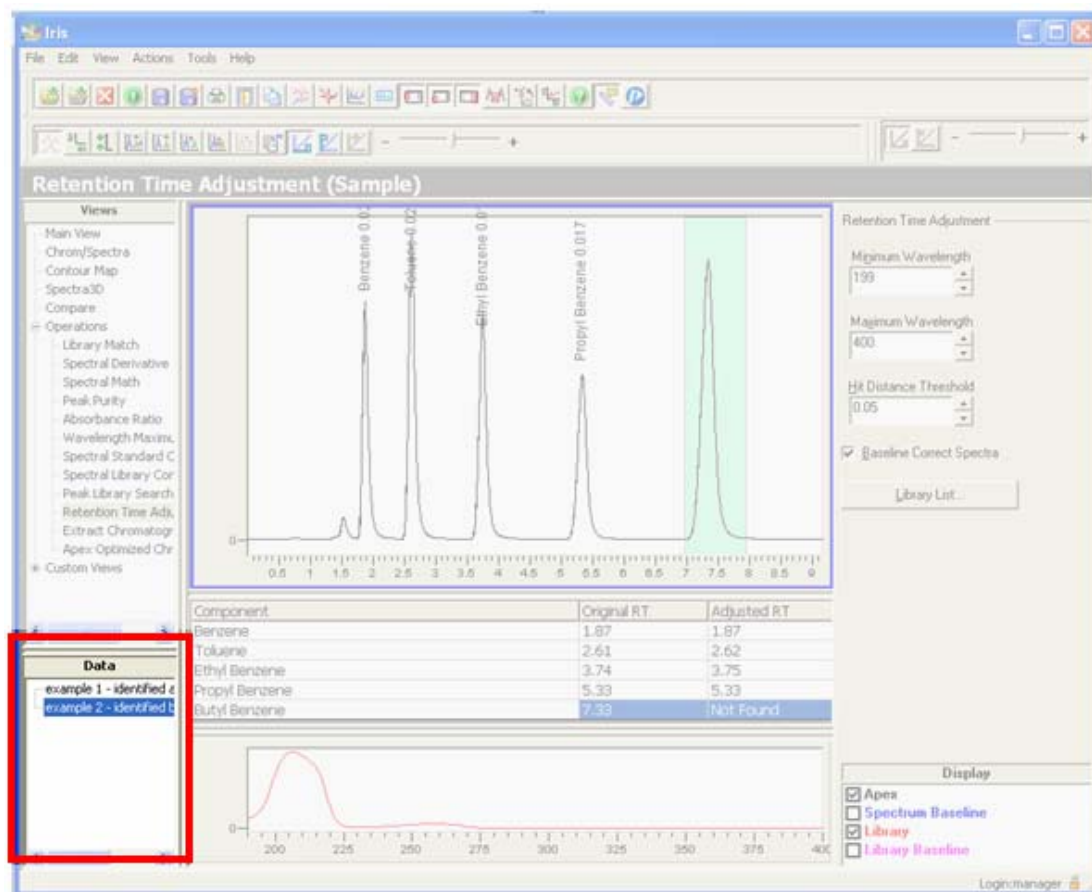
- **Chromatogram Pane** - When a chromatogram is selected on the Data Tree, this pane displays the selected chromatogram. If the selected chromatogram has no component list, then the upper right hand corner of the Chromatogram pane displays the text "Selected chromatogram has no component list". Otherwise, the peaks that have been identified as a result of this operation are labeled with the component name that matched a named spectrum in the library/ies.

  As different chromatograms are selected the chromatogram, component list, and result graph are updated.
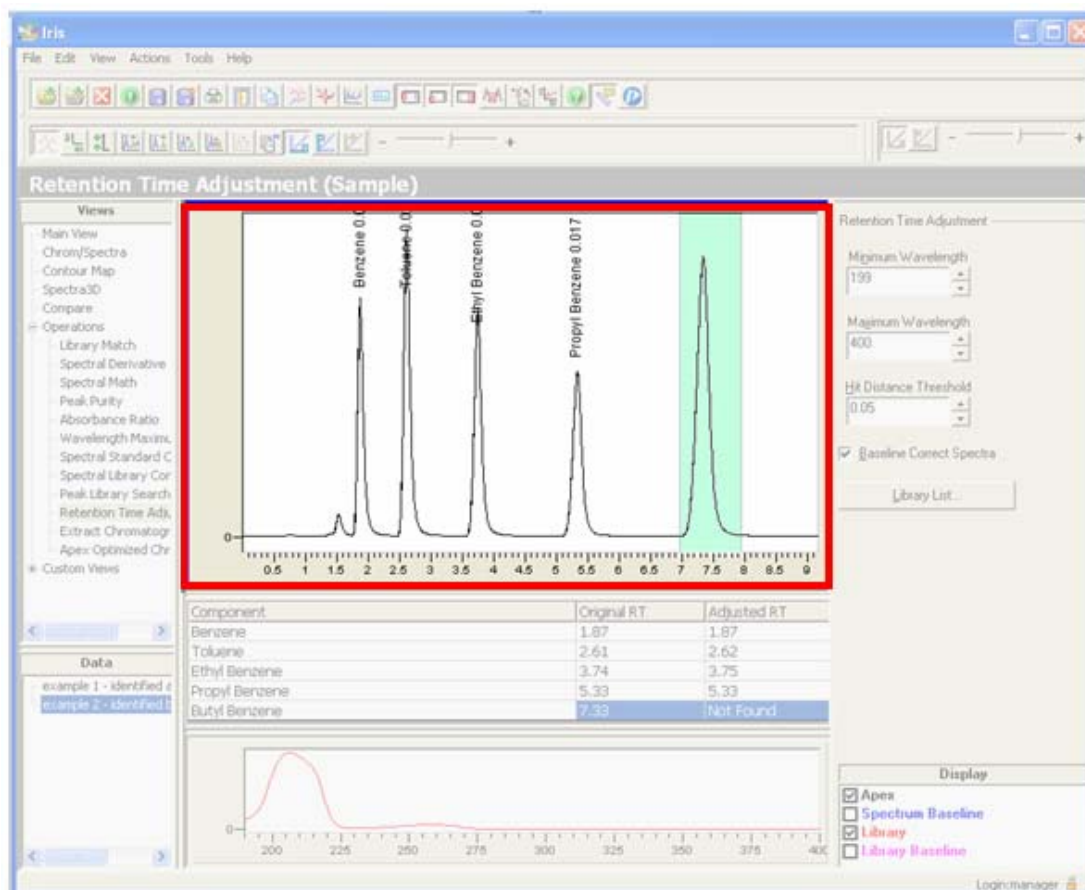
- **Parameters Pane** - The Parameters pane displays the parameters that are used to match the library spectra to the peak apex spectra; and when a match is found adjust the retention times.
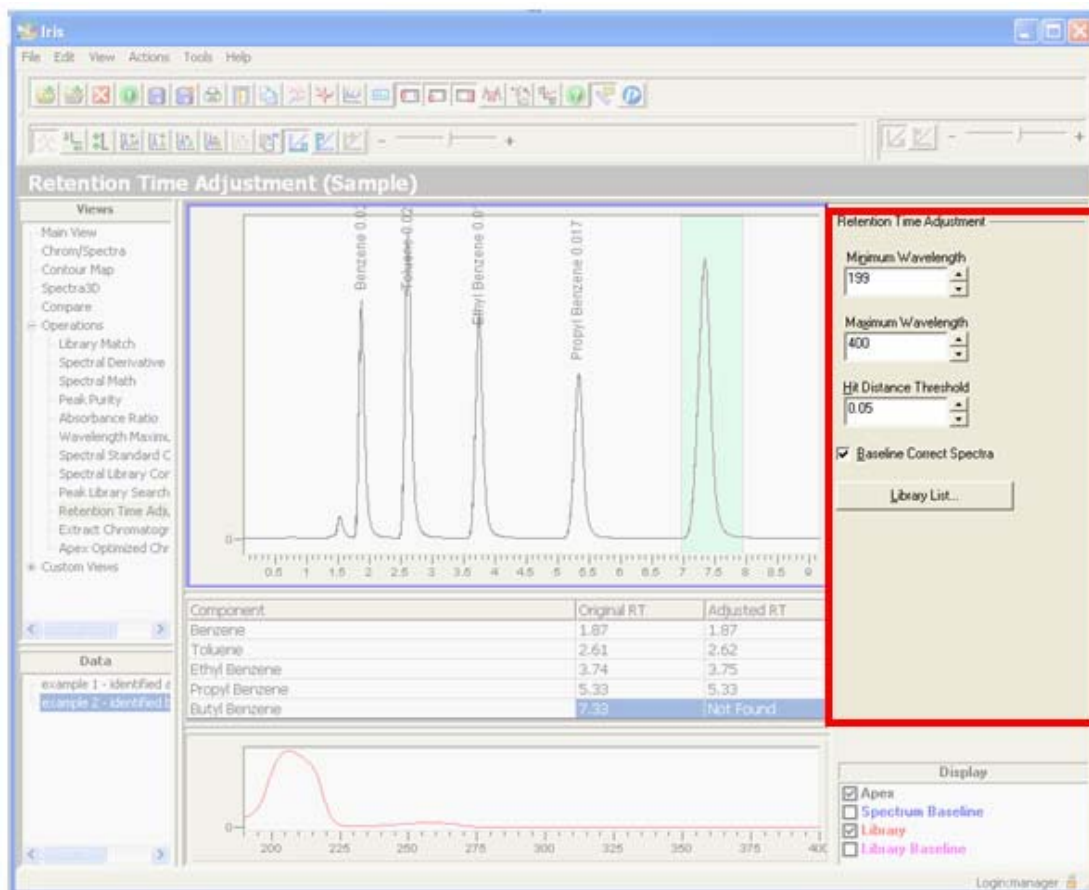
- **Component List** - This list displays the component list as present in the TotalChrom Result File and the original and adjusted retention times.

  When you make a selection in the Component List, the corresponding peak in the chromatogram is highlighted in green, and the spectral details that are checked on the Display List are displayed on the Results pane.
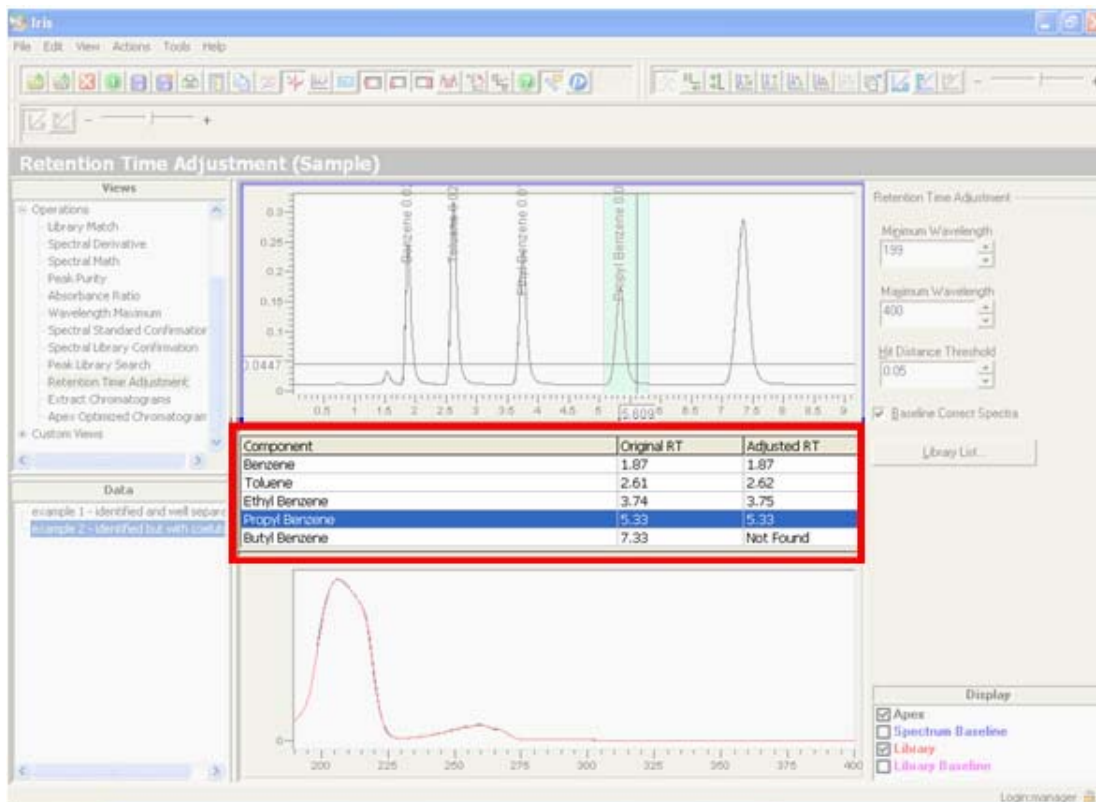
If the component selected in the component list is unmatched, the library spectrum can be displayed, but there is no peak apex spectrum.
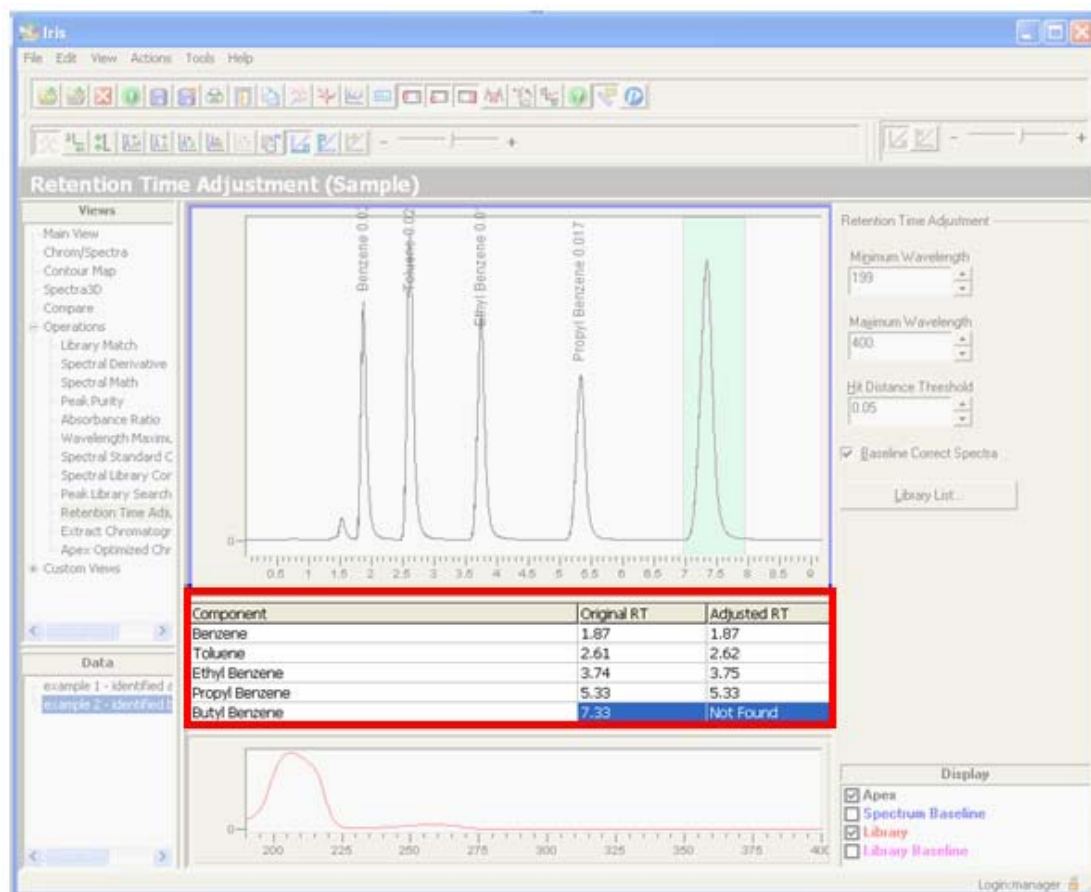
If a component is not matched to a peak, the component in the Component List is labeled as **Not Found**. If a component in the Component List is not present in the spectral library it will be marked a **Not Present in Library**.

- **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 of the currently selected peak.

  Spectrum Baseline - Check this item to display the baseline spectrum for the currently selected peak.

  Library - Check this item to display the library spectrum identified as a match for the selected peak.

  Library Baseline - Check this item to display the baseline spectrum from the library spectrum.
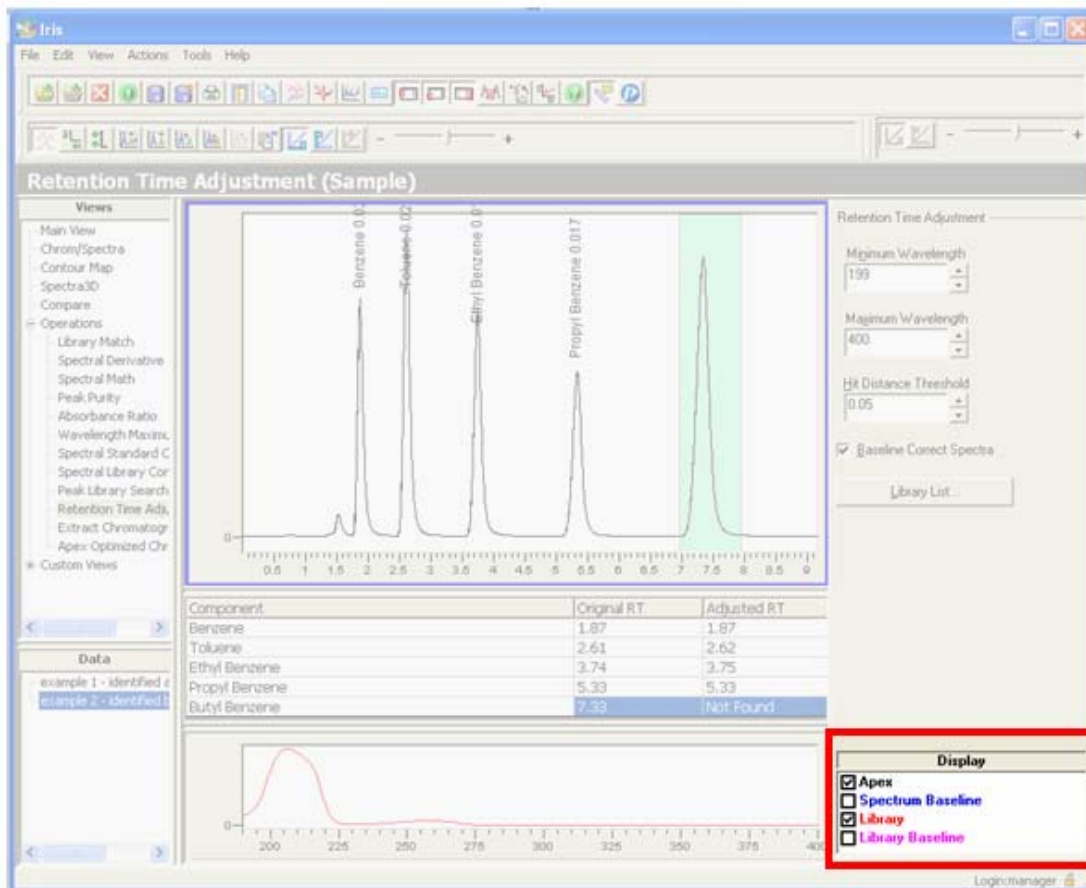
- **Results Pane** - The Results pane displays the items that appear checked on the Display List.

  From this pane you can view the apex spectrum of the currently selected peak and matching library apex spectrum.
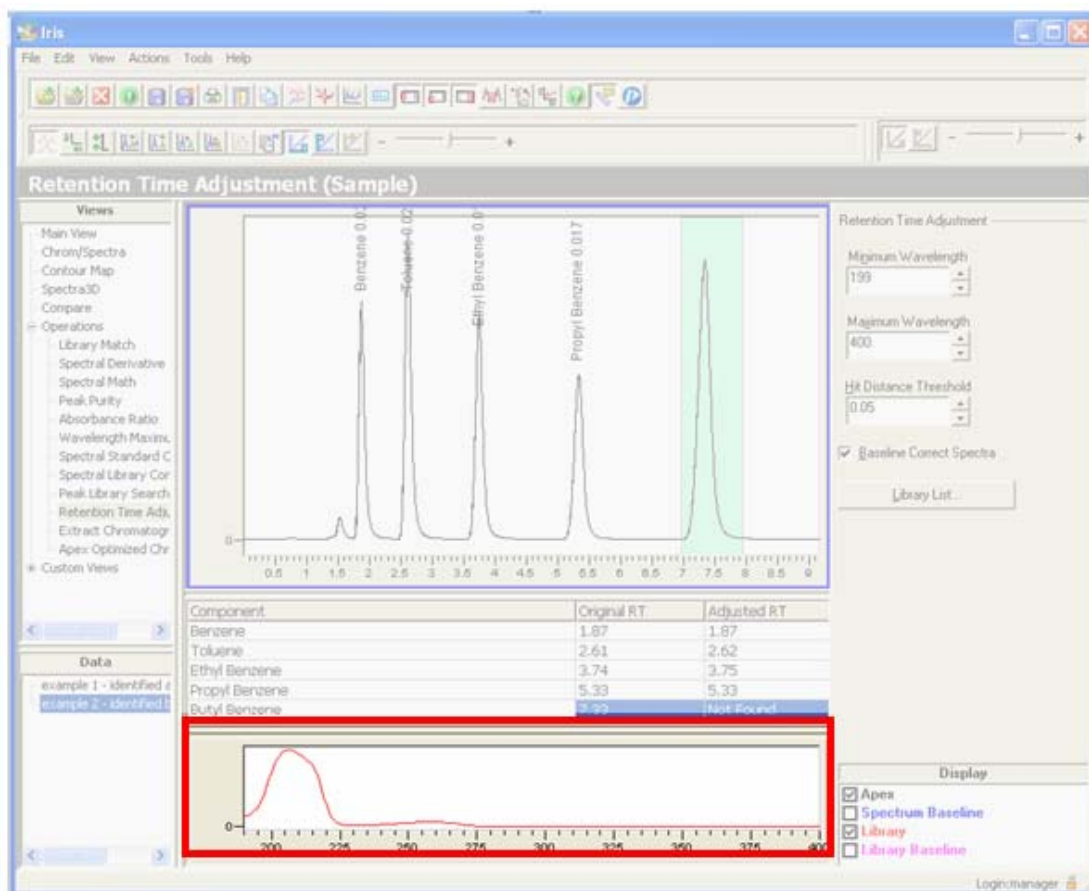
How do I modify the parameters that are used in the spectral matching?

The Parameters pane displays the values that are currently used in the spectral matching. At any time you can modify the parameters that are listed on the on the Retention Time Adjustment page; and IRIS automatically updates the display with the new library matches. 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 in the spectral matching.
Maximum Wavelength	Use the Maximum Wavelength spin box to set the upper limit of the spectrum range to be used in the spectral matching.
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 spectral matching.
Library list	Select this command button to display the Libraries List dialog.

Summary of Operation: Retention Time Adjustment

The following procedure shows you how to correct component retention times and save the results.

To correct component retention times:

Note: *It is not necessary that the chromatographic peaks in TotalChrom are recognized as labeled components or lie within component tolerance windows, nor is it even necessary that the peak are properly identified. It is only necessary that these components exist in the chromatogram's embedded method file (embedded in the chromatogram's result file).*

Note: *For this operation to be successful, it is important that the component names, as labeled by TotalChrom in the chromatogram's embedded method, are spelled exactly as those components listed in the selected spectral libraries. This includes any spacing or hyphens.*

1. First, open the desired chromatogram in IRIS, and select **Retention Time Adjustment**.
   The Retention Time Adjustment operation displays.

2. Click on the **Library List** button.
   The Libraries List dialog appears.

3. From the Libraries List dialog, choose any number of spectral libraries to use for matching.

4. Click **OK**.

5. You are returned to the Retention Time Adjustment operation.

    At this point, the listed components in the chromatogram's embedded method are checked against all the components listed in the selected spectral libraries. For this operation, these components can lie well outside of the TotalChrom method's peak tolerance window and can even have been initially mis-identified in the result file.

    For each component name that is matched, the library spectrum is then matched to the closest fitting peak apex spectrum in the chromatogram. If this spectral match falls within the selected threshold value, peak identification is confirmed.

    Thereupon, the chromatogram is graphically displayed with the properly matched/identified components and includes the annotation of the adjusted retention times (see example, below).
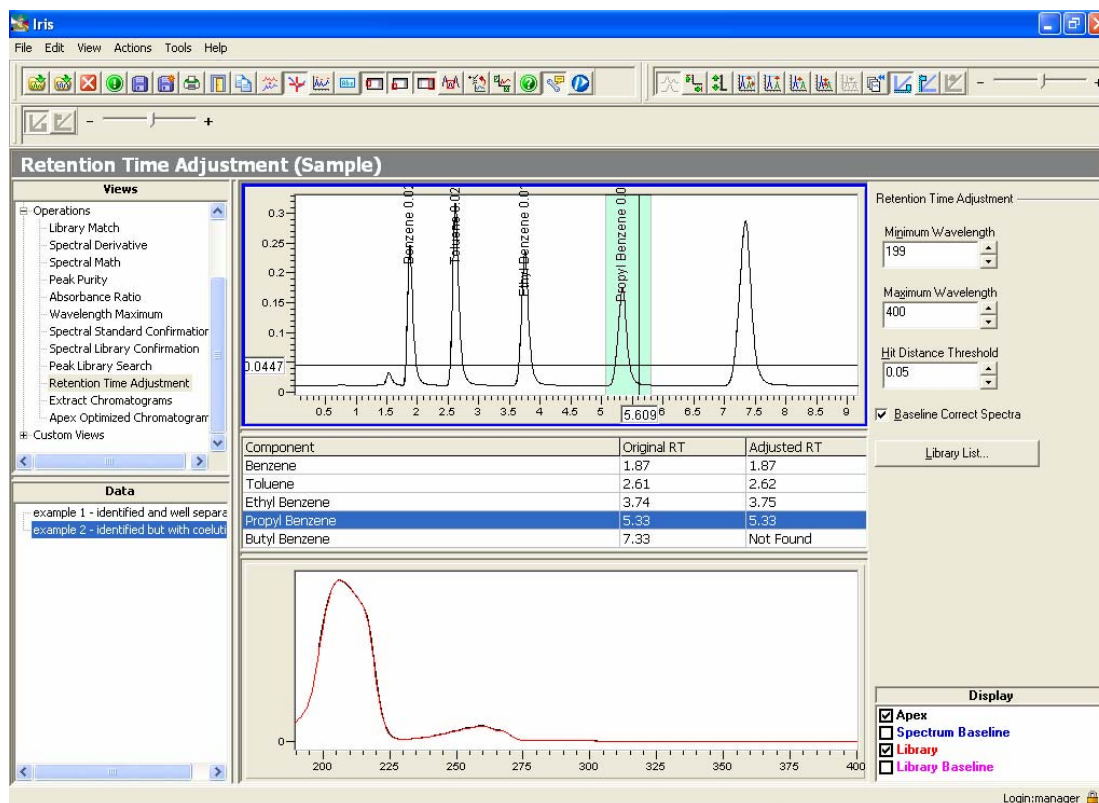
To examine the Retention Time Adjustment values at different wavelengths:

➢   Enter a new value for the **Minimum Wavelength** and/or **Maximum Wavelength**.

    The Wavelength Maximum value labels are updated for every peak in the chromatogram.

To examine the effect of using baseline corrected spectra on the Retention Time Adjustment values:

➢   Check **Baseline Correct Spectra**.

    The Wavelength Maximum value labels are updated for every peak in the chromatogram.

To save the results and parameters used in the calculation:

➢ The corrected retention times can then be saved to the chromatogram's result file by selecting **File > Save Results** from the IRIS menu bar. In addition, when making this selection, the parameters used to correct the retention times are also saved to the chromatogram's spectral method file.

To display the Retention Time Adjustment results in the Main or Chrom/Spectra view:

1. Select **Actions > Label Chromatograms...**

2. Select **Retention Time Adjustment** from the list of available labels.

To review for any chromatogram previously determined Retention Time Adjustment values and the parameters used:

➢ Select this chromatogram again on the Retention Time Adjustment operation.
  The saved results and the calculation parameters are automatically displayed.

Extract Chromatograms

The Extract Chromatogram operation allows you to extract up to 8 chromatograms from a spectral file and automatically process them.

➢ To access this operation, expand the **Operations** node on the IRIS **Views** tree and select **Extract Chromatograms**.
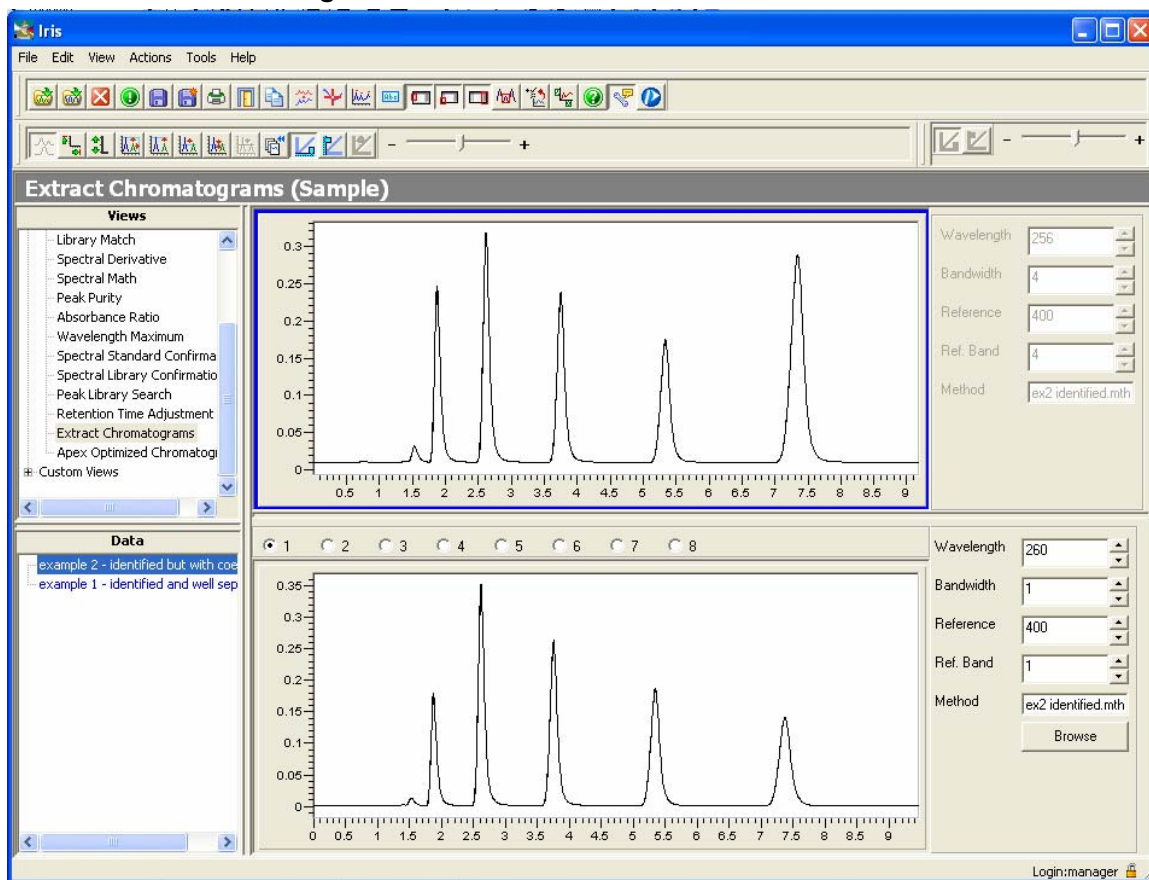

Figure 8-8 The Extract Chromatograms operation

Extracting Chromatograms at Different Wavelengths

To extract chromatograms at different wavelengths you must first select an opened chromatogram from the Extract Chromatograms Data Tree. The selected chromatogram is displayed in the top graph of this page. You can then use the bottom graph to extract up to eight chromatograms. To extract chromatograms all you need to do is enter an Analytical Wavelength for each chromatogram you want to extract. However, this operation also provides you with the ability to specify the following additional parameters for each chromatogram you want to extract: **Bandwidth**, **Reference Wavelength**, and **Reference Bandwidth**.

After you have finished establishing the extraction parameters for up to 8 chromatograms select **File > Save Results** to have IRIS save all of the extraction parameters to the appropriate spectral method files. Once the parameters are saved, you can then use AutoCalc to automate this operation during a TotalChrom sequence that will be processed with the same TotalChrom method that you specified when you extracted the chromatograms. Then, when a run is completed all of the wavelengths you have specified and saved to a method file, are extracted automatically after each run. *For more information, on automating this operation refer to **Chapter 9 AutoCalc: Automating Arithmetic Operations and Chromatographic Extractions**.*

Note: *The RAW files and corresponding RST files, that are created as a result of performing the Extract Chromatograms operation are named with the same name as the original *.raw file used to perform the operation. However, the file names for the extracted chromatograms have the following additional information appended to the file name: the wavelength collected under, the bandwidth, the reference wavelength, and the reference bandwidth.*

In addition, if a file has already been created with the same name, IRIS automatically adds the date and time of the newly extracted chromatogram to the RAW and RST files in order to prevent duplicate file names.

For example...

*In the following example a chromatogram named **Example 2 - identified but with coelution.RST** is selected for the operation, its raw file is named Example1_coelution.raw; and the following extraction parameters are specified to produce 1 extracted chromatogram:*
Analytical Wavelength** of **260
Bandwidth** of **1
Reference Wavelength** of **400
Reference Bandwidth** of **1

*Then, selecting **File > Save Results** causes IRIS to create the following files: Example1_coelution_360_1_400_1.RAW (this is the RAW file for the newly extracted chromatogram) and Example 2 - identified but with coelution_360_1_400_1.RST (this is the result file that is automatically created for the newly extracted chromatogram.).*

Tell me about the Extract Chromatograms Operation.

The Extract Chromatograms operation is comprised of the following areas:

- **Views Tree** - This Views Tree provides you access to the Extract Chromatograms operation.

  You can access this operation by expanding the **Operations** node and selecting **Extract Chromatograms**.
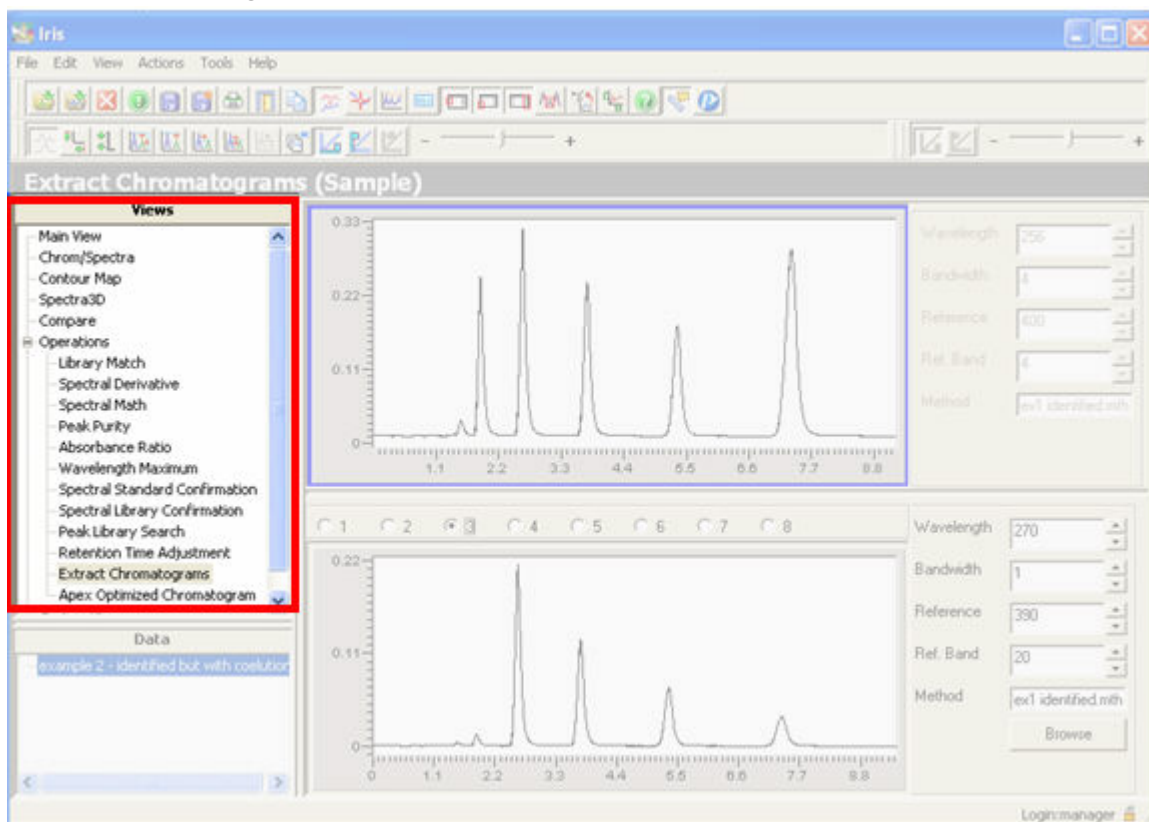
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatograms that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.
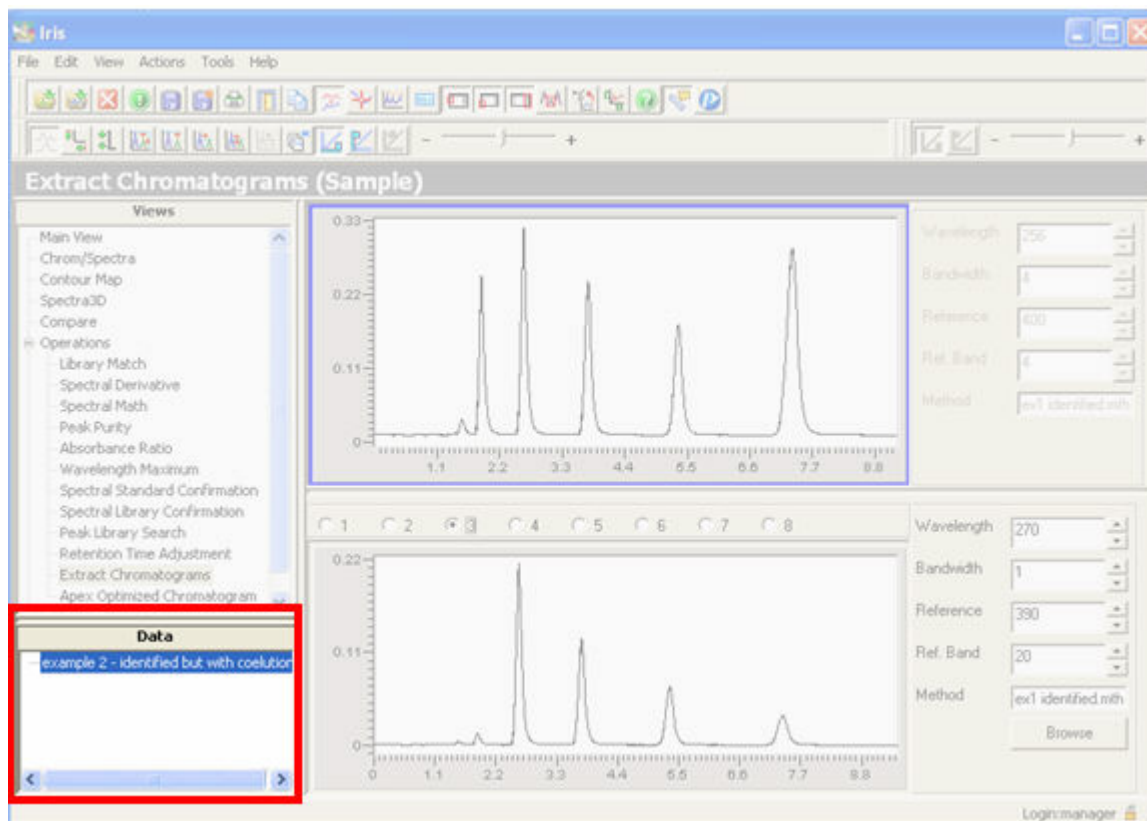
- **Chromatogram Pane** - When you select a chromatogram from the Data Tree, the selected chromatogram is displayed here. To the right of the displayed chromatogram are the parameters under which the chromatogram was originally collected.
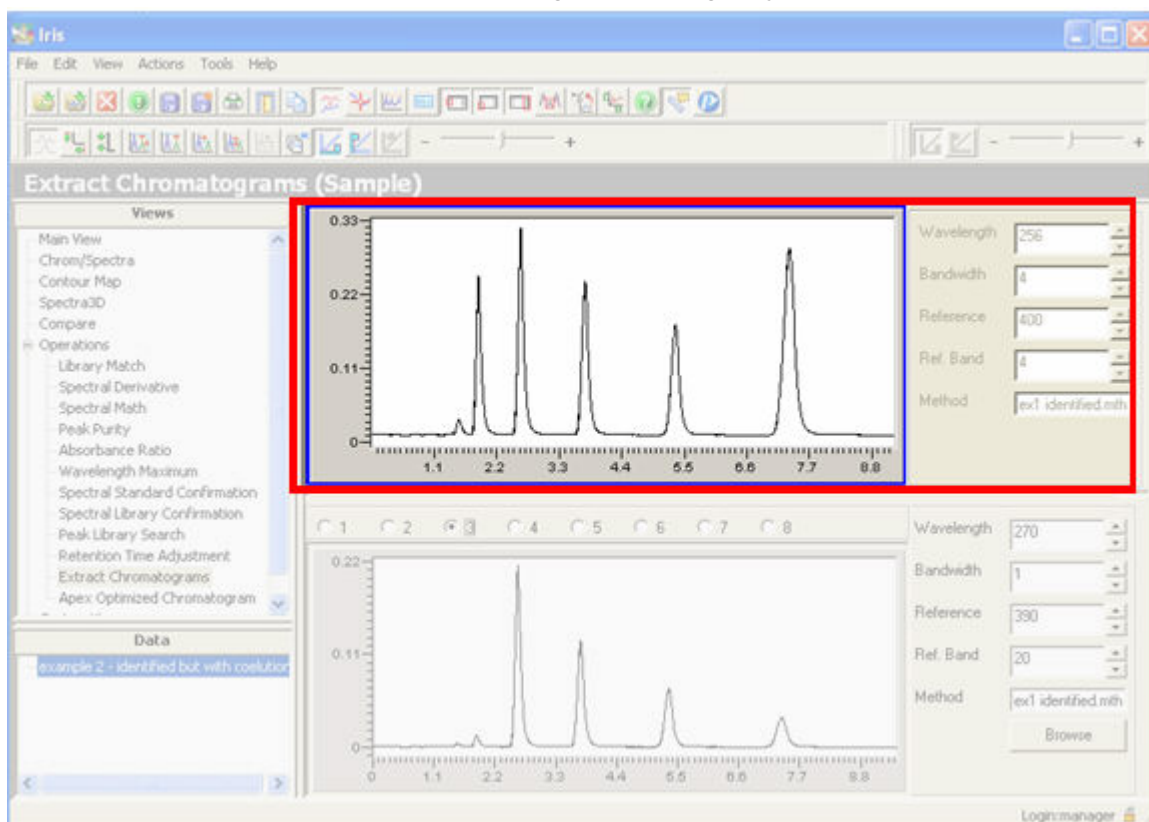
- **Extracted Chromatograms Pane** – Once you have selected a chromatogram from the Data Tree, you can use the Extracted Chromatograms pane to view the sample chromatogram at different wavelengths.

  To extract a single chromatogram click on one of the radio buttons located on the top of this pane (there are eight radio buttons displayed on the page) and then specify the Analytical **Wavelength**, **Bandwidth**, **Reference** Wavelength, and **Reference Bandwidth** for the chromatogram you want to extract.

  If desired, you can also click the **Browse** button to specify a different TotalChrom method for the extracted chromatogram. By default, the Method field for the Extracted Chromatogram contains the same method as specified for the chromatogram selected on the IRIS Data Tree.

  As soon as you enter a valid (analytical) wavelength, the extracted chromatogram is displayed on this pane.

  If you want to extract additional chromatograms, select the next radio button and once again enter the parameters for the chromatogram. Even without saving the results, IRIS will remember the parameters you specified on each radio button you selected to create an extracted chromatogram until you select a new chromatogram from the Data Tree or leave the Extract Chromatograms operation.

  You can save the extracted chromatograms and the parameters for the extracted chromatogram (which can then be used by AutoCalc to automatically create multiple chromatograms during a TotalChrom sequence) by selecting **File > Save Results** from the IRIS menu bar. *For more information on using AutoCalc, refer to **Chapter 9 AutoCalc: Automating Arithemtic Operations and Chromatographic Extractions***.
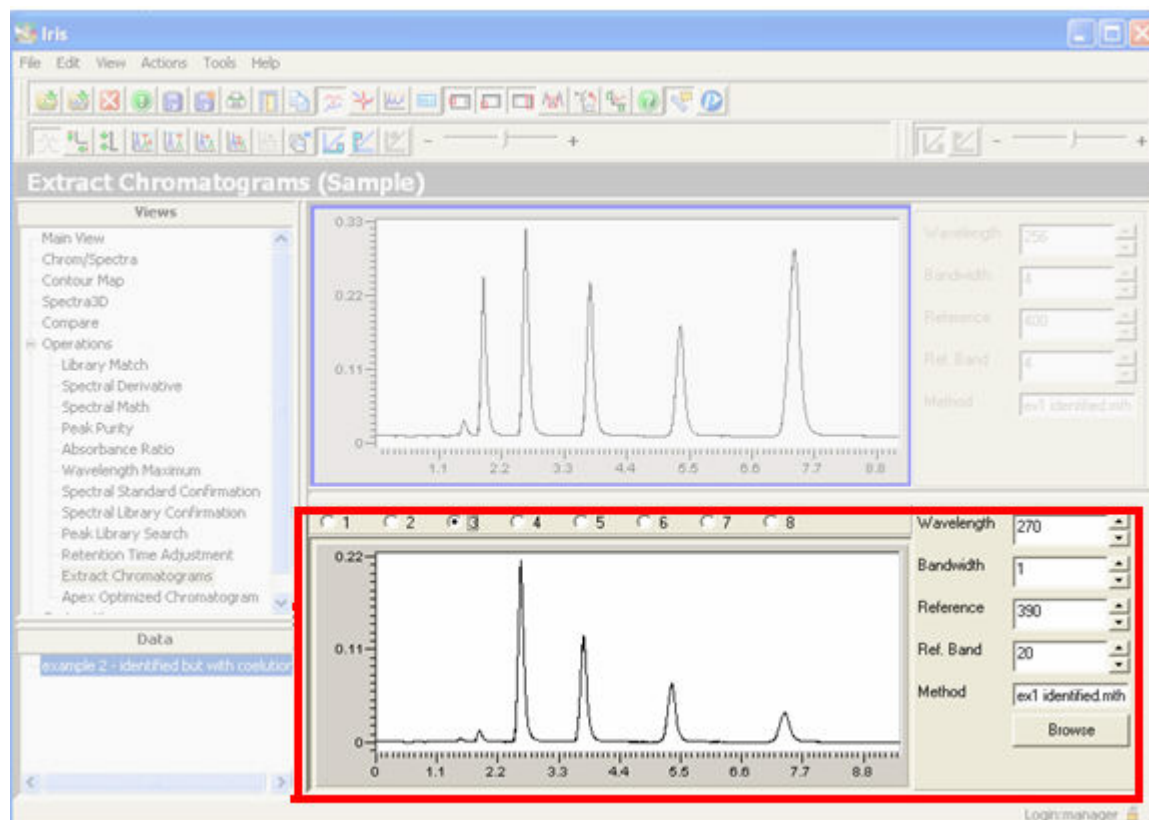
How do I modify the parameters that are used to Extract Chromatograms?

Each time you select a radio button on the Extract Chromatogram pane you can specify the following parameters for the chromatogram you want to extract:

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.*
Method	This text box displays the selected TotalChrom method. This method will be used to process the new chromatogram to generate a new result files. The file that appears here defaults to the same method that was used for the original chromatogram, but you can select a different method to be used by clicking on the **Browse…** button and selecting a method from the dialog box that appears, or by typing the name of the method directly into the text box.
Browse	Select the Browse... button to display a standard Windows file selector. From the file selector dialog you select the method file that will be associated with the chromatogram you are extracting.

Summary of Operation: Extract Chromatograms

The following procedure shows you how to extract multiple chromatograms, from a single result file, at wavelengths other than the two specified by the photodiode detector.

1. From the IRIS **Views Tree** expand the **Operations** node and select **Extract Chromatograms**.

   The Extract Chromatograms operation displays.

2. Select an opened chromatogram from the Extract Chromatograms Data Tree.

   The selected chromatogram is displayed in the top graph of this page.

   OR

   If necessary open the required chromatogram using **File** > **Open** > **Chromatogram**...

3. To extract multiple chromatograms click on one of the radio buttons located on the top of this pane (there are eight radio buttons displayed on the page).

4. Specify the **Analytical Wavelength**, **Bandwidth**, **Reference Wavelength**, and **Reference Bandwidth** for the chromatogram you wish to extract.

   As soon as you enter a valid Analytical Wavelength, the extracted chromatogram is displayed.

5. If you want to extract additional chromatograms, select the next radio button; and, once again, enter the parameters for the chromatogram. IRIS will remember the parameters you specify on each radio button you selected. These parameters will be displayed each time you select the corresponding radio button until you select a new chromatogram from the data tree or leave the Extract Chromatograms page.

6. You can save the extracted chromatograms by selecting **File > Save Results...** from the IRIS menu bar.

   IRIS automatically creates RAW files for each extracted chromatogram. These raw files are automatically reprocessed by IRIS and TotalChrom Result files (.RST) of the same name are created. The RAW files and corresponding RST files that are automatically created are named with the same name as the original result file, with the following additional information appended to the file name: the wavelength collected under, the bandwidth, the reference wavelength, and the reference bandwidth.

Apex Optimized Chromatogram

The **Apex Optimized Chromatogram** operation allows you to create a chromatogram with the optimum wavelength set for each peak. The optimum wavelength for a peak is defined as the wavelength maximum of the apex spectrum of that peak.

➢ To access this operation, expand the **Operations** node on the **Views** tree and select **Apex Optimized Chromatogram**.
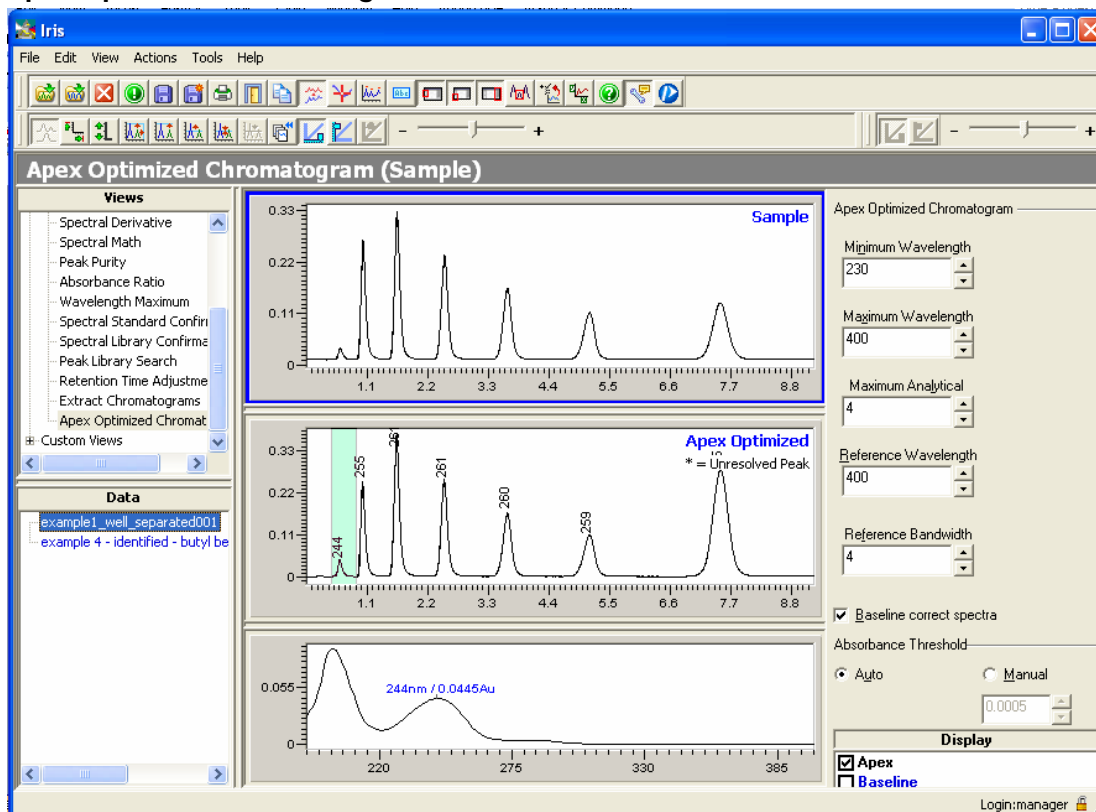

Figure 8-9 The Apex Optimized Chromatogram operation

Rules for setting the wavelength for a peak

The Apex Optimized Chromatogram operation is a specialized version of the Extract Chromatogram operation. In the Extract Chromatogram operation, you specify the wavelength at which to extract the chromatogram from the spectral file. The same wavelength is used for the complete chromatogram. In the Apex Optimized Chromatogram operation, the wavelength used for extraction is the wavelength maximum of the chromatographic peak in the peak apex spectrum (the same value as is return by the Wavelength Maximum function). This is determined for each peak in the chromatogram and the extraction wavelength adjusted for each peak. The final chromatogram is therefore the sum of each of these extracted sections.

The rules for creation of the Apex Optimized Chromatogram are:

1. The wavelength used for a peak that is baseline separated from any previous peak is the wavelength maximum of the apex spectrum of that peak. The peak is labelled with its detection wavelength.

2. If the peak is not baseline separated from the previous peak then the wavelength of the previous peak is used. Instead of the detection wavelength the peak is labeled with an "*" to indicate that it is unresolved from the earlier peak.

3. Use the **View > Chromatogram Baselines** to see how TotalChrom has treated each peak.

Tell me about the Apex Optimized Chromatogram operation.

The Apex Optimized Chromatogram operation is comprised of the following areas:

- **Views Tree** – The Views Tree provides you access to the Apex Optimized Chromatogram operation. You can access this operation by expanding the Operations node and selecting Apex Optimized Chromatogram.
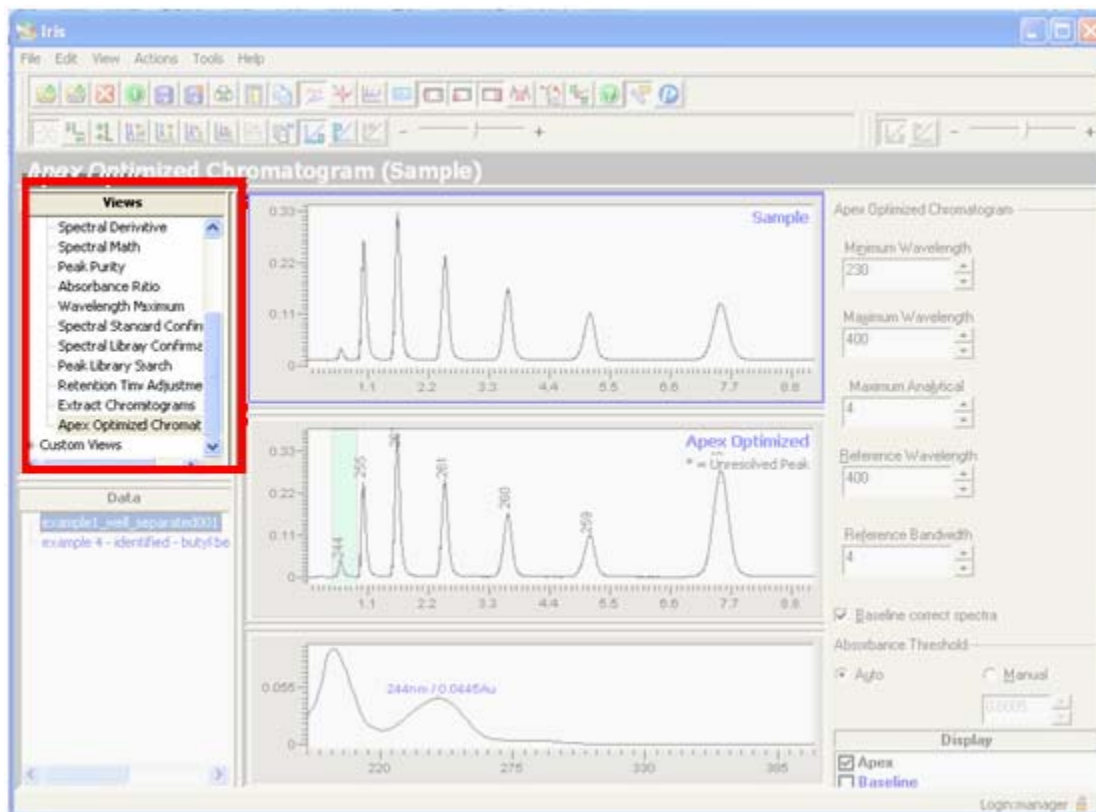
- **Data Tree** - The Data Tree displays a list of currently loaded chromatograms. You use the Data Tree to select the chromatogram that you want to use in the operation. The chromatogram you select from the Data Tree is displayed in the upper pane (Chromatogram pane) for this operation.

Note: *When you select a chromatogram from the Data Tree, IRIS checks that the TotalChrom method used to generate the result file from the raw file can still be found. This TotalChrom method will be used later to process the optimized chromatogram. If the method cannot be found, an error message is displayed that informs you that the raw Apex Optimized chromatogram can be saved but not the result file.*
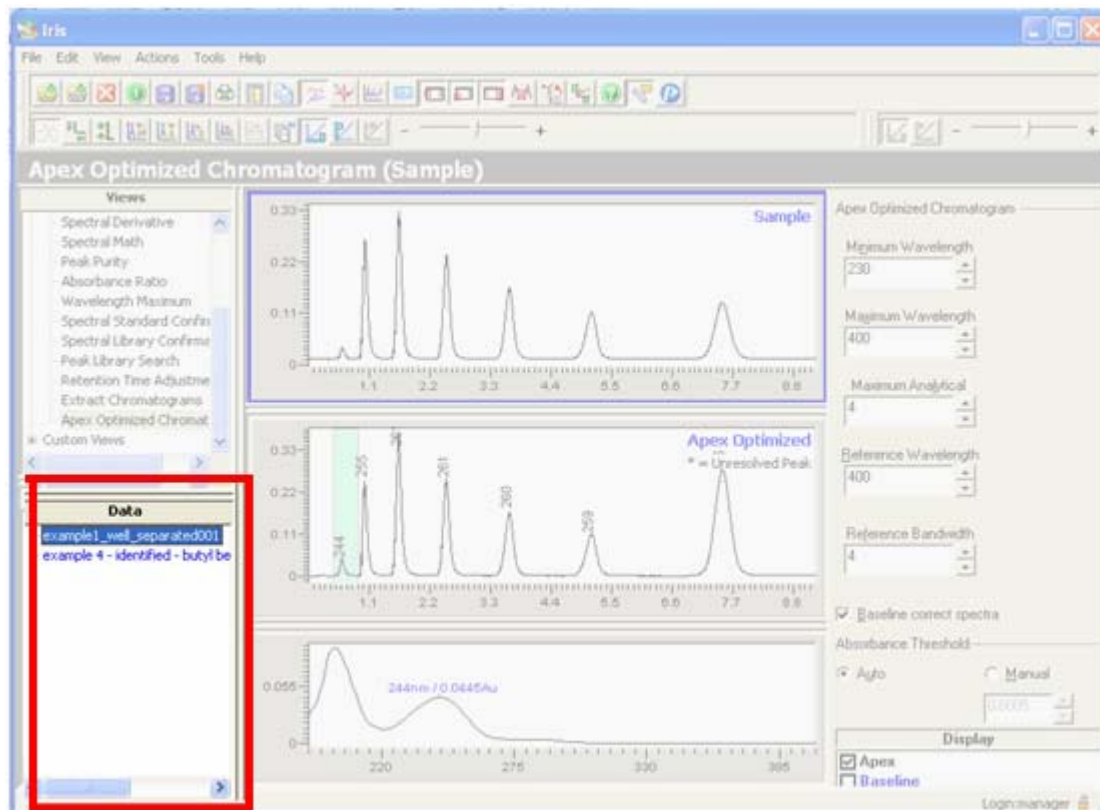
- **Sample Chromatogram Pane** - This pane displays the chromatogram that you selected from the Data Tree. It should be noted that unlike the chromatogram panes in other operations, you cannot select chromatographic peaks on this pane.
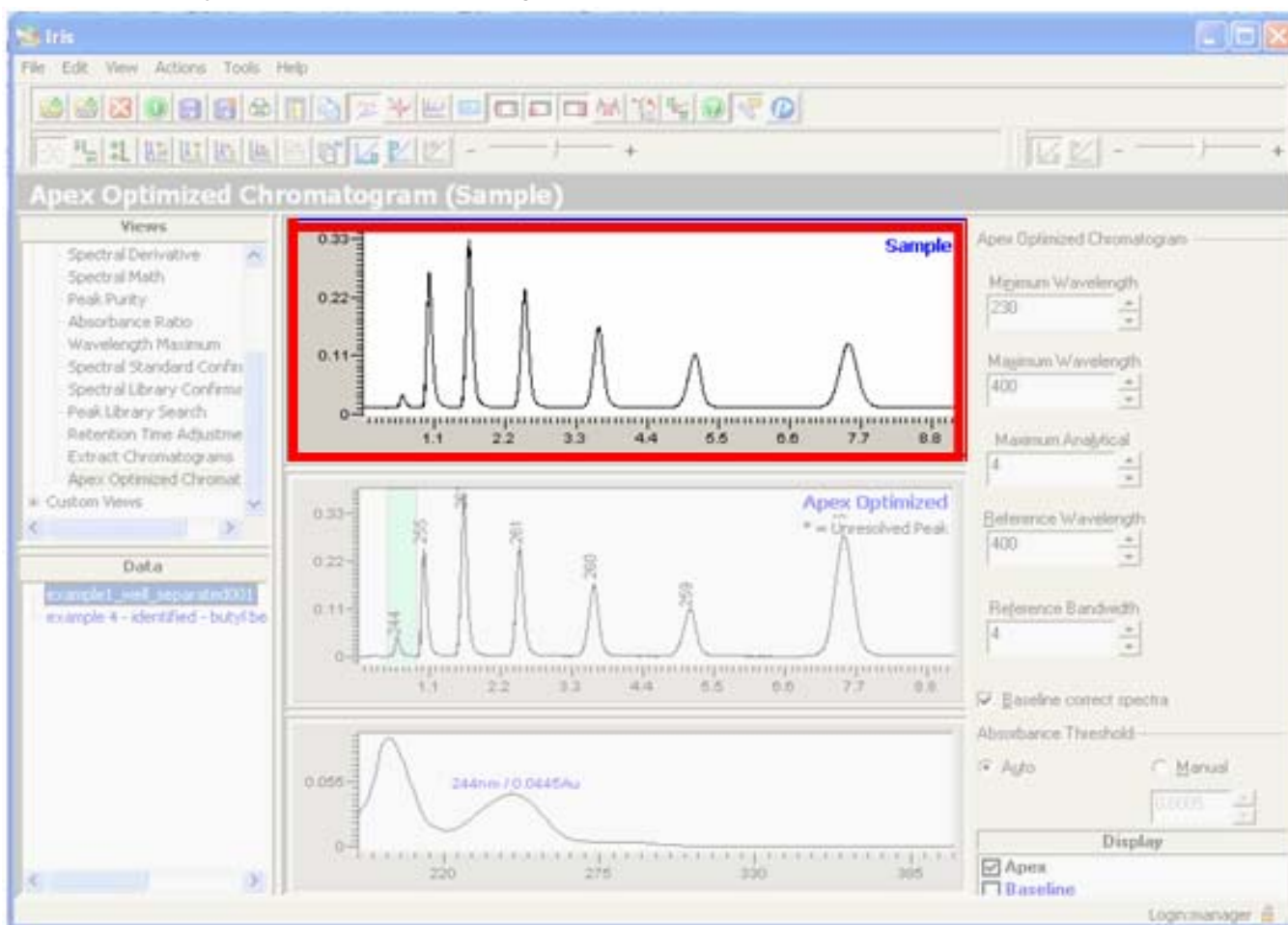
- **Apex Optimized Chromatogram Pane** - As soon as you select the sample chromatogram from the Data Tree, IRIS calculates the optimum wavelength for each peak, and extracts the chromatogram at these different wavelengths and displays the Apex Optimized Chromatogram in this pane. The first peak on the apex optimized chromatogram is automatically selected and appears highlighted in pale green. The apex spectrum for the selected peak is shown in the bottom Spectra pane. To select a different peak, simply click on that peak.
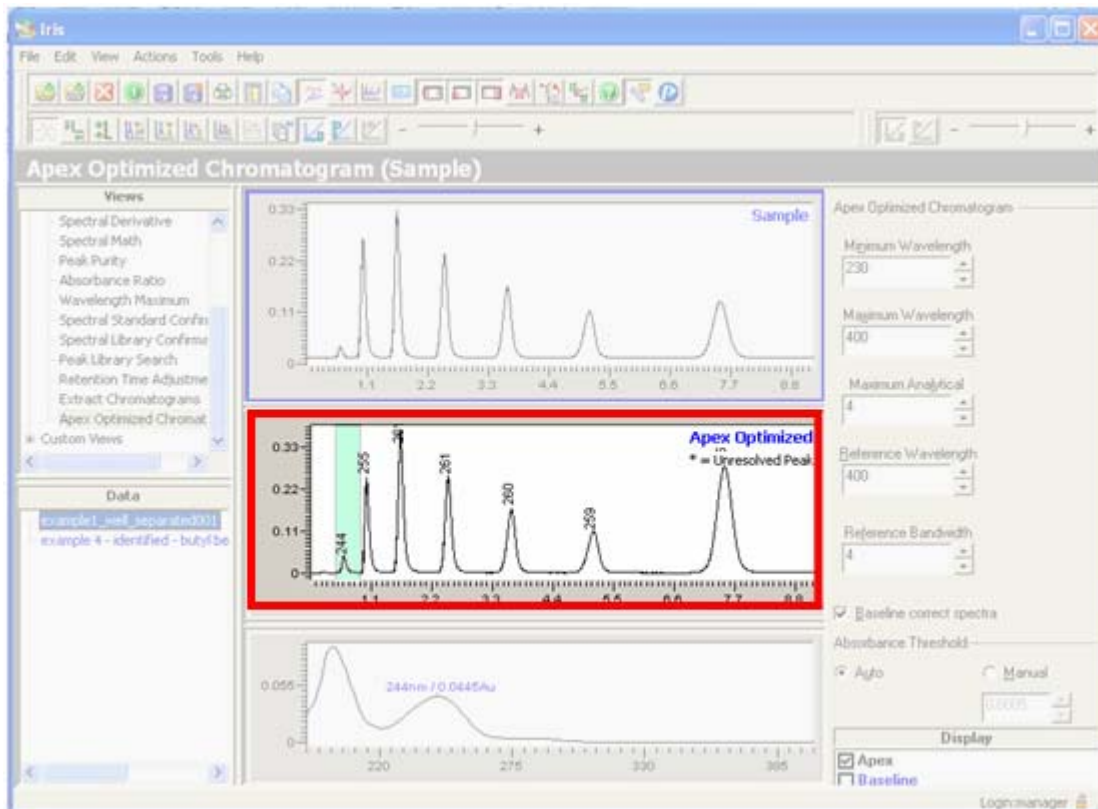
- **Parameters Pane** - This pane is where you set and investigate the various parameters used to calculate the Apex Optimized Chromatogram. Whenever you change a parameter displayed on this operation, and have a chromatogram selected on the Data Tree, IRIS automatically updates the display with the new Apex Optimized Chromatogram.
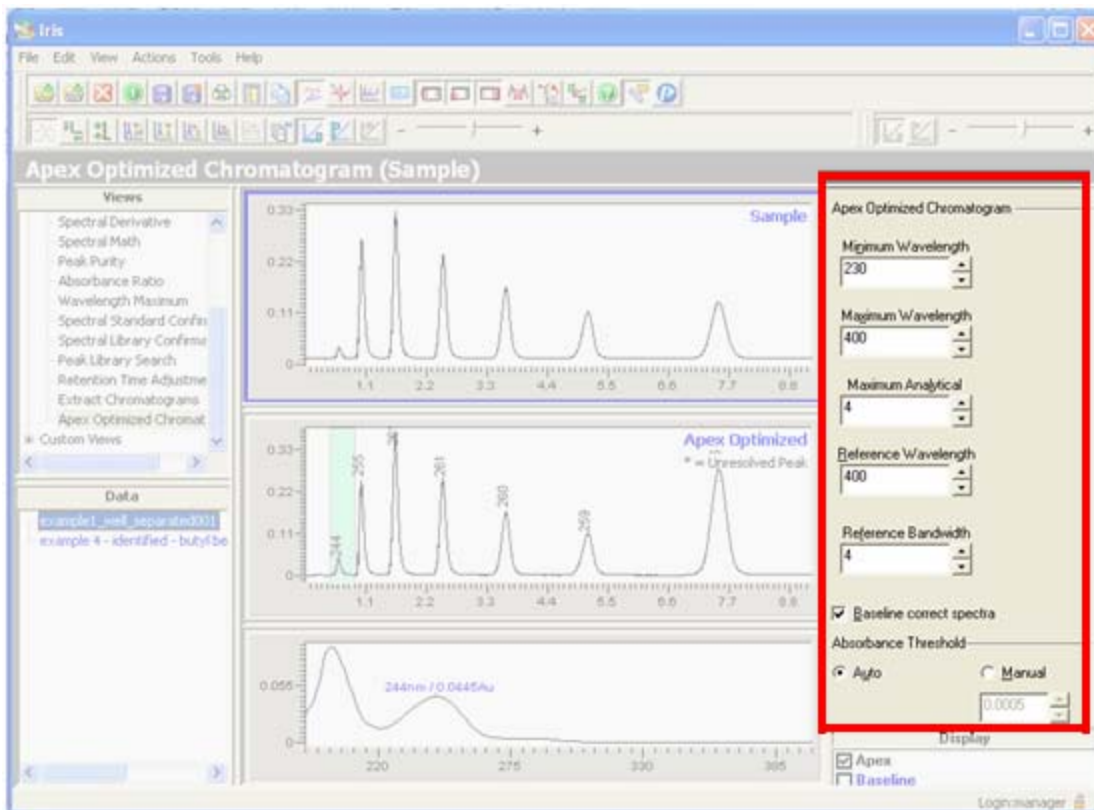
- **Results Pane** - This pane displays the items that appear checked on the Display List. When **Apex** is checked on the Display List, this pane shows the apex spectrum for the peak currently selected on the Apex Optimized Chromatogram pane. The apex spectrum is labeled with the wavelength actually used in extracting that region of the chromatogram. This label will show the actual extraction wavelength. It is then possible to observe the difference between the extraction wavelength and the true optimum wavelength, if the wavelength cannot be optimized, e.g. the peak is not baseline separated from the earlier peak.
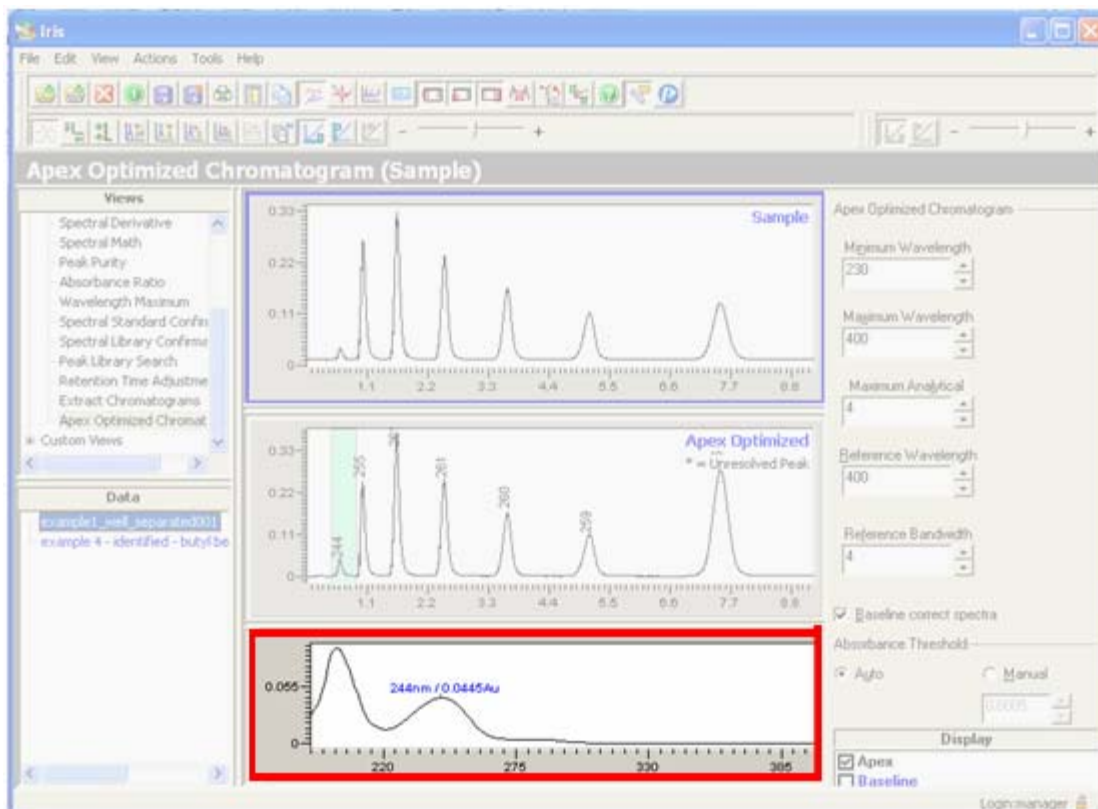
- **Display List** - You use the Display List to select the information you want displayed on the Results pane. When items appear checked on this list, they are displayed in the Results Pane.

  Meanwhile, unchecked items are not displayed.

  The following items are available for selection on the Display List:

  Apex - When this item is checked, the Peak Apex Spectrum pane displays the spectrum taken at the maximum point of the selected chromatogram peak. The peak maximum of the spectrum is labeled with the wavelength and absorbance.

  Baseline - When this item is checked, the baseline spectrum for the selected chromatogram peak is displayed on the Peak Apex Spectrum.
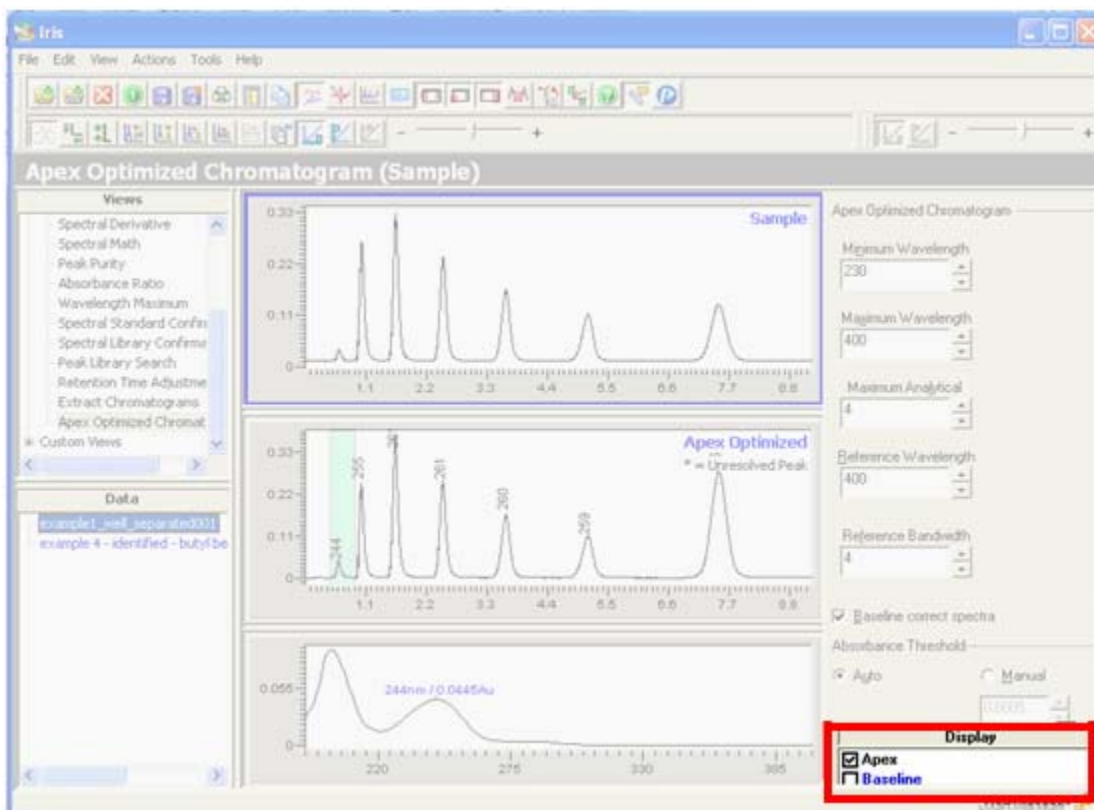
How do I modify the parameters that are used to create a chromatogram with the optimum wavelength set for each peak?

The Parameters pane is where you set and investigate the various parameters for creating a chromatogram with the optimum wavelength set for each peak. At any time you can optimize/adjust the parameters that are listed on the Apex Optimized Chromatogram operation; and the Apex Optimized Chromatogram value labels are updated for every peak in the chromatogram.

Below is a list of the parameters you can modify:

Control	Description
Minimum Wavelength	Enter the Minimum Wavelength to set the lower limit of the spectrum range to be used for the calculation of the wavelength maximum.
Maximum Wavelength	Enter the Maximum Wavelength to set upper limit of the spectrum range to be used in the calculation of the wavelength maximum.
Maximum analytical bandwidth	Enter the maximum analytical bandwidth that will be used to create the optimized chromatogram. If the analytical wavelength falls too close to either end of the range, the full range may not be available.
Reference wavelength	Enter the Reference Wavelength to be used in creating the optimized chromatogram.
Reference bandwidth	Enter the Reference Bandwidth to be used in creating the optimized chromatogram… ***Note:*** *If you set this field to 0, then the Reference Channel is turned off.*
Baseline corrected	Check this option to use the baseline corrected spectrum for the calculation of the wavelength maximum.
Auto	Select this radio button to have IRIS automatically set the absorbance minimum. The maximum wavelength will not be calculated in any area below this threshold in order to prevent erroneous assigning maxima to random noise.
Manual	Select this radio button if you want to manually set the absorbance minimum value. The maximum wavelength will not be calculated in any area below the value you specify. This prevents erroneous assigning maxima to random noise.

Summary of Operation: Apex Optimized Chromatogram

The summary below illustrates how to create a chromatogram with the optimum wavelength set for each peak.

To create a chromatogram with the optimum wavelength set for each peak in a chromatogram:

1. Expand the **Operations** node on the **Views Tree** and select **Apex Optimized Chromatogram**.

    The Apex Optimized Chromatogram operation is displayed.

2. Select a chromatogram from the Data Tree.

    The chromatogram is displayed on the Sample Chromatogram pane with the first peak selected; and IRIS calculates the optimum wavelength for each peak, extracts the chromatogram at these different wavelengths and displays the apex optimized chromatogram in the pane directly below the sample chromatogram.

3. If you are interested in viewing the apex spectrum for a specific peak in the apex optimized chromatogram, click on the peak.

    The apex spectrum for the currently selected peak is displayed in the bottom Spectra Pane; the peak maximum of the displayed spectrum is labeled with the wavelength and absorbance.

Note: *If you select a different chromatogram the apex optimized chromatogram is recalculated and re-displayed. If you change any of the parameters used in this calculation, the apex optimized chromatogram is updated.*

4. You can add result spectra to the Data Tree by checking Apex and or Baseline on the Display List and then selecting **Actions > Add All to view**.

    The selected spectra is added to the Data Tree under the parent chromatogram. The added spectra is labeled on the data tree with the Retention Time followed by "-Apex" or "-Baseline".

To create a TotalChrom chromatogram from the apex optimized chromatogram:

1. From the menu bar select **File > Save Results**.

    A save dialog appears that asks you to confirm whether or not you want to save the changes to the chromatogram's result file (.RST) and the changes to its associated spectral method files (.TSM).

2. Click **Yes**.

    If the TotalChrom method is present, a result file is generated.   The apex optimized chromatogram that is generated is named after the original .raw file name followed by "_Apex Optimized" plus a time/date stamp if the name already exists.

Note: *The conditions used to create the chromatogram can only be found on the Apex Optimized Chromatogram View and are not stored with the chromatogram in any way. Therefore, if you use the Save Results... command, it is your responsibility to ensure that you have documentation on how the chromatogram was created. For this reason this operation cannot be performed by AutoCalc.*

Saving the Results of a Calculation

The **File > Save Results** command allows you update a chromatogram's result file with the results of any operation performed on a chromatogram in IRIS; thereby allowing you to include these results in a TotalChrom report.   The parameter values used to obtain these results are saved in the Chromatogram's spectral method file and the Process Spectral Method associated with the chromatogram.

To save the results of an operation:

1. After you have performed an operation on a chromatogram, select **File > Save Results…** from the IRIS menu bar.

   The Save Results confirmation dialog appears.

Note: *The Save Results dialog differs slightly depending upon the type of operation you have performed.*

2. From the **Save Results** dialog you are prompted to confirm whether or not you want to save the new values to the chromatogram's result file (.RST); as well as update the chromatograms spectral method files (.TSM) with the parameter values that were to determine the results of the operation you just performed.

   If the operation you performed could only be performed on Identified peaks, skip to step **4**. Operations that can only be performed on identified peaks are: Spectral Standard Confirmation, Spectral Library Confirmation, and Peak Library Search.

3. Select **Currently identified peaks** to only save results for peaks that are currently identified (named) in TotalChrom. If you select this option results are not saved for unidentified peaks.

   OR

   Select **Include unidentified peaks** to save results for identified and unidentified peaks.

   If you select this option, any unidentified peaks that have results calculated are added to the results file as Unknown 1, Unknown 2, etc. Any unidentified peaks which do not have results are not added to the file.

4. Click **Yes** to save the results and calculation parameters.

   The results are added to the TotalChrom result file and can now be included in a TotalChrom printed report. *See the section titled **How do I include the results of chromatographic operations performed by IRIS/AutoCalc in a TotalChrom report?** on page **312**.*

Note: *The report format **AppValues.rpt**, which is present in the installed example data directory, can be used to print out the saved results in the .RST file from all of the operations in IRIS/AutoCalc.*

How IRIS saves Information to TotalChrom

The results of any of the operations you perform on a chromatogram in IRIS can be saved to the chromatogram's result file (.RST), and the parameters that were used calculate the results are saved the associated spectral method files (.TSM). The saved results can to be included in a printed TotalChrom report.

These results are stored in the Application Value fields in the component list associated with the result file. This requires that the peaks in TotalChrom be identified (named). In addition, because these fields can only contain numerical data, the IRIS information must sometimes be encoded. Listed below is how the IRIS operation saves its information to TotalChrom.

Note: *The Save Results dialog appears automatically whenever you have performed an operation on a chromatogram and selected another chromatogram/operation, or attempt to close the application.*

Peak Purity

- Pass results are reported as the normal Purity Index value.

- Failed results are reported as a negative Purity Index.

- Peaks which are not already identified in TotalChrom and for which the Purity cannot be calculated (which are reported as 0 within IRIS) are not identified and are never reported within TotalChrom.

- Peaks which are identified in TotalChrom and for which the Purity cannot be calculated (which are reported as 0 within IRIS) are reported within TotalChrom with the value -888.

Spectral Standard Confirmation

- Pass results are reported as the normal Absorbance Index value.

- Failed results are reported as a negative Absorbance Index.

- Named peaks in the sample for which no match can be found in the standard/reference chromatogram are reported as -999.

- Named peaks in the sample for which the Spectral Standard Confirmation cannot be calculated are reported as -888.

Wavelength Maximum and Absorbance Ratio

- Peaks for which the Wavelength Maximum and Absorbance Ratio values can be calculated are reported with the same value as in IRIS.

- Peaks which are already identified in TotalChrom and for which the Wavelength Maximum and Absorbance Ratio values cannot be calculated are reported in TotalChrom as -888.

- Peaks which are not already identified in TotalChrom and for which the Wavelength Maximum and Absorbance Ratio values cannot be calculated are not identified and are not reported in TotalChrom.

Spectral Library Confirmation

- Pass results are reported as the normal Hit value.

- Failed results are reported as a negative Hit value.

- Named peaks in the sample for which no match can be found in the library are reported as -999.

Peak Library Search

- The component name found by IRIS will be entered as the Component Name in the Component List. The Hit value will reported. This test has no failure results. Peaks that are not matched in the library are not recorded in TotalChrom.

Note Carefully: *Saving the results of this test to TotalChrom will delete any existing Component List. These results should only be saved if a component list does not already exist for if you want to completely rewrite the component list. Any information, either from TotalChrom or IRIS, stored in an existing component list will be lost.*

Extract Chromatograms

This is a new chromatogram, whose creation does not affect the parent chromatogram/result file.

Retention Time Adjustment

- A component in the component list, which is in the library, and for which a match if found, will have the hit value placed in the App Value.
  The value will be negative if the Hit Value is greater than the Hit Distance Threshold.

- A component in the component list, which is not in the library, is not reported in the result file.

- An unidentified peak has no app value associated with it.

Apex Optimized Chromatogram

This is a new chromatogram, whose creation does not affect the parent chromatogram/result file.

How do I include the results of chromatographic operations performed by IRIS/AutoCalc in a TotalChrom report?

Once you have saved the results of an IRIS/AutoCalc operation, the results can be included in a TotalChrom report. For your convenience we have provided you with a report template named **AppValues.rpt**, which can be found in the installed example data directory for IRIS. This report template includes all of the parameters that you can possibly add from IRIS/AutoCalc to a result file. However, if you do not want to use the AppValues.rpt, you can also modify the Default Report and add only the IRIS/AutoCalc results that you choose to the Default Report template.

Note: For more information on modifying the Default Report, refer to the TotalChrom Help file.

To use the AppValue.rpt:

1. In TotalChrom Reprocess Results open the result file that you want to use to generate a report.

2. Select **File > Report Format**.

3. Locate and select the **AppValues.rpt** as the report format.
   The report information display changes according to the new format you selected.

Chapter 9. AutoCalc: Automating Arithmetic Operations, Extractions and Apex Spectra Printing

What is AutoCalc?

AutoCalc is a user program that is installed with your IRIS Spectral Processing Software. During a TotalChrom (TC) sequence, this user program allows you to automatically perform any combination of the arithmetic operations (with the exception of Apex Optimized Chromatogram) provided by IRIS (*such as Peak Purity and Spectral Standard Confirmation*). This program also allows the automated extraction of up to 8 chromatograms and printing of chromatograms annotated with apex spectra. This is all achieved by specifying **AutoCalc.exe** as a user program in the Process section of a TotalChrom (TC) method and entering the desired series of AutoCalc commands in the command line.

It is important to note that, as part of either a real-time or reprocessed sequence, the results of the automated operations are automatically saved to the TotalChrom Result file (.RST) and can automatically be included in a TotalChrom report

Points to consider when using AutoCalc for automated operations in TotalChrom

AutoCalc uses the parameter values that are stored in an IRIS Process Spectral Method to perform any of the automated operations described in this chapter. Therefore, it is important that you understand how to have IRIS automatically generate a Process Spectral Method, and then update that method with all of the parameter values that will be used by AutoCalc for automated operations.

As explained in ***Chapter 2 Getting Started***, the Process Spectral Method (along with the Chromatogram's Spectral Method) is created when a chromatogram is first opened in IRIS. The Process Spectral Method is given the same name and stored in the same location as the TotalChrom Method that was used to create the result file that you opened in IRIS; however the file extension is .tsm instead of .mth. Although the Process Spectral Method is created upon opening a chromatogram in IRIS for the first time, it cannot be used by AutoCalc, each operation that you want to automate is first performed in IRIS on a chromatogram that has been processed by the same TotalChrom method that will be used to process the future sequence, and the results saved.

Note: *All chromatograms that are opened in IRIS and have been processed by the same TotalChrom method share the same Process Spectral Method.*

Following are detailed instructions on how to have IRIS generate a Process Spectral Method that can be used by AutoCalc during a running sequence.

Note: *For more information on Spectral Methods, see the section titled **About Spectral Methods** on page **28**. For more information on arithmetic operations an chromatographic extractions, refer to **Chapter 8. Performing Operations on Chromatograms**.*

Before specifying which operations you want to be performed, you must do the following:

1. In IRIS, open a chromatogram that has been processed with the same TC method that will be used to run or reprocess the sequence.

2. Next, verify the name of the chromatogram's associated TC method. To do this, first, right mouse click on the chromatogram in the IRIS Data Tree and select **Information** from the context menu that appears.

   A Chromatogram Information dialog appears that shows the TC method associated with the selected chromatogram. **Note this method name.**

3. Now, exit the Chromatogram Information dialog box.

4. From the IRIS menu bar select **File > Open > Method**, and open the spectral method having the same name and file location as the TC method just noted.

   The View Method dialog appears. This dialog allows you to view the parameters for the method you just opened.

5. In the opened Method Viewer, review the spectral method's individual parameters by selecting all the desired operation(s) from the list, at left.

6. Once you have reviewed the parameters of the spectral method, you can modify any of these parameters by doing the following:

   - First, exit the Method Viewer dialog box.

   - Then, for the chromatogram opened in Step 1, select any of the desired operations that are listed under Operations in the IRIS Views tree.

   - Make any required changes to the parameters listed for the operation you selected.

   - Select the next operation to which you want to make changes.

     When you select a different operation, a dialog appears that prompts you to select if you want to save the changes. From this dialog select the desired save mode and click **Yes**.

   - After completing the changes for the last operation, save the results via **File > Save Results.**

     The Process Spectral Method is then updated with all the modified parameter values. Moreover, for AutoCalc, these parameters can now be used by any chromatograms processed by the TC Method having the same name.

Note: For information on using AutoCalc to print automatically the Apex Spectra display for each sample in a running sequence, see the section titled **Using AutoCalc to print automatically the Apex Spectra display for each sample in a running sequence** on page **320** .

How do I use AutoCalc to automatically perform the desired operations during a TotalChrom sequence?

In general, you specify which arithmetic operations, chromatographic extractions and/or apex spectra print-outs you wish to automate via a user command line in the Process section of a TotalChrom method.

Note: *Normally you will not need to specify a specific IRIS Process Spectral Method as part of the command line. By default, IRIS will use the Process Spectral Method with the same name as the TotalChrom method that will be used to process the sequence. If the TotalChrom Method is unavailable, then AutoCalc operations will use the default spectral method.*

In order to implement AutoCalc:

1. From TotalChrom's main window (the "Navigator" screen), open the required method using Method Editor.

2. Select **Process > User Programs** from the menu bar.

3. Under **<Drive>\Program Files\PerkinElmer Instruments\Iris**, select **AutoCalc.exe** as a user program under **Program Name and Options**. *Note: The directory path may be somewhat different; depending on any changes opted during software installation.*
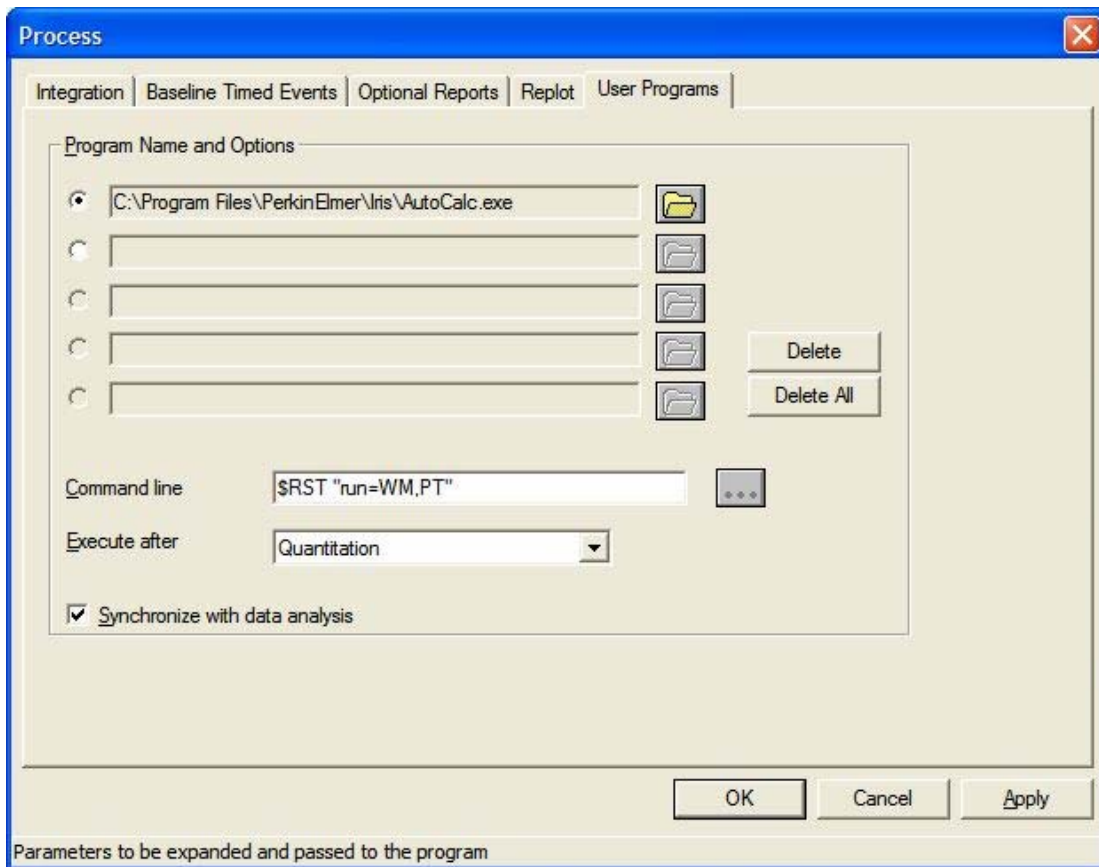
4. In the Command line text box, type **$RST "run="**, followed by any combination of operations codes, depending on the automated operations that are desired (see example, above).   Always end the last operation with a quote. For the corresponding entry codes, see the table below.

   When the program is executed by TotalChrom during an active sequence or Batch operation, $RST will prompt TotalChrom to pass the name and path of the current result file to AutoCalc. From the current result file, IRIS determines the name and path of the TotalChrom processing method used to create it.

Note: *The following format must be used when specifying which operations you wish to run in conjunction with an analysis:* ***$RST "run= X, Y, Z"*** *Where X, Y, Z represent various operations you can specify.*

Following is a list of the operations you can specify:

Codes for "run=	Description
PT	Peak Purity
AR	Absorbance Ratio
WM	Wavelength Maximum
SC	Spectral Standard Conformation
LC	Spectral Library Conformation
RT	Retention Time Adjustment
LM	Library Match
EC	Extract Chromatograms
Print	Print Apex Spectra with chromatogram (Spectra are not baseline corrected.) ***Note:*** *For more information on using this command, see page **320**.*
All	Executes all available calculations.

5. From the **Execute after** drop down, select **Quantitation**.

6. Click **OK** when finished.

7. Save the method.

   Now that you have saved the method, AutoCalc will be invoked for any sequence that is run, which uses this method.

Command Line Examples

Following is a list of example operations you can specify:

Command Line Examples	Description
$RST "run=PT,WM,Print"	Command for performing Peak Purity, Wavelength Maximum and Printing Apex Spectra.
$RST "run=SC,LC,EC"	Command for performing Spectral Standard Conformation, Spectral Library Conformation and Extract Chromatograms.
$RST "Method=C:\<path\file name>.tsm" "run=PT"	Command for executing a specified IRIS Process Spectral Method and performing Peak Purity."
$RST "debug=yes" "run=pt,print"	Command for displaying the debug window when AutoCalc runs. These command allow you to check that AutoCalc is performing as you requested. When these commands are specified the debug window will be displayed after the Peak Purity operation is performed and after the apex spectra output is printed.

Using AutoCalc to print automatically the Apex Spectra display for each sample in a running sequence

Using AutoCalc it is possible to print automatically the Apex Spectra display for each sample in a running sequence. This is accomplished by specifying a **Print** command in the AutoCalc command line in the user Program section of a TotalChrom Method. However, before you add the Print command to the AutoCalc Command line, you must follow the steps outlined in this section to specify the printing parameters that AutoCalc will use during a running sequence.

Steps you must perform before you can automate the printing of chromatograms annotated with apex specta

As with all AutoCalc parameters, the printing details are stored in an IRIS Process Spectral Method. Therefore, before you can use AutoCalc to automatically print the apex spectra display for each sample in a sequence, you must first update the IRIS Process Spectral Method, that has the same name as the TotalChrom Method that will be used to process the sequence, with certain details of that output including what printer should be used.

To update an IRIS Process Spectral Method that will be used by AutoCalc for automated printing of apex spectra in TotalChrom:

1. In IRIS, open a chromatogram that has been processed with the same TotalChrom method that will be used to run or reprocess the sequence.

2. Next, verify the name of the chromatogram's associated TotalChrom method. To do this, first, right mouse click on the chromatogram in the IRIS Data Tree and select **Information** from the context menu that appears.

   A Chromatogram Information dialog appears that shows the TotalChrom method associated with the selected chromatogram. **Make sure that this method name is the same name as the TotalChrom method that will be used to process the sequence.**

3. Now, exit the Chromatogram Information dialog box.

4. Make sure that the chromatogram that you just selected in step 2 is still selected (highlighted in blue) on the Data Tree.

5. From the IRIS menu bar select **File > AutoCalc Print Setup…**
   The AutoCalc Print Setup dialog appears.
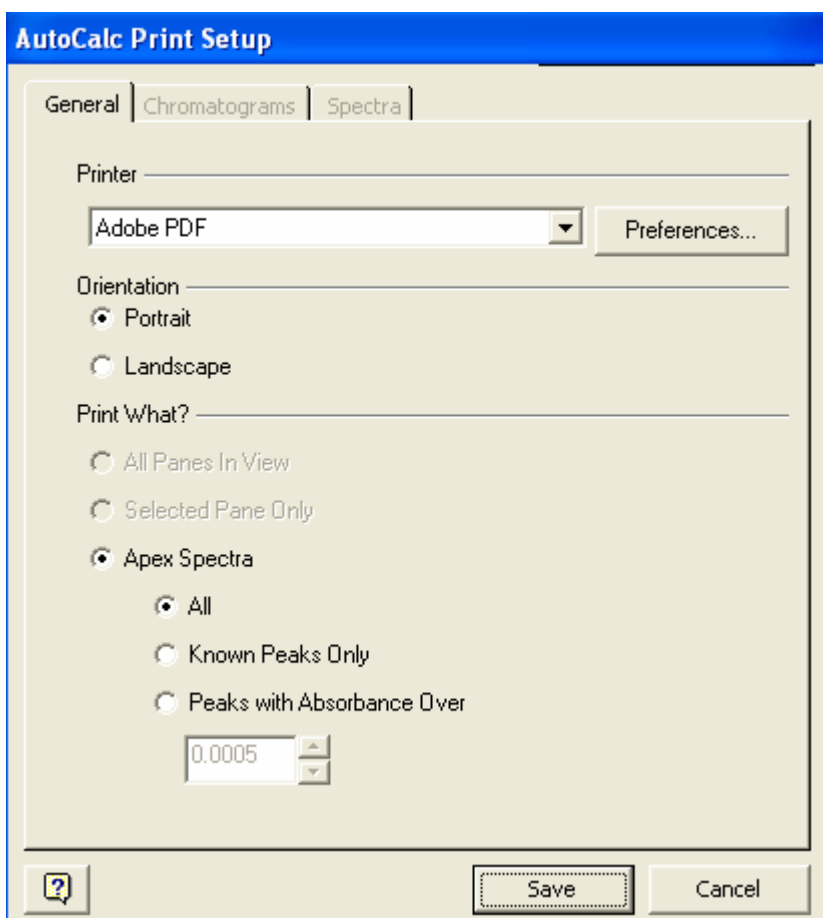
6. Select a printer from the **Printer** drop down list and then, if desired click on the **Preferences** button to define the settings for the printer.

7. Select one of the following Orientation options: **Portrait** or **Landscape**.

8. Under the **Print What** section, notice that the Apex Spectra radio button is the only option enabled on this dialog. From this section you define which apex spectra will be included during a running sequence that will be processed by the same TotalChrom method that was used to process the chromatogram you opened in Step 1.

    You can select 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 a specific absorbance limit.**

9. Click **Save** when are finished specifying the printing parameters.

    The printing parameters are saved to the Process Spectral Method that you identified in Step 2. Moreover, for AutoCalc, these parameters can now be used by any chromatograms that will be processed by the TotalChrom Method having the same name as the Process Spectral Method you just updated.

Chapter 10. Printing and Presentations

Printing and Presentation Options

IRIS lets you easily create high-quality presentation output.

This chapter shows you how to:

- Add your own annotations to IRIS views

- Copy chromatograms and spectra to other programs

- Print chromatograms and spectra at high resolution and in color

Annotations

IRIS allows you to 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 relative to the left and top edges of the pane.

Note: It should be noted that annotations are associated with a pane; annotations on a chromatogram are not associated with that chromatogram. For example, annotations on a chromatogram pane, in the Chrom/Spectra View, will not be displayed in the any other view. In addition, when you leave a view any annotations you had placed on a pane in that view are cleared.

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 on the pane.

4. Click and drag the annotation 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, style, and text of any annotation you have placed on the screen 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.

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 by selecting the annotation you wish to delete and then selecting **Actions > Annotations > Delete** from the menu bar.

➢ If you wish to delete all annotations that appear on a page select **Actions > Annotations > Delete All**.

Copying IRIS Images to other Locations

IRIS allows you to 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 paste the image into an application such as Paint.

How do I export a chromatogram or spectrum in numerical format for use in spreadsheet programs?

To export a displayed chromatogram or spectrum in numerical format for use in spreadsheet programs:

You can export a displayed chromatogram or spectrum to spreadsheet programs such as Microsoft Excel by using the **Edit > Copy Object** command. This command copies the chromatogram or spectrum in numerical format to the clipboard.

1. If you are using a stacked display, click on the chromatogram or spectrum that you want to copy.

   OR

   If you are using an overlaid display, where only one chromatogram or spectrum is currently visible, click on that pane.

2. From the menu bar select **Edit > Copy Object**.

   The chroatogram or spectrum is copied in numerical format 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.
   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 from IRIS

On any view or operation in IRIS you can select **File > Print** to specify the format for printing chromatograms and spectra that are currently displayed on the IRIS window.

Note: *We recommend that you use a color printer, since a black and white printer will not allow you to distinguish between multiple plots, if more than one chromatogram or spectrum is being printed.*

➢ To print chromatograms and spectra from a view or operation select **File > Print...** from the IRIS menu bar.

The Print dialog displays. On this dialog you define the format and printer that will be used to generate a print out of data currently displayed in the IRIS window.
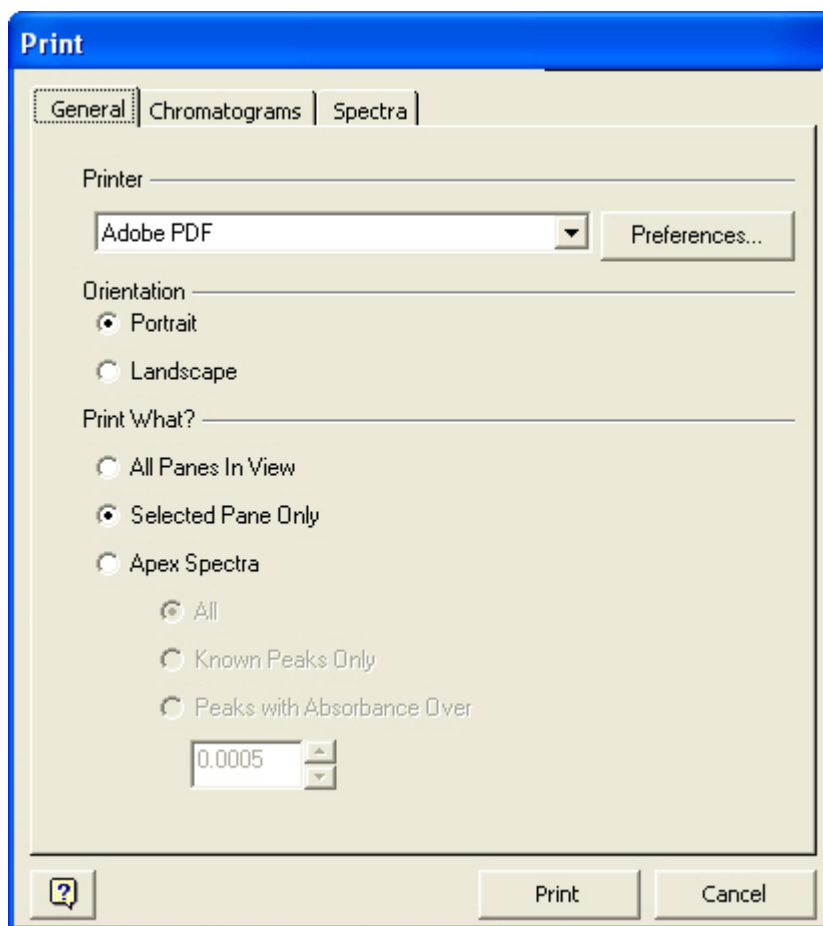
Figure 11-1 The Print dialog

What information is included on printouts?

After you have defined your printing preferences on the Print dialog and clicked on the **Print** button, a print out of your selections is generated. On the first page of each print out the following information is provided:

- A title for the document being printed.

  This title is set by IRIS.

- The complete color-coded 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.

Note: *This page does not apply to Apex Spectra printouts.*

- 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*

Operation:	Chrom/Spectra View	
Date:	07/11/2006, 12:57:18 AM Eastern Standard Time	
User:	manager	
Page:	1 of 2	

Chromatograms

■	Filename:	example 1 - identified and well separated.rst
	Path:	c:\iris\data\data\data\data\

Spectra

■	Filename:	example 1 - identified and well separated.rst - 3.696
	Path:	c:\iris\data\data\data\data\
■	Filename:	example 1 - identified and well separated.rst - 5.082
	Path:	c:\iris\data\data\data\data\
■	Filename:	example 1 - identified and well separated.rst - 2.383
	Path:	c:\iris\data\data\data\data\

Figure 11-2 An example of the first page that is printed when a user selects to print spectra currently displayed on the Chrom/Spectra View

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*.

Operation: Chrom/Spectra View
Date: 07/11/2006, 01:03:23 AM Eastern Standard Time
User: manager
Page: 2 of 2
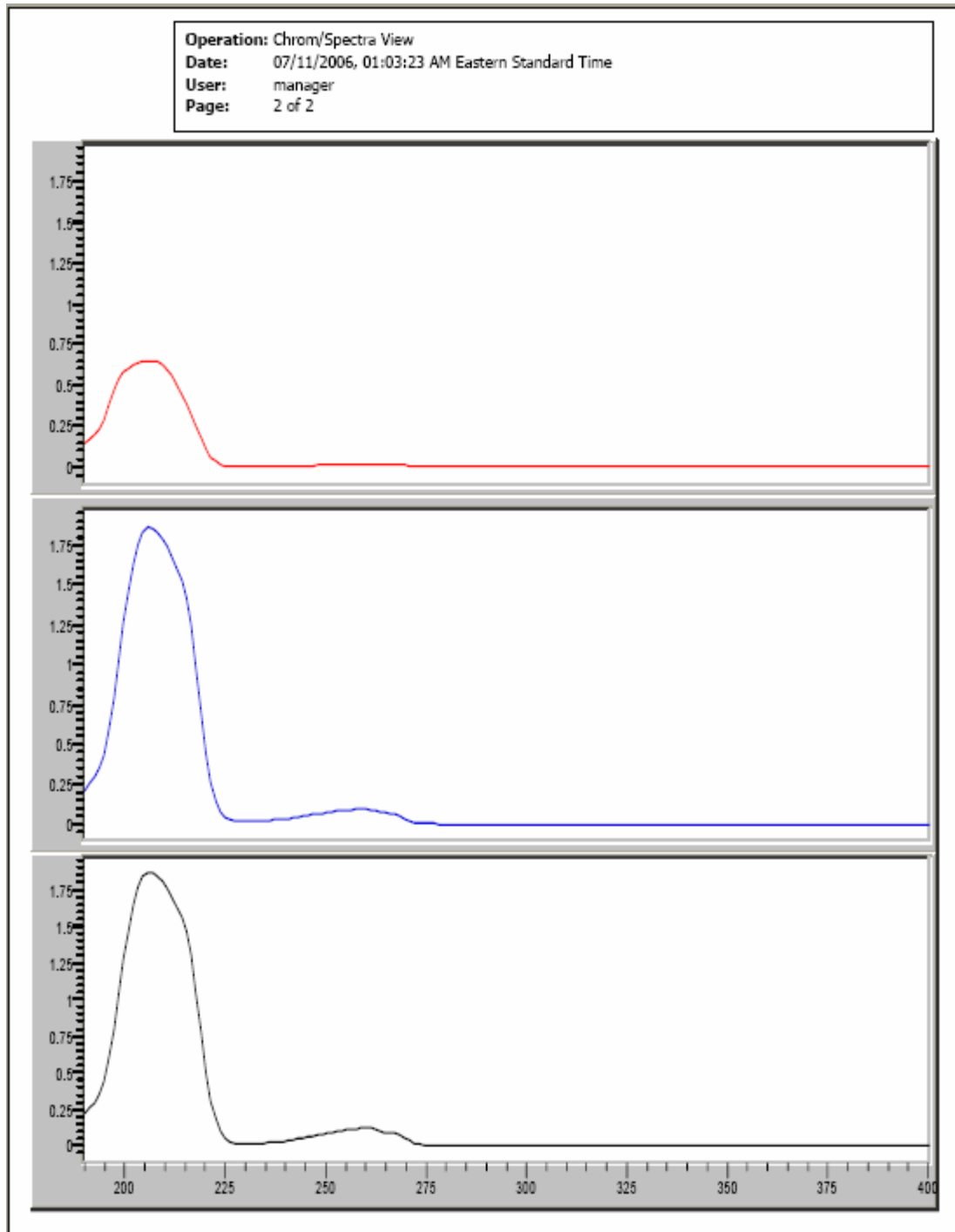
Figure 11-3 Example showing the second page of a print out, where a user selected to print the contents in the currently selected Spectra pane from the Chrom/Spectra View using a Stacked display.

Tell me about the Print dialog.

When you select **File > Print…** from the IRIS menu bar the Print dialog displays. This dialog consists of three tab pages. The **General** tab page is always enabled and from this tab page you can define the printer you want to use as well as the orientation for your print out, and define what you want to print. If you select **Selected Pane** only on the General tab page, the **Chromatograms** tab page and **Spectra** tab page are enabled; and these tab pages allow you to define the format for printing the contents of a chromatogram pane or a spectra pane.

Note: *The settings defined on the Chromatograms tab page apply to print outs only when a Chromatogram pane is currently selected on the view from where the Print dialog was accessed. The settings defined on the Spectra tab page apply only when a Spectra pane is currently selected on the view from where the Print dialog was accessed.*

Following is an overview of the setting available to you on the Print dialog.

General tab page

This tab page is always enabled and allows you to select where and what you would like to print.
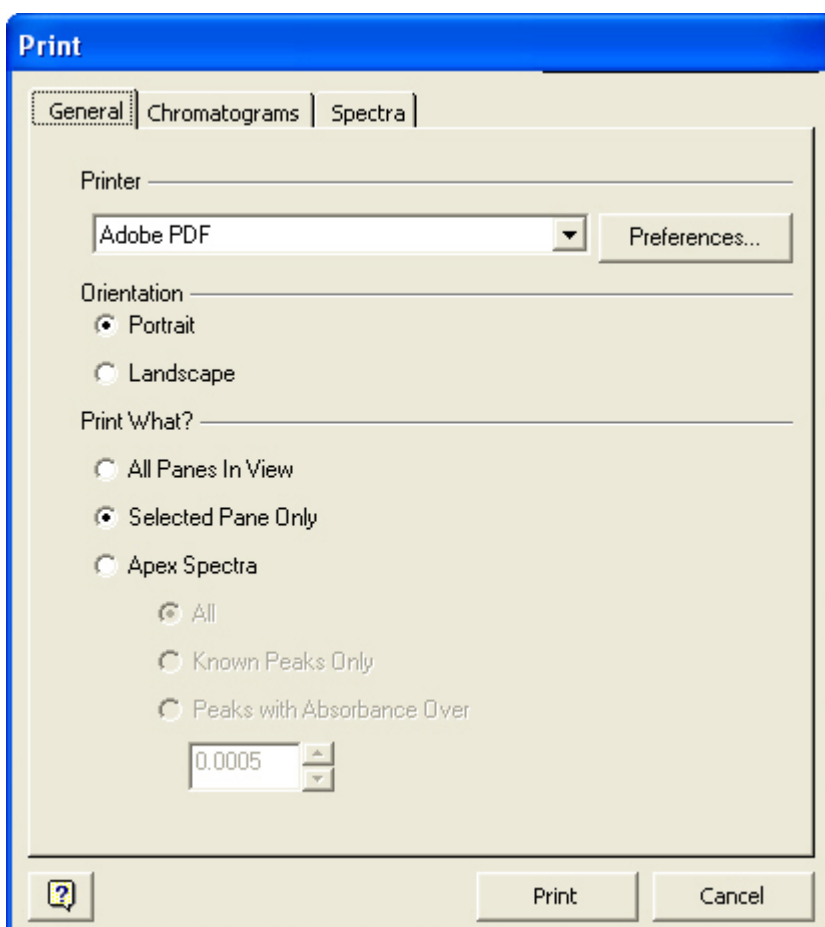
Figure 11-4 The General tab page

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.

Orientation – Select to print either in Portrait mode or Landscape mode.

Print What - Select one of the following radio buttons:

All Panes in View- Prints the contents of the current IRIS window, except for the Views Tree and Data Tree.

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. These tab pages allow you to define how you want the chromatograms and spectra to be presented on the print-out.

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 spectra that are above the set absorbance limit.

Note: *Spectra are always printed over their complete range, 190-400, 400-700, or 190-700.*

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 and displayed on the IRIS window.

    Example: In the example below, we selected a Chromatogram pane on the Chrom/Spectra View that contained two overlaid chromatograms. We then selected **File > Print**, **Selected Pane Only**. From the **Chromatogram** tab page on the **Print** dialog, we then seelcted the **As View** option. The first page of the print out contained a color coded key identifying each chromatogram included on the print out, and the second page is shown below:

Overlaid - Prints the chromatograms, which are displayed on the currently selected Chromatogram pane, using an overlaid display.

For an example of what the Overlaid print out would look like, refer to the previous example for As View.

Stacked - Prints the chromatograms, which are displayed on the currently selected chromatogram pane, using a stacked display.

Example: In the example below, we selected a Chromatogram pane on the Chrom/Spectra View that contained two overlaid chromatograms. We then selected **File > Print**, **Selected Pane Only**. From the **Chromatogram** tab page on the **Print** dialog, we then seelcted the **Stacked** option. The first page of the print out contained a color coded key identifying each chromatogram included on the print out, and the second page is shown below:
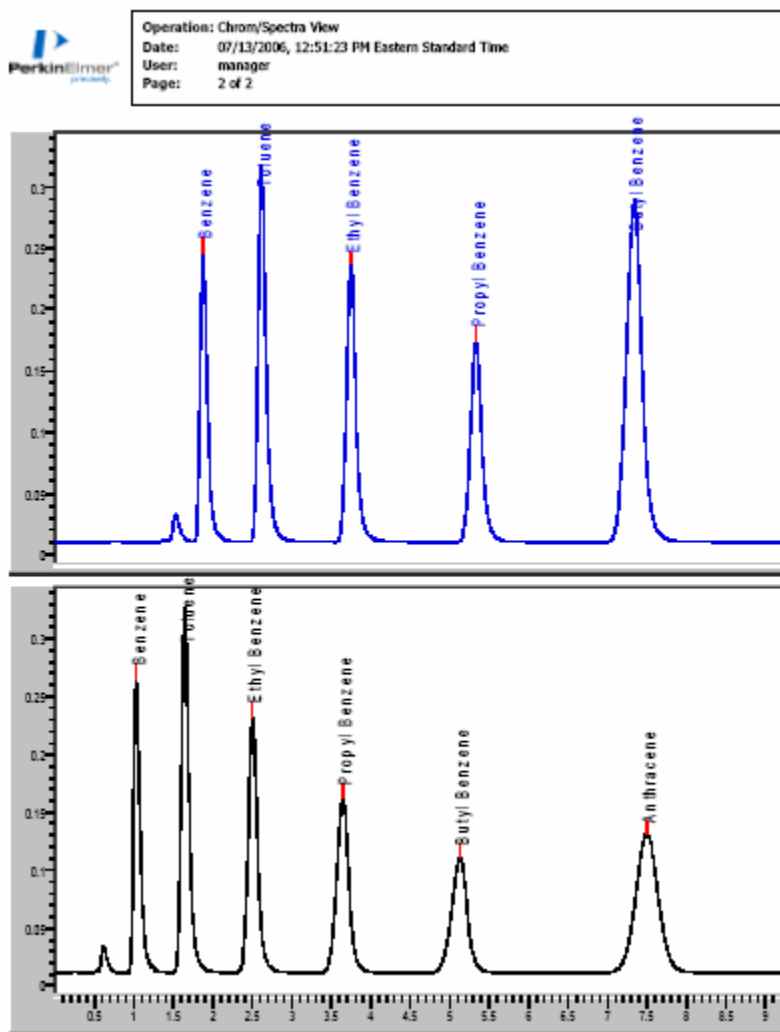
Note: *This option will print all of the chromatograms that are displayed in the selected pane on one page. However, you can use the **Separate Plots** option to define exactly how many chromatograms you want displayed on a printed page.*

Separate Plots - Prints each chromatogram that is displayed on the currently selected Chromatogram pane on separate plots. When this option is selected the **per page** spin box is enabled; and from this spin box you specify the number of plots that will be printed on each page.
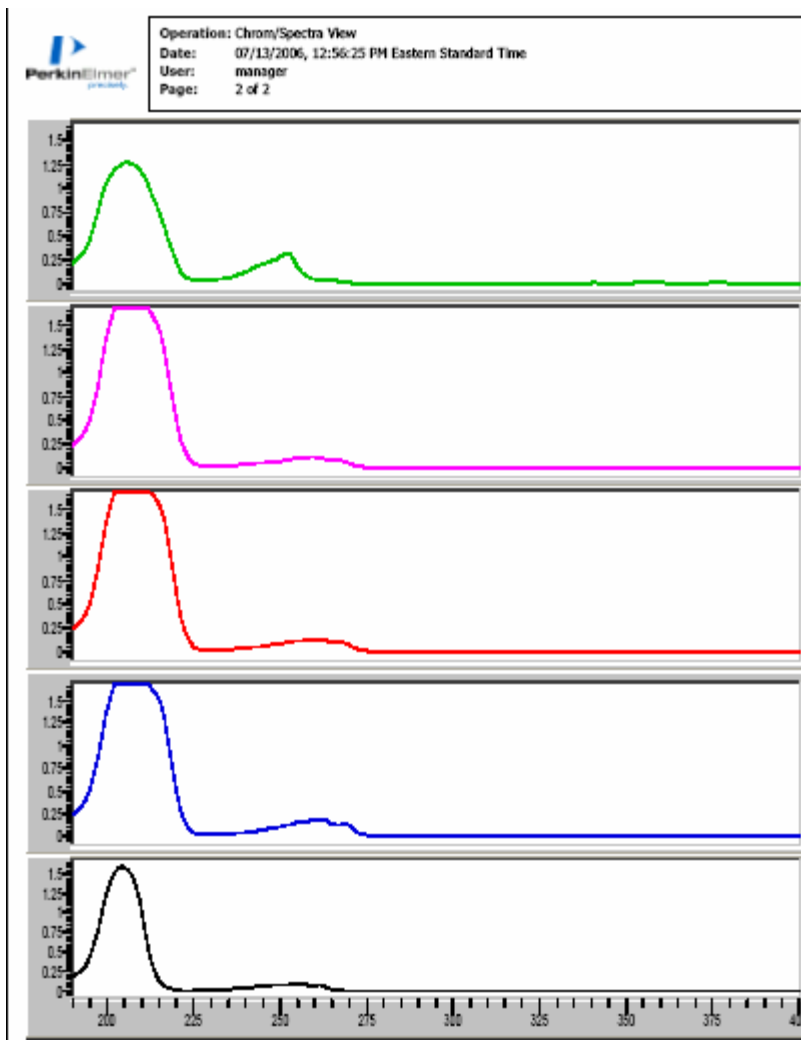
Example: In the example below, we selected a Chromatogram pane on the Chrom/Spectra View that contained two overlaid chromatograms. We then selected **File > Print**, **Selected Pane Only**. From the **Chromatogram** tab page on the **Print** dialog, we then seelcted the **Separate Plots** option with **2 plots per page**. The first page of the print out contained a color coded key identifying each chromatogram included on the print out, and the second page is shown below:

Note: If you had more than two chromatograms that you wanted to print, and you selected to print 2 Separate Plots per page, then your report would consist of multiple pages, with two chromatograms displayed on each page.
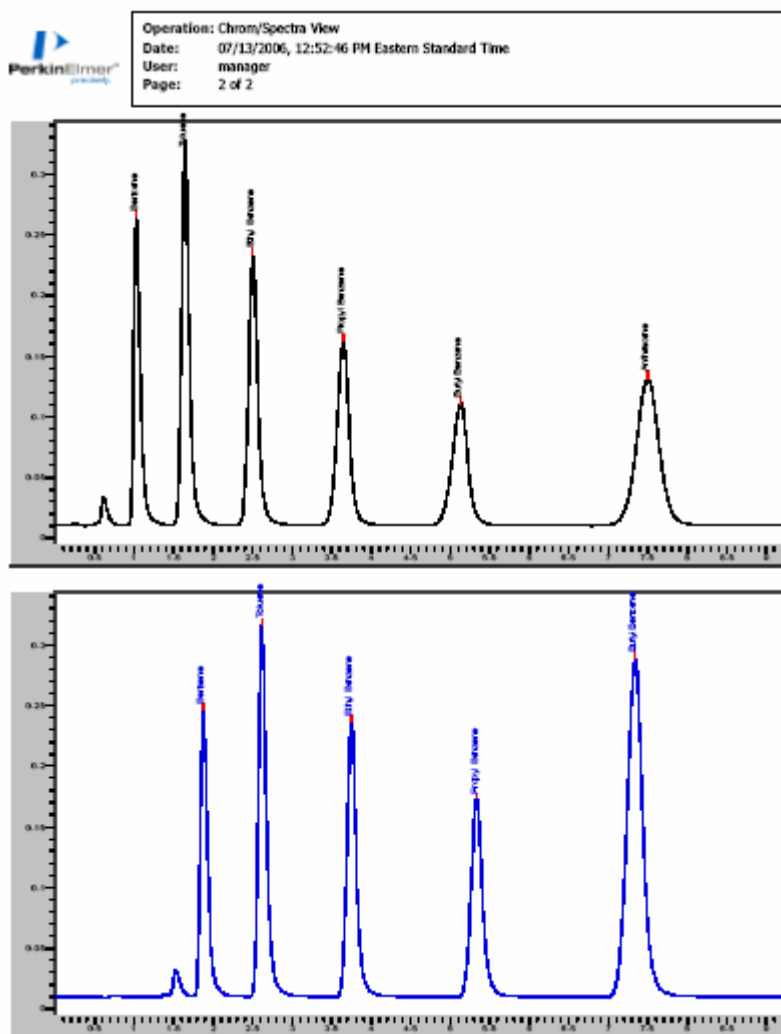
Labels - From this section you specify which label will be displayed on the printout.

> **As View** - Prints the label currently displayed on the view for the selected Chromatogram pane.

> **Specified** - Enables a drop down menu from where you select a specific label for the printout.

Spectra tab page

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.
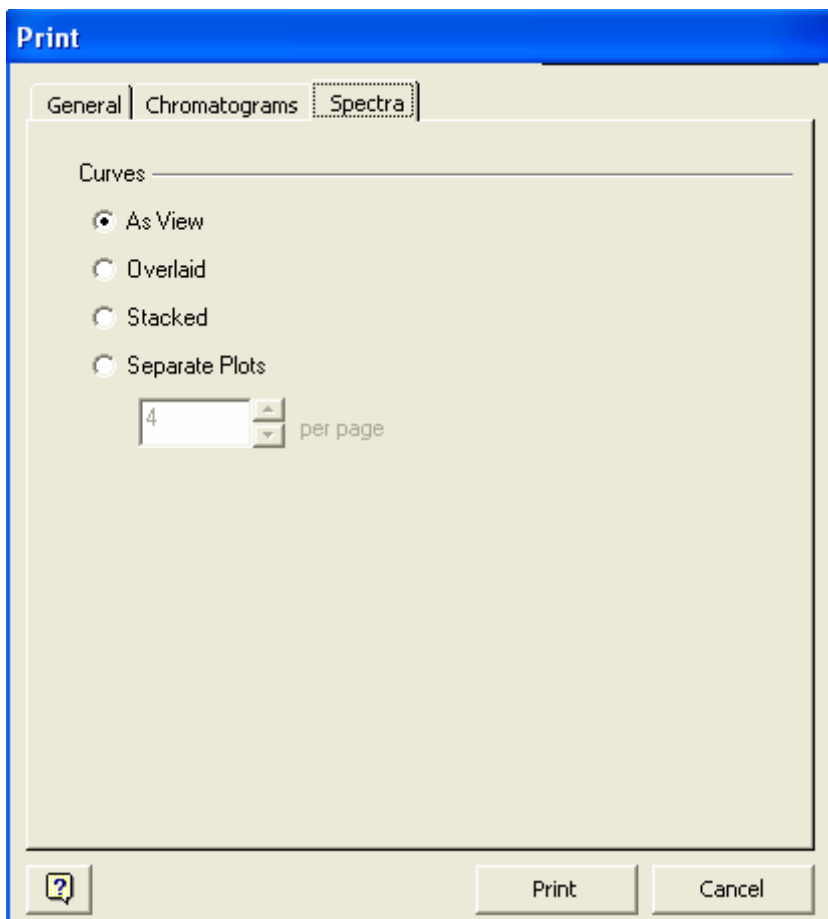
Figure 11-6 The Spectra tab page is enabled when you select the Selected Pane only radio button on the General tab page; and can be used when a Spectra pane is selected on the current view

The following options are available to you on the Spectra tab page:

As View - Prints an image of the Spectra pane that is currently selected and displayed on the IRIS window.

Example: In the example below, we selected a Spectra pane on the Chrom/Spectra View that contained five overlaid spectra. We then selected **File > Print**, **Selected Pane Only**. From the **Spectra** tab page on the **Print** dialog, we then selected the **As View** option. The first page of the print out contained a color coded key identifying each spectrum included on the print out, and the second page is shown below:

Overlaid - Prints the spectra, which are displayed on the currently selected Spectra pane, using an overlaid display.

Stacked - Prints the spectra, which are displayed on the currently selected Spectra pane, using a stacked display.

Example: In the example below, we selected a Spectra pane on the Chrom/Spectra View that contained five overlaid spectra. We then selected **File > Print**, **Selected Pane Only**. From the **Spectra** tab page on the **Print** dialog, we then selected the **Stacked** option. The first page of the print out contained a color coded key that identifying each spectrum included on the print out, and the second page is shown below:

Separate Plots - Prints each spectrum that is displayed on the currently selected Spectra pane on separate plots. When this option is selected the **per page** spin box is enabled; and from this spin box you specify the number of plots that will be printed on each page.

Example: In the example below, we selected a Spectra pane on the Chrom/Spectra View that contained five overlaid spectra. We then selected **File > Print**, **Selected Pane Only**. From the **Spectra** tab page on the **Print** dialog, we then selected the **Separate Plots** option and selected to print **2** spectra **per page**. The first page of the print out contained a color coded key identifying each spectrum included on the print out, and the second page is shown below (this report also contained a third page displaying two spectra and a fourth page displaying the final spectrum):

Note: *Since the example above contained more than two spectra the report consisted of four pages. The first page contained a color coded key identifying each spectrum in the print out. The second and third pages contained two spectra per page, and the last page contained the firth spectrum that we selected to print.*
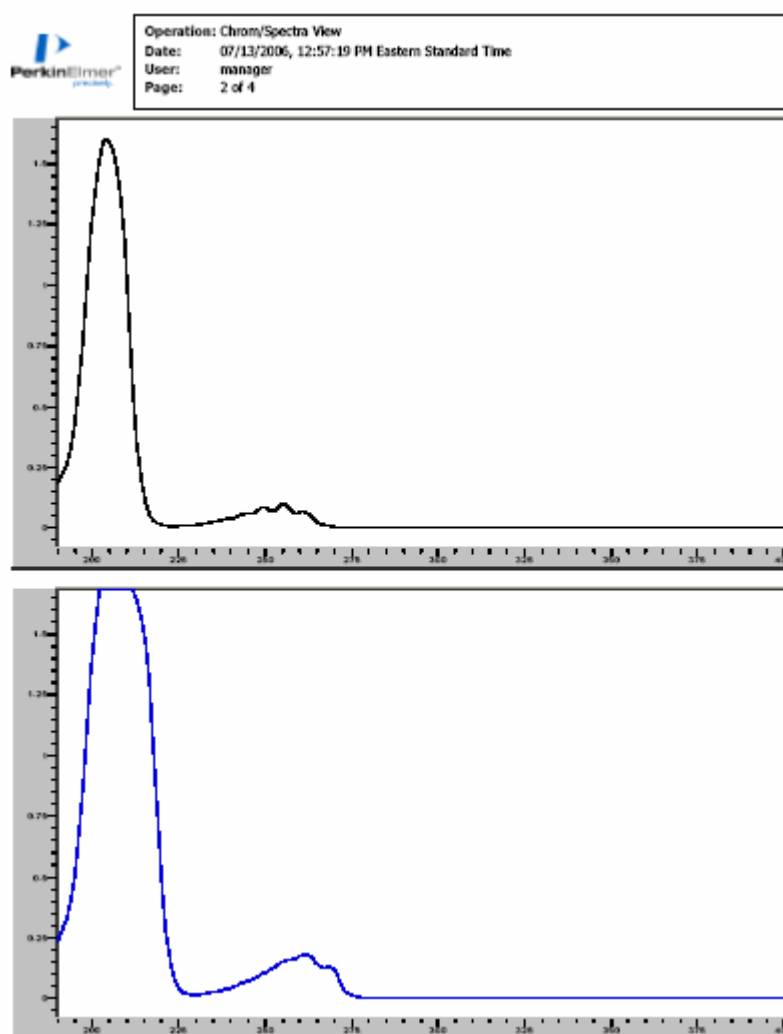
How do I print the current view or operation?

If you want to print the contents of the current view or operation:

You can select to print the contents of the current IRIS window, except for the Views Tree and Data Tree by doing the following:

1. From the current view/operation select **File > Print…**
   The Print dialog displays.
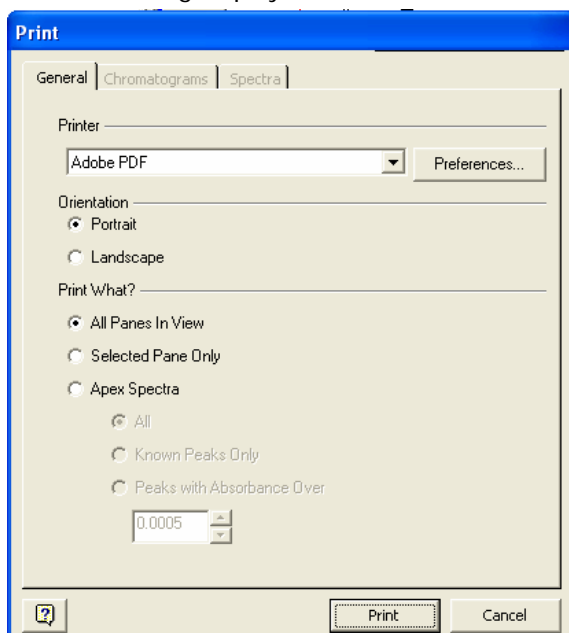
2. Under the **Printer** drop down menu, select a printer.

3. Click on the **Preferences** button to define the settings for the printer.

4. Select the Orientation for the print out as either **Portrait** or **Landscape.**

5. Select **All Panes in View** to print the contents of the current IRIS window, expect for the Views Tree and Data Tree.

Note: *If you are printing the display from an operation, the only option that is enabled is **All Panes in View***

6. Click **Print** when you are finished.

How do I print the contents of a single pane from any of the IRIS views?

On any of the IRIS views you can select to print only the contents of the currently selected pane. If you have selected a Chromatogram pane, or a Spectra pane, you can define how the contents of the currently selected pane will be printed. On the other hand, if the pane you have currently selected is a 3D Plot pane or a Contour Map pane, the display, currently shown on the IRIS window for the selected pane, is printed when you select **File > Print** and select the **Selected Pane Only** option on the Print dialog.

To print the contents of the currently selected pane:

1. From the current view select the pane you want to print by clicking anywhere inside the white space of the pane.

   A blue border appears around the selected pane.

2. From the IRIS menu bar select **File > Print…**

   The Print dialog displays.
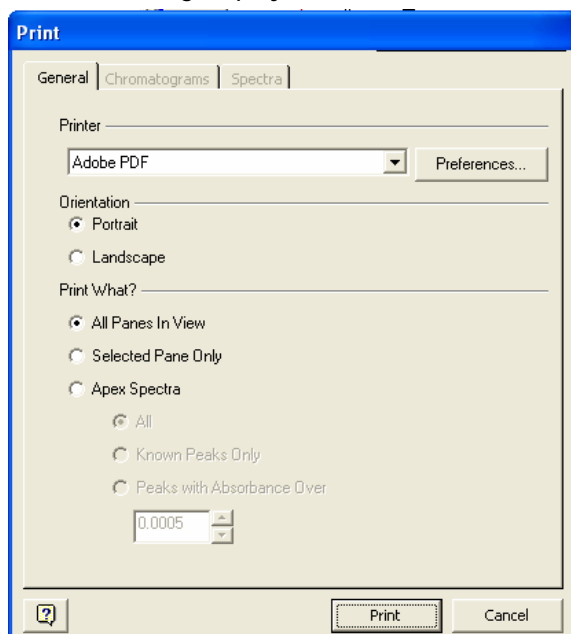
3. Under the **Printer** drop down menu, select a printer.

4. Click on the **Preferences** button to define the settings for the printer.

5. Select the Orientation for the print out as either **Portrait** or **Landscape.**

6. Select **Selected Pane Only** to print the contents of the pane that is currently selected.

   When this option is selected, the **Chromatograms** and **Spectra** tab pages are enabled. These tab pages allow you to define how you want the chromatograms and spectra to appear on the printnout, if a Chromatogram pane or Spectra pane is the currently selected pane.

7. If a **Chromatogram pane** is currently selected click on the **Chromatograms** tab page and skip to the section titled: *To define the format for printing the currently selected chromatogram pane:* page *343*.

   OR

   If a **Spectra pane** is currently selected, click on the **Spectra** tab page and skip to the section titled: *To define the format for printing the currently selected spectra pane:*on page *344*.

   OR

   If a Contour Map pane or 3D Plot pane is selected, click on the **Print** button to print the current display for the selected pane.

To define the format for printing the currently selected chromatogram pane:

This section applies only to views where a chromatogram pane is currently selected.

1. From the current view click on the Chromatogram pane that contains the chromatograms that you want to print.
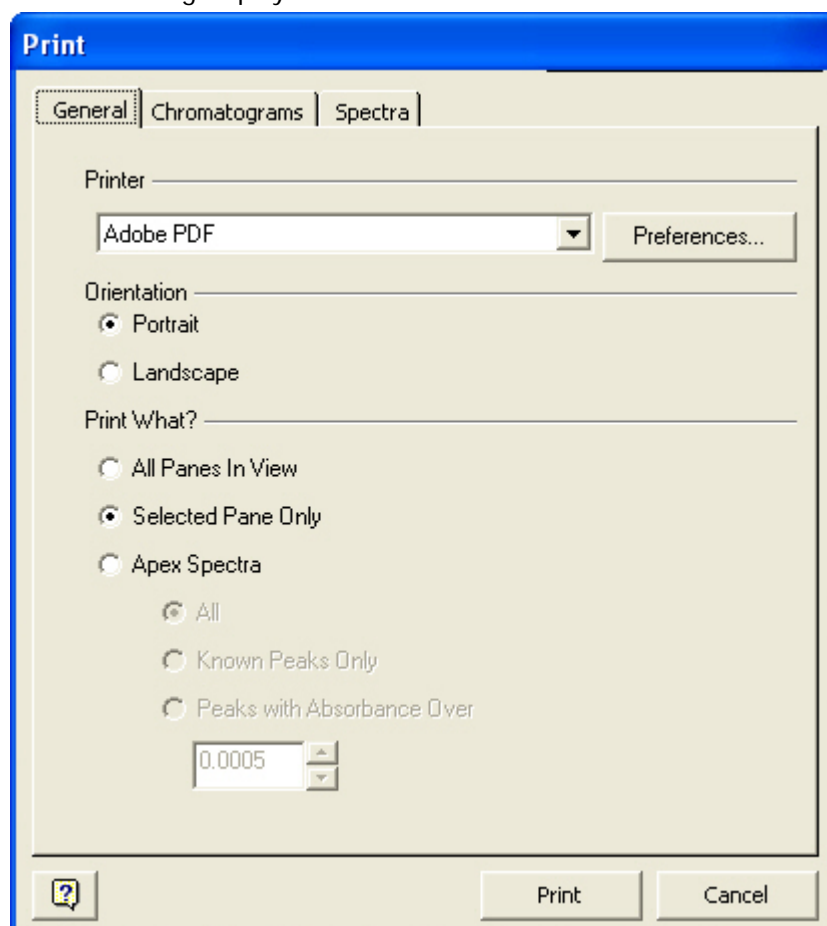
2. Click **File > Print**.
   The Print dialog displays.

3. On the Print dialog click **Selected Pane** only.

   The Chromatograms tab page is enabled.

4. Click on the **Chromatograms** tab page.

   The Chromatograms tab page displays:
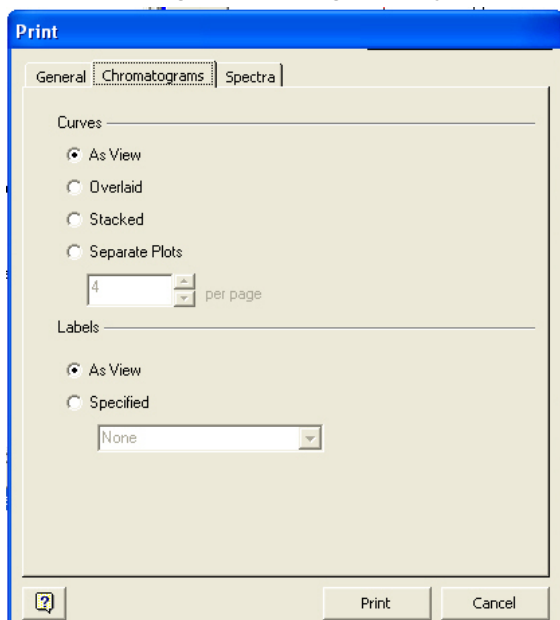
From the Chromatograms tab page you can select whether or not you want the chromatograms to be printed as overlaid on one plot, stacked on one plot, or printed on separate plots. You can also specify which label will be displayed on the printed chromatograms.

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 the contents of the current chromatogram pane that is selected on IRIS window.e and Data Tree.

> **Overlaid** - Prints the chromatograms, which are displayed on the currently selected chromatogram pane, using an overlaid display.

> **Stacked** - Prints the chromatograms, which are displayed on the currently selected chromatogram pane, using a stacked display. All of the chromatograms are displayed on one page.

> **Separate Plots** - Prints each chromatogram on a separate plot. When this option is selected the **per page** spin box is enabled; and from this spin box you specify the number of individual graphs 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 IRIS workspace for the selected chromatogram pane.

> **Specified** - Enables a drop down menu from where you select a specific label for the printout.

5. When finished, click **Print**.

To define the format for printing the currently selected spectra pane:

This section applies only to views where a Spectra pane is currently selected.

1. From the current view click on the Spectra pane that contains the spectra that you want to print.
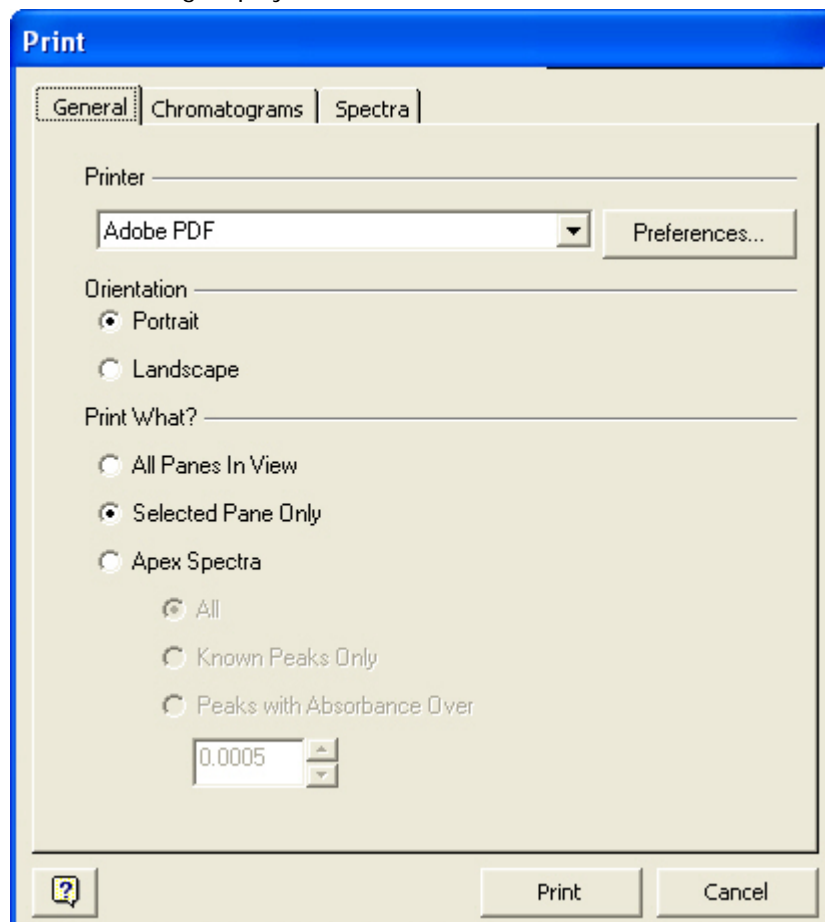
2. Click **File > Print**.

   The Print dialog displays.

3. On the Print dialog click **Selected Pane** only.

   The Chromatograms tab page is enabled.

4. Click on the **Spectra** tab page.

   The Spectra tab page displays.
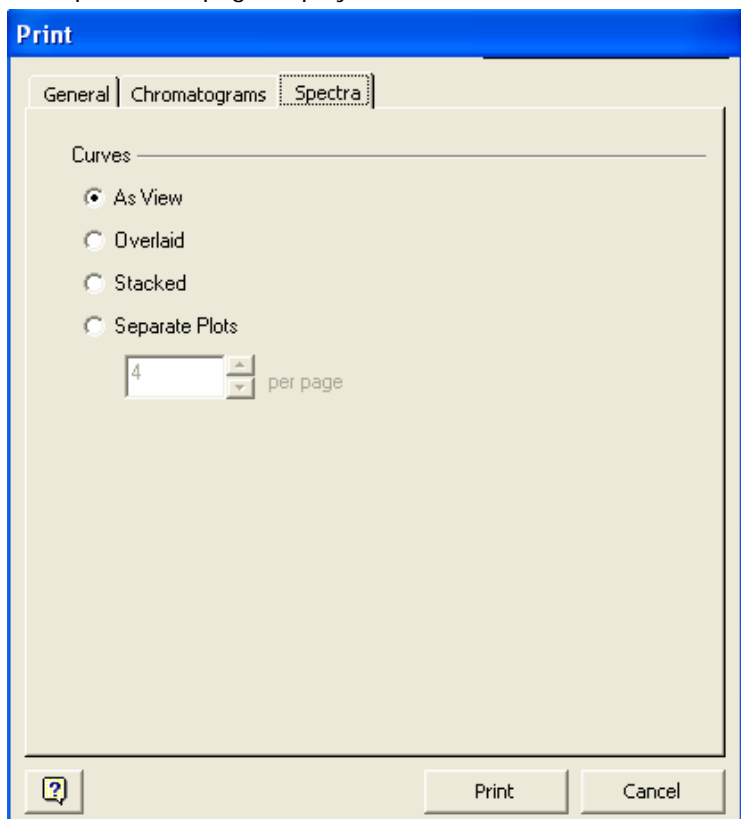
From the Spectra tab page you can define the print layout for the spectra displayed in the currently selected pane. You can select to print the currently displayed spectra overlaid on one plot, or stacked on one plot, or you can select to print separate plots for each currently displayed spectrum.

The following options are available to you on the Spectra tab page:

As View - Prints an image of the Spectra pane that is currently selected and displayed on the IRIS window.

Overlaid - Prints the spectra, which are displayed on the currently selected Spectra pane, using an overlaid display.

Stacked - Prints the spectra, which are displayed on the currently selected Spectra pane, using a stacked display. All of the spectrta are printed on one page.

Separate Plots - Prints each spectrum that is displayed on the currently selected Spectra pane on separate plots. When this option is selected the **per page** spin box is enabled; and from this spin box you specify the number of plots that will be printed on each page.

5. When finished, click **Print**.

How do I print a chromatogram and its associated apex spectra?

1. From the Data Tree click on the chromatogram you want to print along with its associated apex spectra from the Data Tree.

2. From the IRIS menu bar select **File > Print...**
   The Print dialog appears.
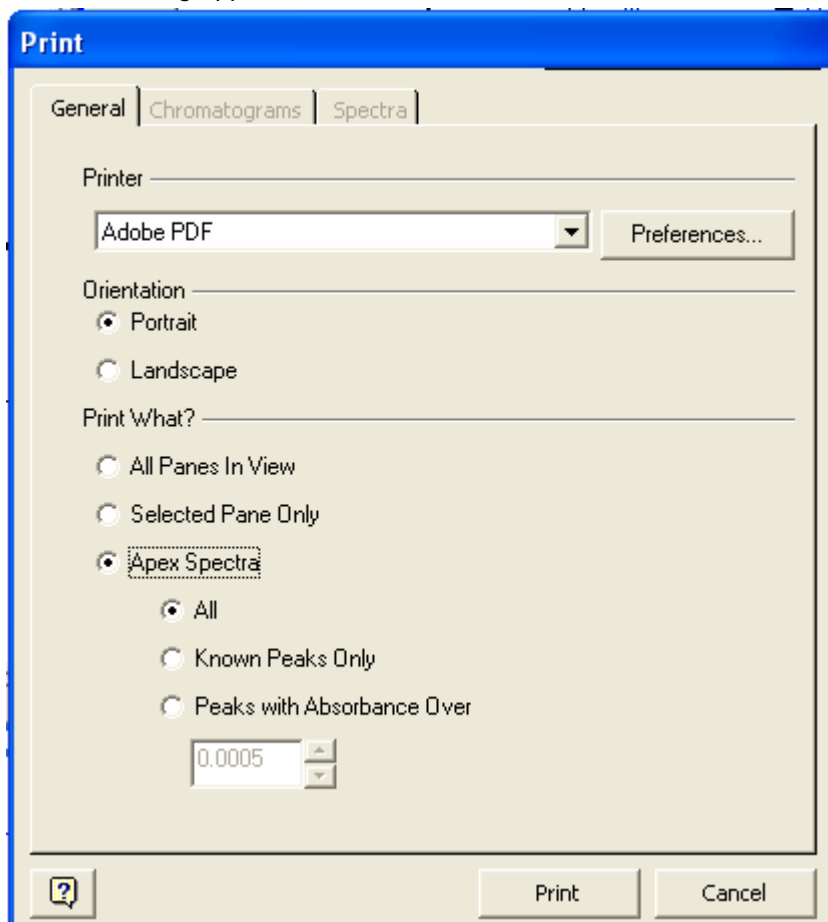
3. Click on **Apex Spectra** to print the chromatogram that is currently selected on the Data Tree and its associated apex spectra.

4. When **Apex Spectra** 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 spectra that are above the set absorbance limit**.

Note: *Spectra are always printed over their complete range, 190-400, 400-700, or 190-700.*

5. When you have finished defining the print output, click **Print**.

A report similar to the screen shown below is generated:
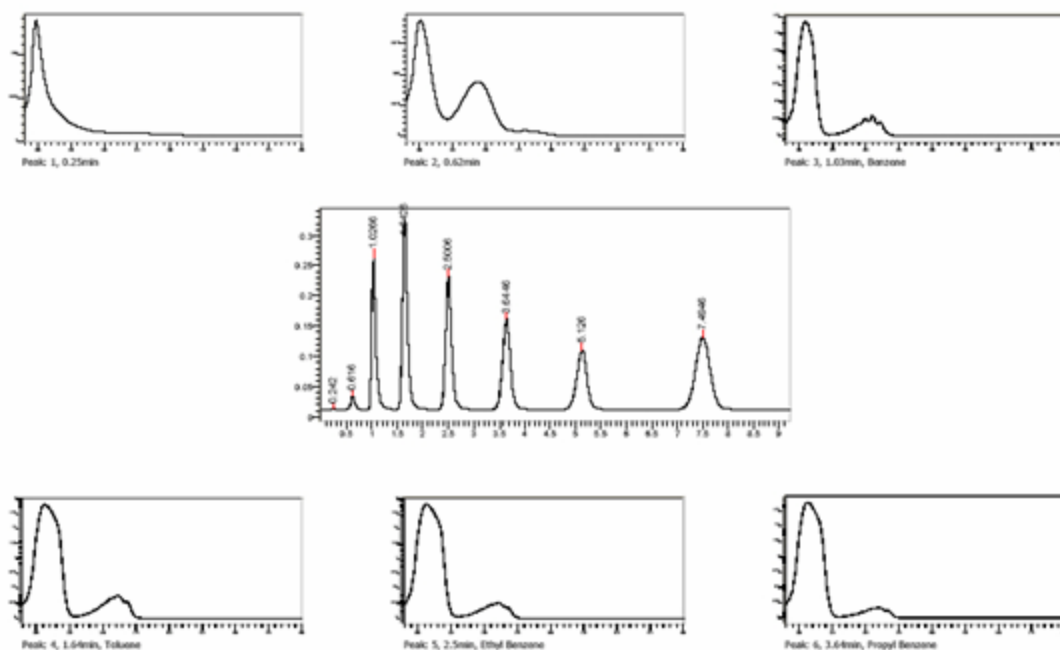
Appendix 1: Calculation Algorithms

Calculation Algorithms

This Appendix includes information on two major calculation algorithms used in IRIS:

- IRIS Purity Algorithm

- Euclidean Distance Algorithm

IRIS Peak Purity Algorithm[1]

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 IRIS, you can therefore select where on the peak you want to take the two spectra.

To check the peak purity, IRIS 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 IRIS.

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[1] *Poile, A.F. and R.D. Conlon,* J. Chrom., **204** *(1981): 149–152.*

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 within IRIS, 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.

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