Agilent OpenLAB and Agilent EZChrom *Elite*

PDA Analysis

Agilent Technologies

Contents

1   Using This Guide

Introduction
The PDA software provides functionality to extract and analyze spectral data from Diode Array Detectors.

Who Should Read This Guide?
This document is designed for users of the PDA analysis software.

2   About the PDA Software

The PDA software enables you to view and analyze spectra generated with a photo diode array detector on your LC. In order to use the PDA analysis software, you must have control software installed for your LC that contains PDA detector capabilities.

To turn on the PDA analysis option,

1. Open an instrument configuration.
2. In the **Instrument Configuration** window, click **Configure**.
3. In the available/configured modules configuration window, click **Options.**
4. Select **PDA** and then click **OK**.
5. Configure a PDA detector for your LC instrument. For details on configuration of your particular instrument, see the control help for that instrument.
6. In the **Instrument Window** choose **PDA Options** from the **Method** menu to set post-run PDA analysis parameters. Note that data acquisition parameters for the method may need to be set in **Instrument Setup** according to the requirements of the control software for your PDA detector.

Once the PDA analysis software has been turned on, you can

- View spectra and chromatograms in a variety of formats.

- Create and search spectral libraries.

- Perform on-demand peak purity calculations and program peak purity calculations to occur as part of analysis.

- Set up spectral filtering and extract spectra from 3D data.

- Set Multi-Chromatogram Options

- Set up chromatogram ratios.

- Create custom PDA reports.

3   About PDA Views

The PDA views provide a variety of ways to view PDA data. You can choose to display one view at a time, or a combination of views, such as 3D and Contour or Mixed with 3D.

To select the view, choose **PDA View** from the **View** menu or by selecting **Views** from the PDA Toolbar. A right mouse click within any of the view windows provides a unique menu of options for that view. You must have a data file open in order to access PDA Views.

Mixed View

3D Views

Contour View

Max Plot View

Chromatogram View

Multi-Chromatogram View

Spectrum View

Ratio View

About the PDA Toolbar

Whenever one of the PDA Views is selected, a toolbar will appear with one or more menus and buttons that enable you to manipulate the view or perform various actions.
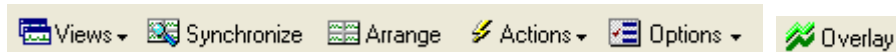
Click **Views** to select or change the current PDA View.

Click **Synchronize** to synchronize the axis and zoom limits with the other two plots. When the contour plot is zoomed, the time range of the chromatogram and the wavelength range of the spectrum plot are automatically adjusted to the same range as the contour. When synchronized zooming has been performed, the x axis plot limits for the chromatogram and spectrum plot are changed. To unzoom these plots to the limits of the data, you must select Full Unzoom from the contour plot context menu.

Click **Arrange** to restore the view windows to default positions. Use this after you have resized the windows by clicking and dragging the window borders.

The **Overlay** button appears when a Multi-chromatogram view containing multiple chromatograms is displayed. This toggles between chromatogram overlay view and separate chromatogram view.

Click **Actions** to open the Actions menu.

Click **Options** to open the Options menu.

About the 3D View

The 3D View provides a three-dimensional view of absorbance versus time and wavelength. Wavelengths of appreciable absorbance and interference, which may be invisible in a single wavelength plot, are easy to locate with the 3D View. The plot can be elevated and rotated around its axis for display from any angle.
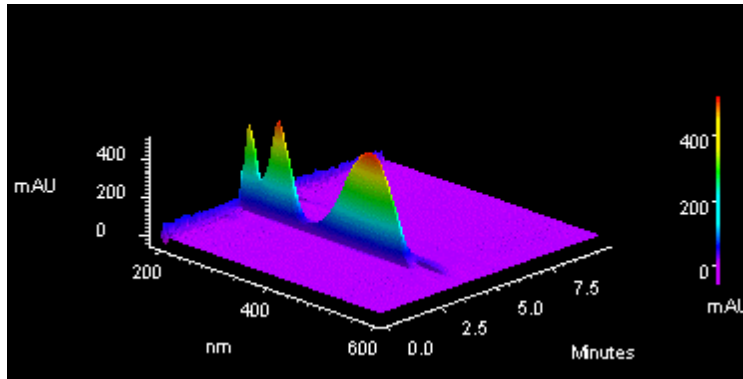
Note: During a run, the user must manually refresh this display. It does not update automatically. Right-click inside the 3D view to display the pop-up menu that gives you access to plot rotation and axis setup features.

3D Plot movement selections

Click on the type of plot movement you wish to use (elevate, roll, rotate, rotate XYZ, or spin). A checkmark will appear next to the selected movement option. Once you have selected one of the options, you will be returned to the plot and the cursor will indicate the type of movement selected.
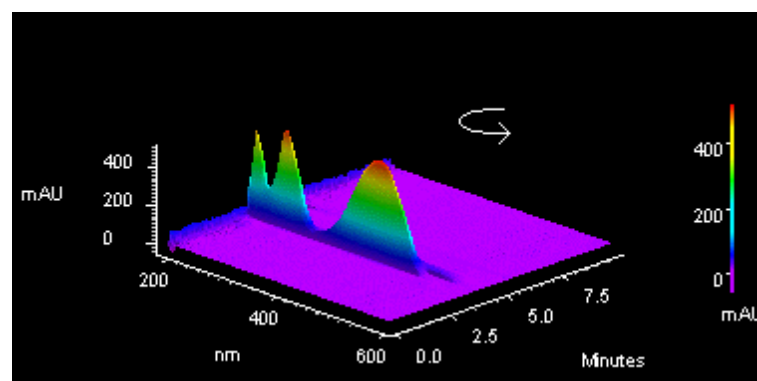
Hold down the left mouse button and move the cursor in the direction you wish to move the plot. The plot will move as you move the cursor. The movement option will remain in effect until you turn it off. When finished, click the right mouse button, and de-select the movement option. If you wish to return to the original view, select the **Reset** command.

Note: These values can be viewed or changed using the 3DPlot/Properties dialog.

Set the 3D Properties

To set or change the properties of the 3D data display,

7. From **View** menu, select **PDA View.**

8. Select **Mixed View** or one of the other views that includes **3D**.

9. Do a right mouse click on the 3D view and select **Properties**.

10. Click the **General** tab to enter or change the properties for the 3D plot. Click the **Axis Setup** tab to set or change the axis parameters.
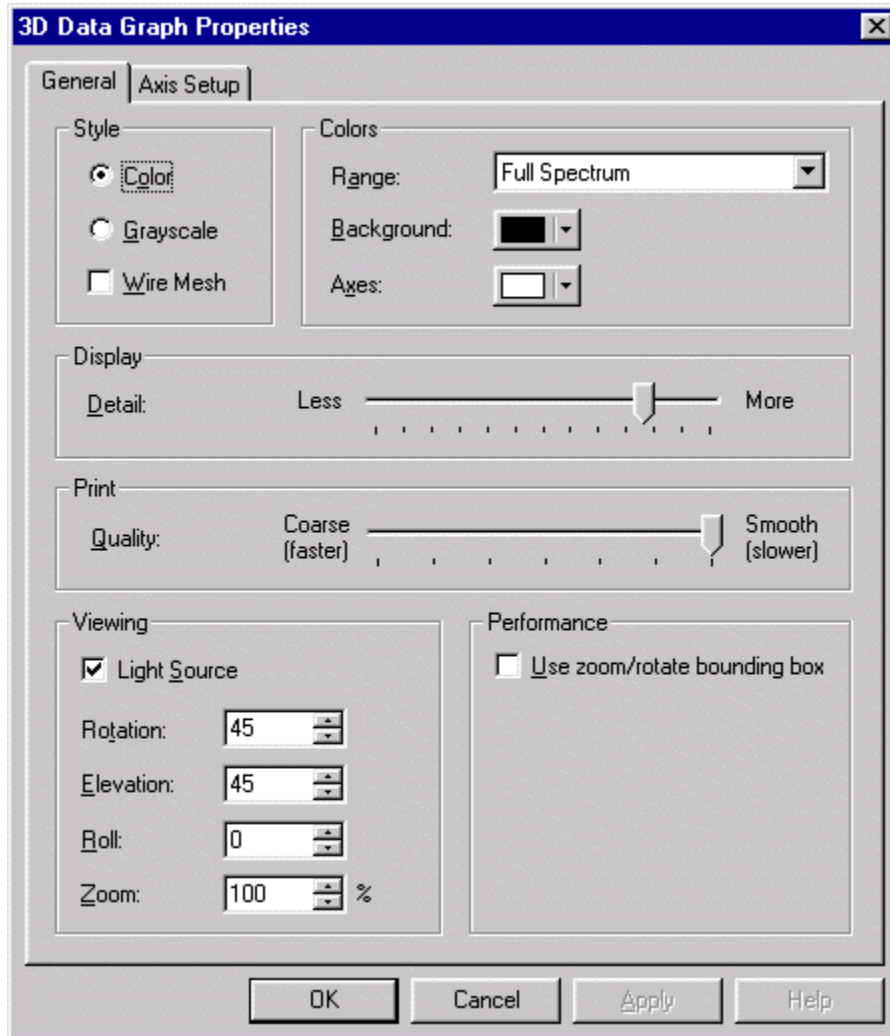
Style

Select **Color or Grayscale** for the plot. Checking the Wire Mesh box will cause the data to be rendered as a wire frame plot, rather than as a solid fill plot.

Colors

Range

Select how you want the coloration on the plot to appear. When **Light & Dark Range** is selected, alternating light and dark bands are used. When **Full Spectrum** is selected, then a continuous color spectrum is used.

Background

Select the color for the background of the plot.

Axes

Select the color for the plot axes.

Display

This specifies the relative quality of the displayed contour plot. Lower Display Detail will result in faster drawing of the plot.

Print

This specifies the relative quality of the printed contour plot. Lower Print Quality will result in faster printing.

Viewing

Use these fields to view current rotation settings, or to set them manually.

Light source

Selecting this option causes the plot to be shaded as if an external light source were shining upon it.

Rotation

This reports the current level of rotation (front to back) in the aspect position of the plot. When a new value is entered, the plot is redrawn to reflect the new value upon exiting the dialog.

Elevation

This reports the current level of tilt (forward or backward) in the aspect position of the plot. When a new value is entered, the plot is redrawn to reflect the new value upon exiting the dialog.

Roll

This reports the current level of roll (side to side) in the aspect position of the plot. When a new value is entered, the plot is redrawn to reflect the new value upon exiting the dialog.

Zoom

This reports the current level of magnification in the plot. When a new value is entered, the plot is redrawn to reflect the new value upon exiting the dialog.

Performance - Use Zoom/Rotate Bounding Box

When this box is checked, the plot will be temporarily replaced by a box during zoom and rotation operation. When the operation is completed, the plot will be redrawn. Checking this box will increase performance on computers with slower graphic subsystems.
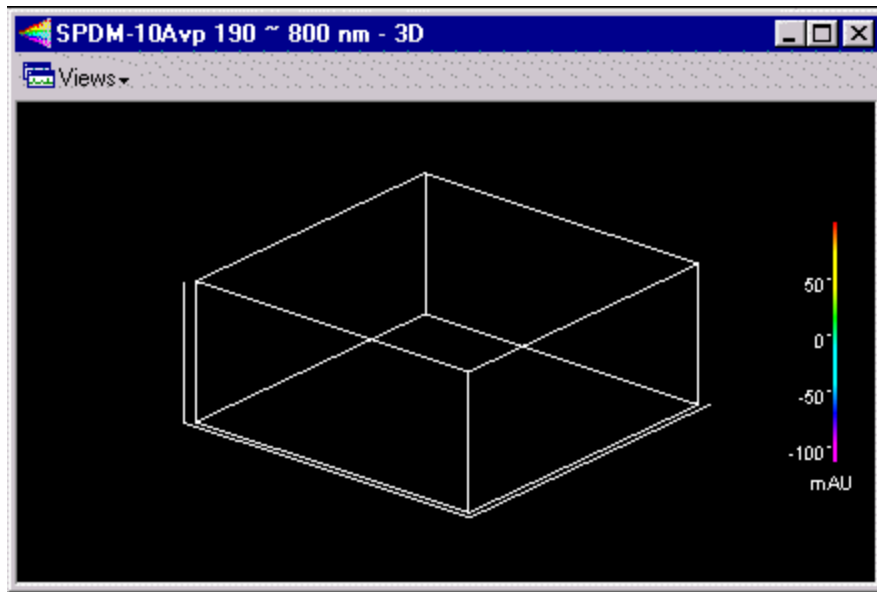
Set the 3D Axis Properties

To set the axis properties for the 3D plot,

1. From **View** menu, select **PDA View.**

2. Select **Mixed View** or one of the other views that includes **3D**.

3. Do a right mouse click on the 3D view and select **Properties**.

4. Click the **Axis Setup** tab to enter or change the axis properties for the 3D plot.

3D Data Graph Properties

General | Axis Setup

Limits

Time

Min: 0.00 ⇕ Max: 11.99 ⇕ Minutes

☐ Autoscale
Get Limits

Wavelength

Min: 200 ⇕ Max: 400 ⇕ nm

☑ Autoscale
Get Limits

Absorbance

Min: -0.29 ⇕ Max: 51.11 ⇕ mAU

☑ Autoscale
Get Limits

Labels

Font: Arial ▾ Size: 7 ⇕

Color: ☐ ▾ Style: **B** / U

OK | Cancel | Apply | Help
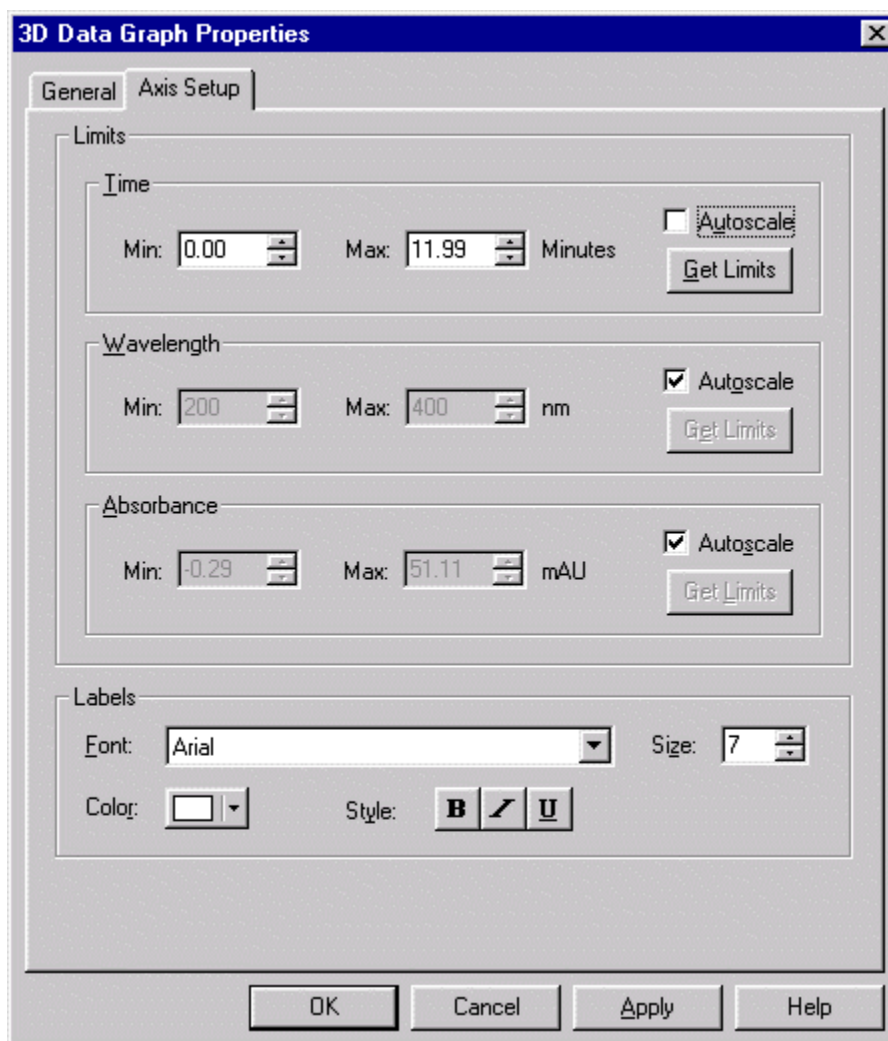
Limits

Enter the limits for the 3D plot.

Time

Click **Autoscale** if you want to have the software automatically scale the time axis to the maximum values. To enter a manual range, de-select the **autoscale** box, and add your own limits, or click the **Get Limits** button to enter the limits displayed on the current 3D graph.

Wavelength

Click **Autoscale** if you want to have the software automatically scale the wavelength axis to the maximum values. To enter a manual range, de-select the **autoscale** box, and add your own limits, or click the **Get Limits** button to enter the limits displayed on the current 3D graph.

Absorbance

Click **Autoscale** if you want to have the software automatically scale the absorbance axis to the maximum values. To enter a manual range, de-select the **autoscale** box, and add your own limits, or click the **Get Limits** button to enter the limits displayed on the current 3D graph.

Labels

You can customize the labeling of the 3D plot using the parameters in this area. You can select font, size, color and style using the selections provided.

Rotate the 3D plot

A right mouse click on the 3d plot provides a menu containing various rotation options. When you select one of these rotation options, a checkmark will appear next to the option and that rotation option will become active on the plot.
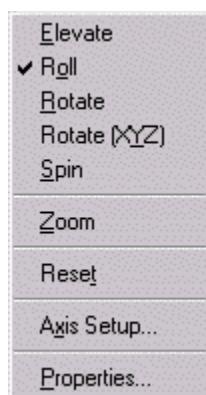
The cursor will change to indicate the option is in effect.
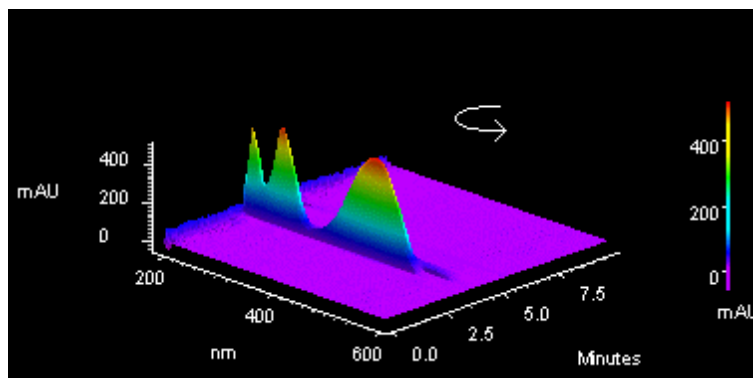
While the option is active, you can move the plot in the designated way by holding down the left mouse button and moving the mouse in the desired direction on the plot. When you have the plot in the desired position, click the right mouse button and turn off the option by selecting it again (checkmark removed). If you want to return to the original plot view, click the right mouse button and select **Reset**.

About the Contour View

The Contour Plot (also referred to as an Isoabsorbance Plot) provides an aerial view of the absorbance of the sample at each wavelength versus time. The contour view supplies quick and easy-to-assimilate information about those wavelengths at which the sample exhibits appreciable absorbance. With contour view, it is also possible to generate a Chromatogram View for an individual wavelength and a Spectrum View for a given point in time.
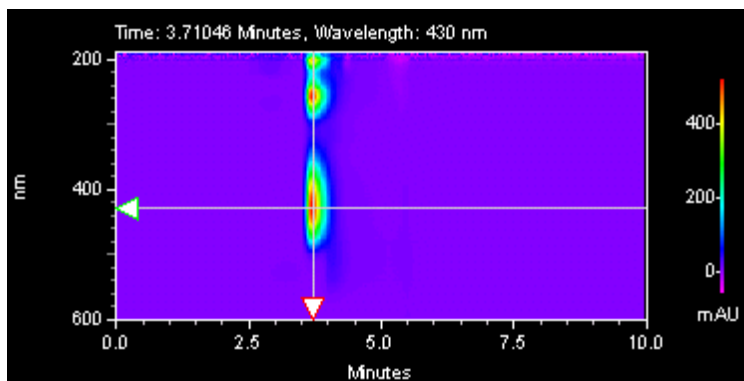
Right-click inside the window to display the pop-up menu. Select **Properties** to display the Contour Properties dialog box.

To generate a chromatogram view from contour view of Mixed View or Mixed View w/ 3D:

1. Select **PDA Views** from the **View** menu, and then select **Mixed View** to display the Contour Map, Chromatogram, and Spectrum.

11. Move the cursor to the triangle-shaped handle located on the left- hand wavelength axis of the Contour Map and press the left mouse button.

12. Drag the cursor up or down to the desired wavelength and release the mouse button.

13. The chromatogram associated with the specified wavelength is displayed in the Chromatogram View.

To generate a spectrum view from contour view of Mixed View or Mixed View w/ 3D:

14. Select **PDA Views** from the **View** menu, and then select **Mixed View** to display the Contour Map, Chromatogram, and Spectrum.

15. Move the cursor to the triangle-shaped handle located on the time axis of the Contour Map and press the left mouse button.

16. Drag the cursor to the desired peak and release the mouse button

17. The Spectrum associated with the specified retention time value is displayed in the Spectrum View.

Set the Contour Properties

To set the properties of the Contour View,

18. Do a right mouse click anywhere on the contour plot and then select **Properties**.

19. Click the **General** tab and set or change the properties for the Contour plot.
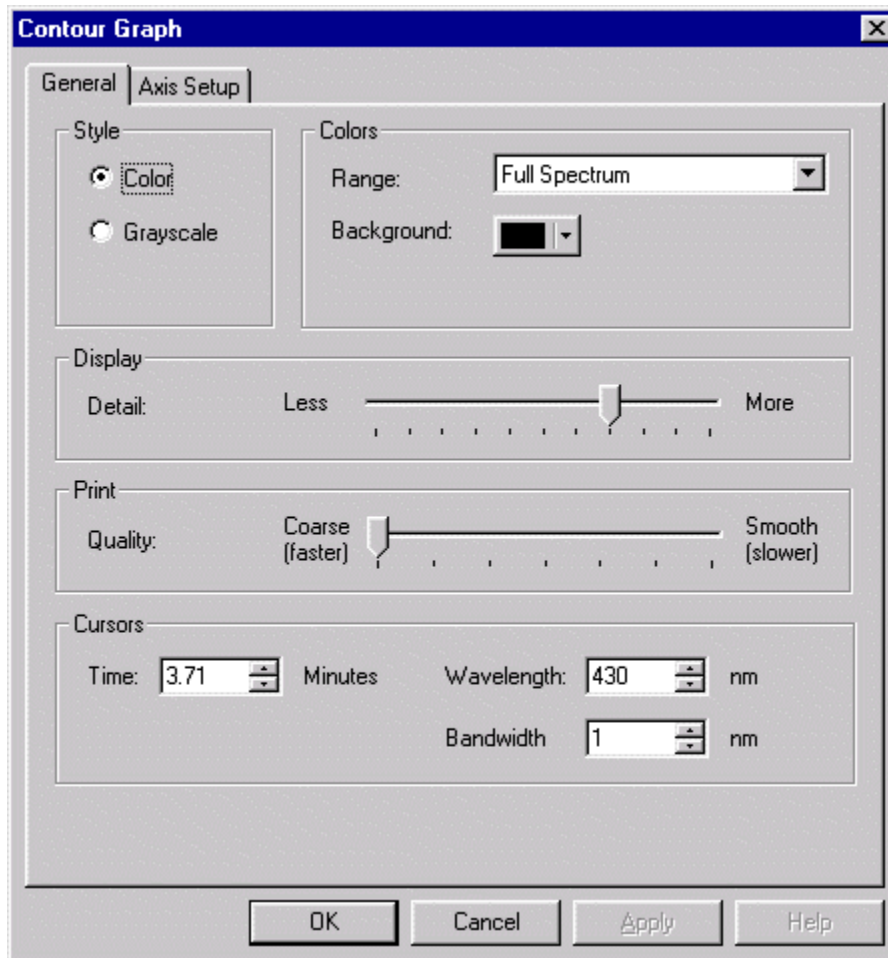
Style

Select how you want the plot to appear, **Grayscale** or **Color**.

Colors

Range: Use this to select how the coloration of the plot is to be displayed. When **Light & Dark Range** is selected, alternating light and dark bands are used. When **Full Spectrum** is selected, then a continuous color spectrum is used.

Background: Select the color to be used for the background of the plot.

Display

This specifies the relative quality of the displayed contour plot. Less Display Detail will result in faster rendering of the plot.

Print Quality

This specifies the relative quality of the printed contour plot. Coarse Print Quality will result in faster printing.

Cursors

The values in these boxes reflect the current contour cursor positions. When you change the values, the cursors on the plot will be changed when you exit the dialog or when you click the Apply button.

Time

This specifies the time position (X Value) of the cursor. When a value is entered, the X cursor is updated to the new position upon exiting the dialog.

Wavelength

This specifies the wavelength position (Y Value) of the cursor. When a value is entered, the Y cursor is updated to the new position upon exiting the dialog.

Bandwidth

This specifies the wavelength band that will be averaged when a chromatogram is extracted from the contour plot. The extracted chromatogram is an average of the absorbances at each wavelength in the wavelength band. The wavelength band is equal to the selected wavelength (see above) +/- one half of the bandwidth.

Set up the Contour Axis

To set or change the Axis Setup of the Contour Plot,

1. Do a right mouse click anywhere on the contour plot and then select **Properties**.

2. Click the **Axis Setup** tab and set or change the axis properties for the Contour plot.

If the Autoscale box is selected, the software will automatically set the axis parameter. If you want to enter the parameter manually, clear the Autoscale box, then enter the range values, or click **Get Limits** to bring in the limits from the current view.
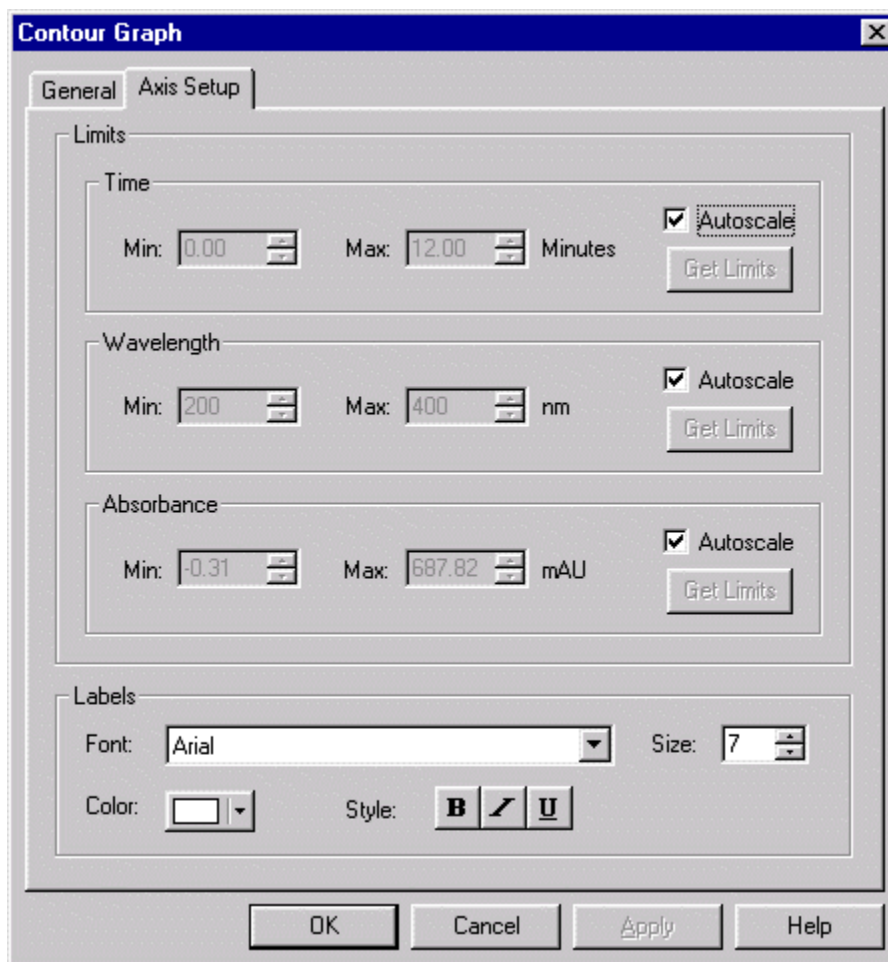
Time

Click **Autoscale** if you want to have the software automatically scale the time axis to the maximum values. To enter a manual range, de-select the **autoscale** box, and add your own limits, or click **Get Limits** to enter the limits displayed on the current contour graph.

Wavelength

Click **Autoscale** if you want to have the software automatically scale the wavelength axis to the maximum values. To enter a manual range, de-select the **autoscale** box, and add your own limits, or click **Get Limits** to enter the limits displayed on the current contour graph.

Absorbance

Click **Autoscale** if you want to have the software automatically scale the absorbance axis to the maximum values. To enter a manual range, de-select the **autoscale** box, and add your own limits, or click **Get Limits** to enter the limits displayed on the current contour graph.

Labels

You can customize the labeling of the contour plot using the parameters in this area. You can select font, size, color and style using the selections provided.

About the Mixed View

This view displays the contour, chromatogram and spectrum views along with the fourth pane displaying similarity, purity and peak profile.
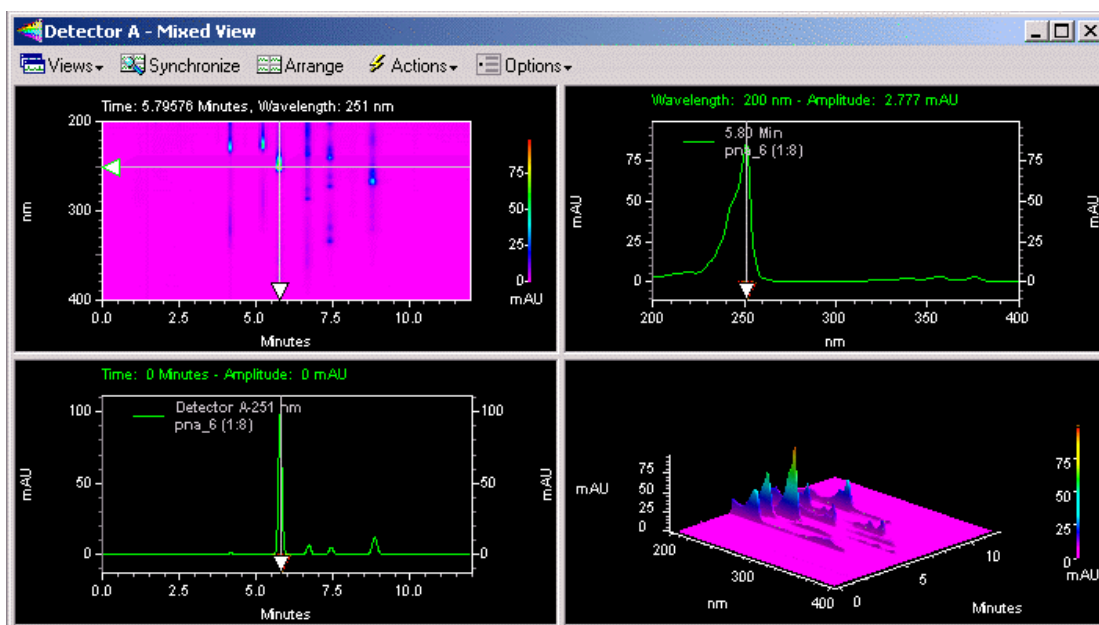
To zoom in on a portion of either the contour plot, chromatogram or spectrum, hold the left mouse button down, move the mouse over the plot until the area of interest is highlighted and release the mouse button.

To quickly move to the previous level of zoom, double-click on the plot.

To Unzoom to the full plot after multiple zooming operations, use Ctrl+Z, or use Shift + double-click in the window, or click the right-mouse button anywhere in the window and select Full Unzoom from the pop-up menu.

Note:	You can synchronize the axis and zoom limits with the other two plots using the Synchronize button on the PDA toolbar. When the contour plot is zoomed, the time range of the chromatogram and the wavelength range of the spectrum plot are automatically adjusted to the same range as the contour. When synchronized zooming has been performed, the x axis plot limits for the chromatogram and spectrum plot are changed. To unzoom these plots to the limits of the data, you must select Full Unzoom from the contour plot context menu.

Mixed View Options Button

This button presents options that enable you to toggle the view for the lower right pane of the mixed view:

- Show 3D Plot
- Show Peak Similarity and Threshold Plot
- Show Peak Purity Plot
- Show Spectrum Similarity Table

Mixed View Actions Button

This button presents the following actions:

Search Library...

Opens the Spectral Library Search window.

Overlay Spectra...

Allows you to select a spectrum to overlay in the Spectrum view pane.

Select Peak for Similarity/Purity Display...

Enables you to select a peak for display in the Purity view pane.

Add to Multi-Chromatogram Table...

Enables you to select a chromatogram to add to the Multi-Chromatogram table in the PDA options.

Add Spectrum to Spectral Library

Selecting this menu item allows you to create a new library entry from the spectrum currently displayed in the spectrum pane. The spectrum will be added to the currently open library (opened by selecting File > Spectrum Library > Open). A name of the form "Spectrum at time xx.xx" will be used as the Compound Name for the new library entry.

About Mixed View w/ 3D

Select Mixed View w/ 3D from the **PDA Views** menu to display the contour, chromatogram and spectrum views along with a fourth pane containing a three dimensional plot of PDA data.

About Chromatogram View

The **Chromatogram View** (also called the **Working Chromatogram**) may be used alone or as part of the Mixed View or Mixed View with 3D displays.

To generate a working chromatogram from the Contour plot, click and drag the Y-Axis arrow to the wavelength of the desired chromatogram.

To generate a working chromatogram from the Spectrum plot, click and drag the X-Axis arrow to the wavelength of the desired chromatogram.

To add a chromatogram from the Contour or Spectrum plot, click and drag the arrow as described above to select the wavelength, and then select **Actions** followed by **Add to multi-chromatogram table** and then click **Add** followed by **Close**.

The following operations can be performed using the working chromatogram:

To select a peak for the **Similarity/Purity** display, hold down the CTRL key and click the mouse on the apex of the desired peak. This function requires that the chromatogram has been analyzed first by clicking the **Analyze** button or selecting **Analyze** from the **Analysis** menu.

Right-click within the chromatogram window to display the pop-up menu. This menu contains the same options as the basic chromatogram window for all detector types, and in addition enables you to overlay chromatograms from different wavelengths and change the Gallery view.

About the Max Plot

To view a Max Plot

1. From the **Channel Selection** box on the toolbar, click the arrow and then select **Spectrum Max Plot**.

A Max Plot is a chromatogram with each point plotted at its maximum absorbance. This plot gives an indication of the appearance of the chromatogram when the wavelengths are optimized for each peak. This plot is useful in selecting an appropriate Analysis Channel for each peak. Note: The Max Plot is not an analysis channel and is not used to analyze chromatographic data.
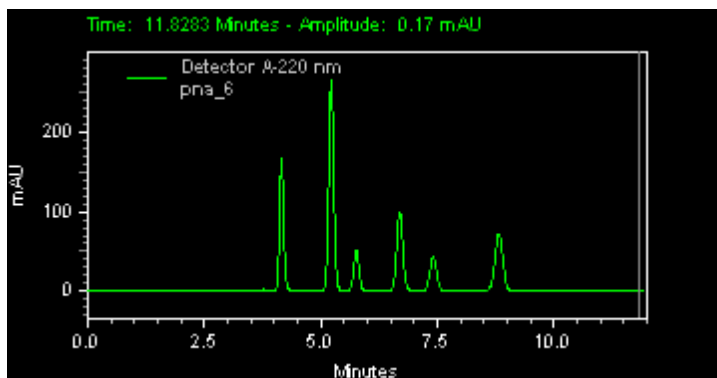
About the Multi-Chromatogram View

This view displays multiple chromatographic plots of absorbance versus time, each at a different wavelength. This displays all of the chromatograms that were specified in the **Multi-Chromatogram** tab of the **PDA Options**.

Click **Overlay** on the PDA Toolbar to toggle between separate chromatograms and chromatograms overlaid on a single axis.

Right-click within the window to display the data system menu for chromatogram window options.
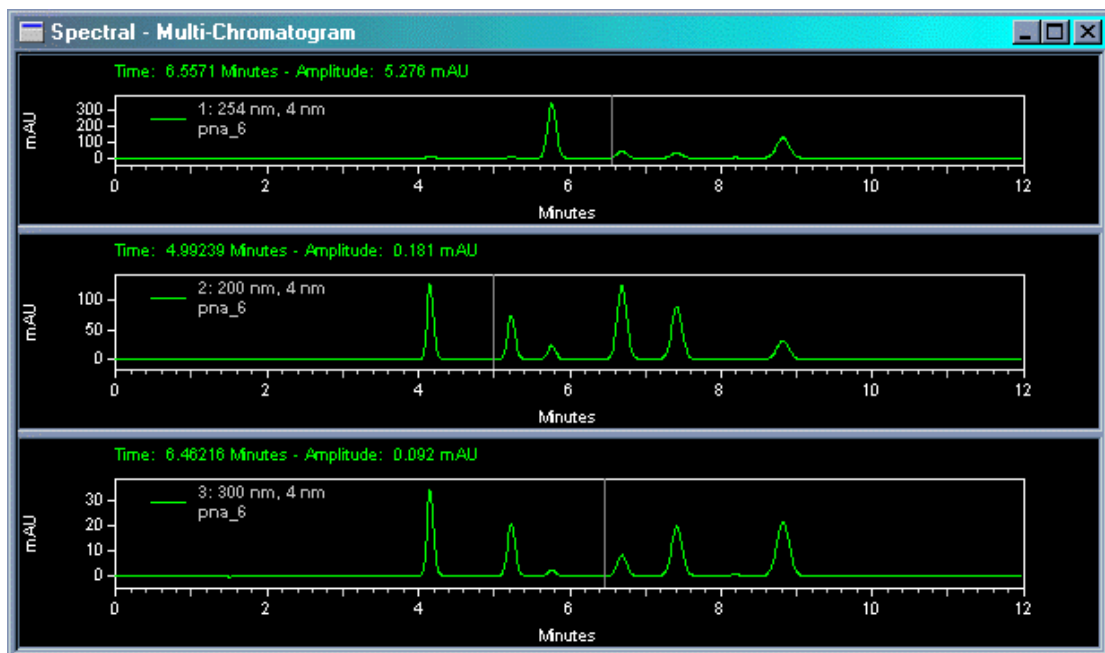
About the Spectrum View

This view displays the spectrum associated with a time on the chromatogram.

To change the displayed spectrum,

1. From the **View** menu, select **PDA View** and then choose **Mixed View**, or select **Views** from the PDA Toolbar, and then select **Mixed View.**

2. Drag the X-Axis arrow on the Contour plot to a time where the desired spectrum is displayed in the Spectrum pane.

To change the working chromatogram,

1. From the **Mixed View** click and drag the arrow in the **Spectrum** pane to the desired wavelength. The chromatogram associated with this wavelength will be displayed in the Chromatogram pane.
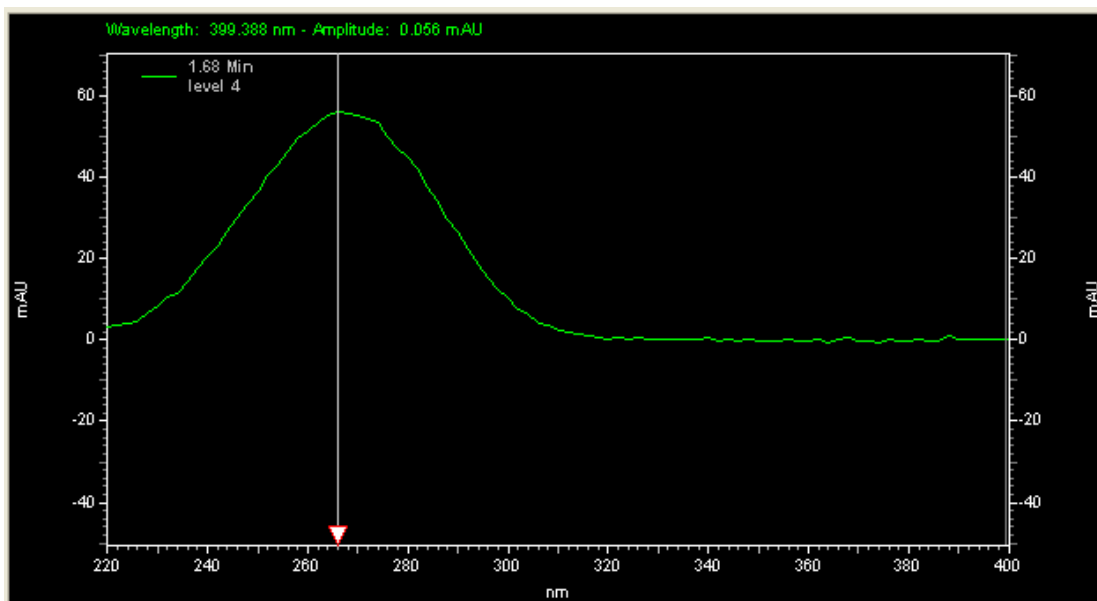
Change the Spectrum Properties

Do a right mouse click in the Spectrum pane, and then select **Properties** to access the standard data system trace properties dialog. This enables you to add another trace to the view, or change scaling. It also allows you to selectively remove overlaid traces from the view.

Spectrum Background Correction

A background correction can be performed if the current spectrum has been extracted from an integrated peak of the working chromatogram.

To perform a spectrum background correction,

1. Do a right mouse click in the **Spectrum** window and then select **Operations** followed by **Background Correction**.

2. Click the spectrum on which you wish to perform the calculation. A background correction for the displayed spectrum will be performed based on the working chromatogram. The background-corrected spectrum is then displayed along with the original spectrum.

Refer to **Background Correction** in the **PDA Analysis and Calculations** section for details on how this calculation is performed.

Notes:

Background Correction may only be performed once on a spectrum. If a second background correction is attempted on an already corrected spectrum, a message box is displayed and the operation ignored.

If the current spectrum has not been extracted from an integrated peak of the working chromatogram, selecting this menu item will have no effect on the spectrum.

Background subtraction must always be the first operation performed on a spectrum. If a background correction is attempted on a spectrum after another operation has already been performed (including operation specified in the **Spectrum** tab of **PDA Options**), a message box is displayed and the background correction request ignored.

Interpolate Spectrum

A spectrum interpolation can be performed if the current spectrum has been extracted from an integrated peak of the working chromatogram.

To perform a spectrum background correction,

1. Do a right mouse click in the **Spectrum** window and then select **Operations** followed by **Interpolate**.
2. Click the spectrum on which you wish to perform the calculation. A 10:1 interpolation will be performed on the spectrum. The new interpolated spectrum is then displayed along with the original spectrum.

   Refer to **Spectrum Interpolation** in the **PDA Analysis and Calculations** section for details on how this calculation is performed.

Export a Spectrum
To export a spectrum,

1. In the **Spectrum** pane, do a right mouse click. Select **Utilities** followed by **Export.** A check-mark will appear next to the **Export** command.
2. Click on the spectrum to be exported as an ASCII file.
3. The **Export Spectrum As** dialog box will appear. Browse to the folder where you want to save the exported spectrum and then type a name for the file in **File Name** and then click **Save**.

Overlay Spectra
To overlay more than one spectra in the Spectrum pane,

1. Click **Actions** from the **PDA** toolbar, followed by **Overlay Spectra...**
2. In the dialog box presented, enter a time for which you want the spectrum displayed and then click **Overlay**, or drag the cursor on the Contour or Chromatogram View to the time for which you want the spectrum displayed.

To remove the spectra you have added to the view,

1. Do a right mouse click in the Spectrum pane, and then select **Clear overlays**.

You can also clear selected spectra using the Trace Setup dialog accessed by doing a right mouse click in the view and then selecting Properties.
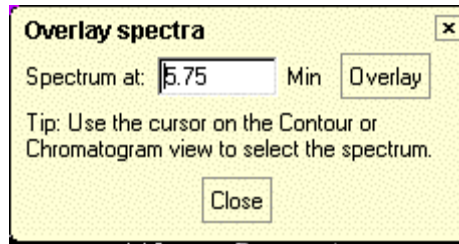
About the Peak Purity Plot

To show the Peak Purity Plot in the 4th pane of the **Mixed View**

1. From the View menu select PDA View followed by Mixed View.
2. Click Options followed by Show Peak Purity Plot.
3. Click the Action button, and choose Select Peak for Purity/Similarity Display to select a peak for purity display.

The Total Peak Purity view displays the purity profile for a chromatogram extracted from the 3D data. The view displays purity information for the Max Plot chromatogram that is displayed in the Chromatogram pane of the Mixed View. The pane will be blank until the data has been analyzed, and a peak has been selected for the purity calculation.
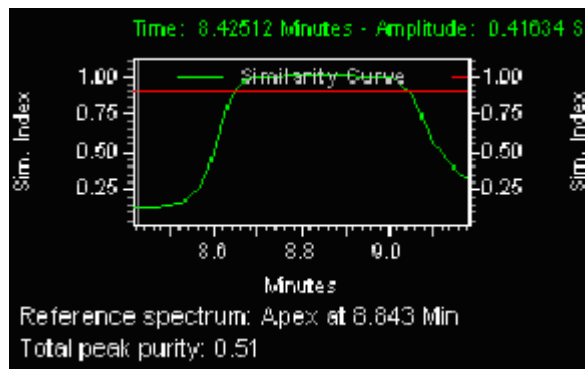
Purity View Properties

To set up the properties for the Purity View, do a right mouse click in the **Purity View**, and select **Properties**.
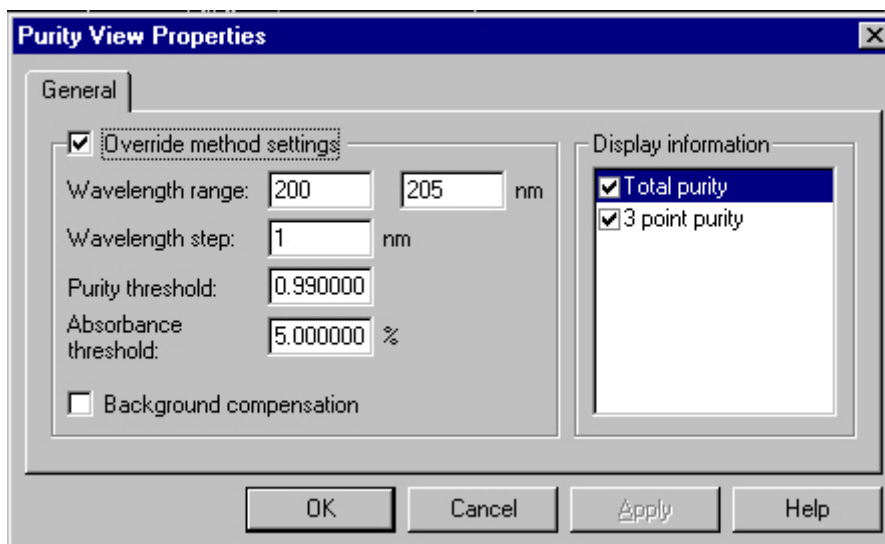
By default, the Similarity View will use the current method settings from the **Purity** tab of **PDA Options**. To override the method settings, click the Override method settings box, then enter the values you wish to use.

Wavelength range

Specify the wavelength range over which the purity calculations will be performed, for example, from 200nm to 400nm.

Wavelength Step

Specify the wavelength spacing (in nm) to be used when purity calculations are performed.

Purity threshold

The comparison of two spectra gives a Similarity Index (SI), the closer to 1.00 number is the more similar the spectra are. The purity threshold is used to eliminate spectra that do not match. If the SI is greater than the purity threshold, the spectra will be considered pure. In general, a spectra with an SI greater than 0.9900 would have a high probability of being the same as the apex spectra. A SI greater than 0.9000 but less than 0.9900 shows some similarity but would need to be evaluated with care and caution. SI less then 0.9000 should be considered non-similar.

Absorbance threshold

This value represents the percentage of peak height that spectra will included in purity calculation. Spectra in sections of the peak that do not exceed this threshold will not be included in the purity calculation. This provides a method of eliminating spectra where the concentration of the compound is so low that the solvent spectrum interferes.

Background compensation

Checking this box will cause spectra to be corrected for background using the peak baseline prior to being used in the calculation of purity.

Select Peak for Purity Similarity Display
To select the reference spectrum for the purity calculation,

1. Make sure the data has been analyzed. (If you are not sure, click the analyze button on the toolbar.)

2. Click **Actions** followed by **Select Peak for Purity/Similarity Display**.

3. Select a peak by holding down the CTRL button and then clicking on a peak from the Chromatogram or Contour pane. The retention time of the reference spectrum is displayed along with a value indicating peak purity.

4. Continue to select or change the peak. When you are finished, click **Close**.

Similarity View Properties

To set up the properties for the Similarity View,

1. In the **Peak Similarity and Threshold** Plot, do a right mouse click, and then select **Properties**.
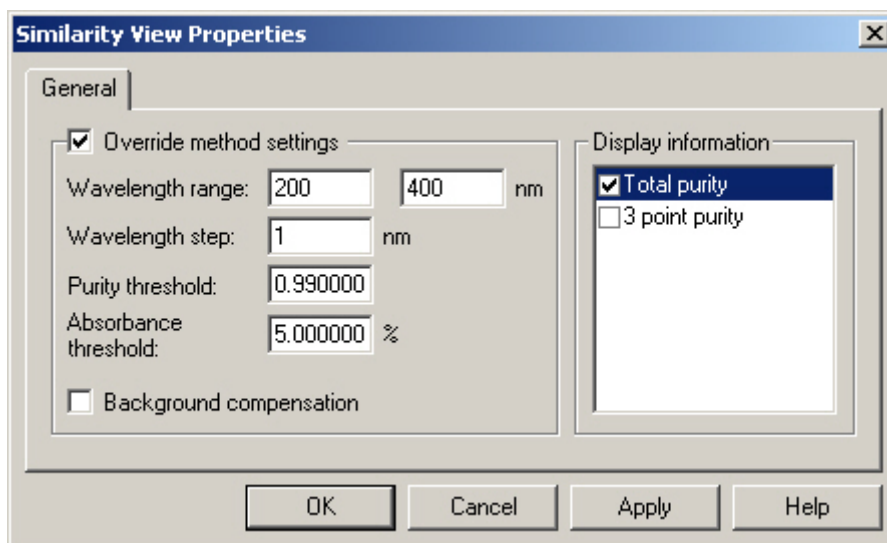
By default, the Similarity View will use the current method settings. To override the method settings, click the Override method settings box, then enter the values you wish to use.

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Specify the wavelength range over which the purity calculations will be performed, for example, from 200nm to 400nm.

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Specify the wavelength spacing (in nm) to be used when purity calculations are performed.

Purity threshold

The comparison of two spectra gives a Similarity Index (SI), the closer to 1.00 number is the more similar the spectra are. The purity threshold is used to eliminate spectra that do not match. If the SI is greater than the purity threshold, the spectra will be considered pure. In general, a spectra with an SI greater than 0.9900 would have a high probability of being the same as the apex spectra. A SI greater than 0.9000 but less than 0.9900 shows some similarity but would need to be evaluated with care and caution. SI less then 0.9000 should be considered non-similar.

Absorbance threshold

This value represents the percentage of peak height that spectra will included in purity calculation. Spectra in sections of the peak that do not exceed this threshold will not be included in the purity calculation. This provides a method of eliminating spectra where the concentration of the compound is so low that the solvent spectrum interferes.
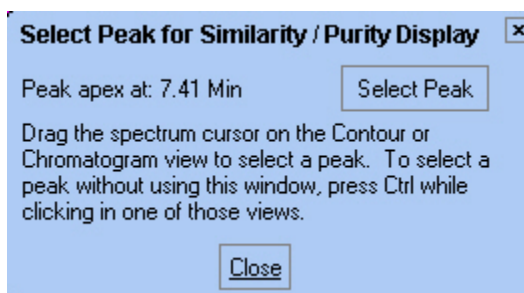
Background compensation

Checking this box will cause spectra to be corrected for background using the peak baseline prior to being used in the calculation of purity.

Select Peak for Purity Similarity Display

To select the reference spectrum for the purity calculation,

1. Make sure the data has been analyzed. (If you are not sure, click the analyze button on the toolbar.)

2. Click **Actions** followed by **Select Peak for Purity/Similarity Display**.

3. Select a peak by holding down the CTRL button and then clicking on a peak from the Chromatogram or Contour pane. The retention time of the reference spectrum is displayed along with a value indicating peak purity.

4. Continue to select or change the peak. When you are finished, click **Close**.

About the Ratio View

To show the Ratio Plot

1. From the **View** menu select **PDA View** followed by **Ratio Plot.**

The Ratio view displays two PDA wavelength channels and the ratio of those two channels. These may be viewed during real-time acquisition as well as during post-run analysis. The flat tops on the ratio peaks are a preliminary indication of peak purity. The ratio wavelengths and parameters are set in the **Ratio** tab of **PDA Options**.

The Y-axis of the ratio chromatogram is auto-scaled to 1.
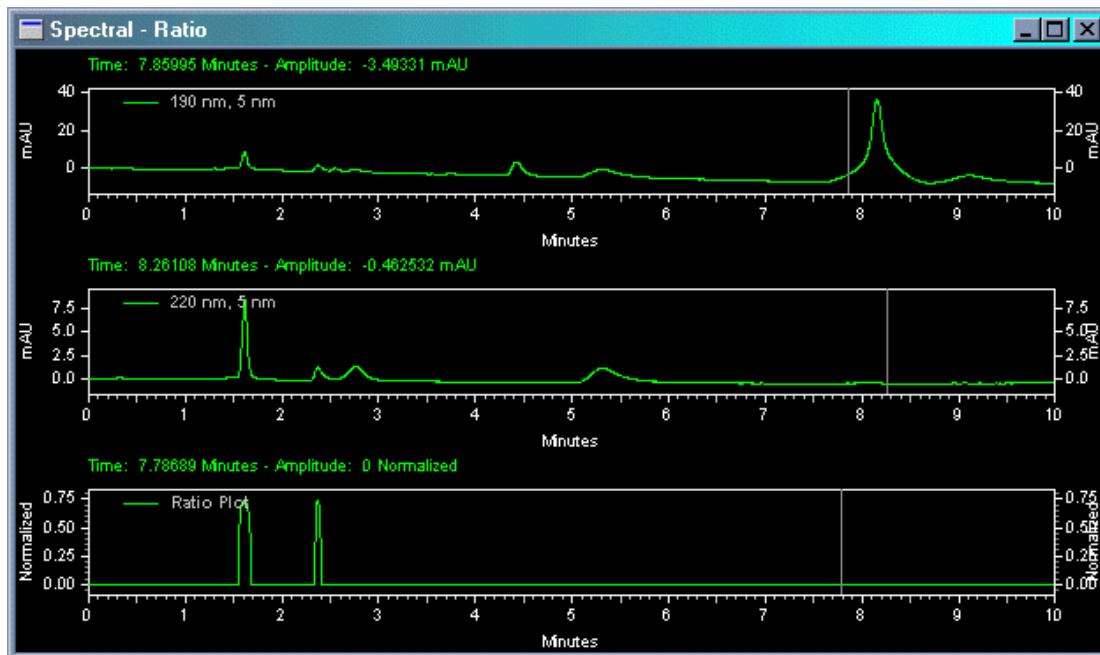
Right-click within the window to display the pop-up context menu. The context menu is the same as for a standard chromatogram graph window.

PDA Utilities

To access the **Utilities** menu,

1. Do a right mouse click from any PDA View and then select **Utilities**.

The Utilities menu allows displayed spectra to be printed, copied, saved or exported. Some or all of the following options will be presented.

Print

Select Print to automatically print the currently displayed spectra.

Copy to Clipboard

Select Copy to Clipboard to copy the displayed spectra to the Clipboard. The contents of the Clipboard may then be pasted into other software.

Save trace

Select Save trace to save the spectrum to a file with an .spc extension for later inclusion in a library or report.

Export

Select Export to enable export of the item in the method.

About the Spectrum Similarity Table

The Spectrum Similarity table is displayed in the fourth pane of the PDA Mixed View when the Options Mode is set to Show Spectrum Similarity Table.

To view the Spectrum Similarity Table

1. In the Mixed View, click Options.
2. Select **Show Spectrum Similarity Table.**

Spectra are automatically added to and deleted from this table whenever a spectrum is added to and deleted from the Spectrum pane of the Mixed View.

Spectrum Name

This column displays identifications for each of the spectra added to the table. For spectra extracted from the 3D data, the identification includes the time. For spectra extracted from a chromatogram based on a peak name, the identification includes the peak name. For spectra loaded from a file, the identification includes the retention time.

A check mark next to the colored line for a spectrum indicates that the spectrum will serve as the Reference Spectrum for the similarity calculation.

Spectra may be added to the table in the Mixed View by selecting the **Add to Similarity Table** from the **Actions** button menu.

Similarity

This column displays the similarity of the spectrum on that row relative to the Reference Spectrum. The Reference Spectrum is determined by double-clicking on a row of the table or by highlighting a row and pressing the 'Set Reference' button.

Until a reference spectrum has been selected the similarity for all spectra in the table will be zero.

Refer to the PDA Analysis and Calculations section for details on how this calculation is performed.

Source

This column displays the source of the spectrum. (Current Data) refers to working spectra extracted form the current 3D data. When spectra come form a stored data file, this column will display the source filename.

Set Reference

Click this button will designate the spectrum in the currently highlighted row of the table as the new reference spectrum. The similarities for all of the rows will be recalculated based on the new reference spectrum.

Print

Click this button will output a simple text report representation of the table to the default printer.

Properties

Click this button will display a dialog with parameters related to the calculation of similarity.

Set the Similarity Table Properties

To set the properties for the Similarity Table

1. From the Spectrum Similarity Table, click **Properties** to display the table properties dialog box.

2. Enter the wavelength range and data point spacing (in nm) for similarity calculations.
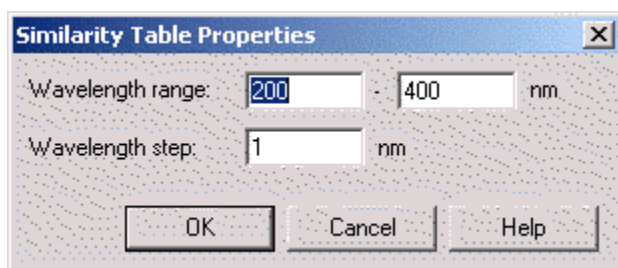
Note: During analysis, it is possible that a portion of this wavelength range will be outside the range of the acquired data. In that case, the wavelength range will be truncated to the limits of the acquired data.

Extract a Chromatogram

To generate a **chromatogram view** (extract a working chromatogram) from contour view of Mixed View or Mixed View w/ 3D:

1. From the **View** menu, select **PDA View** followed by **Mixed View**, or click **Views** from the **PDA Toolbar** and then select **Mixed View.** This will display the Contour Map, Chromatogram, and Spectrum.

2. Move the cursor to the triangle-shaped handle located on the left-hand wavelength axis of the Contour Map and press the left mouse button.

3. Drag the cursor up or down to the desired wavelength and release the mouse button.

4. The chromatogram associated with the specified wavelength will be displayed in the Chromatogram View.

4   About PDA Method Options

The PDA Options enable you to set up a variety of analysis options for use with a configured photo diode array detector. To access the PDA Options for your method,

5. From the **Method** menu, select **PDA Options...**

6. Click the desired tab to set the parameters for data analysis.

Tab	Used for
Library	Enabling spectral libraries and specifying search parameters.
Purity	Entering parameters for purity calculations.
Spectrum	Defining filtering and per-peak spectrum parameters
Multi-chromatogram	Enabling multi-wavelength chromatograms
Ratio	Setting up ratio plots

Set Library Options

To set the PDA Library options,

1. From the **Method** menu, select **PDA Options** and then click the **Library** tab.

Using this tab, you can enter library parameters for spectral library searches that will be saved as part of your method. When you do a spectral library search "using method parameters", these parameters will be used.

Library/Enabled

Enter the spectral library to be searched or select from available libraries by clicking the Library field followed by clicking the file button. You can select more than one library. To enable the library for searching, click the **Enabled** box. If this list is empty, or no library is enabled, no hits will be returned when a search is performed.

Search parameters

Enter the parameters to use for the search.

Wavelength range

Enter the wavelength range to search

Wavelength step

Enter a step number for the search. Larger numbers will make the search faster, but if you use too large of a step, spectral details may not be picked up.

Max hits

Specify the number of hits that will be reported in the results of a library search. Note that this works in conjunction with the *Similarity Threshold* parameter to limit the number of hits reported.

Similarity threshold

Enter a number for threshold of similarity. The library search results will only display matches whose similarity to the unknown exceeds this value.

Pre-filters

The options in this group allow you to specify search pre-filters that will be performed on library spectra prior to the test for similarity. All pre-filters are optional.

Retention time range

When values are entered in these fields, PDA limits its search to library spectra obtained from peaks whose apex are within the specified retention time range. This pre-filter is optional and may be left blank.

Lambda max

When one or more of these values is specified, library search will be restricted to those library entries containing lambda max value(s) within 5nm of all of the specified values. Entries without matching lambda max value(s) are automatically excluded from the search (no similarity calculation is made). Entering values for lambda max is optional.

Compound name filter

If you are searching only for spectra that contain a certain name, enter the name here. Only spectra containing that name will be searched.

PDA Options Purity

To set the PDA Purity options,

1. From the **Method** menu, select **PDA Options** and then click the **Purity** tab.

The Purity tab is used to set the parameters necessary to perform on-demand peak purity calculations and peak purity calculations that occur as part of analysis.
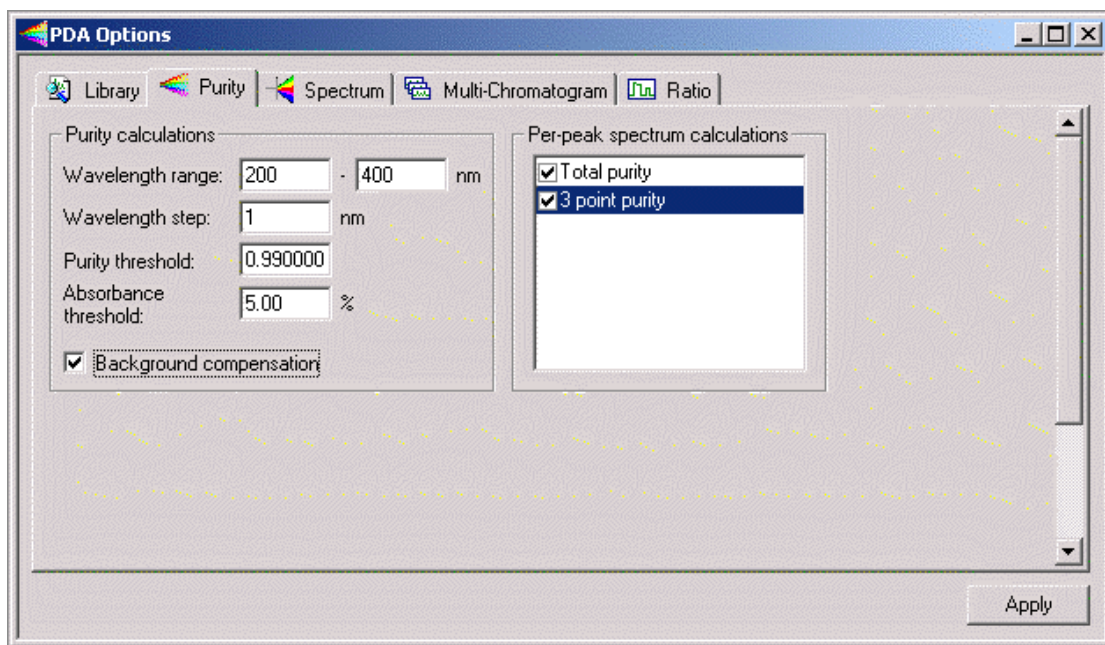
Purity Calculations

From/To – wavelength range

Specify the wavelength range over which the purity calculations will be performed, for example, from 200nm to 400nm.

Step

Specify the wavelength spacing (in nm) to be used when purity calculations are performed.

Purity threshold

The comparison of two spectra gives a Similarity Index (SI), the closer to 1.00 number is the more similar the spectra are. The purity threshold is used to eliminate spectra that do not match. If the SI is greater than the purity threshold, the spectra will be considered pure. In general, a spectra with an SI greater than 0.9900 would have a high probability of being the same as the apex spectra. A SI greater than 0.9000 but less than 0.9900 shows some similarity but would need to be evaluated with care and caution. SI less than 0.9000 should be considered dissimilar.

Absorbance threshold

This value represents the percentage of peak height that spectra will included in purity calculation. Spectra in sections of the peak that do not exceed this threshold will not be included in the purity calculation. This provides a method of eliminating spectra where the concentration of the compound is so low that the solvent spectrum interferes.

Background compensation

Checking this box will cause spectra to be corrected for background using the peak baseline prior to being used in the calculation of purity.

Per-peak spectrum calculations

Checking any of these boxes indicates that the indicated value will be calculated on a per-peak basis during analysis. The result of this calculation will then be available in reports and as chromatogram annotations.

Unchecking values that are not of interest will speed up analysis. If a box is unchecked and the field appears in a run report, it will be reported as zero

Total Purity

Checking this box will cause total peak purity to be calculated on a per-peak basis during analysis. The result of this calculation will then be available in reports and as chromatogram annotations. If this box is unchecked and a report contains the field, a zero will be reported.

3 Point Purity

Checking this box will cause 3-point peak purity to be calculated on a per-peak basis during analysis. The result of this calculation will then be available in reports and as chromatogram annotations. If this box is unchecked and a report contains the field, a zero will be reported.

PDA Options Spectrum

To set the PDA Spectrum options,

1. From the **Method** menu, select **PDA Options** and then click the **Spectrum** tab.

The Spectrum tab is used to specify the types of filtering and processing to be performed on spectra that are extracted from the 3D data during analysis as well as to spectra displayed in the PDA Spectrum View.

Note: The processing specified on this page is performed prior to any use of the spectra in the software, including display, searching and reporting.
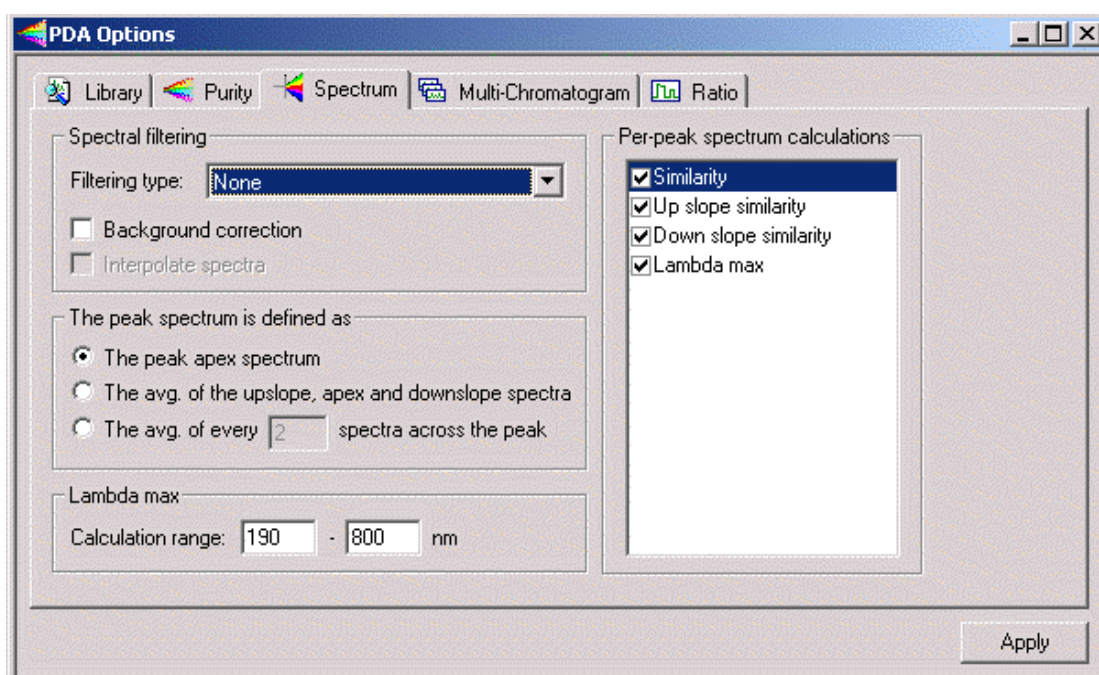
Spectral filtering

In this area you designate how spectral filtering (if any) will be performed.

Filtering type

Choose the type of filtering for the spectral plot. Choices are None, Smooth, 1st Derivative, and 2nd Derivative. Selecting one of these smoothing algorithms can remove noise from the spectrum.

Background correction

If this box is checked, then, prior to display, a correction for spectral background is made, as follows:

1. The spectra from the baseline start and baseline stop times for the peak are extracted from the data.

2. For each spectrum in the peak, a corresponding background spectrum is generated by linear interpolation between the baseline start and baseline stop spectra.

3. This background spectrum is subtracted from the original spectrum.

Note: The baseline start and stop times that are used in calculating background compensation are based on the detected peaks for the channel currently selected.

Interpolate spectra

Select this check box to automatically perform 10:1 interpolation of spectra displayed in the spectrum window using a cubic spline curve fit. This interpolation is performed after the applying any spectral filtering option (1st derivative, 2nd derivative or smooth) to the display spectrum.

Note: Interpolated spectra may not be stored or added to a library.

The peak spectrum is defined as

This specifies how the spectra extracted from the 3D data are averaged before being exported or used in the Spectrum Report.

Spectrum Averaging is the calculation of a spectrum whose absorbance values are the average of the absorbances.

The peak apex spectrum

When this is selected, the spectrum at the apex of the peak will be used, without averaging.

The average of upslope/apex/downslope spectra

When this is selected, three spectra (the spectra at the upslope inflection point, the apex and the downslope inflection point) are averaged.

The average of every n spectra across the peak

When this is selected, enter a value for **n**. Every nth spectrum from the peak is extracted, beginning at the start of the peak and continuing to the end of the peak. These extracted spectra are averaged and become the spectrum that is used.

Per-peak spectrum calculations

Checking any of these boxes indicates that the indicated value will be calculated on a per-peak basis during analysis. The result of this calculation will then be available in reports and as chromatogram annotations.

Unchecking values that are not of interest will speed up analysis. If a box is unchecked and the field appears in a run report, it will be reported as zero

Similarity

Checking this box causes the Peak Apex Similarity to the reference spectrum to be calculated on a per-peak basis during analysis. The result of this calculation will then be available in reports and as chromatogram annotations. If this box is unchecked and a report contains the field, a zero will be reported.

Peak Apex Similarity applies only to named peaks and its use requires that a reference spectrum be included in the peak table.

Upslope Similarity

Checking this box causes upslope similarity to be calculated on a per-peak basis during analysis. Upslope similarity compares the peak apex spectrum to the spectrum at the peak inflection point on the upslope side (to the left of the apex). The result of this calculation will then be available in reports and as chromatogram annotations. If this box is unchecked and a report contains the field, a zero will be reported.

Downslope Similarity

Checking this box causes downslope similarity to be calculated on a per-peak basis during analysis. Downslope similarity compares the peak apex spectrum to the spectrum at the peak inflection point on the downslope side (to the right of the apex). The result of this calculation will then be available in reports and as chromatogram annotations. If this box is unchecked and a report contains the field, a zero will be reported.

Lambda Max

Checking this box indicates causes the lambda max (wavelength at which the highest absorbance occurs) to be computed for each peak of each multi-chromatogram extracted from the 3D data during analysis. The result of this calculation will then be available in reports and as chromatogram annotations. If this box is unchecked and a report contains the field, a zero will be reported.

Lambda Max Calculation Range

This allows the user to restrict the wavelength range over which the Spectrum Max Plot will be calculated. Default is the detector acquisition range.

Set Multi-Chromatogram Options

To set the PDA Multi-Chromatogram options,

1. From the **Method** menu, select **PDA Options** and then click the **Multi-Chromatogram** tab.

The Multi-Chromatogram tab is used to specify data channels within the 3D data for integration and quantitation. It allows you to select wavelengths to display on the Multi-Chromatogram plot. When you select an **Enabled** box, enter a wavelength and bandwidth to be used for one plot. After you have set up several wavelengths, you can disable them temporarily by clicking the **Enabled** box such that the check mark is not displayed. The Multi-Chromatogram display is selected from the View Gallery. The

Multi-Chromatogram view is not functional unless one or more valid wavelengths are enabled in the Multi-Chromatogram Definition tab.
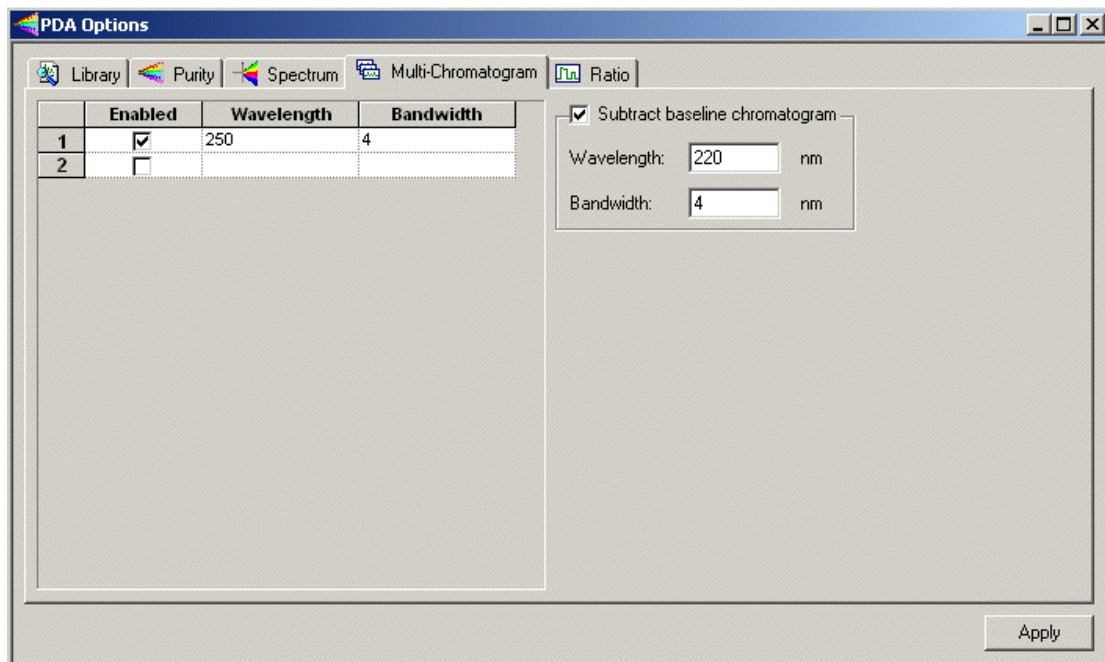
Enabled

To enable a wavelength, click the checkbox. To disable a wavelength, click the checkbox again to remove the checkmark. Only enabled wavelengths will appear in the multi-chromatogram view.

Wavelength

Enter the wavelength you want to view.

Bandwidth

Specify the nm range to be averaged in generating the analog signal for each channel.

The multi-chromatogram data is calculate by averaging the data points of chromatograms across the wavelength range of "wavelength - 1/2*bandwidth" to "wavelength + 1/2*bandwidth".

For example, given a wavelength of 600 nm and a bandwidth of 4 nm, for each sample time, the data points from the chromatograms at 598, 599, 600, 601 and 602 nm are added, and the sum divided by 5.

If, in the example above, the detector's range is 190 - 600 nm (inclusive), the software only averages the points across chromatograms at 598, 599 and 600 nm (dividing by 3). The range would not be exceeded.

Subtract baseline chromatogram

Click this to enable baseline subtraction of a designated chromatogram to be extracted from the 3D data.

Wavelength

Enter the wavelength at which you wish to extract a chromatogram to be used for baseline subtraction.

Bandwidth

Specify a bandwidth range to be averaged in generating the extracted chromatogram.

PDA Options Ratio

To set the PDA Ratio options,

1. From the **Method** menu, select **PDA Options** and then click the **Ratio** tab.

The Ratio tab is used to set the displays of the Ratio Plot View. The Ratio View displays two PDA wavelength channels and the ratio of those two channels. These may be viewed during real-time acquisition as well as during post-run analysis. The flat tops on the ratio peaks are a preliminary indication of peak purity.
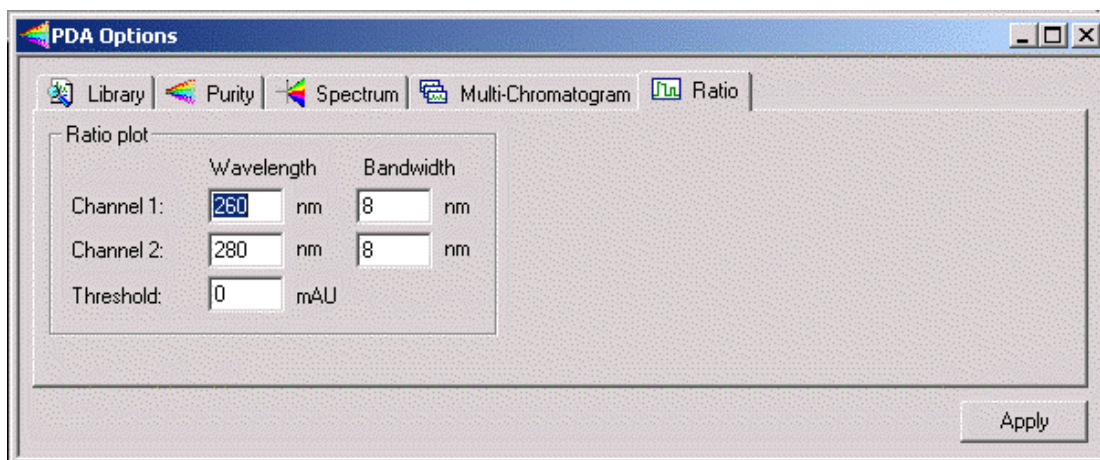
Ratio plot

These controls specify the wavelength of the ratio multi-chromatogram channels. The extracted chromatograms will be centered about the specified wavelength.

Channel 1, Channel 2

Wavelength

Enter the wavelength of the chromatogram channel.

Bandwidth

Enter the nm range to be averaged in generating the chromatogram channel.

Threshold

Enter the ratio threshold. The threshold is the minimum absorbance value required in the chromatograms of both channels for calculation of the ratio. It is a method of eliminating ratio calculation at minor absorbance values, such as those that occur with noise. If the threshold value is not met, the ratio plot will be zero.

5 Spectral Library Search

Define a Spectral Library

In order to define a library, you must have the spectra to be added saved on disk as spectra files (.spc) or you can create a library from the spectra obtained during the current run. To define a spectral library

1. From the **File** menu, select **Spectral Library** followed by **New** to create a new spectral library. Select **Open** to browse for and open an existing library to modify.

2. Click **Spectrum Data Source** and select **Current Data, Spectrum File** or **Named Peak.**

If you select **Current Data**, a dialog box will appear where you enter the retention time for the spectra to be added to the library.

If you select **Spectrum File**, browse to the spectrum file to be added to the library and then click **OK**.

If you select **Named Peak**, chose a peak whose spectrum is to be added to the spectral library from the dialog box presented. (The current chromatogram must be **Analyzed** first in order to use this option.)
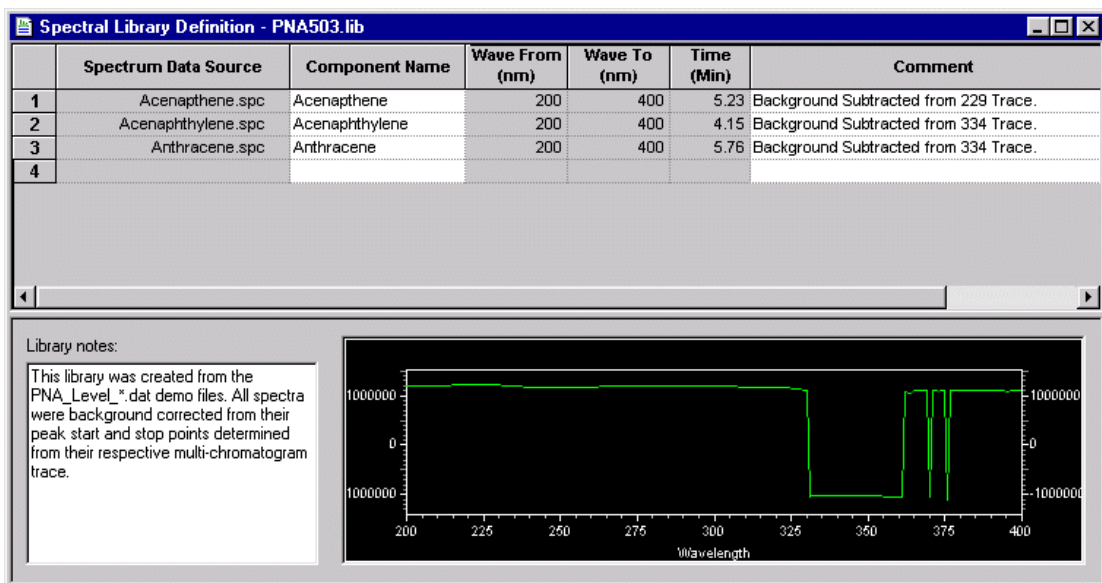
Spectrum Data Source

Click on the arrow in this field to select either **Current Data**, **Spectrum File**, or **Named Peak** to be used to select the spectrum to be added to the library.

To add a spectrum from a spectrum file, the spectrum must have been previously saved as a file with the .spc extension, for example, using the **Save Trace** function accessed by doing a right mouse click in the Spectrum pane followed by **Utilities**.

If you chose to enter a spectrum from the current data file, a dialog box will appear where you must select the retention time at which you wish to select the spectrum.

If you chose to enter a spectrum from a named peak, a dialog box will appear where you must select the named peak from which the spectrum will be extracted.

Component Name

Enter the chemical name or description of the spectrum. This field is used for display and search pre-filter purposes.

Lambda Max From ... To

The wavelength range of the spectrum over which the software will calculate the lambda (absorbance) max value is displayed in these columns.

Retention time

When the spectrum was extracted from a chromatographic run, the software automatically identifies the retention time of the spectrum and enters the value in this field. When desired, the value in this field may be edited. This field can also be used as a search pre-filter.

Comment

Enter any descriptive information desired related to the spectrum. This field is used only for display and documentation purposes.

Library Notes

This field is used to document relevant information about the library as a whole, e.g., documentation about run conditions or general sample information.

Note: Fields in existing library entries may be edited by selecting the fields with the cursor. Right-click on a row in the library table to display a pop-up menu used to cut, paste, copy, and insert and delete lines from the library as necessary.

Search the Spectral Library

To open **Spectral Library Search**,

1.   From Mixed View, click Actions followed by Search Library.

From here you can perform a library search on a spectrum you have selected in the contour map, and you can also select a spectrum from the current data or stored spectrum file to search.
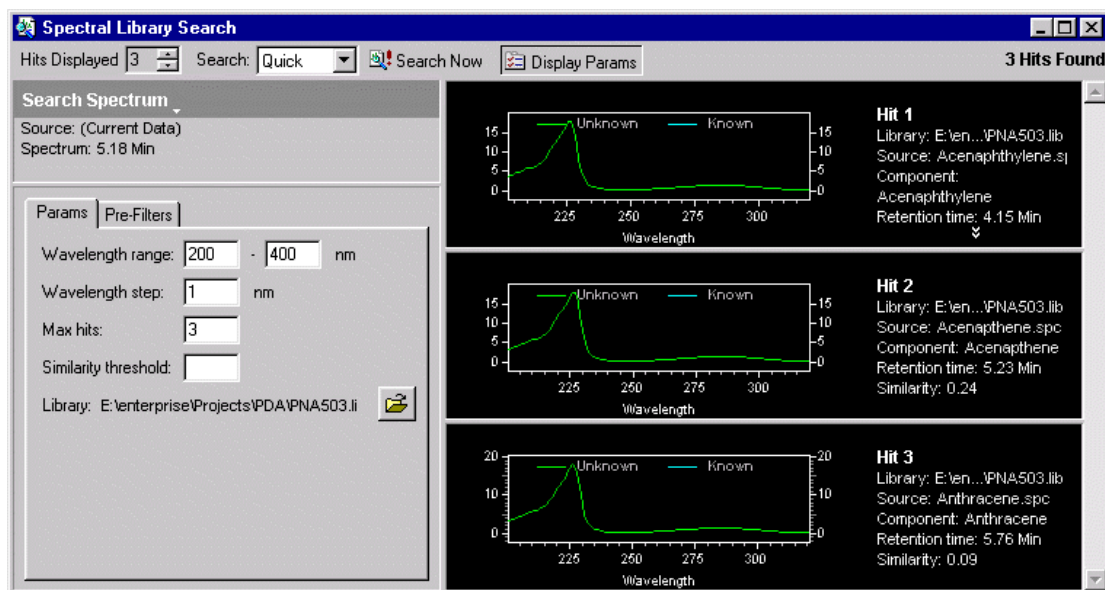
Hits Displayed

Select the number of hits you wish to display. Hits will be displayed in order of similarity.

Search

Select **Method** if you want to use the parameters from the current method for your search. Select **Quick** if you want to enter or change the parameters yourself before the search.

Display Params

Click this button if you want to display the parameters. It toggles between display and no display. If you always use the method parameters, you may not wish to display them when you search.

Search Now

When you click this button, a library search will be performed on the selected spectra using the parameters, and the number of hits requested will be displayed.

Search Spectrum

Click the arrow to select a new spectrum to search on. You can choose from the current data, or from a stored spectrum file. The current spectra source will be displayed.

Params

This tab contains search parameters. If you have elected to use the Method search parameters, the parameters displayed will be those in your current method in the **Library** tab of the **PDA Options**. If you have elected to do a Quick search, the parameters will be default parameters.

Wavelength Range

These specify the wavelength range over which the library search will be performed.

Wavelength Step

This specifies the data point spacing to be used when a library search is performed.

Max Hits

This specifies the number of hits that will be reported in the results of a library search. Note that this works in conjunction with the **Similarity Threshold** parameter to limit the number of hits reported.

Similarity Threshold

Specifying a value for this control will cause the library search results to only display matches whose similarity to the unknown exceeds this value. Note that this control works in conjunction with the **Max Hits** parameter to limit the number of hits reported.

Library

Displays the library from the method, or for Quick enables you to select a library to be used.

Pre-Filters

When **Quick** is selected as the *Search* Mode, the items on this tab allow the user to specify search pre-filters that will be performed on library spectra prior to the test for similarity. All pre-filters are optional. If **Method** is selected as the **Search** Mode, the items on this tab are read-only and reflect the parameters values on the **Library** tab of **PDA Options.**

Retention Time Range

When a Retention Time Range is specified, library search will be restricted to those library entries whose retention time is within the specified range. Entries outside this range are automatically excluded from the search (no similarity calculation is made). Entering a value for this is optional.

Lambda Max

When one or more of these values is specified, library search will be restricted to those library entries containing a lambda max within 5 nm of one of the specified values. Entries without a matching lambda max are automatically excluded from the search (no similarity calculation is made). Entering values for this is optional.

Compound Name Filter

When a Compound Name Filter is specified, library search will be restricted to those library entries whose name contains the specified string as a case-insensitive sub-string. Entries without a matching sub-string are automatically excluded from the search (no similarity calculation is made). Entering a value for this is optional.

Choose a Spectrum to Search

To chose a spectrum to use for the library search,

1. From the **Mixed View**, click **Actions** and then select **Search Library.**
2. Click **Search Spectrum**.
3. Select **Current Data**, **Spectrum File**, or **Named Peak**.

4.   Enter the **Retention Time**, **Spectrum File**, or **Named Peak** to be used for the search.
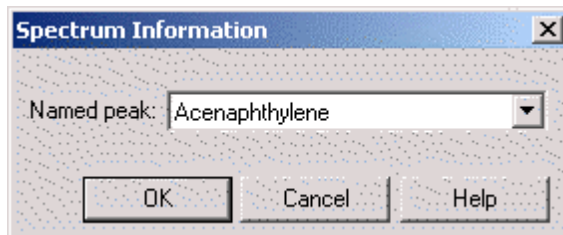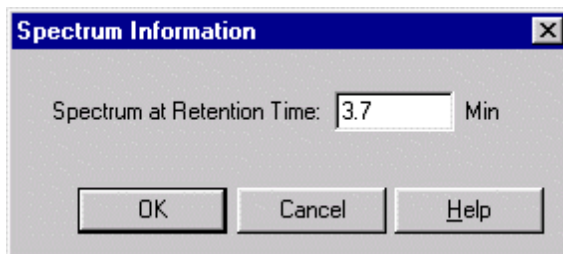
6   How to Perform a Manual Library Search

1.   In the Contour pane of the Mixed View window, drag the vertical cursor to the apex of the peak of interest to display the corresponding spectrum in the spectrum pane.

2.   Click on **Actions** and then click **Search library...** to open the **Spectral Library Search** dialog box. On the toolbar **Search** selection, click either **Method** to use the library parameters from the method, or **Quick** to enable you to modify the search parameters. Before you do the search, make sure you have either selected a library in your method, or have opened a library to do a quick search.

3.   Click **Search Now** to display the Library Search Results window, showing the three closest matches in the specified library. When appropriate, click on the >> or << button to display additional matches.

7   How to Collect Spectra for a Library

5.   Perform an acquisition using a sample with known components or standards to be placed in the library.

6.   Perform an analysis using the appropriate integration parameters. (Ideally, Spectral Background Correction would be checked in the **Spectrum** tab of **PDA Options**.)

7.   Using the **Contour** plot in the **Mixed View** window, drag the vertical cursor to the apex of a peak to display the corresponding spectrum in the spectrum view. See also About Spectra Extracted from the Contour View.

8.   Right-click within the spectrum view to display the Spectrum pop-up menu, select **Utilities** followed by **Save Trace**. A check-mark will appear next to **Save Trace**. Click on the spectrum you wish to save.   Browse to the location where you want to save the spectrum file, and enter a file name in the dialog box and then click **Save**. Repeat this procedure for each

spectrum to be added to the library. Files are saved automatically with the .spc extension.

Note: To add the 1st or 2nd Derivative of a spectrum to the library, select the appropriate filter in the **Spectrum** tab of **PDA Options** and repeat steps 3 and 4.

Reports and Peak Table

8 About PDA Report Items

When the PDA option is enabled, the following report items will be available to insert into a custom report: Library Search Report, Library Definition Report, Purity Report, and Spectrum Report.

Custom Report Graph Items for PDA

The Insert Graph menu has the following additional items available when the PDA Option is enabled: 3D Data Graph, and Contour Graph.

3D Data Graph

The 3D View provides a three-dimensional view of absorbance versus time and wavelength. Wavelengths of appreciable absorbance and interferences, which may be invisible in a single wavelength plot, are easy to locate with the 3D View. Select this function to automatically enter the 3D map into the report. Click the right-mouse button within the 3D map and select Properties to display the 3D Properties dialog box and enter appropriate changes

The 3D map can be elevated and rotated around its axis for display from any angle. These functions work the same as in the 3D view window.

Insert Contour Graph

A data file containing PDA data may include a Contour Plot in a custom report. The contour view (also referred to as Isoabsorbance plot) provides an aerial view of the absorbance of the sample at each wavelength vs. time. The contour view supplies quick and easy–to-assimilate information about those wavelengths at which the sample exhibits appreciable absorbance.

Changing parameters for the contour graph in a custom report works exactly as in the Contour view.

9 Custom Report PDA Parameters

A variety of PDA information can be placed in a custom report. These items are inserted in the report by placing the cursor at the location where you want to insert the item, then do a right mouse click to access the custom report menus.

Create a Library Search Report

To create a Library Search Report, or add a Library Search Report to your custom report,

1. Click **Edit Custom Report** 🖼️ to open the custom report.
2. On the custom report, do a right mouse click and select **Insert Report** followed by **Library Search Report.** The Library Search Report template will be inserted at the location of the cursor in the custom report.

To modify the parameters of the report, do a right mouse click in the report table, then select **Properties**.
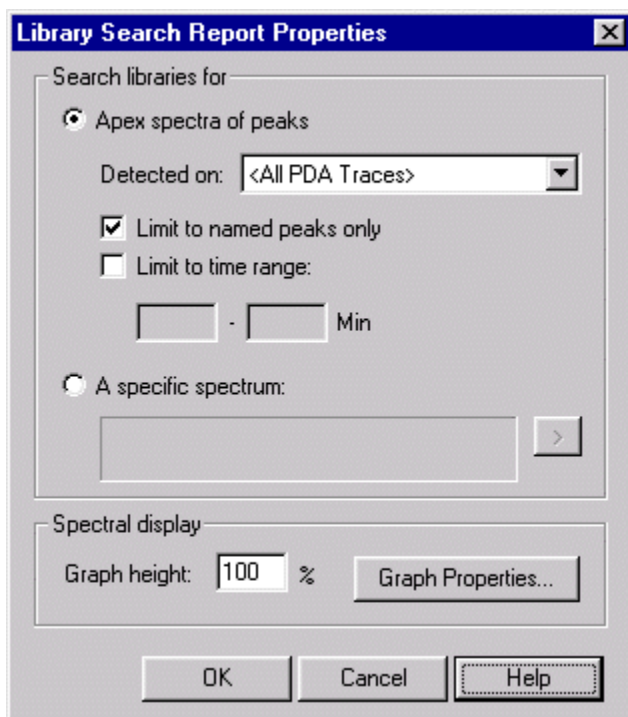
Search Libraries for

This specifies what peaks should be searched for the report. When **Apex Spectra of Peaks** is selected, the **Specific Spectrum** button is disabled. When **A Specific Spectrum** is selected, the other controls of this group are disabled.

Apex spectra of peaks

Choose this option to enable search based on the apex of peaks detected by criteria you select.

Detected on

Select the detection criteria for the peaks to be searched. Choices include **All Multi-Chroms**, **All PDA Traces**, **Max Spectrum Plot**, or the available wavelength channels.

Limit to named peaks only

This allows the user to restrict the peak selection (made above) to a time range. When checked, only peaks within the specified time range will be searched When this box is unchecked, the Time Range edit fields are disabled.

Limit Peaks to time range

This allows you to restrict the peak selection (made above) to a time range. When checked, only peaks within the specified time range will be searched. When this box is unchecked, the Time Range edit fields are disabled.

A Specific Spectrum

Select this to specify the spectrum to be searched. When this is selected, click the ⬜ button to select the spectrum source. The menu contains the following choices: Current Data, Named File, or Spectrum Peak.

Spectral Display

The in this group specify the layout of the search results graphs.

Graph Height

This specifies the relative height of a search results graph. A value of 100% corresponds to a standard sized graph. A larger value may be selected to provide more a detailed plot.

Graph Properties

Pressing this button displays the graph properties dialog as found on the standard Spectrum graph.

Create a Library Definition Report

To create a Library Definition Report, or add a Library Definition Report to your custom report,

1. Click **Edit Custom Report** 🖼 to open the custom report.
2. On the custom report, do a right mouse click and select **Insert Report** followed by **Library Definition Report.** The Library Definition Report template will be inserted at the location of the cursor in the custom report.

To specify parameters for the report, do a right mouse click in the report table, then select **Properties**. The Library Definition Report Properties dialog will appear.
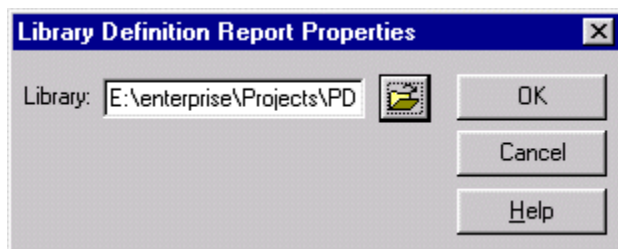

Enter or select the library you wish to include in the report, then click OK.

Create a Purity Report

To create a Purity Report, or add a Purity Report to your custom report,

1. Click **Edit Custom Report** 🖼 to open the custom report.
2. On the custom report, do a right mouse click and select **Insert Report** followed by **Purity Report.** The Purity Report template will be inserted at the location of the cursor in the custom report.

Selecting this option will insert a Purity Report into your custom report. To specify parameters for the report, do a right mouse click in the report table, then select **Properties**.
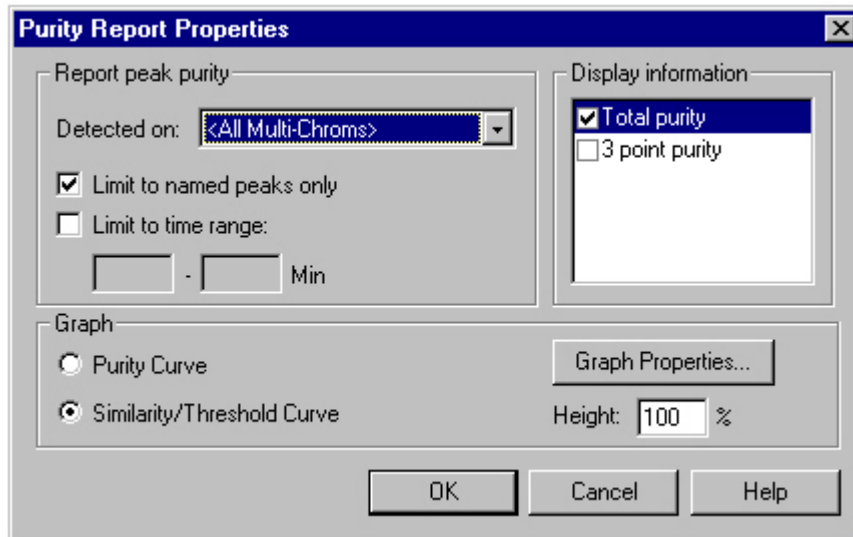
Detected on

This allows the user to select the channel or channels from which peaks will be detected.

Limit to named peaks only

This allows the user to restrict the peak selection (made above) to named peaks.

Limit to time range

This allows the user to restrict the peak selection (made above) to a time range. When checked, only peaks within the specified time range will be searched. When this box is unchecked, the Time Range edit fields are disabled.

Graph

Select the type of curve you want to graph - Purity Curve or Similarity/Threshold Curve.

Height %

This value will enable you to "zoom" the graph. A value of 100% will maximize the curve to the page.

Graph Properties...

Pressing this button displays the graph properties dialog as found on the standard graph.

Create a Spectrum Report

To create a Spectrum Report, or add a Spectrum Report to your custom report,

1. Click **Edit Custom Report** to open the custom report.
2. On the custom report, do a right mouse click and select **Insert Report** followed by **Spectrum Report.** The Spectrum Report template will be inserted at the location of the cursor in the custom report.

The report will display a table showing spectra extracted from peaks of the chromatogram. Based on the selection on the **Spectrum** tab of **PDA Options**, the report

will contain either the apex spectrum, an average of the upslope, apex and downslope spectra, or several averaged spectra.

To set parameters for this report, do a right mouse click on the table, and then select **Properties**.
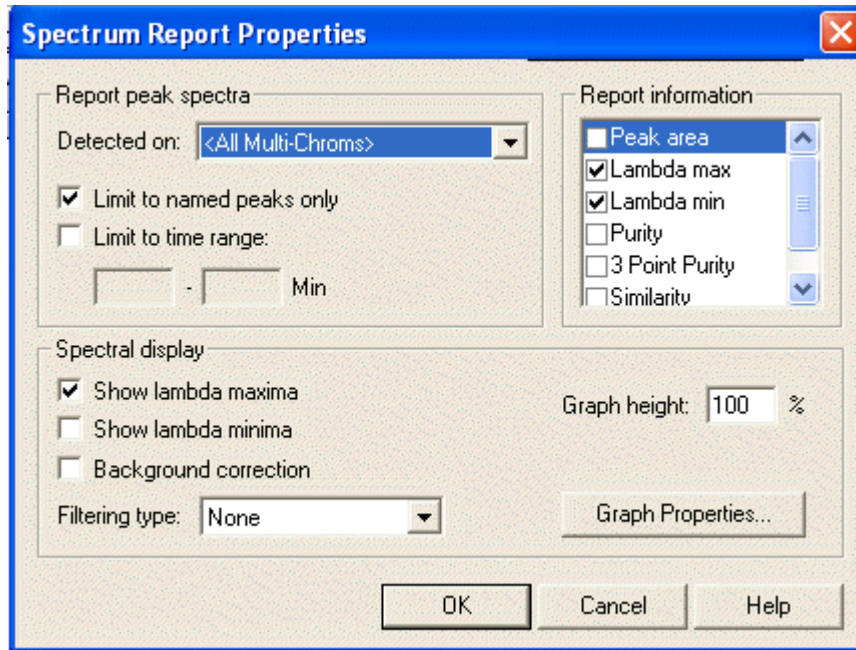
Report peak spectra

Detected on

This allows the user to select the channel or channels from which peaks will be detected.

Limit to named peaks only

This allows the user to restrict the peak selection (made above) to a time range. When checked, only peaks within the specified time range will be searched When this box is unchecked, the Time Range edit fields are disabled.

Limit Peaks to time range

This allows the user to restrict the peak selection (made above) to a time range. When checked, only peaks within the specified time range will be searched. When this box is unchecked, the Time Range edit fields are disabled.

Time Range

This allows the user to restrict the peak selection (made above) to a time range. When the *Limit to time range* box is unchecked, these fields are disabled.

Report information

Checking any of these indicates that the indicated value will be calculated and printed to the right of the graph. Unchecking values that are not of interest will speed up analysis.

Peak Area

Checking this indicates that the peak area should be printed to the right of the spectrum graph.

Lambda Max

Checking this indicates that the three largest lambda max values should be printed to the right of the spectrum graph.

Lambda Min

Checking this indicates that the three largest lambda min values should be printed to the right of the spectrum graph.

Total Purity

Checking this indicates that total peak purity should be printed to the right of the spectrum graph.

3 Point Purity

Checking this indicates that 3 point peak purity should be printed to the right of the spectrum graph.

Similarity

Checking this indicates that peak apex similarity to the reference spectrum should be printed to the right of the spectrum graph. The use of this value requires that a reference spectrum be included in the peak table. See the section on **Analysis and Calculations** for details.

Spectral Display

The selections in this group specify the content and labeling of the spectrum graphs.

Show Lambda Maxima

Checking this box will annotate each spectrum graph with the 3 largest lambda max absorbance.

Show Lambda Minima

Checking this box will annotate each spectrum graph with the 3 largest lambda minima absorbance.

Background Correction

Checking this will cause each apex spectrum to be corrected for background using the chromatographic baseline prior to being used elsewhere in analysis. Refer to the **PDA Analysis and Calculations** section for details on the formula used.

Filtering Type

This allows the user to specify a mathematical filtering function to be performed on all spectra extracted from the 3D data during analysis. Refer to the **Analysis and Calculations** section for details on the formula used for each filter.

Graph Height

This specifies the relative height of the spectrum graph. A value of 100% corresponds to a standard sized graph. A larger value may be selected to provide more a detailed plot.

Graph Properties

Click this button to display the graph properties dialog as found on the standard Spectrum graph.

10 Changes to the Peak Table for PDA Option

When using the PDA option, certain columns will be added to the peak table that enable you to analyze peaks from the PDA detector.

Detection

Select the basis for the identification of the peak. If you choose **Ret Time**, only the retention time will be used for identification of the peak. If you choose **Ret Time with Spectral Confirm**, the Similarity of the peak's spectrum to that of a designated reference spectrum will be used in addition to the retention time as the basis of peak identification.

Spectrum

If you want Similarity to be used as a basis for peak identification, then click on the arrow to the right of this field to specify the stored reference spectrum to be used for comparison. During identification, this reference spectrum is compared to the peak apex spectrum and a similarity index is computed. A peak is considered identified if this calculated similarity index is at least the value specified in the Similarity column of the peak table.

If Similarity is not specified as a basis for peak identification, then this field is ignored.

Similarity

If Similarity is specified as a basis for peak identification, then this field specifies required minimum similarity index for a peak to be considered identified. During identification, the reference spectrum (see previous section) is compared to the peak apex spectrum and a similarity index is computed. A peak is considered identified if the calculated similarity index is at least the value in this field.

If Similarity is not specified as a basis for peak identification, then this field is ignored.

Analysis Channel

Specify which Diode Array wavelength channel is to be used for analysis of the peak. The choices will be those specified in **Instrument Setup/DAD**.

11 PDA Analysis and Calculations

This section describes the various types of calculations that are related to PDA data and how the data is analyzed.

General

In order to maintain accuracy during the application of multiple operations, all calculations are performed using double-precision floating-point numbers.

Analysis spectra

Background correction

About Chromatograms Extracted from the Contour View

Two types of chromatograms may be extracted from the 3D data:

- [Multi-Chromatogram Channels](#): One or more chromatograms defined on the **Multi-Chromatogram** tab.

- [Working Chromatogram](#): A single chromatogram extracted from the Contour plot on the **Mixed View** display. (This chromatogram is displayed in the **Chromatogram View)**

To generate a **chromatogram view** (extract a chromatogram) from contour view of Mixed View or Mixed View w/ 3D:

3. Select **PDA Views** from the **View** menu, and then select **Mixed View** to display the Contour Map, Chromatogram, and Spectrum.
4. Move the cursor to the triangle-shaped handle located on the left- hand wavelength axis of the Contour Map and press the left mouse button.
5. Drag the cursor up or down to the desired wavelength and release the mouse button.
6. The chromatogram associated with the specified wavelength is displayed in the Chromatogram View.

About Multi-Chromatogram Channels

The following apply to multi-chromatogram channels:

- In the Channel Selection drop down list, each multi-chromatogram channel have a unique identifier that includes the wavelength and bandwidth of the channel.

- Each Multi-Chromatogram Channel have a separate Integration Events Table, Manual Integration Table, Export Table and Performance Table associated with it.

- All Multi-Chromatogram Channels share a common peak and group table. Within the peak and group tables, any channels may be selected as the analysis channel for quantitative information.

- When an analysis is performed, all multi-chromatogram channels are automatically analyzed.

- Each multi-chromatogram channel is an average of the absorbances monitored at each wavelength in the wavelength range. The wavelength range is equal to the selected wavelength +/- one half of the bandwidth.

About the Working Chromatogram

The following apply to the working chromatogram:

- In the Channel Selection drop down list, a single entry is added that identifies the channel as applying to the working chromatogram.

- This channel has a separate Integration Events Table, Manual Integration Table, Export Table and Performance Table associated with it. These are separate from the tables of the Multi-Chromatogram Channels (above).

- When an analysis is performed, the working chromatogram will automatically be analyzed.

- The working chromatogram is an average of the absorbances monitored at each wavelength in the wavelength range. The wavelength range is equal to the selected wavelength +/- one half of the bandwidth.

About Spectra Extracted from the Contour View

Two types of spectra may be extracted from the 3D data:

- Analysis Spectra: One or more spectra automatically extracted from the 3D data during analysis and used for peak identification, similarity and/or library search.

- Working Spectrum: A spectrum extracted from the Contour plot on the *Mixed View* display. (This chromatogram is displayed in the *Spectrum* View)

To generate a **spectrum view** (working spectrum) from the contour view of Mixed View or Mixed View w/ 3D:

7. Select **PDA Views** from the **View** menu, then select **Mixed View** to display the Contour Map, Chromatogram, and Spectrum.
8. Move the cursor to the triangle-shaped handle located on the time axis of the Contour Map and click the left mouse button.
9. Drag the cursor to the desired peak and release the mouse button
10. The spectrum associated with the specified retention time value is displayed in the Spectrum View.

About Analysis Spectra

The following applies to the analysis spectra:

- Prior to being used elsewhere, all analysis spectra extracted from the data are filtered according to the settings on the *Spectrum* tab of *PDA Options*.

About Working Spectrum

The following apply to the working spectrum:

- Working spectra extracted from the 3D data are never filtered according to the settings on the **Spectrum** tab of **PDA Options**.

- The working Spectrum is not available as a trace in Custom Reports.

Background Correction

Background Correction may only be performed on a spectrum as a result of the settings on the **Spectrum** tab of **PDA Options**, the settings on the **Purity** tab of **PDA Options**, or by setting the properties of the Peak Purity Pane.

Background subtraction is always the first operation performed on a spectrum. If a background correction is attempted on a spectrum after another operation has already been performed (including an operation specified in the **Spectrum** tab of **PDA Options**), a message box is displayed and the background correction request ignored.

Background subtraction may only be performed once on a spectrum. If a second background correction is attempted on a spectrum after the operation has already been performed (including the operation specified in the **Spectrum** tab of **PDA Options**), a message box is displayed and the background correction request ignored.

A background correction is performed as follows:

1. The spectra from the baseline start and baseline stop times for the peak are extracted from the 3D data. The Max Plot is used to determine the peak that is used.

2. For each spectrum in the peak, a corresponding background spectrum is generated by linear interpolation between the baseline start and baseline stop spectra.

3. These background spectra are subtracted from the original spectra.

Note: While compensation for background will provide more precise purity calculations, it can slow down re-analysis of large data files.

Spectrum Interpolation

Spectrum interpolation may be performed on a spectrum as a result of the settings on the **Spectrum** tab of **PDA Options** or by doing a right mouse click in the **Spectrum** window and then selecting **Operations** followed by **Interpolate**.

Interpolated spectra may not be stored in spectrum libraries.

The calculation is performed by doing a 10:1 interpolation of the spectrum data points using a cubic spline curve fit. This interpolation is performed after the applying any spectral filtering option (1st derivative, 2nd derivative or smooth) to the spectrum.

Upslope and Downslope Spectra

The upslope and downslope spectra of a peak are identified by calculating the second derivative of the portion of the chromatogram containing the peak. The two times at which the second derivative plot crosses zero are known as the inflection points. For normal peaks (i.e. non-Negative peaks), the Upslope Spectrum is the spectrum at the time represented by the first inflection point, while the Downslope Spectrum is the spectrum at the time represented by the second inflection point.

Spectrum Operations

The following table describes what operations can be done on what type of spectra

	Raw Spectra (no filtering or operations)	Smoothed, 1st Derivative and 2nd Derivative Spectra	Interpolated Spectra
Search against Libraries	Yes		
Similarity	Yes		
Peak Identification using spectral confirmation	Yes, but generate an error if the operations that were performed on the reference spectrum do not match the current method.		
Appear in Reports	Yes	Yes, but label must include what operations were performed	
Purity	Yes	No.	
Add to Spectrum Library	Yes		No

Spectrum Smoothing

Spectrum smoothing may be performed on a spectrum as a result of the settings on the **Spectrum** tab of **PDA Options** or by doing a right mouse click in the **Spectrum** window, and then selecting **Operations** followed by **Smooth**.

Smoothing may be performed repeatedly on a spectrum.

The calculation is performed by doing a 9 point Savitsky-Golay smooth on the spectrum data points.

Spectrum Derivatives

Calculation of the 1st and 2nd derivatives of a spectrum may be performed on a spectrum as a result of the settings on the **Spectrum** tab of **PDA Options** or by doing a right mouse click in the **Spectrum** window, and then selecting **Operations** followed by **1st or 2nd *D*erivative** .

Derivatives may be computed repeatedly on a spectrum.

The absorbance values of the 1st derivative of a spectrum are computed by calculating the differences between adjacent absorbance values to create a new spectrum. The 2nd

derivative of a spectrum is defined as the 1st derivative of the 1st derivative of the spectrum.

Library Search Calculations

General

During analysis, if one or more search libraries are defined on the **Library** tab of **PDA Options**, then an automated library search is performed on every integrated peak of every PDA analysis channel.

Note: Unless a **Library Search Results** object is part of the method custom report, no automated library searching will done when analysis is performed.

In this section, a **Query Spectrum** is defined the unknown spectrum that is being searched. A **Reference Spectrum** is defined as a spectrum from a spectrum library file.

During a search, the apex spectrum of the peak (the query spectrum) is compared to each spectrum contained in the libraries (reference spectra) to determine the similarity of the query spectrum to the reference spectrum. The similarity is quantified through the calculation of a Similarity Index for each query/reference pair. The Similarity Indices are used to generate a hit list of the best matching entries. A perfect match will have a Similarity Index of 1.0000. Similarity indices less than 1 indicate differences in the spectral patterns.

If the query spectrum and the reference spectrum have different wavelength ranges, then the intersection of the two ranges is used in the similarity calculation.

If the query spectrum and the reference spectrum have different wavelength steps, then the higher resolution spectrum is de-resolved to match the other spectrum before being used in the similarity calculation.

Pre-Filters

A pre-filter is a criterion on a reference spectrum that must be met before that spectrum is used in similarity calculations. One or more pre-filters may be specified in the search parameters. When multiple pre-filters are specified, a reference spectrum must meet all of the individual pre-filters in order to be considered for similarity calculations.

Reference spectra that do not meet all of the pre-filter criteria are automatically excluded from calculations and from being a candidate for a hit list. No similarity calculation is performed on these spectra.

The following pre-filters are supported:

Retention Time Range: When a Retention Time Range is specified, a reference spectrum is excluded unless the retention time for the spectrum in the library is within the specified range.

Lambda Max: Up to three Lambda Max values may be specified. When one or more Lambda Max values are specified, a reference spectrum is excluded unless all of the specified maxima are within 5 nm of a maxima for the spectrum in the library.

Compound Name: When a Compound Name is specified, a reference spectrum is excluded unless the specified string is a case-insensitive sub-string of the compound name for the spectrum in the library.

Ratio Chromatogram Calculation

The ratio chromatogram plot consists of data points calculated as follows:

Ratio Pt. = abs_1 / sqrt(abs_1 * abs_1 + abs_2 * abs_2)

where: abs_1 = the absorbance in chromatogram 1 at this wavelength

abs_2 = the absorbance in chromatogram 2 at this wavelength

If the absorbance of any point in chromatogram 1or chromatogram 2 is less than the threshold, the Ratio Pt. for that wavelength is set to 0.

Similarity Calculations

The Similarity Index (SI) compares two spectra across the wavelength range defined in **Method > PDA Setup > Library**. A Library Search is performed using the Similarity Index, determined as follows:

A spectrum is considered as an array of absorbances at each wavelength.

$(a(\lambda_1), a(\lambda_2), ..., (a(\lambda_n))$

Where $a(\lambda_i)$ is the absorbance at λ_i

The spectrum can also be represented as an n-dimensional vector, with each absorbance corresponding to one dimension of the vector, as follows:

$S = (a(\lambda_1), a(\lambda_2), ..., (a(\lambda_n))$

When two spectra are obtained from the same compound, the ratio between corresponding elements in S1 and S2 is constant; consequently, the vectors have the same direction. In such a case, the angle between the two vectors would be 0. As a general rule, the greater the angle between the two vectors, the less similarity they have. This relationship is calculated as follows:

$$SI = \cos(\theta)$$

where :

θ = The angle between the two vectors

The closer the SI value is to 1, the more similar two spectra are considered to be. Two spectra are considered to be identical when the SI value is close to 1.

Lambda Max/Min Calculations

A lambda max (min) is defined as a local maximum (minimum) of the absorbance values of a spectrum. The user must define the wavelength range over which the calculation should be performed.

To compute n lambda max (min) values for a spectrum, the software will find the n local maxima (minima) with the largest (smallest) absorbance values.

The calculation range of Lambda Min/Max is based on the X-axis settings for the graph in the *Axis Setup...* context menu item of the graph.

Peak Purity Calculations

The purity of a peak is calculated by comparing each spectrum in the peak to the apex spectrum using the calculation given in the Similarity Calculation section. To derive a purity number, the peak area under the spectra whose similarity meets the purity threshold will be divided by the total area of the peak.

Calculating Total Purity

Total purity for an integrated peak is calculated as follows:

1. The purity calculation range of the peak is determined by starting from the peak apex and working toward peak start and peak end. Each chromatographic point in the peak is compared to the absorbance threshold. The start and stop values of the range are defined as the point at which the absorbance drops below the absorbance threshold.

2. For each point in the calculation range, a similarity index is calculated by comparing the spectrum for that point to the peak apex spectrum.

3. All spectra whose similarity index meets or exceeds the Purity Threshold will be considered similar to the apex spectra.

4. A sum is calculated of the chromatographic area represented spectrally similar points.

5. The Purity Index is calculated by dividing this sum by the total area over the calculation range of the peak.

6. The purity index will have a range of between 0.000000 and 1.000000.

Three Point Purity

Three Point Purity is calculated by comparing the spectrum at the apex of a peak (Point 1) with the spectra at the up-slope (Point 2) and down-slope (Point 3) of the peak. The up-slope spectrum is defined as the spectrum located 20% of the distance from the peak start to the peak apex. The down-slope spectrum is defined as the spectrum located 20% of the distance from the peak end to the peak apex. These two similarity indices are then averaged to get the 3-point purity value.

$$\text{Time}_{up} = \text{Time}_{start} + (\text{Time}_{apex} - \text{Time}_{start}) * 1/5$$

$$\text{Time}_{down} = \text{Time}_{apex} + (\text{Time}_{end} - \text{Time}_{apex}) * 4/5$$

Similarity indices are generated for the up-slope and down-slope spectra, relative to the apex. Since spectra taken at different points along a pure peak will look identical, they will have high similarity indices. The closer the Similarity Index is to 1.0000, the more similar or more pure the peak is deemed to be.

While it is easy to decide that a peak with a very low Purity value (0.0000-0.8900) is indeed not pure, it is more difficult to decide whether peaks with purity values between 0.9000 and 0.9500 are actually contaminated. The user's judgment is required to make this determination and may necessitate using other methods to determine purity, such as overlaying the spectra or comparing first and second derivatives.

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