Empower PDA Software

Getting Started Guide

NOTICE

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Preface

The *Empower PDA Software Getting Started Guide* describes the basics of how to use the Empower™ PDA option to develop a PDA processing method for peak purity and library matching and to review PDA results.

This guide is intended for a wide variety of users whose familiarity with computers and software ranges from novice to expert.

Organization

This guide contains the following:

Chapter 1 describes the Empower PDA software.

Chapter 2 describes the basics of viewing PDA data.

Chapter 3 describes how to derive chromatograms and how to develop a peak purity processing method.

Chapter 4 describes how to create a spectral library and match spectra to the library.

Chapter 5 describes the basics of printing reports.

Appendix A, Default PDA Reports shows examples of various PDA reports available in Empower software.

Related Documentation

***Waters Licenses, Warranties, and Support*:** Provides software license and warranty information, describes training and extended support, and tells how Waters handles shipments, damages, claims, and returns.

Online Documentation

***Empower Help*:** Describes all Empower windows, menus, menu selections, and dialog boxes for the base software and software options. Also includes reference information and procedures for performing all tasks required to use Empower software. Included as part of the Empower software.

***Empower Read Me File*:** Describes product features and enhancements, helpful tips, installation and/or configuration considerations, and changes since the previous version.

***Empower LIMS Help*:** Describes how to use the Empower LIMS Interface to export results and import worklists.

Empower Toolkit Professional Help: Describes how to use the common-object-model, message-based protocol to communicate with the Empower software from a third-party application.

Printed Documentation for Base Product

Empower Software Getting Started Guide: Provides an introduction to the Empower software. Describes the basics of how to use Empower software to acquire data, develop a processing method, review results, and print a report. Also covers basic information for managing projects and configuring systems.

Empower Software Data Acquisition and Processing Theory Guide: Provides theories pertaining to data acquisition, peak detection and integration, and quantitation of sample components.

Empower System Installation and Configuration Guide: Describes Empower software installation, including the stand-alone Personal workstation, Workgroup configuration, and the Enterprise client/server system. Discusses how to configure the computer and chromatographic instruments as part of the Empower System. Also covers the installation, configuration, and use of acquisition servers such as the LAC/E^{32} module, the busLAC/E™ card, and interface cards used to communicate with serial instruments.

Empower System Upgrade and Configuration Guide: Describes how to add hardware and upgrade the Empower software using an import-and-export upgrade method.

Empower Software System Administrator's Guide: Describes how to administer the Empower Enterprise client/server system and Workgroup configuration.

Empower Software Release Notes: Contains last-minute information about the product. Also provides supplementary information about specific Empower software releases.

Printed Documentation for Software Options

Empower System Suitability Quick Reference Guide: Describes the basics of the Empower System Suitability option and describes the equations used by the System Suitability software.

Empower PDA Software Getting Started Guide: Describes the basics of how to use the Empower PDA option to develop a PDA processing method and to review PDA results.

Empower GC Software Getting Started Guide: Describes how to use the Empower GC option to develop a GC processing method and to review GC results.

***Empower GPC Software Getting Started Guide*:** Describes how to use the Empower GPC option to develop a GPC processing method and to review GPC results.

***Empower GPCV Software Getting Started Guide*:** Describes how to use the Empower GPCV option to develop a GPCV processing method and to review GPCV results.

***Empower Light Scattering Software Getting Started Guide*:** Describes how to use the Empower Light Scattering option to develop a light scattering processing method and to review light scattering results.

***Empower ZQ Mass Detector Software Getting Started Guide*:** Describes installation, configuration, calibration, and tuning methods, as well as how to operate the ZQ Mass Detector with Empower software.

***Empower Chromatographic Pattern Matching Software Getting Started Guide*:** Describes how to use the Chromatographic Pattern Matching option to develop a pattern matching processing method and to review pattern matching results.

***Empower Dissolution System Software Quick Start Guide*:** Describes how to operate the Alliance® Dissolution System using Empower software.

***Empower Toolkit Programmer's Reference Guide*:** Describes how to use the common-object-model, message-based protocol to communicate with Empower software from a third-party application.

***Waters Integrity System Getting Started Guide*:** Describes features of the Waters Integrity® System and provides step-by-step tutorials that guide a user through the use of the Empower Mass Spectrometry (MS) option.

***Empower AutoArchive Software Installation and Configuration Guide*:** Describes how to install and configure the Empower AutoArchive option.

Documentation on the Web

Related product information and documentation can be found on the World Wide Web. Our address is http://www.waters.com.

Related Adobe Acrobat Reader Documentation

For detailed information about using Adobe® Acrobat® Reader, see the *Adobe Acrobat Reader Online Guide*. This guide covers procedures such as viewing, navigating, and printing electronic documentation from Adobe Acrobat Reader.

Printing This Electronic Document

Adobe Acrobat Reader lets you easily print pages, page ranges, or the entire document by selecting **File > Print**. For optimum print quantity, Waters recommends that you specify a PostScript® printer driver for your printer. Ideally, use a printer that supports 600 dpi print resolution.

Documentation Conventions

The following conventions can be used in this guide:

Convention	Usage
Purple	Purple text indicates user action such as keys to press, menu selections, and commands. For example, "Click **Next** to go to the next page."
Italic	Italic indicates information that you supply such as variables. It also indicates emphasis and document titles. For example, "Replace *file_name* with the actual name of your file."
Courier	Courier indicates examples of source code and system output. For example, "The SVRMGR> prompt appears."
Courier Bold	Courier bold indicates characters that you type or keys you press in examples of source code. For example, "At the LSNRCTL> prompt, enter **set password oracle** to access Oracle."
<u>Underlined Blue</u>	Indicates hypertext cross-references to a specific chapter, section, subsection, or sidehead. Clicking this topic using the hand symbol brings you to this topic within the document. Right-clicking and selecting **Go Back** from the shortcut menu returns you to the originating topic. For example, "<u>Section 4.1.2, Steps in Library Matching</u>, shows the steps used for library matching."
Keys	The word *key* refers to a computer key on the keypad or keyboard. *Screen keys* refer to the keys on the instrument located immediately below the screen. For example, "The A/B screen key on the 2414 Detector displays the selected channel."
…	Three periods indicate that more of the same type of item can optionally follow. For example, "You can store *filename1*, *filename2*, … in each folder."
>	A right arrow between menu options indicates you should choose each option in sequence. For example, "Select **File > Exit**" means you should select **File** from the menu bar, then select **Exit** from the File menu.

Notes

Notes call out information that is helpful to the operator. For example:

Note: Record your result before you proceed to the next step.

Attentions

Attentions provide information about preventing damage to the system or equipment. For example:

STOP *Attention: To avoid damaging the detector flow cell, do not touch the flow cell window.*

Cautions

Cautions provide information essential to the safety of the operator. For example:

Caution: To avoid burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.

Caution: To avoid electrical shock and injury, turn off the detector and unplug the power cord before performing maintenance procedures.

Caution: To avoid chemical or electrical hazards, observe safe laboratory practices when operating the system.

Chapter 1
PDA Software Overview

This chapter describes basic features of Empower™ software for the PDA detector, an overview of this tutorial, and the process for restoring sample PDA data on your workstation or client. Once you have loaded the sample data, you can view and manipulate the data in a variety of ways, as described in .

1.1 What Is PDA Software?

The Empower Photodiode Array (PDA) software enables you to use Empower software to acquire and process spectral and chromatographic data. You use the PDA software with the Waters® 996 PDA Detector and/or the Waters 2996 PDA Detector.

Features of the PDA Software

The Empower System is a total chromatography and results management system that you can adapt to your individual chromatography requirements. The Empower System consists of the following components:

- A computer that runs Empower software. Basic hardware configurations are:
  - Personal – A standalone workstation running Empower software
  - Workgroup – A small network running Empower software with a maximum of four computers
  - Enterprise – A client/server network running Empower software
- Empower software
- Empower database

The Empower PDA software is an integrated part of Empower software. PDA data acquisition, processing, and reporting with the PDA software requires use of the base LC Empower software.

Features of the Base LC Empower Software

Empower software provides a graphical, icon-based user interface to acquire, process, and manage chromatographic data. Empower software runs on Windows® 2000 Professional and Windows XP Professional operating systems. Empower software also supports multitasking operations, providing you with the ability to have multiple windows

open at the same time. You can view a real-time data acquisition run while simultaneously producing summary results of previously acquired data.

The base LC Empower software provides tools for:

- Creating projects
- Configuring chromatographic systems
- Developing instrument methods to control chromatographic systems
- Acquiring data from samples and standards
- Developing a processing method to perform integration, calibration, and quantitation
- Processing data and obtaining results
- Creating report methods to generate custom reports
- Viewing and printing reports
- Backing up, deleting, restoring, and copying the contents of an individual project

PDA Software Functions

The Empower PDA software supports the following:

- **PDA Instrument dialog box** – Allows you to define control parameters for the 996 PDA Detector and the 2996 PDA Detector.
- **Real-time PDA data acquisition displays** – Allow you to plot the latest acquired spectra and wavelengths in Run Samples for up to four individual real-time data acquisition plots.
- **3D blank subtraction** – Allows you to use a method set to subtract a labeled 3D chromatogram (a solvent blank) from a second 3D chromatogram (a sample or standard) and display the difference in Review. This feature is useful for subtracting the effects of a mobile phase containing strong UV absorbing compounds.
- **PDA calibration** – Allows you to examine the wavelength calibration, the reference spectrum and dark current, and the effects of exposure time on photodiode saturation.
- **PDA diagnostics** – Allow you to run internal and external diagnostics on the 996 PDA Detector and the 2996 PDA Detector.
- **Review** – Allows you to view your chromatograms and spectra by using the Contour plot, which allows you to extract, integrate, and process 2D chromatograms and spectra (from the 3D data), and the Spectrum Index plot, which displays selected spectra for the peaks shown in the Chromatogram plot. Displays the maximum impurity for multiple PDA purity passes. Also displays the Review Results window which displays purity and library match results.

- **Spectrum Review Spectral plot** – Allows you to display library, peak, and extracted spectra in Review, search against library spectra, create derived spectra, and create and maintain spectral libraries.
- **Spectrum Review tables** – Allow you to display spectral and match results data in Review, such as the:
  - Spectra table with an image of all extracted spectra and peak apex spectra with complete data on each spectrum including wavelength range and the resolution used to acquire PDA data
  - Library Match table with the results of the library search
  - Spectrum Points table with the wavelength and absorbance data points

1.2 Tutorial Overview

This guide does *not* cover acquiring data, optimizing integration, generating calibration curves, quantitating unknowns, obtaining results for 2D chromatograms, or performing specific PDA procedures. For details on these topics, see the *Empower Software Getting Started Guide* or the *Empower Help*.

This tutorial shows you how to:

- Restore PDA data from the Empower Software Options CD-ROM. This data is used in all the examples in this guide.
- Extract chromatograms and spectra.
- Develop a processing method.
- Calculate peak purity and examine peak purity results.
- Build a UV spectral library and match unknown spectra to spectra in the library and examine library match results.
- Generate a report.

Before You Begin

Note: This guide uses the Empower Pro interface. If you do not have access to this interface, see your system administrator.

Before you perform the procedures described in this guide, make sure that:

- You installed or upgraded the Empower software as described in the *Empower System Installation and Configuration Guide* or the *Empower System Upgrade and Configuration Guide*.
- You reviewed and followed the basic operating procedures, including data acquisition, described in the *Empower Software Getting Started Guide*.

- You read the *Waters 996 PDA Detector Operator's Guide* or the *Waters 2996 PDA Detector Operator's Guide.*
- Your printer is properly configured for use with Empower software.

1.3 Restoring the PDA Project

To run through the tutorial, you will restore the PDA example project, called PDA_Default. This project contains the sample PDA data used in all the examples in this guide. Use the Restore Project Wizard in Configuration Manager to restore the project data to your computer. The PDA project is located on the Empower Options CD-ROM.

To restore the PDA project PDA_Default:

1. Start Empower software by double-clicking the **Empower Login** desktop icon. Alternatively, select **Start > Programs** (for Windows XP, **All Programs) > Empower > Empower Login**. The Empower Login dialog box appears (Figure 1-1).

Personal System	Workgroup or Enterprise System
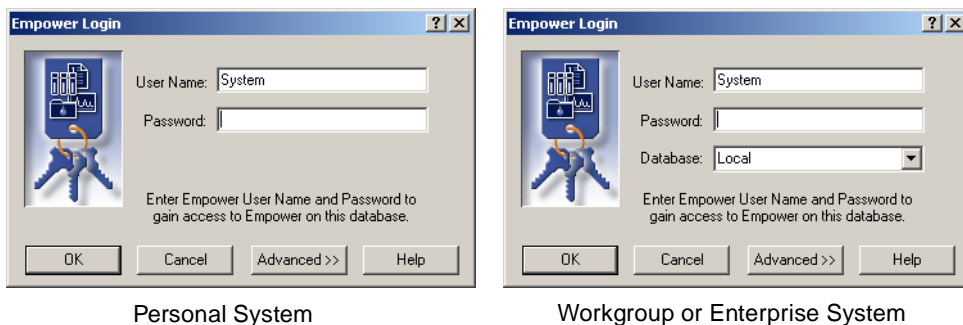
Figure 1-1 Empower Login Dialog Box

2. Enter your user name and password. If you do not know your user name or password, see your system administrator.

3. If you are using an Empower Enterprise system, select the correct database from the Database list. This list appears only when you are connected to a client/server system.

4. Click **Advanced** and verify that the Requested Interface field is set to Pro. If you cannot select the Empower Pro interface, see your system administrator.

5. Click **OK**. The Empower Pro window appears with the name of the logged-in user displayed (Figure 1-2).
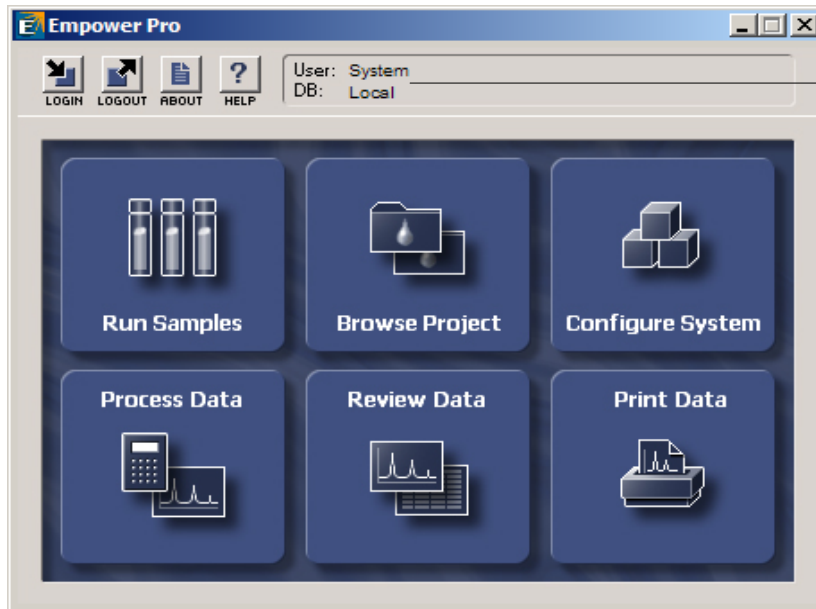
Figure 1-2 Empower Pro Window

6. Click **Configure System** ([Figure 1-2](#)) to open Configuration Manager with the available projects displayed in the view table ([Figure 1-3](#)).

Restore Project(s) Tool

Figure 1-3 Configuration Manager

7. Click (Restore Project(s)) ([Figure 1-3](#)) to start the Restore Project Wizard ([Figure 1-4](#)).

8. Click the **Browse** button to select the path of your CD-ROM drive as shown in Figure 1-4. The drive letter can differ from the one in Figure 1-4.

Browse Button
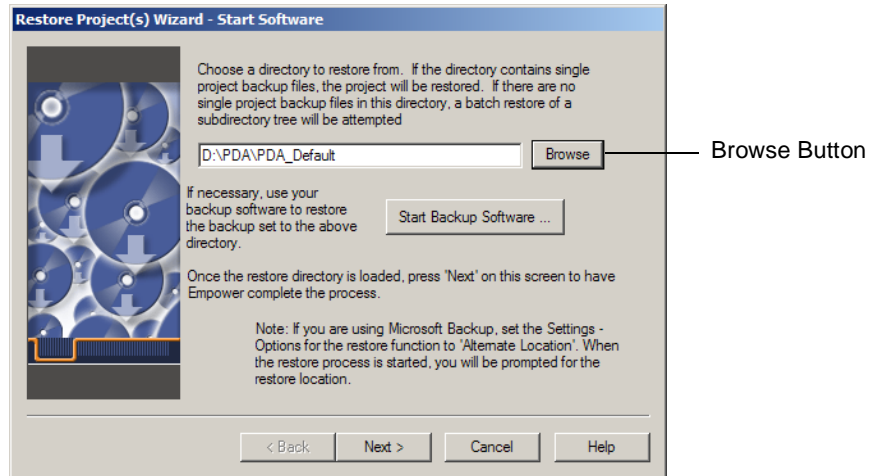
Figure 1-4 Restore Project Wizard - Start Software Page

9. Click **Next** and follow the instructions on each page to complete the wizard.

    Note: *If a message indicates that the restored project needs to be updated, click* ***OK***.

10. Click **Finish** on the last page, Restore Display, to complete the restoration. The restored project, PDA_Default, appears in the Configuration Manager Projects view table (Figure 1-5). If the project does not appear, select **View > Refresh**.

11. Select **File > Exit** to close Configuration Manager.

Figure 1-5 Configuration Manager with Restored PDA_Default Project

You are now ready to view the PDA data using Empower software.

Chapter 2
Viewing PDA Data

This chapter provides step-by-step procedures for viewing chromatograms and spectra.

2.1 Tutorial Overview

This tutorial shows you the basics of using Empower software to view the data in the PDA_Default project. Now that you have restored PDA sample data to your Empower workstation, you can examine it in a variety of ways. The procedures in this chapter are the building blocks for using the PDA software and are a foundation for the procedures in Chapter 3, Peak Purity Processing, and Chapter 4, Library Matching. This chapter describes the simple tasks you can do to view the PDA data that comes with Empower software, including:

- Using Review and viewing the Contour plot
- Displaying the 3D plot
- Zooming in on plots
- Extracting a chromatogram
- Extracting a spectrum
- Annotating chromatograms and spectra

2.2 Viewing Data in Review

Review is the Empower software application that lets you view and manipulate 2D and 3D chromatographic data brought in from the Project window. Review consists of menus, toolbars, the Chromatogram plot, the Channel table, the Contour plot, the Spectrum Review Spectral plot, and the Spectrum Review tables. Each item is explained in this section.

To view a PDA data file in Review:

1. In the Empower Pro window (Figure 2-1), click **Browse Project** to open the Browse Project dialog box (Figure 2-2).
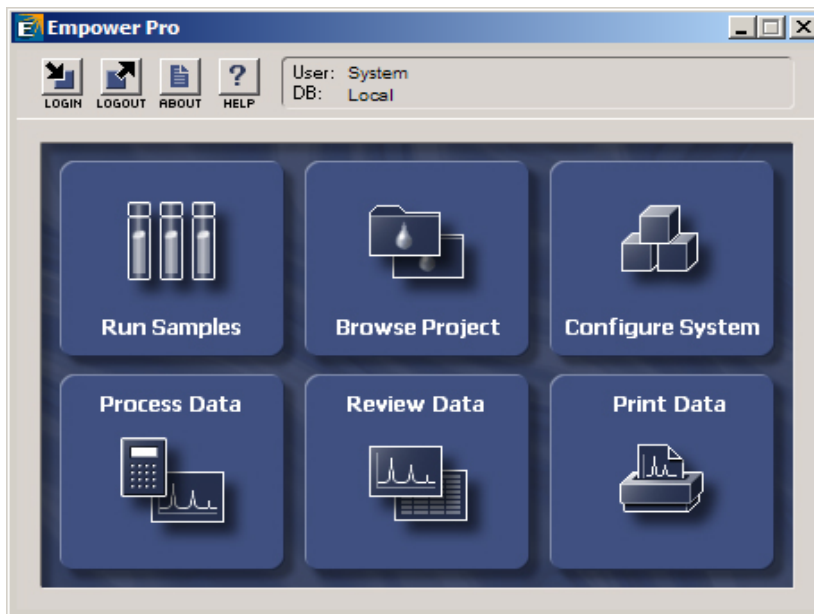
Figure 2-1 Empower Pro Window
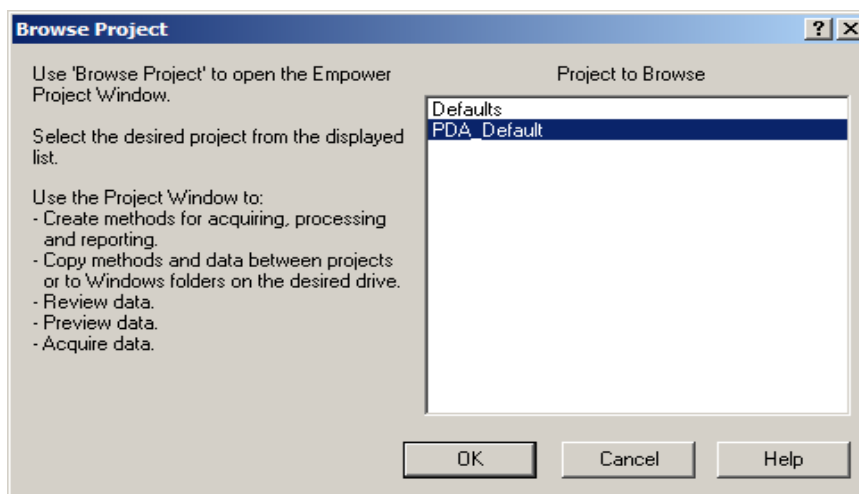
Figure 2-2 Browse Project Dialog Box

2. In the Project to Browse list, select **PDA_Default** and click **OK**. The project window opens (Figure 2-3).

Review Button   Channels Tab

Channels
View Table

Figure 2-3 Project Window

3. Click the **Channels** tab in the Project window, then select **Mixture** in the Channels View table. Click 🖼 (Review) or select **Tools > Review** (Figure 2-3). Review appears with the unprocessed data in a screen similar to the one in Figure 2-4. (If your screen does not resemble Figure 2-4, select **Window > Main Window**.)

Figure 2-4 Review with PDA Data, no Chromatogram Extracted

4. If the Review window is not maximized, click ▭ (maximize) ([Figure 2-4]). Maximize both the main window and the daughter window.

    *Note: If you do not see the Contour plot and Spectrum Review, select **View > 3D Layout***.

    [Figure 2-5] displays Review with the Review Main window maximized. The Contour plot is an overhead view of the three-dimensional data file in which the x-axis plots time and the y-axis plots wavelength. The chromatogram plot displays extracted chromatograms. Spectrum Review displays extracted spectra.

    Note: No chromatograms or spectra are extracted at this time.
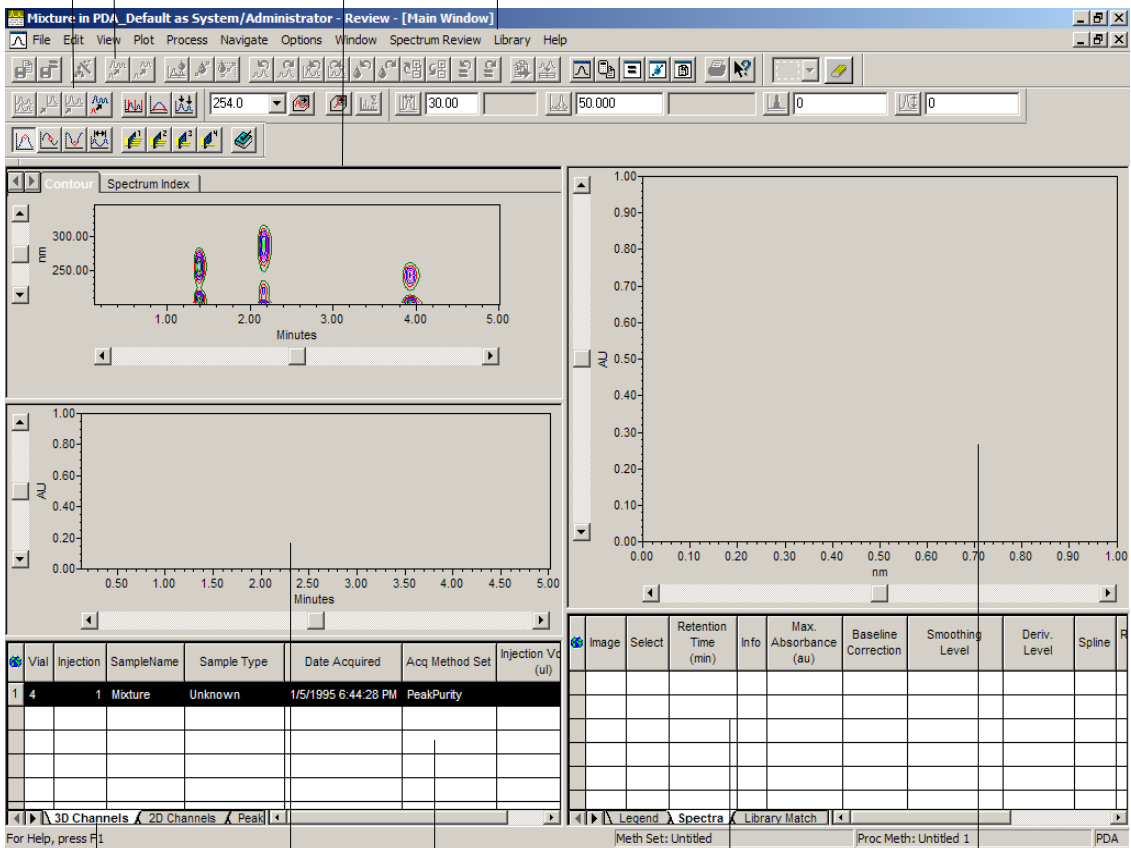
Figure 2-5 Maximized Review Main Window in 3D Layout

5. Click the **3D Channels** tab at the bottom of Review (Figure 2-5) to view information regarding the data file, such as sample name, type, date, time acquired, and so on.

6. Figure 2-6 shows the tools you can use as shortcuts in Review. This figure shows the toolbar separated into two rows in order to label the tools.

Review Toolbar

Save Processing Method

Save All

Processing Method Wizard

Full View

Unzoom

Integrate

Calibrate

Quantitate

Previous 2D Channel

Next 2D Channel

Review Tools

Review Main
Window

Processing Method

Results

Method Set

Annotation Tools
Drop-Down List

Clear Annotation
Tool

Apply Method Set

Next 3D Channel

Previous 3D Channel

3D Plot

Empower Help

Print

Overlay

Extract Choices Drop-Down List

Extract Chromatogram

Extract Spectrum

Library Match

Maximum Impurity-Pass 4

Maximum Impurity-Pass 3

Maximum Impurity-Pass 2

Maximum Impurity-Pass 1

Peak Offsets

Peak Valleys

Peak Inflection

Peak Apex

Set Peak Width,
Set Threshold,
Set Minimum Area,
and
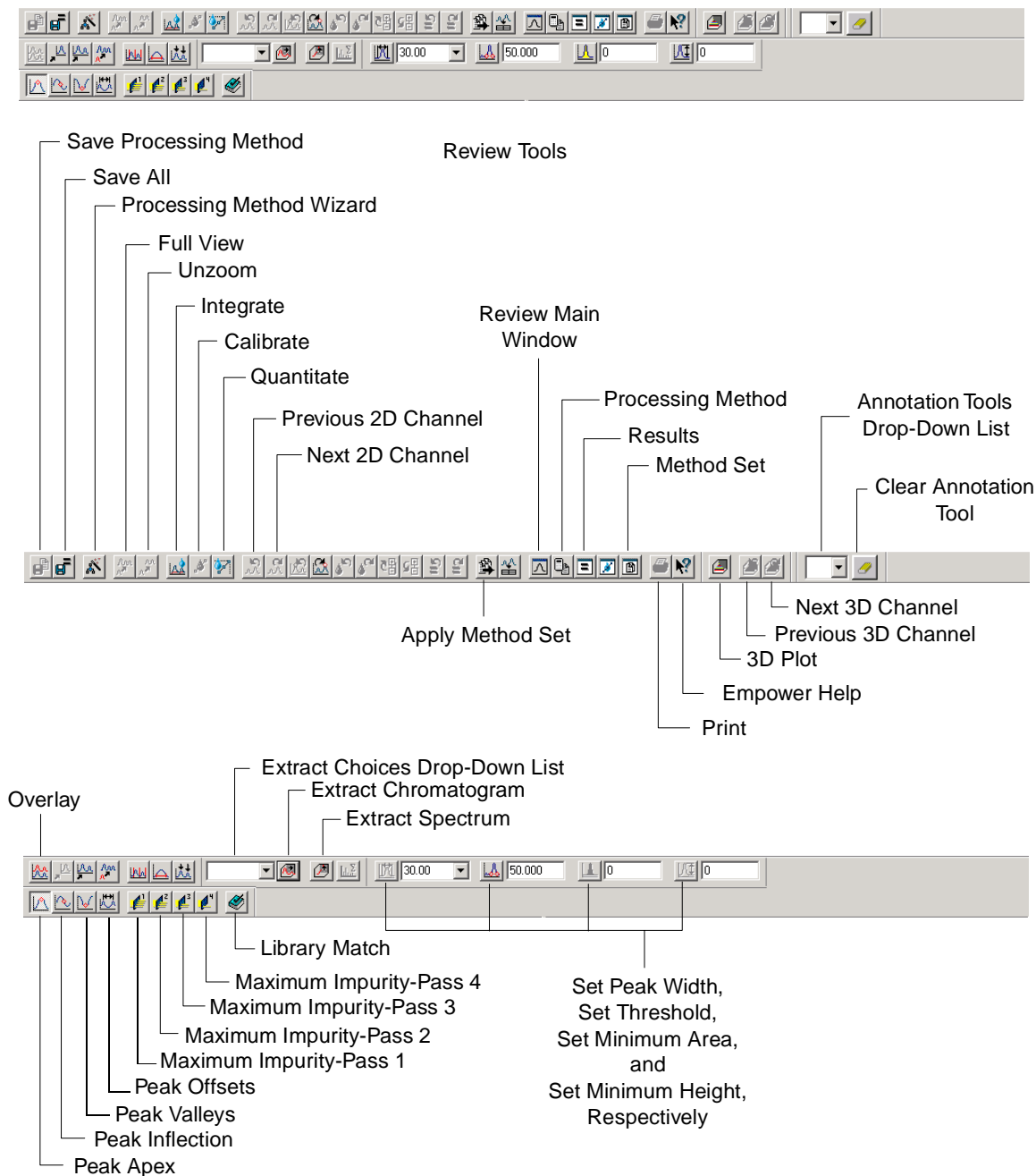Set Minimum Height,
Respectively

Figure 2-6 Review Toolbar Buttons

2.3 Displaying the 3D Plot

The 3D plot provides a three-dimensional view of PDA data. The data is plotted on three axes:

- **X-axis** – Represents time.
- **Y-axis** – Represents absorbance units.
- **Z-axis** – Represents wavelength.

You can rotate the 3D plot to view the data from the following different perspectives:

- The front view displays a chromatogram with time plotted on the x-axis and absorbance units plotted on the y-axis.
- The side view displays the UV spectra with wavelength plotted on the x-axis and absorbance units plotted on the y-axis.
- The top view displays a contour plot with time plotted on the x-axis and wavelength plotted on the y-axis.

To display the 3D plot:

1. With the PDA data open in Review, click the **Legend** tab on the lower-right side of Review to display the legend for the absorbance. The legend defines the colors used in the Contour plot and the 3D plot.
2. Click ⌨ **(3D Plot)** or select **Window > 3D Plot** (Figure 2-6) to view the 3D plot.
3. You can rotate the handle on the 3D plot (Figure 2-7) to view it from one of the following perspectives:
   - **Front** – Move the handle to the upper center of the window.
   - **Side** – Move the handle to the upper-right corner of the window.
   - **Top** – Move the handle to the bottom center of the window.

Handle for Rotating 3D Plot

Close Button

X-Axis Represents Time

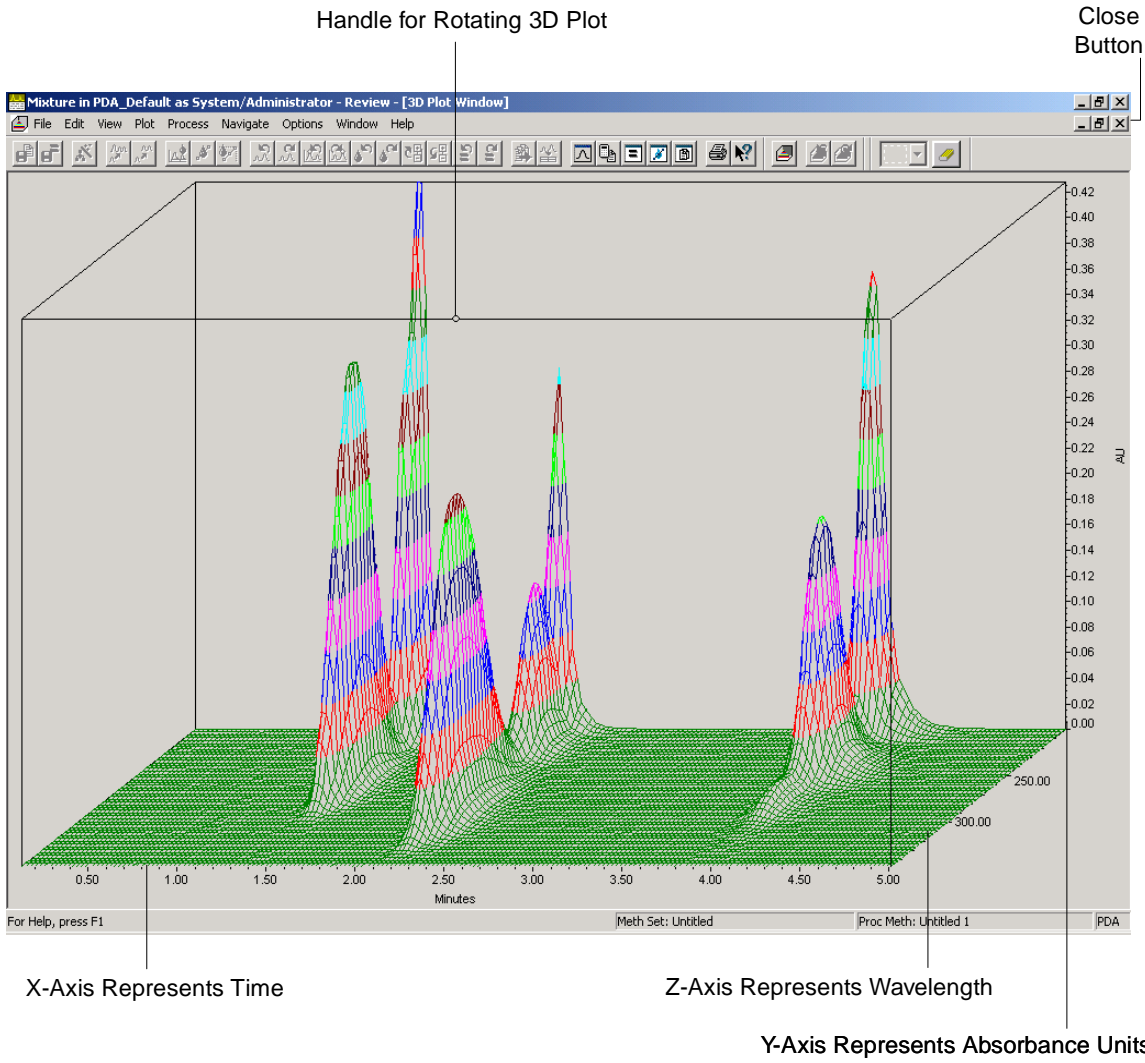Z-Axis Represents Wavelength

Y-Axis Represents Absorbance Units

Figure 2-7 Sample 3D Plot Window

4.   To exit the 3D Plot window and return to the Review Main window, click ⊠ (Close) (the lower close button) in the upper-right corner of the daughter (not the main) window.

2.4 Zooming In on Plots

You can examine graphical plot features in detail by using a zoom box. You can zoom in on the Chromatogram plot, Contour plot, and Spectral plot. You also use this technique to view baseline noise in a chromatogram.

To create a zoom box, hold down the left mouse button and drag the mouse around the features you want to enlarge. A box appears around the area of interest (Figure 2-8). When the box is in the desired size and position, release the left mouse button.
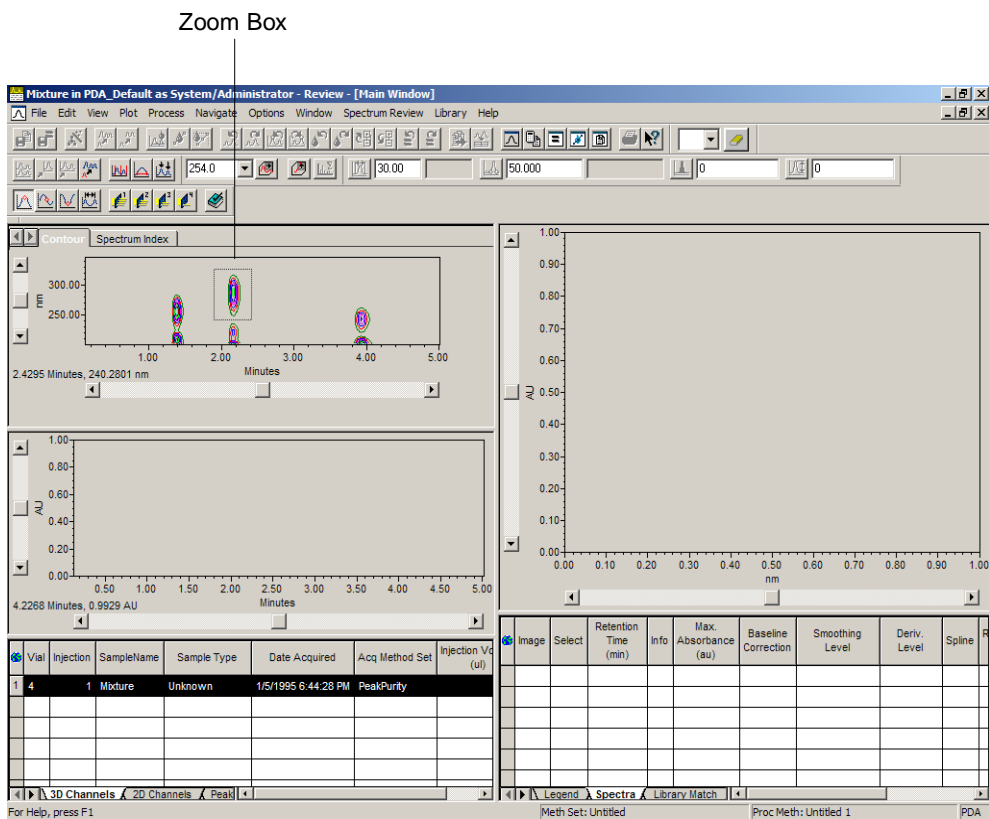

Figure 2-8 Creating the Zoom Box

Figure 2-9 shows the area after zooming.

Note: If you view the 3D plot after zooming on the Contour plot, the 3D plot is also zoomed to the same region of data. There is no zoom capability directly in the 3D Plot window. See Section 2.3, Displaying the 3D Plot, for information on viewing the 3D plot.

Full View Tool

Unzoom Tool
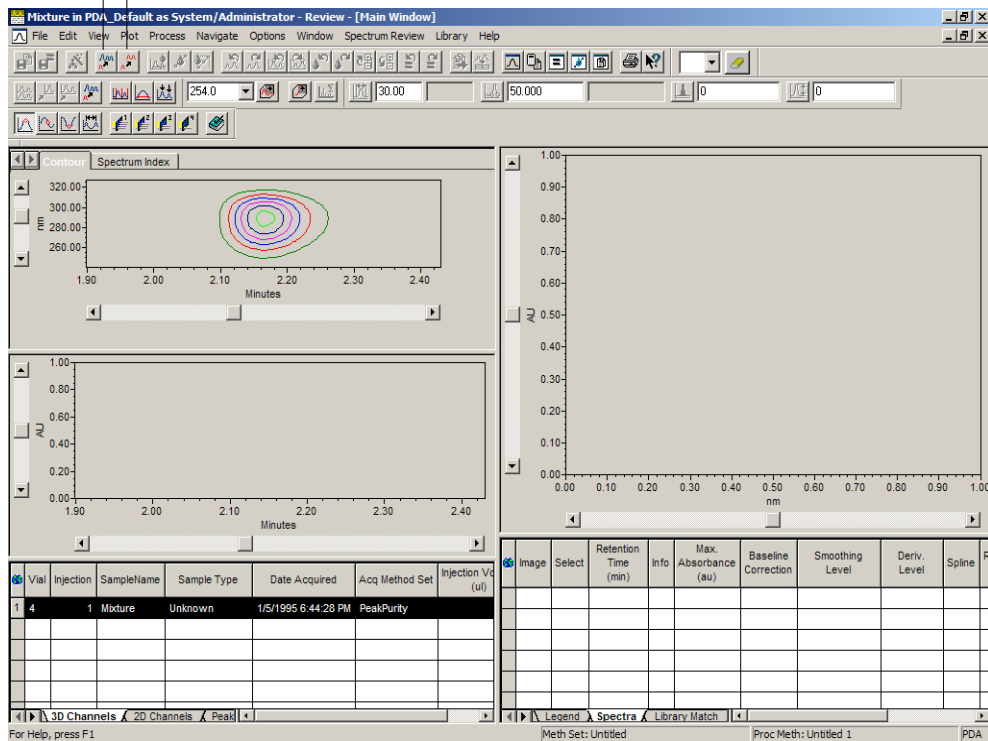
Figure 2-9 Zoomed View of the Contour Plot

Rescaling the Plot

You can revert to the unzoomed plot in one of the following ways:

- **Full View** – Reverts the plot to its original scale. To activate Full View, click ▦ (Full View) or right-click in the Contour plot and select **Full View** (Figure 2-9).

- **Unzoom** – Returns to the previous zoom when you have zoomed repeatedly. To activate Unzoom, click ▦ (Unzoom) or right-click in the Contour plot and select **Unzoom** (Figure 2-9).

Saving Zoom Parameters

If you repeatedly want to see the same zoomed area, you can save the parameters of the zoom box. To save the zoom parameters:

1. Right-click the desired plot, then select **Properties** to access the Plot Properties dialog box.

2. Click the **Scaling** tab, then click **Get Values from Plot** to fill in values from the plot in the Scaling section of the tab (Figure 2-10). Alternatively, enter the X-start, X-end, Y-start, and Y-end values manually.

3. Click **OK** to apply the settings.

Note: For details on how to customize display settings, see the "Customizing the Display of Data in Review" topic in the Empower Help.
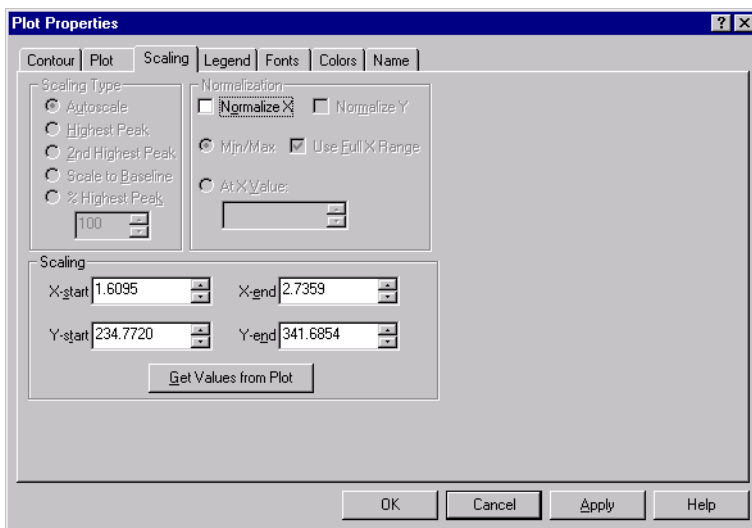

Figure 2-10 Scaling Tab

2.5 Extracting a Chromatogram

Extracting a chromatogram manually allows you to see what the chromatogram looks like at any wavelength across the collected wavelength range. You can extract chromatograms from several wavelengths and then overlay them for further comparison.

To manually extract a chromatogram:

1. With the PDA data displayed in Review, select **254.0** from the **Extract Choices** list to display a wavelength marker and an extracted 254.0-nm chromatogram in the chromatogram plot (Figure 2-11).

   Note: The 254.0 nm selection is a default value that automatically appears in the list.

Extract Choices
Drop-Down List

Wavelength
Marker

2D Channels Tab

Spectra Tab

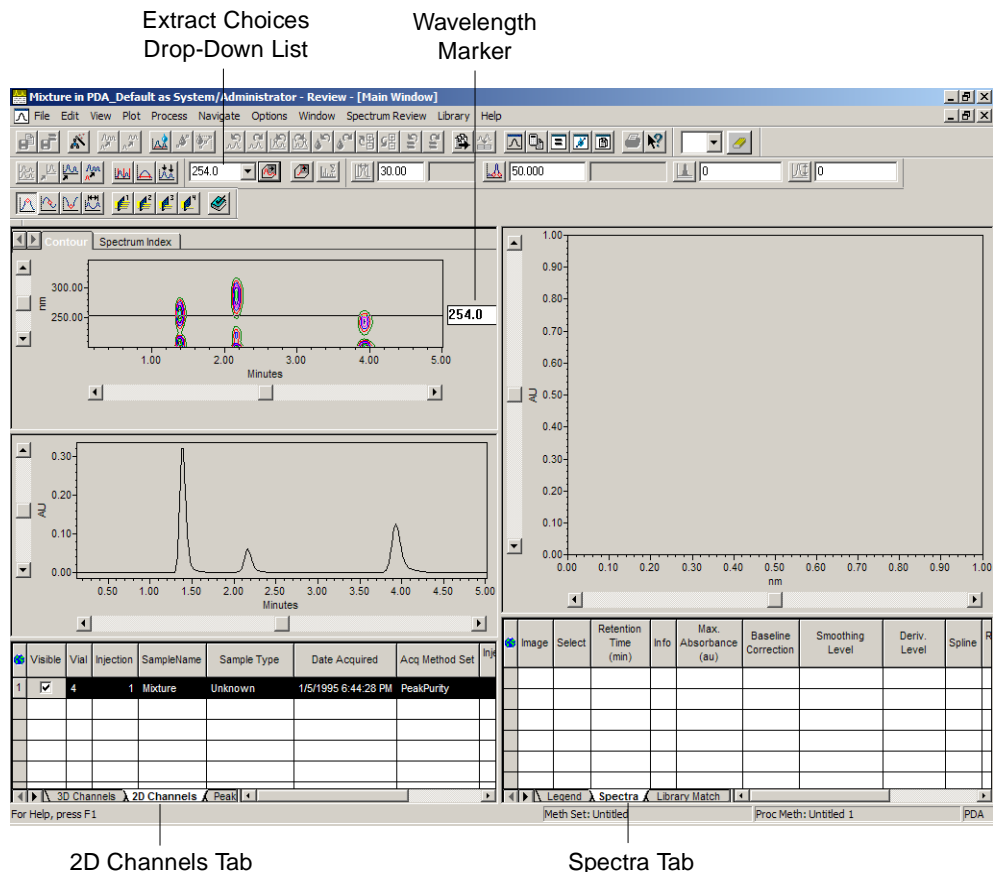Figure 2-11 Extracted Chromatogram at Wavelength 254 nm

2. View a chromatogram at a different wavelength using one of these methods:

   • In the Extract Choices list, select the empty choice at the top. Drag the wavelength marker to the desired wavelength, for example, **280.0**. As you drag the wavelength marker, the chromatogram automatically updates in the Chromatogram plot (Figure 2-12).

- In the Extract Choices list, select the empty choice at the top of the list. Double-click the wavelength marker and enter the desired wavelength, for example, **280.0**, and press **Enter** (Figure 2-12).

- Enter the desired wavelength, for example, **280.0** in the Extract Choices list, and click ⬚ (Extract Chromatogram) (Figure 2-12).

Extract Chromatogram
Tool

Extract Choices
Drop-Down List

Wavelength Marker

2D Channels Tab

Chromatogram Plot

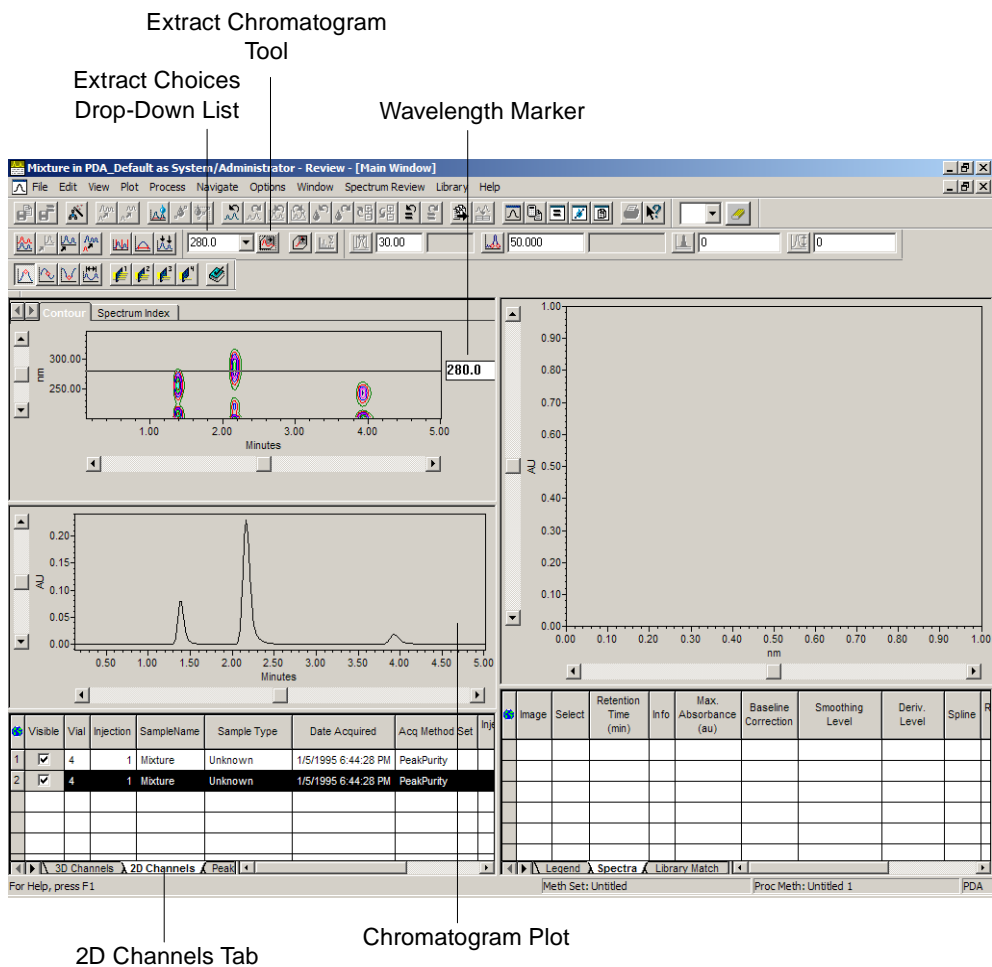Figure 2-12 Extracted Chromatogram at Wavelength 280 nm

3. To overlay the chromatograms, click ⬚ (Overlay) or select **Plot > Overlay**. The overlaid chromatograms appear in the Chromatogram plot as shown in Figure 2-13. The 2D Channels tab lists each extracted chromatogram. The Channel Description field indicates the extracted wavelength (you might need to scroll to the right side of the table to see this field).

4. Click ![Overlay icon] (Overlay) or select **Plot > Overlay** again to toggle back to a single chromatogram. The extracted chromatogram that is selected in the 2D Channels tab is displayed.

Two Markers Indicate a Chromatogram
Extracted at Two Different Wavelengths

Overlay Tool

Overlaid
Chromatograms

Channel
Description

Figure 2-13 Overlaid Chromatograms at Wavelengths 254 and 280 nm

2.6 Extracting a Spectrum

When you extract a spectrum manually, you can see what the spectrum looks like at any time in the chromatographic run. You can extract a spectrum at several elution times and then overlay them for further comparison. You can also use extracted spectra to build spectral libraries.

To manually extract a spectrum:

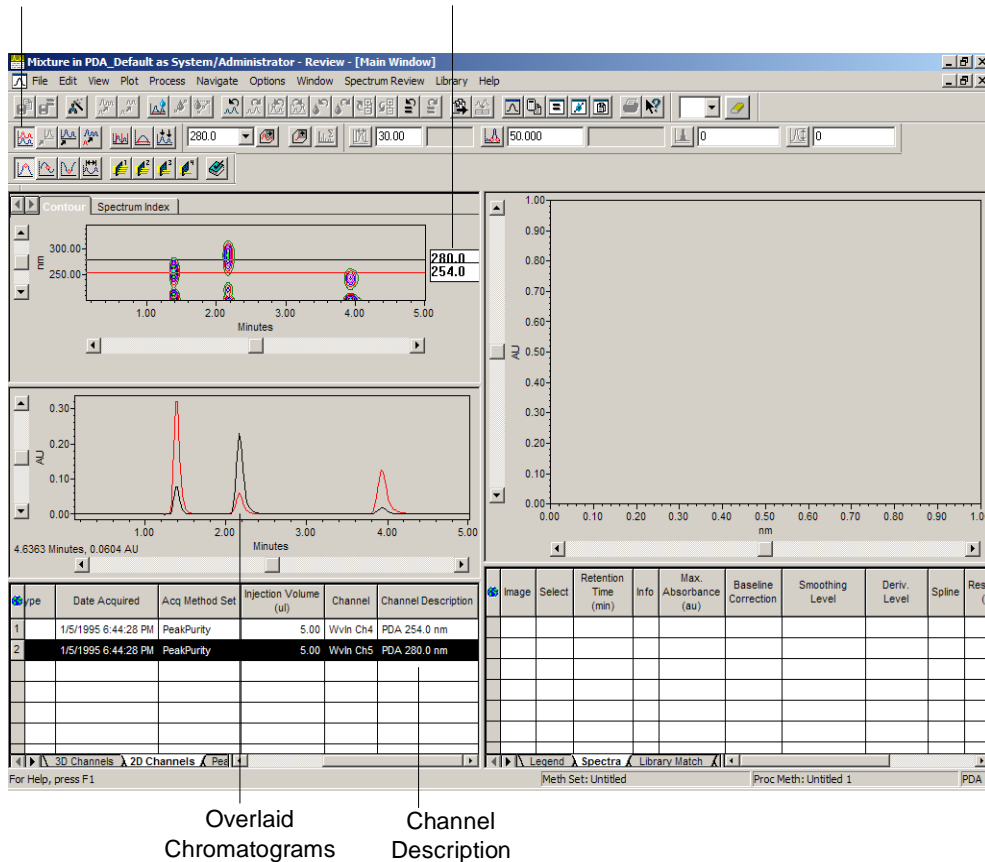1. With the PDA data displayed in Review, click ![icon] (Extract Spectrum) to display a spectrum marker in the lower-left corner of the Contour plot and to display an extracted spectrum in Spectrum Review.

2. Change the spectrum marker using one of the following methods:

   • Drag the spectrum marker to the desired time, for example, **1.388** (the retention time of the first peak apex). Notice as you drag the marker, the spectrum automatically updates in Spectrum Review (**Figure 2-14**).

   • Double-click the spectrum marker and enter the desired time, for example, **1.388**, then press **Enter** (**Figure 2-14**).

   • Right-click the spectrum marker and select **Edit**. Enter the desired time, for example, **1.388**, then press **Enter** (**Figure 2-14**).

Extract Spectrum Tool   Spectrum Review
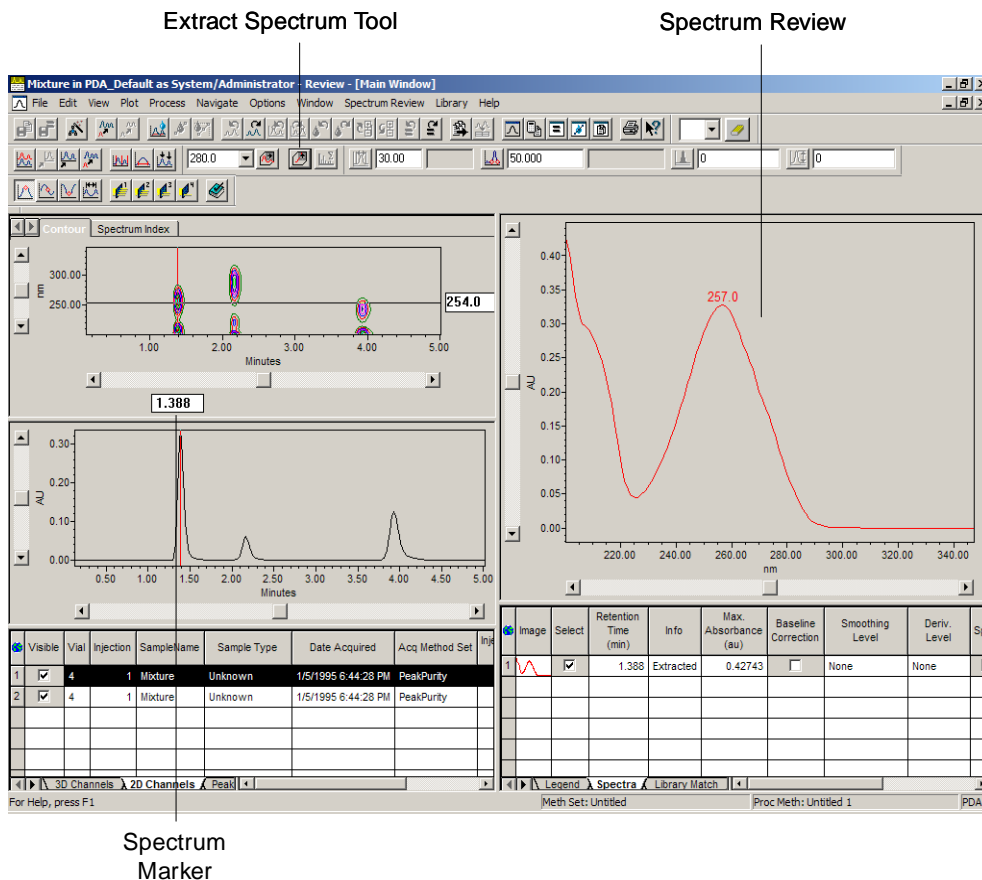
Spectrum
Marker

Figure 2-14 Extracted Spectrum at 1.388 Minutes

3. Click ![Extract Spectrum icon] (Extract Spectrum) to extract another spectrum.

4. Change the spectrum marker to **2.159** (the retention time of the second peak apex). The next spectrum is overlaid with the previous spectrum in Spectrum Review (Figure 2-15).
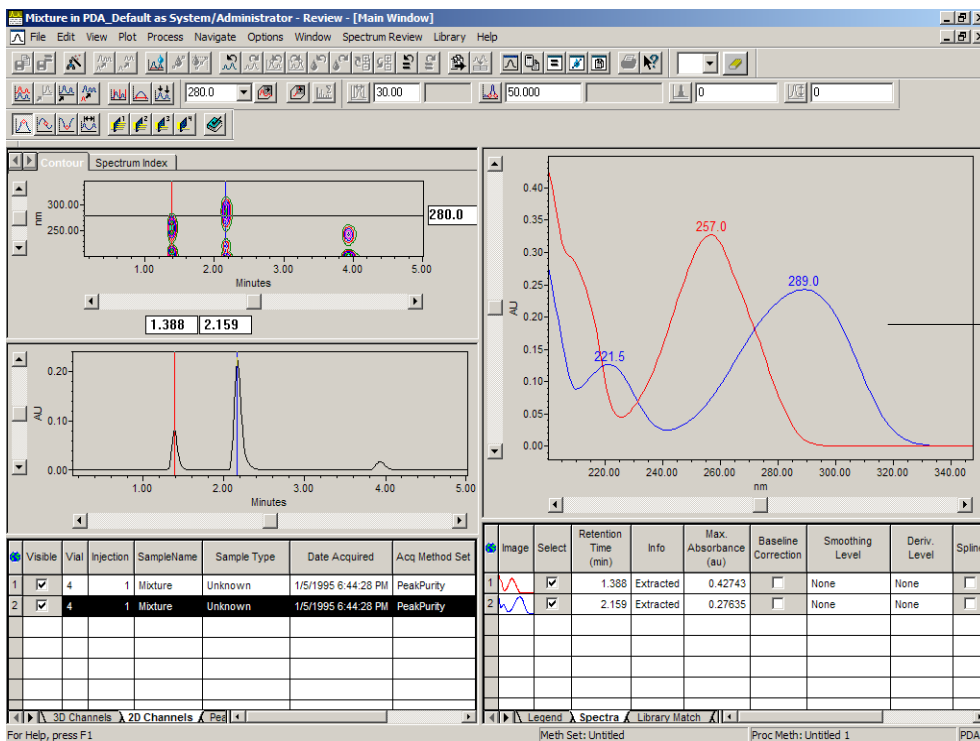
Figure 2-15 Overlaid Extracted Spectra

5. Click the **Spectra** tab on the lower-right side of the window (Figure 2-16) to view information about the spectra in Spectrum Review.
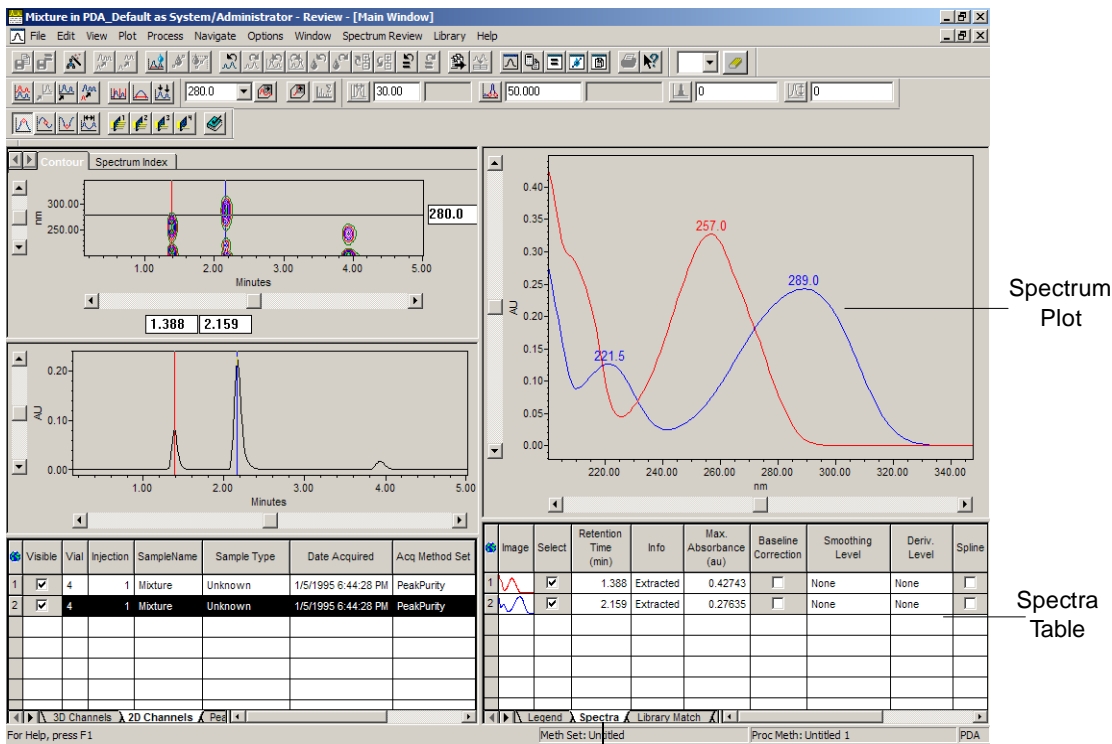
Figure 2-16 Spectra Table

6. To view the overlaid spectra in a normalized fashion, double-click inside the Spectrum plot. Note that the y-axis does not display absorbance units. Viewing the normalized spectra corrects for concentration differences and allows you to compare the UV spectra based on their shape differences (Figure 2-17).

You can restore the default scaling (autoscaling) by double-clicking again inside the Spectrum plot.
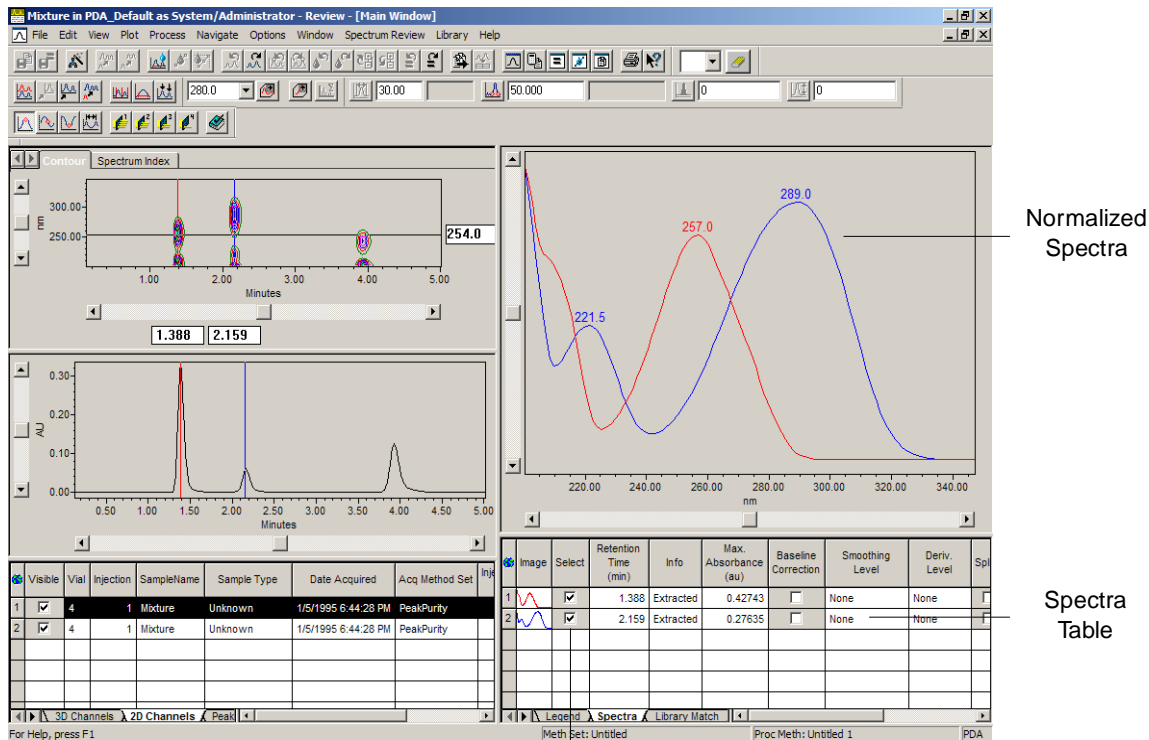
Figure 2-17 Normalized Spectra

7. In the Spectra table below Spectral Review, clear all but one spectrum by selecting the **Select** check box (Figure 2-17).

8. Scroll in the area containing the tabs below the Spectra table and click the **Spectrum Points** tab to display the raw data points of the spectrum, as shown in Figure 2-18.
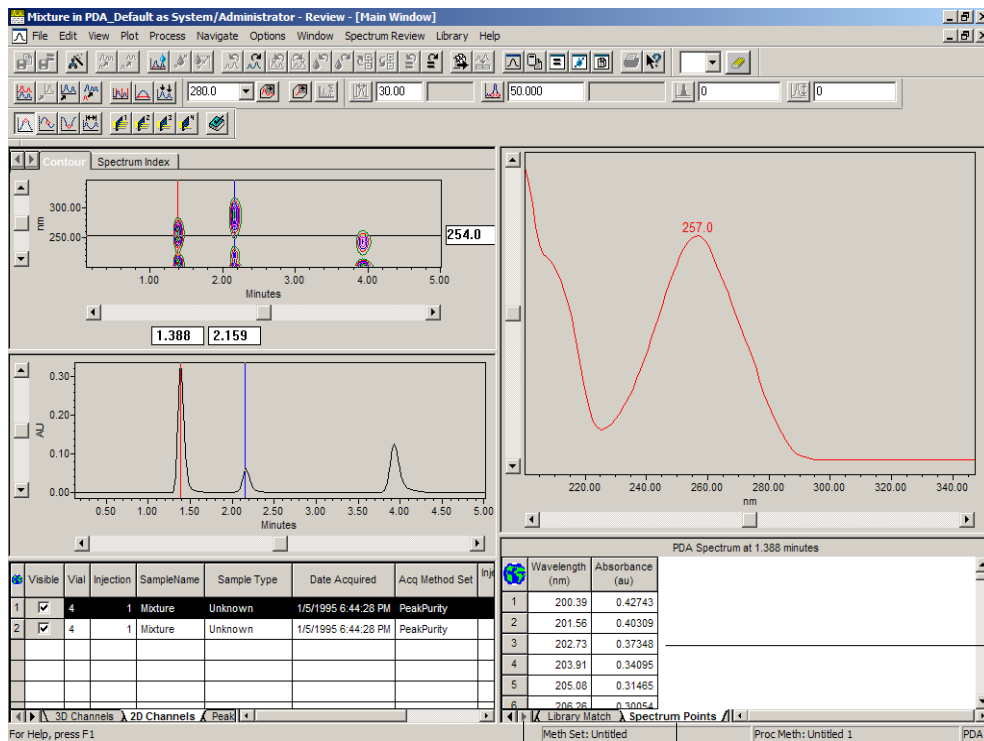
Spectrum
Points
Table

Spectrum Points Tab

Scroll Here Until the Spectrum Points Tab Appears

Figure 2-18 Viewing Spectrum Points: Absorbances Versus Wavelengths

2.7 Annotating Chromatograms and Spectra

Empower software has a set of Annotation tools. In Review, these tools allow you to add notes to chromatograms and spectra when you want to:

- Copy one to a document or presentation
- Print one for reference

Note: Annotations are not saved when you exit Review.

2.7.1 Adding Annotations

In the following example, you will annotate the chromatogram by drawing an arrow and adding supporting text.

Drawing an Arrow

To draw an arrow on the chromatogram:

1. With the PDA data displayed in Review, select ⟵ (arrow tool) from the Annotation Tools list (Figure 2-19).
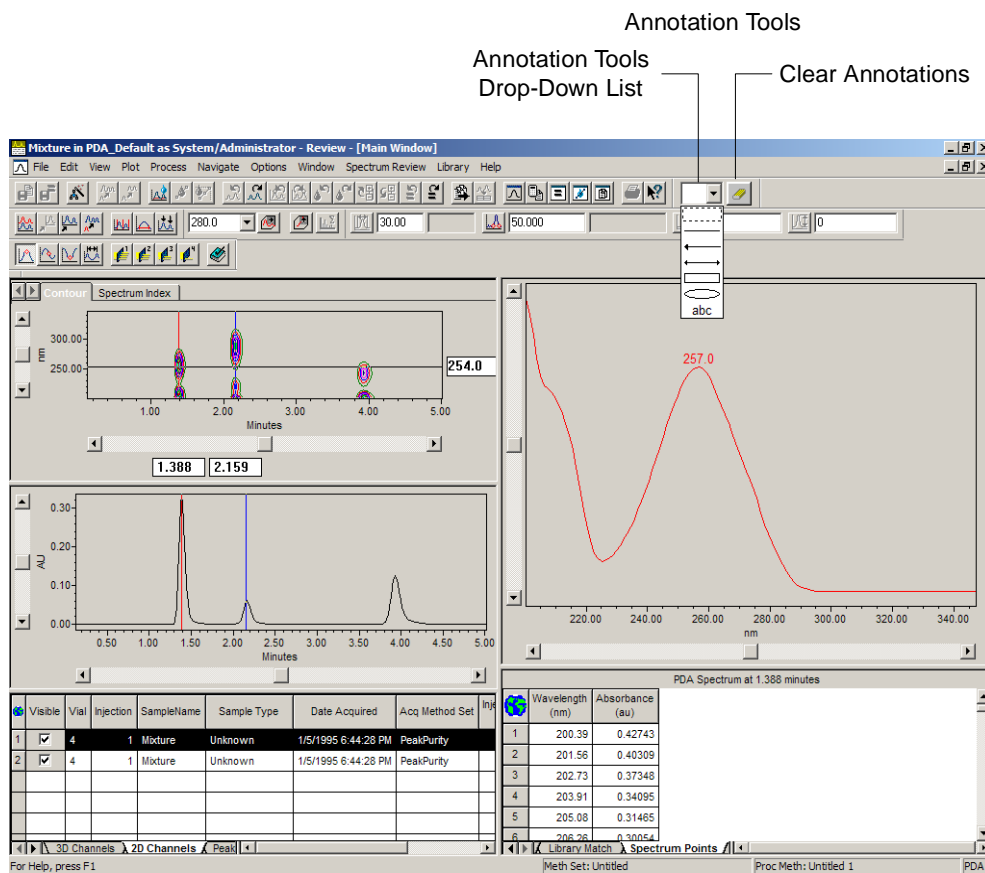
Figure 2-19 Annotation Tools

2. Move the mouse to the Chromatogram plot. The pointer changes to an arrow tool.

3. Hold the left mouse button while drawing an arrow to the tallest peak. When you finish drawing, release the mouse button.

4. Click the arrow to select it. The pointer changes to a pointing finger and the arrow is selected (black handles appear on each end). When the arrow is selected, you can:

   • Move it to another location by clicking a part of the arrow other than the black handles and dragging it to the desired location.

   • Rotate the arrow by holding one of the black handles and turning it in the desired direction.

   • Resize the arrow by pulling one of the black handles.

   • Delete the arrow by pressing the **Delete** key.

   If you do not want to change the arrow, click elsewhere in the Chromatogram plot to deselect it.

5. Right-click the arrow and select **Annotations > Properties** to open the Annotation Object Properties dialog box (Figure 2-20). From this dialog box you can change the style, thickness, and/or color of the arrow.

Figure 2-20 Annotation Object Properties Dialog Box for an Object

Adding Text

To add text to the chromatogram:

1. From the Annotation Tools list, select abc (text tool).

2. Move the mouse to the Chromatogram plot. The pointer changes to **+abc**.

3. Click the area of the plot where you want to add text to open the Annotation Object Properties dialog box ([Figure 2-21](#)).
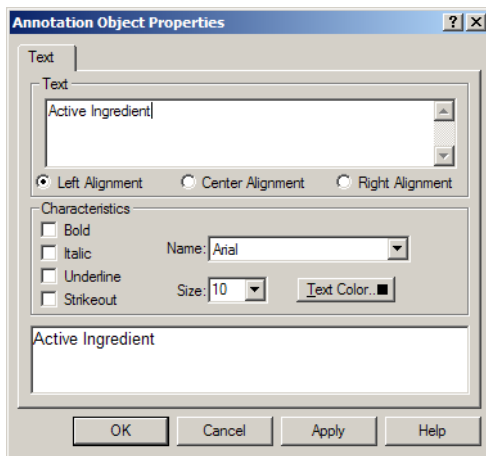
Figure 2-21 Annotation Object Properties Dialog Box for Text

4. In the Text field, type the text you want to add to the chromatogram. From this dialog box you can also specify text properties such as alignment, font, and size. When you are finished, click **OK** to add the text to the chromatogram.

5. Click the text to select it. The pointer changes to a pointing finger and the text is selected (black handles appear on the corners). When the text is selected, you can:

   • Move the text to another location by clicking a part of the text other than the black handles and dragging to the desired location.

   • Resize the text box (if all of your text is not displayed) by pulling one of the black handles.

   • Delete the text by pressing the **Delete** key.

   If you do not want to make these changes to the text, click elsewhere in the plot to deselect the text.

6. To change the text and/or the properties of the text, right-click the text and select **Annotations > Properties** to open the Annotation Object Properties dialog box

shows an example of an annotation in the Chromatogram plot.
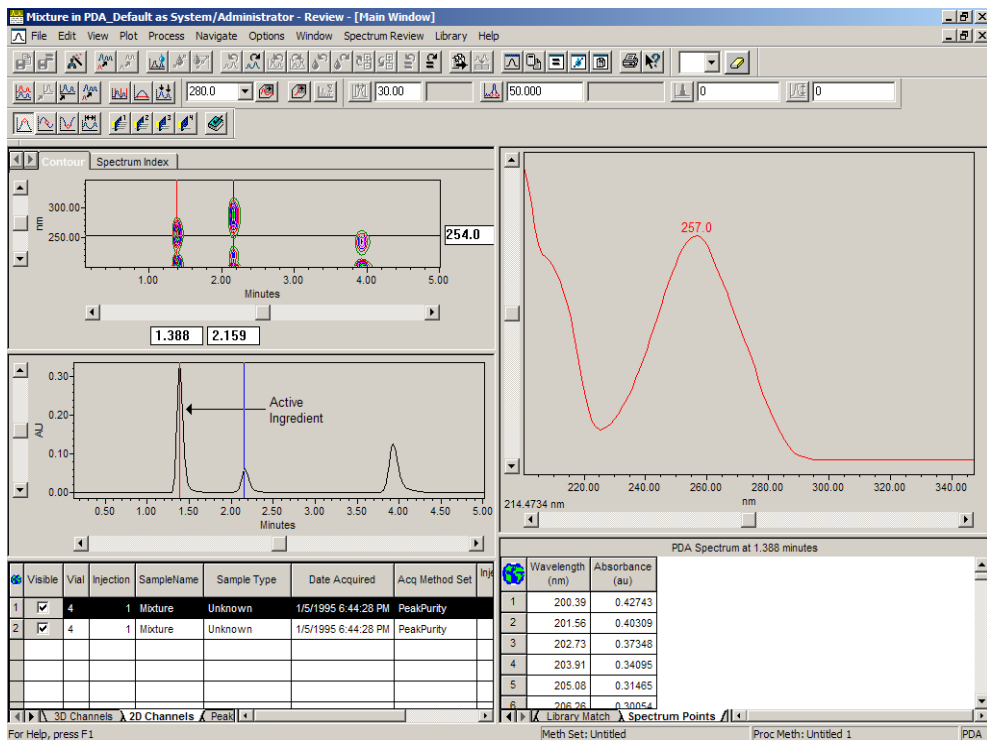
Figure 2-22 Annotation Example

2.7.2 Erasing Annotations

Click [icon] (Clear Annotations) to remove *all* annotations. You can only remove individual annotations by selecting them and pressing the **Delete** key.

2.7.3 Annotation Tools

The remaining Annotation tools, other than the text tool, function the same way as the arrow. This list describes each Annotation tool and the action it performs or the object it creates.

Tool	Action or Object
——	Draws a line.
←——	Draws a line with an arrowhead on one end.
←——→	Draws a line with arrowheads on both ends.
▭	Draws a rectangle.
⬭	Draws an ellipse.
abc	Adds text.

Exiting Review

To exit Review:

1. Click **X** **(Close)** or select **File > Exit** (Figure 2-18). A dialog box warning that the modified method set cannot be saved appears.

2. Click **OK**.

Next Steps

Now that you have examined the PDA_Default data in Review, you have the following options:

- You can develop a processing method for peak purity, as described in Chapter 3, Peak Purity Processing.

- You can create a spectral library and develop a processing method to match unknown spectra against the library, as described in Chapter 4, Library Matching.

Chapter 3
Peak Purity Processing

This chapter provides a tutorial with step-by-step procedures for developing a PDA processing method to determine peak purity.

3.1 Tutorial Overview

This tutorial familiarizes you with Empower PDA software tools and the procedures used to develop a PDA processing method for determining peak purity. You calculate peak purity to determine if a peak is spectrally homogeneous. Spectral heterogeneity can indicate the presence of a coelution. A coelution of two or more spectrally distinct compounds can produce a spectrally heterogeneous peak.

This tutorial shows you how to:

- Derive chromatograms.

- Develop a processing method to determine peak purity using the Processing Method Wizard, which is where you define key parameters for integration and peak purity determination.

- Examine peak purity results in Spectrum Index.

- View peak purity results in the Purity table and Purity plot of the Results window.

Steps to Compute Peak Purity

The following are the steps used to compute peak purity:

1. Bring the PDA data from the PDA_Default Project into Review (Section 3.2).
2. Derive chromatograms (Section 3.2).
3. Build a PDA processing method (Section 3.3).

4. View results (Section 3.4).

- Peak apex spectrum
- Maximum impurity pass 1 spectrum
- Purity table
- Purity plot and points of maximum impurity

3.2 Deriving Chromatograms

Before developing a processing method that computes peak purity, you must derive a chromatogram. A derived chromatogram is the two-dimensional chromatogram extracted from the three-dimensional PDA data at the wavelength that you specify.

Note: A Max Plot chromatogram is a derived chromatogram which plots the maximum spectral absorbance measured at each time point. It allows you to see all the chromatographic peaks in the sample regardless of each peak's lambda max (the wavelength at maximum absorbance). The Max Plot chromatogram is useful in determining the appropriate noise interval for your processing method. It is also necessary to check the absorbance range of the Max Plot chromatogram. When obtaining purity results, it is important that your sample does not exceed 1.0 AU in any region of the 3D data (see step 4 in Section 3.2). However, data extracted at one particular wavelength is typically used to set processing parameters when developing a processing method and for quantitative purposes. Because Max Plot uses the absorbances from all wavelengths in the range selected during data acquisition, it is not meaningful to use it to generate a calibration curve and quantitate data.

To derive a chromatogram:

1. Open the Project window, which you opened in Chapter 2, Viewing PDA Data, by selecting the PDA_Default project in the Windows taskbar.

2. In the Channels tab of the Project window (Figure 3-1), select **Mixture**. Click [image] **(Review)** or select **Tools > Review**. The Review window appears.

Review
Button

Figure 3-1 Project Window of the PDA_Default Project

3. From the Extract Choices list in Review, select **MAX Plot** to extract a Max Plot chromatogram. The chromatogram appears in the Chromatogram plot (Figure 3-2).

Select MAX Plot from the
Extract Choices Drop-Down List
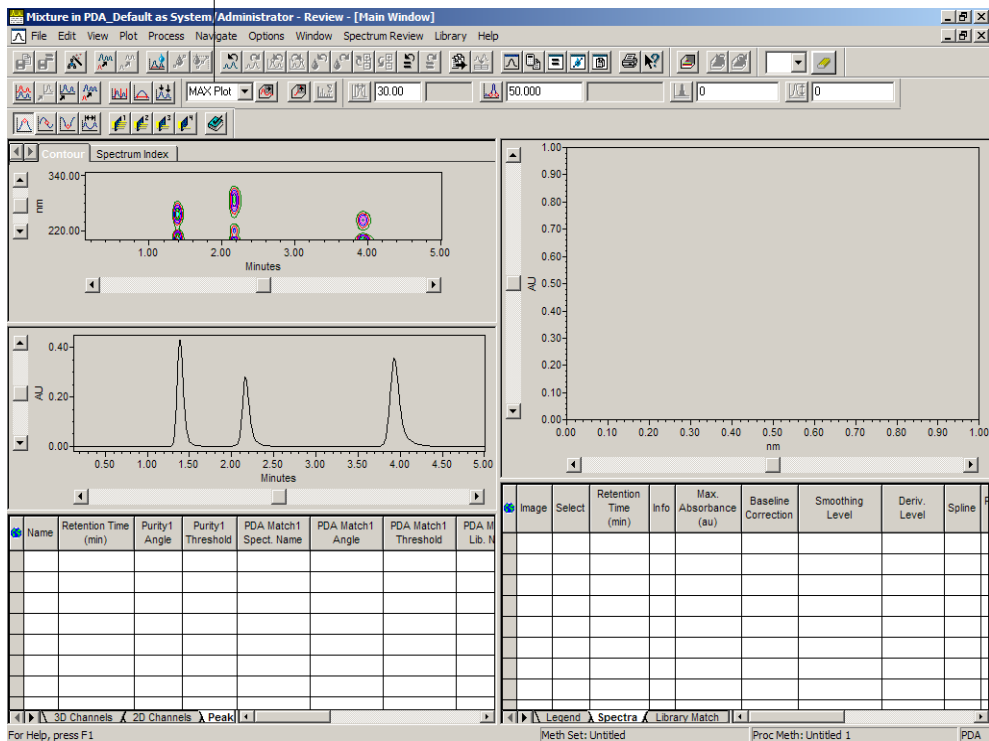
Figure 3-2 Review Main Window with a Max Plot Chromatogram

4. Ensure that the absorbance of all peaks in the chromatogram is less than 1 AU (absorbance unit). This criteria is necessary when calculating purity results in order for these results to be valid. If the absorbance is less than 1 AU, proceed with the next step. If the absorbance of any peak is greater than 1 AU, dilute the sample appropriately and acquire it again.

5. From the Extract Choices list, select **254** to extract a chromatogram at 254.0 nm. The Max Plot chromatogram is replaced with the newly extracted chromatogram (Figure 3-3). We will use the 254-nm extracted channel to determine the optimal integration parameters.

Select 254 from the
Extract Choices Drop-Down List
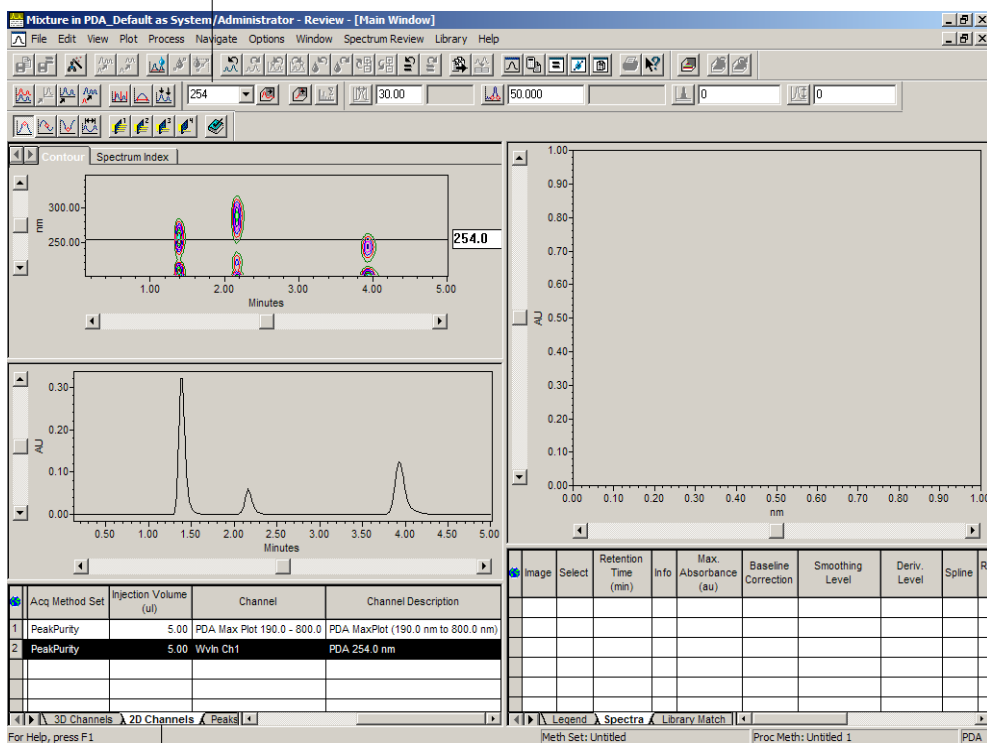
2D Channels Tab

Figure 3-3 Review Main Window with a Chromatogram at 254 nm

3.3 Developing a PDA Processing Method

Now that you have verified that your absorbance for each peak is less than 1 AU and you have extracted a chromatogram at the desired wavelength, you can build a processing method to integrate the chromatograms and assess peak purity. The simplest way to build a PDA processing method is to use the Processing Method Wizard.

Note: This procedure assumes that the Use V3.0X Style Peak Width and Threshold Determination system policy is enabled (the default setting). If this system policy is not enabled on your system, the Processing Method Wizard will look slightly different. For more information on this system policy, see the Empower Help.

This procedure uses the traditional integration algorithm rather than the ApexTrack integration algorithm. For more information on these processing algorithms, see the Empower Software Acquisition and Processing Theory Guide *or the* ApexTrack Integration: Theory and Application *white paper (go to www.waters.com).*

Note: *Ensure that the 254-nm channel is selected in the Channels tab (Figure 3-3).*

To build a PDA processing method:

1. Click ![icon] (Processing Method Wizard) or select **File > New > Processing Method** to start the wizard and open the Processing Method Wizard dialog box (Figure 3-4).

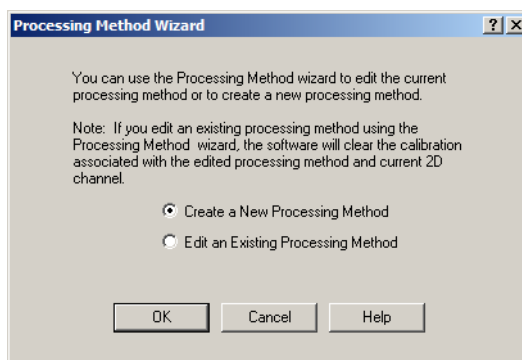

Figure 3-4 Processing Method Wizard Dialog Box

2. Click **Create a New Processing Method** and click **OK** to open the New Processing Method dialog box (Figure 3-5).
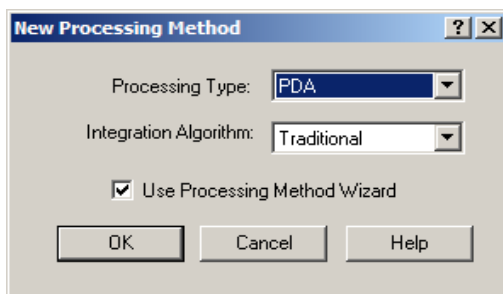
Figure 3-5 New Processing Method Dialog Box

3. From the Processing Type list, select **PDA**. If the Integration Algorithm list is present, select **Traditional**. Make sure the **Use Processing Method Wizard** check box is selected, then click **OK** to go to the Integration - Peak Detection 1 page (Figure 3-6).

Note: The Integration Algorithm list appears only if ApexTrack integration is enabled in your system policies and ApexTrack is enabled in your PDA_Default project.
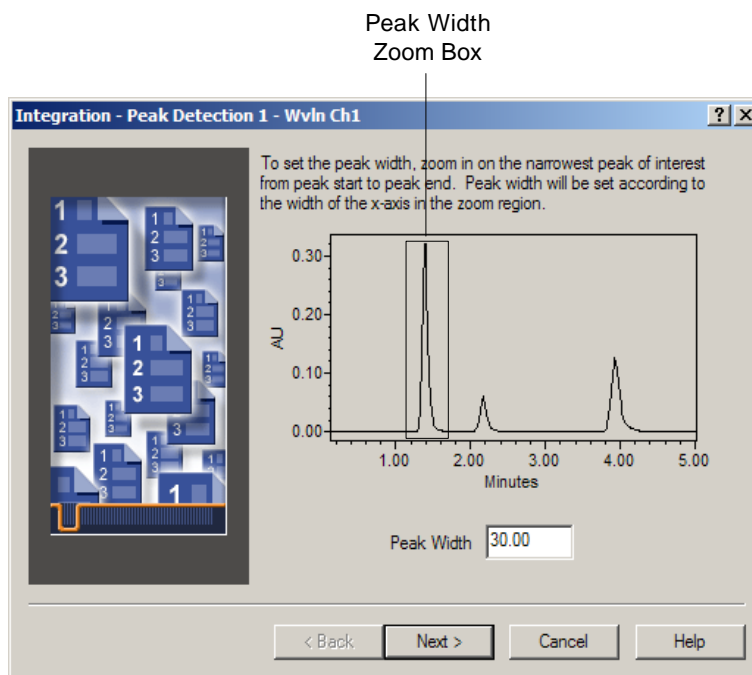
Figure 3-6 Integration - Peak Detection 1 Page, Full View

This page allows you to set the peak width parameter in your processing method. Peak width determines the number of points bunched together to act as a filter and to produce a single point for peak detection (although all data points are used during integration).

4. Follow the wizard instructions and use the mouse to draw a zoom box that encloses the liftoff and touchdown of the narrowest peak of interest (peak 1 at 1.39 minutes). The software displays the zoomed selection and determines the appropriate peak width (Figure 3-7).

Note: Right-click the plot to access the Full View/Unzoom shortcut menu selections. If you are unsure which peak is the narrowest, you can check different peaks by zooming and unzooming repeatedly on each peak. Each time you zoom, the software calculates and displays the appropriate peak width. Zoom properly (and perhaps multiple times) so that the peak start is at the beginning of the displayed x-axis in the plot and the peak end is at the end of the displayed x-axis in the plot.

The peaks in this data have nearly identical widths and all yield a Peak Width setting of 15 seconds when zoomed correctly. It therefore does not matter which peak is chosen, however, some data does contain peaks of varying widths. In this case, the appropriate peak should be used.

Figure 3-7 Setting the Peak Width Parameter

5.   Click **Next** to go to the Integration - Peak Detection 2 page ().
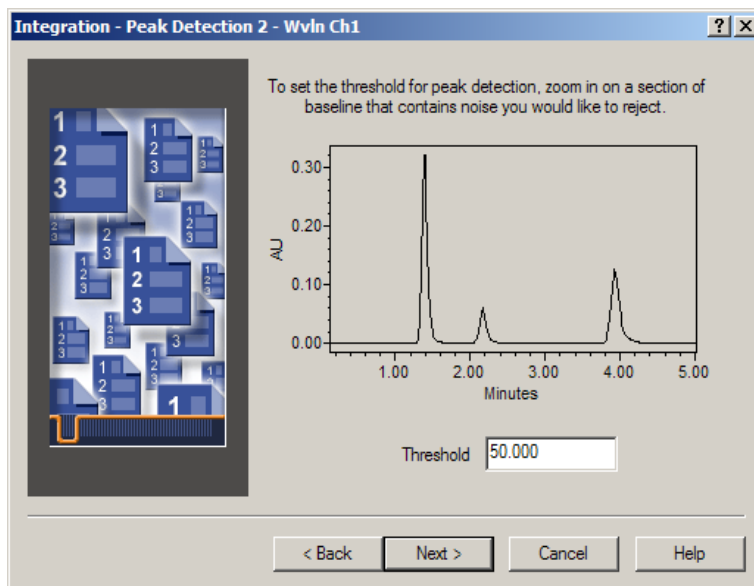
Figure 3-8 Integration - Peak Detection 2 Page

This page allows you to set the threshold parameter in your processing method based on a selected area of baseline. Threshold is a slope setting that defines the peak liftoff and touchdown points by determining whether the slope across three data points exceeds the determined slope threshold. It is used to reject baseline noise.

6. Follow the wizard instructions and use the mouse to draw a zoom box that encloses the area of the baseline that contains the greatest amount of noise and no peaks of

interest (approximately 0.2 to 1.2 minutes). The software displays the zoomed selection and determines the appropriate threshold value (Figure 3-9).

Note: In general, you might need to zoom the baseline several times to ensure that the selected area is free of peaks of interest.
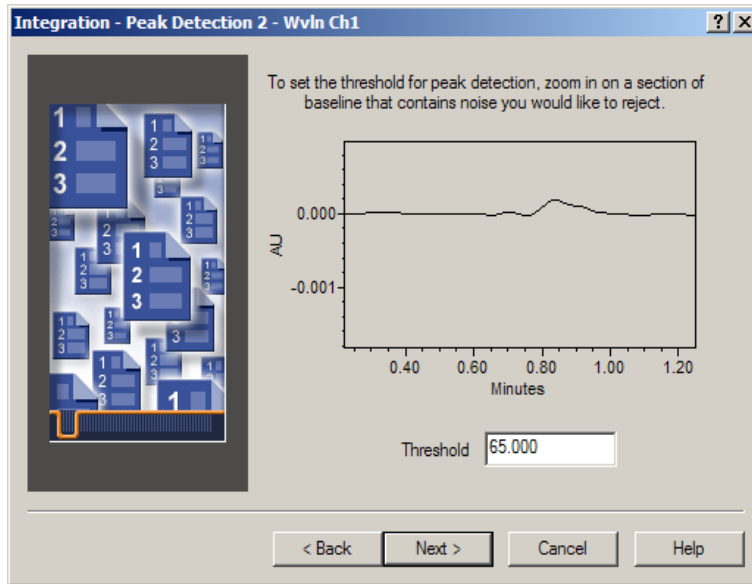
Figure 3-9 Setting the Threshold Parameter

7. Click **Next** to go to the Integration - Integration Region page (Figure 3-10).
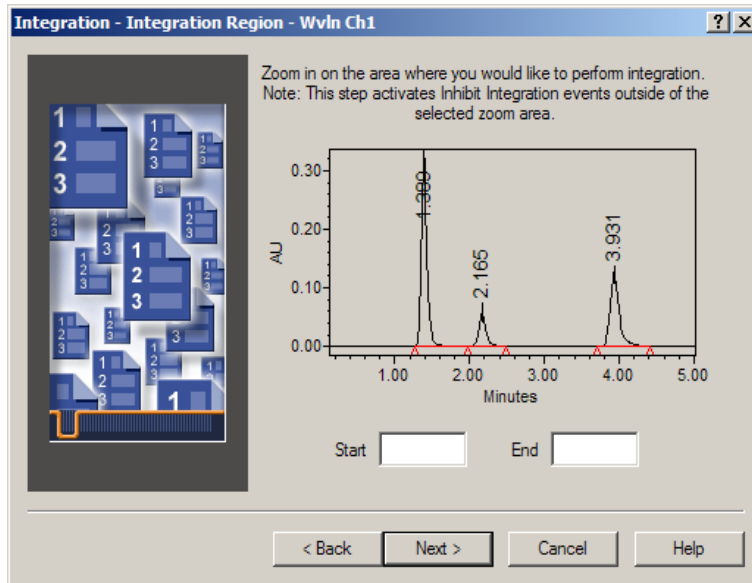
Figure 3-10 Integration - Integration Region Page

This page allows you to refine your integration by using an Inhibit Integration event to specify a time region where no integration occurs.

8. If you want to inhibit integration, follow the wizard instructions and use the mouse to draw a zoom box that encloses the area where you want integration to occur. Integration is inhibited in the area *outside* of the zoom box. The software displays the zoomed selection. Click **Next** to go to the Integration - Peak Rejection page (Figure 3-11).

This step is not necessary with this particular data. If you do not want to inhibit integration, do nothing in this dialog box and click **Next**.
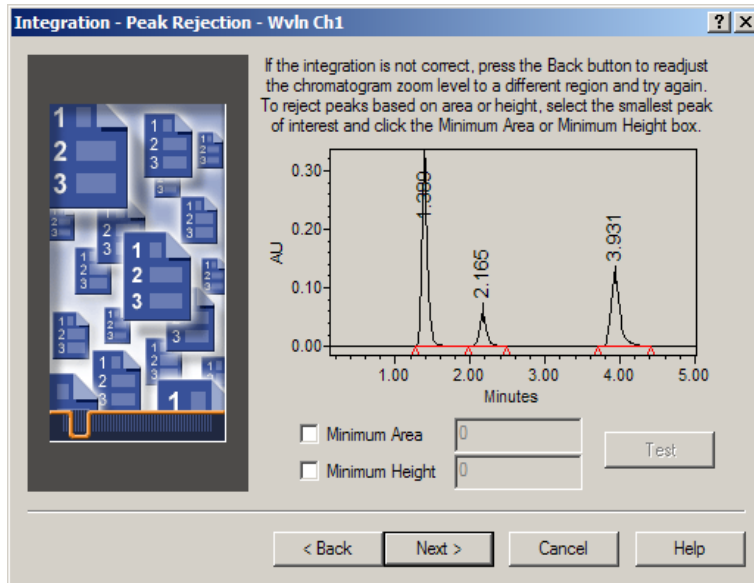
Figure 3-11 Integration - Peak Rejection Page

Use this page to reject small peaks that are not of interest. This step is not necessary with this particular data as there is no unwanted baseline noise that is integrated. Click **Next** to go to the Calibration - General page.

Note: To use this setting, select the smallest peak of interest to highlight it in red. Select the Mimimum Area or Minimum Height check box to set the minimum area or height to 95% of the selected peak.

When it is necessary to use the Minimum Area or Minimum Height parameter, you typically use one or the other, but not both. For more information see the "Minimum Area" and "Minimum Height" topics in the Empower Help.

9. Accept the default settings and click **Next**. A message indicates that channels require channel names in order to perform cross-channel internal standard processing. Click **No** in this dialog box. The Names and Retention Times page appears (Figure 3-12).
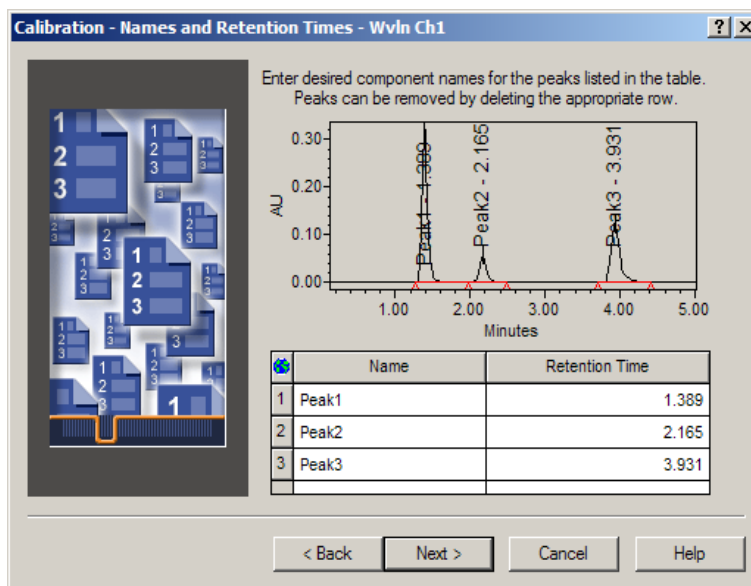
Figure 3-12 Names and Retention Times Page

10. Click **1** to the left of the Name column in the first row. Press **Shift** and click **3** to the left of the Name column in the third row. All rows are highlighted. Press **Delete** on your keyboard to remove all entries from this table. Click **Next** on this page and successive pages until the PDA Purity/Matching page appears (**Figure 3-13**).

Note: Entering component names and amounts is necessary only when you intend to process standards, generate calibration curves, and quantitate unknowns. Since this guide focuses on generating purity and library match data, this information is unnecessary. For information about calibration and quantitation, see the Empower Software Getting Started Guide *or the* Empower Help*.*

Figure 3-13 PDA Purity/Matching Page

11. Ensure **Yes** is selected for the question "Do you wish to perform peak purity testing on all peaks?". For the question "Do you wish to match spectra against PDA library spectra?" click **No** (Figure 3-13). Click **Next** to go to the PDA Spectral Contrast page (Figure 3-14).

Figure 3-14 PDA Spectral Contrast Page

Use this page to set the noise interval by selecting a segment of the baseline that is free of peaks. You must select a segment of the baseline that is at least one-half minute in length.

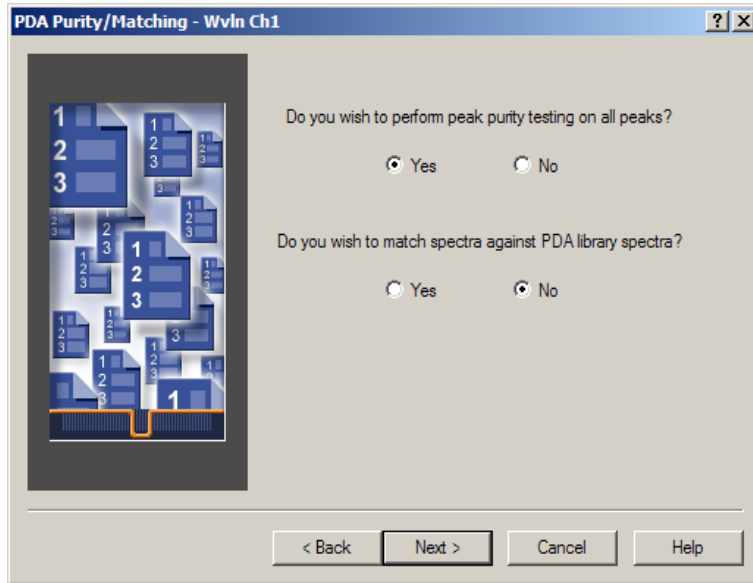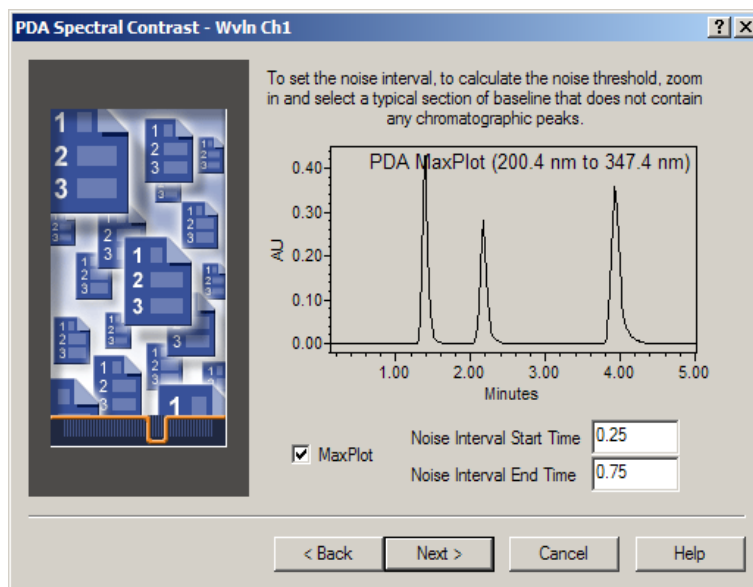*Note: The MaxPlot check box is selected by default and allows you to display the Max Plot chromatogram in the PDA Spectral Contrast page regardless of the type of chromatogram that was extracted before starting the Processing Method Wizard. It is recommended to use the Max Plot chromatogram when setting the noise interval to ensure that this parameter is set to a region of the baseline where no compound-related spectral absorbance occurs.*

When the MaxPlot check box is selected, the software displays the Max Plot chromatogram for the entire wavelength range in which the data was acquired. If you need to view the Max Plot chromatogram without using the entire acquired wavelength range, you must create a Max Plot chromatogram with the appropriate Start and End Wavelength by creating a derived channel in your method set and then applying the method set before starting the Processing Method Wizard. The Start and End Wavelength Limits are set in the Purity tab of the processing method. (These settings are not available when using the Processing Method Wizard. You must view the processing method by clicking (Processing Method) or by selecting Window > Processing Method.)

It is often desirable to define the wavelength start range above 190 nm to eliminate the effect of mobile phase absorption.

12. Select the segment of the baseline from approximately 3.00 to 3.50 minutes (Figure 3-15).

You can select the baseline area by using the mouse to zoom in on the desired area, or you can specify the baseline area by typing the start and end time of the desired area in the Noise Interval Start Time and Noise Interval End Time text boxes, respectively.

When zooming, you can right-click and select **Fullview** or **Unzoom** to move between zoom levels. Do this to view multiple sections of baseline before deciding on the correct region for the noise interval.

For details about selecting the noise interval, see the "Determining the Noise Interval" topic in the *Empower Help*.
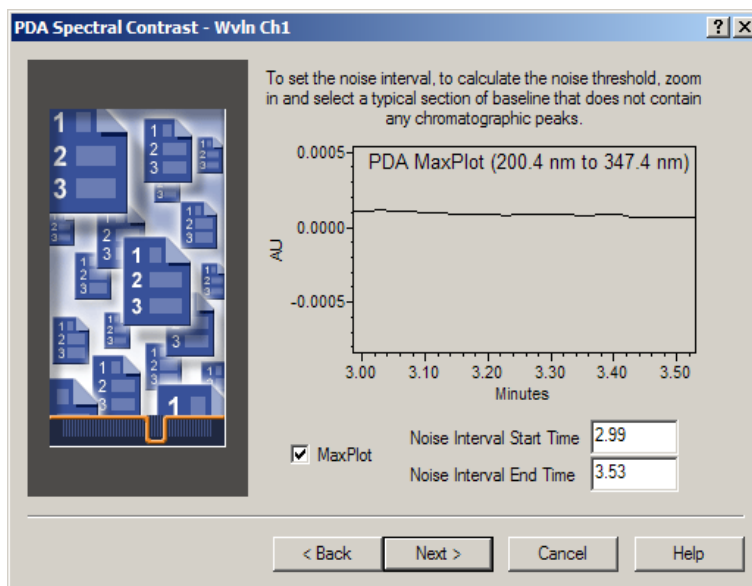
Figure 3-15 Setting the Noise Interval

13. Click **Next** to go to the Processing Method Name page (Figure 3-16).
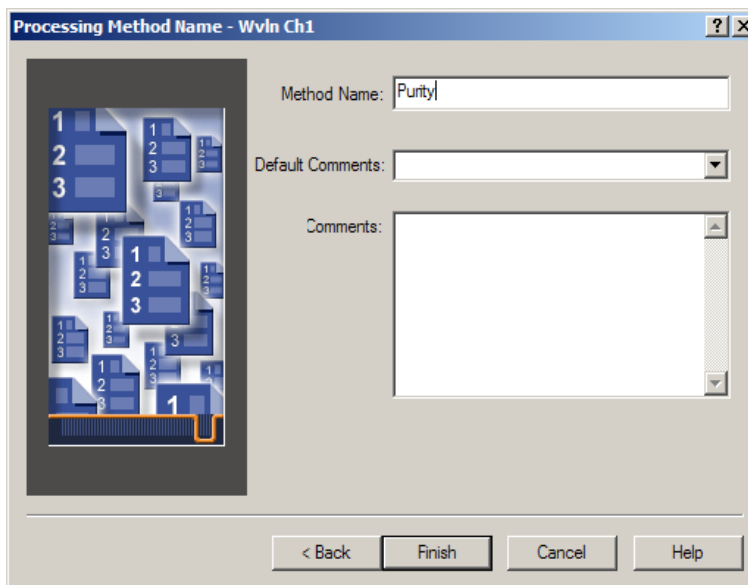
Figure 3-16 Processing Method Name Page

14. In the Method Name field, enter a processing method name, for example, **Purity**, then click **Finish**. The processing method is saved and the Review Main window appears with the chromatogram processed (). The name of the processing method is indicated in the right side of the status bar.

Note: The Empower PDA software automatically sets the Threshold Criteria in the processing method to AutoThreshold. For details, see the "Threshold Criteria Considerations for Spectral Contrast (PDA)" topic in the Empower Help.

This procedure assumes that the Automatically Apply Method Set and New Method Set: Create a new method set when starting Review options are enabled in Review. To verify or change these settings, select **Options > Method Set Options**. *If the Automatically Apply Method Set option is not enabled, your chromatogram will not be automatically processed. To process it, click* ▓ *(Apply Method Set) or select* **Process > Apply Method Set.**

Figure 3-17 Integrated Chromatogram in Review

When working with a PDA processing method, you must specify an extracted channel(s) and a processing method(s) to use when processing the extracted data. You also have the ability to specify multiple extracted channels and to use different processing methods for each, if desired. When you extract channels as you did in Section 3.2, Deriving Chromatograms, they are automatically added to your method set. No further action is required, however, it is recommended to verify the information in your method set and to rename the derived channel from the generic name that the software assigns to a name that is more meaningful to you.

3.3.1 Viewing, Modifying and Saving the Method Set

1. Click 📄 (Method Set) or select **Window > Method Set** to open the Method Set Editor window (Figure 3-18).
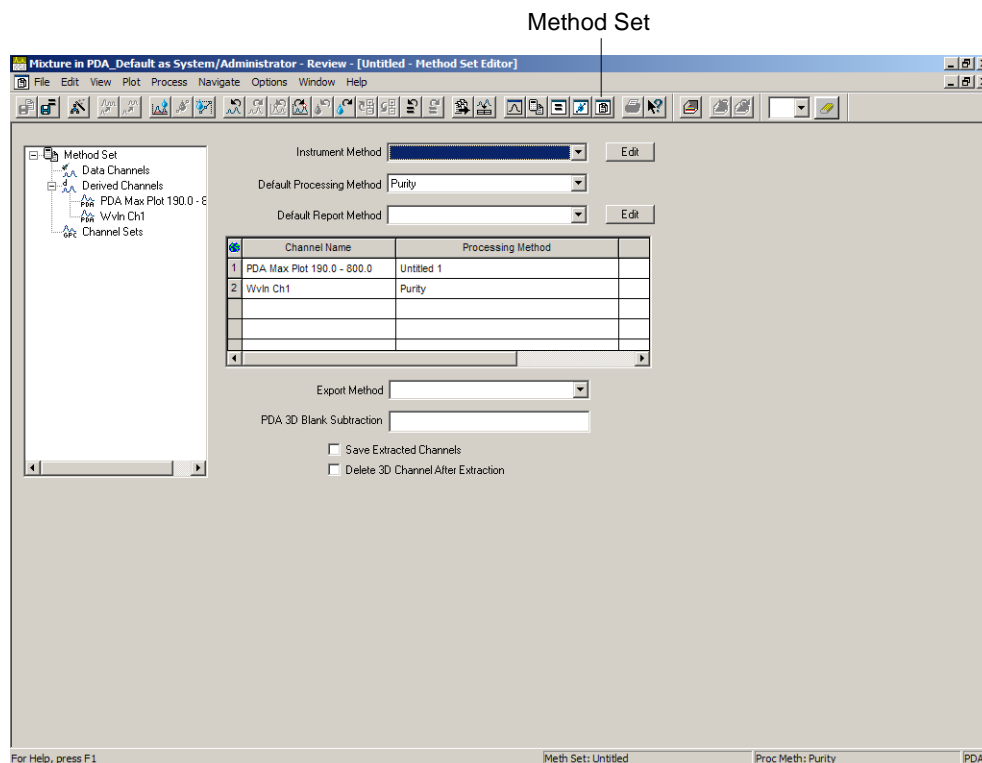
Method Set

Figure 3-18 Method Set Editor Window

Notice that the Max Plot and 254 nm (named Wvln Ch1) chromatograms that you extracted earlier are listed in the Channel Name column of the Channel table. The processing method that you just created has also been specified in this table for the 254-nm chromatogram.

2. Right-click the **Wvln Ch1** derived channel in the tree pane on the left side of the Method Set Editor window. Select **Rename** from the shortcut menu. Wvln Ch1 is selected (Figure 3-19). Enter a new name for this derived channel, for example, **254nm**.
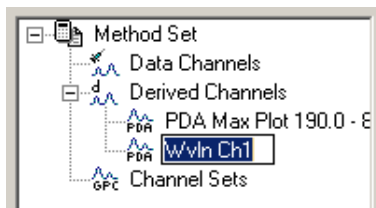
Figure 3-19 Method Set Tree Pane

3. Click the first row in the Channel table to select the Max Plot chromatogram (Figure 3-18). Press **Delete** on your keyboard to remove this row from the table.

4. Select **File > Save As > Method Set** to open the Save current Method Set dialog box (Figure 3-20).
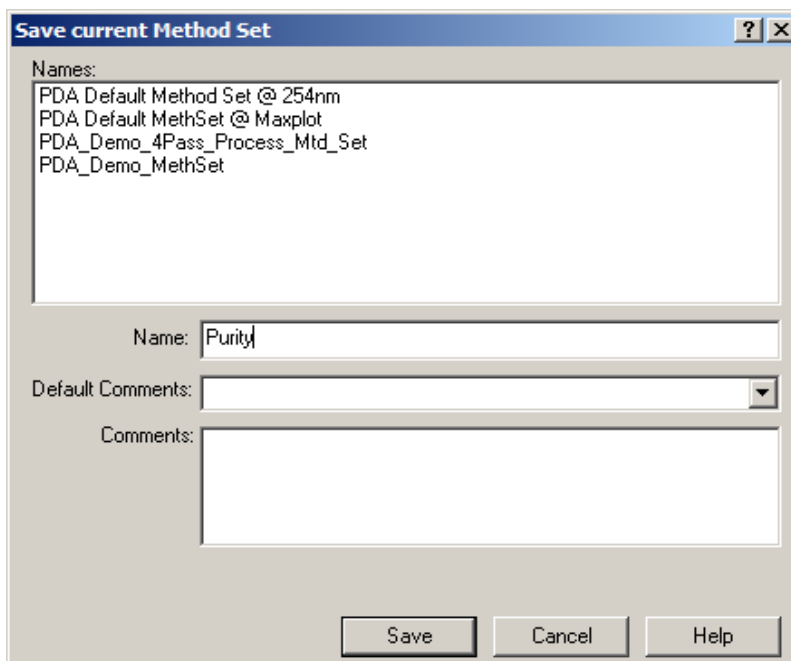
Figure 3-20 Save Current Method Set Dialog Box

5. In the Name field, type a name, for example, **Purity**, then click **Save**. The name of the method set appears in the title bar of the Method Set Editor window and should look as in Figure 3-21.

Method Set
Name Appears in
Title Bar
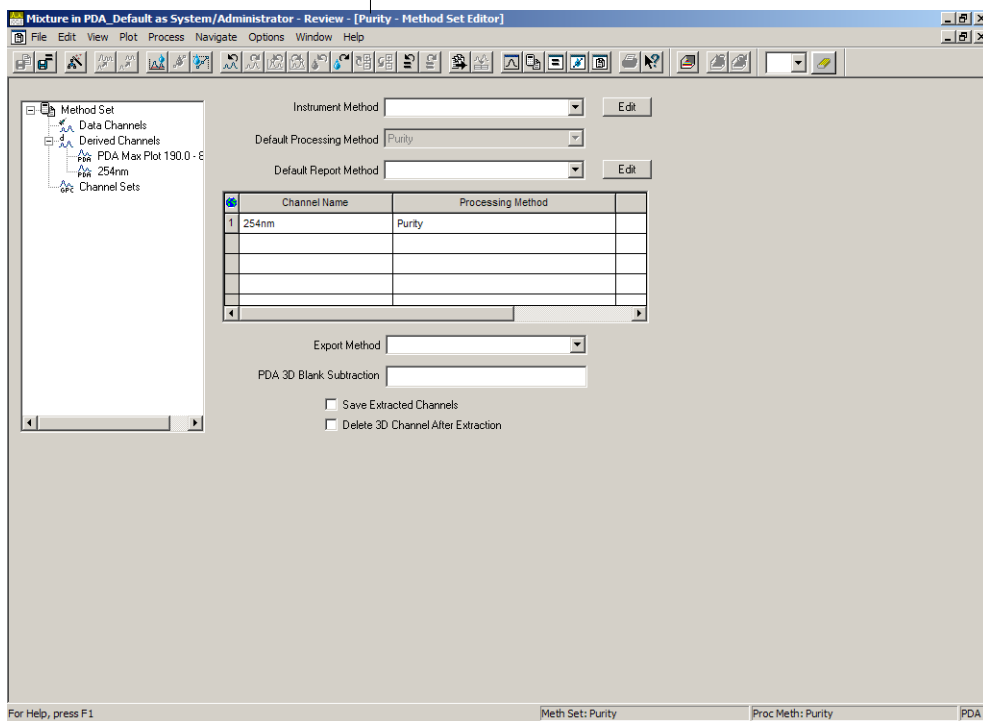
Figure 3-21 Method Set Editor Window after Modifying and Saving

3.3.2 Viewing the Peak Purity Calculation

1. Click ![icon] (Review Main Window) or select **Window > Main Window** to return to the Review Main window.

2. Click ![icon] (Apply Method Set) or select **Process > Apply Method Set** to extract and process the channel specified in the method set you created (Figure 3-22).
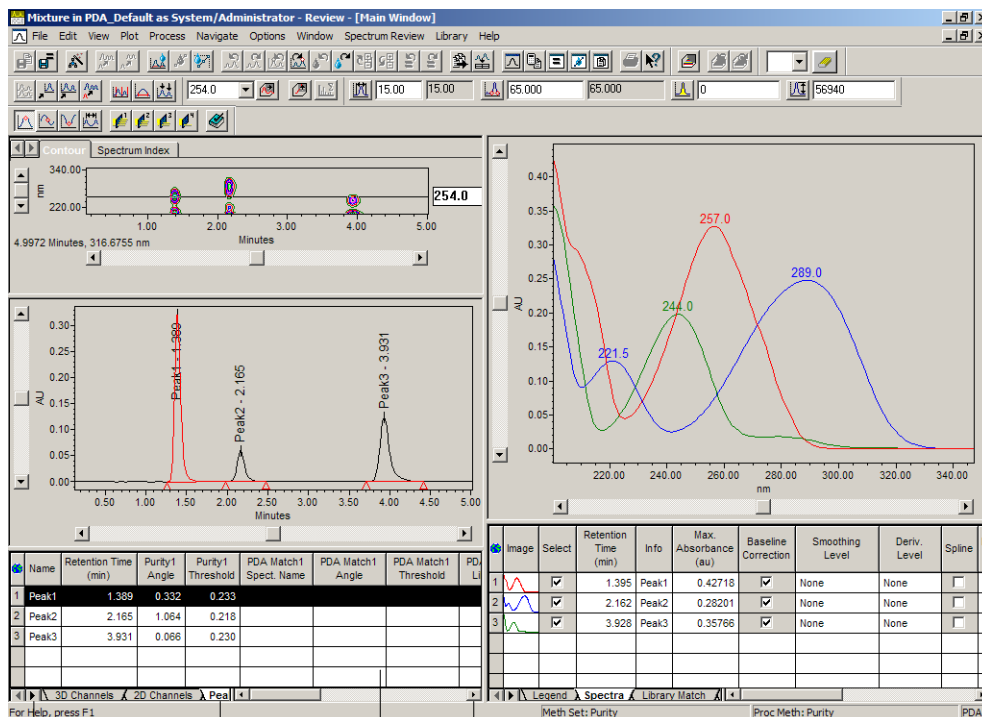
Figure 3-22 Purity Angle and Purity Threshold Values in the Peaks Table

3. Select the scroll arrows where indicated at the bottom left of the window to make the Peaks tab visible (Figure 3-22). To view the numeric results of the peak purity calculation, click the **Peaks** tab at the bottom of the Review Main window.

4. Using the mouse, move the Chromatogram plot up or down by dragging the split bar to size the Chromatogram plot and the Peaks table. The Peaks table displays the Purity Angle and Purity Threshold values (Figure 3-22). If the Purity Angle and Purity Threshold values are not visible, scroll within the Peaks table to reveal them.

5. If the Purity Angle is less than the Purity Threshold, the peak is spectrally homogeneous. Scroll down in the table to view the values for every integrated peak. Note that Peaks 1 and 2 are not spectrally homogeneous; whereas Peak 3 is spectrally homogeneous. For details on interpreting peak purity results, see the "Interpreting Peak Purity Results (PDA)" topic in the *Empower Help*.

3.4 Reviewing Peak Purity Results

Once you have calculated peak purity, you can view the results in different ways. For example, you can examine the apex spectrum and the maximum impurity spectrum in Spectrum Index (see Section 3.4.1). Alternatively, you can use the Results window to examine the Purity plot and to view the peak purity results (see Section 3.4.2).

3.4.1 Using Spectrum Index

Spectrum Index allows you to view the apex spectra of the integrated peaks. To view Spectrum Index:

1. In the Review Main window, click the **Spectrum Index** tab (Figure 3-23). The apex spectrum for each integrated peak appears and additional Spectrum Index tools appear in the toolbar if they were not already present.

   Note: *If the Spectrum Index tools do not appear, right-click the toolbar and select* ***Spectrum Index*** *or select* ***View > Toolbar > Spectrum Index****.*

3
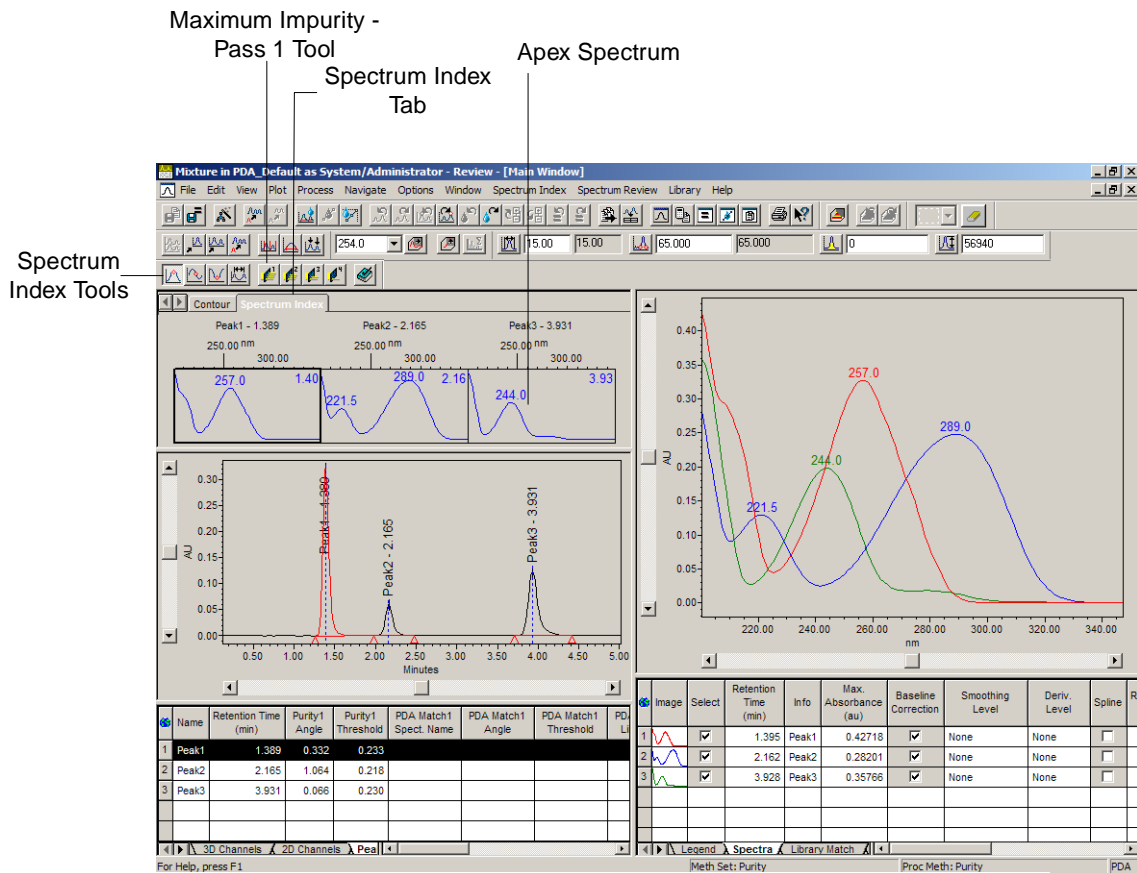
Figure 3-23 Apex Spectrum

2. Click ![icon] (Maximum Impurity - Pass 1) (Figure 3-23) to display the maximum impurity spectrum in the Spectrum Index plot (Figure 3-24). You are now viewing the apex spectrum overlaid with the spectrum within the integrated peak that differs most from the apex spectrum. This is displayed for every integrated peak. The dotted line in the Chromatogram plot indicates the location within each peak from where these spectra are taken. The lambda max, which is the wavelength of the maximum absorbance, for each spectrum is labeled in the Spectrum Index plot by default.

Spectrum Index Plot
(y-axis is absorbance)
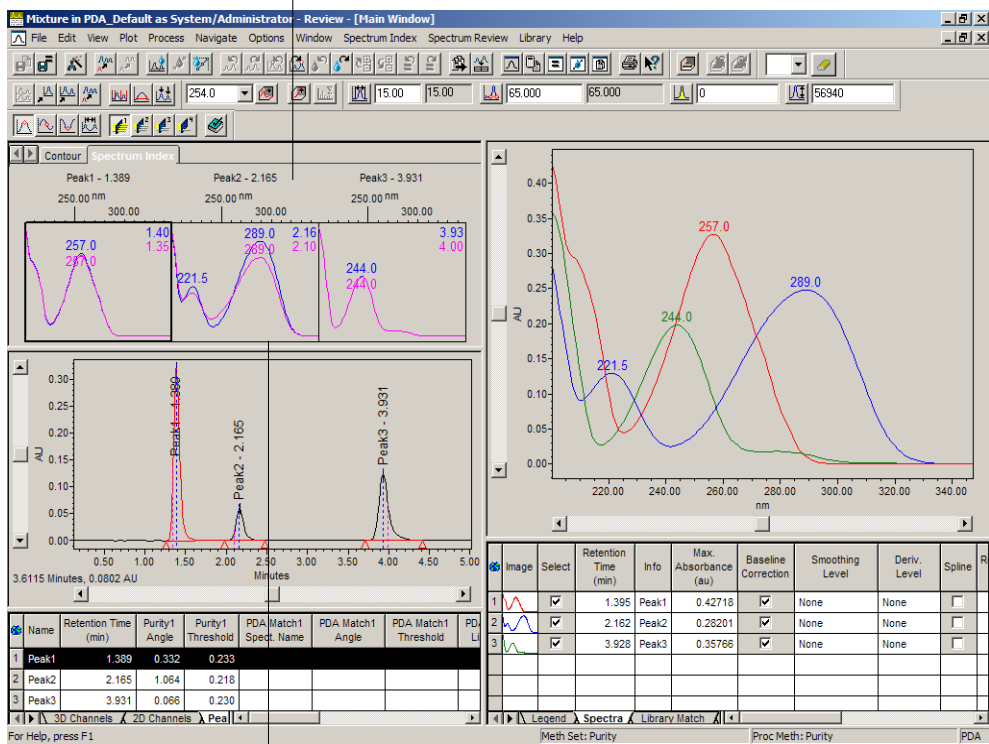
X-Axis Is Wavelength

Figure 3-24 Apex Spectra Overlaid with Maximum Impurity Spectra

3. In the Chromatogram plot, zoom in on the first peak and examine the overlaid spectra and note the slight differences between the spectra (Figure 3-25). Recall that this peak is not spectrally pure.
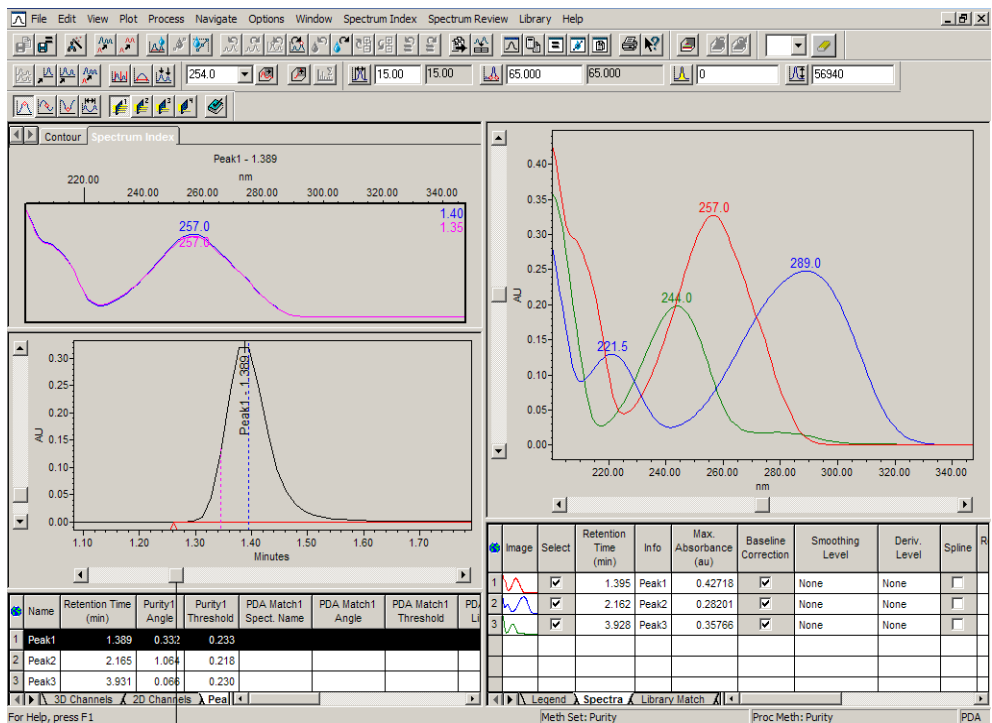
Scroll to the Right to Examine
Subsequent Peaks

Figure 3-25 Maximum Impurity Spectrum with Peak 1 Spectra Zoomed

4. Scroll to the right and examine the spectra from the second peak and note the significant differences between the spectra (Figure 3-26). Recall that this peak is not spectrally pure.
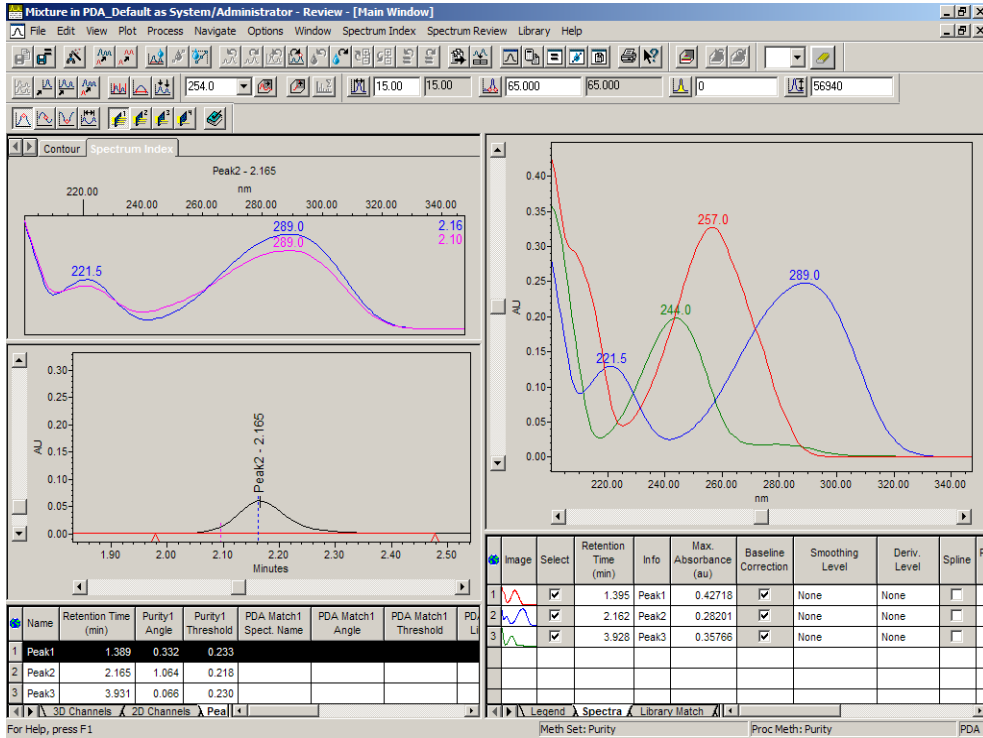
Figure 3-26 Maximum Impurity Spectrum with Peak 2 Spectra Zoomed

5. Scroll to the right and examine the spectra from the third peak (Figure 3-27). The spectra overlay well.

   The first and second peaks do not appear spectrally homogeneous and require further investigation. The third peak appears spectrally homogeneous. This validates the purity results discussed in step 5 of "Viewing the Peak Purity Calculation".

Results Tool
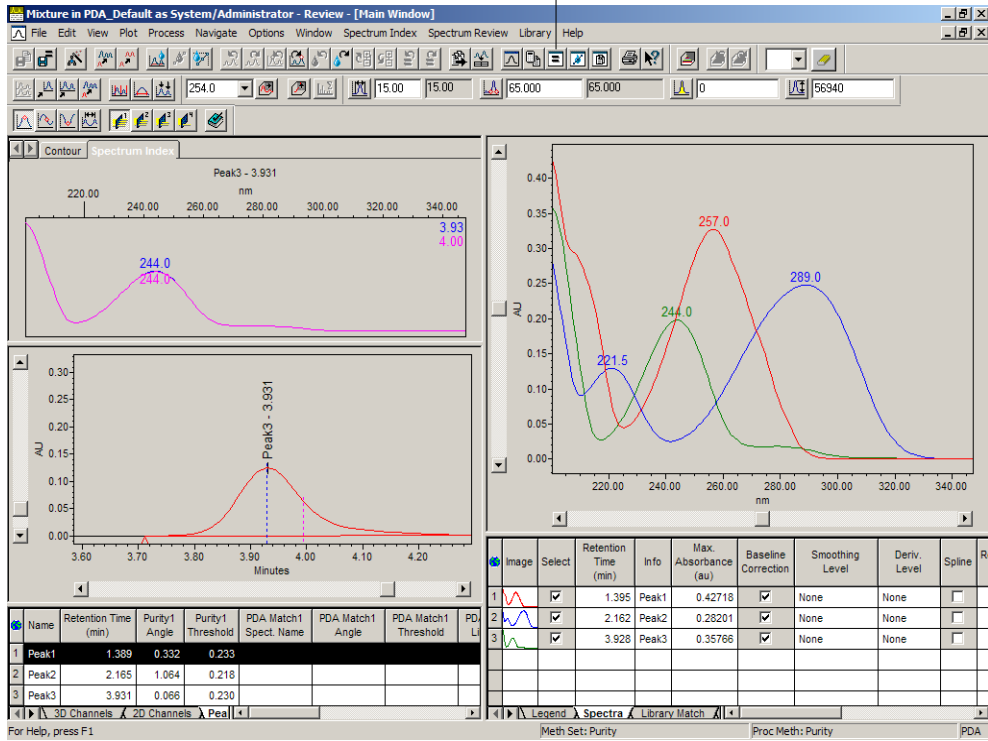
Figure 3-27 Maximum Impurity Spectrum with Peak 3 Spectra Zoomed
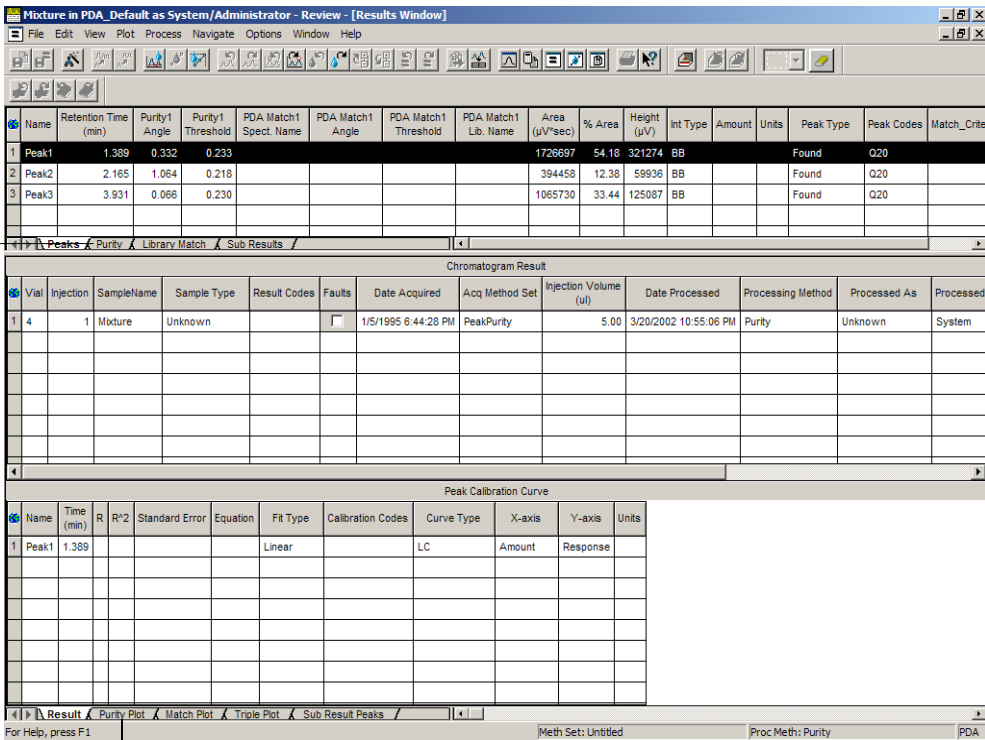
3.4.2 Using the Results Window

To view the Purity plot in the Results window:

1. Click ▣ (Results) or select **Window > Results Window** (Figure 3-27) to open the Results window (Figure 3-28). If necessary, maximize the Results window or size the different panes by dragging the split bars.

Purity
Tab

Purity Plot Tab

Figure 3-28 Results Window

2. Click the **Purity** tab in the top pane and the **Purity Plot** tab in the bottom pane of the Results window. The Purity plot (Figure 3-29) displays the currently active (selected) chromatographic peak and plots the Purity Angle and the Purity Threshold values across the entire peak. Note that the left y-axis is in absorbance units and the right y-axis is in Spectral Contrast degrees. The Purity tab displays the purity information for each peak in a numerical format. Select each peak using the Peak list (Figure 3-29).
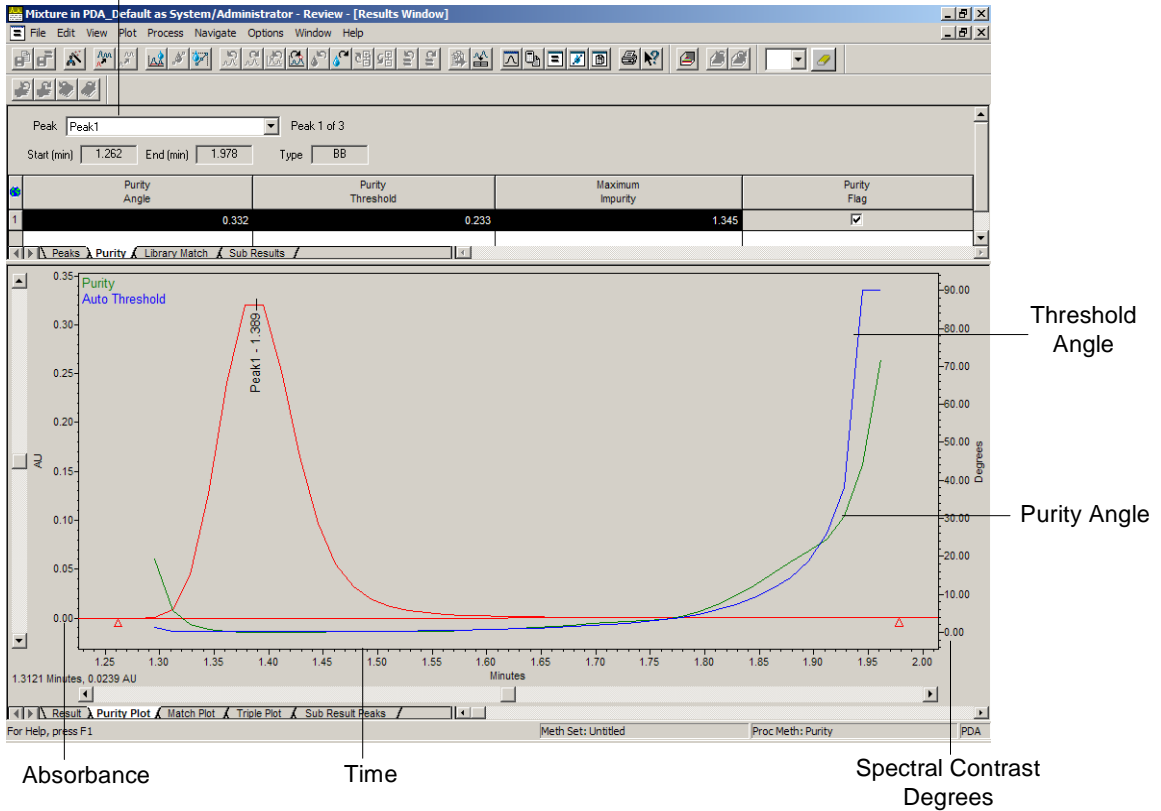
Figure 3-29 Purity Plot

For a spectrally homogeneous peak, the Purity Angle always remains below the Threshold Angle. For the peak at 1.389 minutes, the Purity Angle is above the Threshold Angle in the early region of the peak, which indicates that the peak is not spectrally homogeneous in this region.

The Purity Plot plots the Spectral Contrast Angle between the peak apex spectrum and all other peak spectra. For details, see the "Spectral Contrast Angle (PDA)" topic in the *Empower Help*. The Purity Angle increases in the peak tails due to the effects of baseline noise.

3. Select the next peak in the Peak list ([Figure 3-29](#)) to display the Purity plot for the next peak at 2.165 minutes. The Purity Angle is above the Threshold Angle in the early region of the peak, which indicates that the peak is not spectrally homogeneous in this region.

4. Select the next peak in the Peak list ([Figure 3-29](#)) to display the Purity plot for the next peak at 3.931 minutes. The Purity Angle is below the Threshold Angle throughout the entire peak, which indicates that the peak is spectrally homogeneous.

5. Right-click the Purity plot, then select **Properties** to open the Plot Properties dialog box ([Figure 3-30](#)).
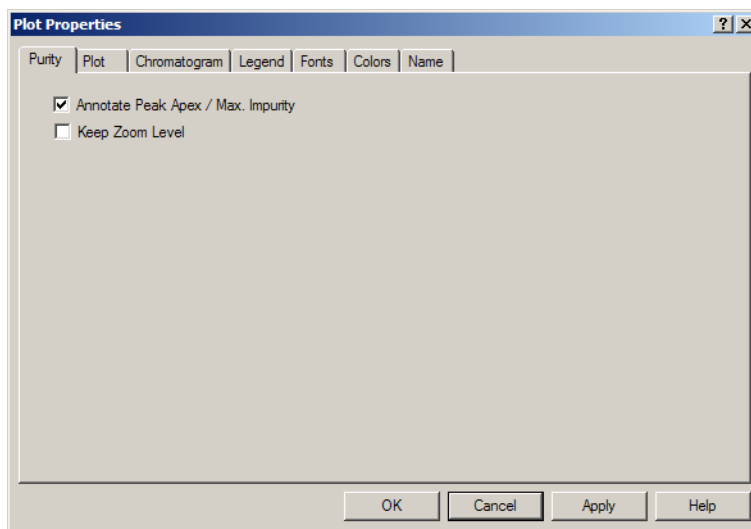
Figure 3-30 Plot Properties Dialog Box

6. In the Purity tab, select the **Annotate Peak Apex/Max. Impurity** check box, then click **OK**. The point of maximum impurity appears in the Purity plot and is marked with an "M" ([Figure 3-31](#)). The peak apex is marked with a vertical line drawn from the apex of the peak perpendicular to the baseline.
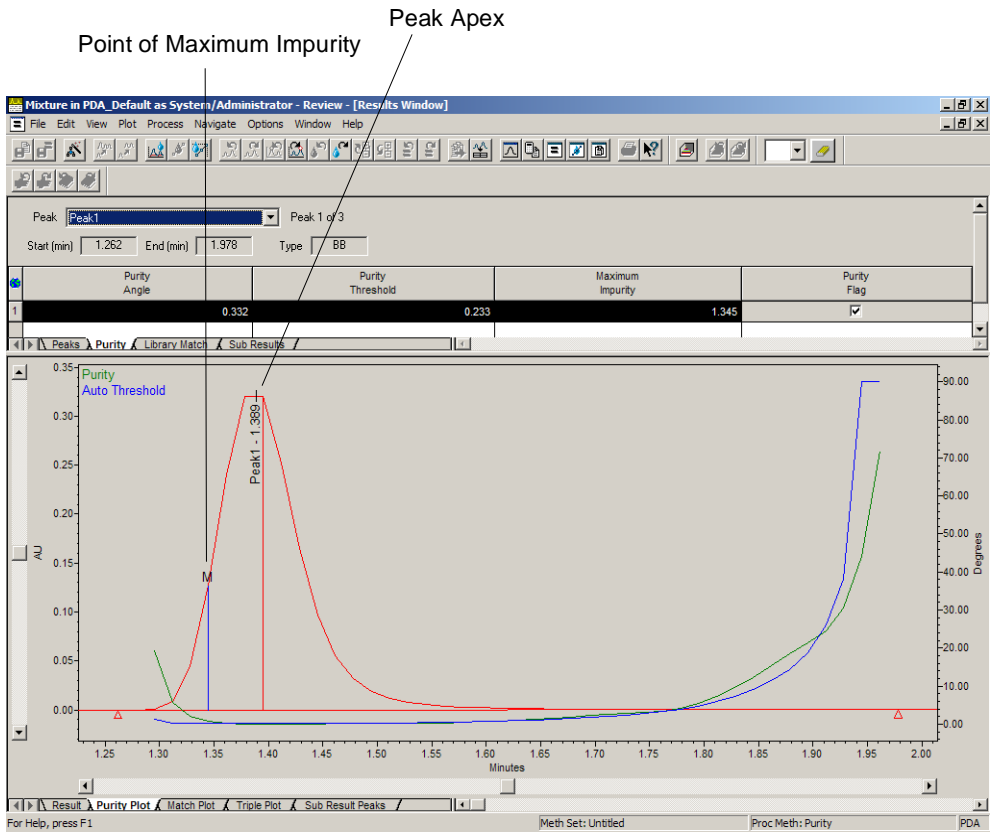
Figure 3-31 Purity Plot with Maximum Impurity Indicator

7. Save the result so you can print it in a report. To save the results, select **File > Save > Result**. If **Save > Result** is not available, select **Save > Processing Method** first.

8. Exit Review by clicking ⊠ (Close) or selecting **File > Exit**.

Next Steps

Now that you have developed a processing method to assess peak purity, you have the following options:

- Modify the existing processing method to include library matching, as described in Chapter 4, Library Matching.

- Modify the processing method for multicomponent peak purity. For details, see the "Multicomponent Peak Purity Testing (PDA)" topic in the *Empower Help*.

- Print your results, as described in Chapter 5, Printing Reports.

3

Chapter 4
Library Matching

This chapter provides step-by-step tutorials describing the procedures for creating a library and matching unknown or acquired spectra to spectra in a library.

4.1 Tutorial Overview

This tutorial familiarizes you with Empower software tools and procedures used to create a library and to match spectra to a library. Library matching allows you to identify peaks by comparing spectra from unknown peaks to spectra from known peaks. These tutorials show you how to:

- Create a library from existing standards.

- Match unknown spectra to spectra in a library.

- Review library matching results.

Section 4.1.1, Steps in Creating a Library, lists the steps used to create a library. Section 4.1.2, Steps in Library Matching, shows the steps used for library matching. If you followed the tutorial in Chapter 3, Peak Purity Processing, you can use the processing method that you developed and modify it to include library matching.

4.1.1 Steps in Creating a Library

The following steps in creating a library are outlined in Section 4.2:

1. Select PDA 3D Channels of data.
2. Bring data into Review.
3. Open an existing method set.
4. Create a new library.
5. Add spectra to the library.

4

4.1.2 Steps in Library Matching

```
                          ┌──────────┐
                          │  Start   │
                          └──────────┘
                               │
                               ▼
                          ╱────────────╲
                         ╱ Do You Want to ╲        Yes: With Peak Purity
                        ⟨ Do Library Matching ⟩──────────────────────────┐
                         ╲ and Peak Purity? ╱                            │
                          ╲────────────╱                                 │
                               │                                         │
                      No: Library Match Only                             │
                               ▼                                         ▼
              ┌────────────────────────────┐        ┌────────────────────────────┐
              │  Bring PDA data into Review │        │  Bring PDA data into Review │
              │       (Section 4.3.1)       │        │       (Section 4.3.1)       │
              └────────────────────────────┘        └────────────────────────────┘
                               │                                         │
                               ▼                                         ▼
              ┌────────────────────────────┐        ┌────────────────────────────┐
              │    Derive a Chromatogram    │        │  Open an Existing Method Set │
              │       (Section 4.3.2)       │        │       (Section 4.3.1)       │
              └────────────────────────────┘        └────────────────────────────┘
                               │                                         │
                               ▼                                         ▼
              ┌────────────────────────────┐        ┌────────────────────────────┐
              │  Set Integration Parameters │        │   Enable Library Matching   │
              │       (Section 4.3.2)       │        │       (Section 4.3.1)       │
              └────────────────────────────┘        └────────────────────────────┘
                               │                                         │
                               ▼                                         │
              ┌────────────────────────────┐                            │
              │      Set Noise Interval     │                            │
              │       (Section 4.3.2)       │                            │
              └────────────────────────────┘                            │
                               │                                         │
                               ▼                                         │
              ┌────────────────────────────┐                            │
              │   Enable Library Matching   │                            │
              │       (Section 4.3.2)       │                            │
              └────────────────────────────┘                            │
                               │                                         │
                               ▼                                         │
              ┌────────────────────────────┐                            │
              │  Integrate Chromatogram and │◄───────────────────────────┘
              │      Calculate Results      │
              │       (Section 4.3.3)       │
              └────────────────────────────┘
                               │
                               ▼
              ┌────────────────────────────┐
              │        View Results         │
              │        (Section 4.4)        │
              └────────────────────────────┘
                               │
                               ▼
                          ┌──────────┐
                          │   End    │
                          └──────────┘
```
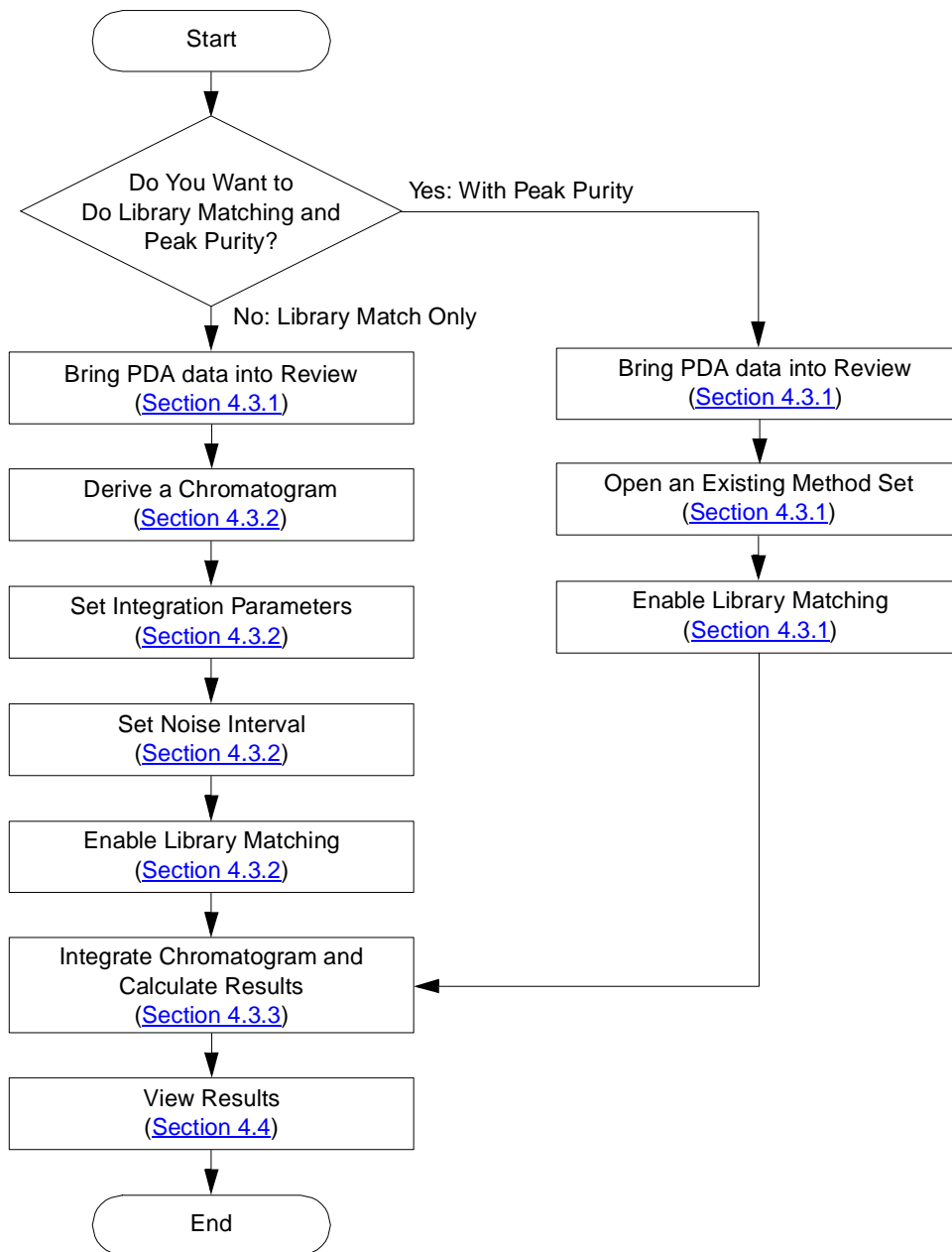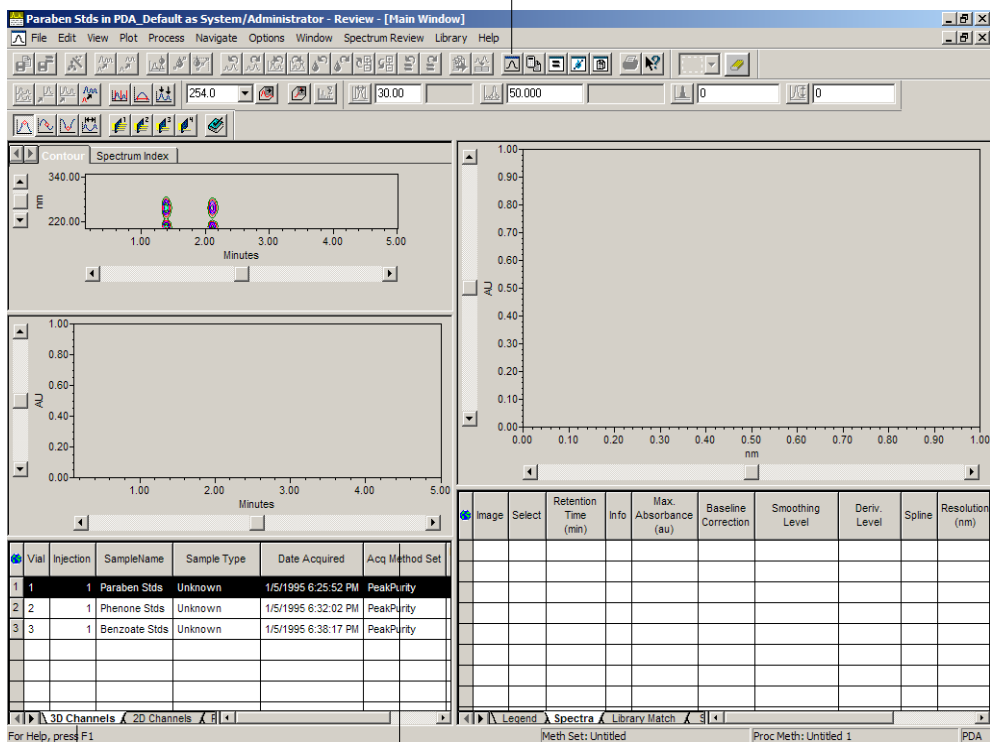
Figure 4-1 Steps in Library Matching

4.2 Creating a New Library

You must create a library of known spectra before you can match unknown spectra to them. The first step in creating a library is to choose the spectra to add to the library. In this procedure, you add the spectra for Paraben Stds, Phenone Stds, and Benzoate Stds to the library.

To create a library:

1. In the Project window Channels view, which you opened in Chapter 2, Viewing PDA Data, select **Paraben Stds**, **Phenone Stds**, and **Benzoate Stds**, then click (Review) or select **Window > Review** (Figure 4-2) to open the Review Main window (Figure 4-3).

   Note: In Empower software, multiple items can be selected simultaneously by selecting them in the table with a Ctrl-click (multiple noncontiguous rows) or a Shift-click (multiple contiguous rows).

   If the Review Main window is not displayed, click (Review Main Window) or select Window > Main Window (Figure 4-3).

Review Tool

Figure 4-2 Selecting Data for a Library

Review Main Window Tool

Figure 4-3 Review Main Window

2. At the bottom of the Review Main window, click the **3D Channels** tab (Figure 4-3). Make sure Paraben Stds is selected.

3. To enlarge the 3D Channels table if necessary, use the mouse to drag the split bar above the 3D Channels table higher on the screen, as shown in Figure 4-3.

4. Select **File > Open > Method Set** to open the Open an existing Method Set dialog box (Figure 4-4).
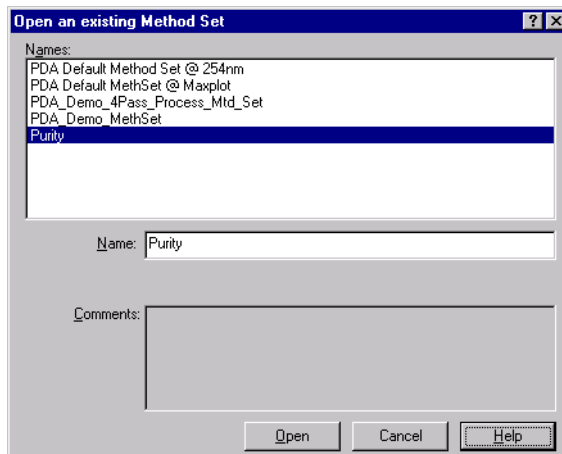
Figure 4-4 Open an Existing Method Set Dialog Box

5. In the Names list, select **Purity** or the method set that you created in Chapter 3, Peak Purity Processing, then click **Open**. The Purity method set is automatically applied to the data in the Review window. The chromatogram at 254 nm for Paraben Stds appears in the Review Main window (Figure 4-5) and the apex spectra for the peaks appear in Spectrum Review.

*Note: If you did not create a method set, select **PDA_Demo_MethSet**. A method set is required in order to extract a chromatogram during processing.*

*If the Automatically Apply Method Set check box is not enabled in the Method Set Options dialog box (the default setting found by selecting **Options > Method Set Options**), the method set is not automatically applied. To apply the method set, click ⬛ (Apply Method Set) or select **Process > Apply Method Set**.*

Apply Method Set Tool
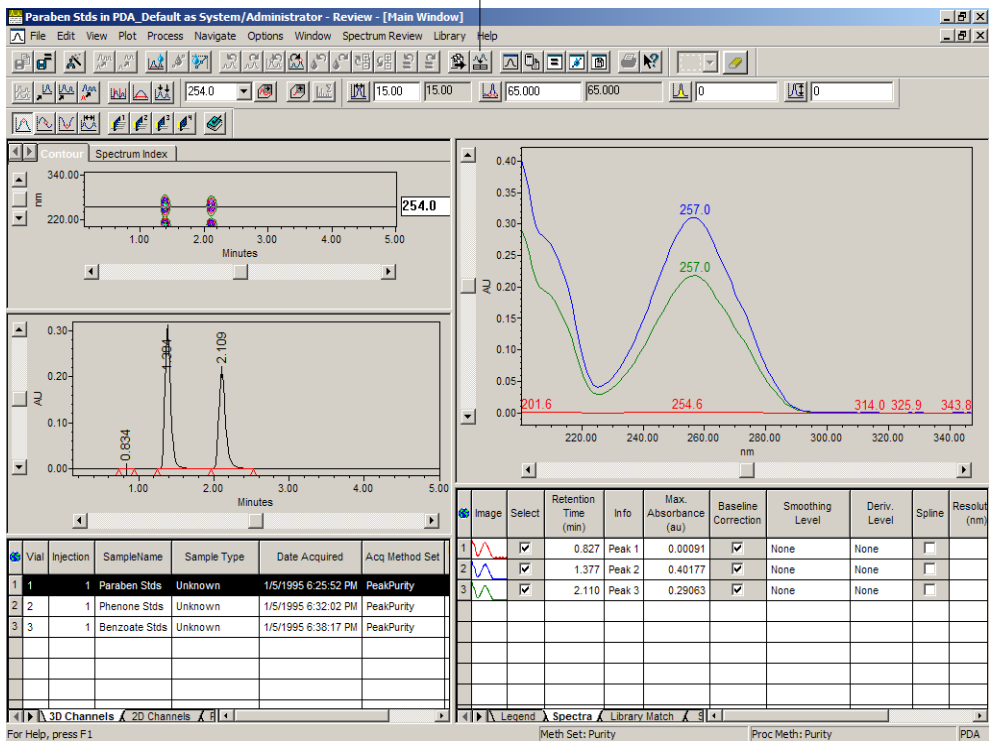
Figure 4-5 Paraben Stds Chromatogram

6. Select **Library > New Library** to open the Create a new Library dialog box
   ([Figure 4-6](#)).

   Note: *The retention times of the peaks do not match the retention times displayed in
   the Spectra tab. The apex spectrum is extracted using a method that differs slightly
   from that used to determine retention time.*

4
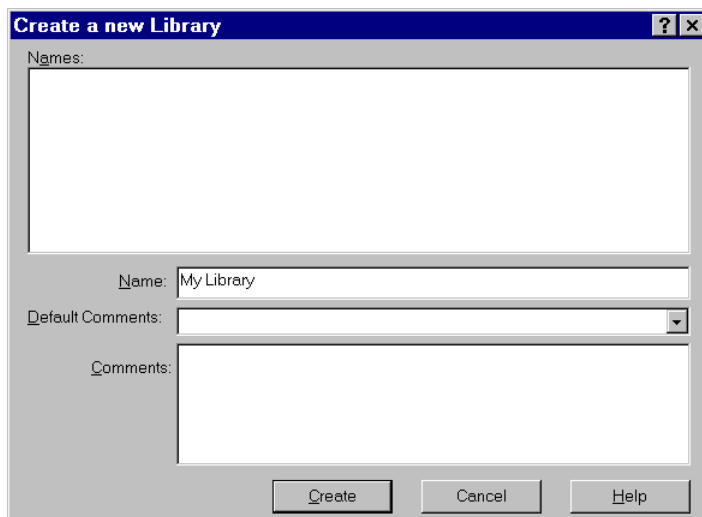
Figure 4-6 Create a New Library Dialog Box

7. In the Name field, type a name for the new library, for example, **My Library**, then click **Create** to create the new library.

Adding Spectra to the Library

1. In the Spectra table, make sure that the check box in the Select column is only selected for the two main peaks as shown in <u>Figure 4-7</u>. The two spectra appear in Spectrum Review.

    Note: When addding spectra to a library, choose your spectra by using the Select column in the Spectra table. Spectra that do not have the Select field selected are not added to the library.

Spectra Plot
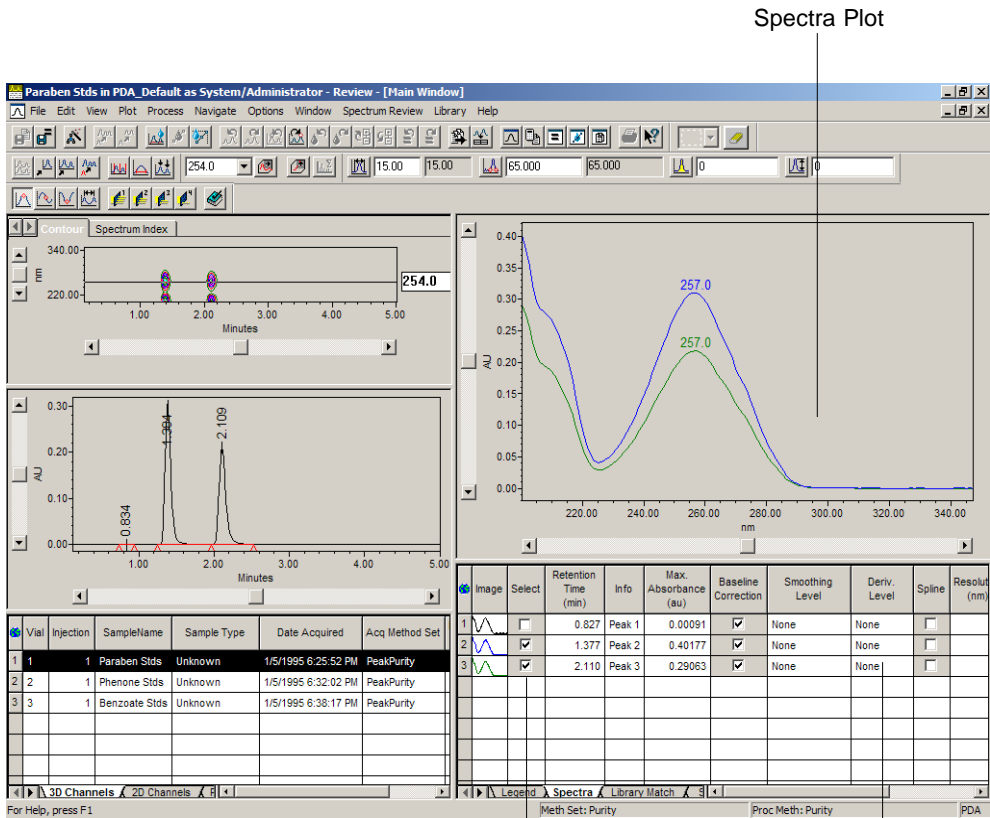
Figure 4-7 Spectrum Review with Spectra Selected

Select Check Boxes   Spectra Table

2. Select **Library > Add to library My Library** to open the Add Spectrum to Library
    dialog box (Figure 4-8).

3. In the Name text box, change the name from the default of *Peak 2* to **Methyl
    Paraben**, then click **OK**. The Add Spectrum to Library dialog box reappears for the
    next spectrum.
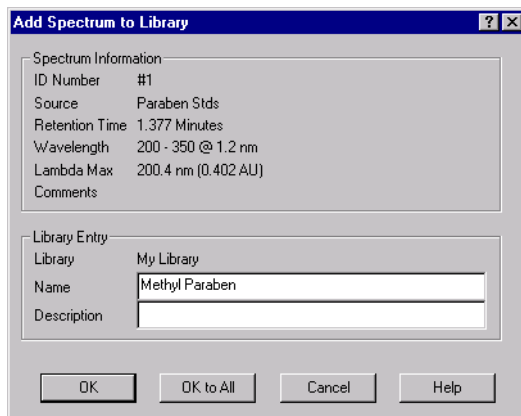
Figure 4-8 Add Spectrum to Library Dialog Box

4. Change the name from the default of *Peak 3* to **Ethyl Paraben** in the Name text box, then click **OK**. The spectra are added to the library and appear (unselected) in the Spectra table.

5. In the 3D Channels table, select **Phenone Stds**. The processed chromatogram for Phenone Stds appears. Repeat steps 1 and 2 for Peaks 2 and 3 in this sample.

6. In the Name text box, change the name from the default of *Peak 2* to **Acetophenone**, then click **OK**. The Add Spectrum to Library dialog box reappears for the next spectrum.

7. Change the name from the default of *Peak 3* to **Propriophenone** in the Name text box, then click **OK**. The spectra are added to the library and appear (unselected) in the Spectra table.

8. In the 3D Channels table, select **Benzoate Stds**. The processed chromatogram for Benzoate Stds appears. Repeat steps 1 and 2 for Peaks 1 and 2 in this sample.

9. In the Name text box, change the name from the default of *Peak 1* to **Ethyl Paba**, then click **OK**. The Add Spectrum to Library dialog box reappears for the next spectrum.

10. Change the name from the default of *Peak 2* to **Benzoic Acid** in the Name text box, then click **OK**. The spectra are added to the library and appear (unselected) in the Spectra table.

11. Select **File > Exit**.

4.3 Matching Spectra to a Library

Now that you have created a spectral library, you can compare unknown spectra to spectra in the library. As Figure 4-1 shows, you can create a processing method in one of the following ways:

- If you plan to use peak purity and library matching, you can modify the existing processing method that you created in Chapter 3, Peak Purity Processing, to include library matching. See Section 4.3.3, Modifying an Existing Processing Method for Library Matching.
- If you plan to do library matching only, you can create a new processing method for library matching alone. See Section 4.3.2 Creating a New Processing Method for Library Matching.

When you have finished creating a processing method, proceed to Section 4.3.3, Performing Library Matching.

4.3.1 Modifying an Existing Processing Method for Library Matching

To modify the existing processing method:

1. In the Project window (Figure 4-9), which you opened in Chapter 2, Viewing PDA Data, select **Mixture** from the Channels view, then click ![Review icon] (Review) or select **Tools > Review** to open the Review Main window.

Figure 4-9 Project Window of the PDA_Default Project

The Review window appears with the unprocessed data (Figure 4-10).
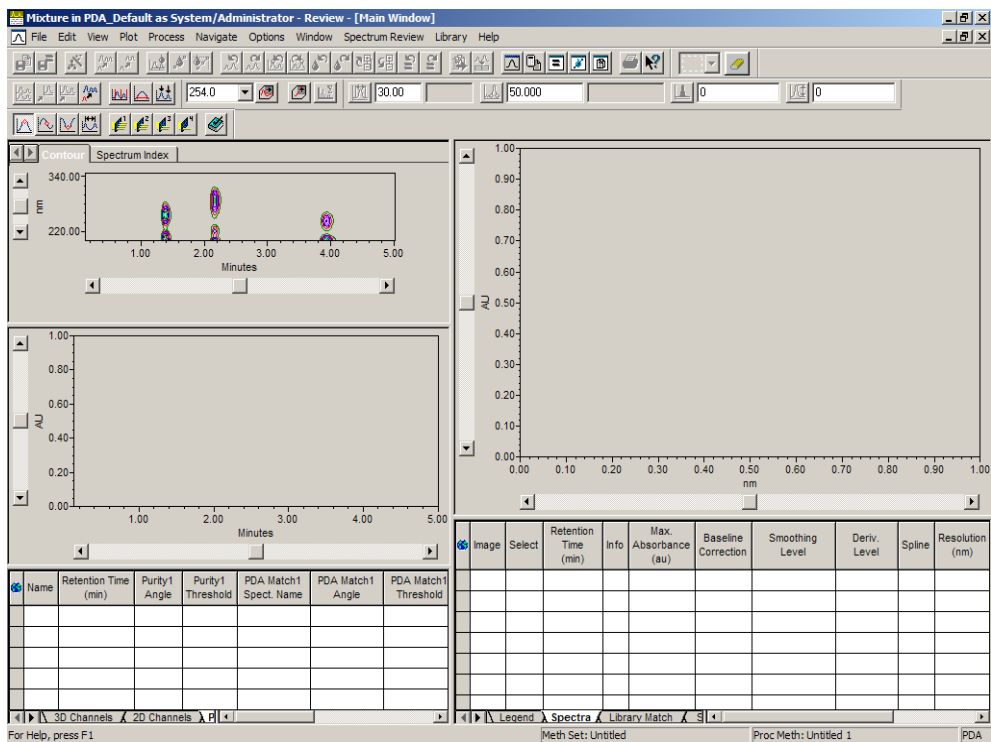
Figure 4-10 Review Main Window

2. Select **File > Open > Method Set** to open the Open an existing Method Set dialog box.

3. In the Names list, select **Purity** or the method set that you created in Chapter 3, Peak Purity Processing, then click **Open**. The Purity method set is automatically applied to the data in the Review window. The chromatogram at 254 nm for Mixture appears in the Review Main window (Figure 4-11) and the apex spectra for the peaks appear in Spectrum Review.

   *Note: If you did not create a method set, select **PDA_Demo_MethSet**. A method set is required in order to extract a chromatogram during processing.*

   *If the Automatically Apply Method Set check box is not enabled in the Method Set Options dialog box (the default setting found by selecting **Options > Method Set Options**), the method set is not automatically applied. To apply the method set, click ▨ (Apply Method Set) or select **Process > Apply Method Set**.*

Processing Method Tool
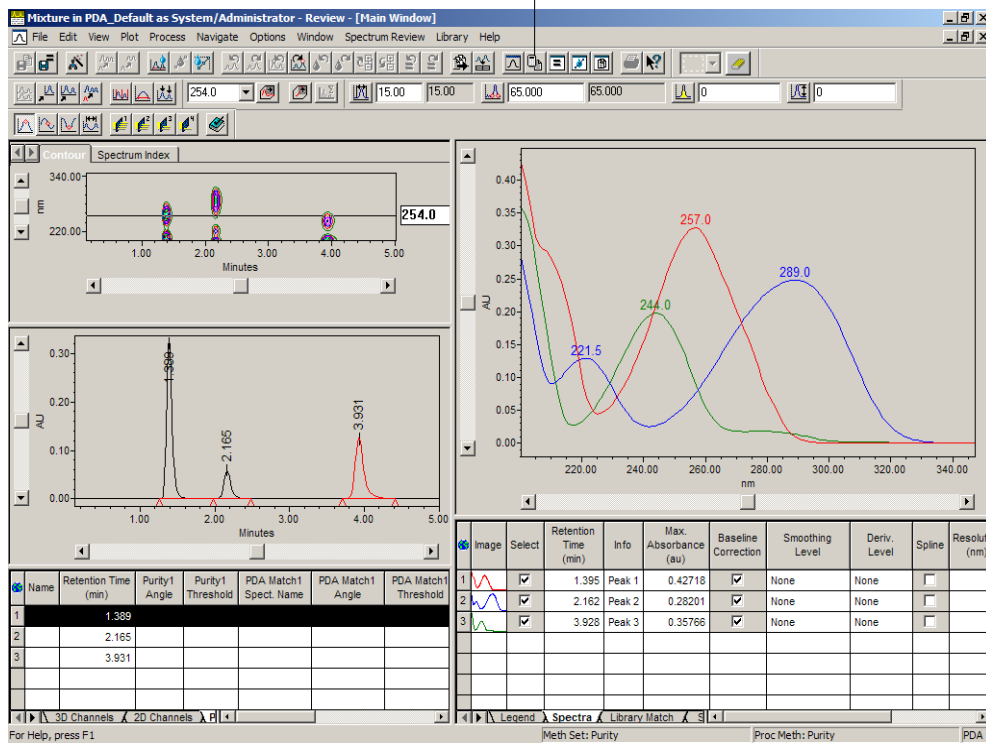
Figure 4-11 Integrated 254 nm Chromatogram of Mixture

4. Click ![icon] (Processing Method) or select **Window > Processing Method** to open the Processing Method window and modify the existing processing method for library matching (Figure 4-12). If necessary, maximize the window.

PDA Library Search Tab
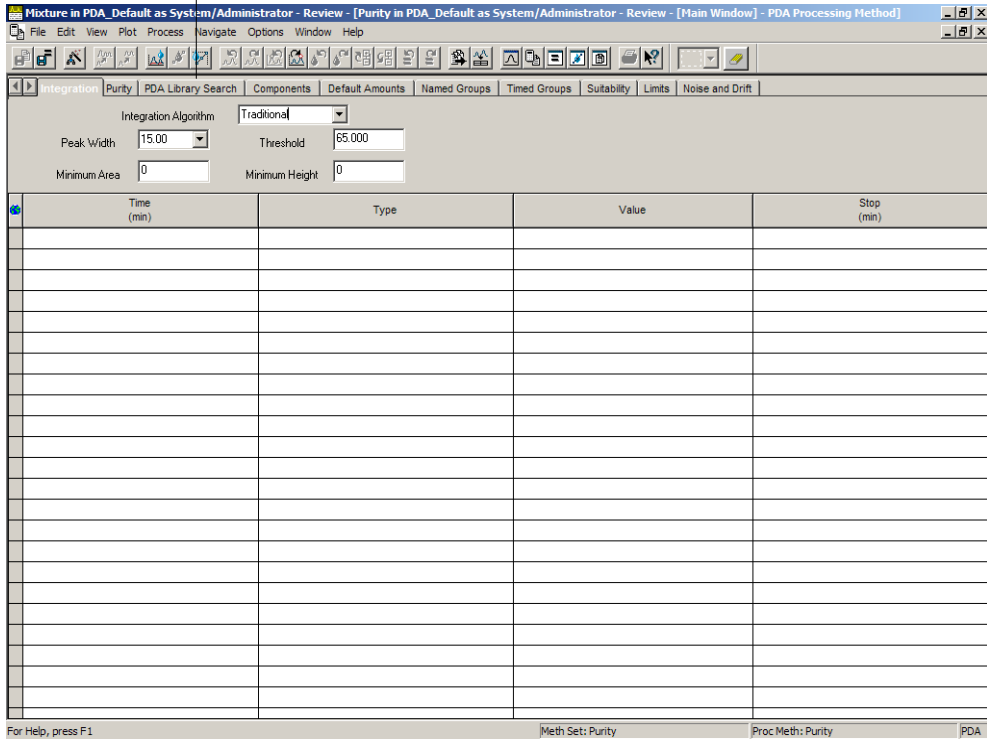
Figure 4-12 Processing Method Window

5. Click the **PDA Library Search** tab. In the Library list box, select the check box of the library you want to search, for example, **My Library** (Figure 4-13).

    Note: For information on setting a solvent angle, see the "Guidelines for Estimating the Solvent Angle (PDA)" topic in the Empower Help. *For this demonstration, we will leave the solvent angle set to 1.0. In general, this value needs to be determined empirically and set accordingly.*

4

Library List Box
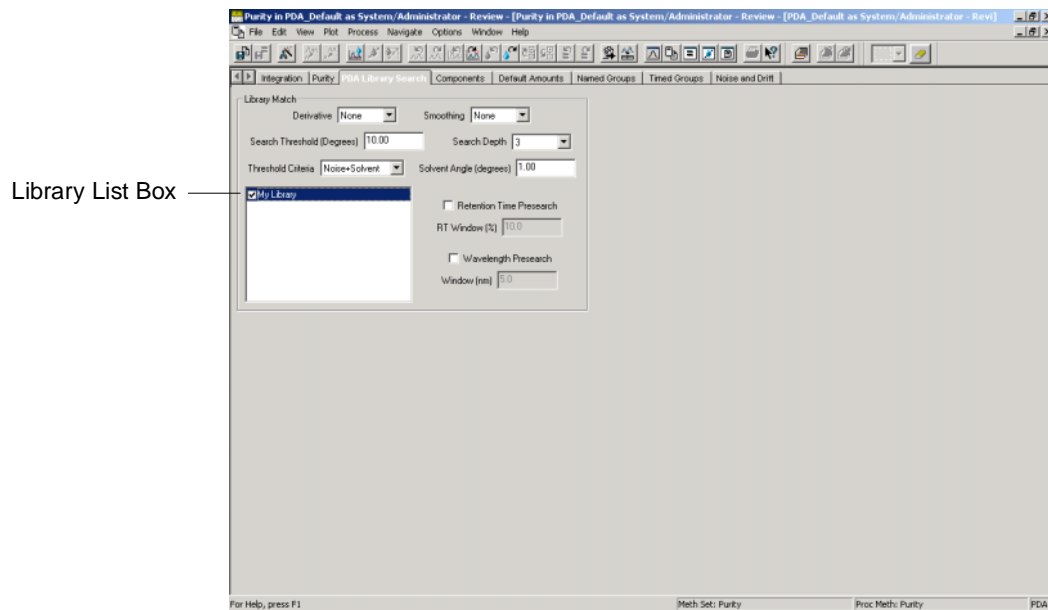
Figure 4-13 PDA Library Search Tab

6. Click [icon] (Review Main Window) or select **Window > Main Window** to return to the Review Main window.

7. To save the changes to the existing processing method, select **File > Save > Processing Method**.

    To save the modified method under a different name, select **File > Save As > Processing Method**. Type a name in the Name field, for example, **Purity and Library Match**, then click **Save**.

8. To save the modified method set under a different name, select **File > Save As > Method Set**. Type a name in the text box, for example, **Purity and Library Match**, then click **Save**.

    Note: After modifying a processing method or method set, you should click [icon] *(Method Set) or select* **Window > Method Set** *and verify that the desired processing method(s) are specified in the Channel table for the appropriate channel(s). Save the method set if you make any modifications.*

9. Proceed to Chapter 4.3.3, Performing Library Matching, to complete the steps for library matching.

4

4.3.2 Creating a New Processing Method for Library Matching

If you plan to do library matching without peak purity, you can build a processing method for library matching alone. The simplest way to build a PDA processing method is by using the Processing Method Wizard.

Note: This procedure assumes that the Use V3.0X Style Peak Width and Threshold Determination system policy is enabled (the default setting). If this system policy is not enabled on your system, the Processing Method Wizard will look slightly different.

To build a PDA processing method for library matching:

1. Before you start, you must derive a chromatogram as described in Section 2.5, Extracting a Chromatogram.

2. Click ![icon] (Processing Method Wizard) or select **File > New > Processing Method** to start the wizard and open the Processing Method Wizard dialog box (Figure 4-14).
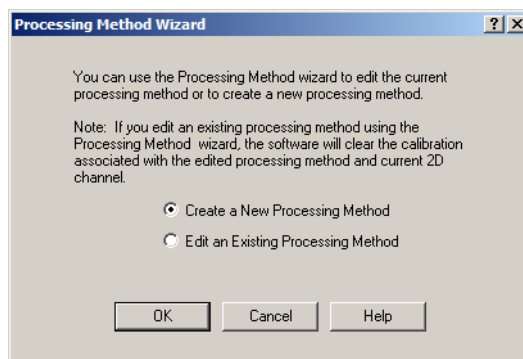
Figure 4-14 Processing Method Wizard Dialog Box

4

3. Click **Create a New Processing Method** and click **OK** to open the New Processing Method dialog box (Figure 4-15).
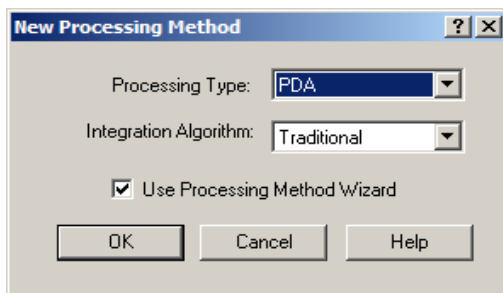
Figure 4-15 New Processing Method Dialog Box

4. From the Processing Type list, select **PDA**. If the Integration Algorithm list is present, select **Traditional**. Make sure the **Use Processing Method Wizard** check box is selected, then click **OK** to go to the Integration - Peak Detection 1 page (Figure 4-16).

Note: The Integration Algorithm list appears only if ApexTrack integration is enabled in your system policies and ApexTrack is enabled in your PDA_Default project.

4
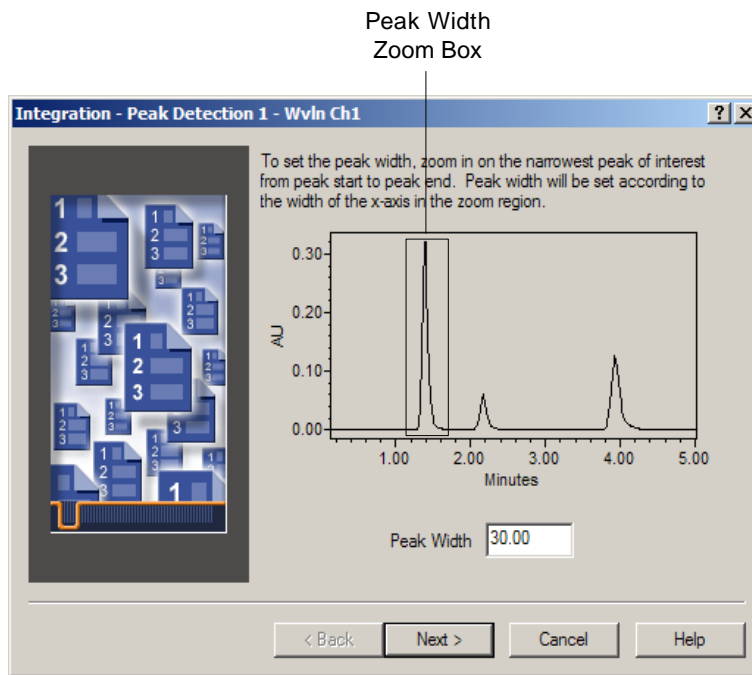
Figure 4-16 Integration - Peak Detection 1 Page, Full View

This page allows you to set the peak width parameter in your processing method. Peak width determines the number of points bunched together to act as a filter and to produce a single point for peak detection (although all data points are used during integration).

5. Follow the wizard instructions and use the mouse to draw a zoom box that encloses the liftoff and touchdown of the narrowest peak of interest (peak 1 at 1.39 minutes). The software displays the zoomed selection and determines the appropriate peak width (Figure 4-17).

    Note: Right-click the plot to access the Full View/Unzoom shortcut menu selections. If you are unsure which peak is the narrowest, you can check different peaks by zooming and unzooming repeatedly on each peak. Each time you zoom, the software calculates and displays the appropriate peak width. Zoom properly (and perhaps multiple times) so that the peak start is at the beginning of the displayed x-axis in the plot and the peak end is at the end of the displayed x-axis in the plot.

    The peaks in this data have nearly identical widths and all yield a Peak Width setting of 15 seconds when zoomed correctly. It therefore does not matter which peak is chosen, however, some data does contain peaks of varying widths. In this case, the appropriate peak should be used.

Figure 4-17 Setting the Peak Width Parameter

6.   Click **Next** to go to the Integration - Peak Detection 2 page ([Figure 4-18](#)).
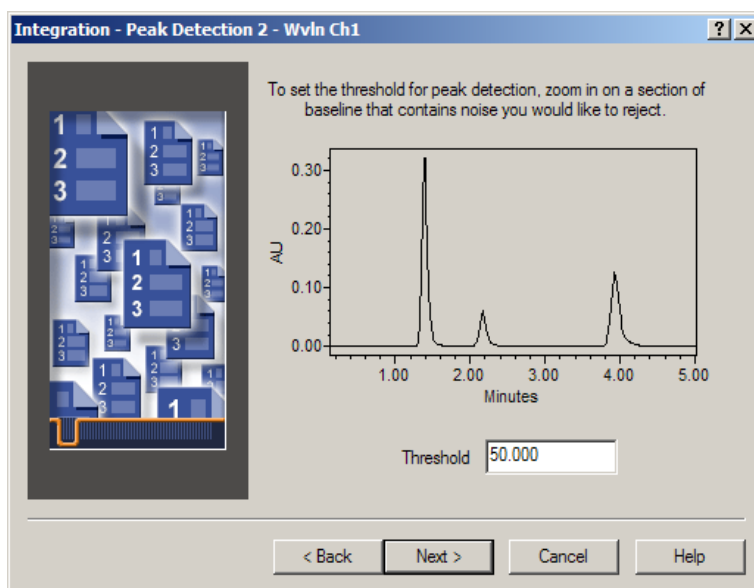
Figure 4-18 Integration - Peak Detection 2 Page

This page allows you to set the threshold parameter in your processing method based on a selected area of baseline. Threshold is a slope setting that defines the peak liftoff and touchdown points by determining whether the slope across three data points exceeds the determined slope threshold. It is used to reject baseline noise.

7. Follow the wizard instructions and use the mouse to draw a zoom box that encloses the area of the baseline that contains the greatest amount of noise and no peaks of interest (approximately 0.2 to 1.2 minutes). The software displays the zoomed selection and determines the appropriate threshold value (Figure 4-19).

Note: In general, you might need to zoom the baseline several times to ensure that the selected area is free of peaks of interest.
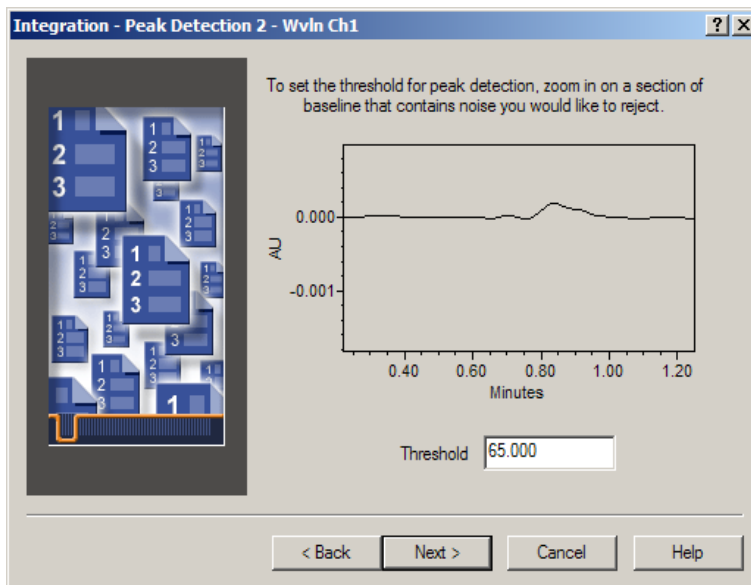
Figure 4-19 Setting the Threshold Parameter

8. Click **Next** to go to the Integration - Integration Region page (Figure 4-20)
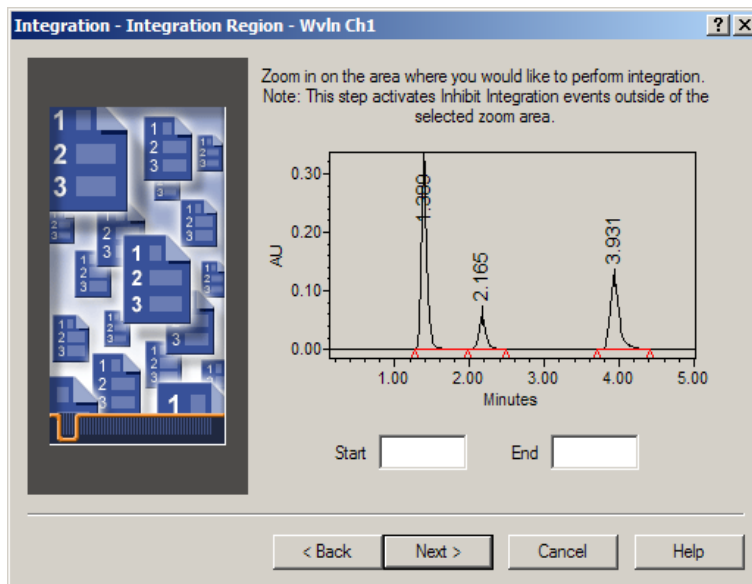
Figure 4-20 Integration - Integration Region Page

This page allows you to refine your integration by using an Inhibit Integration event to specify a time region where no integration occurs.

9. If you want to inhibit integration, follow the wizard instructions and use the mouse to draw a zoom box that encloses the area where you want integration to occur. Integration is inhibited in the area *outside* of the zoom box. The software displays the zoomed selection. Click **Next** to go to the Integration - Peak Rejection page (Figure 4-21).

This step is not necessary with this particular data. If you do not want to inhibit integration, do nothing in this dialog box and click **Next**.

4
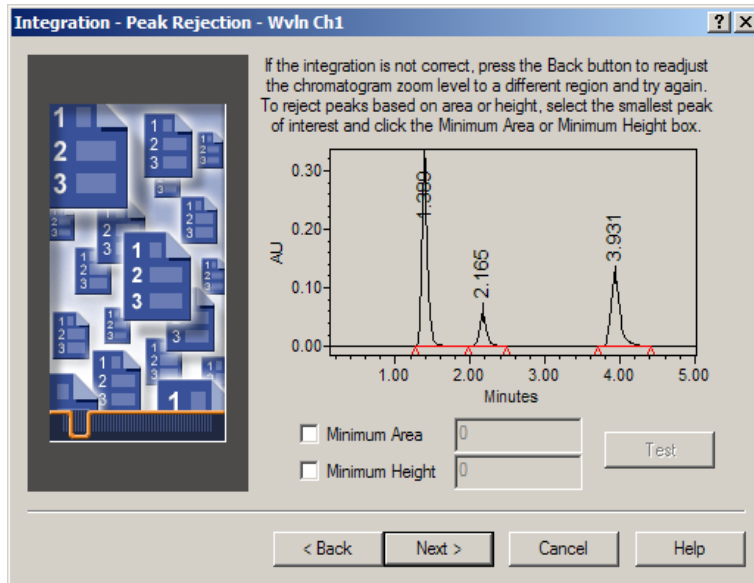
Figure 4-21 Integration - Peak Rejection Page

Use this page to reject small peaks that are not of interest. This step is not necessary with this particular data as there is no unwanted baseline noise that is integrated. Click **Next** to go to the Calibration - General page.

Note: To use this setting, select the smallest peak of interest to highlight it in red. Select the Mimimum Area or Minimum Height check box to set the minimum area or height to 95% of the selected peak.

When it is necessary to use the Minimum Area or Minimum Height parameter, you typically use one or the other, but not both. For more information see the "Minimum Area" and "Minimum Height" topics in the Empower Help.

10. Accept the default settings and click **Next**. A message indicates that channels require channel names in order to perform cross-channel internal standard processing. Click **No** in this dialog box. The Names and Retention Times page appears (Figure 4-22).

4
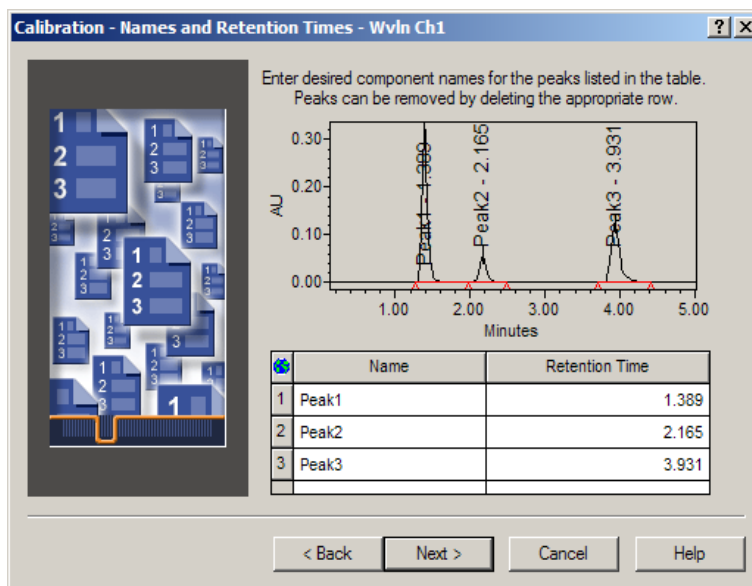
Figure 4-22 Names and Retention Times Page

11. Click **1** to the left of the Name column in the first row. Press **Shift** and click **3** to the left of the Name column in the third row. All rows are highlighted. Press **Delete** on your keyboard to remove all entries from this table. Click **Next** on this page and successive pages until the PDA Purity/Matching page appears (**Figure 4-23**).

   Note: Entering component names and amounts is necessary only when you intend to process standards, generate calibration curves, and quantitate unknowns. Since this guide focuses on generating purity and library match data, this information is unnecessary. For information about calibration and quantitation, see the Empower Software Getting Started Guide *or the* Empower Help*.*

4
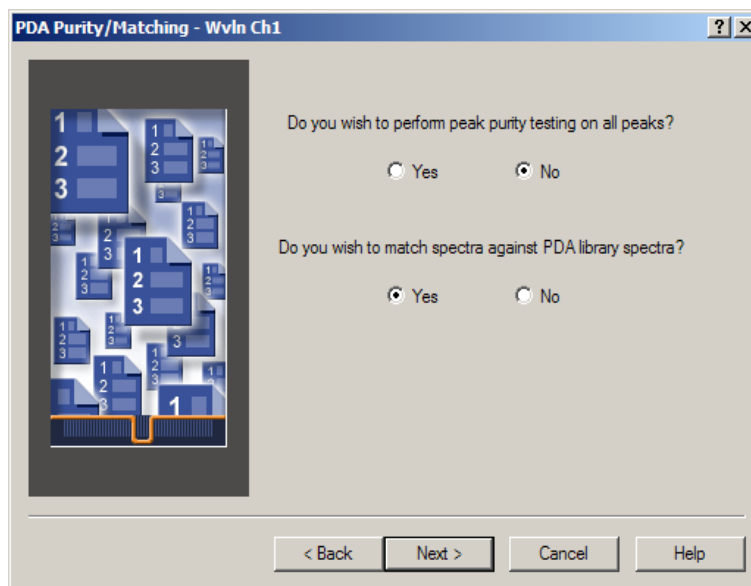
Figure 4-23 PDA Purity/Matching Page

12. For the question "Do you wish to perform peak purity testing on all peaks?", click **No**. For the question "Do you wish to match spectra against PDA library spectra?", click **Yes**. Click **Next** to go to the PDA Spectral Contrast page (Figure 4-24).

4
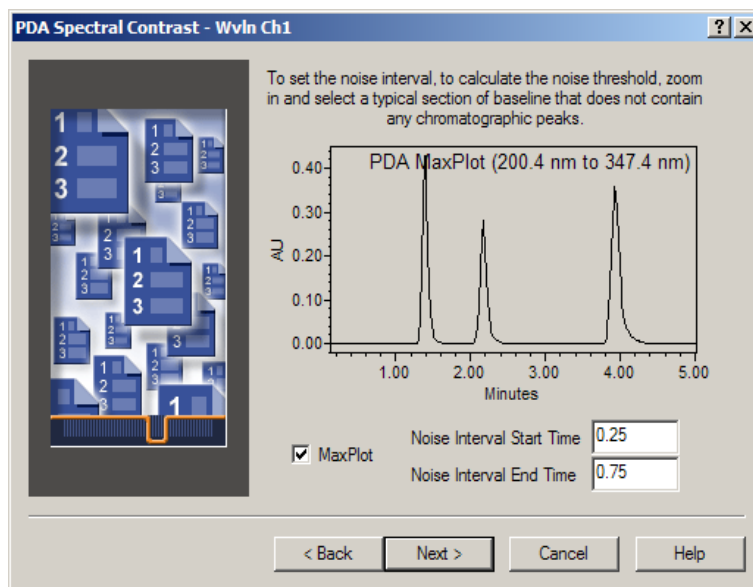
Figure 4-24 PDA Spectral Contrast Page

Use this page to set the noise interval by selecting a segment of the baseline that is free of peaks. You must select a segment of the baseline that is at least one-half minute in length.

Note: The MaxPlot check box is selected by default and allows you to display the Max Plot chromatogram in the PDA Spectral Contrast page regardless of the type of chromatogram that was extracted before starting the Processing Method Wizard. It is recommended to use the Max Plot chromatogram when setting the noise interval to ensure that this parameter is set to a region of the baseline where no compound-related spectral absorbance occurs.

When the MaxPlot check box is selected, the software displays the Max Plot chromatogram for the entire wavelength range in which the data was acquired. If you need to view the Max Plot chromatogram without using the entire acquired wavelength range, you must create a Max Plot chromatogram with the appropriate Start and End Wavelength by creating a derived channel in your method set and then applying the method set before starting the Processing Method Wizard. The Start and End Wavelength Limits are set in the Purity tab of the processing method. (These settings are not available when using the Processing Method Wizard. You must view the processing method by clicking (Processing Method) or by selecting Window > Processing Method.)

It is often desirable to define the wavelength start range above 190 nm to eliminate the effect of mobile phase absorption.

13. Select the segment of the baseline from approximately 3.00 to 3.50 minutes ([Figure 4-25](#)).

    You can select the baseline area by using the mouse to zoom in on the desired area, or you can specify the baseline area by typing the start and end time of the desired area in the Noise Interval Start Time and Noise Interval End Time text boxes, respectively.

    When zooming, you can right-click and select **Fullview** or **Unzoom** to move between zoom levels. Do this to view multiple sections of baseline before deciding on the correct region for the noise interval.

    For details about selecting the noise interval, see the "Determining the Noise Interval" topic in the *Empower Help*.

    By default, the Empower PDA software sets the Threshold Criteria to Noise plus Solvent with the Solvent Angle set to one degree. For details, see the "Threshold Criteria Considerations for Spectral Contrast (PDA)" topic in the *Empower Help*.
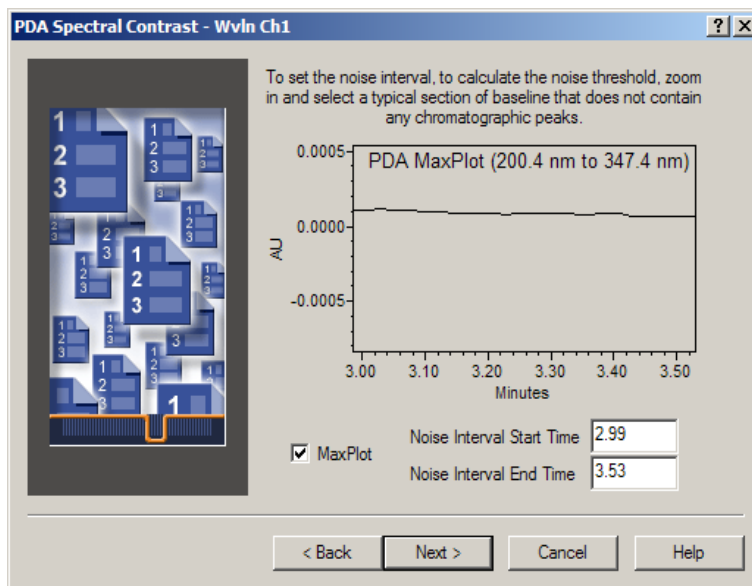
Figure 4-25 Setting the Noise Interval

14. Click **Next** to go to the PDA Match Library page ([Figure 4-26](#)).
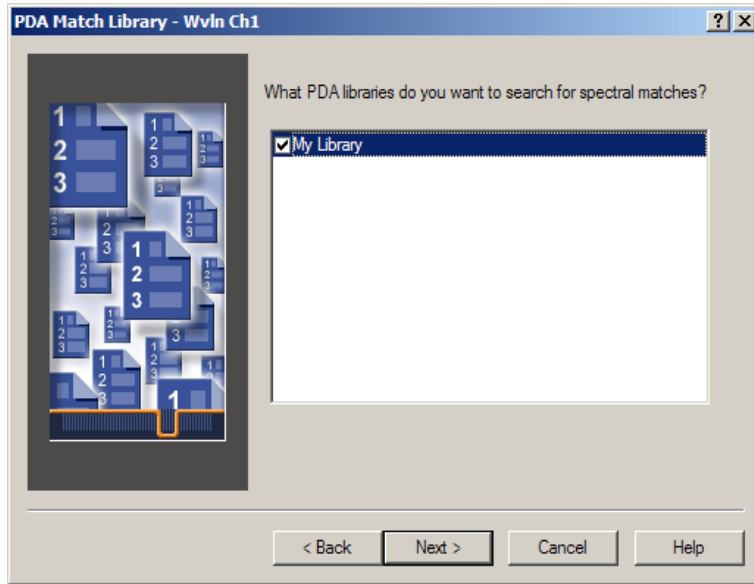
Figure 4-26 PDA Match Library Page

15. Select the check box for the library(s) you want to match against, for example, **My Library**, then click **Next** to go to the Processing Method Name page (Figure 4-27).

4
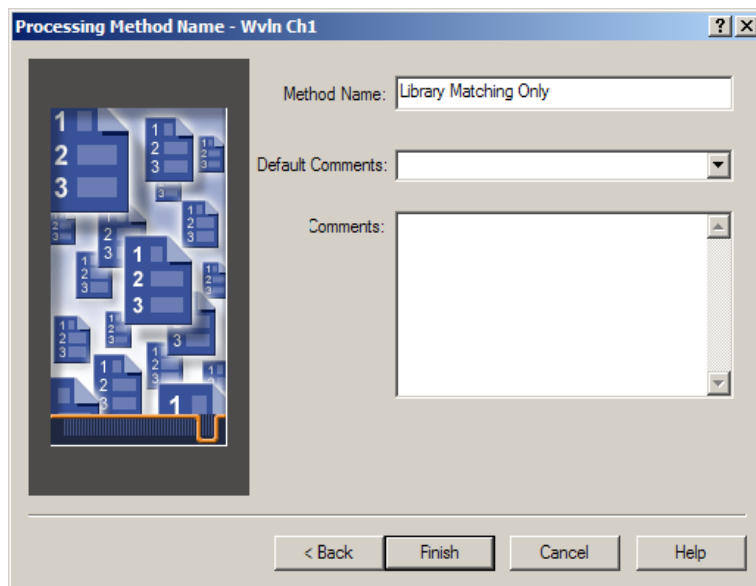
Figure 4-27 Processing Method Name Page

16. In the Method Name field, enter a processing method name, for example, **Library Matching Only**, then click **Finish**. The processing method is saved and the Review Main window appears with the Max Plot chromatogram processed. The name of the processing method appears in the right side of the status bar.

When working with a PDA processing method, you must save both the processing method and method set. You can then apply the saved method set to subsequent data to determine library matching.

Saving the Method Set

1. Select **File > Save As > Method Set** to open the Save current Method Set dialog box.

2. In the Name field, type a name, for example, **Library Matching Only**, then click **Save**. The processing method is saved and the Review Main window appears with the chromatogram processed (Figure 4-28). The name of the processing method is indicated in the right side of the status bar.

   Note: This procedure assumes that the Automatically Apply Method Set and New Method Set: Create a new method set when starting Review options are enabled in Review. To verify or change these settings, select Options > Method Set Options. If the Automatically Apply Method Set option is not enabled, your chromatogram will not be automatically processed. To process it, click ▓ (Apply Method Set) or select Process > Apply Method Set.
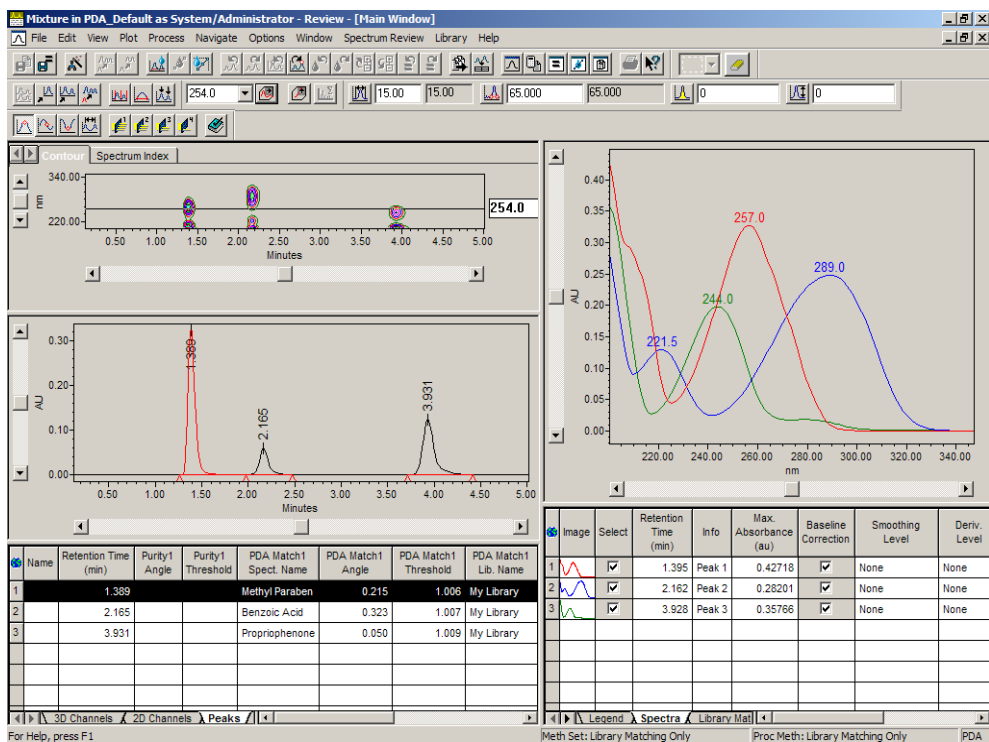
Name of Method Set and
Processing Method
Indicated Here

Figure 4-28 Processed Chromatogram with Library Match Results

4.3.3 Performing Library Matching

Now that you have created or modified a processing method, you can perform library matching. Apply an appropriate method set to compute the library Match Angle and Threshold Angle for each peak.

1. If the Review Main window is not displayed, click $\boxed{\Lambda}$ (Review Main Window) or select **Window > Main Window** (Figure 4-29).

2. Click $\boxed{\mathbb{B}}$ **(Apply Method Set)** or select **Process > Apply Method Set**.

Figure 4-29 Integrated Chromatogram with Library Match Results
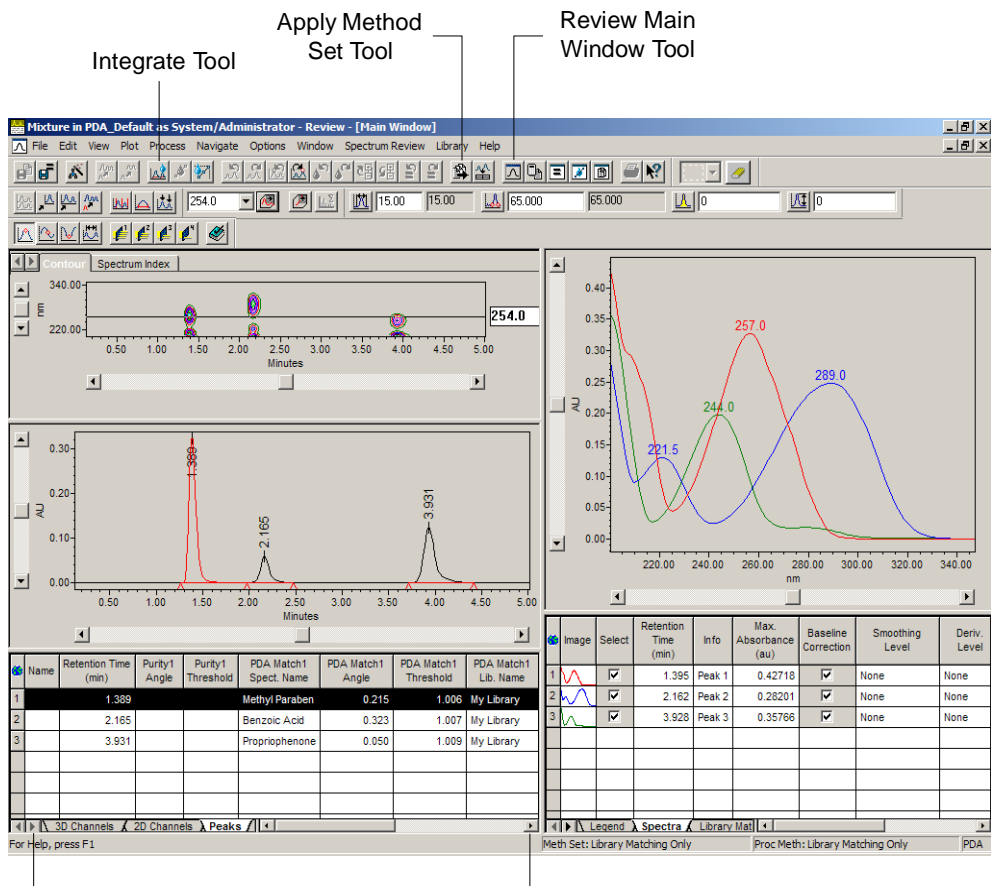
3. Use the arrows at the bottom of the Peaks table to scroll until the Peaks tab appears (Figure 4-29). Click the **Peaks** tab. The Peaks table displays the results of library matching.

4. Scroll to the right of the Peaks table (Figure 4-29) to view the portion of the table that lists the match results, if necessary.

A Match Angle less than a Match Threshold indicates a good match. A Match Angle greater than the Match Threshold indicates a poor match. Match 1 is the closest (best) match to a library spectrum; Match 2 is the next closest; Match 3 is the third closest. For details, see the "Interpreting PDA Library Matching Results" topic in the *Empower Help*.

4.4 Reviewing Library Matching Results

Once you have determined library matching, you can view the results in different ways. For example, you can examine the apex spectra and the Triple plot by using the Spectrum Index and the Results window, respectively.

To view the library matching results:

1. Click the **Spectrum Index** tab to view Spectrum Index in the Review Main window (Figure 4-30).

Spectrum
Index Tab
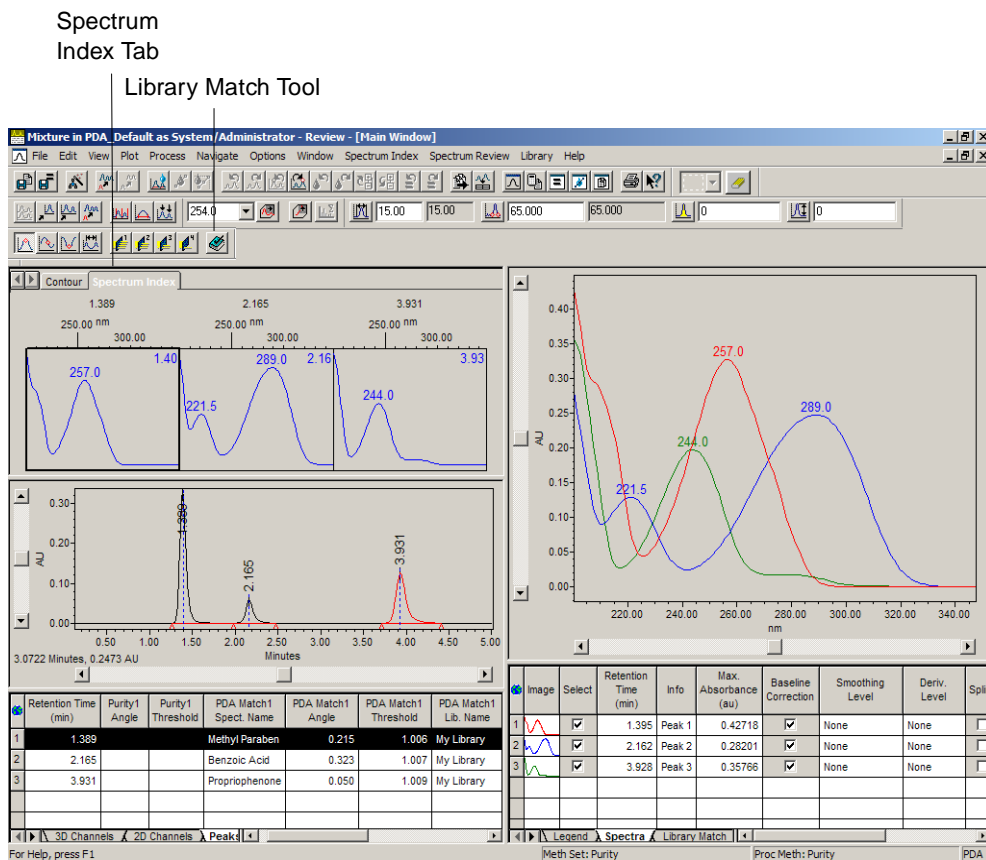
Library Match Tool

Figure 4-30 Spectrum Index with Library Matching

2. Click ![library match icon] (Library Match) to overlay the library match spectrum with the peak spectrum in the Spectrum Index plot (Figure 4-31).

Figure 4-31 Overlaid Library Match Spectra

By default, the black spectra are the library spectra and the blue spectra are the peak apex spectra. If these types of spectra overlay well, this is a qualitative indication of a good match.

Note: The colors displayed in your software can differ from those described here.

3. For another view of the library match results, click ▣ **(Results)** or select **Window > Results** to open the Results window, which contains the Library Match table and Match Plot (Figure 4-32). If necessary, maximize the Results window or size the different panes by dragging the split bars.

4. In the Results window, click the **Match Plot** tab (Figure 4-32). The Library Match plot displays the peak spectrum overlaid with any possible matches to the library spectra. The name of the library spectra is displayed in the upper-right side of the plot. When you select a specific peak from the Peaks tab in the top pane of this

window, the Match plot displays the corresponding match data for the selected peak. Up to three matches can be displayed for each apex spectrum.
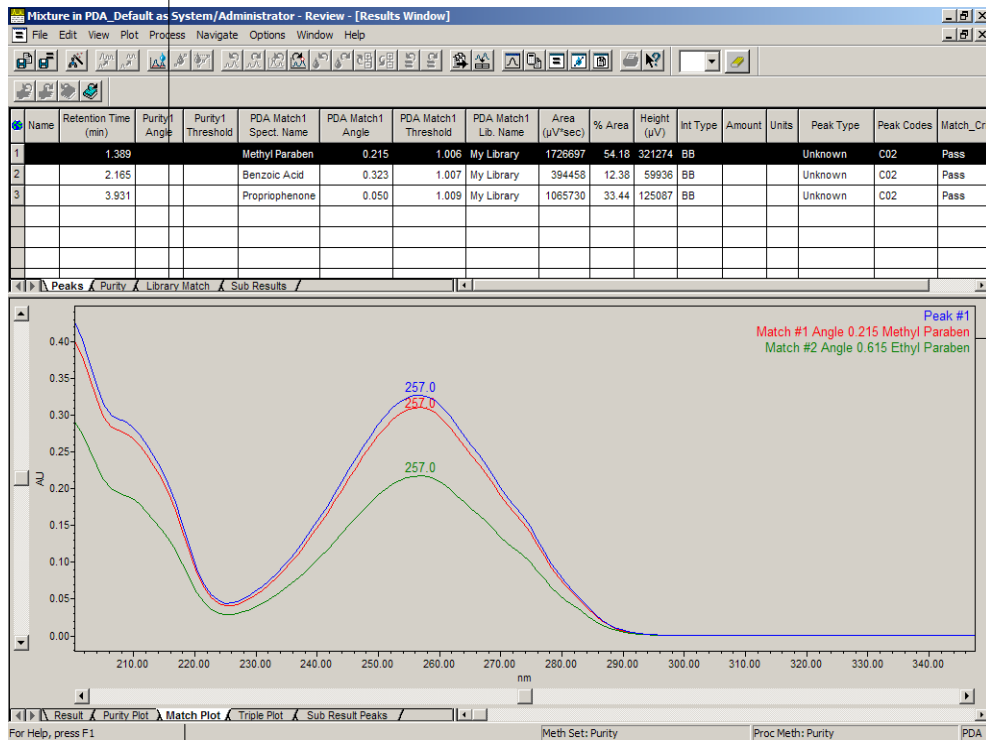
Library Match Tab

Names of Library Spectra

Match Plot Tab

Figure 4-32 Results Window Displaying Library Match Plot

5. Click the **Library Match** tab in the upper table (Figure 4-33). The Library Match tab displays the match information for each peak in a numerical format. Select each peak using the Peak drop-down list. Up to three matches can be displayed for each apex spectrum.
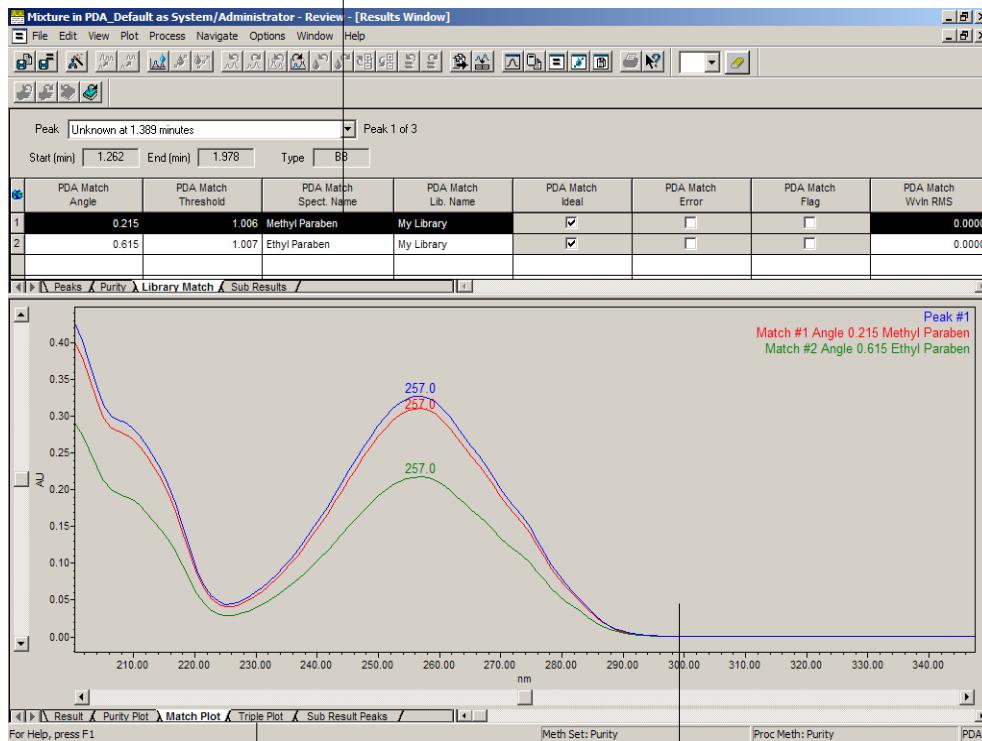
Library Match Table

Figure 4-33 Library Match Plot and Library Match Tab

*Note: You can normalize the spectra to visually check the match. Right-click in the Library Match plot, then select **Properties** to open the Plot Properties dialog box. Click the **Scaling** tab, then select **Normalize Y**, and click **OK**.*

6. In the lower pane, click the **Triple Plot** tab (Figure 4-34). The Triple plot shows the peak spectrum, the library spectrum, and the difference spectrum, which shows the difference between the peak spectrum and the library spectrum. Again, you can navigate between peaks using the Peak drop-down list in the Library Match tab or by selecting the appropriate row in the Peaks tab.
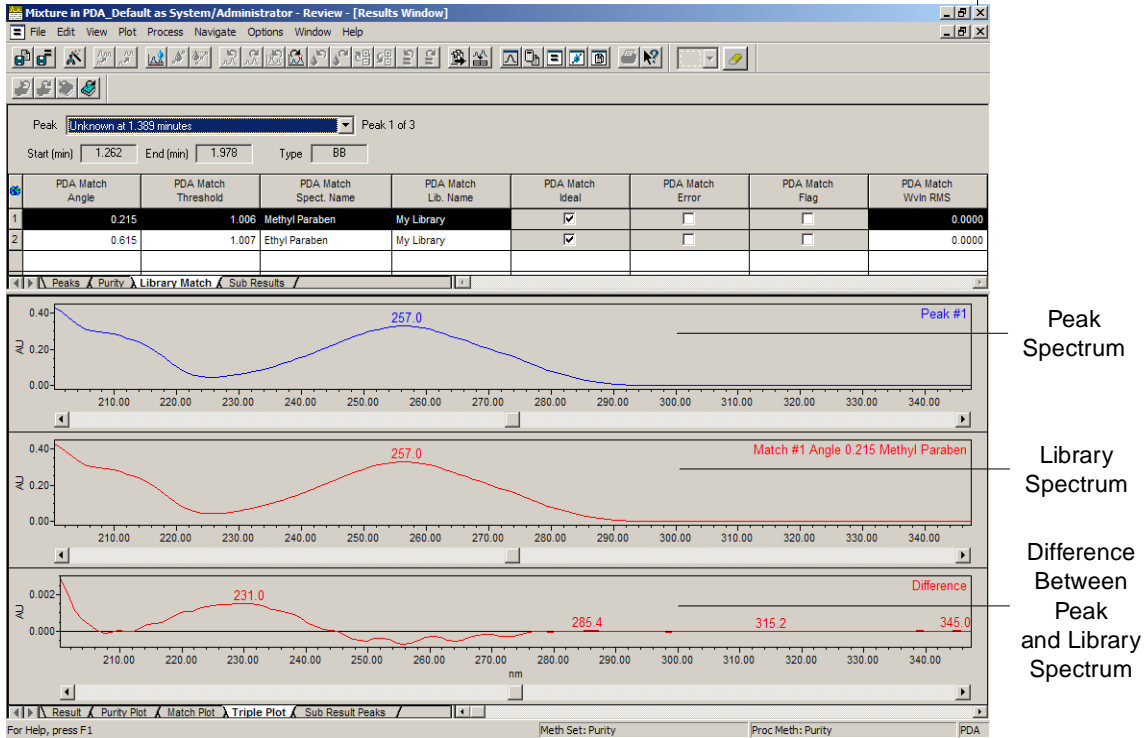
Figure 4-34 Triple Plot: Library Match

7. Save the result so you can print it in a report. To save the result, select **File > Save > Result**. If **Save > Result** is not available, select **Save > Processing Method** first.

8. Exit Review by clicking ☒ **(Close)** or selecting **File > Exit**.

Next Step

You can now proceed to Chapter 5, Printing Reports, to print your results.

Chapter 5
Printing Reports

When you want to print a report with PDA data (using Empower software), you can:

- Preview the report before printing it.
- Print in the background.

5.1 Previewing a Report

To preview a report before printing it:

1. Go to the Project window (Figure 5-1), which you opened in Chapter 2, Viewing PDA Data. If the Project window is minimized in the Windows taskbar, maximize it.

Preview Tool   Results Tab

Figure 5-1 Project Window

2. Click the **Results** tab (Figure 5-1) to view the Results view in the Project window.

3. Select the data you want to print, then click **(Preview)** or select **Tools > Preview/Publisher** to open the Open Report Method dialog box ().
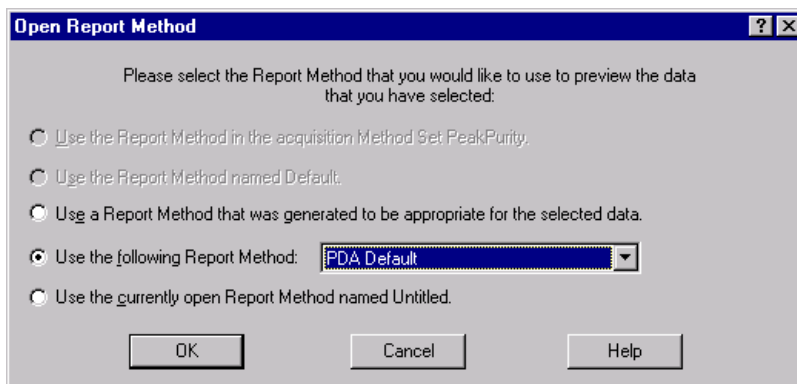
Figure 5-2 Open Report Method Dialog Box

4. Click the **Use the following Report Method** option button, then select **PDA Default** from the list, and click **OK** to view a preview of the report ().

5
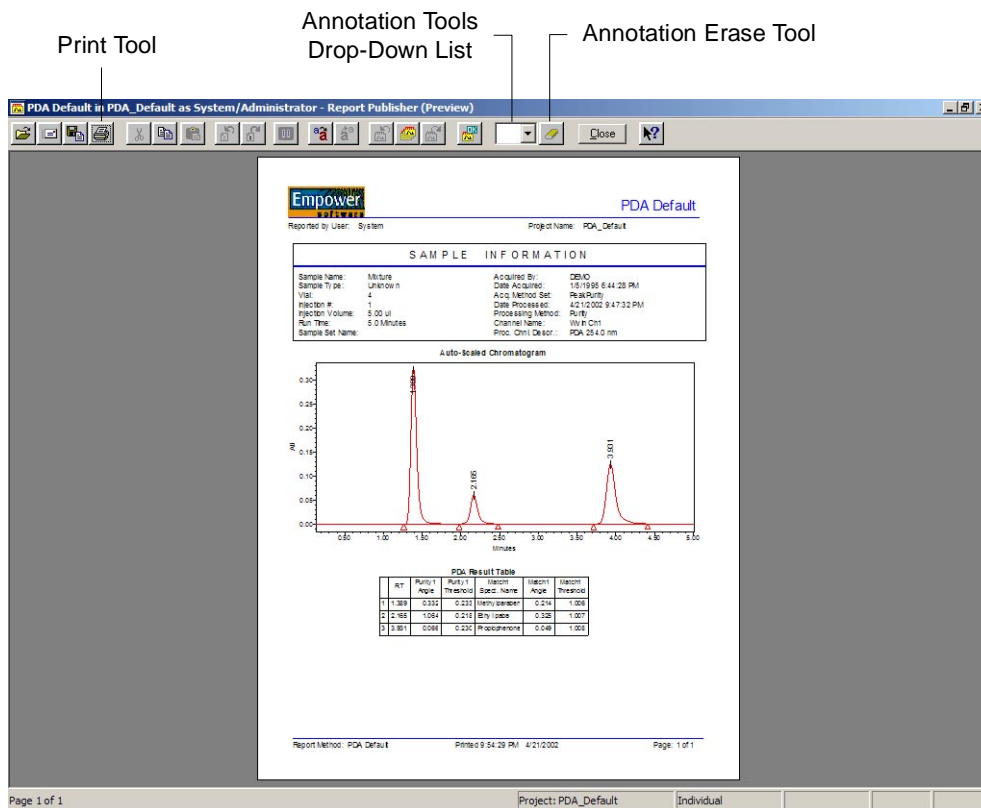
Figure 5-3 Preview of an Example Report

5. From Preview, you have the following options:

- You can add annotations. For more information on using the Annotation tools, see Section 2.7, Annotating Chromatograms and Spectra. When annotations are added in Preview, they appear on the report when the report is saved and subsequently printed. However, annotations do not become part of the report method and are not saved with the method.

Note: To modify a method so that all reports that use that method contain the annotations, use the drawing objects in the report group tree of Report Publisher. For more information on these tools, see the Empower Help*.*

- If the report looks the way you want it, click 🖨 **(Print)** (Figure 5-3). The Print dialog box appears. Make sure that the correct settings are selected, then click **OK**. A report similar to the one in Figure 5-6 prints.

5
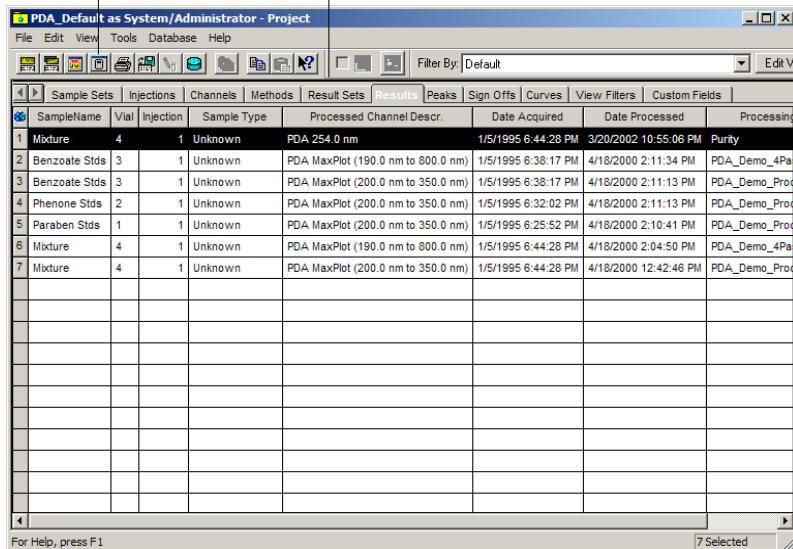
- If you want to change the way your report looks, you can modify it by using Report Publisher. For details, see the "Report Publisher" topic in the *Empower Help.*

- To exit without printing, click **X** **(Close)**, or select **File > Exit**.

5.2 Background Printing

To print in the background:

1. Go to the Project window (Figure 5-4), which you opened in Chapter 2, Viewing PDA Data. If the Project window is minimized in the Windows taskbar, maximize it.

Print Tool   Results Tab

Figure 5-4 Project Window

2. Click the **Results** tab. The Results view appears in the Project window.

3. Select the data you want to print, then click 🖨 **(Print)** in the Project window (Figure 5-4). The Background Processing and Reporting dialog box appears (Figure 5-5).
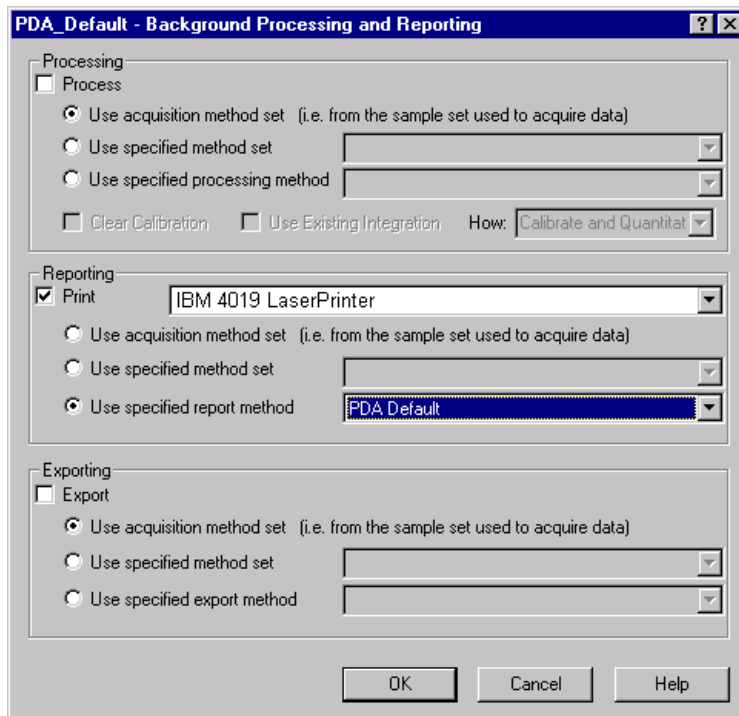
5

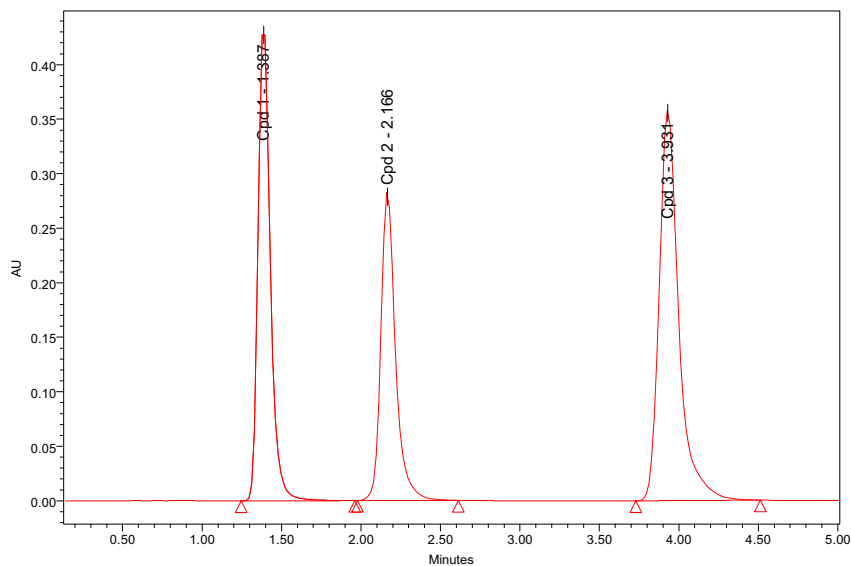Figure 5-5 Background Processing and Reporting Dialog Box

4. In the Reporting section, make sure that **Print** and **Use specified report method** are selected.

5. From the drop-down list next to the Use specified report method option button, select **PDA Default**, then click **OK**. A report similar to the one in Figure 5-6 prints.

Empower software

PDA Default

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture
Sample Type:	Unknown
Vial:	4
Injection #:	1
Injection Volume:	5.00 ul
Run Time:	5.0 Minutes
Sample Set Name:	

Acquired By:	DEMO
Date Acquired:	1/5/1995 6:44:28 PM
Acq. Method Set:	PeakPurity
Date Processed:	8/20/2001 10:05:42 PM
Processing Method:	PDA_Demo_4Pass_Processing
Channel Name:	PDA Max Plot 190.0 - 800.0
Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

PDA Result Table

	Name	RT	Area	Purity (#1) Angle	Purity (#1) Threshold	Purity Flag	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold	PDA Match Flag
1	Cpd 1	1.387	2319720	0.303	0.233	Yes	Methylparaben	0.316	0.206	Yes
2	Cpd 2	2.166	1816222	1.049	0.220	Yes	Ethyl paba	0.325	0.207	Yes
3	Cpd 3	3.931	3059836	0.066	0.230	No	Propiophenone	0.049	0.209	No

Report Method: PDA Default   Printed 10:41:14 PM   4/24/2002   Page: 1 of 1

Figure 5-6 Sample Report

Printing Reports 119

Appendix A
Default PDA Reports

A

There are nine default reports available in the PDA Default project. These reports are available for your direct use or could be used as a starting point to create a customized report. These reports can be copied from the PDA Default project and pasted into other projects. You can also transfer these reports by dragging them from one project and dropping them into another project.

Examples of the sample reports are provided in this appendix. The following reports are in the PDA Default project:

- 1 Pass Peak Purity Report
- 254 nm Extraction Example Report
- 3D Plot Report
- 4 Pass Peak Purity Report
- LC Default Individual Report
- PDA Default Report
- PDA Purity Lib Match Report
- PDA Purity Plot Report
- PDA Spectrum Index Plot Report

A.1 1 Pass Peak Purity Report

Empower
software

<div style="text-align:right">1Pass Peak Purity</div>

Reported by User :   System   Project Name :   PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name :	Mixture	Acquired By :	DEMO
Sample Type :	Unknown	Date Acquired :	1/5/1995 6:44:28 PM
Vial :	4	Acq. Method Set :	PeakPurity
Injection # :	1	Date Processed :	8/20/2001 10:06:11 PM
Injection Volume :	5.00 ul	Processing Method :	PDA_Demo_Processing
Run Time :	5.0 Minutes	Channel Name :	Maxplot 200 350nm
Sample Set Name :		Proc. Chnl. Descr. :	PDA MaxPlot (200.0 nm to 350.0 nm)

Purity Plot
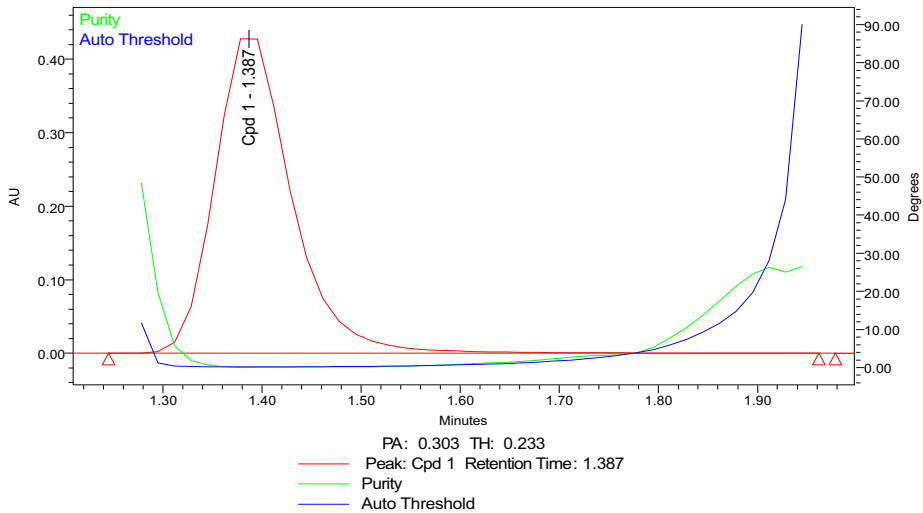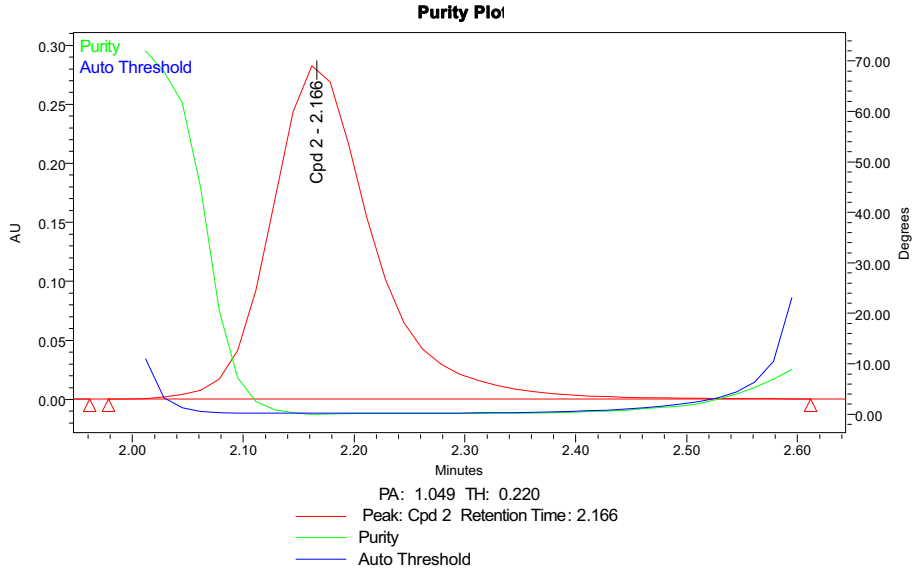
PA: 0.303 TH: 0.233

Peak: Cpd 1 Retention Time: 1.387
Purity
Auto Threshold

Report Method : 1Pass Peak Purity   Printed 10:34:38 PM   4/24/2002   Page: 1 of 3

Empower software

1Pass Peak Purity

Reported by User: System   Project Name: PDA_Default

Purity Plot

PA: 1.049 TH: 0.220
Peak: Cpd 2 Retention Time: 2.166
Purity
Auto Threshold

Report Method: 1Pass Peak Purity   Printed 10:34:38 PM   4/24/2002   Page: 2 of 3

Empower software

1Pass Peak Purity

Reported by User: System

Project Name: PDA_Default

Purity Plot
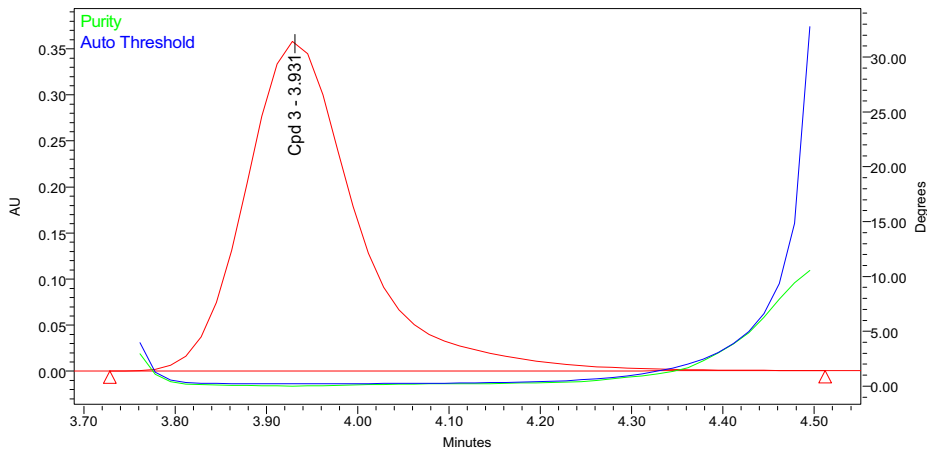
PA: 0.066 TH: 0.230
Peak: Cpd 3 Retention Time: 3.931
Purity
Auto Threshold

PDA Result Table

	Name	RT	Purity (#1) Angle	Purity (#1) Threshold
1	Cpd 1	1.387	0.303	0.233
2	Cpd 2	2.166	1.049	0.220
3	Cpd 3	3.931	0.066	0.230

Report Method: 1Pass Peak Purity   Printed 10:34:38 PM   4/24/2002   Page: 3 of 3

A.2 254 nm Extraction Example Report

Empower software

254 nm Extraction Example

Reported by User: System

Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:06:11 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_Processing
Run Time:	5.0 Minutes	Channel Name:	Maxplot 200_350nm
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (200.0 nm to 350.0 nm)

254 nm Chromatogram
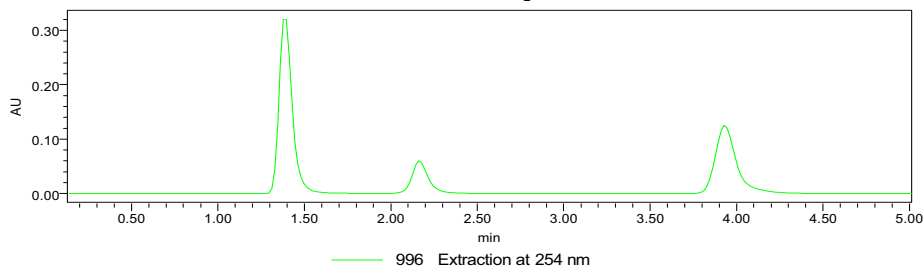
996 Extraction at 254 nm

Example Extraction Table
Time: 4.000 min
Channel: 996

#	Wavelength (nm)	Absorbance (au)	#	Wavelength (nm)	Absorbance (au)	#	Wavelength (nm)	Absorbance (au)
1	220.37	0.01562	15	236.87	0.07518	29	253.41	0.06030
2	221.55	0.01787	16	238.05	0.07940	30	254.60	0.05359
3	222.72	0.02056	17	239.23	0.08295	31	255.78	0.04675
4	223.90	0.02371	18	240.41	0.08582	32	256.96	0.04002
5	225.08	0.02739	19	241.59	0.08802	33	258.15	0.03372
6	226.26	0.03135	20	242.78	0.08946	34	259.33	0.02809
7	227.44	0.03558	21	243.96	0.08996	35	260.51	0.02323
8	228.62	0.04013	22	245.14	0.08941	36	261.70	0.01919
9	229.80	0.04506	23	246.32	0.08782	37	262.88	0.01592
10	230.97	0.05023	24	247.50	0.08525	38	264.07	0.01338
11	232.15	0.05539	25	248.68	0.08181	39	265.25	0.01147
12	233.33	0.06044	26	249.87	0.07751	40	266.44	0.01012
13	234.51	0.06545	27	251.05	0.07242	41	267.62	0.00921
14	235.69	0.07043	28	252.23	0.06662	42	268.81	0.00863

Report Method: 254 nm Extraction Example   Printed 10:35:47 PM   4/24/2002   Page: 1 of 2

Empower software

254 nm Extraction Example

Reported by User: System

Project Name: PDA_Default

Example Extraction Table
Time: 4.000 min
Channel: 996

	Wavelength (nm)	Absorbance (au)
43	269.99	0.00828
44	271.18	0.00810
45	272.36	0.00802
46	273.55	0.00804
47	274.74	0.00809
48	275.92	0.00815
49	277.11	0.00822
50	278.30	0.00827
51	279.48	0.00827
52	280.67	0.00821
53	281.86	0.00808
54	283.05	0.00785
55	284.23	0.00758
56	285.42	0.00730
57	286.61	0.00695
58	287.80	0.00661
59	288.99	0.00622
60	290.17	0.00576
61	291.36	0.00526
62	292.55	0.00471
63	293.74	0.00414
64	294.93	0.00355
65	296.12	0.00302
66	297.31	0.00255
67	298.50	0.00211
68	299.69	0.00180

A.3 3D Plot Report

Empower software

3D Plot

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:06:11 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_Processing
Run Time:	5.0 Minutes	Channel Name:	Maxplot 200_350nm
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (200.0 nm to 350.0 nm)

3D Plot

Report Method: 3D Plot   Printed 10:36:51 PM   4/24/2002   Page: 1 of 1

A.4 4 Pass Peak Purity Report

Empower software

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:05:42 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_4Pass_Processing
Run Time:	5.0 Minutes	Channel Name:	PDA Max Plot 190.0 - 800.0
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

Purity Plot

PA: 0.303 TH: 0.233   PA: 0.062 TH: 0.234

PA: 0.026 TH: 0.235   PA: 0.019 TH: 0.235

Peak: Cpd 1 Retention Time 1.387
Purity
Auto Threshold

Report Method: 4 Pass Peak Purity   Printed 10:38:10 PM   4/24/2002   Page: 1 of 3

Empower
software

4 Pass Peak Purity

Reported by User: System   Project Name: PDA_Default

Purity Plot

PA: 1.049 TH: 0.220

PA: 0.119 TH: 0.220

PA: 0.040 TH: 0.221

PA: 0.018 TH: 0.222

— Peak: Cpd 2 Retention Time 2.166
— Purity
— Auto Threshold

Report Method: 4 Pass Peak Purity   Printed 10:38:10 PM   4/24/2002   Page: 2 of 3

Empower software

4 Pass Peak Purity

Reported by User: System   Project Name: PDA_Default

Purity Plot

PA: 0.066 TH: 0.230

PA: 0.033 TH: 0.231

PA: 0.018 TH: 0.232

PA: 0.016 TH: 0.232

—— Peak: Cpd 3 Retention Time 3.931
—— Purity
—— Auto Threshold

PDA Result Table

	Name	RT	Purity (#1) Angle	Purity (#1) Threshold	Purity (#2) Angle	Purity (#2) Threshold	Purity (#3) Angle	Purity (#3) Threshold
1	Cpd 1	1.387	0.303	0.233	0.062	0.234	0.026	0.235
2	Cpd 2	2.166	1.049	0.220	0.119	0.220	0.040	0.221
3	Cpd 3	3.931	0.066	0.230	0.033	0.231	0.018	0.232

PDA Result Table

	Purity (#4) Threshold	Purity (#4) Angle
1	0.235	0.019
2	0.222	0.018
3	0.232	0.016

Report Method: 4 Pass Peak Purity   Printed 10:38:10 PM   4/24/2002   Page: 3 of 3

A.5 LC Default Individual Report

Empower software

LC Default Individual Report

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:05:42 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_4Pass_Processing
Run Time:	5.0 Minutes	Channel Name:	PDA Max Plot 190.0 - 800.0
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

	Peak Name	RT	Area	% Area	Height
1	Cpd 1	1.387	2319720	32.24	431888
2	Cpd 2	2.166	1816222	25.24	281385
3	Cpd 3	3.931	3059836	42.52	357284

Report Method: LC Default Individual Report   Printed 10:39:53 PM   4/24/2002   Page: 1 of 1

A.6 PDA Default Report

Empower software

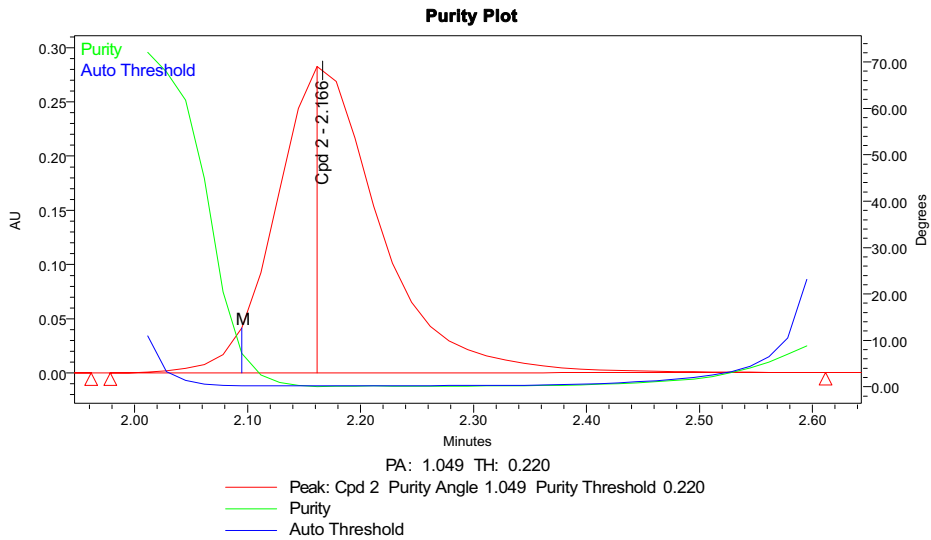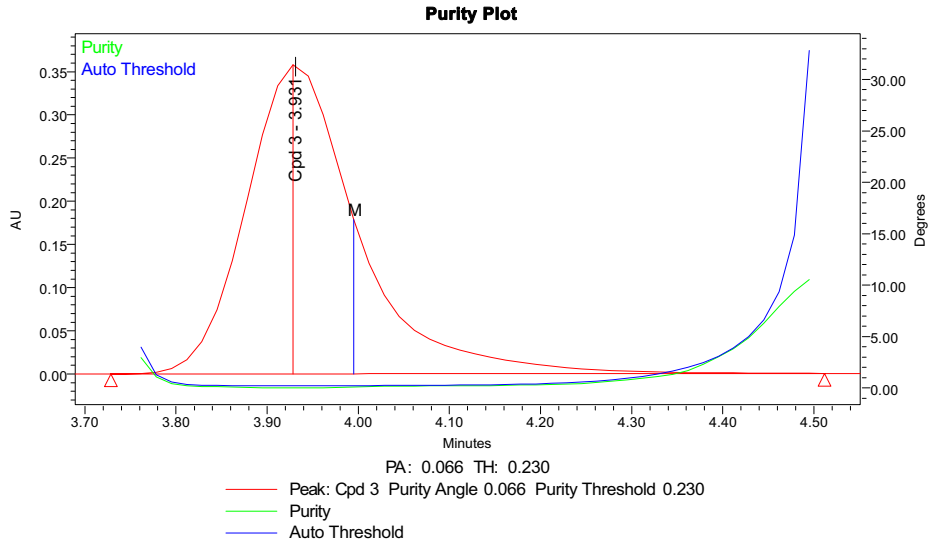PDA Default

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture
Sample Type:	Unknown
Vial:	4
Injection #:	1
Injection Volume:	5.00 ul
Run Time:	5.0 Minutes
Sample Set Name:	

Acquired By:	DEMO
Date Acquired:	1/5/1995 6:44:28 PM
Acq. Method Set:	PeakPurity
Date Processed:	8/20/2001 10:05:42 PM
Processing Method:	PDA_Demo_4Pass_Processing
Channel Name:	PDA Max Plot 190.0 - 800.0
Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

PDA Result Table

	Name	RT	Area	Purity (#1) Angle	Purity (#1) Threshold	Purity Flag	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold	PDA Match Flag
1	Cpd 1	1.387	2319720	0.303	0.233	Yes	Methylparaben	0.316	0.206	Yes
2	Cpd 2	2.166	1816222	1.049	0.220	Yes	Ethyl paba	0.325	0.207	Yes
3	Cpd 3	3.931	3059836	0.066	0.230	No	Propiophenone	0.049	0.209	No

Report Method: PDA Default   Printed 10:41:14 PM   4/24/2002   Page: 1 of 1

A.7 PDA Purity Lib Match Report

Empower software

PDA Purity Lib Match Report

Reported by User: System   Project Name: PDA_Default

SAMPLE INFORMATION

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:05:42 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_4Pass_Processing
Run Time:	5.0 Minutes	Channel Name:	PDA Max Plot 190.0 - 800.0
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

Purity Plot

PA: 0.303 TH: 0.233
- Peak: Cpd 1
- Purity
- Auto Threshold

Library Match Plot

Cpd 1
Match #1 Angle 0.316 Methylparaben

PDA Result Table

	Name	RT	Area	Purity Angle	Purity Threshold	Purity Flag	Match Spect. Name	Match Angle	Match Threshold	Match Flag
1	Cpd 1	1.387	2319720	0.303	0.233	Yes	Methylparaben	0.316	0.206	Yes

Report Method: PDA Purity Lib Match Report   Printed 10:43:13 PM   4/24/2002   Page: 1 of 3

Empower software

PDA Purity Lib Match Report

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:05:42 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_4Pass_Processing
Run Time:	5.0 Minutes	Channel Name:	PDA Max Plot 190.0 - 800.0
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

Purity Plot

PA: 1.049 TH: 0.220
- Peak: Cpd 2
- Purity
- Auto Threshold

Library Match Plot

Cpd 2
Match #1 Angle 0.325 Ethyl paba

PDA Result Table

	Name	RT	Area	Purity Angle	Purity Threshold	Purity Flag	Match Spect. Name	Match Angle	Match Threshold	Match Flag
1	Cpd 2	2.166	1816222	1.049	0.220	Yes	Ethyl paba	0.325	0.207	Yes

Report Method: PDA Purity Lib Match Report   Printed 10:43:13 PM   4/24/2002   Page: 2 of 3

Empower software

PDA Purity Lib Match Report

Reported by User: System

Project Name: PDA_Default

SAMPLE INFORMATION

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:05:42 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_4Pass_Processing
Run Time:	5.0 Minutes	Channel Name:	PDA Max Plot 190.0 - 800.0
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

Purity Plot

Purity
Auto Threshold

Cpd 3 - 3.931

M

PA: 0.066 TH: 0.230

— Peak: Cpd 3
— Purity
— Auto Threshold

Library Match Plot

Cpd 3
Match #1 Angle 0.049 Propiophenone

PDA Result Table

	Name	RT	Area	Purity Angle	Purity Threshold	Purity Flag	Match Spect. Name	Match Angle	Match Threshold	Match Flag
1	Cpd 3	3.931	3059836	0.066	0.230	No	Propiophenone	0.049	0.209	No

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Report Method: PDA Purity Lib Match Report   Printed 10:43:13 PM   4/24/2002   Page: 3 of 3

A.8 PDA Purity Plot Report

Empower software

<div style="text-align:right">PDA Purity Plot</div>

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture	Acquired By:	DEMO
Sample Type:	Unknown	Date Acquired:	1/5/1995 6:44:28 PM
Vial:	4	Acq. Method Set:	PeakPurity
Injection #:	1	Date Processed:	8/20/2001 10:05:42 PM
Injection Volume:	5.00 ul	Processing Method:	PDA_Demo_4Pass_Processing
Run Time:	5.0 Minutes	Channel Name:	PDA Max Plot 190.0 - 800.0
Sample Set Name:		Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

Purity Plot

PA: 0.303 TH: 0.233

Peak: Cpd 1 Purity Angle 0.303 Purity Threshold 0.233
Purity
Auto Threshold

Report Method: PDA Purity Plot   Printed 10:44:06 PM   4/24/2002   Page: 1 of 4

Empower software

PDA Purity Plot

Reported by User :   System

Project Name :   PDA_Default

Purity Plot

PA: 1.049 TH: 0.220
Peak: Cpd 2 Purity Angle 1.049 Purity Threshold 0.220
Purity
Auto Threshold

Report Method: PDA Purity Plot   Printed 10:44:06 PM   4/24/2002   Page: 2 of 4

Empower software

PDA Purity Plot

Reported by User: System   Project Name: PDA_Default

Purity Plot

PA: 0.066 TH: 0.230

—— Peak: Cpd 3 Purity Angle 0.066 Purity Threshold 0.230
—— Purity
—— Auto Threshold

Report Method: PDA Purity Plot   Printed 10:44:06 PM   4/24/2002   Page: 3 of 4

Empower
software

PDA Purity Plot

Reported by User: System   Project Name: PDA_Default

PDA Result Table

	Name	RT	Purity (#1) Angle	Purity (#1) Threshold	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold
1	Cpd 1	1.387	0.303	0.233	Methylparaben	0.316	0.206
2	Cpd 2	2.166	1.049	0.220	Ethyl paba	0.325	0.207
3	Cpd 3	3.931	0.066	0.230	Propiophenone	0.049	0.209

A

Empower software

PDA Spectrum Index Plot

Reported by User: System   Project Name: PDA_Default

S A M P L E   I N F O R M A T I O N

Sample Name:	Mixture
Sample Type:	Unknown
Vial:	4
Injection #:	1
Injection Volume:	5.00 ul
Run Time:	5.0 Minutes
Sample Set Name:	

Acquired By:	DEMO
Date Acquired:	1/5/1995 6:44:28 PM
Acq. Method Set:	PeakPurity
Date Processed:	8/20/2001 10:05:42 PM
Processing Method:	PDA_Demo_4Pass_Processing
Channel Name:	PDA Max Plot 190.0 - 800.0
Proc. Chnl. Descr.:	PDA MaxPlot (190.0 nm to 800.0 nm)

Spectrum Index Plot

Cpd 1 - 1.387   Cpd 2 - 2.166   Cpd 3 - 3.931

PDA Result Table

	Name	RT	Area	Purity (#1) Angle	Purity (#1) Threshold	Purity Flag	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold	PDA Match Flag
1	Cpd 1	1.387	2319720	0.303	0.233	Yes	Methylparaben	0.316	0.206	Yes
2	Cpd 2	2.166	1816222	1.049	0.220	Yes	Ethyl paba	0.325	0.207	Yes
3	Cpd 3	3.931	3059836	0.066	0.230	No	Propiophenone	0.049	0.209	No

Report Method: PDA Spectrum Index Plot   Printed 10:45:14 PM   4/24/2002   Page: 1 of 1

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