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PolyView2000™

Operation Manual



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9065 PolyView Users

Please see the PolyView Operator's Manual, 03-914738-00.

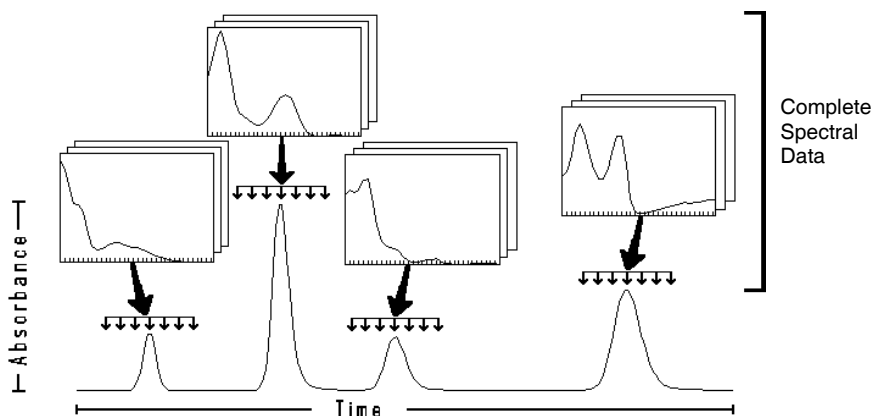
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Overview

The PolyView2000™ Spectral Processing application, in conjunction with the Varian Star Workstation, provides post-run analysis of data from the 330 and the 9065 Diode Array Detectors. Unlike other chromatographic UV absorbance detectors that provide data in two domains, time and absorbance, the Diode Array Detectors yields data in the additional domain of wavelength.



Domains of 330 Diode Array Data

While the data from a diode array detector is obviously more complex than a typical UV absorbance detector, this complexity can provide the chromatographer with considerably more information as well, when it is presented in an easily understandable form.

The PolyView2000 application is designed to handle this more complex data structure, enabling the user to extract a spectrum from any point in time within a chromatogram, or to generate an absorbance chromatogram at any wavelength (or set of wavelengths) within the wavelength range of the Diode Array detector. In this way, PolyView2000 can provide the user with qualitative information regarding an eluting compound. When PolyView2000 is used to generate an absorbance chromatogram, the Diode Array detector functions in much the same way as any other UV absorbance detector, yielding a concentration profile that changes with time. When PolyView2000 is used to examine UV absorbance spectra, this information, in conjunction with the retention time of the eluting compound, provides qualitative information that can be used to identify the compound of interest. In addition, the Multi-Component Analysis process (MCA) combines accurate quantitative analysis and purity evaluation, even in cases of coelution.

PolyView2000 Functions

The principal functions of the PolyView2000 Spectral Processing application are described in the following paragraphs.

Plotting a Chromatogram

The PolyView2000 application can provide plots of absorbance as a function of time at any of the available wavelengths within the range of the Diode Array Detector. While the absorbance chromatogram is the mainstay of chromatographic analysis, and as such, is often the starting point within the PolyView2000 application, UV absorbance chromatograms are not the only plots provided within PolyView2000. Additional treatments of the 330 data are also available, including plots of Purity Parameter™, absorbance ratios, wavelength maxima, and absorbance maxima. Just as an absorbance chromatogram provides absorbance as a function of time, these other parameters are also generated as a function of time. The results are a series of time dependent profiles that provide qualitative information on the spectral characteristics of eluting peaks. All chromatogram plots can be obtained both interactively and under automation.

Examining and Analyzing Spectra

As mentioned earlier, through the PolyView2000 application, all of the spectra that constitute a raw data file are available for examination and analysis. Spectra can be viewed individually, or up to seven spectra can be overlaid and viewed at the same time. This spectral plot can be expanded to visually show detailed information about spectra. In addition, these spectra can be operated on arithmetically, enabling you to create a composite spectrum that is the sum or difference of several spectra. Most spectral functions are performed interactively, but reports comparing sample spectra to reference spectra are produced as part of the library search and MCA automated commands.

Building, Editing, and Searching Spectral Libraries

A particularly useful feature of PolyView2000 is the ability to create and maintain libraries of spectra. Within a spectral library, you can archive those spectra that are relevant to a particular chromatographic application. PolyView2000 can then provide ordered searches of these libraries, comparing a spectrum of interest within a chromatogram to the spectra stored within one or more spectral libraries. Library building and editing are interactive tasks. Library searching can be performed interactively or under automation.

Determining the Purity of a Chromatographic Peak

Co-elution is often a problem with samples composed of structurally similar constituents. PolyView2000 provides several means of determining the purity of a chromatographic peak by displaying the spectral characteristics across the width of the peak. As an example, the Purity Parameter of a UV spectrum uniquely distinguishes it from other similar spectra. By plotting the Purity Parameter as a function of time, slight differences in the UV spectra across a peak can be determined, indicating the presence of impurities. Similarly, absorbance ratio and wavelength maxima plots can be used to indicate spectral dissimilarities across the width of a peak.

Purity Evaluation by Multicomponent Analysis

Multicomponent Analysis also provides a powerful tool to assess the spectral homogeneity of a peak or the presence of unexpected impurities. All purity features are available both interactively and under automation.

Preparing Reports of Diode Array Data

An important function of PolyView2000 is the ability to generate reports that contain the results of the data analyses. These reports are available in a variety of formats and cover most of the operations that are possible with PolyView2000. You can generate reports while working at the Star Workstation itself (interactive reports) or at the end of a run and some time later in an automated fashion (non-interactive reports).

Recalculating the Peak Sense Events in a Diode Array Raw Data File

Associated with every UV absorbance chromatogram obtained with the Diode Array detector is a series of peak sense events. These events mark important points within a chromatogram, and include peak start, peak end, peak apex and peak upslope and downslope. At times, the peak sense events that are calculated and set at the time of acquisition of a diode array data file may need to be redetermined. PolyView2000, in conjunction with the Method Builder application of the Star Workstation, provides the ability to recalculate these peak sense events.

Quantitative Analysis by Multicomponent Analysis

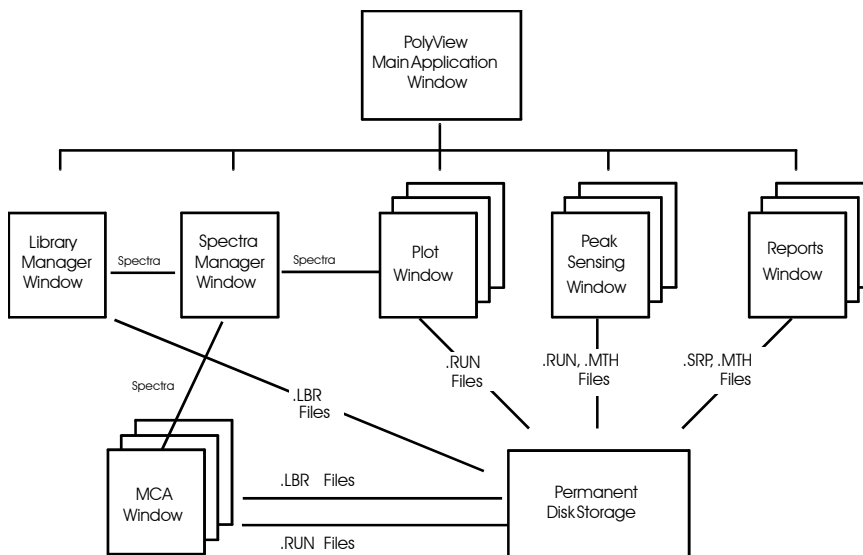
By integrating a group of fused peaks at each wavelength, one obtains an Area Spectrum. This Area Spectrum is the baseline-corrected spectrum of an elution fraction. If the spectra of all components are known, the Area Spectrum can be decomposed by a simple mathematical process known as Multicomponent Analysis. This process is routinely used in spectrophotometers. In PolyView2000, this process is used to obtain precise quantitative results from overlapping peaks.

Creation of Reduced Data Files

PolyView2000 can reduce a data file to a few channels of data corresponding to time-programmed wavelengths or wavelength ranges, while preserving full spectra for the events detected by peak sensing. These channels can also be created while preserving all spectral data. These files can be created both interactively and under automation.

PolyView2000 Windows

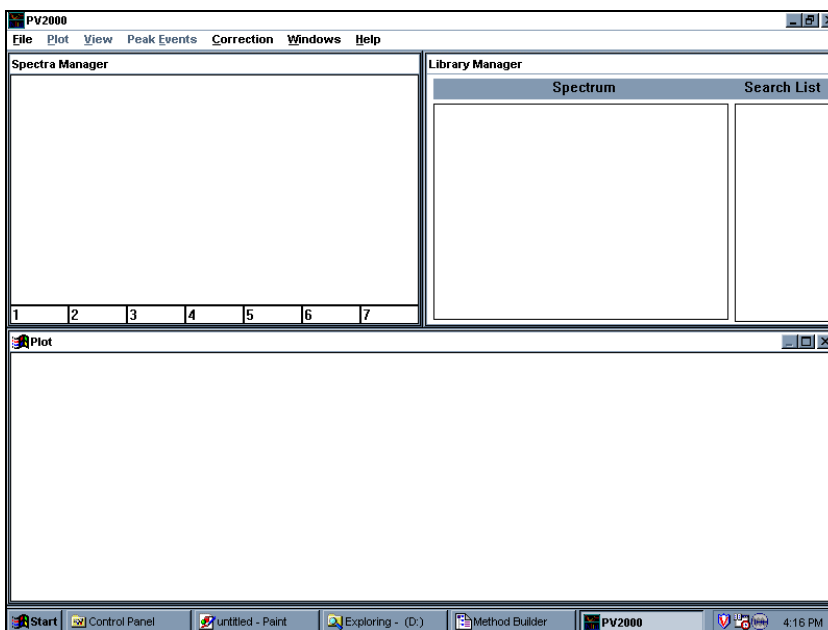
The PolyView2000 Spectral Processing application is organized into several distinct child windows within the PolyView2000 main application window. These include the Plot window, Spectra Manager window, Library Manager window, Reports window, Peak Sense window, and the MCA window. The following illustration shows the organization of the PolyView2000 windows and the transfer of data between the windows. Multiple copies of several of the windows are available as indicated.



Organization of the PolyView2000 Windows

The three windows used most in PolyView2000 functions are the Plot, Spectra Manager, and Library Manager Windows. When PolyView2000 is first started, the screen is split between these three windows.

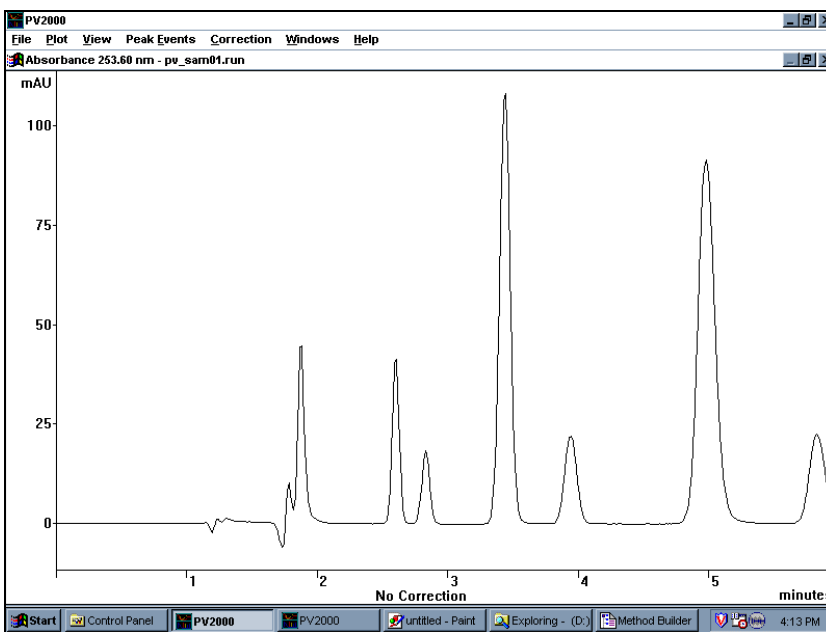
You will find that many of the data analyses performed using PolyView2000 requires only these three windows and you will not need to alter the screen configuration or create additional windows. Of course, if your work requires the use of additional windows, they are easily created and the screen configuration can be easily altered. Following is a brief description of the six PolyView2000 windows. For more detail on the functions available in each and the operation of On-Line Help, refer to the operation section.



PolyView2000 Configuration Upon Start-Up

Plot Window

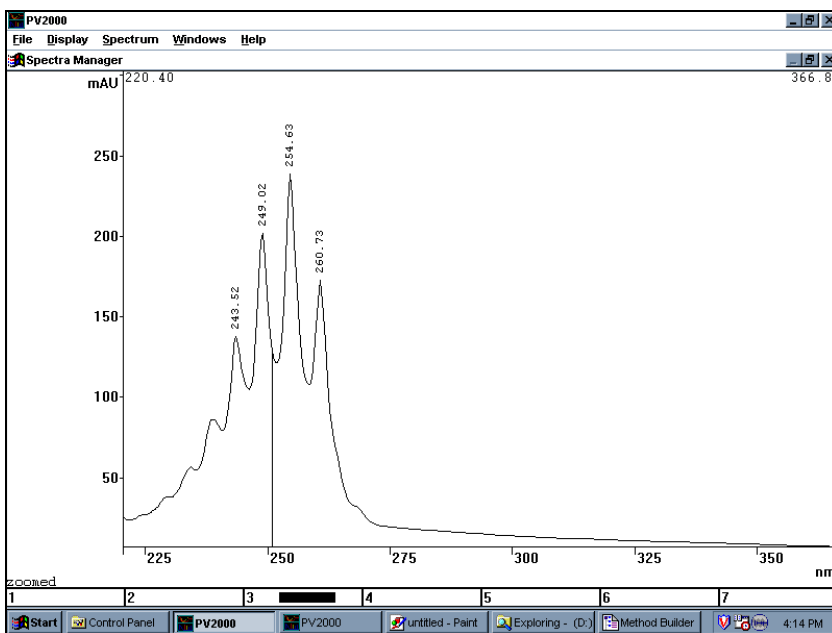
The Plot window is used to plot chromatograms from diode array raw data files (.RUN files). The plots can display the absorbance, Purity Parameter, absorbance ratio, absorbance maxima or wavelength maxima, each as a function of run time. Once displayed, regions of the plot can be enlarged (zoomed) and a variety of spectral correction methods can be selected. Only one chromatogram at a time can be displayed in the window. However, several plot windows can be displayed on the screen at the same time for comparison of different .RUN files or to compare different plot conditions on the same .RUN file. Additional features in the plot window include the ability to display peak events (peak start, peak end, apex, etc.) and to transfer selected spectra from the displayed raw data file to the Spectra Manager window.



The Plot Window

Spectra Manager Window

The primary function of the Spectra Manager window is the display and analysis of UV spectra from selected points in a chromatogram. A single spectrum can be displayed or up to seven spectra can be overlaid. Information about the spectra, such as compound name, operator, and method name, can be added or edited in this window. This information becomes a part of that particular spectrum record and is transferred with it between the various PolyView2000 windows.

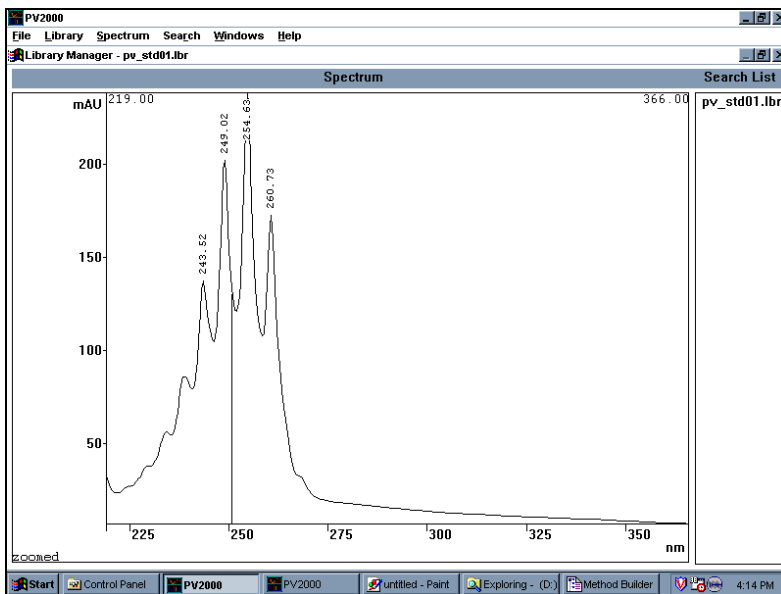


The Spectra Manager Window

A number of data analysis functions can be performed in the Spectra Manager window such as the calculation of Purity Parameters, the determination of their statistics, and the arithmetic combination of spectra. Also, the wavelength range over which the Purity Parameter is calculated can be optimized. Several different reports of the spectra and the spectral analyses can be prepared and printed directly from the Spectra Manager window. From this window, spectra can be transferred to the Library Manager window for use in spectral library functions.

Library Manager Window

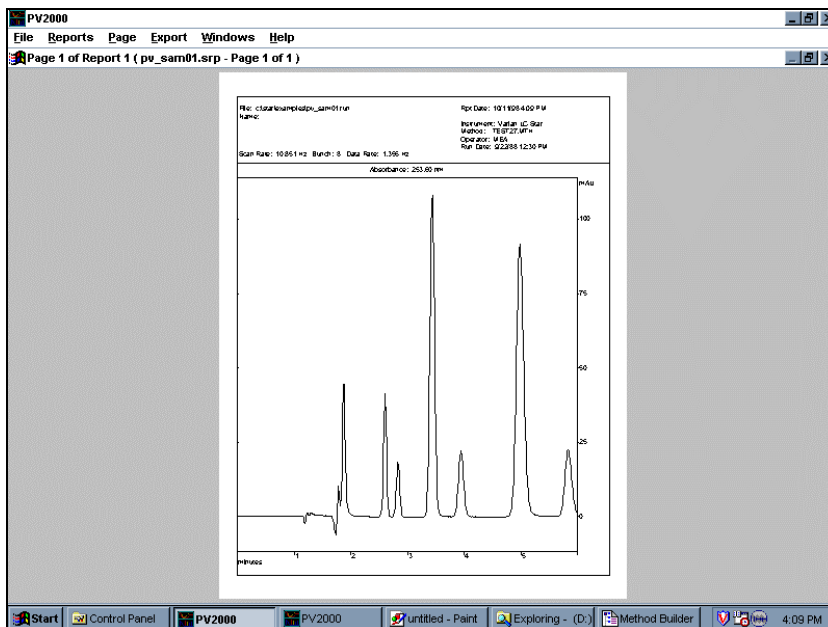
A variety of spectral library functions are performed within the Library Manager window. These include building and editing libraries, conducting library searches, and preparing reports of the search results. Any spectrum transferred into the Library Manager window from the Spectra Manager is held in a spectrum register. The Library Manager window has the ability to hold only one spectrum. Information about the spectrum (name, operator, etc.) can be added to this spectrum in the same manner as it was in the Spectra Manager window. In addition, the Library Manager provides for the management of disk libraries of up to **120** spectra each. Library management functions include adding, editing, and deleting spectra in libraries. Additionally, key word searches of the contents of a library can be made to quickly locate a selected spectrum. In addition to the library management functions, library searches are performed in the Library Manager window. To conduct a search, a spectrum is transferred from the Spectra Manager window into the Library Manager window. One or several disk libraries are specified and the search is conducted based on a number of selectable criteria including Purity Parameter, retention time, and wavelength. The search results are ordered according to the similarity between the target spectrum and the match spectrum from the library. Reports can then be printed to document the results of the library search.



The Library Manager Window

Reports Window

A wide variety of printed reports can be prepared in PolyView2000. Most operations within PolyView2000 windows can be documented through an “interactive” report obtained either by selecting **Print...** from the File menu or by selecting the **Print** pushbutton appearing in many of the dialog boxes. This is just one way to obtain a printed report. Reports can also be prepared in an automated, unattended fashion. In PolyView2000, a separate window, the Reports window, is used to prepare and manage these reports. The Reports window is not activated at the time of start-up and must be created using the **New Reports Window** command item under the Window menu. A variety of different reports can be prepared in this window including chromatograms, library searches, peak purity surveys, and tables of data characterizing the peak events in a data file. The contents of a report are first specified in the Method Builder application and then prepared in the Reports window. Once prepared, the report is saved as a disk file. It can then be loaded in the Report Window, viewed on the screen, edited, printed, or exported in a variety of formats to other windows applications.

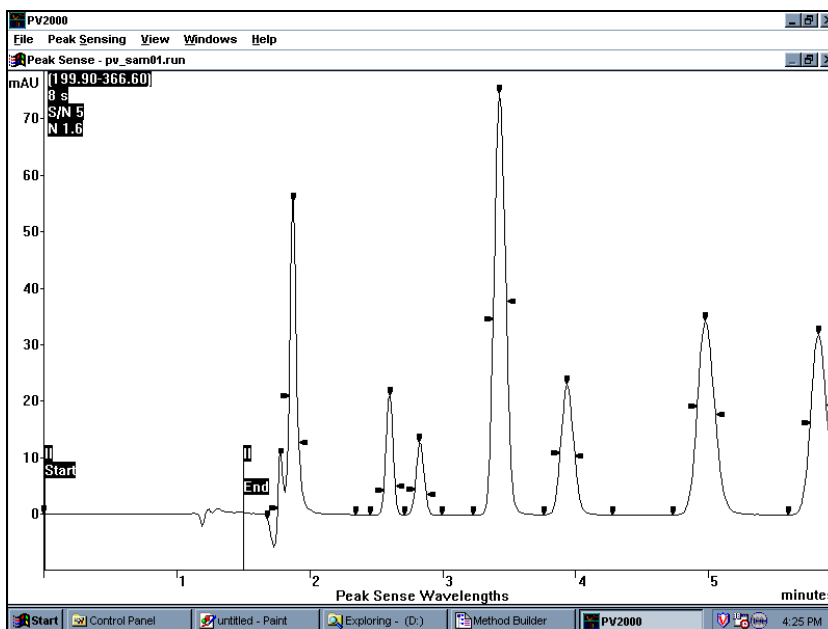


The Reports Window

Peak Sense Window

A number of functions within PolyView2000 require peak detection to determine the various peak events (peak start, peak end, inflection points, and apices). For instance, to provide efficient spectral correction to account for mobile phase absorbance, the peak start and ending points must be accurately determined. This ensures that the spectra obtained for an eluting peak accurately reflect the compound of interest, free from background interference. At the time of data collection, PolyView2000 peak sensing is automatically performed on diode array files, provided a PolyView2000 section is included in the Workstation method file (.MTH) under which the data is collected. However, if no PolyView2000 section was included or if the peak detection was not accurate (as indicated by misplaced peak events or baseline), then peak sensing may need to be repeated.

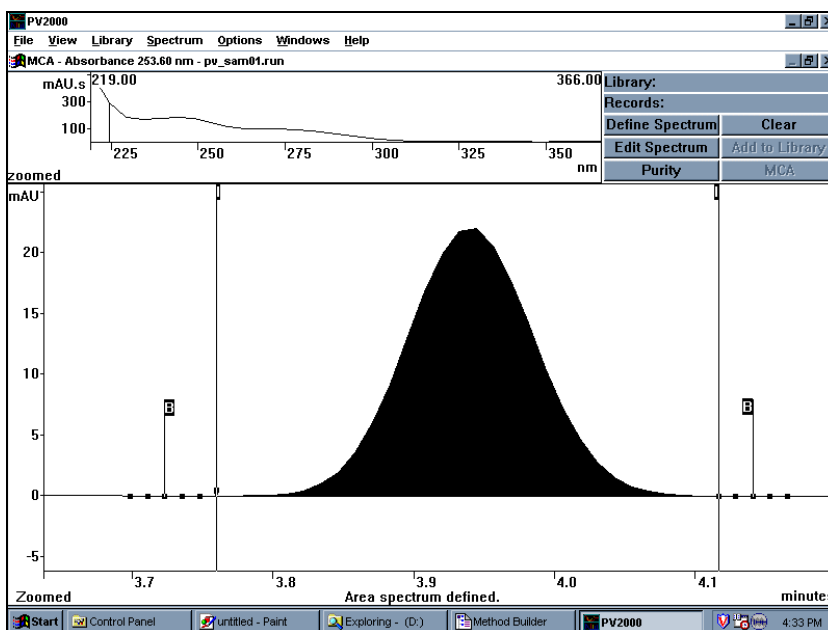
The Peak Sense window is used to perform a peak sense recalculation on a raw data file. Like the Reports window, the Peak Sense window must be created using the **New Peak Sense Window** menu item under the Windows menu. In the Peak Sense window, the absorbance chromatogram is displayed at the wavelength used for peak sensing. To perform peak sensing, the method to be used (wavelength, peak width, etc.) is specified in the Method Builder application and then used in the Peak Sense window. Additional features include the ability to view and edit the noise data acquired by the Workstation during the diode array monitor period. The noise data is used in conjunction with the S/N ratio parameter to detect peaks, and editing of this data may be required in cases where there was excessive detector noise during the monitor period.



The Peak Sense Window

MCA Window

The Multicomponent Analysis window contains all the functions required to perform MCA Quantitative Analysis and Purity Evaluation. The MCA window is composed of 3 areas. The bottom half displays a Chromatogram at a user selectable wavelength, which is used to select the integration limits. The upper right quarter contains 6 push buttons that implement the main commands, and the upper left quarter shows the spectrum of the integrated chromatogram region.



The MCA Window

PolyView2000 and Other Star Workstation Applications

The PolyView2000 application software is integrated into the Star Workstation to enable the Star Workstation to conduct automated tasks that include PolyView2000 functions. For instance, at the end of a chromatographic run, the Star Workstation can automatically conduct PolyView2000 peak sensing on the .RUN data file and prepare selected PolyView2000 reports, all without your actually having to start the PolyView2000 application.

Upon installation, a PolyView2000 Icon is added to the Star Workstation's Star Tool Bar. PolyView2000 interacts with two other Star Workstation applications, Method Builder and System Control/Automation. The .MTH file is used to specify the peak sensing conditions (wavelength, peak width, etc.) or the type of reports needed. Method Builder is used to build and manage the .MTH files that are used to perform these functions. In PolyView2000, the Method Builder application can be accessed directly, without going through the Star Tool Bar. When accessed in this manner, after the method editing session is completed and the Method Builder application is closed, control is returned to PolyView2000, right where you left off.

NOTE: See the *Data Acquisition with LC Control Operation Manual* for more details on the functions and operation of the Method Builder application.

The other Star Workstation application that interacts with PolyView2000 is System Control/Automation. However, the interaction is not readily apparent to the user, as it involves internal processing within the Star Workstation via the AutoLink protocol. When a diode array detector is operating under the control of the System Control/Automation application, post-run PolyView2000 processing and reporting is performed on any diode array file based on the PolyView2000 section in the active method. If no PolyView2000 section exists in the method, default peak sensing conditions are used, and no reports are created.

The results of the peak sensing and/or reporting performed under System Control/ Automation are exactly the same as if these functions had been performed interactively in PolyView2000. System Control/Automation is also used if a series of .RUN files need to have peak sensing or reporting repeated on them. In this case, the recalculation capability of System Control/Automation is used to conduct PolyView2000 peak sensing or reporting on a series of .RUN files.

NOTE: See the *Data Acquisition with LC Control Operation Manual* for more details on the operation of the System Control/Automation application.

Operation

The functions and operation of PolyView2000 are described in this section. PolyView2000 is a very powerful software program and often there are several different ways to accomplish the same task. It is not the purpose of this section to fully describe every operation available within PolyView2000. Rather, the primary functions will be described along with a series of short Examples to assist you in getting started using PolyView2000; in particular, learn to use the On-Line Help which contains both Reference and Procedural information. This may help you to accomplish a particular function in a different way than that described in the Tutorials. Use the commands that are best suited to your particular needs or application. The primary PolyView2000 functions covered in this section are:

- [Configuring PolyView2000 Windows](#)
- [Plotting A Chromatogram](#)
- [Examining Spectra from a Chromatogram](#)
- [Performing Library Functions](#)
- [Reporting Data](#)
- [Determining Peak Purity](#)
- [Purity Parameter](#)
- [Performing Peak Sensing](#)
- [Automating PolyView2000 Operations](#)
- [Using On-Line Help](#)
- [Quantitative Analysis by MCA](#)
- [Purity Evaluation by MCA](#)
- [Creating Workstation Channels](#)

Following the description of each of these functions, one or more Tutorials are presented to help you get started. Read the functional description section, work through the Tutorials, and then refer to the section or On-Line Help if further information is needed on a particular window or menu item.

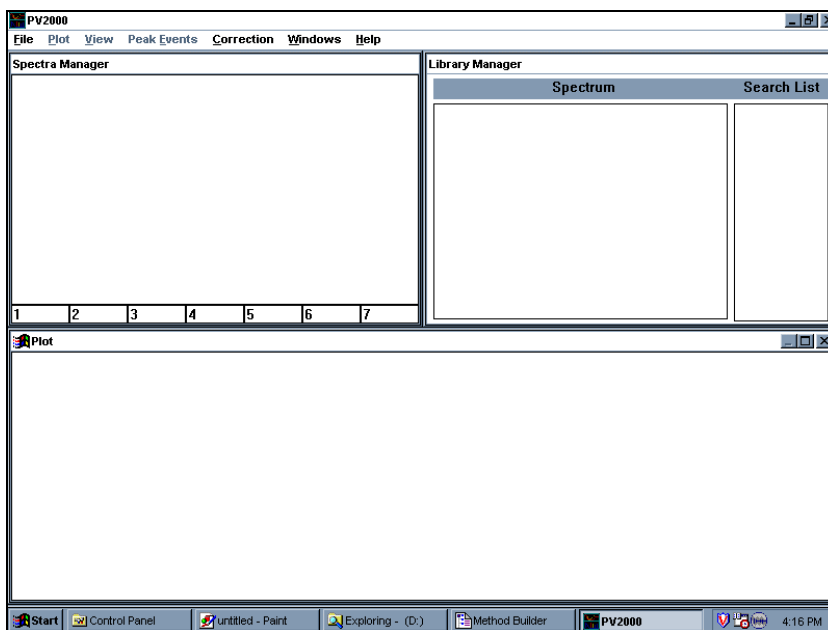
Configuring PolyView2000 Windows

PolyView2000 operates under Microsoft Windows Multiple Document Interface (MDI). The MDI tracks and reflects changes in the menus associated with each child window within a main application window. The menu at the top of the main application window changes as the nature of the active child window changes. For example, within the PolyView2000 main application window, if you move from a child window that is used to manage spectra, to a child window used to manage libraries, spectrum-specific menus will be replaced with library-specific menus. When a number of child windows are present in the main application window, a child window is made the **active window** by moving the mouse cursor to that window and clicking on its title bar. The title bar changes color (or contrast) to indicate that it is now the active window. The menu names and items reflecting the functions and operation of the active child window are then displayed within the menu bar of the main application window at the top of the screen. Organization of the child windows within the main application window can easily be accomplished using the commands under the Window menu. Individual windows can be created, hidden from view, recalled, and arranged in a variety of different configurations. In addition, all of the window sizing controls are available as they are in most Windows applications.

NOTE: If you require further explanation of the operation of the MDI within Windows, or additional detail on Microsoft Windows itself, refer to the *Star Workstation Operation Manual*, or the Microsoft Windows User's Guide.

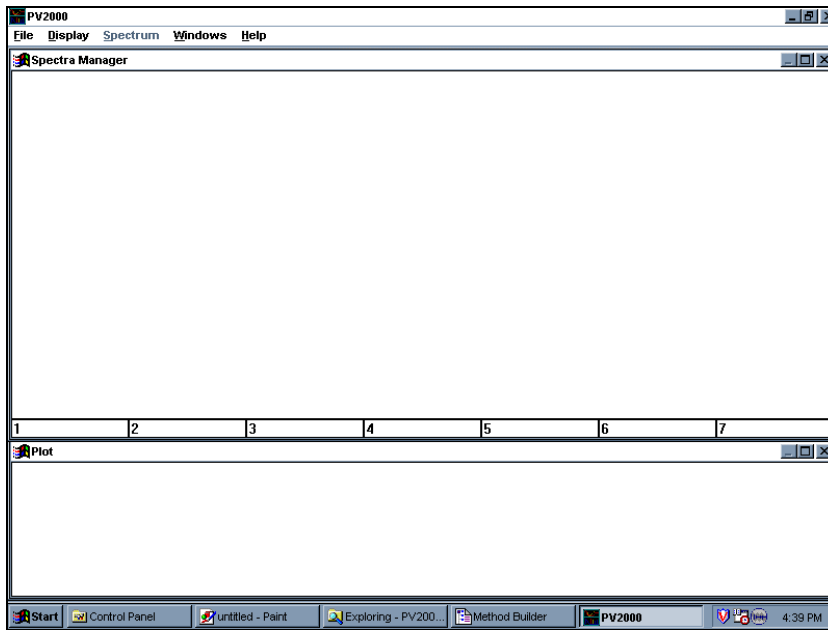
Tutorial: Configuring PolyView2000 Windows

On the Star Tool Bar, double-click on the PolyView2000 icon. The application opens with the screen split between the Spectra Manager, Library Manager and Plot windows with the focus on the Plot Window.



PolyView2000 Configuration Upon Start-Up

All of the windows can be sized using the border controls and the sizing control in the upper right corner of each child. In addition, the main application window can be sized using its borders and controls when it is not maximized. The one restriction is that all the child windows must always remain within the confines of the main application window. Practice some of these window sizing functions and make the screen appear as shown below.



*PolyView2000 Window Configured to Increase
Spectra Manager Area*

The number and type of windows displayed within the PolyView2000 main application window are controlled using the Window menu. This menu is visible and available at all times.

- Create a Reports window by selecting **New Reports Window** under the Window menu.
- Repeat this process, but this time select a Peak Sense window. These windows, like all the others, can be moved and sized to any configuration desired using standard Windows methods. But, don't do that now. There is a much easier way as you will see in a minute.

Any of the currently active windows within PolyView2000 can be instantly accessed and displayed full screen.

- Drop down the Window menu and note that the five PolyView2000 windows are listed at the bottom of the menu.

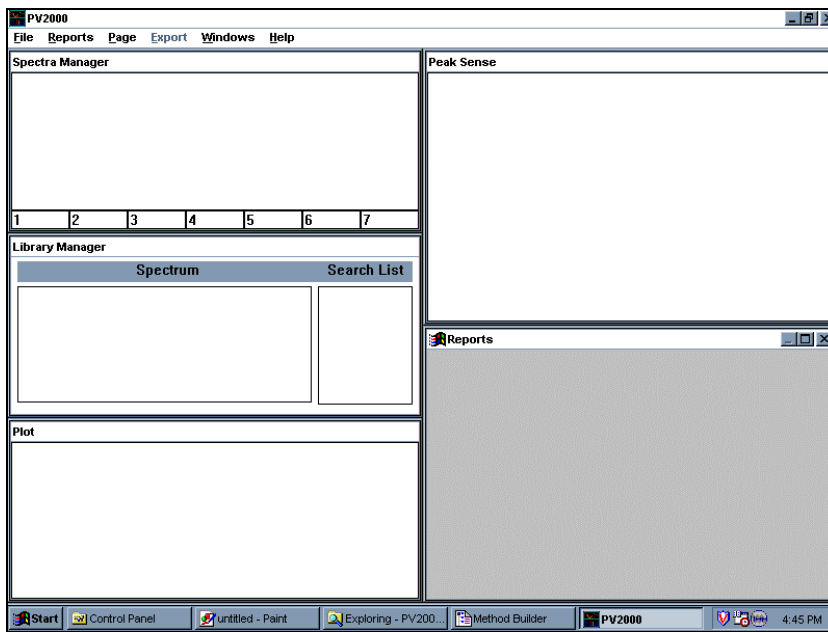


Windows Menu Listing the Currently Active Windows

- Click on Spectra Manager in the Window menu and note that this window is brought forward.
- You may maximize any window to the full size of the PolyView2000 window simply by double-clicking on the title bar of the window you wish to maximize. Try this now, to maximize the Spectra Manager window.
- Any of the open windows can be accessed instantly in this manner. Multiple copies of the Plot, Peak Sense, and Reports windows can be active simultaneously and all of these multiple copies will be listed under the Window menu. This allows you to rapidly select from a number of chromatograms that may be currently active within PolyView2000.

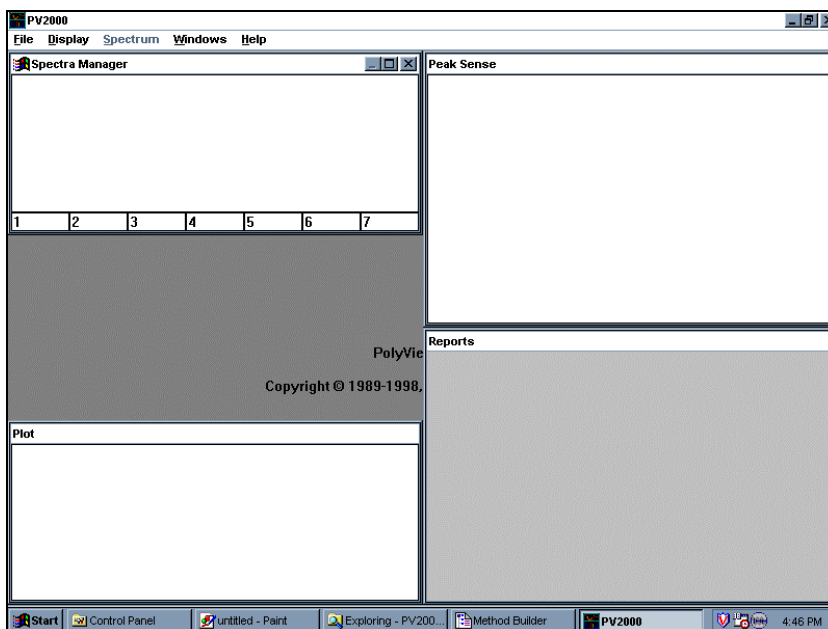
A rapid method is provided that allows you to view all the currently active windows.

- Drop down the Window menu and select "Arrange All". The screen is split between all the windows under the Window menu.



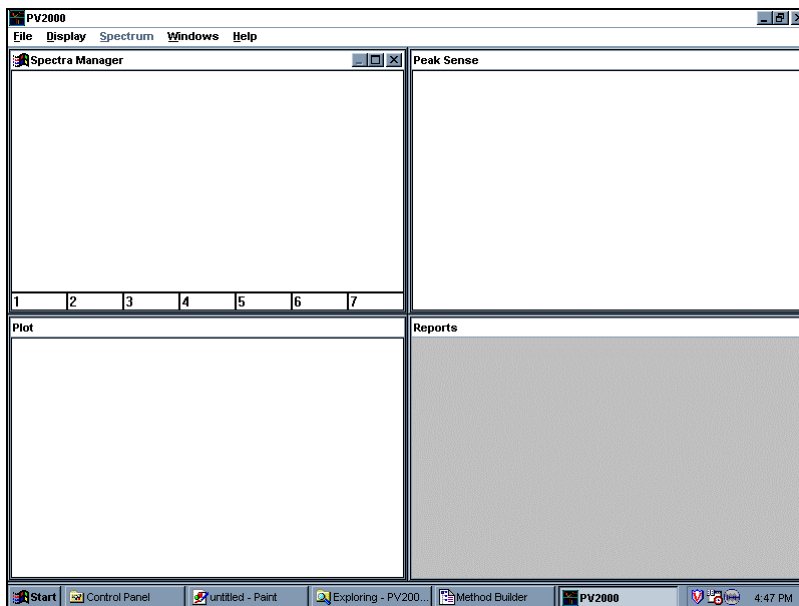
Screen Split Among Five PolyView2000 Windows

- Individual windows can be hidden from view also. Click on the title bar of the Library Manager window to make it the active window, drop down the Window menu and select Hide. Notice that the Library Manager is hidden from view, but it is not removed from the list of active windows (i.e., it still appears in the list under the Window menu).



PolyView2000 with Library Manager Hidden from View

- Now, the remaining windows can be organized. Select “Arrange Visible” under the Window menu and note that the screen is partitioned between the four remaining visible windows.



PolyView2000 Screen Split Between the Four Visible Windows

- In this way the screen can be rapidly configured to meet the needs of the PolyView2000 function you are performing.
- Also try “Arrange Plots”.

Except for the Spectra Manager and Library Manager windows, the individual child windows within PolyView2000 can be closed.

- Drop down the Window menu and select Arrange All.
- Close the Reports, Plot, and Peak Sense windows by double-clicking in their Control Menu boxes. Note that the Control Menu box does not appear in a window's title bar until that window has been selected as the active window by clicking within it. You may go ahead and double-click where the Control Menu box would be and the window will be both selected and closed at the same time.
- Drop down the Window menu and note that these three windows are no longer listed. It is important to remember the difference between a window that is merely hidden and one

that is closed. All data in a window is lost when it is closed, but simply hiding the window does not destroy the data.

- Close PolyView2000 by double-clicking on the Control Menu box in the main application window.

Plotting a Chromatogram

With the PolyView2000 application, raw data files transmitted from the Diode Array detector and collected at the Star Workstation can be plotted, examined on the display screen of the Star Workstation, and then printed out. The Plot Window, within the PolyView2000 main application window, is used to perform these functions. There are a variety of different plot types available in PolyView2000, each expressing the raw data in a different format. See On-Line Help (Help:Plot Manager) for an explanation of the different types of plots available and their uses. In the following Tutorial, the basics of chromatogram plotting are covered. In subsequent Tutorials, some of the more functional aspects of the chromatogram plots, such as their use in the determination of peak purity, will be addressed.

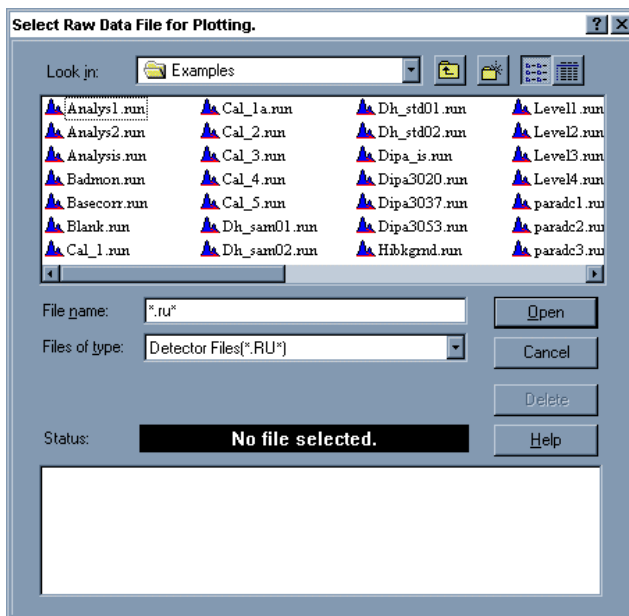
NOTE: It is not the purpose of this Tutorial to cover every possible function in the Plot window. However, the basic functions are covered. For information on those functions not covered, such as those under the Peak Events menu, refer to On-Line Help.

In the following Tutorial, some of the files in the EXAMPLES directory will be modified and/or created. Prior to performing this Tutorial, you should ensure that you are working with original copies of the data files called for. If you are not sure whether these files have been modified by previous use of the Tutorial, reinstall the PolyView2000 software from the CD using your serial number.

Tutorial: Plotting a Chromatogram

On the Star Tool Bar, double-click on the PolyView2000 Spectral Processing icon. The application starts with the screen split between the Spectra Manager, Library Manager and Plot windows with the focus on the latter. To plot a chromatogram:

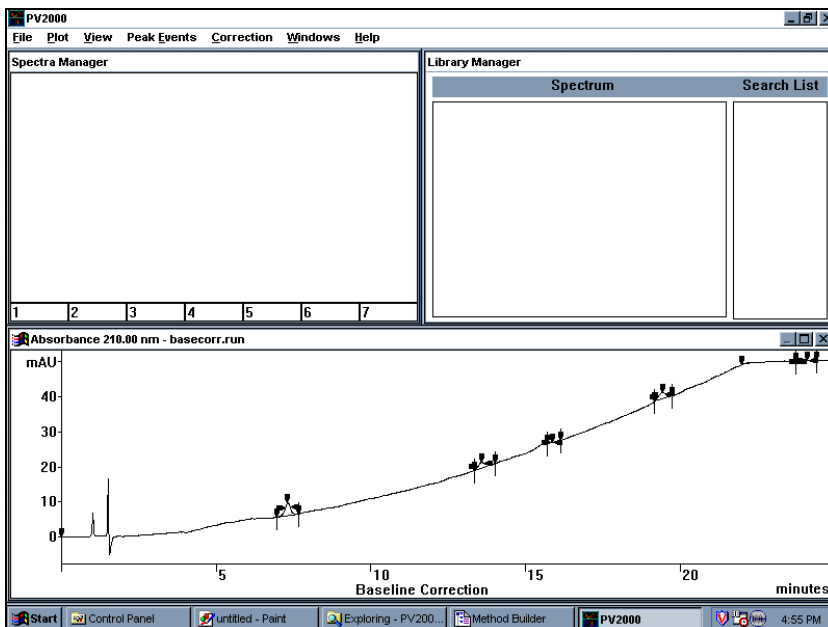
- Drop down the File menu and select Open File... . A dialog box is displayed that lists directories and files with the extension .RUN. Press the “Help” button for more information on the operation of file selection dialog boxes.



File Selection Dialog Box

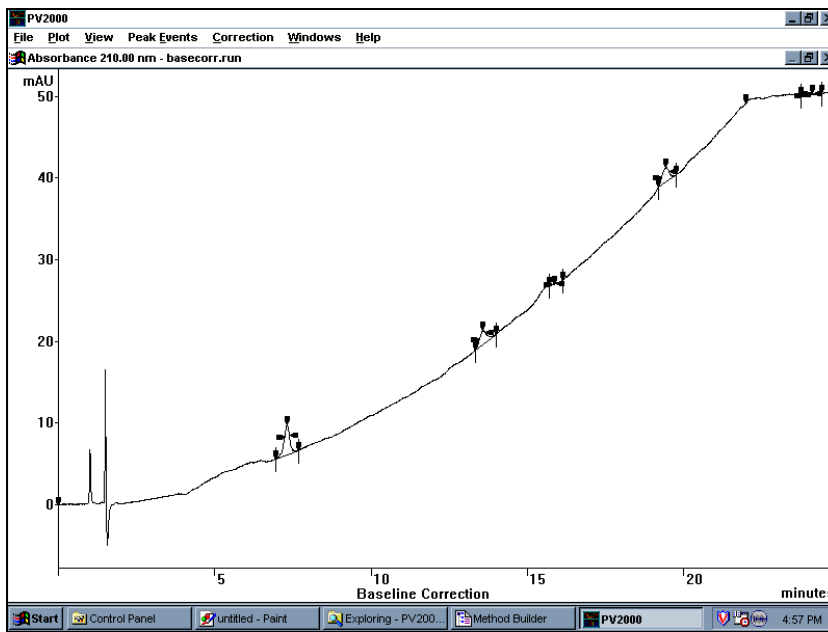
- Select the EXAMPLES directory from the list and note that the *Path* field should display: C:\Star\Examples.
- Click once on a .RUN file. Try BASECORR.RUN. Notice that the fields at the bottom of the screen fill with information about this file. The Status field indicates that a valid file has been selected. If you had selected a non-diode array file, an error message in red would have been displayed instead.

- Click on OK to accept this file. The absorbance chromatogram at 253.6 nm is plotted in the Plot window at the bottom of the screen. The plot is autoscaled on the Y-axis and is plotted from time 0.0 to the end of the run
- Use the Select Plot Parameters command in the Plot menu to plot the absorbance at 210 nm instead. If the plot goes off scale, double click to the left of the absorbance scale when a double headed arrow appears.



BASECORR.RUN in the Plot Window

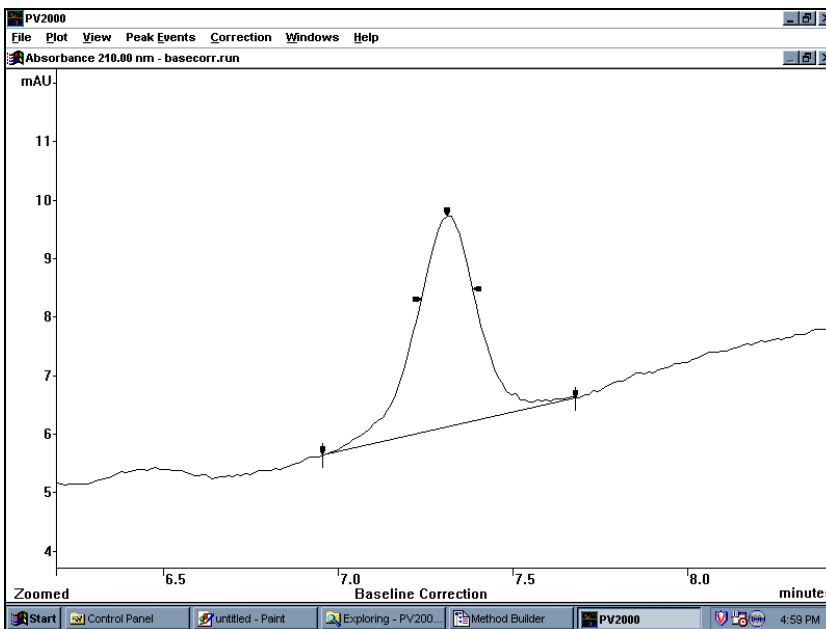
Many PolyView2000 functions can be performed with the window in this configuration, but there are many ways to alter the viewing of the chromatogram. Maximize the Plot window (click on the box with a solid line above it in the upper right hand corner of the Plot window). This enlarges the chromatogram to full screen.



Full Chromatogram

More detail in the chromatogram can be seen by utilizing the zoom functions. Zooming in PolyView2000 is accomplished by the *drag* method, the same as in the Interactive Graphics application.

- Using the mouse, position the cursor below the baseline just to the left of the peak at 7.2 minutes. Press the left mouse button and hold it down as you drag the mouse diagonally toward the upper-right portion of the peak. The portion to be zoomed will appear in reverse video and when the mouse button is released, the plot will be zoomed.



Plot Window Zoomed in on a Peak

- The plot can be zoomed again to observe even more detail. Try zooming the top portion of the peak.
- Pressing the Escape (ESC) key during the *drag* process aborts the zoom process.

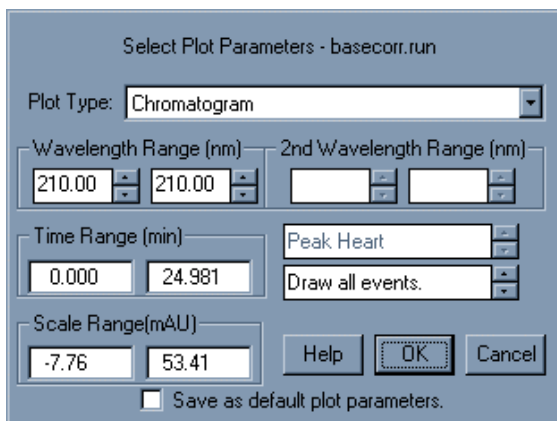
To restore the scaling in effect prior to the latest zoom, drop down the View menu and select Previous. The plot is returned to the full peak. Selecting Previous again restores the zoomed top portion of the peak and thus functions as a *toggle* to switch between two zoom selections.

The full time range can be restored by positioning the cursor just below the time axis and double-clicking with the left mouse button. Note that the cursor changes to a double-headed arrow indicating that the plot will be restored to its full X-axis scaling. Similarly, the full Y-axis scaling can be restored by double-clicking the mouse with the cursor positioned to the left of the Y-axis. Both can be restored simultaneously by double-clicking in the lower left corner. Try these functions now and restore the

scaling to its original setting when the chromatogram was first displayed.

NOTE: Chromatogram zooming can also be controlled using the commands under the View menu. See On-Line Help (Help:Commands) for an explanation of these menu items.

To change the plot configuration (type, wavelength, etc.), drop down the Plot menu and click on the Select Plot Parameters menu item. This displays a dialog box that is used to specify the type of plot and features. The X and Y scales can be set here, the peak events can be turned on and off, and the wavelength range can be selected. Many of these features will be covered in future Tutorials.



The Select Plot Parameters Dialog Box

For this exercise:

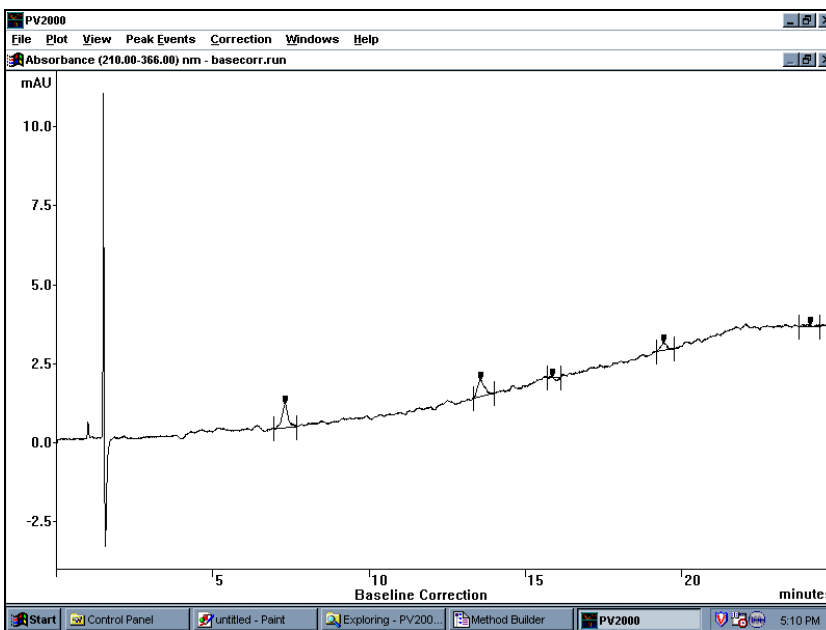
- Change the events list box in the lower right corner to Draw Apex Events.
- Set the wavelength range to 220-366 nm using the Wavelength Range scroll boxes by either scrolling to the proper number or typing the number. Type the left hand lower wavelength first and then the right hand higher wavelength.

NOTES: For many PolyView2000 functions where either a single wavelength or a wavelength range can be set, setting the right hand scroll box with cause both boxes to have the same wavelength. Setting the right hand scroll box will not effect the left hand box. Therefore, to set a single wavelength you only need to set the right hand scroll box.

Checking the “Save as default parameters dialog” check box will save this plot type and condition (except time and scale ranges) and make them the default conditions for any plot windows created later, even in other sessions of PolyView2000. Do not check it at this time.

- Click on the OK pushbutton. The chromatogram will be plotted using the average absorbance in the range of 220.00-366.00 nm with only peak apex events drawn. Notice that the original absorbance scaling was maintained.

To autoscale, simply double-click in the left margin of the Plot window. To prepare for the next step, zoom in on the peak at 19.3 minutes.

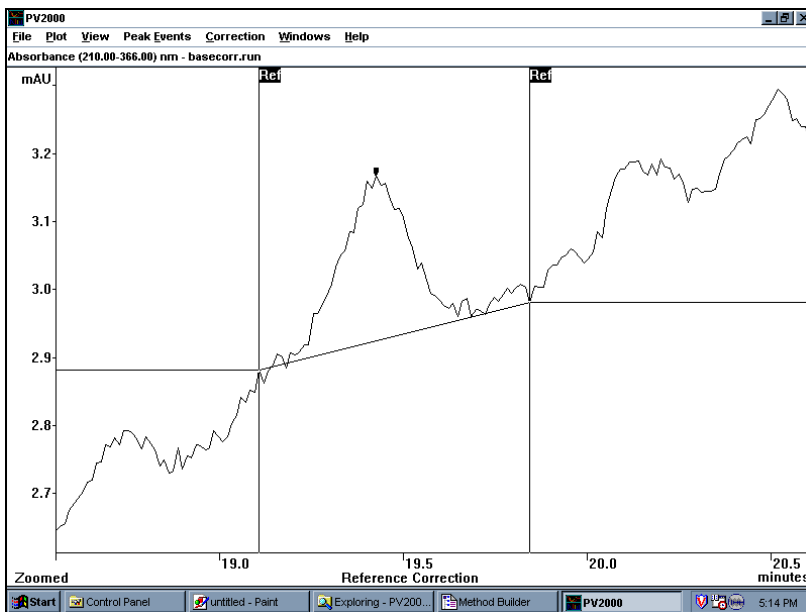


BASECORR.RUN Displayed at 220.00-366.00

When spectra are extracted from a chromatogram, PolyView2000 automatically corrects them for background absorbance. Several different types of correction are available and are set in the Correction menu.

The default is *baseline* but other types are available, including the selection of one or more spectra in the file to use as a reference to which all others are corrected. The current correction method is indicated below the time axis. Let's try that function now:

- Drop down the Correction menu and select Reference. A dialog box appears that prompts you to select the reference spectra.
- Move the cursor to any point of the chromatogram left of the peak at 19.3 minutes, and press the left mouse button once. A vertical line is drawn to indicate the reference spectrum. Define another reference spectrum right of the peak, then press OK to indicate that you are done defining the reference spectra. The baseline is redrawn to indicate the change. The correction is interpolated between reference spectra, and uses the outermost reference spectra to correct spectra extracted from the corresponding regions. The correction method can easily be changed to a different mode by making a different selection in the Correction menu.



BASECORR.RUN with Reference Correction

- Make the correction method **Peak Start** and note that the red lines under each peak originate from the peak start event and project horizontally.

The effect of the baseline correction mode on extracted spectra will be demonstrated in the next Tutorial.

Associated with each .RUN file is information that describes the conditions under which it was collected (date, time, etc.). Within PolyView2000, some of this information can be viewed and edited.

- Under the File menu, select Edit File Information. A dialog box will appear that allows you to edit selected fields. Fields that can be edited are enclosed in boxes and they are edited by standard Windows techniques.

Edit File Information - basecorr.run

c:\star\examples\basecorr.run

Name:	MIXTURE		
Run Date:	2/15/89 9:59 AM	Spectra:	2034
Time Range:	0.00 - 24.98 minutes	Events:	32
Operator:	MEA	Scan Rate:	10.851 Hz
Method:	LASTCHAN.M	Bunch:	8
Instrument:	SEA WOLF	Data Rate:	1.356 Hz
Notepad:	AMOBARE 2.5 ug/ML, THEOPHYLLINE 1 ug/ML, NAPA 1 ug/ML 0 - 37* MeOH GRADIENT IN 20 MIN, MN COLUMN		

The Edit File Information Dialog Box

- Type your name in the Operator field and add any comments into the Notepad.
- Click on OK to write the new information into the disk file for BASECORR.RUN.

To print a chromatogram from PolyView2000, select the Print Plot command under the File menu and the chromatogram will be printed on the current Star Workstation printer. See [Appendix A](#) for an example of this interactive plot report.

Close PolyView2000 by double-clicking on the Control Menu box in the main application window.

Examining Spectra from a Chromatogram

The primary advantage of a diode array detector such as 330 is the availability of spectra at all points in the chromatogram. These spectra provide valuable qualitative data on the nature of an eluting peak. Using the PolyView2000 advanced baseline correction routines, the spectra extracted from a chromatogram are free of errors due to any background solvent absorbance and reflect only the spectra of the eluting compound. Within PolyView2000, the primary spectra handling utilities are provided in the Spectra Manager window. This window provides a temporary storage location for up to seven UV spectra. The spectra that are held in these spectrum registers are available for a variety of spectral processing and reporting functions. In addition, the window serves as a temporary storage location for spectra before they are transferred to the Library Manager window for inclusion in a disk library or as the target of a library search.

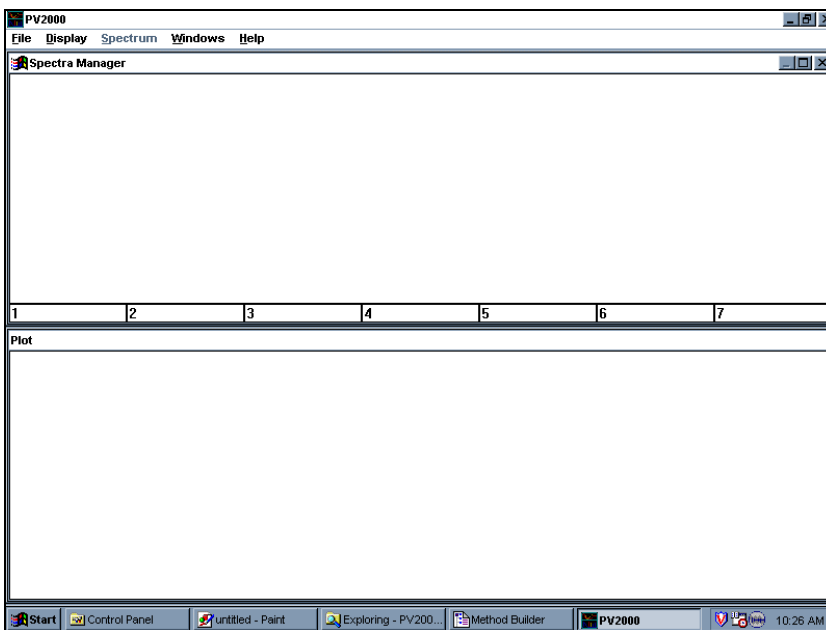
The spectral processing functions available include the calculation of Purity Parameter statistics, the optimization of Purity Parameter spectral ranges and the arithmetic combination of spectra. Details on these functions can be found in On-Line Help (Help:Commands:Spectrum).

The following two Tutorials cover the Spectra Manager functions. As you are going through these Tutorials, keep in mind that there may be more than one way to perform many of these functions. An attempt has been made to present the most straightforward method. With experience, you may find that an alternate way of accomplishing the function may be more suited to your application. Feel free to explore these alternate command paths.

Tutorial: Spectral Plots

On the Star Workstation Star Tool Bar, double-click on the PolyView2000 Spectral Processing icon. The application starts with the screen split between the Spectra Manager, Library Manager and Plot windows with the focus on the latter.

Configure the screen to display the Spectra Manager window and the Plot window.

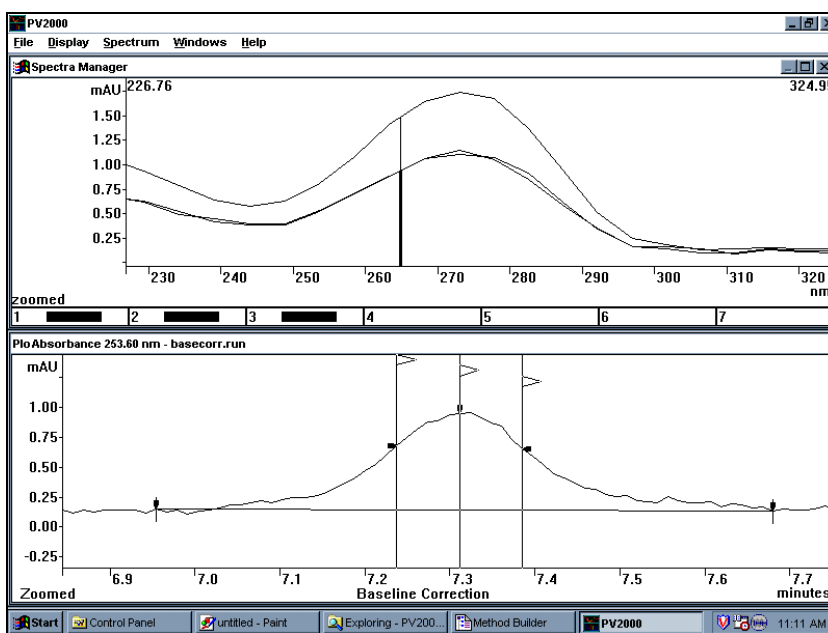


PolyView2000 Screen without Library Manager

Plot out the data file BASECORR.RUN from the EXAMPLES directory in the Plot window at 210 nm with Apex and Inflections events displayed (see the Tutorial: Plotting a Chromatogram).

Spectra are extracted from the chromatogram and transferred to the Spectra Manager window by clicking the mouse at the desired point in the chromatogram. Try that now:

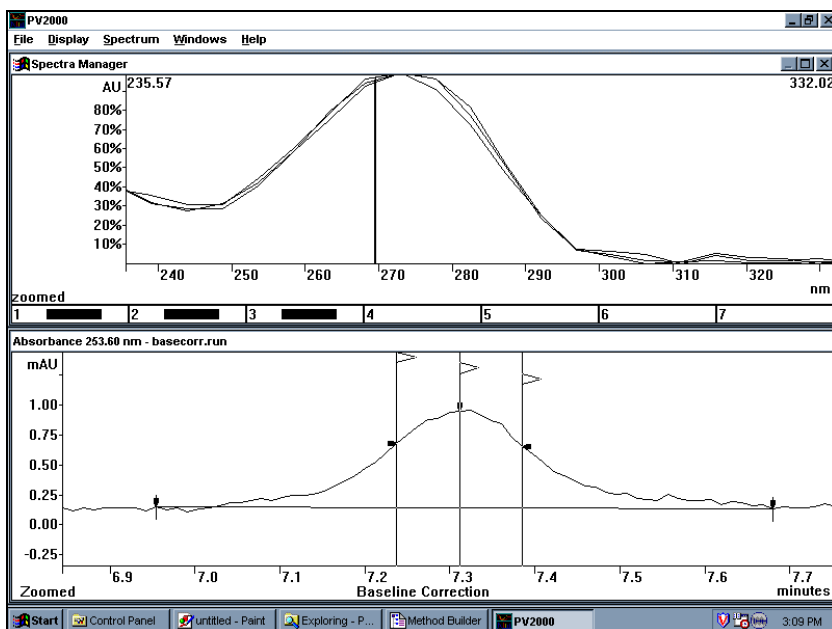
- Zoom in on the peak at 7.3 minutes and extract three spectra at the apex and the front and back inflection points. Notice that each time you click on the chromatogram, a spectrum for that peak appears in the Spectra Manager window and the point in the chromatogram where the spectrum was extracted is marked with a corresponding colored line. Move the cursor to the Spectra Manager display and zoom on the spectra until your screen should look similar to the figure below.



Overlaid Spectra, and Markers on the Chromatogram

The graphics display in the Spectra Manager window is controlled through a set of commands in the Display menu. Try some of these now:

- First, click on the title bar of the Spectra Manager window to make it the active window.



Normalized Spectra

- Drop down the Display menu and select Normalized. Notice that the three spectra are now normalized over their entire wavelength range. You can compare them over a limited range by zooming on the range as shown. This allows you to compare their shapes.
- Drop down the Display menu again and select Single Plots. In this mode, only one spectrum is displayed at the time. Use the numbered colored buttons at the bottom of the Spectrum Manager Window to choose which spectrum you want to display.

The wavelength scale for the Spectra Manager window is variable depending on the wavelength of data that was collected. For the 9065, this is 190 to 367 nm (the limits of the 9065 diode array detector). For the 330 Diode array detector, this is the range over which the data was taken. If spectra from different diode array runs are compared where the range of wavelengths for the data is different, the widest range needed to encompass all of the data is used. When the spectra is zoomed, the entire screen is considered the working range. The wavelength flags

that appear at the top of the Spectra Manager display indicate this range. This is the range considered when normalizing plots and in performing some of the math functions addressed in the next Tutorial. The range can be quickly altered by zooming in the Plot window. Try that now:

- Zoom (using the *click and drag* method) the Spectra Manager window in the region between 250 and 300 nm.

The seven numbered boxes at the bottom of the Spectra Manager window screen represent the seven spectra storage registers in this window. When a spectrum is in one of the spectrum registers, a colored rectangle appears in the box. A filled rectangle means that the spectrum is currently displayed, while an empty rectangle indicates that the register position contains a spectrum but it is not currently displayed. You can turn on and turn off which spectra are displayed by clicking in the numbered boxes containing spectra.

Try that now. Point to the second box and click the mouse. Notice that the first spectrum was turned off, since Single Plots was selected. If Multiple Plots had been currently selected, this action would simply turn on and off that particular spectrum. Double-clicking on a particular box calls up that spectrum for editing. This feature will be covered in the *Tutorial: Building and Editing a Library*.

Spectra can be removed from the Spectra Manager window using the controls under the Spectrum menu.

Drop down the Spectrum menu and select Delete Displayed Spectra.

NOTE:	All spectra in the display area are removed. A deleted spectrum is actually removed from the window and cannot be recalled without going back to the Plot window and extracting it again.
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- Drop down the Spectrum menu and select Delete All Spectra. Note that all of the boxes at the bottom of the screen are empty, indicating that there are no spectra left in the window.

Now let's utilize the display functions you have learned to address a practical problem: the determination of peak purity. Using these spectrum display controls, you can quickly compare different spectra from within one eluting peak for an indication of peak purity.

- In the Plot window, open PV_SAM01.RUN and zoom in on the fourth peak in the chromatogram at 3.45 minutes.
- Transfer spectra from the front and back portions of this peak to the Spectra Manager window.
- In the Spectra Manager window, configure the display for Multiple plots, 190.00-366.60 nm and Normalized. (Remember to click on the title bar to activate the Spectra Manager window.)
- Turn on both spectra and note that they do not overlay perfectly, indicating the presence of co-eluting compounds in what appeared to be a single homogeneous peak. While visual overlays like this are useful for peaks with substantial impurities, numerical approaches, detailed in the next Tutorial, are less subjective and can be statistically validated.
- To see the results of this exercise on a pure peak, repeat this on the peak eluting at approximately 5.0 minutes and observe how well the spectra are overlaid.

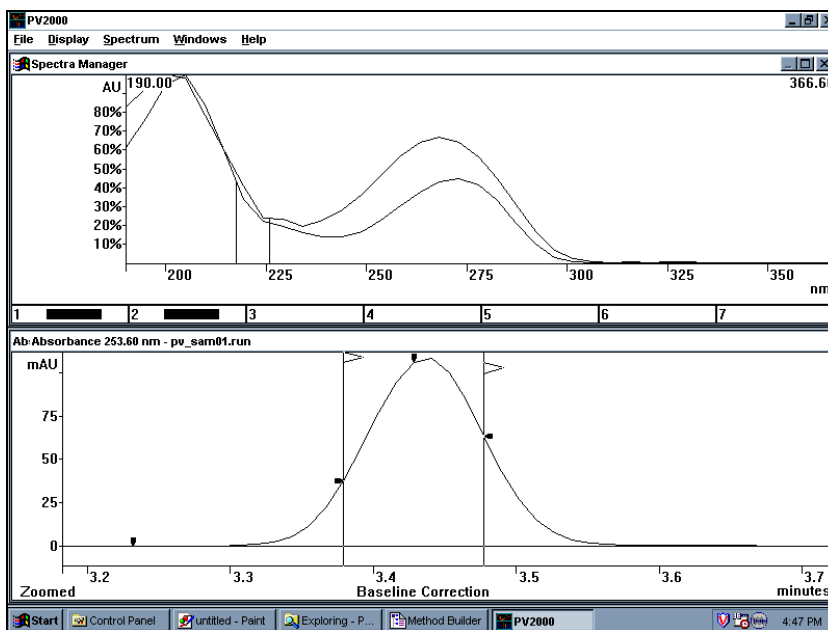
To print reports of spectra extracted from a chromatogram, click on the Spectra Manager title bar, then drop down the File menu and select Print Spectra. The report will either be a single spectrum report (if the window currently is displaying one spectrum) or a spectral overlay, if multiple spectra are currently displayed on the screen. (See [Appendix A](#) for example of the two types of reports).

Do not close PolyView2000. Instead, go on to the next Tutorial.

Tutorial: Spectral Math Functions

A variety of spectral processing functions are available in the Spectra Manager window that help in the evaluation of spectral data.

- Using the procedures from the previous Tutorial, transfer two spectra from the upslope and downslope portions from the impure peak at 3.45 minutes in PV_SAM01.RUN to the Spectra Manager window.
- Configure the window to display multiple plots between 190 and 367 nm with the 'Normalized' setting. The vertical lines in the Spectra Manager window correspond to the Purity Parameter calculated for each spectrum.



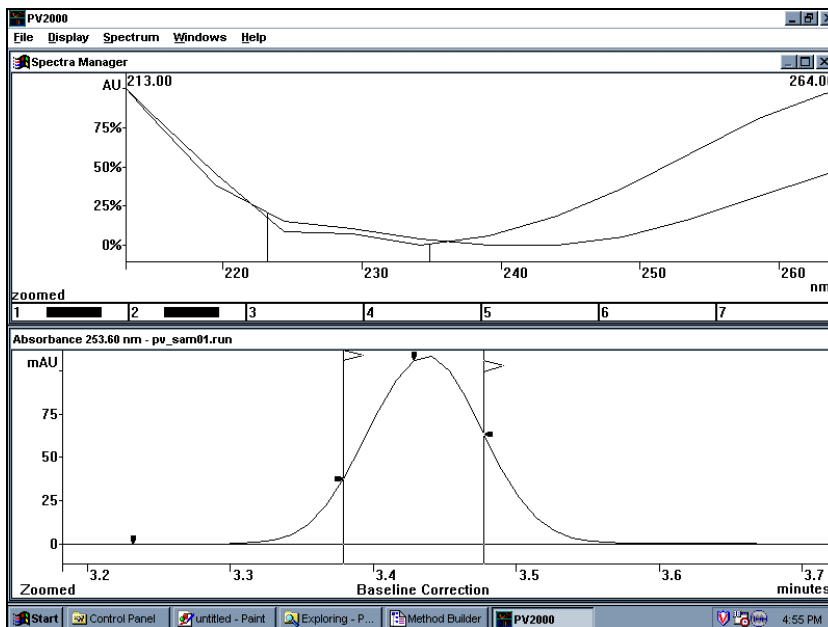
*Upslope and Downslope Spectra
from the Peak at 3.45 Minutes in PV_SAM01.RUN*

- Display the numerical value for these Purity Parameters by dropping down the Spectrum menu and selecting View PuPs. In the dialog box, you can change the wavelength range for the Purity Parameter calculation and the values are instantly recalculated.

NOTE: In PolyView2000, Purity Parameter is often abbreviated PuP.

- Close the Purity Parameters dialog box by double-clicking on its Control menu box.

The Purity Parameter in PolyView2000 is a primary means to distinguish between different spectra. However, the ability to discriminate between spectra is highly dependent on the wavelength range over which the calculation is made. PolyView2000 provides a means to optimize the wavelength range used in this calculation. Drop down the Spectrum menu and select Optimize PuP. Notice that the cursor is replaced with the hourglass, indicating that the Star Workstation is calculating. Following completion of the calculation, select Yes in the informational pop-up and the wavelength range in the Spectra Manager window is set to the values that result in the optimum separation of Purity Parameter values for the displayed spectra.

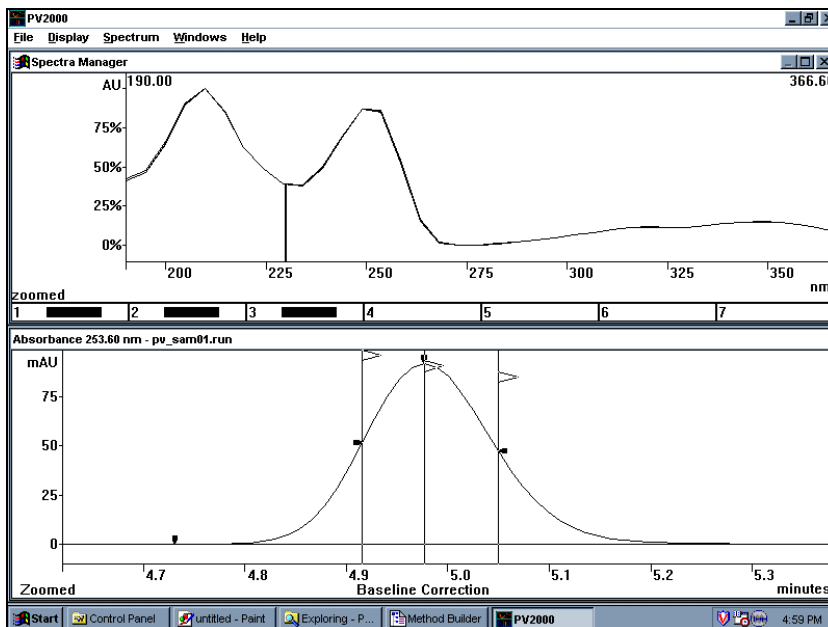


Screen Display Following Optimization of the Purity Parameter

The optimum range for your spectra may not exactly match that shown, since it is dependent on exactly where in the peak that the spectra were extracted.

The Optimize PuP function is particularly useful for determining the proper wavelength range to use in assessing peak purity using Purity Parameter plots (see [Determining Peak Purity on page 71](#)).

For the next two exercises, replace the spectra in the Spectra Manager window with three spectra corresponding to the upslope, downslope, and apex of the pure peak at 5.0 minutes in PV_SAM01.RUN. Configure the Spectra Manager window to display Multiple Plots, Normalized in the range 190-367 nm. The screen should appear as shown below.



*Screen Display of the Three Spectra
Extracted from the Peak at 5.0 Minutes in PV_SAM01.RUN*

The statistical variation in the Purity Parameters for a number of spectra can be calculated.

- Drop down the Spectrum menu and select Do Statistics.
- A dialog box appears that lists all the spectra in the window, their correlation values, average and standard deviation. Initially, the wavelength range is set to what is selected in the spectrum window. Statistics over alternate ranges can also be calculated.

PuP Target

Name:

PuP: nm Standard Deviation: nm

Wavelength Range(nm)

No	Filename	tR (min)	Spectrum Type	Correction	PuP (190.00->366.60 nm)
1	pv_sam01.run	4.915	Upslope	Baseline	230.069
2	pv_sam01.run	4.977	PeakApex	Baseline	229.922
3	pv_sam01.run	5.050	DownSlope	Baseline	229.711
Best Correlation: 1 and 2 Sim: 0.999980 Dissim: 0.006394					
Worst Correlation: 1 and 3 Sim: 0.999899 Dissim: 0.014219					
Average PuP = 229.901 nm Standard Deviation = 0.147 nm					

Listbox not full. ☐ Accumulate Info

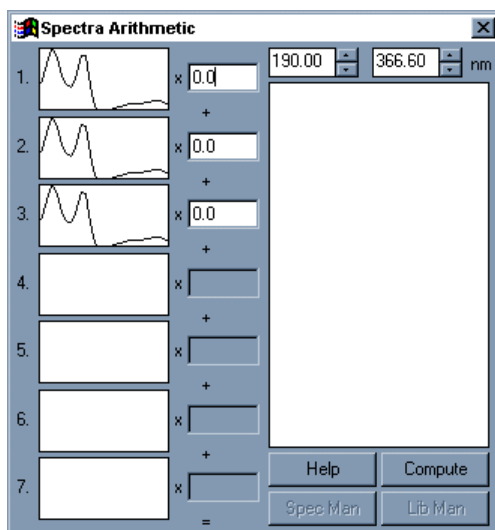
The Purity Parameter Statistics Dialog

- Check the Accumulate Info box, change the wavelength range to 220-366.6 nm, and click on the Calculate pushbutton. Data for the new range is added to the original data.
- Try several more ranges and note that the statistics report lengthens. The entire report can be viewed using the scrolling controls to the right of the report.
- Select the Print pushbutton to obtain a printed report of the calculations (see [Appendix A](#) for an example of the PuP statistics report) for an example of the PuP statistics report).
- The Purity Parameter statistics can be used to help in deciding the significance of subtle differences in values among several different spectra and for establishing expected correlation values for library searches.
- By entering a Purity parameter target and/or a standard deviation in the fields composing the PuP Target group, you can express the purity parameter statistics relative to a standard. If filled, the Name field will be added to the report.

- Close the Statistics dialog box by double-clicking on its Control menu box.

Spectra can be combined arithmetically in the Spectra Manager window.

- With the screen configured as above, drop down the Spectrum menu and select Do Arithmetic. The Spectra Arithmetic dialog box will appear with all current spectra displayed.



The Spectra Arithmetic Dialog Box

- Edit the factor fields just to the right of each spectrum by replacing the 0.0 with 0.333.
- Set the wavelength range to 190-367 nm and select Compute.
- The spectrum that appears in the large graphics display is the average spectrum, since factors of 0.333 were used for each of the three source spectra. From here, the calculated spectrum can be sent to the Library Manager or the next storage location in the Spectra Manager.

Close the PolyView2000 application by double-clicking on the Control Menu box in the main application window.

NOTE: Spectra arithmetic functions are particularly useful for preparing spectral libraries which contain averaged spectra resulting from several different injections. To do this, transfer apex or area spectra from several different injections of a standard solution to the Spectra Manager window. Average the spectra using the spectra arithmetic functions and transfer the resulting spectrum to the Library Manager for inclusion in the library.

Performing Library Functions

PolyView2000 incorporates extensive Spectral Library functions. These capabilities allow the user to match spectra resulting from the injection of unknown samples with reference spectra archived in spectral libraries. The Library functions in PolyView2000 include the ability to build a library from a .RUN file, edit the library records, search the list of library entries for a specific name or key word associated with the record, and conduct spectral searches of the library. Spectral searches can either involve searching the library for a specific spectrum picked from a .RUN file or searching a .RUN file for a specific spectrum from the library. Initially, the library search can be narrowed down by restricting the retention time and Purity Parameter match windows. Then, the library spectra that meet these first criteria are considered and their spectral similarity with the target spectrum is quantified, providing "Dissimilarity" and "Similarity" indices. The library operations are some of the most complex in PolyView2000. They routinely involve the use of three different windows (Plot, Spectra Manager, and Library Manager) with movement of data (spectra) between the three windows.

Like similar operations in other related disciplines (Mass Spectrometry, Infrared Spectrometry), the library functions in PolyView2000 provide qualitative information about an eluting peak by correlating an unknown "target" spectrum with a known reference spectrum. In UV spectrometry, this task is somewhat more difficult, since the information content of UV spectra is low (nearly identical spectra can be obtained from different compounds) and the spectra obtained can be influenced by a number of factors (pH, ionic strength, detector non-linearity, mobile phase absorbance, etc.). Consequently, care must be taken in the interpretation of data from a Diode Array Detector. At best, the spectral correlations obtained from library searches should be used as a means to confirm the identity of an eluting

peak after taking into account other criteria such as the retention time comparison with a known standard. In very few cases is it possible to draw conclusions about the identity of an unknown based on the UV spectrum alone without reference to a known standard. The quality of library matches will depend greatly on the conditions under which the library spectra were saved. It is important to use the same HPLC conditions when constructing a library as are to be used in the analysis of unknowns, particularly if the analytes are sensitive to minor solvent effects. Care must be taken to insure that the detector and other components of the system are in good working order to obtain the best library search results. If in doubt about the quality of the spectra contained in one of your libraries, it is probably best to reinject known reference standards and obtain new spectra.

NOTE:	For a detailed discussion on how library searches are conducted in PolyView2000 and how to interpret the results of a library search, see Library Searches on page 105. for an example of the PuP statistics report.
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In the following Tutorials, the primary library functions will be covered. Initially, you must "Create" a library disk file for storage of spectra. A Tutorial will be presented on Building and Editing that covers the techniques available to place spectra in a library and to add information to each library record that is helpful in characterizing it. PolyView2000 provides extensive editing capabilities for management of your disk libraries. Only the primary capability of locating a particular library record and editing its information section will be covered in this Tutorial. In the last Tutorial, the spectral search capabilities will be covered. It is important to keep in mind that the results actually obtained in library searches depend heavily on the nature of the analytes and the analytical conditions. It is important for each user to develop criteria based on their particular application for the interpretation of library search results.

The Tutorials are based on hypothetical standard and sample chromatograms. The Standard (PV_STD01.RUN) contains several peaks for which spectra have been archived in the spectral library provided with PolyView2000 (PV_STD01.LBR). In the Tutorial, you will be asked to create your own library and to store spectra in it from PV_STD01.RUN. Your library should be nearly identical to the one provided (PV_STD01.LBR). Next,

you will be asked to determine if the peaks in the Hypothetical sample (PV_SAM01.RUN) match the peaks in the standard. Both the library you build and the one provided will be searched.

PolyView2000 can have different types of libraries. Because of the way the spectra are stored in a library, spectra collected with different wavelength ranges and different bandwidths **MUST** be stored in different libraries. (Data collected at different data rates can be stored in the same library because data rates do not effect spectral characteristics.) For example, data collected with a wavelength range of 200 to 400 nm could not be stored in a library with data collected over a wavelength range 200 to 500 nm. Also, data collected from 2 different diode arrays, e.g. the 9065 and the 330, cannot be stored in the same library.

Even though data with different acquisition parameters must be kept separate in different libraries, any library can be used to with any spectrum. The wavelength ranged searched with the wavelength range of overlap between the particular library and the target spectrum. For example, if the library has a wavelength range from 250 to 400 nm and the spectrum was taken from 300 to 450 nm, the target spectrum will be reduced to a spectrum from 300 to 400 nm and the library search will occur.

Therefore, it is desirable for most standards which will be used in a library to take data with the same wavelength and bandwidth.

Tutorial: Creating a Library

From the Star Tool Bar, double-click on the PolyView2000 icon. PolyView2000 will open with the three primary windows displayed.

- Click on the Library Manager title bar to make it the active window and select *Create Library* under the Library menu.
- In the file selection dialog box that is displayed, select the EXAMPLES directory(double-click on it) and then edit the File name field to assign a name for the library you are about to create. Name the library **MYLIB.LBR** and select *OK*. If the file already exists, you need to delete it first. This can be done by pressing the *Cancel* button, then executing *Library: Open* to select MYLIB.LBR, then *Library: Delete* to delete it.

After the name for the new library is selected, the required disk file is created and the library becomes the Current Library in the Library Manager window. A dialog box is displayed that allows you to add information to the library header. In the dialog box, add whatever information you like to the three fields and select *OK*.

The screenshot shows a dialog box titled "Edit Library Information - mylib.lbr". The path "c:\star\examples\mylib.lbr" is displayed at the top. Below the title bar are three text input fields labeled "Library Name", "Library Notepad", and "Library Owner". At the bottom left, there is a table with library statistics:

Date Library Created:	10/13/98 9:19 AM
Date Library Last Changed:	10/13/98 9:19 AM
Number of Records in Library:	0, 0 deleted, 120 limit

At the bottom right, there are three buttons: "Help", "OK", and "Cancel".

The Edit Library Information Dialog

Don't close PolyView2000. Instead, go on to the next Tutorial.

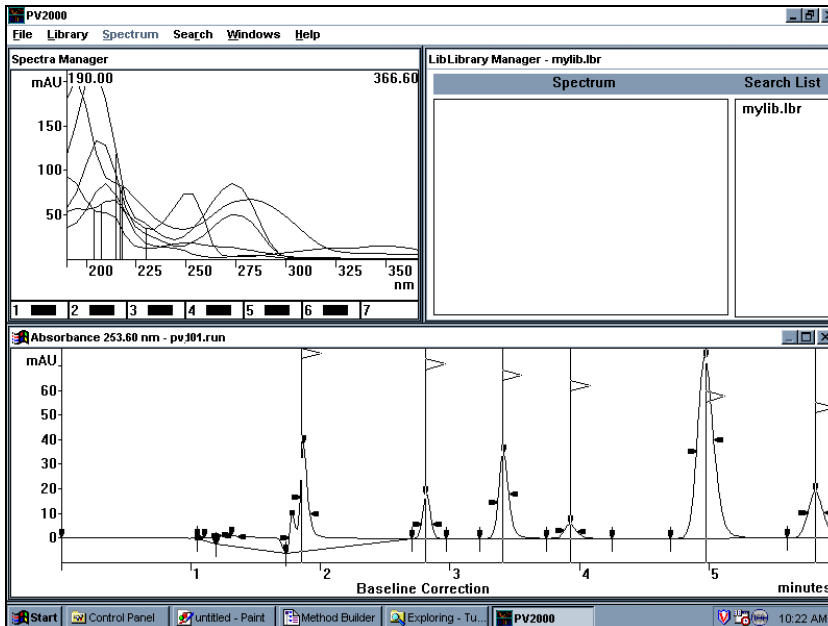
NOTE: It is important to distinguish how the Library Manager addresses different libraries. All libraries are maintained as disk files just as any other DOS file in the Star Workstation. The Library Manager maintains a list of those .LBR files to address during searches, called the Search List. In addition, one library can be designated as the "Current Library" for the Library Manager window. The Current Library is the one that is currently open for adding, editing, and deleting spectra, and is listed in the title bar of the Library Manager window. Libraries are added to the search list using the Add to List command in the File Selection dialog box. Also, when a new library is created, it becomes the current library and is added to the search list. A shortcut to making one of the libraries in the search list into the Current Library is to double-click on its name in the search list.

Tutorial: Building and Editing a Library

Be sure to complete the previous Tutorial on creating a library. The screen should now display the three primary PolyView2000 windows and MYLIB.LBR should be the only library in the Search List in the Library Manager window.

To place a spectrum into a library, you must first extract it from the .RUN file and transfer it to the Spectra Manager window.

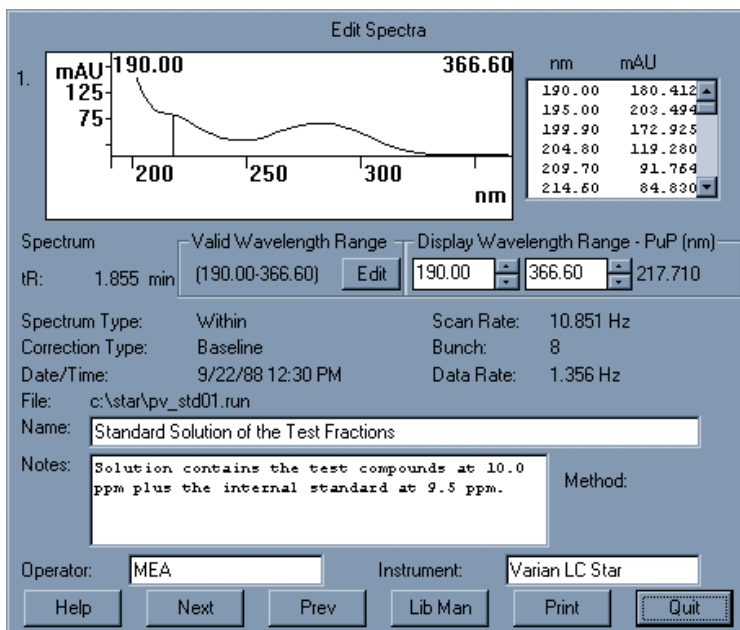
- Click on the title bar of the Plot window to make it the active window.
- Plot out the absorbance chromatogram of PV_STD01.RUN at 253.6 nm (refer to *Plotting a Chromatogram* on page 2.
- Transfer a spectrum from the apex of each peak to the Spectra Manager window *Examining Spectra from a Chromatogram* on page 35.



Screen Configuration for Building a Library File

- Click on the title bar of the Spectra Manager window to make it the active window.
- Select Edit Spectra in the Spectrum menu.

A dialog box is displayed, that allows you to view and edit a variety of information that is attached to each spectrum in the Spectra Manager. This information, along with the spectrum itself constitutes the "Spectrum Record" that can be transferred between the various PolyView2000 windows and becomes a permanent part of the disk file when it is stored in the Library. The number in the upper left corner of the dialog box indicates which of the seven spectrum registers you are currently viewing and editing.



The Edit Spectra Dialog Box

- Initially, change the Valid wavelength range to 200-366.6 nm (select Edit in the Valid Wavelength range box and set the new range in the small dialog box that appears).

- Add or alter any information you would like in the five edit fields (Name, Note, Operator, Method and Instrument). Name the peak Compound #1.
- Once this has been completed, you can obtain a printed report of the spectrum and its associated information. Select the Print pushbutton and wait for the report to be printed.

Select Next to access the next spectrum in the Spectra Manager window. Note that you must verify the edited changes. Select Yes. Continue editing the spectrum information sections for each of the six spectra in the Spectra Manager window. At this point in the Tutorial, all of the spectra and their associated information sections are temporarily stored in the Spectra Manager window. They are not yet part of the library.

After the last spectrum in the Spectra Manager window has been edited, select Quit. You are now ready to begin adding these spectra to the library (MYLIB.LBR).

- In the Spectra Manager window, select Single Plots under the Display menu. The spectrum in register position #1 is displayed.
- Next, select To Library Manager under the Spectrum menu. The displayed spectrum in the Spectra Manager window is transferred to the single spectrum register in the Library Manager window (i.e., it becomes the Current Spectrum in the Library Manager). Add it to the Library by selecting Add Spectrum to Library in the Library menu.

NOTE: In PolyView2000, multiple spectra can be transferred from the Spectra Manager window to the Library Manager window and added to the current library in a single step.

- To add the other spectra in the Spectra Manager window to the library, go back to the Spectra Manager window and select Multiple Plots under the display menu. Then, click on the boxes under the spectra to de-select spectrum # 1 and select spectra #2 through #6.
- Next, select To Library Manager under the Spectrum menu. You will be told that since multiple spectra are selected, you cannot change the Library Manager's current spectrum, and

asked if you want to add the 5 selected spectra to the current library. Answer Yes.

NOTE:	After the transfer of multiple spectra, a small message window informs you of the success of the operation and disappears after a few seconds. You can close this message window earlier by pressing the Enter key or clicking on the window.
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At this point, the library should contain spectra for all the peaks in PV_STD01.RUN. In the Library Manager window, select Select/Edit Library Record under the Edit menu. Notice that all of the spectra added to the library are listed.

A library can be searched for key words (in the Name and Notepad fields of each record) as a rapid means of locating a desired record. Try that now:

- Select Change Parameters.
- Type a key word in the Search Text field that can be found in the Name or Notepad fields of only one of the records.
- Check the Search in Name and Search in Notepad boxes and select Do Search.

Notice that the list of records is updated to include only those with the specific key word. The other records are still in the library, but are not displayed in the list.

Individual records in the library can be edited or deleted.

- First, list the entire library by doing a key word search with only a space (use the space bar) in the Search Text field. Then, select one of the records from the list in the List Library Records dialog box by pointing and clicking with the mouse.
- Select Edit Record. A dialog box appears that is very similar to the Edit Spectra dialog box in the Spectra Manager window. This is your interface to the actual data stored on disk in the library. You can alter the information stored for that particular spectrum or delete the record from the library altogether. For more information on the functions available in this dialog box, press the "Help" button.

Library Records - mylib.lbr

#	tR (min)	File	Name
1.	1.855	pv_std01.run	Compound #1
2.	2.814	pv_std01.run	Compound #2
3.	3.428	pv_std01.run	Compound #3
4.	3.920	pv_std01.run	Compound #4
5.	4.977	pv_std01.run	Compound #5
6.	5.787	pv_std01.run	Compound #6

Options

Select and edit record or change parameters or print list.

Search Parameters

Search Text:

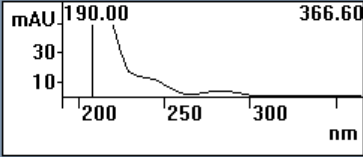
☐ Search in Name
 ☐ Search in Notepad

Records: 6

MYLIB.LBR after addition of spectra

Edit Library Records - mylib.lbr

4 of 6



nm	mAU
190.00	53.286
195.00	56.688
199.90	55.304
204.80	57.673
209.70	64.324
214.60	65.564

Spectrum: Valid Wavelength Range: Display Wavelength Range - PuP (nm)

tR: 3.920 min (190.00-366.60) 190.00 366.60 207.48nm

Spectrum Type: Within Scan Rate: 10.851 Hz

Correction Type: Baseline Bunch: 8

Date/Time: 9/22/88 12:30 PM Data Rate: 1.356 Hz

File: c:\star\pv_std01.run

Name:

Notes:

Method:

Operator: Instrument:

The Edit Library Records Dialog Box

- Select Quit to close the dialog box.

Don't close PolyView2000. Instead, go on to the next Tutorial to learn how to conduct spectral library searches.

NOTE: In the above description on building a library, the information associated with each spectrum (Name, Operator, etc.) was added to the spectrum in the Spectra Manager window prior to its transfer to the Library Manager. Alternately, this information could have been added after the spectrum was transferred to the Library Manager window. The spectrum information fields are accessible after the spectrum becomes a part of the disk library and this information could have been edited after all spectra were in the library (see the above Tutorial). Also, the information is accessible when the spectrum is the Current Spectrum in the Library Manager window (i.e., after it is transferred from the Spectra Manager window but before it is added to the library). To access the Current Spectrum in the Library Manager, select Edit Spectrum under the Spectrum menu. When the desired information has been added, the spectrum can be added to the library directly. In building libraries, use whatever method seems the most straightforward for editing the information associated with the spectra and adding them to the library.

Tutorial: Conducting a Spectral Library Search

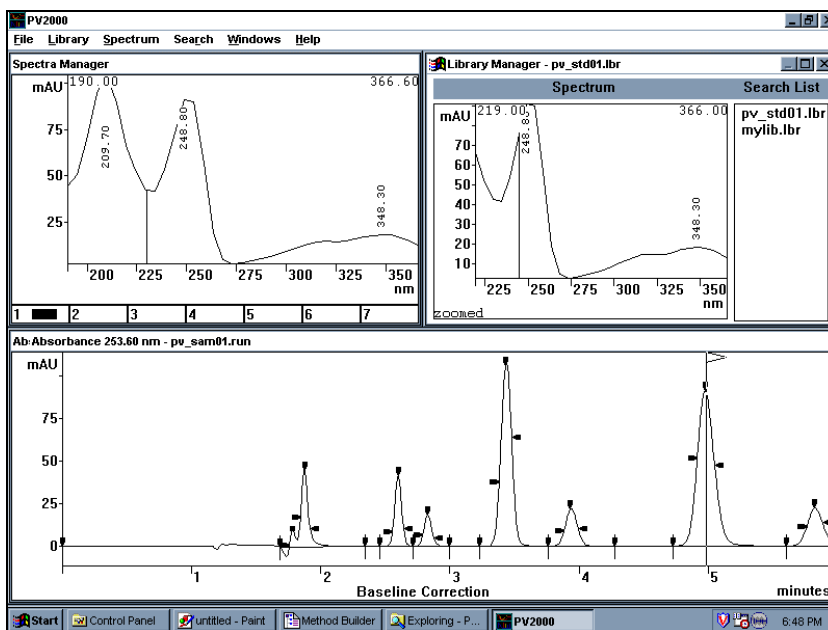
The first step in conducting a library search is to extract a spectrum for the peak of interest from the .RUN file.

- In the Plot window, select Open File under the File menu.
- In the file select dialog box, locate PV_SAM01.RUN and double-click on it. The file will be plotted in the window and for this Tutorial, the default plot conditions can be used.
- In the Spectrum Manager, select **Delete All Spectra** in the **Spectrum** menu, then set the **Display** mode to **Single Plot**.
- The target spectrum for all library searches is the Current spectrum in the Library Manager window. The spectrum is transferred to this window in the same manner as was used in the previous Tutorial (i.e., by transfer through the Spectra Manager window). Using the cursor, extract the apex spectrum from the peak at 5.0 minutes and transfer it to the Spectra Manager window. Activate the Spectra Manager

window and select To Library Manager under the Spectrum menu. You will not be asked if you want to add the spectrum to the current library because the Spectrum Manager was in the 'Single Plot' Display mode.

In the Library Manager, a search list of disk libraries must be built. The library you created in the previous Tutorial should already be listed in the search list. Let's add the library provided with PolyView2000.

- Click on the Library Manager title bar to make it the active window and select Open Library under the Library menu.
- Select PV_STD01.LBR from the list and activate the Add to List pushbutton. Click on the Cancel pushbutton to close the dialog box. Note that it becomes the current library and is added to the Search List.



Screen Configuration for Library Search

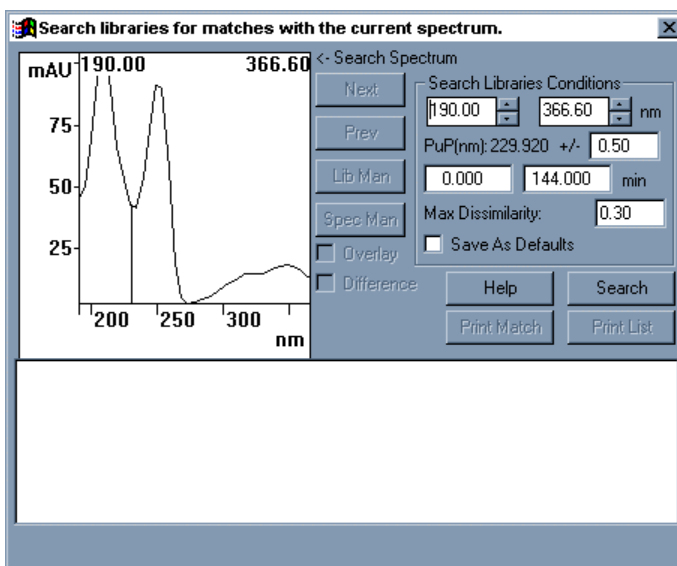
To send a single spectrum to the Library Manager without being asked about adding it to the library, set the Spectra Manager to the 'Single Plot' mode.

NOTE: Management of the contents of the Search List is accomplished with the controls under the Search menu. For more information on the use of these controls, refer to On-Line Help (Help:Commands:Search). Also, any of the libraries in the list can be designated the Current library by double-clicking on its name in the list. That library is then opened for editing. Note that the Current library is always listed in the title bar of the Library Manager window.

Now that the target of the search is the Current Spectrum in the Library Manager, and the search list of libraries is built, you can conduct the search.

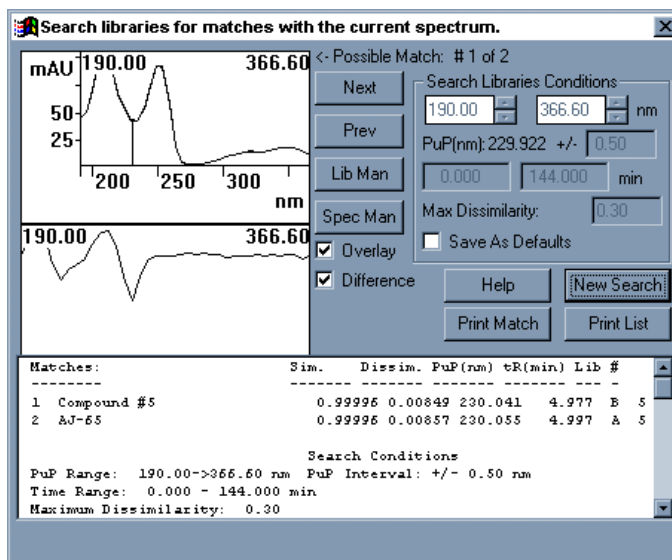
Select Search for Spectrum under the Search menu.

The Library Search dialog box is displayed that is used to set the search parameters and display the results. Refer to [Library Searches](#) on page 105 for more information on the way in which library searches are conducted and search parameters are set.



The Library Search Dialog Box

For now, simply change the wavelength range to 200-366.6 nm and leave all the other controls at their default settings. Select the Search pushbutton. The search is conducted and, after a short period of time, the results are displayed on the screen.



Results of a Library Search

Following the search, a wide variety of additional functions are available. Using the controls in the list box at the bottom of the screen, view the entire library search text report. This report lists the best 5 library matches (sometimes fewer if there were not 5 library spectra that met the retention time and Purity Parameter criteria.) and the search conditions, followed by the information pertaining to the target and match spectra. This report can be printed (Print List) and an example is shown in [Appendix A](#). Other controls in the dialog box enable you to access all of the matches individually (Next and Prev) and obtain a detailed report for each (see [Appendix A](#) as an example).

NOTE: The library search results are ranked in order of decreasing similarity. For a discussion of the indices used to quantify the match between two spectra, see [Library Searches](#) on page 105, or refer to On-Line Help.

By default, the result of the search (if successful) is to display the target spectrum overlay with the best match. Since these two spectra are often very close, their difference is displayed as a spectrum in the lower portion of the spectrum plot. To not display this difference, click on the check box titled Difference to uncheck it. This provides you with a larger size spectral overlay.

When you are finished using this particular target spectrum, close the search dialog box by double-clicking on its Control Menu box. To search any of the other peaks in the chromatogram, simply transfer a spectrum from the peak to the Library Manager (through the Spectra Manager window) and select Search for Spectrum. Try that now. Determine the name of the compound eluting at 8 minutes in PV_SAM01.RUN by searching the two libraries.

Close PolyView2000 by double-clicking on the Control Menu box in the main application window.

Reporting Data

PolyView2000 has two modes of operation: the ***interactive mode*** where the user is actually sitting at the Star Workstation, and the ***non-interactive mode***, where functions and reports are generated automatically using a set of pre-defined conditions contained in a PolyView2000 reports method. Most of the windows and dialog boxes in PolyView2000 permit you to document your work through an interactive printed report (select either the Print command under the File menu or the Print pushbutton in dialog boxes). Examples of these reports are presented in **Appendix A**. Most of the functions (and reports) that can be performed interactively can also be conducted in the non-interactive mode. In **Automating PolyView2000 Operations** on page 90 you will learn how to automate these data processing and reporting functions. In PolyView2000, your primary control over these non-interactive reports is through the Reports window. Non-interactive reports are specified using the Method Builder application (see the LC Star Workstation Manual) which can be accessed directly from the Reports window in PolyView2000. When the Method Builder application is accessed in this manner, control is passed directly back to PolyView2000 when it is closed. Once a report method is specified, the report can be generated and stored as a disk file. Then, you can view

or print the report at some later time. The following Tutorial is broken into two parts. The first part covers the viewing and printing of an existing report file on the disk. The second part covers the specification and generation of a report file.

NOTE: In the following Tutorial, some of the files in the EXAMPLES directory will be modified and/or created. Prior to performing this Tutorial, you should ensure that you are working with original copies of the data files called for. If you are not sure whether these files have been modified by previous use of the Tutorial, re-install PolyView2000 from the CD.

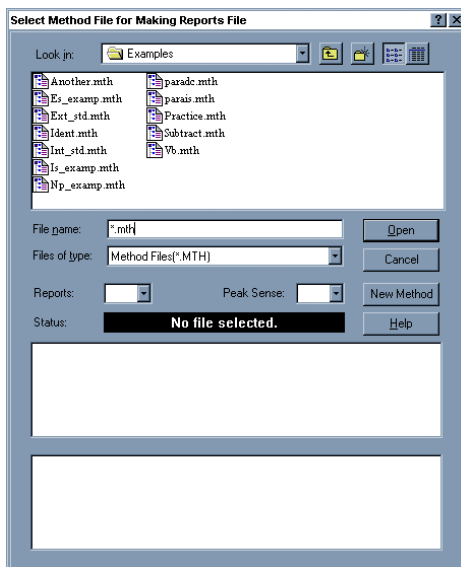
Tutorial: Making a Report

The first step in making a report is to construct a report method in the Method Builder application.

Activate the Reports window (if not already active).

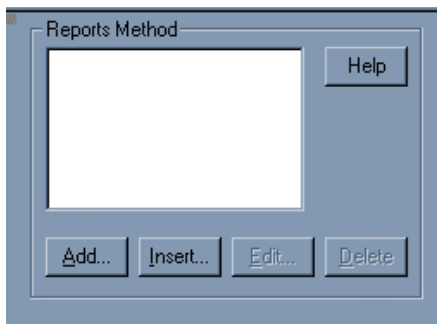
Drop down the Reports menu and select Edit Method.

The Method Selection dialog box allows you to create a new method or edit an existing method. Let's create a new method.



The Method Selection Dialog Box

Select the New Method pushbutton and accept the default bus address of 4, the address used in collecting the Example data files. Control is transferred to the Method Builder application and the window used to specify reports is displayed.

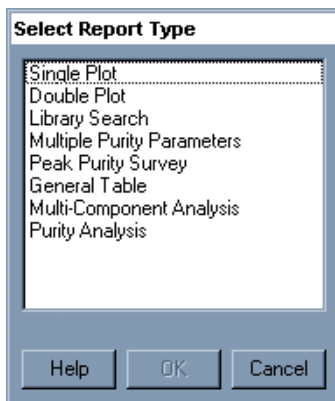


The PolyView2000 Reports Method Window

NOTE: For more details on the operation of the Method Builder application, refer to the Data Acquisition with LC Control Operation Manual.

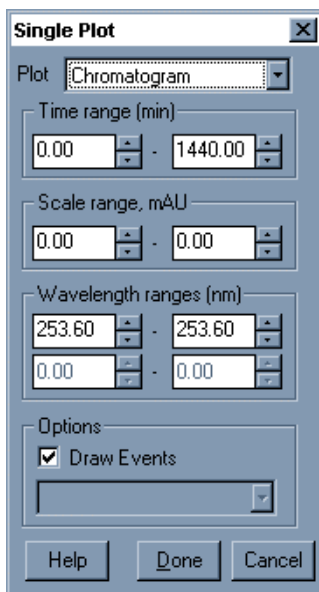
The Report Method dialog box is used to manage the list of report types called for in the .MTH file. First, let's call for the generation of a Single Plot report.

Select Add and a list of the reports that can be generated is shown.



Reports that can be generated automatically

Select the Single plot report and press OK. Note that a dialog box is displayed that allows you to specify the parameters for the report.

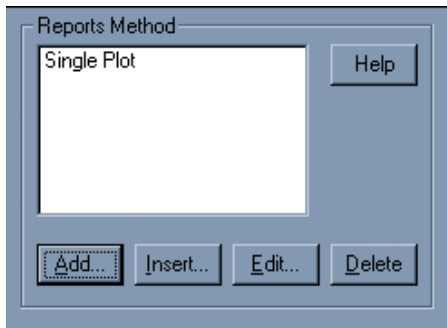


Single Plot Report Parameters

Leave the Plot type at Chromatogram (the default), set the wavelength range to 220-311 nm, and leave the Time and Scale ranges at their default settings.

These parameters will generate a report of the entire data file using the average absorbance from 220 to 311 nm with the absorbance range autoscaled.

Click on Done at the bottom of the window. Notice that the Single Plot is included as the first report specified.



A Single Plot Report was added to the Report List

Now, let's include a library search in the report.

Click on Add and select Library Search.

Note that the Select Library File dialog box is displayed. You now specify the libraries that you want to use for a library search. Once you have specified the library, you can specify the parameters to use in conducting an automated library search. Let's duplicate the search that was conducted in the Tutorial, Conducting a Spectral Library Search.

Leave the Report Type set to Short Graphics.

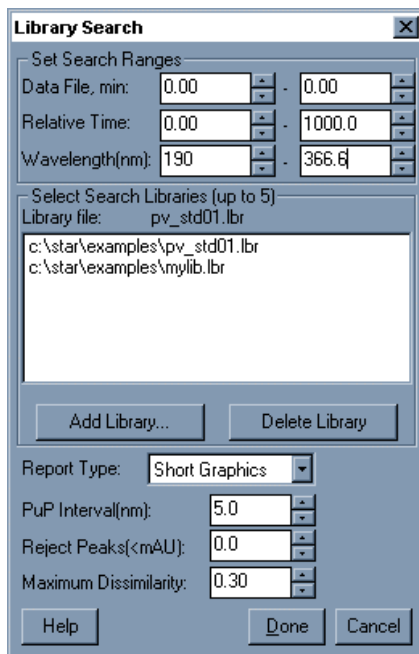
Set the wavelength range to 190-366.6` nm and leave all of the other fields at their default settings.

Now, build a list of libraries to search.

Select Add Library and the Library Select dialog box is displayed.

Select MYLIB.LBR. Select Open and the library will be added to the search list. Repeat this for the PV_STD01.LBR file.

Back in the Report Method window, select Add to append the Library Search report to the list of reports.



The Library Search Report, showing the list of libraries to be searched

NOTE: Once a series of reports have been entered into the list, the controls in the Report Method window are then used to manage the list. You can edit or delete selected reports and rearrange the order that the reports will be generated (and appear in the disk file). For details on the operation of these list management controls, see On-Line Help (search for Report Method).

Now that the types of reports have been specified, you need to assign the .MTH file a name and save it to the disk.

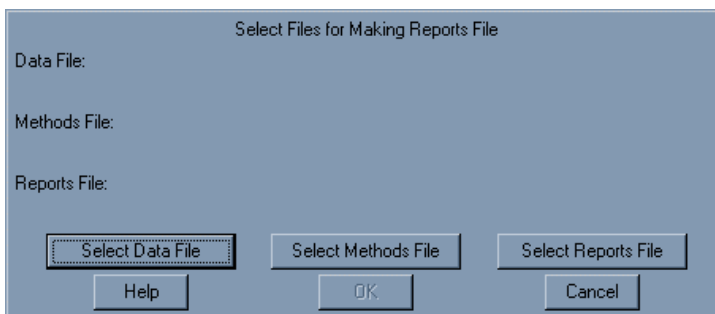
Drop down the File menu and select the Save As command.

In the dialog box, assign it the name MYMETH. You do not need to add the .MTH extension. It is automatically added to the method name.

Close the Method Builder application by clicking on the Control Menu box in the main application window.

When the Method Builder application is exited, control is returned to PolyView2000, just where you left off (in the Reports Window). You can now generate a report using the .MTH file you just created. To generate the report, you must specify the .RUN file that is the source of the raw data, the .MTH file that will be used to process the data, and the .SRP file that becomes the disk file containing the report.

Drop down the Reports menu and select Create Report.



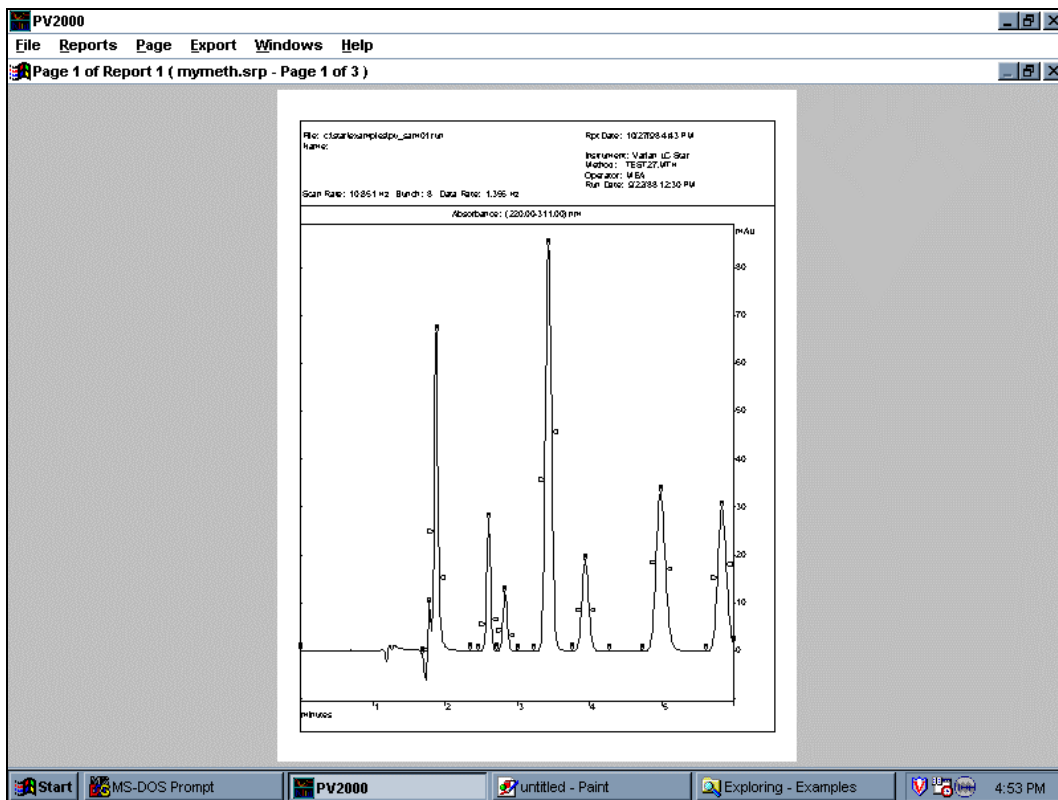
Report Creation Dialog Box

Press *Select Data File* and choose PV_SAM01.RUN as the raw data file from which to construct the report.

Press *Select Methods File* and choose MYMETHOD.MTH as the method to construct the report.

Press *Select Reports File*, type MYREPORT into the File name field and select OK..

Press OK to start the report generation. A dialog box is displayed that informs you of the progress. After the report is completed, it is automatically displayed in the Reports window. It can now be accessed or printed just as any other existing report file.



MYREPORT.SRP displayed in Reports Window

Close PolyView2000 by clicking on the Control Menu box in the main application window.

NOTE: The above Tutorial covered the building of a *new* report generation method. PolyView2000 also allows you to edit an existing report method by selecting Edit Method under the Report menu and specifying an existing .MTH file in the File Select dialog box. You can then add new report types to the file or edit the ones already in the .MTH file.

You may also extract the report method directly from an existing .SRP file even if the original .MTH file used to generate it doesn't exist on the disk. To do this, select Edit Current Method under the Report menu. The dialog box that appears allows you to generate a .MTH file with the same root as the .SRP file. You may choose another name if desired.

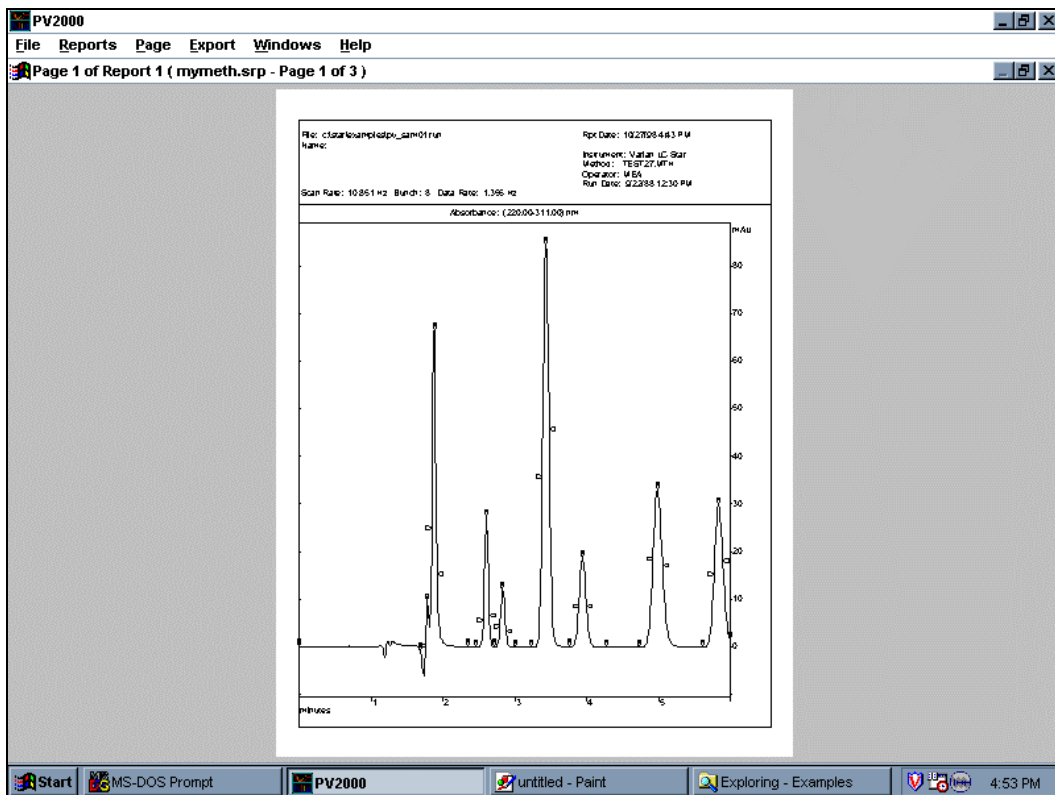
Tutorial: Viewing and Printing of an Existing Report

If you have not already executed the previous Tutorial, do so to create a report file.

If PolyView2000 is not already running, start PolyView2000 now.

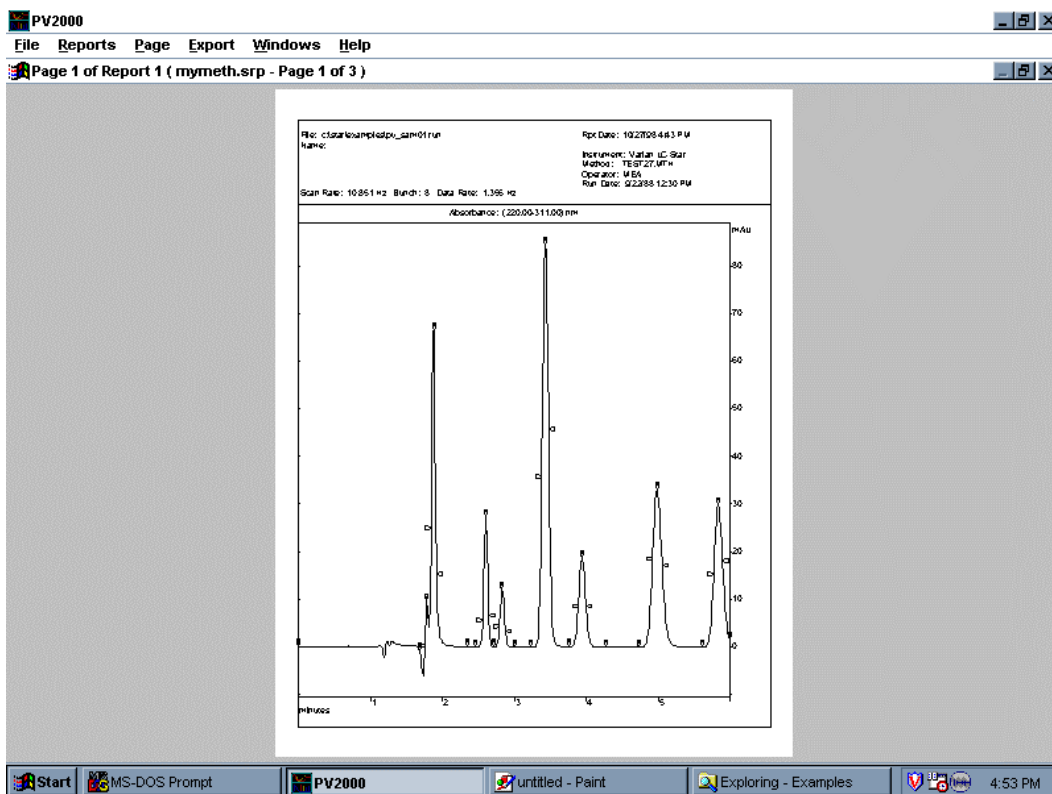
- Select New Reports Window under the Windows menu.

To view a report file, select Open Report under the Reports menu and select MYREPORT.SRP from the EXAMPLES directory. Notice that as you select the disk file, the pages in the report are listed. This enables you to confirm the contents of the report before it is called into the window. Select OK to confirm your selection.



Report window displaying an .SRP file

The first page of the specified report is displayed in the window. Initially, it is displayed in color, and the full page is reduced in size to fit within the window. Access the next page of the report by selecting Next Page under the Page menu. The commands under this menu can be used to access or delete sequentially the individual pages in the report. The Next Page and Previous Page commands can also be given through the Page Up and Page Down keys in the numeric keypad of your keyboard. The report can be viewed in monochrome by selecting that mode under the Page menu.



Library Search Report Zoomed in

Next, drop down the **Report** menu and select **View Page List**. This allows you to view the method that was used to generate the report and the resulting pages. Any of the individual pages can be accessed by selecting them from the page list and

selecting the View Page pushbutton. Select a page of the Library Search Report.

Particular details in the report can be viewed by moving the cursor to the desired region of the report by a click and drag operation of the mouse. Zoom in on the region of the report dealing with a library match. Notice that the remainder of the report can still be accessed using the scrolling controls at the bottom and right of the screen. Clicking the left mouse button anywhere within the report un-zooms the display.

Drop down the File menu and select Print Page. The currently displayed page is printed on the Star Workstation printer. If desired, all the pages in the report can be printed using the Print File command in this menu.

Determining Peak Purity

A primary feature of a Diode Array Detector such as 330 is the ability to provide information on the purity of chromatographic peaks. These peak purity determinations are made by assessing the degree of difference in the UV spectra across the width of a peak. There are a number of mathematical parameters that can be used to compare spectra. The Purity Parameter used in PolyView2000 is perhaps the most sensitive to minor co-eluting impurities (contaminants) within a peak of interest (the analyte).

Purity Parameter

For a given wavelength range, the Purity Parameter is the average wavelength weighted by the square of the absorbance.

$$\text{PuP} = \frac{\sum A_i^2 \cdot \lambda_i}{\sum A_i^2} \quad (\text{Absorbance } A_i \text{ @ wavelength } \lambda_i)$$

Consequently, by examining the Purity Parameter values across a peak, you can quickly determine the presence of contaminants. Rather than examining a table of Purity Parameter values, PolyView2000 provides the ability to plot the Purity Parameter as a function of time. If the peak is free of co-eluting compounds, the Purity Parameter will not change over the course of the peak and it will appear with a flat top. However, if impurities are present, the top of the peak will slope, indicating that the Purity

Parameter is not consistent across the width of the peak. In addition to the Purity Parameter, other qualitative parameters associated with UV spectra can be plotted and provide information on peak purity, including the wavelength of maximum absorbance and the ratio of absorbances at two different wavelengths. Like the Purity Parameter plot, a flat topped peak indicates that the parameter is not changing with time and provides a measure of peak homogeneity.

The accuracy of all the measures of peak purity are dependent on a number of factors. There must be some degree of separation between the analyte and contaminant to create a difference in the UV spectra across the width of the peak. If the two (or more) compounds exactly co-elute, then the UV spectra will not change across the peak and it will appear to be pure. Care must be taken to maximize the efficiency of your HPLC system. A Diode Array Detector cannot make up for low efficiency columns and/or inadequate separation techniques. In addition, the degree of difference in the UV spectra between the analyte and the contaminant will have a profound effect on the peak purity determination. If spectral differences are small, it will be very difficult to determine impurities. However, the Purity Parameter is very sensitive to even slight spectral differences and this will not often be a problem for PolyView2000. A final consideration in peak purity determinations is the wavelength range used in specifying the plots. It is possible for the spectra of an analyte and a contaminant to be very similar over a certain portion of the UV spectrum and different over another portion. It is important to carefully consider the wavelength range used to assess peak homogeneity. To assist you in selecting the proper wavelength range for plotting the Purity Parameter, you can use the optimization routine in the Spectra Manager under the Spectrum menu.

PolyView2000 provides several ways to construct Purity Parameter plots. They can be plotted interactively in the plot window or generated automatically in a non-interactive report. In addition, the non-interactive Peak Purity Survey report presents the same information in a slightly different format. In this report, the Purity Parameters of all spectra within a peak are compared with the Purity Parameter of the apex spectrum and, if different, the peak is marked. With experience, you will find the technique that best suits your application.

In the previous tutorials, you have already learned the techniques that are required to conduct these Peak Purity determinations. The basic technique for plotting a .RUN file was covered in *Plotting a Chromatogram* on page 25 and preparing non-interactive reports was covered in *Reporting Data* on page 61. Consequently, the details of these operations will not be covered here. Instead you will be asked to refer to these previous tutorials. The data file used in this tutorial, PV_SAM01.RUN, has several impure peaks that should become obvious as you work through this tutorial.

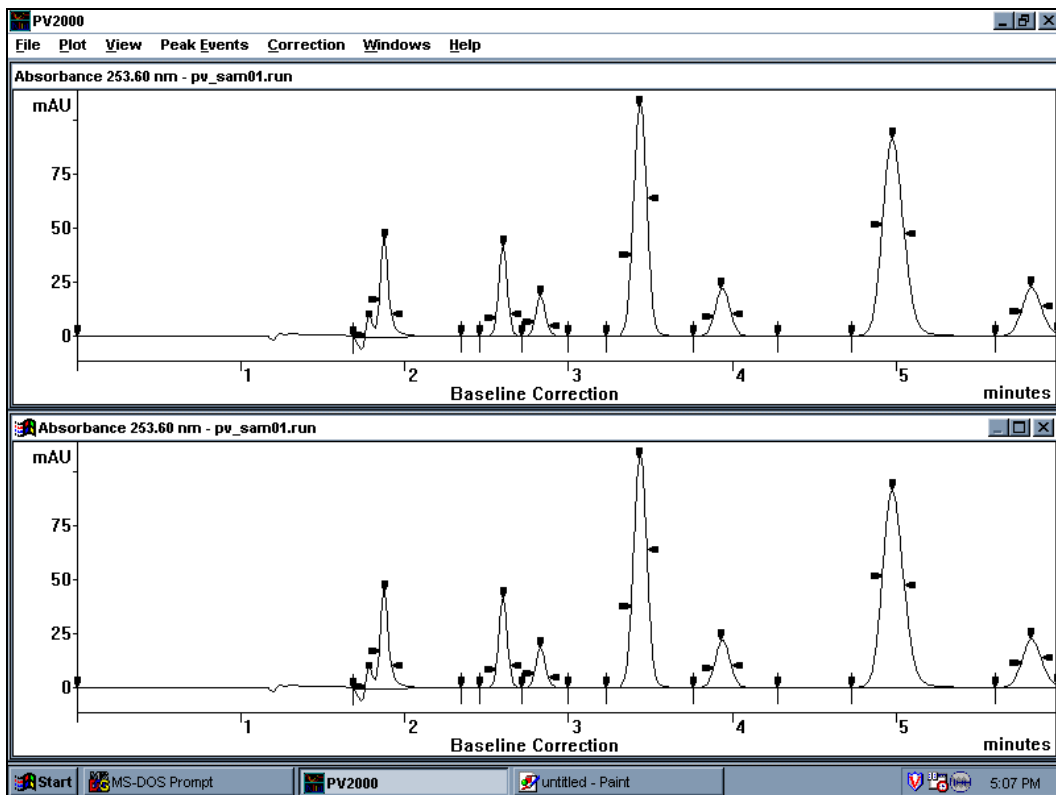
Note that Multicomponent Analysis can be also used to assess the purity of a peak, as detailed in *Purity Evaluation by MCA* on page 100.

Tutorial: Purity Parameter Plots

The first step is to configure your screen to compare two plots.

- At the Workstation Star Tool Bar, open PolyView2000 by double-clicking on the PolyView2000 icon.
- Configure PolyView2000 with only the Plot window active, hiding both the Spectra Manager and Library Manager windows.
- In the Plot window, plot out PV_SAM01.RUN at 254 nm.
- Drop down the Plot menu and select Duplicate Plot Window. Wait for the plot to appear and then select Arrange Visible under the Window menu.

Your screen should display two identical plots of the file. This is the most convenient screen configuration for determining peak purity because you can view the absorbance chromatogram in one window and the purity information in another.

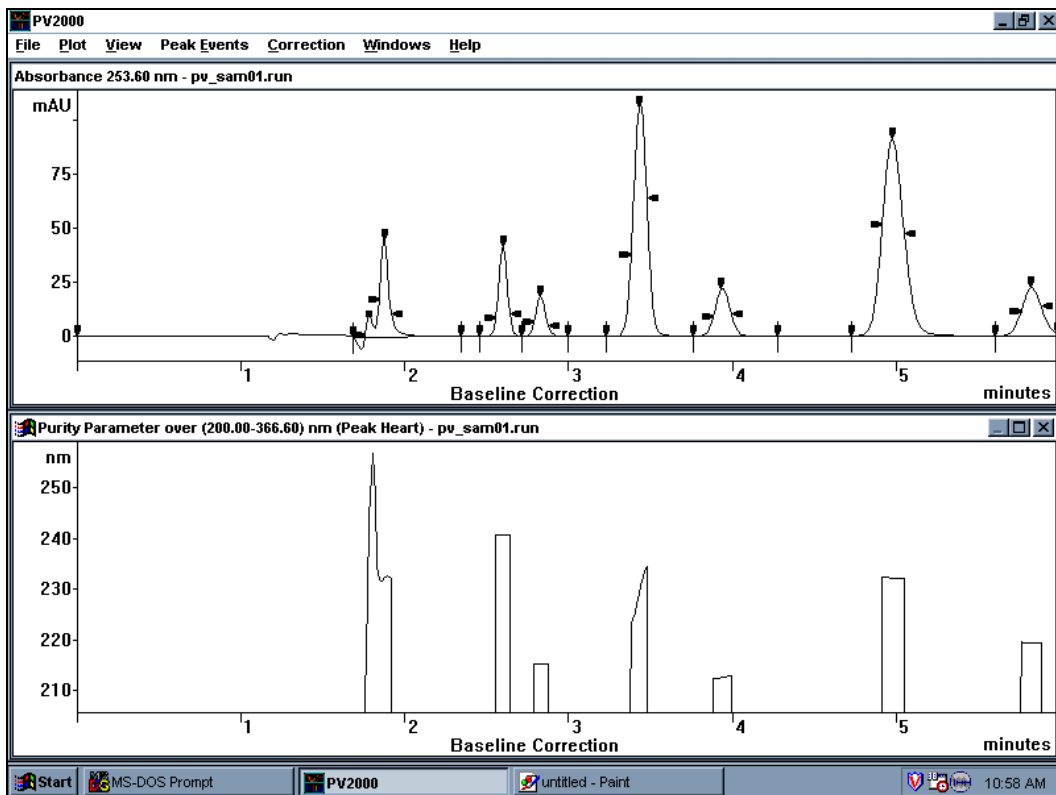


Duplicated Plot Window

You must now specify the Purity Parameter plot.

- With the focus on the bottom window, drop the Plot menu and select Plot Parameters.
- Specify a Purity Parameter plot with a wavelength range of 200-366 nm. Accept the defaults for all of the other parameters.

The Purity Parameter is now plotted as a function of time between the upslope and downslope inflection points of each peak. Notice that the peak at 3.5 minutes has a sloping top, indicating that the Purity Parameter is changing across the width of the peak.



*Purity Parameter Plot indicates impure peak
at 3.5 minutes*

You can easily verify the spectral dissimilarities in the impure peak by transferring the upslope and downslope spectra to the Spectra Manager window. In either Plot window, extract spectra corresponding to the upslope and downslope of the peak at 3.5 minutes. Activate the Spectra Manager window and observe the differences in the two spectra.

NOTE: The peak at 3.9 minutes in PV_SAM01.RUN also has a significant impurity that can be clearly detected by plotting the Purity Parameter over the range of 224-278 nm.

Don't close PolyView2000. Instead, go on to the next tutorial.

Tutorial: Peak Purity Survey Report

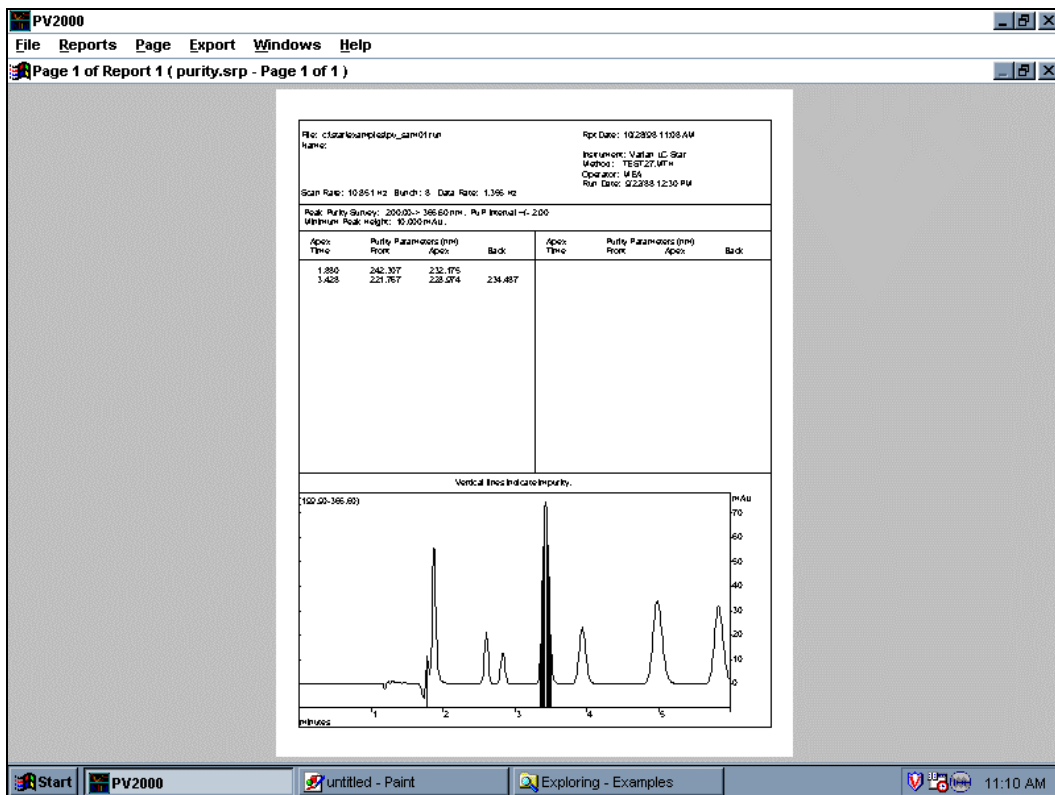
The Peak Purity Survey report is a non-interactive report and therefore must be specified in the Method Builder application.

- Call up the Reports window and create a new report method (see *Tutorial: Making a Report* on page 62).
- In the Method Builder application, select Add and set the type of report to Peak Purity Survey and Say OK.

In the dialog box, you can specify the conditions for report generation. For an example of a peak purity survey report, see *Appendix B*.

- Set the PuP Interval field to 2 nm.
- Set the wavelength range to 200-367 nm and leave all other parameters at their default conditions.
- Select Done and assign the report method the name of PURITY.MTH. (Use the Save As command under the File menu.)
- Close the Method Builder application by clicking on the Control menu in the main application window.

Now, let's use the report method to determine peak purity on a file. Back in the PolyView2000 Reports window, create a report of the data file PV_SAM01.RUN using the PURITY.MTH method. Call the report file PURITY.SRP (see *Tutorial: Making a Report* on page 62). The report displays the absorbance chromatogram (at the wavelength used for peak sensing) with impure peaks shaded. (Note that the peak at 3.5 minutes is indicated as being impure.



*Peak Purity Survey Report showing
impure peak at 3.5 minutes*

Close PolyView2000 by double-clicking on the Control Menu box in the main application window.

Performing Peak Sensing

Several functions within PolyView2000 rely on an accurate determination of peaks in a chromatogram. To provide corrected spectra that accurately reflect eluting peaks, the baseline profile must be determined with precision. Also, many of PolyView2000's automated functions require the determination of peak placement within a chromatogram. It is the job of the peak sensing algorithm to accurately determine where a baseline segment starts and stops and where eluting peaks are located.

There are a number of user-selectable parameters that must be set to insure accurate peak sensing. As with most data systems, including the Interactive Graphics application in the Star Workstation, you must set the peak width and S/N value (the threshold parameter) according to the peak width and size you expect. In addition, since you are dealing with multi-channel data, in PolyView2000 the wavelength used for peak sensing must be specified. A single wavelength or a wavelength range can be specified. All of the peak sensing parameters can be time programmed to accommodate changing conditions over the course of a chromatographic run.

Like the reports method, a separate portion of the Star Workstation .MTH file is set aside for the PolyView2000 peak sensing method. This enables peak sensing to be performed automatically under the control of the System Control/Automation application. Along with this, the Method Builder application is used to specify and manage the peak sensing method. Within PolyView2000, a separate window is provided in which to manage the peak sensing functions. In this Peak Sense window you can view a chromatogram at the peak sensing wavelength, view and alter the detector noise monitor data, access the Method Builder application, and conduct peak sensing on a file.

The following tutorials cover the basic operations of constructing a peak sensing method and performing peak sensing on a data file. In the last tutorial, the procedure for defining a new noise monitor will be covered.

NOTE: For more detailed information on peak sensing in PolyView2000, including procedures to follow for troubleshooting peak sensing, refer to *Peak Sensing* on page 109.

In the following Tutorial, some of the files in the EXAMPLES directory will be modified and/or created. Prior to performing this tutorial, you should ensure that you are working with original copies of the data files called for. If you are not sure whether these files have been modified by previous use of the Tutorial, reinstall the PolyView2000 software from the CD using your serial number.

Tutorial: Building a Peak Sensing Method

From the Star Workstation Star Tool Bar, double-click on the PolyView2000 icon to activate the application.

Drop down the *Windows* menu, activate a New Peak Sense Window and maximize it.

You are now ready to build a new peak sensing method.

- Under the Peak Sensing menu, select *Edit Method*.
- To define a new .MTH file, select New Method in the dialog box. Accept the default bus address (all methods sections are attached to an address, used under automated operation). Control will be transferred over to the Method Builder application and the Peaksense Time Program dialog box will appear. In this window, the default peak sensing time program is in the list box at the bottom of the window. The operation of the edit controls in this window is similar to other windows within the Method Builder application. Modify the Peak Sensing Method as follows.

Peaksense Options/Value

Peak Width (sec): 8

Range (nm): 253.60 - 253.60

Add Delete Help

Time (min) Event

0.00 Peaksense Options/Value

0.00 Peaksense	253.60 - 253.60 nm 8 sec
0.00 S/N Threshold	5
0.00 Inhibit Integrate	0.01

The Peak Sense Time Program Window

- Click on the first line 0.00 Peaksense..., and alter the wavelength range fields to 273-273 nm. Note that, unlike previous versions of the Method Builder, the numbers change immediately in the Method listing and that there is no Change button. However, the parameters do not actually change in the method until you save the method.
- Click on the second line 0.00 S/N Threshold... and change the S/N value to 6.
- Click on the third line 0.00 Inhibit Integrate... and change the end time to 2.00 minutes. This ensures rejection of the region containing the void peak.
- Then, click on Add twice. This creates two new lines in the method.
- Click on the second of the new lines and change the event to Peaksense, the time to 16.0, and the wavelength range to 215-215.
- Click on the first of the new lines and change the event to *S/N Threshold*, the time to 19.5 and the S/N value to 10. Your screen should look like the following.

S/N Threshold Options/Value

S/N Threshold: 10

Add Delete Help

Time (min) Event

19.5 S/N Threshold Options/Value

0.00 Peaksense	273.00 - 273.00 nm	8 sec
0.00 S/N Threshold	6	
0.00 Inhibit Integrate	2.00	
16.00 Peaksense	215.00 - 215.00 nm	8 sec
19.50 S/N Threshold	10	

The New Peak Sensing Time Program

Next, assign a name to the method and save it to the disk.

- Drop down the File menu and select Save As.
- Save your method as PSENSE.MTH in the EXAMPLES directory.
- Close the Method Builder application and note that control is passed back to PolyView2000 right where you left off.

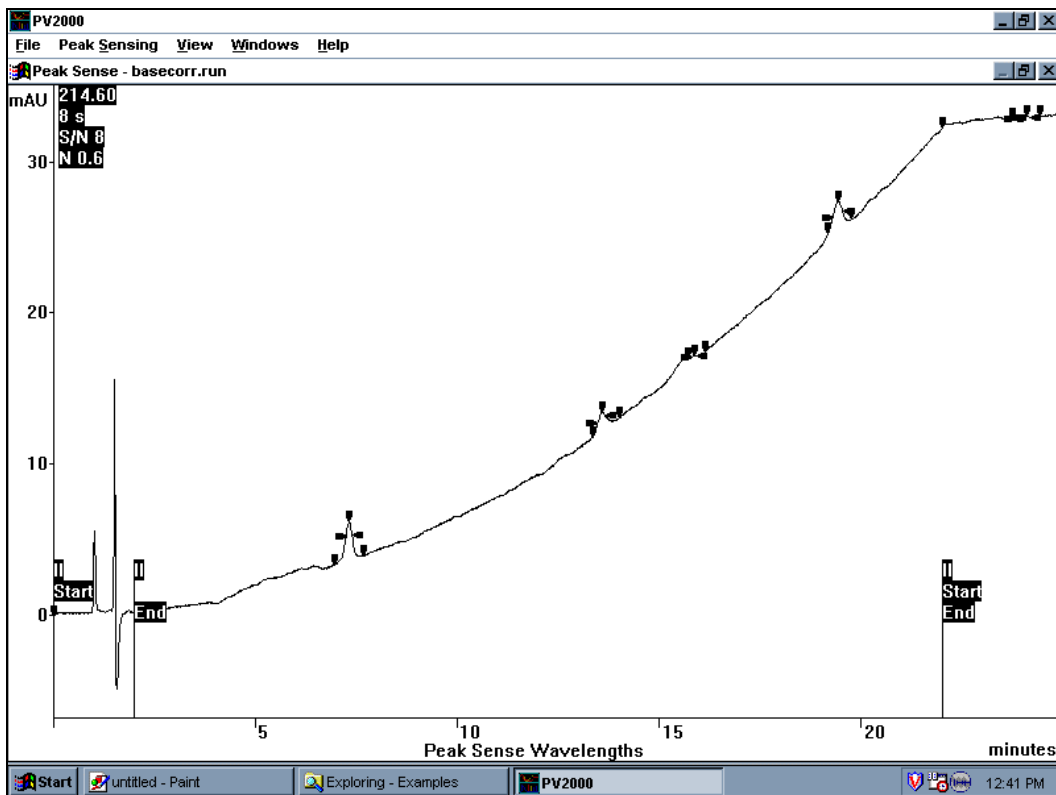
Don't close PolyView2000. Go on to the next tutorial to learn how to use the method you built to perform peak sensing.

NOTE: The above tutorial covered the building of a NEW peak sensing method. PolyView2000 also allows you to edit an existing peak sensing method by selecting Edit Method under the Peak Sensing menu and specifying an existing .MTH file in the File Select dialog box. Control is transferred to the Method Builder application with the specified .MTH file already loaded. It can then be edited and saved for use later.

A similar feature allows you to extract the peak sensing method directly from an existing .RUN file even if the original .MTH file used to generate it doesn't exist on the disk. To do this, select Edit Current Method under the Peak Sensing menu. The dialog box that appears allows you to generate a .MTH file with the same root as the .RUN file. Simply select OK in the dialog box that appears and control will be transferred to the Method Builder application with the .MTH file just created loaded into the window. If a file by that name already exists on the disk in the current directory, you will be given the opportunity to verify your selection to prevent inadvertently overwriting it.

Tutorial: Conducting Peak Sensing

To begin this tutorial, your screen should be configured as in the last tutorial, with a single Peak Sense window. The task will be to perform peak sensing on BASECORR.RUN using the method you built in the last tutorial. Drop down the *File* menu and select *Open File*. Select BASECORR.RUN in the EXAMPLES directory and then *OK*. The file is plotted in the window.

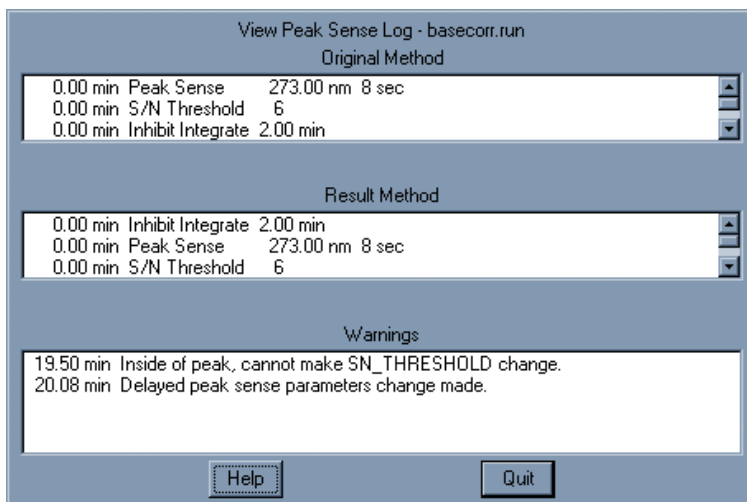


BASECORR.RUN in Peak Sense window

NOTE: The peak events are displayed in both the Peak Sense window and the Plot window (optional). These events are color coded as follows: White-Apex, Red-Peak Start, Yellow-Peak End, Purple-Inflection points, Dark Blue-Valley points, and Lt. Blue-Shoulder. The placement of peak events in a chromatogram indicates how well the peak sensing method was tailored to the actual chromatogram. For more information on how PolyView2000 conducts peak sensing and how to assess peak sensing results, see [Peak Sensing](#) on page 109.

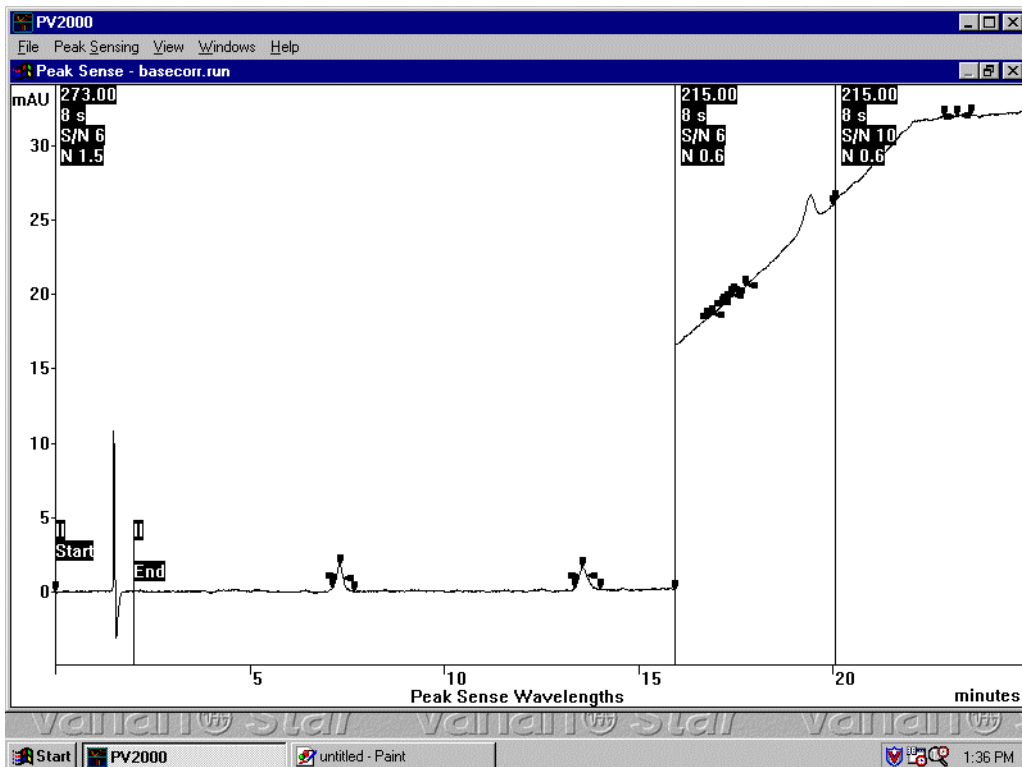
Now, let's perform the peak sensing operation. Select *Do Peak Sensing* under the *Peak Sensing* menu. In the dialog box, select the file you created, PSENSE.MTH, in the EXAMPLES directory. Notice that the peak sensing method is listed on the screen. Select OK. Peak sensing is automatically performed.

Often, the peak sensing method you specify cannot be executed exactly as it is called for in the .MTH file. For instance, time-programmed changes in peak sense parameters (wavelength, peak width, etc.) cannot be made within a peak. When this occurs, a dialog box appears that informs you of this situation. This is the case for the BASECORR.RUN file and the View Peak Sense Log dialog box is displayed on the screen. The Peak Sense Log shows the original method you specified, the resulting method (as executed), and the reasons why it was modified. After viewing the information, select Quit.



Peak Sense Log showing changes to method

Following the peak sensing function, the display is updated to reflect the new peak sense results.



New Peak Sense Results

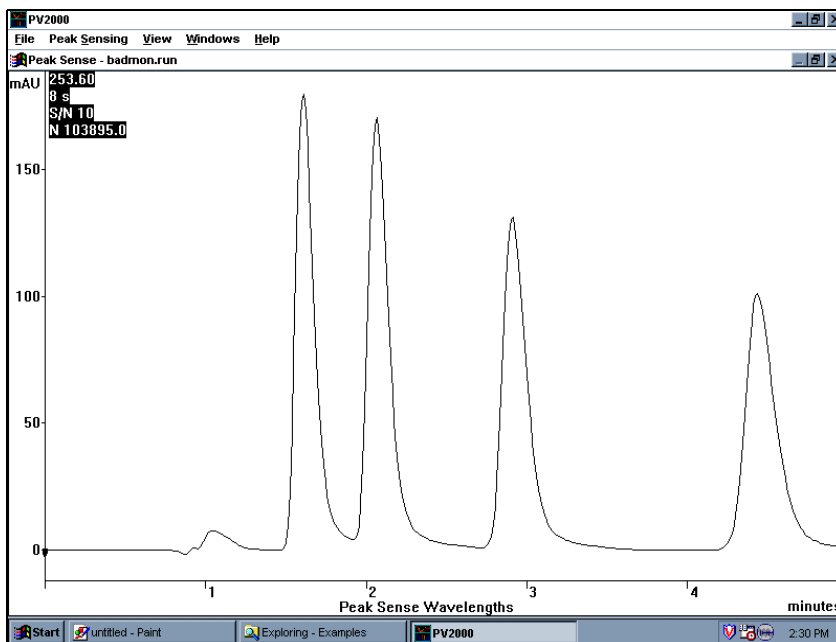
The chromatogram display in the Peak Sense window is very similar to the display in the Plot window, but it only displays the time-programmed absorbance specified in the Peak Sensing Time Program. Flags at the top part of the screen indicate where time-programmed peak sense events were executed. By default, the whole file is displayed and automatically scaled. You can use the mouse and the View menu to modify the time and amplitude settings.

Close PolyView2000 by double-clicking on the Control Menu box in the main application window.

Tutorial: Troubleshooting Peak Sensing

Attached to all .RUN files is noise data transmitted to the Star Workstation from 330 when it is in the MONITOR state, just prior to the start of the run. During the peak sensing operation, PolyView2000 determines the level of chromatographic noise from this monitor data and differentiates peaks from noise using the S/N ratio you set in the peak sensing time program. Occasionally, the run monitor associated with the .RUN file may not be accurate, possibly due to the presence of a late eluting peak from a previous run or a gradient program that had not yet stabilized prior to the run monitor period. PolyView2000 provides the ability to redefine the data used as the noise monitor in these situations and this function will be illustrated here. Configure PolyView2000 with a single Peak Sense window and call up BADMON.RUN from the disk.

NOTE: See **Peak Sensing** on page 109 for more information on how PolyView2000 conducts peak sensing and how to set the peak sensing parameters.

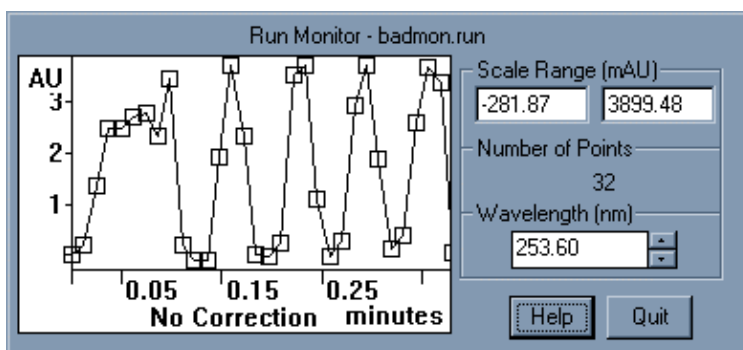


BADMON.RUN showing no detected peaks

If the file is in the original state, you will notice that the peak sensing function did not detect any peaks and the noise value at the upper left corner of the screen is very high. If this is not the case, this tutorial has already been executed on the file; restore the file to its original state by executing the *Remove User Monitor* command in the *Peak Sensing* menu.

You can examine the run monitor:

- Drop down the File menu and select *View Run Monitor*. You will see that several high absorbance spikes occurred during the monitor.



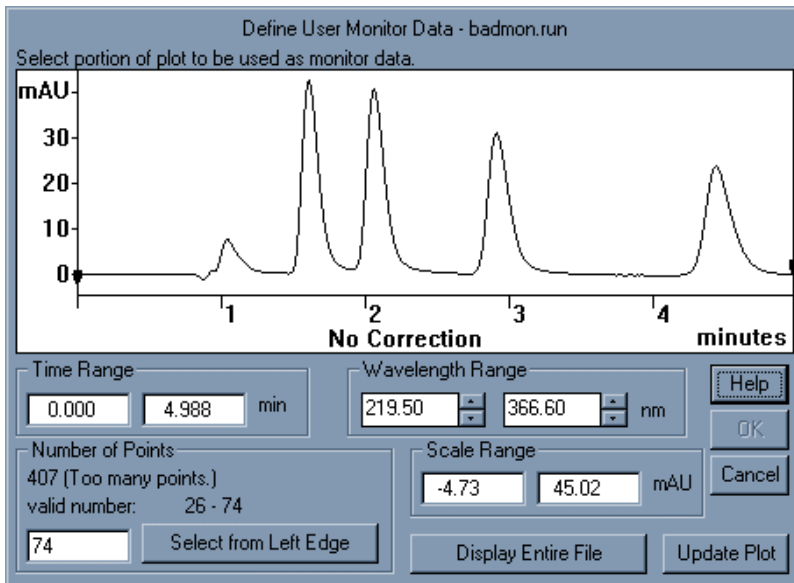
The Run Monitor dialog box

- Using the wavelength control, you can view the run monitor data at any of the diodes which were used to collect the data. Select Quit to close the dialog box.

If a file has a noise monitor that is corrupted or inappropriate, like BADMON.RUN, you can select a portion of the baseline in the chromatogram to be used for the noise calculation. This is called the User Monitor and after it is specified, peak sensing is automatically repeated.

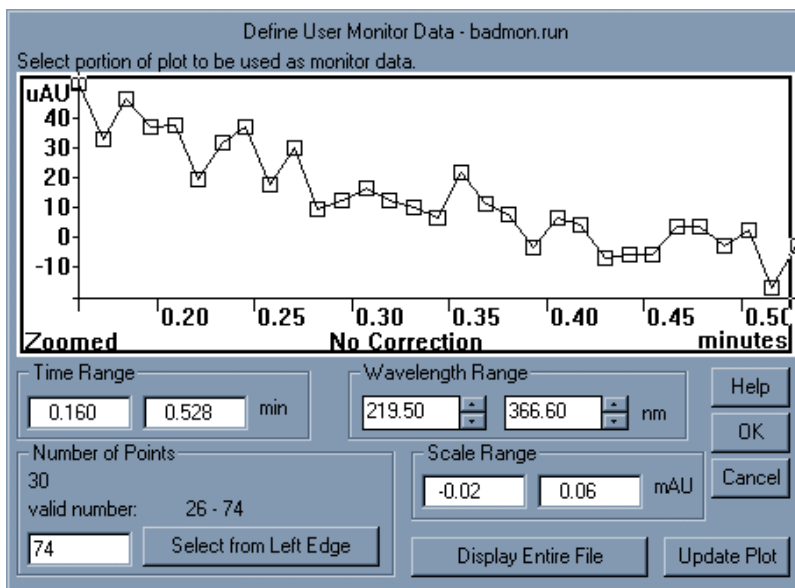
- Drop down the Peak Sensing menu and select Define User Monitor.

The controls in the dialog box that appears are used to select a portion of the file as the noise monitor.



The Define User Monitor dialog box

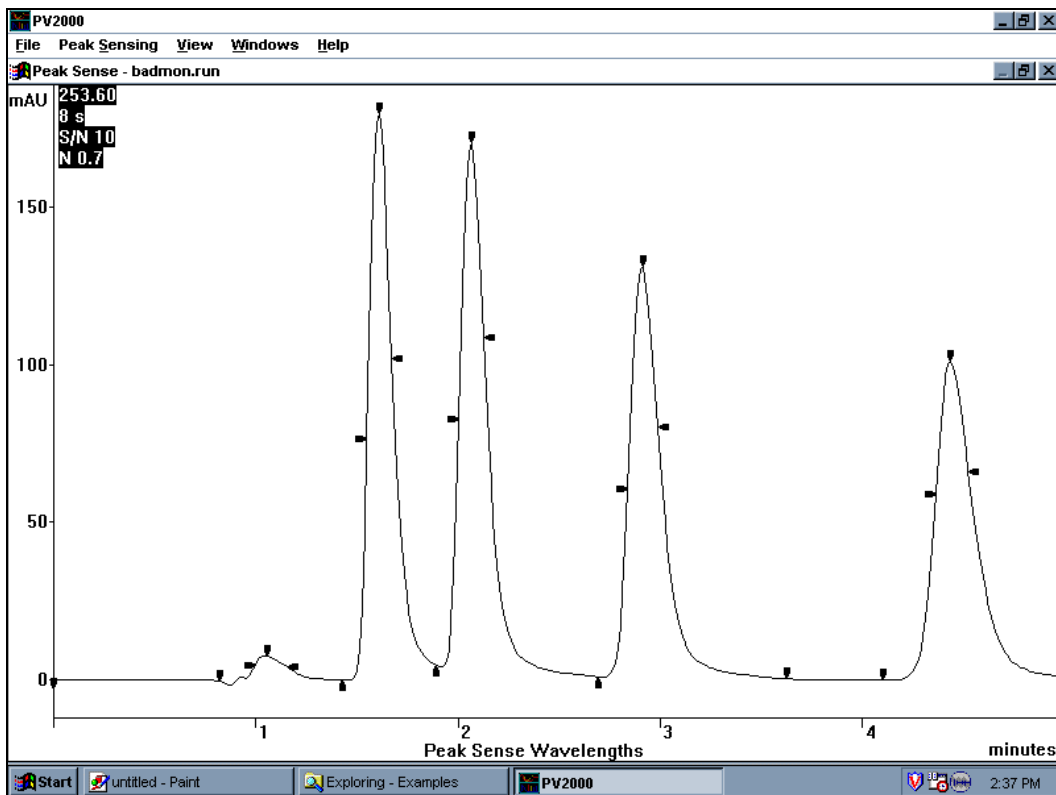
- Select a portion of the baseline between 0 and 1 minute using the click and drag method. When you do this, the plot will zoom and a message will be displayed in the Number of Points field. If the piece of baseline is too large, *Too many points* will be displayed and you will need to select a smaller portion of the baseline until the proper number of points is selected. Use *Select from Left Edge*. Try for a number between 26 and 74. When assigning the User Monitor, be sure to use a flat portion of the baseline, free from eluting peaks. To autoscale the plot after selecting a new region, double-click left of the Absorbance axis, where the cursor becomes a double-headed vertical arrow.



Section of Baseline chosen as User Monitor

- After the number of points is refined, select OK and peak sensing will automatically be performed using this new monitor.

When the plot is displayed, you will note that normal peak detection has occurred (see the following figure). Also, the new noise value is displayed in the upper left corner of the screen. The new monitor data can be viewed in the same manner as before. This time, select View User Monitor under the File menu. The dialog box that is displayed is very similar to the View Run Monitor dialog box.



*BADMON.RUN with proper Peak Sensing
after definition of a User Monitor*

Close PolyView2000 by double-clicking on the Control Menu box
in the main application window.

Automating PolyView2000 Operations

One of the primary features of the PolyView2000 Spectral Processing Application is the ability to conduct automated analyses of the spectral data from the 330 or 9065 diode array detectors. Instead of having to process the data manually after it is collected, the Workstation has the ability to perform many of these functions automatically, immediately after data collection. The PolyView2000 functions that can be performed in this manner include peak sensing to locate the peaks in a chromatographic run and any of the non-interactive reports. The functions in the non-interactive reports allow you to perform sophisticated analyses of diode array spectral data in an automated fashion.

You can automatically add PolyView2000 functions to the processing of a diode array data file at the end of each chromatographic run by adding a PolyView2000 method associated with the detector (by means of the bus address) will be executed. During an automated sequence of injections, the PolyView2000 reports that are generated are written to a permanent disk file with a .SRP extension. These reports will also be printed automatically if the Automated Printing is enabled in System Control (at the bottom of the *Automation* menu). If you don't wish these reports to be printed automatically, simply disable the autoprint function. The .SRP files will still be generated and will be available for viewing in the Reports window. In addition to the generation of PolyView2000 report files during live runs, these .SRP files can be generated during a Recalculation sequence in System Control.

Using On-Line Help

There are three ways to access On-Line Help in PolyView2000: the F1 key, the Help menu, and the Help push button which is present in most dialog boxes.

Tutorial: Using On-Line Help

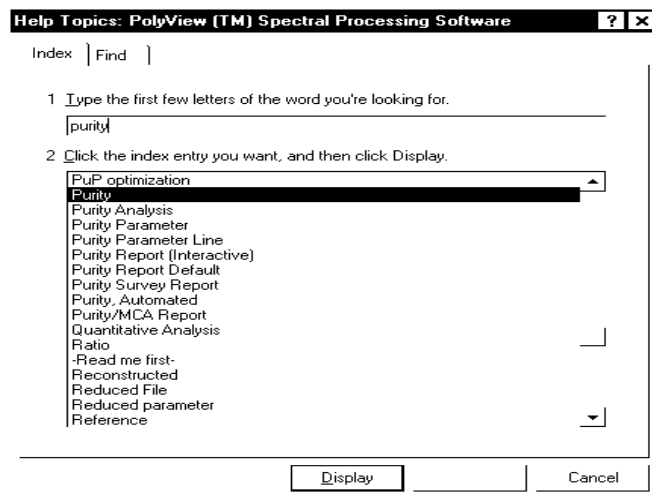
Pull down the Help menu. Select *Using Help* to display the information file provided with Windows. Learn the basics, then close the Help window. Also try Index, Commands, Keyboard, Procedures.

Select the MCA Manager window, then press the F1 key. The Help window appears, displaying information relevant to the MCA Manager. Maximize the Help window. Note that the abbreviation MCA is underlined with a dotted line. This indicates a defined term. Move the cursor over the word MCA. Note that the cursor turns to an index. Click the mouse, and the definition is displayed as a pop-up definition. Click again, and the pop-up disappears. Now move down to terms with a solid underline. These represent Hypertext jumps, which means that selecting such an item by a single click will send you to another topic. Try *Computing Area Spectra*. You can come back to the previous topic using the *Back* pushbutton above the help text.

You can also print any topic by using the *Print Topic* command in the *File* menu of the Help window.

Display the Preferences dialog box by pulling down the File menu and selecting Preferences. Press the Help pushbutton. Maximize the Help window and scroll through the information. There is such a Help pushbutton in most dialog boxes in PolyView2000.

Try a keyword search. Press the *Index* button in the Help window, and start typing a keyword, for example Purity. Note that as you enter more characters, a list of keywords is scrolled to the point where it best matches what you requested.



The Keyword Search Dialog Box

Now select one of the keywords and press the *Display* button inside the dialog box (or press the Enter key).

Quantitative Analysis by MCA

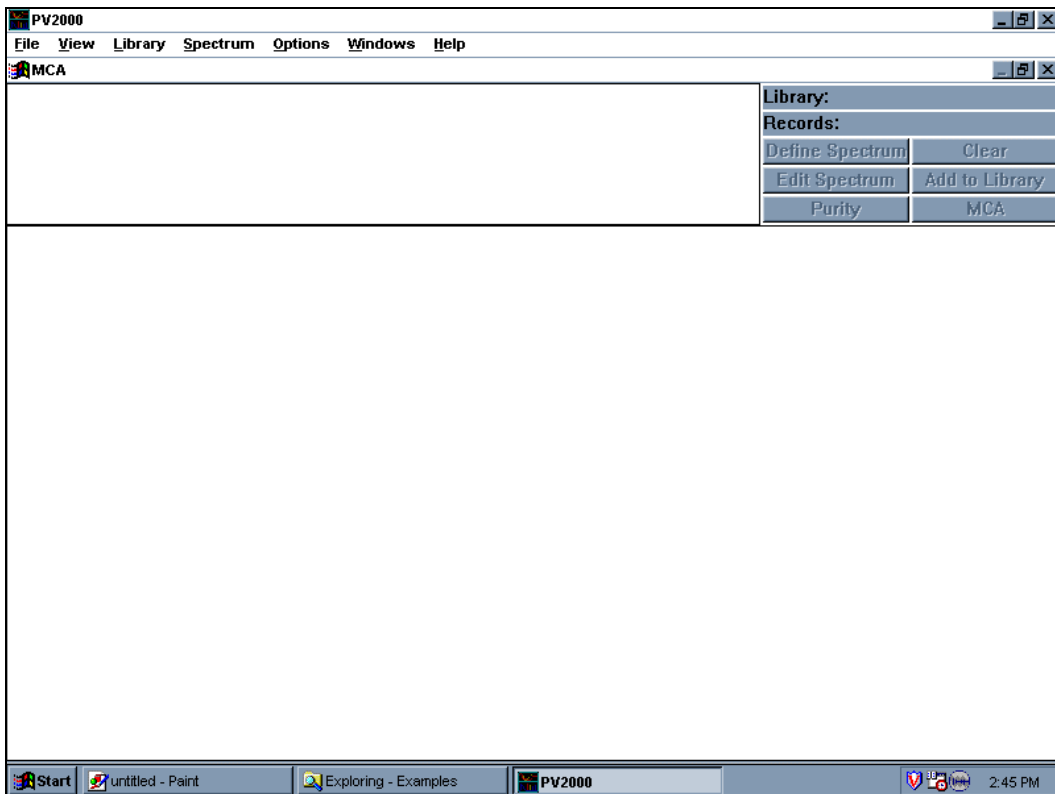
Multicomponent Analysis is a proven technique, routinely used in spectrophotometry to determine the composition of a mix of known components in unknown amounts. In PolyView2000, an original adaptation of the technique provides easy quantitative analysis of groups of up to six overlapping peaks, regardless of the chromatographic resolution (*J. Chrom*, 631 (1993) 15-21).

This requires that the components have different enough spectra. If this is not the case, the report will let you know. The one-page MCA report provides amounts, peak profiles, and an evaluation of the quality of the decomposition. MCA can be invoked interactively or under automation.

In PolyView2000, you can also define a peak as an internal standard to refine the quantitation results. This process is documented in On-Line Help and is only available in the non-interactive MCA report. You can obtain such reports interactively by using the PolyView2000 Reports window to create and display a report including MCA commands specifying the use of the Internal Standard.

Tutorial: Using the MCA Window for Quantitative Analysis

Create an MCA window, using the *New...* command in the *Windows* menu. The MCA window will automatically fill up the PolyView2000 workspace.



The MCA Window

Pull down the *File* menu and select *Open File*. Select DIPA3020.RUN in the EXAMPLES directory. A chromatogram is displayed on the bottom half of the window. It contains one major peak of DIQUAT, which we will use as a reference.

To integrate this peak, press the *Define Spectrum* pushbutton. Click in front of the peak, on the peak start marker at about 1.8 minutes, then double-click on the peak end marker at about 3.3 minutes. This defines integration limits and baseline correction spectra for this peak (for more details, see On-line help on computing Area Spectra). A dialog box requests you to provide information to be saved along with this spectrum. Enter Diquat in the peak name field, 10 for the Amount and ug for the unit (Note that the flow rate was read from the file). Press *OK* after all the fields have been updated. The MCA window now shows the area spectrum and the integrated region is highlighted.

Enter Area Spectrum Values

Name: 0.1D

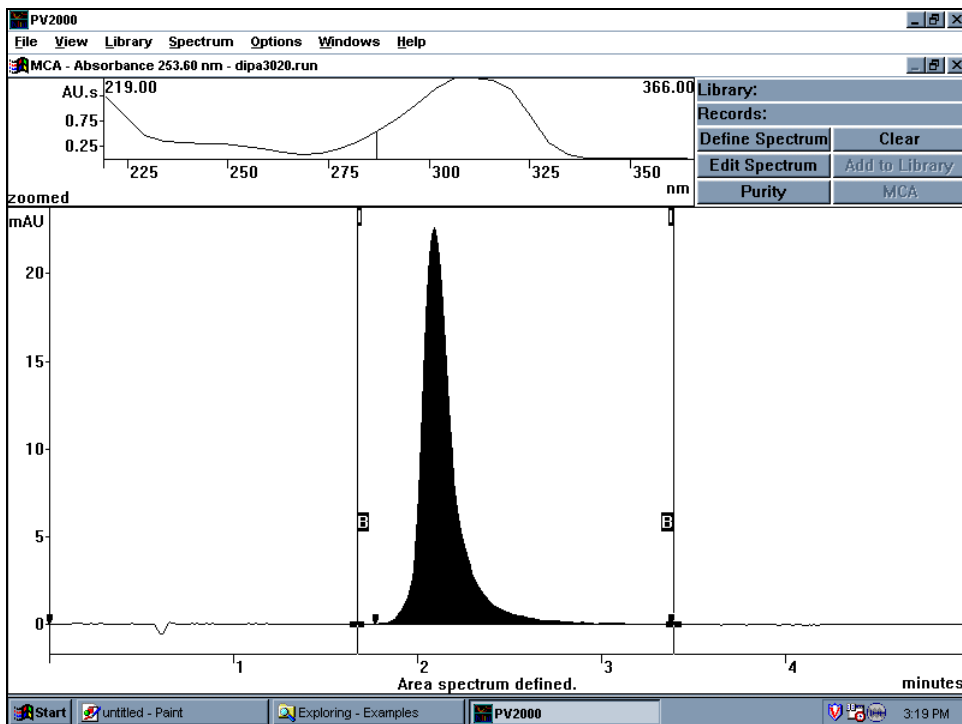
Flow Rate: 2.000 ml/minute **Flow read from PUMP Method Log**

☐ Reference Amount

Amount: 1 Units: X

Help OK

The Amounts Dialog



The MCA window, showing the integrated region and the Area Spectrum

Create a new library, using the *Create Library* command in the *Library* menu. Name it PQDQ.LBR. We will now compute area spectra and add them to this library.

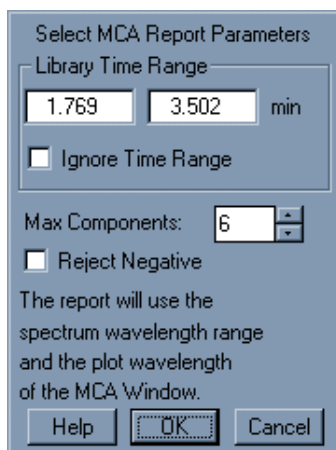
Add the spectrum to the library by pressing *Add To Library*.

Now repeat the process with DIPA3037.RUN. The compound name is Paraquat, the amount 10 and the unit ug. Add the spectrum to the library. Create a new library, PQ.LBR, and press *Add to Library* again. At this point, you have created 2 libraries: PQDQ.LBR contains Paraquat and Diquat spectra, while PQ.LBR contains Paraquat only.

Now repeat the integration process described in step 3 with DIPA3053.RUN. This is an unknown mix, so do not enter any values in the Amount and Unit fields. Instead, just press OK.

Pull down the Library menu and select Open Library. Select PQDQ.LBR.

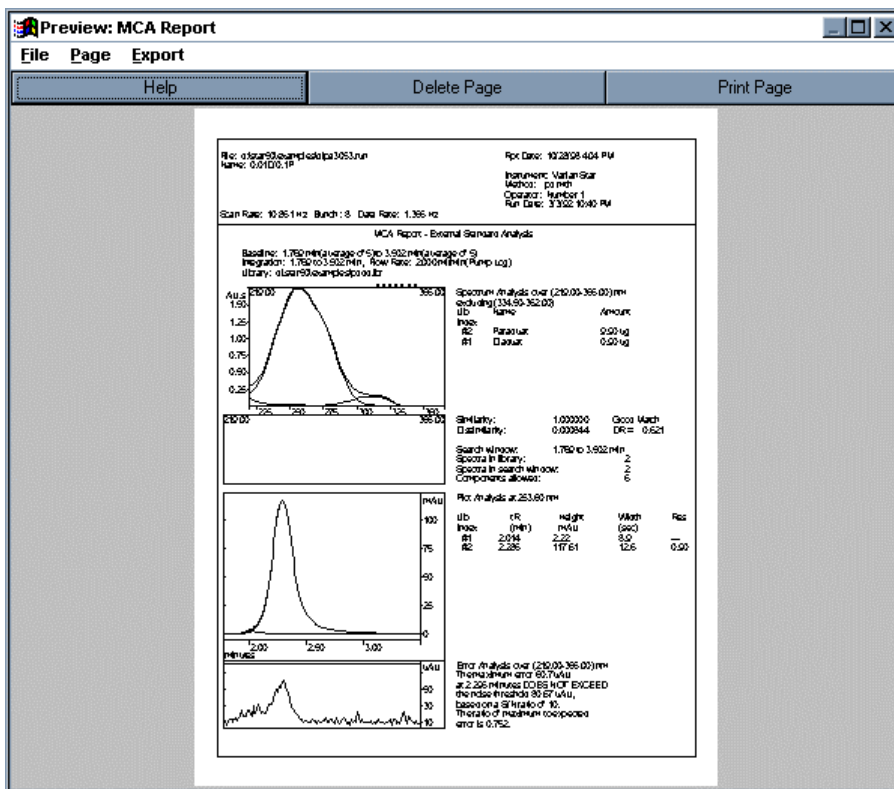
Press the MCA button. Press OK in the dialog box requesting additional parameters. This displays an MCA Report in a Preview window. This report has 3 main components: the Spectrum Analysis, the Plot Analysis and the Error Analysis. You can use the mouse to zoom in on a particular region of the report. To do this click on the left mouse button and drag to highlight the area of interest. Double-click to restore. Note that the right half of the Spectrum Analysis provides quantitative results, while the right half of the Plot Analysis provides qualitative results such as retention times and resolution. The Error Analysis compares the residual error in each point of the chromatogram to the noise. See On-Line Help, Interpreting MCA reports for more details. Press Print Page if you want to obtain a hardcopy, then Delete Page to terminate the Preview.



The MCA Report Parameters

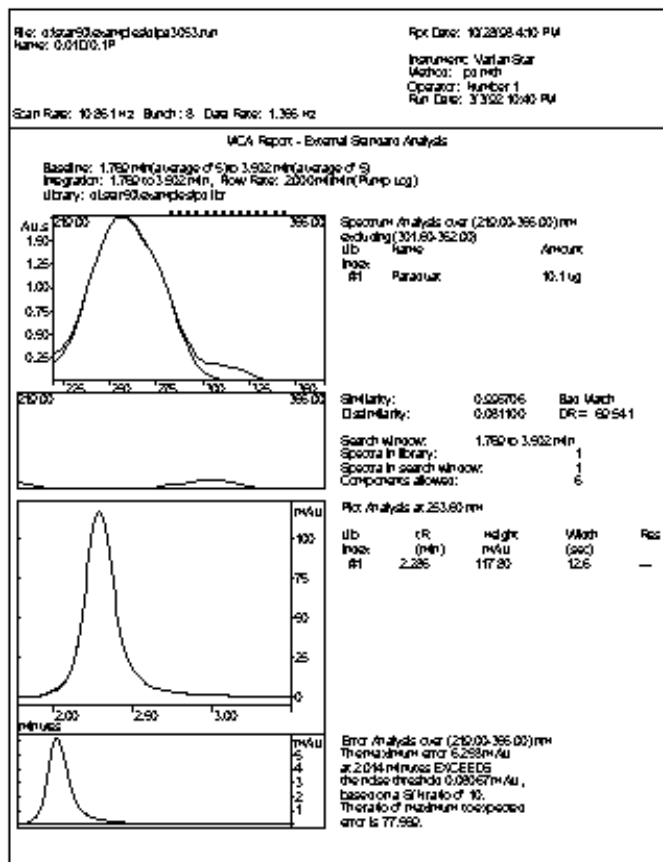
NOTE: The MCA process uses up to six spectra from the library which match the specified time range. If more than six spectra fit in the range, only the closest to the center of the range are used. As all component spectra must be independent, avoid placing multiple copies of the same spectrum in a library.

When only one component is specified, the best match in the time range is determined based on spectral comparison, not retention time.



MCA report in the absence of unexpected impurities

Now select Library PQ.LBR (Library: Open Library), which contains only Paraquat, and press MCA. Press OK, and observe the resulting report. The MCA process fails to account for all of the fused pair of peaks, since not all components were in the library. The Error plot indicates the location and the peak profile of the impurity, which greatly exceeds the noise level. The quantitation of the compound that was present, Paraquat was correct as the amount in the mixture was 10 ug. The MCA process is able to detect any significant variation in the spectra. Close the preview window.



MCA Report showing major impurity

Tutorial: Automating the Quantitative Analysis

Create a Report window using the *New Reports Window* command from the *Windows* menu. Pull down the Reports menu, select *Edit Method*, and press *New Method*. Click on *OK* in the dialog box to get to the Method Builder Application. In the Method Builder Application, set the report type to *Multicomponent Analysis* by pressing the Add button and selecting Multi-Component Analysis. Your screen should look like the figure below after you select the PQDQ.lbr.

The screenshot shows the 'Multi-Component Analysis' dialog box with the following settings:

- Peak Time Range (min): 0.00 - 0.00
- Reject Peaks (<mAU): 0.00
- Baseline Determination: # of spectra: 5
- Spectrum Range (nm): 495.00 - 495.00
- Plot wavelength (nm): 495.00
- Default flow rate (ml/min): 1.000
- Report Type: Text + Graphics
- Select Library: File: pqdq.lbr, Select... button, d:\star50\examples\pqdq.lbr
- Max. number of components: 6
- Relative Retention Time Range: 0.80 - 1.20
- ☐ Reject Negative Amounts
- ☐ Use Internal Standard
- Buttons: Help, Done, Cancel

The MCA Report Window in the Method Builder

Set the peak time range to 1.5 to 5 minutes and the wavelength range to 219 to 366nm and the Plot wavelength to 254nm. Select Done, save the method as MCA.MTH and close the Method Builder application.

In the Reports window, pull down the Reports menu and select Create Report. Select file DIPA3053, method MCA.MTH, and set the report name to MCA.SRP. The report produced is very similar to the one obtained interactively. Close the Report window.

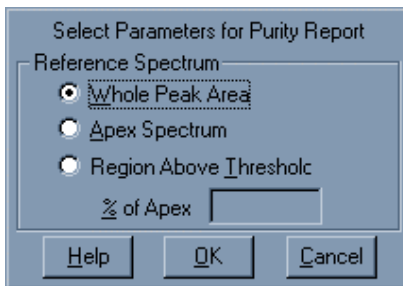
Note that unlike the interactive MCA, in which you decide how to integrate the peak, the automated MCA process relies on the PolyView2000 Peak Sensing for the determination of the peak start and end. Bad peak sensing would result in poor MCA analyses. See [Peak Sensing](#) on page 109.

Purity Evaluation by MCA

In the absence of reference spectra, a peak can be analyzed by MCA on its average spectrum, its Apex spectrum, or a heart cut based on a height threshold. This is used to assess Peak Purity. The Error Analysis portion of the Purity Report will report any significant fluctuation of the shape of the spectrum across the peak.

Tutorial: Using the MCA Window to Evaluate Purity

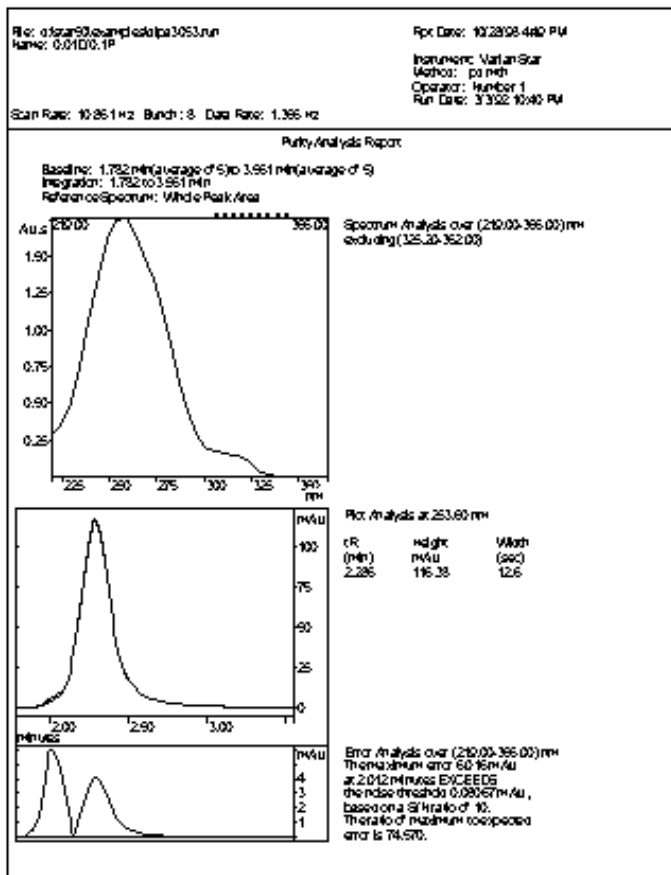
In the MCA window, open file DIPA3053.RUN. Integrate the peak as described in previous tutorials, then press *Purity*. A dialog box lets you choose options for the Purity Report. The purpose of the second and third options is to avoid lumping in too much baseline noise in the reference spectrum, which might be a problem at low levels or with a long tailing peak. When you select *Whole Peak Area*, the resulting report analyzes the peak on its average spectrum. The Error Analysis plot will show whether or not the peak is spectrally homogeneous. Print the report if desired and close the Preview window.



Purity Report Parameters

For more information, see On-Line Help on Interpreting MCA Reports.

Note that you should use this feature to check that a reference spectrum comes from a pure peak PRIOR to adding it to a library. Always screen your standards in this manner prior to adding them to a library.



MCA Purity Report

The Purity Report can also be produced under automation. Select *Purity Analysis* as the report type in the Method Builder.

In PolyView2000, a computation of molar extinction coefficients has been added as an option to the Purity Report. Based on a chromatographic run, this feature provides in a single step the determination of the extinction coefficients at all wavelengths monitored and documents the purity (or impurity!) of the sample. Search On-Line Help for 'Extinction Coefficient'.

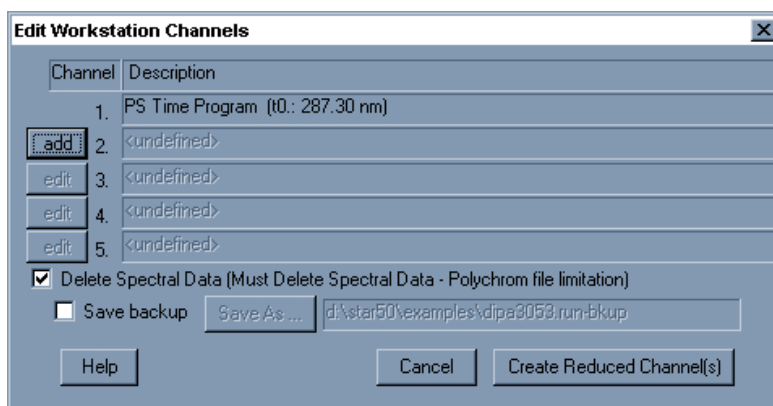
Creating Workstation Channels

You can create a reduced file containing 1 to 5 channels of time-programmed absorbance data, and full spectra for the original peak events.

A reduced file will take up much less storage space than a complete diode array file. Also, a reduced file will allow the workstation software to process one or more chromatograms where the processed wavelength changes during the run. Usually only the monitor channel data will be automatically processed by the data handling on the workstation.

In the 9065, the workstation channels replace the original data, as it does not have full spectral data for each time point on the chromatogram. It does have enough data to calculate the purity parameter on the upslope, peak apex and downslope as well as do library searches and spectral comparisons. It does not have enough data to do MCA quantitation or the peak purity survey. For 330 data, the workstation channels are stored independently of the spectral data. You still have the option of deleting the spectral data if reducing file size is desired.

To create workstation channels, pull down the File menu item and select Create workstation channels.



Edit workstation channels dialog box.

By default, the first and only channel is defined by the current peak sensing tune program. The other four channels can be time programmed using the button. Once this is programmed, using the Create Reduced Channels button creates a file containing the reduced channels.

The file name used for the reduced channels is derived from the original filename by appending :"_bkup" to the filename, e.g., mydata.run will be saved as mydata_bkup.run. In order to save the original file with all of the spectral data, check the Save Backup check box. This will rename the existing file with the extension run-bkup. This file can be reprocessed as any other diode array file.

Library Searches

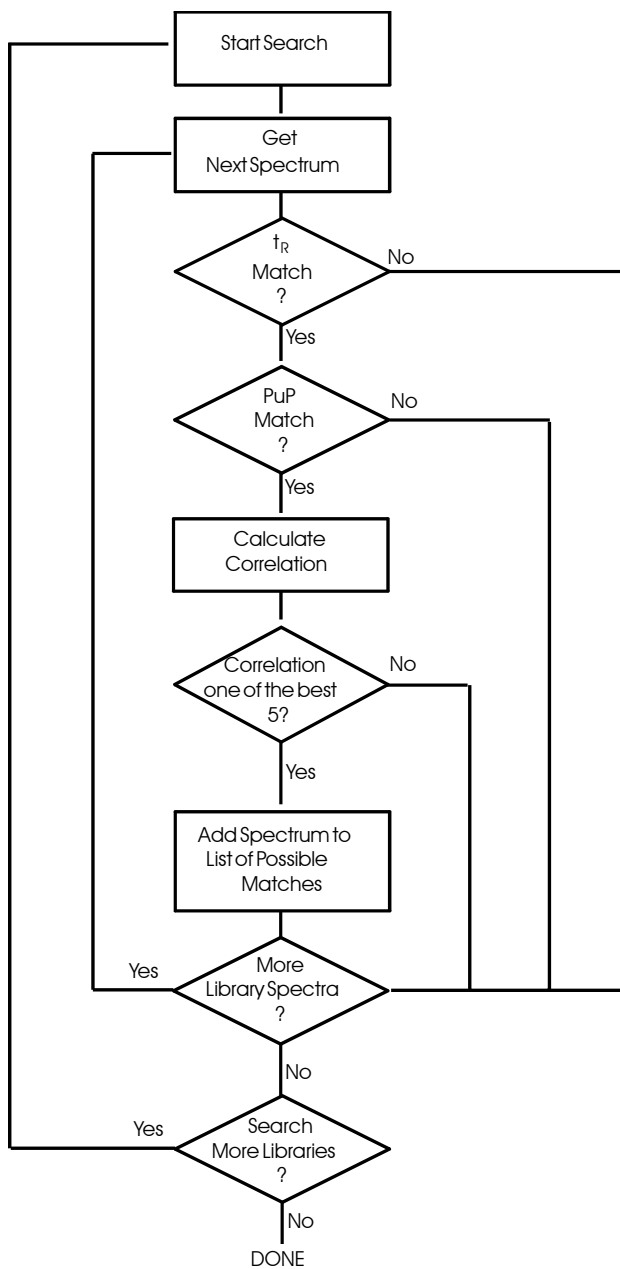
The advanced spectral searching capabilities in PolyView2000 allow you to match unknown spectra from eluting peaks with spectra stored in a spectral library. This provides valuable information for confirmation of peak identity. The spectral similarity indices provided during the library search along with retention time information can be used to quantify the degree of confidence in identifying the unknown peak. With a little experience, you will be able to develop criteria based on your particular application for the identification of eluting peaks based on these indices.

Library searches in PolyView2000 are quite flexible, allowing the user to establish a number of criteria to narrow down the search. Prior to conducting the search, you are allowed to set windows for retention time, Purity Parameter (PuP) and dissimilarity within which the search will be conducted. For instance, if you are using the exact same method for the analysis of unknowns as for the standards from which the library was built, you may decide that the unknowns should have the same retention time ($\pm 10\%$) as the standards. You would then set a relative retention time tolerance of 0.1. For Non-interactive reports, set the Relative Time field to 0.9-1.1 and for Interactive searches set the search window to $\pm 10\%$ of the retention time of the target spectrum. Similarly, a Purity Parameter window can be defined. When a library search is conducted, PolyView2000 considers all those spectra in the search libraries that are within the retention time and Purity Parameter windows. Those spectra meeting these first two criteria are considered as possible matches and similarity and dissimilarity indices are computed. Matches exceeding the dissimilarity threshold are discarded. They are then ranked according to the similarity index and the best five are listed in the report (except the short reports, where only the best match is reported).

The figure below illustrates the processes involved in conducting a library search for a spectrum in PolyView2000.

The similarity and dissimilarity indices are the primary means of assessing the degree of match between the target and library spectra. There are a wide variety of factors that can influence the match quality. Listed below are a number of factors that can lead to poor spectral matches in PolyView2000:

- Spectral shifts due to solvent differences (pH, ionic strength, type of solvent, etc.).
- Concentration differences between the target spectrum and library spectra.
- Chromatographic noise at low S/N ratios.
- Co-eluting compounds within the analyte peak
- Inaccuracies in the gradient composition and/or badly sloping baselines during gradient separations.
- Spectral characteristics of the analyte (i.e., presence or absence of unique features in the UV spectra).

*PolyView2000 Library Searches*

Following the library search, the degree of match is quantified from the similarity and dissimilarity. It is up to the user to establish guidelines unique to their particular application for the interpretation of the match criteria presented. The statistics report obtained from the Spectra Manager window can be used to assist the user in formulating guidelines for similarity and dissimilarity values, by providing the PuP mean and standard deviation, as well as the best and worst similarity and dissimilarity for a set of spectra. Using these computations, you can determine the degree of expected correlation for your particular application. Then, based on these numbers, you can set up the library search parameters to best meet your needs.

Under routine conditions, the following table can be used as a guide for interpreting similarity and dissimilarity values in library searches. Keep in mind that a number of factors can lead to poorer match indices for identical peaks. You should qualify the expected values based on your particular application, using the statistical functions in the Spectral Manager window.

Table 1 Library Matches in PolyView2000

Match Quality	Similarity Range	Dissimilarity Range
Good	1.0 - 0.998	0.0 - 0.06
Poor	0.998 - 0.980	0.06 - 0.20
Bad	<0.980	>0.20

Note that in the MCA reports, the goodness of fit is compared to a predicted threshold deduced from the monitored noise values. Search for "Interpreting MCA Reports" in the On-Line Help to obtain the detail of the algorithm use. The On-Line Help also details how to customize the match criteria and disable the good/poor/bad match estimation made by the software.

Peak Sensing

A number of functions in PolyView2000 require an accurate means of detecting the presence of peaks in a chromatogram. It is the function of the Peak Sensing algorithm to determine the profile of the baseline and the presence of peaks on the baseline. The peak sensing in PolyView2000 is separate from the Workstation's peak sensing due to the different needs of the two programs. For peak sensing, the user must specify the wavelength (or range) to be considered, the peak width (in seconds at half height) and the desired threshold (expressed as a S/N ratio). All of these parameters are time-programmable to meet the changing characteristics of the data file. Keep in mind that, unlike peak sensing in the Data Handling application of the Workstation, peak width in PolyView2000 is not updated automatically and updates must be time programmed into the peak sensing method. This Peak Sense Time Program is built in the Method Builder program and then used to perform the peak sensing function either interactively in PolyView2000's Peak Sense window or non-interactively under the System Control/Automation program. If the peak sensing parameters have been properly set, accurate peak detection will result. For many applications, you may find that the following peak sensing program gives adequate peak detection:

Wavelength: 220-367 nm

Peak Width: either 4 or 8 sec

S/N: either 5 or 10

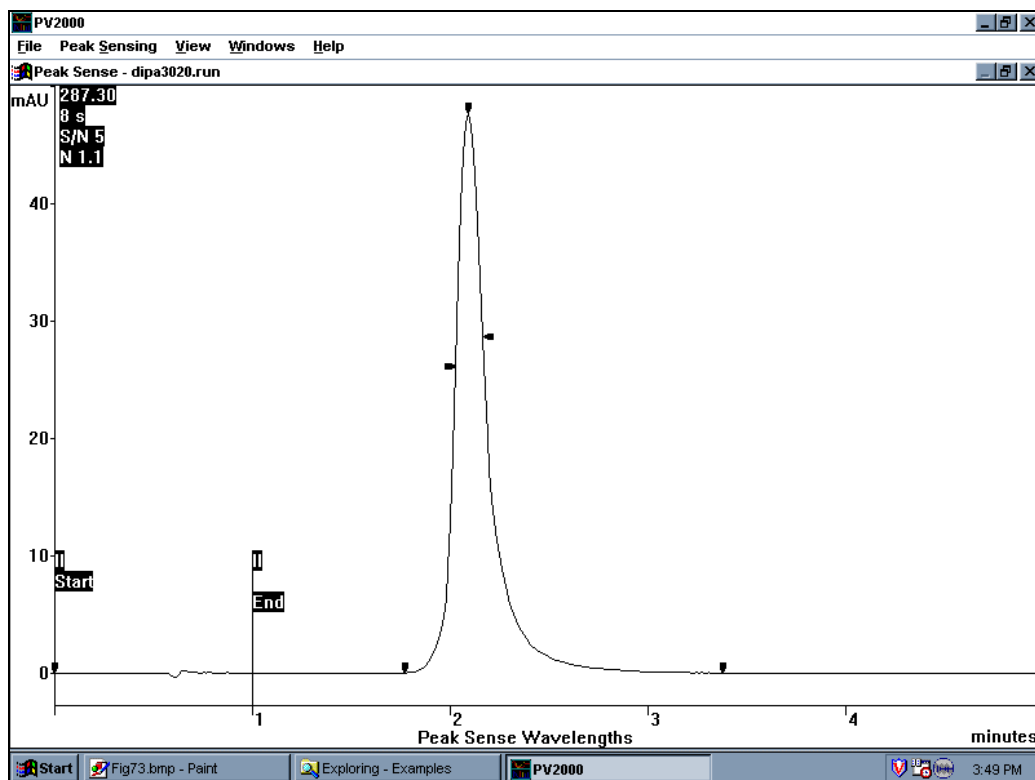
The best indication as to the accuracy of the peak sensing (and therefore the appropriateness of the method used) is the location of the peak event markers in the chromatogram. The Peak Apex events should be accurately placed at the true apex, the upslope and downslope events should be approximately midway along the peak and the peak start and end points should be in the flat portion of the baseline immediately adjacent to the peak. Often, the peak width parameter doesn't have to be set exactly for accurate peak event placement. Also, since peak events in PolyView2000 are used only to perform baseline corrections of spectra, not to compute peak areas, only peak start and peak end events are critical.

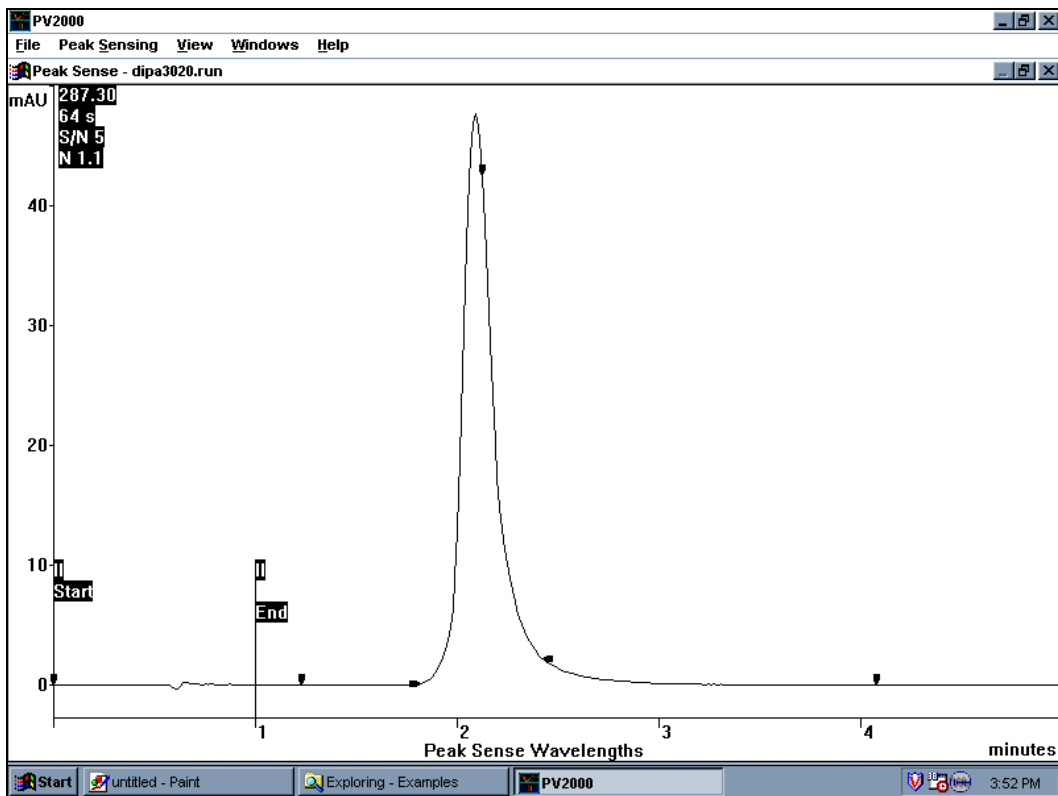
Most of the time, you will find that, if the peak width is set to the width at half height of your chromatographic peaks and a reasonable S/N value is set (use S/N=5 whenever possible), peak event placement will be similar to the figure below. In some situations, such as sloping baselines and/or very small peaks relative to noise, you may find that events are not placed correctly. In these situations, you will need to experiment with different peak sensing programs until you find one suitable for your particular application. *Table 2 Peak Sense Troubleshooting* can be used as a guide in troubleshooting peak sensing in PolyView2000. Following are two examples illustrating poor peak sensing results and the required change in the peak sensing program to improve the results. Keep in mind that the noise values calculated from the diode array run monitor data can influence peak sensing and, if corrupted, may need to be recalculated using the Define User Monitor function. The Peak Sense window displays the value of the noise in arbitrary units. Noise values above 2 usually indicate a problem. For more information, search On-Line Help for 'Troubleshooting'.

Note that the Inhibit Integrate event (II) can be used to inhibit peak retention during a portion of the chromatogram.

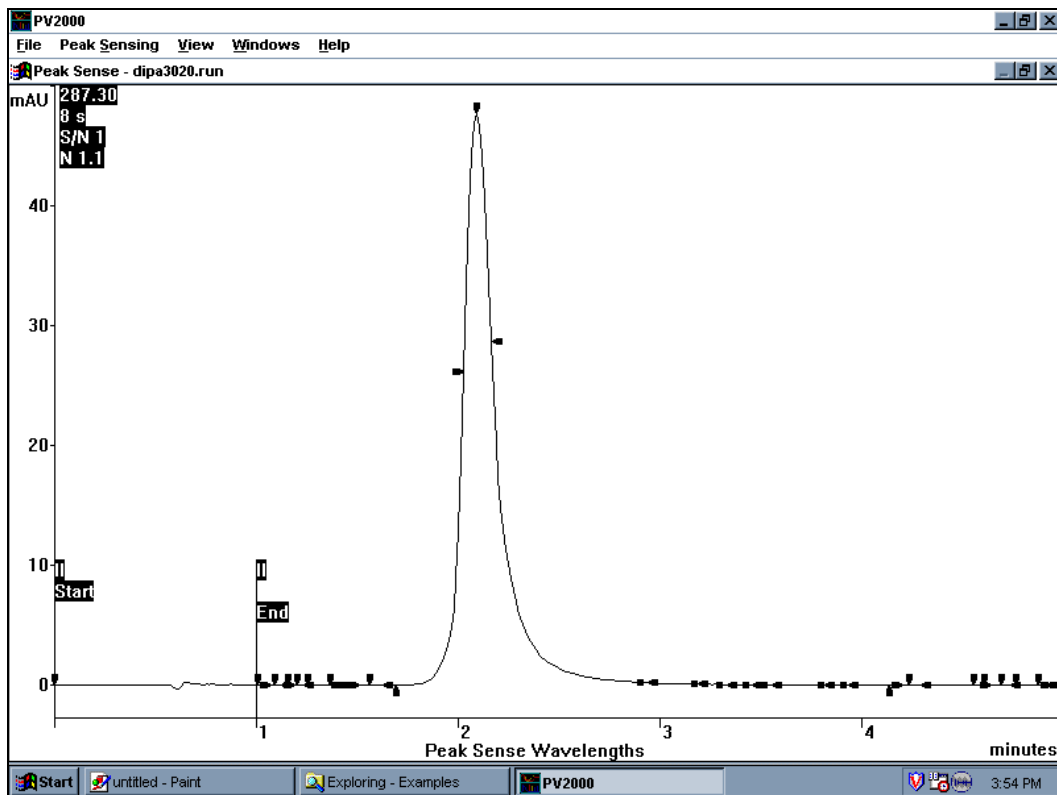
Table 2 Peak Sense Troubleshooting

Condition	Symptom	Solution
S/N set correctly (usually 5)	Misplaced peak events. Inflections are significantly below 60% of the peak height on a symmetrical peak.	Use smaller peak width
S/N set correctly (usually 5)	Peaks not detected.	Use larger peak width.
Peak width set to width at 1/2 height.	Small peaks not detected.	Use smaller S/N
Peak width set to width at 1/2 height.	Many peaks events found in baseline.	Use larger S/N

*Correct Placement of Peak Sensing Events*



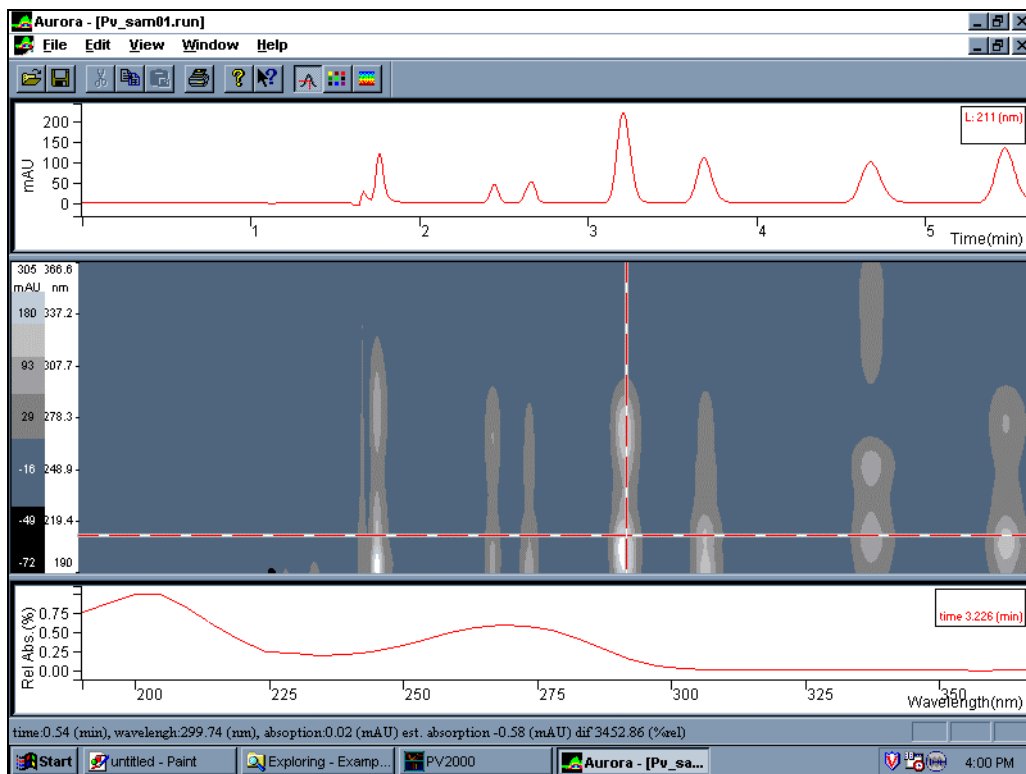
Effect of an excessive width setting



Effect of a too small S/N setting

Aurora

Aurora displays and prints isoabsorbance curves for diode array data files. The figure below shows a typical monochrome output for Aurora. Color output is supported on qualified printers (consult you sales representative). Aurora is installed in the Star folder as part of the PolyView2000 installation. A diode array file can be opened into Aurora using the file menu.



Aurora Report, Exported

Aurora has three windows, a chromatogram window at the top of the display, an isogram window in the center and a spectrum window on the bottom. These Windows can be sized by moving the bars that separate the three windows.

A chromatogram and spectrum for display are chosen by moving the cursor to the wavelength and time that you would like to display and right clicking the mouse. Many different chromatograms and spectra can be selected by right clicking on different points of the display. The existing chromatograms and spectra can be moved "grabbing" either the horizontal or vertical lines with the cursor by left clicking the mouse while the "hand" is on that particular line.

The color intensities on the isogram are scaled logarithmically. The colors can be changed and can be set to gray scale to more easily see the result of printing the isogram on a monochrom printer.

Appendix A

PolyView2000 produces a wide variety of printed reports, as most data processing functions can be documented by means of a printed report. The reports generated one at the time while you are working at the Workstation are termed **Interactive Reports**. The reports obtained in an unattended, automated fashion, as the result of the execution of a method are called **Non-Interactive Reports**. Both types of reports can be annotated in color if printed on a color printer such as the HP 600/700/800 or 900 series color printers. In general, the colors that you see on the screen will be reproduced on the page, with the exception of yellow and white which may be printed in black. Of course, if you don't have a color printer available, the prints are produced in black and white. The reports have been designed so no information content is lost if they are printed in black and white only. For routine work, you may want to turn off the color option in the printer set-up window because usually shorter printing times will be achieved if the print is not in color.

The reports on the following pages will give you an example of what your reports will look like. Changes in the parameters you enter for the report, the number of wavelengths that you have collected and the version of software that you have will effect the report itself. However, all of the data will be on the report.

Interactive Reports

This appendix lists examples of PolyView2000 Interactive Reports. Refer to [Appendix B](#) for examples of PolyView2000 Non-Interactive Reports.

Page 1 - PV_SAM01.RUN Peak Sense Log
File: c:\star\pv_sam01.run
Name: Sample #1
Sample #1 with the internal standard added at Instrument: Varian LC Star 9.5 ppm.
Method: TEST27.MTH
Operator: MEA
Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz
Original Peak Sense Time Program Method
0.00 min Peak Sense 200 - 367 nm 8 sec
0.00 min S/N Threshold 5
0.01 min Inhibit Integrate 1.50 min
3.00 min Peak Sense 254 nm 8 sec

Result Peak Sense Time Program Method
0.00 min Peak Sense 200 - 367 nm 8 sec
0.00 min S/N Threshold 5
0.01 min Inhibit Integrate 1.50 min
3.07 min Peak Sense 254 nm 8 sec

Peak Sense Warnings
3.00 min Inside of peak, cannot make PEAK SENSE change.
3.07 min Delayed peak sense parameters change made.

Run Monitor: 64 spectra.

nm	Noise	nm	Noise	nm	Noise	nm	Noise
190	6.891	239	1.138	287	1.416	335	1.717
195	2.295	244	1.261	292	1.641	339	2.442
200	1.490	249	1.171	297	1.385	344	1.768
205	1.622	254	1.242	302	1.301	348	2.633
210	1.174	259	1.312	306	1.815	353	1.828
215	1.235	263	1.632	311	1.739	358	2.264
220	1.054	268	1.622	316	1.532	362	2.730
224	1.145	273	1.332	321	2.013	367	2.654
229	1.375	278	1.203	325	1.666		
234	1.105	283	1.554	330	1.408		

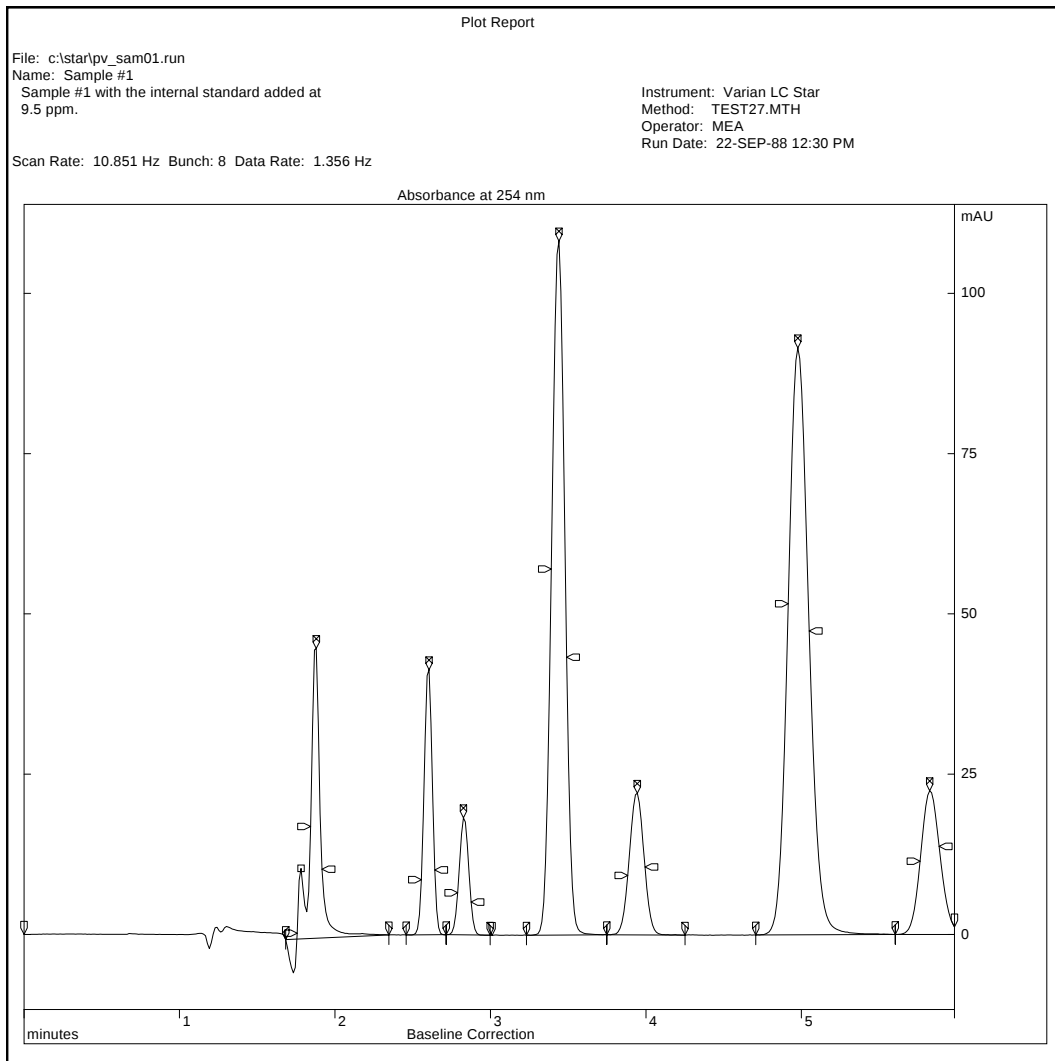
Events between 0.000 - 5.984 minutes.

tR (min)	Events	tR (min)	Events	tR (min)	Events
1.	0.000 Baseline	13.	2.716 Peak Start	25.	3.944 Peak Apex
2.	1.683 Peak Start	14.	2.789 Upslope	26.	3.993 Downslope
3.	1.757 Upslope	15.	2.826 Peak Apex	27.	4.252 Peak End
4.	1.782 Fr Shoulde	16.	2.875 Downslope	28.	4.706 Peak Start
5.	1.843 Upslope	17.	2.998 Peak End	29.	4.915 Upslope
6.	1.880 Peak Apex	18.	3.010 Baseline	30.	4.977 Peak Apex
7.	1.917 Downslope	19.	3.232 Peak Start	31.	5.050 Downslope
8.	2.347 Peak End	20.	3.391 Upslope	32.	5.603 Peak Start
9.	2.458 Peak Start	21.	3.441 Peak Apex	33.	5.763 Upslope
10.	2.556 Upslope	22.	3.490 Downslope	34.	5.824 Peak Apex
11.	2.605 Peak Apex	23.	3.748 Peak Start	35.	5.886 Downslope
12.	2.642 Downslope	24.	3.883 Upslope	36.	5.984 Baseline

Peak Sense Log Report

Windows and Command path:

Plot Window: File: Print Peak Sense Log
Peak Events: List Peak Events: Print List Button



Plot Report

Windows and Command path:

Plot Window:

File: Print Plot

File: c:\star\pv sam01.run

Name: Sample #1

Sample #1 with the internal standard added at 9.5 ppm.

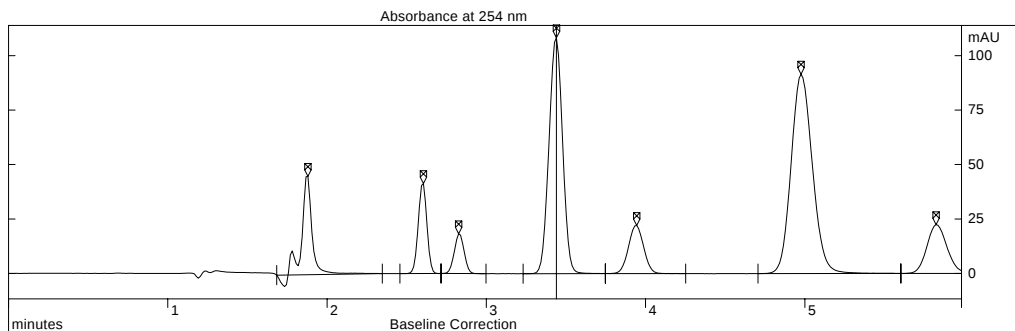
Instrument: Varian LC Star

Method: TEST27.MTH

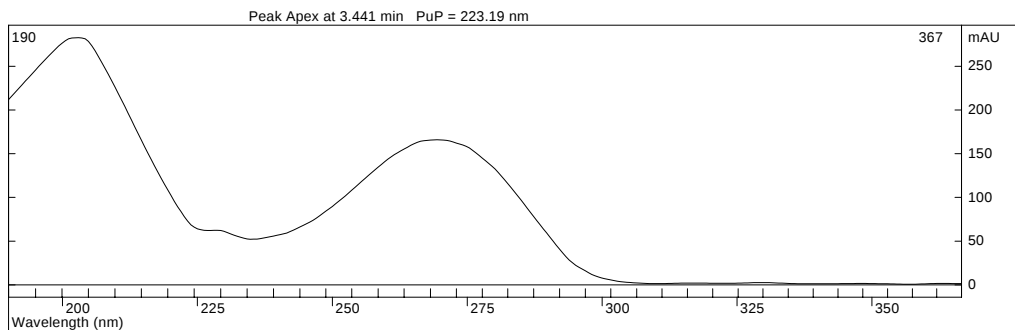
Operator: MEA

Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz



Absorbance Table							
nm	mAU	nm	mAU	nm	mAU	nm	mAU
190	211.813	229	62.279	268	165.528	306	2.231
195	245.728	234	52.480	273	162.186	311	1.501
200	276.279	239	55.891	278	144.949	316	2.098
205	278.452	244	66.206	283	115.750	321	1.877
210	226.368	249	84.260	287	78.149	325	1.967
215	165.596	254	108.252	292	40.820	330	2.657
220	108.615	259	134.642	297	16.007	335	1.658
224	66.077	263	155.326	302	5.686	339	1.242



Peak Event Report

Windows and Command path:

Plot Manager:

Peak Events: List Peak Events: Print Button

Page 1 - Chromatogram Search Report

Match	Similarity	Dissimilarity	PuP (nm)	Event	tR (min)
1	1.00000	0.00147	229.907	Peak Apex	4.977
2	0.99999	0.00508	230.039	Upslope	4.915
3	0.99996	0.00948	229.685	Downslope	5.050

Search Conditions

PuP Range: 190->367 nm PuP Interval: +/- 5.00 nm
 Time Range: 0.000 - 5.984 min
 Maximum Dissimilarity: 0.30

File: c:\star\pv_sam01.run

Name: Sample #1

Sample #1 with the internal standard added at Instrument: Varian LC Star
 9.5 ppm.

Method: TEST27.MTH

Operator: MEA

Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

Search Spectrum

File: c:\star\pv_sam01.run

tR: 4.964 min PuP (190->367 nm) = 229.941 nm

Name: Sample #1

Sample #1 with the internal standard added at Instrument: Varian LC Star
 9.5 ppm.

Method: TEST27.MTH

Operator: MEA

Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

Detector Range: 190->367 nm Valid Range: 190->367 nm

Spectrum Type: Within Peak Correction Type: Baseline

Chromatogram Search Report

Search Conditions

PuP Range: 200->367 nm PuP Interval: +/- 5.00 nm
Time Range: 0.000 - 5.984 min

File: c:\star\pv_sam01.run

Name: Sample #1

Sample #1 with the internal standard added at
9.5 ppm.

Instrument: Varian LC Star

Method: TEST27.MTH

Operator: MEA

Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

Search Spectrum, Scaled by 1.780

File: c:\star\pv_sam01.run

IR: 4.915 min PuP (200->367 nm) = 232.289 nm

Name: Sample #1

Sample #1 with the internal standard added at
9.5 ppm.

Instrument: Varian LC Star

Method: TEST27.MTH

Operator: MEA

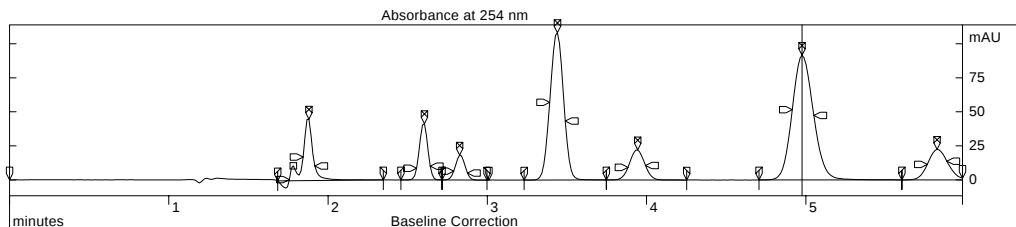
Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

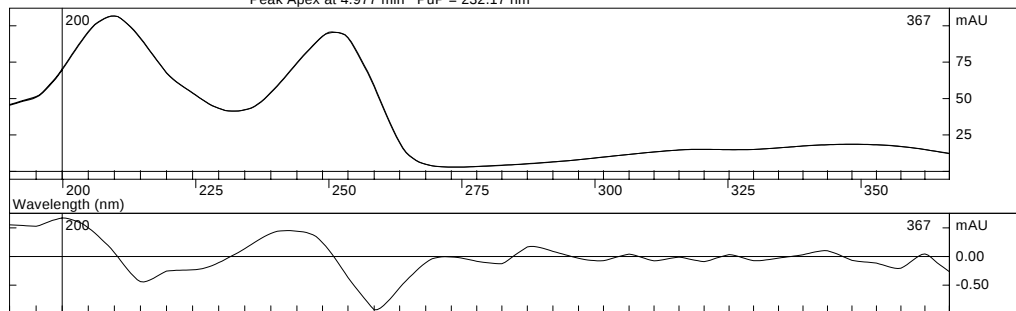
Detector Range: 190->367 nm Valid Range: 190->367 nm

Spectrum Type: Upslope

Correction Type: Baseline



Match #2 of 3 Matches Similarity: 0.99998 Dissimilarity: 0.00620
Peak Apex at 4.977 min PuP = 232.17 nm

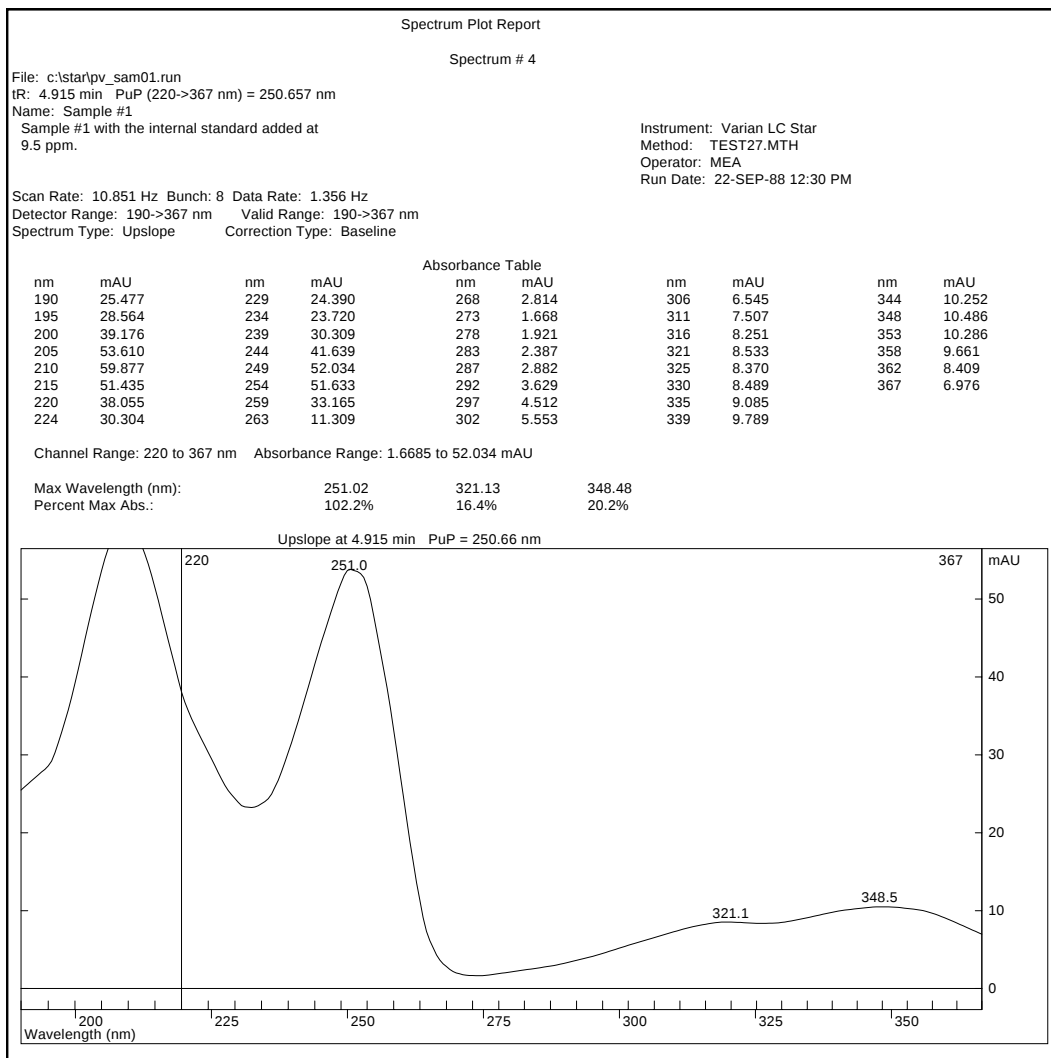


Chromatogram Search Report (Graphics)

Windows and Command path:

Plot Manager:

Peak Events: Search Peak Events: Print button

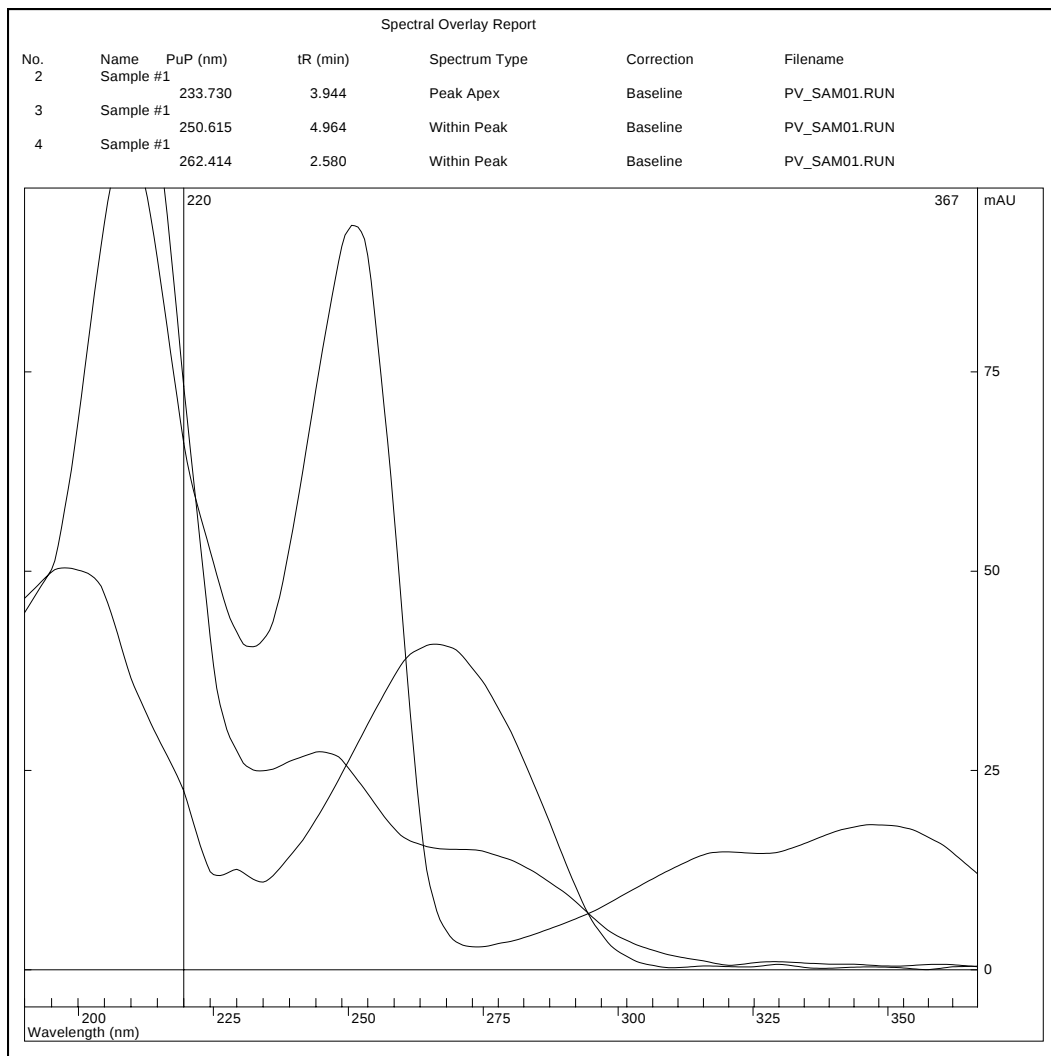


Spectrum Plot Report (Single)

Windows and Command path:

Spectra Manager:

Spectrum: Print Spectra (single displayed)



Spectrum Plot Report (Multiple)

Windows and Command path:

Spectra Manager:

File: Print Spectra (multiple spectra displayed)

PuP Statistics Report

No	Filename	tR (min)	Spectrum Type	Correction	PuP (220->367 nm)
1	PV_SAM01.RUN	4.903	Within Peak	Baseline	250.593
2	PV_SAM01.RUN	4.940	Within Peak	Baseline	250.645
3	PV_SAM01.RUN	4.977	Peak Apex	Baseline	250.597
4	PV_SAM01.RUN	5.001	Within Peak	Baseline	250.617
5	PV_SAM01.RUN	5.026	Within Peak	Baseline	250.581
6	PV_SAM01.RUN	5.063	Within Peak	Baseline	250.633
Best Correlation:					4 and 5 Sim: 0.999996 Dissim: 0.002719
Worst Correlation:					1 and 6 Sim: 0.999878 Dissim: 0.015613
Average PuP =					250.611 nm Standard Deviation = 0.023 nm

Target:

PuP: 250.611 nm

No	Filename	tR (min)	Spectrum Type	Correction	PuP (220->367 nm)
1	PV_SAM01.RUN	4.903	Within Peak	Baseline	250.593 (-0.018 nm)
2	PV_SAM01.RUN	4.940	Within Peak	Baseline	250.645 (+0.034 nm)
3	PV_SAM01.RUN	4.977	Peak Apex	Baseline	250.597 (-0.014 nm)
4	PV_SAM01.RUN	5.001	Within Peak	Baseline	250.617 (+0.006 nm)
5	PV_SAM01.RUN	5.026	Within Peak	Baseline	250.581 (-0.030 nm)
6	PV_SAM01.RUN	5.063	Within Peak	Baseline	250.633 (+0.022 nm)
Best Correlation:					4 and 5 Sim: 0.999996 Dissim: 0.002719
Worst Correlation:					1 and 6 Sim: 0.999878 Dissim: 0.015613
Average PuP =					250.611 nm Standard Deviation = 0.023 nm

Target:

PuP: 250.611 nm Standard Deviation: 0.002 nm

No	Filename	tR (min)	Spectrum Type	Correction	PuP (220->367 nm)
1	PV_SAM01.RUN	4.903	Within Peak	Baseline	250.593 (-7.80 SD)
2	PV_SAM01.RUN	4.940	Within Peak	Baseline	250.645 (+14.86 SD)
3	PV_SAM01.RUN	4.977	Peak Apex	Baseline	250.597 (-6.12 SD)
4	PV_SAM01.RUN	5.001	Within Peak	Baseline	250.617 (+2.77 SD)
5	PV_SAM01.RUN	5.026	Within Peak	Baseline	250.581 (-13.07 SD)
6	PV_SAM01.RUN	5.063	Within Peak	Baseline	250.633 (+9.71 SD)
Best Correlation:					4 and 5 Sim: 0.999996 Dissim: 0.002719
Worst Correlation:					1 and 6 Sim: 0.999878 Dissim: 0.015613
Average PuP =					250.611 nm Standard Deviation = 0.023 nm

PuP Statistics Report

Windows and Command path:

Spectra Manager:

Spectrum: Do statistics: Print Button

Page 1 - pv_std01.lbr Library Index Report

File: c:\star\pv_std01.lbr
Name: Test Fractions
Note: Contains spectra from PV_STD01.RUN
Operator: MEA
Last Modified: 14-MAR-94 2:34 PM

No.	tR (min)	Filename	Name
1.	1.888	PV_STD01.RUN	Trimonreal
2.	2.825	PV_STD01.RUN	Dimonreal
3.	3.430	PV_STD01.RUN	AS-98
4.	3.948	PV_STD01.RUN	AH-187
5.	4.997	PV_STD01.RUN	AJ-65
6.	5.848	PV_STD01.RUN	AS-96

Library List Report

Windows and Command path:

Library Manager:

File: Print Library Index

Library: Select/Edit Library Records: Print button

Page 1 - Library Search Report

Matches:	Sim.	Dissim.	PuP(nm)	tR(min)	Lib #
-----	-----	-----	-----	-----	-----
AJ-65	0.99997	0.00735	232.282	4.997	A 5

Search Conditions

PuP Range: 200->367 nm PuP Interval: +/- 5.00 nm
 Time Range: 0.000 - 1440.000 min
 Maximum Dissimilarity: 0.30

Libraries:

A: c:\star\pv_std01.lbr

Search Spectrum

File: c:\star\pv_sam01.run
 tR: 4.964 min PuP (200->367 nm) = 232.198 nm
 Name: Sample #1
 Sample #1 with the internal standard added at Instrument: Varian LC Star
 9.5 ppm. Method: TEST27.MTH
 Operator: MEA
 Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz
 Detector Range: 190->367 nm Valid Range: 190->367 nm
 Spectrum Type: Within Peak Correction Type: Baseline

Possible Match #1

File: c:\star\tutorial\pv_std01.run
 tR: 4.997 min PuP (200->367 nm) = 232.282 nm
 Name: AJ-65
 Test fraction @ 10.0 ppm. Instrument: Varian LC Star
 Method: TEST27.MTH
 Operator: MEA
 Run Date: 22-SEP-88 12:30 PM

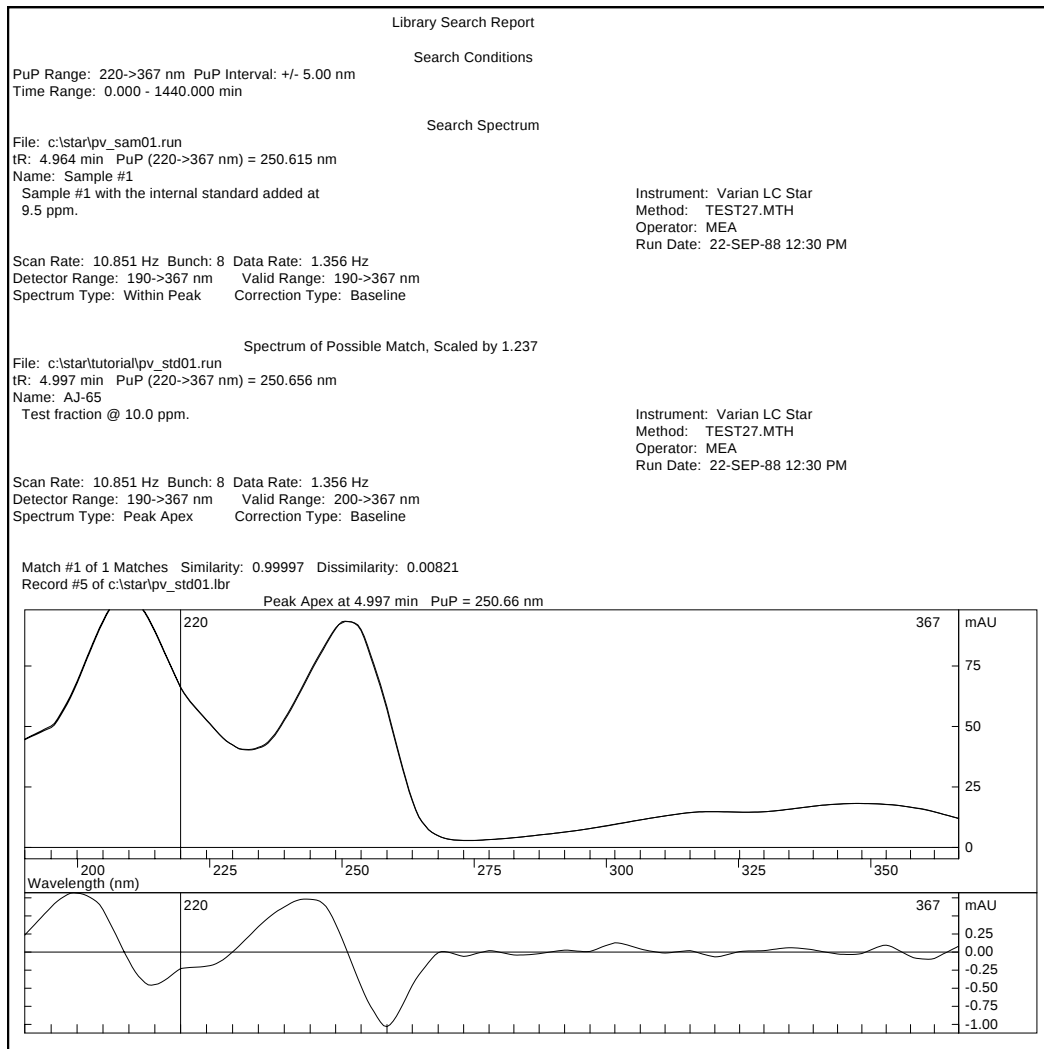
Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz
 Detector Range: 190->367 nm Valid Range: 200->367 nm
 Spectrum Type: Peak Apex Correction Type: Baseline

Library Search Report (Text)

Windows and Command path:

Library Manager:

Search: Search for Spectrum: Search button: Print List button

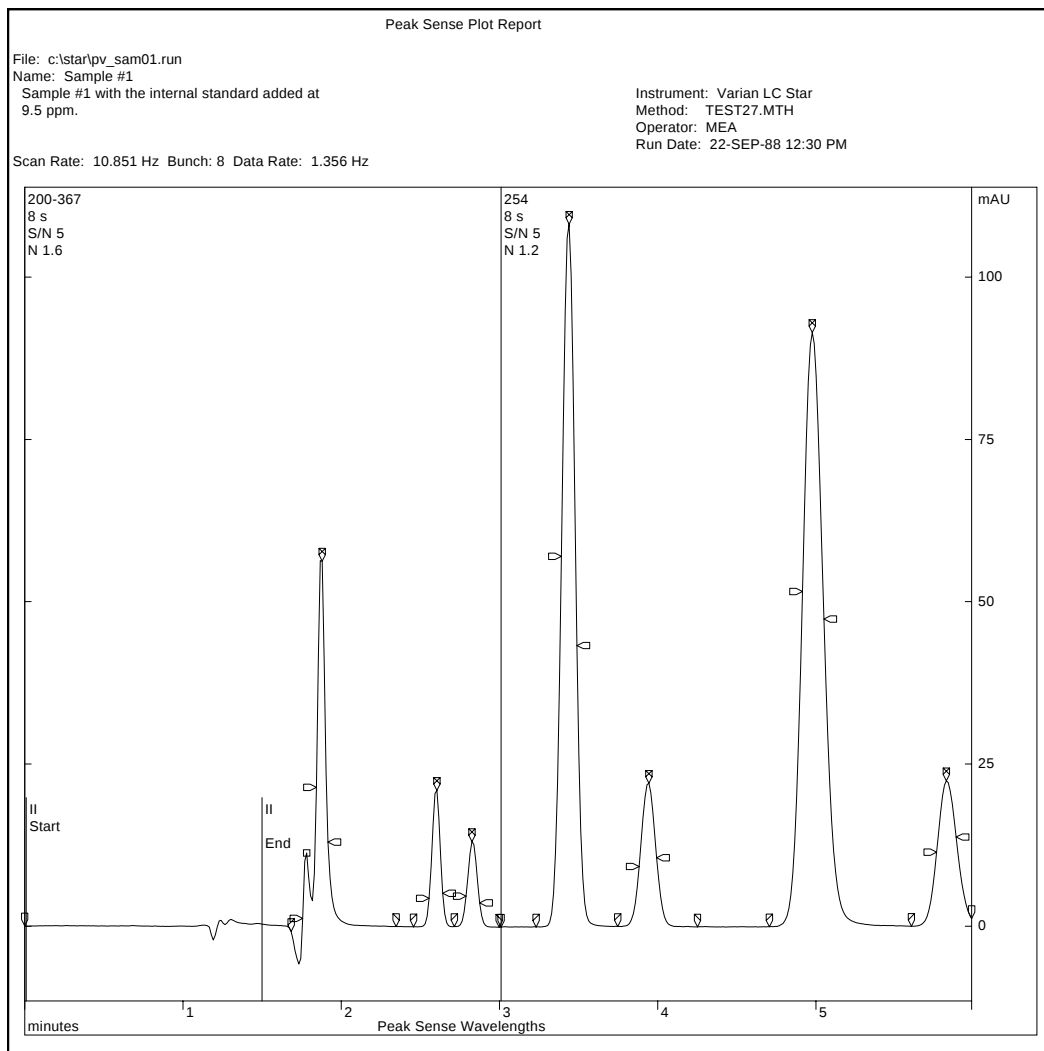


Library Search Report (Graphics)

Windows and Command path:

Library Manager:

Search: Search for Spectrum: Search button: Print Match button



Peak Sense Plot Report

Windows and Command path:
 Peak Sense Manager:
 File: Print Plot

File: c:\star\tutorial\dipa3053.run
Name: 0.01D/0.1P

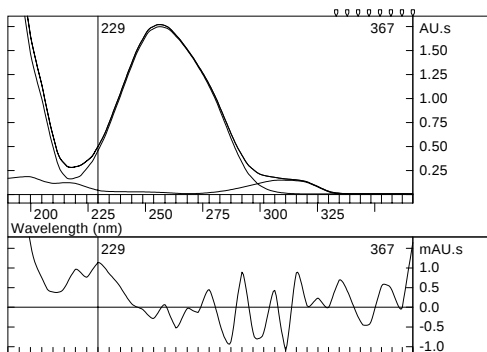
Rpt Date: 28-JUN-94 9:30 PM

Instrument: Varian Star
Method: pq.mth
Operator: Number 1
Run Date: 3-MAR-92 10:40 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

MCA Report - External Standard Analysis

Baseline: 1.757 min (average of 5) to 3.502 min (average of 5)
Integration: 1.757 to 3.502 min, Flow Rate: 2.000 ml/min (Pump Log)
Library: c:\star\pqcalhi.lib

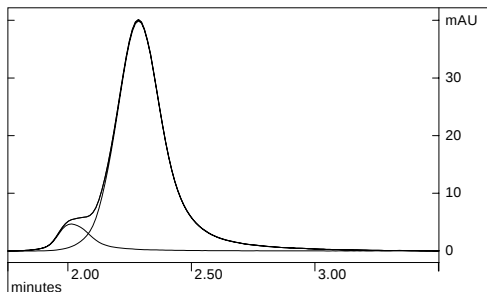


Spectrum Analysis over 229-367 nm
excluding 335-367

Lib	Name	Amount
Index		
#1	Paraquat	9.89 ug
#2	Diquat	0.91 ug

Similarity: 1.00000
Dissimilarity: 0.00072 (0.001352)

Search window: 1.757 to 3.502 min
Spectra in library: 2
Spectra in search window: 2
Components allowed: 6



Plot Analysis at 287 nm

Lib	tR	Height	Width	Res
Index	(min)	mAU	(sec)	
#2	2.014	4.67	8.9	---
#1	2.286	39.89	12.6	0.90

Error Analysis over 229 - 367 nm

The maximum error 40 uAU
at 2.291 minutes DOES NOT EXCEED
the noise threshold 80 uAU.
The ratio of maximum to expected
error is 0.487.

Multicomponent Analysis Report

Windows and Command path:

MCA Manager:

MCA button

File: c:\star\tutorial\dipa3053.run
Name: 0.01D/0.1P

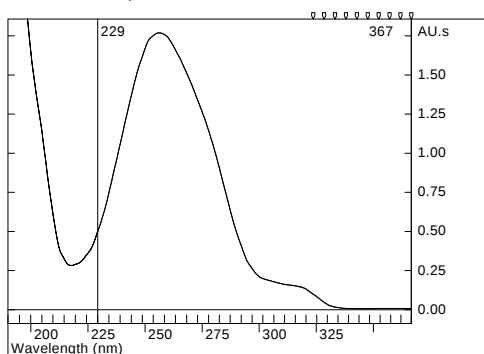
Rpt Date: 28-JUN-94 9:31 PM

Instrument: Varian Star
Method: pq.mth
Operator: Number 1
Run Date: 3-MAR-92 10:40 PM

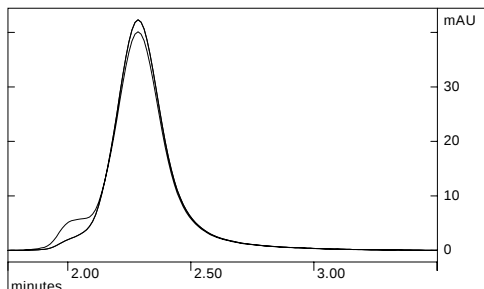
Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

Purity Analysis Report

Baseline: 1.757 min (average of 5) to 3.502 min (average of 5)
Integration: 1.757 to 3.502 min
Reference Spectrum: Whole Peak Area

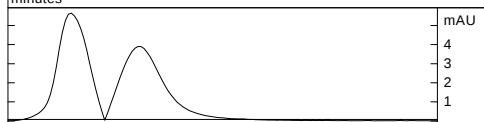


Spectrum Analysis over 229-367 nm
excluding 325-367



Plot Analysis at 287 nm

tR (min)	Height mAU	Width (sec)
2.286	42.32	12.6



Error Analysis over 229 - 367 nm

The maximum error 6 mAU
at 2.012 minutes EXCEEDS
the noise threshold 0.08 mAU.
The ratio of maximum to expected
error is 67.004.

Purity Analysis Report

Windows and Command path:
MCA Manager:
Purity button

File: c:\star\pitcon94\p94012.run
Name: DiNitroPhenol

Rpt Date: 28-JUN-94 9:29 PM

Instrument: LC System Research
Method: ISOCRAT.MTH
Operator: Chris Kellogg
Run Date: 18-FEB-94 6:05 PM

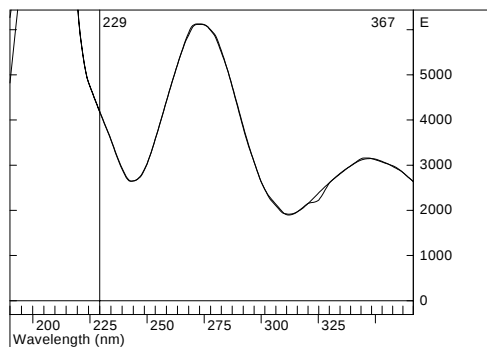
Scan Rate: 10.851 Hz Bunch: 4 Data Rate: 2.713 Hz

Molar Extinction Coefficient Report: Dinitrophenol

Baseline: 2.482 min (average of 5) to 3.146 min (average of 5)

Integration: 2.482 to 3.146 min

Reference Spectrum: Region above 50.00% of Apex, 2.599 to 2.716 min



Spectrum Analysis over 229-367 nm

Flow Rate: 1.000 ml/min (Pump Log)
Path Length: 0.60 cm
Amount: 0.0012 micromole

E (254 nm) = 3580 /M-cm

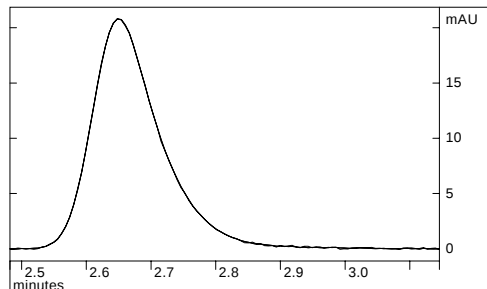
E_{max} (273.5 nm) = 6130 /M-cm

E_{max} (346.3 nm) = 3160 /M-cm

Reference vs. Whole Area Spectrum

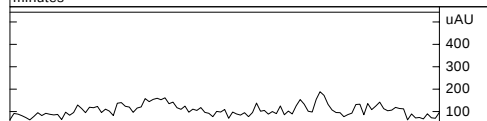
Similarity: 0.99996

Dissimilarity: 0.00941



Plot Analysis at 254 nm

tR (min)	Height mAU	Width (sec)
2.650	20.80	6.7



Error Analysis over 229 - 367 nm

The maximum error 2e+002 uAU
at 2.962 minutes DOES NOT EXCEED
the noise threshold 5e+002 uAU.
The ratio of maximum to expected
error is 0.347.

Extinction Coefficient Report

Windows and Command path:

MCA Manager:

Purity button

(Extinction Coeff. computation is enabled in Options:Report Options)

Appendix B

PolyView2000 produces a wide variety of printed reports, as most data processing functions can be documented by means of a printed report. The reports generated one at the time while you are working at the Workstation are termed **Interactive Reports**. The reports obtained in an unattended, automated fashion, as the result of the execution of a method are called **Non-Interactive Reports**. Both types of reports can be annotated in color if printed on a color printer such as the Canon BJC-600. In general, the colors that you see on the screen will be reproduced on the page, with the exception of yellow and white which may be printed in black. Of course, if you don't have a color printer available, the prints are produced in black and white. The reports have been designed so no information content is lost if they are printed in black and white only. For routine work, you may want to turn off the color option in the printer set-up window because usually shorter printing times will be achieved if the print is not in color.

Non-Interactive Reports

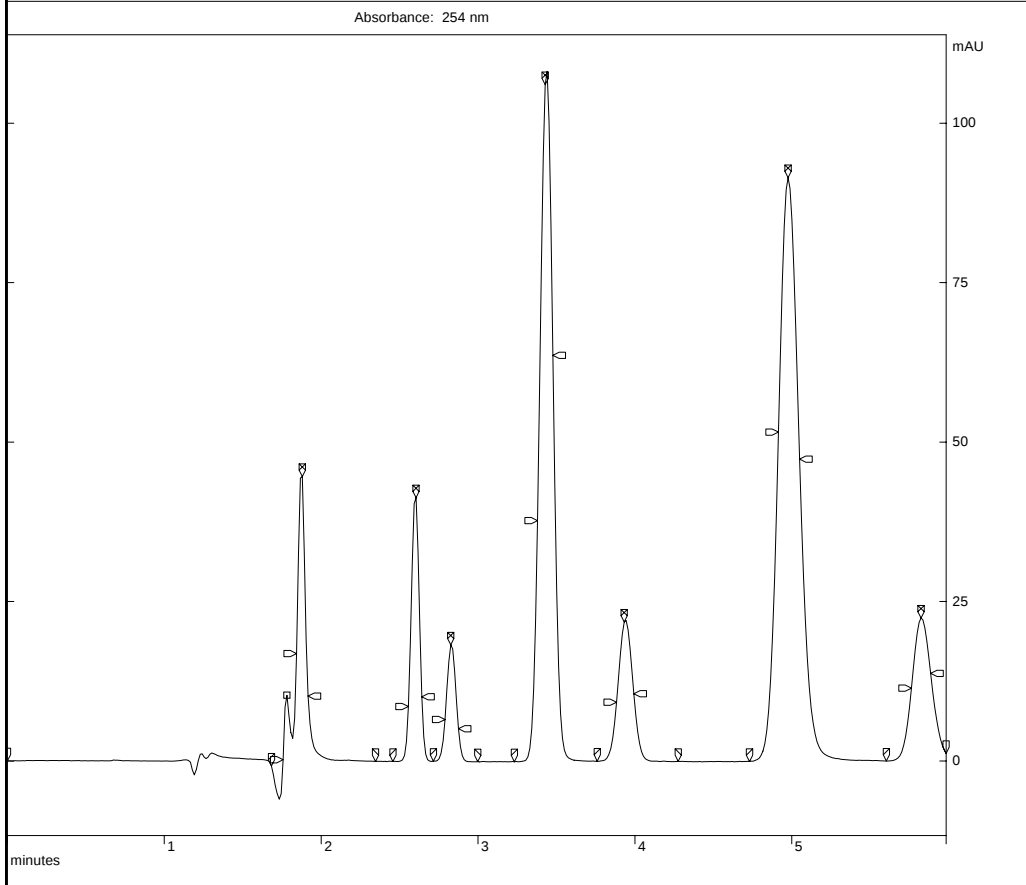
This appendix lists examples of PolyView2000 Non-Interactive Reports. Refer to [Appendix A](#) for examples of PolyView2000 Interactive Reports.

File: c:\star\tutorial\pv_sam01.run
Name: Sample #1
Sample #1 with the internal standard added at
9.5 ppm.

Rpt Date: 28-JUN-94 7:04 PM

Instrument: Varian LC Star
Method: TEST27.MTH
Operator: MEA
Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz



Single Plot Report

File: c:\star\tutorial\pv_sam01.run Rpt Date: 28-JUN-94 7:04 PM
 Name: Sample #1
 Sample #1 with the internal standard added at 9.5 ppm. Instrument: Varian LC Star
 Method: TEST27.MTH
 Operator: MEA
 Run Date: 22-SEP-88 12:30 PM
 Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

General Table Report - Page 1
 Events between 0.000 and 5.984 minutes

Time	Event	Absorbance	Max Absorbance	Purity	Ratio
		254->254 nm mAU	220->367 nm mAU	220->367 nm nm	254->254 nm /224->224 nm
1.683	PeakStart	-0.793	-0.504	*****	*****
1.757	Upslope	0.191	8.548	223.598	0.1581
1.782	FShoulder	10.315	19.907	273.197	0.6449
1.843	Upslope	16.836	40.884	262.922	0.5032
1.880	Peak Apex	44.607	105.878	263.670	0.5102
1.917	Downslope	10.167	25.140	263.314	0.5058
2.347	Peak End	-0.076	0.000	*****	*****
2.458	PeakStart	-0.076	0.229	*****	*****
2.556	Upslope	8.542	11.337	262.413	1.5995
2.605	Peak Apex	41.284	54.427	262.415	1.6018
2.642	Downslope	10.063	13.318	262.416	1.6010
2.716	PeakStart	-0.040	0.313	*****	*****
2.789	Upslope	6.495	9.592	247.354	1.1924
2.826	Peak Apex	18.198	26.732	247.367	1.1909
2.875	Downslope	5.050	7.538	247.343	1.1851
2.998	Peak End	-0.114	0.000	*****	*****
3.232	PeakStart	-0.122	0.267	*****	*****
3.379	Upslope	37.678	74.099	261.460	1.0250
3.428	Peak Apex	106.050	168.610	262.114	1.3294
3.477	Downslope	63.600	91.466	262.351	1.4825
3.760	PeakStart	-0.021	0.153	*****	*****
3.883	Upslope	9.200	38.892	230.608	0.4120
3.932	Peak Apex	21.791	75.919	233.044	0.5024
3.993	Downslope	10.536	31.829	235.205	0.5850
4.276	Peak End	-0.069	0.000	*****	*****
4.731	PeakStart	-0.076	0.191	*****	*****
4.915	Upslope	51.570	52.034	250.657	1.4131
4.977	Peak Apex	91.479	92.874	250.612	1.4131
5.050	Downslope	47.330	48.422	250.549	1.4120
5.603	PeakStart	-0.015	0.168	*****	*****
5.763	Upslope	11.416	35.615	255.623	0.5189
5.824	Peak Apex	22.410	68.912	255.721	0.5238
5.886	Downslope	13.735	41.612	255.850	0.5301

General Table Report

File: c:\star\tutorial\pv_sam01.run
 Name: Sample #1
 Sample #1 with the internal standard added at
 9.5 ppm.

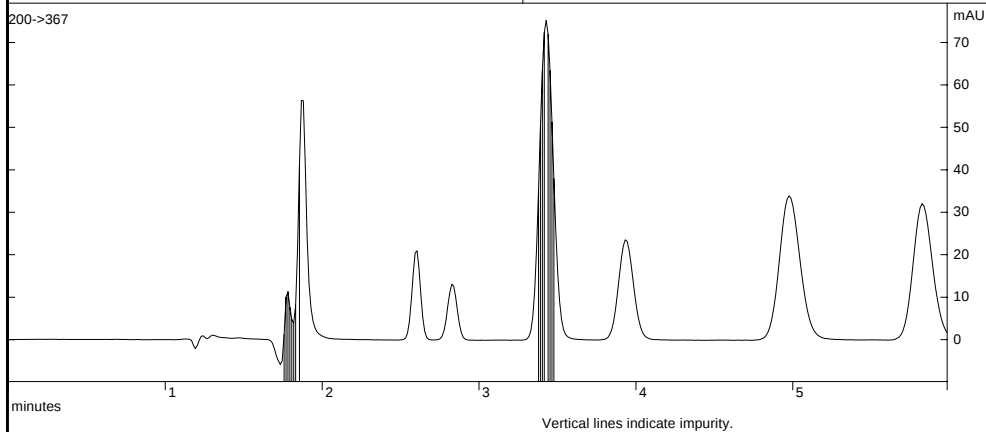
Rpt Date: 28-JUN-94 7:04 PM

Instrument: Varian LC Star
 Method: TEST27.MTH
 Operator: MEA
 Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

Peak Purity Survey: 200->367 nm. PuP Interval +/- 0.50 nm.
 Minimum Peak Height: 10.000 mAU.

Apex Time	Purity Parameters (nm)			Apex Time	Purity Parameters (nm)		
	Front	Apex	Back		Front	Apex	Back
1.880	208.067	232.316	232.185				
3.428	223.089	228.963	234.327				



Peak Purity Survey Report

```

File: c:\star\tutorial\pv_sam01.run      Rpt Date: 28-JUN-94 7:04 PM
Name: Sample #1
Sample #1 with the internal standard added at 9.5 ppm.
Instrument: Varian LC Star
Method: TEST27.MTH
Operator: MEA
Run Date: 22-SEP-88 12:30 PM
Scan Rate: 10.851 Hz   Bunch: 8   Data Rate: 1.356 Hz
  
```

Multiple Purity Parameters Report - Page 1
Events between 0.000 and 5.984 minutes

Time	Event	210->367 nm		190->249 nm		224->278 nm		273->367 nm	
		MaxAbs	PuP	MaxAbs	PuP	MaxAbs	PuP	MaxAbs	PuP
		mAU	nm	mAU	nm	mAU	nm	mAU	nm
1.683	PeakStart	-0.5	*****	-0.7	*****	-0.7	*****	-0.5	*****
1.757	Upslope	14.1	214.959	20.5	201.139	5.7	229.157	0.5	274.429
1.782	FShoulder	25.6	257.948	29.2	208.509	17.1	246.030	10.3	315.668
1.843	Upslope	47.2	251.646	106.2	201.503	34.7	252.837	33.8	287.754
1.880	Peak Apex	123.1	252.339	286.1	201.034	91.0	253.951	91.4	287.136
1.917	Downslope	28.9	252.098	69.8	200.554	21.3	253.434	21.3	287.386
2.347	Peak End	0.0	*****	0.0	*****	0.0	*****	0.0	*****
2.458	PeakStart	0.2	*****	0.0	*****	-0.0	*****	0.2	*****
2.556	Upslope	11.3	254.189	14.0	205.660	11.3	261.903	10.6	278.280
2.605	Peak Apex	54.4	254.179	67.2	205.636	54.4	261.904	50.6	278.282
2.642	Downslope	13.3	254.161	16.5	205.688	13.3	261.901	12.4	278.276
2.716	PeakStart	0.3	*****	0.0	*****	0.0	*****	0.3	*****
2.789	Upslope	19.6	226.701	34.4	200.446	6.8	251.018	4.9	280.651
2.826	Peak Apex	54.5	226.714	96.7	200.401	19.1	251.019	13.7	280.666
2.875	Downslope	15.3	226.785	27.0	200.451	5.4	250.991	3.8	280.691
2.998	Peak End	0.0	*****	0.0	*****	0.0	*****	0.0	*****
3.232	PeakStart	0.3	*****	-0.0	*****	-0.0	*****	0.3	*****
3.379	Upslope	137.2	239.448	163.9	204.420	74.1	262.394	74.1	278.144
3.428	Peak Apex	248.9	245.592	304.1	204.503	168.6	262.374	167.2	278.169
3.477	Downslope	107.9	250.057	137.3	204.774	91.5	262.192	87.8	278.209
3.760	PeakStart	0.2	*****	0.0	*****	0.1	*****	0.2	*****
3.883	Upslope	54.9	218.595	62.2	204.496	22.4	238.209	5.9	282.686
3.932	Peak Apex	112.8	219.472	139.0	203.693	43.4	240.695	14.7	282.021
3.993	Downslope	49.6	220.278	66.0	202.968	18.1	242.663	7.4	281.657
4.276	Peak End	0.0	*****	0.0	*****	0.0	*****	0.0	*****
4.731	PeakStart	0.2	*****	0.0	*****	0.0	*****	0.2	*****
4.915	Upslope	59.9	238.663	59.9	219.445	52.0	245.820	10.5	335.613
4.977	Peak Apex	106.7	238.632	106.7	219.419	92.9	245.760	18.6	335.538
5.050	Downslope	55.5	238.605	55.5	219.346	48.4	245.689	9.6	335.377
5.603	PeakStart	0.2	*****	0.1	*****	0.1	*****	0.2	*****
5.763	Upslope	77.3	230.226	80.0	207.025	29.9	259.177	29.9	278.869
5.824	Peak Apex	150.6	230.303	156.5	206.944	58.3	259.235	58.3	278.830
5.886	Downslope	91.5	230.423	95.5	206.837	35.6	259.310	35.6	278.796

Multiple Purity Parameters Report

File: c:\star\tutorial\pv_sam01.run Rpt Date: 28-JUN-94 7:04 PM
 Name: Sample #1
 Sample #1 with the internal standard added at 9.5 ppm.
 Instrument: Varian LC Star
 Method: TEST27.MTH
 Operator: MEA
 Run Date: 22-SEP-88 12:30 PM
 Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

 Library Search Report (report best 5 matches or no match) - Page 1
 Search Parameters: PuP Interval: +/-5.00 nm, Wavelength Range: 200->367 nm
 Data File Time Range: 2.199 - 4.497 min.
 Library Relative Time Range: 0.000 - 1000.000
 Minimum Peak Height: 0.000 mAU, Maximum Dissimilarity: 0.30
 Libraries:
 A: c:\star\tutorial\pv_std01.lbr

Target Spectrum: Peak Apex, PuP(nm): 240.714, tR(min): 2.605
 Peak Height: 67.218 mAU at 200 nm

No Matches

Target Spectrum: Peak Apex, PuP(nm): 215.179, tR(min): 2.826
 Peak Height: 68.307 mAU at 200 nm

Possible Matches:	Sim.	Dissim.	PuP(nm)	tR(min)	Lib	#
-----	-----	-----	-----	-----	---	-
Dimonreal	1.00000	0.00220	215.211	2.825	A	2
AS-98	0.96376	0.26677	219.558	3.430	A	3
AS-96	0.96203	0.27293	219.509	5.848	A	6

Target Spectrum: Peak Apex, PuP(nm): 228.963, tR(min): 3.428
 Peak Height: 304.074 mAU at 205 nm

No Matches

Target Spectrum: Peak Apex, PuP(nm): 212.576, tR(min): 3.932
 Peak Height: 115.909 mAU at 200 nm

Possible Matches:	Sim.	Dissim.	PuP(nm)	tR(min)	Lib	#
-----	-----	-----	-----	-----	---	-
AH-187	0.98600	0.16676	212.096	3.948	A	4
Dimonreal	0.97866	0.20550	215.211	2.825	A	2

Library Search Report (Long Text Format)

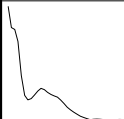
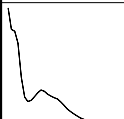
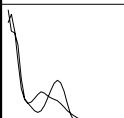
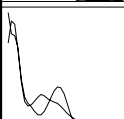
File: c:\star\tutorial\pv_sam01.run Rpt Date: 28-JUN-94 7:04 PM
Name: Sample #1
Sample #1 with the internal standard added at 9.5 ppm. Instrument: Varian LC Star
Method: TEST27.MTH
Operator: MEA
Run Date: 22-SEP-88 12:30 PM
Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

Library Search Report (report best match or no match) - Page 1
Search Parameters: PuP Interval: +/-5.00 nm, Wavelength Range: 200->367 nm
Data File Time Range: 2.199 - 4.497 min.
Library Relative Time Range: 0.000 - 1000.000
Minimum Peak Height: 0.000 mAU, Maximum Dissimilarity: 0.30

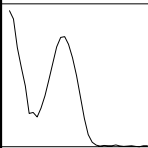
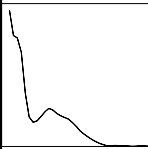
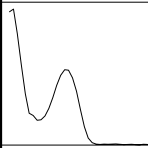
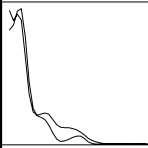
Libraries:
A: c:\star\tutorial\pv_std01.lbr

Target Apex(min)	Best Possible Match	Sim.	Dissim.	tR(min)	Lib	Entry #
-----	-----	-----	-----	-----	---	-
2.605	No Match					
2.826	Dimonreal	1.00000	0.00220	2.825	A	2
3.428	No Match					
3.932	AH-187	0.98600	0.16676	3.948	A	4

Library Search Report (Short Text Format)

File: c:\star\tutorial\pv_sam01.run Name: Sample #1 Sample #1 with the internal standard added at 9.5 ppm.		Rpt Date: 28-JUN-94 7:04 PM Instrument: Varian LC Star Method: TEST27.MTH Operator: MEA Run Date: 22-SEP-88 12:30 PM	
Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz			
Library Search Report (report best 5 matches or no match) - Page 2 Search Parameters: PuP Interval: +/-5.00 nm, Wavelength Range: 200->367 nm Data File Time Range: 2.199 - 4.497 min. Library Relative Time Range: 0.000 - 1000.000 Minimum Peak Height: 0.000 mAU, Maximum Dissimilarity: 0.30 Libraries: A: c:\star\tutorial\pv_std01.lbr			
	Target Spectrum: Peak Apex, tR: 2.826 min, PuP: 215.179 nm Peak Height: 68.307 mAU at 200 nm		
	Possible Match: (Library: A, # 2) Dimonreal Note: Internal standard peak at 9.5 ppm. Similarity Dissimilarity PuP(nm) tR(min) 1.00000 0.00220 215.211 2.825		
	Possible Match: (Library: A, # 3) AS-98 Note: Test fraction @ 10.0 ppm Similarity Dissimilarity PuP(nm) tR(min) 0.96376 0.26677 219.558 3.430		
	Possible Match: (Library: A, # 6) AS-96 Note: Test fraction @ 10.0 ppm. Similarity Dissimilarity PuP(nm) tR(min) 0.96203 0.27293 219.509 5.848		

Library Search Report (Long Graphics Format)

File: c:\startutorial\pv_sam01.run Name: Sample #1 Sample #1 with the internal standard added at 9.5 ppm.		Rpt Date: 28-JUN-94 7:04 PM Instrument: Varian LC Star Method: TEST27.MTH Operator: MEA Run Date: 22-SEP-88 12:30 PM	
Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz			
Library Search Report (report best match or no match) - Page 1 Search Parameters: PuP Interval: +/-5.00 nm, Wavelength Range: 200->367 nm Data File Time Range: 2.199 - 4.497 min. Library Relative Time Range: 0.000 - 1000.000 Minimum Peak Height: 0.000 mAU, Maximum Dissimilarity: 0.30			
Libraries: A: c:\startutorial\pv_std01.lbr			
	Peak Apex, tR: 2.605 min, PuP: 240.714 nm Peak Height: 67.218 mAU at 200 nm No Match		
	Peak Apex, tR: 2.826 min, PuP: 215.179 nm Peak Height: 68.307 mAU at 200 nm Possible Match: (Library: A, # 2) Dimonreal Note: Internal standard peak at 9.5 ppm. Similarity Dissimilarity PuP(nm) tR(min) 1.00000 0.00220 215.211 2.825		
	Peak Apex, tR: 3.428 min, PuP: 228.963 nm Peak Height: 304.074 mAU at 205 nm No Match		
	Peak Apex, tR: 3.932 min, PuP: 212.576 nm Peak Height: 115.909 mAU at 200 nm Possible Match: (Library: A, # 4) AH-187 Note: Test fraction @ 10.0 ppm Similarity Dissimilarity PuP(nm) tR(min) 0.98600 0.16676 212.096 3.948		

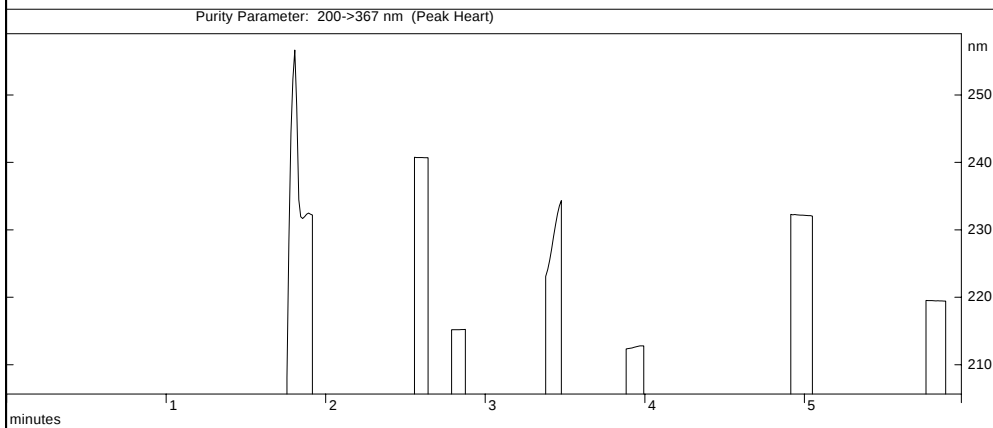
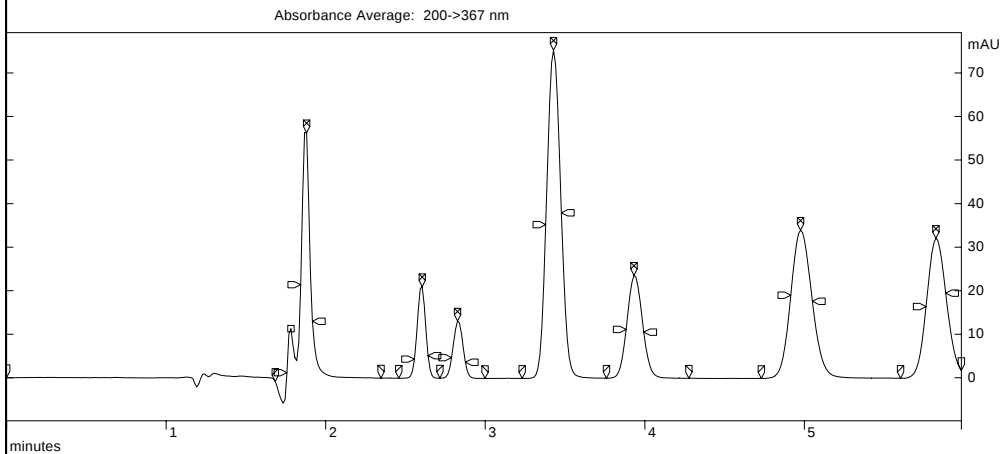
Library Search Report (Short Graphics Format)

File: c:\star\tutorial\pv_sam01.run
Name: Sample #1
Sample #1 with the internal standard added at
9.5 ppm.

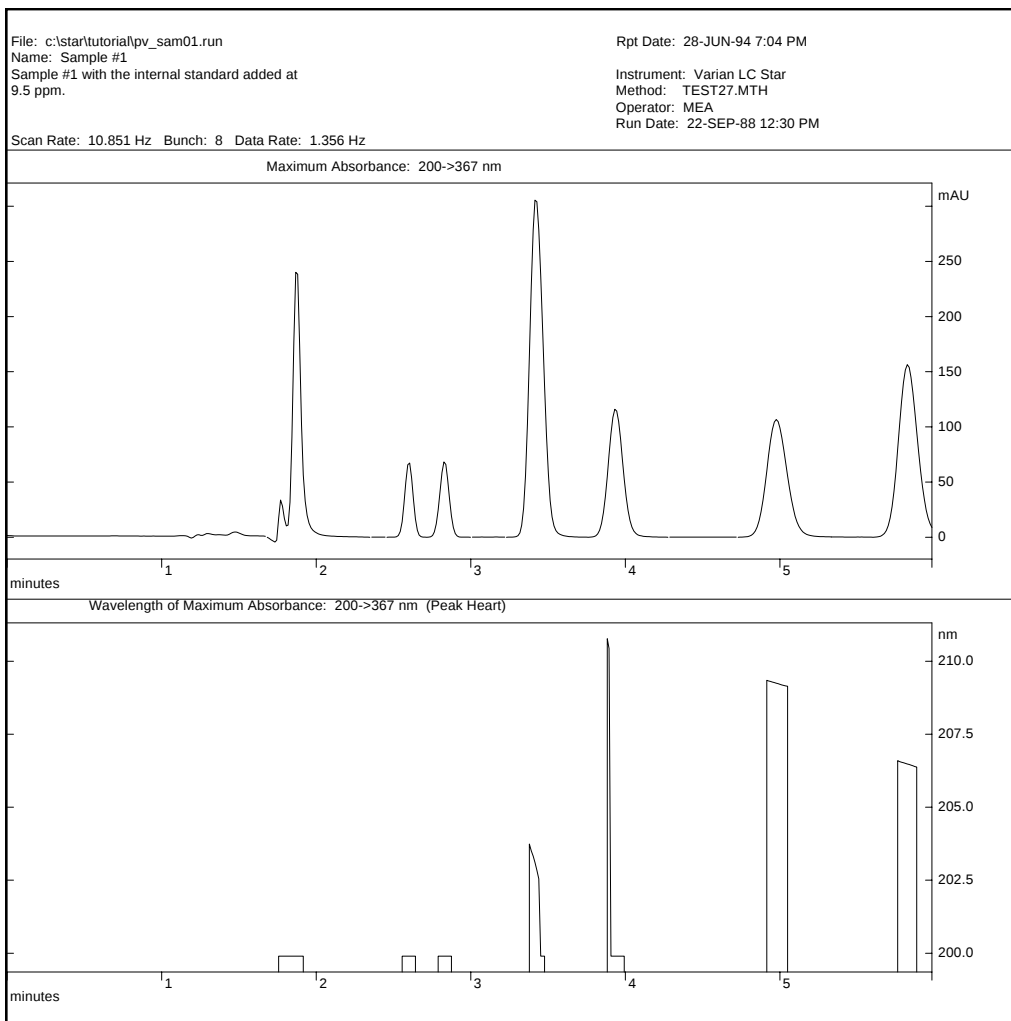
Rpt Date: 28-JUN-94 7:04 PM

Instrument: Varian LC Star
Method: TEST27.MTH
Operator: MEA
Run Date: 22-SEP-88 12:30 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz



Double Plot Report (Example 1)



Double Plot Report (Example 2)

File: c:\star\tutorial\dipa_is.run
 Name: 0.01Diquat/0.1Paraquat
 Same as DIPA3053.RUN, but added
 DiMethylPhtalate peak at 1.2 minutes as
 Internal Standard

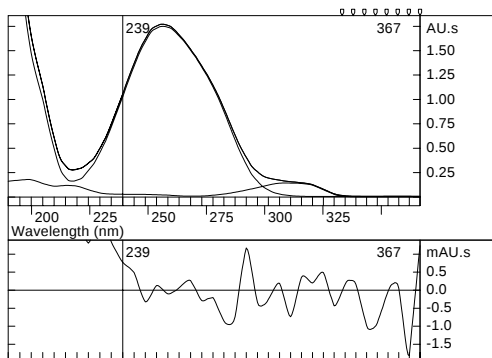
Rpt Date: 28-JUN-94 7:10 PM

Instrument: Varian Star
 Method: pq.mth
 Operator: Number 1
 Run Date: 3-MAR-92 10:40 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

MCA Report - External Standard Analysis

Baseline: 1.794 min (average of 5) to 3.563 min (average of 5)
 Integration: 1.794 to 3.563 min, Flow Rate: 2.000 ml/min (Pump Log)
 Library: c:\star\tutorial\dipa_is.lib



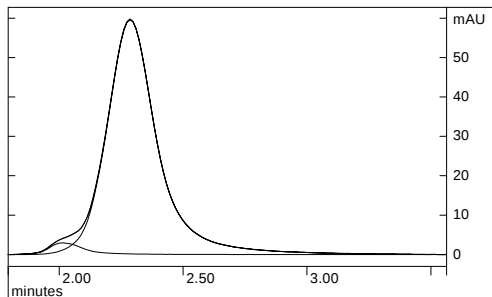
Spectrum Analysis over 239-367 nm

excluding 335-367

Lib	Name	Amount
Index		
#1	Paraquat	9.90 ug
#2	Diquat	0.88 ug

Similarity: 1.00000
 Dissimilarity: 0.00074 (0.001303)

Search window: 1.828 to 2.743 min
 Spectra in library: 3
 Spectra in search window: 2
 Components allowed: 6



Plot Analysis at 283 nm

Lib	tR	Height	Width	Res
Index	(min)	mAU	(sec)	
#2	2.014	2.94	8.8	---
#1	2.286	59.62	12.6	0.90

Error Analysis over 239 - 367 nm

The maximum error 30 uAU
 at 2.291 minutes DOES NOT EXCEED
 the noise threshold 80 uAU.
 The ratio of maximum to expected
 error is 0.356.

Multicomponent Analysis Report (External Standard)

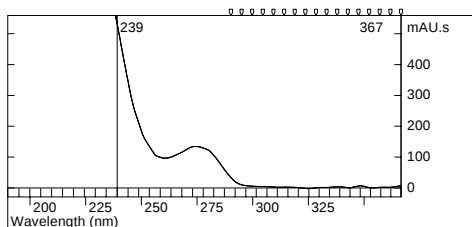
File: c:\star\tutorial\dipa_is.run
Name: 0.01Diquat0.1Paraquat
Same as DIPA3053.RUN, but added
DiMethylPhthalate peak at 1.2 minutes as
Internal Standard

Rpt Date: 28-JUN-94 7:10 PM
Instrument: Varian Star
Method: pq.mth
Operator: Number 1
Run Date: 3-MAR-92 10:40 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

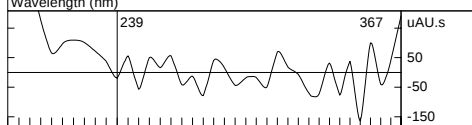
MCA Report - Internal Standard Calibration

Baseline: 1.020 min (average of 5) to 1.376 min (average of 5)
Integration: 1.020 to 1.376 min, Flow Rate: 2.000 ml/min (Pump Log)
Library: c:\star\tutorial\dipa_is.lbr



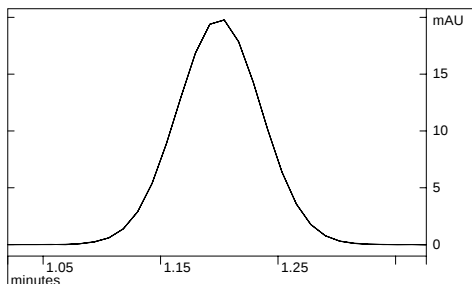
Lib Index	Name	Amount
#3	Caffeine - IS	1.01 ug

Expected IS Amount: 1.000000 #3
IS Correction Factor: 1.005416

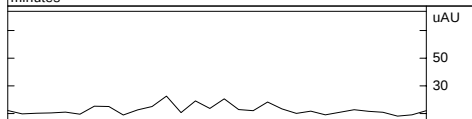


Similarity: 1.00000
Dissimilarity: 0.00048 (0.002723)

Search window: 0.963 to 1.445 min
Spectra in library: 3
Spectra in search window: 1
Components allowed: 1



Lib Index	tR (min)	Height mAU	Width (sec)	Res
#3	1.200	19.77	5.7	---



Error Analysis over 239 - 367 nm

The maximum error 20 uAU
at 1.154 minutes DOES NOT EXCEED
the noise threshold 80 uAU.
The ratio of maximum to expected
error is 0.269.

Multicomponent Analysis Report (Internal Standard Calibration)

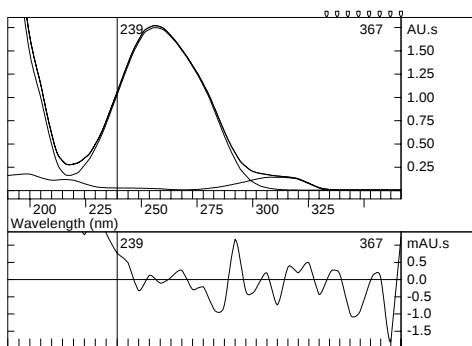
File: c:\star\tutorial\dipa_is.run
 Name: 0.01Diquat/0.1Paraquat
 Same as DIPA3053.RUN, but added
 DiMethylPhthalate peak at 1.2 minutes as
 Internal Standard

Rpt Date: 28-JUN-94 7:10 PM
 Instrument: Varian Star
 Method: pq.mth
 Operator: Number 1
 Run Date: 3-MAR-92 10:40 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

MCA Report - Internal Standard Analysis

Baseline: 1.794 min (average of 5) to 3.563 min (average of 5)
 Integration: 1.794 to 3.563 min, Flow Rate: 2.000 ml/min (Pump Log)
 Library: c:\star\tutorial\dipa_is.lbr

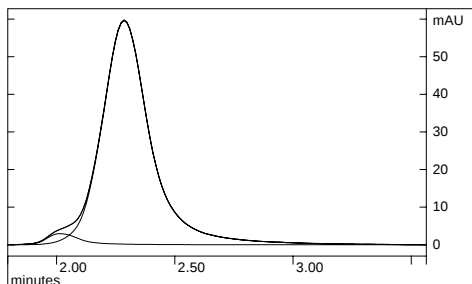


Spectrum Analysis over 239-367 nm
 excluding 335-367

Lib	Name	Amount
#1	Paraquat	9.84 ug
#2	Diquat	0.87 ug

IS Correction Factor: 1.005416

Similarity: 1.00000
 Dissimilarity: 0.00074 (0.001303)
 Search window: 1.828 to 2.743 min
 Spectra in library: 3
 Spectra in search window: 2
 Components allowed: 6



Plot Analysis at 283 nm

Lib	tR	Height	Width	Res
Index	(min)	mAU	(sec)	
#2	2.014	2.94	8.8	---
#1	2.286	59.62	12.6	0.90

Error Analysis over 239 - 367 nm

The maximum error 30 uAU
 at 2.291 minutes DOES NOT EXCEED
 the noise threshold 80 uAU.
 The ratio of maximum to expected
 error is 0.356.

Multicomponent Analysis Report (Internal Standard Analysis)

File: c:\star\tutorial\dipa_is.run
Name: 0.01Diquat/0.1Paraquat
Same as DIPA3053.RUN, but added
DiMethylPhtalate peak at 1.2 minutes as
Internal Standard

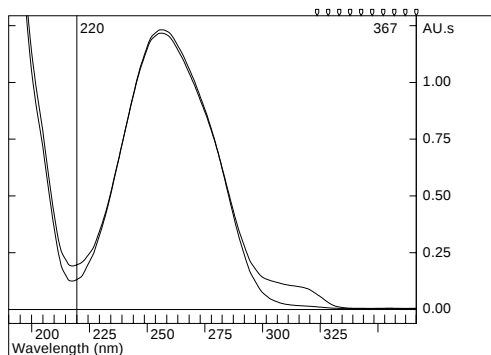
Rpt Date: 28-JUN-94 7:10 PM

Instrument: Varian Star
Method: pq.mth
Operator: Number 1
Run Date: 3-MAR-92 10:40 PM

Scan Rate: 10.851 Hz Bunch: 8 Data Rate: 1.356 Hz

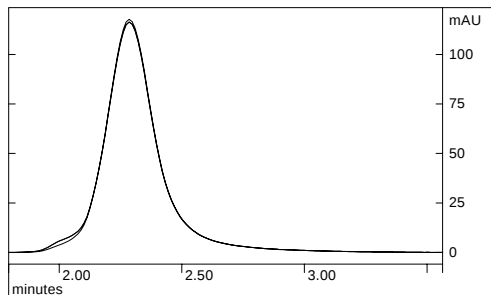
Purity Analysis Report

Baseline: 1.794 min (average of 5) to 3.563 min (average of 5)
Integration: 1.794 to 3.563 min
Reference Spectrum: Region above 50.00% of Apex, 2.175 to 2.396 min



Spectrum Analysis over 220-367 nm
excluding 325-367

Reference vs. Whole Area Spectrum
Similarity: 1.00000
Dissimilarity: 0.00000



Plot Analysis at 254 nm

tR (min)	Height mAU	Width (sec)
2.286	116.39	12.6



Error Analysis over 220 - 367 nm

The maximum error 6 mAU
at 2.012 minutes EXCEEDS
the noise threshold 0.08 mAU.
The ratio of maximum to expected
error is 69.061.

Purity Analysis Report