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Agilent Technologies

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Star Chromatography Workstation
Version 6

Data Handling and Reports

Tutorials

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Table of Contents

Introduction

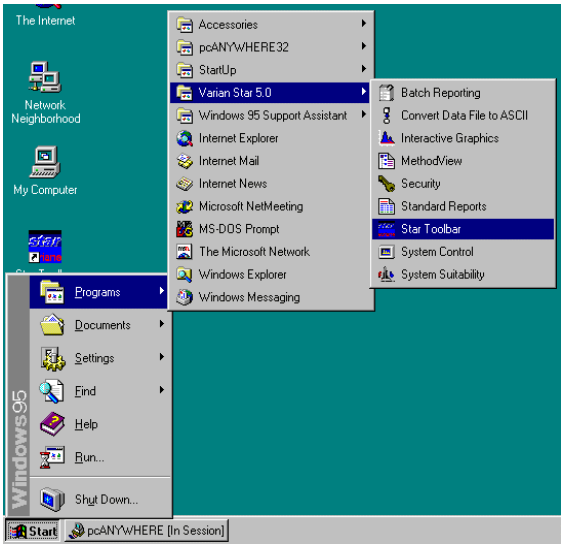
Overview of the Star Chromatography Workstation

The Star Chromatography Workstation is composed of four main applications; Method Editor (METHED97.EXE), System Control/ Automation (CHEMIS32.EXE), Report (REPORT32.EXE), and Interactive Graphics/Data Handling (NGIG.EXE). Each of these main applications is represented by an icon in the Varian Star program group, as well as by a button on the Star Bar (STARBAR.EXE).

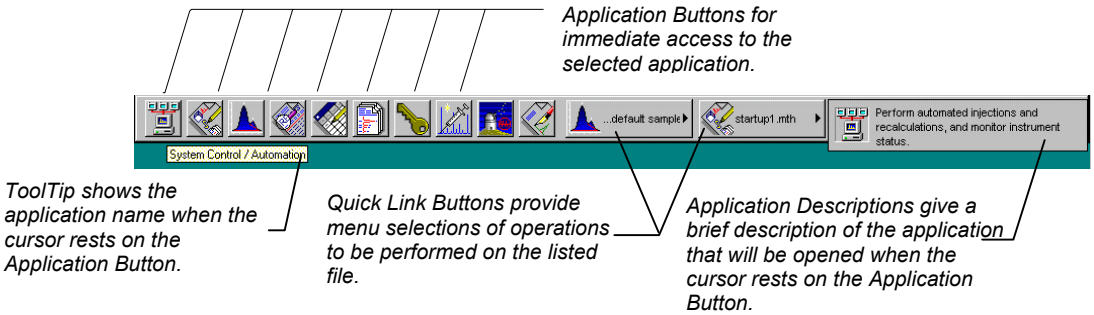
Using the Star Toolbar

Star Chromatography Workstation is a suite of applications for controlling chromatographs, collecting data from chromatograph detectors, and analyzing that data. The Star Toolbar provides quick and easy access to the Star Chromatography Workstation applications. When activated the Star Toolbar behaves very much like the Windows Taskbar. It can be docked on any of the four sides of the display screen and other Windows programs will not cover or go behind it when they are opened in full screen mode.

If the Star Toolbar is not already opened on your Star Workstation, you can start it from the Windows Start Menu.



Elements of the Star Toolbar



Application Buttons

Used to monitor instrument status, perform automated injections, and perform batch recalculations.

Used to view and edit instrument operation, data acquisition, and data handling methods.

Used to review chromatograms, interactively edit data handling parameters, and recalculate results.

Used to preview standard chromatogram and results reports.

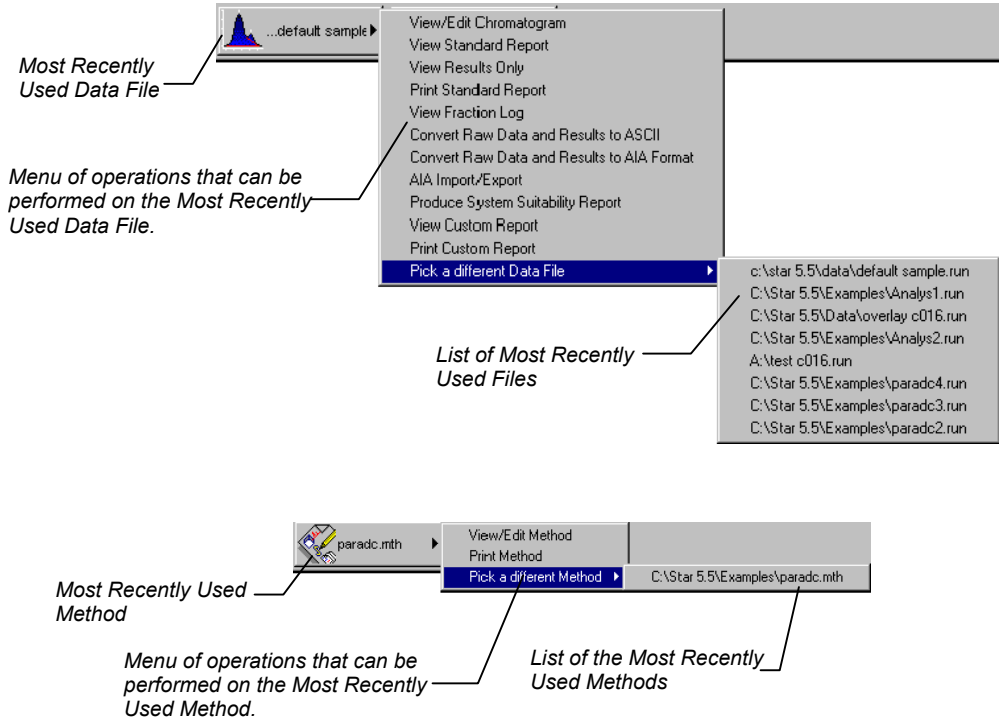
Used for offline editing of SampleLists, RecalcLists and Sequences.

Used to generate standard reports for a group of Data Files by dragging and dropping them on the Batch Report Window.

Used to set Star Workstation security options and passwords.

Other application buttons may be added to the Star Toolbar when you install additional Star Workstation Options, such as StarFinder, Star Custom Report Writer, and PolyView2000.

Quick Link Buttons



Using the Tutorials

These tutorials are intended for demonstrating the data handling capabilities of the Star Workstation by having the user follow a set of step by step procedures. Each procedure is followed by a picture to verify that the user has performed the actions correctly.

After running the tutorials the user should have a good idea of how to use the Workstation to reprocess chromatography data after it is collected.

Example Files

When you install the Star Chromatography Workstation software the Examples directory will be created in the directory where your workstation software is installed. The Examples directory contains files which can be used for learning about the Star Chromatography Workstation data handling capabilities. These include calibrated Methods, a Sequence, a RecalcList, and data files. These files can be used for comparison and to see various components of the system, such as calibration curves, without having to build them yourself.

Enabling the ADC Board Driver

The ADC Board Driver must be enabled to use the example files in the tutorial manual. To verify that the ADC Board Driver is installed and enabled, click on the Star Bar with the right mouse button and select “Enable / Disable instrument modules”. Make sure that the ADC Board Driver appears in the topmost window list box. If the ADC Board is in the bottom list box, re-enable the driver by highlighting the “ADC Board” and click on the “enable” button. If the ADC Board does not appear in either window, reinstall the Star Workstation with control software containing the ADC Board Driver.

Reinstalling the Tutorial Files

If the tutorials have been run previously, you may wish to reinstall them so that you can follow the specific instructions of the tutorials. To do this,

1. Insert the Star Workstation CD-ROM into the CD-ROM drive.

   If the CD program does not start automatically, run *Install.exe* from the CD.

2. From the CD Browser click on the “Install” button, then click on “Refresh Tutorials”.

3. When prompted from the optional components dialog box, choose to install “Example Data Files and Methods”. (You can unclick the checkboxes for the other optional components)

4. Click on the “Next” button.

The tutorials are arranged in a series that will help you utilize the data handling capabilities of the Star Workstation. Upon completing these tutorials, you should be familiar with how to recalculate, reintegrate and calibrate data collected with the Star Workstation.

Tutorial Basics

These tutorials make the following assumptions:

- You have installed the Star Chromatography Workstation to the Star Directory and Windows is installed to the Windows directory.
- You are generally familiar with Microsoft Windows and understand the terms Click, Click and drag, Double-click, etc.
- Your PC is powered up and you are running Windows.

Tutorials with Access Control and Audit Trail Software

If Access Control and Audit Trail software is installed on the WS, you will be subject to its regulation as you go through the tutorials.

- You will need the right to Batch Recalc data.
- You will need the right to run instruments with standard.
- You will need the right to modify methods.

Some of the steps described in the tutorial may be modified by the presence of Access Control and Audit Trail software. Also, some of the screens in the tutorial will look different with Access Control software.

Tutorial 1 Recalculating Results

The Star Chromatography Workstation allows you to recalculate results in Interactive Graphics and in System Control. Both applications offer unique advantages for reprocessing data files. Interactive Graphics lets you see the changes to the chromatogram in an interactive environment. System Control lets you easily recalculate large groups of data files as part of a sequence.

Topics Discussed

- Reintegration with Moved Start/Ends
- Reintegrating
- Method Editing
- Recalculating in System Control

Preparing a Data File for Use with this Tutorial

Because PRACTICE.RUN is used for several tutorials, you should copy this data file to RECALC.RUN for use with this tutorial.

In Windows Explorer.

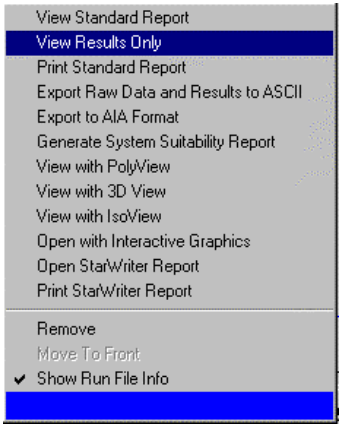
1. View the C:\STAR\EXAMPLES directory.
2. Use the right mouse button to click on PRACTICE.RUN, and select *Copy*.
3. Select EDIT→Paste. A copy of this file is added at the end of the list.
4. Use the right mouse button to click on the new file. Select Rename, and type RECALC.RUN.

Opening a Data File and Method in Interactive Graphics

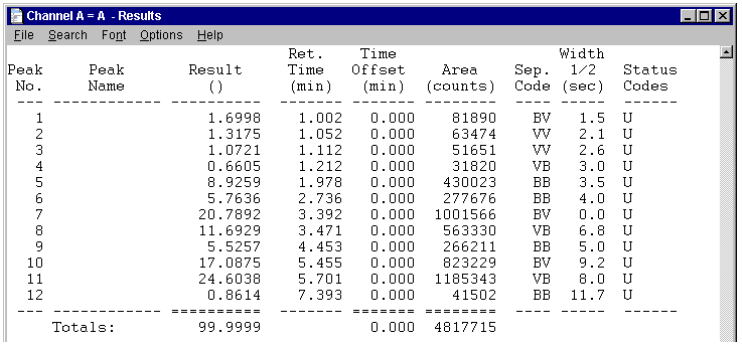
Begin by opening the Interactive Graphics application, selecting a chromatogram to reprocess, and a method to use for recalculation.

1. From the Star Bar, open the Interactive Graphics/Data Handling application. The Open Multiple Data Files dialog box appears.
2. Double-click on RECALC.RUN in the EXAMPLES directory to add it to the list on the lower right side of the dialog box, and click on *Open File(s)*.
3. When the Open Method File dialog appears, click on *OK* to "Build Method from Data File".
4. Select FILE→Open Method... . The Open Method dialog box appears.
5. Find and select the method called ANOTHER.MTH in the EXAMPLES directory.

6. To open the results file **click on the chromatogram trace with the right mouse button**. Select "View Results only" as seen in the menu below.



The results for the data file appear. Note the areas for the peaks at 3.392 and 3.471 minutes. The results should appear as shown below.



Channel A = A - Results

File Search Font Options Help

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.6998	1.002	0.000	81890	BV	1.5	U
2		1.3175	1.052	0.000	63474	VV	2.1	U
3		1.0721	1.112	0.000	51651	VV	2.6	U
4		0.6605	1.212	0.000	31820	VB	3.0	U
5		8.9259	1.978	0.000	430023	BB	3.5	U
6		5.7636	2.736	0.000	277676	BB	4.0	U
7		20.7892	3.392	0.000	1001566	BV	0.0	U
8		11.6929	3.471	0.000	563330	VB	6.8	U
9		5.5257	4.453	0.000	266211	BB	5.0	U
10		17.0875	5.455	0.000	823229	BV	9.2	U
11		24.6038	5.701	0.000	1185343	VB	8.0	U
12		0.8614	7.393	0.000	41502	BB	11.7	U
	Totals:	99.9999		0.000	4817715			

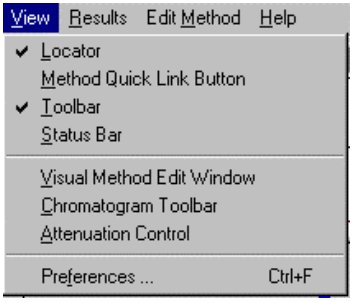
7. Close the Results window. Click on the ☒ in the upper right corner of the window.

8. In Interactive Graphics Select VIEW→PREFERENCES. Select the TRACE SETTINGS TAB. Click on the Radio Button called SHOW ALL EVENTS under SHOW / HIDE PEAK EVENTS section.

9. Select the VIEW→Locator Window. (make sure it is checked)

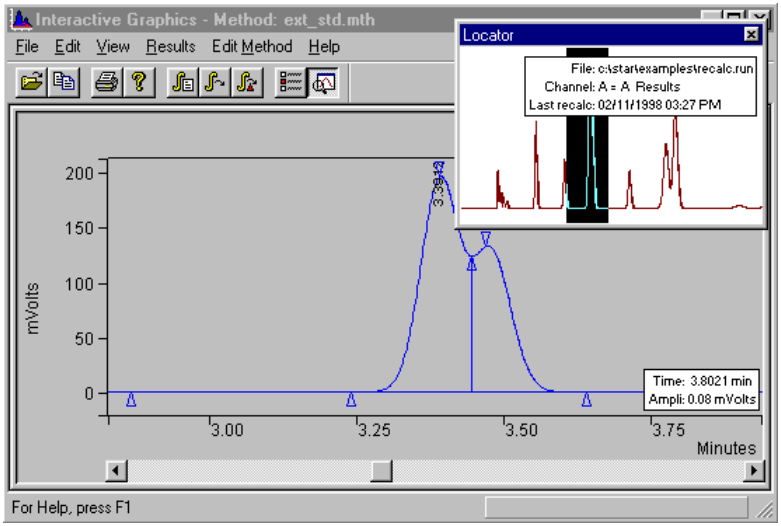
10. Select VIEW→Chromatogram toolbar Button (make sure it is checked)

    Your settings should look as follows



11. Now, zoom in on the center of the fused peak eluting at about 3.4 minutes. To zoom, highlight the desired area in the locator window or highlight the region in the main window from 2.5 to 4.0. (Areas of the display can be highlighted by clicking on the upper left hand corner of the region you wish to zoom with the left mouse button, dragging the mouse to the lower right hand corner of the area you wish to display, and releasing the mouse button.)

Your display should look as follows:



This enlarges the valley area between these overlapped peaks.

Moving a Peak Event

In the Interactive Graphics/Data Handling application, you have the ability to move peak events manually. With this technique, you can accurately define the placement of baseline peak events in instances where the automatic placement of events was not optimum. You move a baseline event by clicking and dragging the event triangle to a new location.

When you are adjusting the position of a start or end point, you may find it more convenient and accurate to display the actual data points from the file on the screen.

To hide the locator window:

1. Select VIEW→Preferences →Locator (make sure it is unchecked)

2. Click on the *Trace Settings* tab

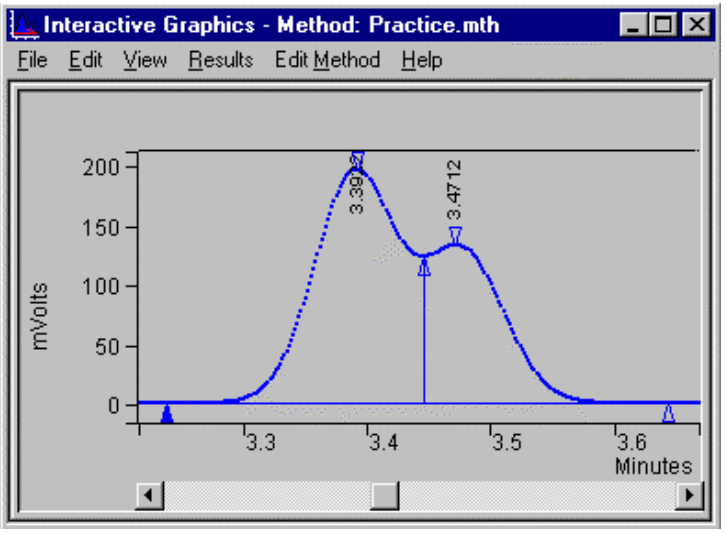
3. Click on the *Point* option button under Plot Type. Click *OK*. The chromatogram is now drawn as a series of dots representing the individual data points from the file.

4. Place the cursor over the valley point between the two peaks.

5. Click the left mouse button, hold it down, and drag the valley point slightly to the left.

6. When the cursor is at the new valley point location, release the mouse button to move the valley point there

   Your window should look similar to this:



Note that the peak event triangle for the valley point is now drawn as a solid triangle indicating a moved peak event. Now that the point is repositioned, you can recalculate the file using the moved baseline event.

Recalculating the Data File

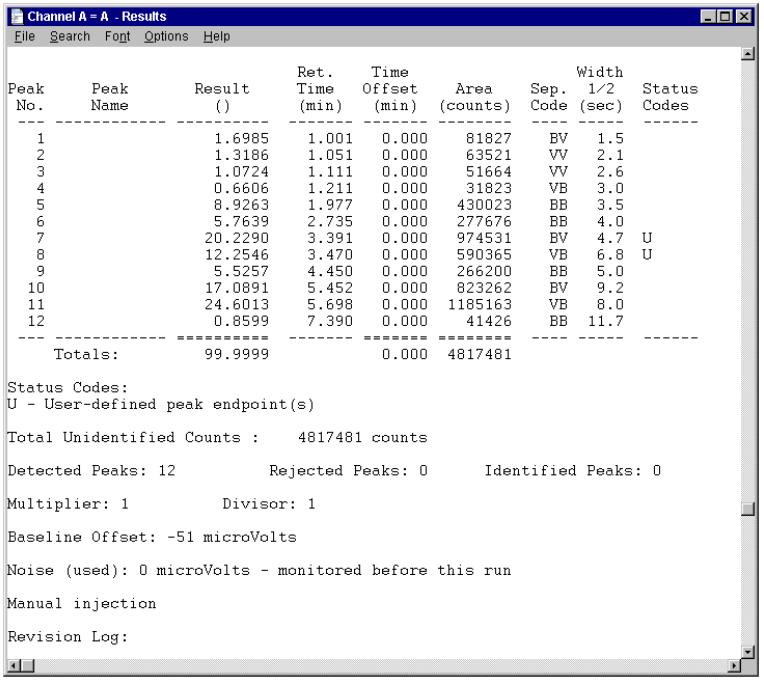
1. Select RESULTS→Reintegration List... . A check mark appears in the Run DH column for the data file. This indicates that the file is selected for recalculation.

NOTE: **Before reintegration, you can return the event triangle to its original position by selecting RESULTS→Reintegrate now/Clear Moved Events, or you can use a right mouse click on the event triangle that you have moved then select Reset to Original Position from the displayed menu.**

2. Click on Calculate Results.
   A message appears on the screen, asking if you wish to save moved start/end points, click on *Yes*.

3. To open the results file **click on the chromatogram trace with the right mouse button**. Select “View Results only”.

The areas for the peaks at 3.391 and 3.470 minutes have changed as a result of the change in the valley point between them. Compare your values to those shown below. Note that the two peaks have been flagged as using User-defined peak endpoint.



Channel A = A - Results

File Search Font Options Help

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.6985	1.001	0.000	81827	BV	1.5	
2		1.3186	1.051	0.000	63521	VV	2.1	
3		1.0724	1.111	0.000	51664	VV	2.6	
4		0.6606	1.211	0.000	31823	VB	3.0	
5		8.9263	1.977	0.000	430023	BB	3.5	
6		5.7639	2.735	0.000	277676	BB	4.0	
7		20.2290	3.391	0.000	974531	BV	4.7	U
8		12.2546	3.470	0.000	590365	VB	6.8	U
9		5.5257	4.450	0.000	266200	BB	5.0	
10		17.0891	5.452	0.000	823262	BV	9.2	
11		24.6013	5.698	0.000	1185163	VB	8.0	
12		0.8599	7.390	0.000	41426	BB	11.7	
	Totals:	99.9999		0.000	4817481			

Status Codes:
U - User-defined peak endpoint(s)

Total Unidentified Counts : 4817481 counts

Detected Peaks: 12 Rejected Peaks: 0 Identified Peaks: 0

Multiplier: 1 Divisor: 1

Baseline Offset: -51 microVolts

Noise (used): 0 microVolts - monitored before this run

Manual injection

Revision Log:

When you reintegrate a data file after moving peak start and end points, the Workstation uses the new points in the peak processing algorithm. So any manual adjustments you make to these points are preserved.

Editing the Method

Now let's look at some of the changes you can make to the method and how they affect the results. The Results window contains the chromatographic results calculated for this data file. In addition to the injection time and date, the report lists the time and date of the last recalculation done and the method used to do it. Close the Results window.

The Results window can be positioned, scrolled, and sized.

Now, let's change the method so that the peaks are measured in units of peak height rather than peak area.

1. Select EDIT METHOD→Integration Parameters... . The Integration Parameters dialog box is displayed.

   The Integration Parameters dialog box is partitioned into sections associated with the various functions of peak detection, measurement, and result calculation. Notice that the Results window is automatically hidden while you work on the method.

2. Click on *Peak Height* within the Peak Measurement section of the Integration Parameters dialog box. The Peak Height radio button becomes dark (selected).

3. Click on *Save.*

   This saves the settings for this window temporarily, but it does not write them to the method. You must save the method itself to save the changes permanently.

4. To reintegrate the file according to the new method, select RESULTS→Reintegrate Now.

   When the calculations are complete, open the Results window as previously described. Notice that both the Result (%) and the number of counts (now in peak height units) have changed for each peak.

5. Close the Results window and return to the Interactive Graphics/Data Handling window.

6. To end your Interactive Graphics session, select FILE→Exit. Do not save changes to the method or the data file.

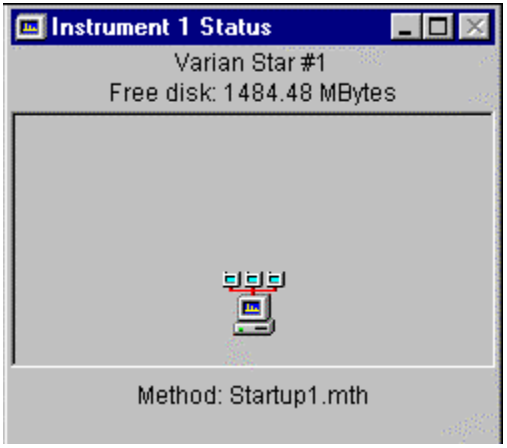
Recalculating in System Control

You can use a sequence in System Control to reprocess one or more chromatographic data files. This lets you adjust chromatographic conditions after you've made a sequence of injections.

Because recalculations do not require the use of any instrument modules, you can recalculate with any instrument, even one with no modules assigned to it.

1. Open System Control.

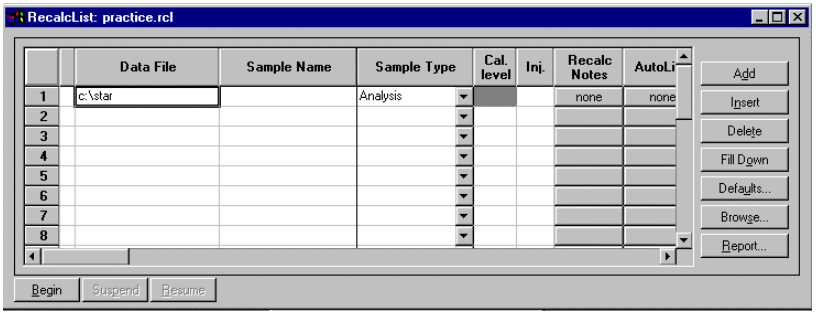
2. Select an unused instrument from the Instrument menu in System Control or double-click in the box for that instrument in the Instrument Configuration window. The Instrument's System Control window opens and displays the Instrument's Status.



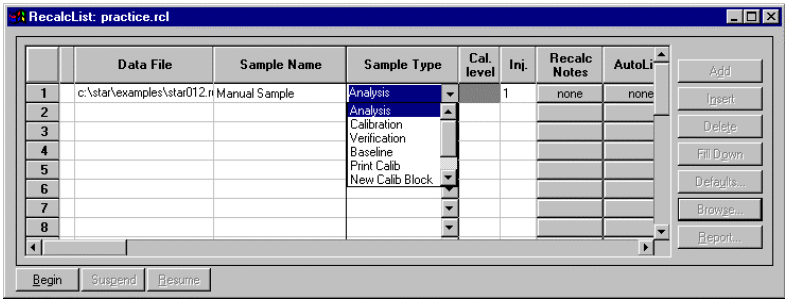
Creating a Recalc List for Recalc

1. Select FILE→New Recalc List
2. Type in practice.rcl into the edit field that contains the file name.
3. Select the EXAMPLES directory and Click on SAVE.
4. Double-click in the first cell in the first column and then click on any other entry in the first line.

The cell becomes active.



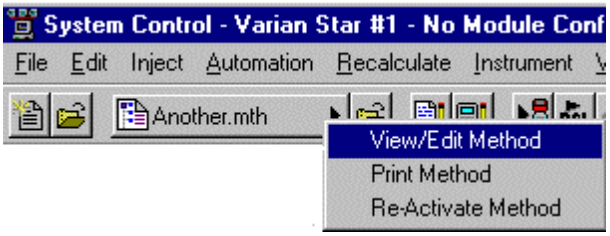
5. Click on *Browse*... . This opens the Open Data File dialog box.
6. Find and open the data file called STAR012.RUN in the Tutorial directory. This file is added to the RecalcList spreadsheet. Click on the arrow in the Sample Type field. A drop-down menu opens.



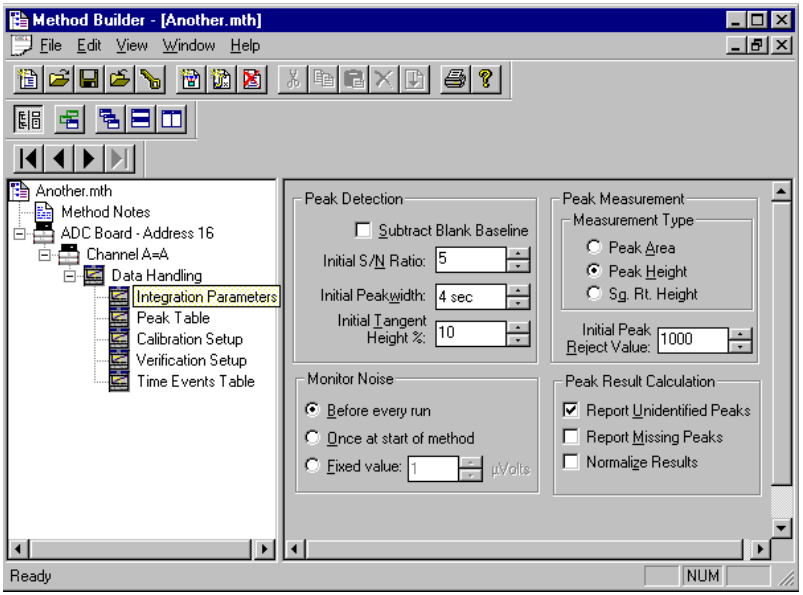
7. Click on *Analysis*. Leave the other fields set to their default values.

Preparing to Recalculate the Results

1. Select FILE→Activate Method
2. Choose the Method ANOTHER.MTH from the EXAMPLES directory. Click on the Open button
3. Click on the Active Method on the Toolbar, and select View / Edit Method.



4. From Method View highlight the Integration Parameters Section of the Method.

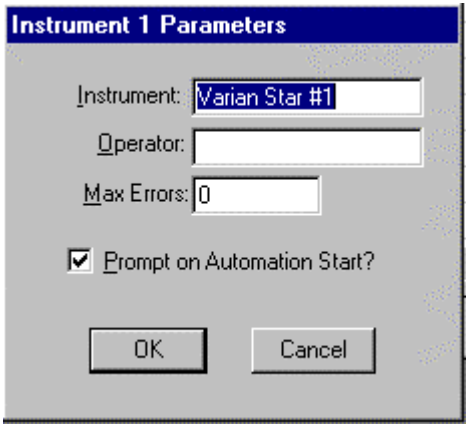


5. The Integration Parameters window opens, showing the current settings. Peak measurement is by height, and Report Unidentified Peaks is checked.
6. Click on Peak Area in the peak measurement box.
7. Select FILE→Save
8. Close Method View

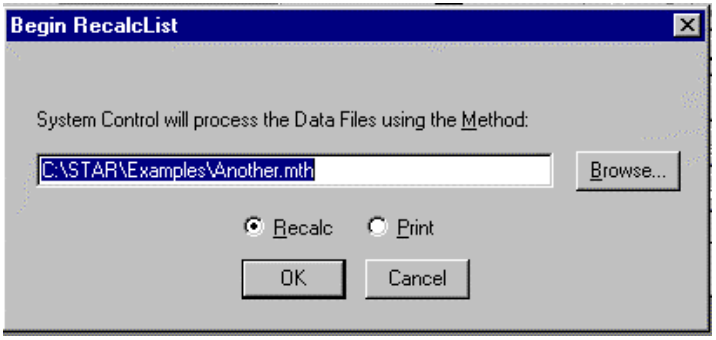
Recalculating the Results

1. Select RECALCULATE→Begin Recalc List
2. If previously set to Prompt on Sequence start, the Instrument Parameters dialog box appears.
3. Click in the Operator text box, type your name, then click OK. (If the Parameters dialog box does not appear, you can open it by selecting Instrument/Configuration, and click on Instrument n Parameters).

Note: **If Access Control software is installed, the entries for instrument and operator will be preset and not changeable.**



A message from System Control appears stating that it will be recalculating samples in RECALC.SMP using the method ANOTHER.MTH.



4. Click the *OK* button to carry out the operation.

   A message appears at the bottom of the window to inform you that System Control is doing the recalculation. The sample name, the injection number, and the name of the data file are listed. Wait for the message “End of automation reached.” This message appears when the recalculation has been completed.

5. Click the *Report* button in the Active Recalc List window.

   The results report is displayed. Look at the Results column. Notice that the result for each peak is expressed as a percentage of the total area for all the peaks.

Tutorial 2 Changing Peak Detection Parameters

The Workstation provides several ways to change how peaks are detected after a run. You can modify settings in the Peak Detection area of the Integration Parameters window, or you can use the Time Events Table to program changes in Peak Width or inhibit integration. All such changes require reintegration with the new method. Both System Control and Interactive Graphics/Data Handling allow these changes. For this tutorial, you will use Interactive Graphics.

Topics Discussed

- Changing the Initial Peak Width
- Changing the Signal-to-Noise Ratio
- Changing the II and WI Time Events

Preparing the Data File for Use with this Tutorial

Because PRACTICE.RUN is used for several tutorials, you should copy this data file to DETECT.RUN for use with this tutorial.

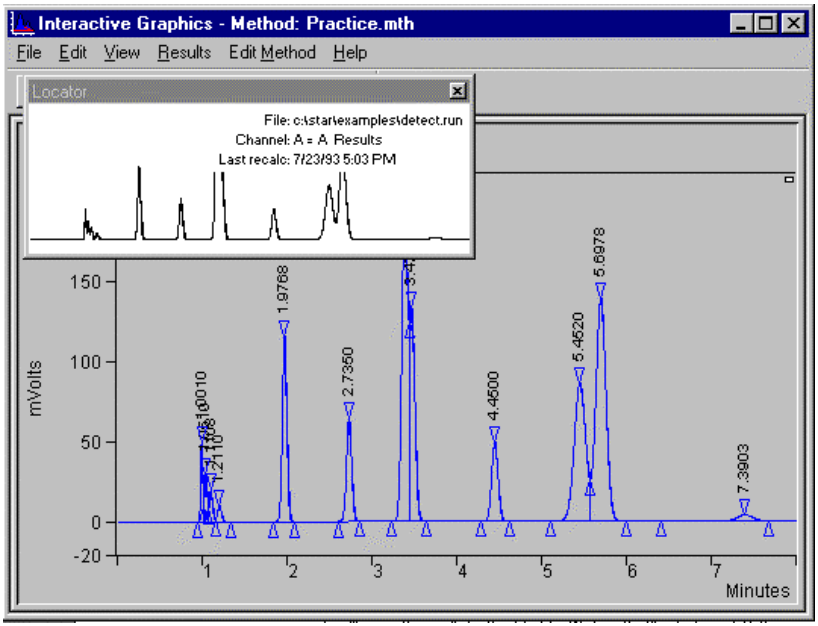
In Windows Explorer.

1. View the C:\STAR\EXAMPLES directory.
2. Use the right mouse button to click on PRACTICE.RUN, and select *Copy*.
3. Select EDIT→Paste. A copy of this file is added at the end of the list.
4. Use the right mouse button to click on the new file. Select Rename, and type DETECT.RUN.

Opening the Data File and Method

1. Click on the Interactive Graphics/Data Handling bar on the Star Bar.
2. From the EXAMPLES directory, double-click on the DETECT.RUN file.
3. Click on *Open File(s)* and *OK* to build Method from data file.
4. Select FILE→Open Method, then double-click on PRACTICE.MTH.

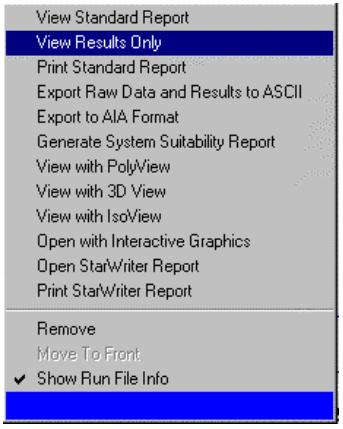
Notice the title bar of the Interactive Graphics/Data Handling window. The Method is identified as PRACTICE.MTH.



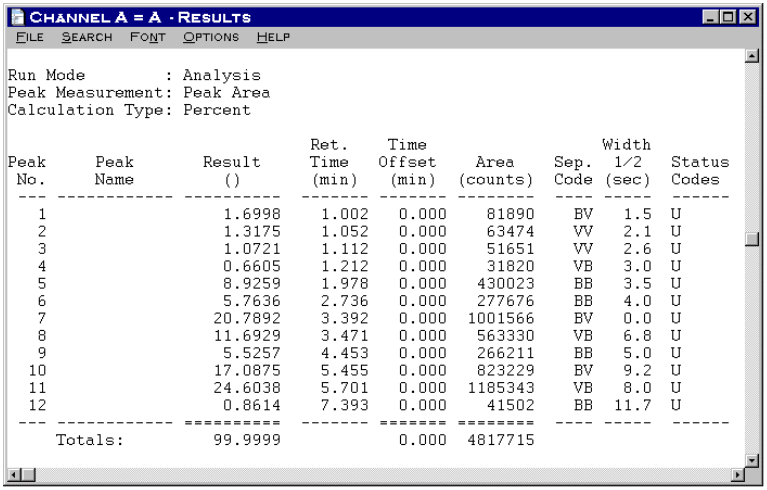
Changing the Initial Peak Width

You can now edit the data handling parameters for the method. Before you make any changes, though, look at the results for the run as they appear initially.

1. To open the results file **click on the chromatogram trace with the right mouse button**. Select “View Results only” as seen in the menu below.



The results for the data file appear. Note the areas for the peaks at 3.392 and 3.471 minutes. The results should appear as shown below.



CHANNEL A = A - RESULTS

FILE SEARCH FONT OPTIONS HELP

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.6998	1.002	0.000	81890	BV	1.5	U
2		1.3175	1.052	0.000	63474	VV	2.1	U
3		1.0721	1.112	0.000	51651	VV	2.6	U
4		0.6605	1.212	0.000	31820	VB	3.0	U
5		8.9259	1.978	0.000	430023	BB	3.5	U
6		5.7636	2.736	0.000	277676	BB	4.0	U
7		20.7892	3.392	0.000	1001566	BV	0.0	U
8		11.6929	3.471	0.000	563330	VB	6.8	U
9		5.5257	4.453	0.000	266211	BB	5.0	U
10		17.0875	5.455	0.000	823229	BV	9.2	U
11		24.6038	5.701	0.000	1185343	VB	8.0	U
12		0.8614	7.393	0.000	41502	BB	11.7	U
Totals:		99.9999		0.000	4817715			

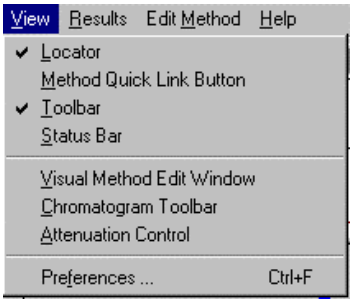
Now, let's take a look at the Initial Peak Width setting and its effect on how the peaks are processed.

2. Close the Results window.

3. Select the VIEW→Locator Window. (make sure it is checked)

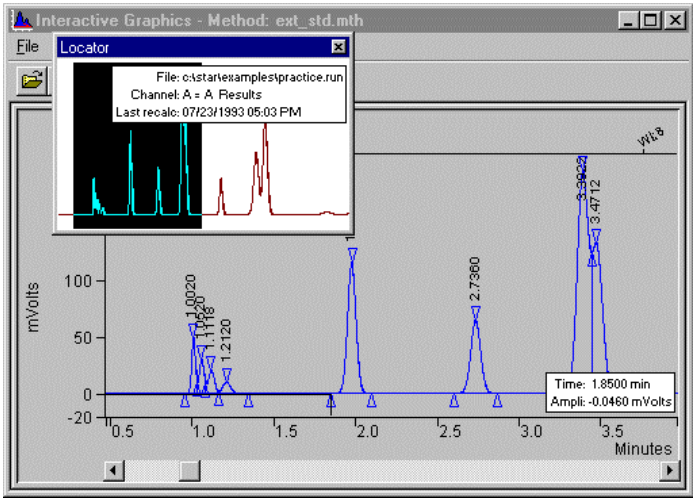
4. Select VIEW→Toolbar (make sure it is checked)

   Your settings should look as follows



5. Zoom in on the area from 0.5 to 4.0. To zoom, highlight the desired area in the locator window or highlight the region in the main window from 0.5 to 4.0. (Areas of the display can be highlighted by clicking on the upper left hand corner of the region you wish to zoom with the left mouse button, dragging the mouse to the lower right hand corner of the area you wish to display, and releasing the mouse button.)

   Notice the placement of baselines and peak event markers.



6. Select EDIT METHOD→Integration Parameters... .
   The Integration Parameters dialog box appears.

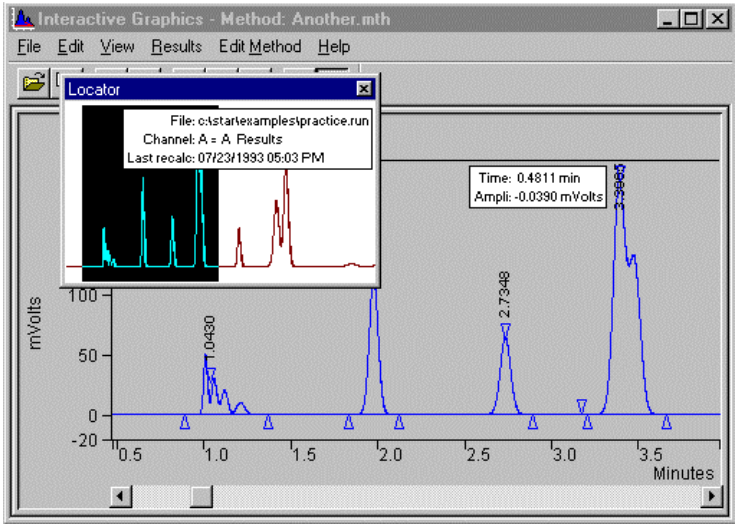
7. Increase the Initial Peak Width value from 4 to 32 by clicking on the up arrow until 32 appears in the box.

8. Choose *Save* to close the Integration Parameters dialog box and return to the Interactive Graphics/Data handling window.

9. Select RESULTS→Reintegration List...
   Note that a check mark appears in the Run DH check box for the data file.

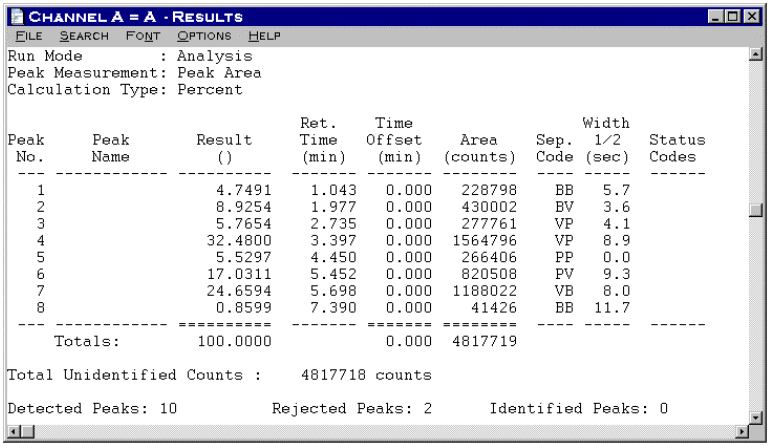
10. Click on Calculate Results.

11. After peak processing has been completed, examine the new set of peak events displayed in the Zoom window. Notice that for the narrow, early eluting peaks the event markers have shifted to the right and that three of the peaks do not have apex peak event triangles on them. This suggests that the Initial Peak Width value is too large relative to the narrow widths of these peaks. Obviously, it is important to set the Initial Peak Width small enough so that narrow, early eluting peaks are properly detected and their peak events are accurately marked.



12. Again, view the results by accessing the report by clicking on the mouse button with the right button and selecting “view results only”.

13. Maximize the Results window and examine this new set of results.



Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		4.7491	1.043	0.000	228798	BB	5.7	
2		8.9254	1.977	0.000	430002	BV	3.6	
3		5.7654	2.735	0.000	277761	VP	4.1	
4		32.4800	3.397	0.000	1564796	VP	8.9	
5		5.5297	4.450	0.000	266406	PP	0.0	
6		17.0311	5.452	0.000	820508	PV	9.3	
7		24.6594	5.698	0.000	1188022	VB	8.0	
8		0.8599	7.390	0.000	41426	BB	11.7	
Totals:		100.0000		0.000	4817719			

Total Unidentified Counts : 4817718 counts

Detected Peaks: 10 Rejected Peaks: 2 Identified Peaks: 0

Compare this to the original results. You will notice that the retention times are slightly shifted and only 8 peaks are reported. The fused peaks at about 3.4 minutes are now reported as one

peak. Also, only one peak was detected between 1.0 and 1.5 minutes. The total number of detected peaks is now 10.

When experimenting with peak processing in the Interactive Graphic/Data Handling application, it is convenient to leave the Results window active. Then, after any Reintegration, this window is automatically displayed.

Changing the Signal-to-Noise Ratio

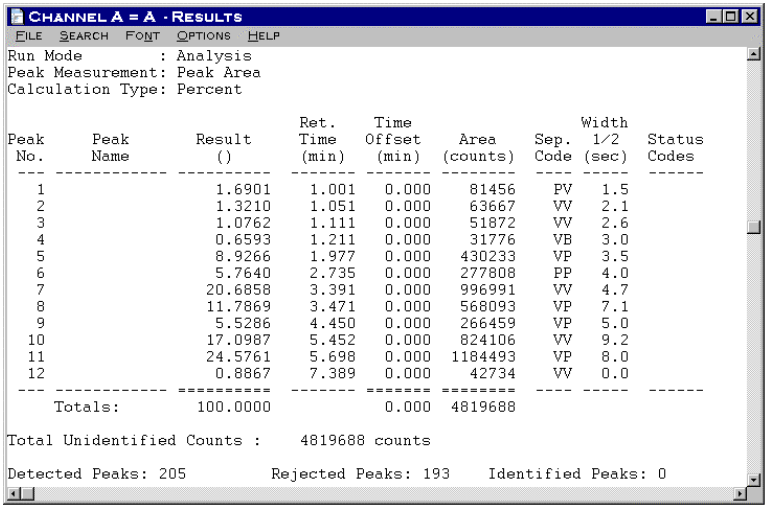
1. Select EDIT METHOD→Integration Parameters... .
2. Decrease the S/N Ratio from 5 to 1.
3. Set the Initial Peak Width back to 4.
4. Choose Save to close the Integration Parameters window.

   You can reintegrate the data file quickly if you know that you want to use the settings in the Reintegration List that you used last time.
5. Choose RESULTS→Reintegrate Now.

   Many peak event markers for small peaks have appeared. The lower S/N Ratio caused peak processing to detect the smaller signals as peaks.

   Check the final Results of this change in the S/N Ratio.
6. Maximize the Results window and take a look at the new set of Results.

They should match the ones below. Note the high number of detected peaks.



CHANNEL A = A - RESULTS

FILE SEARCH FONT OPTIONS HELP

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.6901	1.001	0.000	81456	PV	1.5	
2		1.3210	1.051	0.000	63667	VV	2.1	
3		1.0762	1.111	0.000	51872	VV	2.6	
4		0.6593	1.211	0.000	31776	VB	3.0	
5		8.9266	1.977	0.000	430233	VP	3.5	
6		5.7640	2.735	0.000	277808	PP	4.0	
7		20.6858	3.391	0.000	996991	VV	4.7	
8		11.7869	3.471	0.000	568093	VP	7.1	
9		5.5286	4.450	0.000	266459	VP	5.0	
10		17.0987	5.452	0.000	824106	VV	9.2	
11		24.5761	5.698	0.000	1184493	VP	8.0	
12		0.8867	7.389	0.000	42734	VV	0.0	
	Totals:	100.0000		0.000	4819688			

Total Unidentified Counts : 4819688 counts

Detected Peaks: 205 Rejected Peaks: 193 Identified Peaks: 0

Now, the detected peaks correspond to all signals larger than the new S/N Ratio. The change in S/N Ratio affects the placement of peak events and baselines, the accuracy of which are necessary for reliable chromatographic quantitation. Setting the correct S/N Ratio is particularly important in percent calculations, where the results for each peak are expressed as a percentage of the total area or height counts for all the peaks.

Note: **To restore the data handling on this file for the next tutorial, increase the S/N Ratio from 1 to 5 and reintegrate the data file.**

Changing the II Time Events

A method can include a set of time-programmable events to tailor the integration and peak area allocation functions for a particular run. In this section, we will examine how Inhibit Integrate (II) affects peak detection. Other Time-Programmable events are discussed in a later tutorial.

1. Double-click in the Chromatogram window to restore the Zoom window to the full range of the run file.

In addition to the positions of the peak events, Interactive Graphics also indicate the positions of time events to help you

interpret how your chromatogram is being processed. Since no time events have been programmed yet, none of these markers should appear.

2. Select OPTIONS→Preferences.

3. Make sure the Show Time Events box is checked. Click on *OK* to confirm the choice and close the dialog box.

4. Select EDIT METHOD→Time Events. The Time Events Table window is displayed.

Note: For an exercise in how to graphically enter Timed Events, see *Tutorial 5 Using the II, SR, and VB Time Events.*

Now, let's add a time event to inhibit integration at the beginning of the chromatogram.

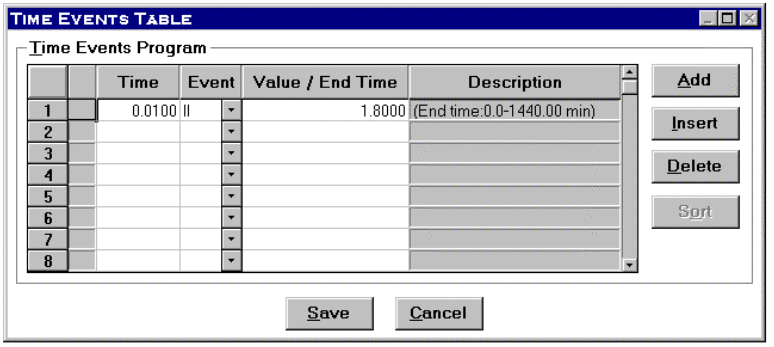
1. Click on *Add*, to add a new line of default entries.

2. Click the arrow at the right of the Event box.

3. Click the up or down scroll arrow until II appears. Click the II event.

4. Change the start time and end times. Enter 0.01 into the Time column and 1.80 into the Value/End Time column.

   Your display should look like the following.



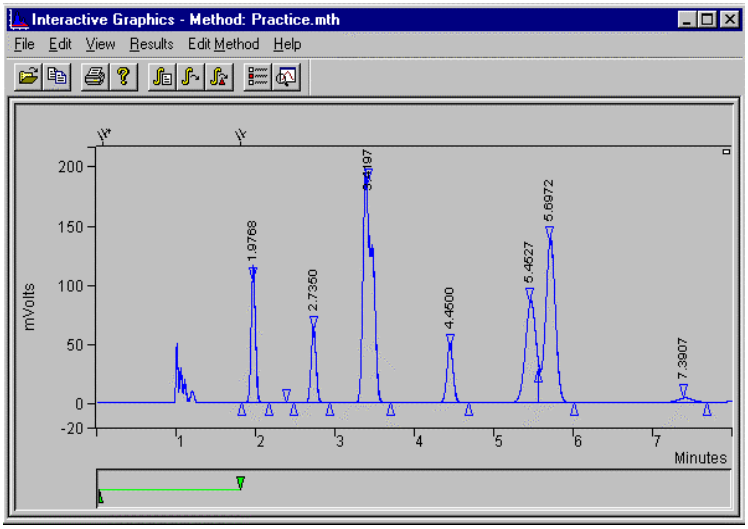
5. Choose *Save* to close the Time Events Table window.

6. Select RESULTS→Reintegrate Now.

7. Select VIEW→View Method Edit Window. This will display the Time Event Window below the chromatogram trace.

You must reintegrate with the new settings because the II Time Event affects peak integration.

After the calculation, the results will automatically be displayed and the plot will be updated to reflect the changes in the time program. The figure below shows the updated chromatogram. Notice that no peak event markers appear at the beginning of the chromatogram. Also, the Time Events annotation on the display has been updated with green boxes near the baseline at the start and end times for the II event.



Changing the WI Time Event

Now, suppose you decided not to use the II event but wanted to program some changes in the peak width.

1. Open the Time Events Table window again by selecting EDIT METHOD→Time Events. The II line in the spreadsheet is selected (active).

2. Click the down arrow at the right of the Event box.

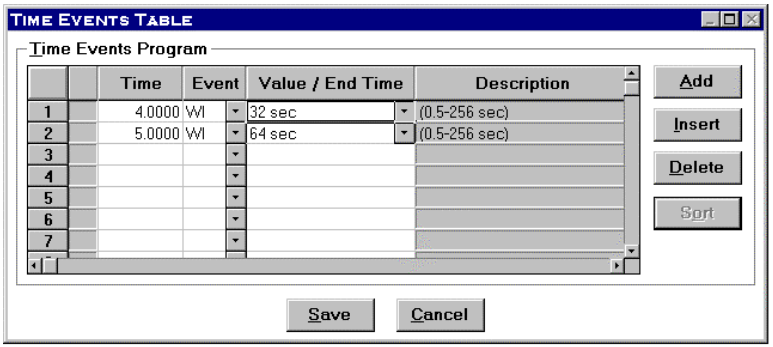
3. Press the down arrow and select WI to replace the II event.

4. Change the start Time to 5.0 and the Value/End Time to 64.

5. Click on *Add*. Set the new WI event start Time to 4.0 and the Value/End Time to 32.

   When you add a line for an earlier time event, use Sort to move it to the correct position in the table.

6. Click on *Sort*. Your display should look like the following display.



7. Select and Delete the WI event at 5.00 min.

8. Choose *Save* to close the Time Events Table window.

9. Select RESULTS→Reintegrate Now.

   You must Reintegrate as before because you made changes that affect peak integration. After the calculation, the results will automatically be displayed and the plot will be updated to reflect the changes in the time program. Notice that the Time Events annotation on the display has been updated with a light blue box near the baseline at the start time for the first WI event.

10. Click on the WI Time Event marker.

    The Time Event information box is displayed in the upper left corner of the Zoom window, showing the event type, its program time, and the actual time of execution.

11. When you are done viewing this information, double-click on the Control menu box.

    The small peaks at the beginning of the file are once again detected and included in the report. Their peak event markers should be correctly placed. The peaks that eluted after four minutes were not integrated correctly because the Peak Width setting was too high. Peak processing does not correct for this because the automatic Peak Width updating was turned off once you made a WI time program. Whenever you time program Peak Widths, you must make all the appropriate changes over the length of the run.

    The other Time Events can be programmed just as the II and WI events were.

12. Close the Results window to return to the Interactive Graphics/ Data Handling window.

13. Close Interactive Graphics and do not save changes to either the method or the data file.

Tutorial 3 Filling a Peak Table

Peak tables contain the peak-specific information necessary for the execution of most data handling, peak processing, and quantitative operations. Using Interactive Graphics, you can easily create a peak table for any chromatographic data gathered with the Workstation. This tutorial covers the basic steps for filling the Peak Table.

Topics Discussed

- Opening the Peak Table window
- Adding peaks
- Naming peaks
- Designating peak functions
- Entering amounts for calibration levels
- Editing the Peak Table
- Setting calibration options

Preparing a Data File for Use with this Tutorial

Because PRACTICE.RUN is used for several tutorials, you should copy this data file to TABLE.RUN for use with this tutorial.

Open Windows Explorer.

1. View the C:\STAR\EXAMPLES directory.
2. Use the right mouse button to click on PRACTICE.RUN, and select *Copy*.
3. Select EDIT→Paste. A copy of the file is added at the end of the list.
4. Use the right mouse button to click on the new file. Select Rename, and type TABLE.RUN.

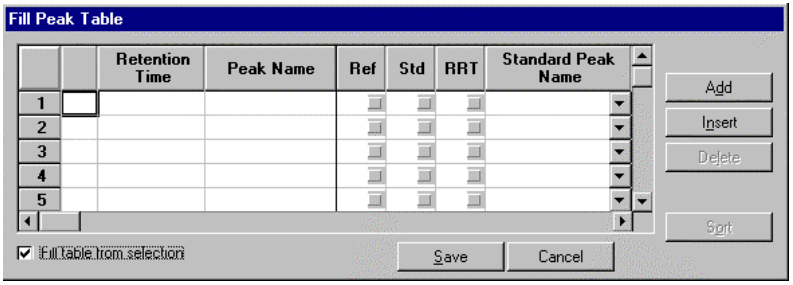
Opening the Fill Peak Table Window

1. Open Interactive Graphics by clicking on the Interactive Graphics bar in the Star Bar.
2. Double-click on TABLE.RUN from the EXAMPLES directory and click on *Open Files(s)*. When the Open Method file dialog box appears, click *OK* to build Method from data file.
3. Select FILE→Open Method.
4. Choose the EXAMPLES directory and double-click on PRACTICE.MTH.

After you make TABLE.RUN the active chromatogram and PRACTICE.MTH the active method, you can open the Fill Peak Table window.

1. Select EDIT METHOD→Fill Peak Table.

   The Fill Peak Table window appears.



The Fill Peak Table window can be moved anywhere on the window. Just click on the title bar and drag it to the desired position. For now, move the Fill Peak Table window to the upper part of the screen.

Adding Peaks with the cursor

Now, you can begin to add peaks to the Peak Table. Notice the small, triangular event markers on the chromatogram indicating each peak start, peak apex, and peak end. You can add a peak to the Peak Table by clicking anywhere between its peak start and peak end event markers.

1. Move the cursor to the Zoom window, and click anywhere between the start and end markers of the first peak eluting at about 1.0 minutes.

   The first line of the Peak Table now displays the retention time for this peak. The Peak Name field contains the default name.

2. Click between the peak start and peak end markers for the peak eluting at about 2.8 minutes. Check to see that the peak is entered.

3. Now, click on the peak eluting at about 2.0 minutes.
4. Continue filling the Peak Table until all the peaks are listed.
5. Do not enter the peaks at 1.05, 1.11 and 3.47 and 7.4 minutes.
6. Click on *Sort*. When you look at the Peak Table, you will see that the peaks have been reordered according to their retention times.
7. Select *Save*.

Adding Peaks from a selection

You can also add all peaks contained within a selection. A selection is the area selected during zooming.

1. Click on the box marked “fill table from selection” which is located on the “fill peak table” menu.
2. Move the cursor to the Zoom window, click on the left upper corner of the area you wish to select (start at .5 minutes) and click and hold left mouse button. Drag the mouse to the lower right hand corner of the area you wish to select (end at 6 minutes) and release the mouse button.

Peaks are automatically entered into the peak table. Care should be used when using this command because any integrated peak within the selected range will be added to the peak table.

Naming Peaks

Select EDIT METHOD→Peak Table. This displays the Peak Table Window. Maximize the Peak Table window by clicking on the full-screen icon (square box) in the upper right corner.

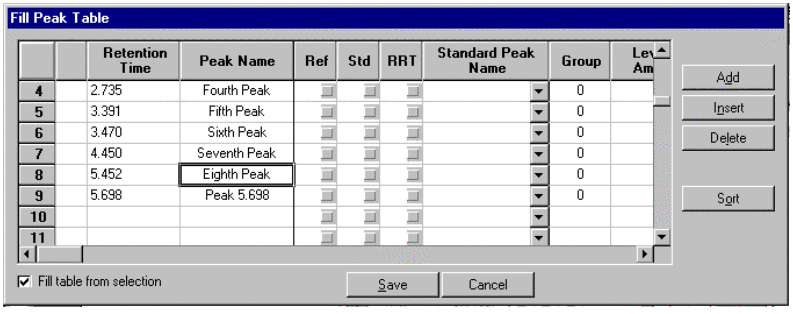
As you added peaks to the table, the software supplied unique default names based on the peak retention times. When you have added all the peaks to the Peak Table, you can edit each Peak Name and supply more descriptive names if you wish.

1. Click on the Peak Name field for the first peak.
2. Type First Peak.

   As you type, the Peak Name field is cleared and the name First Peak replaces it.
3. Press the Down Arrow key on your keyboard. The Peak Name field for the second peak is now highlighted. Type Second Peak. Press the Down Arrow Key again.

   This is a faster way of entering data than to select fields by a click of the mouse.
4. Rename the third peak Internal Standard. Press the Down Arrow key.
5. Continue renaming the remaining peaks.

Your table should look like the one shown.



Designating Peak Functions

You can designate certain peaks to perform specific functions. The check boxes in the Peak Table are used to select the following functions:

Heading	Peak Type	Function
Ref	Reference Peak	Used to adjust the peak identification retention time windows for changes in chromatographic conditions that may cause retention times to drift.
Std	Internal Standard Peak	Used as an internal standard peak for the calculation of results with an internal standard or by normalized %.
RRT	Relative Retention Time Peak	Used as a reference from which the relative retention times of other peaks are calculated.

Reference Peak(s)

1. Click in the Ref check box for the peak named Sixth Peak.

   A check mark appears in the Ref box to indicate that it has been selected.

2. Click the Ref box for the peak named Internal Standard.

   The Ref box for this peak is also selected. You may choose to have more than one reference peak.

Note: **If more than one peak appears in the reference peak time window, the largest peak in the window is selected as the Reference Peak.**

Internal Standard Peak(s)

An internal standard peak is used in the calculation of results according to the internal standard or normalized percent procedure. You can designate up to eight peaks as Internal Standard Peaks.

Note: Refer to *Tutorial 7 Calibrating with Internal Standards & Getting Around in the Curve Manager* for further information on calibrating with multiple internal standards.

Click on the Std box for the third peak, named Internal Standard. It is now indicated in the Standard Peak Name column that the third peak is designated as the Internal Standard peak for the calculation of results for all other peaks in the table.

Relative Retention Time Peak

The RRT peak is used, in association with the unretained peak time, to calculate relative retention times for all identified and reported peaks. Only one peak can be designated the Relative Retention Time Peak.

- Click the RRT box for the peak named First Peak.

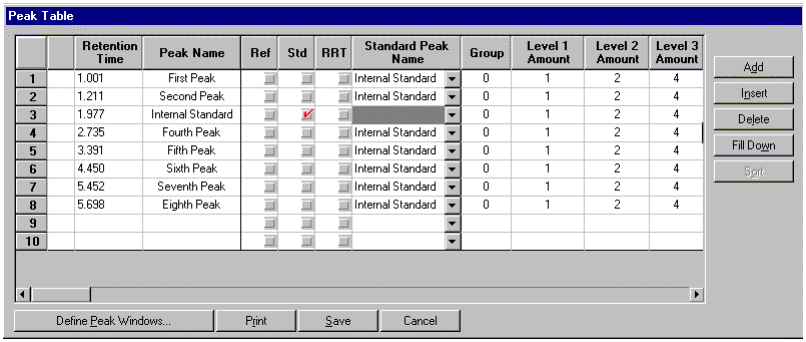
The RRT box is now marked for the first peak.

Entering Amounts for Calibration Levels

Use the horizontal scroll bar to view the Amount columns. You can examine and enter amounts for up to ten different calibration levels. Each level corresponds to a calibration mixture. The value entered for each peak is the known, measured amount of that compound in the standard. The calibration levels are used in verification and calibration runs.

1. Click the horizontal scroll bar to move through the ten levels.
2. Click on the Level 1 Amount cell for the first peak. The default value is highlighted.
3. Click on the Level 2 Amount cell for the first peak in the peak table.
4. Enter 2.0.
5. To set all peaks to the same values as the first peak, you can enter the value for each peak, or take advantage of the Fill Down function: Click on the box labeled *'Level 2 Amount'*. The whole column is now highlighted. Click on the *'Fill Down'* button and observe that the value for the first peak is copied to all peaks.
6. Repeat the previous operations to set Level 3 to 4.0, Level 4 to 8.0, Level 5 to 16.0, and Level 6 to 32.0 for each peak in the peak table.

The peak table should look like the one shown.



Editing the Peak Table

On the right side of the Peak Table there are three edit buttons: Delete, Add, and Insert. These buttons allow you to edit the Peak table.

1. Click anywhere on the line for the peak named First Peak.
2. Now click the *Insert* button.

   A new line is displayed just before the selected line. Each level for the inserted peak has been assigned a default value of 1.
3. Click anywhere on the line for the Internal Standard peak.
4. Click the *Insert* button.

   A new line is now displayed just before the Internal Standard entry, with a Retention Time of 1.977 and default values of 1 for all Amounts levels.
5. Click the Delete button. The selected Peak Table entry is removed.

6. Select the entry inserted at the top of the Peak Table by clicking anywhere in that line.

7. Click the *Delete* button again. The entry inserted at the top of the Peak Table is deleted.

   Now, save the contents of the Peak Table you just created.

8. Click the Save button to close the Peak Table Window.

9. Select FILE→Save Method As.

10. Name the method TABLE.MTH.

11. Click *OK*.

If you were to use this method to execute any data handling or quantitative operation, the Peak Table you have just created would be used.

Close Interactive Graphics and do not save changes to either the method or the data file.

Tutorial 4 Identifying Peaks

A chromatographic data file contains all of the raw data points collected for one injection. The Workstation's data handling system identifies any peaks detected in the raw data based on their retention times. The workstation allows you to create and adjust time windows, which define ranges of retention times in which peaks are to be identified. Time windows can also be used to eliminate from reports those peaks that have no analytical significance.

Topics Discussed

- Peak Windows
- Time Windows
- Showing Peak Windows
- Changing Peak Functions
- Peak Reject
- Reporting Unidentified Peaks

Preparing a Data File for Use with this Tutorial

Because TABLE.RUN is used for several tutorials, you should copy this data file to IDENT.RUN for use with this tutorial.

In Windows Explorer:

1. View the C:\STAR\EXAMPLES directory.
2. Use the right mouse button to click on TABLE.RUN, and select Copy.
3. Select EDIT→Paste. A copy of this file is added at the end of the list.
4. Use the right mouse button to click on the new file. Select Rename, and type IDENT.RUN.

Peak Windows

Since chromatographic retention times are not absolutely precise, you specify a window of time (a peak window) for the Workstation to identify a particular peak. The peak window is the actual span of time on the chromatogram that the software searches. The software will only identify a peak if it falls within the peak window.

1. Open the Interactive Graphics/Data Handling application by clicking on its bar in the Star Bar.
2. Double-click on IDENT.RUN in the EXAMPLES directory and click on *Open Files(s)*. When the open method file dialog box appears, click *OK* to build Method from data file.
3. Select FILE→Open Method...
4. Select IDENT.MTH from the EXAMPLES directory.

   The title bar reads “Graphics - IDENT.RUN Channel A-A Method: IDENT.MTH.”

5. Choose EDIT METHOD→Peak Table...

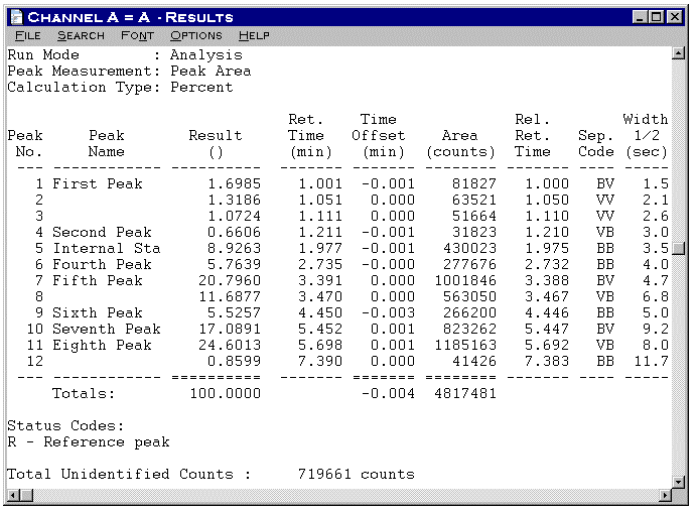
   The Peak Table associated with this data file appears. Notice that eight peaks are listed in the Peak Table.

6. Choose *Save* or *Cancel*, and return to the Interactive Graphics/Data Handling window.

7. Select Results→Reintegrate Now. Save the Method before reintegration.

8. To view the results, click on the chromatogram trace with the right mouse button. Select VIEW RESULTS ONLY.

Your window should look like the one below.



CHANNEL A = A - RESULTS

FILE SEARCH FONT OPTIONS HELP

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel. Ret. Time	Sep. Code	Width 1/2 (sec)
1	First Peak	1.6985	1.001	-0.001	81827	1.000	BV	1.5
2		1.3186	1.051	0.000	63521	1.050	VV	2.1
3		1.0724	1.111	0.000	51664	1.110	VV	2.6
4	Second Peak	0.6606	1.211	-0.001	31823	1.210	VB	3.0
5	Internal Sta	8.9263	1.977	-0.001	430023	1.975	BB	3.5
6	Fourth Peak	5.7639	2.735	-0.000	277676	2.732	BB	4.0
7	Fifth Peak	20.7960	3.391	0.000	1001846	3.388	BV	4.7
8		11.6877	3.470	0.000	563050	3.467	VB	6.8
9	Sixth Peak	5.5257	4.450	-0.003	266200	4.446	BB	5.0
10	Seventh Peak	17.0891	5.452	0.001	823262	5.447	BV	9.2
11	Eighth Peak	24.6013	5.698	0.001	1185163	5.692	VB	8.0
12		0.8599	7.390	0.000	41426	7.383	BB	11.7
	Totals:	100.0000		-0.004	4817481			

Status Codes:
R - Reference peak

Total Unidentified Counts : 719661 counts

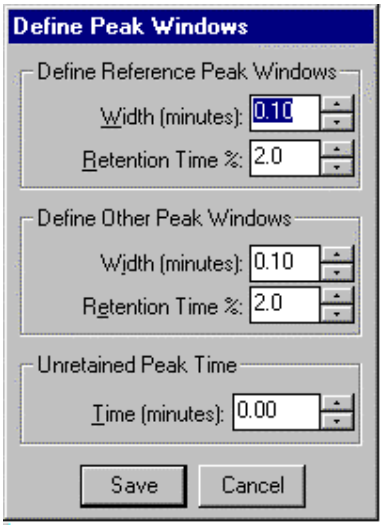
All peaks in the Peak Table are identified. There are 719661 unidentified counts for peaks not listed in the Peak Table.

Define Peak Windows

Each peak window is determined by a time window that you enter. The time windows are both added to and subtracted from the expected retention times to define the peak windows. You set the time window sizes in the Define Peak Windows dialog box.

The workstation recognizes two types of time windows; one for reference peaks and one for all other peaks. Peaks in the two window types are identified differently. For reference peaks, the largest peak in the window is considered the reference peak. For other peaks, the one closest to the center of the peak window is identified as the peak.

1. Close the Results window.



2. Choose EDIT METHOD→Peak Table...

3. Click on the *Define Peak Windows...* button to open the Define Peak Windows dialog box.

   The time window used to identify a peak is an absolute width in minutes plus a relative width expressed as a percentage of the peak's retention time. You may set either the absolute or relative time window to zero.

4. Verify a time window width of 0.1 minutes plus 2% of the Retention Time for the reference peaks.

5. Verify the same settings for the “Width” and “Retention Time %” for Other Peaks.

6. Click on *Save* to exit to the Peak Table window.

7. Click *Save* again to return to Interactive Graphics.

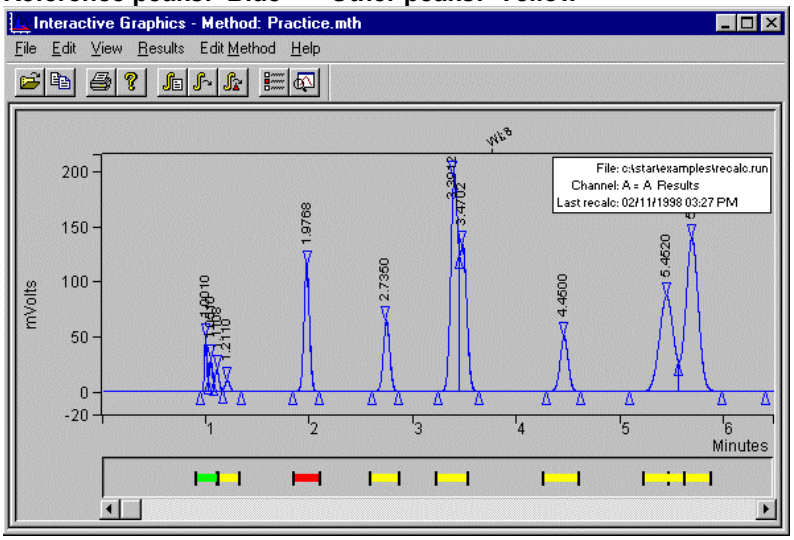
Showing Peak Windows

In Interactive Graphics, you have the option of displaying the peak window for each of the peaks in the Peak Table.

1. Select VIEW→Visual Method Edit Window, to show the peak window for each of the peaks in the peak table.

The chromatogram should look like the one shown. Identifying color bars appear under each peak that has been entered in the Peak Table. The default colors for the different peak window bars are:

Standard peaks: Red **Relative Retention Time peak: Green**
Reference peaks: Blue **Other peaks: Yellow**



Changing Peak Functions

You can change the peak functions easily in the Peak Table.

1. Select EDIT METHOD→Peak Table.
2. Click in the Ref. box for the fourth peak to make it the reference peak.
3. Click *Save* to return to the Interactive Graphics window.

   Look at the color bars under the peaks. The fourth peak now has a blue Reference Peak indicator bar. The third peak, a standard peak that is also a reference peak, is marked with stripes of blue and red.
4. Select EDIT METHOD→Peak Table, deselect the Ref box for the internal standard peak.
5. Click *Save* to return to the Graphic window. The third peak is now marked by a red bar.

Peak Reject

Often, over the course of a chromatographic run, numerous peaks appear that are not of interest to you. While these peaks might be detected, there is no need to report them. There are several ways to tailor a peak processing method so that only the peaks of interest are reported. One of these is Peak Reject.

Set the Peak Reject value such that peaks smaller than a specified size are eliminated from the final report.

1. Select EDIT METHOD→Integration Parameters.
2. Set the Initial Peak Reject value field to 50000.

   Now, peaks smaller than 50000 counts will not be included in the Results file.

   Choose *Save*.

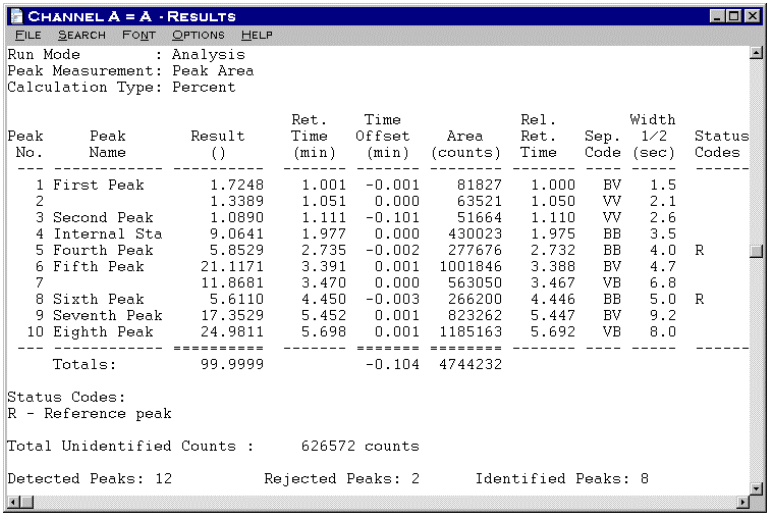
You'll need to recalculate the Results file so that this new Peak Reject value is used in the results.

3. Select RESULTS→Reintegrate Now. (You can also select the “Reintegrate Now” Function from the Toolbar)

   Take a look at the Results now.

4. Open the Results Report by clicking with the right mouse button on the chromatogram trace.

When the Results window appears, enlarge it so that you can see the results of this recalculation.



CHANNEL A = A - RESULTS

FILE SEARCH FONT OPTIONS HELP

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel. Ret. Time	Sep. Code	Width 1/2 (sec)	Status Codes
1	First Peak	1.7248	1.001	-0.001	81827	1.000	BV	1.5	
2		1.3389	1.051	0.000	63521	1.050	VV	2.1	
3	Second Peak	1.0890	1.111	-0.101	51664	1.110	VV	2.6	
4	Internal Sta	9.0641	1.977	0.000	430023	1.975	BB	3.5	
5	Fourth Peak	5.8529	2.735	-0.002	277676	2.732	BB	4.0	R
6	Fifth Peak	21.1171	3.391	0.001	1001846	3.388	BV	4.7	
7		11.8681	3.470	0.000	563050	3.467	VB	6.8	
8	Sixth Peak	5.6110	4.450	-0.003	266200	4.446	BB	5.0	R
9	Seventh Peak	17.3529	5.452	0.001	823262	5.447	BV	9.2	
10	Eighth Peak	24.9811	5.698	0.001	1185163	5.692	VB	8.0	
	Totals:	99.9999		-0.104	4744232				

Status Codes:
R - Reference peak

Total Unidentified Counts : 626572 counts

Detected Peaks: 12 Rejected Peaks: 2 Identified Peaks: 8

Unidentified Peaks

Often, a chromatogram will contain peaks that are not of analytical interest but are as large as, or larger than those peaks that are of interest. In these cases, adjusting the Peak Reject value is inappropriate; you would lose both important and unimportant peaks from the report. The Interactive

Graphics/Data Handling application provides another means to focus a report on the peaks of interest. You can turn off reporting of unidentified peaks.

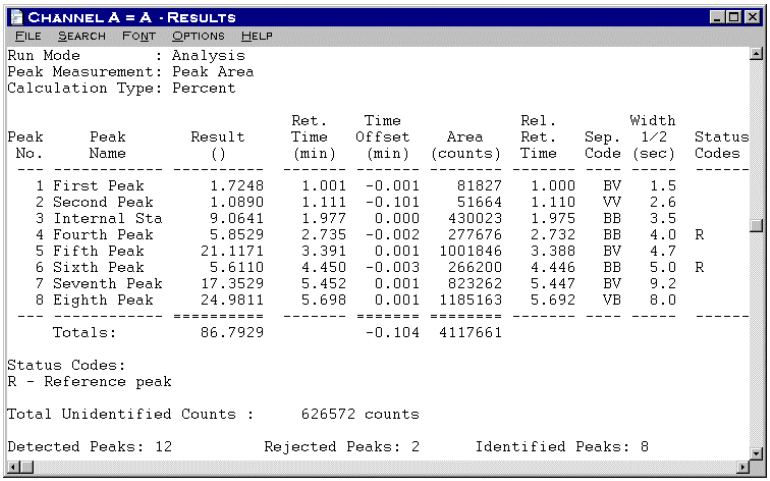
1. Select EDIT METHOD→Integration Parameters again.

2. Click once in the box labeled Report Unidentified Peaks so that this option is no longer selected. Press *Save*.

   Only identified peaks will now appear in the Results file.

3. Select RESULTS→Reintegrate Now to recalculate the data file using this new set of parameters.

The results should appear.



The list of reported peaks is considerably shorter now. It includes only those peaks corresponding to identified peaks in the Peak Table and does not include other peaks, even those that are larger than the Peak Reject value. Here, only peaks identified within their respective retention time search windows are included in the final results file.

4. Close Interactive Graphics and do not save changes to either the method or the data file.

Tutorial 5 Using the II, SR, and VB Time Events

You can use the Time Events Table to program several peak processing changes over the time of a chromatogram. The Inhibit Integrate (II), Solvent Reject (SR), and Valley Baseline (VB) time events can help you optimize peak detection and calculation of results. Both System Control and Interactive Graphics/Data Handling allow these changes. For this tutorial, you will use Interactive Graphics.

Topics Discussed

- Inhibiting Integration
- Using Solvent Reject
- Using Valley Baseline
- The effects of Other Peak Processing Events on VB

Inhibiting Integration

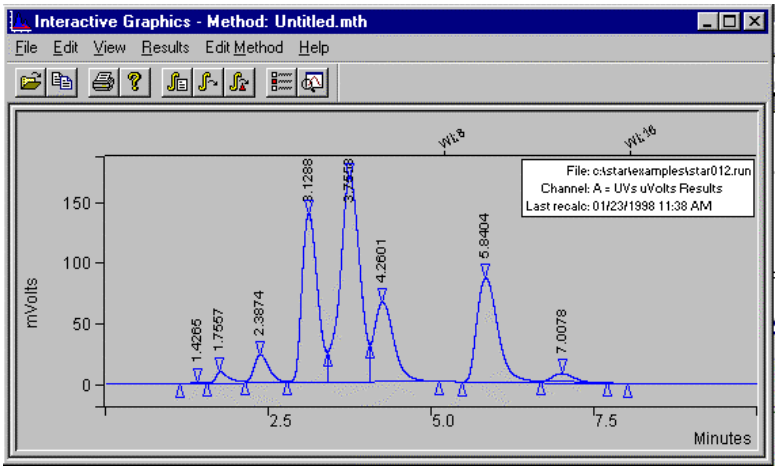
An Inhibit Integrate (II) event is a time programmable event that can be used to turn off integration in selected regions of the chromatogram. The Inhibit Integrate event is used to:

- Eliminate from a report those peaks that are not of interest.
- Avoid improper baseline assignment during periods when the baseline might be distorted, such as during the switching of sampling valves.
- Force or create a baseline where needed in the chromatogram.

The Inhibit Integrate event suppresses integration of peak area or height between the start and end time of the event. The II event forces the baseline to be drawn to the point where the II event starts and the baseline to start drawing at the point where the II event stops. When II is active, peak processing is disabled. This does not mean that raw data is not being stored; it simply means that it is not being integrated. If you remove the II event, integration is turned back on when you recalculate the stored data file.

1. From the Star Bar, open the Interactive Graphics/Data Handling application.
2. Find and open the EXAMPLE directory from the Open Multiple Data Files dialog.
3. Double-click on STAR012.RUN to select this data file, then click on *Open File(s)*. When the open method file dialog box appears, click *OK* to build Method from data file.

The data file now appears, and the chromatogram should look like the one shown.



4. You will use a method created from the method stored in the data file. This has already been built.

   You will edit the Time Events Table to see how Inhibit Integrate works. This example illustrates how the II event critically affects the placement of the chromatographic baseline. The baseline used in this example is not intended to represent proper baseline placement.

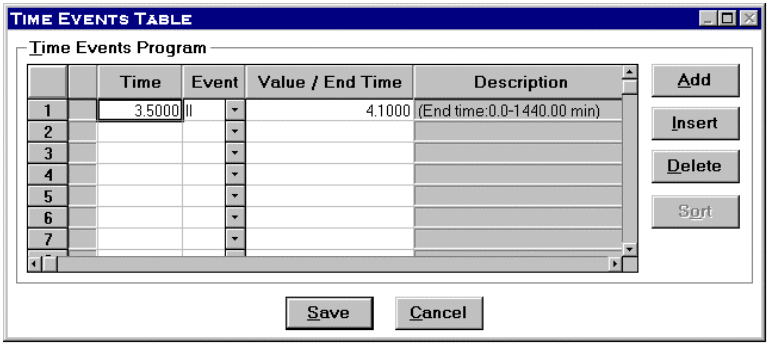
5. Select EDIT METHOD→Time Events. The Time Events dialog box appears.

Editing a Time Event from the Menu

Now, let's program the Inhibit Integrate (II) event to start at 3.5 minutes and stop at 4.1 minutes.

1. Click on *Add*.
2. Click on the down arrow at the right of the Event box.
3. Scroll through the Event types and select the II. Set the Start time to 3.5 and the End time to 4.1.

   The Time Events Table should match the following figure.



4. Choose *Save* to close the Time Events Table window and return to the Interactive Graphics/Data Handling window.

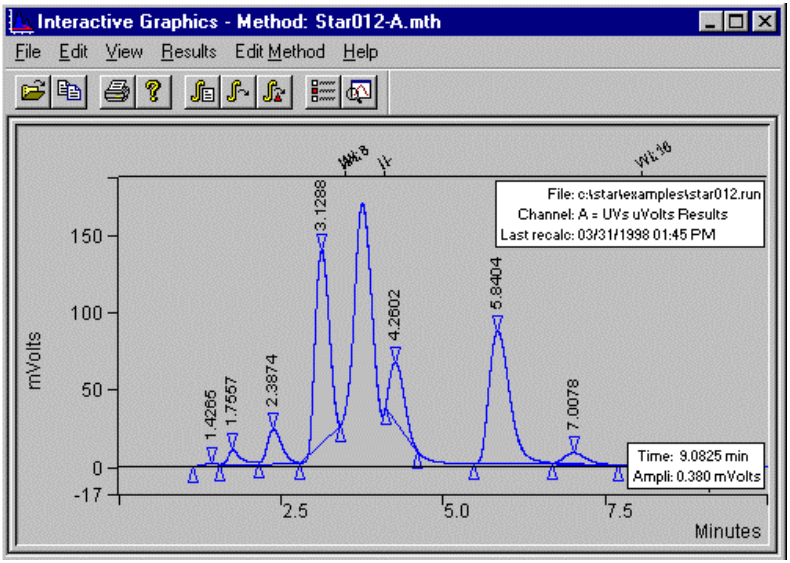
   Changes to the II event must be followed by a Reintegration.

5. Select RESULTS→Reintegration List.

   The Run DH check box for STAR012.RUN is checked.

6. Click on *Calculate Results.*

The Workstation begins processing the file. After Reintegration is complete, look at how the new II event has affected the chromatographic data file. A baseline has been forced at the points where II is turned on and off. Also, notice that the peak at about 3.7 minutes is not detected and does not have peak event marks.

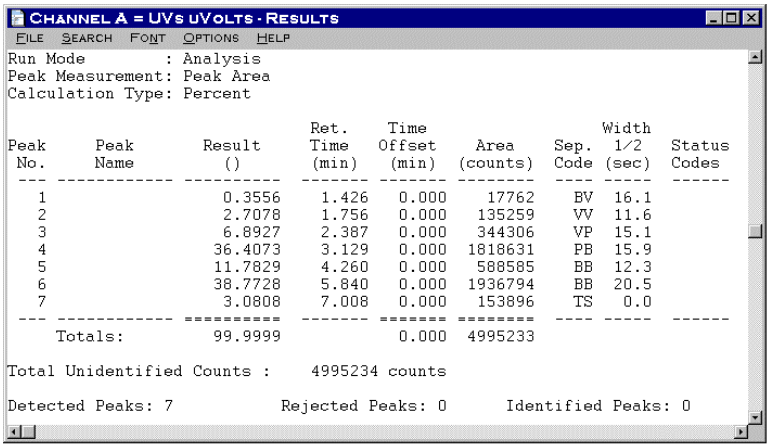


Changes in the II event also alter the final Results for a chromatographic data file.

7. Select RESULTS→View Data File Report.

8. Enlarge the Results window and examine the Results.

They should appear as in the following figure.



CHANNEL A = UVs uVOLTS - RESULTS

FILE SEARCH FONT OPTIONS HELP

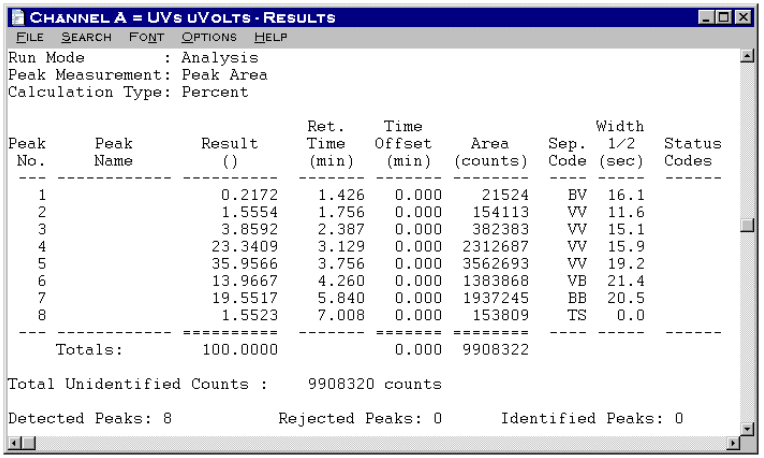
Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0.3556	1.426	0.000	17762	BV	16.1	
2		2.7078	1.756	0.000	135259	VV	11.6	
3		6.8927	2.387	0.000	344306	VP	15.1	
4		36.4073	3.129	0.000	1818631	PB	15.9	
5		11.7829	4.260	0.000	588585	BB	12.3	
6		38.7728	5.840	0.000	1936794	BB	20.5	
7		3.0808	7.008	0.000	153896	TS	0.0	
Totals:		99.9999		0.000	4995233			

Total Unidentified Counts : 4995234 counts

Detected Peaks: 7 Rejected Peaks: 0 Identified Peaks: 0

Compare this to the original results shown in the following figure. Observe that the peak originally detected at about 3.7 minutes is now eliminated from the report. Also, notice how the area counts have changed as a result of the new baseline placement.



CHANNEL A = UVs uVOLTS - RESULTS

FILE SEARCH FONT OPTIONS HELP

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0.2172	1.426	0.000	21524	BV	16.1	
2		1.5554	1.756	0.000	154113	VV	11.6	
3		3.8592	2.387	0.000	382383	VV	15.1	
4		23.3409	3.129	0.000	2312687	VV	15.9	
5		35.9566	3.756	0.000	3562693	VV	19.2	
6		13.9667	4.260	0.000	1383868	VB	21.4	
7		19.5517	5.840	0.000	1937245	BB	20.5	
8		1.5523	7.008	0.000	153809	TS	0.0	
Totals:		100.0000		0.000	9908322			

Total Unidentified Counts : 9908320 counts

Detected Peaks: 8 Rejected Peaks: 0 Identified Peaks: 0

If you were to delete the II event from the Time Events Table and then perform a Reintegration, the peak at 3.7 minutes would again be detected.

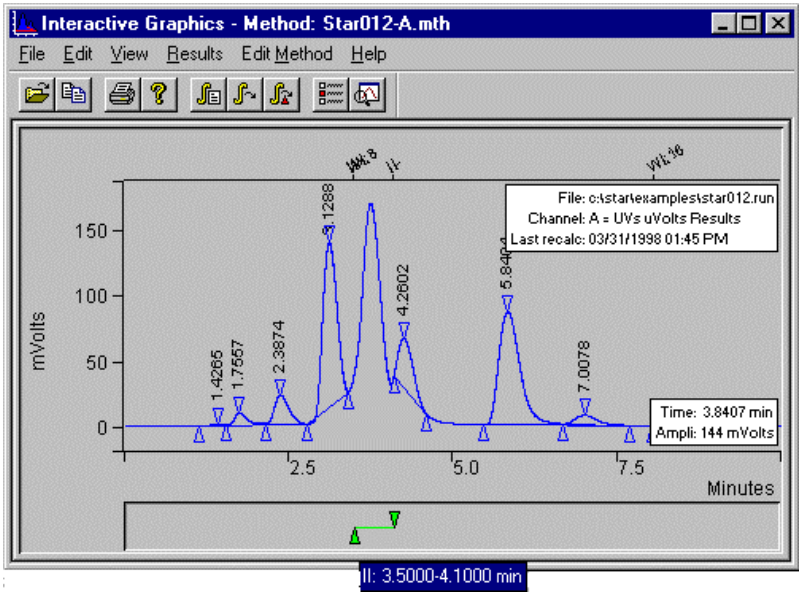
Graphical Placement of a Time Event

You can graphically edit the II Event by selecting Interactive Time Events from the Time Events menu. When the timed events window is shown below the chromatogram, try using the left and right mouse buttons to edit the events.

Close the results window.

1. Select TIME EVENTS→Visual Method Edit Window. The Interactive window now appears below the chromatogram.

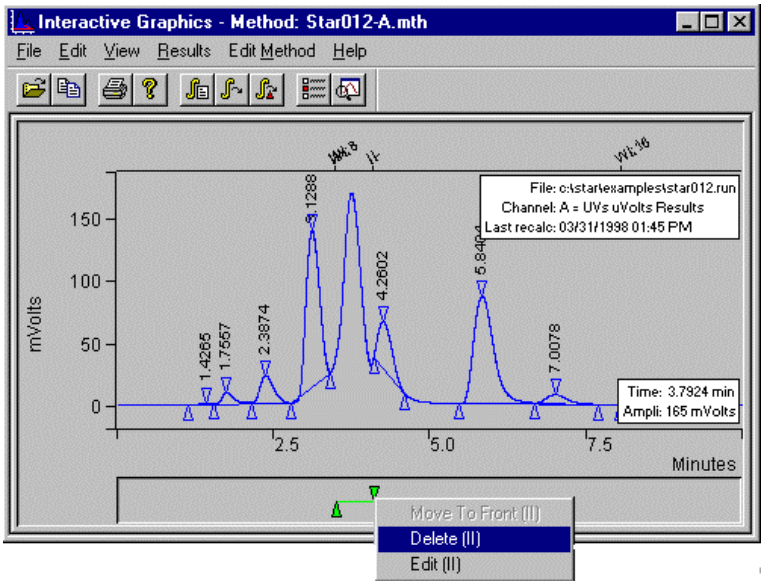
2. Place the cursor on the II event in the interactive window. A small window is displayed providing you with information on the event.



You can move the II event by clicking and dragging the triangles marking the start and end times.

Use the right mouse button to click on either the triangle or the connecting line marking the II event.

3. Select Delete (II) from the menu. The II event is deleted from the Interactive Time Event window and also from the Time Events Table. Now you can reintegrate the data file without the II event.



4. Select RESULTS→Reintegrate Now.

   Notice that the peak at 3.7 minutes is now detected in the chromatogram and included in the Results report.

Using Solvent Reject

In this section, you will see how the Inhibit Integrate (II) function differs in performance from the Solvent Reject (SR) function.

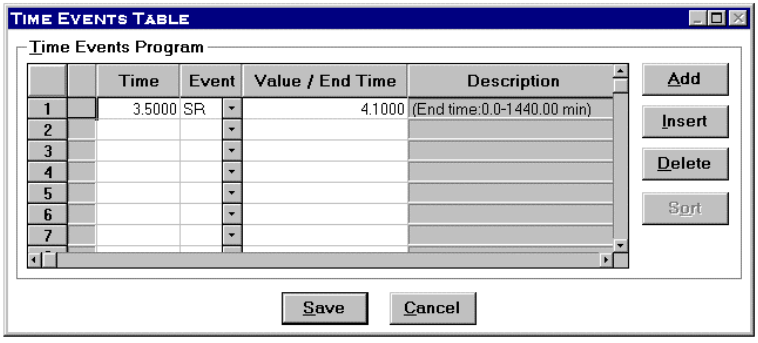
1. Double-click on the Interactive Time Events window bar.

   When the Time Events dialog box appears, note that the II event is no longer listed.

2. Click on *Add* to enter a new event into the table.

3. Click on the down arrow in the Event box. Scroll through the Event types and select Solvent Reject (SR). Set the start and end times again to 3.5 and 4.1 minutes.

   The table should look like the one in the following Figure.

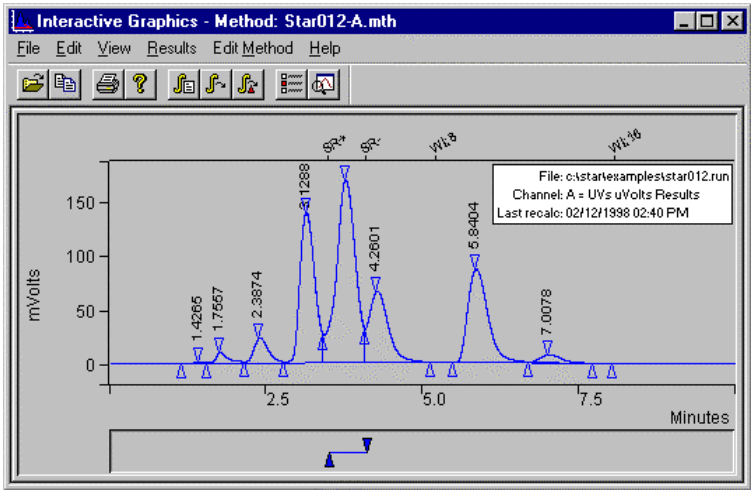


4. Choose *Save* to close the Time Events Table window.

It's time to implement the new change to the peak processing conditions. Since the settings in the Reintegration list would be the same as they were the last time you used it, you can use the Reintegrate Now shortcut.

5. Select RESULTS→Reintegrate Now.

After peak processing has been completed, the plot will be updated to reflect the changes in the time program.

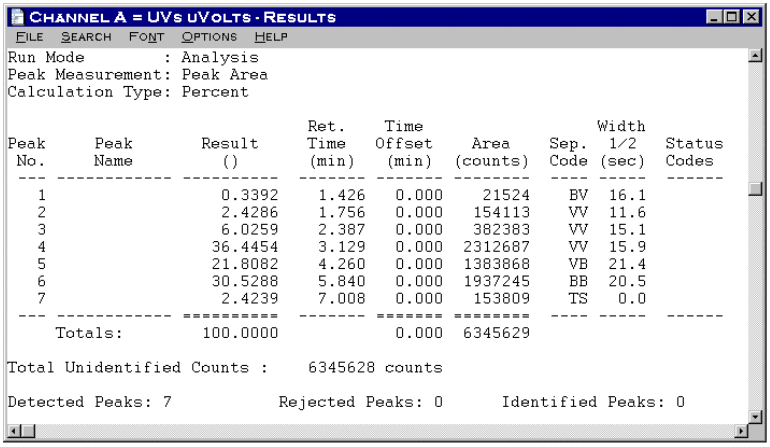


Notice that the SR function does not affect baseline placement or peak detection. Thus, the plot looks similar to the original data file used at the beginning of this tutorial. Solvent Reject eliminates peaks from reports but does not influence their detection, or affect baseline placement.

6. To open the results file click on the chromatogram trace with the right mouse button. Select “View Results only”.

7. Enlarge the Results window and take a look at the previously missing peak.

8. The results should look like those in the following figure.



CHANNEL A = UVs uVOLTS - RESULTS

FILE SEARCH FONT OPTIONS HELP

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0.3392	1.426	0.000	21524	BV	16.1	
2		2.4286	1.756	0.000	154113	VV	11.6	
3		6.0259	2.387	0.000	382383	VV	15.1	
4		36.4454	3.129	0.000	2312687	VV	15.9	
5		21.8082	4.260	0.000	1383868	VB	21.4	
6		30.5288	5.840	0.000	1937245	BB	20.5	
7		2.4239	7.008	0.000	153809	TS	0.0	
Totals:		100.0000		0.000	6345629			

Total Unidentified Counts : 6345628 counts

Detected Peaks: 7 Rejected Peaks: 0 Identified Peaks: 0

Solvent Reject operates as a post-integration filter. It rejects detected peaks whose apices fall within the SR window. It will not affect tangent separations, baseline placement, or Peak Width updating. In contrast, II affects all three of these functions by changing where baselines are established.

9. Close the Results window.

Using Valley Baseline

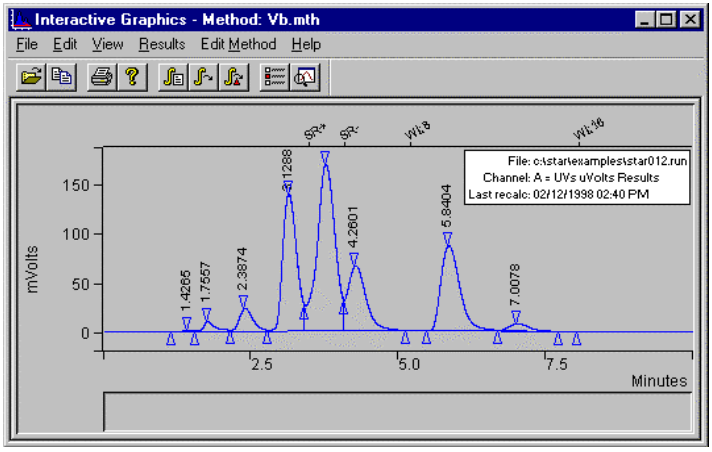
A Valley Baseline (VB) event creates a time window within which all valley points are forced to behave as baseline points for peak integration. Now, take a look at how the VB event functions.

1. Select FILE→Open Method.
2. Select the method file VB.MTH from the EXAMPLES directory.

   To ensure that the integration for STAR012.RUN is at the proper starting point, reintegrate this data file with the new method.

3. Select RESULTS→Reintegrate Now.

The chromatogram should look like the one shown.



Use the Interactive Time Events to add a Valley Baseline (VB) event, and to set its start and end times.

4. With the right mouse button click on the Visual Method Edit Window.

5. Click on VB: Add Valley Baseline.

   The VB event is now displayed in the Visual Method Edit Window below the chromatogram. The event is also entered in the Time Events Table. Now edit the start and end times.

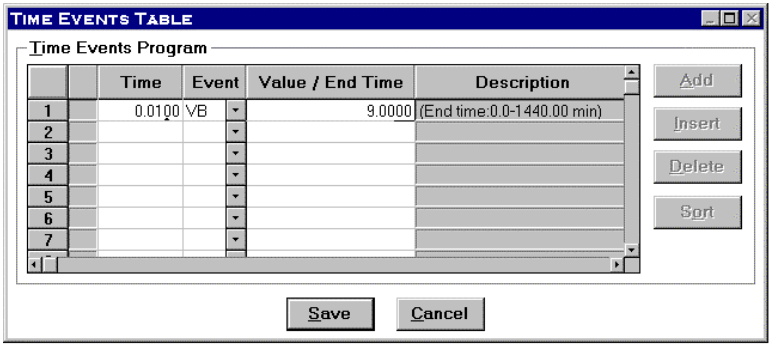
6. Use the right mouse button to click on either the triangles or the connecting line marking the VB event.

7. Click on Edit (VB).

   The displayed Time Events Table now lists the VB event.

8. Set the start Time to 0.01 minutes and the Value/End Time to 9.00 minutes.

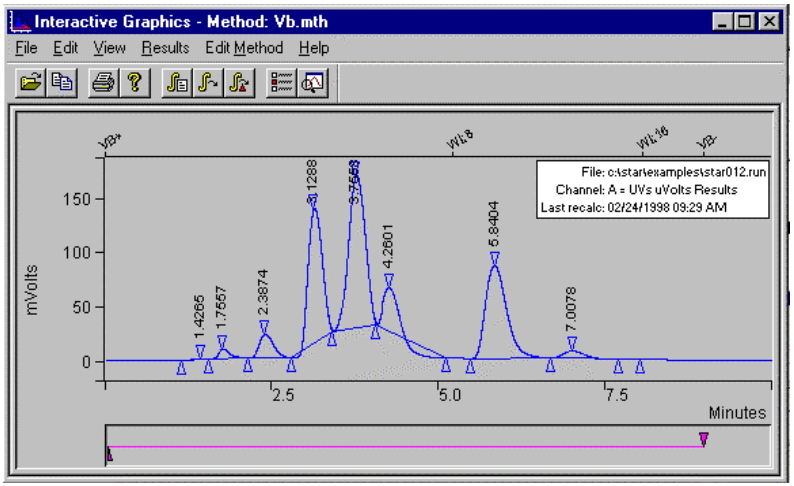
Your table should look like the figure shown.



9. Choose Save.

10. Select RESULTS→Reintegrate Now.

The chromatogram should look like the Figure below.



Notice that the baseline has been drawn to all valley point events. This is not a good baseline assignment, however, so change the VB event to achieve a better integration of this chromatogram.

11. Open the Time Events Table window again.

12. Change the VB time event Start from 0.01 minutes to 0.5 minutes. Change the End time from 9.00 minutes to 3 minutes.

13. Select *Save* to exit the Time Events Table window.

14. Select RESULTS→Reintegrate Now as before.

    Observe the change. Only the first three peaks have been forced to baseline resolution.

Other peak processing events can affect the Valley Baseline parameter and the baseline assignment. Now, take a look at the effect of changes in the tangent height threshold (T%) on the baseline assignment.

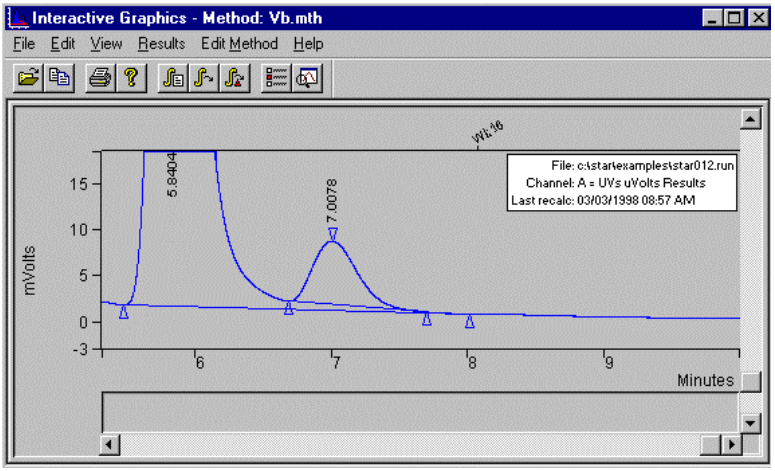
The Effects of Other Peak Processing Events on VB

1. Zoom the chromatogram to expand the area between about 5.5 and 8.5 minutes as shown below.

2. Select VIEW → PREFERENCES. Select the tab for TRACE settings and click on "Show Cursor / Peak information. Click on OK.

3. Select OPTIONS→Show Peak Event Info.

The current Tangent Height Percent threshold is 10%. Notice that, at this setting, the peak at 7 minutes is skimmed as a tangent peak.



4. Move the cursor to the peak event marker at about 7.7 minutes. Notice that this event is identified as the end of a tangent peak (Tangent Pk. End).

5. Select VIEW→Visual Method Edit Window.

6. With the right mouse button click on the Visual Method Edit Window.

7. Click on VB: Add Valley Baseline.

   A new VB event is now displayed in the Interactive window and also added to the Time Event Table.

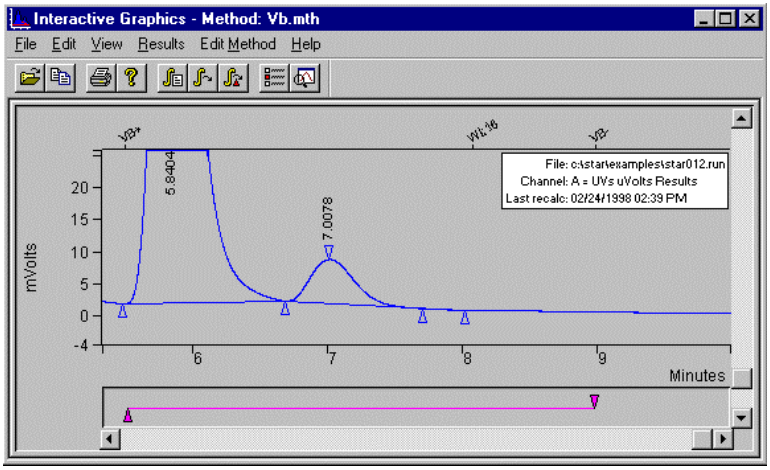
8. Use the right mouse button to Click on one of the triangles or the connecting line marking the new VB event.

9. Click on Edit (VB).

10. In the displayed Time Events Table, set the start Time for the new VB event to 4.5 minutes and the End Time to 9.0 minutes. Choose *Save*.

11. Select RESULTS→Reintegrate Now.

    Notice that the baseline for the peak at about 7 minutes is now drawn from the valley point to the peak end event, and the Tangent Pk. End event is essentially ignored.



So, when you use a VB timed event in an area with skimmed tangent peaks, be aware that the tangent peak events are treated as non-events and that an improper baseline assignment may be drawn.

Changing the Signal-to-Noise Ratio (S/N Ratio) can affect integration by changing the current peak sensing events or introducing new peak sensing events. This can occur whether a VB event is present or not.

12. Select “View” → “Chromatogram Toolbar”. (Make sure that this menu item is checked).

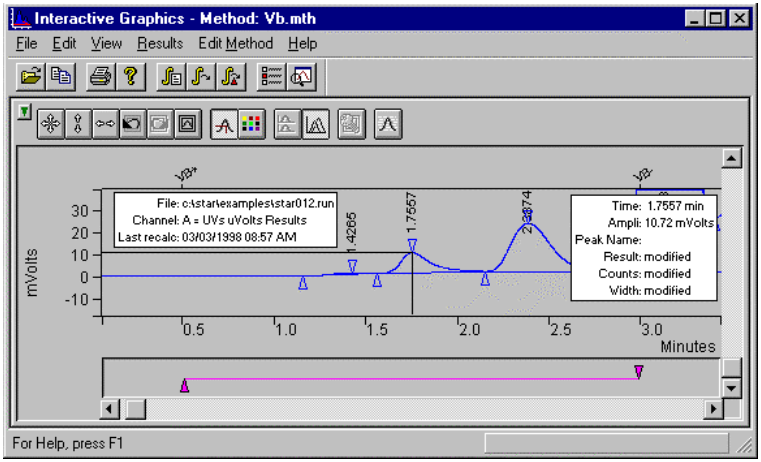
13. Click on the icon below to normalize both the x and y axis of the plot.

14. Zoom the chromatogram from about 0.5 to 3.5 minutes.

15. Select VIEW→Preferences. Select the tab for “Trace Settings” and then check the box named “Show cursor / peak information”. Then click on the “OK” button.

16. Move the cursor to the peak at 1.75 minutes.

The Chromatogram should look like the figure shown.



17. Click on the peak event marker at about 1.6 minutes and notice that it is a valley point.

18. Select EDIT METHOD→Integration Parameters.

19. Decrease the S/N Ratio to 1 and choose *Save.*

20. Select RESULTS→Reintegrate Now. Notice that many baseline noise events are now present.

    A low S/N Ratio can introduce noise-produced peak events, which can affect the integration of the peaks of interest. Likewise, a high S/N setting can change a peak event to a different type of event, which can then affect integration.

21. Select EDIT METHOD→Time Events. Delete the first VB event.

22. Increase the S/N Ratio back to 5.

23. Reintegrate again.

24. Close Interactive Graphics and do not save changes to either the method or the data file.

Tutorial 6 Calibrating with an External Standard

Calibrating with an external standard is a two-step process. First, you perform calibration runs with known amounts of the analytes of interest. This determines the response curve for the analytes with the detector to be used for the analysis. Coefficients for the calibration curve are calculated during this run and saved in the method. After this is done, you can make analysis runs with unknown amounts of the calibration analyte to determine the composition of your sample. The response curve for each analyte is based on an absolute amount of injected material; it is not relative to any other component in the run.

Topics Discussed

- Generation of Calibration Data
- The Calibration Curve

Generation of Calibration Data

Calibration with an External Standard requires that calibration data for each compound be present in the method peak table. This data is generated in calibration runs and saved in the method. The method is then used to perform an analysis of the sample. As many as ten concentration levels can be used to generate a calibration curve for the analytes of interest. This tutorial uses only four different levels in five data files.

1. Open Interactive Graphics from the Workstation Star Bar.

   The Open Multiple Data Files window appears.

2. Choose the chromatographic data file CAL_1.RUN in the EXAMPLES directory.

3. Select *Add To List*.

   You may double-click on a data file to quickly add it to the list.

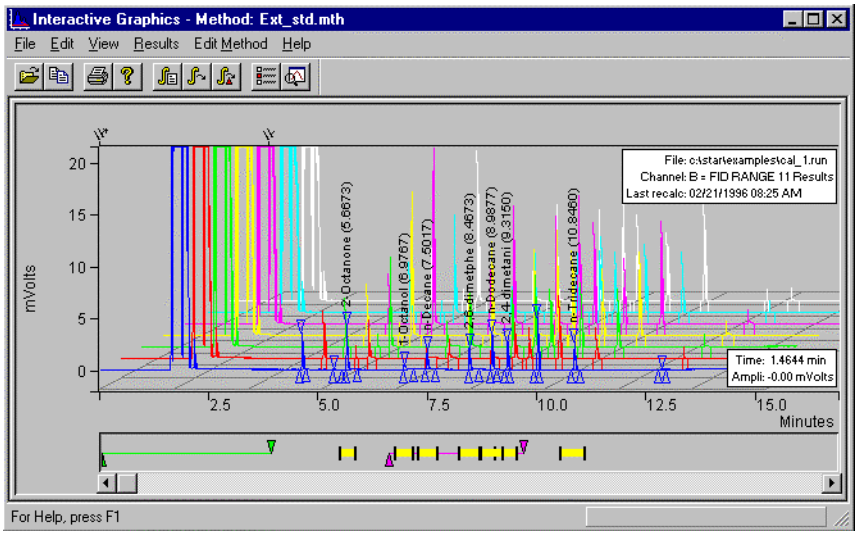
4. Continue adding the following data files to the list: CAL_1A.RUN, CAL_2.RUN, CAL_3.RUN, CAL_4.RUN.

   Channel B should be selected for all data files in this series.

The analytical run for this tutorial is called ANALYSIS.RUN. The file called VERIF_3.RUN is a verification run for this series.

5. Add ANALYSIS.RUN and VERIF_3.RUN to the list and press Open File(s). When the Open Method file dialog box appears, click *OK* to build method from data file.

The IG screen should look similar to the figure shown.



Next, you will load the method file which will be used for the analysis. You will use the method file, EXT_STD.MTH, located in the EXAMPLE directory. The information in the peak table is necessary to generate the coefficients of the calibration curve.

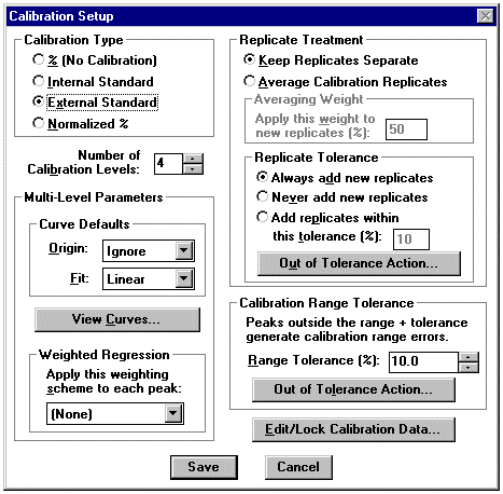
6. Select File→Open Method and select EXT_STD.MTH, press Open.

   The title bar for the Interactive Graphics window now lists EXT_STD.MTH as the active method.

Now, verify the calibration parameters to be used, as follows:

7. Select EDIT METHOD→Calibration Setup.

The Calibration Setup window opens. This window should look like the one shown below. The Calibration Type should be External Standard. The Number of Calibration Levels should be set to 4. The Curve settings should be Linear, Ignore.

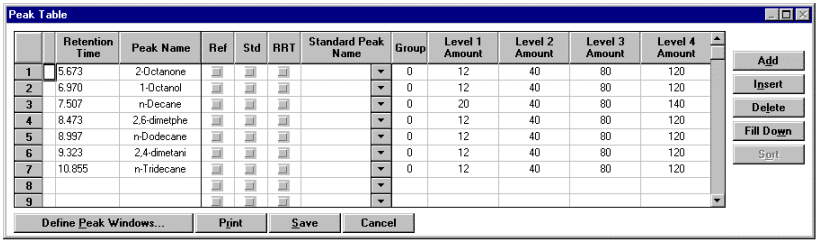


8. Select *Save* to return to the Interactive Graphics window.

   Now, verify the Peak Table settings.

9. Select EDIT METHOD→Peak Table.

   The window should look like the one shown below. Verify the seven peaks have the amounts shown for Levels 1-4.



10. Click the *Save* button to close the Peak Table window.

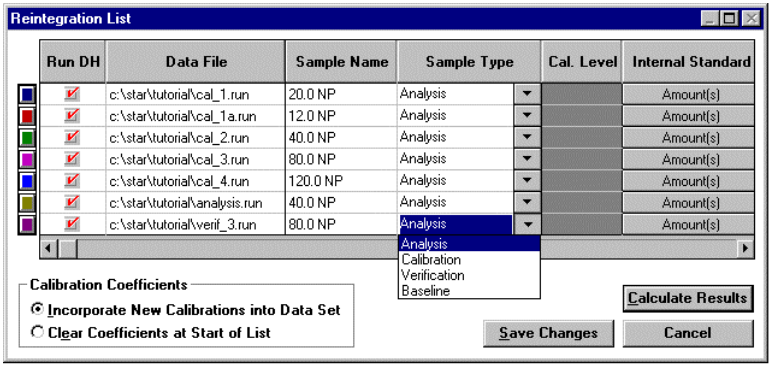
Next, you will set the Verification Deviation Tolerance for the VERIF_3.RUN example.

11. Select EDIT METHOD→Verification Setup. Set the Deviation Tolerance to 15% and the Out-of-Tolerance Action to No Action. Save this setup.

Now, you will use the Reintegration List to reintegrate the data and create the calibration curves.

12. Select RESULTS→Reintegration List...

The Reintegration List appears.



The data file for each chromatogram displayed in IG appears here. The fourth column in the Reintegration List is the Sample Type, which must be set as Calibration for the first five files, Analysis for the sixth and as Verification for the last one.

13. Set the Sample Type for each of the first five data files to Calibration.

14. Click in the cell for each file and pull down the drop-down list box. Click on *Calibration*.

15. Set the Sample Type for ANALYSIS.RUN to Analysis, and VERIF_3.RUN to Verification.

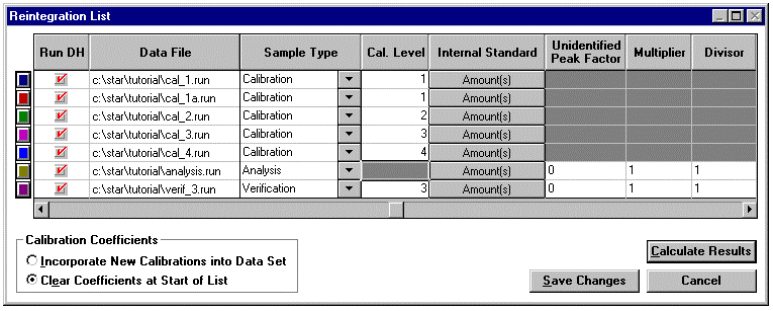
The fifth column is the Calibration Level column. Each value listed here corresponds to a Level column in the peak table. The calibration levels used in this tutorial are numbered 1 through 4

16. Set the Cal. level for CAL_1.RUN and CAL_1A.RUN to 1, CAL_2.RUN to 2, CAL_3.RUN to 3, and CAL_4.RUN to 4. This associates the amounts entered in the Peak Table with the runs that contain those amounts.

17. Set the Cal. Level for the VERIF_3.RUN to 3.

18. Click the scroll bar to scroll past the list of values for the Internal Standard, Multiplier, Divisor, and Unidentified Peak Factor. Leave them set to their default values.

Each chromatogram listed should have its Run DH box checked, which means that the data in that data file will be recalculated. If you want to exclude a file from the recalculation, you can clear its Run DH box by clicking in it. The Reintegration List should look like the example shown.



19. Make sure that the Clear Coefficients at Start of List option is selected.

20. Click the *Calculate Results* button.

21. Select *Yes* to the message, “All calibration coefficients will be cleared - continue?”.

    An information box appears with the message, “Processing CAL_1.RUN.” The message changes as each data file is processed.

The Calibration Curve

When the data has been processed, examine the Calibration Coefficients that have been generated for each peak. These coefficients describe the equation for the calibration curve for each compound.

1. Select EDIT METHOD→Calibration Setup.
2. Click on the *Edit/Lock Coefficients…* button at the bottom of the window.

The Coefficients window should look like the one shown next.

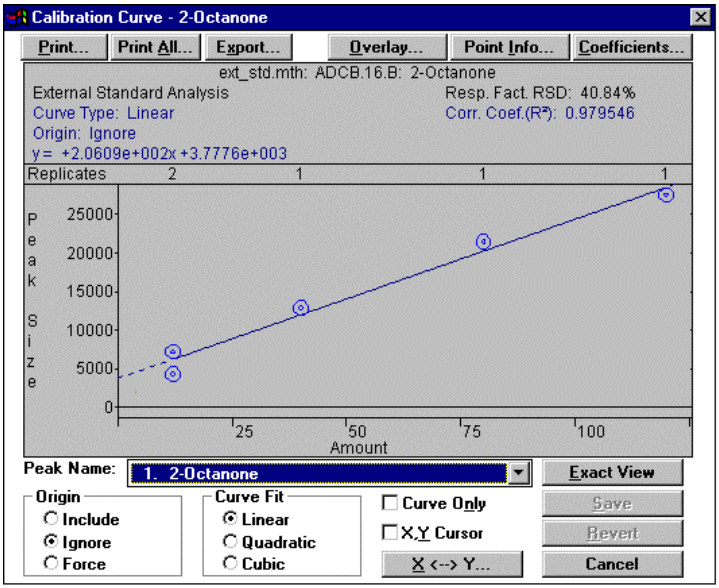
Coefficients

	Retention Time	Peak Name	Lock Coeffs.	X^3	X^2	X	Intercept
1	5.673	2-Octanone		0	0	206.09	3777.6
2	6.970	1-Octanol		0	0	73.15	391.35
3	7.507	n-Decane		0	0	135.56	959.52
4	8.473	2,6-dimetphe		0	0	138.44	2098.3
5	8.997	n-Dodecane		0	0	155.5	4318
6	9.323	2,4-dimetani		0	0	119.48	3333
7	10.855	n-Tridecane		0	0	119.89	3362.8

OK Cancel

3. Click *Cancel* to return to the Calibration Setup.
4. Click on the *View Curves...* button to see the calibration curve for the first peak.

The Calibration Curve window appears. The peak name appears highlighted in the Peak Name area and in the title bar.



The Y axis of the calibration curve corresponds to peak size. The X axis represents the amount of the compound injected.

5. Click the drop-down menu arrow in the Peak Name box.

6. Click on the name of the second peak (1-Octanol).

   The calibration curve for the selected peak appears in the Calibration Curve window. The peak's name appears in the title bar.

7. Continue through the list of peaks until you have seen all the calibration curves.

   Use the drop-down menu or the keyboard Up/Down arrows to select each peak.

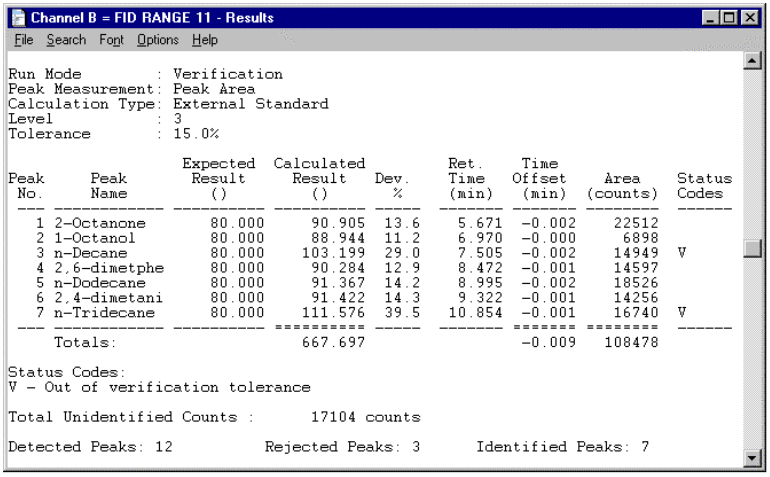
NOTE: Refer to "Tutorial 7 Calibrating with an Internal Standard and Getting Around in the Curve Manager" in this manual, for an exercise in using the other curve functions.

8. Click the *Cancel* button to return to the Calibration Setup. Click *Cancel* again to return to the Interactive Graphics window.

Now you can display the Results Report for any run.

9. Click on the white chromatogram trace with the right mouse button. Select "View Results Only".

   The Verification Report for VERIF_3.RUN appears. Note that Peaks 3 and 7 are marked as "Out of Verification Tolerance".



Channel B = FID RANGE 11 - Results

File Search Font Options Help

Run Mode : Verification
Peak Measurement: Peak Area
Calculation Type: External Standard
Level : 3
Tolerance : 15.0%

Peak No.	Peak Name	Expected Result ()	Calculated Result ()	Dev. %	Ret. Time (min)	Time Offset (min)	Area (counts)	Status Codes
1	2-Octanone	80.000	90.905	13.6	5.671	-0.002	22512	
2	1-Octanol	80.000	88.944	11.2	6.970	-0.000	6898	
3	n-Decane	80.000	103.199	29.0	7.505	-0.002	14949	V
4	2,6-dimetphe	80.000	90.284	12.9	8.472	-0.001	14597	
5	n-Dodecane	80.000	91.367	14.2	8.995	-0.002	18526	
6	2,4-dimetani	80.000	91.422	14.3	9.322	-0.001	14256	
7	n-Tridecane	80.000	111.576	39.5	10.854	-0.001	16740	V
	Totals:		667.697			-0.009	108478	

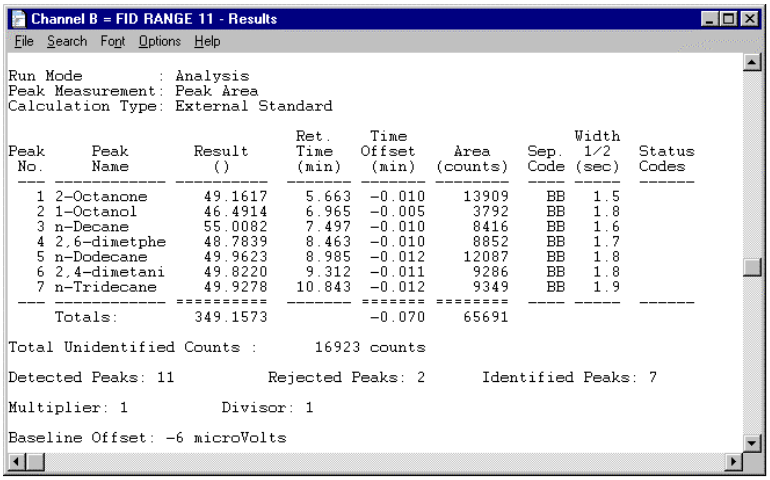
Status Codes:
V - Out of verification tolerance

Total Unidentified Counts : 17104 counts

Detected Peaks: 12 Rejected Peaks: 3 Identified Peaks: 7

10. Close the Results window.

11. Use the right mouse button to click anywhere on the plot trace of the sixth chromatogram, ANALYSIS.RUN (this should be the light blue trace). Select “View Results Only”.



12. Close the Results window.

13. Close Interactive Graphics and do not save changes to either the method or the data file.

Tutorial 7 Calibrating with Internal Standards & Getting Around in the Curve Manager

Calibrating with an Internal Standard allows you to account for variations in sample volumes or for loss during sample preparation. You do this by adding a small, known amount of an additional compound to sets of standards and samples. Then, you perform calibration runs with the standards. When you make an injection, any variation in the sample volume is reflected by a detectable variation in the ratio of areas and amounts for the internal standard. As with external standard runs, the Workstation plots the calibration curve and uses the equation for it to calculate results for the analysis runs. The curve is adjusted to account for the variations in sample volume.

Topics Discussed

- Generation of Calibration Data
- Using the Calibration Curve window
- Using the Calibration Curve options (Curve Manager)

Generation of Calibration Data

Calibration with an Internal Standard requires that calibration data for each compound be present in the method peak table. This data is generated in calibration runs and saved in the method. The method is then used to perform an analysis of the sample. As many as ten concentration levels can be used to generate a calibration curve for the analytes of interest. As many as eight internal standards may be designated. This tutorial uses only four different levels in five data files and uses two internal standards.

1. Open Interactive Graphics from the Workstation Star Bar.

   The Open Multiple Data Files window appears.

2. Choose the chromatographic data file CAL_1.RUN in the EXAMPLES directory.

3. Select *Add To List*.

   You may double-click on a data file to quickly add it to the list.

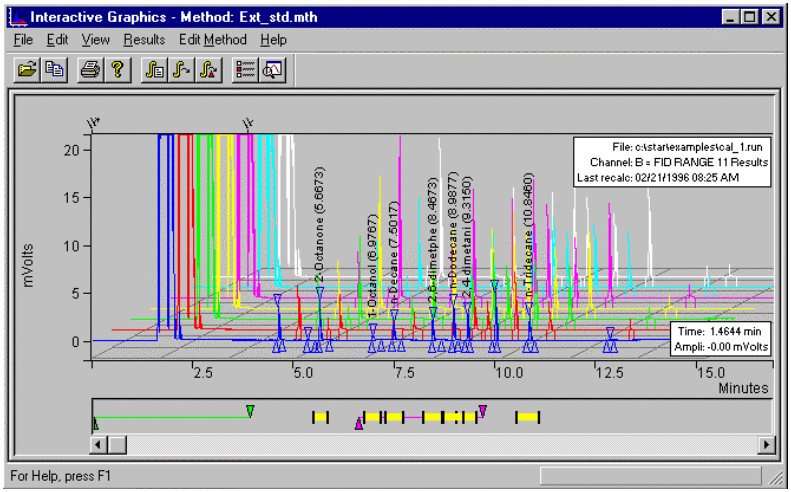
4. Continue adding the following data files to the list: CAL_1A.RUN, CAL_2.RUN, CAL_3.RUN, CAL_4.RUN.

Channel B should be selected for all data files in this series.

The analytical run for this tutorial is called ANALYSIS.RUN. The file called VERIF_3.RUN is a verification run for this series.

5. Add ANALYSIS.RUN and VERIF_3.RUN to the list and press Open File(s). When the Open Method file dialog box appears, click *OK* to build method from data file.

The IG screen should look similar to the figure shown below.



Next, you will load the method file which will be used for the analysis. You will use the method file, INT_STD.MTH, located in the EXAMPLE directory. The information in the peak table is necessary to generate the coefficients of the calibration curve.

6. Select File→Open Method and select INT_STD.MTH, press Open Files.

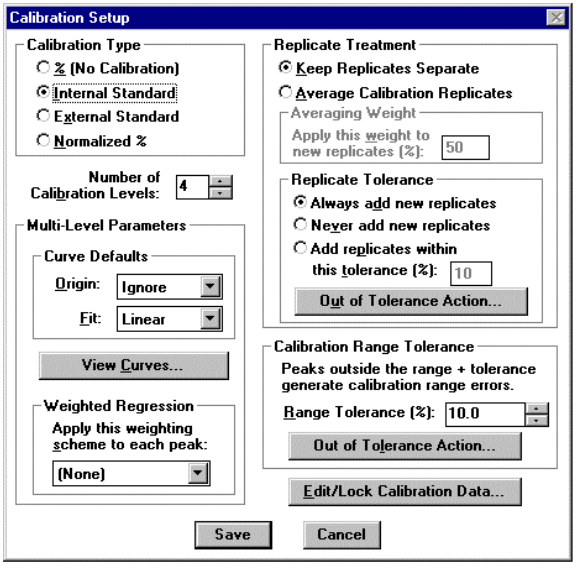
   The title bar for the Interactive Graphics window now lists INT_STD.MTH as the active method.

Now, verify the calibration parameters to be used, as follows:

7. Select EDIT METHOD→Calibration Setup.

   The Calibration Setup window opens.

The window should look like the one shown below. The Calibration Type should be Internal Standard. The Number of Calibration Levels should be set to 4. The Curve settings should be Linear, Ignore.

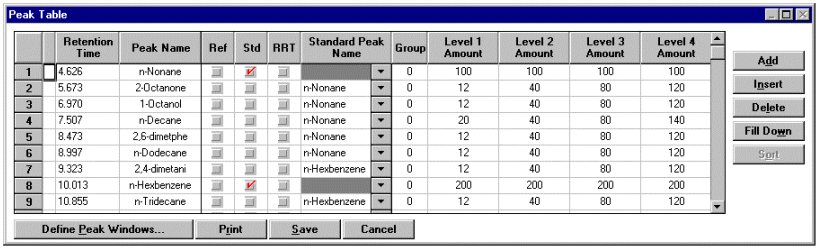


8. Select *Save* to return to the Interactive Graphics window.

Now, verify the Peak Table settings.

9. Select EDIT METHOD→Peak Table.

The window should look like the one shown below. Verify the nine peaks have the amounts shown for Levels 1-4.



Peaks 1 and 8 should be designated as Std peaks. Peaks 2-6 should be assigned Standard Peak Name, n-Nonane, and peaks 7 and 9 should be assigned Standard Peak Name, n-Hexbenzene. Any analyte peak can be assigned to any internal standard.

10. Click the *Save* button to close the Peak Table window.

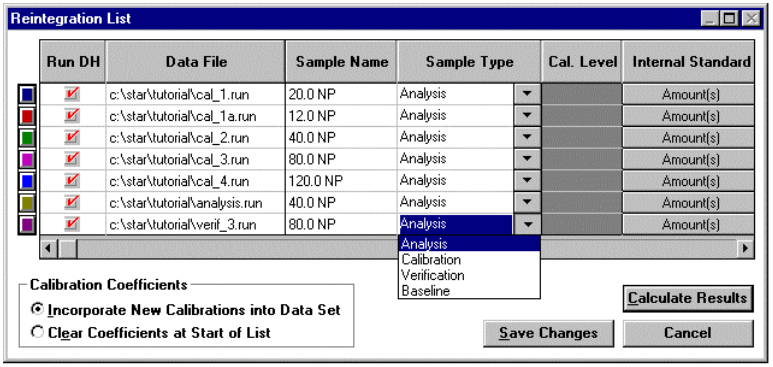
Next, you will set the Verification Deviation Tolerance for the VERIF_3.RUN example.

11. Select EDIT METHOD→Verification Setup. Set the Deviation Tolerance to 15% and the Out-of-Tolerance Action to No Action. Save this setup.

Now, you will use the Reintegration List to reintegrate the data and create the calibration curves.

12. Select RESULTS→Reintegration List...

The Reintegration List appears.



The data file for each chromatogram displayed in IG appears here. The fourth column in the Reintegration List is the Sample Type, which must be set as Calibration for the first five files, Analysis for the sixth and Verification for the last one.

13. Set the Sample Type for each of the first five data files to Calibration.

14. Click in the cell for each file and pull down the drop-down list box. Click on Calibration.

15. Set the Sample Type for ANALYSIS.RUN to Analysis, and VERIF_3.RUN to Verification.

The fifth column is the Calibration Level column. Each value listed here corresponds to a Level column in the peak table. The calibration levels used in this tutorial are numbered 1 through 4.

16. Set the Cal. level for CAL_1.RUN and CAL_1A.RUN to 1, CAL_2.RUN to 2, CAL_3.RUN to 3, and CAL_4.RUN to 4. This associates the amounts entered in the Peak Table with the runs that contain those amounts.

17. Set the Cal. Level for the VERIF_3.RUN to 3.

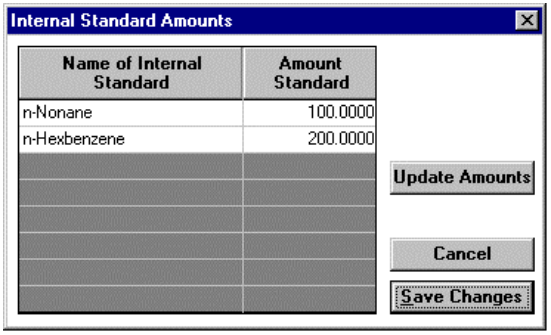
Next, you should verify the amounts for the internal standards referenced in the peak table.

18. Click on the *Amount(s)* button for the first data file.

The Internal Standard Amounts window appears.

If the fields are grayed out, click on the *Update List* button to view the list from the peak table.

Verify the Amount Standard for the two internal standards as shown below.



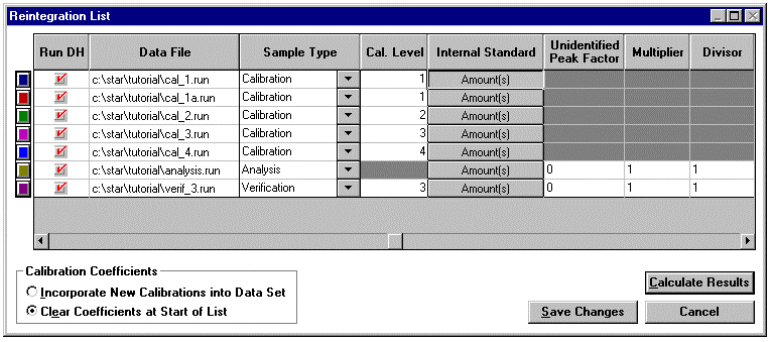
19. Click on the *Save Changes* button.

You will need to do this for each of the data files. The amount of internal standard can vary from sample to sample.

When the data files are reintegrated, these amounts will be placed in the peak table for use in determining the calibration curves.

20. Click the scroll bar to scroll past the list of values for the Multiplier, Divisor, and Unidentified Peak Factor. Leave them set to their default values.

Each chromatogram listed should have its Run DH box checked, which means that the data in that data file will be recalculated. If you want to exclude a file from the recalculation, you can clear its Run DH box by clicking on it. The Reintegration List should look like the example shown.



21. Make sure that the Clear Coefficients at Start of List option is selected.

22. Click the *Calculate Results* button.

23. Select Yes to the message, “All calibration coefficients will be cleared - continue?”.

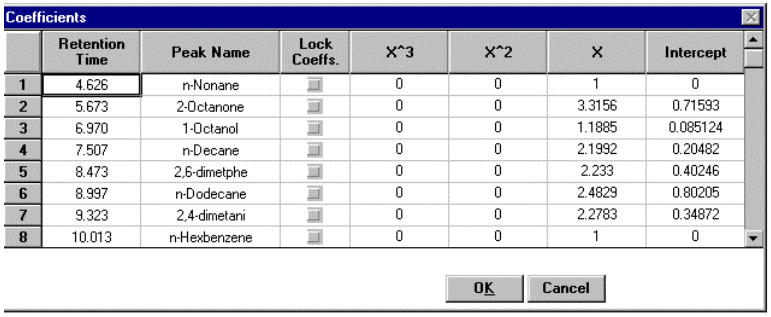
An information box appears with the message, “Processing CAL_1.RUN.” The message changes as each data file is processed.

The Calibration Curve

When the data has been processed, examine the Calibration Coefficients that have been generated for each peak. These coefficients describe the equation for the calibration curve for each compound.

1. Select EDIT METHOD→Calibration Setup.
2. Click on the *Edit/Lock Coefficients* button at the bottom of the window.

The Coefficients window should look like the one shown below.

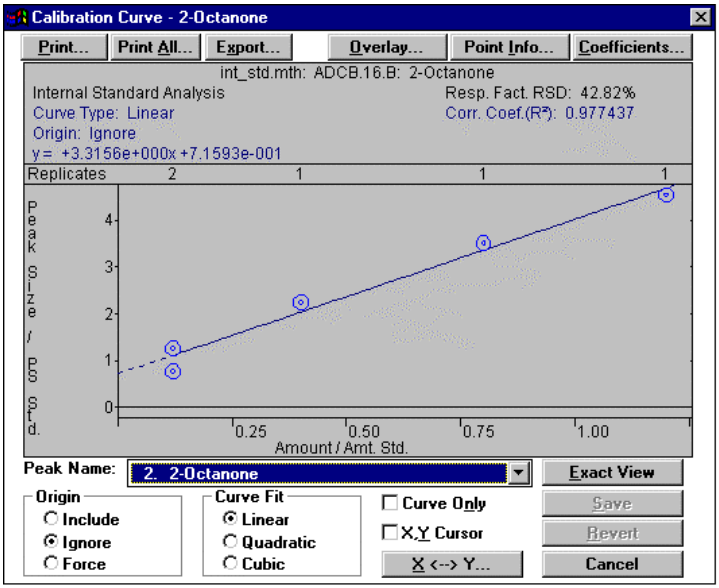


3. Click *Cancel* to return to the Calibration Setup.
4. Click on the *View Curves...* button to see the calibration curve for the first peak (the internal standard, n-Nonane).

The Calibration Curve window appears. The peak name appears highlighted in the Peak Name area and in the title bar.

5. Click the drop-down menu arrow in the Peak Name box.
6. Click on the name of the second peak (2-Octanone).

The calibration curve for the selected peak appears in the Calibration Curve window. The peak's name appears in the title bar.



The Y axis of the calibration curve represents the ratio of the peak size of the analyte compound to the peak size of the internal standard. The X axis represents the ratio of the amount of the analyte compound injected to the amount of internal standard injected.

7. Continue through the list of peaks until you have seen all the calibration curves.

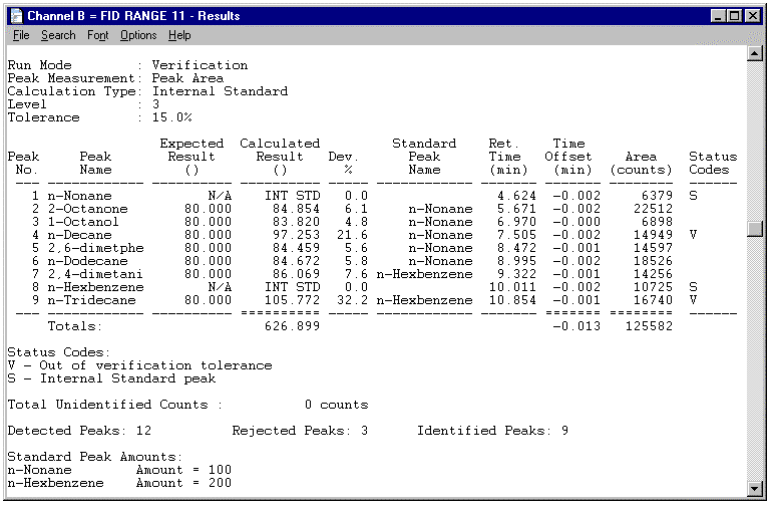
8. Use the drop-down menu or the keyboard Up/Down arrows to select each peak.

9. Click the *Cancel* button to return to the Calibration Setup. Click *Cancel* again to return to the Interactive Graphics window.

Now you can display the Results Report for any run.

10. Click on the white chromatogram trace with the right mouse button. Select “View Results only”.

    The Verification Report for VERIF_3.RUN appears. Note that Peaks 4 and 9 are marked as “Out of Verification Tolerance”.



Channel B = FID RANGE 11 - Results

File Search Font Options Help

Run Mode : Verification
Peak Measurement: Peak Area
Calculation Type: Internal Standard
Level : 3
Tolerance : 15.0%

Peak No.	Peak Name	Expected Result ()	Calculated Result ()	Dev. %	Standard Peak Name	Ret. Time (min)	Time Offset (min)	Area (counts)	Status Codes
1	n-Nonane	N/A	INT STD	0.0		4.624	-0.002	6379	S
2	2-Octanone	80.000	84.854	6.1	n-Nonane	5.671	-0.002	22512	
3	1-Octanol	80.000	83.820	4.8	n-Nonane	6.970	-0.000	6898	
4	n-Decane	80.000	97.253	21.6	n-Nonane	7.505	-0.002	14949	V
5	2,6-dimetphe	80.000	84.459	5.6	n-Nonane	8.472	-0.001	14597	
6	n-Dodecane	80.000	84.672	5.8	n-Nonane	8.995	-0.002	18526	
7	2,4-dimetani	80.000	86.069	7.6	n-Hexbenzene	9.322	-0.001	14256	
8	n-Hexbenzene	N/A	INT STD	0.0		10.011	-0.002	10725	S
9	n-Tridecane	80.000	105.772	32.2	n-Hexbenzene	10.854	-0.001	16740	V
	Totals:		626.899				-0.013	125582	

Status Codes:
V - Out of verification tolerance
S - Internal Standard peak

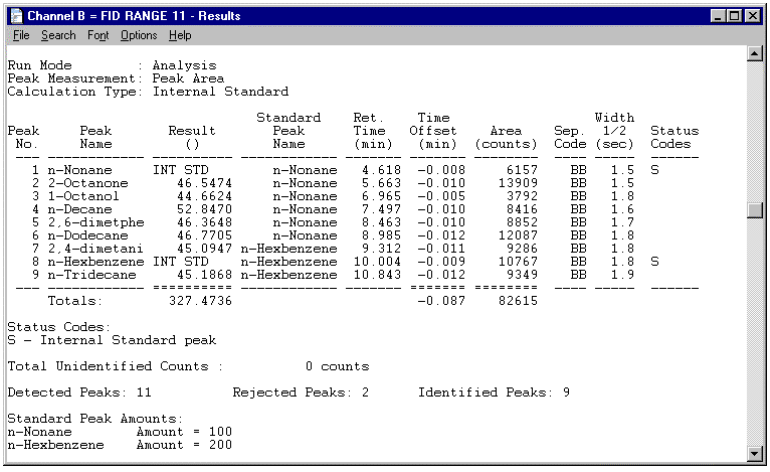
Total Unidentified Counts : 0 counts

Detected Peaks: 12 Rejected Peaks: 3 Identified Peaks: 9

Standard Peak Amounts:
n-Nonane Amount = 100
n-Hexbenzene Amount = 200

11. Close the Results window.

12. Using the right mouse button, click anywhere on the light blue plot trace of the sixth chromatogram, ANALYSIS.RUN. Select *"View Results Only".*



Channel B = FID RANGE 11 - Results

File Search Font Options Help

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Internal Standard

Peak No.	Peak Name	Result ()	Standard Peak Name	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1	n-Nonane	INT STD	n-Nonane	4.618	-0.008	6157	BB	1.5	S
2	2-Octanone	46.5474	n-Nonane	5.663	-0.010	13909	BB	1.5	
3	1-Octanol	44.6624	n-Nonane	6.965	-0.005	3792	BB	1.8	
4	n-Decane	52.8470	n-Nonane	7.497	-0.010	8416	BB	1.6	
5	2,6-dimetphe	46.3648	n-Nonane	8.463	-0.010	8852	BB	1.7	
6	n-Dodecane	46.7705	n-Nonane	8.985	-0.012	12087	BB	1.8	
7	2,4-dimetani	45.0947	n-Hexbenzene	9.312	-0.011	9286	BB	1.8	
8	n-Hexbenzene	INT STD	n-Hexbenzene	10.004	-0.009	10767	BB	1.8	S
9	n-Tridecane	45.1868	n-Hexbenzene	10.843	-0.012	9349	BB	1.9	
	Totals:	327.4736			-0.087	82615			

Status Codes:
S - Internal Standard peak

Total Unidentified Counts : 0 counts

Detected Peaks: 11 Rejected Peaks: 2 Identified Peaks: 9

Standard Peak Amounts:
n-Nonane Amount = 100
n-Hexbenzene Amount = 200

Note the internal standard assignments listed for each peak and the standard amounts documented below the listing of peaks results.

13. Close the Results window.

Using the Calibration Curve Options (Curve Manager)

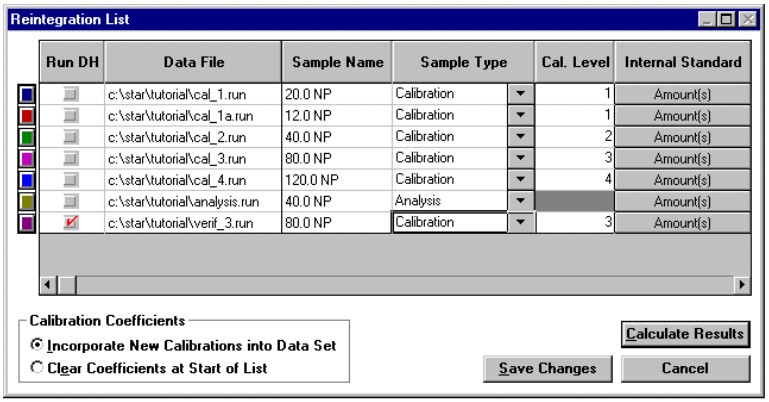
The window which displays the curves and curve options is called the Curve Manager. This step lets you examine the features of some of these options. The following steps will also allow processing of the set of data generated using the Calibrating With External Standards tutorial. Screens and results will be similar.

1. Select RESULTS→Reintegration List...

2. Change the Sample Type of VERIF_3.RUN to Calibration and the Cal. Level to 3.

3. Deselect the Run DH check boxes for all data files except VERIF_3.RUN.

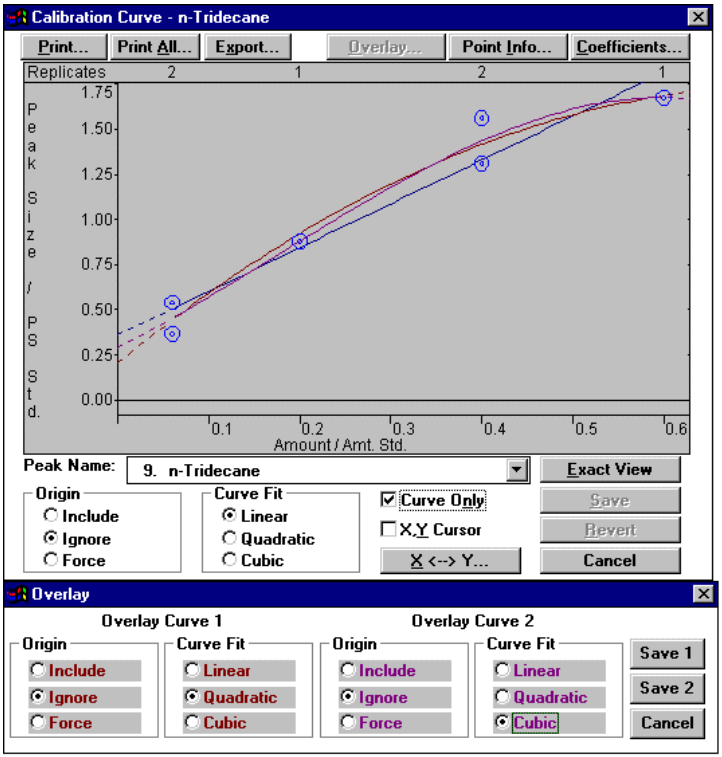
4. Select the “Incorporate New Calibrations into Data Set” option and press *Calculate Results.*



After processing has completed, the data from VERIF_3.RUN will have been added to the previous calibration set. All curves will now show two replicates for level 3.

5. Select RESULTS→View Calibration Curves... to view the added data point. Display the curve for peak 9, n-Tridecane.

6. Select the Curve Only check box. Click on the *Overlay* button. Using the buttons in the Overlay window below the Calibration Curve window, select Quadratic→Ignore for Overlay Curve 1 and Cubic→Ignore for Overlay Curve 2.

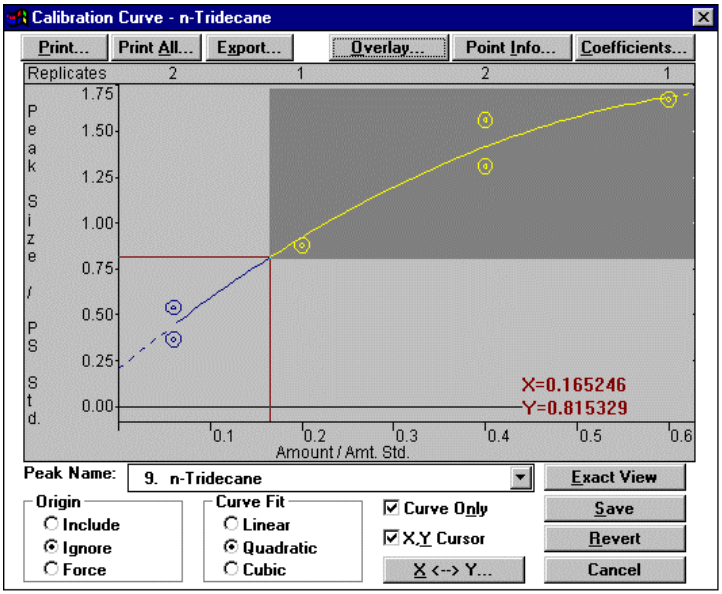


7. Choose to save the first fit by clicking on *Save 1*.

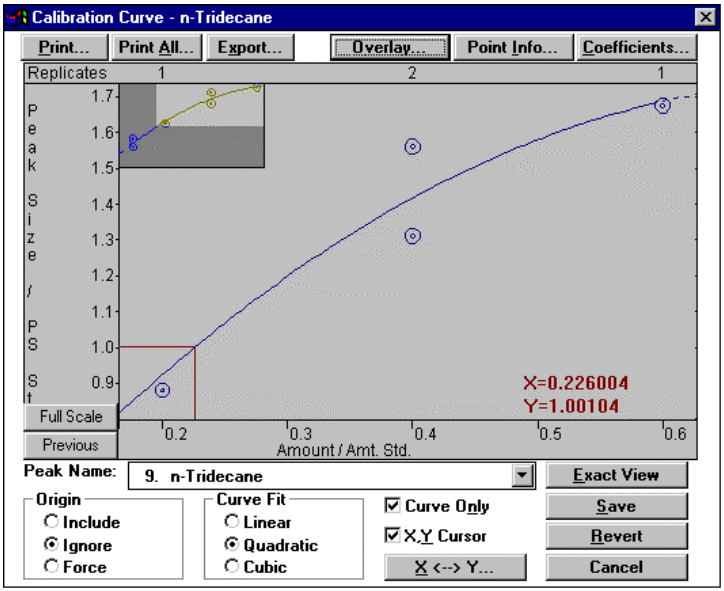
8. Select the X,Y cursor check box and then move the cursor along the calibration curve.

Note the continuous readout of X and Y values. When you are finished, deselect the X,Y cursor check box.

9. Position the cursor in the upper right corner of the graph and, with the left mouse button, click and drag the cursor to a point below the single point as shown in the figure below.



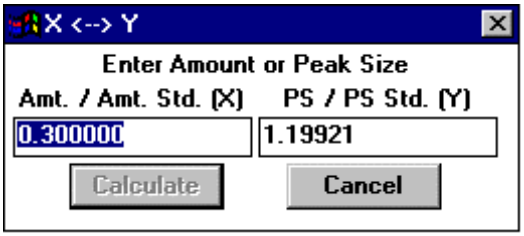
Note that the area highlighted has been magnified and a small window showing the entire graph has been displayed in the upper left corner of the display.



You may continue zooming many times if you wish. At any time you may restore the curve to full scale by clicking on the *Full Scale* button.

10. Click on the *Full Scale* button that appeared after you zoomed (see the lower left corner of the plot area in the figure above).

11. Click on the *X <--> Y...* button and enter a value of 0.3 in the Amount field and then click on *Calculate*.

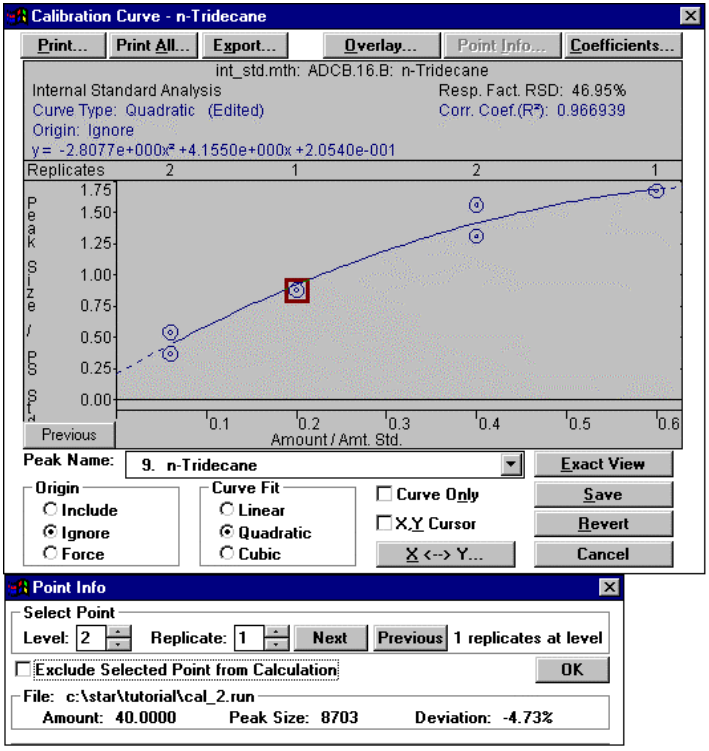


The corresponding Peak Size (PS/PS Std.) is calculated determined by the curve. Enter 1.0 in the Peak Size field and calculate the Amount (Amt./Amt. Std.) determined by the curve.

12. Choose *Cancel* after you are done and deselect the Curve Only box to show the header information.

13. Double-click on any point on the curve. The Point Info window appears. See the figure below.

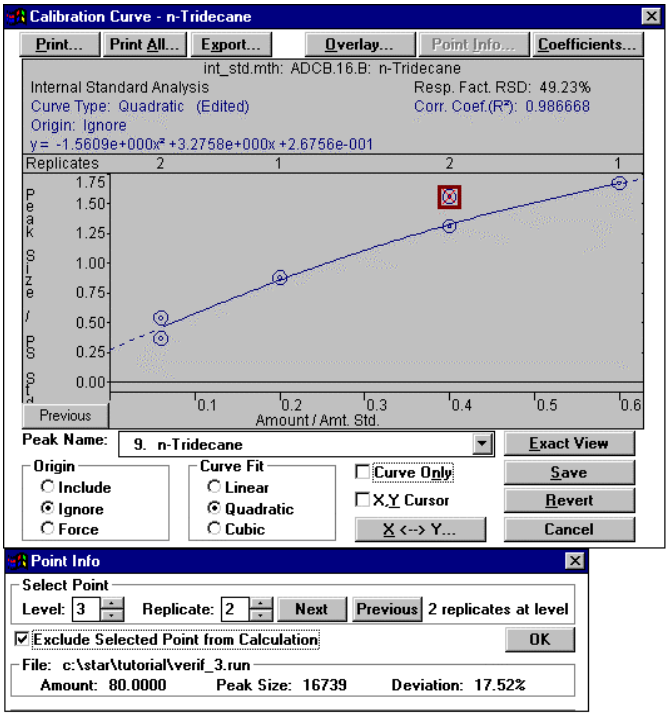
You may also access this window by clicking on the *Point Info* button above the displayed curve.



Various data file information is displayed for that point. You can view information for all the points by using the Level/ Replicate arrows or by clicking on the *Next/Previous* buttons.

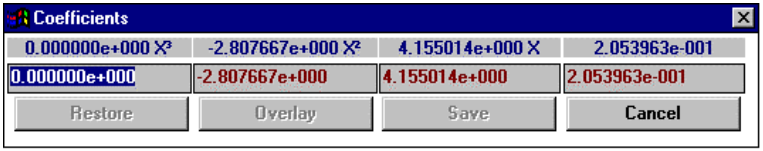
14. Select the point at Level 3, Replicate 2 and exclude this point from the curve fit calculations. Check the "Exclude Selected Point from Calculation" box.

This removes the point from the curve calculations and a new set of coefficients is determined as shown in the next figure.



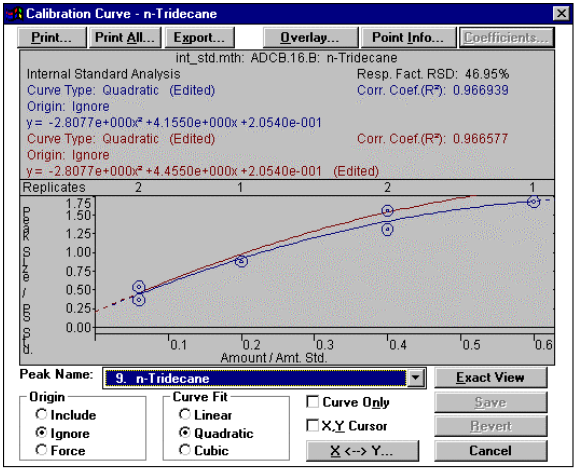
15. Deselect the Exclude Selected Point from Calculation check box and close the Point Info window by clicking on *OK*.

16. Click on the *Coefficients* button. In the Calibration Curve window, change one of the coefficients by a small amount.



17. Click on the *Overlay* button to compare curves for the two sets of coefficients.

Note that the header information displayed at the top of the Curve Manager window indicates 'Edited' after fields that you modified during this tutorial. See the following figure.



18. Close the Curve Manager.

19. Close Interactive Graphics and do not save changes to either the method or the data file.

Appendix A Notes on Printing

The Report Method

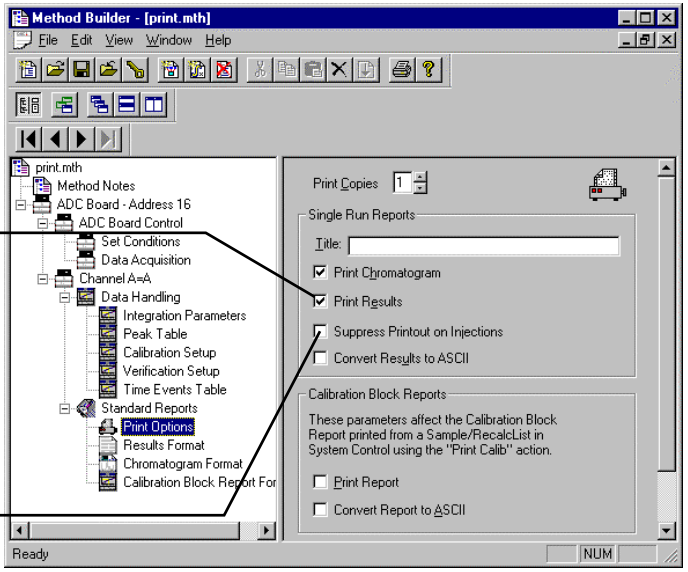
Section Report formatting parameters are specified in the Report section of the Method. The Report Method section is a postrun application section and applies to a specific detector channel. You create a Report Method section for each channel of each detector for which you wish to generate a report.

The Report Method section contains four editing windows. Each one is described.

Only prints when performing recalculations or print actions from System Control. Use this to avoid automated injection delays due to printer problems.

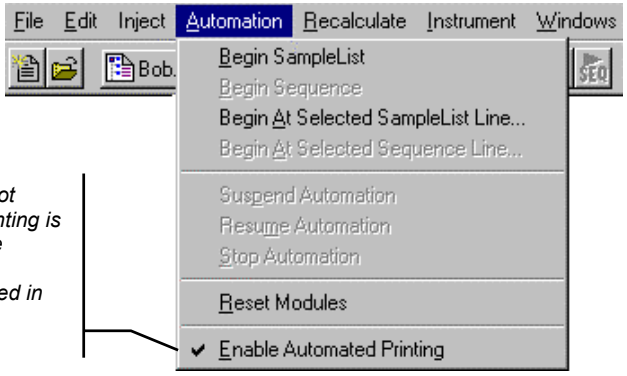
Creates an ASCII file containing the results report. The file is named the same as the Data File with the "TXT" extension.

Check this box if you using any of the Excel Macros contained in the Advanced Applications software.



Automated Printed Report Generation

Once you have added Report sections to your Method, automated printed reports can be generated from System Control after each injection, after recalculations, or by the use of the Print action in the Sequence window.



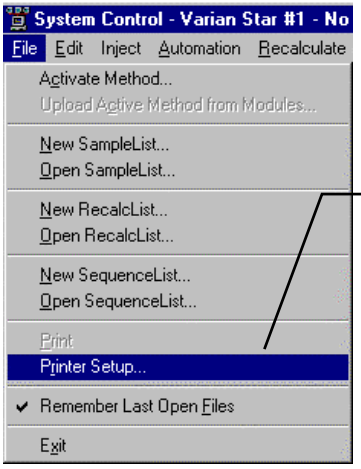
When this item is not checked, report printing is disabled. ASCII file generation is still performed if specified in the Report Method section.

You may choose to disable automated Report printing during the course of an automated sequence of injections or recalculations. You can do this by disabling automated printing from the Automation menu in the Instrument window.

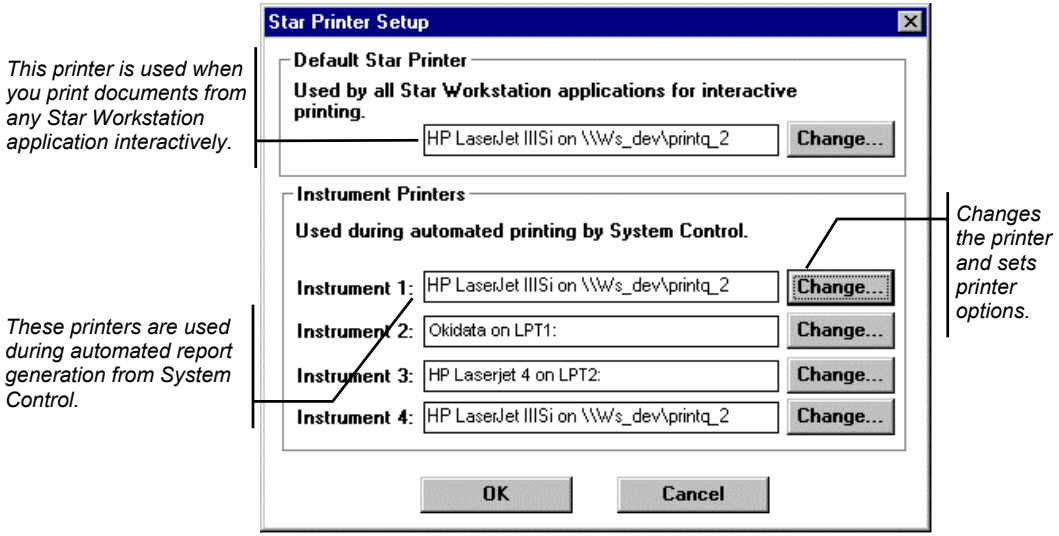
Disabling automated printing is analogous to disconnecting the printer—automation continues but no reports are printed. This is useful if you are about to run out of printer paper but do not wish to suspend automation, or if you have more than one Instrument printing to the same printer and you wish to avoid possible interleaving of reports.

Automated Printing to Multiple Printers

If you are running automation on more than one instrument, you may wish to designate a separate printer for each instrument to avoid interleaving of reports. Pull down the File menu in System Control (or any Star Workstation application).



The Star Printer Setup dialog box is displayed.

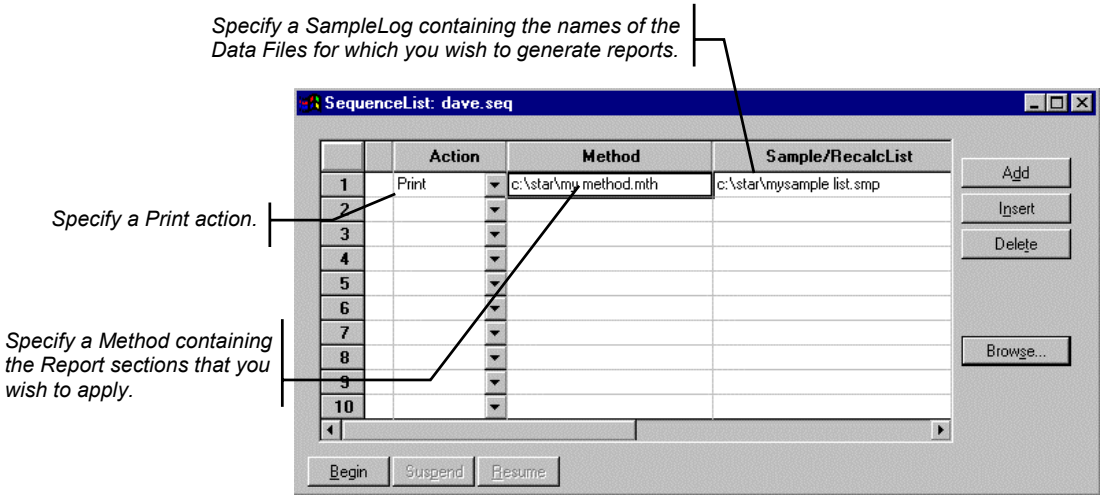


Batch Report Printing without Recalculating

When you perform batch recalculations in System Control, reports are automatically generated using the Report parameters in the active Method. You may wish to print reports for a batch of Data Files without recalculating them.

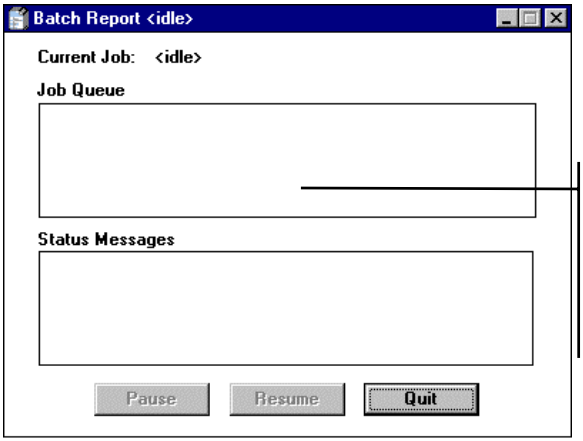
Batch Printing in System Control

Display the active Sequence from the Windows menu in the Instrument window.



Batch Printing with Batch Report

You may print reports for a set of Data Files without using System Control. Run the Batch Report application from the Varian Star 4.5 group in the Program Manager (Window 3.11) or the Programs folder (Windows 95 Start button). The Batch Report window is displayed.



Reports are printed using the formatting options stored in the Method last used to process the Data Files.