Varian, Inc.
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MS Workstation

Tutorial Manual

QUALITY SYSTEM
ISO 9001
CERTIFIED

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Contents

Exercises......7
Overview......7
Executing Exercises......7
    Running an MS Method without a GC Method......8
    Loading an MS File in MS Data Review......9
    Displaying Mass Chromatograms......11
    Getting Spectra from an MS file......15
    Performing a Background Correction......16
    Performing a NIST Library Search......19
    How to Create or Edit NIST libraries......21
    Building a Spectrum List......22
    Editing Spectra......25
    Printing Pictures......26
    Exporting Pictures......27
    Creating and Using a Spooler File......28
    Exporting Spectra......31
    Displaying and Printing Log Information......32

Acquiring a 2000 MS Data File......35
Introduction......35
Starting the Varian MS Workstation......35
Setting Instrument Configuration for Manual Injections......35
    Running an MS Method without a GC Method......37
Adjusting and Tuning the 2000......38
    Adjusting RF Tuning......40
    Adjusting Calibration Gas......40
    Adjusting CI Reagent......41
    Running Auto Tune......42
    Examining Current Module Attributes......43
Preparing a GC/MS Acquisition Method......44
    Creating a New Method......44
    Editing the Method......48
Starting the Acquisition......53
    Activating the GC/MS Method......53
    Starting the GC/MS Run......54
    Injecting 1 µL of the Column Test Mixture......56
    Monitoring Data Acquisition in Progress from System Control......57
    Monitoring the Acquisition in Progress from MS Data Review......59
    Retention Times for Analytes in the Column Test Mix Sample......61
Setting Instrument Configuration for Automated Injections......61
    Configuring the Instrument......61
    Creating the Sample List......66

Qualitative Analysis of GC/MS Data....69
Analyzing GC/MS Data....69
  Demonstration Files....69
  Displaying a Data File....70
  Choosing a Mass Spectrum....71
  Background Correction....71
  Running a Library Search on a Single Spectrum....72
  Creating a Spectrum List....74
  Library Searching a Spectrum List....75
  Performing a Chromatogram Search with a Spectrum List....76

Quantitative Analysis of GC/MS Data....79
Overview of the Quantitative Analysis Tutorials....79
  Demonstration Files....79
  Tutorial Topics....80
Qualitative Identification....81
Editing a Data Handling Method....81
  The Calculations Setup Dialog Box....84
  The Results Treatment Dialog Box....87
  The Compound Table....87
Building a Recalculation List for Calibration....104
Processing a Recalculation List to Add Calibration Data....108
  Examining Calibration Results in Process Data View....108
Reviewing Calibration Results in the Results View....110
  Viewing and Editing Calibration Results in the Method Builder Dialog....115
Processing Analysis Files in a RecalcList....117
  Adding and Processing Entries in an Existing RecalcList....118
Reviewing Analysis Results....124

SIS and MS/MS Acquisitions....127
Introduction Tutorial....127
  Calibration of SIS....127
  MS/MS....130

Run File Tutorials....145
Overview of the Varian MS Workstation for GC Run Files....145
Using the Star Toolbar....145
Using the Tutorials....146
  Example Files....146
  Reinstalling the Tutorial Files....146

Tutorial 1 Recalculating Results....147
Overview....147
Preparing a Data File for Use with this Tutorial....147
Opening a Data File and Method in Interactive Graphics....147
Moving a Peak Event....152
Recalculating the Data File....153
Editing the Method....155
Recalculating in System Control....157

Tutorial 2 Changing Peak Detection Parameters....161
Overview....161

Preparing the Data File for Use with this Tutorial....161
Opening the Data File and Method....161
Changing the Initial Peak Width....163
Changing the Signal-to-Noise Ratio....167
Changing the II Time Events....168
Changing the WI Time Event....171

Tutorial 3 Filling a Peak Table....173
Overview....173
Preparing a Data File for Use with this Tutorial....173
Opening the Fill Peak Table Window....173
Adding Peaks with the cursor....174
Adding Peaks from a selection....174
Naming Peaks....175
Designating Peak Functions....176
    Reference Peak(s)....176
    Internal Standard Peak(s)....176
    Relative Retention Time Peak....176
Entering Amounts for Calibration Levels....177
Editing the Peak Table....177

Tutorial 4 Identifying Peaks....179
Overview....179
Preparing a Data File for Use with this Tutorial....179
Peak Windows....179
Define Peak Windows....180
Showing Peak Windows....181
Changing Peak Functions....182
Peak Reject....183
Unidentified Peaks....184

Tutorial 5 Using the II, SR, and VB Time Events....185
Overview....185
Inhibiting Integration....185
Editing a Time Event from the Menu....187
Graphical Placement of a Time Event....189
Using Solvent Reject....191
Using Valley Baseline....193
The Effects of Other Peak Processing Events on VB....195

Tutorial 6 Calibrating with an External Standard....199
Overview....199
Generation of Calibration Data....199
The Calibration Curve....203

Tutorial 7 Calibrating with Internal Standards....207
Overview....207
Generation of Calibration Data....207
The Calibration Curve....212
Using the Calibration Curve Options (Curve Manager)....214

2000 GC/MS Application Notes 221
Application Note Index...221
Advantage Note Index..225

Exercises

Overview

Varian MS Workstation controls and supports Varian ion trap and quadrupole mass spectrometers. Several Workstation applications generate, process and review Varian MS files.

Method Builder and **System Control** create MS data files in two formats (*.sms and *.xms). **MS Standard Reports** and **MS Custom Reports** produce “Single Run” reports for a data file. Use **MS Data Review** to view single and multiple chromatograms, extract spectra, reprocess data files, view results and search MS libraries. Each application has an entry under the MS Workstation program, and an icon button on the Star Toolbar.

This Tutorial Manual demonstrates the MS Workstation capabilities. While these procedures use the 2000 ion trap, you can adapt them to your Varian platform.

Executing Exercises

Follow these instructions either using a printed manual or using On-Line Help. If you are using On-Line Help, you can use any of three methods to follow the exercise instructions:

I. Print each exercise, then follow the printed instructions.

If you use at a lower screen resolution (800x600) use this method. When printing topics that include graphics, you may need to adjust your printer’s properties to use a fine dithering mode for graphics.

II. Alternate between Help and the Varian MS Workstation Application

Activate both a Varian MS Workstation application (such as MS Data Review, System Control, or Method Builder) and the Exercises or other Tutorials. To alternate between the instructions in the Exercise or Tutorial and the Varian MS Workstation application, click on the desired application in the **Windows Taskbar** (usually at the bottom of the screen). You may also use the keyboard command <**Alt +Tab>** to alternate between applications and Help.

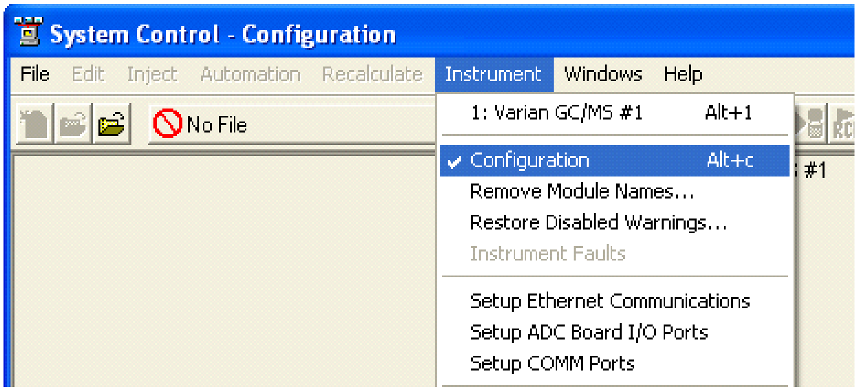
III. Split the screen between MS Data Review and its Help file.

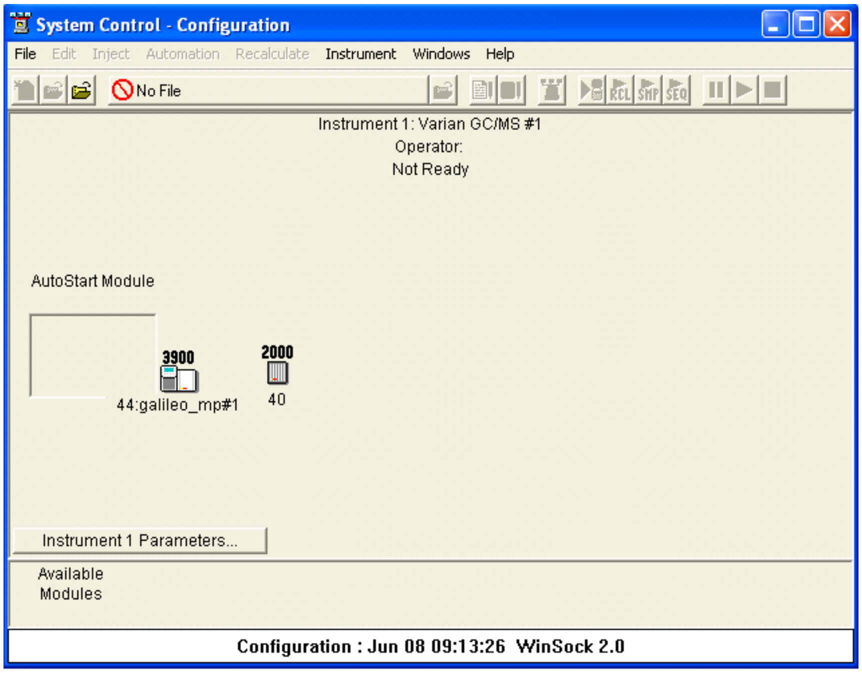
1. Start **MS Data Review**.
2. Minimize or close all applications other than **MS Data Review**.
3. Push the **?** button on **the MS Data Review** toolbar to load On-Line Help. Or, use the Start button in the Windows Taskbar to open Help.
   (Start >Programs >Varian WS >Documentation >MS 2000 Tutorial Help)

4. Split the screen between any one of the Varian MS Workstation application and its On-Line Help by right-clicking in an empty area on the Windows task bar (the one containing the Windows **Start** button) and choosing Tile Horizontally or Tile Vertically.

Running an MS Method without a GC Method

To run an MS method without a GC module or GC method, do the following: From the System Control Menu, under **Instrument,** select **Configuration.**

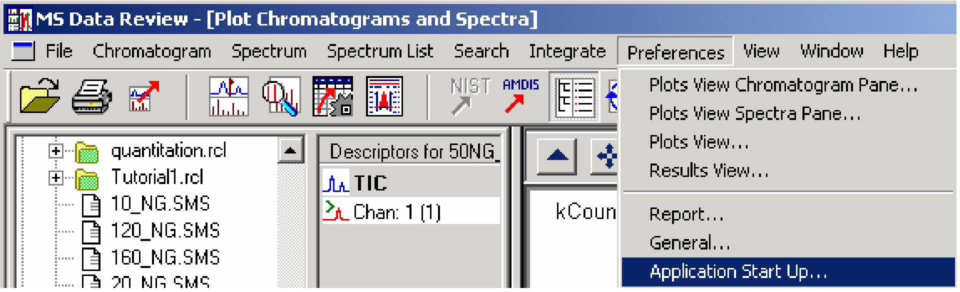


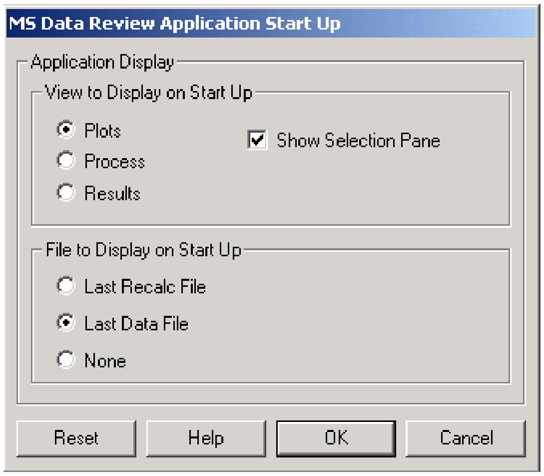


Click on the **GC Module** icon and drag the icon from the **Instrument** area to the **Available Modules** area**.** In addition, if you have an AutoSampler icon, drag it to the **Available Module** area to deactivate it. This will disconnect the GC and the Autosampler modules, and methods will not be downloaded to these modules when an MS method is activated (if you have a CP-8400/8410 AutoSampler, you only need to drag the GC icon as the CP-8400/8410 will be part of the GC Status and Control screen). To activate the GC and Autosampler modules, drag the icons back to the **Instrument** area of the **Configuration** screen.

Loading an MS File in MS Data Review

When MS Data Review is started, the Plot Chromatograms and Spectra window is opened if “Plots” was selected under “View to Display on Start Up” in **the MS Data Review Application Preferences** window (accessed from the menu ***Preferences >Application Start Up***).



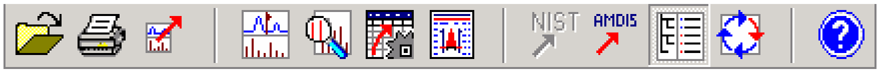


Application Display

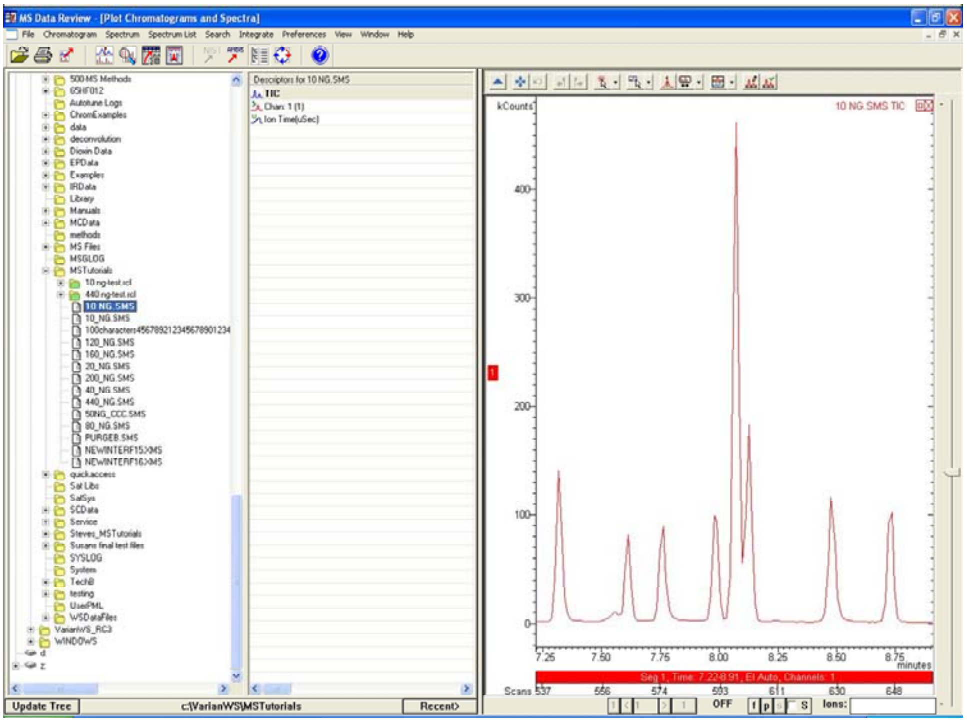
View to Display on Start Up
- ● Plots
- ○ Process
- ○ Results
- ☑ Show Selection Pane

File to Display on Start Up
- ○ Last Recalc File
- ● Last Data File
- ○ None

If “Process” or “Results” was selected under “View to Display on Start Up,” the Plot Chromatograms and Spectra window can be opened by choosing the **Plot Chromatogram and Spectra** button on the MS Data Review toolbar. The Plot Chromatograms and Spectra view displays a chromatogram window as well as Data File and Plot Descriptors panes, which can be shown or hidden at startup if “Show selection pane” is checked/unchecked in the **MS Data Review Application Preferences** dialog. The Data file and Plot Descriptors panes can also be shown/hidden by clicking the **Tree** button in the MS Data Review toolbar.

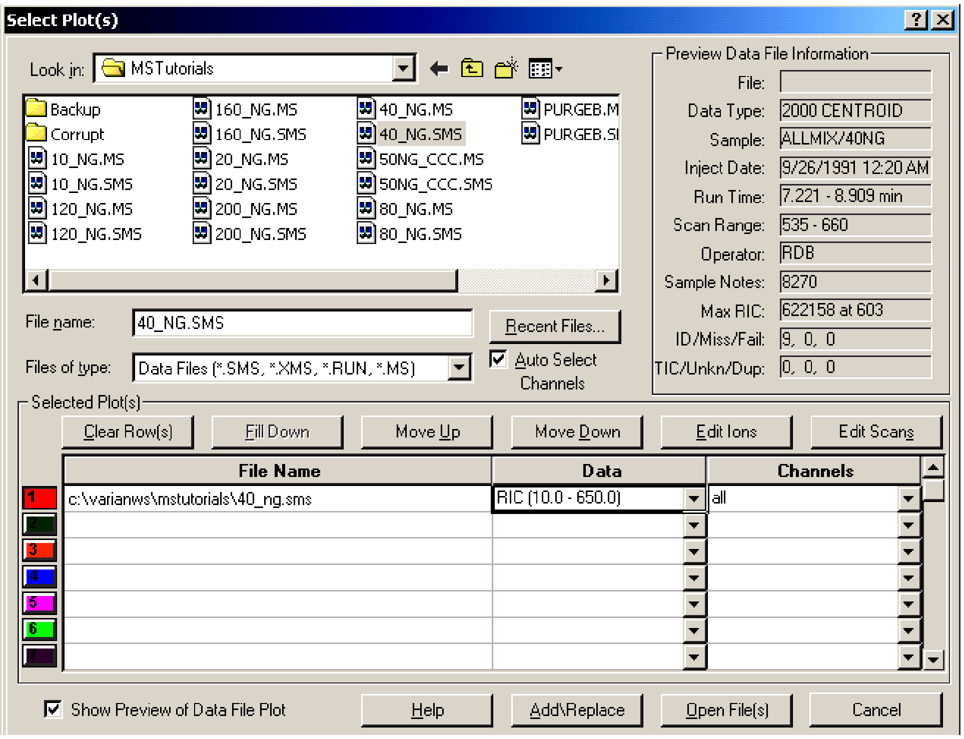


Select a data file and a descriptor to display a chromatogram. For example, to display the TIC (Total Ion Current) chromatogram for the 10_ng.sms file, select C:\VarianWS\MSTutorials\10_ng.sms in the Data File Pane and TIC from the Descriptor Pane.



Use the Descriptors Pane to select chromatograms of specific scan functions used in the data acquisition. In this example, the data file, 10_ng.sms, is a calibration file, which used to quantify an unknown sample (see the Quantitation Tutorial presented later in this Manual). The Descriptors are Method Descriptors, which show the components of the compound table used in the quantitation process. If you open this data file before using it in a calibration process, the method descriptors are not listed in the “Descriptors Pane”. Select the Descriptors you wish to display in the “Descriptors Pane” by right-clicking them.

You can also display Chromatograms by selecting **File >Select Chromatogram Data to Plot** in the MS Data Review menu or the toolbar button.



File name: 40_NG.SMS

Files of type: Data Files (*.SMS, *.XMS, *.RUN, *.MS)

☑ Auto Select Channels

Preview Data File Information

File:	
Data Type:	2000 CENTROID
Sample:	ALLMIX/40NG
Inject Date:	9/26/1991 12:20 AM
Run Time:	7.221 - 8.909 min
Scan Range:	535 - 660
Operator:	RDB
Sample Notes:	8270
Max RIC:	622158 at 603
ID/Miss/Fail:	9, 0, 0
TIC/Unkn/Dup:	0, 0, 0

	File Name	Data	Channels
1	c:\varianws\mstutorials\40_ng.sms	RIC (10.0 - 650.0)	all
2			
3			
4			
5			
6			
7			

☑ Show Preview of Data File Plot

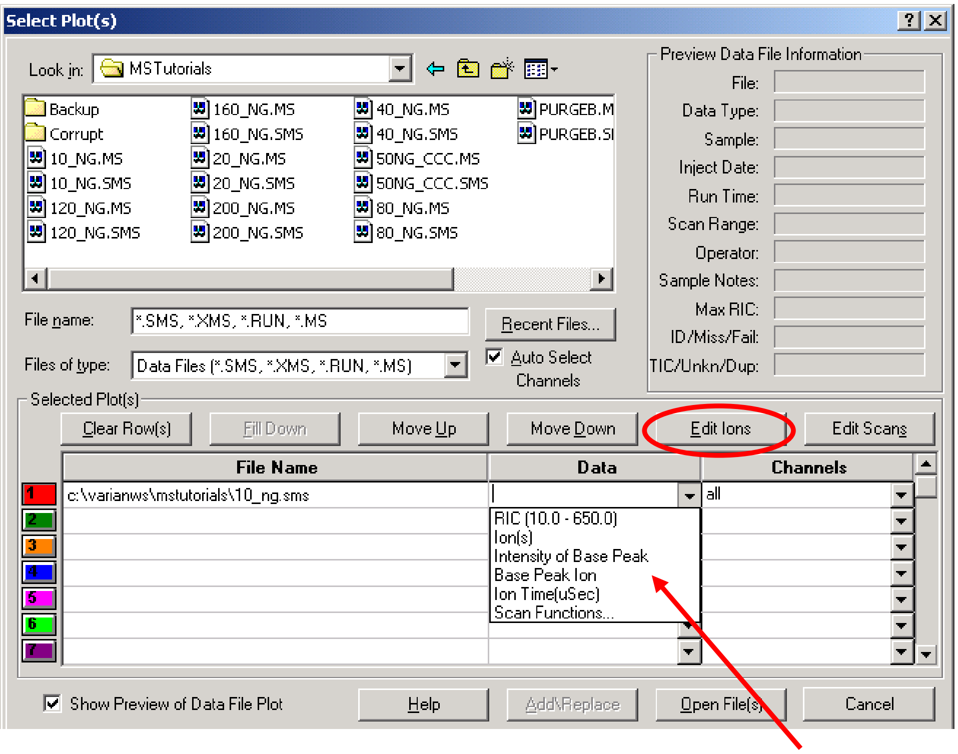
After selecting a file, click **Add\Replace**, then **Open File(s).** The RIC (Reconstructed Ion Current) is displayed if RIC was selected in the Data column. To display chromatograms generated by specific ions, see the topic “How to Display Mass Chromatograms” below.

Opening an MS File from Windows Explorer

Locate data files in either *.ms, *.sms, or *.xms format using Windows Explorer. Double-click on the desired file to open it in MS Data Review. Note that MS Data Review may remain minimized in the Windows Taskbar. When you Restore MS Data Review, the file you selected is displayed. You may also double-click a data file received in an e-mail message to open it in MS Data Review.

Displaying Mass Chromatograms

To display chromatograms generated by specific ions, type the ion(s) m/z values in the *Ions* box located at the lower corner of the chromatogram window, then press **Enter** on the keyboard. When using the command ***File >Select File/Chromatogram..*** in the **MS Data Review** menu or the toolbar button, the following options are available in the Data column besides the RIC value (default):



Look in: MSTutorials

File name: *.SMS, *.XMS, *.RUN, *.MS

Files of type: Data Files (*.SMS, *.XMS, *.RUN, *.MS)

☑ Auto Select Channels

File Name	Data	Channels
c:\varianws\mstutorials\10_ng.sms		all

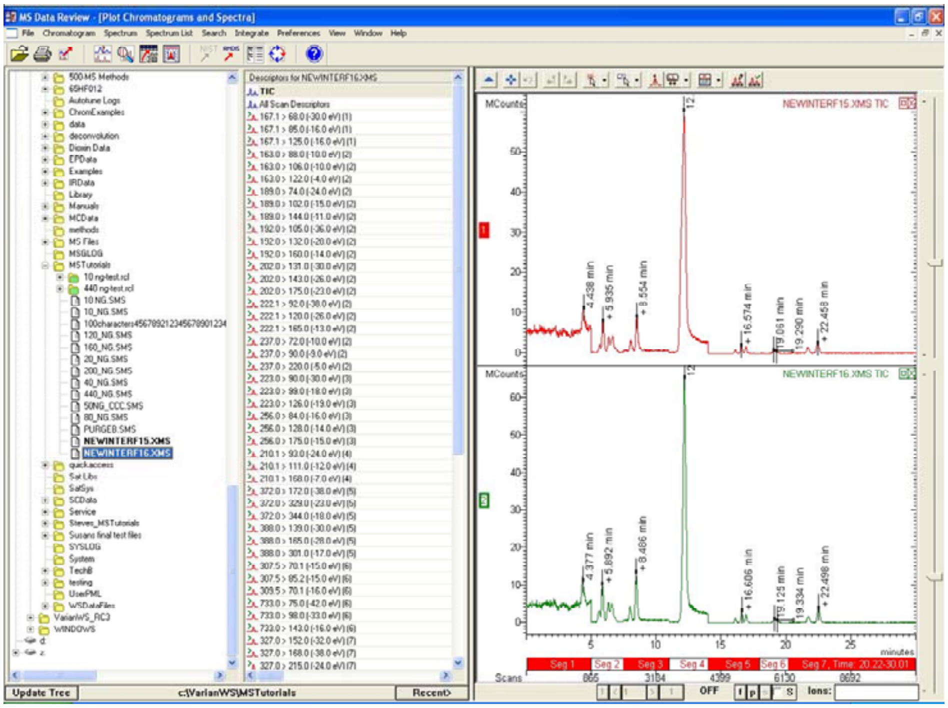
☑ Show Preview of Data File Plot

1. If no file is selected, select a file from the list and click **Add\Replace**.
2. In the Data column for the line corresponding to the desired file, click on the down arrow at the right. This opens the list of options: RIC, Ion(s), etc.
3. Select **Ion(s)** and enter one or several. Repeat the process with other mass combinations, exploring the options described. Note that you can also click on the **Edit Ions** button to change the displayed mass chromatograms.

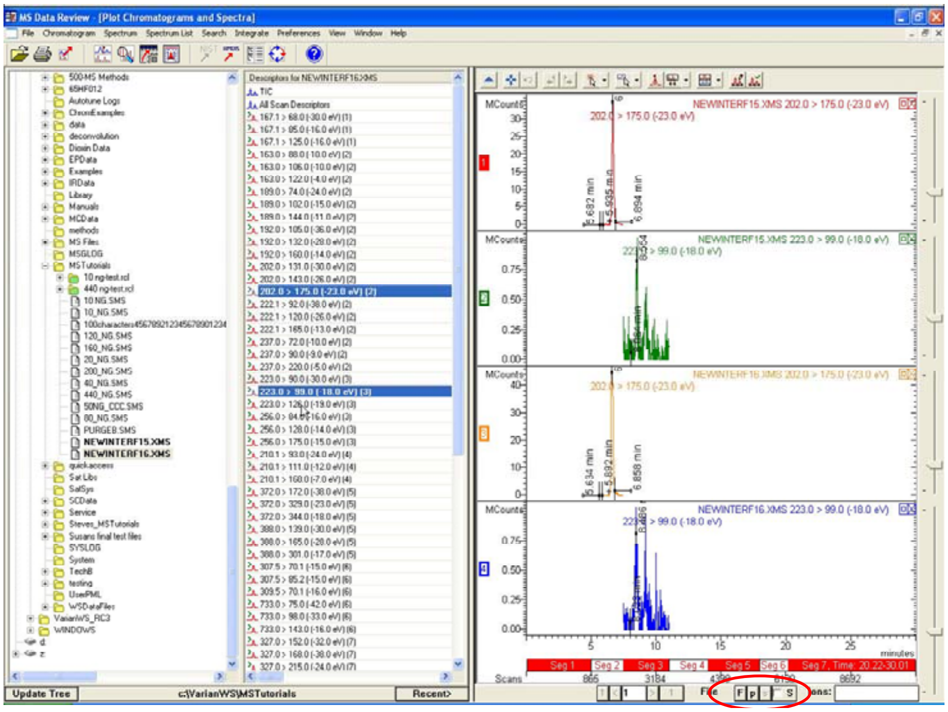
Displaying Multiple Chromatograms

Multiple data file chromatograms can be displayed by selecting the files in the Data File pane while pressing the **Ctrl** key. Multiple chromatograms for different plot descriptors can be displayed by selecting the plot descriptors in the Descriptor Pane while pressing the **Ctrl** key. Selecting plot descriptors in the Descriptor Pane while pressing the **Shift** key will display the chromatograms for all plot descriptors listed between the selected ones.

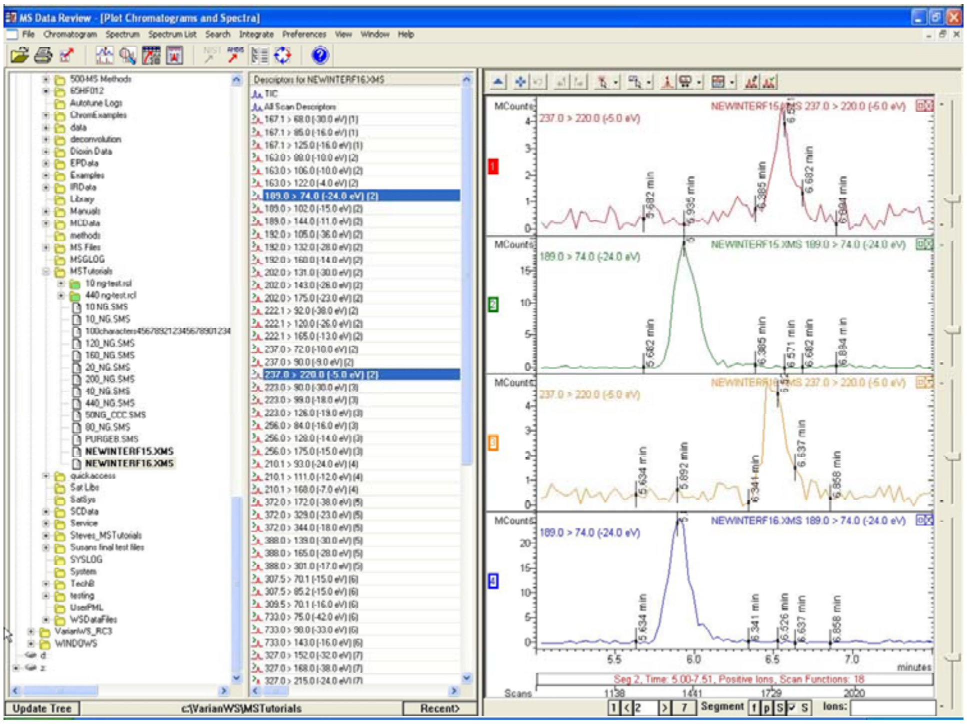
1. Select the NEWINTERF15.XMS file in the C:\VarianWS\MSTutorials directory in the Data File Pane. While pressing the **Ctrl** key select the NEWINTERF16.XMS file in the same directory.



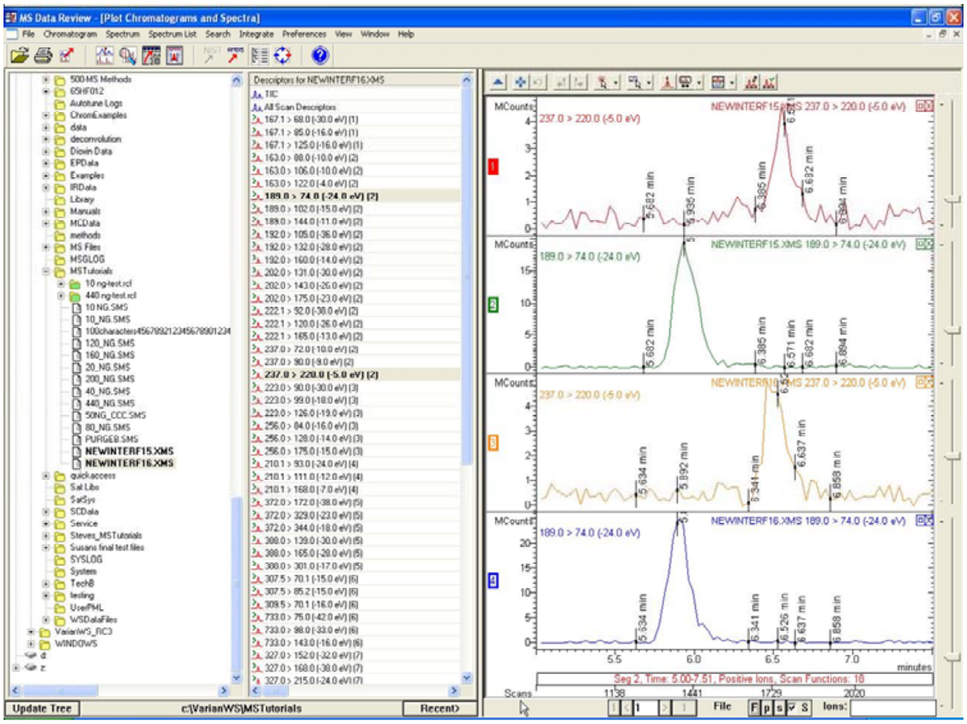
2. While pressing the **Ctrl** key select different descriptors from the Descriptor pane. Chromatograms corresponding to the selected plot descriptors and data files are displayed simultaneously.



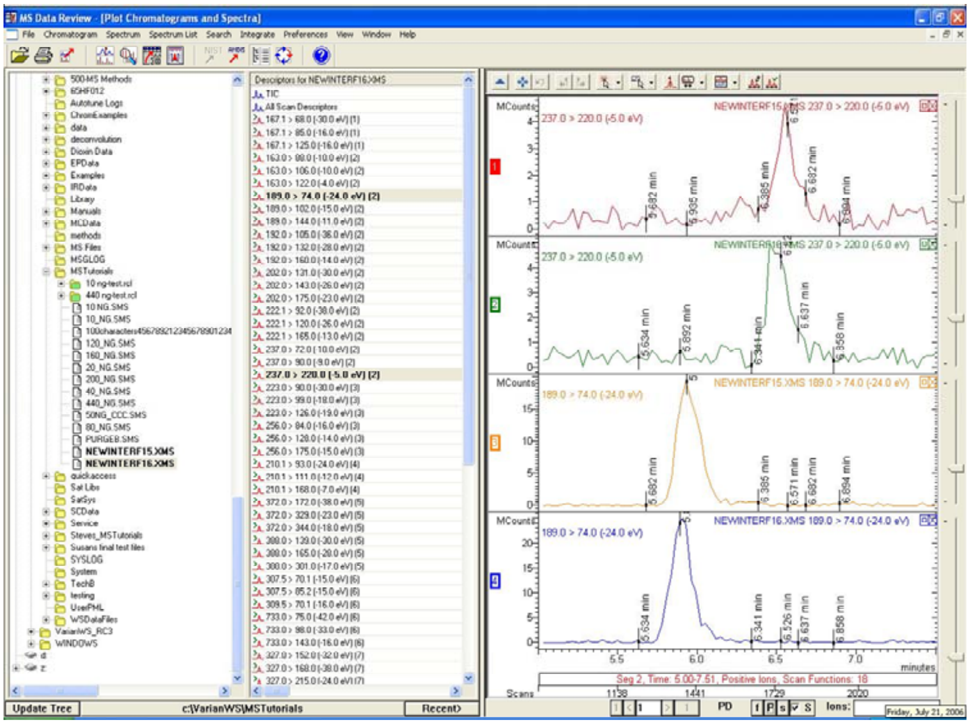
3. Check the box “**S**” at the bottom of the Plot Chromatograms and Spectra window to select the “Segment” mode instead of Full chromatogram. You can display different time segments by clicking the arrows below the chromatogram window.



4. Select the "**f**" button to sort the displayed chromatograms by "Files".



5. Select the "**p**" button to sort the displayed chromatograms by Plot Descriptors.



6. Use the toolbar button in the Plot Chromatograms and Spectra window to switch between Stacked, Overlaid and Normalized Plots. You can also select these options by using the command ***Chromatogram >Set Chromatogram Display*** in the MS Data Review menu.

Alternatively, you can display multiple chromatograms by using the command ***File >Select File/Chromatogram*** in the MS Data Review menu or the toolbar button.

1. Select a file from the list and press the **Add\Replace** button twice to add the same file for two displays. It's preferable to work with the same data files as in the first part of this tutorial (NEWINTERF15.XMS and NEWINTERF16.XMS)
2. Select different ions in the **Data** column and plot descriptors or channels in the **Channels** column for each line. Explore the Plot per Segment option in the **Channels** column.
3. Select a different data file from the list and press **Add\Replace** twice to add another file for multiple displays.
4. Make the same selections in the **Data** and **Channels** columns as in step 2.
5. You can explore the display options again (Segment mode, Sort by File or Plot descriptors, Stacked/Overlaid/Normalized) as described in step 3-5 of the first part of this tutorial.

Getting Spectra from an MS file

In the Chromatogram window, click on a point of interest in the chromatogram. The corresponding spectrum will be displayed in a spectrum window (if the single click action is set to Display Spectrum). You may refine the position of this spectrum by clicking again, or by using the arrow keys or the toolbar buttons to move the pointer tight or left one scan at a time.

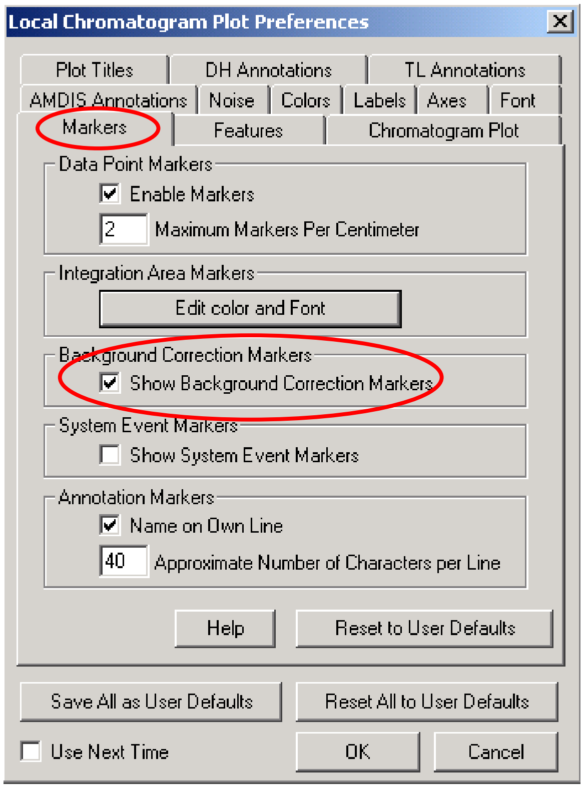
The ***Chromatogram >Set Spectra to Average*** command in the MS data Review menu allows you to display an average of 1 (default), 3, 5, or 7 spectra.

Alternatively, you can use the button in the Chromatogram toolbar to select the number of spectra to be averaged for display.

Spectra may also be background-corrected as described in the next topic, *Performing a Background Correction*.

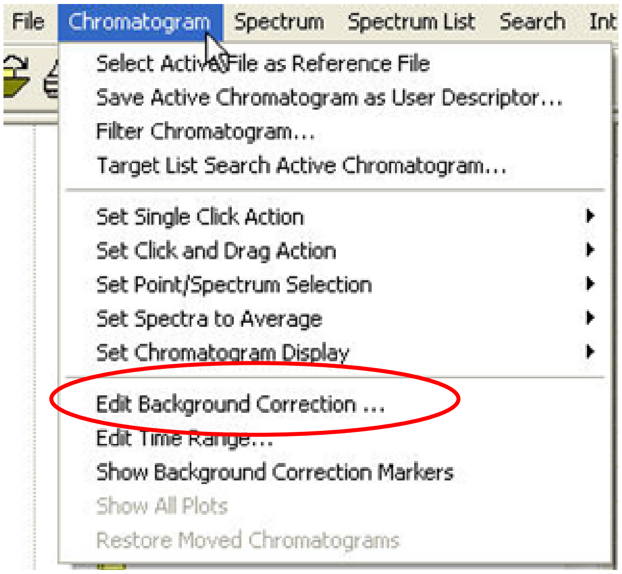
Performing a Background Correction

Load a file of interest and display its TIC or a mass chromatogram. In the ***Chromatogram*** *menu*, verify that the item ***Show Background Correction Markers*** is checked. Alternatively, you may show/hide the background correction markers by using the toolbar button, or by right-clicking anywhere in the chromatogram window, select ***Chromatogram Plot Preferences >Markers*** and check/uncheck **Show Background Correction Markers**.

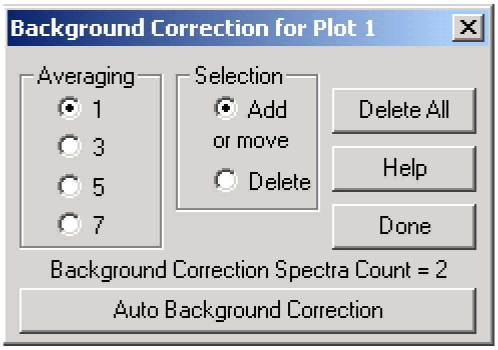


☑ Enable Markers
2 Maximum Markers Per Centimeter
☑ Show Background Correction Markers
☐ Show System Event Markers
☑ Name on Own Line
40 Approximate Number of Characters per Line
☐ Use Next Time

Use the command ***Chromatogram >Edit Background Correction…*** to enter the background selection mode.



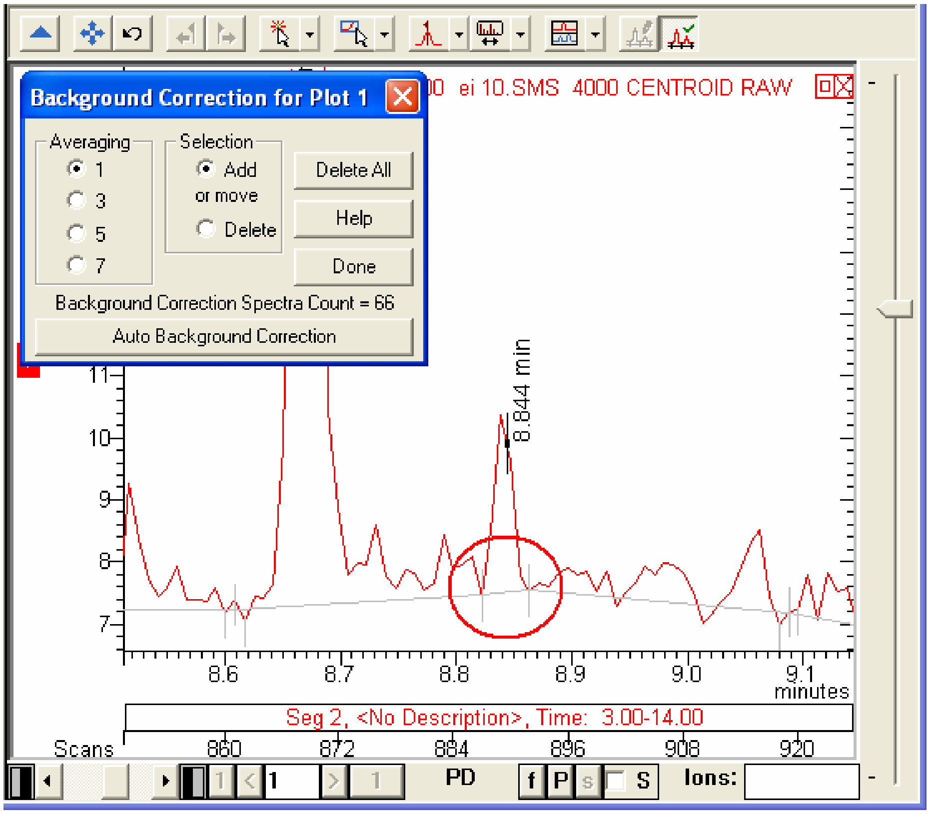
You may also use the toolbar button .



Background Correction Spectra Count = 2

While the Background Correction for Plot 1 dialog is open, if you move the mouse over the chromatogram, the cursor indicates that clicks of the mouse will add background reference points.

Select an isolated peak (you may need to zoom in first) and click on the baseline in front and in the back of the peak of interest.



Background Correction Spectra Count = 66

Markers are added to the plot, indicating the location of the background reference, the number of points averaged, and the baseline level for the plotted abundance. Holding the mouse over one of these points changes the cursor to a hand symbol. Using this “hand tool” you can fine-tune the selection of the background point. After you close the background dialog (using the **Done** button), the reference spectra you have chosen will be used for background-correcting any spectra obtained from this plot.

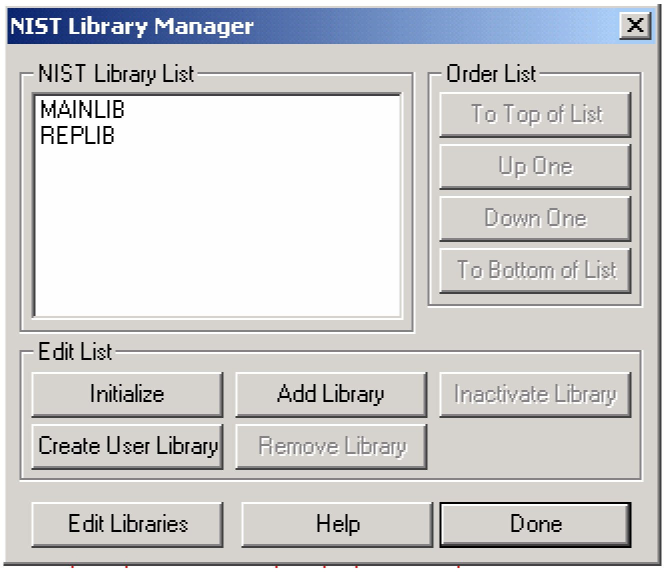
While the Background Correction dialog is displayed, you can delete all the background spectra by using the **Delete All** button, or deleting individual spectra by selecting the **Delete** mode and clicking on the reference spectra to delete.

The ⇧ cursor indicates that clicks of the mouse will delete background reference points. You can also move existing reference spectra by a click and drag operation. This is done by positioning the mouse over the reference point so that the hand tool appears, and then clicking and dragging the point to the desired location.

Performing a NIST Library Search

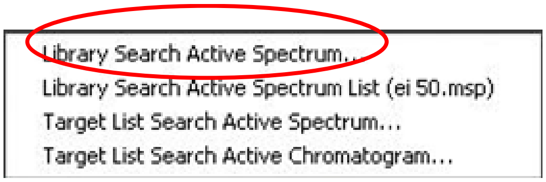
NOTE: This is only available if you own the NIST PC Search program, Version 1.6 or higher.

Select ***Search > Library Manager*** . If no libraries are listed, click **Initialize** and enter the path to your NIST libraries (typically C:\NIST02\MSSEARCH\). This should result in MAINLIB and REPLIB being selected. Close the NIST Library Manager by clicking **Done**.

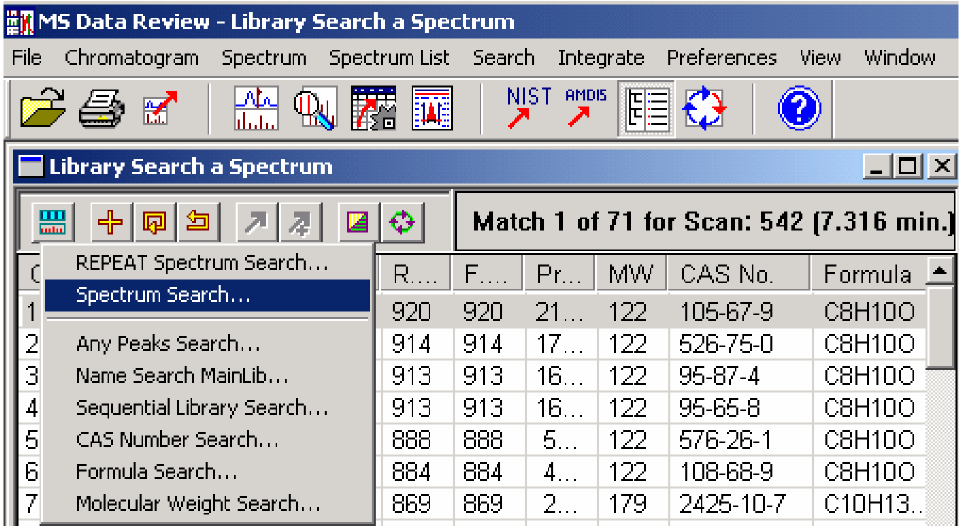


You do not need to repeat the above step for subsequent searches.

After a spectrum of interest is displayed, press the **Search toolbar** button in the MS Data Review window and select **Library Search Active Spectrum**.



The Library Search a Spectrum window displays with a list of matches for the searched spectrum. Press the button and select **Spectrum Search.**

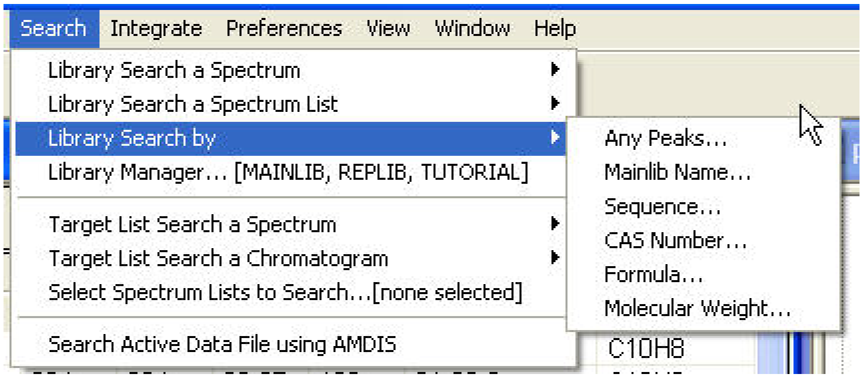


Match 1 of 71 for Scan: 542 (7.316 min.)

C	R....	F....	Pr...	MW	CAS No.	Formula
1	920	920	21...	122	105-67-9	C8H10O
2	914	914	17...	122	526-75-0	C8H10O
3	913	913	16...	122	95-87-4	C8H10O
4	913	913	16...	122	95-65-8	C8H10O
5	888	888	5...	122	576-26-1	C8H10O
6	884	884	4...	122	108-68-9	C8H10O
7	869	869	2...	179	2425-10-7	C10H13..

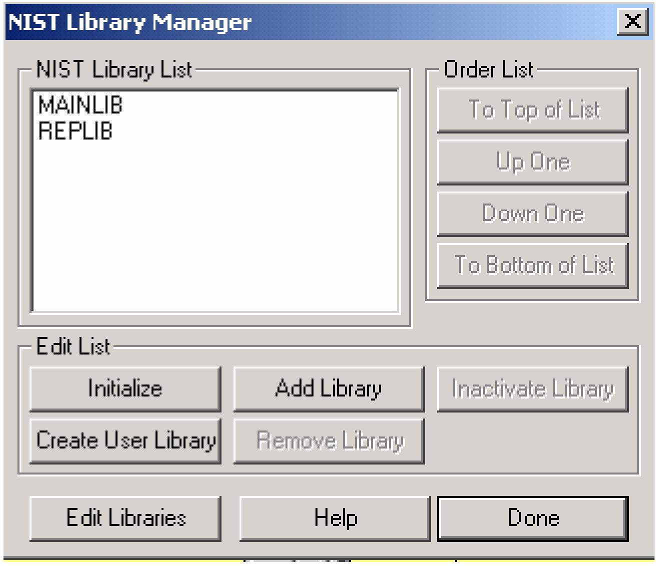
The **NIST Search for Target Spectrum** dialog appears allowing you to change the search parameters and/or edit constraints to better refine your library search.

Other searches (which are not based on a target spectrum) are also available. Select ***Search >Library Search by*** in the MS Data Review menu. The following search options are available:



How to Create or Edit NIST libraries

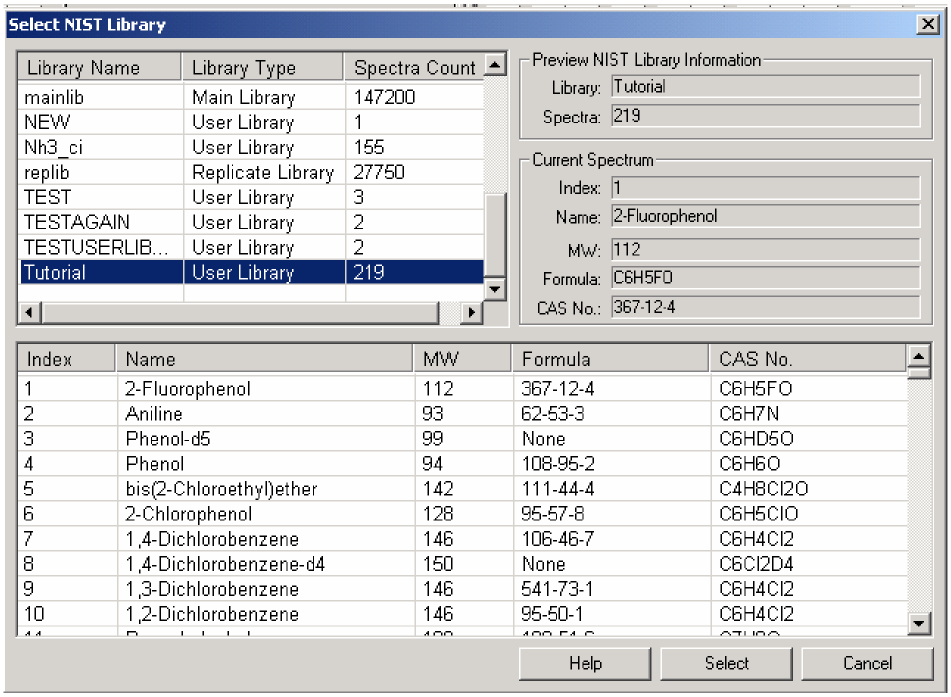
Select ***Search > Library Manager*** in the MS Data Review menu. This selection opens the NIST Library Manager dialog.



MAINLIB
REPLIB

To create a new NIST User Library, press the **Create User Library** button and provide a new name for the library to be created (it is created as a sub-directory of NISTMS).

To select an existing NIST User Library, click **Add Library**. The **Select NIST Library** dialog opens. Click a library name from the list and press the **Select** button.



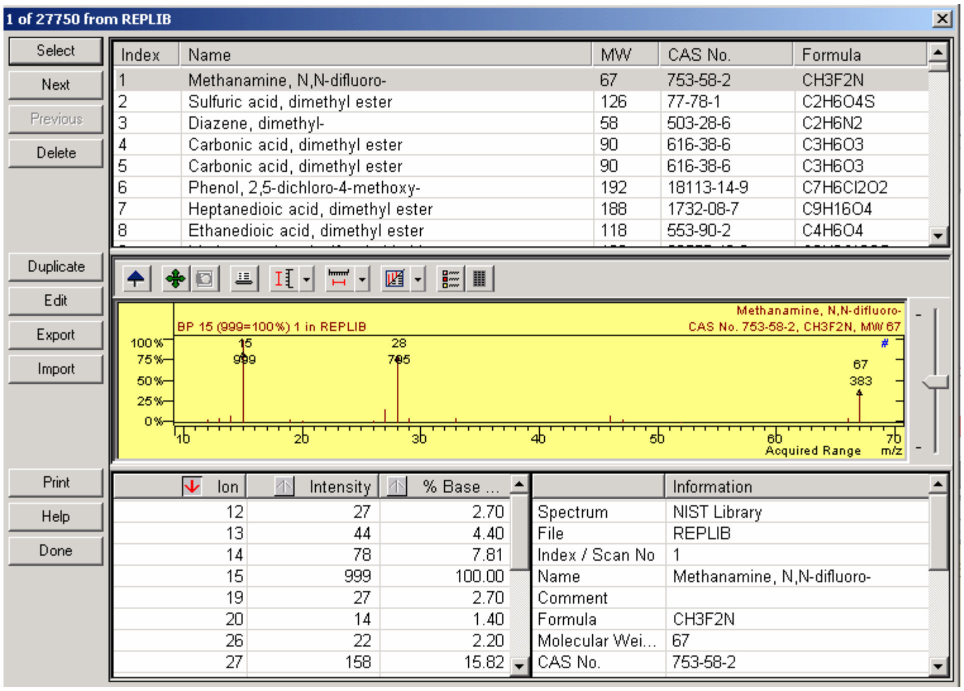
Library Name	Library Type	Spectra Count
mainlib	Main Library	147200
NEW	User Library	1
Nh3_ci	User Library	155
replib	Replicate Library	27750
TEST	User Library	3
TESTAGAIN	User Library	2
TESTUSERLIB...	User Library	2
Tutorial	User Library	219

Library: Tutorial
Spectra: 219

Index: 1
Name: 2-Fluorophenol
MW: 112
Formula: C6H5FO
CAS No.: 367-12-4

Index	Name	MW	Formula	CAS No.
1	2-Fluorophenol	112	367-12-4	C6H5FO
2	Aniline	93	62-53-3	C6H7N
3	Phenol-d5	99	None	C6HD5O
4	Phenol	94	108-95-2	C6H6O
5	bis(2-Chloroethyl)ether	142	111-44-4	C4H8Cl2O
6	2-Chlorophenol	128	95-57-8	C6H5ClO
7	1,4-Dichlorobenzene	146	106-46-7	C6H4Cl2
8	1,4-Dichlorobenzene-d4	150	None	C6Cl2D4
9	1,3-Dichlorobenzene	146	541-73-1	C6H4Cl2
10	1,2-Dichlorobenzene	146	95-50-1	C6H4Cl2

To edit a NIST library, click on the entry in the list and **Edit Libraries**. Click **Select** to choose another library or access a given spectrum in a library. Use **Next** & **Previous** to navigate sequentially the library. Use **Edit** to modify the current spectrum. The spectrum is modified in memory, not in the library. You may put the spectrum back in the same or another library. Use **Delete** to delete the current spectrum. This function is only available in User Libraries. Press **Export >To NIST User Library** to add the current spectrum (modified or not) to a NIST User Library.



Index	Name	MW	CAS No.	Formula
1	Methanamine, N,N-difluoro-	67	753-58-2	CH3F2N
2	Sulfuric acid, dimethyl ester	126	77-78-1	C2H6O4S
3	Diazene, dimethyl-	58	503-28-6	C2H6N2
4	Carbonic acid, dimethyl ester	90	616-38-6	C3H6O3
5	Carbonic acid, dimethyl ester	90	616-38-6	C3H6O3
6	Phenol, 2,5-dichloro-4-methoxy-	192	18113-14-9	C7H6Cl2O2
7	Heptanedioic acid, dimethyl ester	188	1732-08-7	C9H16O4
8	Ethanedioic acid, dimethyl ester	118	553-90-2	C4H6O4

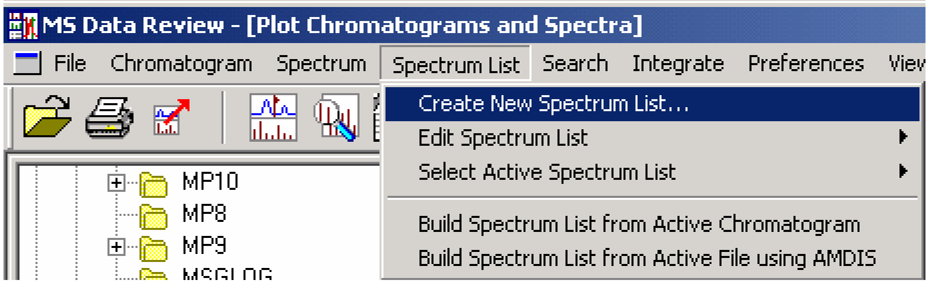
Ion	Intensity	% Base ...
12	27	2.70
13	44	4.40
14	78	7.81
15	999	100.00
19	27	2.70
20	14	1.40
26	22	2.20
27	158	15.82

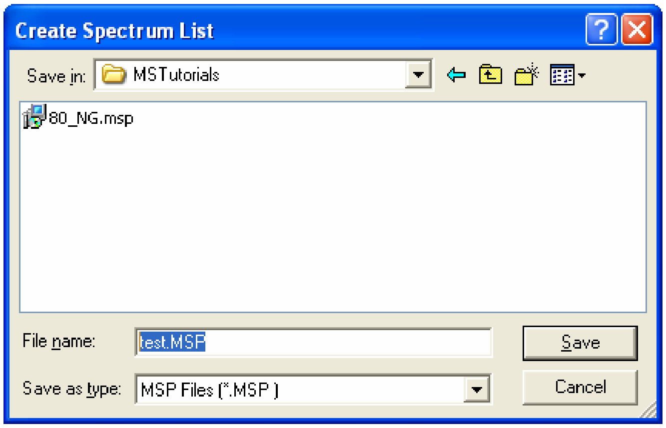
	Information
Spectrum	NIST Library
File	REPLIB
Index / Scan No	1
Name	Methanamine, N,N-difluoro-
Comment	
Formula	CH3F2N
Molecular Wei...	67
CAS No.	753-58-2

Building a Spectrum List

MS Data Review can build lists of spectra in an ASCII format. These lists can be read by the NIST PC Search program. MS Data Review uses Spectrum Lists as an input for searching chromatograms or spectra as well as in the quantitation process.

1. Select ***Spectrum List >Create New Spectrum List***. Type a name and choose a location in the **Create Spectrum List** dialog that is opened and press **Save**. A dialog window is opened that asks if you wish to "Make this file the active file?" Press **Yes**.



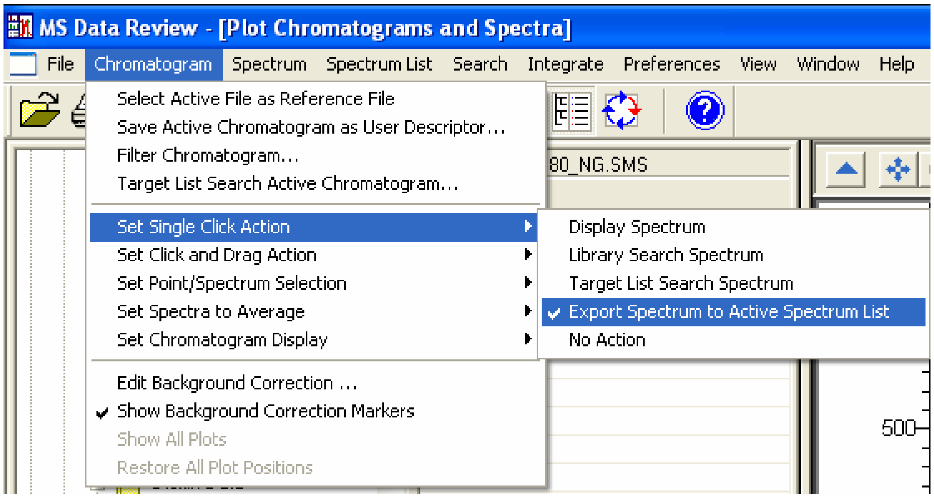


Save in: MSTutorials

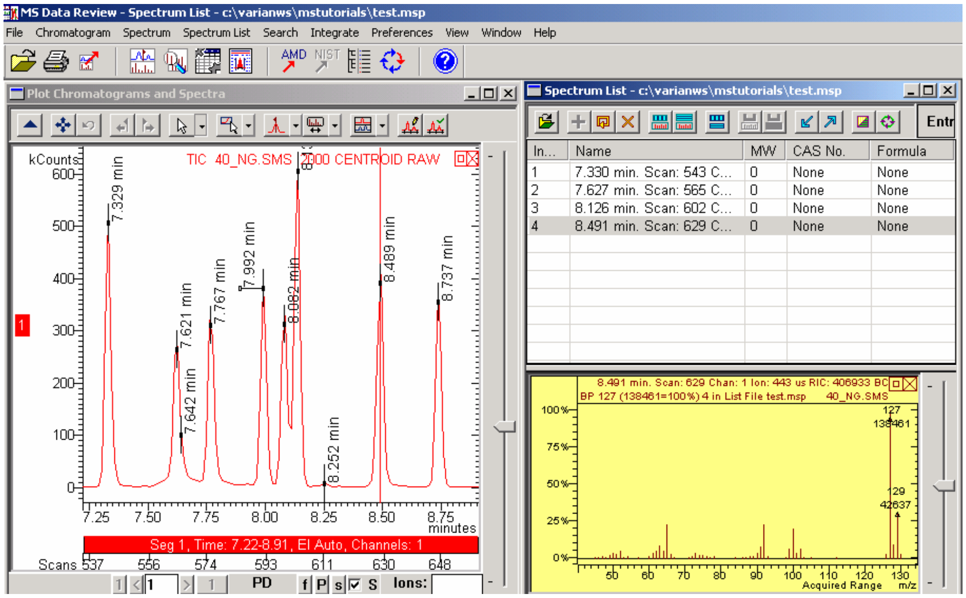
File name: test.MSP

Save as type: MSP Files (*.MSP)

2. In the MS Data Review menu select **Chromatogram >Set Single Click Action >Export Spectrum to Active Spectrum List.**

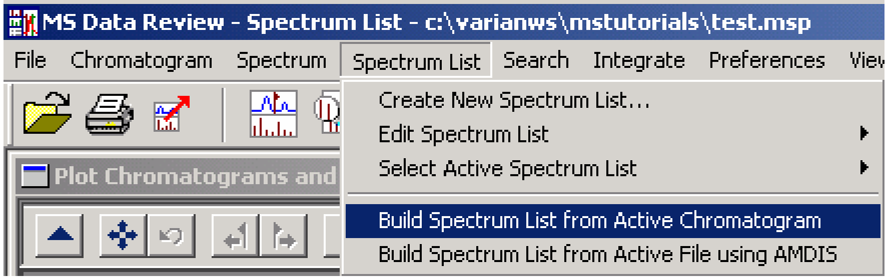


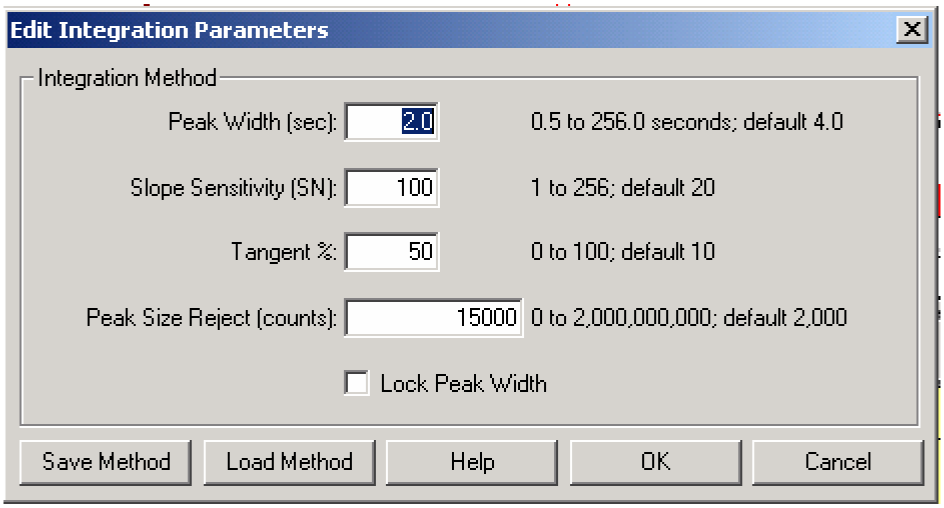
3. Click the scans of interest in the chromatogram window to add the selected spectra to the active spectrum list.



In...	Name	MW	CAS No.	Formula
1	7.330 min. Scan: 543 C...	0	None	None
2	7.627 min. Scan: 565 C...	0	None	None
3	8.126 min. Scan: 602 C...	0	None	None
4	8.491 min. Scan: 629 C...	0	None	None

A spectrum list can also be generated by selecting ***Spectrum List >Build Spectrum List From Active Chromatogram*** in the MS data Review menu. This command opens the **Edit Integration Parameters** dialog.





Peak Width (sec): 2.0 0.5 to 256.0 seconds; default 4.0

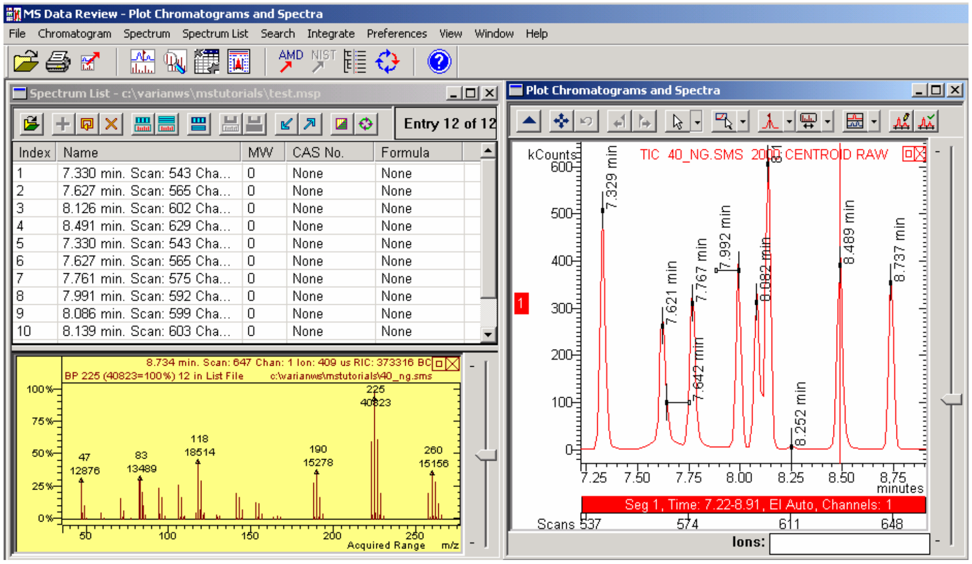
Slope Sensitivity (SN): 100 1 to 256; default 20

Tangent %: 50 0 to 100; default 10

Peak Size Reject (counts): 15000 0 to 2,000,000,000; default 2,000

☐ Lock Peak Width

Press the **Help** button in the **Edit Integration Parameters** window to find the significance of the fields and buttons in this dialog and how are they used to make a spectrum list. Use the default values and press **OK**. The spectrum list is displayed as well as a spectrum window for visualizing each spectrum in the list. Explore the influence of different integration parameters values in the peak detection process performed to build the list.



Index	Name	MW	CAS No.	Formula
1	7.330 min. Scan: 543 Cha...	0	None	None
2	7.627 min. Scan: 565 Cha...	0	None	None
3	8.126 min. Scan: 602 Cha...	0	None	None
4	8.491 min. Scan: 629 Cha...	0	None	None
5	7.330 min. Scan: 543 Cha...	0	None	None
6	7.627 min. Scan: 565 Cha...	0	None	None
7	7.761 min. Scan: 575 Cha...	0	None	None
8	7.991 min. Scan: 592 Cha...	0	None	None
9	8.086 min. Scan: 599 Cha...	0	None	None
10	8.139 min. Scan: 603 Cha...	0	None	None

8.734 min. Scan: 647 Chan: 1 Ion: 409 us RIC: 373316 BC

BP 225 (40823=100%) 12 in List File c:\varianws\mstutorials\40_ng.sms

Seg 1, Time: 7.22-8.91, EI Auto, Channels: 1

Hold the cursor over any button in the Spectrum List toolbar to display a description..

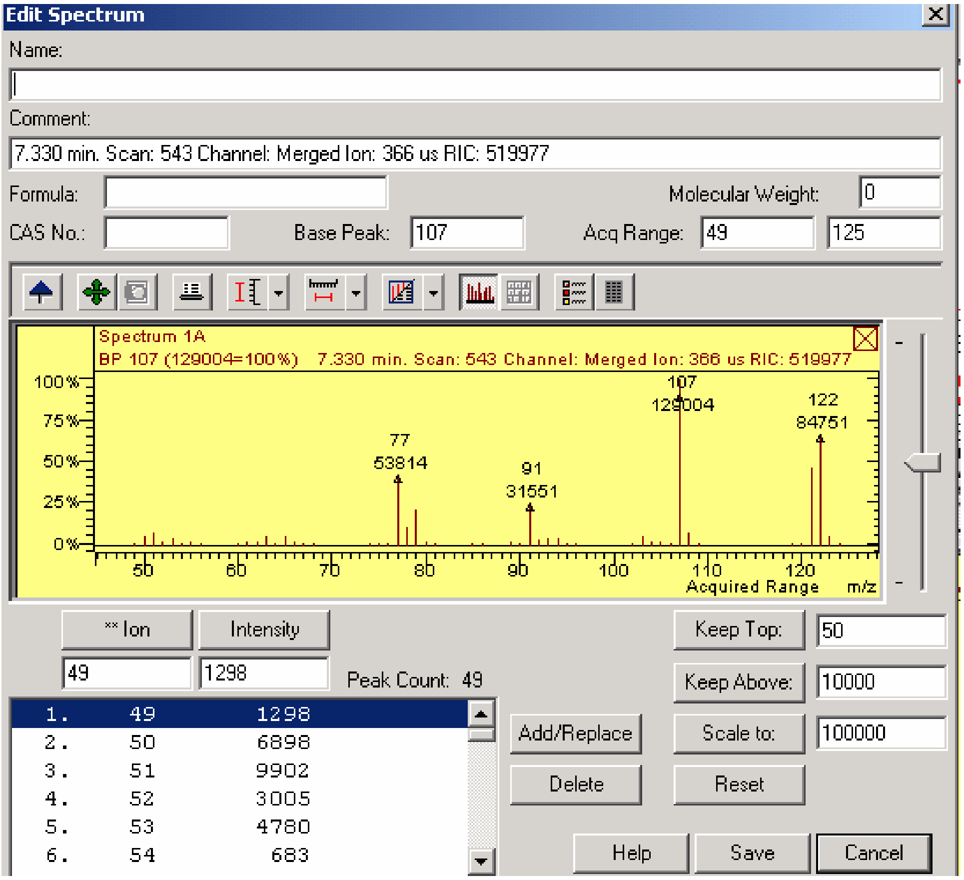
Select the **Library Search Spectrum List** button and then save the search results by pressing the **Update all Searches with Matches** button .

You can add spectra by using the Add Spectrum button and you can delete spectra by using the **Delete Spectrum** button .

Editing Spectra

Select a peak in the Chromatogram window so that a mass spectrum is shown in the Spectrum display. Use the menu command ***Spectrum >Edit Active Spectrum.***

This opens the **Edit Spectrum** dialog.



Edit Spectrum

Name:

Comment: 7.330 min. Scan: 543 Channel: Merged Ion: 366 us RIC: 519977

Formula:

Molecular Weight: 0

CAS No.:

Base Peak: 107

Acq Range: 49 125

Ion: 49

Intensity: 1298

Peak Count: 49

Keep Top: 50

Keep Above: 10000

Scale to: 100000

1.	49	1298
2.	50	6898
3.	51	9902
4.	52	3005
5.	53	4780
6.	54	683

To edit an attribute (name, comment, formula, molecular weight, CAS number, Base Peak), enter the information in the desired field.

To edit mass-intensity pairs, use the **Add\Replace** and **Delete** buttons.

Press **Save** to accept the changes or **Cancel** to reject them.

The changes are now reflected in the Spectrum window.

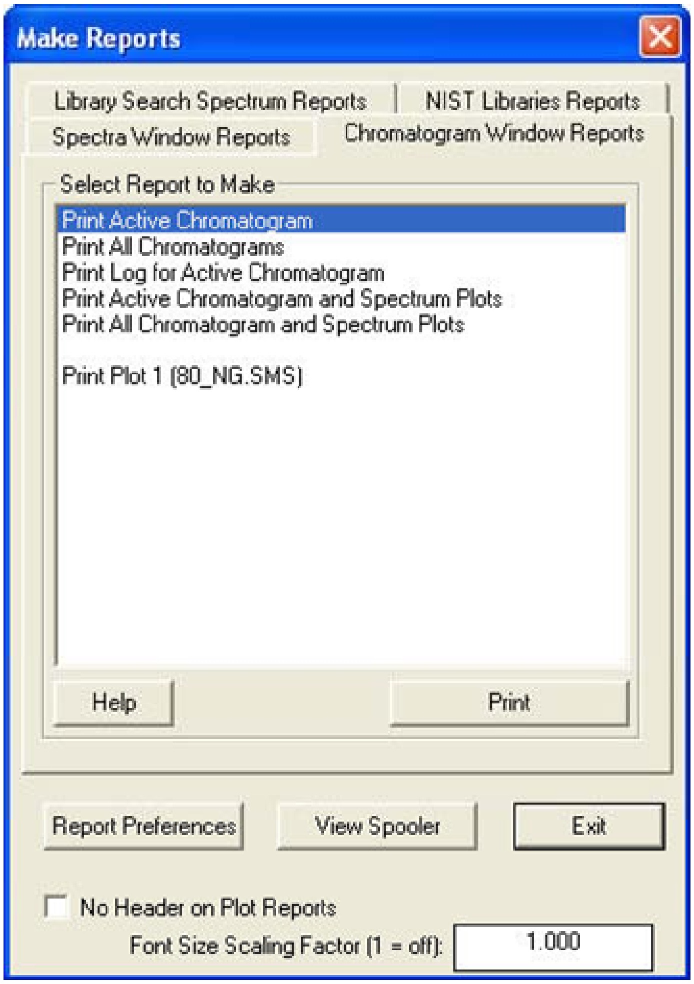
NOTE: Making a change to the ion intensity pairs will flag the spectrum as edited with a '(E)' designation in the spectrum header.

The edited spectrum may now be exported to NIST User Libraries, to a Spectrum List or text file.

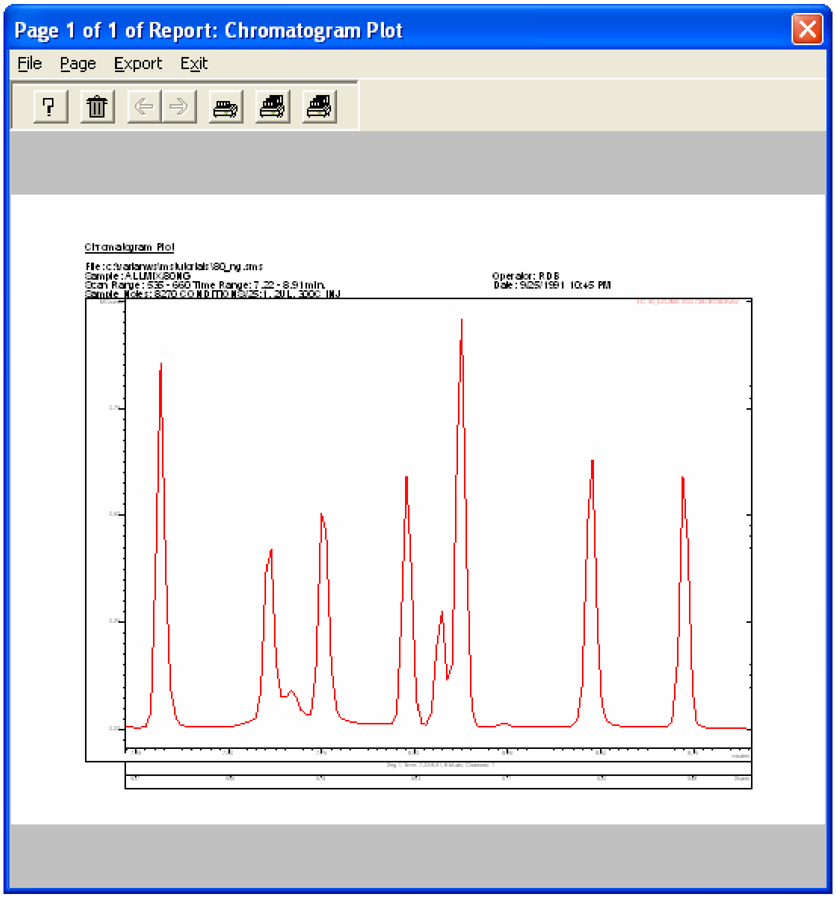
Printing Pictures

Print commands can be issued from the file menu and many windows.

1. Display a spectrum by clicking on a scan of interest in the chromatogram window.
2. In the **File** menu of MS Data Review window select **Preview Reports**.
3. In the MS Data Review menu select ***File >Print…*** .This command opens the Make Reports dialog. Select **Print Active Chromatogram** in the list and click **Print**.



The printout is shown in a preview window that allows you to print, delete or export the page.



Chromatogram Plot

File: c:\varianws\ms\tutorials\80_ng.sms
Sample: ALLMIX80NG
Scan Range: 535 - 660 Time Range: 7.22 - 8.91 min.
Sample Notes: 8270 CONDITIONS/25:1, 2UL, 300C INJ

Operator: RDB
Date: 9/25/1991 10:45 PM

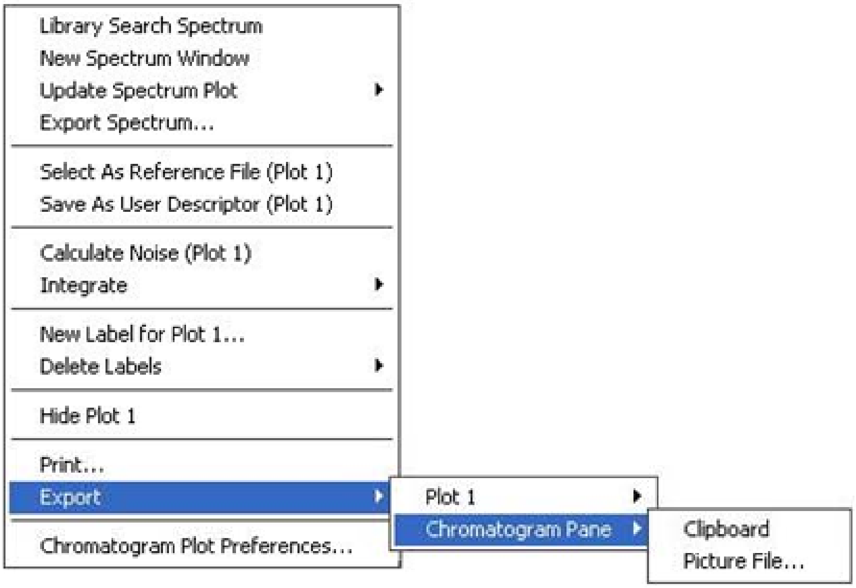
To close the Preview window, click the close box (**X**) in the upper right corner, or click the **Exit** command in the menu.

If **Preview Reports** and **Send Reports to Spooler File** are both unchecked, the Print commands sends the files to the printer.

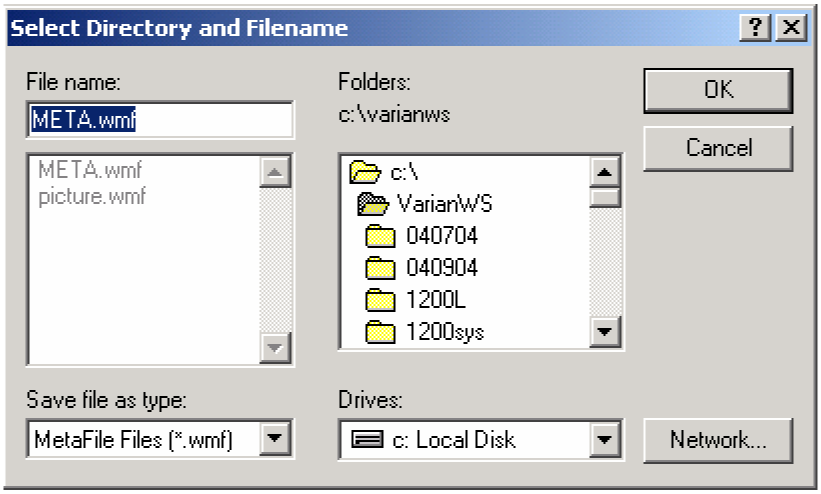
You can also choose to print the active chromatogram and spectrum plot by right-clicking in the chromatogram/spectrum window.

Exporting Pictures

You can exporting to the Clipboard or Picture File to import plots and spectra into third-party word processing and graphics programs. Right click in the chromatogram window and select **Export >Chromatogram Pane >Picture File**



Select a Directory and Filename for the picture you want to export. Click **OK** after making the name and directory selection.



File name: META.wmf

META.wmf
picture.wmf

Folders: c:\varianws

c:\
VarianWS
040704
040904
1200L
1200sys

Save file as type: MetaFile Files (*.wmf)

Drives: c: Local Disk

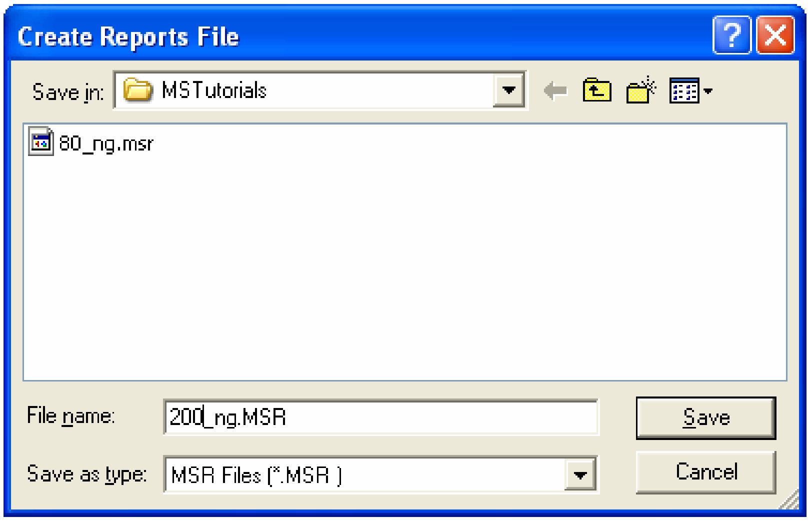
Insert the picture in a Microsoft Word™ document using the **Insert >Picture >From File** The Picture format (vector graphics) leads to much better graphics than the Bitmap format (raster graphics).

Creating and Using a Spooler File

In addition to printing individual reports from Chromatogram, Spectrum, Library Search, and other windows in MS Data Review, the user may also create master files called Spooler Files (also referred to as Reports Files). A Spooler File is a collection of printouts from various screens within the MS Data Review application. Files may contain copies of any screen, which may be printed from MS Data Review.

NOTE: Reports are automatically added to the active Spooler File when the ***Send Reports to Spooler File*** is checked in the MS Data Review **File** menu AND the user selects a print command from the MS Data Review File menu. You may add reports to the Spooler File without printing by simply clicking **Exit** in the Active Spooler File screen (opened with the command ***File >View Spooler File*** in the **MS Data Review** menu). You may stop the automatic additions to the Spooler File by deselecting the ***File >Send Reports to Spooler File*** command.

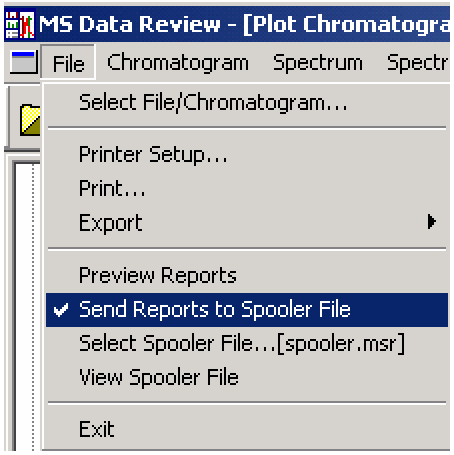
1. To create a Spooler File, open a data file in MS Data Review. Use the command ***File >Select Spooler File*** in the MS Data Review menu. You will be prompted to choose a name for the new Spooler File or to open an existing one.



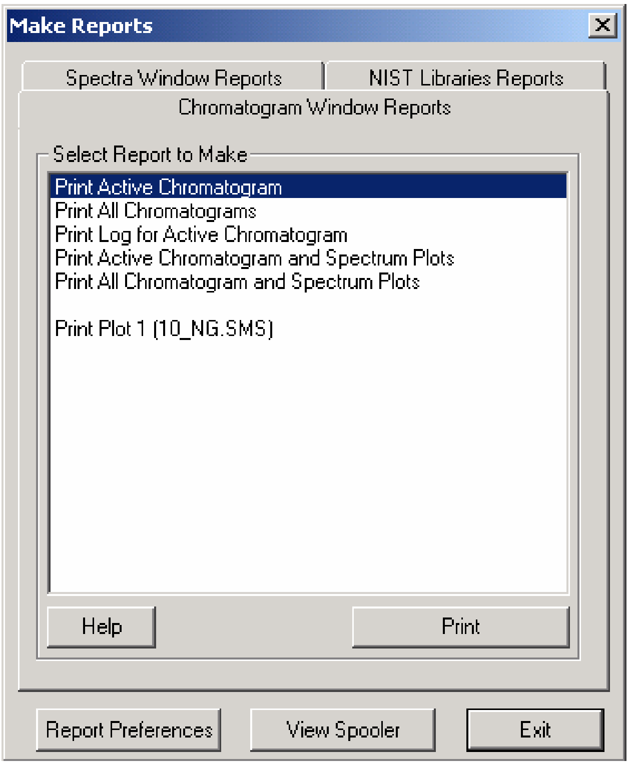
File name: 200_ng.MSR

Save as type: MSR Files (*.MSR)

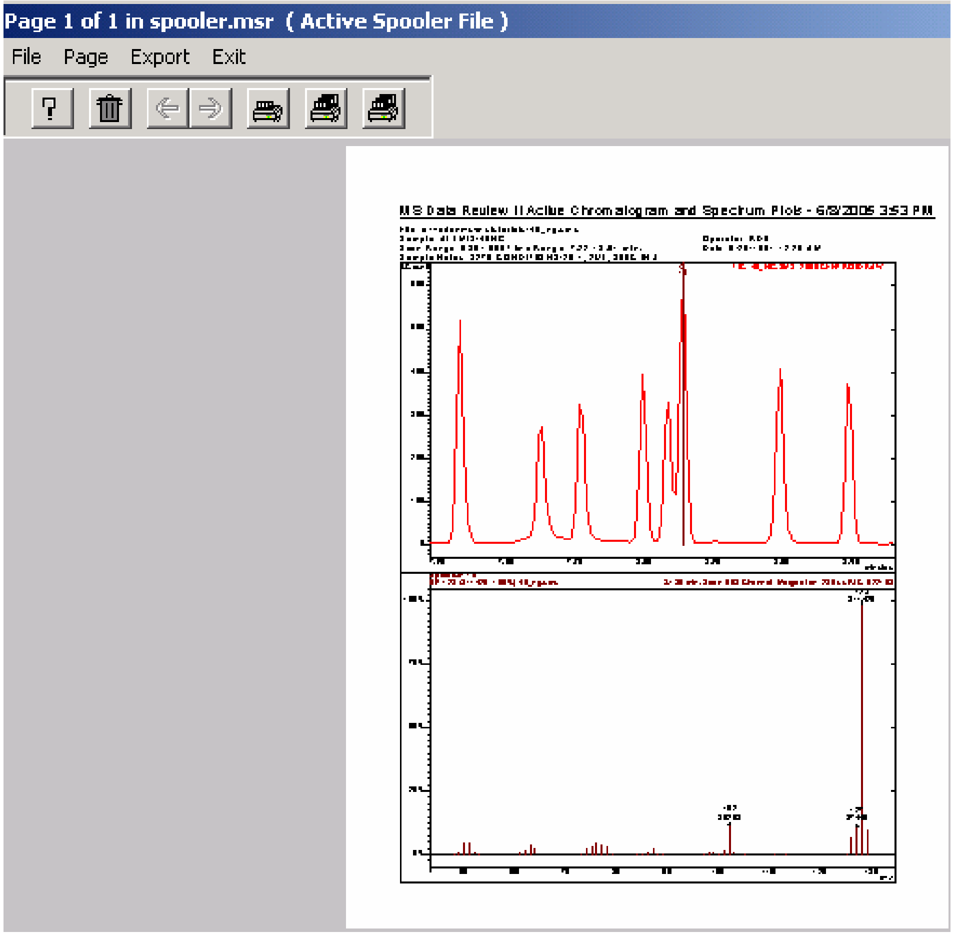
2. Select **File >Send Reports to Spooler File**.



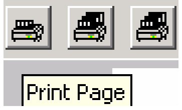
3. Select a spectrum from the active chromatogram. Use the menu commands ***File >Print*** to add entries to this Spooler File. In the **Make Reports** window select **Print Active Chromatogram and Spectrum Plots** and click **Print**.



4. To review the Spooler File contents, use the menu command ***File >View Spooler File***. The last report added to the Spooler File is always shown when the ***View Spooler File*** option is chosen. Use the Next/Previous Page menu commands to navigate the Spooler File. You can click and drag within the page to get a closer view of details.



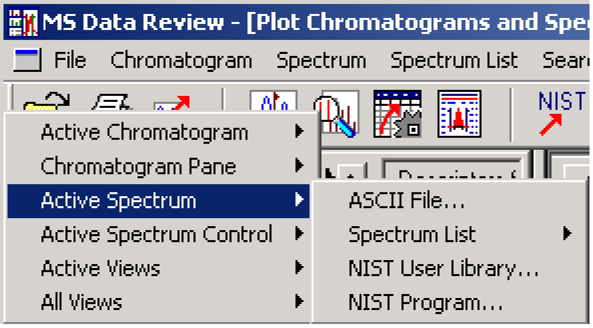
5. If you want to add reports to the Spooler File without printing any of the pages, click **Exit** in the **Active Spooler File** menu.
6. You can print the current page, the current report or the all reports by using the print icons in the toolbar.



Exporting Spectra

Click on a point of interest in the chromatogram plot to display a spectrum.

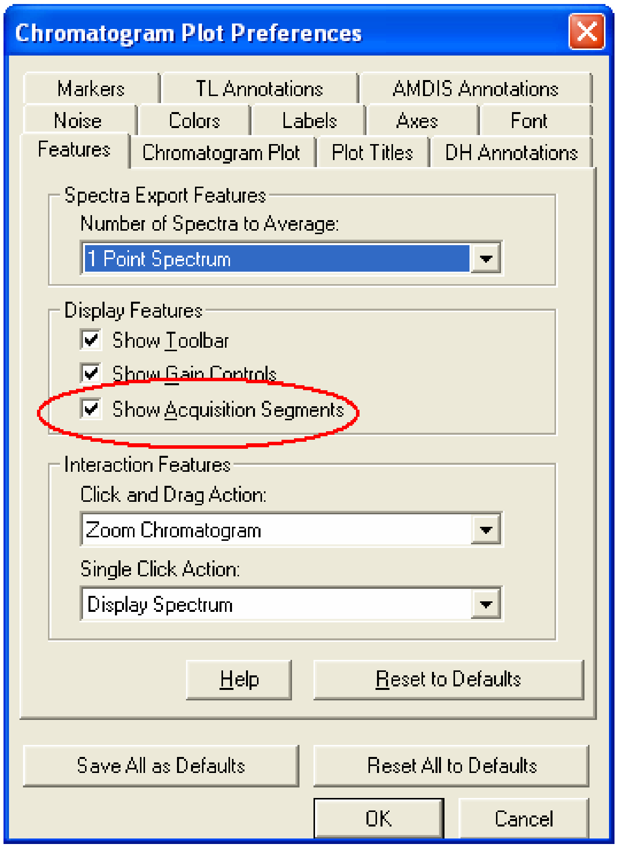
Click on the **Export Chromatograms and Spectra** button in the MS Data Review toolbar and select **Active Spectrum**. You can then make the export destination selection.



Displaying and Printing Log Information

The Log is the section of the data file, which documents the instrument hardware and MS acquisition parameters. This is where you can find out exactly how the data file was acquired. The log information can also be viewed in the data acquisition method used to acquire the file.

In order to view the Log from a plot in MS Data Review, the acquisition segment information needs to be showing on the bottom of the plot window. This is enabled by default, but if it is not showing you will need to right click anywhere in the chromatogram plot and choose the **Chromatogram Plot Preferences**, make sure that the **Show Acquisition Segments** item is checked.



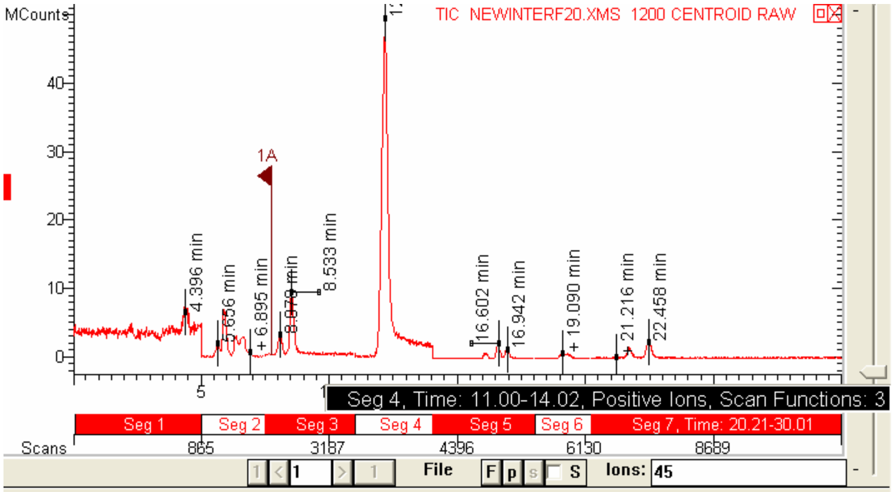
Spectra Export Features
Number of Spectra to Average: 1 Point Spectrum

Display Features
☑ Show Toolbar
☑ Show Gain Controls
☑ Show Acquisition Segments

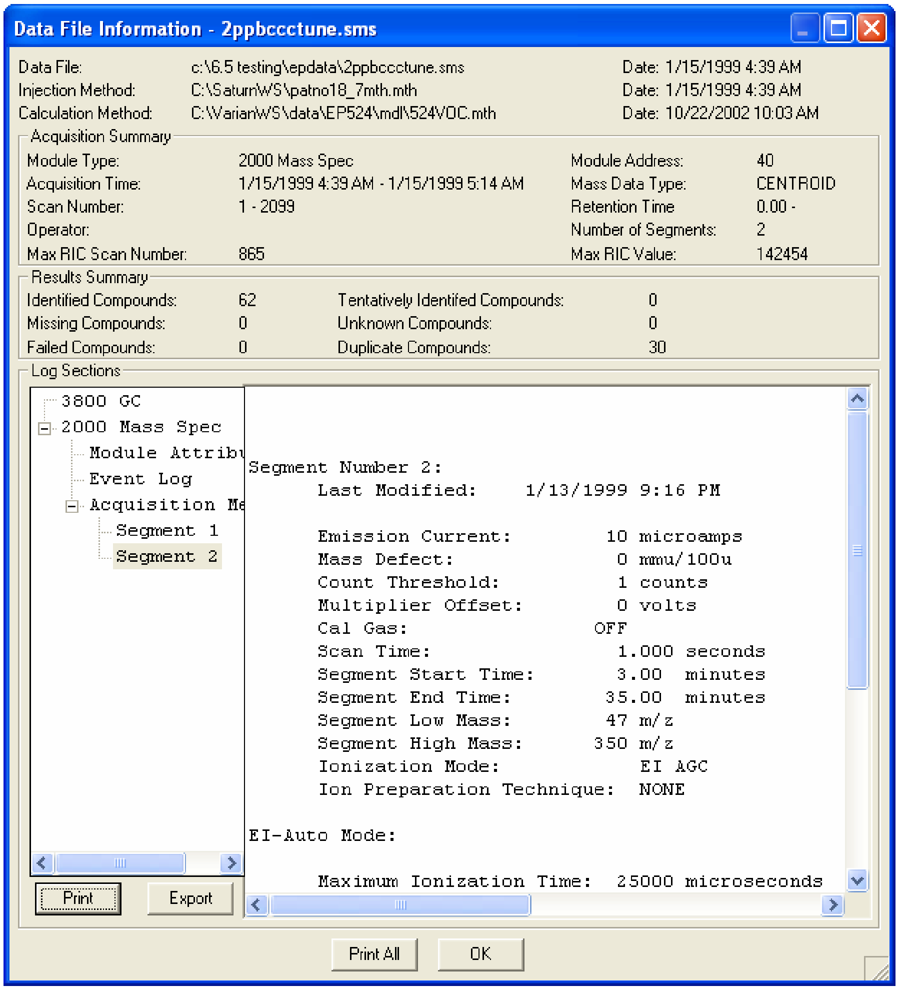
Interaction Features
Click and Drag Action: Zoom Chromatogram
Single Click Action: Display Spectrum

A graphical representation of the acquisition segments is displayed at the bottom of the Chromatogram window.

Place the cursor on this control (the cursor will add a “Log” label), to display log components for the relevant segment.



If multiple plots are displayed, the Segments control refers only to the active plot. Click on or below the segment bar in the chromatogram plot. The **Data File Information** window will be opened.



Data File: c:\6.5 testing\epdata\2ppbccctune.sms Date: 1/15/1999 4:39 AM
Injection Method: C:\SaturnWS\patno18_7mth.mth Date: 1/15/1999 4:39 AM
Calculation Method: C:\VarianWS\data\EP524\mdl\524VOC.mth Date: 10/22/2002 10:03 AM

Acquisition Summary

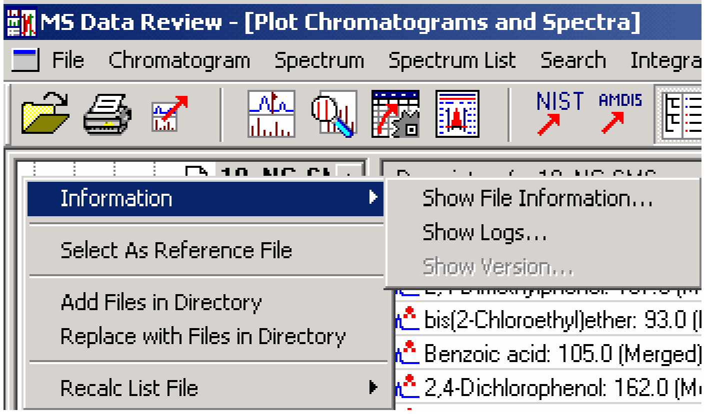
Module Type:	2000 Mass Spec	Module Address:	40
Acquisition Time:	1/15/1999 4:39 AM - 1/15/1999 5:14 AM	Mass Data Type:	CENTROID
Scan Number:	1 - 2099	Retention Time	0.00 -
Operator:		Number of Segments:	2
Max RIC Scan Number:	865	Max RIC Value:	142454

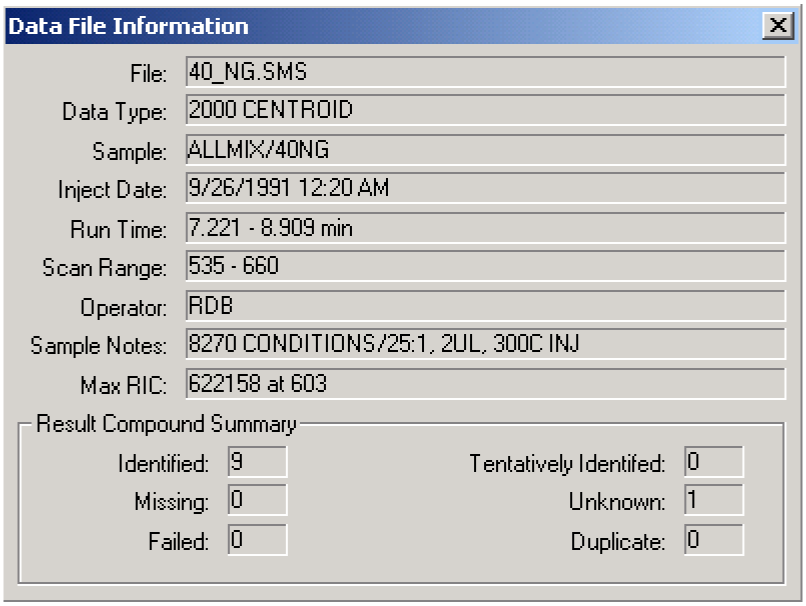
Results Summary

Identified Compounds:	62	Tentatively Identifed Compounds:	0
Missing Compounds:	0	Unknown Compounds:	0
Failed Compounds:	0	Duplicate Compounds:	30

```
Segment Number 2:
      Last Modified:     1/13/1999 9:16 PM

      Emission Current:          10 microamps
      Mass Defect:                0 mmu/100u
      Count Threshold:            1 counts
      Multiplier Offset:          0 volts
      Cal Gas:                  OFF
      Scan Time:                  1.000 seconds
      Segment Start Time:         3.00  minutes
      Segment End Time:          35.00  minutes
      Segment Low Mass:          47 m/z
      Segment High Mass:        350 m/z
      Ionization Mode:              EI AGC
      Ion Preparation Technique:    NONE

EI-Auto Mode:

      Maximum Ionization Time:   25000 microseconds
```

You can also obtain file information by right-clicking on a file name in the Data File Pane and selecting ***Information >Show File Information***.




Data File Information

File: 40_NG.SMS
Data Type: 2000 CENTROID
Sample: ALLMIX/40NG
Inject Date: 9/26/1991 12:20 AM
Run Time: 7.221 - 8.909 min
Scan Range: 535 - 660
Operator: RDB
Sample Notes: 8270 CONDITIONS/25:1, 2UL, 300C INJ
Max RIC: 622158 at 603

Result Compound Summary

Identified: 9
Missing: 0
Failed: 0
Tentatively Identifed: 0
Unknown: 1
Duplicate: 0

Acquiring a 2000 MS Data File

Introduction

This tutorial will familiarize you with the basic operation of the 2000 GC/MS system, including the Varian MS Workstation environment, instrument setup and tuning, GC/MS method building, and acquiring a data file. The GC column used for this tutorial is a fused-silica 30m x 0.25mm ID, 0.25um phase coating of CP-Sil 8 CB (Varian part number CP8751). The VF-5 MS factorFOUR column will give similar results (Varian part number CP8944).The sample used in this tutorial is the Column Test Mix (Varian part number 03-920273-00).

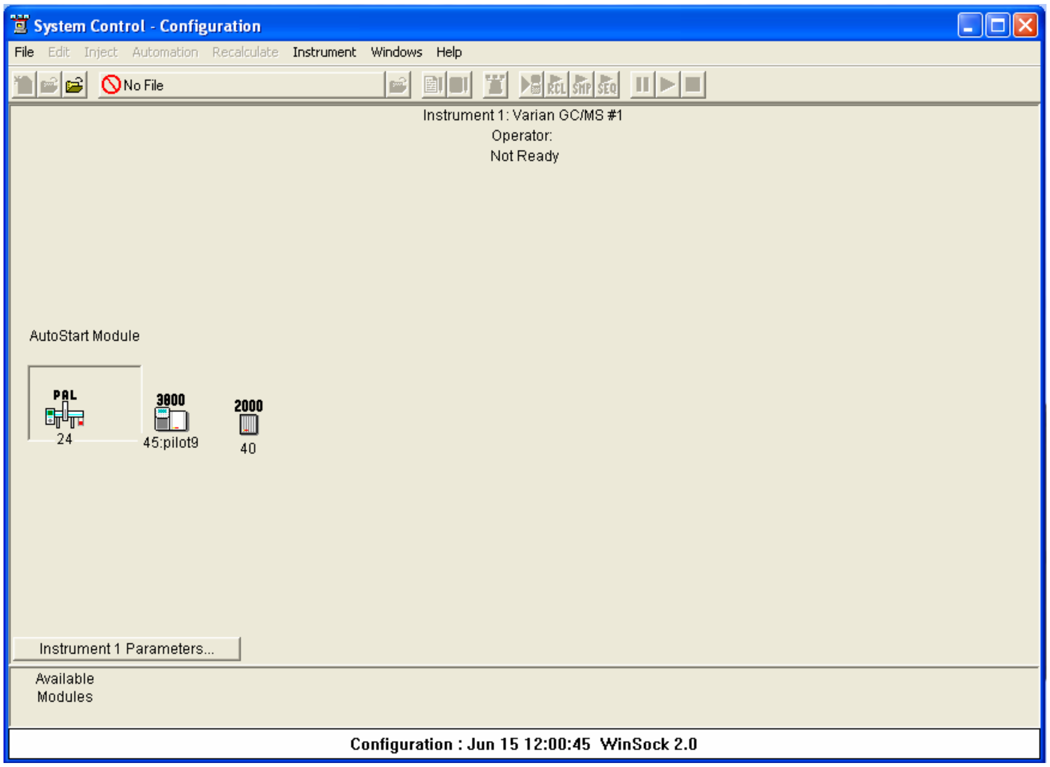
Starting the Varian MS Workstation

To set up the 2000 for acquiring a sample, you should first enter System Control and tune the mass spectrometer. Your Varian MS Workstation software has probably been installed so that the Workstation Toolbar appears at the top of the screen. The Workstation Toolbar is a set of shortcuts to help you navigate the Varian MS Workstation.

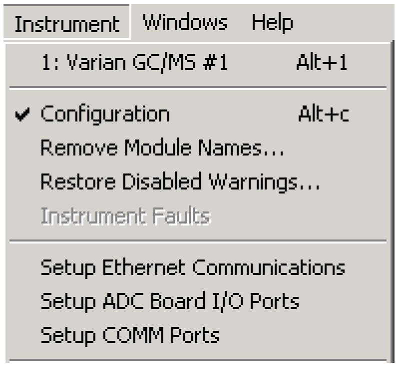
If the Toolbar is not present, open it from the following location: ***Start >Programs >MS Workstation >Workstation Toolbar***. Hold the mouse cursor over each of the buttons in the Workstation Toolbar to display a tool-tip description. You can find more options and complete descriptions in the Help on Star Toolbar accessed by right-clicking in the free area on the right side of the Workstation Toolbar. Click on the **System Control** button on the far left to enter System Control. You may wish to choose the option **Small Buttons on Toolbar** so that the Workstation Toolbar will occupy less space on the screen.

Setting Instrument Configuration for Manual Injections

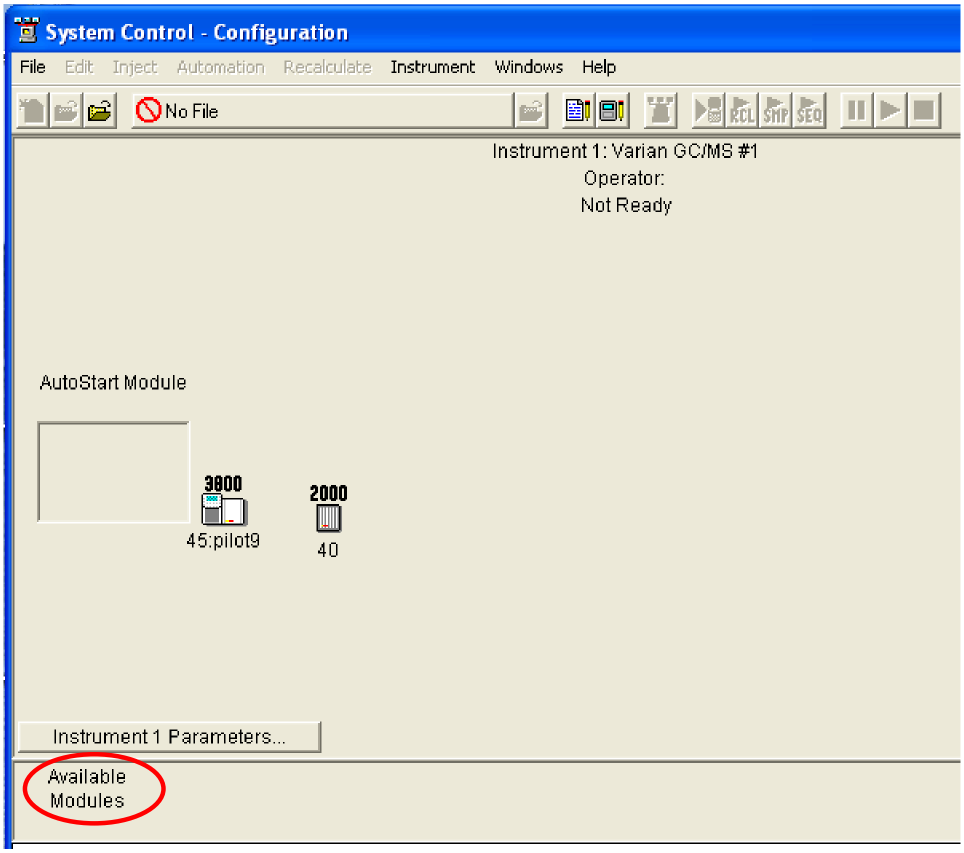
After System Control is started, a new window opens. The components of the System Control window vary depending on the mode of operation. The System Control window title bar (the blue area at the top of the window) should read System Control - Configuration.



If this is not the active window, click ***Instrument >Configuration*** to open the Configuration window.

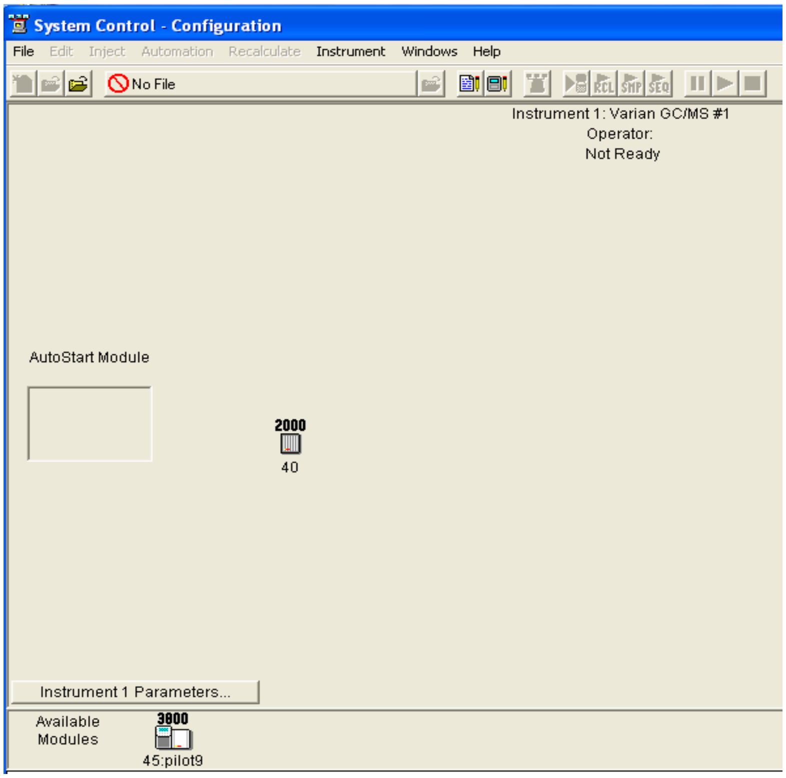


The available instrument modules are shown at the bottom of the System Control Configuration window. The Configuration window shown above indicates that a 3800 GC, a 2000 MS, and a Combi PAL AutoSampler are installed because their Module icons can be observed in the window. For this tutorial we wish to perform manual injections, therefore if you are using a Combi PAL or CP-8400/8410 AutoSampler, you will need to choose the manual injection selection in the Inject Single Sample Dialogue or the Sample List dialogue. This selection is under the Injector Mode selection of the respective tables and is labeled “Manual Injection” or “Manual”. If you are using a 8200 AutoSampler, to configure the instrument for manual injections, use the mouse to click on the 8200 Module (if it is present on your instrument) and drag it into the Available Modules field at the bottom of the System Control window.



Running an MS Method without a GC Method

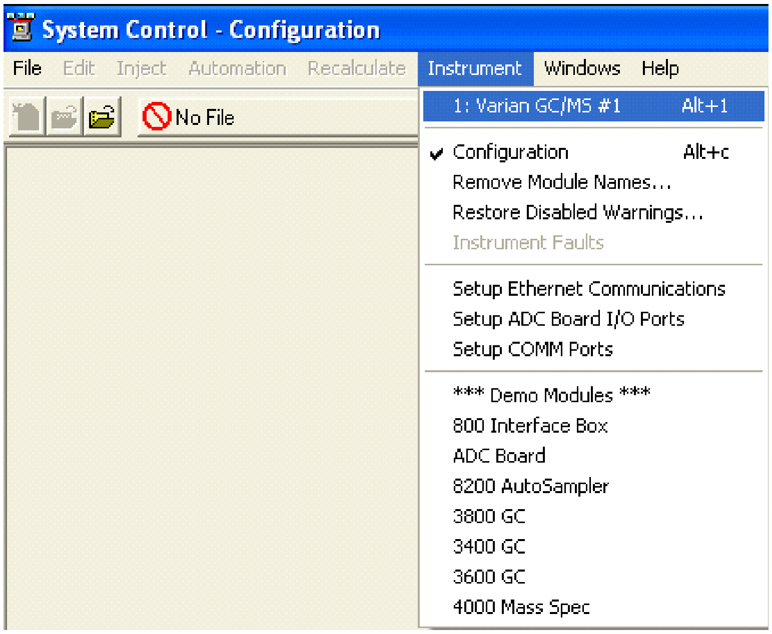
To run an MS method without the GC module or GC method, highlight **Configuration** under the Instrument menu and click the GC Module icon and drag it from mid screen to below the bar labeled **Available Modules.**



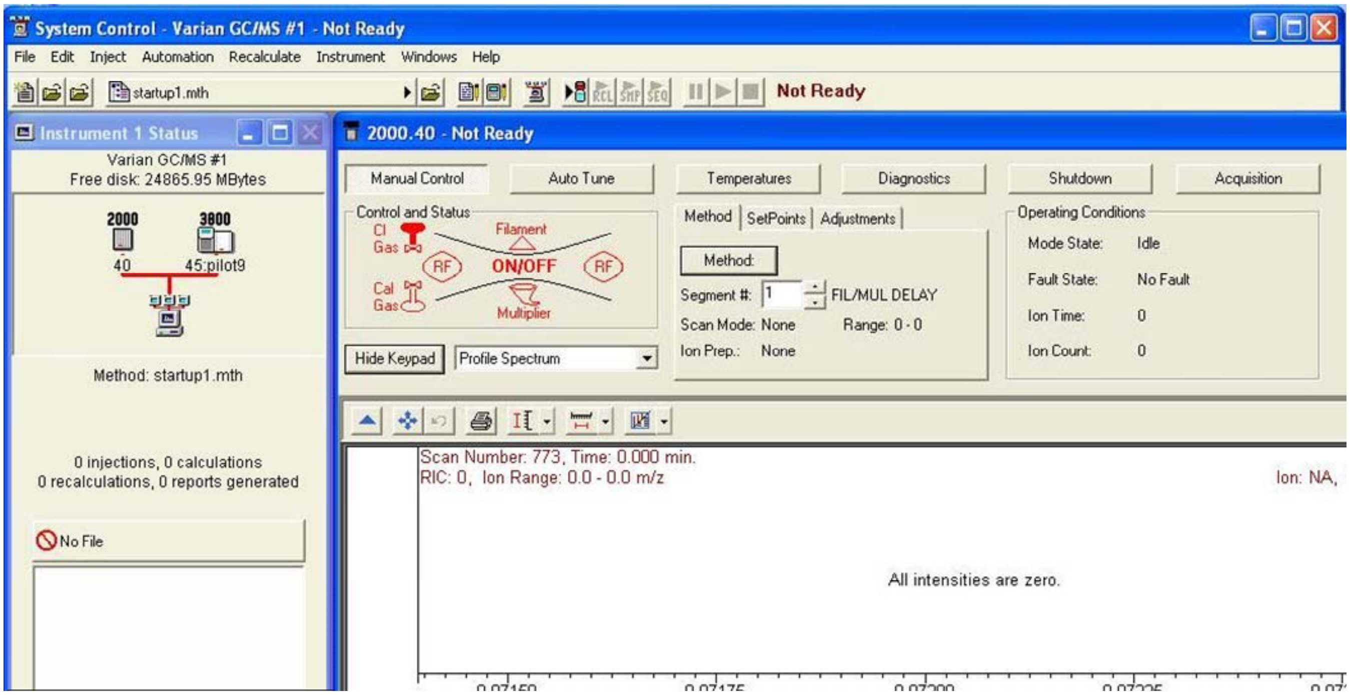
If you have a 8200 AutoSampler, drag and drop the Autosampler icon to the Available module area (note that the CP-8400/8410 is controlled and displayed through the GC Status and Control screen; it requires no special treatment). This removes the GC and the AutoSampler modules from the instrument, and the methods is not downloaded when an MS method is activated. To restore the GC and Autosampler modules, move the icons back into the active field.

Adjusting and Tuning the 2000

To adjust and tune the 2000 MS, display the 2000 Module window by double-clicking the instrument area background or using the Instrument menu to select Varian GC/MS #1.



The window titled “System Control - Varian GC/MS #1” will appear. The 2000 Module Window appears directly within it. If it is iconized, double-click to open it. There are six different modes of operation. By default, the Manual Control mode is active when you enter System Control. Other screens will appear when Auto Tune, Temperatures, Diagnostics, Shutdown, and Acquisition buttons are clicked. Note that the System Control and 2000.40 (2000 MS module) title bars both show **Not Ready**.



Varian GC/MS #1
Free disk: 24865.95 MBytes

Method: startup1.mth

0 injections, 0 calculations
0 recalculations, 0 reports generated

No File

Segment #: 1
Scan Mode: None
Range: 0 - 0
Ion Prep.: None

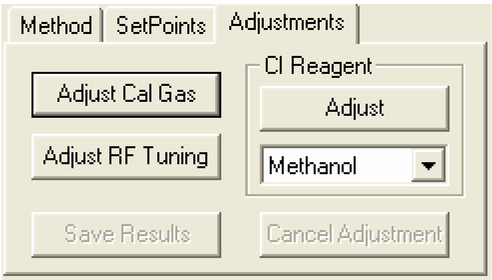
Mode State: Idle
Fault State: No Fault
Ion Time: 0
Ion Count: 0

Scan Number: 773, Time: 0.000 min.
RIC: 0, Ion Range: 0.0 - 0.0 m/z
Ion: NA,

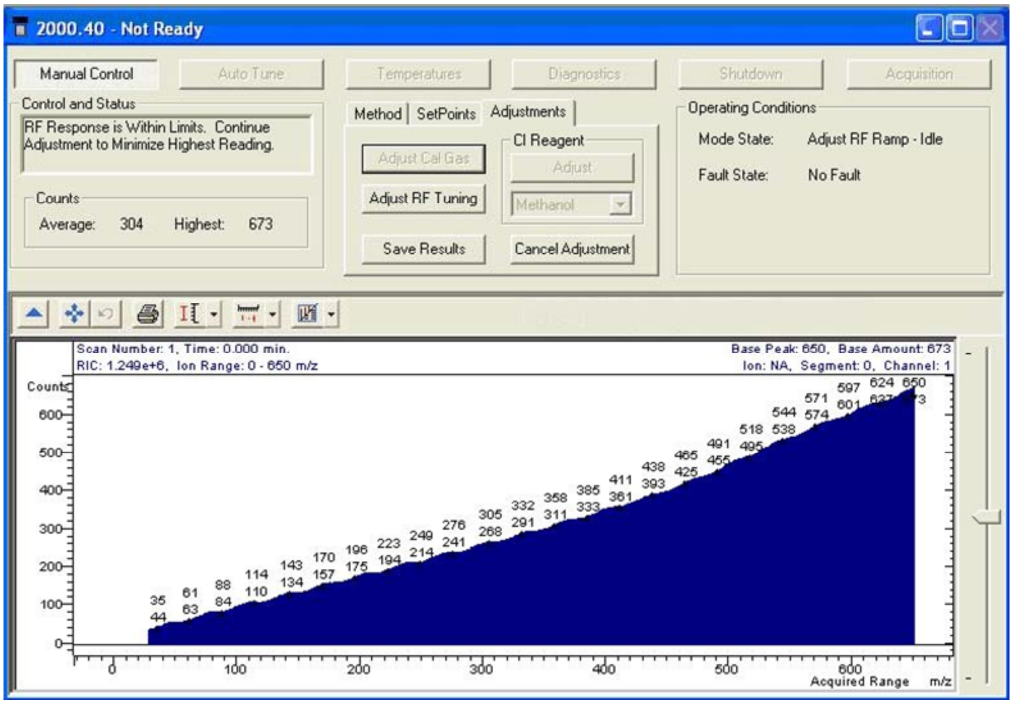
All intensities are zero.

Adjusting RF Tuning

If you are not already in the **Manual Control** section, click it. The first operation is **Adjust RF Tuning**. This adjustment is performed by first clicking the **Adjustments** tab and then selecting the **Adjust RF Tuning** button.



If the RF Ramp is not passing test requirements, open the front door of the MS and find the labeled RF Adjustment screw and adjust it with a flat-blade screwdriver while observing the feedback on the screen. When the Control and Status message reads “RF Response is Within Limits”, click **Save.**



Control and Status

RF Response is Within Limits. Continue Adjustment to Minimize Highest Reading.

Counts

Average: 304 Highest: 673

Operating Conditions

Mode State: Adjust RF Ramp - Idle

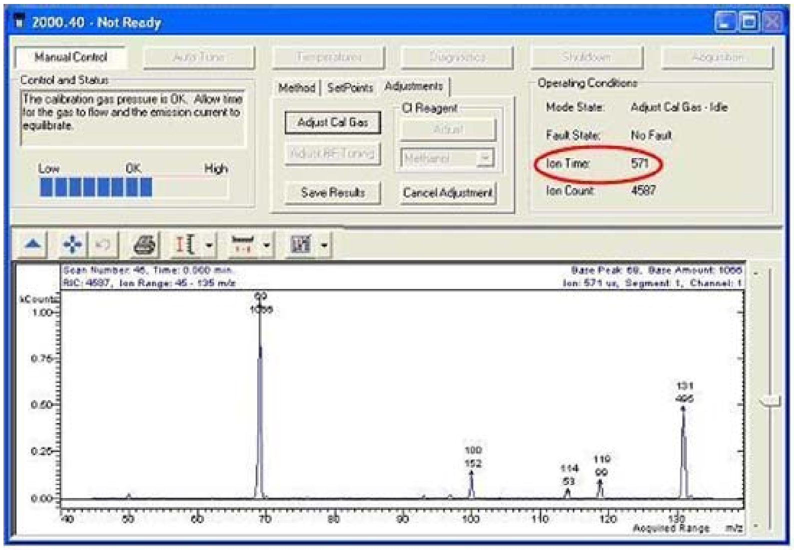
Fault State: No Fault

Scan Number: 1, Time: 0.000 min.
RIC: 1.249e+6, Ion Range: 0 - 650 m/z

Base Peak: 650, Base Amount: 673
Ion: NA, Segment: 0, Channel: 1

Adjusting Calibration Gas

Next, click **Adjust Cal Gas**. Open the front door of the 2000 MS and use the Calibration Gas needle valve to bring the Cal Gas status to **OK**. Note that the acceptable range corresponds to an Ion Time (seen in the Operating Conditions field on the right side of the dialog) of between 300-1000 µSec. When the test passes, click on **Done** to exit the adjustment.



Control and Status: The calibration gas pressure is OK. Allow time for the gas to flow and the emission current to equilibrate.

Operating Conditions

Mode State: Adjust Cal Gas - Idle

Fault State: No Fault

Ion Time: 571

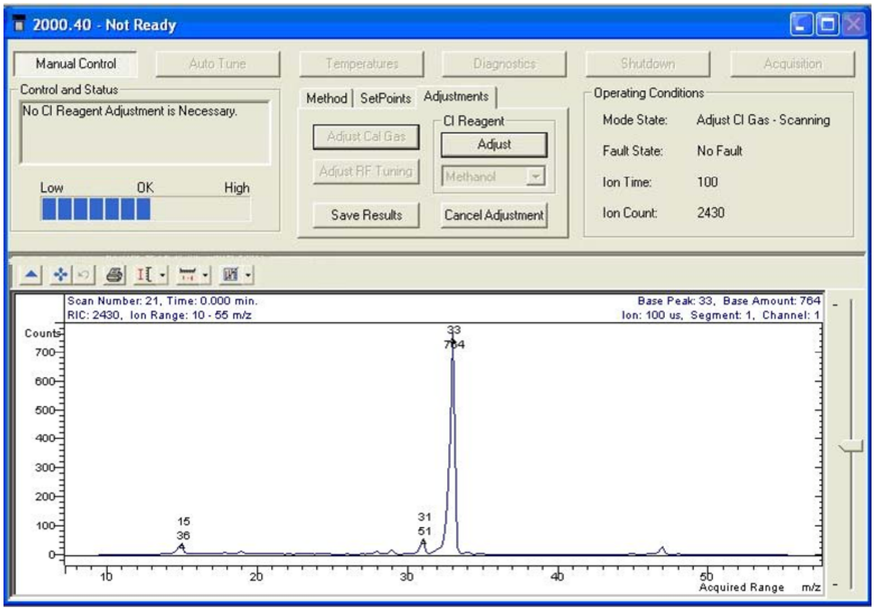
Ion Count: 4587

Scan Number: 46, Time: 0.000 min.
RIC: 4587, Ion Range: 45 - 135 m/z

Base Peak: 69, Base Amount: 1066
Ion: 571 us, Segment: 1, Channel: 1

Adjusting CI Reagent

Click **Adjust** in the **CI Reagent** area. Select the CI gas that your instrument is using by clicking the arrow. Open the front door of the 2000 MS and use the CI Gas needle valve to bring CI reagent status to OK. After the test passes, click **Save Results**.



Control and Status: No CI Reagent Adjustment is Necessary.

Operating Conditions

Mode State: Adjust CI Gas - Scanning

Fault State: No Fault

Ion Time: 100

Ion Count: 2430

Scan Number: 21, Time: 0.000 min.
RIC: 2430, Ion Range: 10 - 55 m/z

Base Peak: 33, Base Amount: 764
Ion: 100 us, Segment: 1, Channel: 1

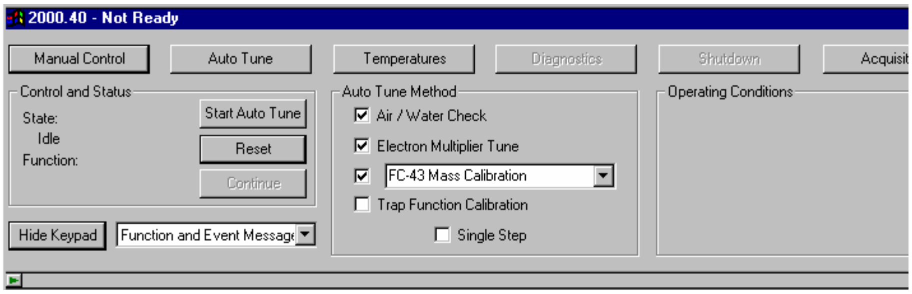
Running Auto Tune

Setting GC Column Temperature for Mass Calibration

Data is usually acquired with a constant flow of about 1mL/min through the GC column. However, some applications require a constant pressure injection and a temperature increase over time. In the constant pressure mode, the column flow rate will decrease as column temperature increases during the run. This will cause a slight shift in the mass axis for the collection of GC/MS data. To assure correct mass assignments, always run Mass Calibration with the GC Column Oven set at or near the high temperature for the column program in your GC/MS method. You can program the temperature from the GC Method (using Method Builder or the GC keyboard). For this tutorial use a constant flow of 1mL/min.

Choosing Auto Tune Methods

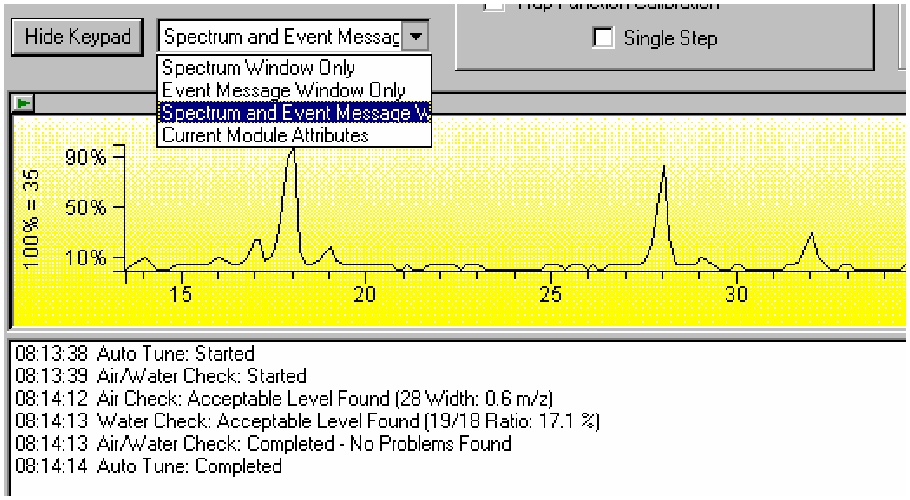
Click **Auto Tune**. Click on the items Air/Water Check, Electron Multiplier Tune, and FC43 Mass Calibration. It is not necessary to run the Trap Function Calibration method unless you wish to perform MS/MS or SIS types of acquisitions. Click on **Start Auto Tune** to run the specified suite of tuning procedures.



State: Idle
Function:

☑ Air / Water Check
☑ Electron Multiplier Tune
☑ FC-43 Mass Calibration
☐ Trap Function Calibration
☐ Single Step

After Auto Tune has started, the following windows should appear:



```
08:13:38  Auto Tune: Started
08:13:39  Air/Water Check: Started
08:14:12  Air Check: Acceptable Level Found (28 Width: 0.6 m/z)
08:14:13  Water Check: Acceptable Level Found (19/18 Ratio: 17.1 %)
08:14:13  Air/Water Check: Completed - No Problems Found
08:14:14  Auto Tune: Completed
```

When the Air/Water check completes, the Multiplier Tune and Mass Calibration methods will be run automatically unless you have selected “Single Step” in the checkbox below. In “Single Step” mode, the tuning will pause between Auto Tune steps so that you may examine the results of each test and then click the **Continue** button in the Status and Control field to initiate the next Auto Tune procedure.

NOTE: The Air/Water Check will use the *last* 10^5 Electron Multiplier Setting and not the manual setting. If the electron multiplier is replaced, the Electron Multiplier Tune procedure must be done *before* the Air/Water Check.

Troubleshooting (Optional)

If any of the steps in the automatic tuning fails, the problem should be corrected before running the tuning procedure again. The system should never be operated if the Air/Water check fails. If in doubt, consult the procedure How to Check for Leaks in the 2000 GC/MS Hardware Maintenance Manual or Help File, both of which contain many procedures to assist you in troubleshooting problems.

NOTE: It is a good idea to become familiar with the advice available in the Troubleshooting section of the 2000 GC/MS Hardware Maintenance Manual or Help File.

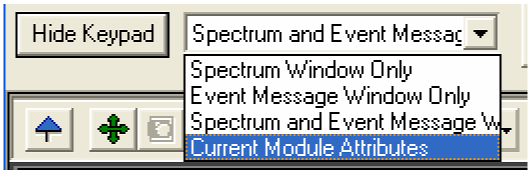
Required Frequency of Tuning

On a daily basis, the only tuning checks that need to be made are (1) Air/Water and (2) Mass Calibration. The electron multiplier voltage setting should be checked biweekly.

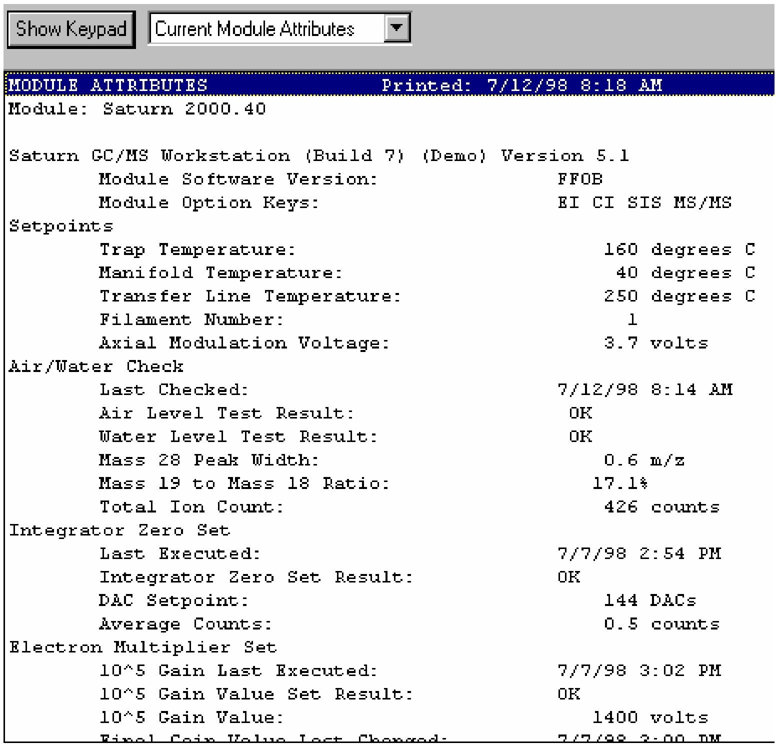
NOTE: The RF voltage ramp should be checked and adjusted, if needed, whenever the trap temperature is changed. Mass calibration should be run again after any adjustment of RF Ramp and/or ion trap temperature. To optimize mass axis stability, mass calibration should be run with the GC column oven at or near the maximum temperature for planned GC/MS runs. However, this precaution is not required if the GC is run in Constant Flow mode.

Examining Current Module Attributes

Note the combo box next to the Show/Hide Keypad button in the **Auto Tune** dialog box.



The Current Module Attributes section of the display shows the current settings and tune status of the instrument. This information is also added to the Segment Log for all acquired data files. Therefore, you can verify before a run, or in examining past archived data files, that the instrument tune is valid.



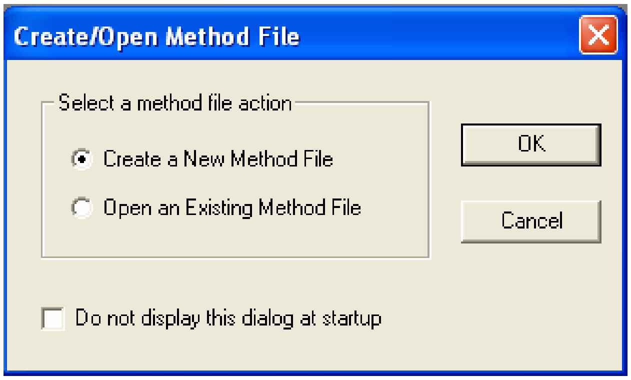
```
MODULE ATTRIBUTES                    Printed: 7/12/98 8:18 AM
Module: Saturn 2000.40

Saturn GC/MS Workstation (Build 7) (Demo) Version 5.1
        Module Software Version:               FF0B
        Module Option Keys:                    EI CI SIS MS/MS
Setpoints
        Trap Temperature:                          160 degrees C
        Manifold Temperature:                       40 degrees C
        Transfer Line Temperature:                 250 degrees C
        Filament Number:                             1
        Axial Modulation Voltage:                  3.7 volts
Air/Water Check
        Last Checked:                          7/12/98 8:14 AM
        Air Level Test Result:                  OK
        Water Level Test Result:                OK
        Mass 28 Peak Width:                        0.6 m/z
        Mass 19 to Mass 18 Ratio:                 17.1%
        Total Ion Count:                           426 counts
Integrator Zero Set
        Last Executed:                         7/7/98 2:54 PM
        Integrator Zero Set Result:            OK
        DAC Setpoint:                              144 DACs
        Average Counts:                            0.5 counts
Electron Multiplier Set
        10^5 Gain Last Executed:               7/7/98 3:02 PM
        10^5 Gain Value Set Result:            OK
        10^5 Gain Value:                          1400 volts
```

Preparing a GC/MS Acquisition Method

Creating a New Method

You may build and edit GC/MS Methods in the Method Builder application. To begin this process, click on the Method Builder button in the Toolbar.

The following dialog box will appear. Choose **Create a New Method File** and click **OK**.



Create/Open Method File

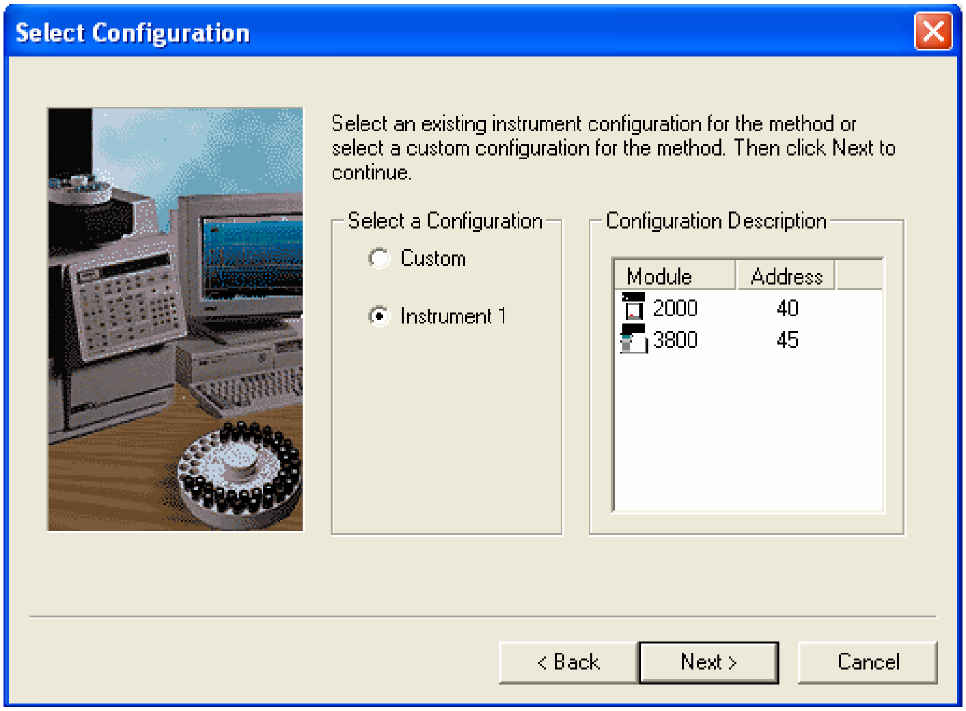
Select a method file action

● Create a New Method File

○ Open an Existing Method File

☐ Do not display this dialog at startup

Choose **Instrument 1** and click **Next** in the **Select Configuration** window.



Select Configuration

Select an existing instrument configuration for the method or select a custom configuration for the method. Then click Next to continue.

Select a Configuration

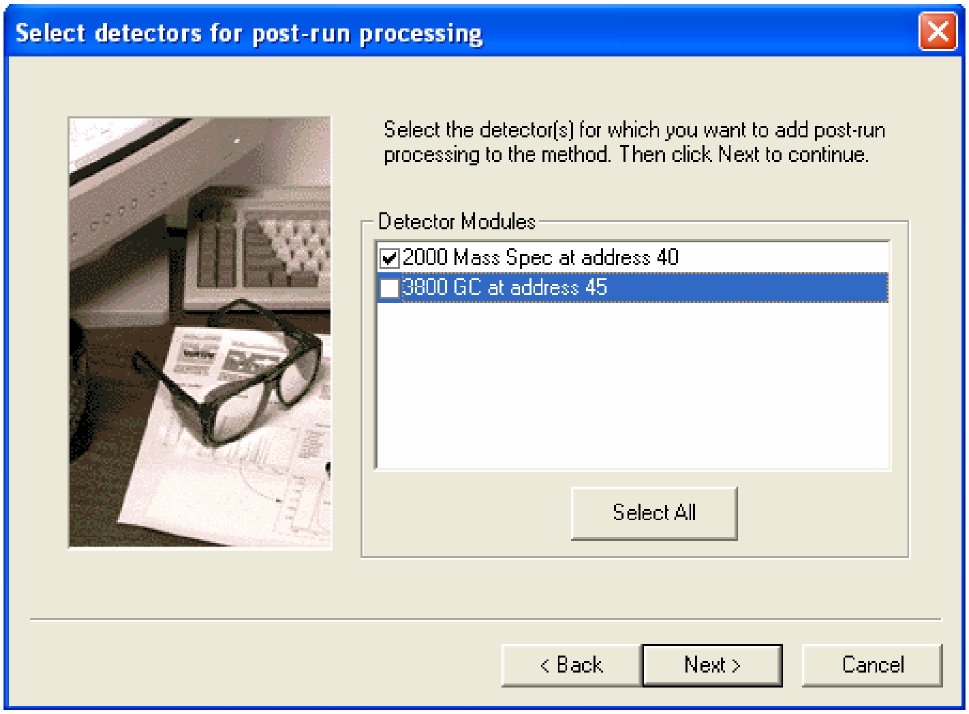
○ Custom

● Instrument 1

Configuration Description

Module	Address
2000	40
3800	45

If the GC was removed from the instrument, it won't be displayed as part of Instrument #1. Refer back to **Setting Instrument Configuration for Manual Injections** earlier in this tutorial section to reconfigure the GC as part of the Instrument.



Select detectors for post-run processing

Select the detector(s) for which you want to add post-run processing to the method. Then click Next to continue.

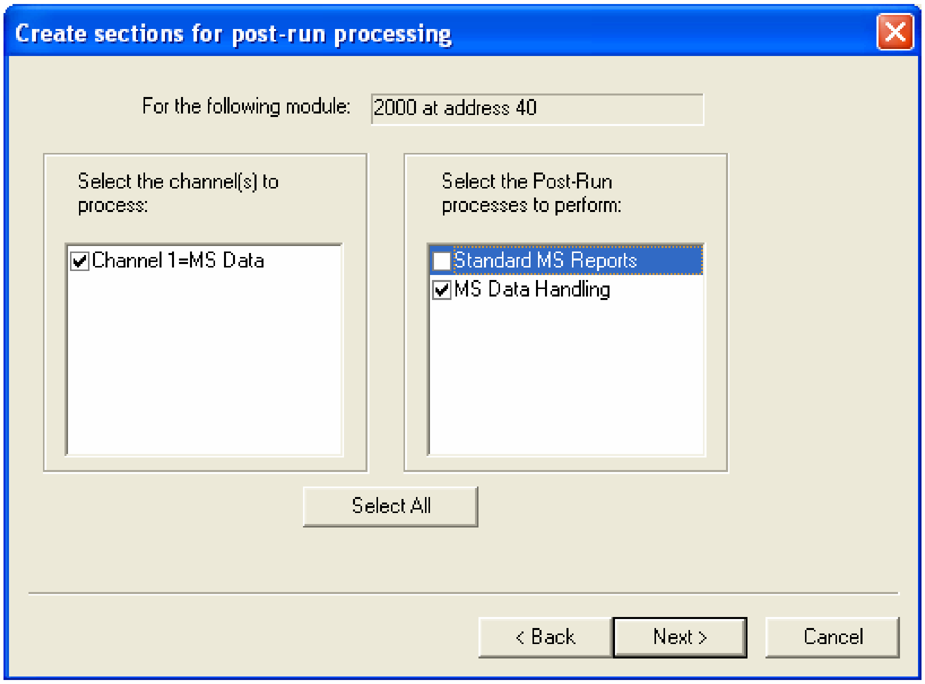
Detector Modules

☑ 2000 Mass Spec at address 40

☐ 3800 GC at address 45

Select only 2000 Mass Spec as the detector and click **Next**.

NOTE: If you were to acquire data with a standard GC detector such as an FID, PFPD, or ECD, you would select the GC detector module here.



For the following module: 2000 at address 40

Select the channel(s) to process:

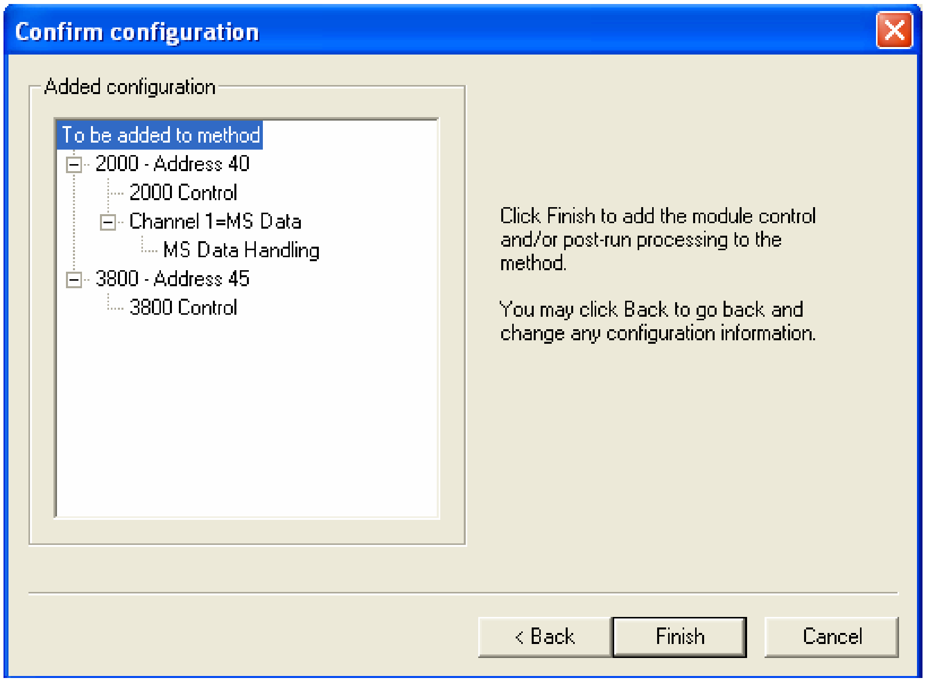
☑ Channel 1=MS Data

Select the Post-Run processes to perform:

☐ Standard MS Reports
☑ MS Data Handling

Select only “Channel 1=MS Data” and **MS Data Handling** for this tutorial, then click **Next**.

NOTE: The MS Data Handling section would be added to perform post-run processing of data files, including Quantitation and automated generation of Custom Reports.



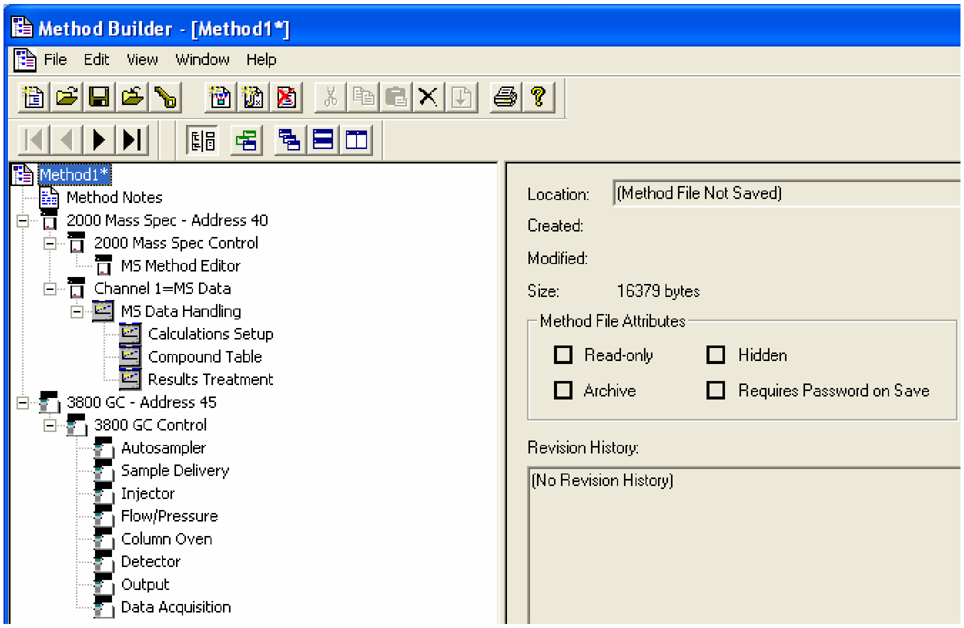
To be added to method
- 2000 - Address 40
  - 2000 Control
  - Channel 1=MS Data
    - MS Data Handling
- 3800 - Address 45
  - 3800 Control

Click Finish to add the module control and/or post-run processing to the method.

You may click Back to go back and change any configuration information.

The **Confirm Configuration** dialog shows that you will create the new method with sections for 3800 GC control and 2000 MS control.

Now click **Finish** and the **Method Builder** dialog box will open with a directory tree on the left side showing an outline of the sections available for editing.



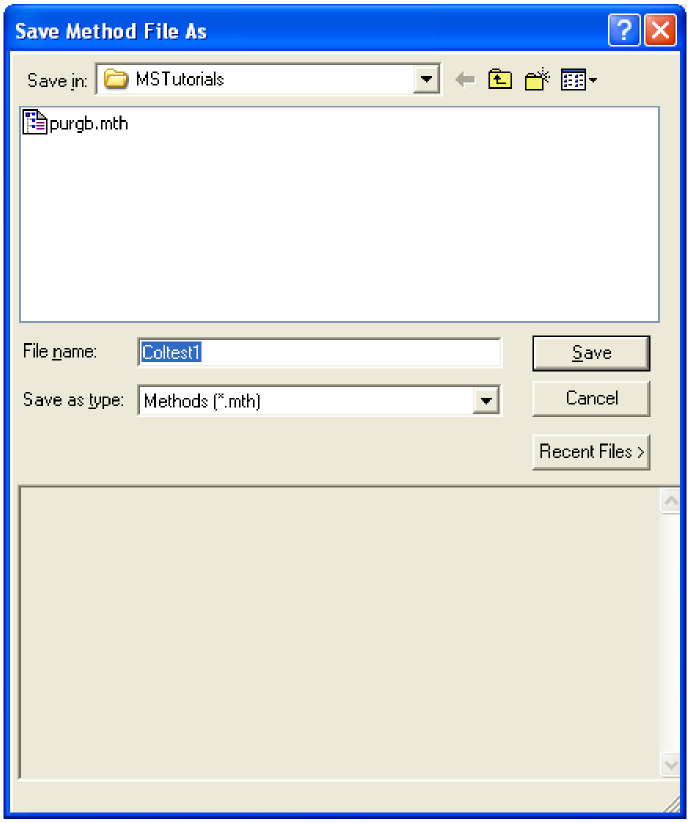
Location: (Method File Not Saved)
Created:
Modified:
Size: 16379 bytes

Method File Attributes

☐ Read-only ☐ Hidden
☐ Archive ☐ Requires Password on Save

Revision History:
(No Revision History)

Editing the Data Handling section will be discussed later in this manual in the Quantitation tutorial. Before you begin editing the method, use the menu command ***File >Save As*** to save the method to the name Coltest1.mth. Enter the name *Coltest1* in the file name field and click **Save.**

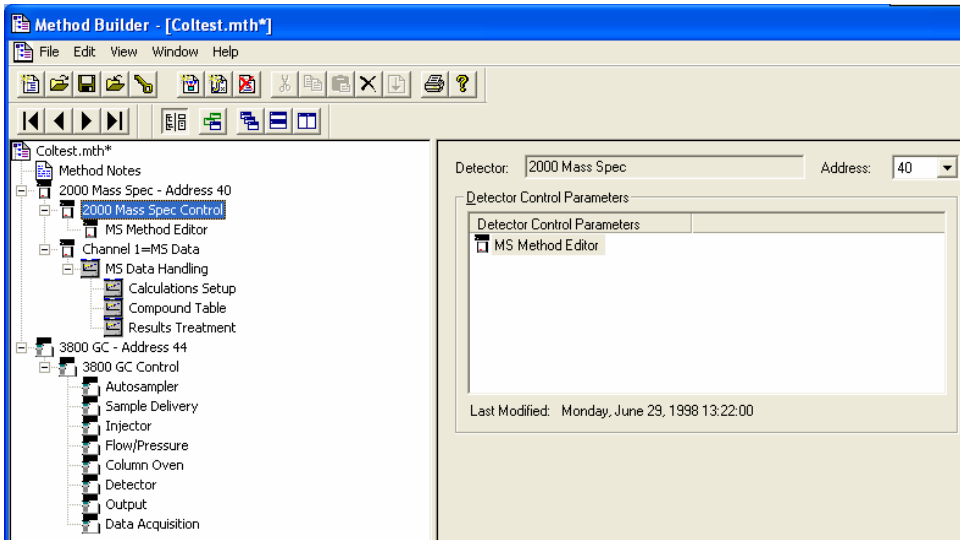


Save in: MSTutorials
File name: Coltest1
Save as type: Methods (*.mth)

Editing the Method

Selecting and Editing the MS Method Section

You are now ready to edit the method so that you can acquire a data file with the Column Test Mix sample. Notice that as you highlight different items in the Method Directory, different screens appear in the right section of the window.



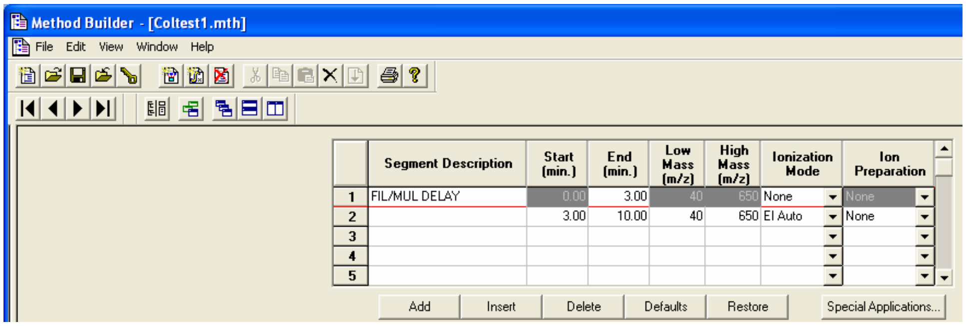
Detector: 2000 Mass Spec

Address: 40

Last Modified: Monday, June 29, 1998 13:22:00

Under the Method Directory item **2000 Mass Spec Control**, click on the item **MS Method Editor**. The display on the right will now show the MS method.

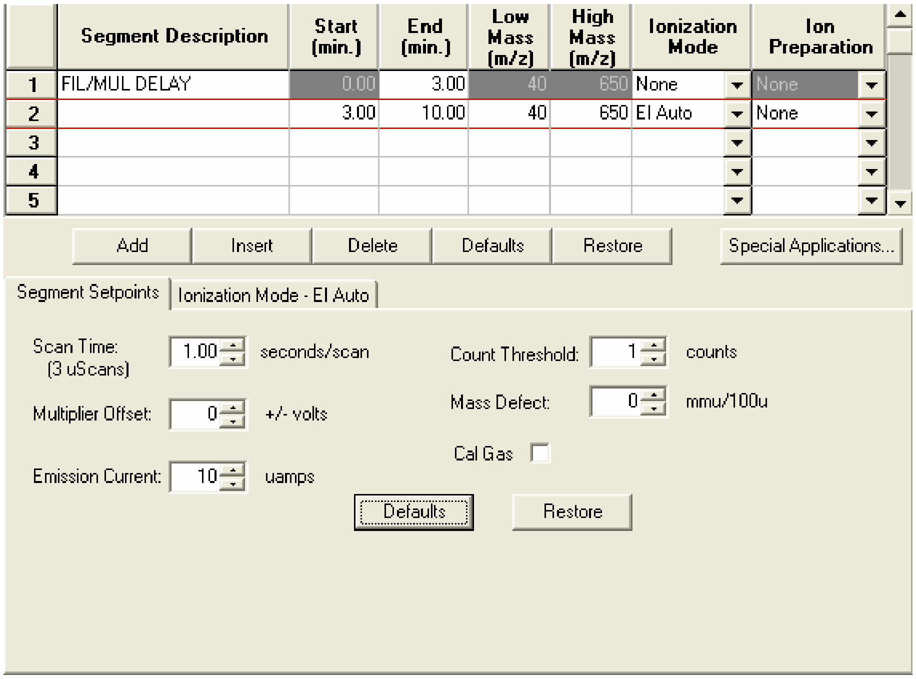
Note that you may click and drag on the splitter bar separating the Method Directory from the Method Display and adjust the position so that the entire MS Method is visible on the screen. Alternatively you may click on the **Show/Hide Method Directory** button in the Method Builder toolbar to hide the Method Directory. The top area of the method screen shows a table of segments for the acquisition.



	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	FIL/MUL DELAY	0.00	3.00	40	650	None	None
2		3.00	10.00	40	650	EI Auto	None
3							
4							
5							

The default table for a new MS section consists of two segments. The first segment is a Filament/Multiplier Delay segment for the first three minutes (Ionization Mode = None). This segment will be acquired with the filament and multiplier turned off to protect the instrument until after the elution of the solvent peak.

The second default segment from 3.00 to 10.00 minutes is Electron Ionization with Automatic Gain Control (EI Auto) to acquire MS data over the m/z range 40-650. This is the standard ionization mode for full-scan EI acquisitions. If you highlight a cell in Segment 2 of the Segment Table, you will see additional tab dialogs for **Segment Setpoints** and **Ionization Mode**.

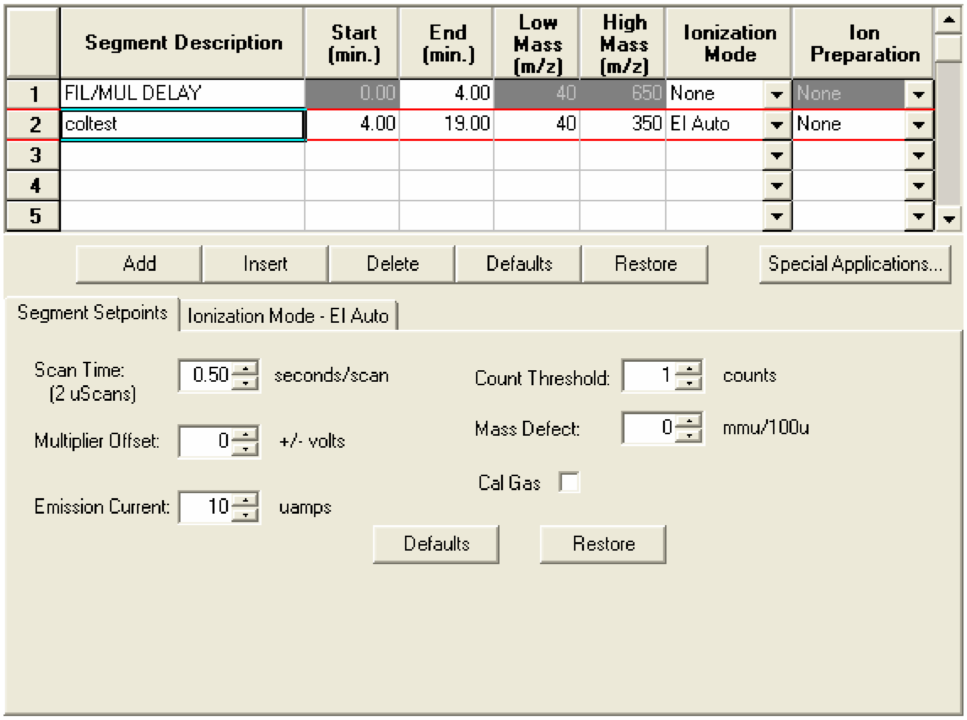


	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	FIL/MUL DELAY	0.00	3.00	40	650	None	None
2		3.00	10.00	40	650	EI Auto	None
3							
4							
5							

Scan Time: (3 uScans) 1.00 seconds/scan
Multiplier Offset: 0 +/- volts
Emission Current: 10 uamps
Count Threshold: 1 counts
Mass Defect: 0 mmu/100u
Cal Gas ☐

Examine the parameters which may be adjusted in the **Segment Setpoints** dialog but do not change them at this time. Click on the tab dialog **Ionization Mode - EI Auto** to see the parameters that may be adjusted there. If you would like more details on the adjustment of these parameters, go to the section **Editing an MS Method** in the **MSWS Software Reference Manual**.

To prepare the MS section of the method for acquiring the Column Test Mix sample, change the end time for Segment 1 by highlighting the **End (min.)** cell and entering 4.00. Then highlight the **Segment Description** cell for Segment 2 and type in “coltest”. Change the end time for Segment 2 to 19 minutes, and then change the m/z range to 40-350. Now the screen should look like the following:



	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	FIL/MUL DELAY	0.00	4.00	40	650	None	None
2	coltest	4.00	19.00	40	350	EI Auto	None
3							
4							
5							

Scan Time: (2 uScans) 0.50 seconds/scan

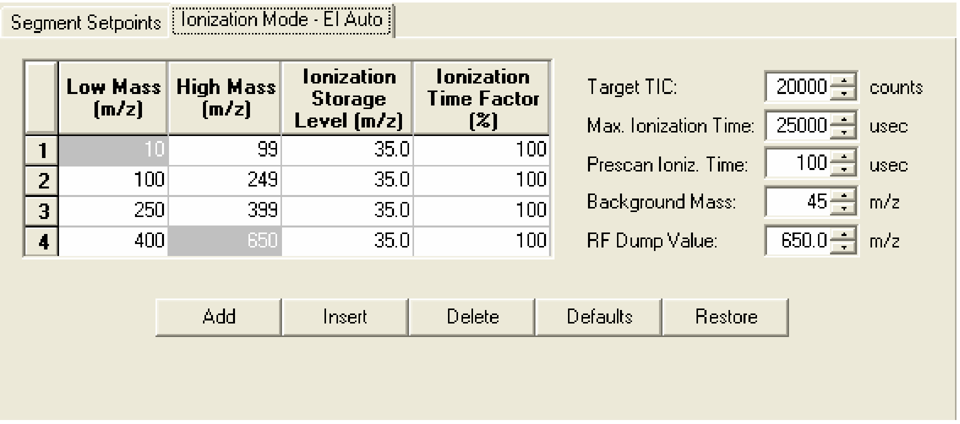
Multiplier Offset: 0 +/- volts

Emission Current: 10 uamps

Count Threshold: 1 counts

Mass Defect: 0 mmu/100u

Cal Gas ☐



	Low Mass (m/z)	High Mass (m/z)	Ionization Storage Level (m/z)	Ionization Time Factor (%)
1	10	99	35.0	100
2	100	249	35.0	100
3	250	399	35.0	100
4	400	650	35.0	100

Target TIC: 20000 counts

Max. Ionization Time: 25000 usec

Prescan Ioniz. Time: 100 usec

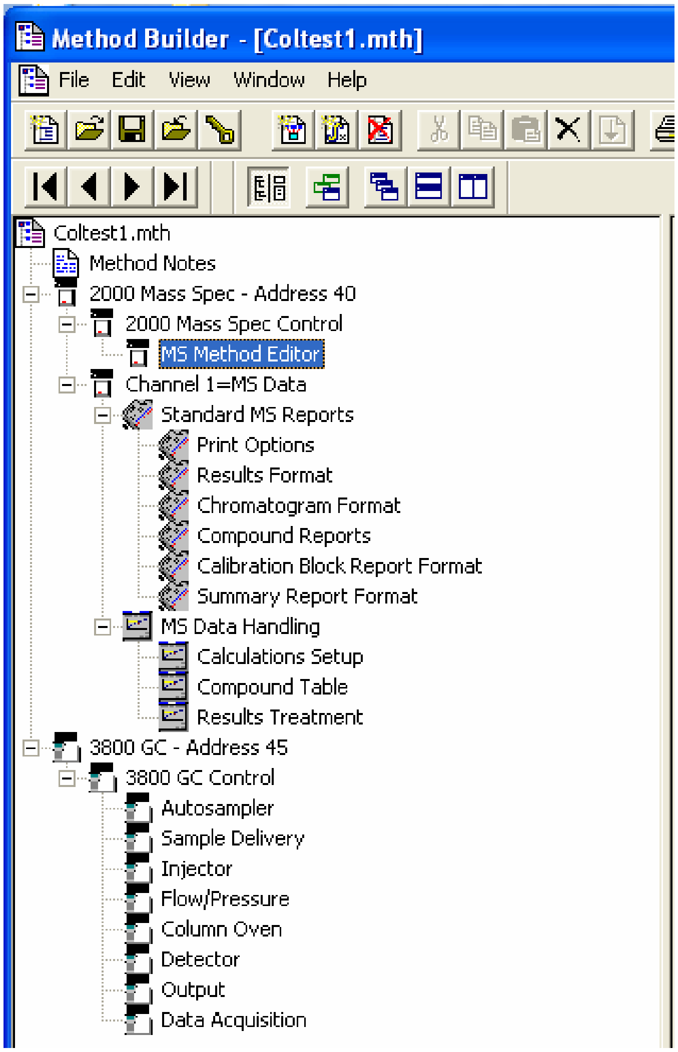
Background Mass: 45 m/z

RF Dump Value: 650.0 m/z

Selecting and Editing the GC Method Section

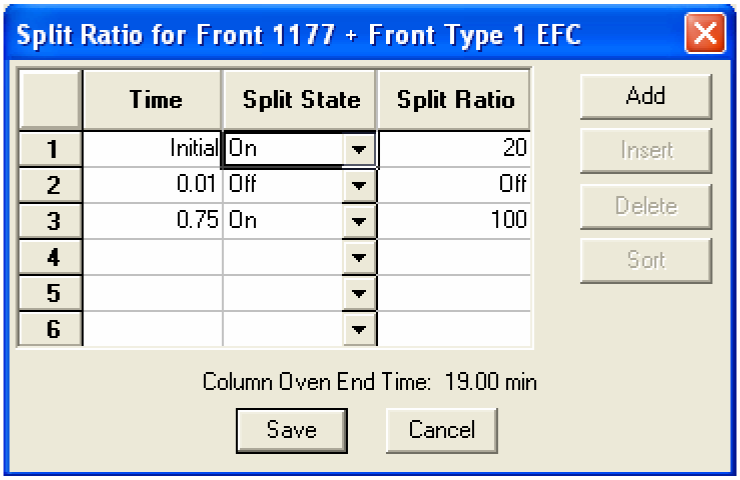
NOTE: This tutorial assumes that you have a 3800 GC. 3900 GC programming is similar.

After completing your examination of the MS section of the method, click on the Injector section of GC Control in the Method Directory. (If you have used the **Show/Hide Directory** button in the toolbar to hide the Method Directory, click on the button to make the directory appear again.)



The Injector section of the 3800 GC method will appear. The tab dialog for the Front injector is shown by default. If your GC column is installed to the Middle or Rear injector positions, click on the appropriate tab and modify the method so that the injector temperature is set correctly.

Set the injector temperature to 250 °C. You may also set the Split Ratio in this section. Click on **Split Ratio** tab and enter the values shown below:

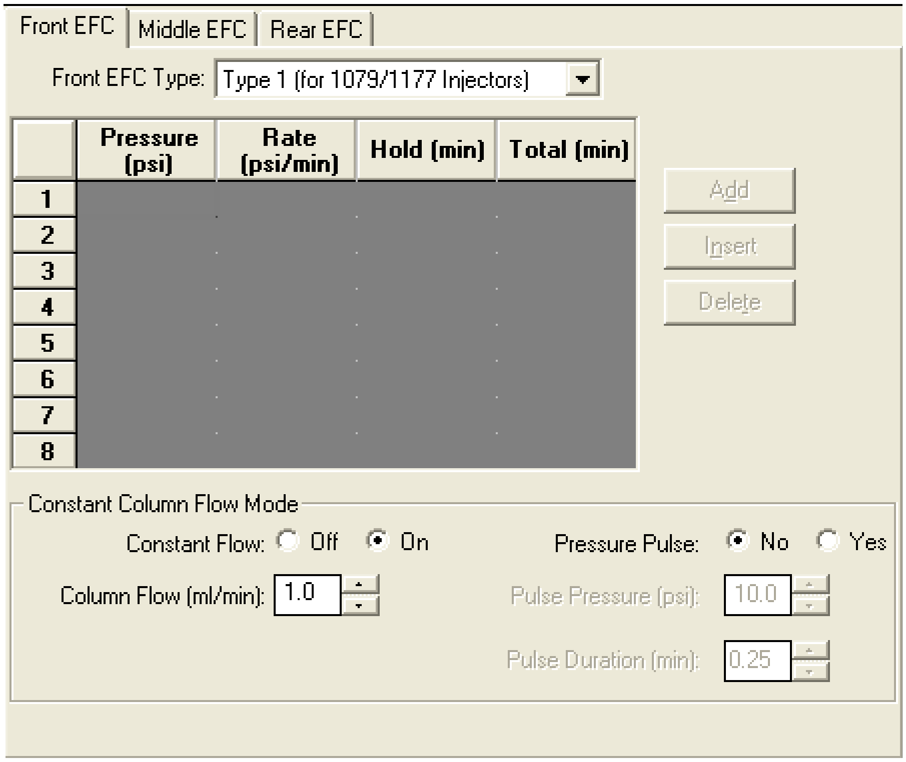


Split Ratio for Front 1177 + Front Type 1 EFC

	Time	Split State	Split Ratio
1	Initial	On	20
2	0.01	Off	Off
3	0.75	On	100
4			
5			
6			

Column Oven End Time: 19.00 min

Now click on the **Flow/Pressure** section of the Method Directory. Select the Front, Middle, or Rear Injector, depending upon where your column is installed. Note that the method is set up for a Type 1 Electronic Flow Control (EFC) in the Front Injector. You may need to modify the EFC Type to match your instrument configuration. The usual mode of acquiring data is constant flow. This can be done by selecting **On** in the **Constant Column Flow Mode** field at the bottom of this dialog. If you wish to use constant flow, choose a flow of 1mL/min.



Front EFC Type: Type 1 (for 1079/1177 Injectors)

	Pressure (psi)	Rate (psi/min)	Hold (min)	Total (min)
1				
2				
3				
4				
5				
6				
7				
8				

Constant Column Flow Mode

Constant Flow: ○ Off ● On

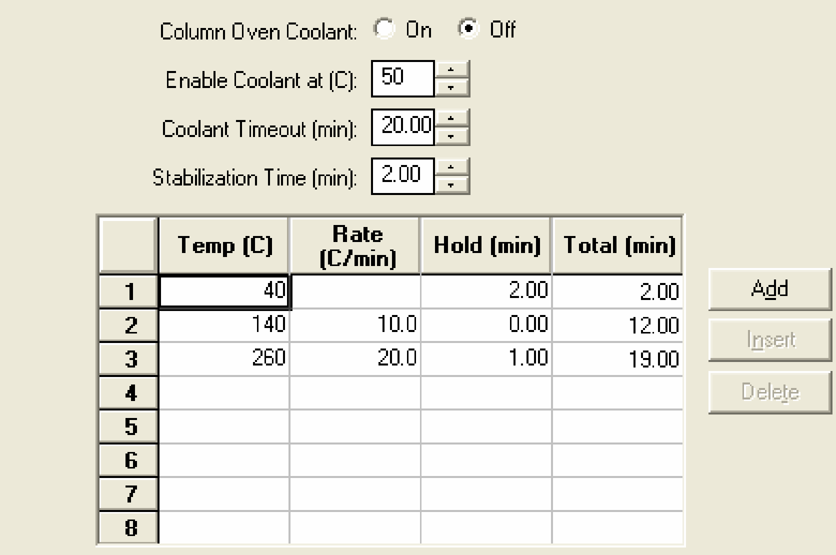
Column Flow (ml/min): 1.0

Pressure Pulse: ● No ○ Yes

Pulse Pressure (psi): 10.0

Pulse Duration (min): 0.25

After examining and editing the **Flow/Pressure** section, click on the **Column Oven** section in the Method Directory. Set the Stabilization Time to 0.50 minutes and set up the Column Temperature Program Table as shown next.



Column Oven Coolant: ○ On ● Off

Enable Coolant at (C): 50

Coolant Timeout (min): 20.00

Stabilization Time (min): 2.00

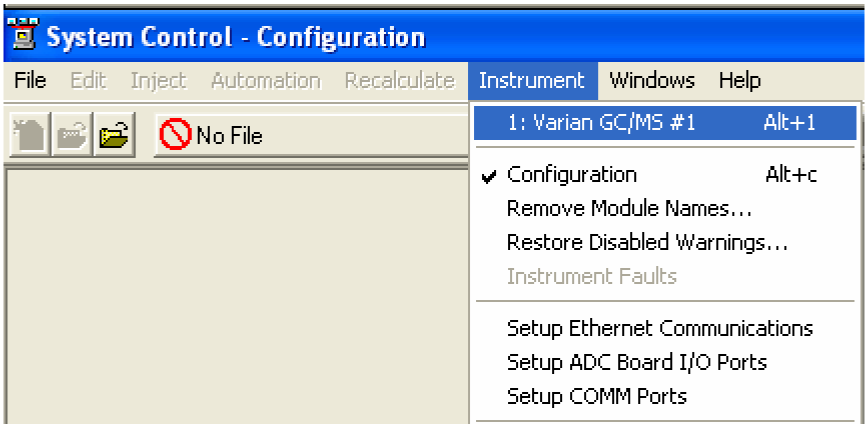
	Temp (C)	Rate (C/min)	Hold (min)	Total (min)
1	40		2.00	2.00
2	140	10.0	0.00	12.00
3	260	20.0	1.00	19.00
4				
5				
6				
7				
8				

You have now completed the examination and editing of the method Coltest1.mth. To Save the method use the pull-down menu **File >Save**. After the method has been saved exit the Method Builder with the menu command ***File >Exit*** or click on the **X** button in the upper right corner of the Method Builder window.

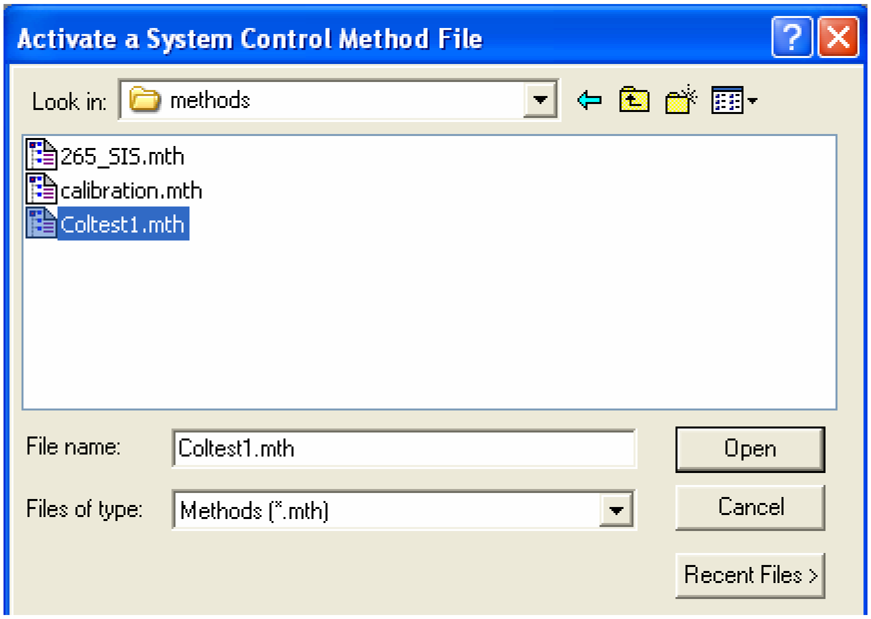
Starting the Acquisition

Activating the GC/MS Method

If System Control is not open, start the application from the Workstation Toolbar. If System Control application opens the Configuration window, select **1:Varian GC/MS #1** from the **Instrument** menu.



Use the menu command ***File >Activate Method*** to display the file selection dialog. Highlight the method Coltest1.mth and click **Open**. The GC and MS portions of the method will be downloaded to the instrument.


Activate a System Control Method File

Look in: methods

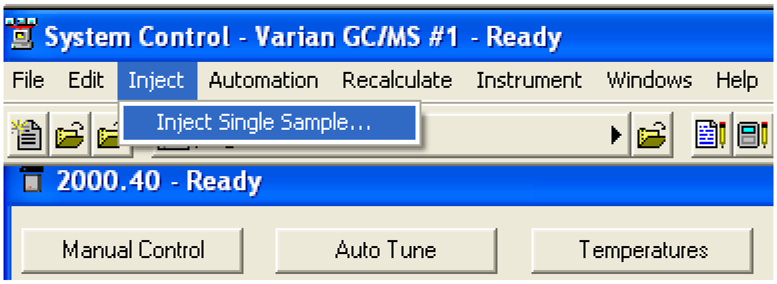
265_SIS.mth
calibration.mth
Coltest1.mth

File name: Coltest1.mth

Files of type: Methods (*.mth)

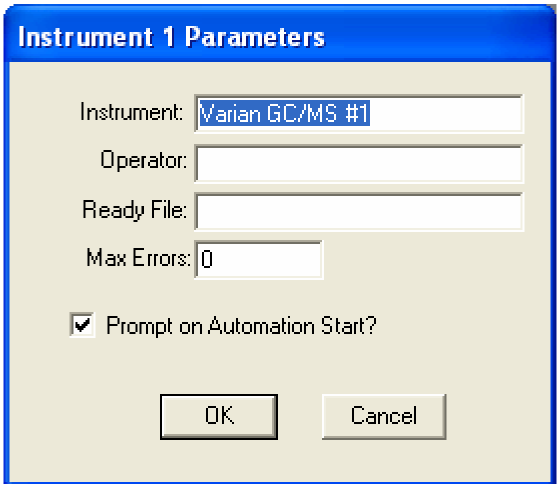
Starting the GC/MS Run

Although you could construct a Sample List and run several samples in Automation, for this tutorial you will learn how to quickly run one sample at a time. When you see the 2000 module come into the Ready state. Click on the ***Inject >Inject Single Sample*** option.


System Control - Varian GC/MS #1 - Ready

2000.40 - Ready

The **Instrument 1 Parameters** dialog box should appear. Enter your name or initials in the *Operator* field and Click the **OK** button.


Instrument 1 Parameters

Instrument: Varian GC/MS #1

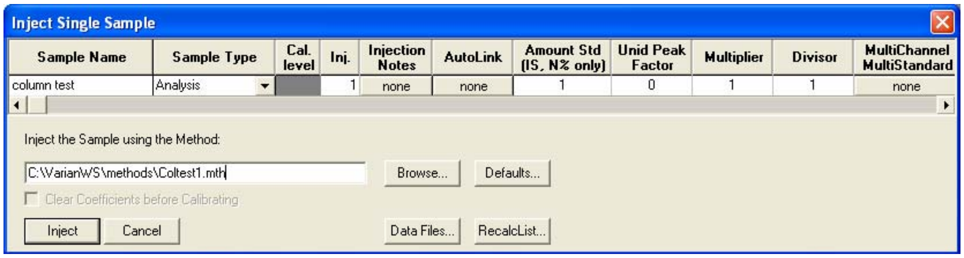
Operator:

Ready File:

Max Errors: 0

☑ Prompt on Automation Start?

The **Inject Single Sample** dialog should appear.



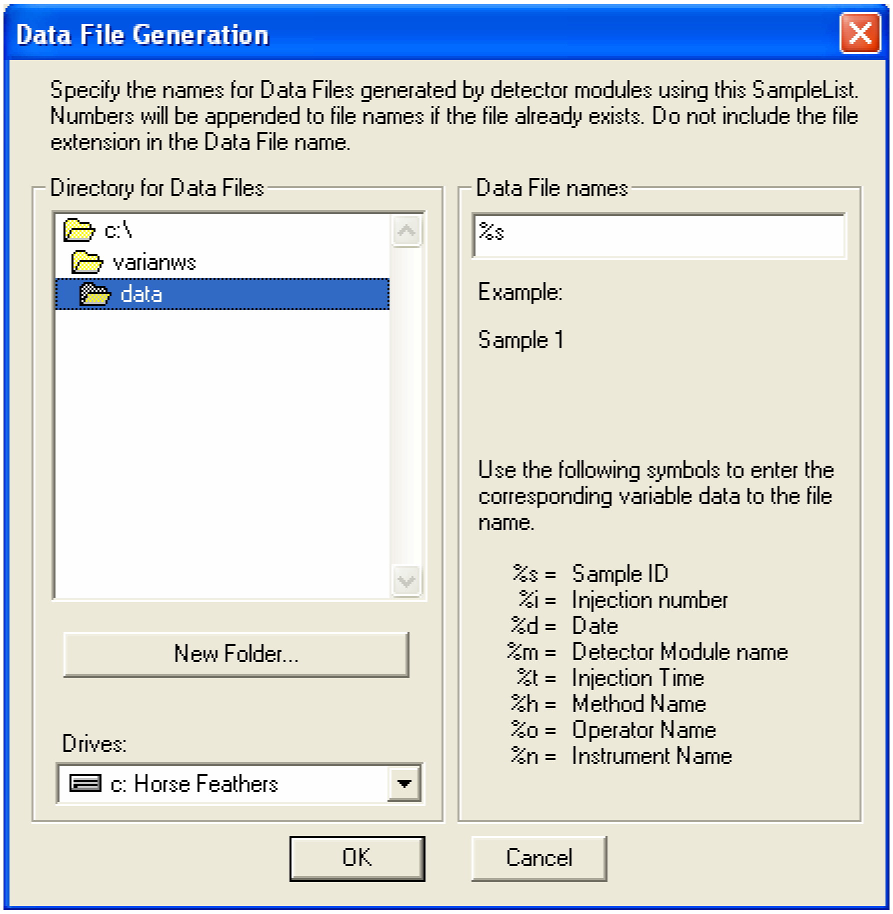
Sample Name	Sample Type	Cal. level	Inj.	Injection Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multiplier	Divisor	MultiChannel MultiStandard
column test	Analysis		1	none	none	1	0	1	1	none

Inject the Sample using the Method:

C:\VarianWS\methods\Coltest1.mth

☐ Clear Coefficients before Calibrating

The active method is displayed in the field **Inject the Sample using the Method**. Type in a name for your sample in the **Sample Name** field. Here we have just chosen the name column test**.** After typing the name, you can activate the **Data Files** button by clicking in any other field. Click on the **Data Files….** button to open the **Data File Generation** dialog.



Specify the names for Data Files generated by detector modules using this SampleList. Numbers will be appended to file names if the file already exists. Do not include the file extension in the Data File name.

%s

Example:

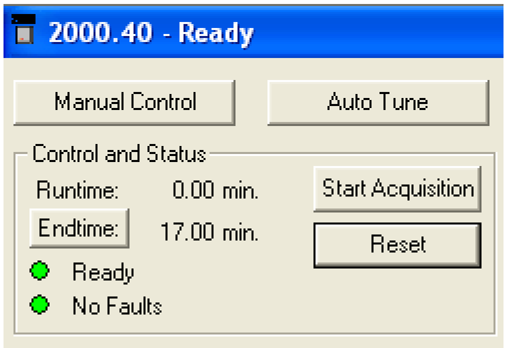
Sample 1

Use the following symbols to enter the corresponding variable data to the file name.

%s = Sample ID
%i = Injection number
%d = Date
%m = Detector Module name
%t = Injection Time
%h = Method Name
%o = Operator Name
%n = Instrument Name

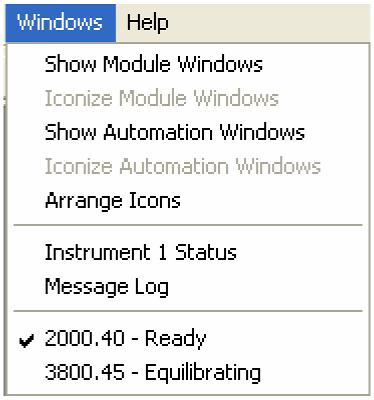
In this dialog, you can choose a directory where you want to save the data file and select the symbols for the labels you want to be added as extensions to the file name.

After you making the file name selections, click the **Inject** button in the lower left of the dialog. Make sure the 2000 MS Module screen is shown (not the GC Module). Verify in the Control and Status area that the 2000 status is “Ready” and “No Faults”.

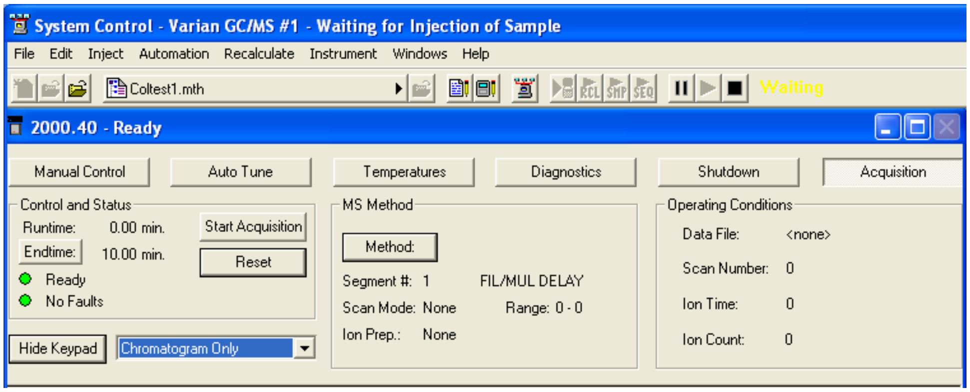


Runtime: 0.00 min.
Endtime: 17.00 min.
● Ready
● No Faults

You may also use the **Windows** menu to check the status of the GC module as well. While the GC injector and column temperatures are being adjusted to those set in the method, the GC module status will be “Equilibrating”.



When the GC has equilibrated and stabilized, the Status Indicator in the System Control Toolbar should read ***Waiting for Injection of Sample***. Choose the option **Chromatogram Only** for the display.



Runtime: 0.00 min.
Endtime: 10.00 min.
● Ready
● No Faults
Chromatogram Only

Segment #: 1 FIL/MUL DELAY
Scan Mode: None Range: 0 - 0
Ion Prep.: None

Data File: <none>
Scan Number: 0
Ion Time: 0
Ion Count: 0

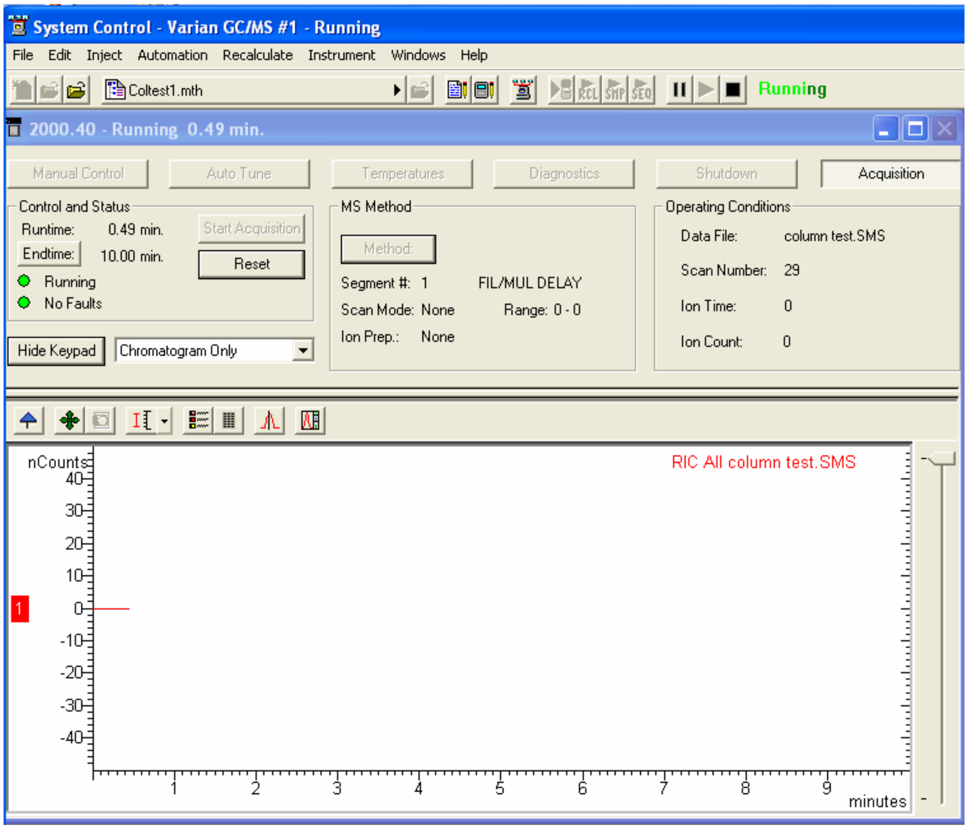
Injecting 1 µL of the Column Test Mixture

Rinse a 10 µL syringe in hexane and expel air bubbles by pumping the syringe plunger up and down several times. Pull the syringe up to the 1.0µL mark to create an upper air gap. Place the needle in the Column Test Mix solution and draw the plunger up to the 2.0 µL mark. Finally, remove the syringe from the solution and draw the plunger up a further 1.0 µL. You can now see exactly how much sample solution is in the syringe. There should be approximately 1.0 µL of air above the sample and 1.0 µL of sample in the barrel of the syringe.

The syringe needle should be left in the injector for 2-3 seconds before depressing the plunger. Inject the sample using an even, consistent injection speed. The optimum speed is about 1 µL/second. As soon as the automatic start switch on the GC injector is depressed, the acquisition will start and you will observe the acquisition of the data file *column test.sms* has begun.

Monitoring Data Acquisition in Progress from System Control

The MS method has specified a 4.00 minute Filament/Multiplier Delay, so, although the chromatogram appears in the display window, there are no peaks observed at first.



System Control - Varian GC/MS #1 - Running

Coltest1.mth

Running

2000.40 - Running 0.49 min.

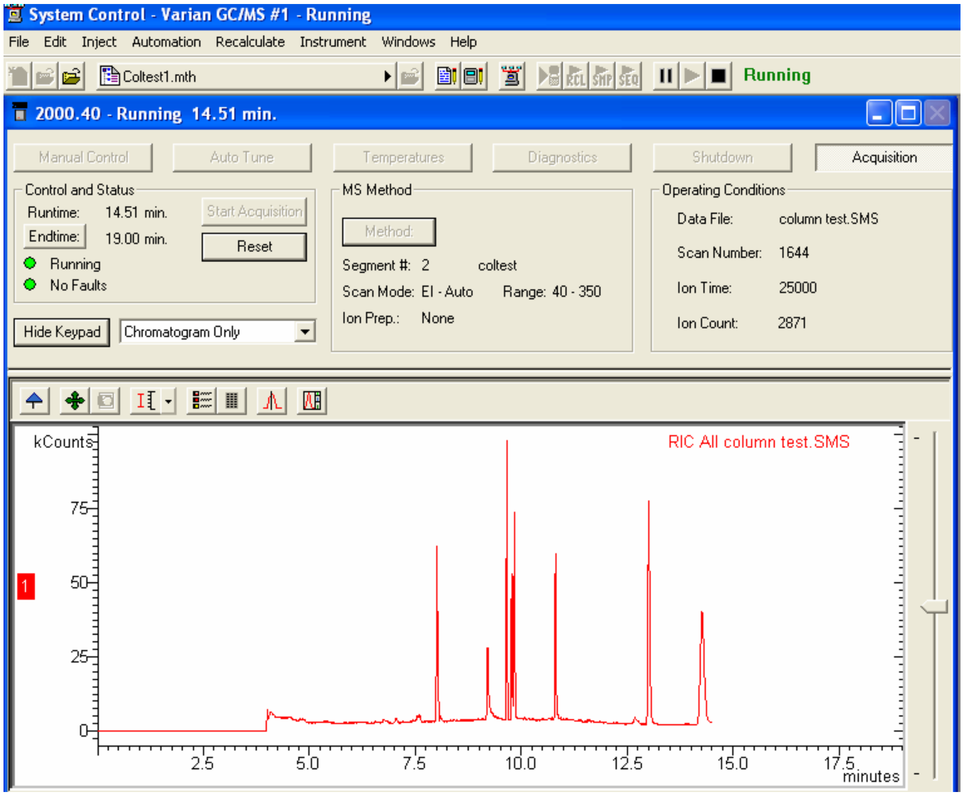
Runtime: 0.49 min.
Endtime: 10.00 min.
● Running
● No Faults

Segment #: 1 FIL/MUL DELAY
Scan Mode: None Range: 0 - 0
Ion Prep.: None

Data File: column test.SMS
Scan Number: 29
Ion Time: 0
Ion Count: 0

RIC All column test.SMS

Examine the features of the 2000 Module during the initial segment. Note that the Status Indicator in System Control now indicates ***Running***. In the Control and Status region, note the progress of the Runtime indicator. In the MS Method region, note the information associated with Segment 1. Now observe the Data File name, Scan Number, Ion Time, and Ion Count in the Operating Conditions region. Click on the **Hide Keypad** button to get a full-screen display of the chromatogram. Click on the **Show Keypad** button to display the keypad again.



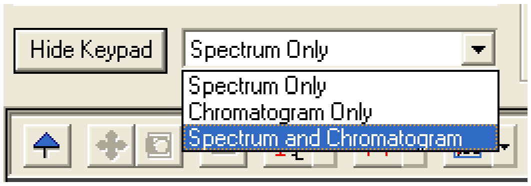
Runtime: 14.51 min.
Endtime: 19.00 min.
● Running
● No Faults

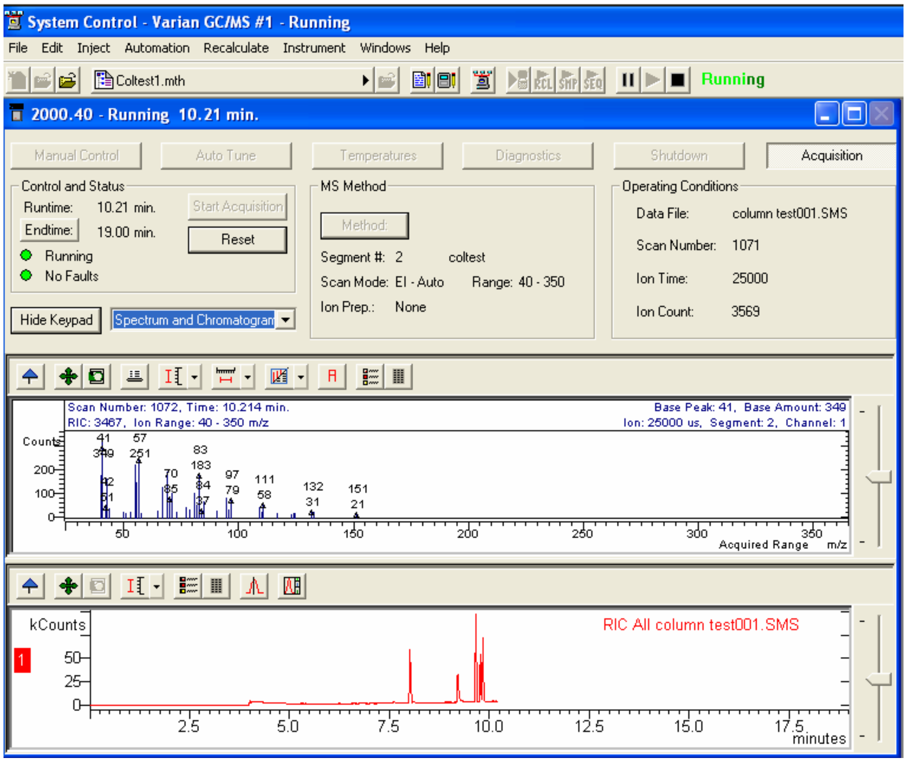
Segment #: 2 coltest
Scan Mode: EI - Auto Range: 40 - 350
Ion Prep.: None

Data File: column test.SMS
Scan Number: 1644
Ion Time: 25000
Ion Count: 2871

When the Runtime reaches 4.0 minutes, Segment 2 will be downloaded and acquisition will switch to EI-Auto mode. Again note the information displayed in the MS Method and Operating Conditions regions. Move the mouse over the buttons in the display toolbars and examine the options available. Choose the **Time Select** button in the Chromatogram toolbar. This button allows you to display the mass spectrum of a particular peak in the chromatogram. You may also click and drag in the chromatogram display to expand a given area for careful examination.

You may look at both the Spectrum and Chromatogram by selecting that option from the drop-down list box.





System Control - Varian GC/MS #1 - Running

2000.40 - Running 10.21 min.

Control and Status
Runtime: 10.21 min.
Endtime: 19.00 min.
● Running
● No Faults

MS Method
Segment #: 2 coltest
Scan Mode: EI - Auto Range: 40 - 350
Ion Prep.: None

Operating Conditions
Data File: column test001.SMS
Scan Number: 1071
Ion Time: 25000
Ion Count: 3569

Scan Number: 1072, Time: 10.214 min. Base Peak: 41, Base Amount: 349
RIC: 3467, Ion Range: 40 - 350 m/z Ion: 25000 us, Segment: 2, Channel: 1

RIC All column test001.SMS

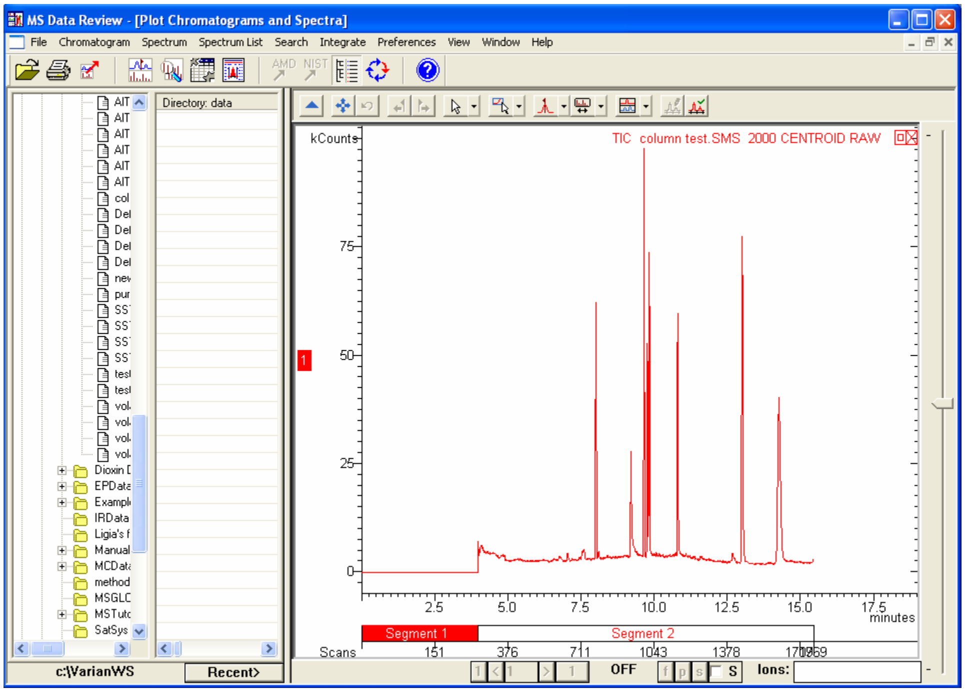
Monitoring the Acquisition in Progress from MS Data Review

Viewing the Chromatogram

The last button on the right of the Chromatogram toolbar in System Control opens **MS Data Review** . When this option is chosen the MS Data Review application opens and the file currently being acquired can be displayed and used for qualitative analysis. Click on this button now to open the **Plot Chromatograms and Spectra** view of MS Data Review.

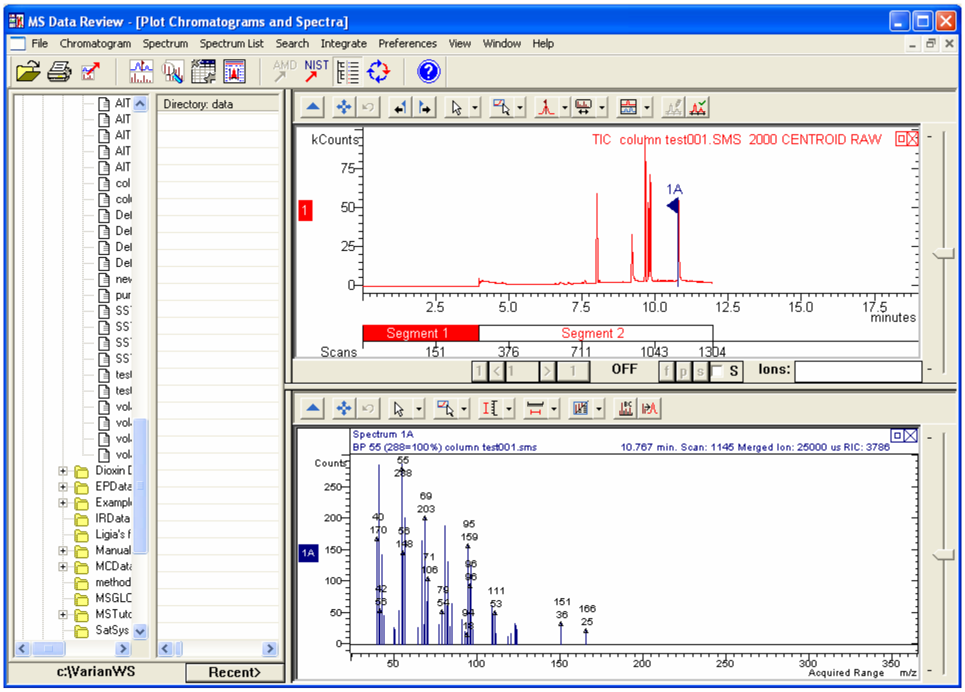
NOTE: The System Control application will remain open and accessible while you are viewing data in MS Data Review. You can return at any time to System Control by Restoring its minimized display from the Windows Taskbar or by clicking **<Alt +Tab>** on the keyboard until System Control is selected.

You will see that the Chromatogram display is set for the full time length of the current method but that only the first portion of the run has been completed. The display is updated every few seconds with additional scans.



Displaying Mass Spectra Peaks of Interest

As you did in the Acquisition dialog in System Control, click on a peak of interest to display the mass spectrum. The MS Data Review spectrum display appears with its own toolbar for user adjustments.



Performing Library Searches

Now that a spectrum has been selected, you can library search it by selecting ***Search >Library Search a Spectrum >1A*** in the **MS Data Review** menu. You can quickly Library Search other spectra by holding down the ‘L’ key on the keyboard while clicking on a peak in the displayed plot.

You can examine and print library search results during data file acquisition. All of the library searching and other Qualitative Identification procedures possible within MS Data Review are discussed in the tutorial “Qualitative Analysis of GC/MS Data”.

Retention Times for Analytes in the Column Test Mix Sample

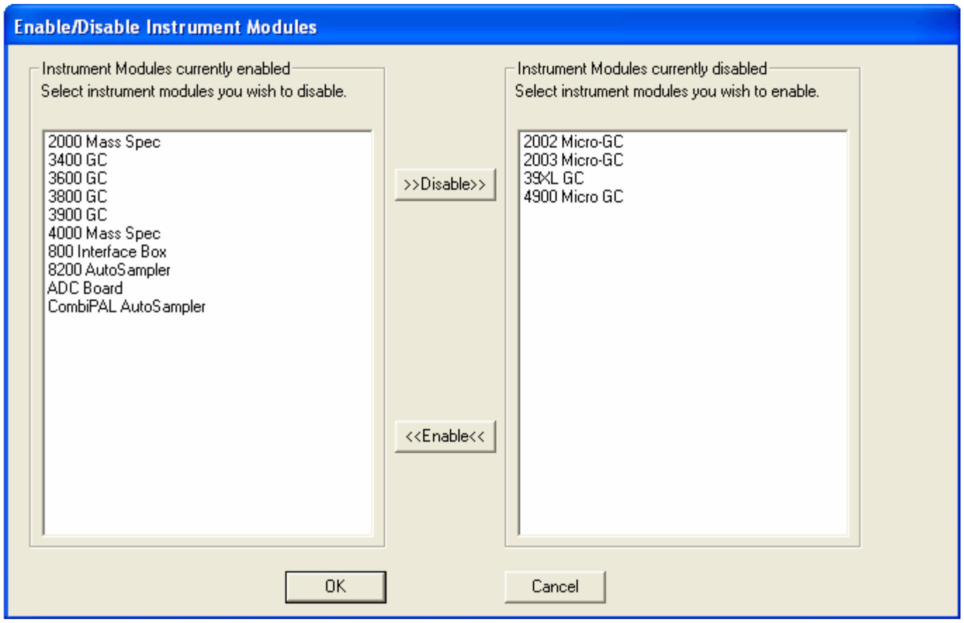
If you wish to perform further qualitative analysis on this data file, you may open it again with MS Data Review at a later time. The components of the Column test mix and their approximate retention times are shown below.

Compound	Retention (min)
Decane	9.063
1-Octanol	10.408
Undecane	10.849
Nonanal	10.966
Phenol, 2,6-dimethyl-	11.074
Quinoline, 1,2,3,4-tetrahydro-	11.457
Benzenamine, 2,6-dimethyl-	12.167
Decanoic acid, methyl ester	14.117
Undecanoic acid methyl ester	15.183
Cyclohexanamine, N-cyclohexyl-	15.275
Dodecanoic acid, methyl ester	16.025
Benzene, hexachloro-	17.667

Setting Instrument Configuration for Automated Injections

Configuring the Instrument

Details on Configuration setup are available in the 2000 Operational Manual. A portion of that information is given here. Before you configure your instrument make sure that required instrument modules are enabled: right click to the right side of the method Quick Link button in the Workstation Toolbar and select **Enable/Disable Instrument Modules**.



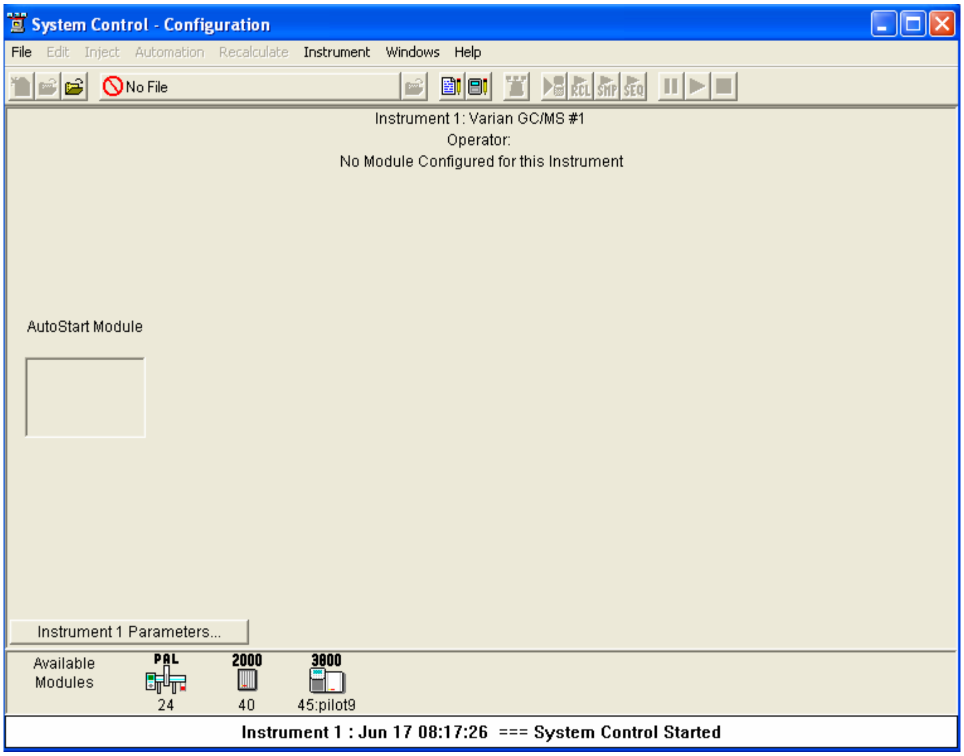
Instrument Modules currently enabled
Select instrument modules you wish to disable.

2000 Mass Spec
3400 GC
3600 GC
3800 GC
3900 GC
4000 Mass Spec
800 Interface Box
8200 AutoSampler
ADC Board
CombiPAL AutoSampler

Instrument Modules currently disabled
Select instrument modules you wish to enable.

2002 Micro-GC
2003 Micro-GC
39XL GC
4900 Micro GC

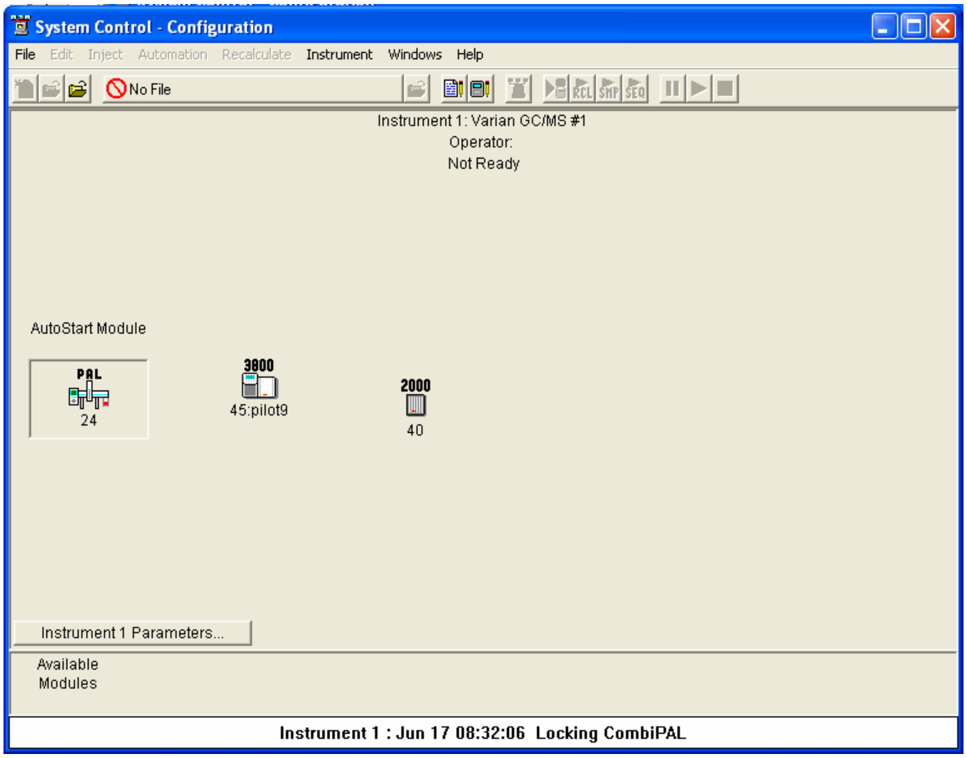
If for example you have a 3800 GC and a Combi PAL AutoSampler, enable the 3800 GC and the Combi PAL AutoSampler. (For users who have a CP-8400/8410 AutoSampler, the autosampler is controlled and displayed through the 3800 or 3900 Status and Control screen. Thus, only the GC needs to be enabled.) When the desired configuration is enabled, click on **OK** and open System Control.



Instrument 1: Varian GC/MS #1
Operator:
No Module Configured for this Instrument

Instrument 1 : Jun 17 08:17:26 === System Control Started

If the instrument has not been previously configured, the 2000, 3800 or 3900 GC, and the Combi PAL AutoSampler (if it exists in your system) will appear at the bottom of this screen in the “Available Modules” section of the window. Use the mouse to click and drag the icons into the center of the screen. If the Combi PAL icon is present, drag it into the **AutoStart Module**. The 2000 and 3800 icons go anywhere to the right of the box, but not in the **AutoStart Module** box.

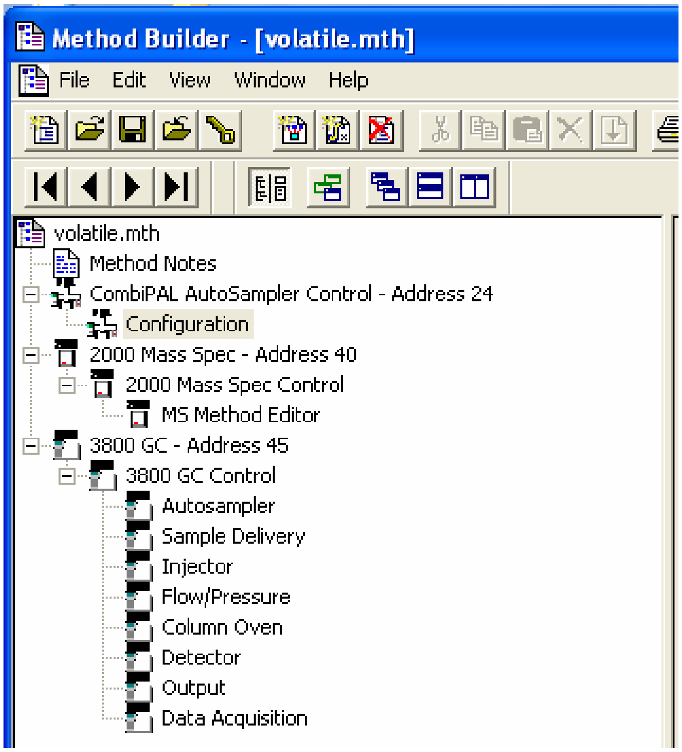


Instrument 1 : Jun 17 08:32:06 Locking CombiPAL

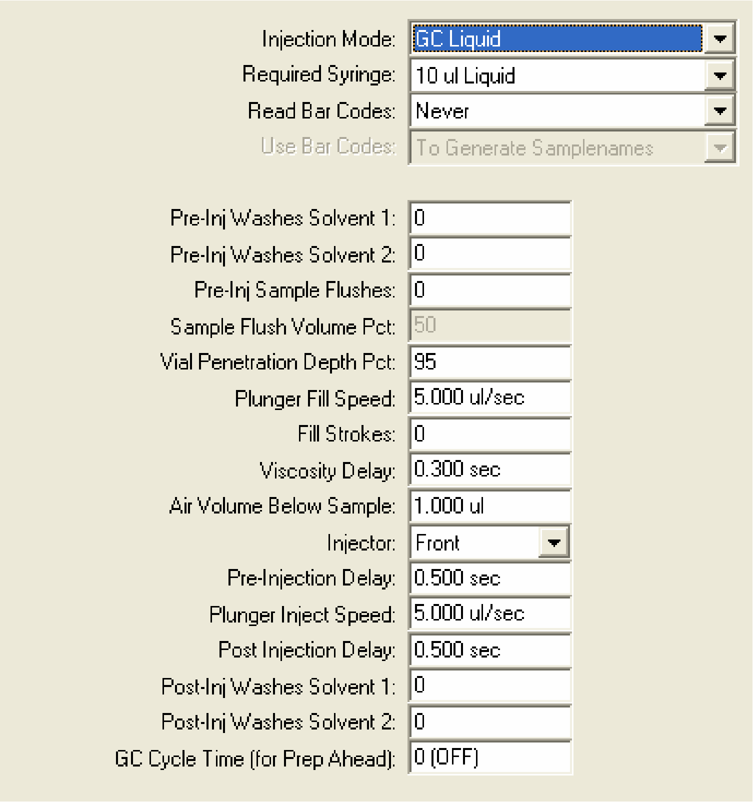
NOTE: If an 8400/8410 AutoSampler is attached to a GC, the GC module should be placed in the **AutoStart Module**.

Once the modules are in place you are ready to proceed.

The AutoSampler is controlled by the parameters set in the Method, which can be opened by clicking on the Method button in the system control screen. Clicking on the AutoSampler section in the Method Directory



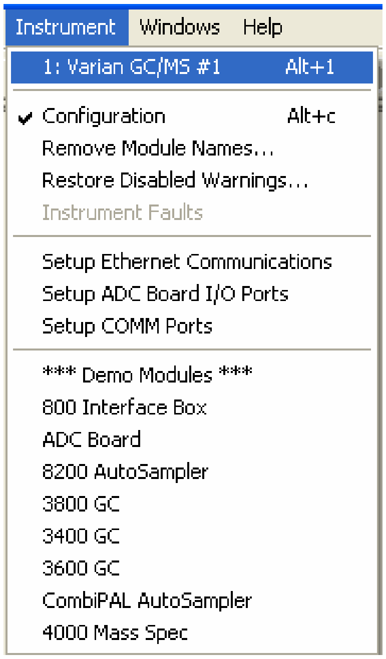
Clicking on the **Configuration** section will give you a screen similar to the following.



Injection Mode: GC Liquid
Required Syringe: 10 ul Liquid
Read Bar Codes: Never
Use Bar Codes: To Generate Samplenames

Pre-Inj Washes Solvent 1: 0
Pre-Inj Washes Solvent 2: 0
Pre-Inj Sample Flushes: 0
Sample Flush Volume Pct: 50
Vial Penetration Depth Pct: 95
Plunger Fill Speed: 5.000 ul/sec
Fill Strokes: 0
Viscosity Delay: 0.300 sec
Air Volume Below Sample: 1.000 ul
Injector: Front
Pre-Injection Delay: 0.500 sec
Plunger Inject Speed: 5.000 ul/sec
Post Injection Delay: 0.500 sec
Post-Inj Washes Solvent 1: 0
Post-Inj Washes Solvent 2: 0
GC Cycle Time (for Prep Ahead): 0 (OFF)

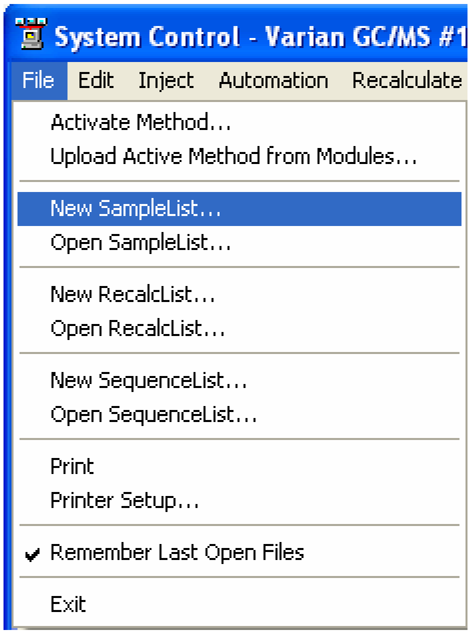
This screen shows parameters for a Combi PAL injection, which can be modified according to the specific applications. Once having the injection parameters set, save the method and open the **System Control** application. If you are in the **Configuration** mode, select the **Instrument** menu and click on **1: Varian GC/MS #1** to open the modules control windows.



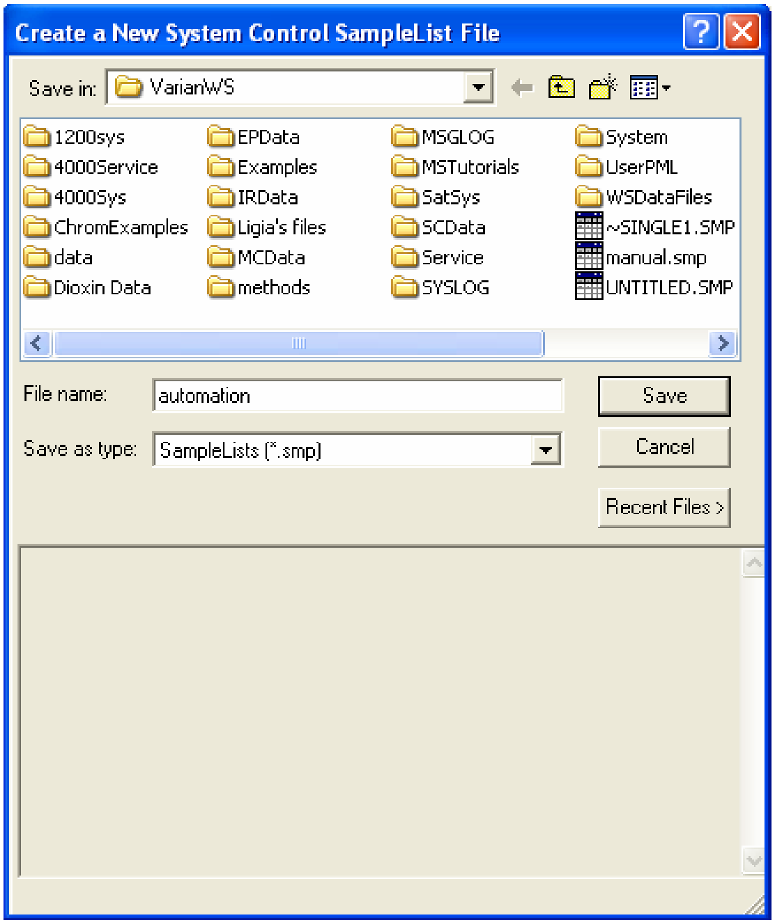
Activate the method that you are going to use for the AutoSampler, GC and MS using the menu command **File >Activate**, select the method and then click **Open**.

Creating the Sample List

Use the menu command **File >New Sample List** in System Control to create a Sample List.



A file selection dialog will be displayed. Type the name “automation” in the *File Name* field and click on **Save**.

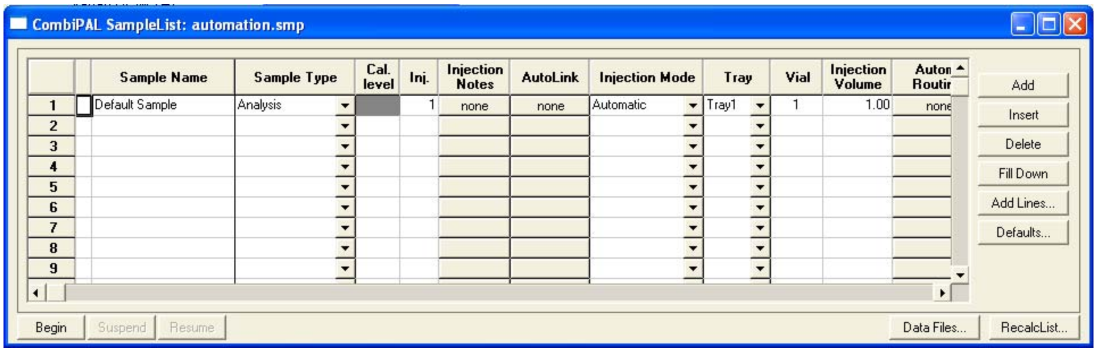


Save in: VarianWS

File name: automation

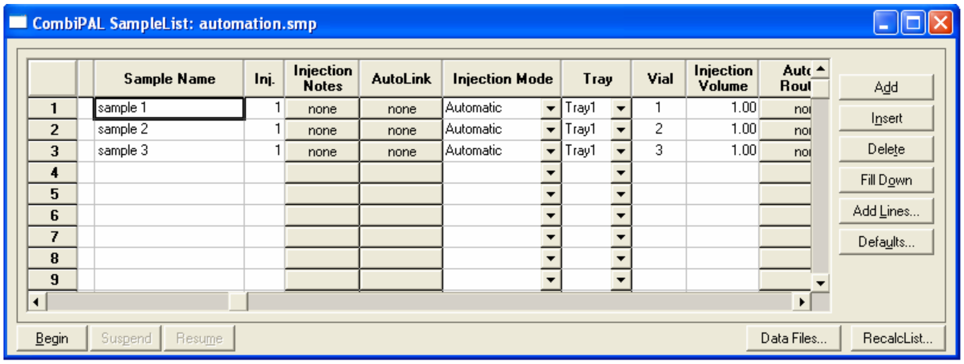
Save as type: SampleLists (*.smp)

The following screen will be displayed.



	Sample Name	Sample Type	Cal. level	Inj.	Injection Notes	AutoLink	Injection Mode	Tray	Vial	Injection Volume	Auton Routir
1	Default Sample	Analysis		1	none	none	Automatic	Tray1	1	1.00	none
2											
3											
4											
5											
6											
7											
8											
9											

Add the samples you wish to run to this list. Type in sample name and vial position information for your samples. Use the pull down arrow to select Analysis under Sample Type.



		Sample Name	Inj.	Injection Notes	AutoLink	Injection Mode	Tray	Vial	Injection Volume	Autc Rout
1		sample 1	1	none	none	Automatic	Tray1	1	1.00	no
2		sample 2	1	none	none	Automatic	Tray1	2	1.00	no
3		sample 3	1	none	none	Automatic	Tray1	3	1.00	no
4										
5										
6										
7										
8										
9										

To start automation, click on the **Begin** button in the bottom left corner. The autosampler will inject the sample contained in vial position 1, followed by the samples in positions 2 and 3.

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Qualitative Analysis of GC/MS Data

Analyzing GC/MS Data

Qualitative analysis to identify compounds present in a data file is one of the most common exercises performed by a GC/MS user. This tutorial will show you some of the basic operations that can be performed with the 2000 software to assist you in this task. The data file you will use for this exercise is found in the directory C:\VarianWS\MSTutorials. The files in this directory are also used in the tutorial Quantitative Analysis of GC/MS Data.

This tutorial is designed to run with the data file 80_ng.sms and the user library tutorial.lbr. The data files are extracts from chromatograms for semivolatile compounds run by split injection on the 2000 GC/MS.

Demonstration Files

The following extracted data files are present in the directory C:\VarianWS\MSTutorials:

10_ng.sms
20_ng.sms
40_ng.sms
50ng_ccc.sms
80_ng.sms
120_ng.sms
160_ng.sms
200_ng.sms

The files contain data on the following nine analytes.

Compound	**Retention Time(min)**
2,4-Dimethylphenol	7.32
bis(2-Chloroethoxy)methane	7.61
Benzoic acid	7.67
2,4-Dichlorophenol	7.75
1,2,4-Trichlorobenzene	7.98
Naphthalene-d8 (Internal Standard)	8.07
Naphthalene	8.13
4-Chloroaniline	8.48
Hexachlorobutadiene	8.72

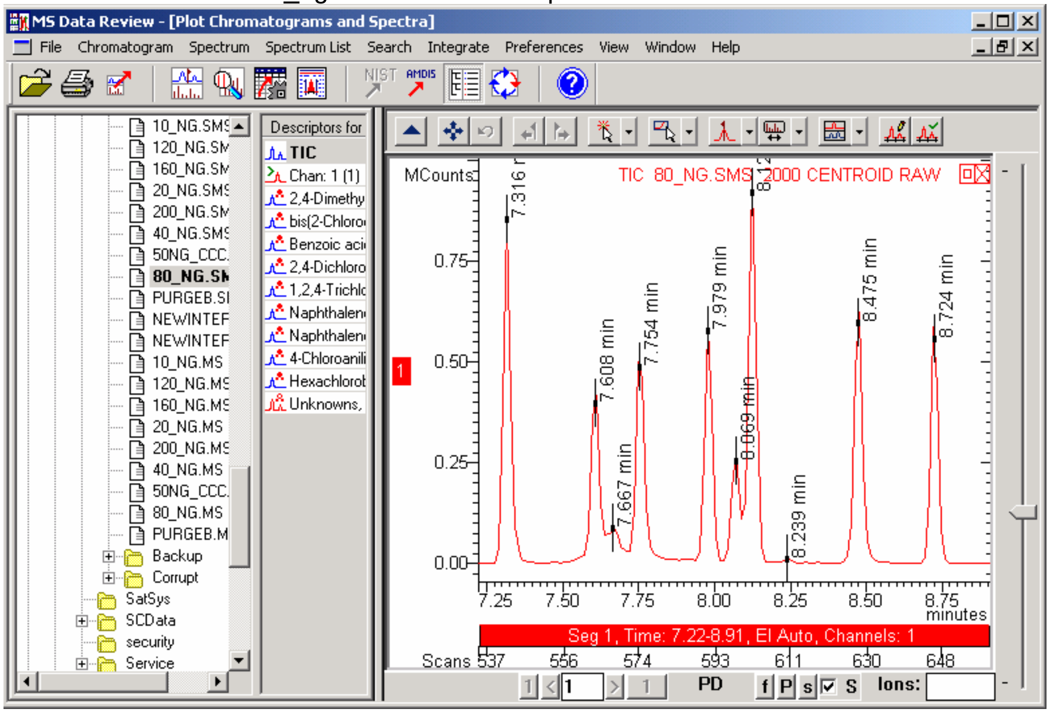
This set of compounds has been chosen to illustrate the principles of GC/MS qualitative identification as applied to real analytical results. For example, benzoic acid coelutes with bis(2-Chloroethoxy)methane and is poorly focused (because the DB-5 column used in the analysis is inappropriate for free acids). We will demonstrate in the tutorials how to deal with coeluting peaks to obtain clean mass spectra for library searching.

The concentration of the internal standard compound (naphthalene-d8) is 40 ng/µL in all data files, whereas the concentrations of the other analytes is indicated by the data file name.

To assist in qualitative identification of compounds in the data files, use the user library file, Tutorial.lbr, which is located in the directory C:\NIST02\MSSEARCH.

Displaying a Data File

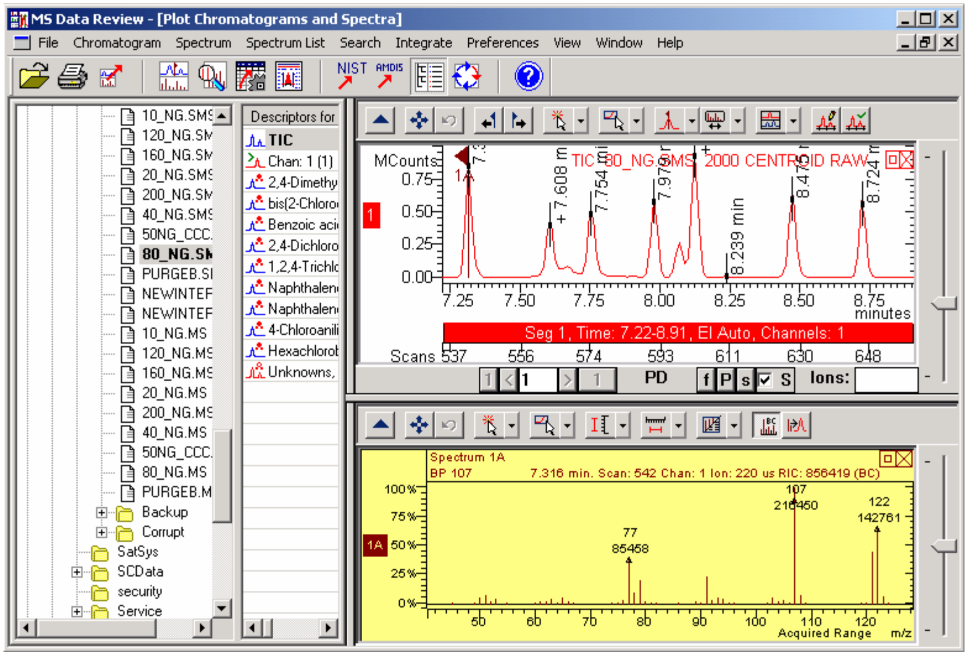
Open the MS Data Review application by clicking the **MS Data Review** button on the MS Workstation Toolbar. The **Plot Chromatograms and Spectra** window will appear on the screen. Select the C:\VarianWS\MSTutorial directory. Select the file 80_ng.sms and click to open it.



Notice that there are two toolbars in this display. The one on top is referred to as the MS Data Review toolbar. The lower toolbar is called the Chromatogram toolbar. If you are not yet familiar with the options in the Chromatogram toolbar, explore some of these features by holding the mouse cursor over each button and observing the tool-tip descriptions. You can select to show/hide the gain control and/or the acquisition segments by right-clicking in the chromatogram plot and choosing **Chromatogram Plot Preferences**. The Chromatogram Plot Preferences dialog opens where you can check/uncheck Show Gain Control and Show Acquisition Segments. Explore the other plot options that you can control in this dialog. Select Zoom Chromatogram as the Click and Drag Action and expand a portion of the chromatogram by clicking and dragging with the mouse. Place the cursor on each axis (you will see that the cursor symbol becomes a double arrow) and double click to restore the full scale on each axis. You can use the **Full Scale** button to restore the full scale on both axis.

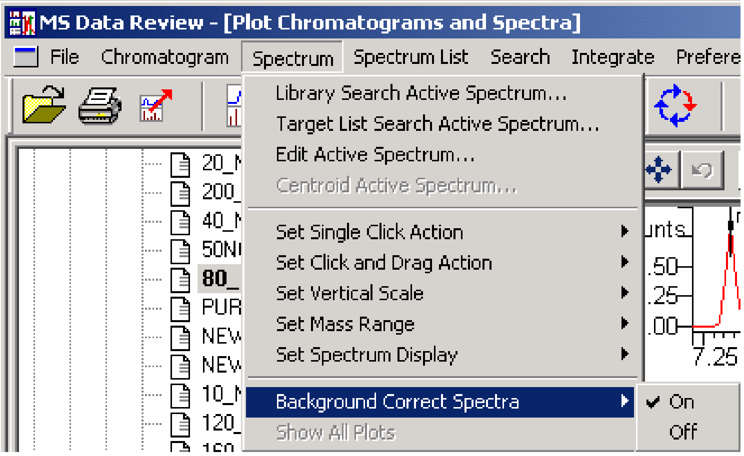
Choosing a Mass Spectrum

Click on the first peak in the chromatogram. The mass spectrum of the scan you selected is now displayed along with the chromatogram.

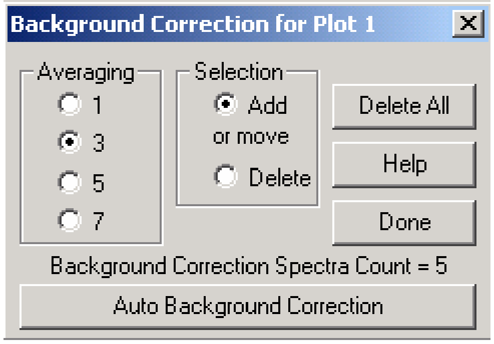


Background Correction

Background correction is a technique used to remove unwanted chemical background ions from a target spectrum to ensure that a more accurate Library Search can be run. Background points can be selected either manually or automatically; both procedures are accessible from the MS Data Review Window. First, make sure that **Background Correct Spectra** option is turned "ON" under the **Spectrum** menu in the MS Data Review window.



Alternatively, you can activate the **Show Background Correction Markers** button in the chromatogram toolbar. Select the command ***Chromatogram >Edit Background Correction*** in the **MS Data Review** menu. The **Background Correction for Plot 1** dialog opens and you can select to do a manual background correction or an automatic one. This process was also described in the **How to Perform a Background Correction** exercise earlier in this Tutorial Manual. Select the **Auto Background Correction** tab for this tutorial and then click **Done**.



Averaging: ○ 1 ● 3 ○ 5 ○ 7

Selection: ● Add or move ○ Delete

Background Correction Spectra Count = 5

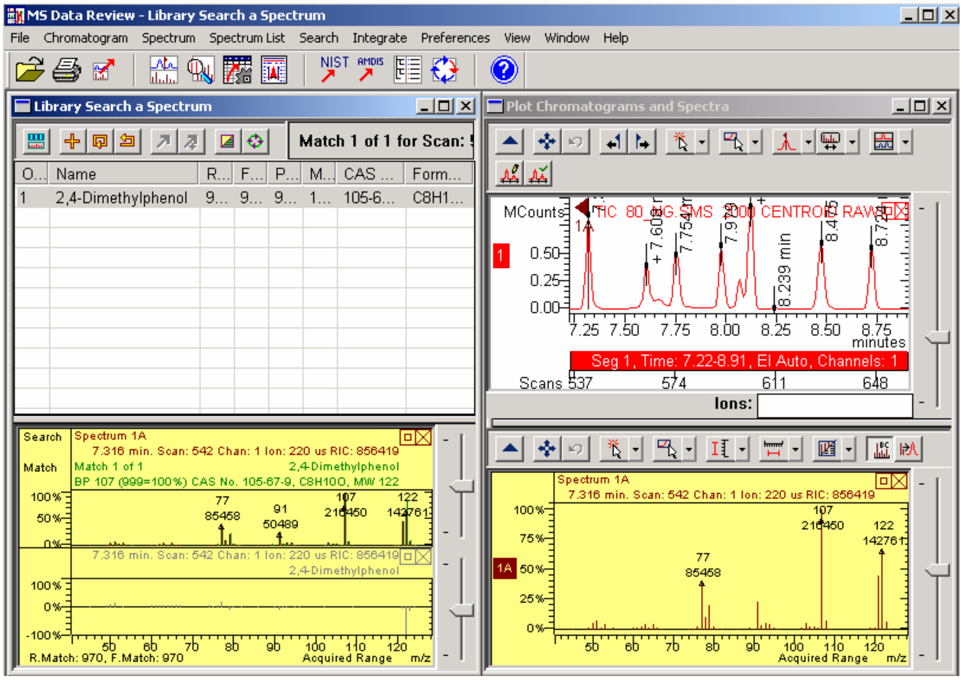
Select a peak of interest in the chromatogram for which you would like to see a spectrum. The label (BC) appears at the end of the scan specifications, displayed in the upper right corner of the spectrum window, indicating that a background corrected spectrum is displayed.

Running a Library Search on a Single Spectrum

Use the MS Data Review menu command ***Search >Select Search Libraries*** to open the NIST Library Manager dialog. Use the **Add Library** button to add the library, Tutorial.lbr. Inactivate any other libraries in the list. Click on **Done** to return to the MS Data Review window. Click on the **Search** button and select **Library Search Active Spectrum**. The results are displayed in the Library Search a Spectrum window.

NOTE: You can quickly Library Search other spectra by holding down the ‘L’ key on the keyboard while clicking on a peak in the displayed plot.

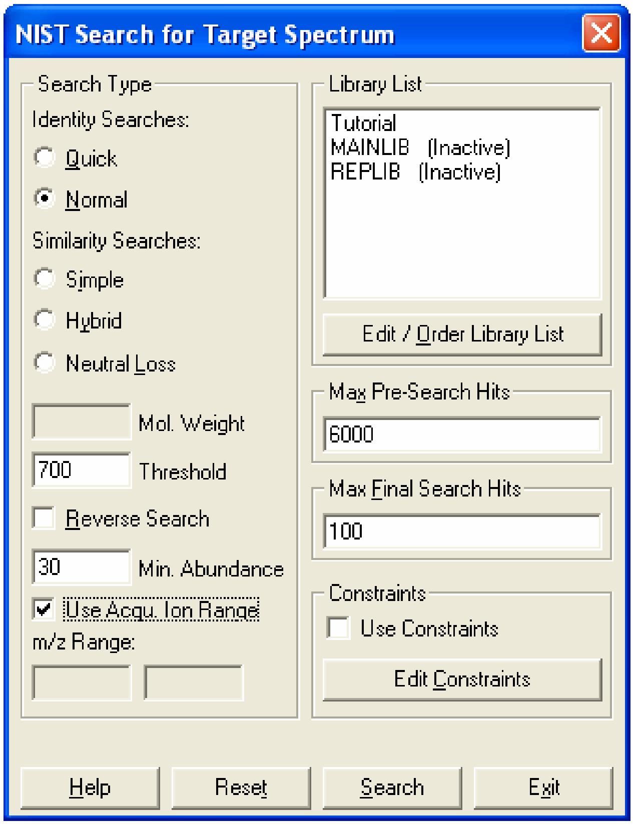
The following screen shows library search results for the first peak in the 80_ng.sms file.



O...	Name	R...	F...	P...	M...	CAS ...	Form...
1	2,4-Dimethylphenol	9...	9...	9...	1...	105-6...	C8H1...

When you select different matches in the list, a spectrum for each match is updated in the spectrum window below the list.

You can repeat the search and edit constraints or change the parameters that affect the identification process by clicking on the **Do NIST Search** button and selecting **Spectrum Search**. This will open the **NIST Search for Target Spectrum** dialog.



NIST Search for Target Spectrum

Search Type

Identity Searches:

○ Quick

● Normal

Similarity Searches:

○ Simple

○ Hybrid

○ Neutral Loss

Mol. Weight:

Threshold: 700

☐ Reverse Search

Min. Abundance: 30

☑ Use Acqu. Ion Range

m/z Range:

Library List

Tutorial
MAINLIB (Inactive)
REPLIB (Inactive)

Max Pre-Search Hits: 6000

Max Final Search Hits: 100

Constraints

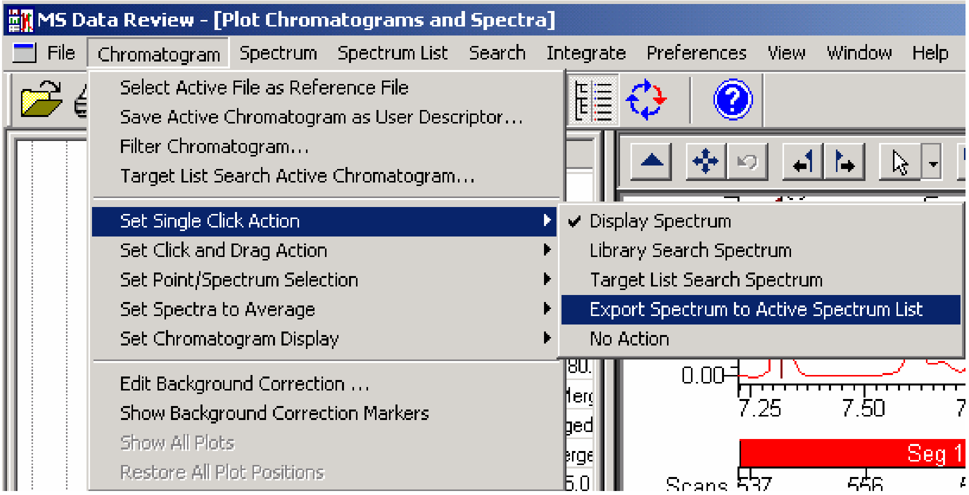
☐ Use Constraints

Creating a Spectrum List

In the MS Data Review menu select ***Spectrum List >Create New Spectrum List***. This command opens the **Create Spectrum List** dialog for typing the spectrum list file name and selecting the directory where it will be saved. When saving the newly created spectrum list you will be asked if you want to make this file the active file. Click **Yes**.

You can build the spectrum list automatically by selecting the command ***Spectrum List >Build Spectrum List from Active Chromatogram*** as described earlier in this manual in the **How to Build a Spectrum List** exercise.

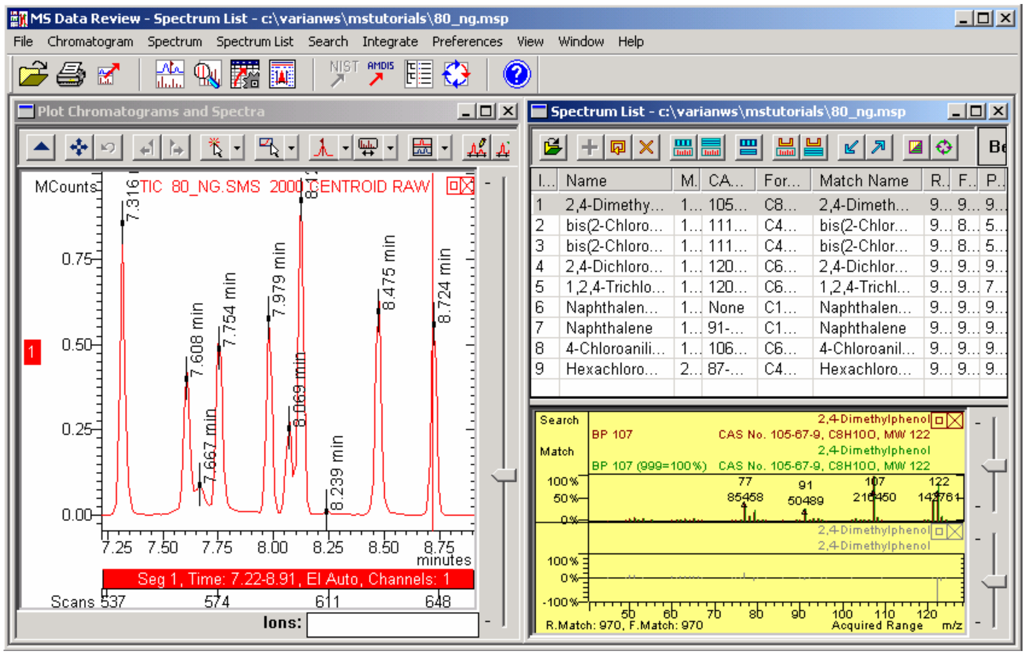
You can also add spectra to the spectrum list one by one by selecting the peaks of interest in the active chromatogram. In the MS Data Review menu select ***Chromatogram >Set Single Click Action >Export Spectrum to Active Spectrum List***.



Click on the first chromatogram peak in the 80_ng.sms. file. The spectrum corresponding to the selected scan is added to the active spectrum list. Similarly, you can add spectra corresponding to all peaks of interest to the active spectrum list. Now that you have created a List file, you can (1) library-search the compounds in the list, (2) search another chromatogram for the presence of those compounds. To stop adding peaks to the active spectrum list, select another **Single Click Action**, such as **Display Spectrum.**

Library Searching a Spectrum List

After all scans of interest were added to the spectrum list, perform a library search of the active spectrum list. In the Spectrum List window select the **Library Search Spectrum List** button . The **NIST Search for Target Spectrum** dialog opens. Use the default values and click **Search**. The search results are displayed in the Spectrum List window. To save the search results in the spectrum list, click the **Update all Searches with Matches** button .



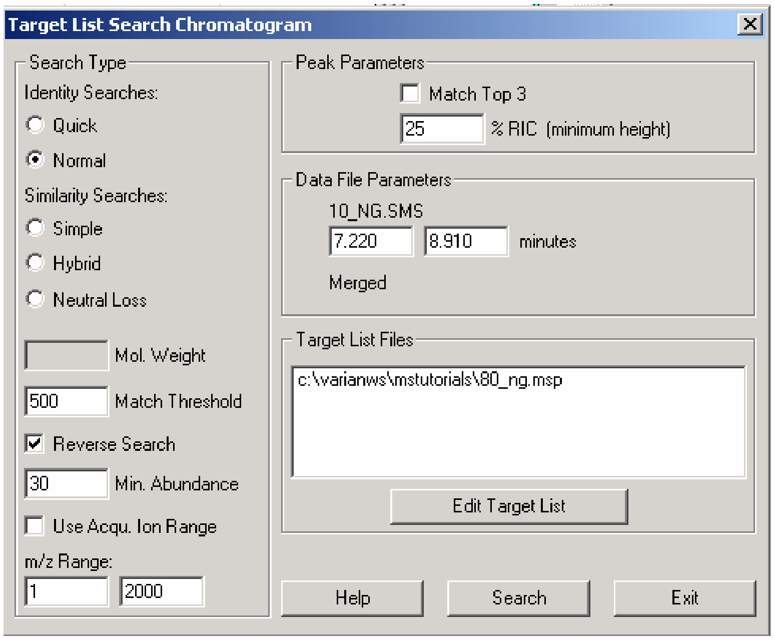
I...	Name	M.	CA...	For...	Match Name	R..	F..	P..
1	2,4-Dimethy...	1...	105...	C8...	2,4-Dimeth...	9...	9...	9...
2	bis(2-Chloro...	1...	111...	C4...	bis(2-Chlor...	9...	8...	5...
3	bis(2-Chloro...	1...	111...	C4...	bis(2-Chlor...	9...	8...	5...
4	2,4-Dichloro...	1...	120...	C6...	2,4-Dichlor...	9...	9...	9...
5	1,2,4-Trichlo...	1...	120...	C6...	1,2,4-Trichl...	9...	9...	7...
6	Naphthalen...	1...	None	C1...	Naphthalen...	9...	9...	9...
7	Naphthalene	1...	91-...	C1...	Naphthalene	9...	9...	9...
8	4-Chloroanili...	1...	106...	C6...	4-Chloroanil...	9...	9...	9...
9	Hexachloro...	2...	87-...	C4...	Hexachloro...	9...	9...	9...

Use the up and down arrows to highlight entries in the table and to examine the mass spectra. Note that you can adjust the splitter bar separating the display areas to size the fields to your preference.

Performing a Chromatogram Search with a Spectrum List

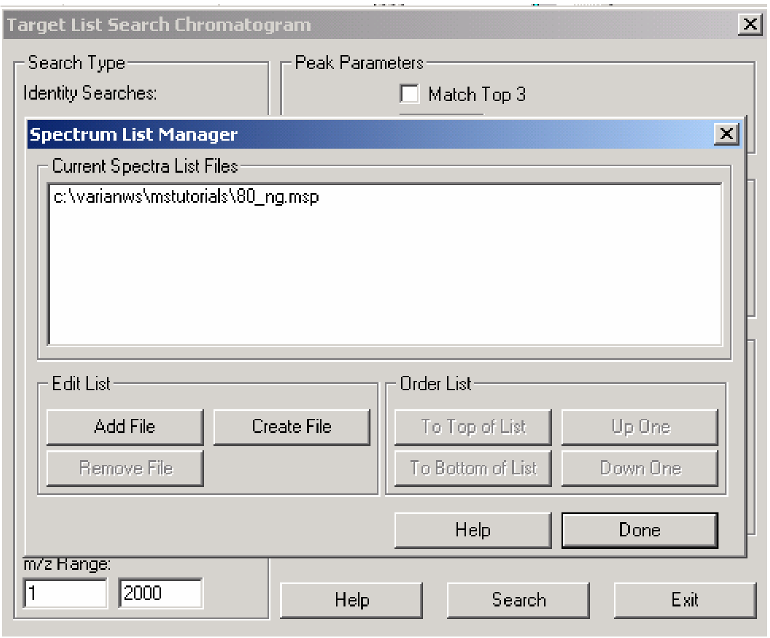
Once a Spectrum List has been created from a calibration data file, you can use the list to quickly determine whether those compounds are present in a GC/MS data file of an unknown. Choose the file *10_ng.sms* to be the unknown chromatogram, because this chromatogram also contains the nine analytes in the list you have just created, but at different concentration levels.

Use the menu command ***Search >Target List Search a Chromatogram >Select a Data File…*** This command will open the dialog **Select Data File** for selecting the chromatogram that you want to search. Select the file *10_ng.sms* and click the **Open File** button. The dialog **Target List Search Chromatogram** appears.



○ Quick
● Normal
○ Simple
○ Hybrid
○ Neutral Loss
Mol. Weight
500 Match Threshold
☑ Reverse Search
30 Min. Abundance
☐ Use Acqu. Ion Range
m/z Range: 1 2000
☐ Match Top 3
25 % RIC (minimum height)
10_NG.SMS
7.220 8.910 minutes
Merged
c:\varianws\mstutorials\80_ng.msp

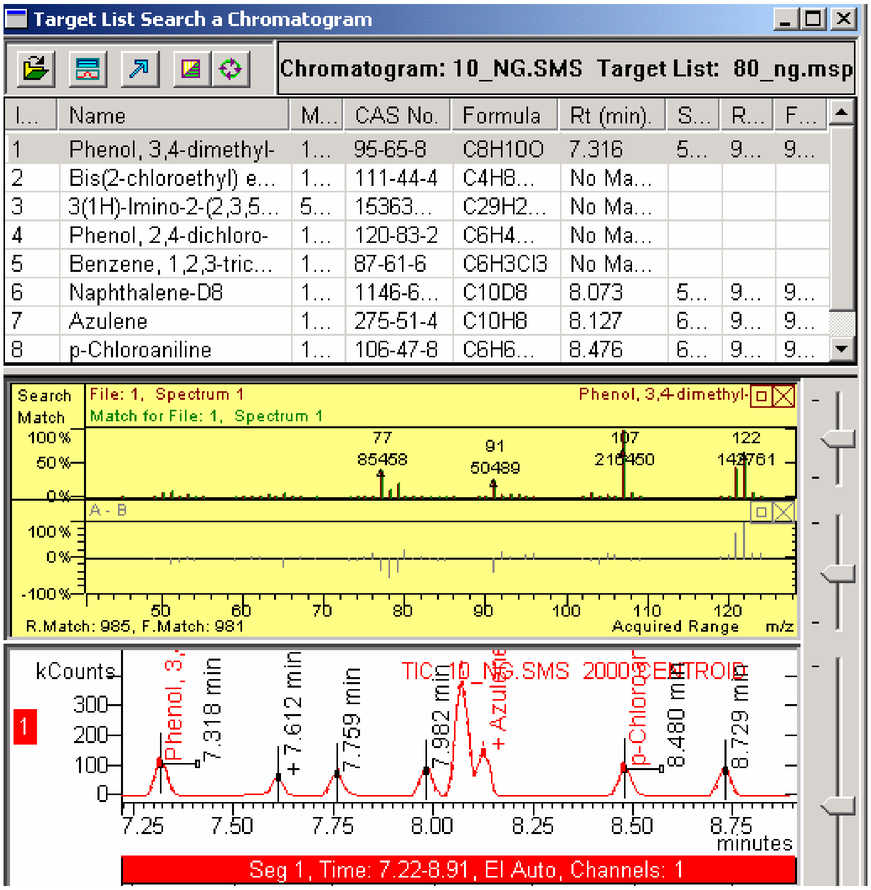
In the Target List Files click the **Edit Target List** button. This will open the **Spectrum List Manager** dialog from which you can select the list(s) used to search the chromatogram.



c:\varianws\mstutorials\80_ng.msp
m/z Range: 1 2000
☐ Match Top 3

Use the **Add File** and/or **Remove File** buttons as needed so that you select only the spectrum list previously created from the chromatogram file *80_ng.sms* (the spectrum list file is *80_ng.msp*). After you select the spectrum list, click **Done** and then **Search** in the **Target List Search Chromatogram** dialog.

The results will appear in the **Target List Search a Chromatogram** window.



Target List Search a Chromatogram

Chromatogram: 10_NG.SMS Target List: 80_ng.msp

I...	Name	M...	CAS No.	Formula	Rt (min)	S...	R...	F...
1	Phenol, 3,4-dimethyl-	1...	95-65-8	C8H10O	7.316	5...	9...	9...
2	Bis(2-chloroethyl) e...	1...	111-44-4	C4H8...	No Ma...			
3	3(1H)-Imino-2-(2,3,5...	5...	15363...	C29H2...	No Ma...			
4	Phenol, 2,4-dichloro-	1...	120-83-2	C6H4...	No Ma...			
5	Benzene, 1,2,3-tric...	1...	87-61-6	C6H3Cl3	No Ma...			
6	Naphthalene-D8	1...	1146-6...	C10D8	8.073	5...	9...	9...
7	Azulene	1...	275-51-4	C10H8	8.127	6...	9...	9...
8	p-Chloroaniline	1...	106-47-8	C6H6...	8.476	6...	9...	9...

Highlight a peak in the list and observe the mass spectrum and the peak location in the displays below. Use **Next** and **Previous** buttons to navigate the list.

Quantitative Analysis of GC/MS Data

Overview of the Quantitative Analysis Tutorials

This section contains eight tutorials which illustrate the concepts of quantitation on the 2000 GC/MS system. The tutorials have been designed to run with a set of data files and the user library *Tutorial.lbr*. The data files are extracts from chromatograms for semivolatile compounds run by split injection on the 2000 GC/MS. The same data files are used repeatedly to demonstrate different steps in the quantitation process.

Use the tutorials along with the text of Quantitation in the 2000 Software Reference Manual to learn how each step of the quantitation process on the 2000 GC/MS is carried out.

Demonstration Files

The following extracted data files are present in the directory C:\VarianWS\MSTutorials.

10_ng.sms
20_ng.sms
40_ng.sms
50_ccc.sms
80_ng.sms
120_ng.sms
160_ng.sms
200_ng.sms

These data files contain an extracted portion of chromatograms which contain data on the following nine analytes. The identities of the analytes and their retention times (in the 80_ng.sms file) are shown below.

Table of Analytes

Compound	Retention Time (min)
2,4-Dimethylphenol	7.32
bis(2-Chloroethoxy)methane	7.61
Benzoic acid	7.67
2,4-Dichlorophenol	7.75
1,2,4-Trichlorobenzene	7.98
Naphthalene-d8 (Internal Standard)	8.07
Naphthalene	8.13
4-Chloroaniline	8.48
Hexachlorobutadiene	8.72

This set of compounds has been chosen to illustrate the principles of GC/MS quantitation as applied to real analytical results. For example, benzoic acid coelutes with bis(2-Chloroethoxy)methane and is poorly focused (because the DB-5 column used in the analysis is inappropriate for free acids). We will demonstrate in the tutorials how to deal with coeluting peaks to obtain accurate quantitation results.

The concentration of the internal standard compound (naphthalene-d8) is 40 ng/µL in all data files, whereas the concentrations of the other analytes vary from 10 ng/µL up to 200 ng/µL, as indicated by the data file name. The inclusion of the internal standard at constant concentration allows us to build an internal standard calibration curve.

To assist in qualitative identification of compounds in the data files, use the user library file Tutorial.lbr which is located in the directory C:\NIST02\MSSEARCH\.

Tutorial Topics

The topics covered in this tutorial section are:

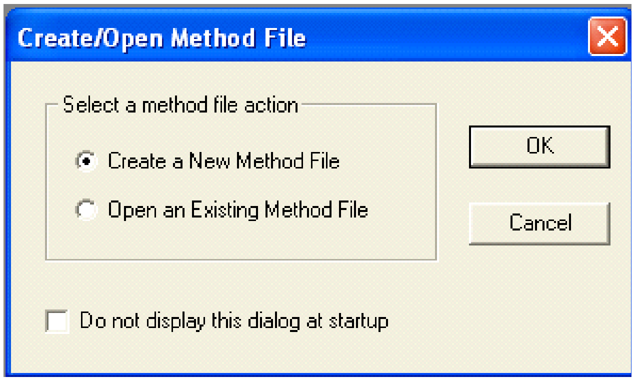
- Qualitative Identification
- Building a Data Handling Method
- Editing a Data Handling Method
- Building a Recalculation List for Calibration
- Processing a Recalculation List to Add Calibration Data
- Reviewing Calibration Results
- Processing Analysis Files in a Recalculation List
- Reviewing Analysis Results

Qualitative Identification

Before you can build a calibration file and quantitate unknown samples, you must analyze a standard sample at a known concentration level and identify the compounds of interest. If you have not already done so, review the tutorial “Qualitative Analysis of GC/MS Data”. If you have completed this tutorial, you have already verified the identity of the nine analytes in the file 80_ng.sms.

Editing a Data Handling Method

Select the **View /Edit Methods** button to create a new data handling method. In the **Create/Open Method File** dialog select **Create a New Method File** and press **OK**.



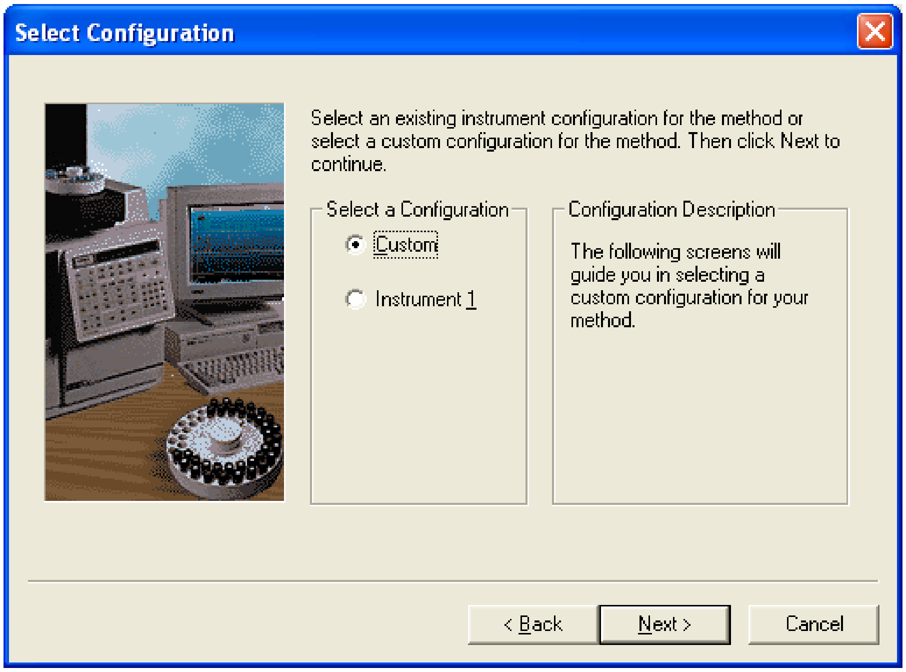
Select a method file action

● Create a New Method File

○ Open an Existing Method File

☐ Do not display this dialog at startup

Select **Custom** in the **Select Configuration** dialog.



Select an existing instrument configuration for the method or select a custom configuration for the method. Then click Next to continue.

Select a Configuration

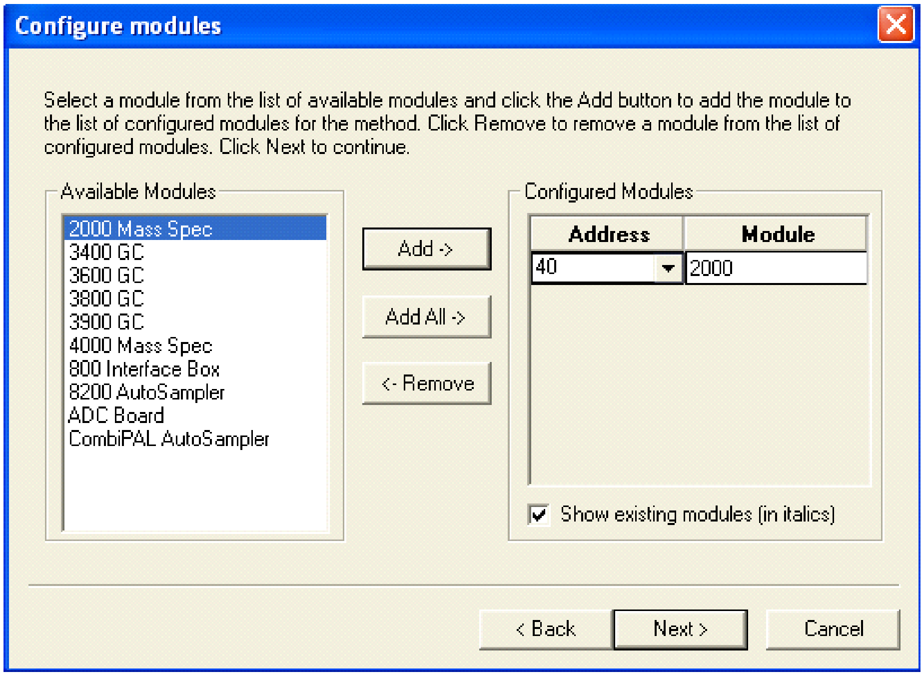
● Custom

○ Instrument 1

Configuration Description

The following screens will guide you in selecting a custom configuration for your method.

Select 2000 Mass Spec from the **Configure Modules** dialog, click the **Add** button and then **Next.**



Select a module from the list of available modules and click the Add button to add the module to the list of configured modules for the method. Click Remove to remove a module from the list of configured modules. Click Next to continue.

Available Modules

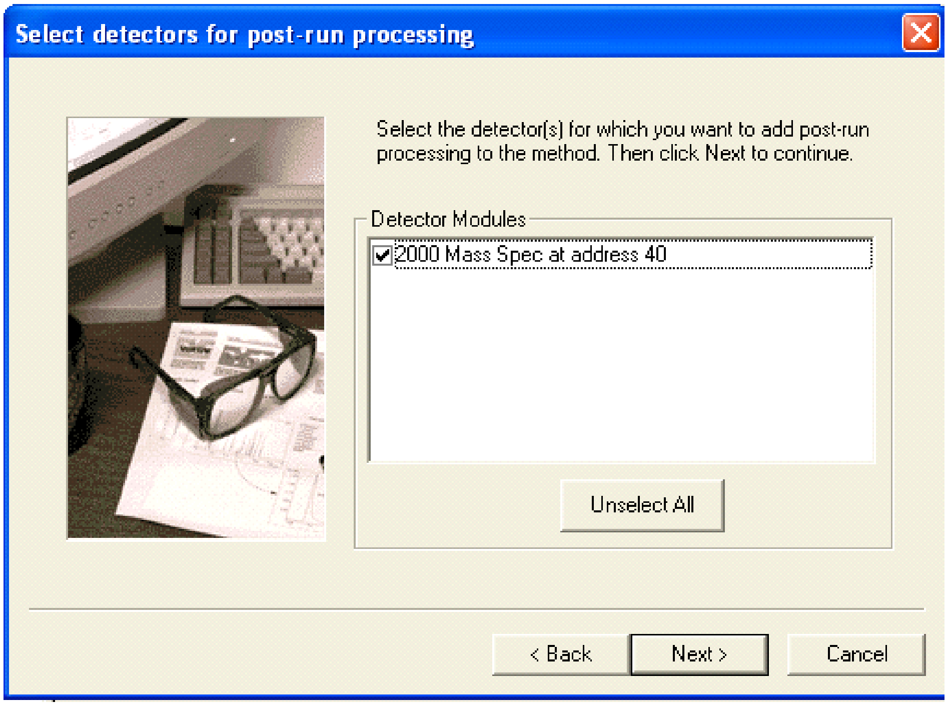
- 2000 Mass Spec
- 3400 GC
- 3600 GC
- 3800 GC
- 3900 GC
- 4000 Mass Spec
- 800 Interface Box
- 8200 AutoSampler
- ADC Board
- CombiPAL AutoSampler

Configured Modules

Address	Module
40	2000

☑ Show existing modules (in italics)

Check 2000 Mass Spec in the Detector Modules field and click **Next**.

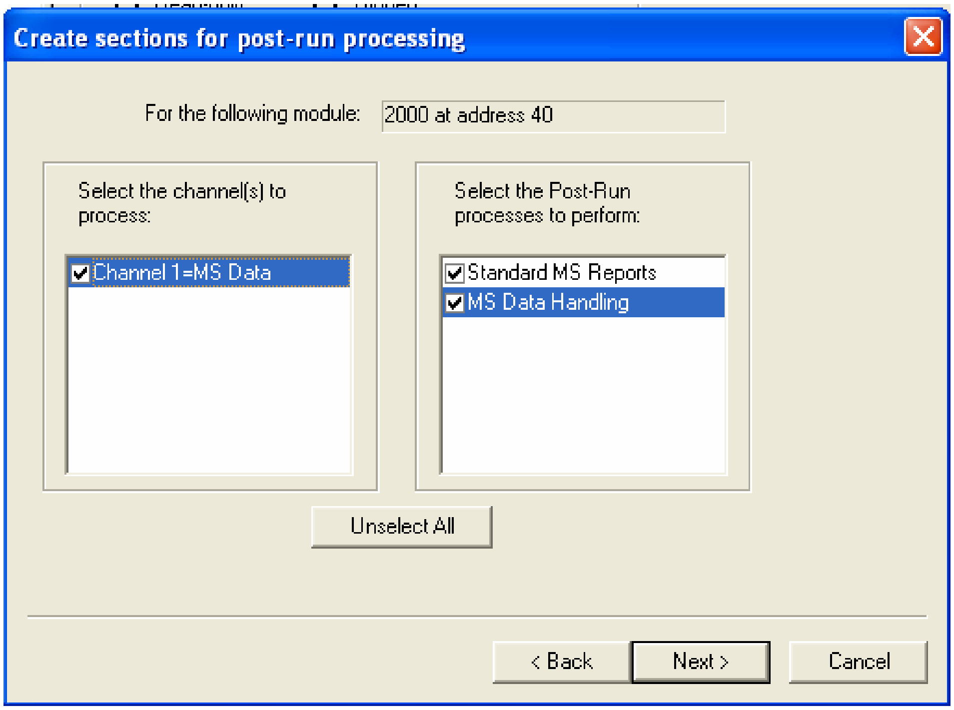


Select the detector(s) for which you want to add post-run processing to the method. Then click Next to continue.

Detector Modules

☑ 2000 Mass Spec at address 40

Select **Channel 1=MS Data**, **Standard MS Reports** and **MS Data Handling** in the **Create sections for post-run processing** dialog and then click **Next.**


Create sections for post-run processing

For the following module: 2000 at address 40

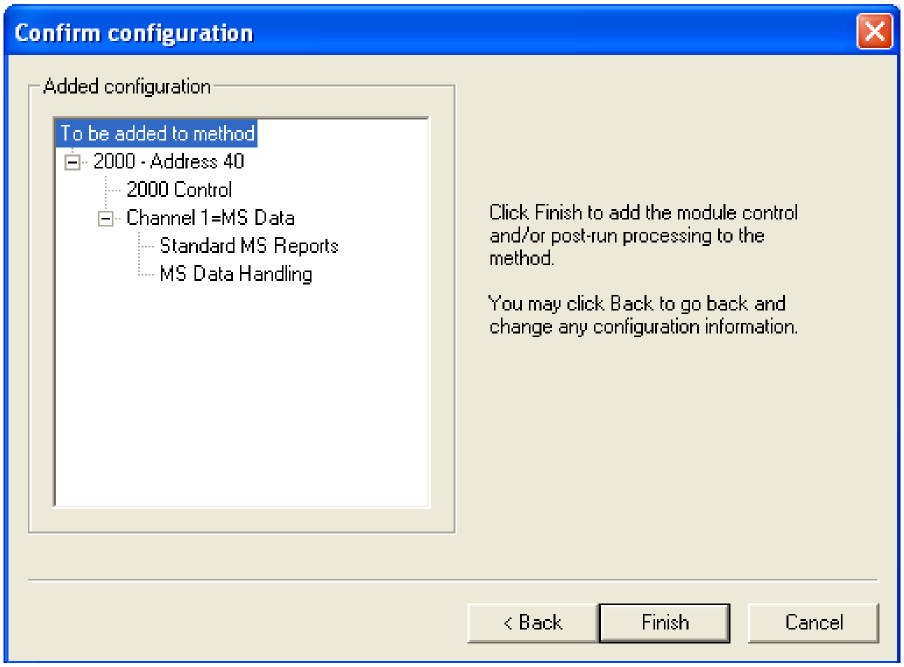
Select the channel(s) to process:

☑ Channel 1=MS Data

Select the Post-Run processes to perform:

☑ Standard MS Reports
☑ MS Data Handling

To confirm configuration and post-run processes, click **Finish** in the next dialog.


Confirm configuration

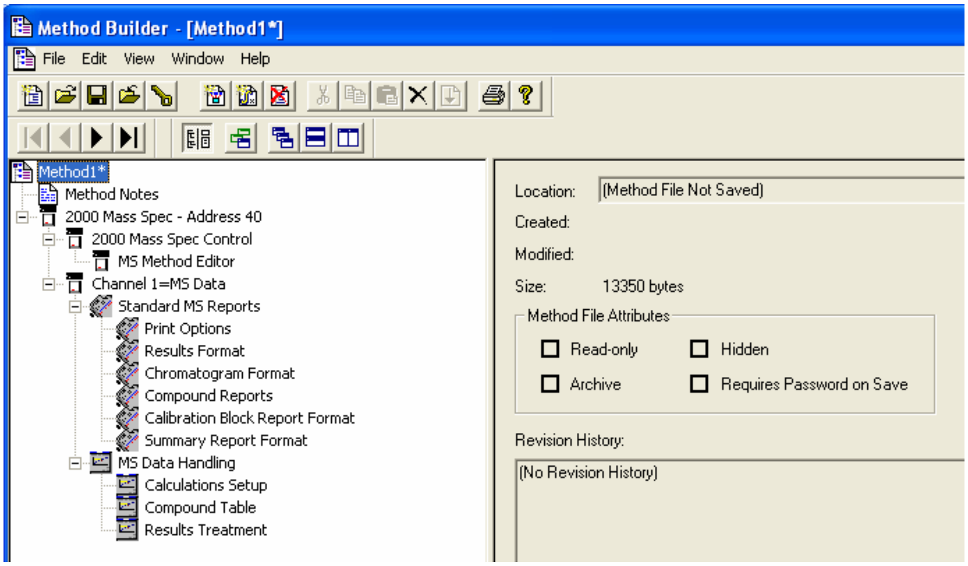
Added configuration

- To be added to method
  - 2000 - Address 40
    - 2000 Control
    - Channel 1=MS Data
      - Standard MS Reports
      - MS Data Handling

Click Finish to add the module control and/or post-run processing to the method.

You may click Back to go back and change any configuration information.

Following these actions, the Method Builder dialog will open with a directory tree on the left side showing an outline of the sections available for editing.



Location: (Method File Not Saved)

Created:

Modified:

Size: 13350 bytes

Method File Attributes

☐ Read-only ☐ Hidden

☐ Archive ☐ Requires Password on Save

Revision History:

(No Revision History)

As shown in the directory tree, the MS Data Handling method contains three sections:

Calculations Setup - where you will set global parameters for calculation type (Area/Height; Internal/External Std.) and for processing of unknown peaks in the chromatogram

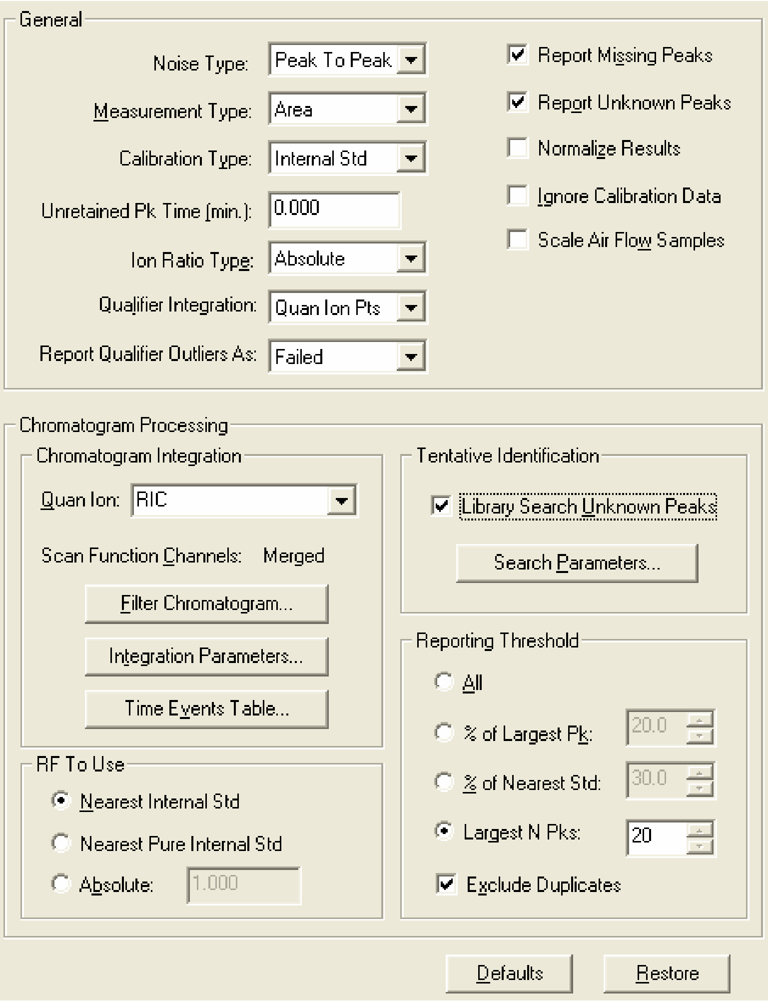
Compound Table - where you will identify internal standards, set calibration concentration levels and adjust quantitation ions and other compound-specific parameters

Results Treatment - where you will set additional rules for Calibration replicates, and set rules for the treatment of failed peaks in Analysis and Verification samples

As you explore the parameters in these data handling method sections, remember that you can discard any edits you have made in a dialog by clicking on the **Restore** button.

The Calculations Setup Dialog Box

Click on the Calculations Setup section. Set the parameters as shown next.



General

Noise Type: Peak To Peak
Measurement Type: Area
Calibration Type: Internal Std
Unretained Pk Time (min.): 0.000
Ion Ratio Type: Absolute
Qualifier Integration: Quan Ion Pts
Report Qualifier Outliers As: Failed

☑ Report Missing Peaks
☑ Report Unknown Peaks
☐ Normalize Results
☐ Ignore Calibration Data
☐ Scale Air Flow Samples

Chromatogram Processing

Chromatogram Integration
Quan Ion: RIC
Scan Function Channels: Merged

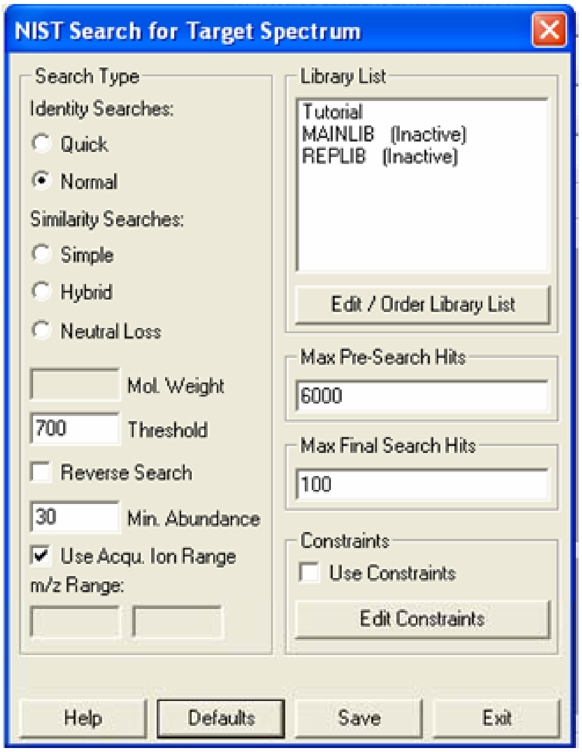
Tentative Identification
☑ Library Search Unknown Peaks

Reporting Threshold
○ All
○ % of Largest Pk: 20.0
○ % of Nearest Std: 30.0
● Largest N Pks: 20
☑ Exclude Duplicates

RF To Use
● Nearest Internal Std
○ Nearest Pure Internal Std
○ Absolute: 1.000

Explore the other features if you like, but use these defaults for this tutorial. The upper **General** section now specifies to do measurements by Peak Area and calibrate via an Internal Standard approach. Missing and Unknown peaks will be reported. In the **Chromatogram Processing** section, observe that the Reconstructed Ion Current (RIC) will be used for integrating unknown peaks and they will be quantified by using the RF of the nearest internal standard. Additionally, the Unknown peaks will be Library-searched using the tutorial.lbr library. Any Unknowns identified by the library search will become Tentatively Identified Compounds (TICs). In data handling Reports, results for TICs will include their library search name and CAS Number (if these are present in the library entry of the match spectrum). Finally, we have chosen to report only the largest 20 Unknown peaks in the chromatogram. The Exclude Duplicates choice prevents peaks which have been identified as Target Compounds from also being reported as unknowns or TICs.

Select **Library Search Unknown Peaks**, click **Search Parameters** and use the same parameters shown next:



NIST Search for Target Spectrum

Search Type

Identity Searches:

○ Quick

● Normal

Similarity Searches:

○ Simple

○ Hybrid

○ Neutral Loss

Mol. Weight:

Threshold: 700

☐ Reverse Search

Min. Abundance: 30

☑ Use Acqu. Ion Range

m/z Range:

Library List

Tutorial
MAINLIB (Inactive)
REPLIB (Inactive)

Max Pre-Search Hits: 6000

Max Final Search Hits: 100

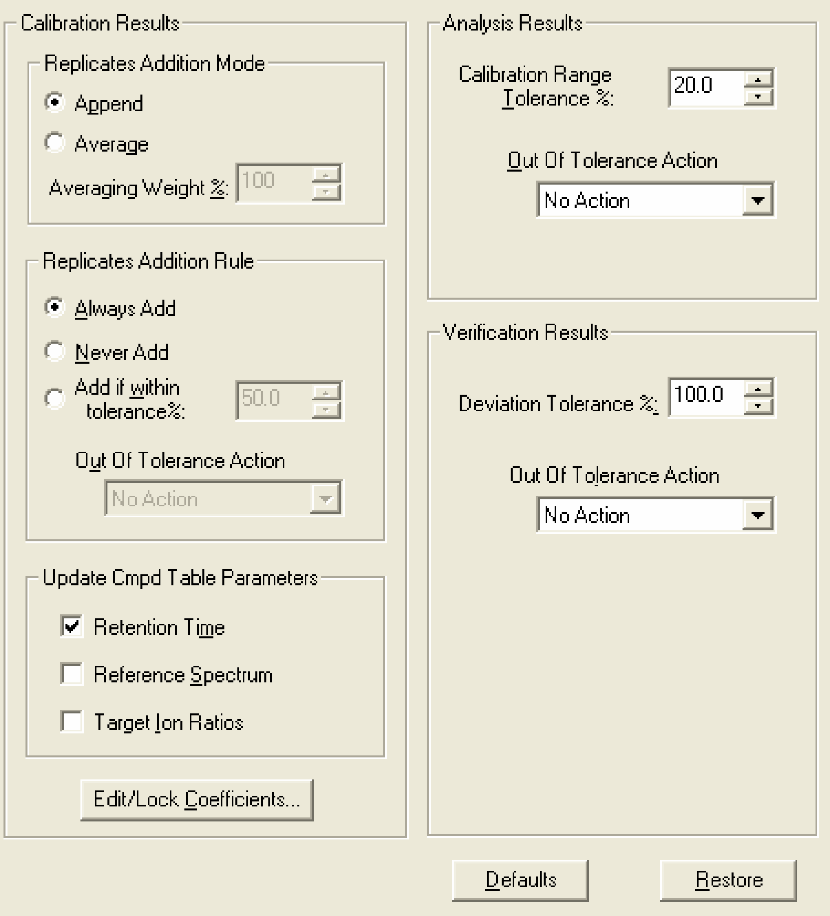
Constraints

☐ Use Constraints

Press **Save** to return to the **Calculations Setup** dialog.

NOTE: Although we have briefly explained the chromatogram processing parameters, this tutorial does not deal with processing unknown peaks. Refer to the Software Reference Manual for an in-depth discussion.

The Results Treatment Dialog Box



● Append
○ Average
Averaging Weight %: 100

● Always Add
○ Never Add
○ Add if within tolerance%: 50.0
Out Of Tolerance Action: No Action

☑ Retention Time
☐ Reference Spectrum
☐ Target Ion Ratios

Calibration Range Tolerance %: 20.0
Out Of Tolerance Action: No Action

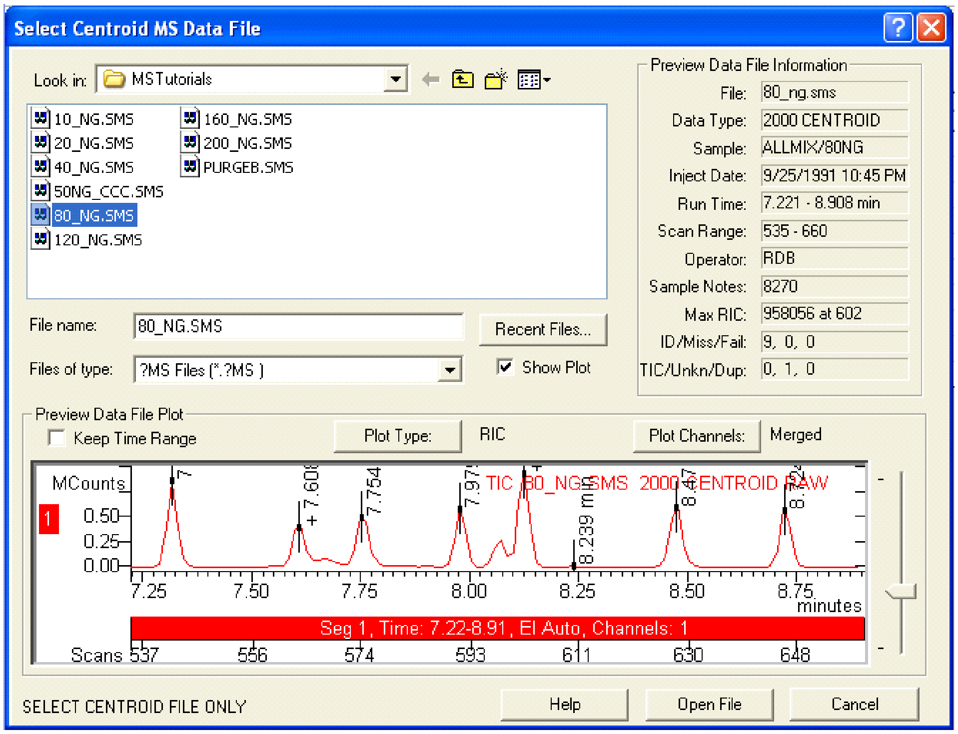
Deviation Tolerance %: 100.0
Out Of Tolerance Action: No Action

Click on the **Results Treatment** dialog in the Method Directory. Explore the options available in each section of the dialog. For this tutorial, use the values shown for each field.

Note that rules have been set for tolerances and Out-of-Tolerance Actions for Calibration, Analysis, and Verification samples.

The Compound Table

When you select the compound table from the directory tree, the compound table dialog is opened as well as the **Select Centroid MS Data File** dialog to open the file used to build the compound table. Select the file 80_ng_sms from the MS Tutorials folder and click on the **Open File** button.



Select Centroid MS Data File

Look in: MSTutorials

10_NG.SMS
20_NG.SMS
40_NG.SMS
50NG_CCC.SMS
80_NG.SMS
120_NG.SMS
160_NG.SMS
200_NG.SMS
PURGEB.SMS

File name: 80_NG.SMS

Files of type: ?MS Files (*.?MS)

☑ Show Plot

Preview Data File Information

File: 80_ng.sms
Data Type: 2000 CENTROID
Sample: ALLMIX/80NG
Inject Date: 9/25/1991 10:45 PM
Run Time: 7.221 - 8.908 min
Scan Range: 535 - 660
Operator: RDB
Sample Notes: 8270
Max RIC: 958056 at 602
ID/Miss/Fail: 9, 0, 0
TIC/Unkn/Dup: 0, 1, 0

Preview Data File Plot

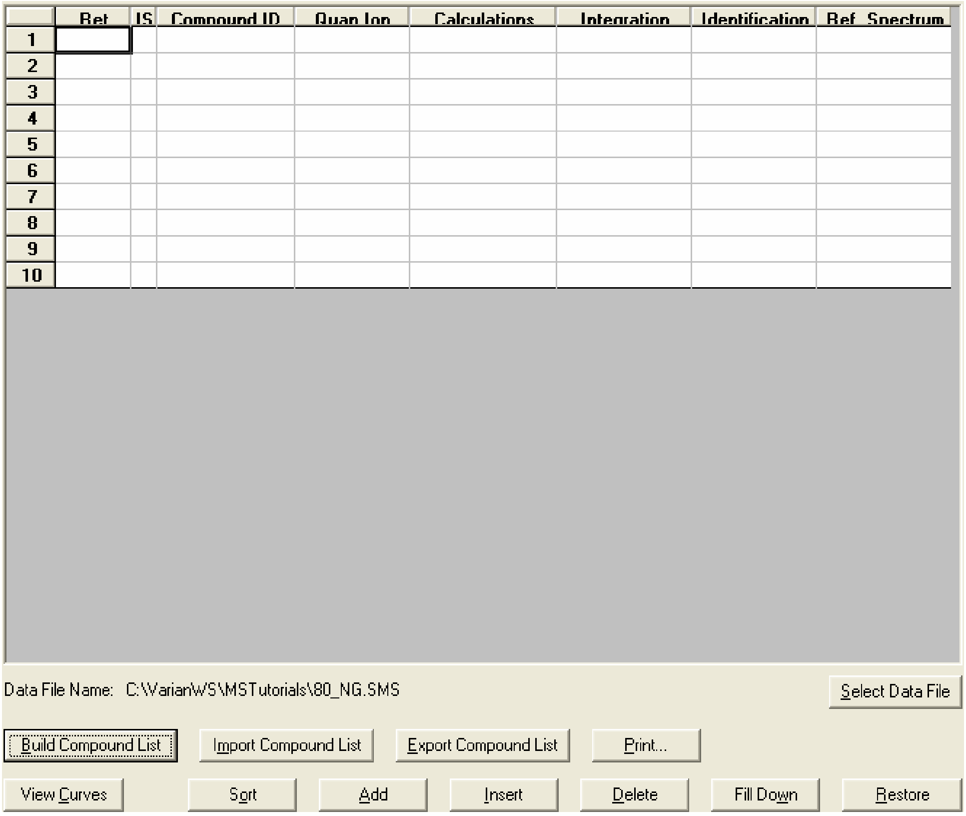
☐ Keep Time Range

Plot Type: RIC

Plot Channels: Merged

SELECT CENTROID FILE ONLY

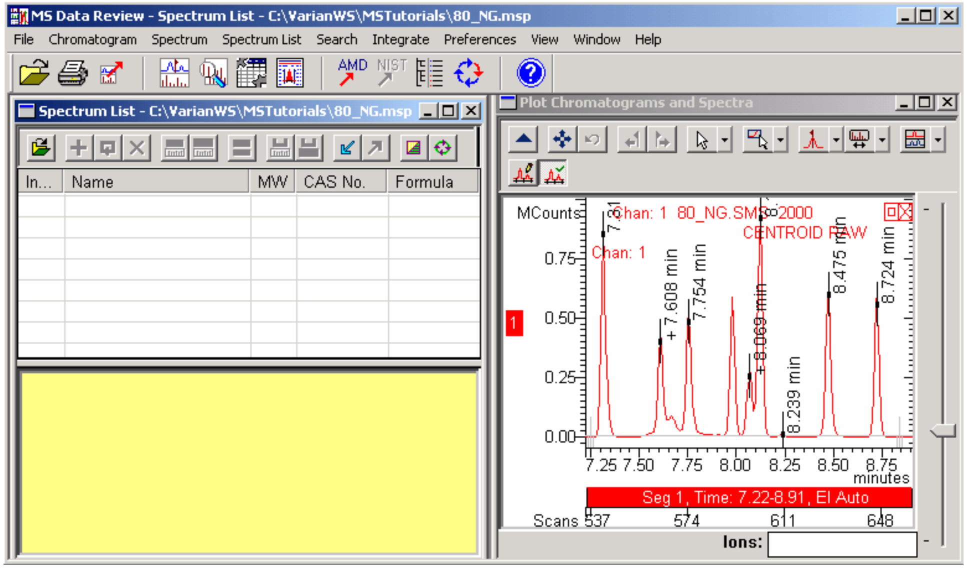
The **Data File Name** field in the compound table dialog shows the file that is being opened:



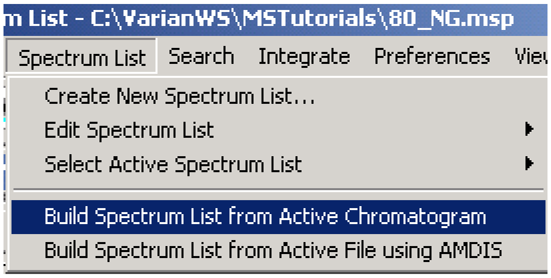
	Ret	IS	Compound ID	Quan Ion	Calculations	Integration	Identification	Ref. Spectrum
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Data File Name: C:\VarianWS\MSTutorials\80_NG.SMS

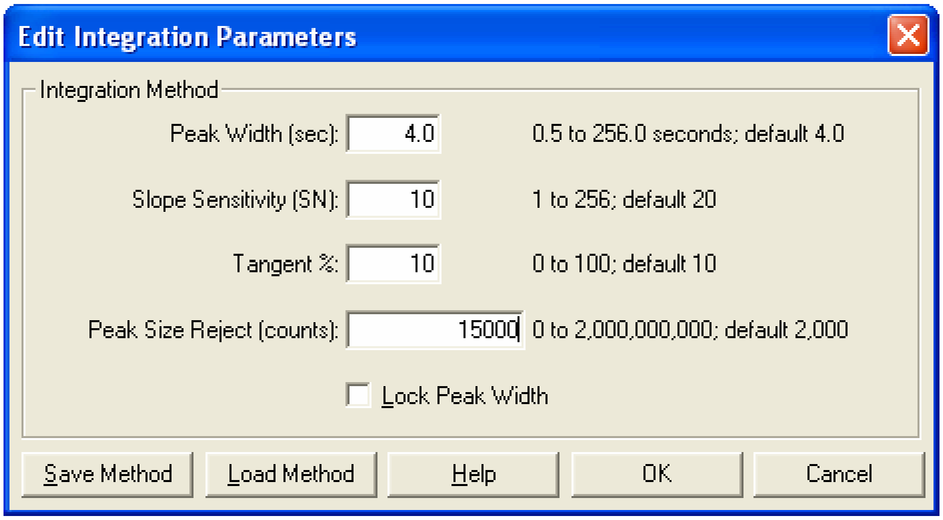
Click on the **Build Compound List** button to create a new compound list. This action opens the MS Data Review application:



Select the **Spectrum List >Build Spectrum List from Active Chromatogram** command.



The **Edit Integration Parameters** dialog is opened. By using the default values, the list will include the compound Hexachlorobutadiene at a retention time of about 8.233 minutes, besides the nine compounds that we use for quantitation in this tutorial. The area of this peak is just below 1500 so, to have the software ignore this peak set the Peak Size Reject (counts) parameter to 15000 and click **OK**. Also, change the Slope Sensitivity(S/N) setting to 10 in order to find the Benzoic Acid peak. Click **OK** to process the file.



Edit Integration Parameters

Integration Method

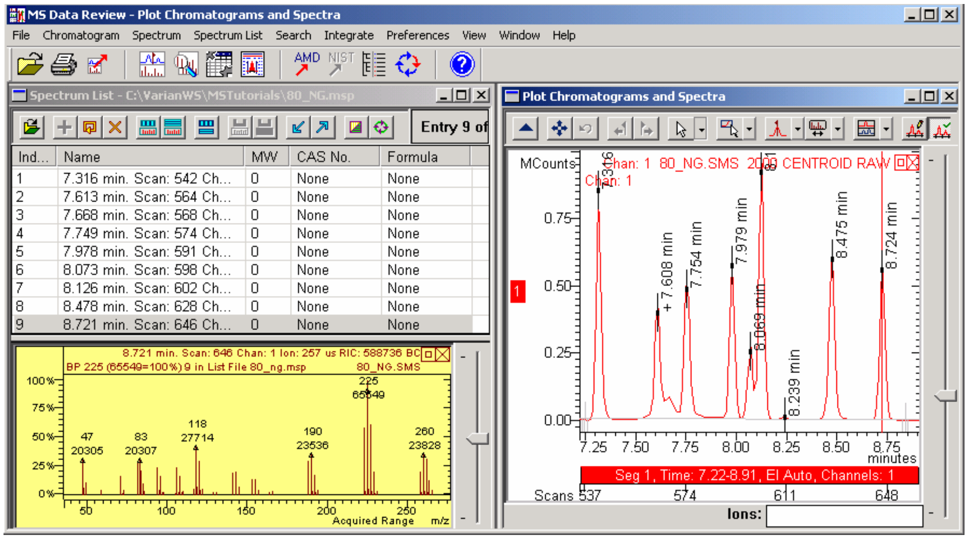
Peak Width (sec): 4.0 0.5 to 256.0 seconds; default 4.0

Slope Sensitivity (SN): 10 1 to 256; default 20

Tangent %: 10 0 to 100; default 10

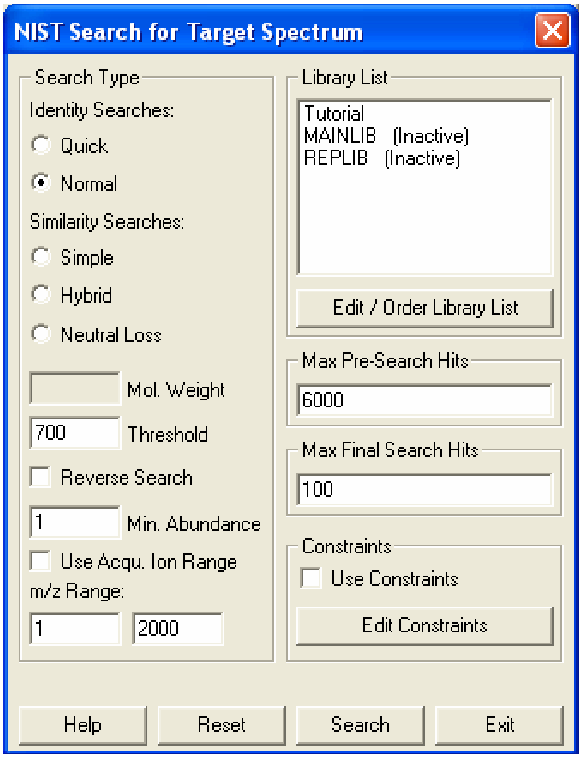
Peak Size Reject (counts): 15000 0 to 2,000,000,000; default 2,000

☐ Lock Peak Width



Ind...	Name	MW	CAS No.	Formula
1	7.316 min. Scan: 542 Ch...	0	None	None
2	7.613 min. Scan: 564 Ch...	0	None	None
3	7.668 min. Scan: 568 Ch...	0	None	None
4	7.749 min. Scan: 574 Ch...	0	None	None
5	7.978 min. Scan: 591 Ch...	0	None	None
6	8.073 min. Scan: 598 Ch...	0	None	None
7	8.126 min. Scan: 602 Ch...	0	None	None
8	8.478 min. Scan: 628 Ch...	0	None	None
9	8.721 min. Scan: 646 Ch...	0	None	None

Select the **Library Search Spectrum List** button in the **Spectrum List** window and use the Tutorial library for searching the spectrum list. In the **NIST Search for Target Spectrum** dialog click the **Edit/Order Library List** tab.

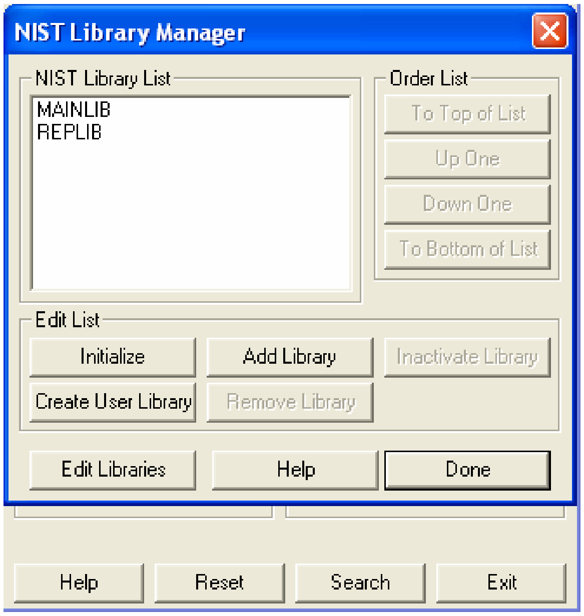


○ Quick
● Normal
○ Simple
○ Hybrid
○ Neutral Loss

Mol. Weight:
Threshold: 700
☐ Reverse Search
Min. Abundance: 1
☐ Use Acqu. Ion Range
m/z Range: 1 2000

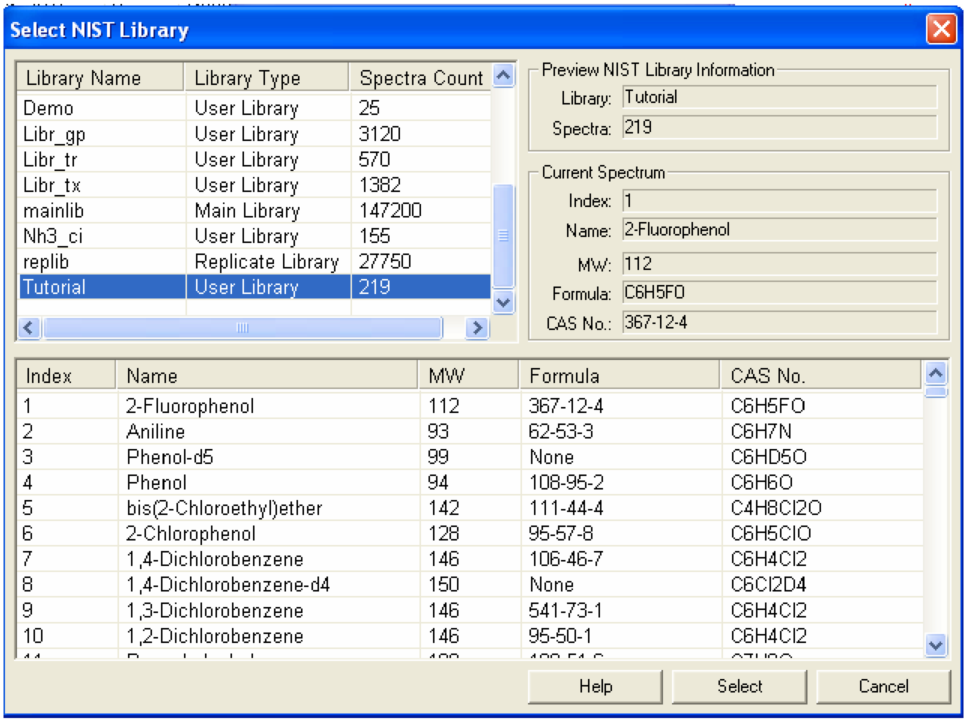
Library List:
Tutorial
MAINLIB (Inactive)
REPLIB (Inactive)

Max Pre-Search Hits: 6000
Max Final Search Hits: 100
☐ Use Constraints



NIST Library List
MAINLIB
REPLIB

If Tutorial library is not listed, click the **Add Libraries** tab. Select the Tutorial Library from the list and click **Select**.



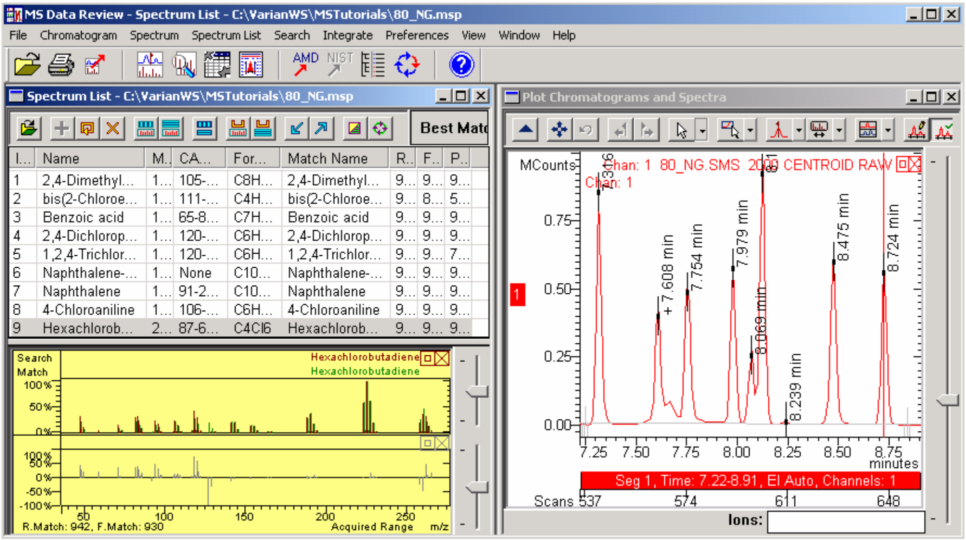
Library Name	Library Type	Spectra Count
Demo	User Library	25
Libr_gp	User Library	3120
Libr_tr	User Library	570
Libr_tx	User Library	1382
mainlib	Main Library	147200
Nh3_ci	User Library	155
replib	Replicate Library	27750
Tutorial	User Library	219

Preview NIST Library Information
Library: Tutorial
Spectra: 219

Current Spectrum
Index: 1
Name: 2-Fluorophenol
MW: 112
Formula: C6H5FO
CAS No.: 367-12-4

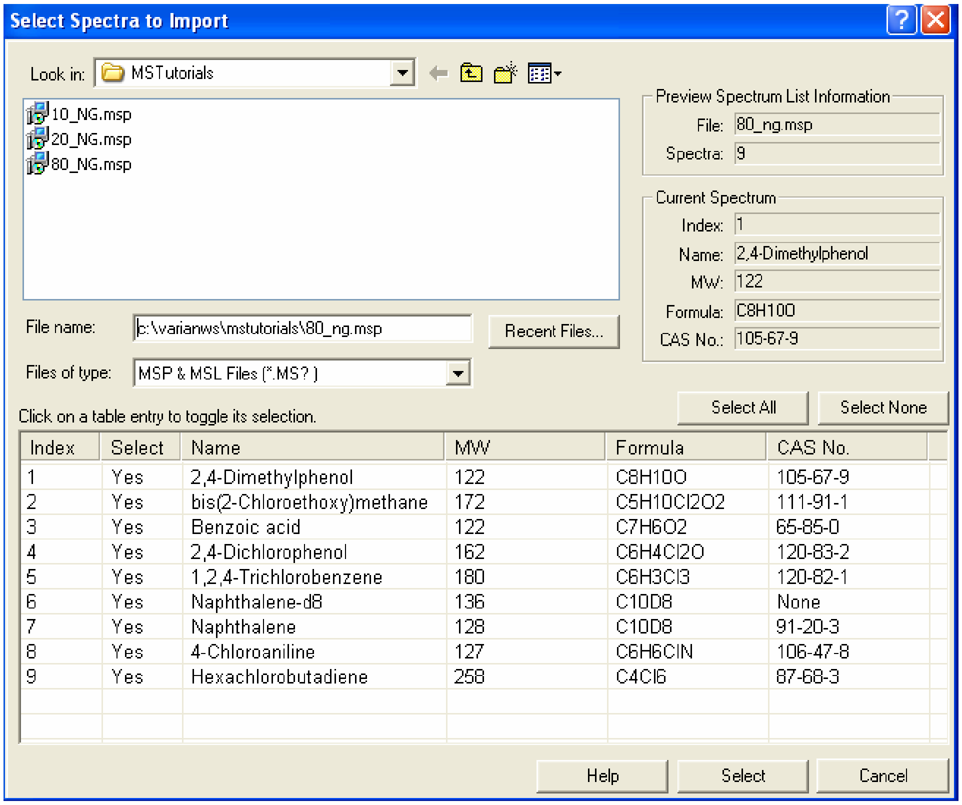
Index	Name	MW	Formula	CAS No.
1	2-Fluorophenol	112	367-12-4	C6H5FO
2	Aniline	93	62-53-3	C6H7N
3	Phenol-d5	99	None	C6HD5O
4	Phenol	94	108-95-2	C6H6O
5	bis(2-Chloroethyl)ether	142	111-44-4	C4H8Cl2O
6	2-Chlorophenol	128	95-57-8	C6H5ClO
7	1,4-Dichlorobenzene	146	106-46-7	C6H4Cl2
8	1,4-Dichlorobenzene-d4	150	None	C6Cl2D4
9	1,3-Dichlorobenzene	146	541-73-1	C6H4Cl2
10	1,2-Dichlorobenzene	146	95-50-1	C6H4Cl2

Select the other libraries listed in the **NIST Library Manager** dialog (in the picture above they are MAINLIB and REPLIB) and click the **Inactivate Library** tab. Press **Done** to close this dialog and then **Search** in the **NIST Search for Target Spectrum** dialog. Save the search results by selecting the **Update All Searches with Matches** button.



I...	Name	M...	CA...	For...	Match Name	R...	F...	P...
1	2,4-Dimethyl...	1...	105-...	C8H...	2,4-Dimethyl...	9...	9...	9...
2	bis(2-Chloroe...	1...	111-...	C4H...	bis(2-Chloroe...	9...	8...	5...
3	Benzoic acid	1...	65-8...	C7H...	Benzoic acid	9...	9...	9...
4	2,4-Dichlorop...	1...	120-...	C6H...	2,4-Dichlorop...	9...	9...	9...
5	1,2,4-Trichlor...	1...	120-...	C6H...	1,2,4-Trichlor...	9...	9...	7...
6	Naphthalene-...	1...	None	C10...	Naphthalene-...	9...	9...	9...
7	Naphthalene	1...	91-2...	C10...	Naphthalene	9...	9...	9...
8	4-Chloroaniline	1...	106-...	C6H...	4-Chloroaniline	9...	9...	9...
9	Hexachlorob...	2...	87-6...	C4Cl6	Hexachlorob...	9...	9...	9...

Close the MS Data Review application. In the **Method Builder** dialog click on the **Import Compound List** button. You will be prompted to a dialog for selecting the spectrum list. The compound list has the name of the file it was created from (in our case it is 80_ng.msp).



Look in: MSTutorials

10_NG.msp
20_NG.msp
80_NG.msp

File name: c:\varianws\mstutorials\80_ng.msp

Files of type: MSP & MSL Files (*.MS?)

Preview Spectrum List Information

File: 80_ng.msp
Spectra: 9

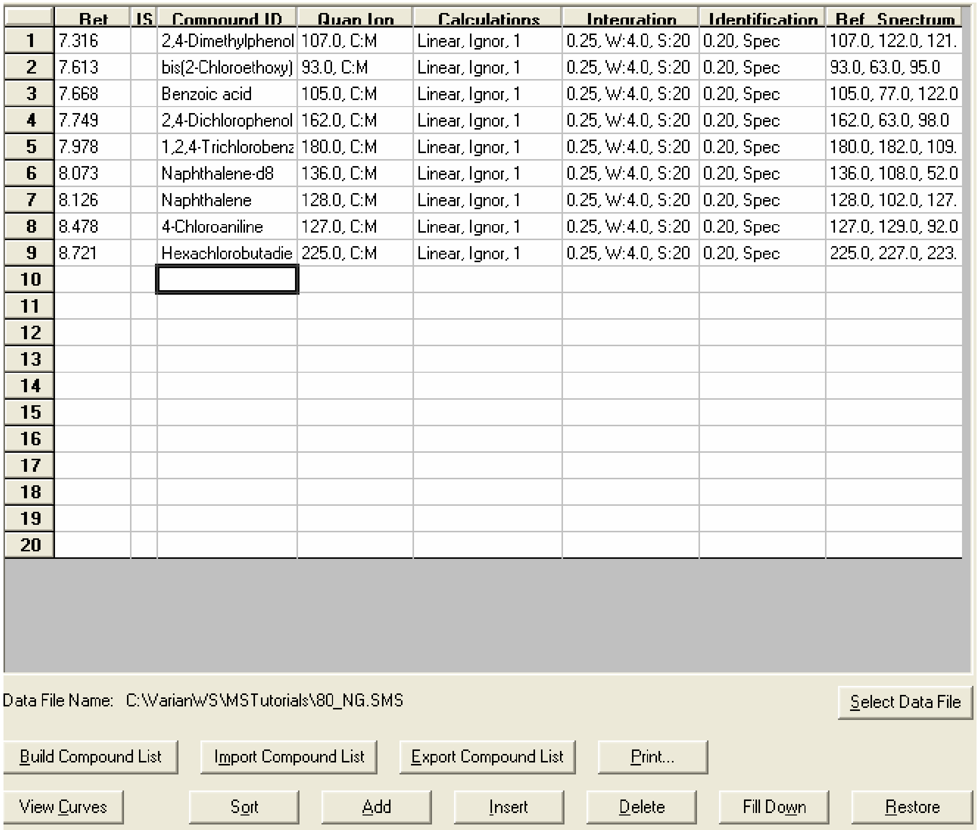
Current Spectrum

Index: 1
Name: 2,4-Dimethylphenol
MW: 122
Formula: C8H10O
CAS No.: 105-67-9

Click on a table entry to toggle its selection.

Index	Select	Name	MW	Formula	CAS No.
1	Yes	2,4-Dimethylphenol	122	C8H10O	105-67-9
2	Yes	bis(2-Chloroethoxy)methane	172	C5H10Cl2O2	111-91-1
3	Yes	Benzoic acid	122	C7H6O2	65-85-0
4	Yes	2,4-Dichlorophenol	162	C6H4Cl2O	120-83-2
5	Yes	1,2,4-Trichlorobenzene	180	C6H3Cl3	120-82-1
6	Yes	Naphthalene-d8	136	C10D8	None
7	Yes	Naphthalene	128	C10D8	91-20-3
8	Yes	4-Chloroaniline	127	C6H6ClN	106-47-8
9	Yes	Hexachlorobutadiene	258	C4Cl6	87-68-3

After you press the **Select** tab, the compound list is imported in the compound table of the **Method Builder** dialog.



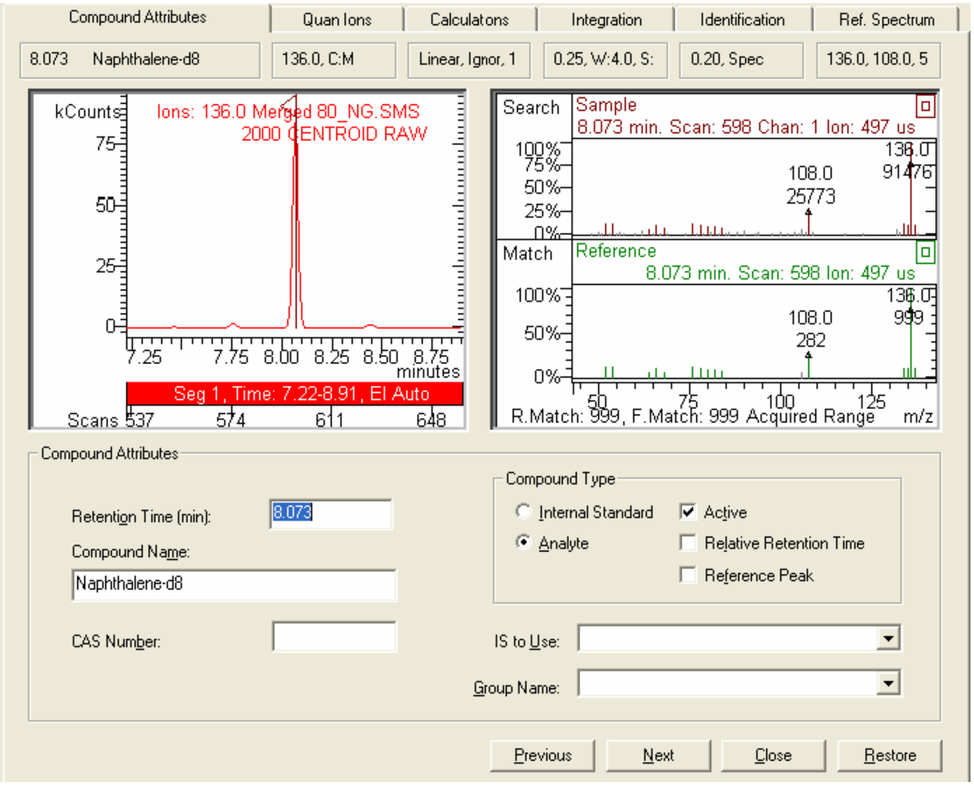
	Ret	IS	Compound ID	Quan Ion	Calculations	Integration	Identification	Ref. Spectrum
1	7.316		2,4-Dimethylphenol	107.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	107.0, 122.0, 121.
2	7.613		bis(2-Chloroethoxy)	93.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	93.0, 63.0, 95.0
3	7.668		Benzoic acid	105.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	105.0, 77.0, 122.0
4	7.749		2,4-Dichlorophenol	162.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	162.0, 63.0, 98.0
5	7.978		1,2,4-Trichlorobenz	180.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	180.0, 182.0, 109.
6	8.073		Naphthalene-d8	136.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	136.0, 108.0, 52.0
7	8.126		Naphthalene	128.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	128.0, 102.0, 127.
8	8.478		4-Chloroaniline	127.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	127.0, 129.0, 92.0
9	8.721		Hexachlorobutadie	225.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	225.0, 227.0, 223.
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

Data File Name: C:\VarianWS\MSTutorials\80_NG.SMS

The compound table consists of eight columns with the following information: Retention Time, Compound ID, Quantitation Ions, Calculations, Integration, Identification, and Reference Spectrum. Double-clicking one of these tab dialog fields for a compound will allow you to view and edit all of the information in the dialog.

Compound ID Tab Dialog

Double-click in the Compound ID field for the compound Naphthalene-d8. You will see the following dialog:



Compound Attributes

Retention Time (min): 8.073

Compound Name: Naphthalene-d8

CAS Number:

Compound Type

○ Internal Standard

● Analyte

☑ Active

☐ Relative Retention Time

☐ Reference Peak

IS to Use:

Group Name:

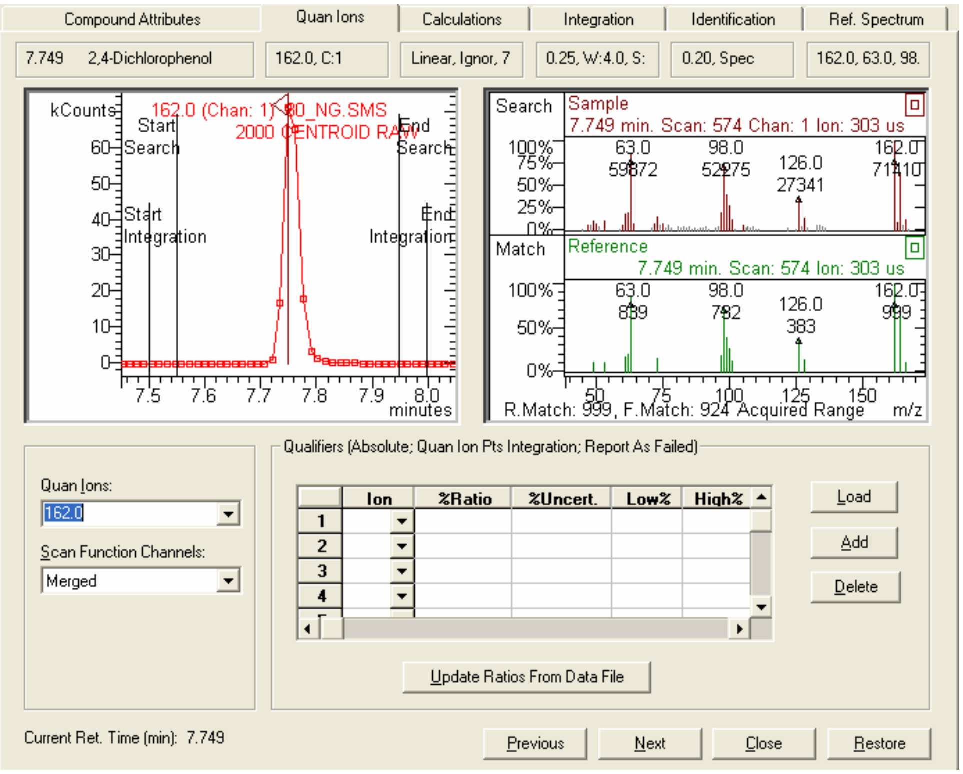
As the display above shows, Naphthalene-d8 will be identified as an analyte; click on Internal Standard instead. The CAS# for this compound is missing. Click in the CAS Number field and enter 1146652 (without using any dashes as separators - note that the dashes are entered automatically for you).

NOTE: In addition to typing in a value, you can change the retention time in this dialog simply by clicking on the chromatogram at the desired time.

Now use the **Next** and **Previous** buttons to check the Compound ID information for the other analytes. You will notice that Naphthalene-d8 is identified as the Internal Standard to use for the other compounds. You may change to other tab dialogs for the current compound by clicking their tabs in the top of the display. Return to the main Compound Table window by clicking the **Close** button.

Quan Ions Tab Dialog

Double-click on the Quan Ions field for the compound 2,4-Dichlorophenol. When peaks were added to the Compound Table in MultiChro, the Quantitation Ion default was the Base Peak (most intense) ion in the mass spectrum for each peak. Note that the plot shows the mass chromatogram for the chosen Quan Ion.

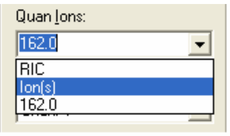


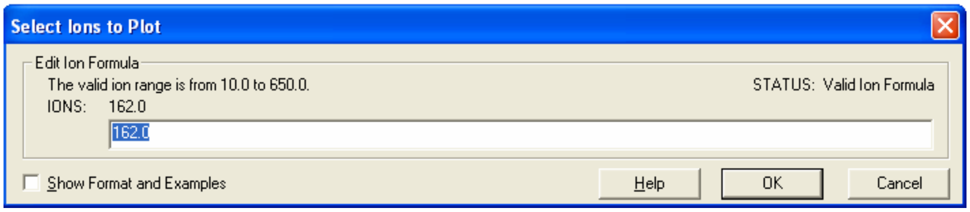
Quan Ions: 162.0

Scan Function Channels: Merged

Current Ret. Time (min): 7.749

You may choose another quan ion, a sum of ions, or a range of ions for quantitation by entering the information in the **Quan Ions** field. Click on the combo box to display the options and click Ion(s).





The valid ion range is from 10.0 to 650.0.

STATUS: Valid Ion Formula

IONS: 162.0

162.0

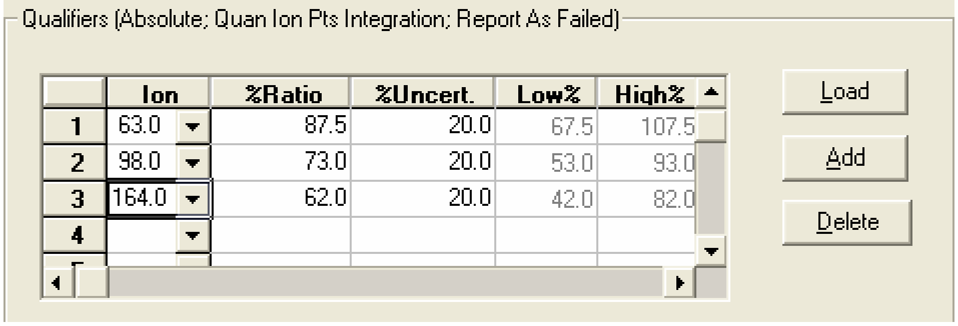
The **Select Ions to Plot** dialog will open. To add two or more ions separate their m/z values by a "+" sign. For example, you might want to add ions 63, 98, and 162 for quantifying 2,4-Dichlorophenol. To do this you would enter 63+98+162 in the *Quan Ions* field. A range of quan ions from 162 to 164 would be entered as 162:164 in the *Quan Ions* field.

NOTE: The Scan Function Channels field is only relevant to MS/MS analysis, in which quantitation may be done on individual Channels of information.

The default base peak Quan Ions are appropriate for quantitation of all of the peaks in this Compound Table. You can use the **Next** and **Previous** buttons to examine the Quan Ion information for other peaks in the Compound Table.

NOTE: You can change the compound retention time in this dialog simply by clicking on the chromatogram at the desired time.

In addition to specifying quantitation ions in this tab dialog, you may also select qualifier ions. Click **Load** to automatically enter the three most intense qualifier ion candidates.



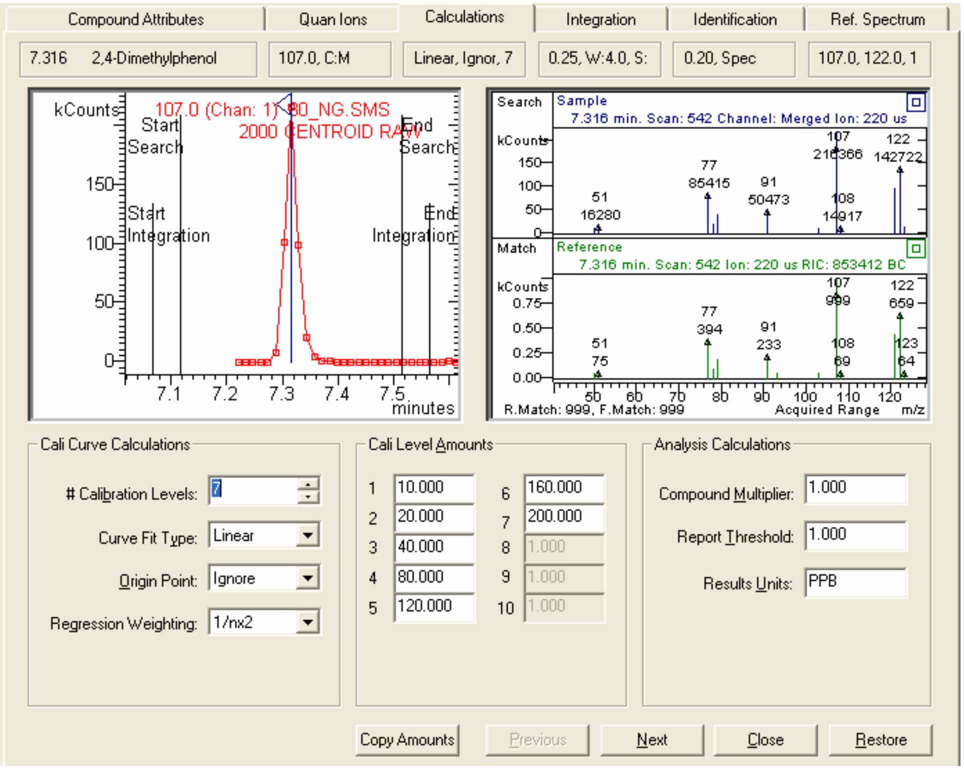
Qualifiers (Absolute; Quan Ion Pts Integration; Report As Failed)

	Ion	%Ratio	%Uncert.	Low%	High%
1	63.0	87.5	20.0	67.5	107.5
2	98.0	73.0	20.0	53.0	93.0
3	164.0	62.0	20.0	42.0	82.0
4					

Note that both the quantitation ions and the allowed qualifier ions must be present in the Reference Spectrum. By clicking **Add** you can add the next most intense reference spectrum ion available. To change a selected qualifier, click the combo box in the *Ion* field to see the available choices. The *Ratio* field shows the intensity of the qualifier ion as a percentage of the chosen Quan Ion(s). The *% Uncertainty* field is the allowed percentage deviation from the Ratio. By default this entry is 20%, but it can be adjusted. Therefore for the top Qualifier shown here, the allowed range for the ratio of the peak area of the Qualifier Ion m/z 63 would be 87.5 ± 20% = 67.5 to 107.5% of the area of the Quantitation Ion m/z 162 if the Ion Ratio Type in the Calculations Setup is *Absolute.*(These values would be 70.0-105.0 if it was *Relative* to the actual Ratio value). When samples are processed as Calibration, Analysis, or Verification runs, the Qualifier Ion tests will be run automatically.

Calculations Tab Dialog

Click on the **Calculations** tab from the current tab dialog. Use the Previous button if necessary to move to the first compound in the list, 2,4-Dimethylphenol. In the Calculations dialog we will enter information about the number of calibration levels and their concentrations. We can enter this information for the first compound in the table, then use the Fill Down feature to apply it to the other analytes in the Compound Table. Although we will not be changing most of the default options in the Calculations dialog for this tutorial, you may wish to explore these options. For example, instead of a Linear Curve Fit calculation, Quadratic and Cubic fits may be chosen. Also, the Origin Point may be the default *Ignore* (do not include in data set), *Include*(add the origin to data set), or *Force*(force curve through zero point).



7.316 2,4-Dimethylphenol	107.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:	0.20, Spec	107.0, 122.0, 1

Cali Curve Calculations

Calibration Levels: 7
Curve Fit Type: Linear
Origin Point: Ignore
Regression Weighting: 1/nx2

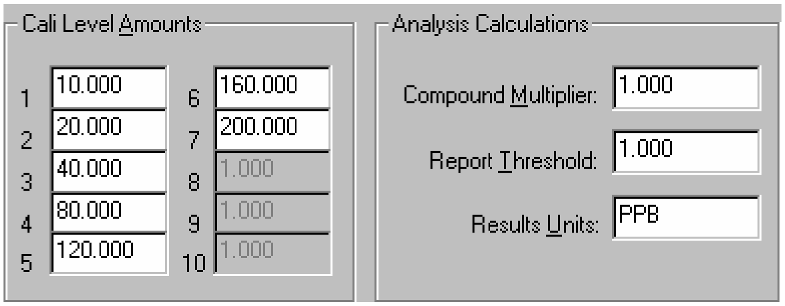
Cali Level Amounts

1	10.000	6	160.000
2	20.000	7	200.000
3	40.000	8	1.000
4	80.000	9	1.000
5	120.000	10	1.000

Analysis Calculations

Compound Multiplier: 1.000
Report Threshold: 1.000
Results Units: PPB

For this tutorial, we will use calibration information at seven different levels. Enter 7 in the # *Calibration Levels* field. You will see the *Cali Level Amounts* field change to allow entry of the different levels. Then, click in the first level in the field *Cali Level Amounts*. Enter the following amounts for Levels 1 through 7: 10, 20, 40, 80, 120, 160, 200. Enter a Report Threshold of 1.000 and Results Units of PPB. Note the Reports Threshold can be set on a per-compound basis. This feature can be used to eliminate reporting of quantitation results for Analysis samples below the Practical Quantitation Limit (PQL), which is generally defined as 5-10 times the Method Detection Limit (MDL). The display should now appear as shown here:



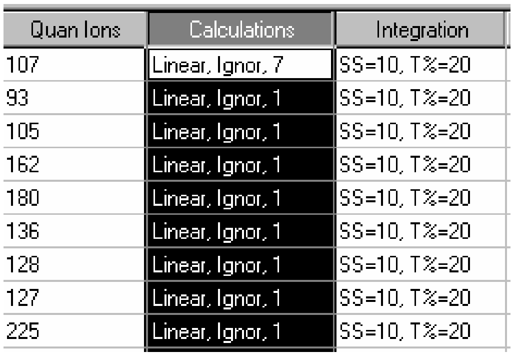
Cali Level Amounts

1	10.000	6	160.000
2	20.000	7	200.000
3	40.000	8	1.000
4	80.000	9	1.000
5	120.000	10	1.000

Analysis Calculations

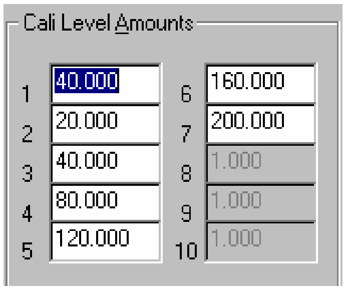
Compound Multiplier: 1.000
Report Threshold: 1.000
Results Units: PPB

Click on the **Close** button. Now the Calculations Information for the first compound is updated in the main Compound Editor dialog. You can fill this information automatically for the other compounds in the table. Click the **Calculations** Tab to highlight all entries, or select the desired block of field cells by clicking and dragging with the mouse.



Quan Ions	Calculations	Integration
107	Linear, Ignor, 7	SS=10, T%=20
93	Linear, Ignor, 1	SS=10, T%=20
105	Linear, Ignor, 1	SS=10, T%=20
162	Linear, Ignor, 1	SS=10, T%=20
180	Linear, Ignor, 1	SS=10, T%=20
136	Linear, Ignor, 1	SS=10, T%=20
128	Linear, Ignor, 1	SS=10, T%=20
127	Linear, Ignor, 1	SS=10, T%=20
225	Linear, Ignor, 1	SS=10, T%=20

Then click the **Fill Down** button. Now the concentrations of the seven calibrations levels are filled in for all analytes. You need to make one further modification to complete the editing process. The internal standard Naphthalene-d8 is actually present at 40 ng/µL in all seven files. Double-click on the **Calculations** tab for Naphthalene-d8 and change the Cali Amounts to 40 for Level 1.



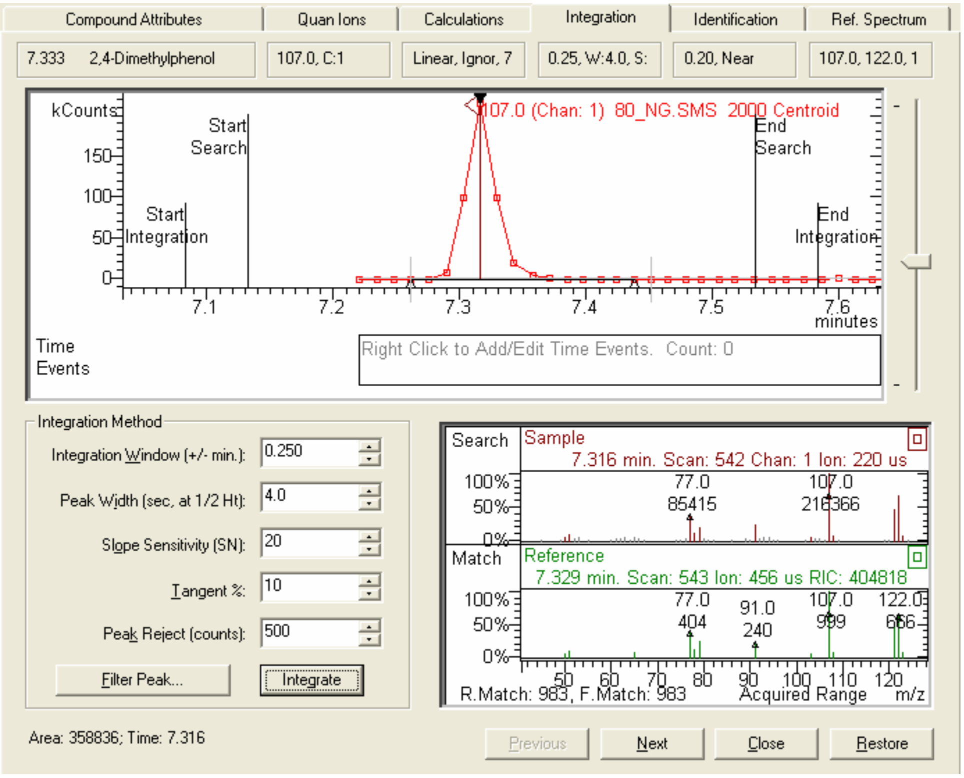
Cali Level Amounts

1	40.000	6	160.000
2	20.000	7	200.000
3	40.000	8	1.000
4	80.000	9	1.000
5	120.000	10	1.000

Finally, Click **Copy Amounts** to enter 40 automatically to the other levels.

Integration Tab Dialog

Click on the **Integration** tab field for 2,4-Dimethylphenol. The default integration parameters for Peak Width, Slope Sensitivity, and Tangent % will provide good integration data for peaks with good shapes and normal widths.



7.333 2,4-Dimethylphenol | 107.0, C:1 | Linear, Ignor, 7 | 0.25, W:4.0, S: | 0.20, Near | 107.0, 122.0, 1

Integration Method

Integration Window (+/- min.): 0.250

Peak Width (sec, at 1/2 Ht): 4.0

Slope Sensitivity (SN): 20

Tangent %: 10

Peak Reject (counts): 500

Area: 358836; Time: 7.316

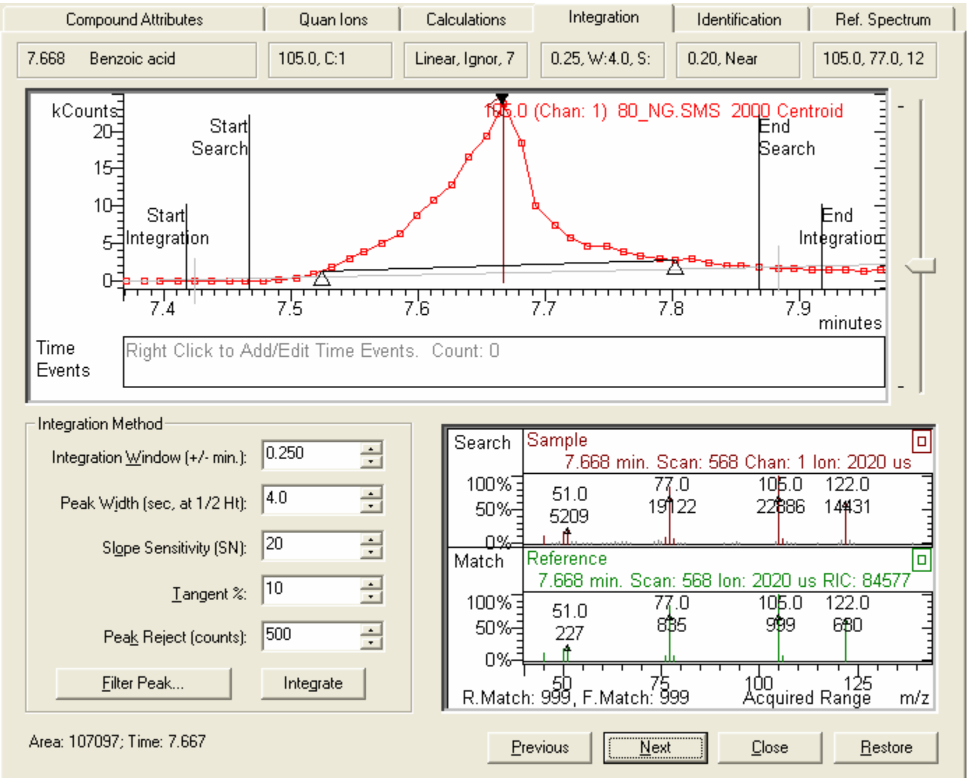
Check how the peak will be integrated by clicking the **Integrate** button. You will see the baseline drawn in the Chromatogram display and the peak area and retention time displayed to the lower left side of the Integration dialog. For this compound the integration process works well with the default integration parameters.

Integrating Difficult Peaks

Because no column phase will provide good peak shapes and resolution for all analytes, you will need to adjust the integration parameters to get good integration on some peaks.

Peak start and end points are detected by comparing the calculated slope at each point to the slope threshold, and therefore are greatly affected by the Peak Width and Slope Sensitivity parameters. The slope is calculated on a segment centered around the point, whose size is derived from the Peak Width parameter value. This means that increasing the Peak Width tends to move the peak start and end points out from the peak apex. This increases the probability that broad peaks will be detected, and tends to smooth out irregular or split peaks during processing (the display is not affected). Typically, a Peak Width value slightly larger than the actual peak width at ½ height works best. In contrast, changing the Slope Sensitivity changes the slope threshold directly. This means that decreasing the Slope Sensitivity also moves the start and end points out from the apex, and increases the probability that small peaks will be detected. Because these parameter have different effects, you may need to vary one or the other or both to get the best results for a given peak. Increasing the Peak Width and/or Slope Sensitivity for broad, poorly shaped, or split peaks often improves the accuracy and reproducibility of integration results.

The benzoic acid peak is a good example for exploring the effects of the individual parameters and their interactions. Click **Next** until the integration data for benzoic acid appears. Now click on the **Integrate** button to integrate the benzoic acid peak.



7.668 Benzoic acid | 105.0, C:1 | Linear, Ignor, 7 | 0.25, W:4.0, S: | 0.20, Near | 105.0, 77.0, 12

Integration Window (+/- min.): 0.250

Peak Width (sec, at 1/2 Ht): 4.0

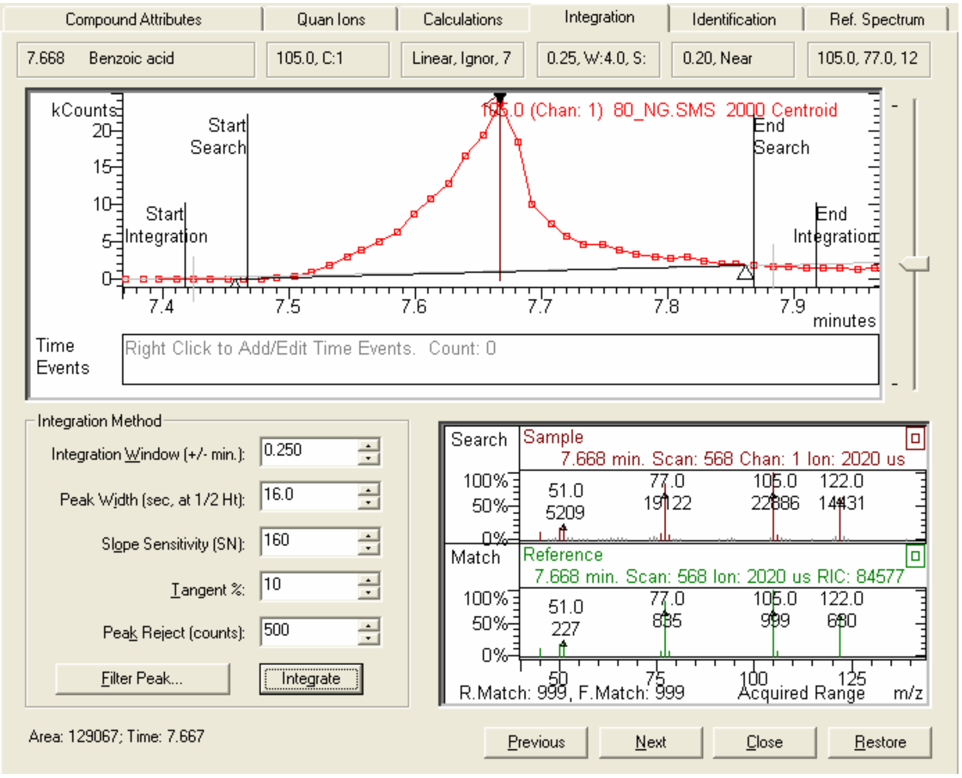
Slope Sensitivity (SN): 20

Tangent %: 10

Peak Reject (counts): 500

Area: 107097; Time: 7.667

Benzoic acid is integrated, but the segment end point is too high. Increase the Peak Width parameter. You will discover that this parameter alone does not allow good control for moving the ends of the segment defining the integration region. When you increase the Peak Width up to 16 sec, both point ends are moved, but they are too far out. Increase the Slope Sensitivity parameter from 20 to 160 and integrate again.



7.668 Benzoic acid | 105.0, C:1 | Linear, Ignor, 7 | 0.25, W:4.0, S: | 0.20, Near | 105.0, 77.0, 12

Integration Window (+/- min.): 0.250

Peak Width (sec, at 1/2 Ht): 16.0

Slope Sensitivity (SN): 160

Tangent %: 10

Peak Reject (counts): 500

Area: 129067; Time: 7.667

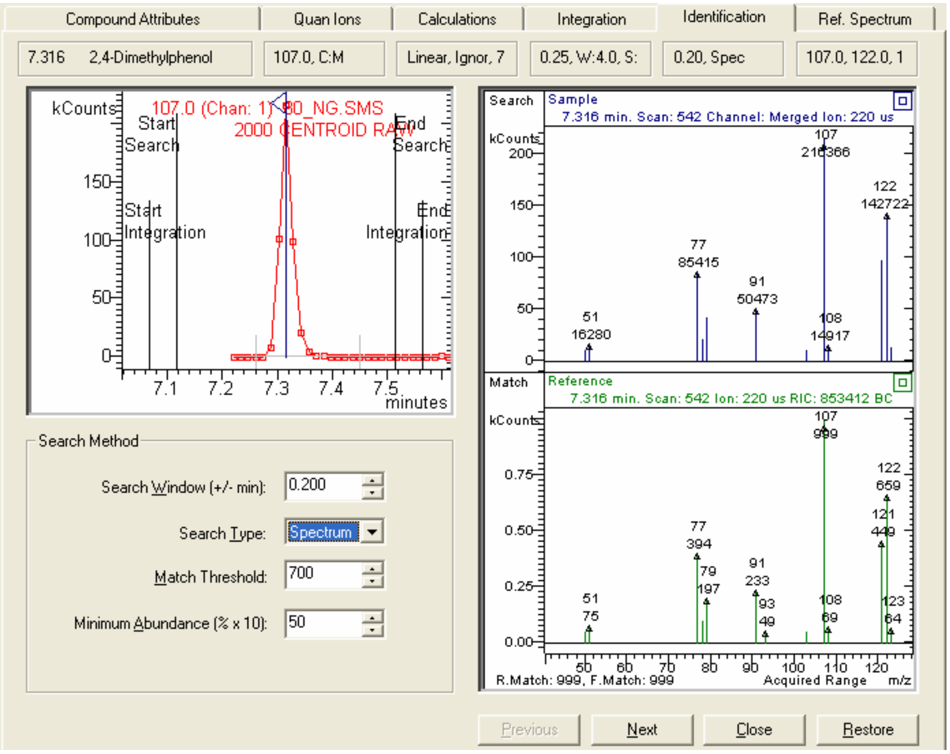
You can have different integration parameters for different compounds in the list, but for a given compound there is only one set of integration parameters for all concentration levels.

After you find a good combination of integration parameters for the mid concentration level data file, 80_ng.sms, confirm that they are acceptable at all calibration levels by re-integrating the compound at several different concentrations. It is particularly important to check the lowest and highest concentration levels. To select a different data file, click **Close** to return to the main table, then click **Select Data File** and select the desired data file, and then return to the Integration page.

When you have finished adjusting the integration parameters for benzoic acid as well as for the other compounds at different calibration levels, re-select the 80_ng.sms data file to continue with the tutorial.

Identification Tab Dialog

Double-click on the *Identification* field for 2,4-Dimethylphenol. Observe the default parameters for peak identification. The default for *Peak Window* is ± 0.200 minutes (± 12 seconds). When the quantitation software is trying to find the 2,4-Dimethylphenol peak and integrate it, a spectrum matching the library fit *Match Threshold* of 700/1000 must be found within this 24 second window bracketing the expected retention time (found in the *Compound Attributes* dialog). Ions in the Reference and Sample spectra below the designated *Minimum Abundence(%x10)* value will be ignored in calculating the library fit. For samples with significant matrix interferences, using *Minimum Abundence(%x10)* values of 50-100 is useful in improving peak identification performance. The integration must also occur entirely within this window. Therefore, always make sure that the peak width is wide enough to allow a good integration to occur. The default value will work fine for normal peaks without excessive tailing.



7.316 2,4-Dimethylphenol | 107.0, C:M | Linear, Ignor, 7 | 0.25, W:4.0, S: | 0.20, Spec | 107.0, 122.0, 1

Search Method

Search Window (+/- min): 0.200

Search Type: Spectrum

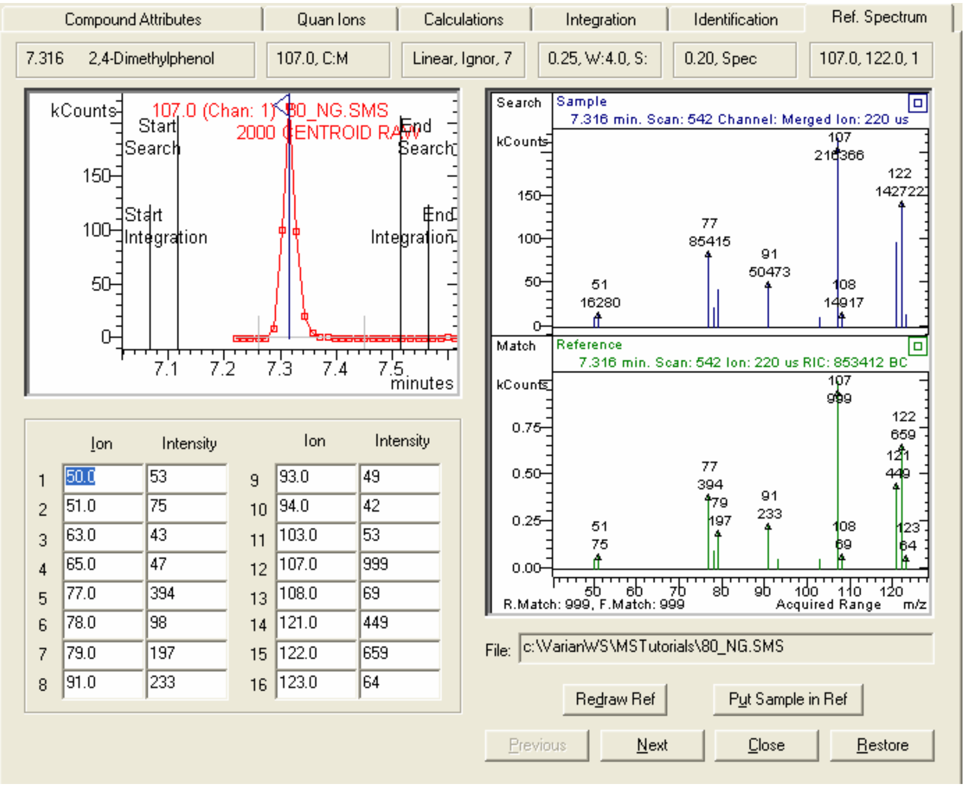
Match Threshold: 700

Minimum Abundance (% x 10): 50

You can use the default Identification parameters for all peaks in the Compound Table except for benzoic acid. For this peak, enter a peak width of 0.400 min.

Reference Spectrum Dialog

Click on the **Reference Spectrum** tab for 2,4-Dimethylphenol. Observe the Sample and Reference mass spectra. Note that the lowest intensity peaks in the Sample spectrum are not included in the Reference spectrum. This is because only the 16 largest peaks are included in the Reference spectrum. Notice that the source data file for the reference spectrum is indicated in the *File:* field. Examine the Ion/Intensity list. You may edit the reference spectrum if desired and redraw the spectrum plot. Editing the reference spectrum is advisable if interference ions (from coelution, background interferences, or column bleed) are observed in the reference spectrum. If the spectrum is edited by the user, the *File:* field will be changed to **Manually Edited**.



7.316 2,4-Dimethylphenol	107.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:	0.20, Spec	107.0, 122.0, 1

	Ion	Intensity		Ion	Intensity
1	50.0	53	9	93.0	49
2	51.0	75	10	94.0	42
3	63.0	43	11	103.0	53
4	65.0	47	12	107.0	999
5	77.0	394	13	108.0	69
6	78.0	98	14	121.0	449
7	79.0	197	15	122.0	659
8	91.0	233	16	123.0	64

File: c:\VarianWS\MSTutorials\80_NG.SMS

Use the **Next** and **Previous** buttons to scroll through the spectra for the analytes in the Compound Table.

Checking Spectral Quality over the Calibration Range

Note that the Reference Spectrum dialog is a convenient place to examine the quality of the mass spectra for the highest and lowest concentration levels of the calibration curve. For example, you can examine the lowest and highest level spectra for the analytes in this tutorial. Click **Close** for this dialog and select the 10_ng.sms using the **Select Data File** button. Look at the comparison of sample to reference for each compound and verify that the reference spectrum (from the 80_ng.sms file first used to build the compound table) is a good match to the spectra in the lowest level sample. The Reverse (R) Match (representing the extent to which the reference spectrum is contained in the sample spectrum), displayed below the Reference spectrum, should be higher than the **Match Threshold** specified in the **Identification** dialog. Remember that the default value of the Match Threshold is 700.

Next, use the **Select Data File** button in the Main Compound Table again to compare spectra from the 200_ng.sms file to the reference spectra. This process of comparison will assure that you have chosen good spectra for identification, specifically ones that will perform well over the entire calibration range. You also can check to see whether a different data file scan would generate a better reference spectrum. When you click on a point in the chromatogram display, the spectrum for that point is shown in the spectrum display. Click on the **Put Sample in Reference** button to generate a new reference spectrum. When you have finished comparing spectra, click on **Close** to return to the main Compound Table dialog.

NOTE: You may take this comparison process one step further by choosing for comparison a data file for an analysis sample (e.g. a spiked matrix extract sample).

Save the Edited Data Handling Method

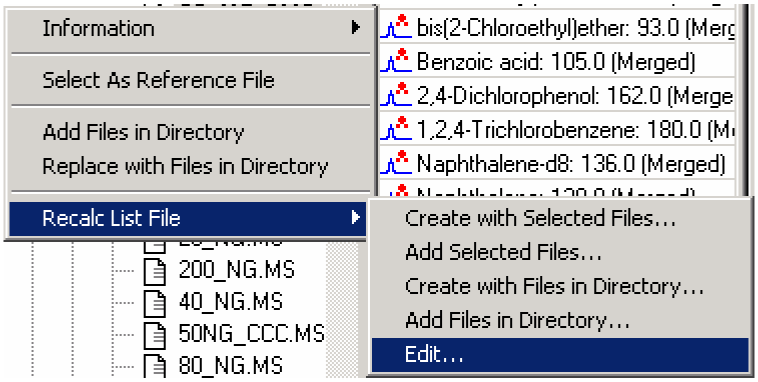
Click on ***File >Save* As..** to save the edited Data Handling method and type a file name in the **Save Method File As** dialog (in our case, the method is named calibration.mth). Click on ***File >Exit*** to close the Method Builder.

You have now finished building and editing a data handling method for the 2000 GCMS Workstation. The next step in the quantitation process is to build a Sample List or Recalc List to add calibration points to the Data Handling method.

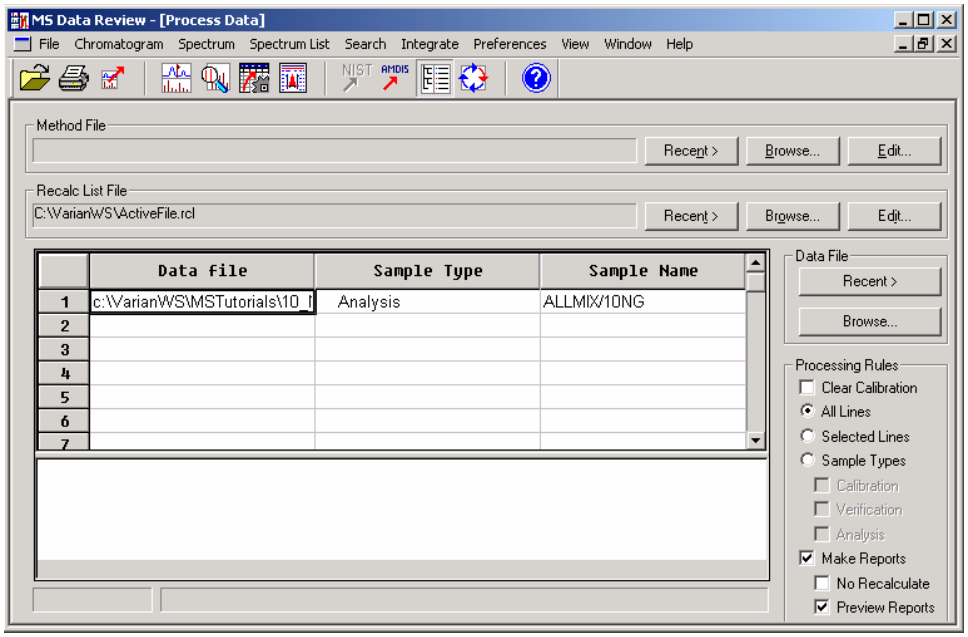
Building a Recalculation List for Calibration

A recalculation list contains all the data files used for calibrating the quantitation (determining the calibration curve) and for analysis. The recalculation list is built in the Automation File Editor that can be accessed in different ways:

1. Click on the **Automation File Editor** application button from the Varian Workstation toolbar.
2. Right click on a data file name (in the Data File Pane of the **MS Data Review** window) and select **Recalc List File >Edit**.



3. Click on the **Process Data** button in the MS Data Review toolbar to open the **Process Data** view



Method File:

Recalc List File: C:\VarianWS\ActiveFile.rcl

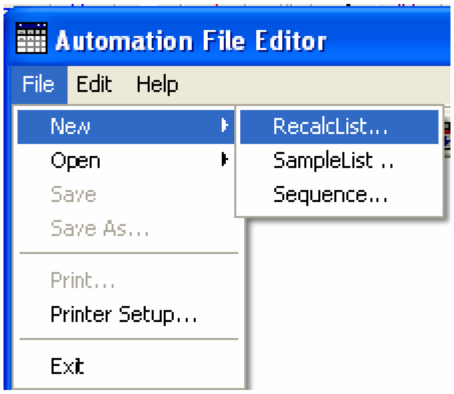
	Data file	Sample Type	Sample Name
1	c:\VarianWS\MSTutorials\10_I	Analysis	ALLMIX/10NG
2			
3			
4			
5			
6			
7			

Processing Rules
- ☐ Clear Calibration
- ● All Lines
- ○ Selected Lines
- ○ Sample Types
  - ☐ Calibration
  - ☐ Verification
  - ☐ Analysis
- ☑ Make Reports
  - ☐ No Recalculate
  - ☑ Preview Reports

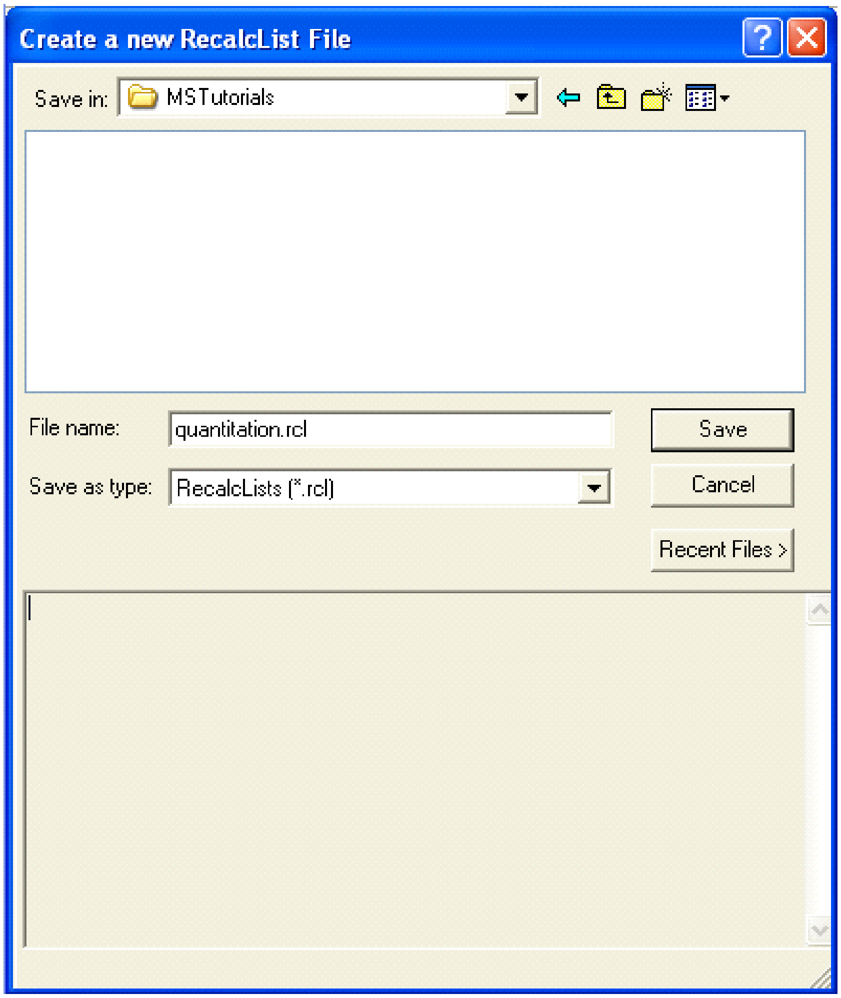
Click the **Edit** tab in the **Recalc List File** field.

All these three actions will open the **Automation File Editor** dialog, where you will build now a new recalculation list.

In the **Automation File Editor** select the menu command **File >New >RecalcList**



Type a file name and choose a directory where you want to save the recalculation list and press **Save** in the **Create a new RecalcList File** dialog.

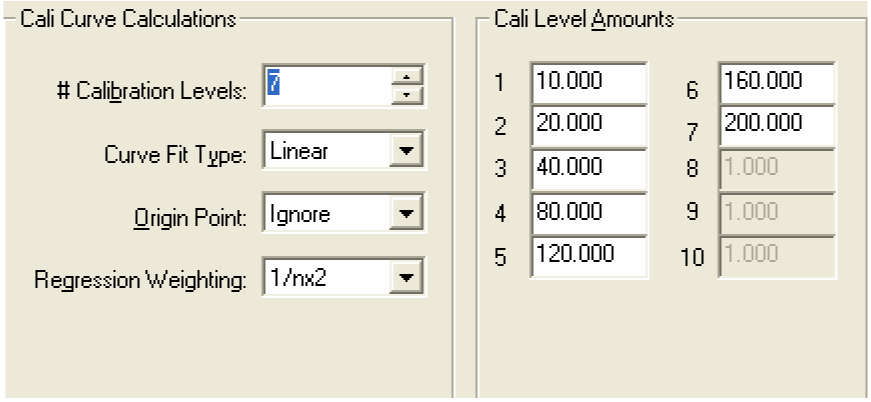


Save in: MSTutorials

File name: quantitation.rcl

Save as type: RecalcLists (*.rcl)

An empty Recalculation List is opened in the **Automation File Editor**. You will now enter the file names for the data files at each concentration level and associate them with the Calibration Levels identified in the Calculations tab dialog in the Compound Table editor (see the previous section of the tutorial under (**Calculations Tab Dialog**). For your convenience the calculation levels entered in that dialog are copied here:


Cali Curve Calculations

Calibration Levels: 7

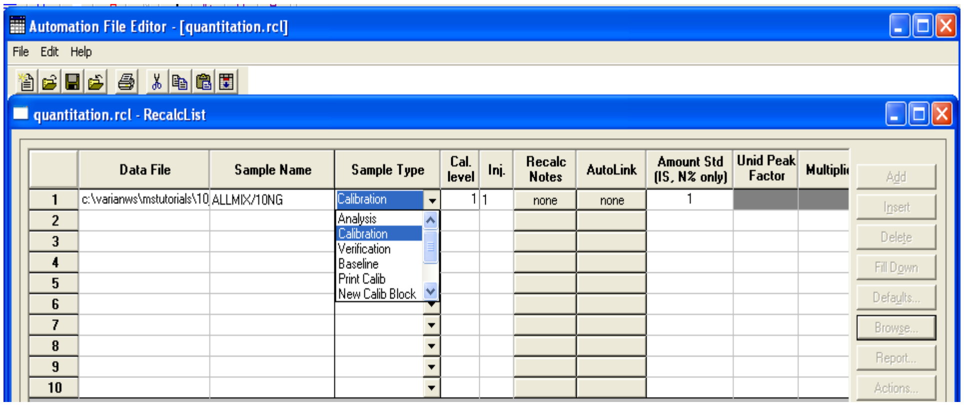
Curve Fit Type: Linear

Origin Point: Ignore

Regression Weighting: 1/nx2

Cali Level Amounts

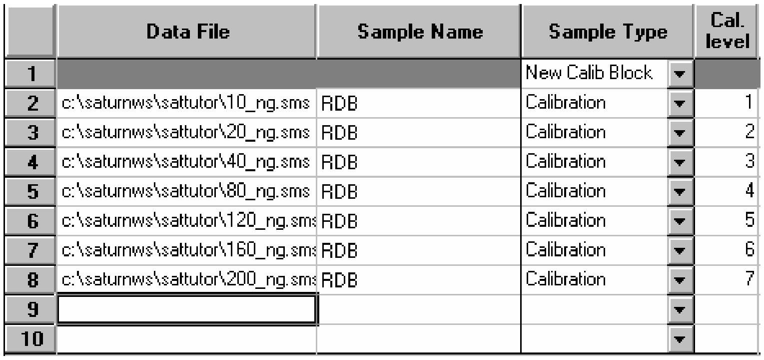
1	10.000	6	160.000
2	20.000	7	200.000
3	40.000	8	1.000
4	80.000	9	1.000
5	120.000	10	1.000



	Data File	Sample Name	Sample Type	Cal. level	Inj.	Recalc Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multipli
1	c:\varianws\mstutorials\10	ALLMIX/10NG	Calibration	1	1	none	none	1		
2										
3										
4										
5										
6										
7										
8										
9										
10										

Position the cursor in the Data File field of Row 1. Click Add and then click Browse... . The **Open Data File** dialog will appear. Select the data file 10_ng.sms in the directory C:\VarianWS\MSTutorials. In the Sample Type field use the pull down arrow and choose Calibration. Then click in the Cal. Level field and enter 1. (Note in the figure above that Calibration Level Amounts 1 is identified as the 10.000 level.)

Use the same procedure to enter the data file 20_ng.sms. Select Calibration in the Sample Type field and enter 2 in the Cal. Level field. Continue to add lines for the 40, 80, 120, 160, and 200 ng level files until all seven levels are entered to the Recalculation List quantitation.rcl. To assure that calibration data are cleared before calculations, highlight the first Row of the list and click Insert and select **New Calibration Block** in the Sample List field. When you are finished, the recalculation list should look like the following:

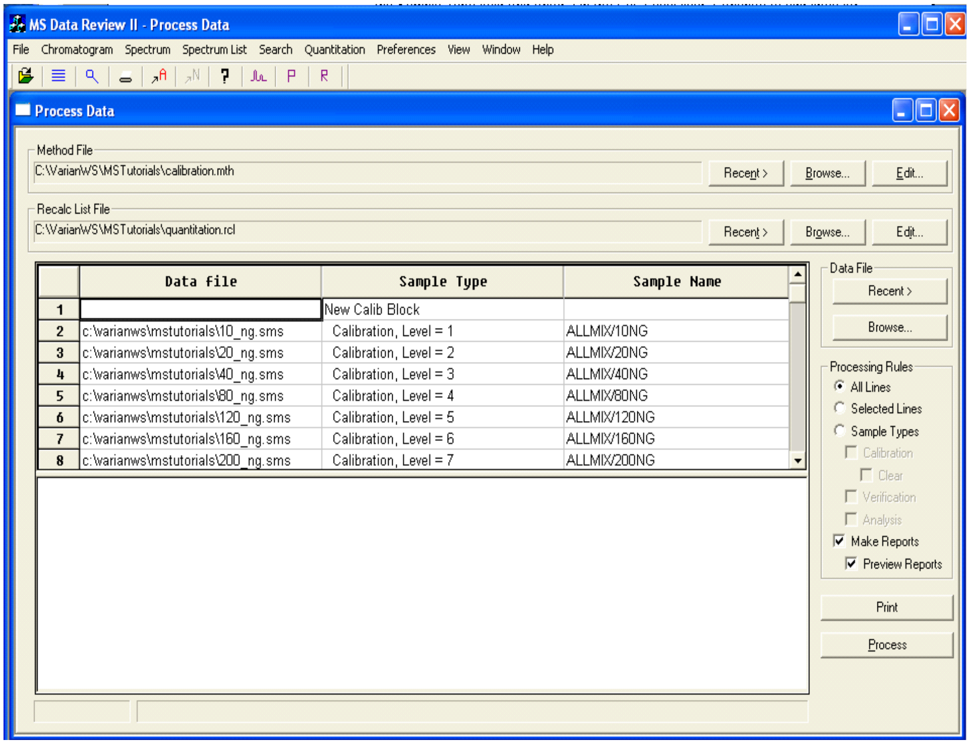


	Data File	Sample Name	Sample Type	Cal. level
1			New Calib Block	
2	c:\saturnws\sattutor\10_ng.sms	RDB	Calibration	1
3	c:\saturnws\sattutor\20_ng.sms	RDB	Calibration	2
4	c:\saturnws\sattutor\40_ng.sms	RDB	Calibration	3
5	c:\saturnws\sattutor\80_ng.sms	RDB	Calibration	4
6	c:\saturnws\sattutor\120_ng.sms	RDB	Calibration	5
7	c:\saturnws\sattutor\160_ng.sms	RDB	Calibration	6
8	c:\saturnws\sattutor\200_ng.sms	RDB	Calibration	7
9				
10				

Use the menu command **File >Save** to save the Recalc List. Click on **File >Exit** to close the Automation File Editor.

Processing a Recalculation List to Add Calibration Data

Open the **Process Data** view in the **MS Data Review** application by clicking the "Process Data" icon. If you opened the **Automation File Editor** from the Process Data view (as described in action 3 in the previous tutorial), you will return automatically here when you close the **Automation File Editor** after saving the recalculation list. The data handling method file should be calibration.mth. Click the **Recent** button in the Recalc List File field and select quantitation.rcl.



Method File: C:\VarianWS\MSTutorials\calibration.mth

Recalc List File: C:\VarianWS\MSTutorials\quantitation.rcl

	Data file	Sample Type	Sample Name
1		New Calib Block	
2	c:\varianws\mstutorials\10_ng.sms	Calibration, Level = 1	ALLMIX/10NG
3	c:\varianws\mstutorials\20_ng.sms	Calibration, Level = 2	ALLMIX/20NG
4	c:\varianws\mstutorials\40_ng.sms	Calibration, Level = 3	ALLMIX/40NG
5	c:\varianws\mstutorials\80_ng.sms	Calibration, Level = 4	ALLMIX/80NG
6	c:\varianws\mstutorials\120_ng.sms	Calibration, Level = 5	ALLMIX/120NG
7	c:\varianws\mstutorials\160_ng.sms	Calibration, Level = 6	ALLMIX/160NG
8	c:\varianws\mstutorials\200_ng.sms	Calibration, Level = 7	ALLMIX/200NG

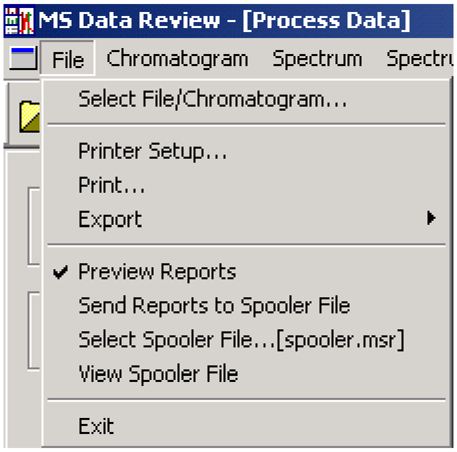
Processing Rules
- ● All Lines
- ○ Selected Lines
- ○ Sample Types
  - ☐ Calibration
    - ☐ Clear
  - ☐ Verification
  - ☐ Analysis
- ☑ Make Reports
  - ☑ Preview Reports

Select **All Lines** in the **Processing Rules** field.

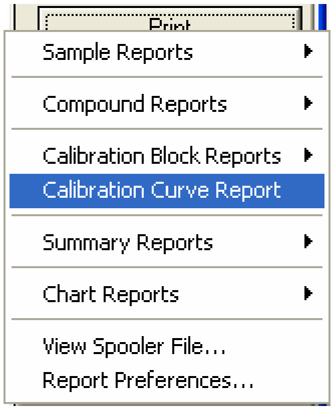
Click on Process. As the Data File list is processed, a message log and a process indicator at the bottom of the display keeps you advised of the progress. The current line in the list is shown followed by the word Processing, until the last line has been completed.

Examining Calibration Results in Process Data View

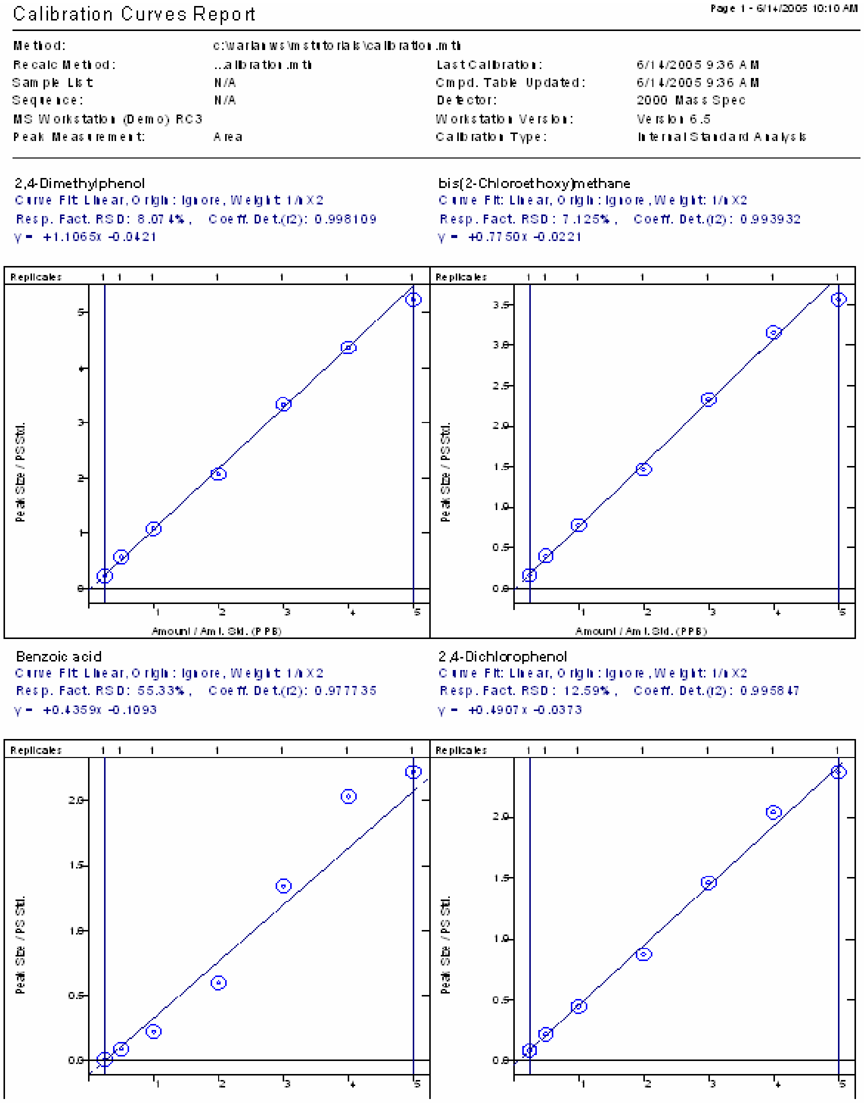
You can view and print calibration reports in the **Process Data** View. Check **Preview Reports** in the **File** menu of the **MS Data Review** window.



You can view calibration block and curve reports as well as summary and chart reports by clicking the **Print** button in the **Process Data** view and making the report selection. If the **Preview Reports** option is not checked in the **File** menu, any print command will send the report file directly to the printer, without opening a preview window. Press **Print** and Select **Calibration Curve Report**



This will bring up a Print Preview screen for the calibration results. Four calibration curves are shown on each page. Use the **Next/Previous Page** buttons ⇦ ⇨ to examine other curves. You can click and drag in the display to expand the screen for better viewing, or you can click the **Print Page or Print Report** buttons 🖨 🖨 to send it to your printer.



Calibration Curves Report

Page 1 - 6/14/2005 10:10 AM

Method:	c:\varianws\mstutorials\calibration.mth		
Recalc Method:	..calibration.mth	Last Calibration:	6/14/2005 9:36 AM
Sample List:	N/A	Cmpd. Table Updated:	6/14/2005 9:36 AM
Sequence:	N/A	Detector:	2000 Mass Spec
MS Workstation (Demo) RC3		Workstation Version:	Version 6.5
Peak Measurement:	Area	Calibration Type:	Internal Standard Analysis

2,4-Dimethylphenol
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
Resp. Fact. RSD: 8.074%, Coeff. Det.(r2): 0.998109
y = +1.1065x -0.0421

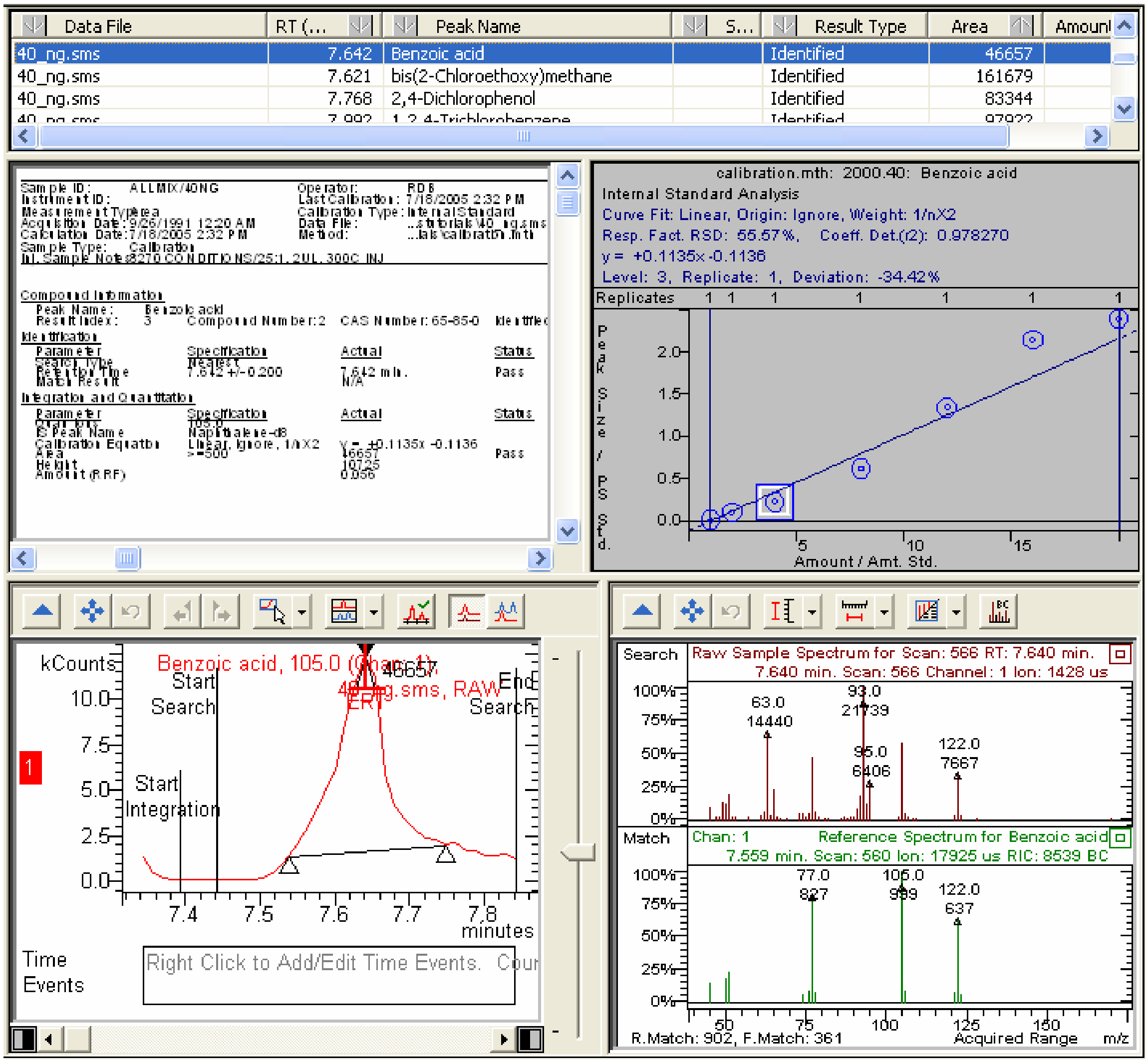
bis(2-Chloroethoxy)methane
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
Resp. Fact. RSD: 7.125%, Coeff. Det.(r2): 0.993932
y = +0.7750x -0.0221

Benzoic acid
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
Resp. Fact. RSD: 55.33%, Coeff. Det.(r2): 0.977735
y = +0.4359x -0.1093

2,4-Dichlorophenol
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
Resp. Fact. RSD: 12.59%, Coeff. Det.(r2): 0.995847
y = +0.4907x -0.0373

Reviewing Calibration Results in the Results View

Open the **View Results** window by clicking the **Results** button in the **MS Data Review** toolbar.



Data File	RT (...	Peak Name	S...	Result Type	Area	Amount
40_ng.sms	7.642	Benzoic acid		Identified	46657	
40_ng.sms	7.621	bis(2-Chloroethoxy)methane		Identified	161679	
40_ng.sms	7.768	2,4-Dichlorophenol		Identified	83344	

Sample ID: ALLMIX/40NG
Instrument ID:
Measurement Type: Area
Acquisition Date: 9/26/1991 12:20 AM
Calculation Date: 7/18/2005 2:32 PM
Sample Type: Calibration
Inj. Sample Notes: 8270 CONDITIONS/25:1, 2UL, 300C INJ
Operator: RDB
Last Calibration: 7/18/2005 2:32 PM
Calibration Type: Internal Standard
Data File: ...\tutorials\40_ng.sms
Method: ...\als\calibration.mth

Compound Information
Peak Name: Benzoic acid
Result Index: 3 Compound Number: 2 CAS Number: 65-85-0 Identified

Identification

Parameter	Specification	Actual	Status
Search Type	Nearest		
Retention Time	7.642 +/- 0.200	7.642 min.	Pass
Match Result		N/A	

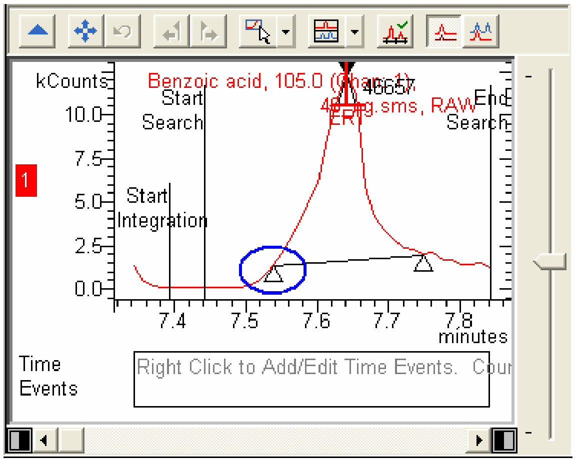
Integration and Quantitation

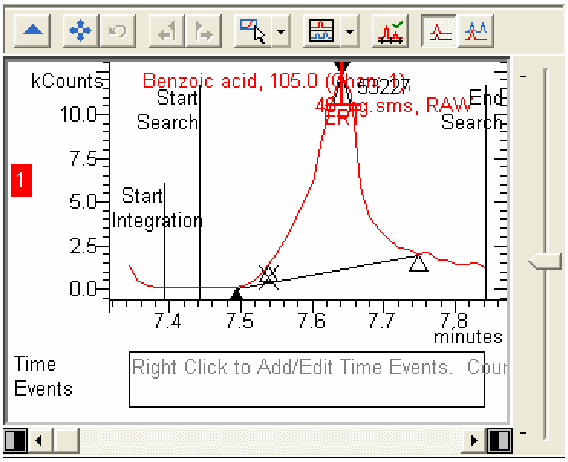
Parameter	Specification	Actual	Status
Quan Ions	105.0		
IS Peak Name	Naphthalene-d8		
Calibration Equation	Linear, Ignore, 1/nX2	y = +0.1135x -0.1136	
Area	>=500	46657	Pass
Height		10725	
Amount (RRF)		0.056	

calibration.mth: 2000.40: Benzoic acid
Internal Standard Analysis
Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2
Resp. Fact. RSD: 55.57%, Coeff. Det.(r2): 0.978270
y = +0.1135x -0.1136
Level: 3, Replicate: 1, Deviation: -34.42%

In the Results view you can examine the calibration and analysis results and interactively adjust the integration parameters for any compound in any data file, which you select from the list displayed on top of the Results view.

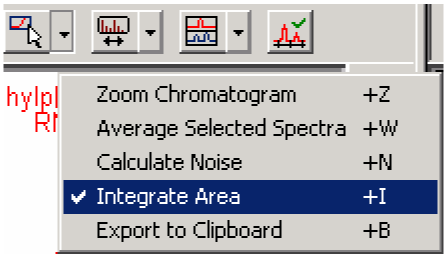
You can manually integrate peaks in the Integration pane by manually moving individual peak event markers. Place the cursor on a peak event(the cursor will change to a hand symbol), then click and drag to the new location.



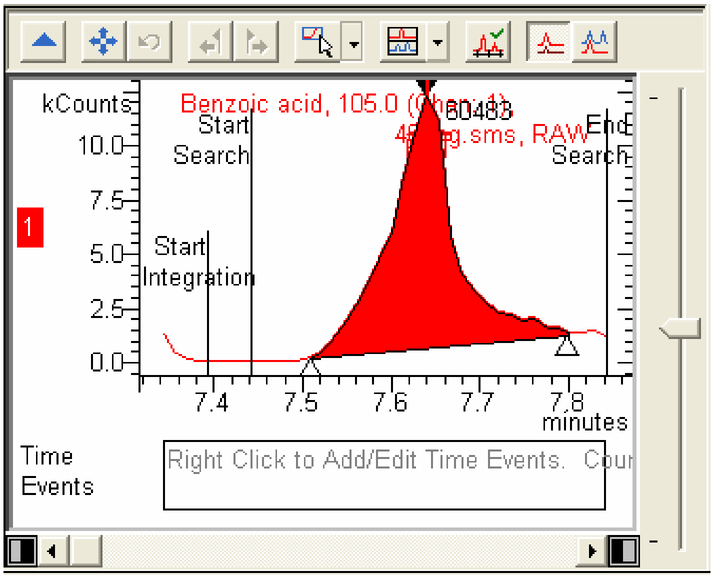


After releasing the mouse drag the peak will be reintegrated and the moved peak event will be shown filled in with the old position still showing, but marked with an “X”. To restore the original peak event position, right-click on the event and select to restore event. The peak will be reintegrated again to the original results.

Alternatively, click on the **Set Click and Drag Action** button in the Integration pane toolbar and select **Integrate Area.**

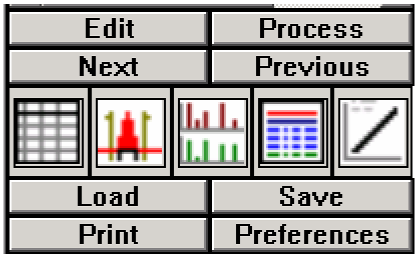


Select the integration region by clicking and dragging along the base of the peak (the newly integrated region will appear red).

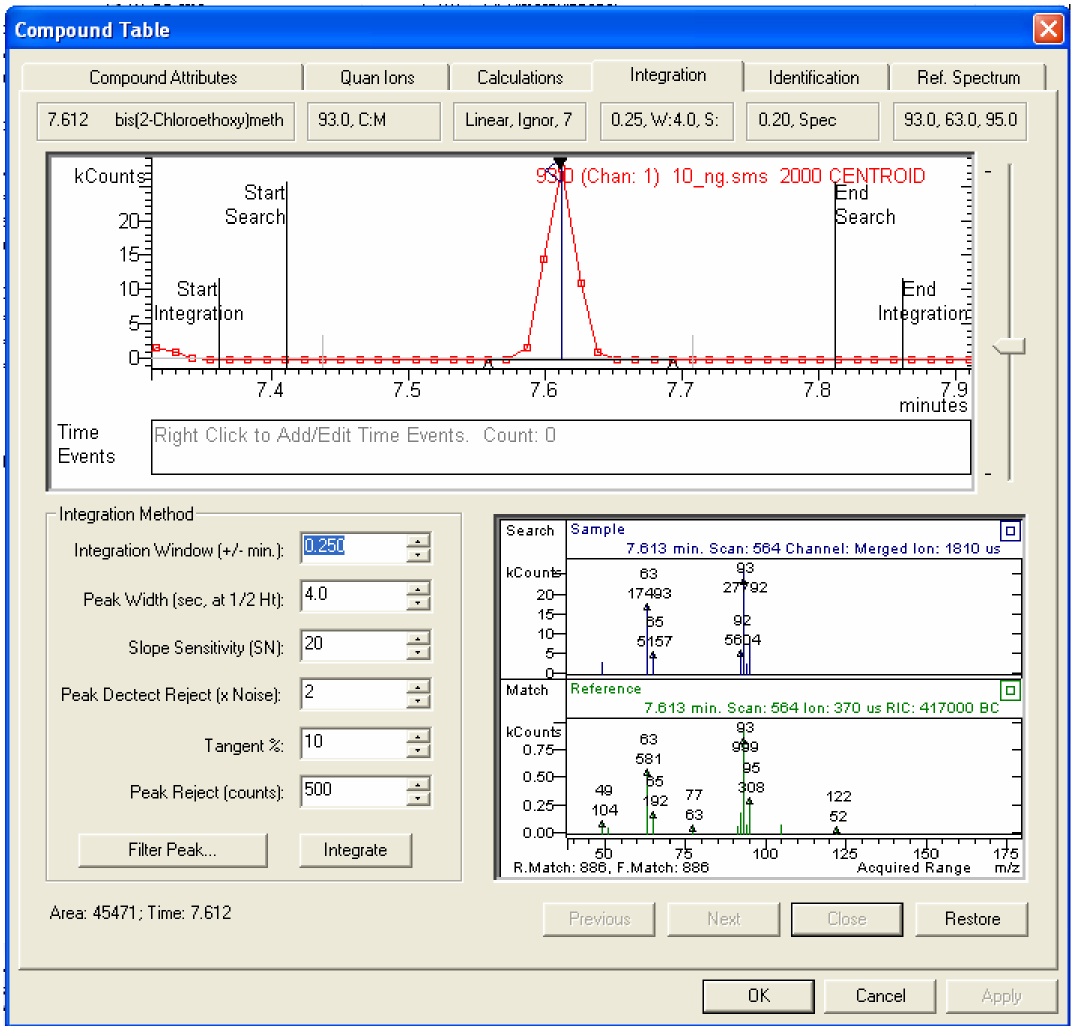


NOTE: This example showed a change made to a calibration data file. This will cause all the calibration data files to be reintegrated to generate a new calibration curve and, in addition, any analysis data files will be reintegrated to generate updated results. If a change is made to an analysis data file, then only that data file will be reintegrated.

You can also modify the integration parameters entered in the data handling method by clicking the **Edit** tab in the lower left side of the Results view.



This action will open the Integration field in the compound table for the compound selected in the Results view.



7.612 bis(2-Chloroethoxy)meth | 93.0, C:M | Linear, Ignor, 7 | 0.25, W:4.0, S: | 0.20, Spec | 93.0, 63.0, 95.0

93.0 (Chan: 1) 10_ng.sms 2000 CENTROID

Time Events: Right Click to Add/Edit Time Events. Count: 0

7.613 min. Scan: 564 Channel: Merged Ion: 1810 us

7.613 min. Scan: 564 Ion: 370 us RIC: 417000 BC

R.Match: 886, F.Match: 886

Integration Method

Integration Window (+/- min.): 0.250

Peak Width (sec, at 1/2 Ht): 4.0

Slope Sensitivity (SN): 20

Peak Dectect Reject (x Noise): 2

Tangent %: 10

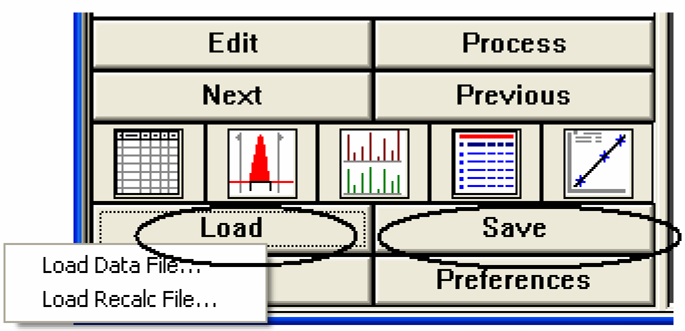
Peak Reject (counts): 500

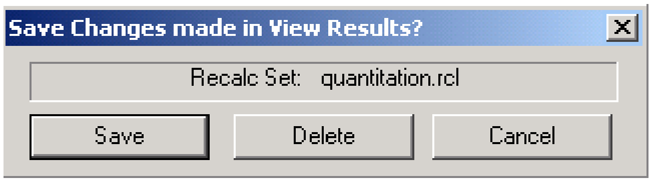
Area: 45471; Time: 7.612

You can change other parameters besides the integration parameters for this compound (in the Identification, Calculations, Ref. Spectrum or Quan Ions fields), but you cannot select another compound in this dialog.

When you make parameter adjustments, the results are automatically updated in the Results view.

NOTE: All changes to the method and data file results in the Results View window are stored to temporary files. These changes will only be saved to the actual files when exiting the Results View window or by clicking on the **Save** button or by loading a new recalclist or a new data file. You will be prompted to save the changes, delete the changes or cancel the save operation.





Recalc Set: quantitation.rcl

Examine the calibration results by selecting the compounds and data files in the list. The *Amount* field in the upper pane displays two types of information, depending on whether it describes a calibration file or an analysis file (the file type information was entered in the *Sample Type* field of the **RecalcList** dialog in the **Automation File Editor**):

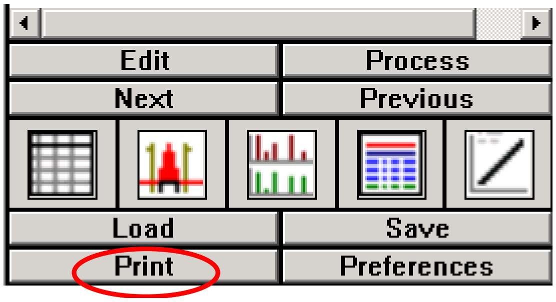
For calibration files, the **Amount** field contains the Relative Response Factor, RRF, defined as:

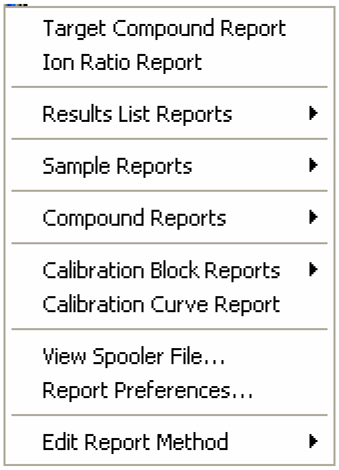
$$\mathbf{RRF=(Peak\ Area/Peak\ Area_{IS})\ x\ (Amount_{IS}/Amount)}$$

Area will be replaced by **Height** in this formula, if height is the selection in the **Measurement Type** field of the **Calculations Setup** section in the **Data Handling Method** (see the section *Editing a Data Handling Method* presented earlier in the tutorial).

For the analysis files, The *Amount* field contains the quantitative amount. Note that for the Internal Standard, Naphthalene-d8, the *Amount* value is 1 for the calibration files.

You can view and print reports from the Results view by pressing the **Print** button in the left lower side of the **View Results** dialog and selecting the type of report.

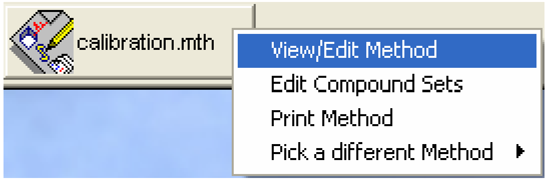




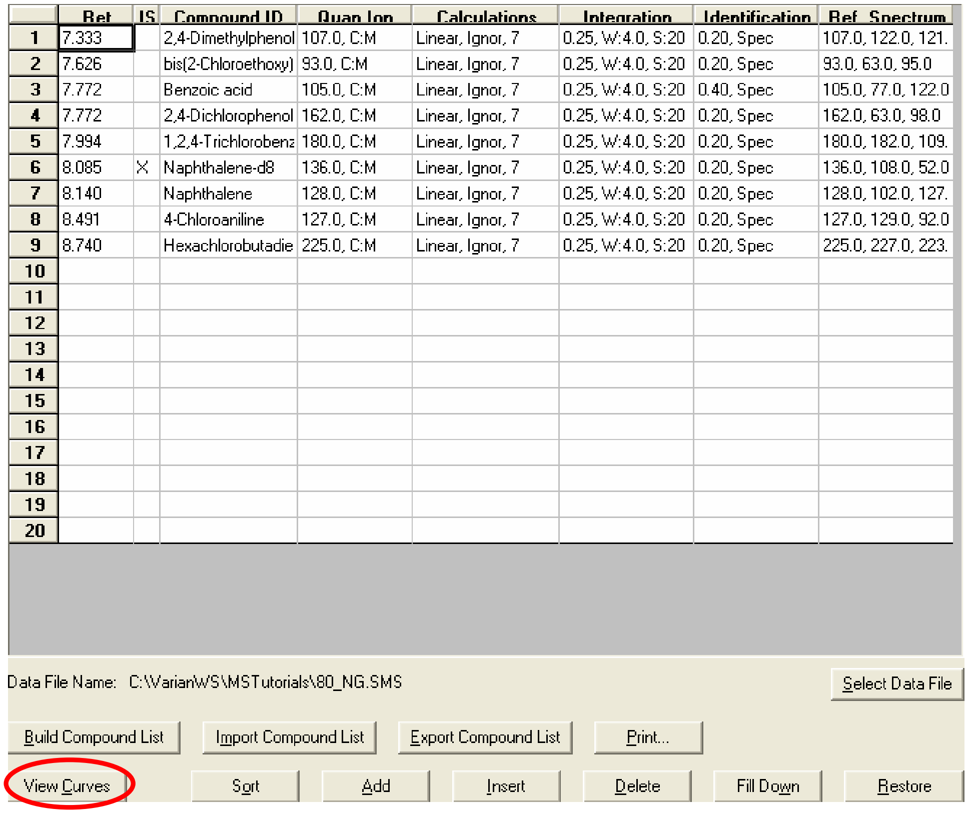
Again, make sure that the **Preview Printed Reports** is checked in the **File** menu of the **MS Data Review** window.

Viewing and Editing Calibration Results in the Method Builder Dialog

You can also view the calibration curves from the Method Builder window. Open the method created and used in this tutorial on quantitation, in our case the method file name is calibration.mth, either by clicking on the **View/Edit Methods** button in the Workstation toolbar, or by using the quick link button.



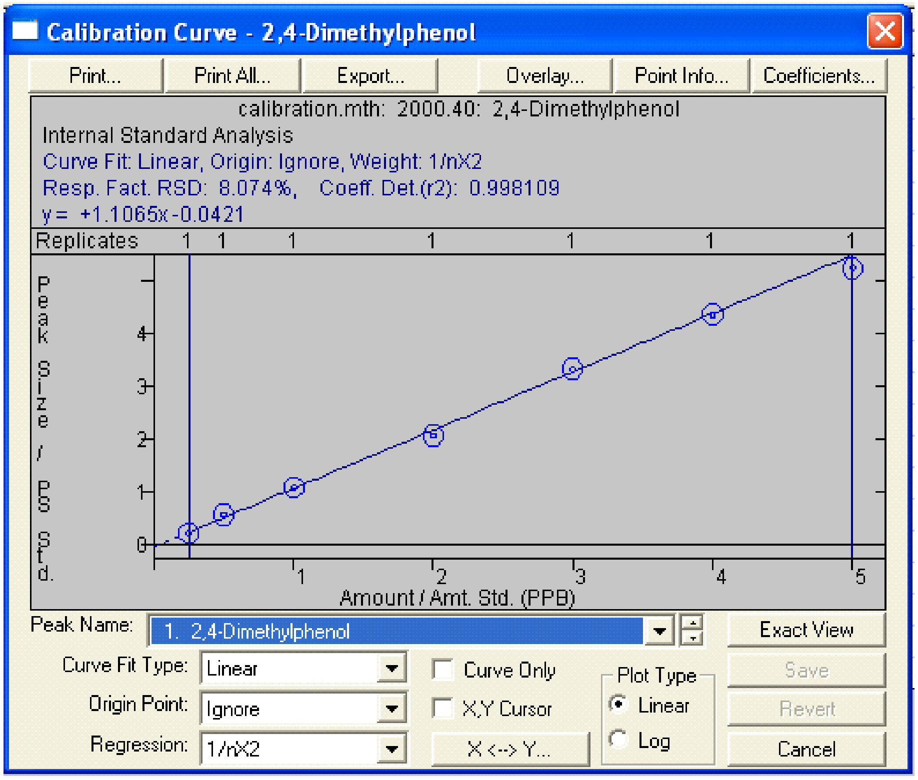
Click in the **Compound Table** section of the Method Directory under MS Data Handling. The Compound Table dialog appears.



	Ret	IS	Compound ID	Quan Ion	Calculations	Integration	Identification	Ref. Spectrum
1	7.333		2,4-Dimethylphenol	107.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	107.0, 122.0, 121.
2	7.626		bis(2-Chloroethoxy)	93.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	93.0, 63.0, 95.0
3	7.772		Benzoic acid	105.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.40, Spec	105.0, 77.0, 122.0
4	7.772		2,4-Dichlorophenol	162.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	162.0, 63.0, 98.0
5	7.994		1,2,4-Trichlorobenz	180.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	180.0, 182.0, 109.
6	8.085	X	Naphthalene-d8	136.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	136.0, 108.0, 52.0
7	8.140		Naphthalene	128.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	128.0, 102.0, 127.
8	8.491		4-Chloroaniline	127.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	127.0, 129.0, 92.0
9	8.740		Hexachlorobutadie	225.0, C:M	Linear, Ignor, 7	0.25, W:4.0, S:20	0.20, Spec	225.0, 227.0, 223.
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

Data File Name: C:\VarianWS\MSTutorials\80_NG.SMS

Click on the **View Curves** button. The calibration curve for the currently selected compound in the Compound Table is displayed.



Calibration Curve - 2,4-Dimethylphenol

calibration.mth: 2000.40: 2,4-Dimethylphenol

Internal Standard Analysis

Curve Fit: Linear, Origin: Ignore, Weight: 1/nX2

Resp. Fact. RSD: 8.074%, Coeff. Det.(r2): 0.998109

y = +1.1065x -0.0421

Peak Name: 1. 2,4-Dimethylphenol

Curve Fit Type: Linear

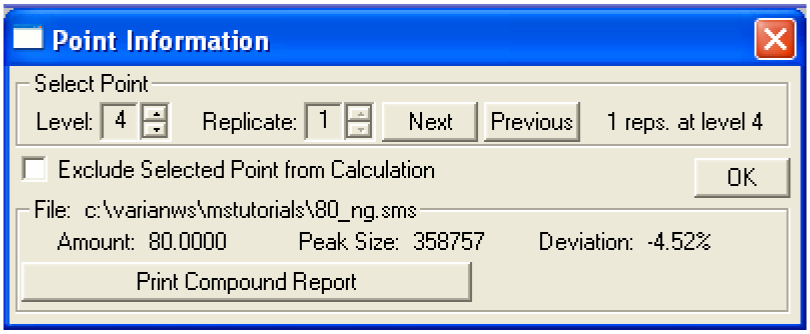
Origin Point: Ignore

Regression: 1/nX2

Notice that the Curve Fit options chosen when the Compound Table was built are defaults for the display (Linear Fit, Ignore Origin Point). The linear fit equation for 2,4-Dimethylphenol is shown above the plot field. Click on the other Origin options, Include Origin Point and Force curve through the origin. Observe the change in the fit equation and the Correlation Coefficient. Now try Quadratic and Cubic curve fits and observe the fit equations and correlation coefficients that result. Note that for most well behaved species analyzed by GC/MS the Linear fit and ignoring the origin point provides the simplest route to good quantitation results.

Click on the up/down arrows in the Peak Name field to observe the curves for the other compounds in the Compound Table. If you have changed the fit options you will be asked whether you wish to keep the edits.

Double-click on one of the data points of the calibration curve graph, for example the 80 ng level for 2,4-Dimethylphenol. Observe the new Point Info dialog appearing below the Calibration Curve dialog.



Level: 4

Replicate: 1

1 reps. at level 4

☐ Exclude Selected Point from Calculation

File: c:\varianws\mstutorials\80_ng.sms

Amount: 80.0000

Peak Size: 358757

Deviation: -4.52%

As you can see in the Calibration Curve plot, this data point has a greater deviation from the fitted line than other data points for the compound. Note the deviation value of –4.52% in the Point Info dialog. Click on the box **Exclude Selected Point from Calculation**. Observe the change in the fit equation, response factor RSD, and the Correlation Coefficient when the point is excluded.

Points may also be excluded from the calculations by right-clicking on the selected data points in the plot. This is a toggle function. Right-clicking on an excluded point will include it again.

NOTE: The process of excluding data points does not remove the data points from the Compound Table. However, exclusion does affect the calibration calculation and the quantitation results.

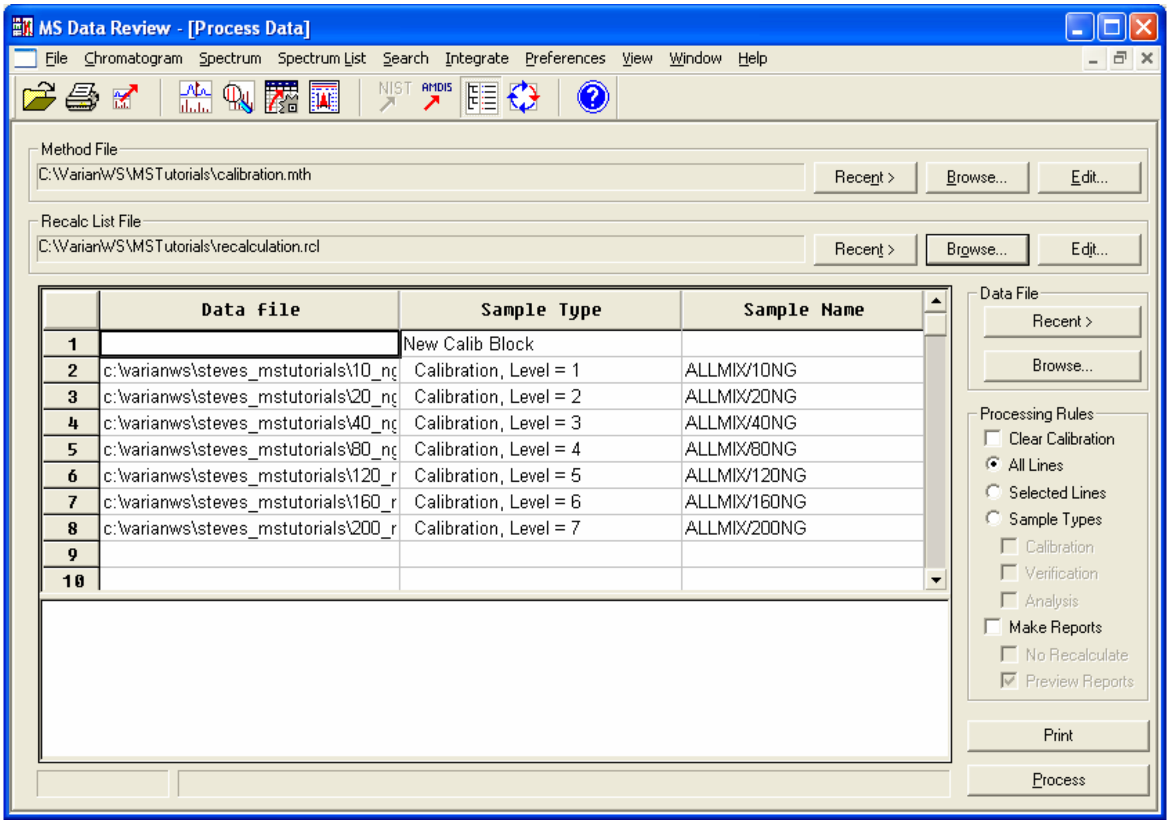
Processing Analysis Files in a RecalcList

Processing of analysis files is performed in the **Process Data** view. There are two ways to process files as Analysis Samples. You can add files to the existing RecalcList used for calibration, or you can just process the currently active file opened in the chromatogram display. Both ways will be exemplified in the following.

Adding and Processing Entries in an Existing RecalcList

You have learned how to process a Calibration Block using a Data Handling method and a RecalcList. Now it is time to process Analysis files in a RecalcList.

Open the **Process Data** view. If they are not already selected, choose the files calibration.mth and quantitation.rcl for the active data handling method and RecalcList, respectively.



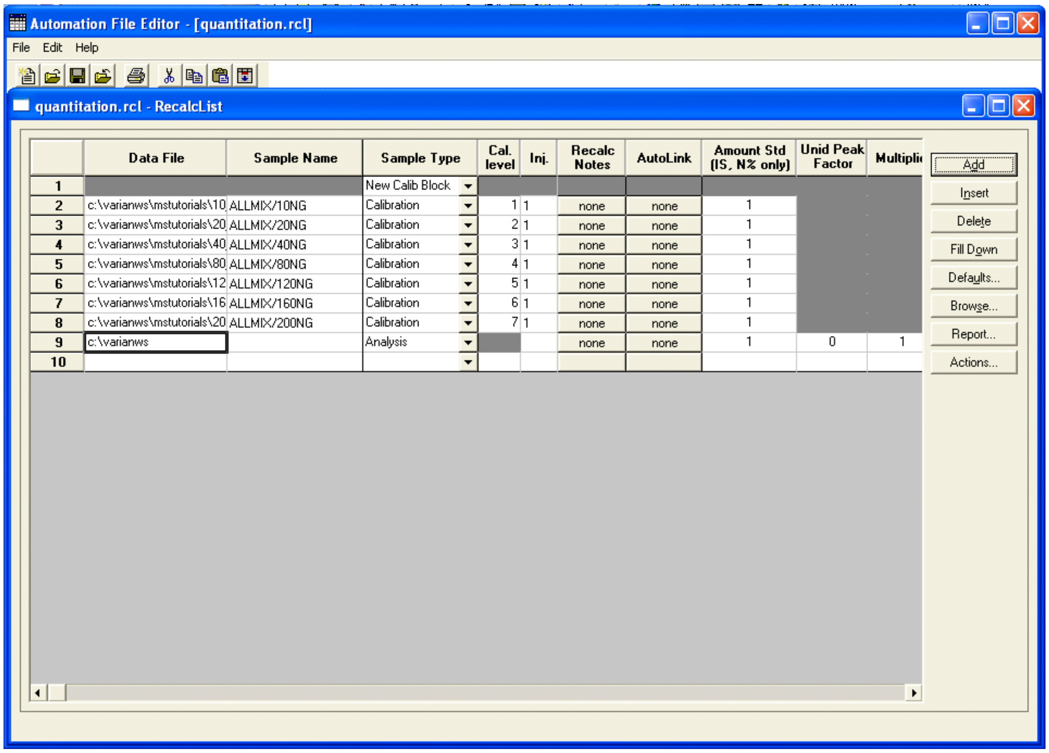
Method File: C:\VarianWS\MSTutorials\calibration.mth

Recalc List File: C:\VarianWS\MSTutorials\recalculation.rcl

	Data file	Sample Type	Sample Name
1		New Calib Block	
2	c:\varianws\steves_mstutorials\10_nç	Calibration, Level = 1	ALLMIX/10NG
3	c:\varianws\steves_mstutorials\20_nç	Calibration, Level = 2	ALLMIX/20NG
4	c:\varianws\steves_mstutorials\40_nç	Calibration, Level = 3	ALLMIX/40NG
5	c:\varianws\steves_mstutorials\80_nç	Calibration, Level = 4	ALLMIX/80NG
6	c:\varianws\steves_mstutorials\120_r	Calibration, Level = 5	ALLMIX/120NG
7	c:\varianws\steves_mstutorials\160_r	Calibration, Level = 6	ALLMIX/160NG
8	c:\varianws\steves_mstutorials\200_r	Calibration, Level = 7	ALLMIX/200NG
9			
10			

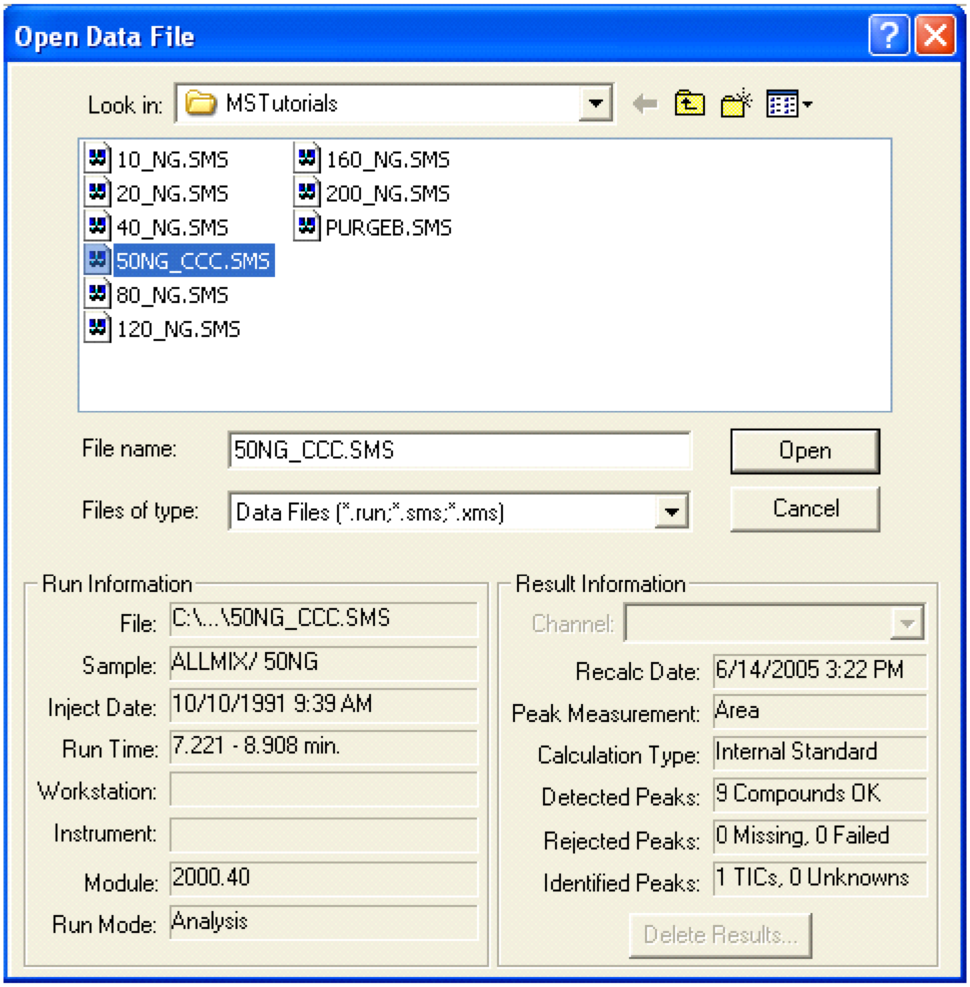
Processing Rules
- ☐ Clear Calibration
- ● All Lines
- ○ Selected Lines
- ○ Sample Types
  - ☐ Calibration
  - ☐ Verification
  - ☐ Analysis
- ☐ Make Reports
  - ☐ No Recalculate
  - ☑ Preview Reports

In the **RecalcList File** line, click **Edit** to enter the **Automation File Editor**. Click **Add** to add a new entry, which will have a default **Sample Type** of **Analysis**.



	Data File	Sample Name	Sample Type	Cal. level	Inj.	Recalc Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multiplie
1			New Calib Block							
2	c:\varianws\mstutorials\10	ALLMIX/10NG	Calibration	1	1	none	none	1		
3	c:\varianws\mstutorials\20	ALLMIX/20NG	Calibration	2	1	none	none	1		
4	c:\varianws\mstutorials\40	ALLMIX/40NG	Calibration	3	1	none	none	1		
5	c:\varianws\mstutorials\80	ALLMIX/80NG	Calibration	4	1	none	none	1		
6	c:\varianws\mstutorials\12	ALLMIX/120NG	Calibration	5	1	none	none	1		
7	c:\varianws\mstutorials\16	ALLMIX/160NG	Calibration	6	1	none	none	1		
8	c:\varianws\mstutorials\20	ALLMIX/200NG	Calibration	7	1	none	none	1		
9	c:\varianws		Analysis			none	none	1	0	1
10										

Click **Browse** to select a file for analysis processing and select the file 50ng_ccc.sms.



Look in: MSTutorials

10_NG.SMS
20_NG.SMS
40_NG.SMS
50NG_CCC.SMS
80_NG.SMS
120_NG.SMS
160_NG.SMS
200_NG.SMS
PURGEB.SMS

File name: 50NG_CCC.SMS
Files of type: Data Files (*.run;*.sms;*.xms)

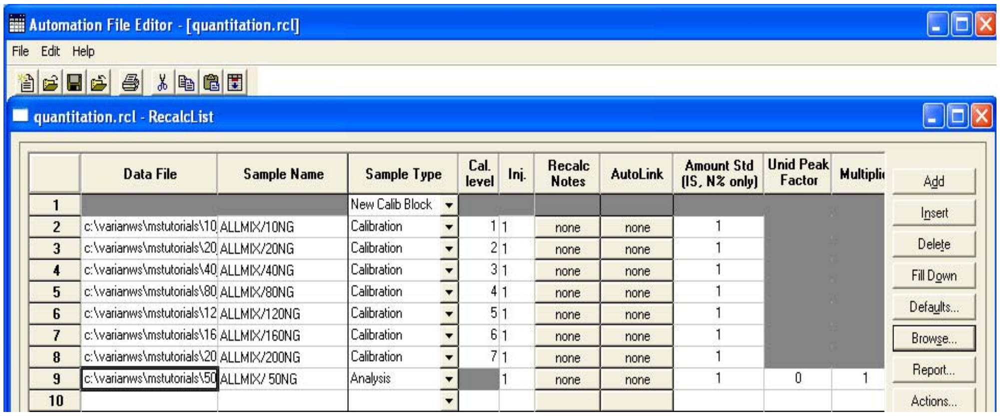
Run Information

File: C:\...\50NG_CCC.SMS
Sample: ALLMIX/ 50NG
Inject Date: 10/10/1991 9:39 AM
Run Time: 7.221 - 8.908 min.
Workstation:
Instrument:
Module: 2000.40
Run Mode: Analysis

Result Information

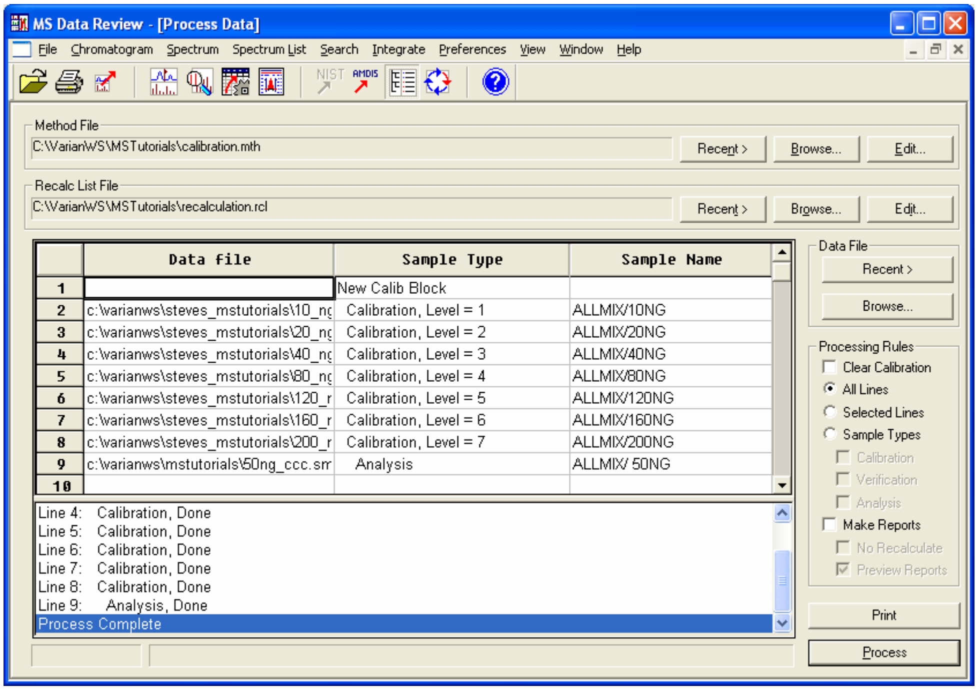
Recalc Date: 6/14/2005 3:22 PM
Peak Measurement: Area
Calculation Type: Internal Standard
Detected Peaks: 9 Compounds OK
Rejected Peaks: 0 Missing, 0 Failed
Identified Peaks: 1 TICs, 0 Unknowns

Click **Open** to close the file selection dialog and enter this file name for the **Analysis** entry in the **RecalcList**.



	Data File	Sample Name	Sample Type	Cal. level	Inj.	Recalc Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multipli
1			New Calib Block							
2	c:\varianws\mstutorials\10	ALLMIX/10NG	Calibration	1	1	none	none	1		
3	c:\varianws\mstutorials\20	ALLMIX/20NG	Calibration	2	1	none	none	1		
4	c:\varianws\mstutorials\40	ALLMIX/40NG	Calibration	3	1	none	none	1		
5	c:\varianws\mstutorials\80	ALLMIX/80NG	Calibration	4	1	none	none	1		
6	c:\varianws\mstutorials\12	ALLMIX/120NG	Calibration	5	1	none	none	1		
7	c:\varianws\mstutorials\16	ALLMIX/160NG	Calibration	6	1	none	none	1		
8	c:\varianws\mstutorials\20	ALLMIX/200NG	Calibration	7	1	none	none	1		
9	c:\varianws\mstutorials\50	ALLMIX/ 50NG	Analysis		1	none	none	1	0	1
10										

If you have already processed the Calibration files, you could delete the **New Calibration Block** entry and the seven Calibration files before Processing the **RecalcList** but this is not necessary. Save the RecalcList and close the **Automation File Editor**. You can now perform the Recalculation immediately by selecting **All Lines** and clicking the **Process** button. You will be able to observe the progress of the recalculation in the processing status field below the Data File Table. At the end of the run the screen will look like this.



Method File: C:\VarianWS\MSTutorials\calibration.mth

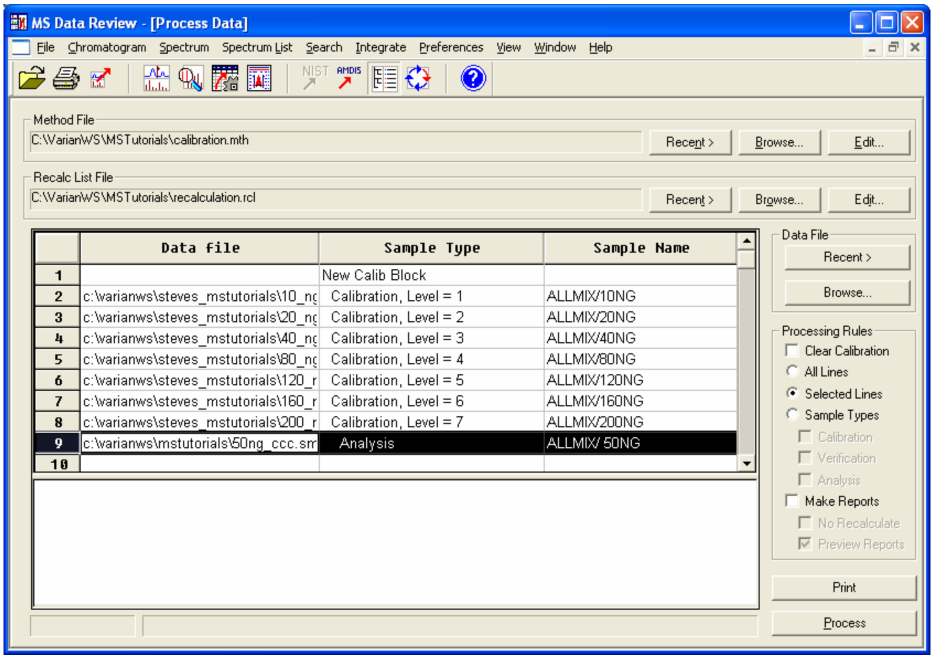
Recalc List File: C:\VarianWS\MSTutorials\recalculation.rcl

	Data file	Sample Type	Sample Name
1		New Calib Block	
2	c:\varianws\steves_mstutorials\10_nç	Calibration, Level = 1	ALLMIX/10NG
3	c:\varianws\steves_mstutorials\20_nç	Calibration, Level = 2	ALLMIX/20NG
4	c:\varianws\steves_mstutorials\40_nç	Calibration, Level = 3	ALLMIX/40NG
5	c:\varianws\steves_mstutorials\80_nç	Calibration, Level = 4	ALLMIX/80NG
6	c:\varianws\steves_mstutorials\120_r	Calibration, Level = 5	ALLMIX/120NG
7	c:\varianws\steves_mstutorials\160_r	Calibration, Level = 6	ALLMIX/160NG
8	c:\varianws\steves_mstutorials\200_r	Calibration, Level = 7	ALLMIX/200NG
9	c:\varianws\mstutorials\50ng_ccc.sm	Analysis	ALLMIX/ 50NG
10			

```
Line 4:   Calibration, Done
Line 5:   Calibration, Done
Line 6:   Calibration, Done
Line 7:   Calibration, Done
Line 8:   Calibration, Done
Line 9:     Analysis, Done
Process Complete
```

Processing Rules:
- ☐ Clear Calibration
- ● All Lines
- ○ Selected Lines
- ○ Sample Types
  - ☐ Calibration
  - ☐ Verification
  - ☐ Analysis
- ☐ Make Reports
  - ☐ No Recalculate
  - ☑ Preview Reports

To only process the 50ng_ccc.sms file, you can select **Selected Lines**, highlight the 50ng_ccc.sms line and then click **Process** (make sure that **Clear Calibration** is not checked)**.**



Method File: C:\VarianWS\MSTutorials\calibration.mth

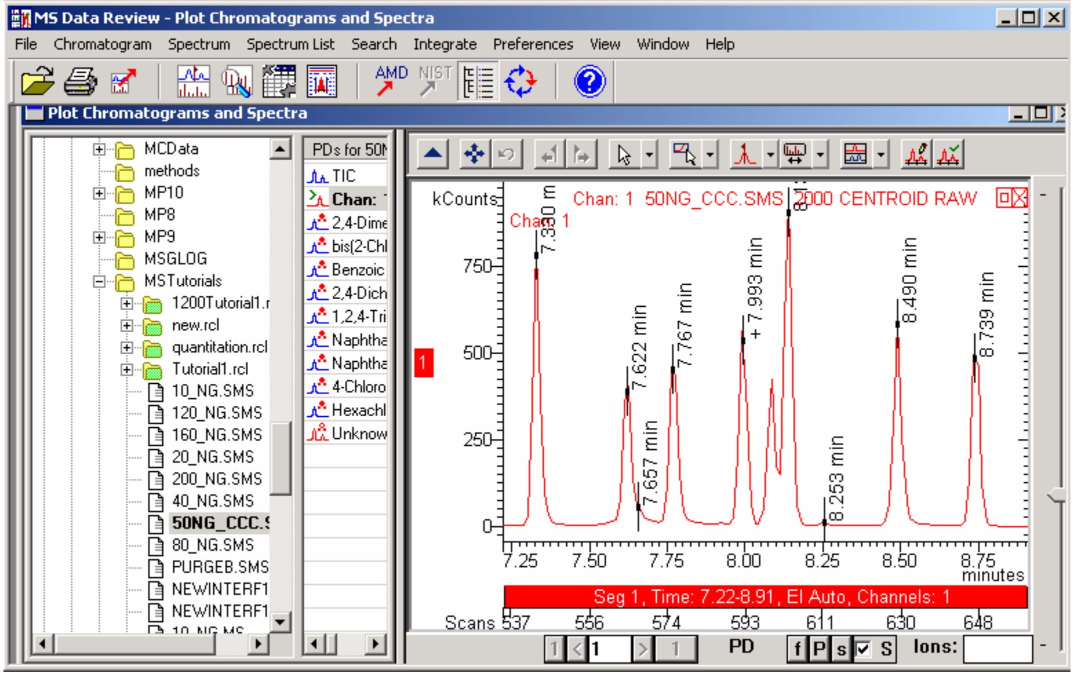
Recalc List File: C:\VarianWS\MSTutorials\recalculation.rcl

	Data file	Sample Type	Sample Name
1		New Calib Block	
2	c:\varianws\steves_mstutorials\10_nç	Calibration, Level = 1	ALLMIX/10NG
3	c:\varianws\steves_mstutorials\20_nç	Calibration, Level = 2	ALLMIX/20NG
4	c:\varianws\steves_mstutorials\40_nç	Calibration, Level = 3	ALLMIX/40NG
5	c:\varianws\steves_mstutorials\80_nç	Calibration, Level = 4	ALLMIX/80NG
6	c:\varianws\steves_mstutorials\120_r	Calibration, Level = 5	ALLMIX/120NG
7	c:\varianws\steves_mstutorials\160_r	Calibration, Level = 6	ALLMIX/160NG
8	c:\varianws\steves_mstutorials\200_r	Calibration, Level = 7	ALLMIX/200NG
9	c:\varianws\mstutorials\50ng_ccc.sm	Analysis	ALLMIX/ 50NG
10			

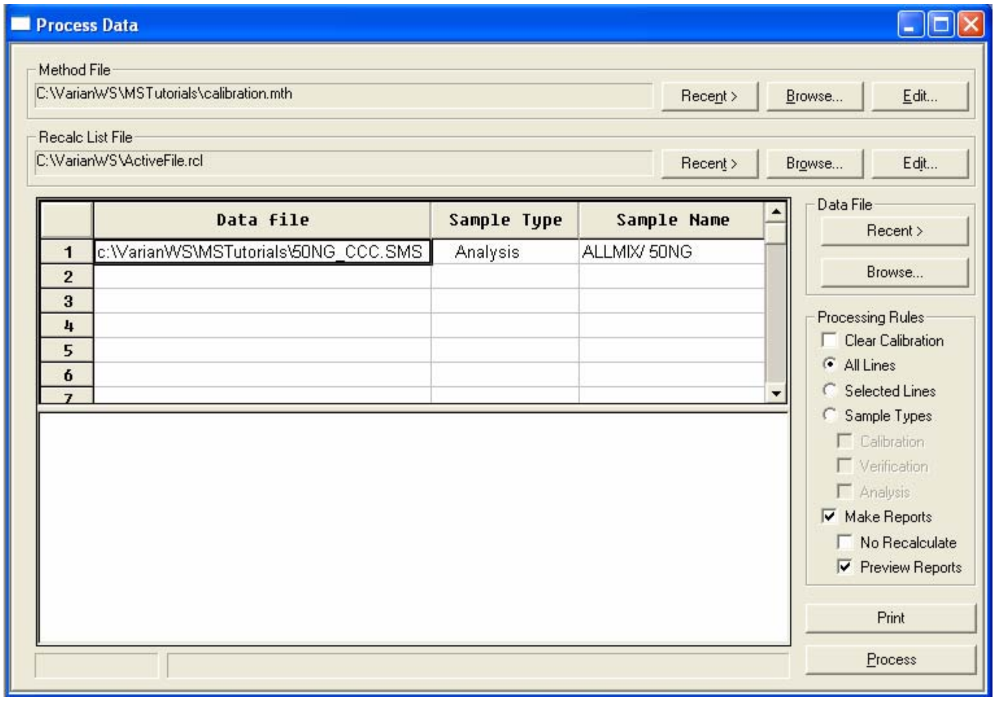
Processing Rules:
- ☐ Clear Calibration
- ○ All Lines
- ● Selected Lines
- ○ Sample Types
  - ☐ Calibration
  - ☐ Verification
  - ☐ Analysis
- ☐ Make Reports
  - ☐ No Recalculate
  - ☑ Preview Reports

Processing the Active Data File

Alternatively, open the chromatogram to be analyzed by selecting the data file name in the Data File pane of the **Plot Chromatograms and Spectra** view**.** Select the same analysis file, 50ng_ccc.sms, as in the previous tutorial.



Select the **Process Data** button in the **MS Data Review** toolbar. This will open the **Process Data** view, which has in the *Method File* field the last method file used (usually to run the Calibration Block) and in the *RecalcList File* field a new recalclist file (named by default ActiveFile.rcl) that contains the currently-active data file.



Process Data

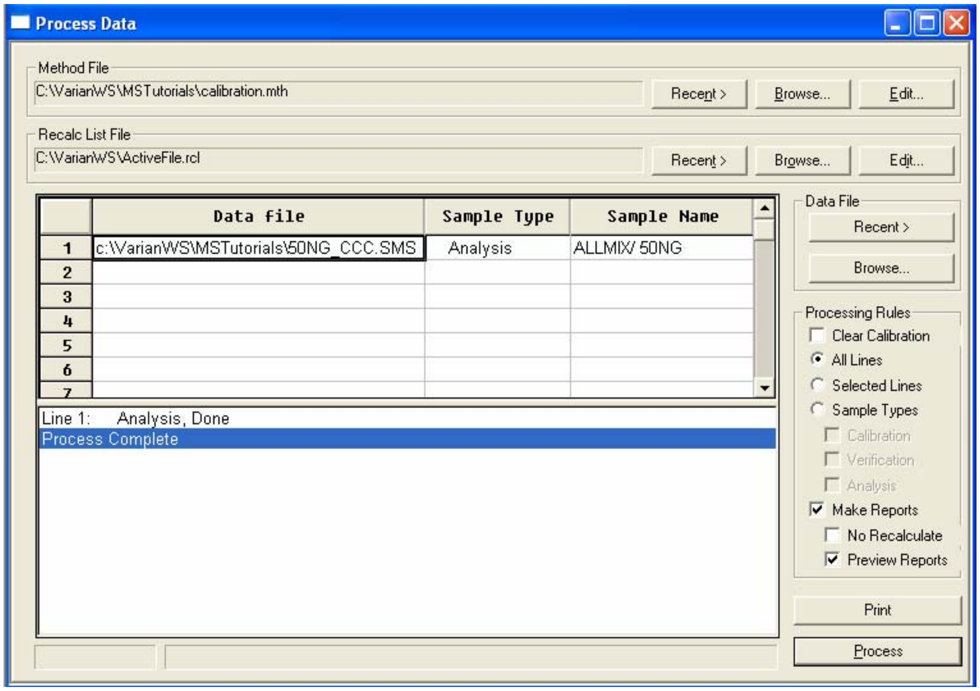
Method File: C:\VarianWS\MSTutorials\calibration.mth

Recalc List File: C:\VarianWS\ActiveFile.rcl

	Data file	Sample Type	Sample Name
1	c:\VarianWS\MSTutorials\50NG_CCC.SMS	Analysis	ALLMIX/ 50NG
2			
3			
4			
5			
6			
7			

Processing Rules
- ☐ Clear Calibration
- ● All Lines
- ○ Selected Lines
- ○ Sample Types
  - ☐ Calibration
  - ☐ Verification
  - ☐ Analysis
- ☑ Make Reports
  - ☐ No Recalculate
  - ☑ Preview Reports

When a calibration is processed, the calibration information including the calibration files that are used, are added to the Calibration Block of the data handling method used for processing. Since you performed the calibration in the previous tutorial, all this information is contained in the method file calibration.mth. To run the analysis, all you have to do is click the **Process** button (make sure that **Clear Calibration** is not checked). As before, the processing status display field will show when the processing is complete.



Process Data

Method File: C:\VarianWS\MSTutorials\calibration.mth

Recalc List File: C:\VarianWS\ActiveFile.rcl

	Data file	Sample Type	Sample Name
1	c:\VarianWS\MSTutorials\50NG_CCC.SMS	Analysis	ALLMIX/ 50NG
2			
3			
4			
5			
6			
7			

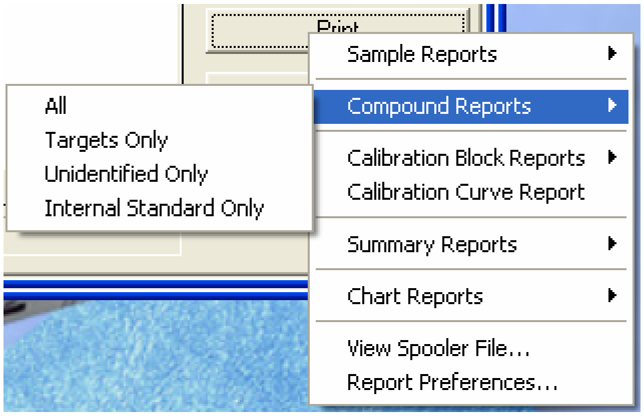
```
Line 1:     Analysis, Done
Process Complete
```

Processing Rules
- ☐ Clear Calibration
- ● All Lines
- ○ Selected Lines
- ○ Sample Types
  - ☐ Calibration
  - ☐ Verification
  - ☐ Analysis
- ☑ Make Reports
  - ☐ No Recalculate
  - ☑ Preview Reports

Reviewing Analysis Results

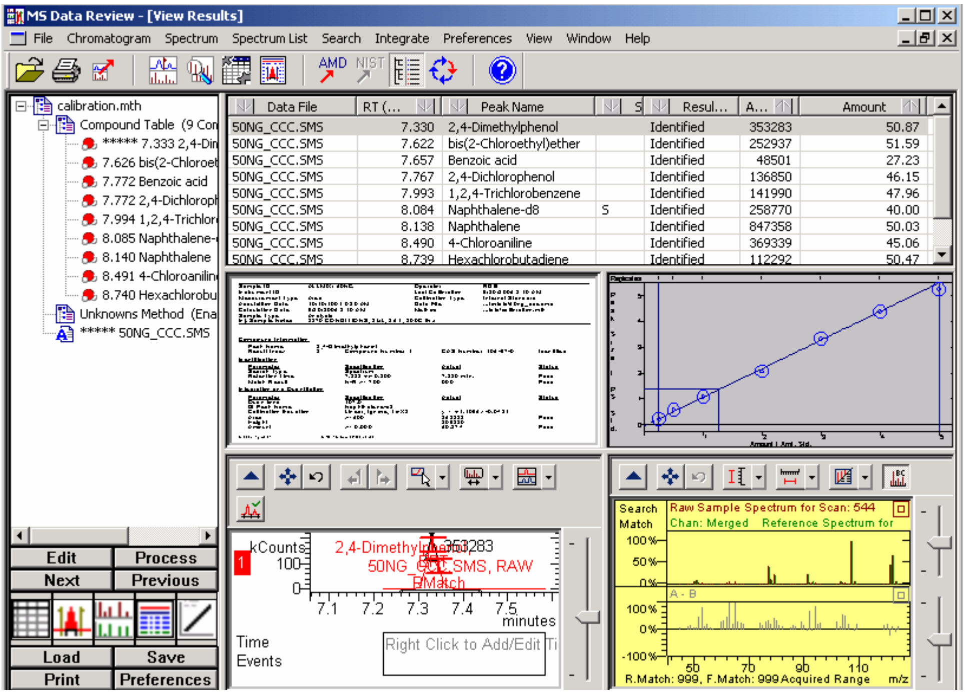
The results for the analysis files you have just processed can be reviewed in the same way as calibration results, from the **Process Data** view or the **Results** view.

In the **Process Data** view, click the **Print** tab, and select among the listed options. Verify that **Preview Printed Reports** is checked in the **File** menu of the **MS Data Review** window, as mentioned in the previous tutorials.



Previews of the selected reports will be opened, which you can examine page-by-page and/or print, as previously described for reviewing the calibration results.

Open the **View Results** dialog by clicking the **View Results** toolbar button . As only the analysis file was processed, the list displayed on top of the Results view contains only the compounds of this data file.

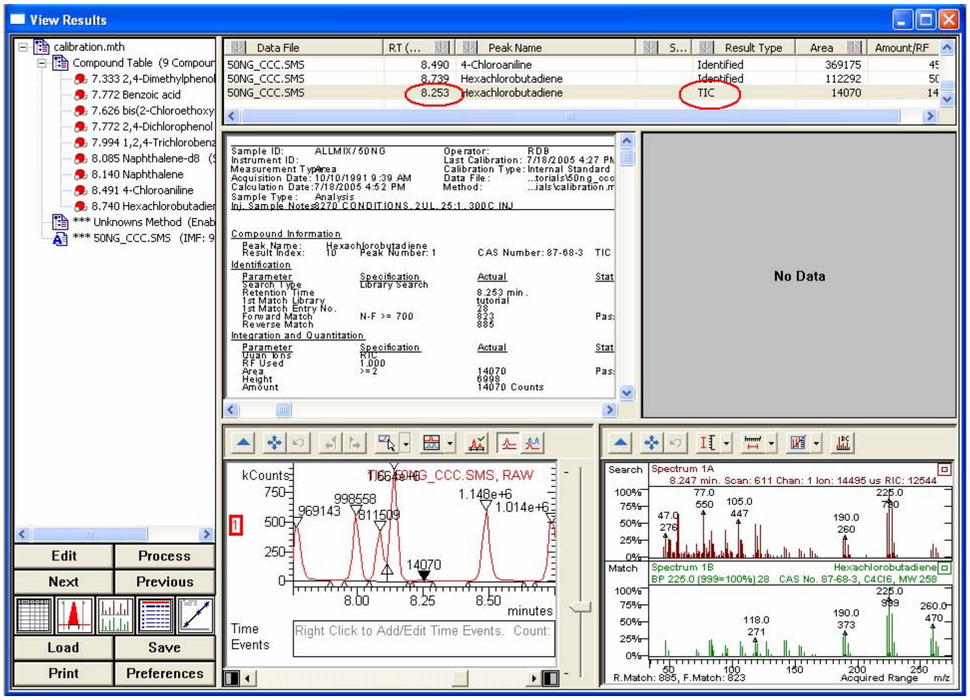


Data File	RT (...	Peak Name	S	Resul...	A...	Amount
50NG_CCC.SMS	7.330	2,4-Dimethylphenol		Identified	353283	50.87
50NG_CCC.SMS	7.622	bis(2-Chloroethyl)ether		Identified	252937	51.59
50NG_CCC.SMS	7.657	Benzoic acid		Identified	48501	27.23
50NG_CCC.SMS	7.767	2,4-Dichlorophenol		Identified	136850	46.15
50NG_CCC.SMS	7.993	1,2,4-Trichlorobenzene		Identified	141990	47.96
50NG_CCC.SMS	8.084	Naphthalene-d8	S	Identified	258770	40.00
50NG_CCC.SMS	8.138	Naphthalene		Identified	847358	50.03
50NG_CCC.SMS	8.490	4-Chloroaniline		Identified	369339	45.06
50NG_CCC.SMS	8.739	Hexachlorobutadiene		Identified	112292	50.47

For the **Analysis** sample type, the **Amount** field reports the quantitative amounts of the compounds, instead of Relative Response Factors that are reported for the

Calibration files. This data file has all of the Target Compounds present at 50 ng levels. All but the Benzoic Acid peak show good quantitative results. Adjusting the integration and curve fit parameters for the benzoic acid species would improve results for this compound. These operations can be performed in the Integration pane of the Results view and in the Method Builder dialog as previously described for adjusting parameters to improve calibration results).

One compound, Hexachlorobutadiene, was also tentatively identified during Chromatogram Processing of unknown peaks. This TIC unknown has a different retention time and lower spectrum match than the compound identified as Hexachlorobutadiene. This suggests that it is in fact a structurally similar, but different compound.



Data File	RT (...	Peak Name	S...	Result Type	Area	Amount/RF
50NG_CCC.SMS	8.490	4-Chloroaniline		Identified	369175	45
50NG_CCC.SMS	8.739	Hexachlorobutadiene		Identified	112292	50
50NG_CCC.SMS	8.253	Hexachlorobutadiene		TIC	14070	14

Sample ID: ALLMIX/50NG
Instrument ID:
Measurement Type: Area
Acquisition Date: 10/10/1991 9:39 AM
Calculation Date: 7/18/2005 4:52 PM
Sample Type: Analysis
Inj. Sample Notes: 8270 CONDITIONS, 2UL, 25:1, 300C INJ
Operator: RDB
Last Calibration: 7/18/2005 4:27 PM
Calibration Type: Internal Standard
Data File: ...torials\50ng_ccc
Method: ...ials\calibration.m

Compound Information
Peak Name: Hexachlorobutadiene
Result Index: 10 Peak Number: 1 CAS Number: 87-68-3 TIC

Identification

Parameter	Specification	Actual	Stat
Search Type	Library Search		
Retention Time		8.253 min.	
1st Match Library		tutorial	
1st Match Entry No.		28	
Forward Match	N-F >= 700	823	Pas
Reverse Match		885	

Integration and Quantitation

Parameter	Specification	Actual	Stat
Quan Ions	RIC		
RF Used	1.000		
Area	>= 2	14070	Pas
Height		6998	
Amount		14070 Counts	

Remember that the parameters for processing unknown peaks are set up in the **Calculations Setup** dialog of the MS Method in the Method Builder application. It can also be accessed from this screen via the **Edit** button.

SIS and MS/MS Acquisitions

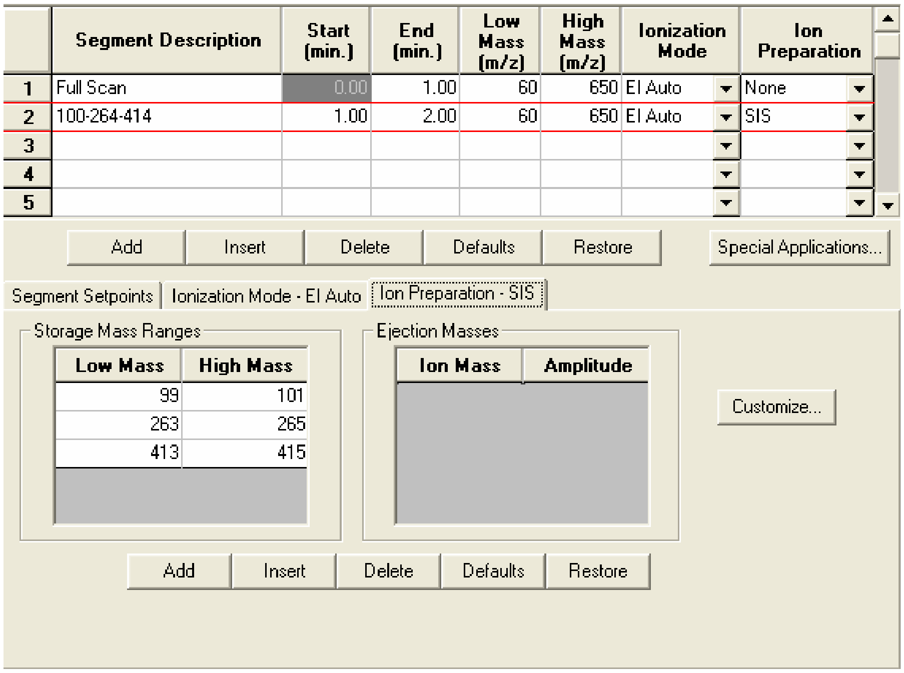
Introduction Tutorial

Calibration of SIS

Using the Selected Ion Storage (SIS) option, you may selectively accumulate or store specific ions in the trap. The ejection of the unwanted ions is performed by applying a multifrequency waveform, which includes those frequencies required to eject the unwanted ions and misses the frequencies corresponding to the stored ions. The waveform amplitude will be adjusted so that there is good ejection of matrix ions while the desired ions are still stored efficiently.

This tutorial assumes that you have a basic knowledge of 2000 GC/MS operation and that your instrument has been tuned.

To perform SIS calibration, you will first build a SIS Method to isolate three ions, m/z=100, 264 and 414 from the calibration gas spectrum. Open the **Method Builder** application from the **Workstation Toolbar**. Open a GC/MS method and edit the MS section. Create a two-segment method as shown below.



	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	Full Scan	0.00	1.00	60	650	EI Auto	None
2	100-264-414	1.00	2.00	60	650	EI Auto	SIS
3							
4							
5							

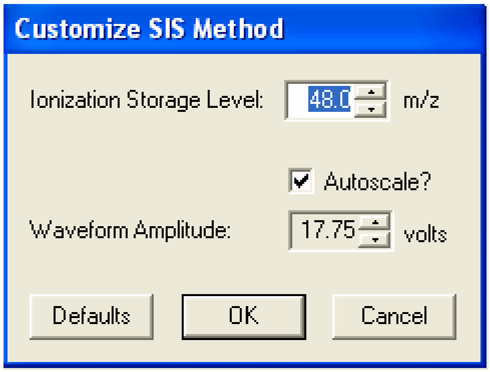
Storage Mass Ranges

Low Mass	High Mass
99	101
263	265
413	415

Ejection Masses

Ion Mass	Amplitude

The first segment detects all ions in the calibration gas in the mass range of 60-650 in the EI Auto mode (no ion preparation). The second segment selectively stores the m/z 100, 264 and 414. To set up the Storage Mass Range for SIS highlight the second segment in the segment table (as shown above), then select the **Ion Preparation - SIS** tab dialog. Under the *Storage Mass Ranges* enter the values shown in the picture. Click the **Customize** button and make sure that the **Autoscale** field is checked.



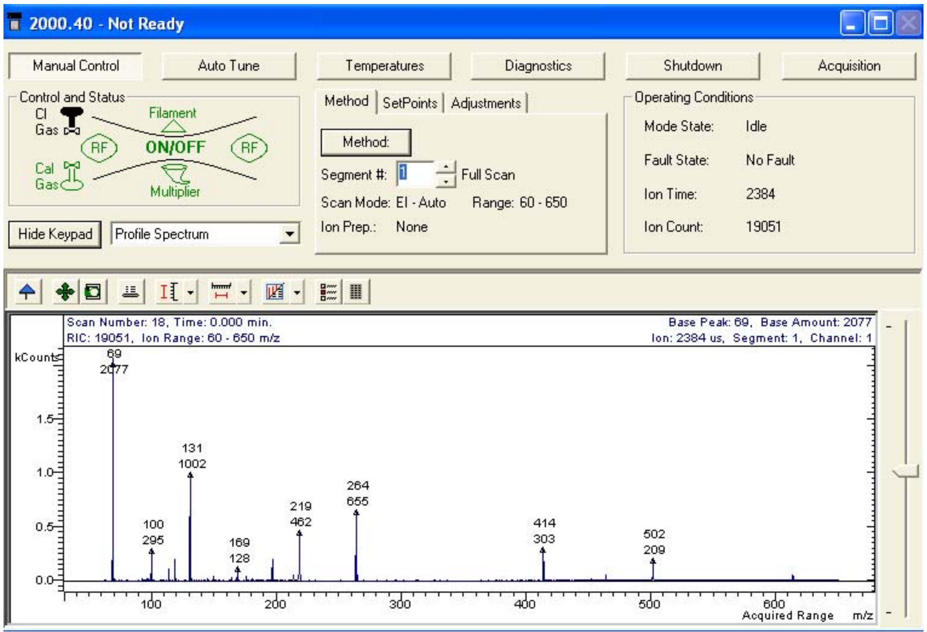
Ionization Storage Level: 48.0 m/z

☑ Autoscale?

Waveform Amplitude: 17.75 volts

This selection will assure that optimum waveform amplitude is calculated, which will eject all the ions except the ones selected for storage. You can further optimize the waveform amplitude value by adjusting the **SIS Amplitude Adjust Factor** in the **Auto Tune** mode in **System Control** as will be described later in this tutorial. Save the method file as CalSIS by using the menu command **File> Save As** and then close the **Method Builder** application.

Select the **System Control** application from the **Workstation Toolbar**. Use the menu command **File >Activate Method**. From the selection dialog select CalSIS.mth and click **Open**. Select the **Manual Control** mode in **System Control**. Turn on the trap and the calibration gas by clicking the **ON/OFF** and **Cal Gas** symbols in the schematic picture in the **Control and Status** field (see picture below). The color of the symbols will turn green showing which components of the instrument are turned on. Use the Segment # field in the Method display to select the desired segments.



Segment #: 1 Full Scan

Scan Mode: EI - Auto Range: 60 - 650

Ion Prep.: None

Mode State: Idle

Fault State: No Fault

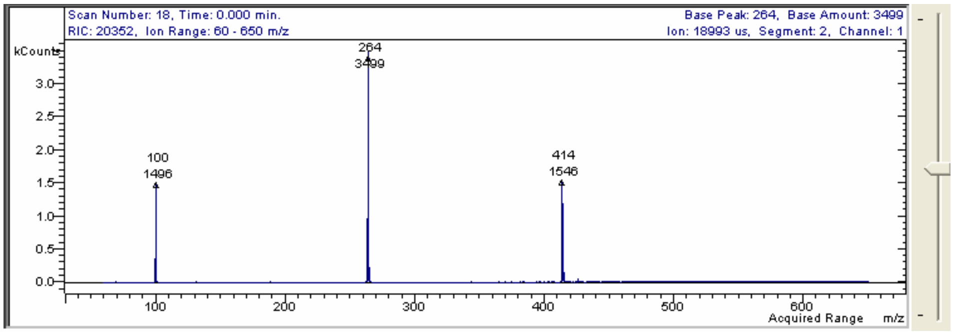
Ion Time: 2384

Ion Count: 19051

Scan Number: 18, Time: 0.000 min.
RIC: 19051, Ion Range: 60 - 650 m/z

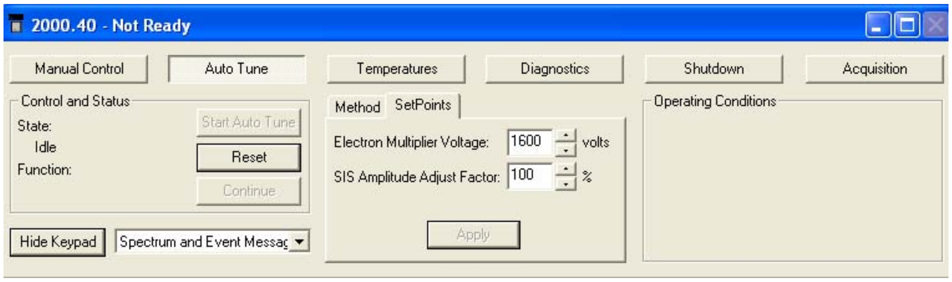
Base Peak: 69, Base Amount: 2077
Ion: 2384 us, Segment: 1, Channel: 1

The mass spectrum displayed in the above picture was acquired with segment 1. It shows all ions in the calibration gas, as there is no ion preparation selected. Select the second segment that uses the SIS ion preparation to store only the m/z 100, 264 and 414. The mass spectrum acquired with segment 2 (SIS on) shows only the selected ions.



A SIS calibration determines the minimum waveform amplitude so that unwanted ions are efficiently eliminated while the peak intensities of the selected ions are maximized. This calibration is performed by finding the lowest SIS Amplitude Adjust Factor. This factor represents the percentage of the waveform amplitude, which was automatically determined by checking the **Autoscale** field in the **Customize SIS Method** dialog, as mentioned previously. The spectrum shown above (SIS on) was acquired with the default value of 100 for the SIS Amplitude Adjust Factor. Note the base peak intensity using the scan display.

Click on **Auto Tune** and select the **Set Points** tab.



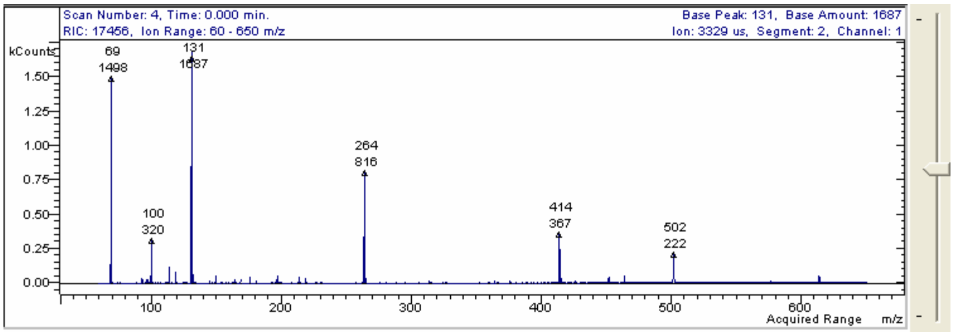
State: Idle

Function:

Electron Multiplier Voltage: 1600 volts

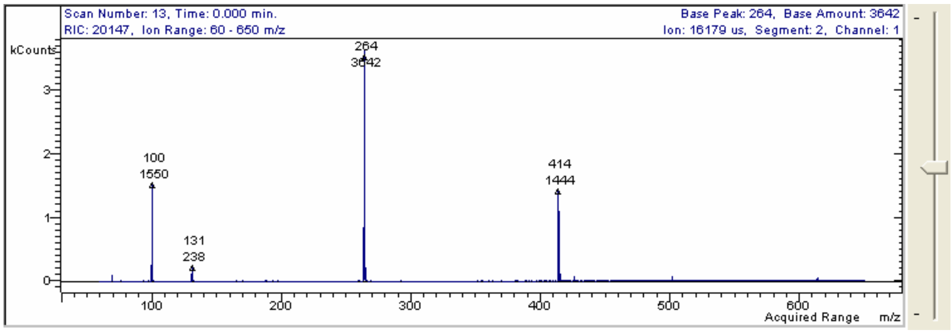
SIS Amplitude Adjust Factor: 100 %

Set the SIS Amplitude Adjust Factor to 50 (the values may be set between 50 and 200) and acquire another spectrum with segment 2.



The waveform amplitude value is too low (50% of the one calculated by choosing Autoscale) and as a result the unwanted ions are not ejected effectively. Note that the base peak ion is 131 and the its intensity is lower compared with the base peak intensity in the first spectrum due to the fact that EI Auto sets an optimum limit in the total number of ions stored in the trap.

Set now the SIS Amplitude Adjust Factor to 90.



The base peak ion is 264 and its intensity increased, however the ejection of the 131 ion is not complete.

For this ion trap system, a SIS Amplitude Adjust Factor of 100 gives the most effective ejection of undesired ions while providing optimum base peak intensity.

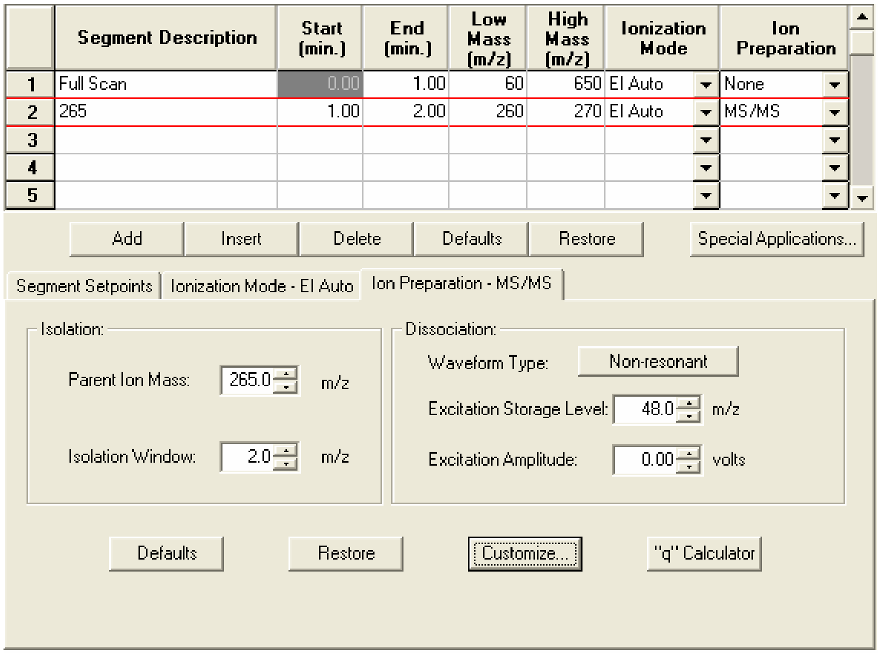
MS/MS

1. Isolating m/z 265 From Cal Gas

In this tutorial you will build an MS/MS ion preparation method that isolates m/z 265 in the cal gas spectrum. Isolating a small peak (m/z 265) in the presence of a large peak (m/z 264) will assist you in learning how to optimize isolation parameters and how the parameters affect the spectrum. The constant source of ions from cal gas facilitates learning how to adjust the MS/MS parameters.

This tutorial represents the first part of a MS/MS method, which is the isolation of the parent ion. To acquire a mass spectrum of the parent ion, we need to set the dissociation amplitude to zero, which means that no fragments are created and only the parent ion is detected.

Open the **View/Edit Method** application from the **Workstation Toolbar** and build a MS/MS method by using the parameters shown next. In the Parent Ion Mass field type 265 and choose an isolation window of 2. Set the Excitation Amplitude to zero in the Dissociation Field.

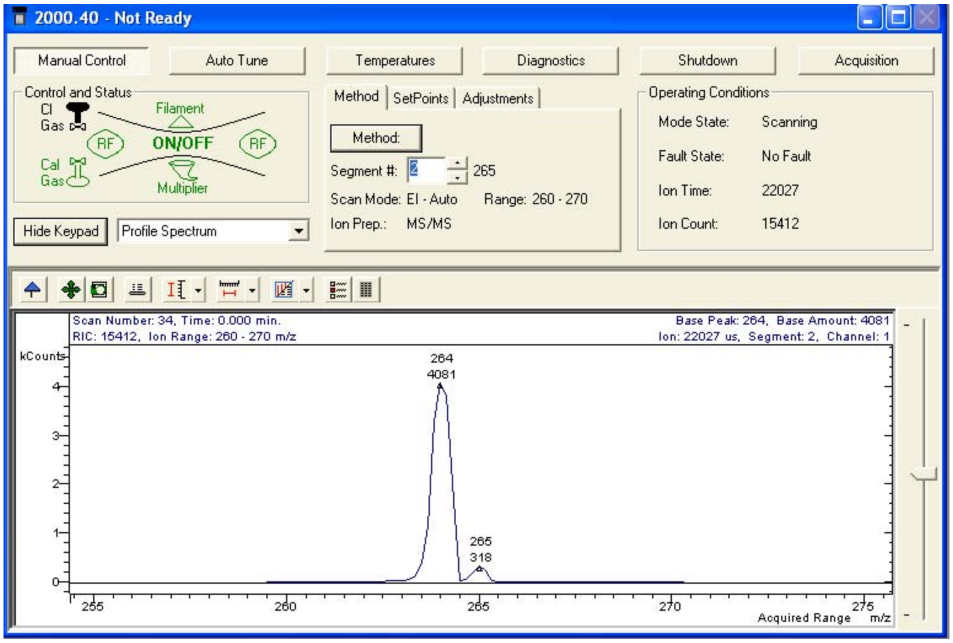


	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	Full Scan	0.00	1.00	60	650	El Auto	None
2	265	1.00	2.00	260	270	El Auto	MS/MS
3							
4							
5							

Isolation:
Parent Ion Mass: 265.0 m/z
Isolation Window: 2.0 m/z

Dissociation:
Waveform Type: Non-resonant
Excitation Storage Level: 48.0 m/z
Excitation Amplitude: 0.00 volts

Save the method by using the command **File >Save As** in the **Method Builder** menu and type a file name in the **Save Method File As** dialog. Close the **View/Edit Method** application and open the **System Control** application. Activate the method you just built by using the command **File >Activate Method**. Turn on the calibration gas and the trap. Observe the spectrum. If any of the peaks are off scale (> 4088 counts), reduce the AGC Target TIC Value in the method. (This can be done quickly by clicking on the **Method** button and making the change. When you close the **Method Builder** dialog you are asked if you want to save the changes and reactivate the method. Click **Yes** in both dialogs.) If the mass spectrum contains both m/z 264 and 265, the isolation window is too large.



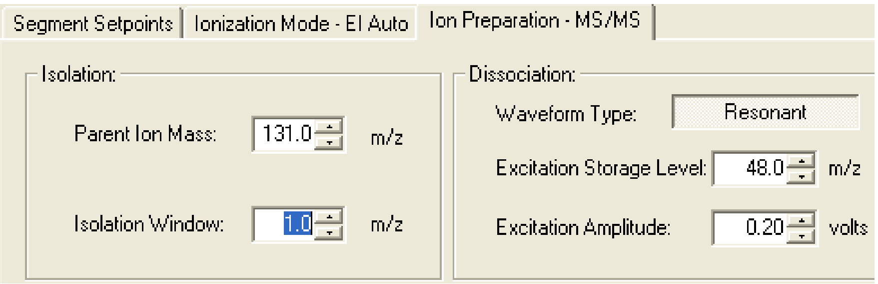
2000.40 - Not Ready

Segment #: 2 265
Scan Mode: El - Auto Range: 260 - 270
Ion Prep.: MS/MS

Operating Conditions
Mode State: Scanning
Fault State: No Fault
Ion Time: 22027
Ion Count: 15412

Scan Number: 34, Time: 0.000 min.
RIC: 15412, Ion Range: 260 - 270 m/z
Base Peak: 264, Base Amount: 4081
Ion: 22027 us, Segment: 2, Channel: 1

In the method, lower the Mass Isolation Window value to 1.



Isolation:

Parent Ion Mass: 131.0 m/z

Isolation Window: 1.0 m/z

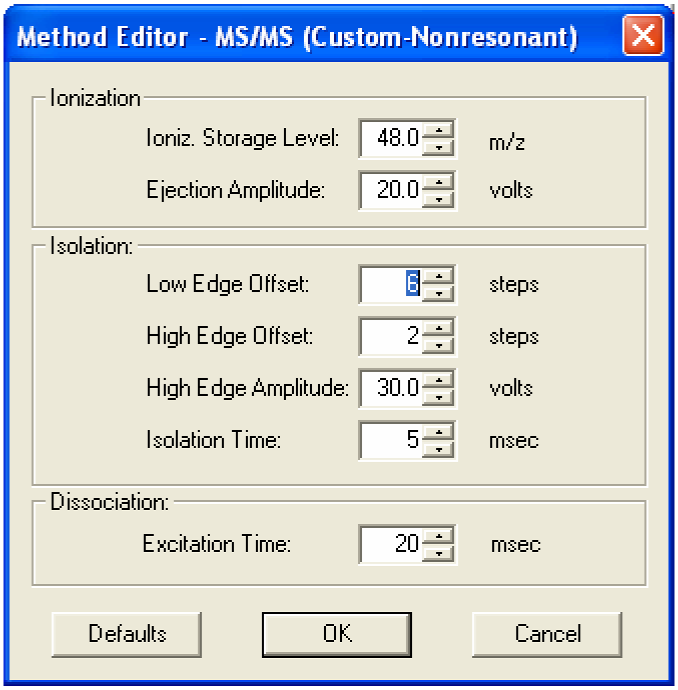
Dissociation:

Waveform Type: Resonant

Excitation Storage Level: 48.0 m/z

Excitation Amplitude: 0.20 volts

Return to System Control and acquire a mass spectrum with these changes. The 264 peak might still be present but its size should be reduced. To further optimize the isolation of m/z 265 from the m/z 264, you can do the following. In the method, click on segment 2 in the list. Click on the **Ion Preparation-MS/MS** tab and then select the **Customize** button.



Ionization

Ioniz. Storage Level: 48.0 m/z

Ejection Amplitude: 20.0 volts

Isolation:

Low Edge Offset: 6 steps

High Edge Offset: 2 steps

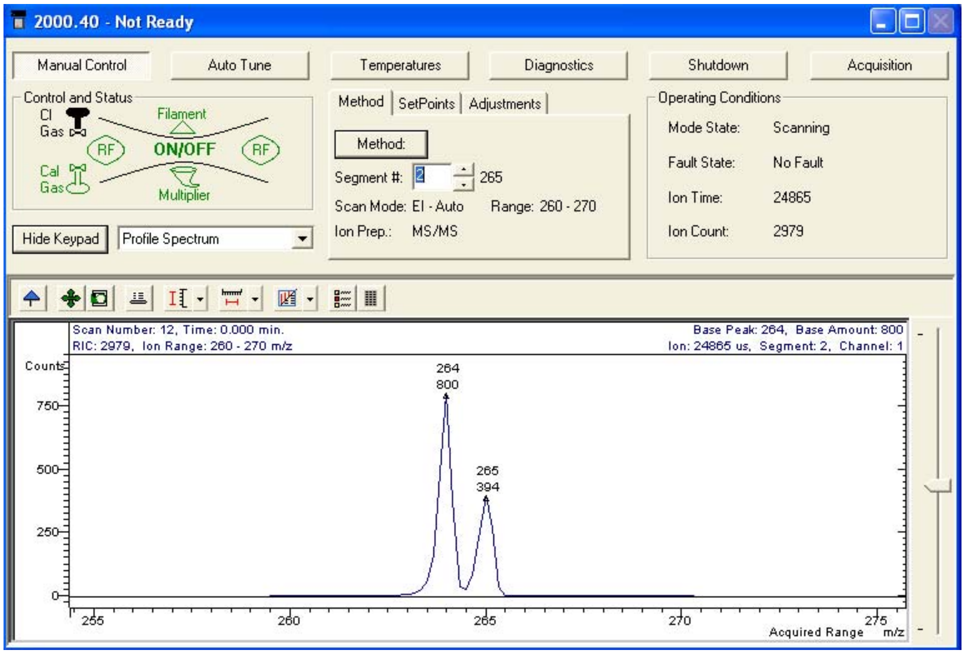
High Edge Amplitude: 30.0 volts

Isolation Time: 5 msec

Dissociation:

Excitation Time: 20 msec

The Low Edge Offset parameter in the Isolation field affects the isolation window on the low mass side of the parent ion. Decreasing the offset (the default value is 6) decreases the window on the low mass side. Set the Low Edge Offset to 4 and close the dialog by pressing **OK**. Keep the Isolation Window value to 1, return to System Control and acquire a mass spectrum with these changes.

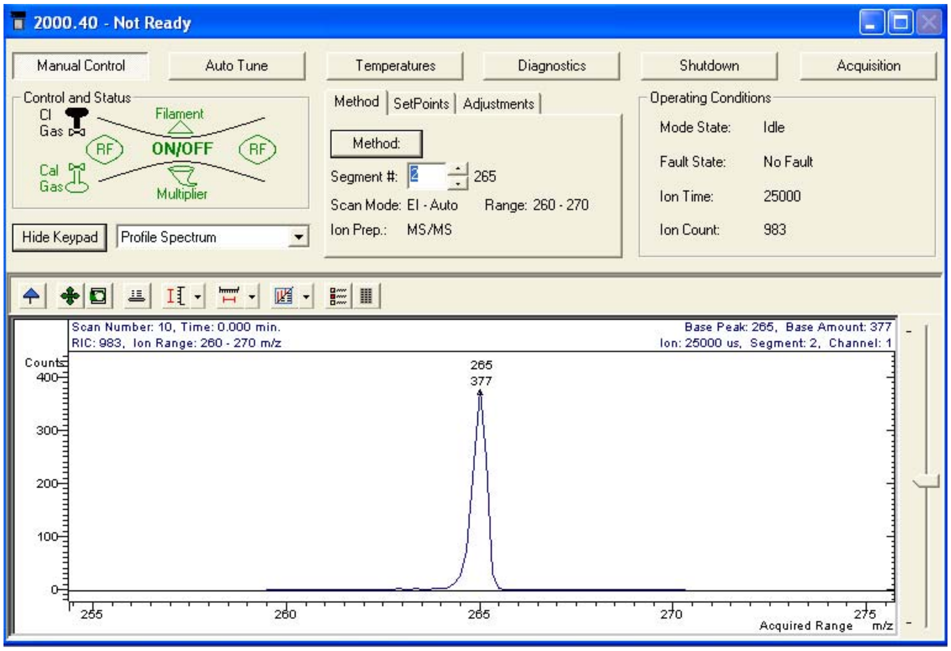


Segment #: 2 265
Scan Mode: EI - Auto Range: 260 - 270
Ion Prep.: MS/MS

Mode State: Scanning
Fault State: No Fault
Ion Time: 24865
Ion Count: 2979

Scan Number: 12, Time: 0.000 min.
RIC: 2979, Ion Range: 260 - 270 m/z
Base Peak: 264, Base Amount: 800
Ion: 24865 us, Segment: 2, Channel: 1

Observe that the m/z 264 peak is further reduced considerably. Now decrease the Low Edge Offset value to 2 and acquire a mass spectrum.



Segment #: 2 265
Scan Mode: EI - Auto Range: 260 - 270
Ion Prep.: MS/MS

Mode State: Idle
Fault State: No Fault
Ion Time: 25000
Ion Count: 983

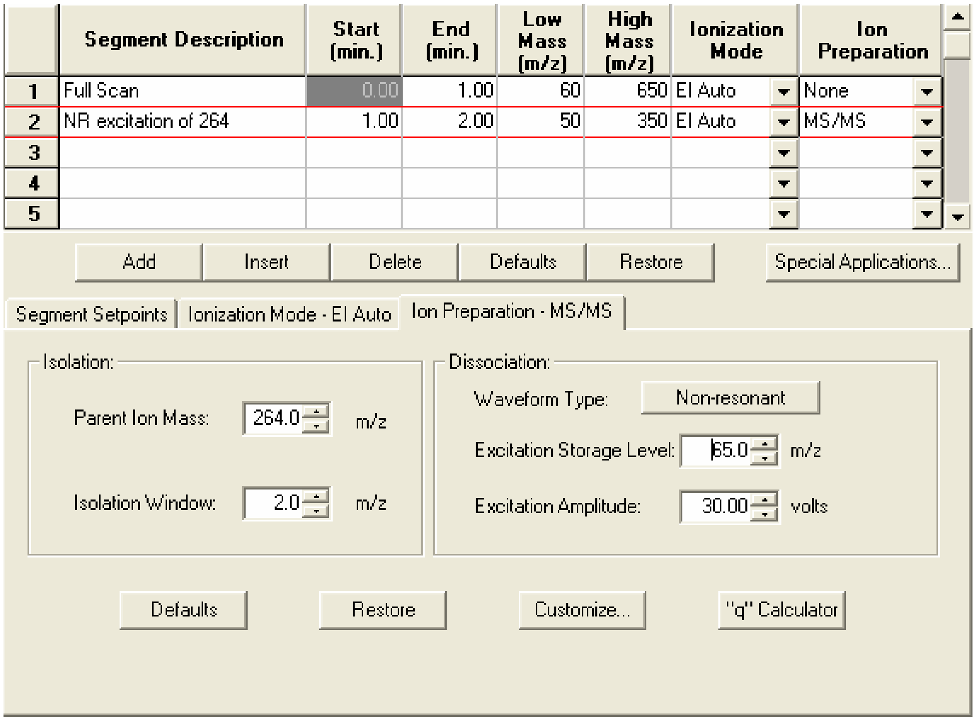
Scan Number: 10, Time: 0.000 min.
RIC: 983, Ion Range: 260 - 270 m/z
Base Peak: 265, Base Amount: 377
Ion: 25000 us, Segment: 2, Channel: 1

The m/z 265 ion has been isolated but its peak intensity has also been slightly reduced due to the fact that the isolation window has become too narrow by decreasing the Low Edge Offset value to 2.

2. CID of M/Z 264 from Cal Gas Using Nonresonant Excitation

In this tutorial you will build a MS/MS method for the m/z 264 ion in the calibration gas using nonresonant excitation. You will optimize the excitation amplitude to achieve a good distribution of product ions. This optimization is performed by acquiring mass spectra with different values of the excitation amplitude.

This tutorial assumes that the trap temperature has been set to 150 °C. Other trap temperatures may require adjustment of the excitation voltages. Open the **Method Builder** application and build a MS/MS method by using the parameters specified below.



	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	Full Scan	0.00	1.00	60	650	EI Auto	None
2	NR excitation of 264	1.00	2.00	50	350	EI Auto	MS/MS
3							
4							
5							

Isolation:

Parent Ion Mass: 264.0 m/z

Isolation Window: 2.0 m/z

Dissociation:

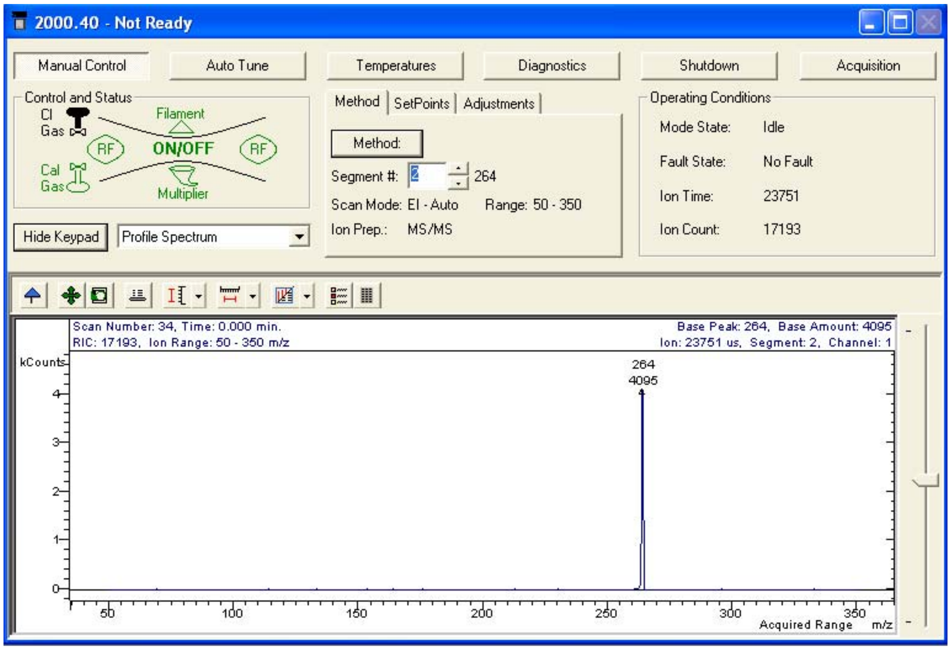
Waveform Type: Non-resonant

Excitation Storage Level: 65.0 m/z

Excitation Amplitude: 30.00 volts

Set the Parent Ion Mass to 264 and select an Isolation Window of 2. The mass range for the MS/MS segment is 50-350. In the Dissociation field set the Waveform Type Non-resonant, the Excitation Storage Level to 65 and the Excitation Amplitude to 30. Click the **Customize** tab and set all the parameters to their default values. In the Segment Setpoint field set the Emission Current to 20 μA. In the Ionization Mode - EI Auto, set the Target TIC value to 10000.

Save the method and close the **Method Builder** application. In System Control activate the method and turn on the calibration gas and the trap.



Segment #: 2 264

Scan Mode: EI - Auto Range: 50 - 350

Ion Prep.: MS/MS

Mode State: Idle

Fault State: No Fault

Ion Time: 23751

Ion Count: 17193

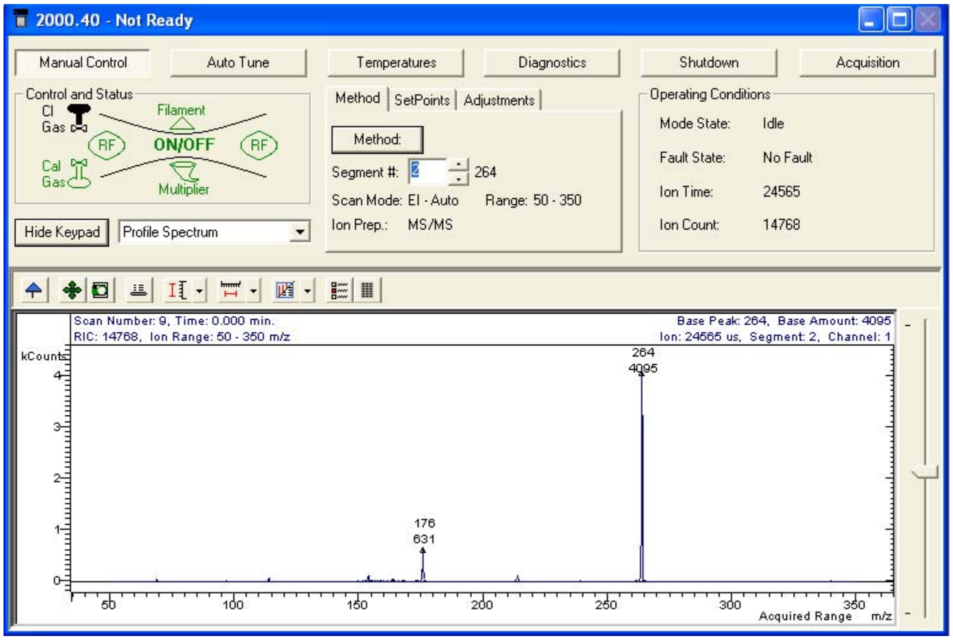
Scan Number: 34, Time: 0.000 min.

RIC: 17193, Ion Range: 50 - 350 m/z

Base Peak: 264, Base Amount: 4095

Ion: 23751 us, Segment: 2, Channel: 1

If the m/z 264 is the only ion in the mass spectrum, a higher voltage is needed to dissociate m/z 264. Click the **Method** button, increase the Excitation Amplitude to 40, save the changes and reactivate the method. Turn on the trap and the calibration gas and observe the spectrum.



Segment #: 2 264

Scan Mode: EI - Auto Range: 50 - 350

Ion Prep.: MS/MS

Mode State: Idle

Fault State: No Fault

Ion Time: 24565

Ion Count: 14768

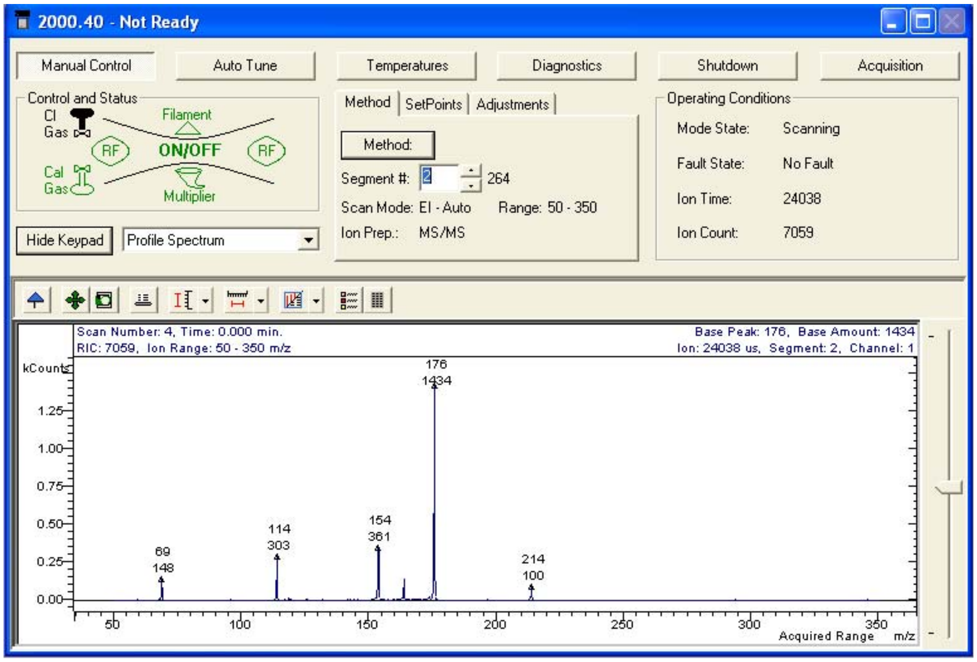
Scan Number: 9, Time: 0.000 min.

RIC: 14768, Ion Range: 50 - 350 m/z

Base Peak: 264, Base Amount: 4095

Ion: 24565 us, Segment: 2, Channel: 1

The m/z 264 peak is still large, the Excitation Amplitude can be increased more. Set the Excitation amplitude to 50 and repeat the steps described above to acquire the mass spectrum.



Profile Spectrum

Segment #: 2 264

Scan Mode: EI - Auto Range: 50 - 350

Ion Prep.: MS/MS

Mode State: Scanning

Fault State: No Fault

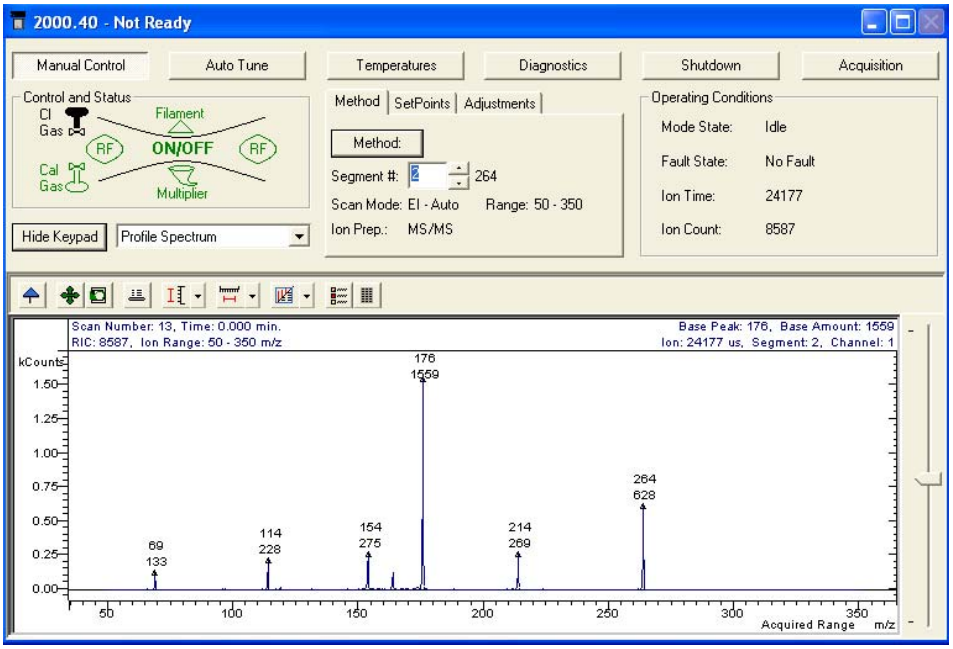
Ion Time: 24038

Ion Count: 7059

Scan Number: 4, Time: 0.000 min.
RIC: 7059, Ion Range: 50 - 350 m/z

Base Peak: 176, Base Amount: 1434
Ion: 24038 us, Segment: 2, Channel: 1

The parent ion m/z 264 is totally absent. You may choose an intermediate value of 45 for the Excitation Amplitude. If the m/z 264 peak is small and the product ions are visible (m/z 214, 176, 164, 154, 114), the voltage will be a good one to use. Set the Excitation Amplitude value to 45 and record the mass spectrum.



Profile Spectrum

Segment #: 2 264

Scan Mode: EI - Auto Range: 50 - 350

Ion Prep.: MS/MS

Mode State: Idle

Fault State: No Fault

Ion Time: 24177

Ion Count: 8587

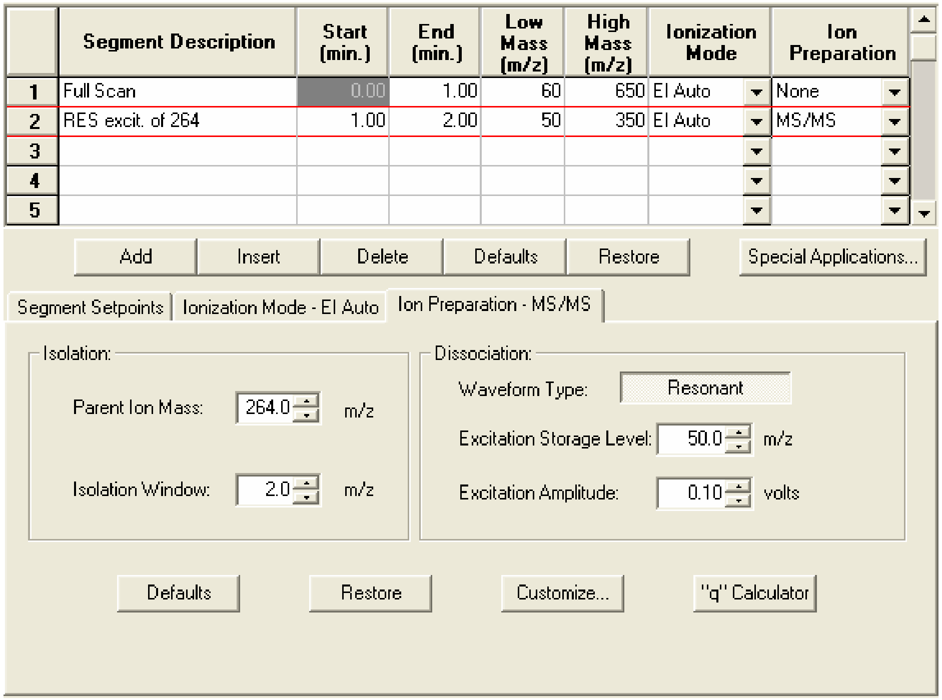
Scan Number: 13, Time: 0.000 min.
RIC: 8587, Ion Range: 50 - 350 m/z

Base Peak: 176, Base Amount: 1559
Ion: 24177 us, Segment: 2, Channel: 1

3. CID of m/z 264 from Cal Gas Using Resonant Excitation

In this tutorial you will build a MS/MS method for the m/z 264 ion of the cal gas using resonant excitation. As in the previous tutorial, you will optimize the Excitation Amplitude to achieve a good distribution of product ions.

This tutorial assumes that you have set the trap temperature to 150 °C. Other trap temperatures may require adjusting the excitation voltages. Open the **Method Builder** and build a MS/MS method by using the parameters specified below:



	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	Full Scan	0.00	1.00	60	650	EI Auto	None
2	RES excit. of 264	1.00	2.00	50	350	EI Auto	MS/MS
3							
4							
5							

Isolation:

Parent Ion Mass: 264.0 m/z

Isolation Window: 2.0 m/z

Dissociation:

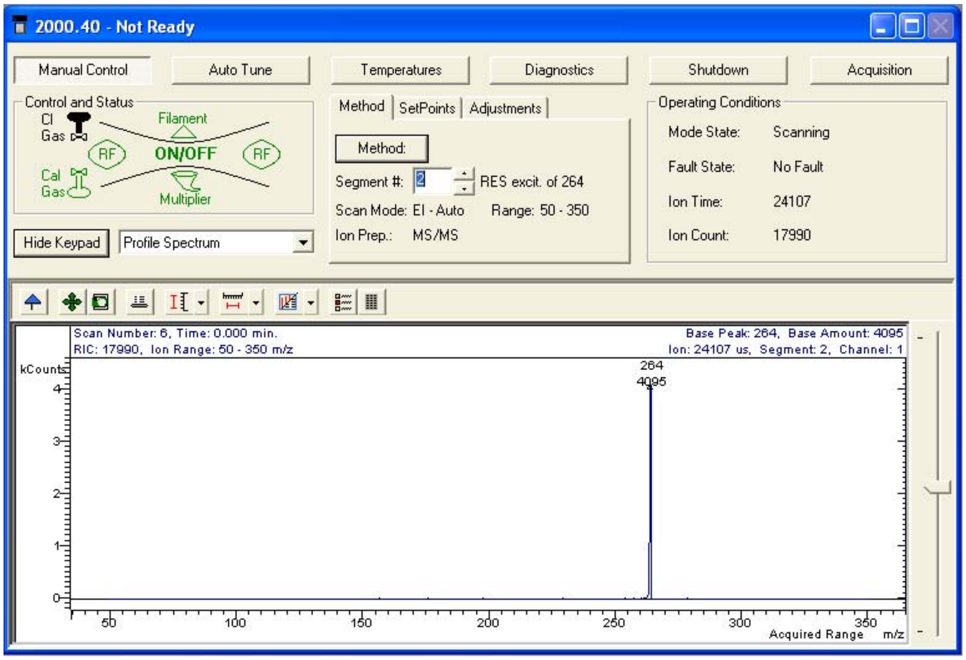
Waveform Type: Resonant

Excitation Storage Level: 50.0 m/z

Excitation Amplitude: 0.10 volts

In the Dissociation field, set the Waveform Type to Resonant, the Excitation Storage Level to 50 and the Excitation Amplitude to 0.1 Click the Customize tab and set all the parameters to their default values. Set the Emission Current to 20 µA and the Target TIC to 10000, as in the previous tutorial.

Save the method and close the **Method Builder** application. In System Control activate the method and turn on the calibration gas and the trap.



2000.40 - Not Ready

Profile Spectrum

Segment #: 2 RES excit. of 264

Scan Mode: EI - Auto Range: 50 - 350

Ion Prep.: MS/MS

Mode State: Scanning

Fault State: No Fault

Ion Time: 24107

Ion Count: 17990

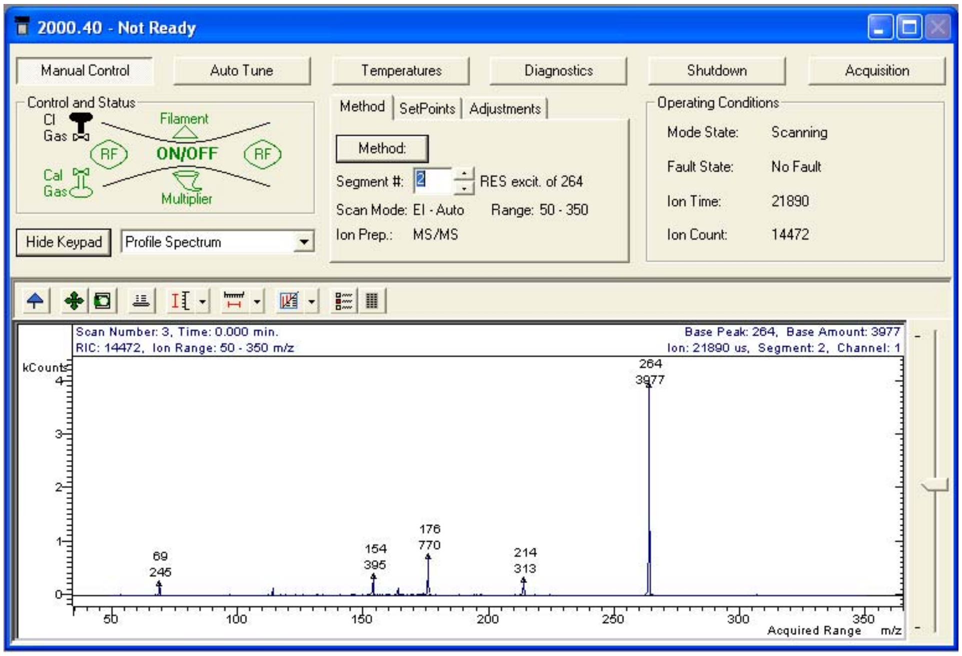
Scan Number: 6, Time: 0.000 min.

RIC: 17990, Ion Range: 50 - 350 m/z

Base Peak: 264, Base Amount: 4095

Ion: 24107 us, Segment: 2, Channel: 1

If m/z 264 is the only ion present in the mass spectrum, a higher voltage will be needed to cause collision-induced dissociation of m/z 264. Set the Excitation Amplitude value to 0.3 and acquire the mass spectrum as described previously.



2000.40 - Not Ready

Profile Spectrum

Segment #: 2 RES excit. of 264

Scan Mode: EI - Auto Range: 50 - 350

Ion Prep.: MS/MS

Mode State: Scanning

Fault State: No Fault

Ion Time: 21890

Ion Count: 14472

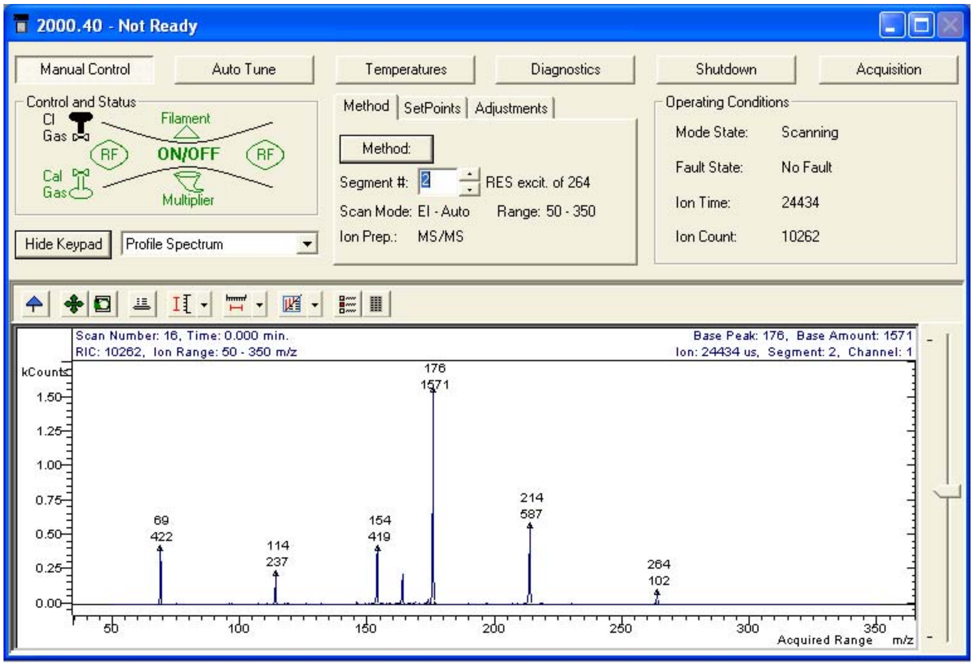
Scan Number: 3, Time: 0.000 min.

RIC: 14472, Ion Range: 50 - 350 m/z

Base Peak: 264, Base Amount: 3977

Ion: 21890 us, Segment: 2, Channel: 1

The height of the m/z 264 peak should be reduced and several product ions should be visible: m/z 214, 176, 164, 154, 114. The voltage may be further increased to convert additional parent ions into product ions. Set the Excitation Amplitude value to 0.5 and record the mass spectrum.



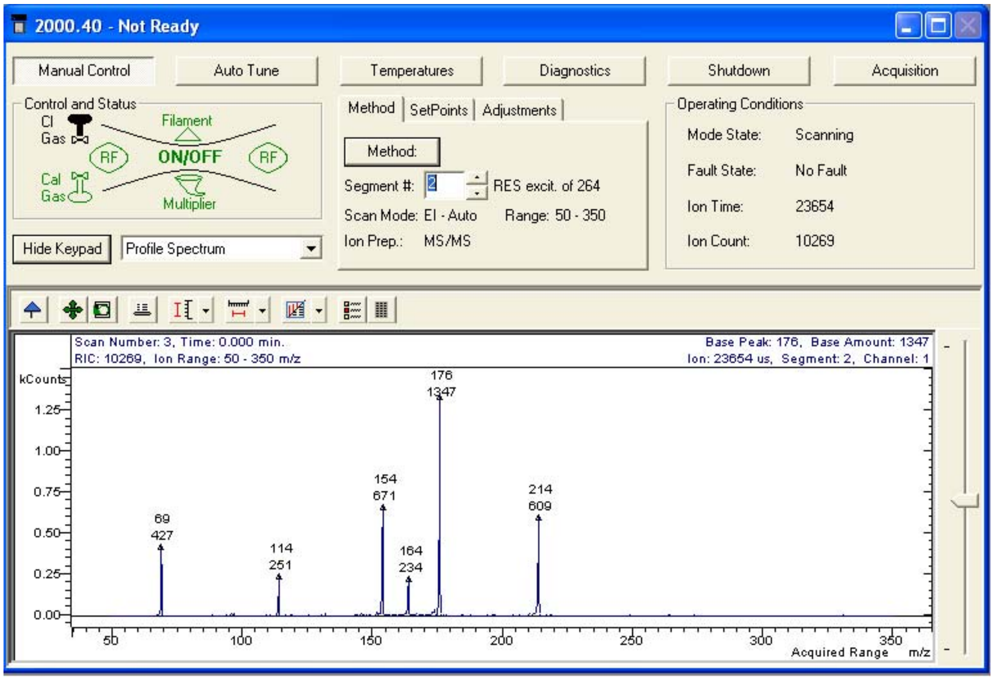
Segment #: 2 RES excit. of 264
Scan Mode: EI - Auto Range: 50 - 350
Ion Prep.: MS/MS

Mode State: Scanning
Fault State: No Fault
Ion Time: 24434
Ion Count: 10262

Scan Number: 16, Time: 0.000 min.
RIC: 10262, Ion Range: 50 - 350 m/z
Base Peak: 176, Base Amount: 1571
Ion: 24434 us, Segment: 2, Channel: 1

If the m/z 264 peak is small and the product ions are visible: m/z 214, 176, 164, 154, 114, the voltage will be a good one to use. For this ion trap system, the optimum value of the Excitation Amplitude is 0.5.

Further increase the Excitation Amplitude to 0.6 and observe the spectrum.



Segment #: 2 RES excit. of 264
Scan Mode: EI - Auto Range: 50 - 350
Ion Prep.: MS/MS

Mode State: Scanning
Fault State: No Fault
Ion Time: 23654
Ion Count: 10269

Scan Number: 3, Time: 0.000 min.
RIC: 10269, Ion Range: 50 - 350 m/z
Base Peak: 176, Base Amount: 1347
Ion: 23654 us, Segment: 2, Channel: 1

If the m/z 264 peak is absent and only the product ions are visible (m/z 214, 176, 164, 154, 114) with smaller peaks than before, the excitation voltage is too high. If only the m/z 264 peak remains, try increasing the modulation range in the **Customize** dialog.

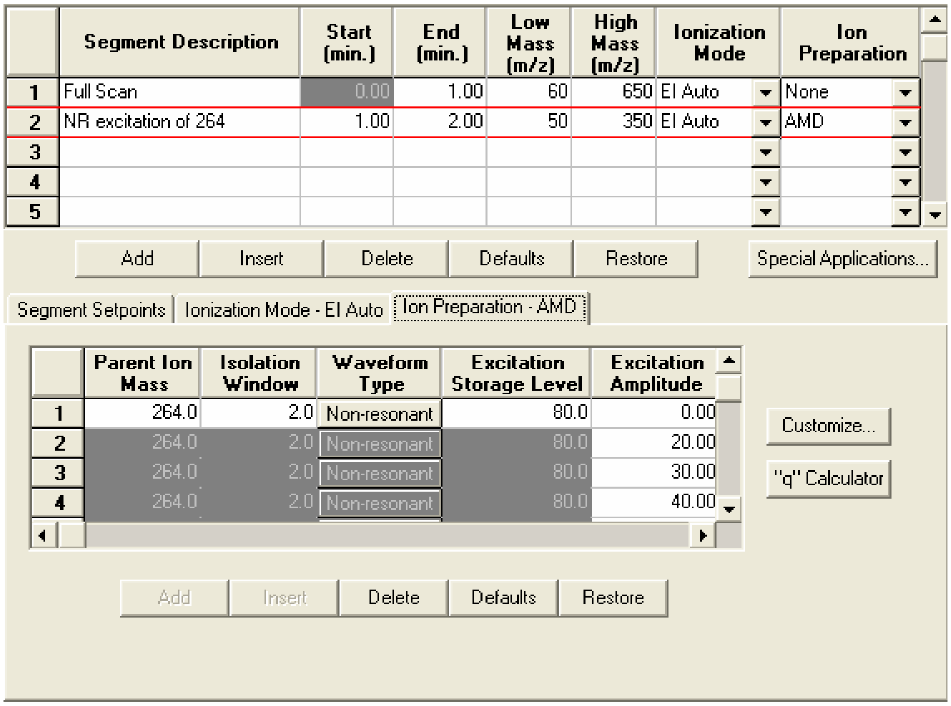
The product ion distribution using resonant excitation is slightly different from the one obtained with nonresonant excitation.

4. Determining the Optimum Excitation Amplitude and Excitation Storage Level for Nonresonant Excitation Using Automated Methods Development (AMD)

In the previous two tutorials we showed how we could optimize the **Excitation Amplitude** manually, by entering different values and recording the mass spectra corresponding to each value. This procedure can by automated by selecting the **Automated Methods Development (AMD)** option under **the Ion Preparation** field in the **Method Builder** dialog.

AMD can be used to automatically optimize both the Excitation Storage Level and the Excitation Amplitude. Follow these steps to optimize the nonresonant excitation by using AMD while using the constant source of ions provided by the calibration gas.

Open the method builder and build a method with the parameters shown below:



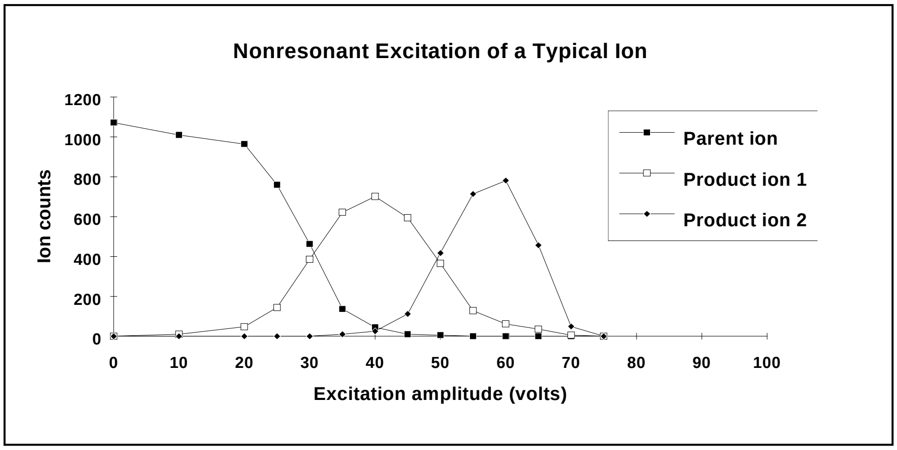
	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	Full Scan	0.00	1.00	60	650	EI Auto	None
2	NR excitation of 264	1.00	2.00	50	350	EI Auto	AMD
3							
4							
5							

	Parent Ion Mass	Isolation Window	Waveform Type	Excitation Storage Level	Excitation Amplitude
1	264.0	2.0	Non-resonant	80.0	0.00
2	264.0	2.0	Non-resonant	80.0	20.00
3	264.0	2.0	Non-resonant	80.0	30.00
4	264.0	2.0	Non-resonant	80.0	40.00

1. For segment 2, select **AMD** in the Ion Preparation field and set the scan range 50-350. Click the **Ion Preparation - AMD** tab and set the Parent Ion Mass to 264, the Isolation Window to 2 and the Waveform Type Non-resonant.
2. The Excitation Storage Level depends on the lowest product ion mass, which in our case is m/z 114. Set the excitation storage level equal to the lowest product ion mass expected divided by 1.4. If the product ions are not yet known, start with the excitation storage level of m/z 48 and an excitation time of 20 ms. In our case a good starting excitation storage level is m/z 80.
3. In the Excitation Amplitude field, enter the first value 0 and then, starting with 20, increment each value by 10. There are a total of 10 entries for the Excitation Amplitude values.

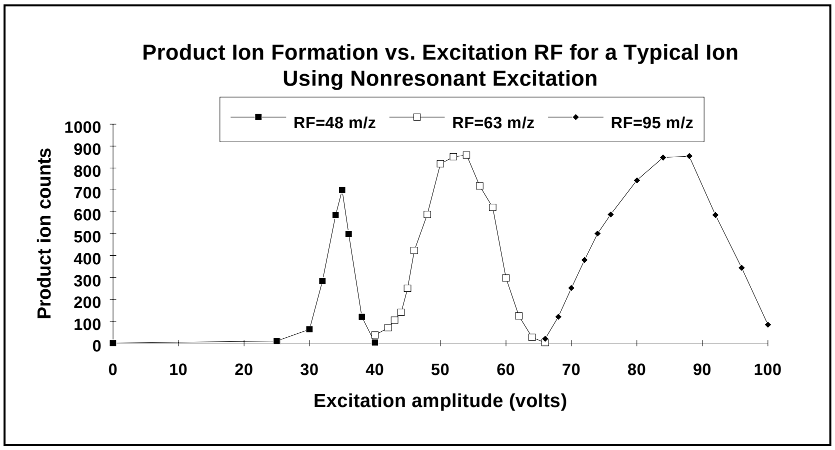
4. Save the method and close the method builder. In **System Control**, activate the method and turn on the trap and the cal gas. Observe the spectrum corresponding to each value of the Excitation Amplitude. If any of the peaks are off scale (> 4088 counts), reduce the Target TIC in the Ionization Mode — EI Auto field. You might need to change the range of variation of the Excitation Amplitude and/or to vary its value by smaller increments for a more accurate optimization. While the spectrum is updated, observe the channel number so that you can identify the excitation amplitude for the optimum distribution of product ions.

For a more accurate optimization you might want to save the data by acquiring the mass spectra in the Acquisition mode. You can then record the peak amplitudes of the parent and product ions for each value of the excitation amplitude and excitation storage level. To determine the optimum excitation amplitude, you can use a different graphics program to plot the peak intensities for the parent and product ions vs. the Excitation Amplitude. An example is given below:



To optimize the excitation storage level, increase its value and repeat the cycle through the excitation amplitudes as described above.

You can determine the optimum excitation storage level by observing the spectra in **Manual Control**. As before, a more accurate optimization can be obtained by saving the data after acquiring it in the **Acquisition** mode. You can determine the optimum excitation storage level by plotting the product ion intensity as a function of excitation amplitude for different storage levels. An example is shown next.

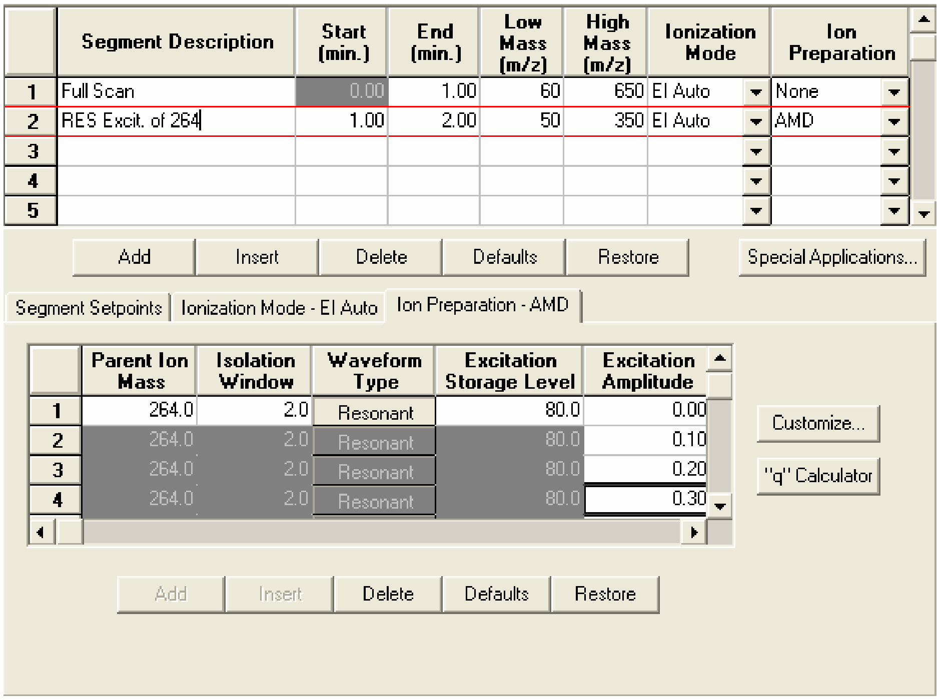


The maximum production of product ions shifts to higher CID (excitation) amplitudes as the excitation storage level is increased.

5. Determining the Optimum Voltage for Resonant Excitation Using Automated Methods Development (AMD)

Follow these steps to determine the optimum voltage for resonant excitation using AMD while using the constant source of ions from the calibration gas.

Open the method builder and build a method using the parameters shown below:



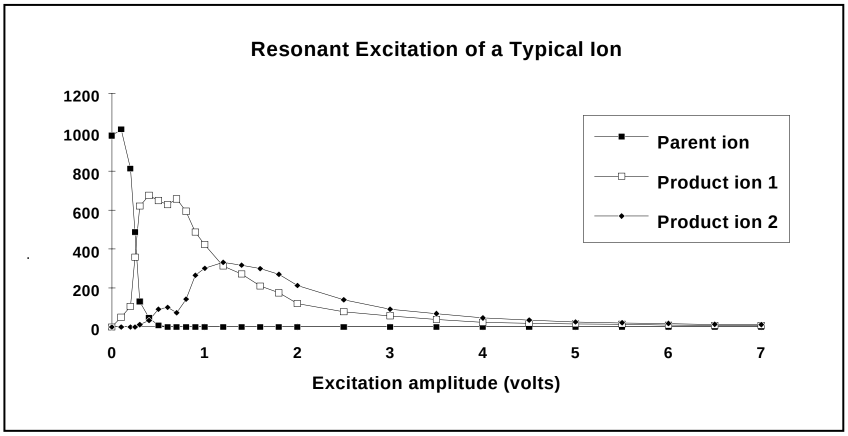
	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	Ionization Mode	Ion Preparation
1	Full Scan	0.00	1.00	60	650	EI Auto	None
2	RES Excit. of 264	1.00	2.00	50	350	EI Auto	AMD
3							
4							
5							

	Parent Ion Mass	Isolation Window	Waveform Type	Excitation Storage Level	Excitation Amplitude
1	264.0	2.0	Resonant	80.0	0.00
2	264.0	2.0	Resonant	80.0	0.10
3	264.0	2.0	Resonant	80.0	0.20
4	264.0	2.0	Resonant	80.0	0.30

1. Set the Parent Ion Mass equal to 264, the Isolation Window to 2 and the Waveform Type Resonant. Set the Excitation Storage Level equal to the lowest mass product ion expected divided by 1.4. We expect the lowest product ion mass to be 114, so that a good start for the excitation storage level is m/z 80. If the product ions are not yet known, start with the excitation storage level of m/z 48 and an excitation time of 20 ms.
2. In the Excitation Amplitude field enter the first value 0 and then, starting with 0.1, increment each value by 0.1. There are 10 entries for the Excitation Amplitude value. Note that much lower amplitude values are needed for resonant excitation compared with nonresonant excitation. This is expected since the transfer of energy is to the parent ion is much more efficient in the resonant case vs. the nonresonant one.

3. Save the method and close the method editor. In **System Control**, activate the method and turn on the trap and the cal gas. Observe the spectrum corresponding to each value of the Excitation Amplitude. If any of the peaks are off scale (> 4088 counts), reduce the Target TIC in the Ionization Mode - EI Auto field. You might need to change the range of variation of the Excitation Amplitude and/or to vary its value by smaller increments for a more accurate optimization. While the spectrum is updated, observe the channel number so that you can identify the excitation amplitude for the optimum distribution of product ions.
4. As mentioned in the previous tutorial, for a more accurate optimization of CID excitation, you might need to record the peak intensities of the parent and product ions and plot them as a function of Excitation Amplitude. For this, you need to save the data from Acquisition mode.

You can plot the peak intensities of the parent and product ions as a function of Excitation Amplitude by using a different graphics software. An example is shown below.



Increase the Excitation Storage Level by increments of 10 m/z and repeat the cycle through the Excitation Amplitudes as described above. Acquire the mass spectra in **Manual Control** and determine the excitation storage and amplitude values for the optimum distribution of product ions.

Run File Tutorials

Overview of the Varian MS Workstation for GC Run Files

The Varian MS Workstation contains 5 Star Workstation applications, which are used to generate, process, and review GC run files. These files are produced by standard GC detectors other than the 2000 MS. The Method Builder and System Control/Automation (Chemis32.exe) applications are used for mass spec data files as well, and are loaded automatically as part of the core Varian MS Workstation software installation. The Report (Report32.exe), Batch Report (Batchr32.exe), and Interactive Graphics/Data Handling (Ngig.exe) applications are loaded if the Chrom Data Handling and Chrom Data Files/Methods options are selected during installation. Each of these applications is represented by an icon in the Varian MS Workstation program folder, as well as by a button on the Star Toolbar (Starbar.exe).

Using the Star Toolbar

The Workstation Toolbar is designed to be a convenient starting place for all of your interactions with the Varian MS Workstation. When you install the MS Workstation, you can select to have the Workstation Toolbar displayed for you automatically, whenever you start Windows. By simply clicking on a Workstation Toolbar button you can invoke its associated application.

If the Star Toolbar is not present, open it by using the mouse to click on ***Start >Programs >MS Workstation >Workstation Toolbar***. Hold the mouse cursor over each of the buttons in the Workstation Toolbar so that you can read the tool-tip descriptions. You can find more options and complete descriptions in the Help on Star Toolbar accessed by right-clicking in the free area on the right side of the Workstation Toolbar.

Clicking on one of the buttons on the Star Toolbar will start or ‘launch’ the corresponding application.

The applications that compose the Varian MS Workstation also allow a certain amount of interaction. System Control can be used to start Method Builder and it uses elements of the Report application when generating results and printing them. From Report you can invoke Interactive Graphics and re-integrate the chromatograms before printing them.

Using the Tutorials

These tutorials are intended for demonstrating the data handling capabilities of the Varian MS 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 Varian MS Workstation software the ChromExamples directory will be created in the directory where your workstation software is installed. The ChromExamples directory contains files, which can be used for learning about the Varian MS 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.

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 Varian MS Workstation CD-ROM into the CD-ROM drive.
2. Select Install to start the Setup Program.
3. Select Update Ver. 6x to Ver. 6.5 MSWS. The installation dialog will walk you through the next steps.
4. When the Product Serial Number dialog is opened, you don’t need to enter the serial numbers (the existing products are highlighted).
5. Accept the Software License Agreement to continue.
6. Select the MS Modules.
7. When prompted from the **Other Selections** dialog, choose to install “Chrom Example Data Files/Methods” and “Chrom Data Handling”.
8. Follow the next steps in the installation process and click Finish in the last dialog. You will be prompted to dialog asking to Restart your Computer. Click **Yes**.

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

Tutorial 1 Recalculating Results

Overview

The Varian MS Workstation allows you to recalculate results acquired with standard GC detectors 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.

View the C:\VarianWS\ChromExamples directory.

Use the right mouse button to click on PRACTICE.RUN, and select *Copy*.

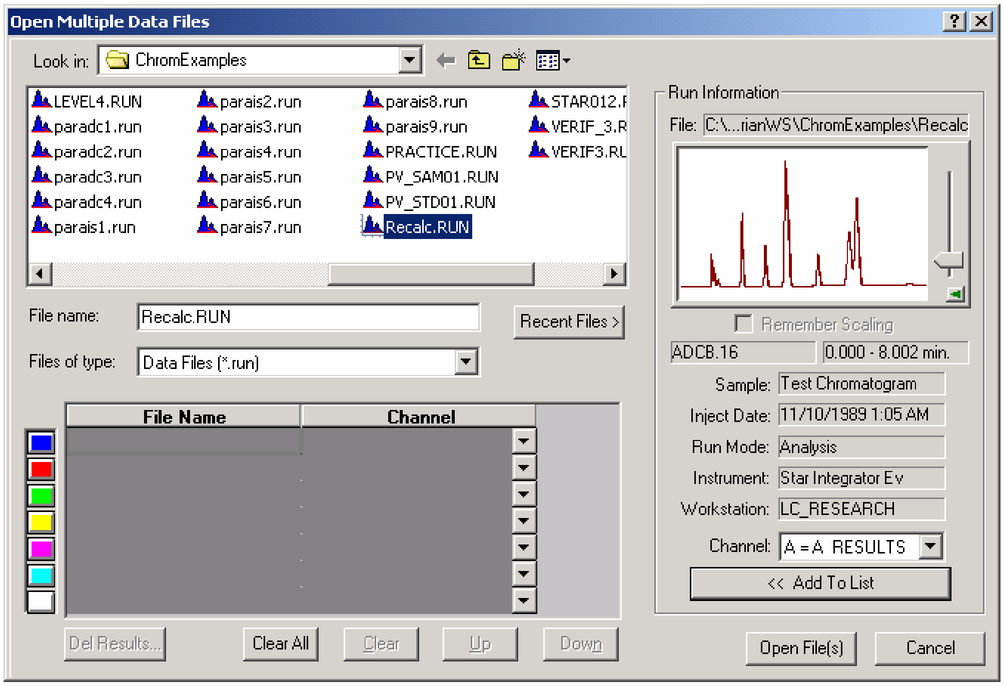
Select ***Edit >Paste***. A copy of this file is added at the end of the list.

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

Opening the Interactive Graphics application by selecting the Review Chromatograms button in the Workstation toolbar.

The Open Multiple Data Files dialog box appears.



Look in: ChromExamples

File name: Recalc.RUN

Files of type: Data Files (*.run)

Run Information

File: C:\...rianWS\ChromExamples\Recalc

ADCB.16 0.000 - 8.002 min.

Sample: Test Chromatogram

Inject Date: 11/10/1989 1:05 AM

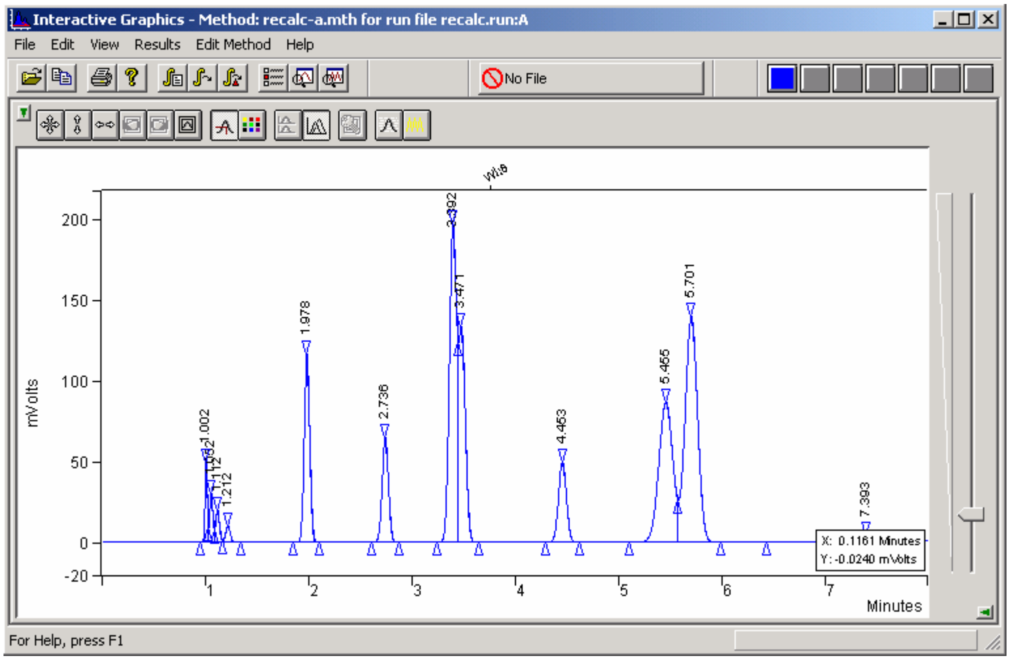
Run Mode: Analysis

Instrument: Star Integrator Ev

Workstation: LC_RESEARCH

Channel: A = A RESULTS

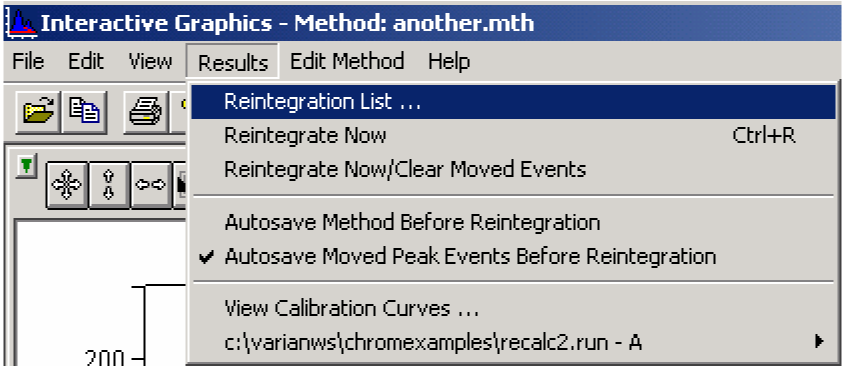
Select the data file Recalc.RUN in the ChromExamples directory, and click the **Open File(s)** tab.



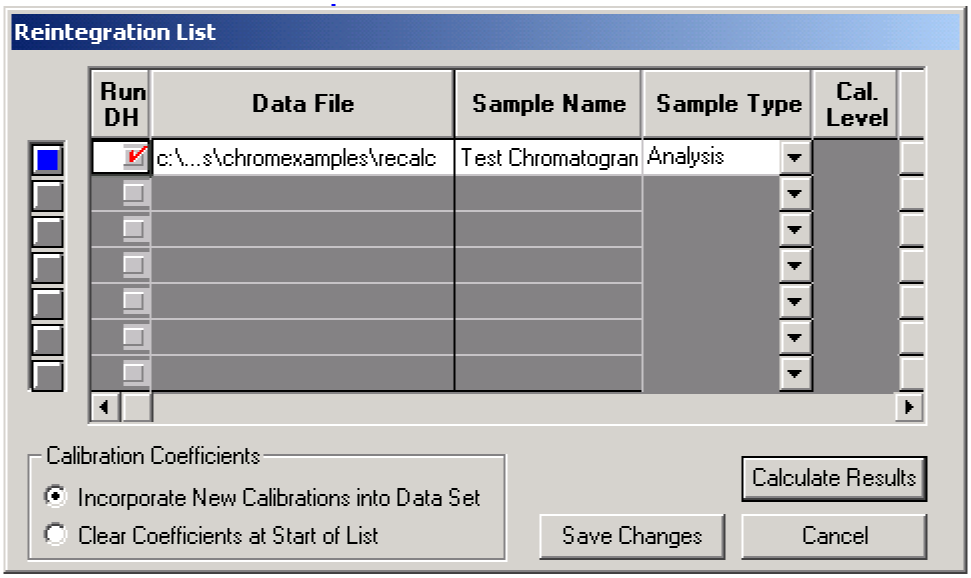
Select ***File >Open Method...*** . The Open Method dialog box appears.

Find and select the method called ANOTHER.MTH in the ChromExamples directory.

Integrate the peaks by selecting the menu command ***Results >Reintegration List…***



The **Reintegration List** dialog opens. A check mark appears in the Run DH column for the data file. This indicates that the file is selected for recalculation.

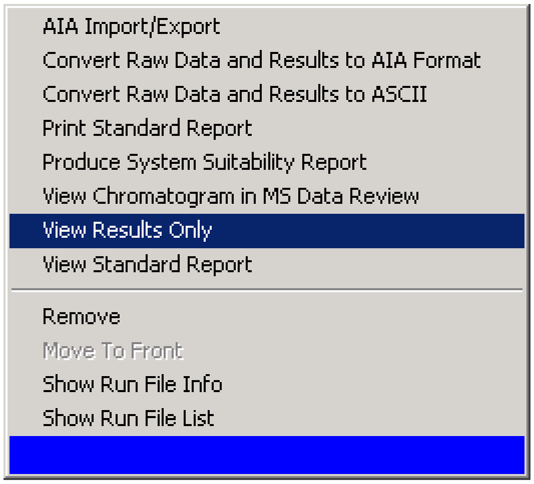


Run DH	Data File	Sample Name	Sample Type	Cal. Level
☑	c:\...s\chromexamples\recalc	Test Chromatogran	Analysis	
☐				
☐				
☐				
☐				
☐				
☐				

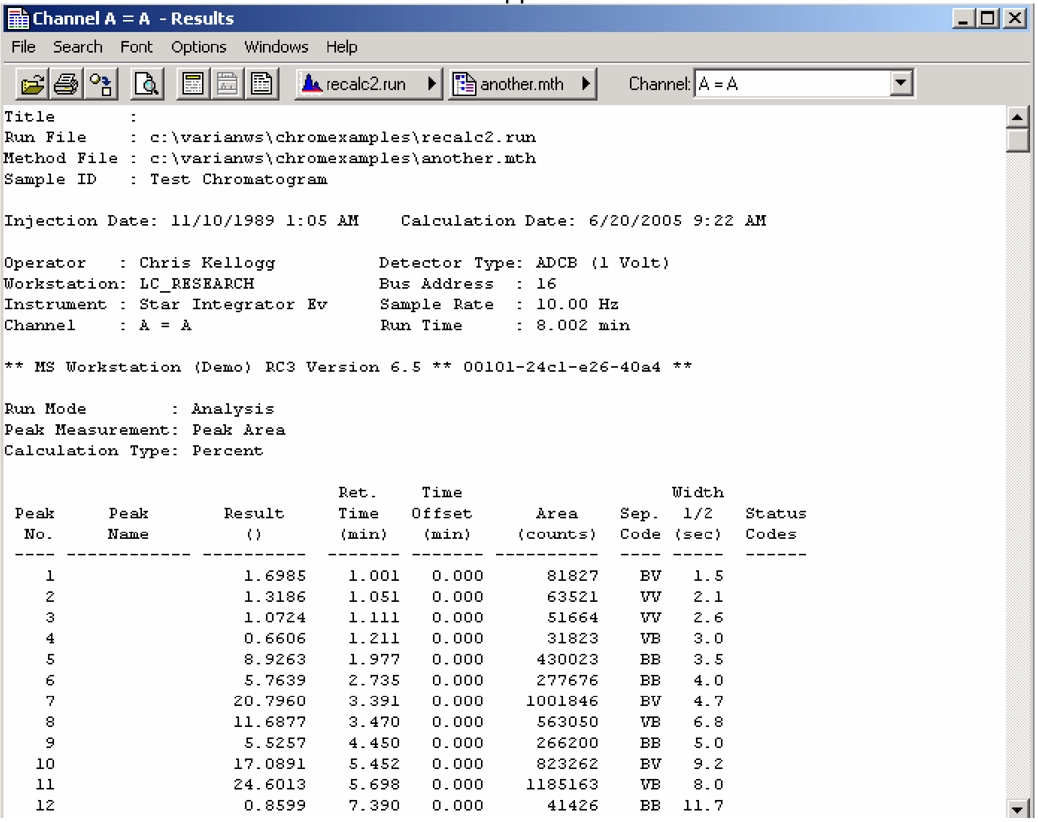
Calibration Coefficients
● Incorporate New Calibrations into Data Set
○ Clear Coefficients at Start of List

Click the **Calculate Results** tab.

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.



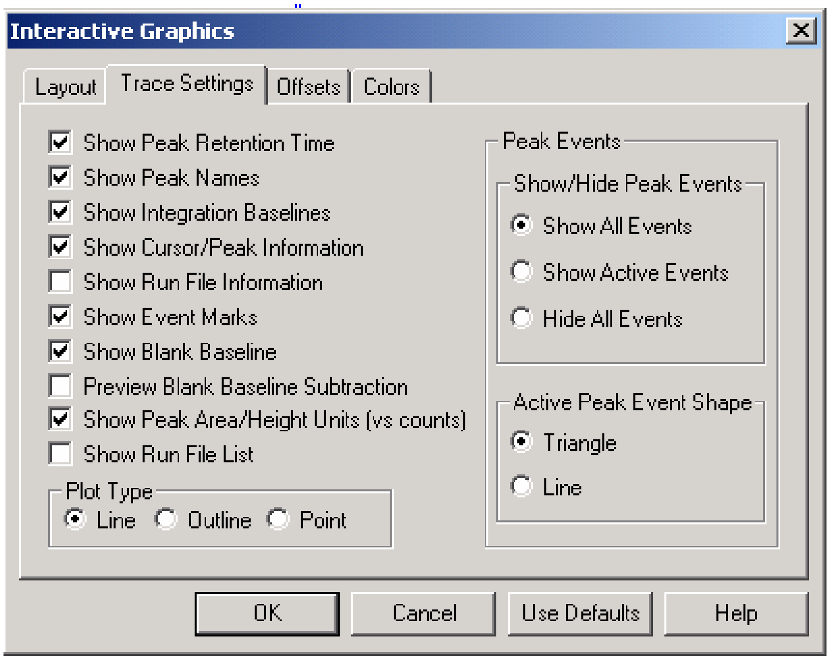
The results for the data file appear. Note the areas for the peaks at 3.392 and 3.471 minutes. The results should appear as shown below.



```
Title       :
Run File    : c:\varianws\chromexamples\recalc2.run
Method File : c:\varianws\chromexamples\another.mth
Sample ID   : Test Chromatogram

Injection Date: 11/10/1989 1:05 AM    Calculation Date: 6/20/2005 9:22 AM

Operator   : Chris Kellogg          Detector Type: ADCB (1 Volt)
Workstation: LC_RESEARCH            Bus Address  : 16
Instrument : Star Integrator Ev     Sample Rate  : 10.00 Hz
Channel    : A = A                  Run Time     : 8.002 min

** MS Workstation (Demo) RC3 Version 6.5 ** 00101-24c1-e26-40a4 **

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

                                Ret.    Time                    Width
 Peak     Peak       Result     Time   Offset      Area    Sep.  1/2   Status
  No.     Name         ()       (min)   (min)    (counts)  Code (sec)  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                 20.7960    3.391   0.000    1001846    BV   4.7
    8                 11.6877    3.470   0.000     563050    VB   6.8
    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
```

Close the Results window by clicking on the ☒ in the upper right corner of the window.

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.



Interactive Graphics

Layout | Trace Settings | Offsets | Colors

☑ Show Peak Retention Time
☑ Show Peak Names
☑ Show Integration Baselines
☑ Show Cursor/Peak Information
☐ Show Run File Information
☑ Show Event Marks
☑ Show Blank Baseline
☐ Preview Blank Baseline Subtraction
☑ Show Peak Area/Height Units (vs counts)
☐ Show Run File List

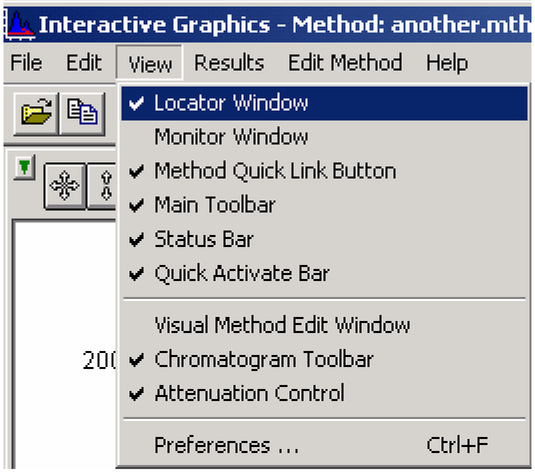
Plot Type
● Line ○ Outline ○ Point

Peak Events

Show/Hide Peak Events
● Show All Events
○ Show Active Events
○ Hide All Events

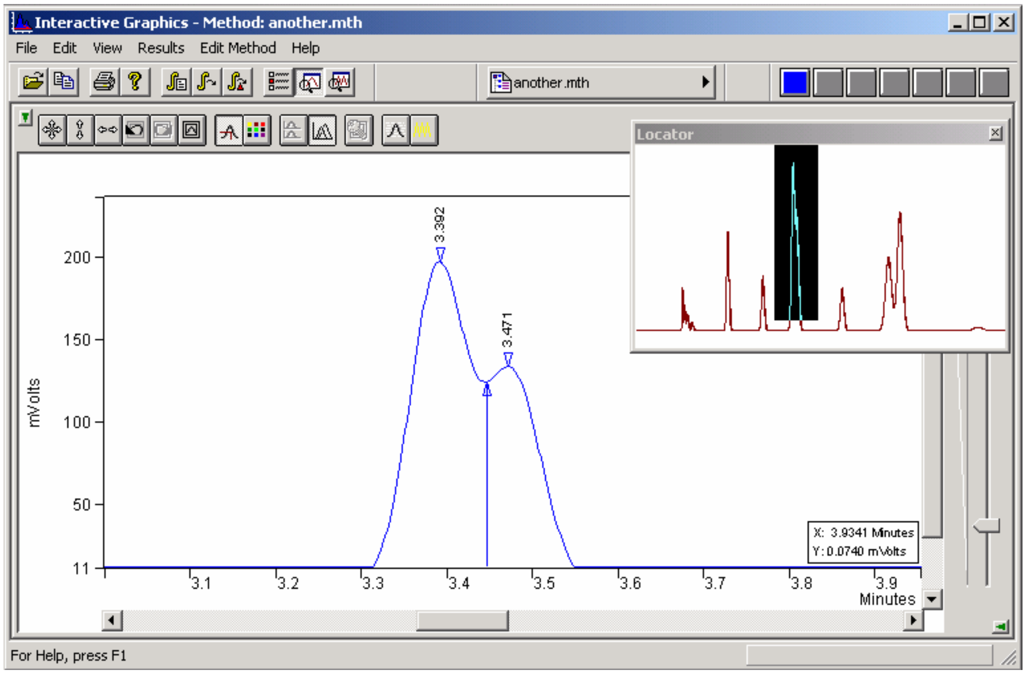
Active Peak Event Shape
● Triangle
○ Line

In the **View** menu of **Interactive Graphics** window check **Locator Window** and Chromatogram Toolbar.



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.

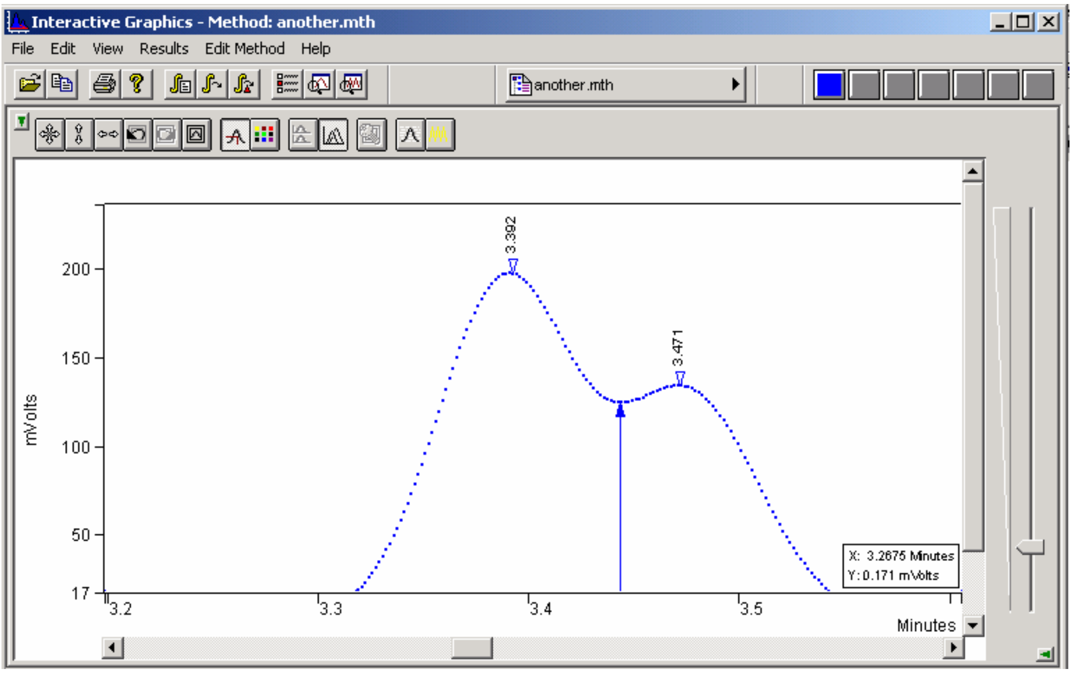
When you are adjusting the position of a start or end point, you may find it more convenient and accurate to display the actual data points from the file on the screen.

To hide the locator window:

1. Uncheck the ***Locator Window*** in the **View** menu and select the command ***Preferences***. Click on the ***Trace Settings*** tab.
2. 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.
3. Place the cursor over the valley point between the two peaks.
4. Click the left mouse button, hold it down, and drag the valley point slightly to the left.

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:

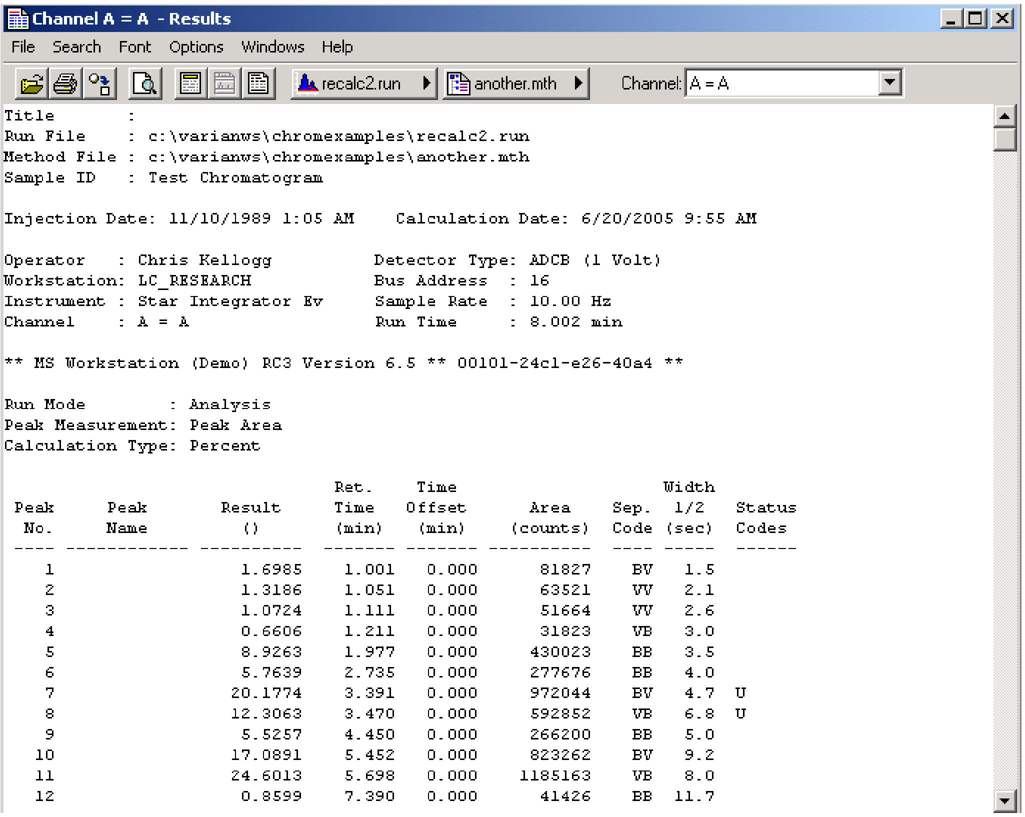


Note that the peak event triangle for the valley point is now drawn as a solid triangle indicating a moved peak event. Now that the point is repositioned, you can recalculate the file using the moved baseline event.

Recalculating the Data File

After moving the valley point to a new position as described in the previous section, reintegrate the peaks by using the command ***Results >Reintegration List…*** and clicking the **Calculate Results** tab.

Open the results by right-clicking on the chromatogram trace and selecting **View Results Only.**

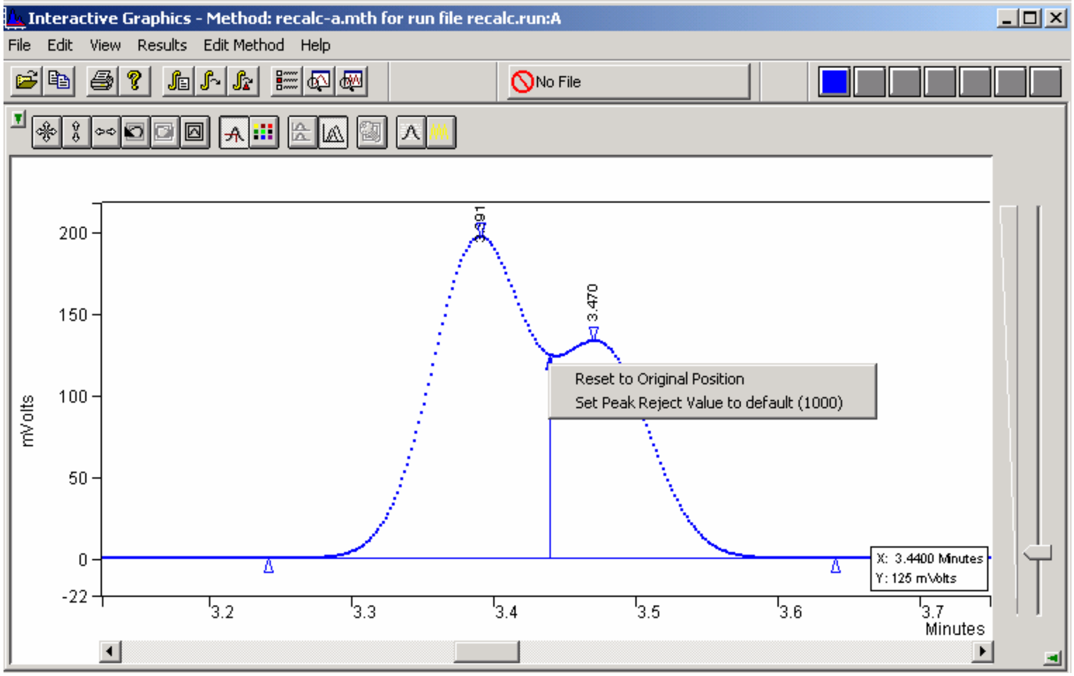


```
Title       :
Run File    : c:\varianws\chromexamples\recalc2.run
Method File : c:\varianws\chromexamples\another.mth
Sample ID   : Test Chromatogram

Injection Date: 11/10/1989 1:05 AM    Calculation Date: 6/20/2005 9:55 AM

Operator    : Chris Kellogg          Detector Type: ADCB (1 Volt)
Workstation: LC_RESEARCH             Bus Address  : 16
Instrument : Star Integrator Ev      Sample Rate  : 10.00 Hz
Channel     : A = A                  Run Time     : 8.002 min

** MS Workstation (Demo) RC3 Version 6.5 ** 00101-24c1-e26-40a4 **

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

                                    Ret.    Time                   Width
 Peak      Peak         Result      Time   Offset      Area   Sep.  1/2   Status
  No.      Name           ()        (min)   (min)   (counts)  Code (sec)  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                  20.1774    3.391   0.000     972044    BV   4.7  U
    8                  12.3063    3.470   0.000     592852    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
```

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.

You can also move the peak start and end points and then reintegrate the peaks. The Workstation uses the new points in the peak-processing algorithm.

You can return the valley point and/or the start and end points to their original positions by using a right mouse click on the event triangle that you have moved and selecting ***Reset to Original Position*** from the displayed menu.



If you reintegrate now after restoring the valley point to its original position, you should get the initial integration results. After examining the reintegration results, close the Results window.

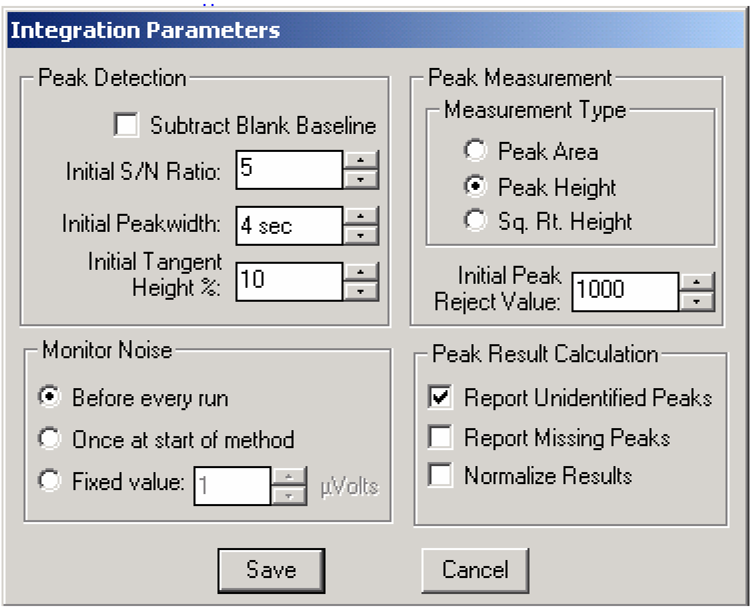
Editing the Method

Now let's look at some of the changes you can make to the method and how they affect the results. This time, leave the Results window open. 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.

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.

Select ***Edit Method >Integration Parameters...*** . The Integration Parameters dialog box is displayed.



Integration Parameters

Peak Detection

☐ Subtract Blank Baseline

Initial S/N Ratio: 5

Initial Peakwidth: 4 sec

Initial Tangent Height %: 10

Peak Measurement

Measurement Type

○ Peak Area

● Peak Height

○ Sq. Rt. Height

Initial Peak Reject Value: 1000

Monitor Noise

● Before every run

○ Once at start of method

○ Fixed value: 1 µVolts

Peak Result Calculation

☑ Report Unidentified Peaks

☐ Report Missing Peaks

☐ Normalize Results

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.

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

Click on **Save***.*

This saves the settings for this window temporarily, but it does not write them to the method.

To reintegrate the file according to the new method, select ***Results >Reintegrate Now***. A dialog box is opened that asks if you want to save the changes to the current method before reintegrating. Click **Yes**.

Open the results window by right-clicking on the chromatogram trace. Notice that both the Result (%) and the number of counts (now in peak height units) have changed for each peak.

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

To end your Interactive Graphics session, select **File >Exit**.

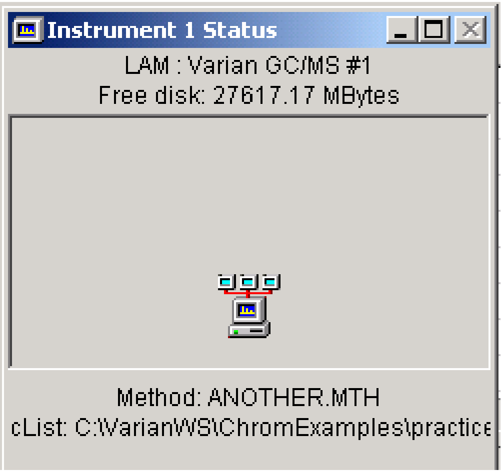
Recalculating in System Control

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. Opening *System Control*.

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.



LAM : Varian GC/MS #1
Free disk: 27617.17 MBytes

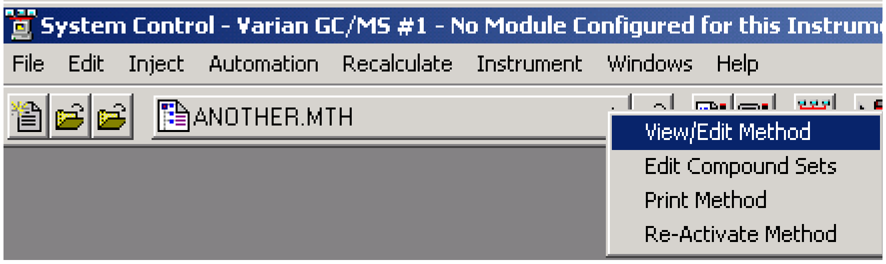
Method: ANOTHER.MTH
cList: C:\VarianWS\ChromExamples\practice

2. Activating and Editing an Existing Method for Recalculation

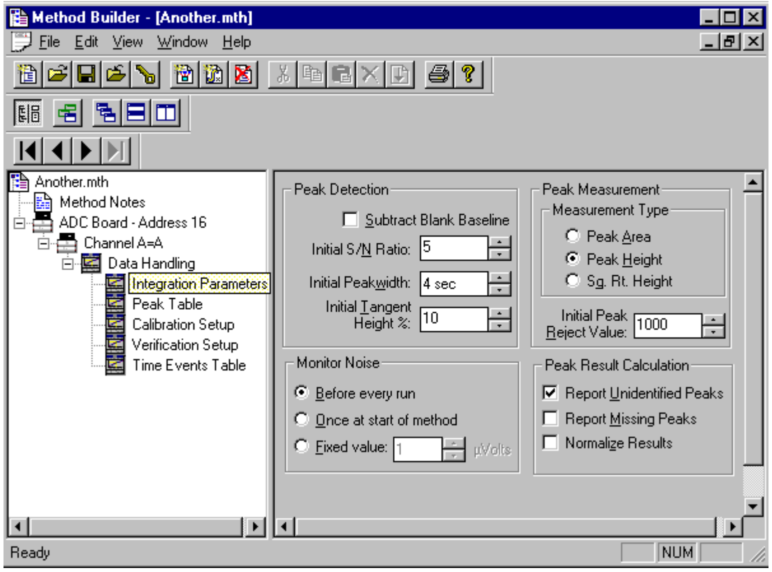
Select ***File >Activate Method***.

The **Activate a System Control Method File** dialog opens. Select the method file ANOTHER.MTH from the ChromExamples directory and click the **Open** button.

Click on the Active Method tab on the Toolbar, which is labeled ANOTHER.MTH in our case, and select ***View / Edit Method***.



From the **Method Builder** dialog, highlight the **Integration Parameters** section of the method.



Another.mth
- Method Notes
- ADC Board - Address 16
  - Channel A=A
    - Data Handling
      - Integration Parameters
      - Peak Table
      - Calibration Setup
      - Verification Setup
      - Time Events Table

Peak Detection
☐ Subtract Blank Baseline
Initial S/N Ratio: 5
Initial Peakwidth: 4 sec
Initial Tangent Height %: 10

Peak Measurement
Measurement Type
○ Peak Area
● Peak Height
○ Sq. Rt. Height
Initial Peak Reject Value: 1000

Monitor Noise
● Before every run
○ Once at start of method
○ Fixed value: 1 µVolts

Peak Result Calculation
☑ Report Unidentified Peaks
☐ Report Missing Peaks
☐ Normalize Results

The **Integration Parameters** window opens, showing the current settings. Peak measurement is by height, and Report Unidentified Peaks is checked.

Select **Peak Area** in the peak measurement field.

Select ***File >Save***.

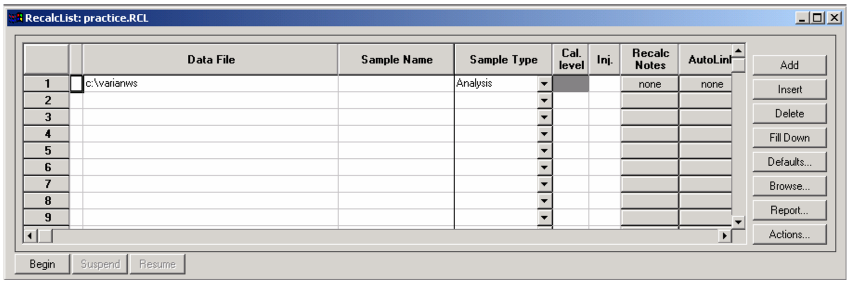
Close Method Builder.

2. Creating a Recalc List for Recalc

Select ***File >New Recalc List***.

Select the ChromExamples directory and type practice.rcl into the file name field.

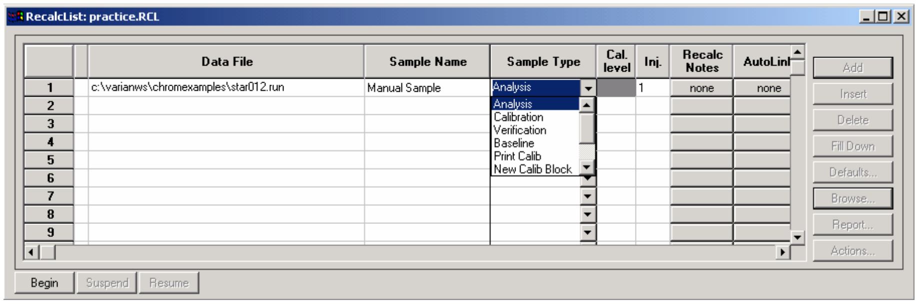
Click the **Save** tab. The **RecalcList** dialog opens.



		Data File	Sample Name	Sample Type	Cal. level	Inj.	Recalc Notes	AutoLinl
1	☐	c:\varianws		Analysis			none	none
2								
3								
4								
5								
6								
7								
8								
9								

Click the ***Add*** tab and then ***Browse...*** .This opens the **Open Data File** dialog box.

Find and select the data file called STAR012.RUN in the ChromExamples directory. Click ***Open****.* This file is added to the RecalcList spreadsheet. Click on the arrow in the Sample Type field. A drop-down menu opens.


RecalcList: practice.RCL

	Data File	Sample Name	Sample Type	Cal. level	Inj.	Recalc Notes	AutoLink
1	c:\varianws\chromexamples\star012.run	Manual Sample	Analysis		1	none	none

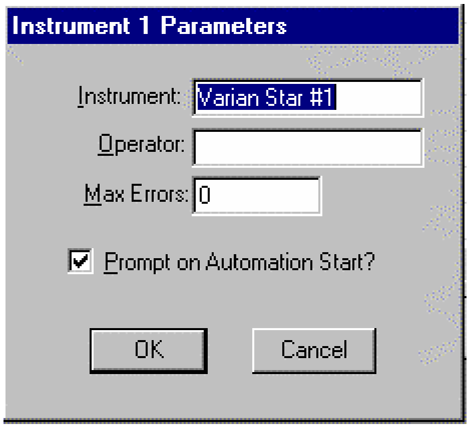
Select ***Analysis***. Leave the other fields set to their default values.

4. Recalculating the Results

Click the **Begin** tab in the **Recalclist** dialog. Alternatively, you can select the menu command ***Recalculate >Begin Recalc List***.

If previously set to “Prompt on Automation Start”, the Instrument Parameters dialog box appears.

Click in the Operator text box, type your name, then click **OK**. (If the **Instrument 1 Parameters** dialog box does not appear, you can open it by selecting Instrument/Configuration, and click on the **Instrument 1 Parameters** tab located in the lower right side of the Configuration window).


Instrument 1 Parameters

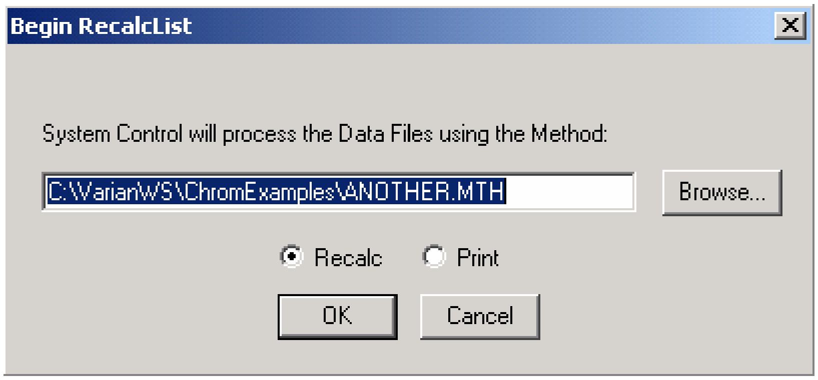
Instrument: Varian Star #1

Operator:

Max Errors: 0

☑ Prompt on Automation Start?

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


Begin RecalcList

System Control will process the Data Files using the Method:

C:\VarianWS\ChromExamples\ANOTHER.MTH

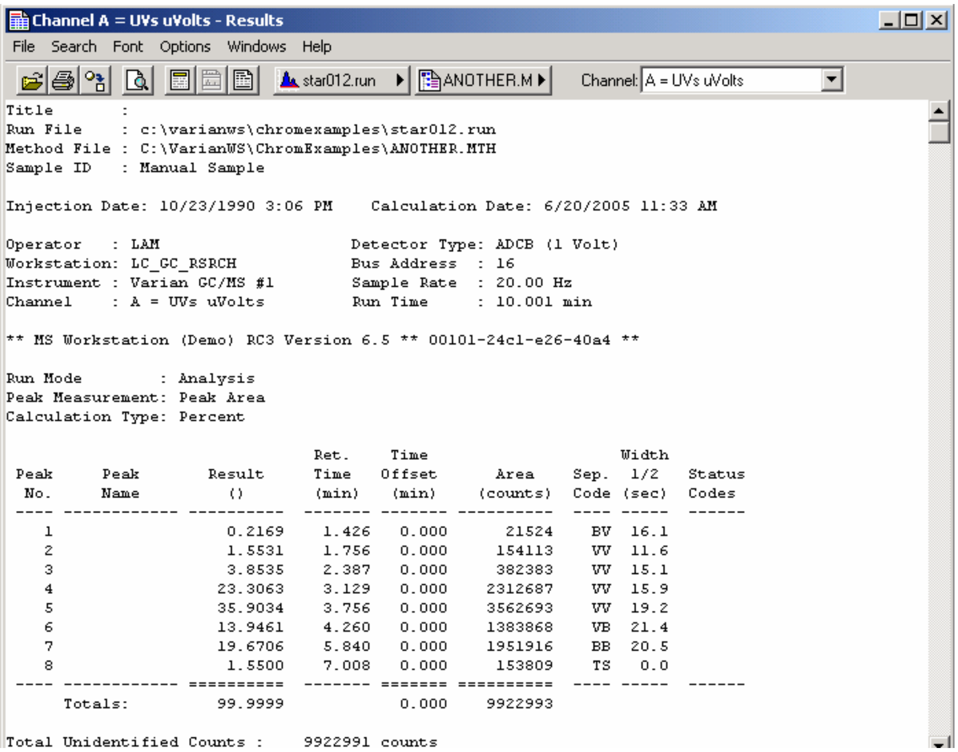
● Recalc ○ Print

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.

Click the ***Report*** button in the active **RecalcList** 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.



```
Title       :
Run File    : c:\varianws\chromexamples\star012.run
Method File : C:\VarianWS\ChromExamples\ANOTHER.MTH
Sample ID   : Manual Sample

Injection Date: 10/23/1990 3:06 PM    Calculation Date: 6/20/2005 11:33 AM

Operator   : LAM                    Detector Type: ADCB (1 Volt)
Workstation: LC_GC_RSRCH            Bus Address  : 16
Instrument : Varian GC/MS #1        Sample Rate  : 20.00 Hz
Channel    : A = UVs uVolts         Run Time     : 10.001 min

** MS Workstation (Demo) RC3 Version 6.5 ** 00101-24c1-e26-40a4 **

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

                                Ret.    Time                    Width
 Peak    Peak       Result     Time  Offset      Area    Sep.  1/2   Status
  No.    Name         ()       (min)   (min)    (counts)  Code (sec)  Codes
 ---- ------------ ----------  ------- ------- ----------  ---- -----  ------
    1                  0.2169    1.426   0.000      21524   BV   16.1
    2                  1.5531    1.756   0.000     154113   VV   11.6
    3                  3.8535    2.387   0.000     382383   VV   15.1
    4                 23.3063    3.129   0.000    2312687   VV   15.9
    5                 35.9034    3.756   0.000    3562693   VV   19.2
    6                 13.9461    4.260   0.000    1383868   VB   21.4
    7                 19.6706    5.840   0.000    1951916   BB   20.5
    8                  1.5500    7.008   0.000     153809   TS    0.0
 ---- ------------ ==========  ------- ======= ==========  ---- -----  ------
      Totals:         99.9999             0.000    9922993

Total Unidentified Counts :    9922991 counts
```

Tutorial 2 Changing Peak Detection Parameters

Overview

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

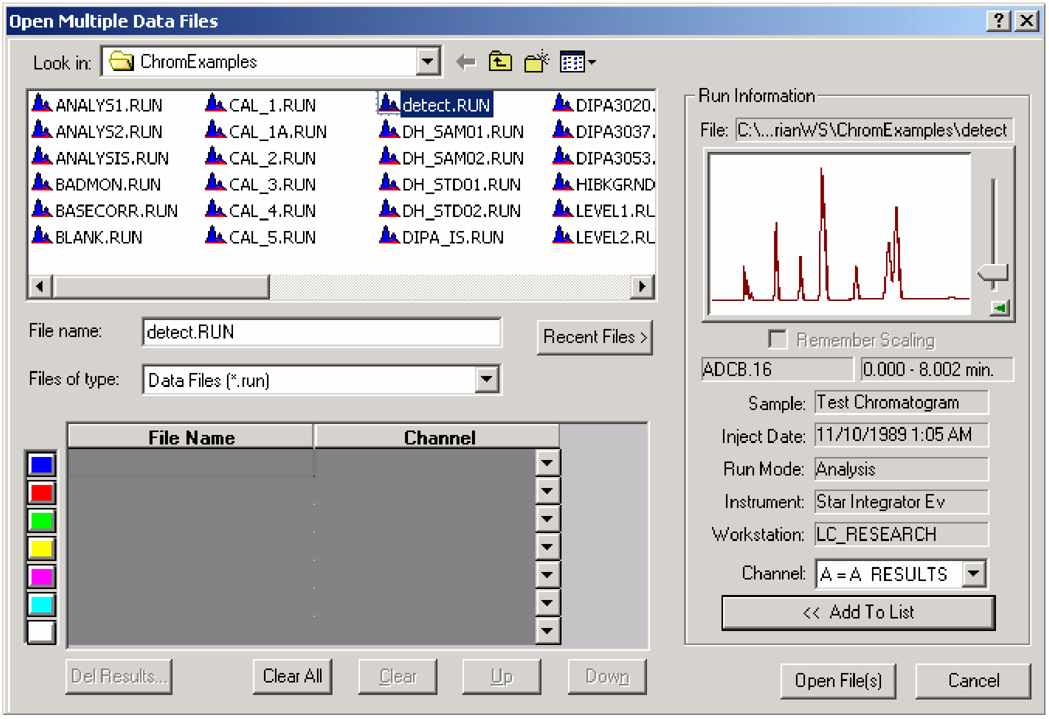
Because PRACTICE.RUN is used for several tutorials, you should copy this data file to DETECT.RUN for use with this tutorial.

In Windows Explorer.

1. View the C:\VarianWS\GCEXAMPLES 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

Click on the **Interactive Graphics/Data Handling** button in the Varian Workstation toolbar. The **Open Multiple Data Files** dialog opens.



Look in: ChromExamples

File name: detect.RUN

Files of type: Data Files (*.run)

File: C:\...rianWS\ChromExamples\detect

ADCB.16

0.000 - 8.002 min.

Sample: Test Chromatogram

Inject Date: 11/10/1989 1:05 AM

Run Mode: Analysis

Instrument: Star Integrator Ev

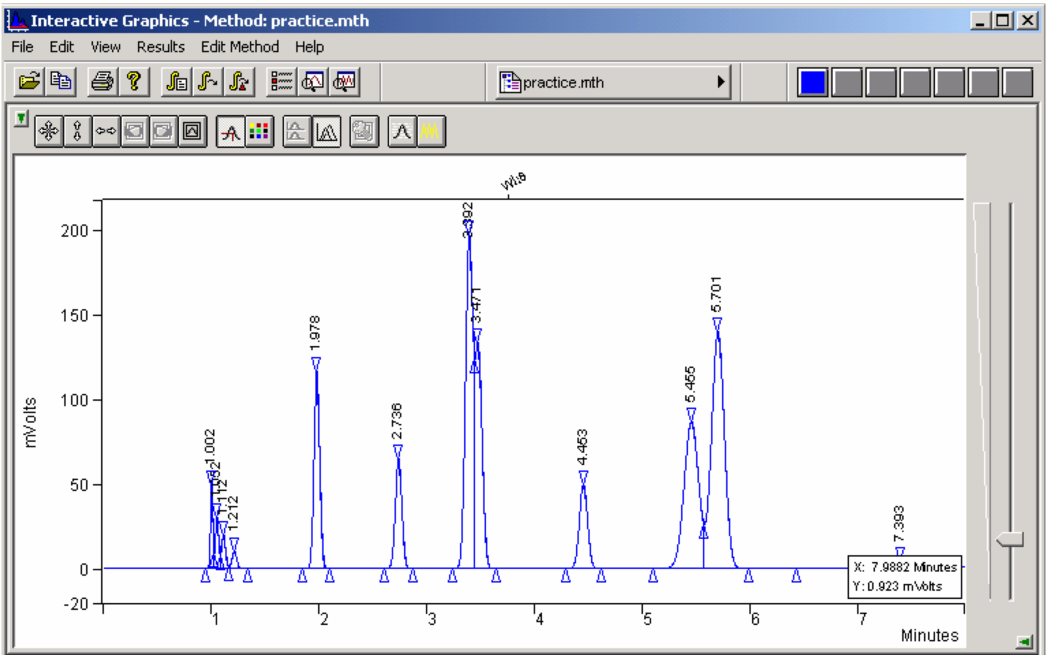
Workstation: LC_RESEARCH

Channel: A = A RESULTS

Select the **detect.RUN** data file from the **ChromExamples** directory and click the **Open File(s)** tab.

1. Select menu command ***File >Open Method*** in the Interactive Graphics window, then double-click on method file **practice.mth**.

Notice the title bar of the **Interactive Graphics/Data Handling** window. The method is identified as **practice.mth**.



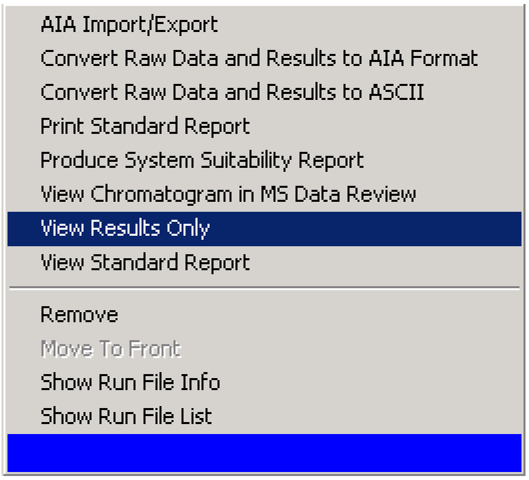
X: 7.9882 Minutes
Y: 0.923 mVolts

Changing the Initial Peak Width

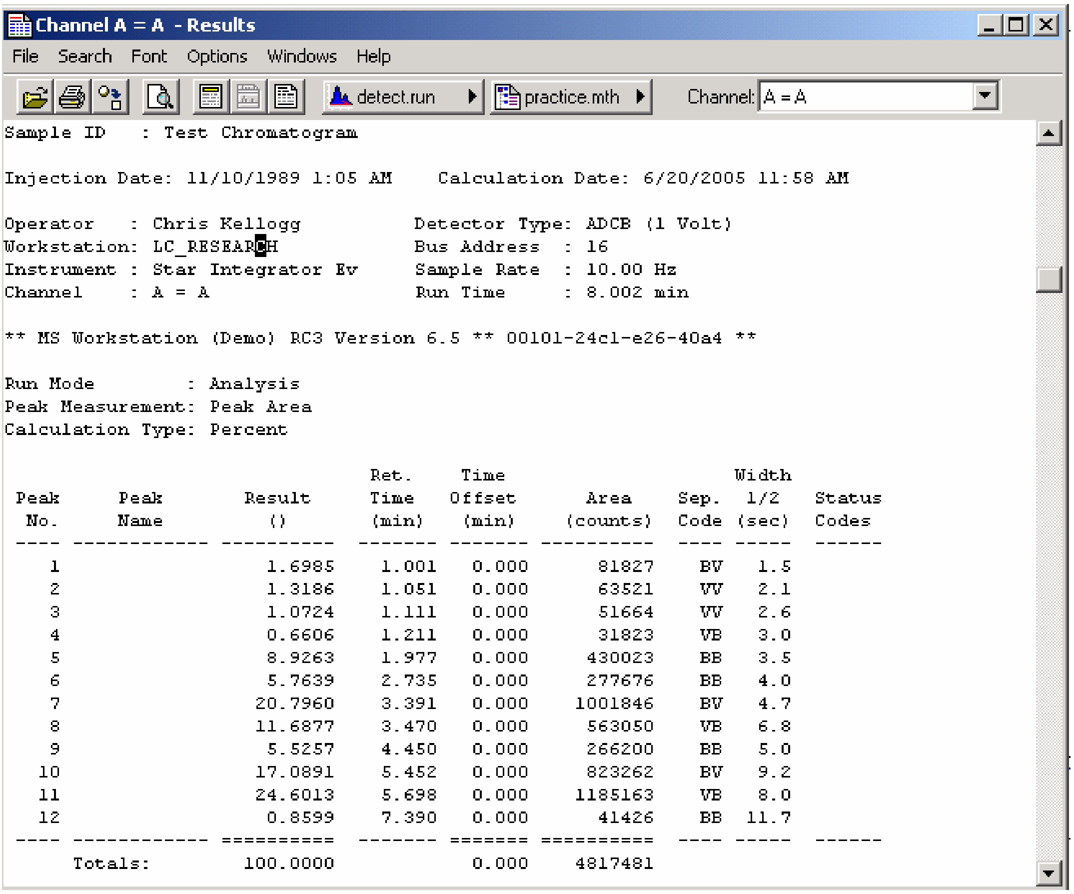
You can now edit the data handling parameters for the method. Before you make any changes, though, integrate the peaks and examine the results for the run as they appear initially.

To integrate, use the menu command ***Results >Integrate Now***.

1. To open the results file click on the chromatogram trace with the right mouse button and select **View Results Only** as seen in the menu below.



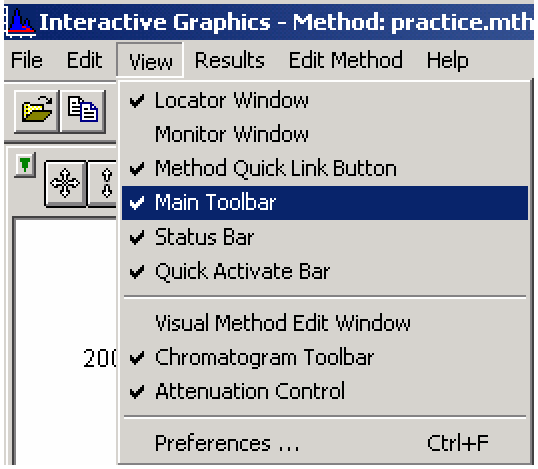
The results for the data file appear. Note the areas for the peaks at 3.392 and 3.471 minutes. The results should appear as shown below.



```
Sample ID   : Test Chromatogram

Injection Date: 11/10/1989 1:05 AM    Calculation Date: 6/20/2005 11:58 AM

Operator    : Chris Kellogg         Detector Type: ADCB (1 Volt)
Workstation: LC_RESEARCH            Bus Address  : 16
Instrument : Star Integrator Ev     Sample Rate  : 10.00 Hz
Channel     : A = A                 Run Time     : 8.002 min

** MS Workstation (Demo) RC3 Version 6.5 ** 00101-24c1-e26-40a4 **

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

                                 Ret.    Time                     Width
 Peak      Peak         Result   Time   Offset      Area     Sep.  1/2   Status
  No.      Name           ()     (min)   (min)    (counts)   Code (sec)  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                  20.7960   3.391   0.000    1001846   BV   4.7
    8                  11.6877   3.470   0.000     563050   VB   6.8
    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:        100.0000          0.000    4817481
```

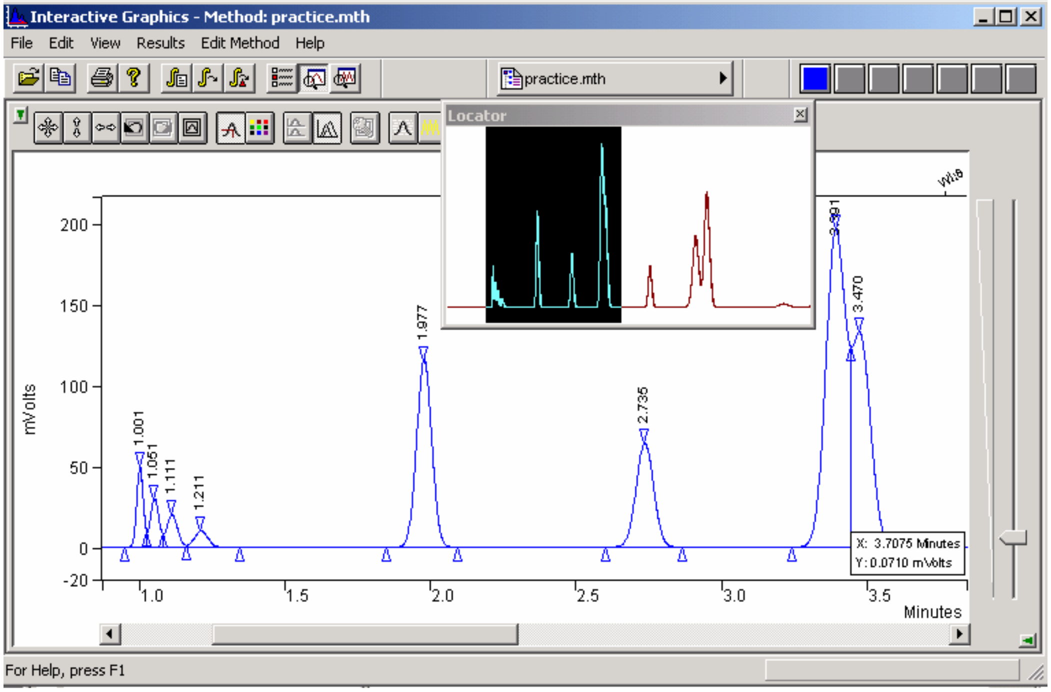
Now, let's take a look at the Initial Peak Width setting and its effect on how the peaks are processed.

2. Close the Results window.
3. Select the menu command ***View >Locator Window*** (make sure it is checked).
4. Select ***View >Main Toolbar*** (make sure it is checked).



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.



Select ***Edit Method >Integration Parameters...*** .

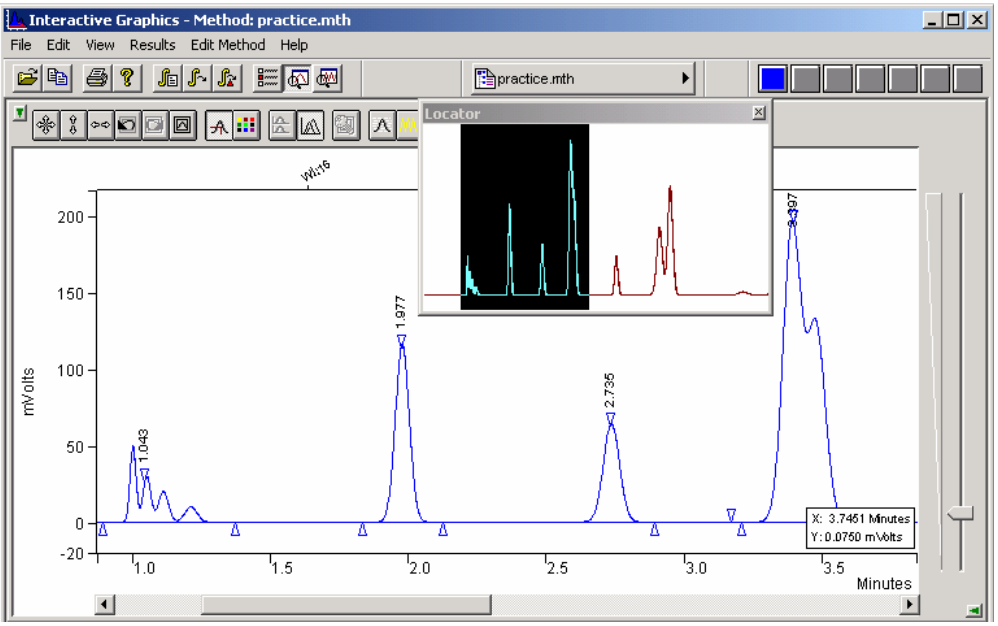
The Integration Parameters dialog box appears.

1. Increase the Initial Peak Width value from 4 to 32 by clicking on the up arrow until 32 appears in the box.
2. Choose Save to close the Integration Parameters dialog box and return to the Interactive Graphics/Data handling window.
3. Select ***Results >Reintegration List...***

Note that a check mark appears in the Run DH check box for the data file.

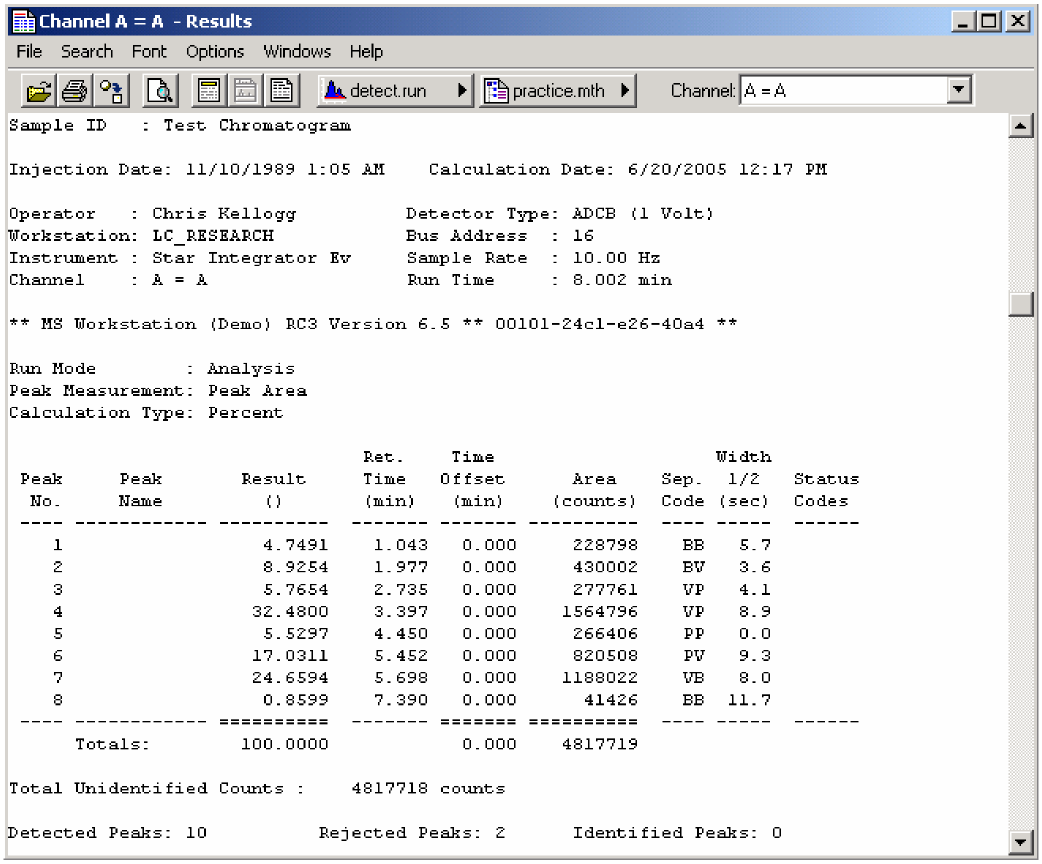
1. Click on the **Calculate Results** tab. A dialog box appears that asks if you want to save changes to the current method before reintegrating. Click Yes.

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.



Again, view the results by right-clicking on the chromatogram trace and selecting **View Results Only**.

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



```
Sample ID   : Test Chromatogram

Injection Date: 11/10/1989 1:05 AM    Calculation Date: 6/20/2005 12:17 PM

Operator    : Chris Kellogg         Detector Type: ADCB (1 Volt)
Workstation: LC_RESEARCH            Bus Address  : 16
Instrument : Star Integrator Bv     Sample Rate  : 10.00 Hz
Channel     : A = A                 Run Time     : 8.002 min

** MS Workstation (Demo) RC3 Version 6.5 ** 00101-24c1-e26-40a4 **

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

                                Ret.    Time                       Width
 Peak      Peak        Result   Time   Offset      Area     Sep.   1/2    Status
  No.      Name          ()     (min)   (min)    (counts)   Code  (sec)   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.

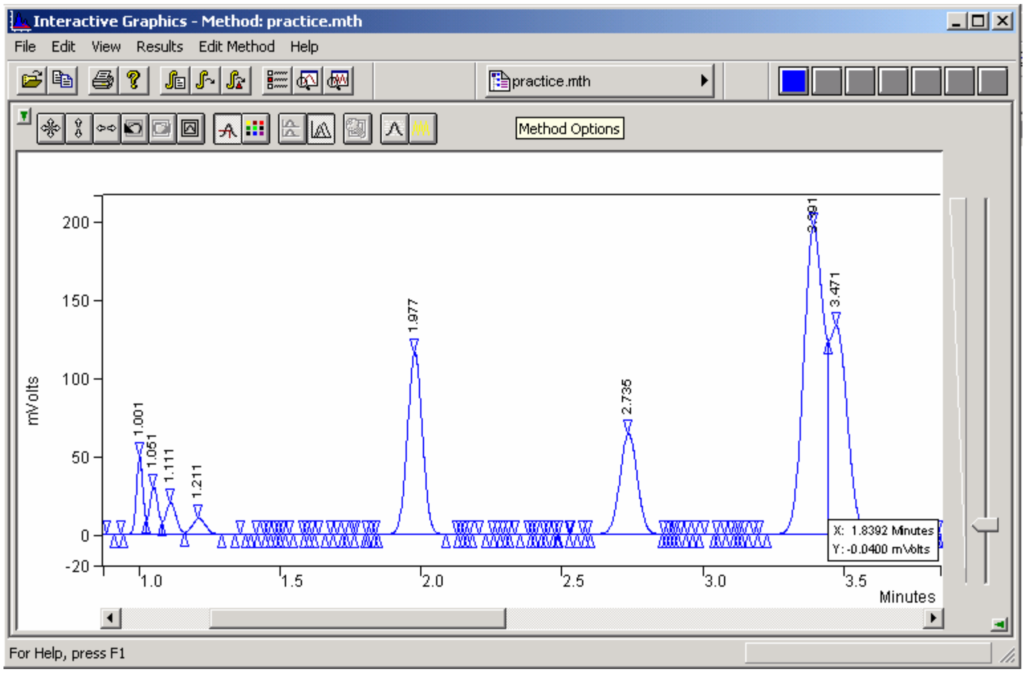
Changing the Signal-to-Noise Ratio

1. Select the menu command ***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.

1. Choose ***Results >Reintegrate Now***.
2. Click on **Save** to save the changed method.

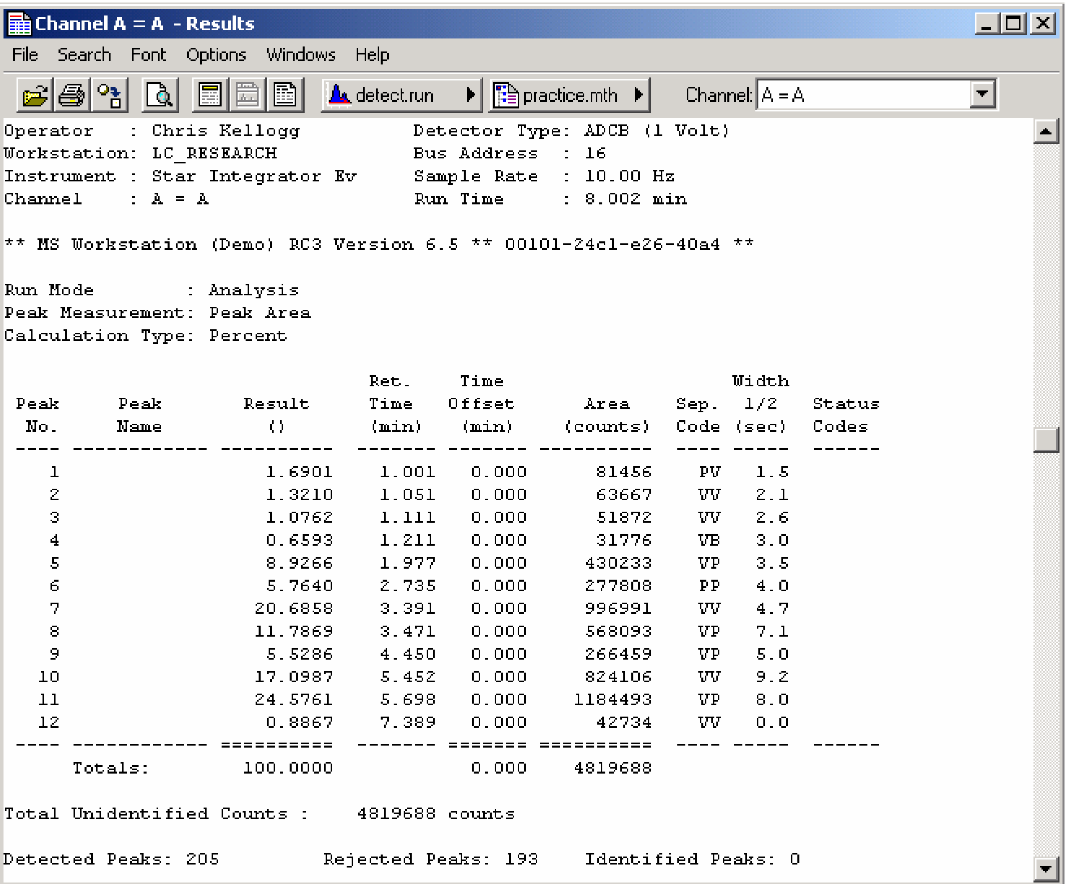
Many peak event markers for small peaks have appeared. The lower S/N Ratio caused peak processing to detect the smaller signals as peaks.



Check the final Results of this change in the S/N Ratio.

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.



```
Operator    : Chris Kellogg          Detector Type: ADCB (1 Volt)
Workstation: LC_RESEARCH             Bus Address  : 16
Instrument : Star Integrator Ev      Sample Rate  : 10.00 Hz
Channel     : A = A                  Run Time     : 8.002 min

** MS Workstation (Demo) RC3 Version 6.5 ** 00101-24c1-e26-40a4 **

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

                                Ret.     Time                     Width
 Peak      Peak        Result    Time    Offset      Area    Sep.   1/2    Status
  No.      Name          ()      (min)    (min)    (counts)  Code  (sec)   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.

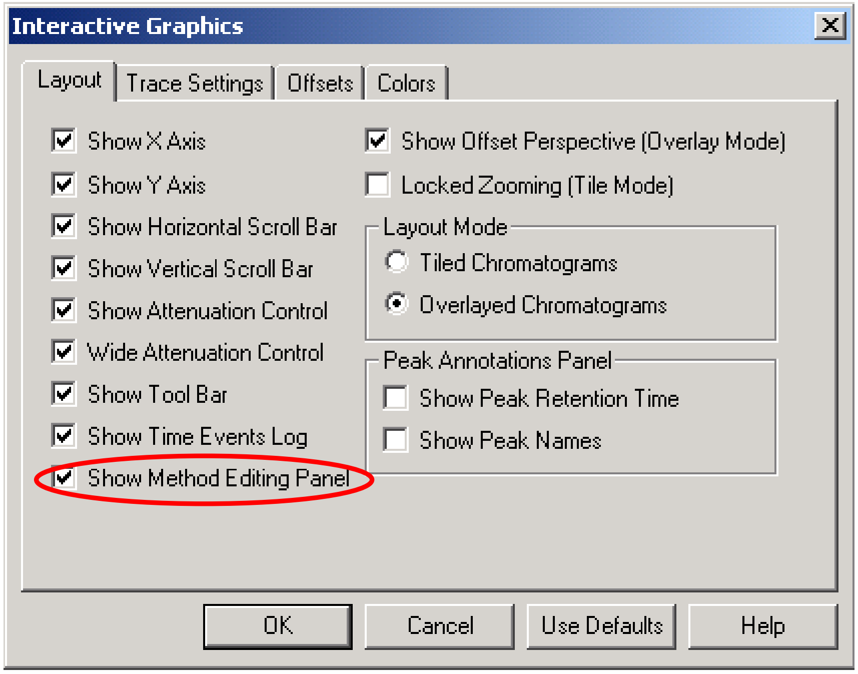
Changing the II Time Events

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.

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.

Select ***View >Preferences***.



☑ Show X Axis
☑ Show Y Axis
☑ Show Horizontal Scroll Bar
☑ Show Vertical Scroll Bar
☑ Show Attenuation Control
☑ Wide Attenuation Control
☑ Show Tool Bar
☑ Show Time Events Log
☑ Show Method Editing Panel
☑ Show Offset Perspective (Overlay Mode)
☐ Locked Zooming (Tile Mode)

Layout Mode
○ Tiled Chromatograms
● Overlayed Chromatograms

Peak Annotations Panel
☐ Show Peak Retention Time
☐ Show Peak Names

Make sure the Show Method Editing Panel box is checked. Click on **OK** to confirm the choice and close the dialog box.

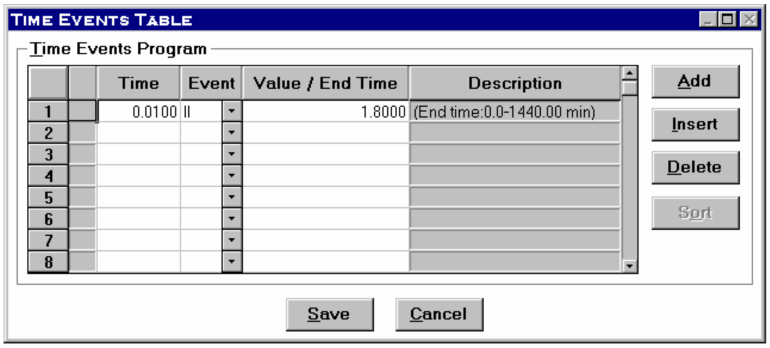
Select ***Edit Method >Time Events***. The Time Events Table window is displayed.

NOTE: For an exercise in how to graphically enter Timed Events, see *Tutorial 5*

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

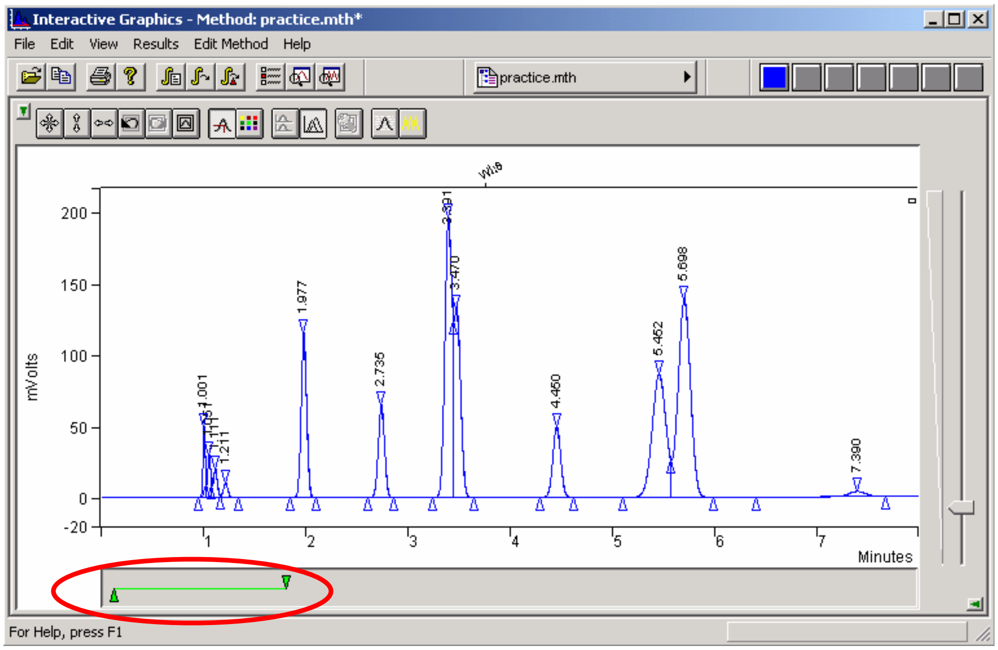
1. Click on **Add**, to add a new line of default entries.
2. Click the arrow at the right of the Event box.
3. Click the up or down scroll arrow until II appears. Click the **II** event.
4. Change the start time and end times. Enter 0.01 into the Time column and 1.80 into the Value/End Time column.

Your display should look like the following.



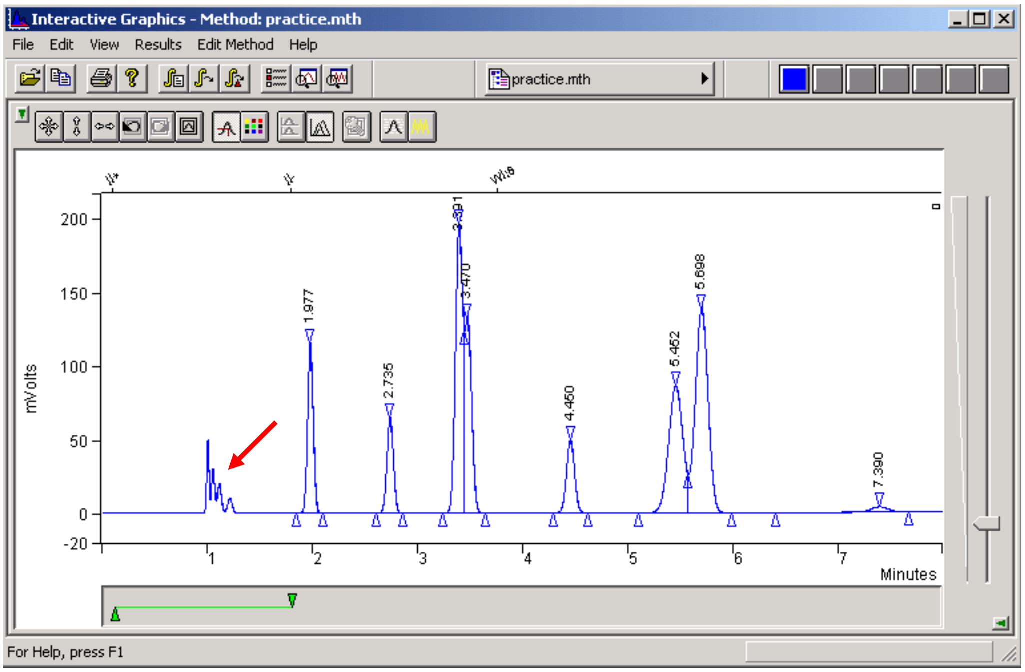
	Time	Event	Value / End Time	Description
1	0.0100	II	1.8000	(End time:0.0-1440.00 min)
2				
3				
4				
5				
6				
7				
8				

Choose **Save** to close the Time Events Table window. This will display the Time Event Window below the chromatogram trace.



Select ***Results >Reintegrate Now***. Save the changed method before reintegration.

After the calculation, the plot will be updated to reflect the changes in the time program. The figure above 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.



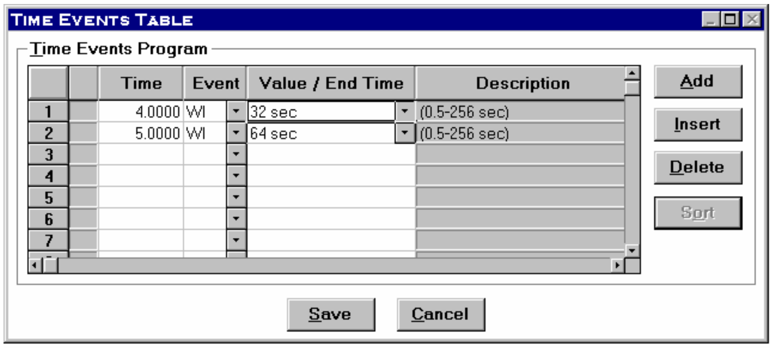
Changing the WI Time Event

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.

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



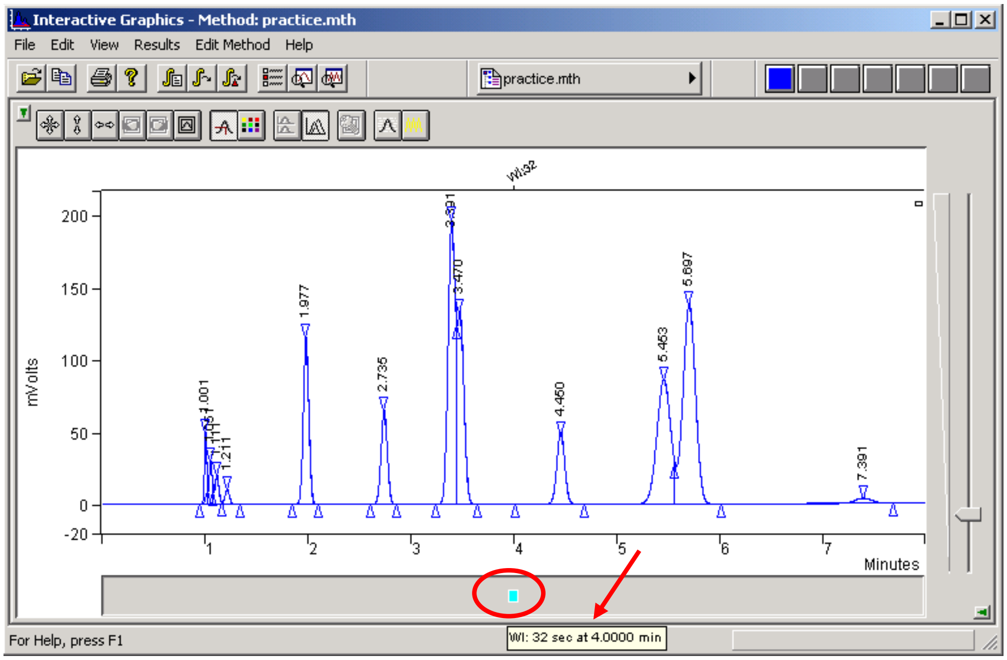
	Time	Event	Value / End Time	Description
1	4.0000	WI	32 sec	(0.5-256 sec)
2	5.0000	WI	64 sec	(0.5-256 sec)
3				
4				
5				
6				
7				

Select and Delete the WI event at 5.00 min.

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

Select ***Results >Reintegrate Now*** and save the changed method before reintegration.

You must Reintegrate as before because you made changes that affect peak integration. After the calculation, 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.



WI: 32 sec at 4.0000 min

For Help, press F1

Place the cursor on the WI Time Event marker.

The Time Event information box is displayed below the marker showing the event type, its program time, and the actual time of execution.

Open the Results window by right-clicking on the chromatogram trace and selecting **View Results Only**. 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.

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

Tutorial 3 Filling a Peak Table

Overview

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.

1. Open Windows Explorer.
2. View the C:\VarianWS\ChromExamples directory.
3. Use the right mouse button to click on Practice.RUN, and select *Copy*.
4. Select ***Edit >Paste***. A copy of the file is added at the end of the list.
5. Use the right mouse button to click on the new file. Select Rename, and type Table.RUN.

Opening the Fill Peak Table Window

Open Interactive Graphics by clicking on the Interactive Graphics bar in the Workstation Toolbar.

Double-click on Table.RUN from the ChromExamples directory and choose **OK**.

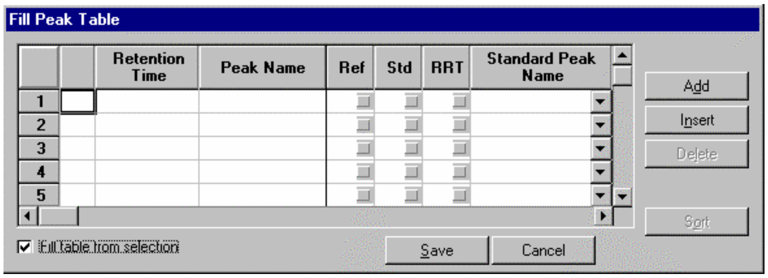
Select ***File >Open Method***.

Choose the ChromExamples 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.

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.

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.

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.

Now, click on the peak eluting at about 2.0 minutes.

Continue filling the Peak Table until all the peaks are listed.

Do not enter the peaks at 1.05, 1.11 and 3.47 and 7.4 minutes.

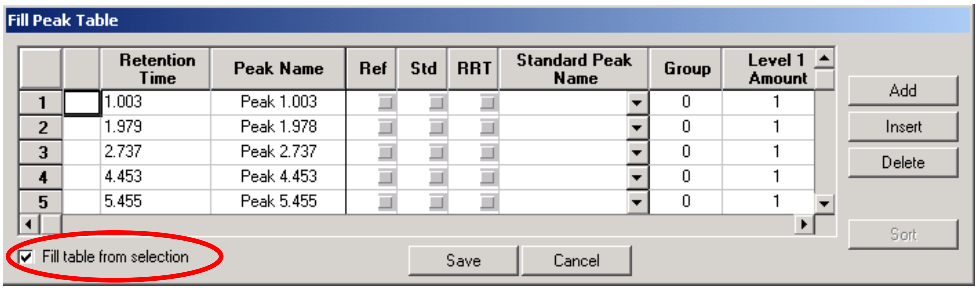
Click on **Sort**. When you look at the Peak Table, you will see that the peaks have been reordered according to their retention times.

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.

Click on the box marked **Fill table from selection** which is located on the Fill Peak Table dialog.



		Retention Time	Peak Name	Ref	Std	RRT	Standard Peak Name	Group	Level 1 Amount
1		1.003	Peak 1.003	☐	☐	☐		0	1
2		1.979	Peak 1.978	☐	☐	☐		0	1
3		2.737	Peak 2.737	☐	☐	☐		0	1
4		4.453	Peak 4.453	☐	☐	☐		0	1
5		5.455	Peak 5.455	☐	☐	☐		0	1

☑ Fill table from selection

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.

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.

Click on the Peak Name field for the first peak.

Type First Peak.

As you type, the Peak Name field is cleared and the name First Peak replaces it.

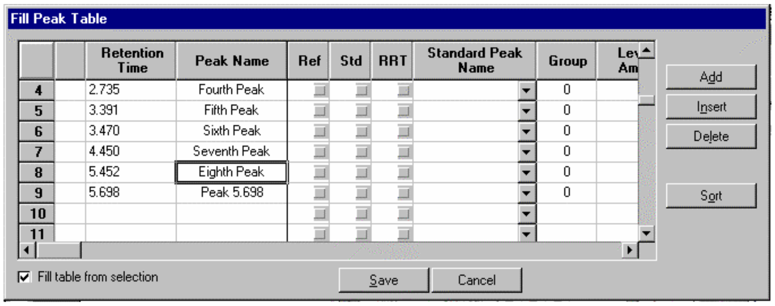
Press the Down Arrow key on your keyboard. The Peak Name field for the second peak is now highlighted. Type Second Peak. Press the Down Arrow Key again.

This is a faster way of entering data than to select fields by a click of the mouse.

Rename the third peak Internal Standard. Press the Down Arrow key.

Continue renaming the remaining peaks.

Your table should look like the one shown.



		Retention Time	Peak Name	Ref	Std	RRT	Standard Peak Name	Group	Lev Am
4		2.735	Fourth Peak	☐	☐	☐		0	
5		3.391	Fifth Peak	☐	☐	☐		0	
6		3.470	Sixth Peak	☐	☐	☐		0	
7		4.450	Seventh Peak	☐	☐	☐		0	
8		5.452	Eighth Peak	☐	☐	☐		0	
9		5.698	Peak 5.698	☐	☐	☐		0	
10				☐	☐	☐			
11				☐	☐	☐			

☑ Fill table from selection

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)

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.

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 calibration process needed for quantitative determinations. You can designate up to eight peaks as Internal Standard Peaks.

NOTE: Refer to *Run File Tutorial* 7 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

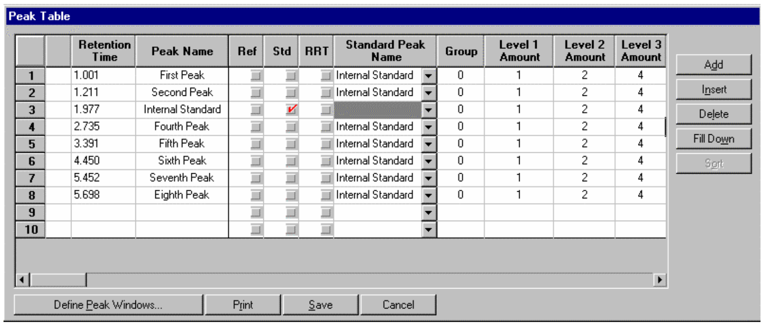
Use the horizontal scroll bar to view the Amount columns. You can examine and enter amounts for up to ten different calibration levels. Each level corresponds to a calibration mixture. The value entered for each peak is the known, measured amount of that compound in the standard. The calibration levels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

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.

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.



		Retention Time	Peak Name	Ref	Std	RRT	Standard Peak Name	Group	Level 1 Amount	Level 2 Amount	Level 3 Amount
1		1.001	First Peak	☐	☐	☐	Internal Standard	0	1	2	4
2		1.211	Second Peak	☐	☐	☐	Internal Standard	0	1	2	4
3		1.977	Internal Standard	☐	☑	☐		0	1	2	4
4		2.735	Fourth Peak	☐	☐	☐	Internal Standard	0	1	2	4
5		3.391	Fifth Peak	☐	☐	☐	Internal Standard	0	1	2	4
6		4.450	Sixth Peak	☐	☐	☐	Internal Standard	0	1	2	4
7		5.452	Seventh Peak	☐	☐	☐	Internal Standard	0	1	2	4
8		5.698	Eighth Peak	☐	☐	☐	Internal Standard	0	1	2	4
9				☐	☐	☐					
10				☐	☐	☐					

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.

Click anywhere on the line for the peak named First Peak.

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.

Click anywhere on the line for the Internal Standard peak.

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.

Click the Delete button. The selected Peak Table entry is removed.

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

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.

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

Select ***File >Save Method As***.

Type the method name, Table.mth, in the file name field and click **Save**.

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.

Tutorial 4 Identifying Peaks

Overview

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:

View the C:\VarianWS\ChromExamples directory.

Use the right mouse button to click on Table.RUN, and select Copy.

Select **EDIT >Paste**. A copy of this file is added at the end of the list.

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.

Open the Interactive Graphics/Data Handling application by clicking on its button in the Workstation Toolbar.

Select the data file Ident.RUN in the ChromExamples directory, and press the **Open File(s)** button.

Select ***File >Open Method*...**

Select IDENT.MTH from the ChromExamples directory.

Choose ***Edit Method >Peak Table*...**

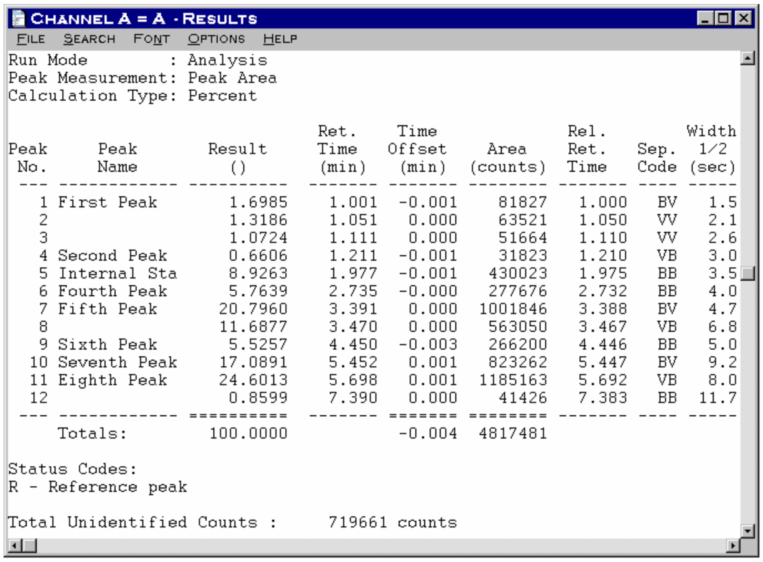
The Peak Table associated with this data file appears. Notice that eight peaks are listed in the Peak Table.

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

Select ***Results >Reintegrate Now***. Save the Method before reintegration.

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.



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

                                    Ret.    Time              Rel.         Width
Peak      Peak          Result      Time   Offset    Area     Ret.   Sep.   1/2
 No.      Name            ()       (min)   (min)   (counts)   Time   Code  (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
```

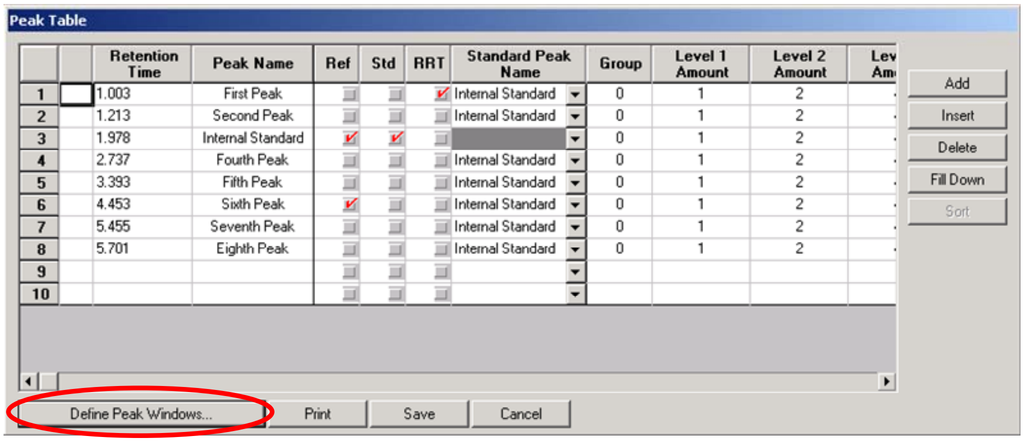
All peaks in the Peak Table are identified. There are 719661 unidentified counts for peaks not listed in the Peak Table.

Define Peak Windows

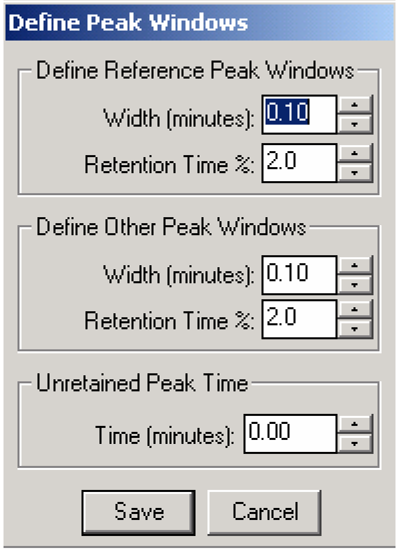
Each peak window is determined by a time window that you enter. The time windows are both added to and subtracted from the expected retention times to define the peak windows.

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.

You set the time window sizes in the **Define Peak Windows** dialog box, which is accessed from the **Peak Table** dialog. Close the Results window. Select the menu command ***Edit Method >Peak Table*** and click on the **Define Peak Windows** tab.



		Retention Time	Peak Name	Ref	Std	RRT	Standard Peak Name	Group	Level 1 Amount	Level 2 Amount	Lev Am
1		1.003	First Peak	☐	☐	☑	Internal Standard	0	1	2	
2		1.213	Second Peak	☐	☐	☐	Internal Standard	0	1	2	
3		1.978	Internal Standard	☑	☑	☐		0	1	2	
4		2.737	Fourth Peak	☐	☐	☐	Internal Standard	0	1	2	
5		3.393	Fifth Peak	☐	☐	☐	Internal Standard	0	1	2	
6		4.453	Sixth Peak	☑	☐	☐	Internal Standard	0	1	2	
7		5.455	Seventh Peak	☐	☐	☐	Internal Standard	0	1	2	
8		5.701	Eighth Peak	☐	☐	☐	Internal Standard	0	1	2	
9				☐	☐	☐					
10				☐	☐	☐					



Define Reference Peak Windows
Width (minutes): 0.10
Retention Time %: 2.0

Define Other Peak Windows
Width (minutes): 0.10
Retention Time %: 2.0

Unretained Peak Time
Time (minutes): 0.00

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.

The default values for both “reference peaks” and “other peaks” are: time window width of 0.1 minutes plus a Retention Time of 2%.

Keep the default values and click the **Save** tab to exit the **Define Peak Windows** dialog and click **Save** again to exit the **Peak Table** window.

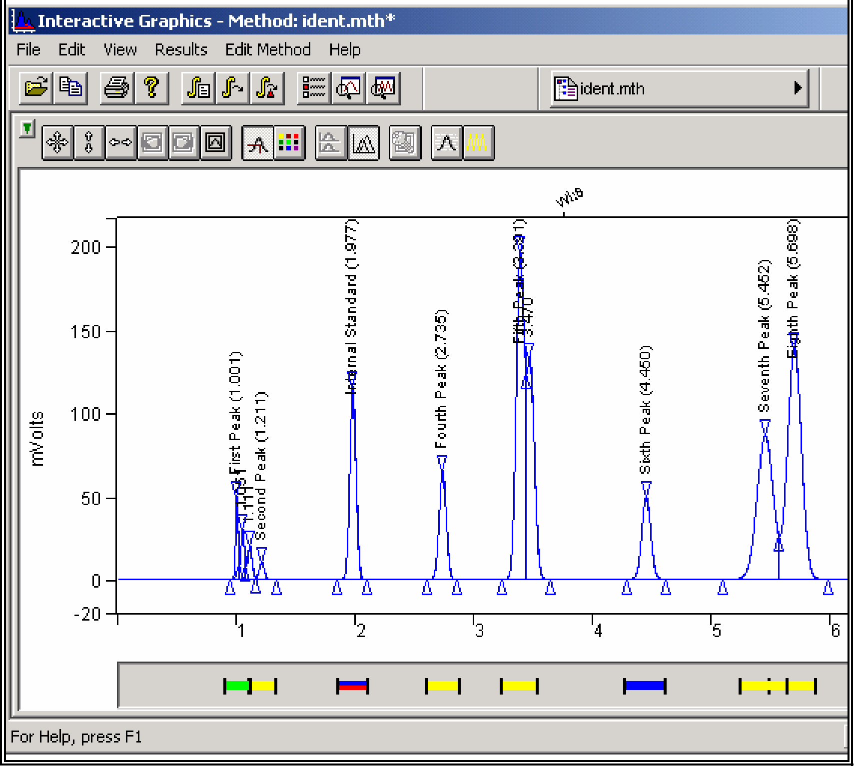
Showing Peak Windows

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

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 Reference peaks: Blue	Relative Retention Time peak: Green Other peaks: Yellow



Changing Peak Functions

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

Select ***Edit Method >Peak Table***.

Click in the Ref. box for the fourth peak to make it the reference peak.

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.

Select ***Edit Method >Peak Table***, deselect the Ref box for the internal standard peak.

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 by adjusting the Peak Reject parameter: peaks smaller than the Peak Reject value are eliminated from the final report.

Select ***Edit Method >Integration Parameters***.

Set the Initial Peak Reject value field to 50000.

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

Press **Save** to exit the **Integration Parameters** dialog.

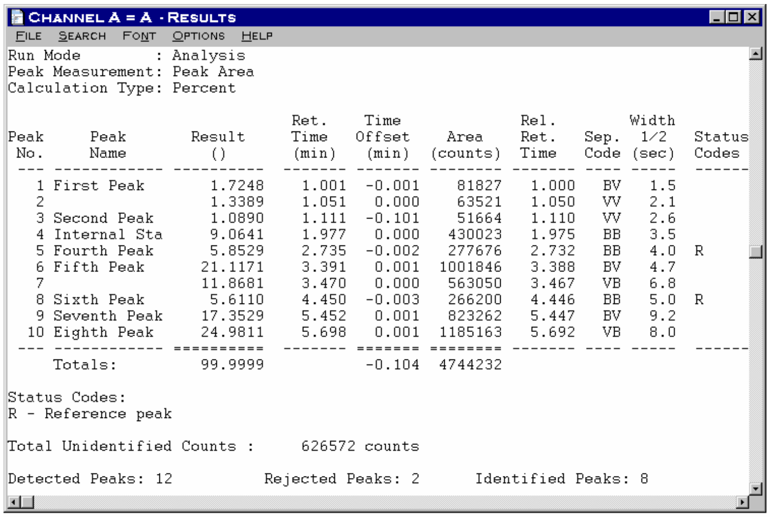
You'll need to recalculate the Results file so that this new Peak Reject value is used in the results.

Select ***Results >Reintegrate Now***. (You can also select the “Reintegrate Now” button from the Toolbar) and save the changes to the current method before reintegrating, in the **Interactive Graphics** dialog that opens.

Take a look at the Results now.

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.



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

                               Ret.    Time             Rel.        Width
Peak     Peak       Result     Time   Offset    Area    Ret.   Sep.  1/2   Status
 No.     Name         ()       (min)   (min)  (counts)  Time   Code (sec)  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.

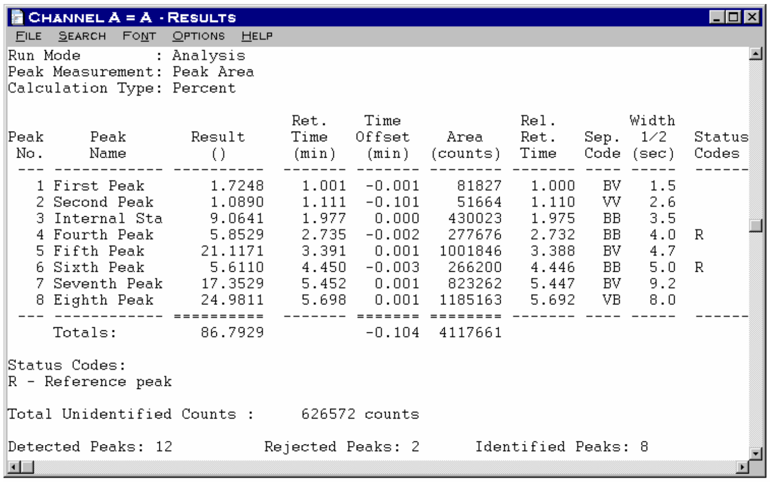
Select ***Edit Method >Integration Parameters*** again.

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.

Select ***Results >Reintegrate Now*** to recalculate the data file using this new set of parameters.

Open the Results window by right-clicking on the chromatogram trace.



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

                               Ret.    Time             Rel.        Width
Peak     Peak       Result     Time   Offset    Area    Ret.   Sep.  1/2   Status
 No.     Name         ()       (min)   (min)  (counts)  Time   Code (sec)  Codes
 --- ------------ ----------  ------- ------- -------- ------- ---- -----  ------
   1 First Peak       1.7248    1.001  -0.001    81827   1.000 BV     1.5
   2 Second Peak      1.0890    1.111  -0.101    51664   1.110 VV     2.6
   3 Internal Sta     9.0641    1.977   0.000   430023   1.975 BB     3.5
   4 Fourth Peak      5.8529    2.735  -0.002   277676   2.732 BB     4.0  R
   5 Fifth Peak      21.1171    3.391   0.001  1001846   3.388 BV     4.7
   6 Sixth Peak       5.6110    4.450  -0.003   266200   4.446 BB     5.0  R
   7 Seventh Peak    17.3529    5.452   0.001   823262   5.447 BV     9.2
   8 Eighth Peak     24.9811    5.698   0.001  1185163   5.692 VB     8.0
 --- ------------ ==========  ------- ======= ======== ------- ---- -----  ------
     Totals:         86.7929           -0.104  4117661

Status Codes:
R - Reference peak

Total Unidentified Counts :     626572 counts

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

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.

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

Overview

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.

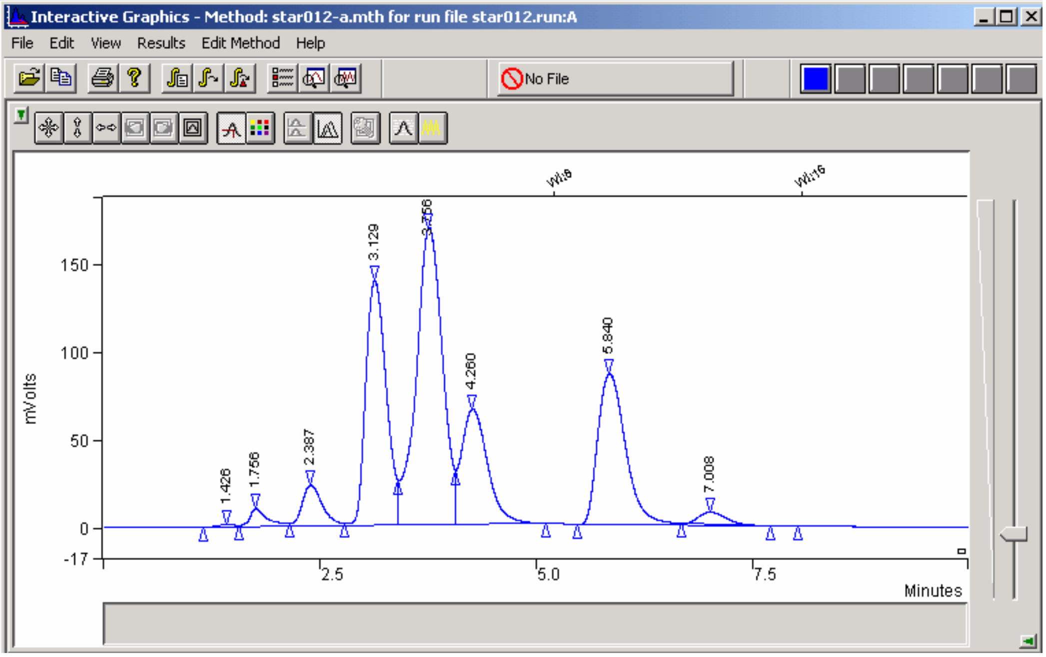
Force or create a baseline where needed in the chromatogram.

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.

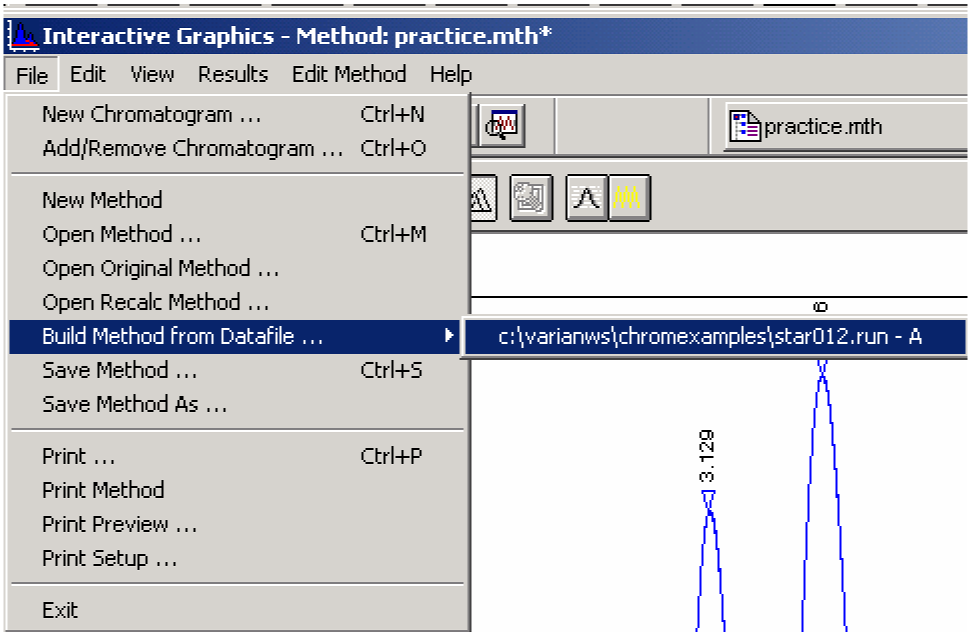
If it is not already opened, select the **Interactive Graphics/Data Handling** application from the Varian Workstation toolbar.

Find and open the data file STAR012.RUN in the ChromExamples directory from the **Open Multiple Data Files** dialog.

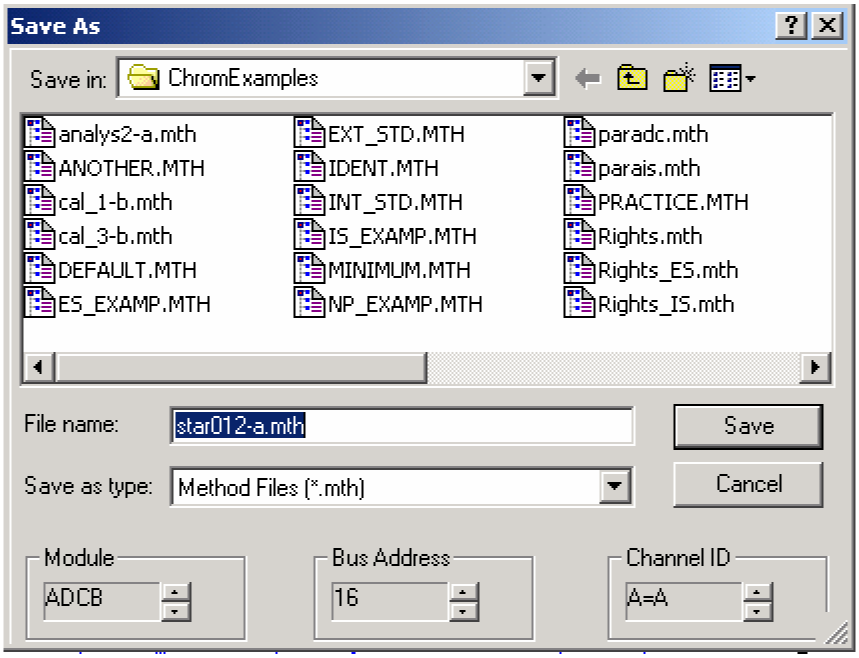
The data file now appears, and the chromatogram should look like the one shown.



Select the menu command ***File >Build Method from Datafile***.



A *Save As* dialog box will ask you to provide a name for the method you are using. Type in star012.mth and click on **OK**.



Save in: ChromExamples

File name: star012-a.mth

Save as type: Method Files (*.mth)

Module: ADCB

Bus Address: 16

Channel ID: A=A

This command opens the Workstation method that was last used to process the data file.

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.

Select ***Edit Method >Time Events***. The Time Events dialog box appears.

Editing a Time Event from the Menu

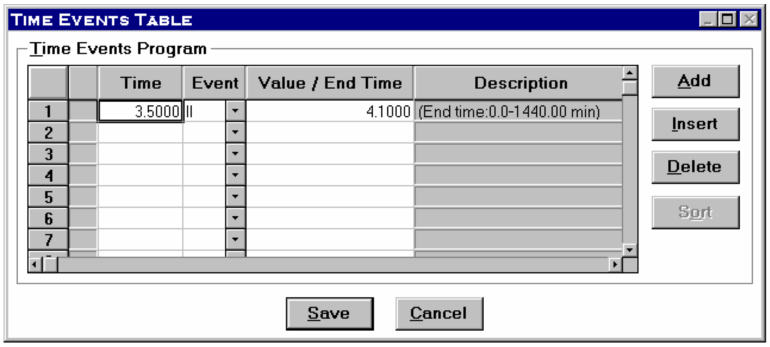
Now, let's program the Inhibit Integrate (II) event to start at 3.5 minutes and stop at 4.1 minutes.

Click on **Add.**

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

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.



		Time	Event	Value / End Time	Description
1		3.5000	II	4.1000	(End time:0.0-1440.00 min)
2					
3					
4					
5					
6					
7					

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

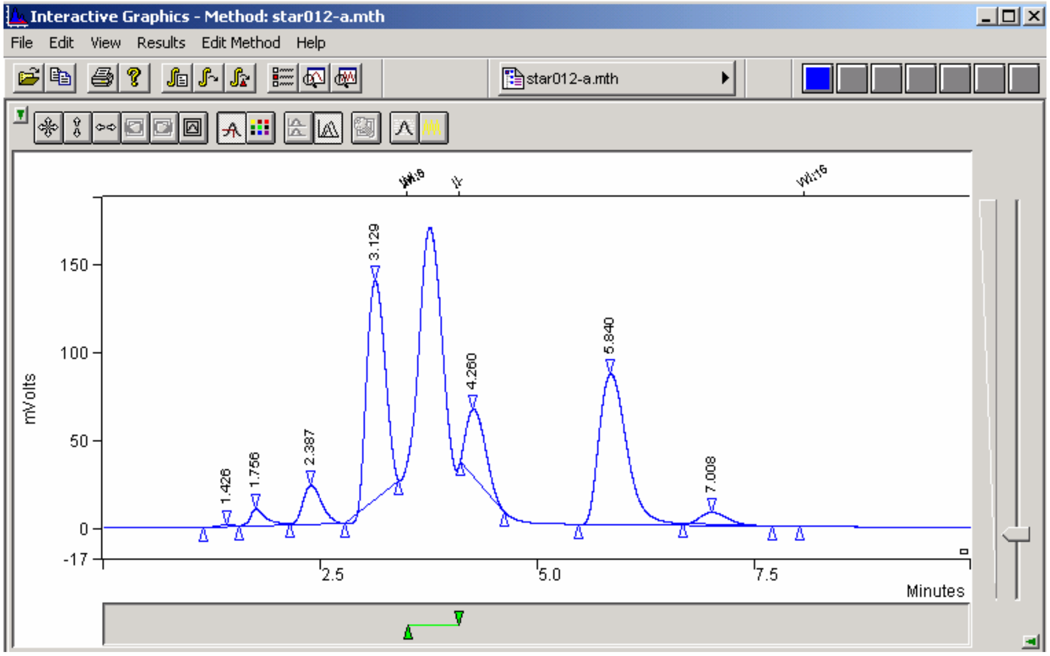
Changes to the II event must be followed by a Reintegration.

Select ***Results >Reintegration List***.

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

Click on the ***Calculate Results*** tab and save the changes to the method in the dialog that opens.

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.

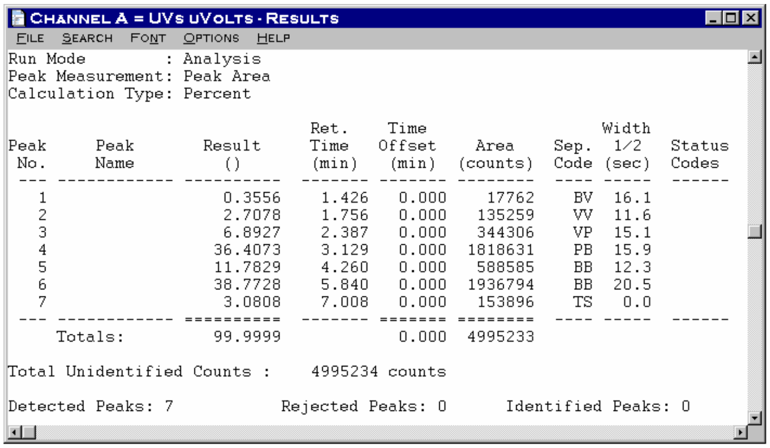


Changes in the II event also alter the final Results for a chromatographic data file.

Open the Results window by right-clicking on the chromatogram trace and selecting **View Results Only.**

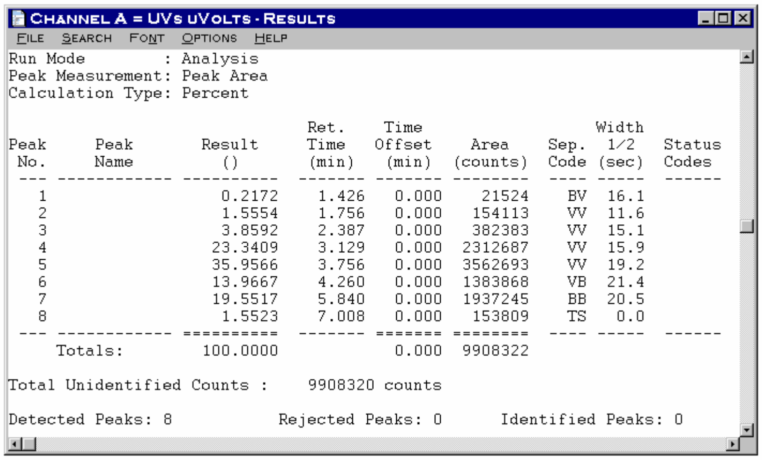
Enlarge the Results window and examine the Results.

They should appear as in the following figure.



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

                                Ret.    Time                    Width
Peak     Peak        Result     Time   Offset    Area    Sep.   1/2   Status
 No.     Name          ()      (min)   (min)   (counts)  Code  (sec)  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.



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

                                Ret.    Time                    Width
Peak     Peak        Result     Time   Offset    Area    Sep.   1/2   Status
 No.     Name          ()      (min)   (min)   (counts)  Code  (sec)  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.

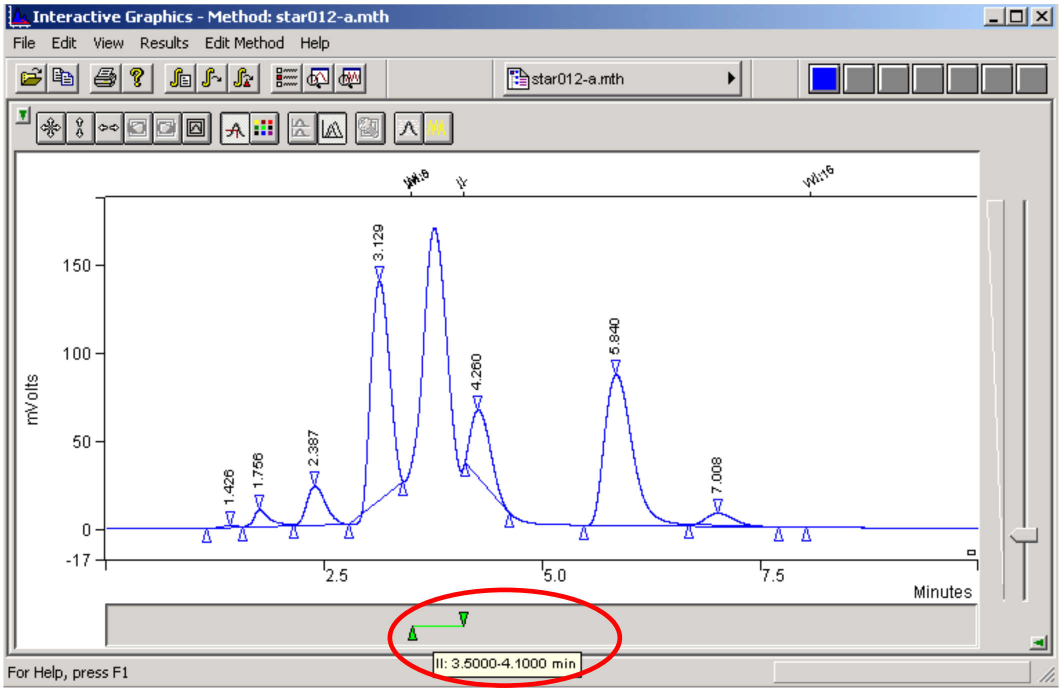
Graphical Placement of a Time Event

You can graphically edit the II Event in the interactive time events window. When the time events window is shown below the chromatogram, try using the left and right mouse buttons to edit the events.

Close the Results window.

Select the menu command ***View*** and check ***Visual Method Edit Window***. The interactive time events window now appears below the chromatogram.

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

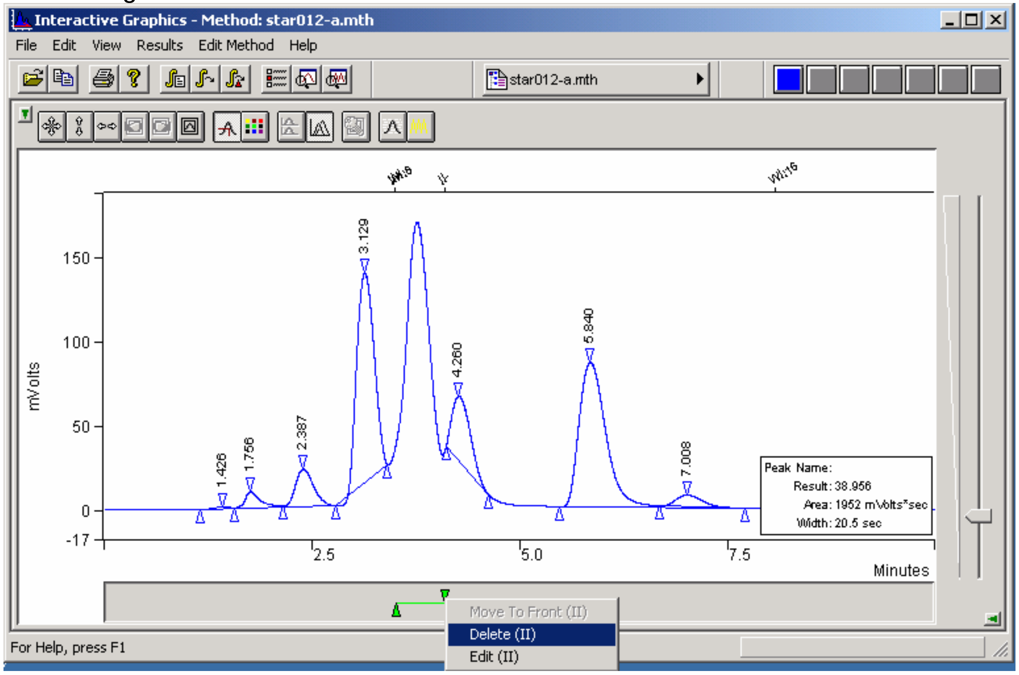


II: 3.5000-4.1000 min

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.

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.



Peak Name:
Result: 38.956
Area: 1952 mVolts*sec
Width: 20.5 sec

Select the menu command ***Results >Reintegrate Now***. Save the changed Method before reintegration.

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.

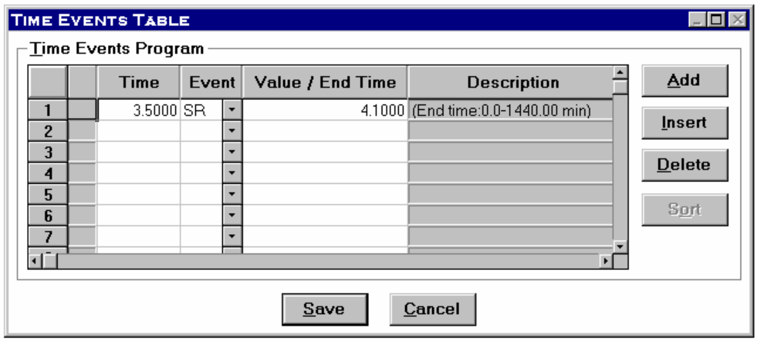
Select the menu command ***Edit Method >Time Events***..

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

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

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.



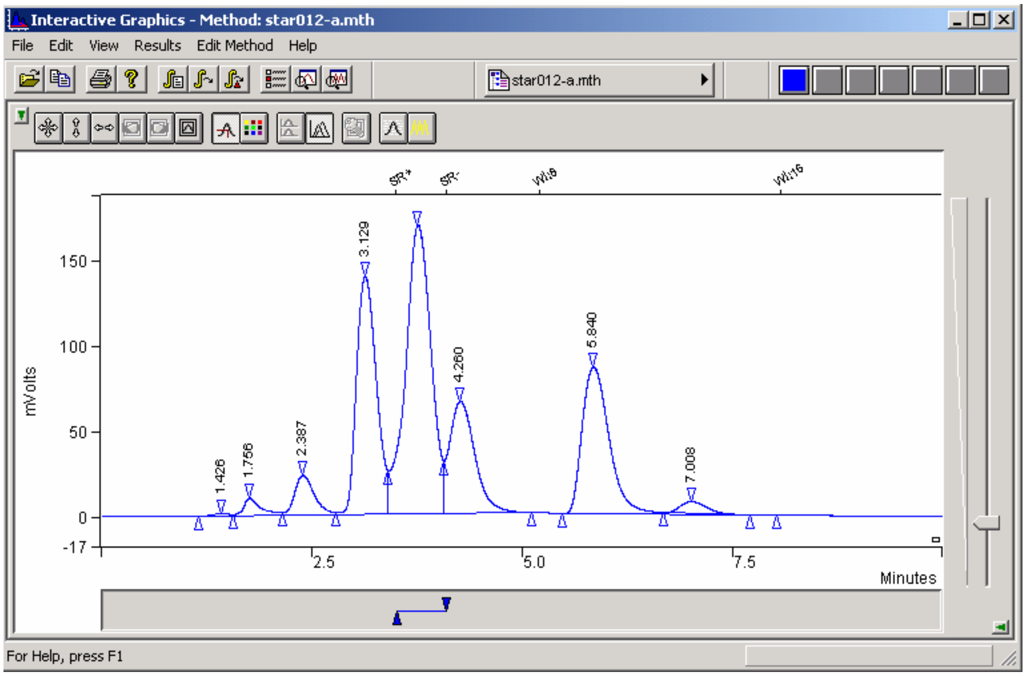
		Time	Event	Value / End Time	Description
1		3.5000	SR	4.1000	(End time:0.0-1440.00 min)
2					
3					
4					
5					
6					
7					

Press **Save** to close the Time Events Table window.

It's time to implement the new change to the peak processing method. 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.

Select the menu command ***Results >Reintegrate Now*** and save the changes to the method before reintegration.

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

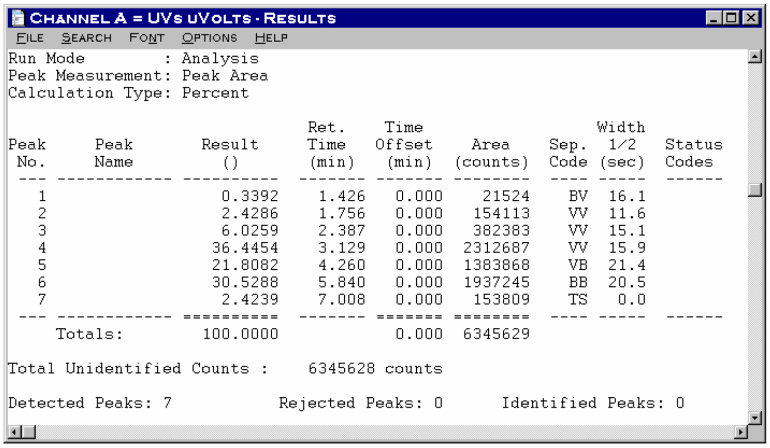


Notice that the SR function does not affect baseline placement or peak detection. Thus, the plot looks similar to the original data file used at the beginning of this tutorial. Solvent Reject eliminates peaks from reports but does not influence their detection, or affect baseline placement.

To open the results file click on the chromatogram trace with the right mouse button and select **View Results Only**.

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

The results should look like those in the following figure.



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

                                   Ret.    Time                     Width
Peak      Peak         Result      Time   Offset     Area    Sep.   1/2    Status
 No.      Name           ()       (min)    (min)   (counts)  Code  (sec)   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.

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.

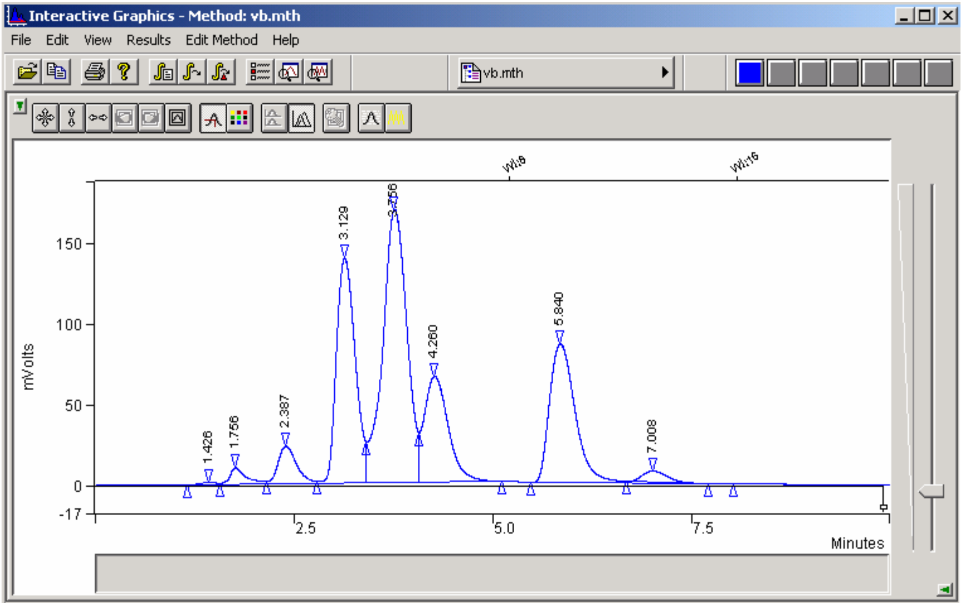
Select ***File >Open Method***.

Select the method file VB.MTH from the ChromExamples directory.

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

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.

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

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.

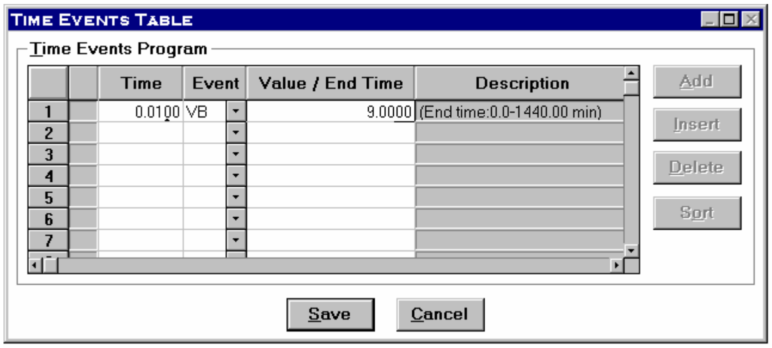
Use the right mouse button to click on either the triangles or the connecting line marking the VB event.

Click on **Edit** (VB).

The displayed Time Events Table now lists the VB event.

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.

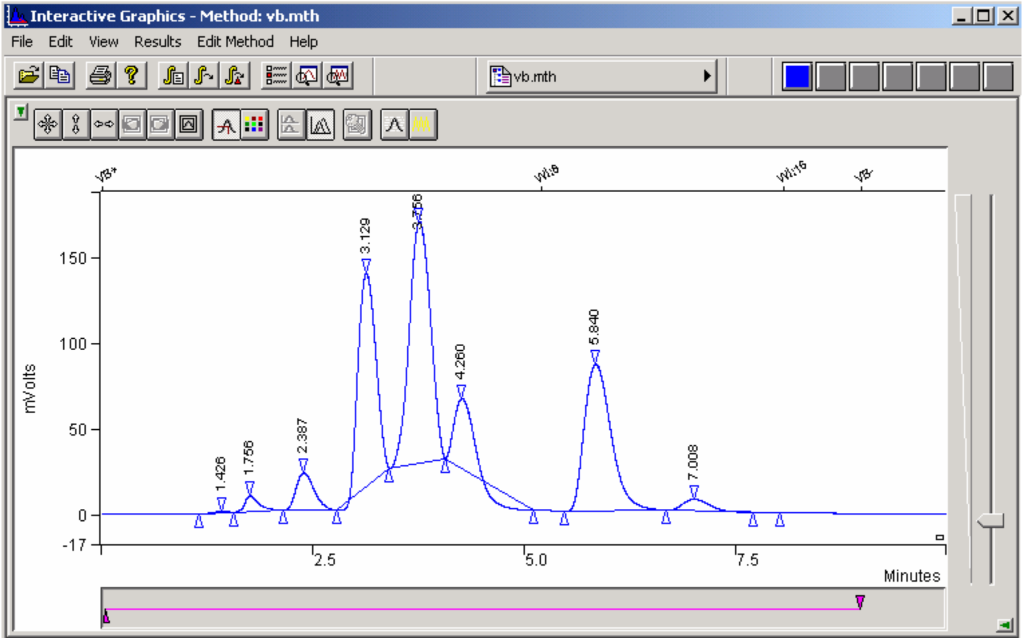


	Time	Event	Value / End Time	Description
1	0.0100	VB	9.0000	(End time:0.0-1440.00 min)
2				
3				
4				
5				
6				
7				

Choose **Save**.

Select the menu command ***Results >Reintegrate Now*** and save the changed method before reintegrating.

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.

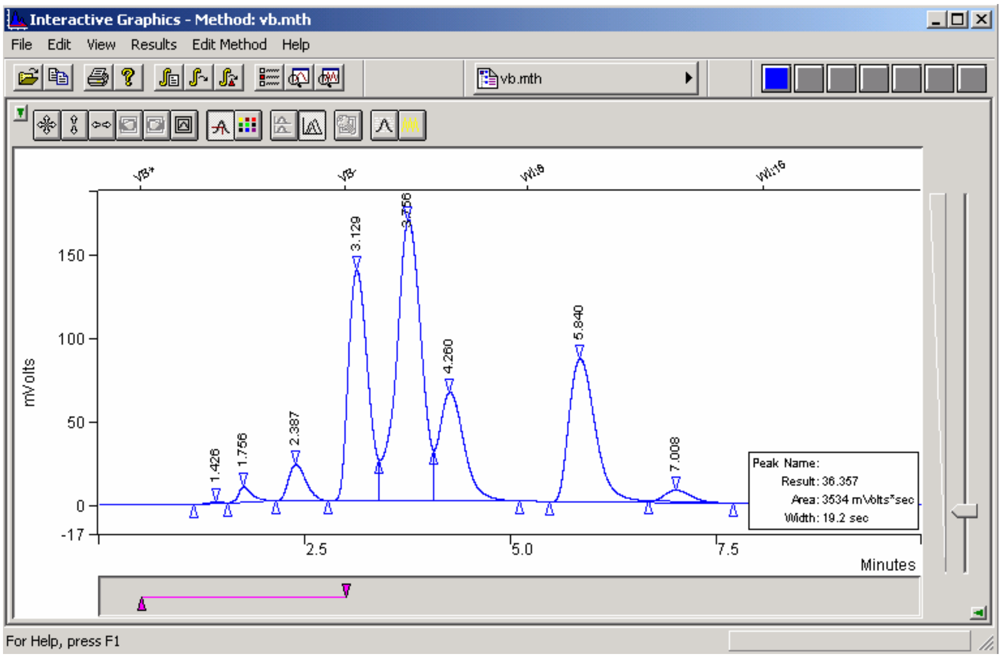
Open the Time Events Table window again.

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.

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

Select ***Results >Reintegrate Now*** as before.

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



Peak Name:
Result: 36.357
Area: 3534 mVolts*sec
Width: 19.2 sec

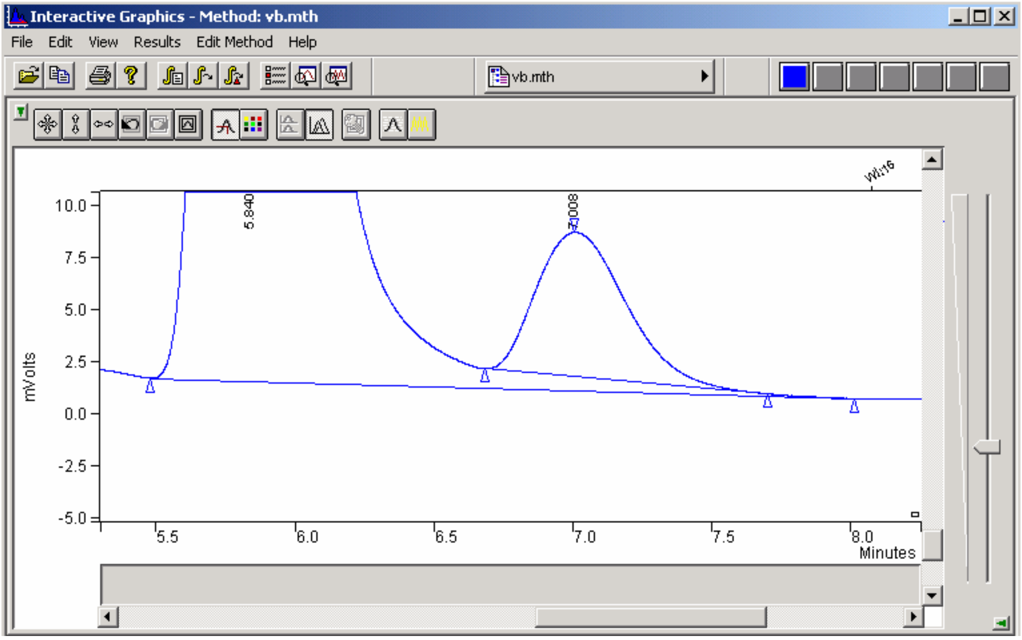
Other peak processing events can affect the Valley Baseline parameter and the baseline assignment. Now, take a look at the effect of changes in the tangent height threshold (T%) on the baseline assignment.

The Effects of Other Peak Processing Events on VB

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

Select the menu command ***View >Preferences***. Click the tab for Trace Settings and check the **Show Cursor >Peak information** field. Click on **OK**.

Select the menu command ***View*** and check **Visual Method Edit Window.**



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

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).

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

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.

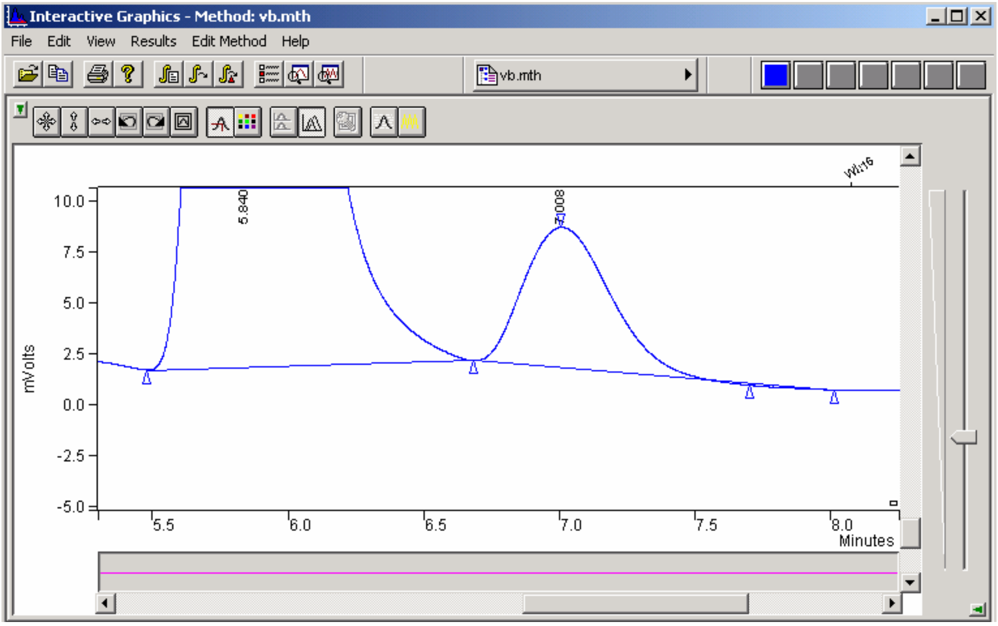
Use the right mouse button to Click on one of the triangles or the connecting line marking the new VB event.

Click on Edit (VB).

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**.

Select ***Results >Reintegrate Now***. Save the Method.

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.

Select ***View >Chromatogram Toolbar***. (Make sure that this menu item is checked).

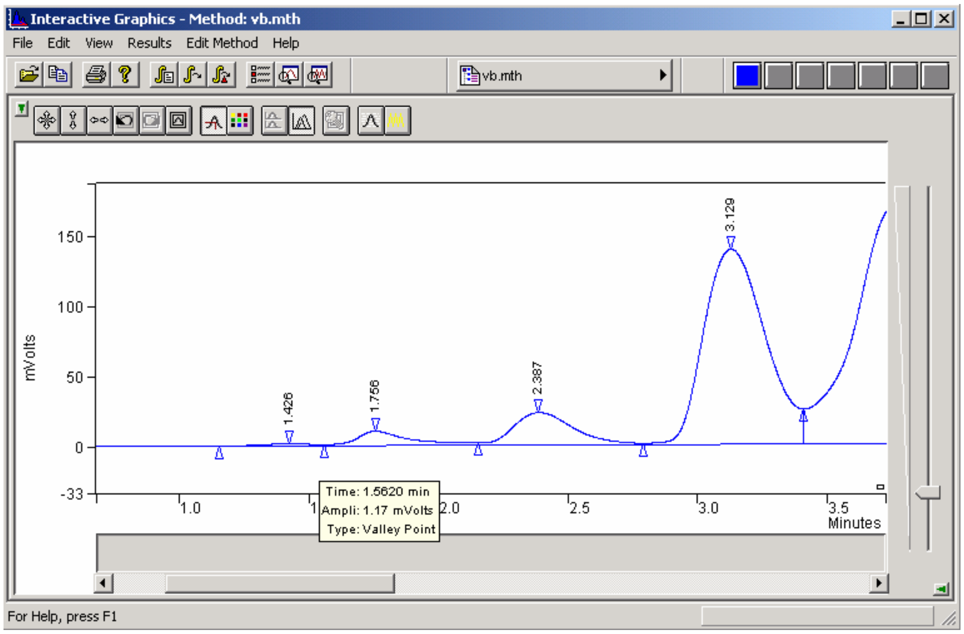
Click on the icon below to normalize both the x and y axis of the plot.

Zoom the chromatogram from about 0.5 to 3.5 minutes.

Select ***View >Preferences***. Select the **Trace Settings** tab and then check the box named **Show cursor / peak information**. Then click on the **OK** button.

Move the cursor to the peak at 1.75 minutes.

The Chromatogram should look like the figure shown.



Interactive Graphics - Method: vb.mth

Time: 1.5620 min
Ampli: 1.17 mVolts
Type: Valley Point

For Help, press F1

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

Select the menu command ***Edit Method >Integration Parameters***.

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

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.

Select ***Edit Method >Time Events***. Delete the first VB event.

Increase the S/N Ratio back to 5.

Reintegrate again.

Close Interactive Graphics.

Tutorial 6 Calibrating with an External Standard

Overview

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.

Open Interactive Graphics from the Workstation Toolbar.

The **Open Multiple Data Files** window appears.

Choose the chromatographic data file CAL_1.RUN in the ChromExamples

Select the **Add To List** tab.

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

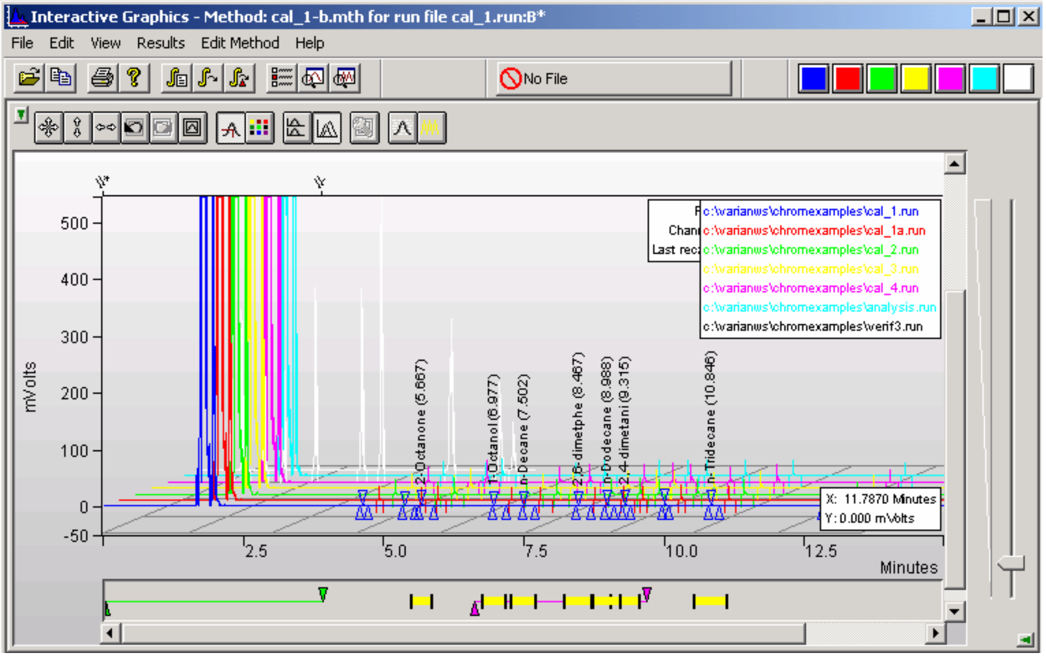
Continue adding the following data files to the list: CAL_1A.RUN, CAL_2.RUN, CAL_3.RUN, CAL_4.RUN.

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

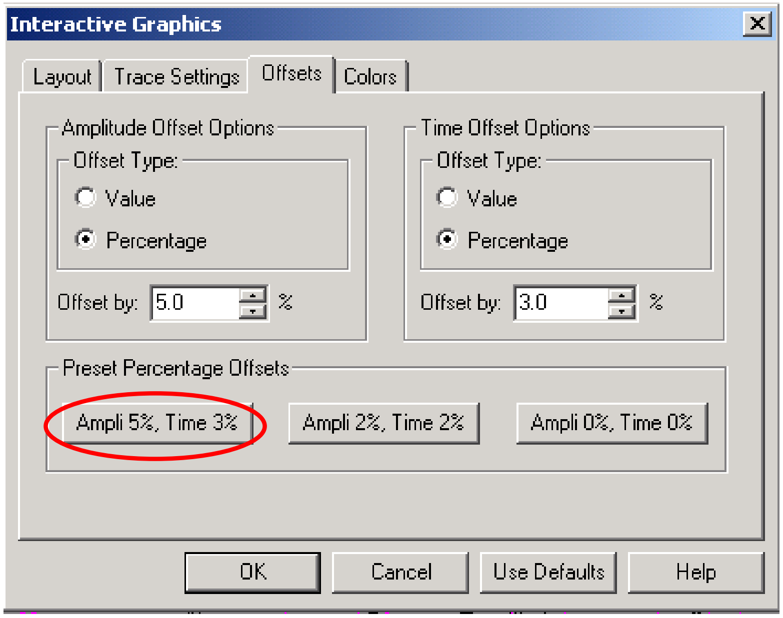
The analytical run for this tutorial is called ANALYSIS.RUN. The file called VERIF_3.RUN is a verification run for this series.

Add ANALYSIS.RUN and VERIF_3.RUN to the list and press **Open Files**.

The IG screen should look similar to the figure shown.



If the chromatograms display looks different, make sure the Overlay Chromatograms button is checked and not the Tile Chromatograms button. Also, select the menu command ***View >Preferences*** and press the Offsets tab in the Interactive Graphics dialog.



Amplitude Offset Options
Offset Type:
○ Value
● Percentage
Offset by: 5.0 %

Time Offset Options
Offset Type:
○ Value
● Percentage
Offset by: 3.0 %

Preset Percentage Offsets
Ampli 5%, Time 3%
Ampli 2%, Time 2%
Ampli 0%, Time 0%

Select the **Ampli 5%, Time 3%** tab and then close the dialog by clicking **OK**.

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 ChromExamples directory. The information in the peak table is necessary to generate the coefficients of the calibration curve.

Select the menu command ***File >Open Method.*** Find and select the method file **EXT_STD.MTH** in the **Open Method** dialog, and press the **Open** tab.

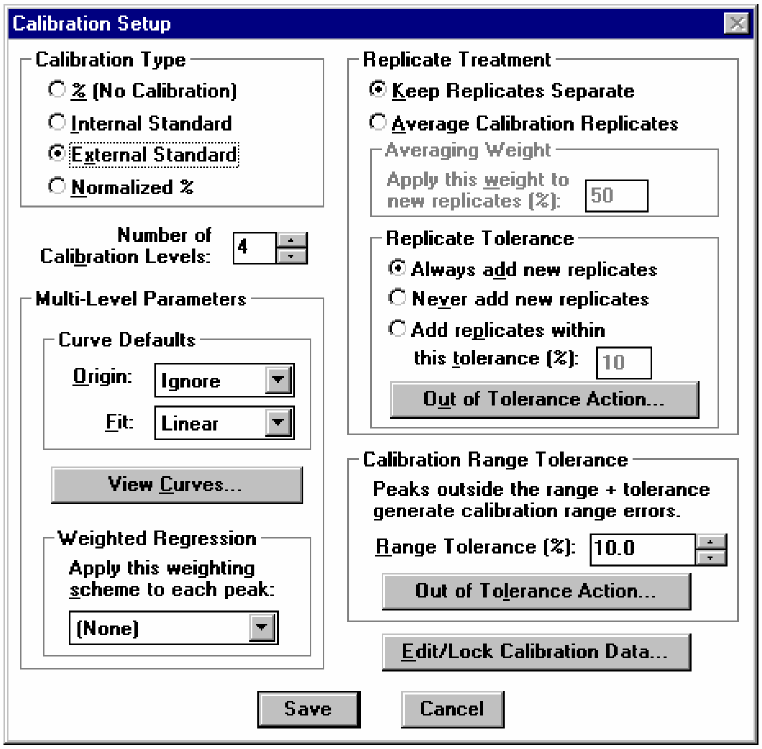
The title bar for the Interactive Graphics window now lists EXT_STD.MTH as the active method.

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

Select ***Edit Method >Calibration Setup***.

The Calibration Setup window opens.

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



Calibration Setup

Calibration Type
○ % (No Calibration)
○ Internal Standard
● External Standard
○ Normalized %

Number of Calibration Levels: 4

Multi-Level Parameters
Curve Defaults
Origin: Ignore
Fit: Linear

Weighted Regression
Apply this weighting scheme to each peak: (None)

Replicate Treatment
● Keep Replicates Separate
○ Average Calibration Replicates
Averaging Weight
Apply this weight to new replicates (%): 50

Replicate Tolerance
● Always add new replicates
○ Never add new replicates
○ Add replicates within this tolerance (%): 10

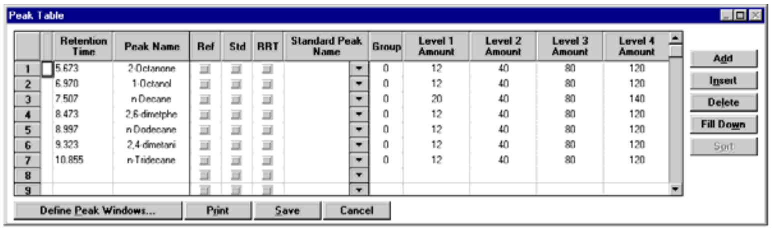
Calibration Range Tolerance
Peaks outside the range + tolerance generate calibration range errors.
Range Tolerance (%): 10.0

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

Now, verify the Peak Table settings.

Select ***Edit Method >Peak Table***.

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



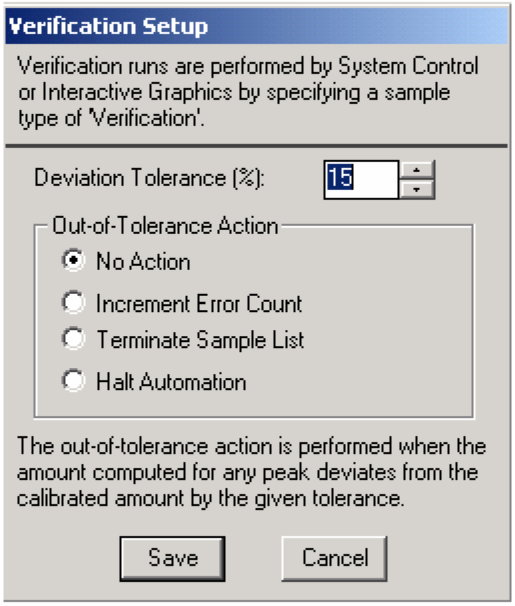
Peak Table

	Retention Time	Peak Name	Ref	Std	RRT	Standard Peak Name	Group	Level 1 Amount	Level 2 Amount	Level 3 Amount	Level 4 Amount
1	5.673	2-Octanone	☐	☐	☐		0	12	40	80	120
2	6.970	1-Octanol	☐	☐	☐		0	12	40	80	120
3	7.507	n-Decane	☐	☐	☐		0	20	40	80	140
4	8.473	2,6-dimetphe	☐	☐	☐		0	12	40	80	120
5	8.997	n-Dodecane	☐	☐	☐		0	12	40	80	120
6	9.323	2,4-dimetani	☐	☐	☐		0	12	40	80	120
7	10.855	n-Tridecane	☐	☐	☐		0	12	40	80	120
8			☐	☐	☐						
9			☐	☐	☐						

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

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

Select ***Edit Method >Verification Setup***. Set the Deviation Tolerance to 15% and the Out-of-Tolerance Action to No Action.



Verification runs are performed by System Control or Interactive Graphics by specifying a sample type of 'Verification'.

Deviation Tolerance (%): 15

Out-of-Tolerance Action
- ● No Action
- ○ Increment Error Count
- ○ Terminate Sample List
- ○ Halt Automation

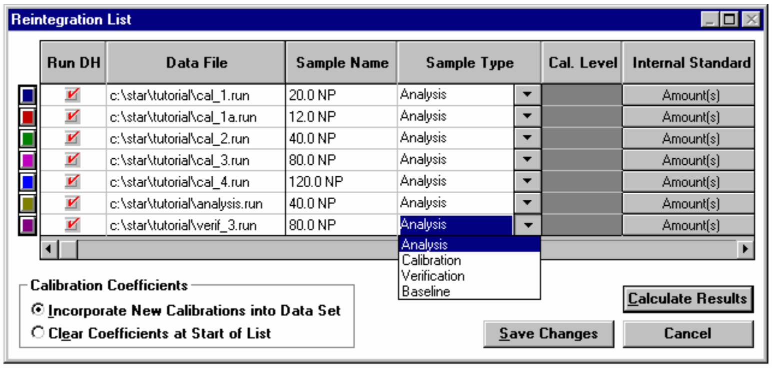
The out-of-tolerance action is performed when the amount computed for any peak deviates from the calibrated amount by the given tolerance.

Save this setup.

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

Select ***Results >Reintegration List*...**

The Reintegration List appears.



Run DH	Data File	Sample Name	Sample Type	Cal. Level	Internal Standard
☑	c:\star\tutorial\cal_1.run	20.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_1a.run	12.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_2.run	40.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_3.run	80.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_4.run	120.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\analysis.run	40.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\verif_3.run	80.0 NP	Analysis		Amount(s)

Calibration Coefficients
- ● Incorporate New Calibrations into Data Set
- ○ Clear Coefficients at Start of List

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.

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

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

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

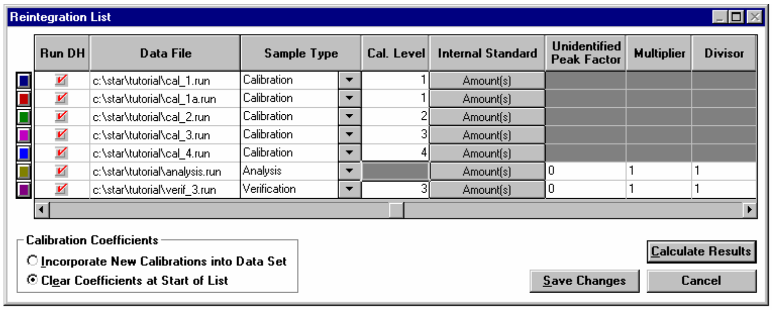
The fifth column is the Calibration Level column. Each value listed here corresponds to a Level column in the peak table. The calibration levels used in this tutorial are numbered 1 through 4

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.

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

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.



Run DH	Data File	Sample Type	Cal. Level	Internal Standard	Unidentified Peak Factor	Multiplier	Divisor
☑	c:\star\tutorial\cal_1.run	Calibration	1	Amount(s)			
☑	c:\star\tutorial\cal_1a.run	Calibration	1	Amount(s)			
☑	c:\star\tutorial\cal_2.run	Calibration	2	Amount(s)			
☑	c:\star\tutorial\cal_3.run	Calibration	3	Amount(s)			
☑	c:\star\tutorial\cal_4.run	Calibration	4	Amount(s)			
☑	c:\star\tutorial\analysis.run	Analysis		Amount(s)	0	1	1
☑	c:\star\tutorial\verif_3.run	Verification	3	Amount(s)	0	1	1

Calibration Coefficients
○ Incorporate New Calibrations into Data Set
● Clear Coefficients at Start of List

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

Click the **Calculate Results** button.

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.

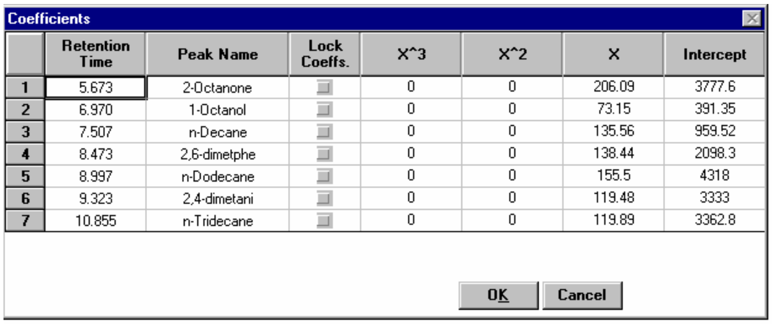
The Calibration Curve

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.

Select ***Edit Method >Calibration Setup***.

Click on the **Edit/Lock Calibration Data**… button at the bottom of the window.

The Coefficients window should look like the one shown next.



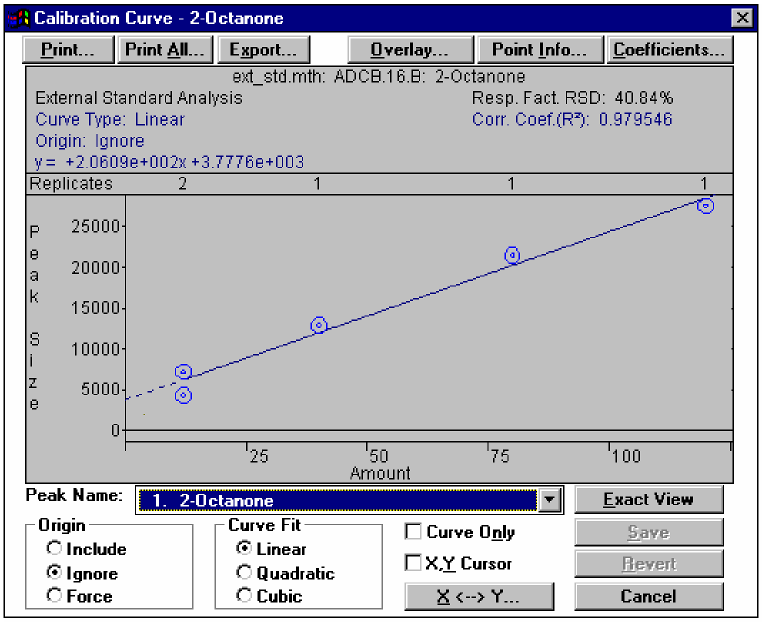
Coefficients

	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

Click **Cancel** to return to the **Calibration Setup** dialog.

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.



Calibration Curve - 2-Octanone

ext_std.mth: ADCB.16.B: 2-Octanone

External Standard Analysis

Curve Type: Linear

Origin: Ignore

y = +2.0609e+002x +3.7776e+003

Resp. Fact. RSD: 40.84%

Corr. Coef.(R²): 0.979546

Peak Name: 1. 2-Octanone

Origin: ○ Include ● Ignore ○ Force

Curve Fit: ● Linear ○ Quadratic ○ Cubic

☐ Curve Only

☐ X,Y Cursor

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

Click the drop-down menu arrow in the Peak Name field.

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.

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.

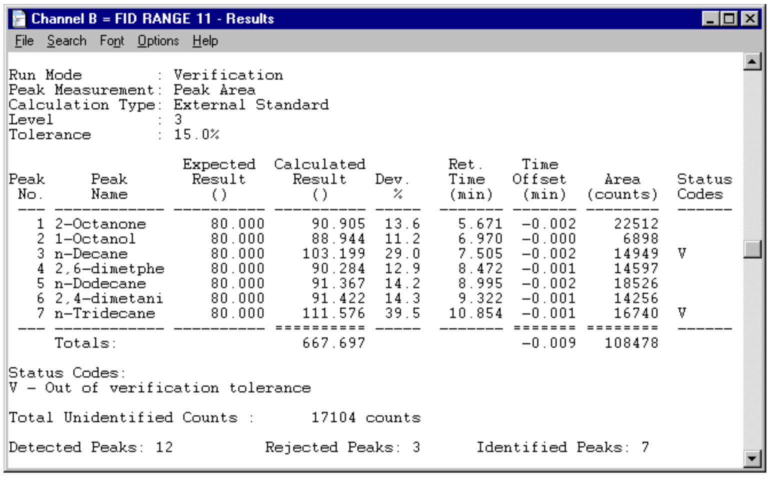
NOTE: Refer to the tutorial, "Calibrating with an Internal Standard and Getting Around in the Curve Manager", for an exercise in using the other curve functions.

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.

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

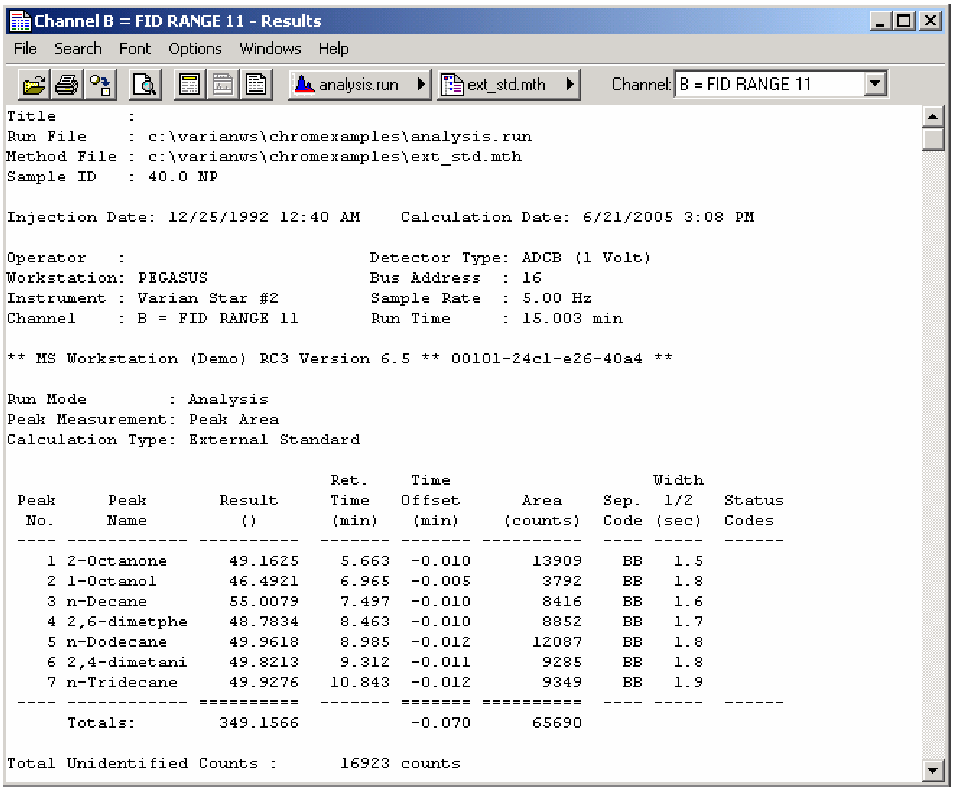
The Verification Report for VERIF_3.RUN appears. Note that Peaks 3 and 7 are marked as “Out of Verification Tolerance”.



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

                      Expected  Calculated        Ret.    Time
Peak      Peak         Result     Result   Dev.   Time   Offset    Area    Status
 No.      Name          ()         ()       %    (min)   (min)   (counts)  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
```

Close the Results window

Right click anywhere on the plot trace of the sixth chromatogram, ANALYSIS.RUN (this should be the light blue trace) and select **View Results Only**.



```
Title       :
Run File    : c:\varianws\chromexamples\analysis.run
Method File : c:\varianws\chromexamples\ext_std.mth
Sample ID   : 40.0 NP

Injection Date: 12/25/1992 12:40 AM    Calculation Date: 6/21/2005 3:08 PM

Operator    :                         Detector Type: ADCB (1 Volt)
Workstation: PEGASUS                  Bus Address  : 16
Instrument : Varian Star #2           Sample Rate  : 5.00 Hz
Channel     : B = FID RANGE 11        Run Time     : 15.003 min

** MS Workstation (Demo) RC3 Version 6.5 ** 00101-24c1-e26-40a4 **

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

                                      Ret.    Time                     Width
 Peak      Peak         Result        Time   Offset      Area    Sep.   1/2   Status
  No.      Name           ()         (min)   (min)     (counts)  Code  (sec)  Codes
 ---- ------------ ----------- ------- ------- ---------- ---- -----  ------
    1 2-Octanone      49.1625    5.663  -0.010      13909   BB    1.5
    2 1-Octanol       46.4921    6.965  -0.005       3792   BB    1.8
    3 n-Decane        55.0079    7.497  -0.010       8416   BB    1.6
    4 2,6-dimetphe    48.7834    8.463  -0.010       8852   BB    1.7
    5 n-Dodecane      49.9618    8.985  -0.012      12087   BB    1.8
    6 2,4-dimetani    49.8213    9.312  -0.011       9285   BB    1.8
    7 n-Tridecane     49.9276   10.843  -0.012       9349   BB    1.9
 ---- ------------ ========== ------- ======= ========== ---- -----  ------
      Totals:        349.1566           -0.070      65690

Total Unidentified Counts :      16923 counts
```

Tutorial 7 Calibrating with Internal Standards

Overview

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.

Open Interactive Graphics from the Workstation Star Toolbar.

The Open Multiple Data Files window appears.

Choose the chromatographic data file CAL_1.RUN in the ChromExamples directory.

Click the **Add To List** tab.

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

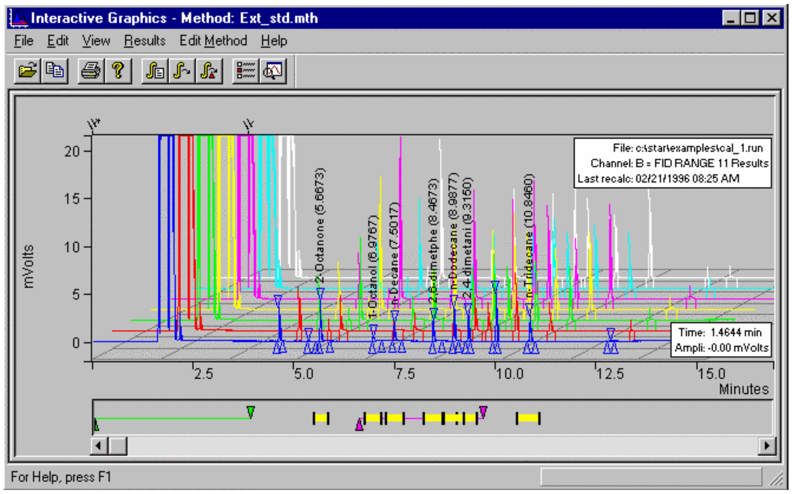
Continue adding the following data files to the list: CAL_1A.RUN, CAL_2.RUN, CAL_3.RUN, CAL_4.RUN.

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.

Add ANALYSIS.RUN and VERIF_3.RUN to the list and press **Open Files**.

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



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 ChromExamples directory. The information in the peak table is necessary to generate the coefficients of the calibration curve.

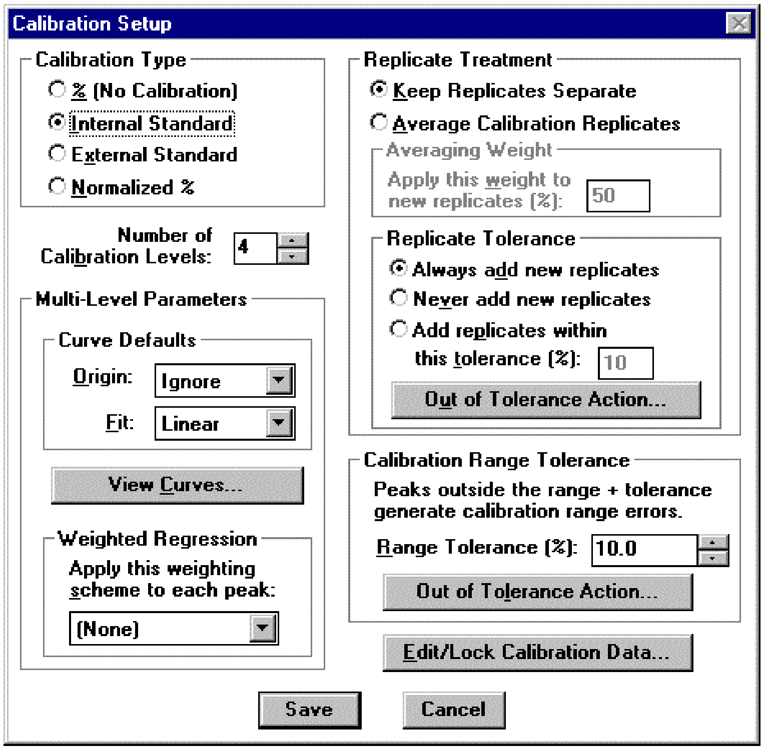
Select ***File >Open Method*** and select **INT_STD.MTH**, press **Open Files**.

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:

Select ***Edit Method >Calibration Setup***.

The Calibration Setup window opens. The window should look like the one shown next.



Calibration Setup

Calibration Type
- ○ % (No Calibration)
- ● Internal Standard
- ○ External Standard
- ○ Normalized %

Number of Calibration Levels: 4

Multi-Level Parameters

Curve Defaults
- Origin: Ignore
- Fit: Linear

Weighted Regression
Apply this weighting scheme to each peak: (None)

Replicate Treatment
- ● Keep Replicates Separate
- ○ Average Calibration Replicates

Averaging Weight
Apply this weight to new replicates (%): 50

Replicate Tolerance
- ● Always add new replicates
- ○ Never add new replicates
- ○ Add replicates within this tolerance (%): 10

Calibration Range Tolerance
Peaks outside the range + tolerance generate calibration range errors.
Range Tolerance (%): 10.0

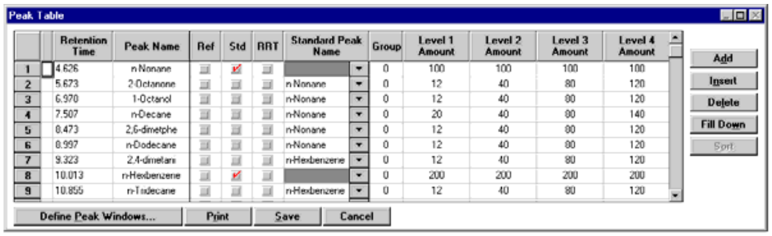
The Calibration Type should be Internal Standard. The Number of Calibration Levels should be set to 4. The Curve settings should be Linear, Ignore.

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

Now, verify the Peak Table settings.

Select ***Edit Method >Peak Table***.

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



Peak Table

	Retention Time	Peak Name	Ref	Std	RRT	Standard Peak Name	Group	Level 1 Amount	Level 2 Amount	Level 3 Amount	Level 4 Amount
1	4.626	n-Nonane	☐	☑	☐		0	100	100	100	100
2	5.673	2-Octanone	☐	☐	☐	n-Nonane	0	12	40	80	120
3	6.970	1-Octanol	☐	☐	☐	n-Nonane	0	12	40	80	120
4	7.507	n-Decane	☐	☐	☐	n-Nonane	0	20	40	80	140
5	8.473	2,6-dimetphe	☐	☐	☐	n-Nonane	0	12	40	80	120
6	8.997	n-Dodecane	☐	☐	☐	n-Nonane	0	12	40	80	120
7	9.323	2,4-dimetani	☐	☐	☐	n-Hexbenzene	0	12	40	80	120
8	10.013	n-Hexbenzene	☐	☑	☐		0	200	200	200	200
9	10.855	n-Tridecane	☐	☐	☐	n-Hexbenzene	0	12	40	80	120

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.

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

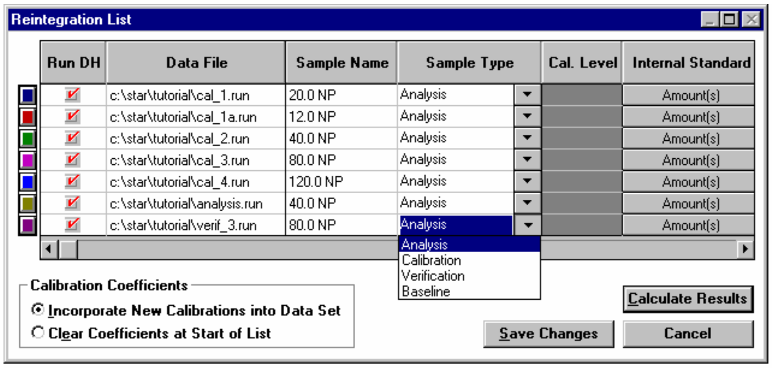
Next, you will set the Verification Deviation Tolerance for the VERIF_3.RUN example.

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.

Select ***Results >Reintegration List...***

The Reintegration List appears.



Run DH	Data File	Sample Name	Sample Type	Cal. Level	Internal Standard
☑	c:\star\tutorial\cal_1.run	20.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_1a.run	12.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_2.run	40.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_3.run	80.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\cal_4.run	120.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\analysis.run	40.0 NP	Analysis		Amount(s)
☑	c:\star\tutorial\verif_3.run	80.0 NP	Analysis		Amount(s)

Calibration Coefficients

● Incorporate New Calibrations into Data Set

○ Clear Coefficients at Start of List

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.

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

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

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.

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.

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

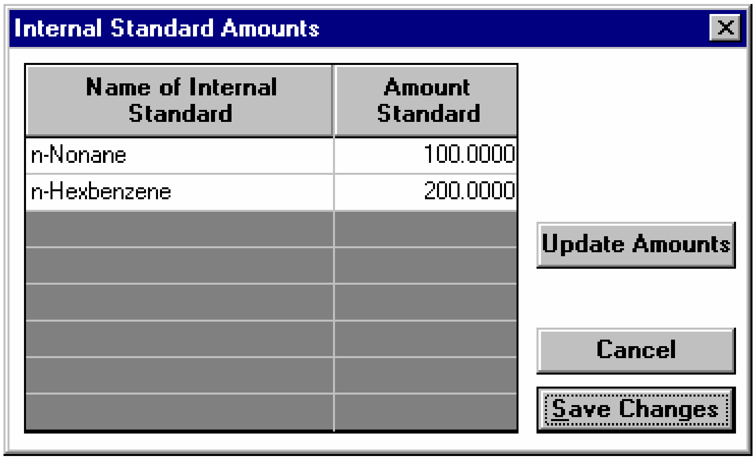
Next, you should verify the amounts for the internal standards referenced in the peak table.

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 next.



Name of Internal Standard	Amount Standard
n-Nonane	100.0000
n-Hexbenzene	200.0000

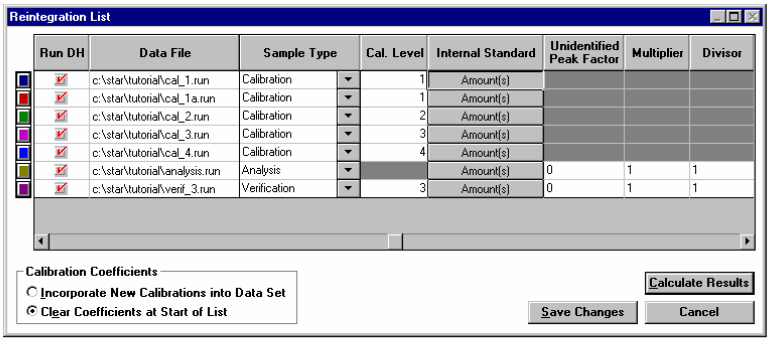
Click on the **Save Changes** button.

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.

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.



Run DH	Data File	Sample Type	Cal. Level	Internal Standard	Unidentified Peak Factor	Multiplier	Divisor
☑	c:\star\tutorial\cal_1.run	Calibration	1	Amount(s)			
☑	c:\star\tutorial\cal_1a.run	Calibration	1	Amount(s)			
☑	c:\star\tutorial\cal_2.run	Calibration	2	Amount(s)			
☑	c:\star\tutorial\cal_3.run	Calibration	3	Amount(s)			
☑	c:\star\tutorial\cal_4.run	Calibration	4	Amount(s)			
☑	c:\star\tutorial\analysis.run	Analysis		Amount(s)	0	1	1
☑	c:\star\tutorial\verif_3.run	Verification	3	Amount(s)	0	1	1

Calibration Coefficients
○ Incorporate New Calibrations into Data Set
● Clear Coefficients at Start of List

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

Click the *Calculate Results* button.

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.

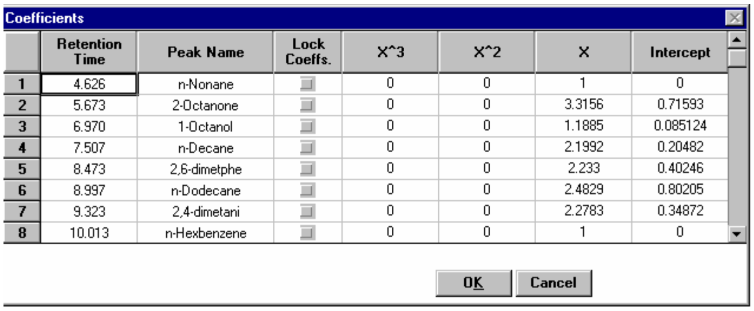
The Calibration Curve

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.

Select ***Edit Method >Calibration Setup***.

Click on the *Edit/Lock Coefficients* button at the bottom of the window.

The Coefficients window should look like the one shown next.



	Retention Time	Peak Name	Lock Coeffs.	X^3	X^2	X	Intercept
1	4.626	n-Nonane	☐	0	0	1	0
2	5.673	2-Octanone	☐	0	0	3.3156	0.71593
3	6.970	1-Octanol	☐	0	0	1.1885	0.085124
4	7.507	n-Decane	☐	0	0	2.1992	0.20482
5	8.473	2,6-dimetphe	☐	0	0	2.233	0.40246
6	8.997	n-Dodecane	☐	0	0	2.4829	0.80205
7	9.323	2,4-dimetani	☐	0	0	2.2783	0.34872
8	10.013	n-Hexbenzene	☐	0	0	1	0

Click **Cancel** to return to the Calibration Setup.

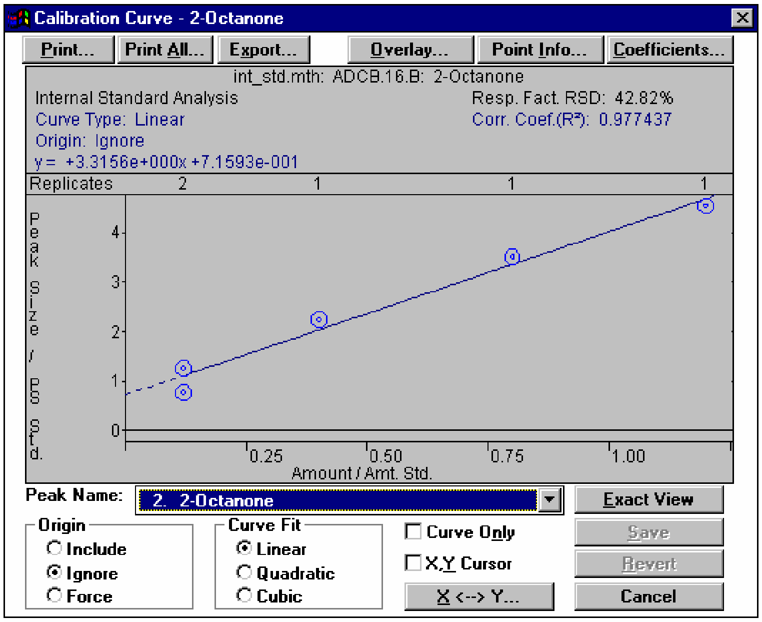
Click on the *View Curves...* button to see the calibration curve for the first peak (the internal standard, n-Nonane).

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

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

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.



int_std.mth: ADCB.16.B: 2-Octanone

Internal Standard Analysis

Resp. Fact. RSD: 42.82%

Curve Type: Linear

Corr. Coef.(R²): 0.977437

Origin: Ignore

y = +3.3156e+000x +7.1593e-001

Peak Name: 2. 2-Octanone

Origin: ○ Include ● Ignore ○ Force

Curve Fit: ● Linear ○ Quadratic ○ Cubic

☐ Curve Only

☐ X,Y Cursor

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.

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

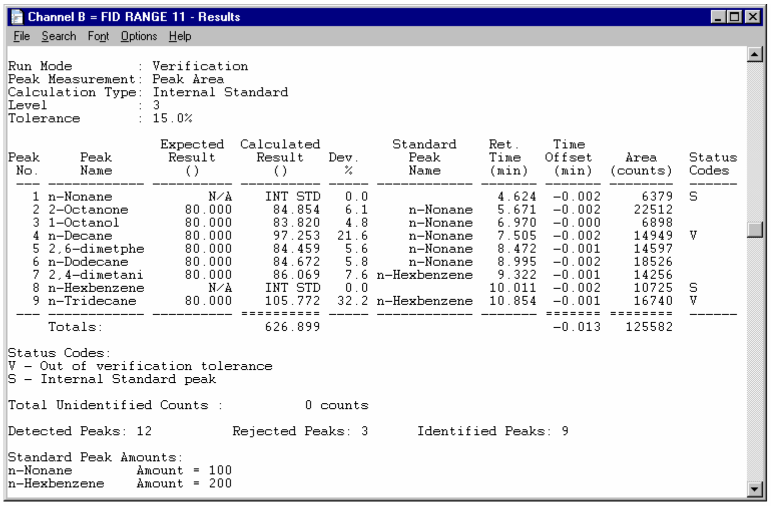
Use the drop-down menu or the keyboard Up/Down arrows to select each peak.

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.

Click on the white chromatogram trace with the right mouse button. Select ***View Results only***.

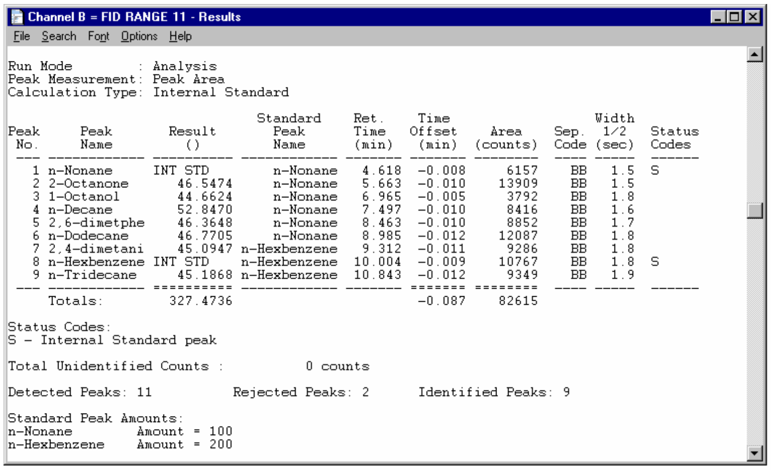
The Verification Report for VERIF_3.RUN appears. Note that Peaks 4 and 9 are marked as “Out of Verification Tolerance”.



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

                   Expected  Calculated         Standard    Ret.    Time
Peak     Peak       Result     Result   Dev.      Peak      Time   Offset    Area    Status
 No.     Name         ()         ()      %        Name      (min)  (min)   (counts)  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
```

Close the Results window.

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



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

                               Standard    Ret.     Time                  Width
Peak     Peak       Result       Peak      Time    Offset    Area    Sep.  1/2   Status
 No.     Name         ()         Name     (min)     (min) (counts)   Code (sec)  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.

Close the Results window.

Using the Calibration Curve Options (Curve Manager)

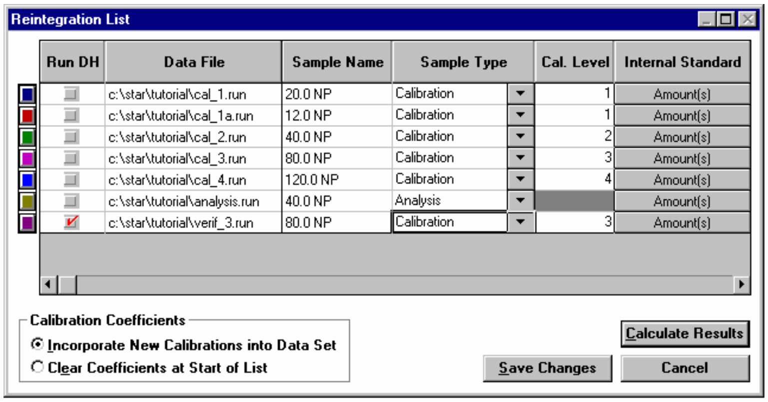
The window which displays the curves and curve options is called the Curve Manager. This step lets you examine the features of some of these options. The following steps will also allow processing of the set of data generated using the Calibrating With External Standards tutorial. Screens and results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

Select ***Results >Reintegration List...***

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

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

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



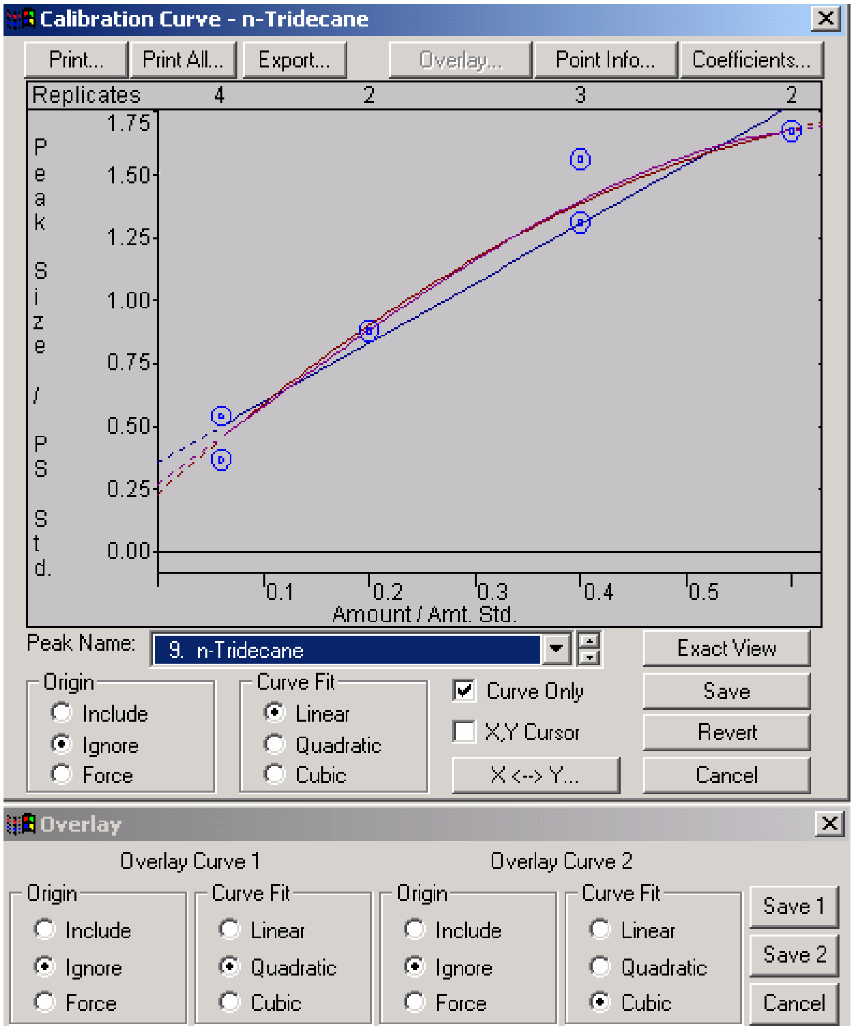
	Run DH	Data File	Sample Name	Sample Type	Cal. Level	Internal Standard
🟦	☐	c:\star\tutorial\cal_1.run	20.0 NP	Calibration	1	Amount(s)
🟥	☐	c:\star\tutorial\cal_1a.run	12.0 NP	Calibration	1	Amount(s)
🟩	☐	c:\star\tutorial\cal_2.run	40.0 NP	Calibration	2	Amount(s)
🟪	☐	c:\star\tutorial\cal_3.run	80.0 NP	Calibration	3	Amount(s)
🟦	☐	c:\star\tutorial\cal_4.run	120.0 NP	Calibration	4	Amount(s)
🟫	☐	c:\star\tutorial\analysis.run	40.0 NP	Analysis		Amount(s)
🟪	☑	c:\star\tutorial\verif_3.run	80.0 NP	Calibration	3	Amount(s)

Calibration Coefficients
● Incorporate New Calibrations into Data Set
○ Clear Coefficients at Start of List

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.

Select the menu command ***Results >View Calibration Curves*...** to view the added data point. Display the curve for peak 9, n-Tridecane.

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.



Peak Name: 9. n-Tridecane

Origin: ○ Include ● Ignore ○ Force

Curve Fit: ● Linear ○ Quadratic ○ Cubic

☑ Curve Only

☐ X,Y Cursor

Overlay Curve 1 — Origin: ○ Include ● Ignore ○ Force; Curve Fit: ○ Linear ● Quadratic ○ Cubic

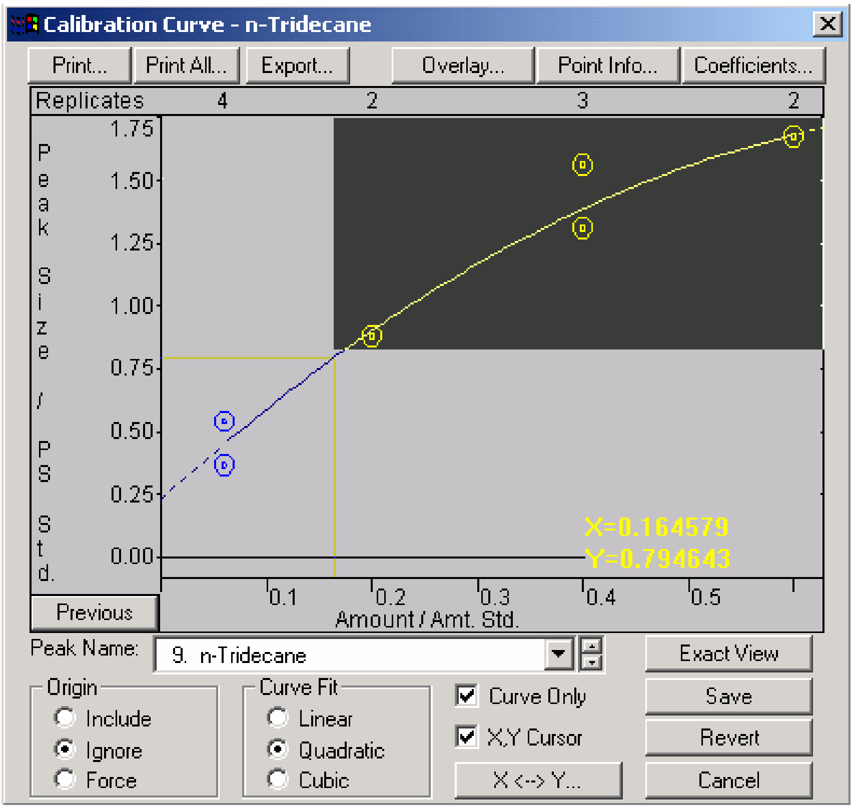
Overlay Curve 2 — Origin: ○ Include ● Ignore ○ Force; Curve Fit: ○ Linear ○ Quadratic ● Cubic

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

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.

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 next figure.



X=0.164579
Y=0.794643

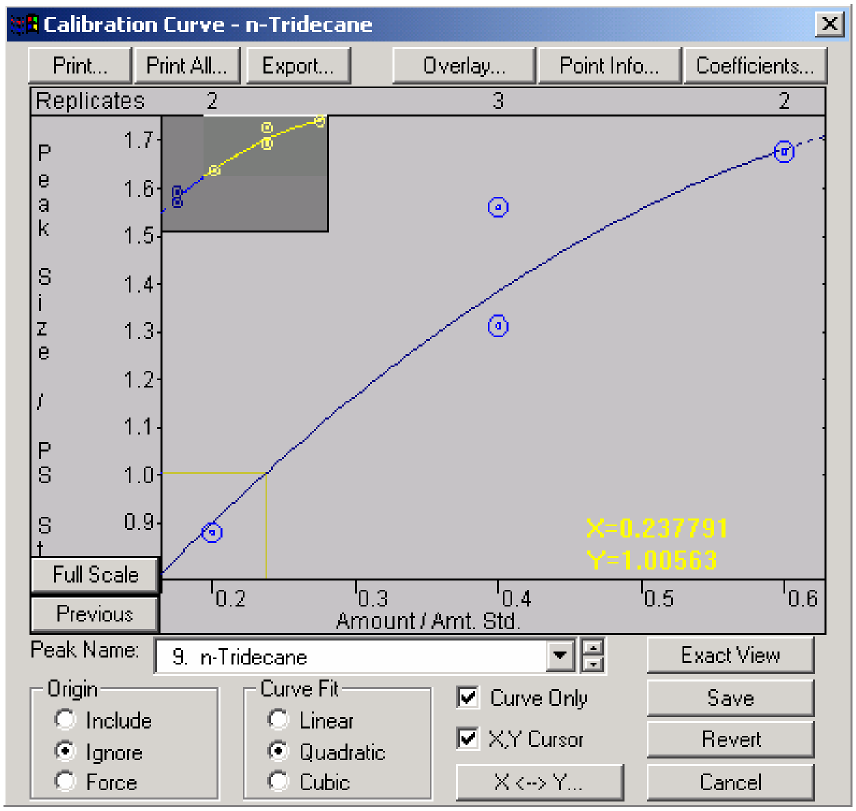
Peak Name: 9. n-Tridecane

Origin
○ Include
● Ignore
○ Force

Curve Fit
○ Linear
● Quadratic
○ Cubic

☑ Curve Only
☑ X,Y Cursor

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**.**



X=0.237791
Y=1.00563

Peak Name: 9. n-Tridecane

Origin
○ Include
● Ignore
○ Force

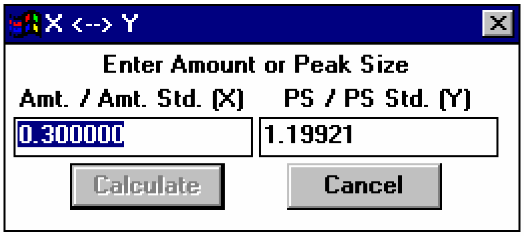
Curve Fit
○ Linear
● Quadratic
○ Cubic

☑ Curve Only
☑ X,Y Cursor

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.

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

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



Enter Amount or Peak Size

Amt. / Amt. Std. (X): 0.300000

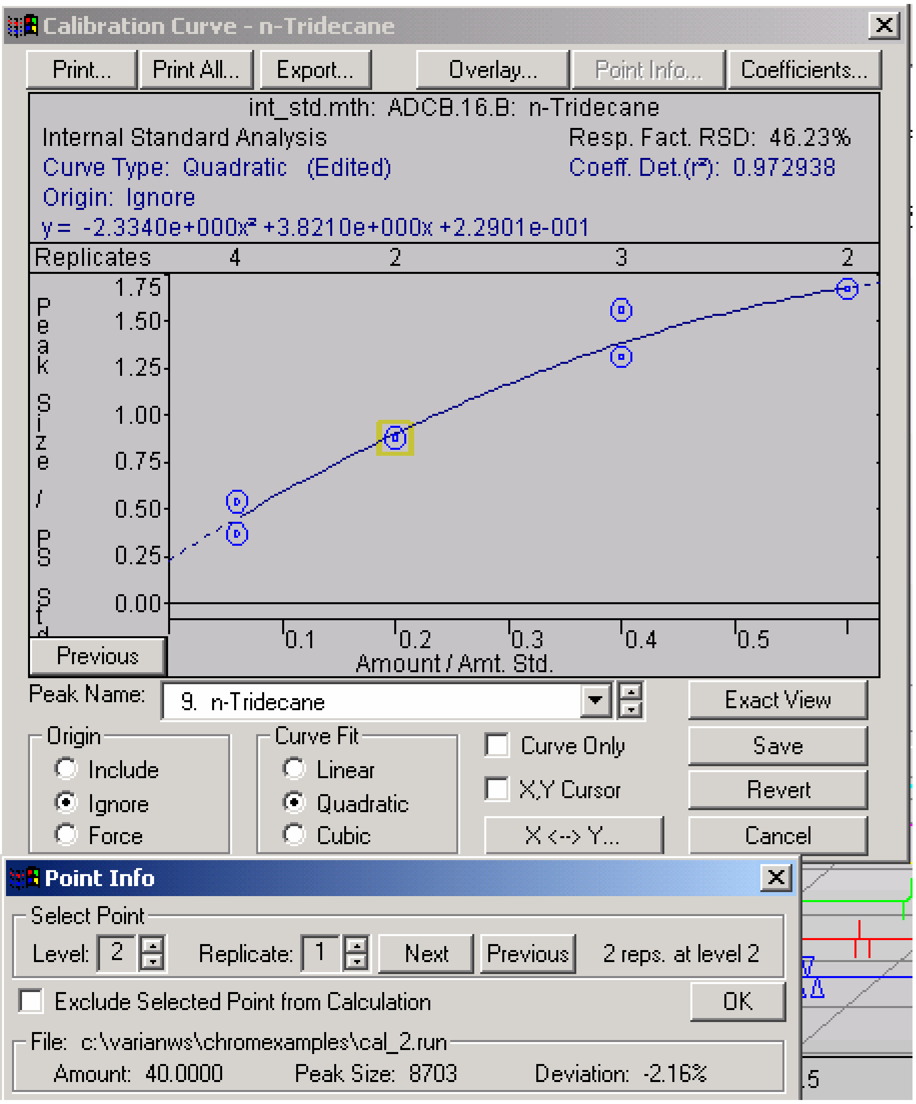
PS / PS Std. (Y): 1.19921

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.

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

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

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



int_std.mth: ADCB.16.B: n-Tridecane

Internal Standard Analysis

Resp. Fact. RSD: 46.23%

Curve Type: Quadratic (Edited)

Coeff. Det.(r²): 0.972938

Origin: Ignore

$y = -2.3340e+000x^2 + 3.8210e+000x + 2.2901e-001$

Peak Name: 9. n-Tridecane

Origin: ○ Include ● Ignore ○ Force

Curve Fit: ○ Linear ● Quadratic ○ Cubic

☐ Curve Only

☐ X,Y Cursor

Select Point

Level: 2 Replicate: 1 2 reps. at level 2

☐ Exclude Selected Point from Calculation

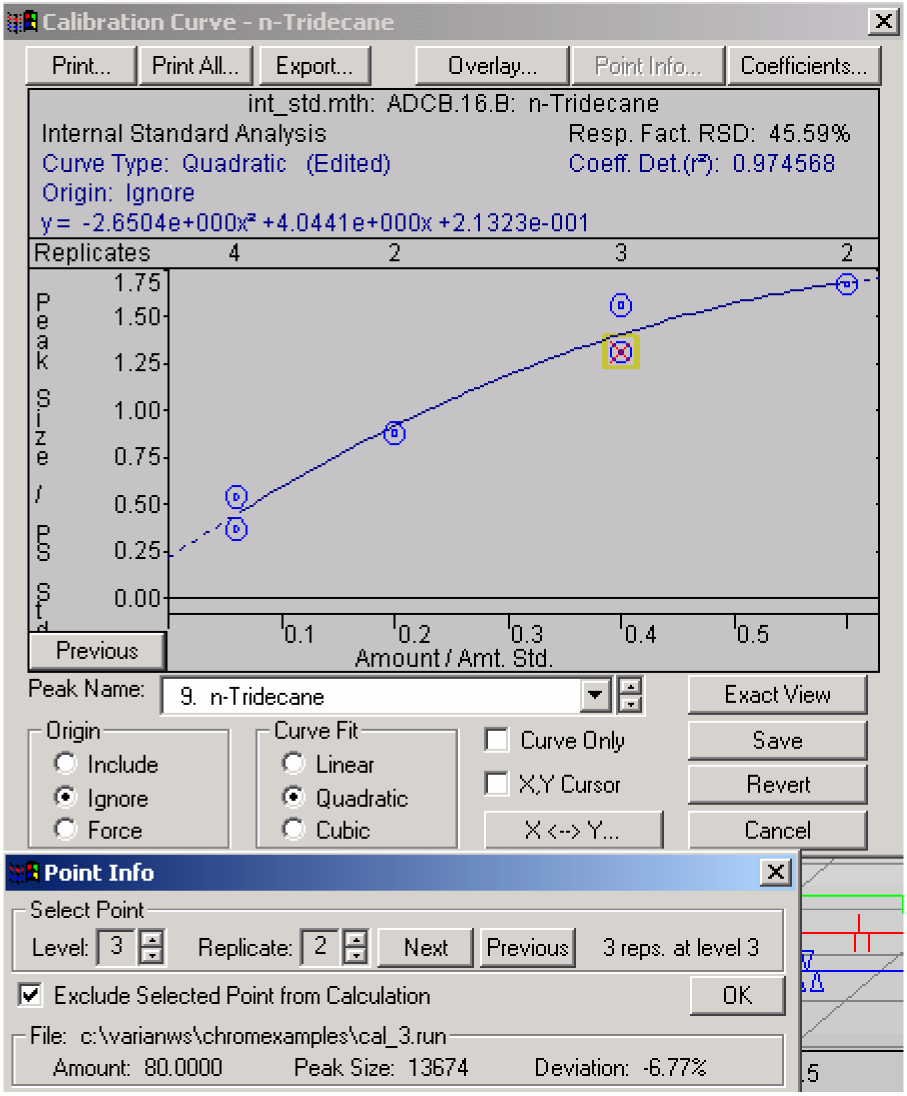
File: c:\varianws\chromexamples\cal_2.run

Amount: 40.0000 Peak Size: 8703 Deviation: -2.16%

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.

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.



int_std.mth: ADCB.16.B: n-Tridecane
Internal Standard Analysis
Resp. Fact. RSD: 45.59%
Curve Type: Quadratic (Edited)
Coeff. Det.(r^2): 0.974568
Origin: Ignore
$y = -2.6504e+000x^2 + 4.0441e+000x + 2.1323e-001$

Peak Name: 9. n-Tridecane

Origin
○ Include
● Ignore
○ Force

Curve Fit
○ Linear
● Quadratic
○ Cubic

☐ Curve Only
☐ X,Y Cursor

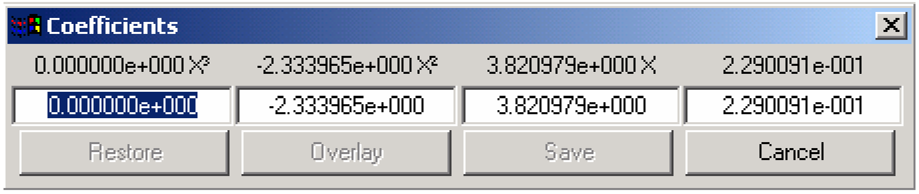
Select Point
Level: 3 Replicate: 2 3 reps. at level 3

☑ Exclude Selected Point from Calculation

File: c:\varianws\chromexamples\cal_3.run
Amount: 80.0000 Peak Size: 13674 Deviation: -6.77%

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

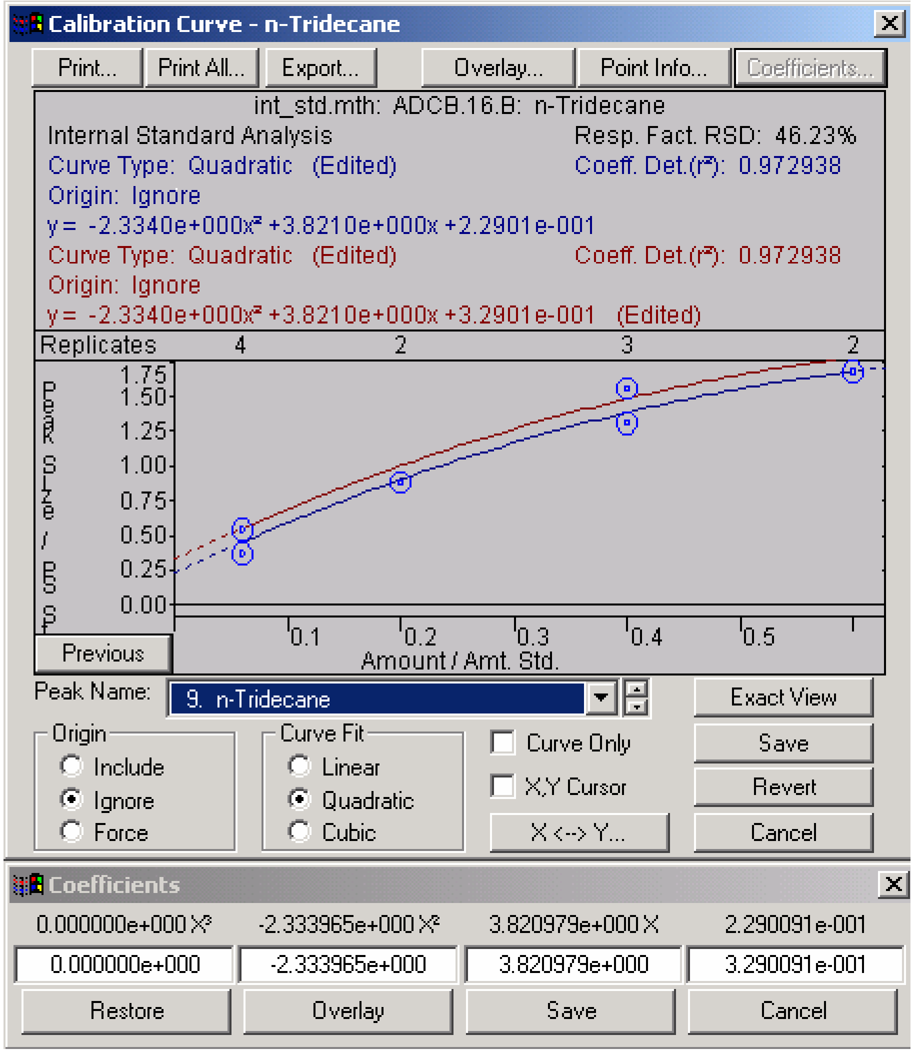
Click on the ***Coefficients*** button. In the **Coefficients** window (next figure), change one of the coefficients by a small amount.



$0.000000e+000\ X^3$	$-2.333965e+000\ X^2$	$3.820979e+000\ X$	$2.290091e-001$
0.000000e+000	-2.333965e+000	3.820979e+000	2.290091e-001

Click on the ***Overlay*** button in the **Coefficients** window 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.



int_std.mth: ADCB.16.B: n-Tridecane

Internal Standard Analysis
Resp. Fact. RSD: 46.23%

Curve Type: Quadratic (Edited)
Coeff. Det.(r^2): 0.972938
Origin: Ignore
$y = -2.3340e+000x^2 +3.8210e+000x +2.2901e-001$

Curve Type: Quadratic (Edited)
Coeff. Det.(r^2): 0.972938
Origin: Ignore
$y = -2.3340e+000x^2 +3.8210e+000x +3.2901e-001$ (Edited)

Peak Name: 9. n-Tridecane

Origin
○ Include
● Ignore
○ Force

Curve Fit
○ Linear
● Quadratic
○ Cubic

☐ Curve Only
☐ X,Y Cursor

0.000000e+000 X^3	-2.333965e+000 X^2	3.820979e+000 X	2.290091e-001
0.000000e+000	-2.333965e+000	3.820979e+000	3.290091e-001

Close the **Calibration Curve** window and exit the **Interactive Graphics** application.

2000 GC/MS Application Notes and Advantage Notes

The following tables list Application Notes and Advantage Notes for the 2000 GC/MS. Copies of these notes are available through your Varian Sales Representative. If you have Internet access, you may use your web browser to examine these notes at:

http://www.varianinc.com

Application Note Index

Note	Title/Description
1	Meeting Mass Spectral Tuning Criteria for EPA Environmental Methodology Key Words: BFB, DFTPP, Tuning, Criteria;
2	Compound Verification and Spectral Integrity Over a Wide Concentration Range with the Varian 2000 GC/MS Key Words: Aldrin, Quadrupole, Linearity, Trace Analysis, Ion Trap
3	The Determination of Semivolatile Organic Compounds in Drinking Water by EPA Method 525 with the Varian 2000 GC/MS Key Words: Drinking Water, Method 525, Empore™, Semivolatiles, Pesticides
4	Tuning the Varian 2000 GC/MS to the EPA Method 625 DFTPP Criteria Key Words: EPA Method 625, DFTPP, Tuning, Semivolatiles
5	2000 GC/MS System Minimizes Spectral Skew Key Words: Spectral Skewing, DFTPP
6	New Generation Ion Trap GC/MS Technology Axial Modulation Key Words: Axial Modulation, Mass Resolution, Sensitivity, Linearity
7	Polychlorinated Biphenyl Analysis and Complex Matrices Key Words: Full Scale Information, Effluent Splitting, High Level Contamination, Coal Tar
8	Determination of Volatile Organic Compounds in Water with the 2000 GC/MS; Key Words: VOCs, Waste Water, Drinking Water, Purge and Trap, Direct Interface
9	Use of the 2000 GC/MS Data System to Separate and Identify Multiple Components in Reconstructed Total Ion Current Chromatographic Peaks Key Words: Coelution, Peak Find Option, Maximized Mass, Biller-Bieman Algorithm, Purge & Trap, Purgeable Organics
10	The Determination of Polyaromatic Hydrocarbons

Note	Title/Description
	Key Words: PAH (Polyaromatic Hydrocarbon), Complex Matrix, Ion Trap, PNA (Polynuclear Aromatic), SPI Injector
11	Narrow Mass Range Scanning Versus Selected Ion Monitoring Key Words: Selected Ion Monitoring, Narrow Mass Range, Enhanced Sensitivity
12	The Determination of Triazine Herbicides at Ultra Trace Levels by Chemical Ionization GC/MS Key Words: Chemical Ionization, Herbicides, Automatic Reaction Control, Isobutane, Molecular Weight Information
13	The Determination of Base/Neutrals in Extracts from Environmental Matrices Key Words: Base/Neutrals, Full Scan, Coelutions, Inert Chromatography
14	The Determination of Phenols in Extracts from Environmental Matrices Key Words: Phenols, Full Scan, Fast Inert Chromatography, Non-target Compounds
15	The Determination of Acid/Base/Neutrals in an Industrial Effluent Key Words: Base Neutrals, Complex Matrix, Ion Clusters
16	Determination of EPA Methods 524.2, 624 and 8260 Analytes with an Open Split Interface to the 2000 GC/MS Key Words: VOC, Purge and Trap, Open Split Interface, EPA Methods 525.2, 624 and 8260
17	Determination of Semivolatile Analytes by US EPA Method 8270 with the 2000 GC/MS; Key Words: Semivolatiles, EPA Method 8270, Split Injection, Waste, Soils, CLP Forms
18	The Determination of Volatile Organic Compounds (VOCs) in Air by the TO-14 Method Using the 2000 II GC/MS; Key Words: VOCs, Air, Method TO-14, Cryotrapping, Full Scan Spectra
19	Direct Split Interface for Analysis of Volatile Organic Compounds Key Words: Direct Split Interface, Non-Cryogenic Method, Purge and Trap, VOC
20	Ozone Precursor Measurements in Ambient Air with the 2000 GC/MS; Key Words: Air, VOCs, Hydrocarbons, Cryotrapping, Full Scan Spectra
21	Ultra Trace Analysis Using Selected Ion Storage GC/MS Key Words: SIS, 2000, GC/MS, Pesticide, SPI, Wave~Board
22	Pesticide Residue Analysis of Bell Pepper Using Selection Ion Storage GC/MS Key Words: Selected Ion Storage, SIS, Pesticide, SPI, 2000
23	Determination of Benzodiazepines in Human Blood Using Wave~Board Technologies of the 2000 3 GC/MS Key Words: Wave~Board, Blood, Diazepam, Nordiazepam, SECI, Drugs
24	Identification of a Fungicide Pollutant at Ultra Trace Levels by Ion Trap GC/MS Key Words: Procymidone, Fungicide, Pesticide, Narrow Mass Range (NMR), Wine, Pesticide Library, SPI
25	The Use of RF Storage Level for Background Elimination with the 2000 GC/MS Key Words: RF Storage Voltage, Pate, Matrix Elimination, Pesticide, 2000
26	Screening for Pesticides in Food with the 2000 GC/MS Key Words: Pesticide, 2000, RF Storage

Note	Title/Description
27	GC/MS/MS with a Benchtop Mass Spectrometer Key Words: 2000 4D, MS/MS, Triple Quad, Timing Diagram
28	GC/MS/MS Analysis of Residual Pesticides in Vegetable Extracts Key Words: 2000 4D, MS/MS, Pesticide, Wave~Board
29	Improved Detection Limits for PCBs in Transformer Oil by Increasing the RF Storage Voltage Key Words: PCBs, Transformer Oil, RF Storage Voltage, Ion Stability, Ionization Time, Improved Detection Limits
30	GC/MS/MS Analysis of Thiabendazole in Grapefruit Extracts Key Words: 2000 4D, MS/MS, Pesticide, Wave~Board
31	GC/MS Analysis of Organotin Compounds in the Environment Key Words: 2000, Organotin, Environmental, Derivative
32	Determination of Polychlorinated Dibenz-p-dioxins and Dibenzofurans in Environmental Samples with the 2000 GC/MS
33	GC/MS for the Detection of Illegal Use of Steroids in the Cattle Industry Key Words: 2000, Steroids, 4-chlorotestosterone, Metabolites
34	Simultaneous Automated Spectral Screening and Quantitation of Picogram Levels of Drugs Key Words: Drug Analysis, THC, Benzodiazepines, Basic Drugs
35	Rapid Simultaneous Quantitation of Triazines, Organophosphates and PAHs Key Words: Triazines, Organophosphates, PAHs, AGC
36	GC/MS/MS Analysis for the Identification of Impurities in Pharmaceutical Products Key Words: 2000 4D, GC/MS/MS, Pharmaceutical
37	The Determination of Trace Level FAMES Using CI Mode GC/MS Key Words: FAMES, Fatty Acids, CI, Isobutane, SPI
38	Analysis of Estrogens in Sheep Liver Extracts Using Selected Ion Storage(SIS) Key Words: Selected Ion Storage, Narrow Mass Range, High Background, Estrogens
39	Quantitation of a Novel Cholinesterase Inhibitor in Human Plasma by GC/MS Key Words: GC/MS, 2000, Cognition Activator, Human Plasma, Cholinesterase Inhibitor
40	GC/MS/MS Analysis for Pesticide Residues in Agricultural Products Key Words: 2000 4D, GC/MS/MS, Pesticide Residues, Food Analysis
41	Reformulated Fuel Analysis by GC/MS: Total Aromatic Hydrocarbons Key Words: 2000 GC/MS, Aromatic Hydrocarbons, Reformulated Fuels, Full Scan Spectra
42	The Determination of Sulfur Gases in Point Source Emissions of a Pulp Mill Key Words: 2000 GC/MS, Sulfur Gases, Emission, Full Scan Spectra
43	GC/MS/MS Analysis of Dinitroaniline Based Herbicides in Fish Key Words: 2000, GC/MS/MS, Herbicides, Fish
44	GC/MS/MS Analysis of Alkylated Polycyclic Aromatic Hydrocarbons Key Words: 2000, GC/MS/MS, PAHs, CI/MS/MS
45	GC/MS/MS Analysis of PAHs in Water Using Large Volume Injections

Note	Title/Description
	Key Words: GC/MS/MS, PAHs, 1078 Injector, Large Volume Injection
46	Determination of Estradiol in Blood by GC/MS/MS Key Words: GC/MS/MS, Estrogen, Estradiol, Large Volume Injections, MS/MS ToolKit, 1078 Injector
47	GC/MS/MS Analysis of Planar PCBs in Biota and Sediment Key Words: 2000, GC/MS/MS, Planar PCBs
48	GC/MS Analysis for Unsaturated Fat Content in Animal Feed Key Words: 2000, Unsaturated Fat, GC/MS, Food, FAMES
49	Analysis of Hydrolyzed Vegetable Protein for Chloropropandiols using Selected Ion Storage Key Words: Selected Ion Storage, Food, Chloropropandiols, GC/MS
50	A Quantitative Comparison of TSD and GC/MS/MS for Atrazine in Surface Water Key Words: Atrazine, TSD, GC/MS/MS, 2000, Environmental
51	GC/MS/MS Analysis of Triazine Herbicide Residues using Multiple Reaction Monitoring Key Words: 2000, GC/MS/MS, ToolKit, MRM, Triazine, Herbicides
52	GC/MS/MS Analysis of β-Damascenone in Rose Oil Key Words: 2000, GC/MS/MS, Flavor and Fragrance, Essential Oils
53	Multi-residue Analysis of Organophosphorus Insecticides by Ion Trap GC/MS/MS Key Words: 2000, MS/MS, Pesticides, Organophosphates
54	Enhanced Selectivity in the Determination of Triazines by Benchtop GC/MS/MS Key Words: 2000, GC/MS/MS, Triazines, Environmental
55	GC/MS/MS Analysis of Cytostatic Drugs in Urine Key Words: Toxicology, Clinical, Drugs
56	Rapid Analysis of Soils for Hazardous Waste by Direct Sample Introduction; Key Words: 2000, Direct Sampling, ChromatoProbe, Herbicides, Soils
57	Chemical Ionization GC/MS for the analysis of Tributyltin Oxide Key Words: TBTO, CI, Environmental 2000
58	App Note 58: Identification of Essential Oil Components EI/MS and Mixed Reagent CI/MS Key Words: Essential Oils, Flavors & Fragrances, Acetonitrile, CI
59	App Note 59: GC/MS Analysis for Morphine and Other Opiates in Urine Key Words: Opiates, Urine, Drugs, 2000
60	GC/MS/MS Analysis for Anabolic Steroids in Urine for Athletic Testing Key Words: Drugs, MS/MS
61	Chlorophenols in Drinking Water using GC/MS/MS Key Words: MS/MS, Water, Phenols, EPA, Environmental
62	App Note 62: Detection of Gasoline in Fire Debris by GC/MS/MS Key Words: MS/MS, Gasoline, Arson, Forensic
63	App Note 63: Tandem-in-Time Mass Spectrometry as a Quantitative Bioanalytical Tool Key Words: MS/MS, 2000

Advantage Note Index

Note	Title / Key Words
1	Advantages of New CI Key Words: New CI Wave~Board, SECI
2	Advantage of CI, A Real Reason to Buy the SECI Option for the 2000 GC/MS; Key Words: SECI, 2000
3	GC/MS/MS Analysis for Target Analytes in a Complex Matrix, Increased Selectivity and Simplified Spectral Interpretation through GC/MS; Key Words: MS/MS, Target Analysis, Alachlor, Pesticide, Complex Matrix, Selectivity
4	GC/MS/MS Analysis for Unknown Compounds, Additional Information About Key MS Fragments for Reconstruction of Structure and Studies of Fragmentation Pathways; Key Words: MS/MS, Qualitative Identification, Malathion, Pesticide, Fragmentation Pathway
5	GC/MS/MS Analysis as a Separations Technique; Key Words: GC/MS/MS, Coelution, Pesticide, MS/MS Separation
6	Multiple CI Gas Capability Enhances Flexibility of Ion Trap GC/MS; Key Words: CI, Reagent Gas, SECI, Programmed Acquisition, External Events
7	GC/MS/MS Using Deuterated Internal Standard; Key Words: GC/MS/MS Deuterated Internal Standard, Drugs
8	GC/MS/MS for Isomer Identification; Key Words: GC/MS/MS, Isomer Identification, Pesticide
9	Hydrocarbon Identification Using the 2000 3 GC/MS; Key Words: Hydrocarbon Identification, 2000, Industrial Application
10	Large Volume Injections for GC and GC/MS Analysis Key Words: GC/MS/MS, 1078 Injector, Large Volume Injection
11	Maximize Information by Splitting Between the Ion Trap Mass Spec and a GC Detector Key Words: Splitter, 2000, GC, Detectors
12	Adv. Note 12: Enhanced Chromatographic Performance with a New Inert Coating for the 2000 Ion Trap Key Words: 2000, SilChrom, Drugs
13	Adv. Note 13: Confirmatory Analysis of Melatonin by EI and CI; Key Words: 2000, CI, Drugs
14	Adv. Note 14: ChromatoProbe - A Simplified Approach to Sample Introduction Key Words: 2000, Drugs, Direct Sampling, ChromatoProbe
15	Enhanced Molecular Weight Confirmation with Deuterated Acetonitrile CI Key Words: LCI, Acetonitrile, FAMEs
16	Confirmation of Saturated Hydrocarbon Molecular Weight with Acetonitrile and d3-Acetonitrile CI Key Words: LCI, Acetonitrile, Hydrocarbons