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**MS Workstation** 

# **Tutorial Manual**



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## **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.
- 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**.

📱 System Control - Config	uration				
File Edit Inject Automation	Recalculate	Instrument	Windows	Help	
🗎 🖻 🚅 🚫 No File		1: Varian	GC/MS #1	Alt+1	
		🗸 Configura	ation	Alt+c	#1
		Remove f Restore [ Instrume	Module Nam Disabled Wa nt Faults	ies irnings	
		Setup Eth Setup AD Setup CO	nernet Com C Board I/C MM Ports	munications ) Ports	
		I			
System Control - Configuration	• • • • •	1			
File Edit Inject Automation Recalculate	Instrument Win	dows Help		le l'uls le l	
	Instrument 1: Va	rian GC(MS #1	RCL SH	PISEQ	
	Opera	itor:			
	Not Re	eady			
AutoStart Module					
3900 2000					
44:galileo_mp#1 ↔0					
Instrument 1 Parameters					
Modules					
Configuration : Jun 08 09:13:26 WinSock 2.0					

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**).

🗱 MS Data Review - [Plot Chron	natograms and Spect	:ra]			
📕 File Chromatogram Spectrum	Spectrum List Search	Integrate	Preferences	View Window	v Help
Image: Constraint of the second s	Descriptors for 50NG		Plots View ( Plots View ) Plots View, Results Vie	Chromatogram Spectra Pane  w	Pane
🔁 10_NG.SMS 🔁 120_NG.SMS 🔁 160_NG.SMS 🖻 20 NG SMS	<mark>∠.</mark> Chan: 1 (1)	kCoun	Report General Application	Start Up	

MS Data Review Application Start Up
Application Display View to Display on Start Up
Plots     Show Selection Pane     Process
C Results
C Last Recalc File
<ul> <li>Last Data File</li> <li>None</li> </ul>
Reset Help OK Cancel
C Results File to Display on Start Up C Last Recalc File C Last Data File None Reset Help OK Cancel

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 **Judd** 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.

Select Plot(s)				<u>?×</u>
Look jn: 🔂 MSTutorials	<b>→</b> ← 🗈	➡	Preview Data F File:	ile Information
Backup         20         160_NG.M5           Corrupt         20         160_NG.SM5           10_NG.M5         20_UG.M5         20_UG.M5           10_NG.SM5         20_UG.M5         20_NG.SM5           120_NG.M5         200_NG.SM5         200_NG.SM5           120_NG.SM5         200_NG.SM5         200_NG.SM5           120_NG.SM5         200_NG.SM5         200_NG.SM5           File name:         40_NG.SM5         40_NG.SM5           Files of type:         Data Files (*.SMS, *.XMS, *.	89 40_NG.MS 89 40_NG.SMS 80 50NG_CCC.MS 80 50NG_CCC.SMS 80 NG.MS 80 NG.SMS 80 NG.SMS	PURGEB.M     PURGEB.SI     PURGEB.SI	Data Type: Sample: Inject Date: Run Time: Scan Range: Operator: Sample Notes: Max RIC: ID/Miss/Fail: TIC/Unkn/Dup:	2000 CENTROID ALLMIX/40NG 9/26/1991 12:20 AM 7.221 - 8.909 min 535 - 660 RDB 8270 622158 at 603 9, 0, 0 0, 0, 0
Selected Plot(s)	Move <u>U</u> p	Move <u>D</u> own	<u>E</u> dit Ions	Edit Scan <u>s</u>
File Name		Data		Channels 🔺
c:\varianws\mstutorials\40_ng.sms		RIC (10.0 - 650.0)		
Show Preview of Data File Plot	<u>H</u> elp	<u>A</u> dd\Replace	Open File(s)	Cancel

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 *lons* 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 **I** toolbar button, the following options are available in the Data column besides the RIC value (default):

Select Plot(s)				? ×
Look in: 🔄 MSTutorials	• 🗧 主	≓ ⊞-	- Preview Data File	File Information
Backup         160_NG.MS           Corrupt         160_NG.SMS           10_NG.MS         20_NG.MS           10_NG.SMS         20_NG.MS           110_NG.SMS         20_NG.MS           110_NG.SMS         20_NG.SMS           110_NG.SMS         20_NG.SMS           110_NG.SMS         200_NG.SMS           110_NG.SMS         200_NG.SMS	) 40_NG.MS ) 40_NG.SMS ) 50NG_CCC.MS ) 50NG_CCC.SMS ) 50NG_CCC.SMS ) 80_NG.MS ) 80_NG.SMS 	♥ PURGEB.M ♥ PURGEB.SI ; <u>Becent Files</u> ✓ Auto Select Channels	Data Type Sample Inject Date Run Time Scan Range Operator Sample Notes Max RIC ID/Miss/Fail TIC/Unkn/Dup	
Selected Plot(s) <u>Clear Row(s)</u> Eill Down	Move <u>U</u> p	Move <u>D</u> own	<u>E</u> dit lons	Edit Scan <u>s</u>
File Name		Data		Channels 🔺
c:\varianws\mstutorials\10_ng.sms		 RIC (10.0 - 650.0)  on(s) Intensity of Base Peak Base Peak Ion Ion Time(uSec) Scan Functions		* * * * * *
Show Preview of Data File Plot	<u>H</u> elp	Add\Replace	<u>O</u> pen File(s	Cancel

- 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, lon(s), etc.
- 3. Select **lon(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 lons** 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.



 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.



 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.



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.

- 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  $\bowtie$  button, or by right-clicking anywhere in the chromatogram window, select *Chromatogram Plot Preferences >Markers* and check/uncheck **Show Background Correction Markers**.

Library Search Spectrum	
New Spectrum Window	
Export Spectrum	
Select As Reference File (Plot 1	)
Save As User Descriptor (Plot 1	)
Calculate Noise (Plot 1)	
Integrate	1
New Label for Plot 1	
Delete Labels	
Hide Plot 1	
Print	
Export	đ
Chromatogram Plot Preference:	s
Chromatogram Plot Preference:	s

Local Chromatogram Plot Preferences				
Plot Titles DH Annotations TL Annotations				
Markers Features Chromatogram Plot				
Data Point Markers				
2 Maximum Markers Per Centimeter				
Integration Area Markers				
Edit color and Font				
Background Correction Markers				
System Event Markers				
Annotation Markers				
Name on Own Line				
Help Reset to User Defaults				
Save All as User Defaults Reset All to User Defaults				
Use Next Time OK Cancel				

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

File	Chromatogram	Spectrum	Spectrum List	Search	Int	
₹ €	Select Active Save Active Filter Chroma Target List S	File as Refe Chromatogr atogram earch Active	erence File am as User Desc Chromatogram	riptor		
	Set Single Cli	ck Action			•	
	Set Click and Drag Action					
	Set Point/Spectrum Selection					
	Set Spectra to Average				•	
	Set Chromatogram Display					
	Edit Backgrou	und Correcti	on			
	Edit Time Rar	ige				
	Show Backgr	ound Correc	tion Markers			
	Show All Plots					
	Restore Mov	ed Chromat	ograms			

You may also use the toolbar button

Background Correction for Plot 1					
Averaging ① 1 ① 3	Selection Add or move	Delete All			
05 07	O Delete	Done			
Background Correction Spectra Count = 2					
Auto Background Correction					

While the Background Correction for Plot 1 dialog is open, if you move the

mouse over the chromatogram, the  ${}^{\parallel} \Phi$  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.



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**.

NIST Library Manag	er	×
NIST Library List		Order List
MAINLIB		To Top of List
		Up One
		Down One
		To Bottom of List
Edit List		
Initialize	Add Library	Inactivate Library
Create User Library	Remove Library	
Edit Libraries	Help	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**.

Spectrum,
Spectrum List (ei 50.msp)
ive Spectrum
ive Chromatogram

The Library Search a Spectrum window displays with a list of matches for the

searched spectrum. Press the button and select **Spectrum Search**.

🕌 MS Data Review - Library Searc	MS Data Review - Library Search a Spectrum					
File Chromatogram Spectrum Spect	trum List	Searc	h Inte	grate	Preferences V	'iew Window
🖻 🎒 🗹   🏦 🔍 🥻	🗁 🍜 😭   🏦 🔍 🎆 🔟   🌿 🎌 🔃 😯					
Library Search a Spectrum						<u>_ D ×</u>
🛗 🕂 🖗 🖄 🗷 🖉	¢	Mate	ch 1 of	i 71 fo	r Scan: 542	(7.316 min.)
C REPEAT Spectrum Search	R	F	Pr	MW	CAS No.	Formula 🔺
1	920	920	21	122	105-67-9	C8H10O
2 Any Peaks Search	914	914	17	122	526-75-0	C8H10O
3 Name Search MainLib	913	913	16	122	95-87-4	C8H100 -
4 Sequential Library Search	913	913	16	122	95-65-8	C8H10O
5 CAS Number Search	888	888	5	122	576-26-1	C8H10O
6 Formula Search	884	884	4	122	108-68-9	C8H10O
7 Molecular Weight Search	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:

Search	Integrate Preferences View	Window Help
Libra Libra	ry Search a Spectrum ry Search a Spectrum List	• N
Libra Libra	ry Search by ry Manager [MAINLIB, REPLIB, 1	Any Peaks ゆ TUTORIAL] Mainlib Name
Targi Targi Selec	et List Search a Spectrum et List Search a Chromatogram :t Spectrum Lists to Search[none	CAS Number Formula Molecular Weight
Sear	ch Active Data File using AMDIS	C10H8

#### 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.

NIST Library Manage	er	×
NIST Library List-		Order List
		To Top of List
HEFEID		Up One
		Down One
		To Bottom of List
Edit List		
Initialize	Add Library	Inactivate Library
Create User Library	Remove Library	
Edit Libraries	Help	Done

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.

Select NIST	Library							×		
Library Name Library Type mainlib Main Library NEW User Library Nh3_ci User Library replib Replicate Library TEST User Library TESTAGAIN User Library TESTUSERLIB User Library Tutorial User Library			Spectri 147200 1 155 27750 3 2 2 2 2 2 219	a Count	Preview NI Library: Spectra: Current Sp Index: Name: MW: Formula: CAS No.:	ST Library In Tutorial 219 ectrum 1 2-Fluorophe 112 C6H5FO 367-12-4	nformation			
Index	Name			MW	Formula	,	CAS No.			
1	2-Fluo	Fluorophenol		2-Fluorophenol		112	367-12-4		C6H5FO	
2	Aniline	)		93	62-53-3		C6H7N			
3	Pheno	l-d5		99	None		C6HD5O			
4	Pheno	I		94	108-95-2		C6H6O			
5	bis(2-0	Chloroethyl)ether		142	111-44-4		C4H8CI20	D		
6	2-Chlo	rophenol		128	95-57-8		C6H5CIO			
7	7 1,4-Dichlorobenzene			146	106-46-7		C6H4Cl2			
8	1,4-Dichlorobenzene-d4			150	None		C6CI2D4			
9	1,3-Dic	chlorobenzene		146	541-73-1		C6H4Cl2			
10	1,2-Dichlorobenzene			146	95-50-1		C6H4Cl2	-		
1.4.4	<b>D</b>			400	400 54 0			لك.		
					Help		Select	Cancel		

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.

1 of 27750 fron	n REPLIB						×		
Select	Index Name		MW	CAS No.	Formula				
Next	1 Methan	amine, N,N-difluoro	-	67	753-58-2	CH3F2N			
	2 Sulfuric	acid, dimethyl este	er	126	77-78-1	C2H6O4S			
Previous	3 Diazene	e, dimethyl-		58	503-28-6	C2H6N2			
Delete	4 Carboni	ic acid, dimethyl es	ter	90	616-38-6	C3H6O3			
	5 Carboni	ic acid, dimethyl es	ter	90	616-38-6	C3H6O3			
	6 Phenol	2,5-dichloro-4-met	hoxy-	192	18113-14-9	C7H6CI2O2			
	7 Heptan	edioic acid, dimethy	yl ester	188	1732-08-7	C9H16O4			
	8 Ethane	dioic acid, dimethyl	ester	118	553-90-2	C4H6O4			
Duplicate	+ + 2	≞ <u>I</u> [ - <u>₩</u> -					_		
Edit					Methan	amine, N,N-difluoro-	- [		
Export	BP 15 (999	=100%) 1 in REPLIB	20		CAS No. 753-58	3-2, CH3F2N, MW 67			
Import	1 100% 15 28 76% 599 765 50% - 26% - 0% - 1b 2b 3b 40 5b 6b 7b - Acquired Range m/z -								
Print	🗸 lon	🔺 Intensity	🔺 % Base 🔺		Information				
Help	12	27	2.70	Spectrum	NIST Library				
	13	44	4.40	File	REPLIB				
Done	14	78	7.81	Index / Scan No	1				
	15	999	100.00	Name	Methanamine,	N,N-difluoro-			
	19	27	2.70	Comment					
	20	14	1.40	Formula	CH3F2N				
	26	22	2.20	Molecular Wei	67				
	27	158	15.82 🗸	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.

 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.

🗱 MS Data Review - [Plot Chromatograms and Spectra]										
🗾 File Chromatogram Spectrum	Spectrum List Search Integrate Preferences	Viev								
🔁 🚑 🛃   🕰 🖬 🕯	Create New Spectrum List									
	Edit Spectrum List									
📄 💼 MP10	Select Active Spectrum List									
	Build Spectrum List from Active Chromatogram									
	Build Spectrum List from Active File using AMDIS									

Create Spectrum List	? 🗙
Save in: 🧀 MSTutorials 💽 🗢 🛍 📑	•
授 80_NG.msp	
File name: test.MSF	<u>à</u> ave
Save as type: MSP Files (*.MSP)	ancel

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

🗱 MS D	ata Review - [Plot Chromatograms and Spec	stra]						
🔄 File	Chromatogram Spectrum Spectrum List Search	Integrate Preferences View Window Help						
	Select Active File as Reference File Save Active Chromatogram as User Descriptor Filter Chromatogram Target List Search Active Chromatogram	E 🔁 📀						
	Set Single Click Action Set Click and Drag Action Set Point/Spectrum Selection	Display Spectrum Library Search Spectrum Target List Search Spectrum						
	Set Spectra to Average Set Chromatogram Display	Export Spectrum to Active Spectrum List     No Action						
	Edit Background Correction Show Background Correction Markers Show All Plots Restore All Plot Positions	500-						

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



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.

🇱 MS Data Review - Spectrum List - c:\varianws\mstutorials\test.msp										
File Chromatogram Spectrum	Spectrum List	Search	Integrate	Preferences	Viev					
Create New Spectrum List Edit Spectrum List Select Active Spectrum List										
	Build Spectrum List from Active Chromatogram Build Spectrum List from Active File using AMDIS									

Edit Integration Parameters	×						
Integration Method							
Peak Width (sec):	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							
Save Method Load Method Help	OK Cancel						

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.

M9	MS Data Review - Plot Chromatograms and Spectra																				
File	Chromatogra	arn S	Spectrum	Spectrum L	ist Sea	ch Integra	ite Prefe	rences Viev	v Wind	ow H	Help										
	😂 🚭 🗹   🔐 😡 🇱 🔤   🎢 🌿 🏭 🔣 🕗																				
5	pectrum Li	st - c	:\varianv	vs\mstute	orials\te	st.msp		_			Plot Chroma	atogra	ms ar	d Spec	tra					- 01:	×
	+ 🗖	×				<u> ×</u> 7 <u></u>	•	Entry 12	of 12		<b>1</b>	÷1	Þ	▶ -		<u>ار</u>	_ ▼ 🙀	•	- 🥂	<b>Ă</b>	
Ind	ex Name				MW	CAS No.	F	ormula	<u> </u>		kCounts	nin	TIC	40_N	G.SMS	3 200	CENT	ROD R	4W 🛛	2 - 1	ſ
1	7.330	min.	Scan: 5	43 Cha	0	None	N	one		ι.	600-	5				1				3	
2	7.627	min.	Scan: 5	35 Cha	0	None	N	one		ι.	1	ιų.				- 1					
3	8.126	min.	Scan: 6	32 Cha	0	None	N	one	- 11	ι.	500-3	· ·			<u> </u>	- 1		nin	_	4	
4	8.491	min.	Scan: 6	29 Cha	0	None	N	one	- 1	ι.	1				5			5	ji j		
5	7.330	min.	Scan: 5	43 Cha	0	None	N	one	- 11	ι.	=			.9	80.	B.		48	2		
6	7.627	min.	Scan: 5	55 Cha	0	None	N	one	- 1	ι.	400-			, <u> </u>	<u></u>	- Å		<sup>co</sup>	-13	1	
17	7.761	min.	Scan: 5	75 Cha	0	None	N	one	- 1	ι.		1		<u>م</u> ع	2	18			<sup>oo</sup>		
8	7.991	min.	Scan: 5	32 Cha	0	None	N	one		1	300			1 21	÷ ۱	Ф			1	4	
9	8.086	min.	Scan: 5	99 Cha	U	None	N	one		17	1000	II.		~							
10	8.139	mın.	Scan: 6	J3 Cha	U	None	N	one	-	ι.	=				.	1			1		
	_	_	8.734	min, Sean: I	547 Chan	: 1 Ion: 409 (	is RIC: 373	316 BC 🖬 🖂	1	ι.	200-	Ц	(			1111			1	4	
	BP 225	(408)	23=100%)	12 in List Fi	le o:\	varianws\mst	utorials\40	_ng.sms	-	ι.		11 -		l fi		1781	_		11		
10	0%-						225	-		ι.	100.3	11		1 6		1141	nin	- 10 -	11		4
							40023			ι.	100	11 -		$\Box$	- 1	U I	51	10	11	٦ĭ	Г
· · ·	-**			440						ι.	-	Ц.,	- 1	M		U 1	12	- /// -	11		
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	12876		13489	1		15278		15156		ι.	t,		1.	·							
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	E L				l. ii							5	Geg 1	Time:	7.22-8	1.91, E	El Auto,	Channe	ls: 1		
	0%				<u> </u>						Scans 53	7		574			611		648		
	50		100		150	200	Acquired	250 Range m/2	-						lo	ons:			-	]- I	

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 **S**.

#### **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:		
l		
Comment:		
7.330 min. Scan: 543 Channel: Merg	ged Ion: 366 us RIC: 519977	
Formula:		Molecular Weight: 0
CAS No.: Ba	ase Peak: 107 Acq Ra	ange: 49 125
<b>▲ ♣ ◙ ≞ I</b> • '	<b>-</b> • <b>- - -</b>	
Spectrum 1A BP 107 (129004=100%) 100% 75% 50% 25% 0%	7.330 min. Scan: 543 Channel: Mergeo 77 53814 91 1 31551 1 31551 70 8b 9b 1d0	1 Ion: 366 us RIC: 519977 107 129004 84751 410 120 Acquired Range m/z
×* Ion Intensity		Keep Top: 50
1 40 1209	Peak Lount: 49	Keep Above: 10000
2. 50 6898	Add/Replace	Scale to: 100000
3. 51 9902	Delete	Beset
4. 52 3005	Delete	116360
5. 53 4780		o Saua Canaal
0. 54 683		p Cancei

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.
- 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.

Make Reports	×
Library Search Spectrum Reports NIST Libraries Report Spectra Window Reports Chromatogram Window Report Select Report to Make Print Active Chromatogram Print All Chromatograms Print Log for Active Chromatogram Print Active Chromatogram and Spectrum Plots Print All Chromatogram and Spectrum Plots Print Plot 1 (80_NG.SMS)	ts   
Help Print	1
Report Preferences View Spooler Exit	
Image: No Header on Plot Reports           Font Size Scaling Factor (1 = off):	

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



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** 

Library Search Spectrum New Spectrum Window Update Spectrum Plot Export Spectrum	•			
Select As Reference File (Plot 1) Save As User Descriptor (Plot 1)	_			
Calculate Noise (Plot 1) Integrate	•			
New Label for Plot 1 Delete Labels	,			
Hide Plot 1				
Print			_	
Export	٠	Plot 1		
Chromatogram Plot Preferences	٦	Chromatogram Pane	•	Clipboard Dicture File

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

Select Directory and Filena	<u>? ×</u>	
File name: META.wmf META.wmf picture.wmf	Folders: c:\varianws C:\ ParianW/S C:\ ParianW/S C: 040704 C: 040904 C: 1200L C: 1200sys	OK Cancel
Save file as type: MetaFile Files (*.wmf)	Drives: 🔲 c: Local Disk	Network

Insert the picture in a Microsoft Word<sup>™</sup> 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.

 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.

Create Repo	rts File				? 🛛
Save in: ն	MSTutorials	•	← 🖻	. 📥	
🗟 80_ng.msr					
File <u>n</u> ame:	200l_ng.MSR				<u>S</u> ave
Save as <u>t</u> ype:	MSR Files (*.MSR )		-		Cancel

2. Select File >Send Reports to Spooler File.



 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.

Make Reports	×
Spectra Window Reports NIST Libraries Reports Chromatogram Window Reports	
Select Report to Make Print Active Chromatogram Print All Chromatograms Print Log for Active Chromatogram Print Active Chromatogram and Spectrum Plots Print All Chromatogram and Spectrum Plots Print Plot 1 (10_NG.SMS)	
Help Print	
Report Preferences View Spooler Exit	

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.

Chromatogram Plot Prefere	nces			
Markers   TL Annotation Noise   Colors   Lab Features   Chromatogram Plot	s   AMDI els   Axes   Plot Titles	6 Annotations Font DH Annotations		
Number of Spectra to Average:           1 Point Spectrum				
Display Features ✓ Show <u>T</u> oolbar ✓ Show <u>Gain Controls</u>				
Interaction Features Click and Drag Action:				
Zoom Chromatogram		•		
Single Click Action: Display Spectrum		•		
Help	<u>R</u> eset to	Defaults		
Save All as Defaults Reset All to Defaults				
	ОК	Cancel		

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

MCount<del>s]</del> TIC NEWINTERF20,XMS 1200 CENTROID RAW ٥X 40-30-1A 20min 4.396 min 533 19.090 min 16.602 min 21.216 min 22.458 min .895 min # 6.942 min 656 min 10-30ł Seq 4. Time: 11.00-14.02. Positive lons. Scan Functions 3187 4396 865 6130 8689 Scans File lons: 45 > F p s S

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.



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

🎇 MS Data Review - [Plot Chroma	atograms and Spectra]
🗾 File Chromatogram Spectrum	Spectrum List Search Integra
📂 🖨 🗹   🏦 🔍 🛛	
Information	Show File Information
Select As Reference File	Show Logs Show Version
Add Files in Directory Replace with Files in Directory	t <sup>a</sup> bis(2-Chloroethyl)ether: 93.0 (I <sup>A</sup> Benzoic acid: 105.0 (Merged)
Recalc List File 🔹 🕨	2,4-Dichlorophenol: 162.0 (M

Data File Inforn	nation	×
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 Compou	ind Summary	
Identifi	ied: 9 Tentatively Identifed: 0	
Missi	ing: 0 Unknown: 1	
Fai	led: 0 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.

Instrument	Windows	Help
1: Varian	GC/MS #1	Alt+1
<ul> <li>Configuration Alt+c</li> <li>Remove Module Names</li> <li>Restore Disabled Warnings</li> <li>Instrument Faults</li> </ul>		
Setup Ethernet Communications Setup ADC Board I/O Ports Setup COMM Ports		

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.
🔋 System Control - Configuration			
File Edit Inject Automation Recalculate I	Instrument	Windows Help	
🐚 📾 🚺 🚫 No File			
		Instrument 1: Varia	an GC/MS #1
		Operato	or:
		Not Rea	ady
AutoStart Module			
3800 2000			
45:pilot9 40			
Instrument 1 Peremetere			
instrument i Parameters			
Available			
MODUES			

### 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 doubleclicking the instrument area background or using the Instrument menu to select Varian GC/MS #1.

System Control - Configuration				
File Edit Inject Automation Recalculate	Instrument Windows Help			
🐚 📾 🔂 No File	1: Varian GC/MS #1 Alt+1			
	<ul> <li>Configuration</li> <li>Alt+c</li> <li>Remove Module Names</li> <li>Restore Disabled Warnings</li> <li>Instrument Faults</li> </ul>			
	Setup Ethernet Communications Setup ADC Board I/O Ports Setup COMM Ports			
	*** Demo Modules *** 800 Interface Box ADC Board 8200 AutoSampler 3800 GC			
	3400 GC 3600 GC 4000 Mass Spec			

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**.



### 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.

Method SetPoints /	Adjustments
	CI Reagent
Adjust Cal Gas	Adjust
Adjust RF Tuning	Methanol
Save Results	Cancel Adjustment

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.** 



## **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  $\mu$ Sec. When the test passes, click on **Done** to exit the adjustment.



#### Adjusting CI Reagent

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

Manual Control	Auto Tune	Temperatures	Diagnostics	Shutdown	Acquisition
ontrol and Status		Method SetPoints A	diustments	Operating Conditions	
o CI Reagent Adjustme	nt is Necessary.		CI Reagent	Mode State: Adj	ust CIGas - Scanning
		Adjust Cal Gas	Adjust	Fault State: No.	Fault
		Adjust RF Tuning	Mathanol -	i dan ordate. I no	, com
Low OK	High			Ion Lime: 100	J
		Save Results	Cancel Adjustment	Ion Count: 243	30
700			/64		
700			784		
600-					
500-			4		-
400-					-
300-			- Hell		
			11		
200-		31			-
200	15	01	11		

### **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.



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



08:13:38 Auto Tune: Started 08:13:39 Air/Water Check: 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<sup>5</sup> 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.

Show Keypad Current Module Attributes	
MODULE ATTRIBUTES Printed: 7/	/12/98 8:18 AM
Module: Saturn 2000.40	
Saturn GC/MS Workstation (Build 7) (Demo) 3	Version 5.1
Module Software Version:	FFOB
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
Rinel Coin Kelve Lost Chenged:	7/7/90 2-00 DM

## **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 10 in the Toolbar.

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



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

Select Configuration			×
	Select an existing instrumen select a custom configuratio continue. Select a Configuration Custom Instrument 1	t configuration for the method or on for the method. Then click Next to Configuration Description Module Address 2000 40 3800 45	
	< Ba	ack Next > Cancel	

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 p	rocessing	×
	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 All	
	< Back Next > Cancel	

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.

Create sections for post-run pro	cessing
For the following module:	2000 at address 40
Select the channel(s) to process:	Select the Post-Run processes to perform:
I Channel 1=MS Data	■ Standard MS Reports IMS Data Handling
S	elect All
	< Back Next > Cancel

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.

Confirm configuration	
Added configuration	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.
	< Back Finish Cancel

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.

🖺 Method Builder - [Method1*]	
🖺 File Edit View Window Help	
	<b>₽ ?</b>
Method 1*   Method Notes   2000 Mass Spec - Address 40   2000 Mass Spec Control   MS Method Editor   Channel 1=MS Data   MS Data Handling   Calculations Setup   Compound Table   Results Treatment   3800 GC - Address 45   Autosampler   Sample Delivery   Injector   Flow/Pressure   Column Oven   Detector   Output   Data Acquisition	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 Metho	d File As		? 🛛
Save jn: 🗀	MSTutorials	• <del>•</del> •	💣 🎟 •
Purgb.mth			
File <u>n</u> ame:	Coltest1		<u>S</u> ave
Save as <u>t</u> ype:	Methods (*.mth)	▼	Cancel
			Recent Files >
,			//

#### **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.

🗎 Method Builder - [Coltest.mth*]			
🗎 File Edit View Window Help			
BFFF BBB XDE	5 <b>8</b>		
Coltest.mth*			
Method Notes	Detector: 2000 Mass Spec	Address:	40 💌
😑 📅 2000 Mass Spec - Address 40	Detector Control Parameters		
😑 🗖 2000 Mass Spec Control			
MS Method Editor	Detector Lontrol Parameters		
🖃 📅 Channel 1=MS Data	MS Method Editor		
🖃 🔛 MS Data Handling			
Calculations Setup			
Results Treatment			
Second C C Control			
Sample Delivery			
Dianpic Delivery	Last Modified: Monday, June 29, 1998 13:22:00		
Column Oven			
Detector			

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.

1	🛍 Method Builder - [Coltest1.mth]											
	File Edit View Window Help											
1												
I												
			Segment De	scription	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	lonizat Mode	ion e	lor Prepara	ation
		1	FIL/MUL DELAY		0.00	3.00	40	650	None	•	None	-
		2			3.00	10.00	40	650	El Auto	-	None	-
		3								-		-
		4								-		-
		5								•		<b>•</b> •
			Add	Insert	Dele	ete C	efaults	Resto	re	Sp	ecial Applic	ations

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 (El Auto) to acquire MS data over the m/z range 40-650. This is the standard ionization mode for full-scan El 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.)	Lo <del>w</del> Mass (m/z)	High Mass (m/z)	lonization Mode		Ion Preparation		
1	FIL/MUL DELAY		3.00			None	•	None 🚽			
2			3.00	10.00	40	650	El Auto	•	None 👻		
3								•	-		
4								•	<b>_</b>		
5								•	· · ·		
Seam	Add Insert Delete Defaults Restore Special Applications										
segn	Segment Setpoints   Ionization Mode - El Auto										
Sca (3	an Time: 1 BuScans)	.00 <u>*</u> sec	onds/scan	Co	unt Thresh	old:	1 🗧 count	ts			
Mu	Itiplier Offset:	0 +/-	volts	Ма	ass Defect:		0 🕂 mmu/	'10	Ou		
Em	ission Current:	10 📩 uar	nps	Ca	al Gas 🥅						
	[Defaults] Restore										

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 - El 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)	lonizatio Mode	lonization Mode		n ration	
1	FIL/MUL DEL	_AY	0.00	4.00	40	) 650	None	•	None	-	
2	coltest		4.00	19.00	40	) 350	El Auto	•	None	-	
3								•		-	
4								•		-	
5								•	]	<b>•</b>	•
	Add	Insert	Delet	e D	)efaults	Resto	re	Sp	ecial Appli	ications	:
Segr	nent Setpoints	Ionization Mo	ode - El Auto								
Sc ( Mi	Scan Time:     0.50 +     seconds/scan     Count Threshold:     1 +     counts       [2 uScans]     0 +     +/- volts     Mass Defect:     0 +     mmu/100u       Multiplier Offset:     0 +     +/- volts     Cal Gas     Emission Current:     10 +       Emission Current:     10 +     uamps     Defaults     Restore										
Segn	nent Setpoints	Ionization Mc	de - El Auto								
	Low Mass (m/z)	High Mass (m/z)	lonization Storage Level (m/z)	loniza Time F (%	ition actor )	Target TI Max. Ioni	C: zation Time:	2	0000 ÷	counts usec	
1	10	99	35.0	)	100	Processel	opia Timo:	F	100		
2	100	249	35.0	)	100	nescarii	ioniz. Time.	-	100 -	usec	
3	250	399	35.0	)	100	Backgrou	und Mass:		45 🚍	m/z	
4	400	650	35.0	)	100	RF Dump	Value:	6	650.0 🛨	m/z	
		Add	Insert	Deleti	e	Defaults	Restore	!			

#### 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		Add				
1	Initial	On '	Ŧ	20		Insert				
2	0.01	Off	•	Off		D.L.				
3	0.75	On ·	•	100		Delete				
4		•	-			Sort				
5		•	•		1					
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   Middle EFC   Rear EFC											
Fre	Front EFC Type: Type 1 (for 1079/1177 Injectors)										
	Pressure (psi)	Rate (psi/min)	Hold (min)	Total (min)		1					
1					/	A <u>d</u> d					
2					[]	nsert					
3					D	alata					
4						eleře					
6											
7											
8											
- Cons	tant Column Flo	w Mode									
	Constant	Flow: 🔿 Off	🖲 On	Pressure F	Pulse:	• No	C Yes				
С	olumn Flow (ml	/min): 1.0	• ]	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									
1	Stabilization Tim	ne (min): 2.00	÷						
	Temp (C)	Rate	Hold (min)	Total (min)					
	Lemb (c)	(C/min)	ποια (πιπη	r otar (min)					
1	40		2.00	2.00	A <u>d</u> d				
1 2	40 140	10.0	2.00 0.00	2.00 12.00	A <u>d</u> d				
1 2 3	40 140 260	10.0	2.00 0.00 1.00	2.00 12.00 19.00	A <u>d</u> d I <u>n</u> sert				
1 2 3 4	40 140 260	10.0	2.00 0.00 1.00	2.00 12.00 19.00	A <u>d</u> d I <u>n</u> sert Dele <u>t</u> e				
1 2 3 4 5	40 140 260	10.0	2.00 0.00 1.00	2.00 12.00 19.00	Add I <u>n</u> sert Dele <u>t</u> e				
1 2 3 4 5 6	40 140 260	10.0	2.00 0.00 1.00	2.00 12.00 19.00	Add I <u>n</u> sert Delete				
1 2 3 4 5 6 7	40 140 260	10.0	2.00 0.00 1.00	2.00 12.00 19.00	Add I <u>n</u> sert Delete				

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 S	ystem Control Method File			? 🗙
Look in: 隘	methods	•	🗈 💣	·
265_SIS.ml	th mth t <mark>h</mark>			
Eile name:	Colleged with		— r	0
rile name.				Upen
Files of type:	Methods (*.mth)		-	Cancel
			F	lecent Files >

#### 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 Single Sample* option.

Ì۳ د	💆 System Control - Varian GC/MS #1 - Ready											
File	Edit	Inject	Automation	Recalculate	Instrument	Windows	Help					
1	This contract Single Sample											
	🖬 2000.40 - Ready											
	Manual Control Auto Tune Temperatures											

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?							
OK Cancel							

The Inject Single Sample dialog should appear.

Inject Single Sample										
Sample Name	Sample Type	Cal. level	lnj.	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 C:WarianWS\method	g the Method: s\Coltest1.mth pefore Calibrating	_	_	Brows	e Defa	ults				
Inject Cancel Data Files RecalcList										

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.

Data File Generation	
Specify the names for Data Files generate Numbers will be appended to file names if extension in the Data File name.	d by detector modules using this SampleList. the file already exists. Do not include the file
Directory for Data Files	Data File names
🗁 varianws 📂 data	Example:
	Sample 1
	Use the following symbols to enter the corresponding variable data to the file name.
	%s = Sample ID %i = Injection number
New Folder	%m = Detector Module name %t = Injection Time
Drives:	%h = Method Name %o = Operator Name %n = Instrument Name
ОК	Cancel

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".



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".

Windows	Help						
Show Module Windows							
Iconize Module Windows							
Show Automation Windows							
Iconize Automation Windows							
Arrange	e Icons						
Instrum	ient 1 Status						
Message Log							
✓ 2000.4	0 - Ready						
3800.45 - 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.

📱 System Control - Varian GC/MS #1 - V	Vaiting for Injection of Sample						
File Edit Inject Automation Recalculate Instrument Windows Help							
■ 🖆 🖻 Collest1.mth 🔸 🖆 🗐 🗐 🎽 Data Sar Asto 💷 🗸 Waiting							
2000.40 - Ready							
Manual Control Auto Tune	Temperatures Diagnostics	Shutdown Acquisition					
Control and Status Runtime: 0.00 min. Start Acquisition Endtime: 10.00 min. Reset Ready No Faults Hide Keypad Chromatogram Only	MS Method Method: Segment #: 1 FIL/MUL DELAY Scan Mode: None Range: 0 - 0 Ion Prep.: None	Dperating Conditions Data File: <none> Scan Number: 0 Ion Time: 0 Ion Count: 0</none>					

## Injecting 1 µL of the Column Test Mixture

Rinse a 10  $\mu$ L syringe in hexane and expel air bubbles by pumping the syringe plunger up and down several times. Pull the syringe up to the 1.0 $\mu$ L 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  $\mu$ L mark. Finally, remove the syringe from the solution and draw the plunger up a further 1.0  $\mu$ L. You can now see exactly how much sample solution is in the syringe. There should be approximately 1.0  $\mu$ L of air above the sample and 1.0  $\mu$ L 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  $\mu$ L/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	
File Edit Inject Automation Recalculate Instrument Windows Help	
🗎 🖆 📴 Coltest1.mth 🛛 👘 🖆 📓 🕅 🗃 🗾 🕅 🛐	II   Running
a 2000.40 - Running 0.49 min.	
Manual Control Auto Tune Temperatures Diagnostics	Shutdown Acquisition
Control and Status	Operating Conditions
Runtime: 0.49 min. Start Acquisition	Data File: column test.SMS
Burning Reset	Scan Number: 29
No Faults     Scan Mode: None Range: 0 - 0	Ion Time: 0
Hide Kounsel Champberger Only	Ion Count: 0
nCounts 40-	RIC All column test.SMS
30-4	
20-4	
10-3	
	4
-10-	4
-20-	4
-30-	
-40	4
123456	7 8 9 minutes -

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.

🚊 System Control - Varian GC/MS #1 - Ru	nning	
File Edit Inject Automation Recalculate Instru	ument Windows Help	
🛍 🖻 📴 Coltest1.mth	🕨 🖬 🖬 🎽 😼 Reil Shir Seq	
a 2000.40 - Running 14.51 min.		
Manual Control     Auto Tune       Control and Status     Runtime: 14.51 min.     Start Acquisition       Endtime:     13.00 min.     Reset       • Running     No Faults       Hide Keypad     Chromatogram Only	Temperatures     Diagnostics       MS Method	Shutdown Acquisition Operating Conditions Data File: column test.SMS Scan Number: 1644 Ion Time: 25000 Ion Count: 2871
1 50- 25- 0-		KIC All column test. SMS
2.5 5.0	7.5 10.0 12.5	15.U 17.5 minutes

When the Runtime reaches 4.0 minutes, Segment 2 will be downloaded and acquisition will switch to El-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.

Hide Keypad	Spectrum Only 🗾
++	Spectrum Only Chromatogram Only Spectrum and Chromatogram

System Control - Varian GC/MS #1 - Running						
File Edit Inject Automation Recalculate Inst	rument Windows Help					
🛅 💕 📴 Coltest1.mth						
2000.40 - Running 10.21 min.						
Manual Control Auto Tune Control and Status Runtime: 10.21 min. Endtime: 19.00 min. Reset No Faults	Temperatures Diagnostics MS Method Method Segment #: 2 coltest Scan Mode: EI - Auto Range: 40 - 350	Shutdown Acquisition Operating Conditions Data File: column test001.SMS Scan Number: 1071 Ion Time: 25000				
Hide Keypad Spectrum and Chromatogram	Ion Prep.: None	Ion Count: 3569				
Soan number: 10/2, Time: 10/214 min. RIC: 3407, Ion Range: 40 - 350 m/z County 340 - 251 83 200 100 100 100 11 100 11 100 10	132 151 31 21 4 4 150 200 250	base Peak 41, Base Amount 349 Ion: 26000 us, Segment 2, Channel: 1 - - - - - - - - - - - - -				
kCounts 50- 25- 0		RIC All column test001.SMS				
2.5 5.0	7.5 10.0 12.5	5 15.0 17.5. minutes				

# 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.

🗰 MS Data Review - [Plot Chromatograms and	l Spectra]	
File Chromatogram Spectrum Spectrum List Se	arch Integrate Preferences View Window Help	- 8 ×
🗲 🖨 🗹   🏦 📢 🛱 🔲   🏸	<sup>₽</sup> ₩ <sup>67</sup>	
AIT AIT		
	kCounts TIC column test.SMS 2000 CENTROID RAW	
		-
		-
□ Del □ Del □ Del	75-	-
🗎 nev		
	<b>1</b> 50-	-
i vol ⊡ - <mark>D</mark> ioxin [	25-	-
EPData		
- Ligia's f		
		-
Barrier MSGLC	2.5 5.0 7.5 10.0 12.5 15.0 17.5 minut	es l
SatSys V	Segment 1 Segment 2 Scans 151 376 711 1043 1378 170959	_
c:\VarianWS Recent>	1 < 1 > 1 OFF fps S lons:	-

#### **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**.

Enable/Disable Instrument Modules			
Enable///isable instrument Modules Instrument Modules currently enabled Select instrument modules you wish to disable. 2000 Mass Spec 3600 GC 3900 GC 3900 GC 4000 Mass Spec 800 Interface Box 8200 AutoSampler ADC Board CombiPAL AutoSampler	>>Disable>>	Instrument Modules currently disabled Select instrument modules you wish to enable. 2002 Micro-GC 2003 Micro-GC 33KL GC 4900 Micro GC	
	< <enable<<< td=""><td>Cancel</td><td></td></enable<<<>	Cancel	

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.

🔋 System Control - Configuration				
File Edit Inject Automation Recalculate Instrument Windows Help				
Instrument 1: Varian GC/MS #1				
Operator: No Modulo Configured for this Instrument				
No module Conligared for this instrument				
AutoStart Module				
In shows and 4. December 20				
Available FRE 2000 3000 Modules Ere III III				
24 40 45:pilot9				
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.

📕 System Control - Configuration							
File Edit Inject Automation Recalcu	ate Instrument	Windows	Help				
👕 📾 🙆 🚫 No File				Ï	B ROL SHIP SEQ		
		Instrumen	t 1: Varia	n GC/	/MS #1		
			Operator	r: alu			
AutoStart Module	<b>)</b> 19	<b>2000</b> 40	NULKEA	Jy			
Instrument 1 Parameters							
Available Modules							
	Instrument 1	: Jun 17	08:32:	06 L	ocking Combi	PAL	

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**.

Create a New S	ystem Control Sa	mpleList File	? 🔀
Save in: 🗀 Vari	anWS	6	• 🖬 📩
i 1200sys 1200service 14000Sys ChromExample: data Dioxin Data	EPData Examples IRData Ligia's files MCData methods	C MSGLOG MSTutorials SatSys SCData SCData SYSLOG	System System UserPML WSDataFiles ~SINGLE1.SMP manual.smp UNTITLED.SMP
<			>
File name: aul	omation		Save
Save as type: Sa	mpleLists (*.smp)	•	Cancel
			Recent Files >
			<u>^</u>
1			

The following screen will be displayed.

CombiPAL SampleList: automation.smp															
		Sample Name	Sample Ty	ре	Cal. level	lnj.	Injection Notes	AutoLink	Injection Mode	Tra	y	Vial	Injection Volume	Auton Routir	Add
1		Default Sample	Analysis	•		1	none	none	Automatic 🗾 🛨	Tray1	-	1	1.00	none	Insert
2	_			-	_				-	-	-		-		Dalaha
3	-			-					<u> </u>		-				Delete
4				÷					÷		-				Fill Down
6				-					t -		-				Add Lines
7				-						1	-				Defaults
8				-					· ·	]	•				
9				-					<u> </u>		•				
• _															
Begin Suspend Resume Data Files										RecalcList					

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.

CombiPAL SampleList: automation.smp													
		Sample Name	Inj.	Injection Notes	AutoLink	Injection M	ode	Trag	y	Vial	Injection Volume	Aute Roul	Add
	1	sample 1	1	none	none	Automatic	•	Tray1	•	1	1.00	noi	Insert
	2	sample 2	1	none	none	Automatic	-	Tray1	•	2	1.00	noi	
	3	sample 3	1	none	none	Automatic	-	Tray1	•	3	1.00	noi	Delete
	4						-		•				Fill Down
	5						-	1	•				
	6					1	-	1	-				Add <u>L</u> ines
	7						-		•				Defaults
1	B					1	-	1	-				
	9						-	1	•			•	
Begin Susgend Resume Data Files RecalcList													

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.

•

# **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	80_ng.sms
20_ng.sms	120_ng.sms
40_ng.sms	160_ng.sms
50ng_ccc.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  $40 \text{ ng/}\mu\text{L}$  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.

MS Data Review - [Plot Chromatograms and Spectra]										
📃 File Chromatogram	Spectrum	Spectrum List	Search	Integral	e Prefere					
	Library : Target I Edit Act	<b>्र</b>								
	Set Sing Set Click Set Vert	l Active Spectru le Click Action < and Drag Actio tical Scale	+ + +	unts. .50- .25-						
	Set Mas Set Spe	is Range ctrum Display		•	.00 7.25					
	Show A	l Plots	scura		Off					

Alternatively, you can activate the Show Background Correction Markers

button A 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**.



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.
📓 MS Data Review - Library Search a Spectrum		
File Chromatogram Spectrum Spectrum List Search Integrate Preference	es View Window Help	
🔎 😂 🗹   🏯 🔍 🧱 🔟   🌿 🎌 🖽 🛟		
Library Search a Spectrum	Plot Chromatograms and Spectra	
📇 🕂 📮 🔰 🥕 🦧 🗖 🐼 Match 1 of 1 for Scan: !		
O Name R F P M CAS Form	AK AK	
1 2,4-Dimethylphenol 9 9 9 1 105-6 CBH1	MCounts 1 0.50 0.25 0.00 7.25 7.50 7.75 8.00 8.25 8.50 8.75 minutes Seg 1, Time: 7.22-8.91, El Auto, Channels: 1 Scans 537 574 611 648 Ions:	
Search         Spectrum 1A           7.316 min. Scan: 542 Chan: 1 lon: 220 us RIC: 95641           Match           Match           D0 %           7.316 min. Scan: 542 Chan: 1 lon: 220 us RIC: 956419           7.316 min. Scan: 542 Chan: 1 lon: 220 us RIC: 956419           7.316 min. Scan: 542 Chan: 1 lon: 220 us RIC: 956419           7.316 min. Scan: 542 Chan: 1 lon: 220 us RIC: 956419           7.316 min. Scan: 542 Chan: 1 lon: 220 us RIC: 956419           100 %           0.4.           2.4-Dimethylphenol           100 %           0.4.           7.316 min. Scan: 542 Chan: 1 lon: 220 us RIC: 956419           100 %           0.4.           0.4.           2.4-Dimethylphenol           100 %           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.           0.4.	Spectrum 1A         7.316 min. Scan: 542 Chan: 1 Ion: 220 us RIC: 858419         I         III         III         III         III         III         III         III         IIII         IIII         IIIII         IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	

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: © <u>Q</u> uick © <u>N</u> ormal Similarity Searches: © Simple	Library List Tutorial MAINLIB (Inactive) REPLIB (Inactive)	
C Hybrid C Neutral Loss Mol. Weight 700 Threshold E Reverse Search	Edit / Order Library List Max Pre-Search Hits 6000 Max Einal Search Hits 100	
30 Min. Abundance ✓ Use Acqu. Ion Range m/z Range:	Constraints Use Constraints Edit <u>C</u> onstraints	
<u>H</u> elp Rese <u>t</u>	<u>S</u> earch E <u>x</u> it	

## **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 E. 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

🞆 MS Data Review - Spectrum List - c:\varianws\mstutorials\80_ng.msp	
File Chromatogram Spectrum Spectrum List Search Integrate Preferences V	View Window Help
🚰 🖨 🗹   🏦 😡 🧱 🔟   🌿 뙛 🖽 🔂   🍳	
Plot Chromatograms and Spectra	Spectrum List - c:\varianws\mstutorials\80_ng.msp
MCounts GTIC 80_NG.SMS 2000 ENTROID RAW	I         Name         M.         CA         For         Match Name         R.         F.         P.           1         2,4-Dimethy         1         105         C8         2,4-Dimeth         9
7.979 min 7.724 min 7.724 min	3         bis[2-Chloro         1         111         C4         bis[2-Chloro         9         8         5           4         2,4-Dichloro         1         120         C6         2,4-Dichlor         9.
	7         Naphthalene         1         91         C1         Naphthalene         9 </td
	Search         2,4-Dimethylphenol□           BP 107         CAS No. 105-67-9, C8H100, MW 122           Match         BP 107 (999=100%)           BP 107 (999=100%)         CAS No. 105-67-9, C8H100, MW 122           100 %         77 91           60 %         26450           91 407         122
0.00 7.25 7.50 7.75 8.00 8.25 8.50 8.75 minutes	2.4-Dimethylphenol
Seg 1, time: 7.22-8,91, El Auto, Channels: 1           Scans 537         574         611         648           Ions:	-100% -100% -100% -100 + 100 + 120 R.Match: 970, F.Match: 970 R.Match: 970, F.Match: 970

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.

Target List Search Chromatog	ram X
Search Type Identity Searches: Quick Normal Similarity Searches: Simple Hybrid Neutral Loss	Peak Parameters Match Top 3 25 % RIC (minimum height) Data File Parameters 10_NG.SMS 7.220 8.910 minutes Merged
Mol. Weight 500 Match Threshold  Reverse Search 30 Min. Abundance Use Acqu. Ion Range m/z Range: 1 2000	Target List Files       c:\varianws\mstutorials\80_ng.msp       Edit Target List       Help     Search

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.

Target List Search Chromatogram		x
Search Type Peak Param	eters	
Identity Searches:	Match Top 3	
Spectrum List Manager		×
Current Spectra List Files		
c:\varianws\mstutorials\80_ng.msp		
Edit List	Order List	
Add File Create File	To Top of List	Up One
Remove File	To Bottom of List	Down One
	Help	Done
m/z Hange: 1 2000 Help	Search	Exit

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.



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 80\_ng.sms

20\_ng.sms 120\_ng.sms

40\_ng.sms 160\_ng.sms

50\_ccc.sms200\_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/ $\mu$ L in all data files, whereas the concentrations of the other analytes vary from 10 ng/ $\mu$ L up to 200 ng/ $\mu$ L, 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.

Create/Open Method File	
Select a method file action Create a New Method File Open an Existing Method File	OK Cancel
Do not display this dialog at startup	J

Select Custom in the Select Configuration dialog.

Select Configuration		×
	Select an existing instrument configuration for the method.         Select a custom configuration for the method.         Select a Configuration         © Lustomi         © Instrument 1    Configuration Description The following screens will guide you in selecting a custom configuration for your method.	to
	< <u>B</u> ack <u>N</u> ext > Can	el

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.
<pre>&lt; Back Next &gt; Cancel</pre>

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

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 Unselect All	
	< Back Next > Cancel	

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 proce	ssing 🛛 🔀	
For the following module: 20	00 at address 40	
Select the channel(s) to process:	Select the Post-Run processes to perform:	
✓ Channel 1=MS Data	Standard MS Reports	
	MS Data Handling	
Unselect All		
< Back Next > Cancel		

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

Confirm configuration	
Added configuration  To be added to method  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.
	< Back Finish Cancel

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.

🖺 Method Builder - [Method1*]	
File Edit View Window Help	
	<b>∌∣?</b> ∣
	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	💽 🔽 Report Mi <u>s</u> sing Peaks
Measurement Type: Area	Report Unknown Peaks
Calibration Type: Internal Std	Normalize Results
Unretained Pk Time (min.): 0.000	- Ignore Calibration Data
Ion Ratio Type: Absolute	▼ Scale Air Flo <u>w</u> Samples
Qualifier Integration: Quan Ion Pts	
Report Qualifier Outliers As: Failed	▼
Chromatogram Processing Chromatogram Integration	Tentative Identification
Scan Function Channels: Merged	I Library Search <u>Unknown Peaks</u> Search <u>P</u> arameters
Integration Parameters	Reporting Threshold
Time Events Table	С <u>А</u> І
	○ % of Largest P <u>k</u> : 20.0 🚔
RE To Use     Nearest Internal Std	C 🔏 of Nearest Std: 30.0 🚔
C Nearest Pure Internal Std	Eargest N Pks: 20 = 1000 = 1000 = 100 = 100 = 100 = 100 = 100 = 100 = 100 = 100 =
C A <u>b</u> solute: 1.000	Exclude Duplicates
	Defaults Restore

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:



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.

Calibration Results	Analysis Results
Replicates Addition Mode	Calibration Range
• Append	
C Average	Out Of Tolerance Action
Averaging Weight <u>%</u> ;   <sup>100</sup>	No Action
Replicates Addition Rule	
Always Add	
C Never Add	Verification Results
Add if within	- · · · - · · · 100.0 -
tolerance%:	Deviation Folerance %: 100.0
Out Of Tolerance Action	Out Of Tolerance Action
No Action 💌	No Action
Update Cmpd Table Parameters	
✓ Retention Time	
Reference Spectrum	
Target Ion Bation	
Edit/Lock Coefficients	
	Defaults Restore

## The Results Treatment Dialog Box

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 🗾 🗲 🗈 📸 🖽 -	Preview Data File Information
10 NG SMS 160 NG SMS	Dista Turpe: 2000 CENTROID
3 20 NG.SMS 3 200 NG.SMS	Sample: ALLMIX/80NG
40_NG.SMS PURGEB.SMS	Inject Date: 9/25/1991 10:45 PM
SONG_CCC.SMS	Pup Time: 7 221 - 8 908 min
80_NG.SMS	Soan Pange: 525 - 660
3 120_NG.5MS	Operator: BDB
	Sample Notes: 8270
	May BIC: 958056 at 602
File name: 80_NG.SMS Recent Files	
Files of type: 2MS Files (* 2MS.)	
	Пеленкильцр. (с. 1, с
Preview Data File Plot	[
Keep Time Range Plot Type: RIC	Plot Channels: Merged
MCounts	IS 2000 €ENTROID AAW
	//\ //\ -
7.25 7.50 7.75 8.00 8.25	8.50 8.75 🖓
Seg 1, Time: 7.22-8.91, El Auto, Cl	hannels: 1
Scans 537 556 574 593 611	630 648 -
SELECT CENTROID FILE ONLY Help	Open File Cancel

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

	Ret	IS	Compound ID	Quan Ion	<u> </u>	alculations		ntegration	Identification	Ref	Snectrum
1											
2					_						
3											
4											
5											
6											
7											
8											
9											
10											
Data Fil	e Name:	C:Wa	arianWS\MSTutoria	ils\80_NG.SM9	6					<u>S</u> e	elect Data File
( <u>B</u> uild	Compoun	d List	Import Comp	oound List	Expor	rt Compound List	t	Print			
View	Curves		Sort	Add		Insert		Delete	Fill Down		Restore

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

🞆 MS Data Review - Spectrum List - C:\VarianWS\MSTutorials\80_N	G.msp
File Chromatogram Spectrum Spectrum List Search Integrate Prefere	ences View Window Help
🗁 🎒 🗹   🏦 😡 🗱 🔟   🎌 🌿 🔠 🛟	
Spectrum List - C:\VarianWS\MSTutorials\80_NG.msp 💶 🗙	Plot Chromatograms and Spectra
<u> </u>	
In Name MW CAS No. Formula	<u>Å</u>
	MCounts , CENTROID F 0.75 CENTROID F 0.75 C

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

m List - C:∖¥ar	'ian₩5\I	MSTutoria	s\80_NG.ms	р				
Spectrum List Search Integrate Preferences								
Create New Spectrum List								
Edit Spectru	ım List			►				
Select Active Spectrum List								
Build Spectrum List from Active Chromatogram								
Build Spectr	um List fr	om Active Fi	le using AMDIS					

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 %: 0 to 100; default 10	
Peak Size Reject (counts): 15000 0 to 2,000,000,000; default 2,000	
□ Lock Peak Width	
Save Method Load Method Help OK Cancel	

👬 MS	Data Review - Plot Chromatogr	ams an	l Spectra				×
File	Chromatogram Spectrum Spectrur	n List - Se	earch Integrate	Preferences Vie	вw	ew Window Help	
	😂 🖌 🛛 🏦 🕄 🗊			i 🗘   🕐		b	
Sp Sp	Spectrum List - C:\VarianWS\MSTutorials\80_NG.msp						
B	+ @ × 📰 📰 🗄		≤⊼⊒€	Entry 9	of	┛ ▲◇♡┙╞╞。╸╩╴ᢥ╵╤╴ळ╴ᄵ	AX.
Ind	. Name	MW	CAS No.	Formula		MCounts Chan: 1.80, NG SMS, 2009 CENTROID RAV IN	I.
1	7.316 min. Scan: 542 Ch	0	None	None		Chan: 1	
2	7.613 min. Scan: 564 Ch	0	None	None			
3	7.668 min. Scan: 568 Ch	0	None	None		0.75	
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			
	8.721 min. Scan: 646	Chan: 1 k	on: 257 us RIC: 588	3736 BC 🗆 🗙 🔔	r l	· 0.25-1	
	BP 225 (65549=100%) 9 in List Fi	le 80_ng.	msp 80_1	NG.SMS			
100	*1		225	. 1			
75	<del>% ]</del>			í -] .			Ч
	118		190	260	LI		
50	% 4/ 83 27714 20305 20307 <b>4</b>		23536	23828 :	ᆔ	7.25 7.50 7.75 8.00 8.25 8.50 8.75	
25	«		t II.	14		minutes	
		ي ال	II. III.			Seg I, Time: 7.22-6.91, El Auto, Unanneis: T	
	*	150	200	250		5cans 53/ 5/4 611 648	
	30 100		Acquired	Range m/z =			1

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.

NIST Search for Target Spectrum						
Search Type Identity Searches: Quick Normal Similarity Searches: Simple	Library List Tutorial MAINLIB (Inactive) REPLIB (Inactive)					
C Hybrid Neutral Loss Mol. Weight 700 Threshold Reverse Search	Edit / Order Library List Max Pre-Search Hits 6000 Max Final Search Hits 100					
1     Min. Abundance       Use Acqu. Ion Range       m/z Range:       1     2000	Constraints Use Constraints Edit Constraints					
Help Reset	Search Exit					

NIST Library Mana	iger	×
NIST Library List		Order List
MAINLIB BEPLIB		To Top of List
		Up One
		Down One
		To Bottom of List
Edit List		
Initialize	Add Library	Inactivate Library
Create User Library	Remove Library	
Edit Libraries	Help	Done
Help R	eset Sear	ch Exit

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

Select NIS	T Library	,									
Library NameLibrary TypeSpectroscolutionDemoUser Library25Libr_gpUser Library312Libr_trUser Library312Libr_txUser Library570Libr_txUser Library138mainlibMain Library147Nh3_ciUser Library155replibReplicate Library219TutorialUser Library219		Spectr 25 3120 570 1382 147200 155 27750 219	a Count		Preview N Library: Spectra: Current Sp Index: Name: MW:	IST Libr Tutoria 219 ectrum 1 2-Fluor 112	rary Information	on			
<			210		~	CAS No.:	367-12	-0 2-4			
1 2 3 4 5 6 7 8 9 10	2-Fluor Aniline Pheno bis(2-C 2-Chlo 1,4-Dic 1,4-Dic 1,3-Dic 1,2-Dic	rophenol I-d5 I Chloroethyl)ether rophenol Chlorobenzene Chlorobenzene-d4 Chlorobenzene Chlorobenzene		MVV           112           93           99           94           142           128           146           150           146           146           146           146		367-12-4 62-53-3 None 108-95-2 111-44-4 95-57-8 106-46-7 None 541-73-1 95-50-1		C61 C61 C61 C61 C61 C61 C61 C61 C61 C61	15F0 17N 1050 160 18C12 15C10 14C12 14C12 14C12 14C12	0	
				400		Help		Selec	t	Cance	:

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

MS Data Review - Spectrum List - C:\VarianWS\MSTutorials\80_NG.msp	
File Chromatogram Spectrum Spectrum List Search Integrate Preferences View Wine	dow Help
🚰 🖨 🗭   🏦 🔍 🗱 🔟   🎌 🌿 🗄 🛟   🎯 🔡	
Spectrum List - C:\VarianWS\MSTutorials\80_NG.msp	Plot Chromatograms and Spectra
	▲★♡୶୲୲ଢ଼୕ୖୢୢୢ୷ୣ୷ୣ୷ୣ୷ୣ୷
I         Name         M.         CA         For         Match Name         R         F         P           1         2,4-Dimethyl         1         105         CBH         2,4-Dimethyl         9         9         9         9         9         2         bis(2-Chloroe         111         C4H         bis(2-Chloroe         9         8         5           3         Benzoic acid         1         65-8         C7H         Benzoic acid         9         <	MCounts Shan: 1 80_NG.SMS 2000 CENTROID RAW 000 Control 0.757 0.757 UE 0.266 UE 752 UE 7
Search         Hexachlorobutadiene         I           Match         Hexachlorobutadiene         I           100 %         I         I         I           100 %         I         I         I         I           100 %         I         I         I         I           198 %         I         I         I         I           198 %         I         I         I         I           100 %         I         I         I         I           100 %         I         I         I         I           100 %         I         I         I         I	0.25 0.00 7.25 7.50 7.75 8.00 8.25 8.50 8.75 minutes Scans 537 574 641 648 Ions:

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).

Select Sp	ectra to li	mport						? 🛛
Look in:	🚞 MSTut	corials 💽 🗸	- 🗈 💣 🗉		<b>D</b>		1 1 1	la face a View
🛃 10 NG	i.msp					eview Spi	ectrum List	Information
20 NG	i.msp					File:	80_ng.msp	)
80 NG	i.msp				S	pectra:	9	
					- Cu	rrent Spe	ctrum	
						Index	1	
						Name:	2.4-Dimeth	ulphenol
						Mute	100	sprionor
						MW.	122 COL100	
File name:	c:\va	rianws\mstutorials\80_ng.msp	Bece	ent Files		ormula:	L8H100	
						AS No.:	105-67-9	
Files of typ	e: MSP	& MSL Files (*.MS? )	•					
Click on a t	able entry to	o toggle its selection.				Sele	ect All	Select None
Index	Select	Name	MW		Formul	a	CAS	S No.
1	Yes	2,4-Dimethylphenol	122		C8H10	0	105-	67-9
2	Yes	bis(2-Chloroethoxy)methane	172		C5H10	CI2O2	111-	91-1
3	Yes	Benzoic acid	122		C7H6C	2	65-8	6-0
4	Yes	2,4-Dichlorophenol	162		C6H4C	120	120-	-83-2
5	Yes	1,2,4-Trichlorobenzene	180		C6H3C	13	120-	-82-1
6	Yes	Naphthalene-d8	136		C10D8		Non	e
7	Yes	Naphthalene	128		C10D8		91-2	0-3
8	Yes	4-Chloroaniline	127		C6H6CIN		106-	-47-8
9	Yes	Hexachlorobutadiene	258		C4CI6		87-8	8-3
				Hel	lp	Se	elect	Cancel

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

	Ret	IS	Comnound ID	Quan Ion	Calculations	Integration	Identification	Ref Snectrum
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 Fi	le Name: (	::W	′arianWS∖MSTutoria	ils\80_NG.SMS				<u>S</u> elect Data File
<u>B</u> uild	Compound	d Lis	t I <u>m</u> port Comp	oound List	Export Compound Lis	t <u>P</u> rint		
View	<u>C</u> urves		S <u>o</u> rt	<u>A</u> dd	Insert	<u>D</u> elete	Fill Do <u>w</u> n	<u>R</u> estore

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	Quan Ions Calc	culations	Integration	Identification	Ref. Spectrum
8.073 Naphthalene-d8	136.0, C:M Linea	r, Ignor, 1 0.2	25, W:4.0, S:	0.20, Spec	136.0, 108.0, 5
kCounts 75 50 25 25 25 7.25 7.75 8 9 7.25 7.75 8 9 7.25 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 8 9 7.75 9 7.75 8 7.75 8 7.75 8 7.75 8 7.75 7.75 8 7.75 8 7.75 8 7.75 8 7.75 8 7.75 8 7.75 8 7.75 8 7.75 8 7.75 7.75	erged 80_NG.SMS 0 CENTROID RAW 3.00 8.25 8.50 8.75 minutes 2 7.22-8.91, El Auto 611 648	Search 100% 75% 50% 25% Match 100% 50% 75% 0%	Sample 8.073 min. 	Scan: 598 Chan: 10 25 11111	1 lon: 497 us         138.0         8.0       91476         773       -         1
Compound Attributes		- Compound	d Type		
Retenti <u>o</u> n Time (min): Compound Na <u>m</u> e: Naphthalene-d8	8.073	⊂ Intern ⊙ Analy	nal Standard yte	<ul> <li>Active</li> <li>Relative Retent</li> <li>Reference Peal</li> </ul>	ion Time k
CAS Num <u>b</u> er:		IS to <u>U</u> se: <u>G</u> roup Name:			•
		<u>P</u> revious	Nex	t <u>C</u> lose	<u>R</u> estore

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 lons Tab Dialog

Double-click on the Quan lons 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.



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

Quan lons: 162.0 RIC lon(s) 162.0			
Select lons to Plot			
Edit Ion Formula The valid ion range is from 10 IONS: 162.0	1.0 to 650.0.		STATUS: Valid Ion Formula
Show Format and Examples		<u>H</u> elp	OK Cancel

The **Select lons 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 lons* field. A range of quan ions from 162 to 164 would be entered as 162:164 in the *Quan lons* 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 lons 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 lon 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.

ualitiers (Absolute; Quan Ion Pts Integration; Report As Failed)							
	lon	1	%Ratio	%Uncert.	Low%	High% 🔺	<u>L</u> oad
1	63.0	•	87.5	20.0	67.5	107.5	
2	98.0	•	73.0	20.0	53.0	93.0	<u>A</u> dd
3	164.0	•	62.0	20.0	42.0	82.0	
4		•					<u>D</u> elete
•						•	

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 gualifier, click the combo box in the lon 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  $87.5 \pm 20\% = 67.5$  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).



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 <u>A</u> mounts	Analysis Calculations
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	Compound <u>M</u> ultiplier: 1.000 Report <u>I</u> hreshold: 1.000 Results <u>U</u> nits: 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.

⊢Ca	li Level <u>A</u> mou	unts-	
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.



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  $\frac{1}{2}$  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.

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.



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  $\pm$  0.200 minutes ( $\pm$  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.



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 lon/Intensity list. You may edit the reference spectrum if desired and redraw the spectrum plot. Editing the references, 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**.



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 if from the Varian Workstation toolbar.
- Right click on a data file name (in the Data File Pane of the MS Data Review window) and select Recalc List File >Edit.

Information •	his(2-Chloroethyl)ether: 93.0 (Merc
Select As Reference File	Contraction de la cidita de la
Add Files in Directory Replace with Files in Directory	1,2,4-Trichlorobenzene: 180.0 (Mi Maphthalene-d8: 136.0 (Merged)
Recalc List File 🔹 🕨	Create with Selected Files
	Add Selected Files
	Create with Files in Directory
	Add Files in Directory
- 1 80 NG.MS	Edit

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

MS Data Review - [Process Data]	list Search Integrate Preference	es View Window Help	
Method File		Recent>	<u>B</u> rowse
C:\VarianWS\ActiveFile.rcl		Recen <u>t</u> >	Browse Edjt
Data file	Sample Type	Sample Name	Data File Recent >
1 c:\VarianWS\MSTutorials\10_1 2	Analysis	ALLMIX/10NG	Browse
3			Processing Rules
6 7			All Lines     O Selected Lines
		·	C Sample Types
			Verification Analysis
			No Recalculate

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

Automation File Editor					
File Edit Help					
New	÷	RecalcList			
Open	F	SampleList			
Save		Sequence			
Save As					
Print					
Printer Setup					
Exit					

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.

Create a nev	v RecalcList File		? 🔀
Save in: 🗀	MSTutorials	• 🗢 主	
File name:	quantitation.rcl		Save
Save as type:	RecalcLists (*.rcl)	•	Cancel
			Recent Files >
			× •

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 Level Amounts				
_				

	Automa	tion File Editor - [qua	ntitation.rcl]											
Fil	e Edit H	lelp												
×	) 🛋 🖪	🖆 😂 🐰 🖻 🛍												
E	quantit	ation.rcl - RecalcList												
Г														
		Data File	Sample Name	Sample Type	e	Cal. level	lnj.	Recalc Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multiplic	A <u>d</u> d	
	1	c:\varianws\mstutorials\10	ALLMIX/10NG	Calibration	•	1	1	none	none	1			Insert	
	2			Analysis Calibration	^								Delete	
	3			Verification	Ξ								Delete	
	4			Baseline Print Calib									Fill D <u>o</u> w	n
	6			New Calib Block	~								Defaylts	
	7				•								Browse.	
	8				•								Depart	-
	9				•								meport.	-
	10	<u> </u>			•								Actions.	

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 <u>Insert</u> 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 III. 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.

<u>л</u> м:	S Data	Review II - Process Data								
File	File Chromatogram Spectrum Spectrum List Search Quantitation Preferences View Window Help									
1	🗳   ≡   Q,   =   , A   , N   🤋   JL   P   R									
= P	Process Data									
	Method File     Recent >     Browse     Edit       C:WairarWSVMSTutorials\calibration.mth     Recent >     Browse     Edit       Recal: List File     C:WairarWSVMSTutorials\quantitation.rcl     Browse     Entit									
ſ		Data file	Sample Type	Sample Name	Data File     Recent >					
	1		New Calib Block							
	2	c:\varianws\mstutorials\10_ng.sms	Calibration, Level = 1	ALLMIX/10NG	Browse					
	3	c:\varianws\mstutorials\20_ng.sms	Calibration, Level = 2	ALLMIX/20NG	Duran in Dular					
	4	c:\varianws\mstutorials\40_ng.sms	Calibration, Level = 3	ALLMIX/40NG	Processing Hules					
	5	c:\varianws\mstutorials\80_ng.sms	Calibration, Level = 4	ALLMIX/80NG	C Calastad Lines					
	6	c:\varianws\mstutorials\120_ng.sms	Calibration, Level = 5	ALLMIX/120NG	C Serected Lines					
	7	c:\varianws\mstutorials\160_ng.sms	Calibration, Level = 6	ALLMIX/160NG	Calibration					
	8	c:\varianws\mstutorials\200_ng.sms	Calibration, Level = 7	ALLMIX/200NG						
					Make Benote					
					Print					
					Process					

Select All Lines in the Processing Rules field.

Click on <u>Process</u>. 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.
	MS D	ata Review - [F	Process Da	ta]
	File	Chromatogram	Spectrum	Spectre
	Se	elect File/Chromat	ogram	
	Pr	rinter Setup		
Г	Pr	rint		
	E	xport		•
L	🗸 Pr	review Reports		
Γ	Se	end Reports to Sp	ooler File	
	Se	elect Spooler File.	[spooler.m	nsr]
	Vi	ew Spooler File		
	E	kit		

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** 

Print	
Sample Reports	۲
Compound Reports	۲
Calibration Block Reports	۰
Calibration Curve Report	
Summary Reports	۲
Chart Reports	۲
View Spooler File	
Report Preferences	

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.



# **Reviewing Calibration Results in the Results View**

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



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.

Edit	Process				
Next	Previous				
Load	Save				
Print	Preferences				

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



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.



Save Changes mad	le in View Results?		×					
Re	Recalc Set: quantitation.rcl							
Save	Delete	Cancel						

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:

#### RRF=(Peak Area/Peak Area<sub>IS</sub>) x (Amount<sub>IS</sub>/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.

•				•	
Edit	Process				
Next	Previous				
Load	Save				
Print	Preferences				

Target Compound Report Ion Ratio Report	
Results List Reports	•
Sample Reports	►
Compound Reports	►
Calibration Block Reports Calibration Curve Report	×
View Spooler File Report Preferences	
Edit Report Method	►

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	Comnound ID	Quan Ion	Calculations	Integration	Identification	Ref Snectrum
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	Х	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 Fi	ile Name:	D:W	'arian₩S\MSTutoria	als\80_NG.SMS				<u>S</u> elect Data File
<u>B</u> uild	l Compoun	d Lis	t Import Com	oound List	Export Compound Lis	t <u>P</u> rint		
View	<u>C</u> urves		S <u>o</u> rt	Add	Insert	<u>D</u> elete	Fill Do <u>w</u> n	<u>R</u> estore

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



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.

Point Information
Select Point Level: 4 🚔 Replicate: 1 🚍 Next Previous 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%
Print Compound Report

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.

ĦN.	MS Dat	a Review - [Process Data]							
	<u>F</u> ile ⊆l	hromatogram Spectrum Spectrum List Sea	arch Integrate Preferences <u>V</u> iew <u>V</u>	⊻indow <u>H</u> elp		- - ×			
	- 5	) 🗹   🏦 🔍 🎆 🔳   🌿	" 🎢 🖽 🔁 🛛 🕐						
[	Method I D:Wariar	File hWS\MSTutorials\calibration.mth			Rece <u>n</u> t > <u>B</u>	rowse			
	necaic i C:Wariar	nWS\MSTutorials\recalculation.rcl			Recen <u>t</u> > B	rowse Edit			
		Data file	Sample Type	Sample M	Name 🔺	Data File Recent >			
	1		New Calib Block						
	2	c:\varianws\steves_mstutorials\10_nç	Calibration, Level = 1	ALLMIX/10NG		Browse			
	3	c:\varianws\steves_mstutorials\20_nç	Calibration, Level = 2	ALLMIX/20NG					
	4	c:\varianws\steves_mstutorials\40_nç	Calibration, Level = 3	ALLMIX/40NG		Frocessing Rules			
	5	c:\varianws\steves_mstutorials\80_nç	Calibration, Level = 4	ALLMIX/80NG					
	6	c:\varianws\steves_mstutorials\120_r	Calibration, Level = 5	ALLMIX/120NG		C Calastad Lines			
	7	c:\varianws\steves_mstutorials\160_r	Calibration, Level = 6	alibration, Level = 6 ALLMIX/160NG					
	8	c:\varianws\steves_mstutorials\200_r	Calibration, Level = 7	ALLMIX/200NG		, Sample Types			
	9								
	10								
						Make Reports			
		Required Reports							
						Print			
[						Process			

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**.

	utoma	tion File Editor - [qua	ntitation.rcl]									
File	Edit H	Help										
1		1 🖆 🎒 🕹 🖻 🖻	s 🗉 🖌									
	nuanti	tation rcl - Recalcl ist										
	quanti	tation. fet - Recatclist										
		Data File	Sample Name	Sample Type	Cal. level	lnj.	Recalc Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multiplic	Add
	1			New Calib Block 💌								Insert
	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			Delete
	4	c:\varianws\mstutorials\40	ALLMIX/40NG	Calibration 👻	3	1	none	none	1			Fill Down
	5	c:\varianws\mstutorials\80	ALLMIX/80NG	Calibration 🔻	4	1	none	none	1			Defaulte
	6	c:\varianws\mstutorials\12	ALLMIX/120NG	Calibration 💌	5	1	none	none				Derdans
	/	c:\varianws\mstutorials\16	ALLMIX/160NG	Calibration -	6	1	none	none				Browse
	8	c: \variariws \mstutoriais \zu	ALLMIX/200NG	Calibration -		1	none	none		0	1	Report
	10	C. Wallariws		Analysis 🔹			none	none	'	U		A = 1 = = =
	10				_							Actions
	•										•	

Click **Browse** to select a file for analysis processing and select the file 50ng\_ccc.sms.

Open Data File	? 🛛
Look in: C MSTutorials 10_NG.SMS 160_NG.SMS 20_NG.SMS 200_NG.SMS 40_NG.SMS PURGEB.SMS 50NG_CCC.SMS 80_NG.SMS 120_NG.SMS 120_NG.SMS	• 🔁 🖆 🖬 •
File name: 50NG_CCC.SMS Files of type: Data Files (*.run;*.sms;*.xms Run Information	Cancel
Sample: ALLMIX/ 50NG Inject Date: 10/10/1991 9:39 AM	Recalc Date: 6/14/2005 3:22 PM Peak Measurement: Area
Mun Time: 7.221 - 0.300 min. Workstation: Instrument: Module: 2000.40	Calculation Type: Internal Standard Detected Peaks: 9 Compounds OK Rejected Peaks: 0 Missing, 0 Failed Identified Peaks: 1 TICs, 0 Unknowns
Run Mode: Analysis	Delete Results

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

utom	ation File Editor - [qua	ntitation.rcl]										
Edit	Help											
quant	itation.rcl - RecalcList											
_				_		-						
	Data File	Sample Name	Sample Type	•	Cal. level	Inj.	Recalc Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multiplic	Add
1			New Calib Block	-		r]				X4 3	0	Incart
2	c:\varianws\mstutorials\10	ALLMIX/10NG	Calibration	•	1	1	none	none	1			Шаск
3	c:\varianws\mstutorials\20	ALLMIX/20NG	Calibration	•	2	1	none	none	1		÷	Delete
4	c:\varianws\mstutorials\40	ALLMIX/40NG	Calibration	-	3	1	none	none	1			Fill Dowr
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			Defa <u>u</u> lts.
7	c:\varianws\mstutorials\16	ALLMIX/160NG	Calibration	-	6	1	none	none	1		7	Browse
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.	Heport
10				*								Actions

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.

MS Dat	a Review - [Process Data]						
🔄 Eile 🖸	🗌 Eile Chromatogram Spectrum Spectrum List Search Integrate Preferences View Window Help 🔤 🖉 🗙						
2	• 🗹   🏦 😡 🌠 🔟   🌿	T 💾 🔂 🛛 🕐					
Method C:\Variar	Method File C/Warian/WS/MSTutorials/calibration.mth Recent > Browse Edit						
Recalc L C:Wariar	.ist File 1WS\MSTutorials\recalculation.rcl			Recent > E	Browse Edjt		
	Data file	Sample Type	Sample	Name	Data File Recent >		
1		New Calib Block					
2	c:\varianws\steves_mstutorials\10_nç	Calibration, Level = 1	ALLMIX/10NG		Browse		
3	c:\varianws\steves_mstutorials\20_nç	Calibration, Level = 2	ALLMIX/20NG		- Processing Dules		
4	c:\varianws\steves_mstutorials\40_nç	Calibration, Level = 3	ALLMIX/40NG		Clear Calibration		
5	c:\varianws\steves_mstutorials\80_nç	Calibration, Level = 4	ALLMIX/80NG		All Lines		
6	c:\varianws\steves_mstutorials\120_r	Calibration, Level = 5	ALLMIX/120NG		C Selected Lines		
7	c:\varianws\steves_mstutorials\160_r	Calibration, Level = 6	ALLMIX/160NG		C Sample Tupes		
8	c:\varianws\steves_mstutorials\200_r	Calibration, Level = 7	ALLMIX/200NG		Colibration		
9	c:\varianws\mstutorials\50ng_ccc.sm	Analysis	ALLMIX/ 50NG		Verification		
10				<b>_</b>			
Line 4:	Calibration, Done			~	E Make Bereda		
Line 5:	Calibration, Done						
Line 6:	Calibration, Done				Review Paparta		
Line 8:	Calibration, Done			=	I™ Treview neports		
Line 9:	Analysis, Done				Print		
Proces	ss Complete			×			
					Process		
	1						

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).

MS Dat	a Review - [Process Data]					
📃 Eile 🖸	hromatogram Spectrum Spectrum List Se	arch Integrate Preferences View Vi	/indow <u>H</u> elp		_ 8 ×	
i 🗲 🖨	🖌 🛛 🏯 😡 🎇 🔳 🖓	🎽 🔁 🔁				
Method I C:Wariar	Method File C:Warian/WS/MSTutorials/calibration.mth					
C:Wariar	List File nWS\MSTutorials\recalculation.rcl			Recent >	Browse Edit	
	Data file	Sample Type	Sample	Name	Data File Recent >	
1		New Calib Block				
2	c:\varianws\steves_mstutorials\10_nç	Calibration, Level = 1	ALLMIX/10NG		Browse	
3	c:\varianws\steves_mstutorials\20_nç	Calibration, Level = 2	ALLMIX/20NG		- Processing Bules	
4	c:\varianws\steves_mstutorials\40_nç	Calibration, Level = 3	ALLMIX/40NG		E Elear Calibration	
5	c:\varianws\steves_mstutorials\80_nç	Calibration, Level = 4	ALLMIX/80NG		C All Lines	
6	c:\varianws\steves_mstutorials\120_r	Calibration, Level = 5	ALLMIX/120NG		Selected Lines	
7	c:\varianws\steves_mstutorials\160_r	Calibration, Level = 6	ALLMIX/160NG		C Sample Tupes	
8	c:\varianws\steves_mstutorials\200_r	Calibration, Level = 7	ALLMIX/200NG			
9	c:\varianws\mstutorials\6Ung_ccc.sm	Analysis	ALLMIX/5UNG		Verification	
10						
					Make Benorts	
					No Recelculate	
					Preview Reports	
					Print	
					Process	

#### 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.

Proces	ss Data			
Method C:Waria Recalc C:Waria	File nWS\MSTutorials\calibration.mth List File nWS\ActiveFile.rcl		Recent >	Browse Edit
	Data file	Sample Type	Sample Name	Data File
1 2 3 4 5 6 7	c:\VarianWS\MSTutorials\5DNG_CCC.SMS	Analysis	ALLMIX/ 50NG	Browse  Processing Rules Clear Calibration All Lines Selected Lines Calibration Verification Analysis Make Reports No Recalculate Print Process

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		Recent >	Browse Edit
Data file	Sample Type	Sample Name	Data File
1       c:\VarianWS\MSTutorials\50NG_CCC.SMS         2       3         3       4         5       6         7       2         Line 1:       Analysis, Done         Process Complete	Analysis	ALLMIX/ 50NG	
			Process

# **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 pageby-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.



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.



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.

	Seament Description	Start	End	Low Mass	High Mass	Ionization	lon	•
		(min. j	(min. j	(m/z)	(m/z)	Mode	Preparation	
1	Full Scan	0.00	1.00	60	650	El Auto 🛛 👻	None 🗸 🗸	
2	100-264-414	1.00	2.00	60	650	El Auto 🛛 👻	SIS 🗸	
3						•	<b>•</b>	
4						-	-	
5						-	-	Ŧ
		1			_	1 .		
	ödd Inset	Dele	ete l	1etaulte	Besto	re I Si	necial & polications	

Segment Setpoints | Ionization Mode - El Auto | Ion Preparation - SIS |

orage Mass Rar	iges		Eject	ion Masses		
Low Mass	High	Mass		lon Mass	Amplitude	
9	9	101		`		Customize
26	3	265				
41	3	415				
1	\dd	Insert	Delete	Defaults	Restore	
	vdd	Insert	Delete	Defaults	Restore	

The first segment detects all ions in the calibration gas in the mass range of 60-650 in the El 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.

Customize SIS Method	
Ionization Storage Level: 48.0 📫	m/z
✓ Autosca Waveform Amplitude: 17.75	ale? volts
Defaults OK Car	icel

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.



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.

🖬 2000.40 - Not Rea	ady				
Manual Control	Auto Tune	Temperatures	Diagnostics	Shutdown	Acquisition
State: Idle Function:	Start Auto Tune Reset Continue	Method SetPoints Electron Multiplier Voltag SIS Amplitude Adjust Fac	e: 1600 × volts stor: 100 × %		
Hide Keypad Spectru	m and Event Messac 💌	Арр	ly.		

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 De	scription	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	lonizatio Mode	n	lon Preparatio	n	-
1	Full Scan			1.00	60	650	El Auto	•	None	-	
2	265		1.00	2.00	260	270	El Auto	•	MS/MS	•	
3								•		-	
4								•		•	
5								•		•	-
	Add	Insert	Dele	ete 🛛 🖸	Defaults	Resto	re	Sp	ecial Applicatio	ons.	
C	and Catalainta Lt	and an effective state of the	<ul> <li>ELAsse</li> </ul>	Lon Prenar	ation - MS7	MC I					

obginorit obtpolitice Torneation introdo Erriato	
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
Defaults Restore	e (Customize) ''q'' Calculator

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				
Manual Control Auto Tune	Temperatures	Diagnostics	Shutdown	Acquisition
Control and Status Class Filament Gas Filament Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	Method SetPoints A Method: Segment #: E Scan Mode: EI - Auto Ion Prep.: MS/MS	djustments   265 Range: 260 - 270	Operating Conditions Mode State: Scar Fault State: No F Ion Time: 2202 Ion Count: 1541	nning iault 27 2
◆         ●         □			Base Peak: 264, Ba Ion: 22027 us, Segme	ise Amount: 4081 -   nt: 2, Channel: 1
kCounts 4 3 2 1 1 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	284 4081	2005 318 4 65		

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

Segment Setpoints   Ionization Mode - El Auto	Ion Preparation - MS/MS
- Isolation:	Dissociation:
Parent Ion Mass: 131.0 - m/z	Waveform Type: Resonant
	Excitation Storage Level: 48.0 😴 m/z
Isolation Window: 1.0 + 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.

Method Editor - MS/MS (Custom-Nonresonar	it) 🔀
_ Ionization	
Ioniz. Storage Level: 48.0 📩 m/z	
Ejection Amplitude: 20.0 📩 volts	
Isolation:	
Low Edge Offset: 🛛 📑 🛫 steps	
High Edge Offset: 2 📩 steps	
High Edge Amplitude: 30.0 🐳 volts	
Isolation Time: 5 📩 msec	
Dissociation:	
Excitation Time: 20 📩 msec	
Defaults OK Can	cel

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.

🖥 2000.40 - Not Ready		
Manual Control Auto Tune	Temperatures Diagnostics	Shutdown Acquisition
Control and Status Cl T Filament Gas Part ON/OFF RF Cal Part Multiplier Hide Keypad Profile Spectrum	Method SetPoints Adjustments Method: Segment #: 265 Scan Mode: El - Auto Range: 260 - 270 Ion Prep.: MS/MS	Operating Conditions Mode State: Scanning Fault State: No Fault Ion Time: 24865 Ion Count: 2979
◆         ●         ■         II         ●         ■		Base Peak: 264, Base Amount: 800
Counts 750 500 250 	264 800 1 265 394 265	270 Acquired Range m/2

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.

🖥 2000.40 - Not Ready			
Manual Control Control and Status Class Control and Status Class Control and Status Class Control and Status Filament ON/OFF RF Cal Profile Spectrum	Temperatures     Diagnostics       Method     SetPoints     Adjustments       Method:	Shutdown     Acquisition       Operating Conditions     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           Count           400-           300-           200-           100-           255           250	E ■ ■ 266 377 ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	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)	lonization Mode	Ion Preparation
1	Full Scan	0.00	1.00	60	650	El Auto 🗖	None 🔻
2	NR excitation of 264	1.00	2.00	50	350	El Auto 🗖	MS/MS 👻
3						•	· 👻
4						•	· 🗸
5							· • •
	Add Insert	Dele	ete [	)efaults	Resto	re S	pecial Applications

Segment Setpoints | Ionization Mode - El Auto | Ion Preparation - MS/MS |

Isolation: Parent Ion Mass: 264.0 $\star$ m/z Isolation Window: 2.0 $\star$ m/z	Dissociation: Waveform Type: Non-resonant Excitation Storage Level: 65.0 in m/z Excitation Amplitude: 30.00 in volts
Defaults Restore	Customize "q" Calculator

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  $\mu$ A. 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.

T 2000.40 - Not Ready		
Manual Control Auto Tune	Temperatures Diagnostics	Shutdown Acquisition
Control and Status Cl T Filament Gas PA ON/OFF RF Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	Method SetPoints Adjustments Method: Segment #: Segment #: Sean Mode: EI - Auto Range: 50 - 350 Ion Prep: MS/MS	Operating Conditions Mode State: Idle Fault State: No Fault Ion Time: 23751 Ion Count: 17193
		Base Peak: 264, Base Amount: 4095
kCounts 4 3 2 2 1 1 5 5 1 1 0 1 100	160 200 200	264 4005 250 300 Acquired Range m/z

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.

🖥 2000.40 - Not Ready		
Manual Control Control and Status Gas ba RF ON/OFF Gas Gas Hide Keypad Profile Spectrum	Temperatures     Diagnostics       Method     SetPoints     Adjustments       Method:	Shutdown Acquisition Operating Conditions Mode State: Idle Fault State: No Fault Ion Time: 24565 Ion Count: 14768
Scan Number: 0, Time: 0.000 min.           RIC: 14768, Ion Range: 60 - 350 m/z           KCounts           4           2           3           4           4           5           10	E III 176 531 ↓ ↓ ↓ 150 200 250	Base Peak: 264, Base Amount: 4095 Ion: 24565 us, Segment 2, Channel: 1 204 4095 

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.

🖥 2000.40 - Not Ready		
Manual Control Auto Tune Control and Status Gas RF ON/OFF RF Cal Gas Hide Keypad Profile Spectrum	Temperatures     Diagnostics       Method     SetPoints     Adjustments       Method:	Shutdown Acquisition Operating Conditions 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           RIC: 7059, Ion Range: 50 - 350 m/z           0.00           1.26           0.50           1.48           0.59           148           0.00           50           100	E III 176 14334 154 381 ↓ 154 154 381 ↓ 150 ↓ 200 280	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.

Manual Control     Auto Tune     Temp       Control and Status     Filament     Method       Ca     RF     ON/OFF     RF       Cal     Multiplier     Scan Mod       Hide Keypad     Profile Spectrum     Ion Prep.:       Scan Number: 13, Time: 0.000 min.     RIC: 6567, Ion Range: 50 - 350 m/z     Kounts	eratures Diagnostics SetPoints Adjustments d: t: 264 le: El -Auto Range: 50 - 350 MS/MS	Shutdown         Acquination           Operating Conditions         Mode State:         Idle           Mode State:         Idle         Fault State:         No Fault           Ion Time:         24177         Ion Count:         8587	isition
Control and Status Cal Sas Control and Status Cal Sas Control and Status RF ON/OFF RF Multiplier Hide Keypad Profile Spectrum Multiplier Scan Mod Ion Prep: Scan Non Scan No	SetPoints Adjustments d: 264 e: El - Auto Range: 50 - 350 MS/MS	Dperating Conditions Mode State: Idle Fault State: No Fault Ion Time: 24177 Ion Count: 8587 Base Peak: 176, Base Amount: 1	659 - I
		Base Peak: 176, Base Amount: 1	669 -
RIC: 8587, Ion Range: 50 - 350 m/z		baser eak. 170, base Amount. 1	-1.4
1.50 - 1.26 - 1.00 - 0.76 - 0.50 - 0.50 - 0.50 - 0.50 - 0.50 - 0.50 - 0.228 - 275 - 0.00 - 0.50 - 0.00 -	176 1659 214 269	204 628	

### 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)	lonizatio Mode	n	Ion Preparation	<b>^</b>
1	Full Scan	0.00	1.00	60	650	El Auto	٠	None 🚽	
2	RES excit. of 264	1.00	2.00	50	350	El Auto	•	MS/MS 🚽	
3							•	-	
4							•	-	
5							•	-	] -
	Add Insert Delete Defaults Restore Special Applications								
Segr	Segment Setpoints Ionization Mode - ELAuto Ion Preparation - MS/MS								
	Isolation:       Parent Ion Mass:       264.0 +       m/z         Isolation Window:       2.0 +       m/z       Excitation Storage Level:       50.0 +       m/z         Isolation Window:       2.0 +       m/z       Excitation Amplitude:       0.10 +       volts         Defaults       Restore       Customize       "q" Calculator								

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  $\mu$ A 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.

a 2000.40 - Not Ready		
Manual Control Control and Status Cl Gas C Gas C Hide Keypad Profile Spectrum	Temperatures     Diagnostics       Method     SetPoints     Adjustments       Method:	Shutdown Acquisition Operating Conditions 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           4           3           2           1           6           5b           100	E ■	Base Peak: 264, Base Amount: 4095 Ion: 24107 us, Segment: 2, Channel: 1 264 995 995 995 995 995 995 995 995 995 99

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		
Manual Control Auto Tune	Temperatures Diagnostics	Shutdown
Control and Status CI Gas Co RF ON/OFF Cal Gas Multiplier Hide Keypad Profile Spectrum	Method SetPoints Adjustments Method: Segment #: Bes excit. of 264 Scan Mode: El - Auto Range: 50 - 350 Ion Prep.: MS/MS	Operating Conditions Mode State: Scanning Fault State: No Fault Ion Time: 21890 Ion Count: 14472
▲         ●         □		Base Peak: 264, Base Amount: 3977
RIC: 14472, Ion Range: 50 - 350 m/z kCounts 	176 154 770 214 395 313 150 200 250	1 204 3977 300 anii 0 350

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.

🖥 2000.40 - Not Ready		
Manual Control Auto Tune	Temperatures Diagnostics	Shutdown Acquisition
Control and Status Cl T Filament Gas ConvOFF RF Cal C NOVOFF RF Gas Multiplier Hide Keypad Profile Spectrum	Method SetPoints Adjustments Method: Segment #: Adjustments FRS excit. of 264 Scan Mode: EI - Auto Range: 50 - 350 Ion Prep.: MS/MS	Operating Conditions Mode State: Scanning Fault State: No Fault Ion Time: 24434 Ion Count: 10262
◆         ◆         II         ►         ✓	1776 1671 154 419 419	Base Peak: 176, Base Amount: 1671 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.

👕 2000.40 - Not Ready										
Manual Control Auto Tune	Temperatures Diagnostics	Shutdown								
Control and Status Class Filament Gas Filament ON/OFF Cal Gas Multiplier Hide Keypad Profile Spectrum	Method SetPoints Adjustments Method: Segment #: 2 - RES excit. of 264 Scan Mode: EI - Auto Range: 50 - 350 Ion Prep.: MS/MS	Operating Conditions Mode State: Scanning Fault State: No Fault Ion Time: 23654 Ion Count: 10269								
▲         ▲         ■										
KCounts 1.25- 1.00- 0.75- 0.50- 427 114 0.26- 0.00- 	176 1347 154 214 671 609 164 234 164 1 164 234	10n: 23004 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.

	Segment Description	Start (min.)	End (min.)	Low Mass (m/z)	High Mass (m/z)	lonizatio Mode	n	lon Preparation	
1	Full Scan		1.00	60	650	El Auto	•	None 🗸 🗸	•
2	NR excitation of 264	1.00	2.00	50	350	El Auto	•	AMD 🚽	·
3							•	-	•
4							•	-	· ]
5							•	-	-
	Add Insert	Dele	ete C	efaults	Resto	re	Sp	ecial Application:	s

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

Segment Setpoints | Ionization Mode - El Auto | Ion Preparation - AMD |

	Parent Ion Mass	Isolation Window	Waveform Type	Excitation Storage Level	Excitation Amplitude	
1	264.0	2.0	Non-resonant	80.0	0.00	Customize
2	264.0	2.0	Non-resonant	80.0	20.00	Customize
3			Non-resonant	80.0	30.00	"g" Calculator
4			Non-resonant	80.0	40.00 🚽	
•					•	
	Add	Insert	Delete	Defaults F	lestore	

- 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 Nonresonant.
- 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 Des	cription	Start (min.)	En (mir	d 1.)	Low Mass (m/z)	High Mass (m/z)	lonizatio Mode	'n	Ion Preparation	
1		Full Scan			0.00		60	650	El Auto	•	None 🚽	
2	2	RES Excit. of 264	RES Excit. of 264 1.00		2.00 50		350	El Auto	•	AMD 🚽		
З	:									•	-	
4	Ļ									•	-	
5	i									•	-	-
		Add Insert Delete		D	)efaults	Resto	e	Sp	ecial Applications	s		
Segment Setpoints   Ionization Mode - El Auto   Ion Preparation - AMD												
		Parent Ion Mass	Isolation Window	Wavef Typ	orm e	E: Stor	xcitation age Leve	Excit Excit	ation 🔺 itude			
	1	264.0	2.0	D Reson	ant		80	.0	0.00		Customize	
	2	264.0		D Reson	iant		80	.0	0.10	-	Customize	
	З	264.0		D Reson	iant		80	.0	0.20		'q'' Calculator	
	4	264.0		D Reson	iant		80	.0	0.30 🚽		·	
	•								•			
		Add	Insert	De	lete	De	efaults	Restore				

- 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



The Open Multiple Data Files dialog box appears.

Look in: ChromExamples Look in: ChromExamples Look in: ChromExamples LeveL4.RUN LeveRIF_3.R Fi LeveL4.RUN L	iun Information ile: [C:\rianWS\ChromExamples\Recalc]
ALEVEL4.RUN         Apparais2.run         Apparais8.run         Apparais7.run         Apparais9.run         Apparais7.run         Apparais9.run         Apparais7.run         Film           Apparadc1.run         Apparais4.run         Apparais9.run         Apparais7.run	tun Information ile: [C:\rianWS\ChromExamples\Recalc
Aparado4.run     Aparais6.run     Aparais7.run     Aparais7.run       Aparais1.run     Aparais7.run     Aparais7.run     Aparais7.run       File name:     Recalc.RUN     Recent Files >       Files of type:     Data Files (*.run)     Image: Parais7.run	Remember Scaling
File Name Channel	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 << Add To List

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...* 

📐 Ir	nterac	tive G	iraphics - Method: another.mth						
File	Edit	View	Results Edit Method Help						
2		<b>a</b> (	Reintegration List						
			Reintegrate Now	Ctrl+R					
I	<b>%</b>	00	Reintegrate Now/Clear Moved Events						
<u> </u>	Ľ		Autosave Method Before Reintegration						
			✓ Autosave Moved Peak Events Before Reintegration						
	200		View Calibration Curves c:\varianws\chromexamples\recalc2.run - A	•					

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.

Reintegration List											
	Run DH	Data File	Sample Name	Sample Type	Cal. Level						
	<b>1</b>	c:\s\chromexamples\recalc	Test Chromatogran	Analysis 🗾 👻							
				-							
				<b>•</b>							
				<u>-</u>							
				-	_						
				<u>-</u>	_						
	•			<u>-</u>	•						
Calib • Ir	ration ncorpo	Coefficients rate New Calibrations into Data \$	Set	Calcul	ate Results						
0.0	lear Co	pefficients at Start of List	Save Ch	anges (	Cancel						

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.

AIA Import/Export	
Convert Raw Data and Results to AIA Format	
Convert Raw Data and Results to ASCII	
Print Standard Report	
Produce System Suitability Report	
View Chromatogram in MS Data Review	
View Results Only	
View Standard Report	
View Standard Report Remove	_
View Standard Report Remove Move To Front	
View Standard Report Remove Move To Front Show Run File Info	
View Standard Report Remove Move To Front Show Run File Info Show Run File List	

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.

File Search Font Options Windows Help         Image: I	📑 Cha	nnel A = A - Re	sults							
Image: Second	File S	earch Font Opt	ions Windows	Help						
Title : Run File : c:\varianus\chromexamples\recalc2.run Hethod File : c:\varianus\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 Kellogy Detector Type: ADCE (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 No. Name () (min) (min) (counts) Code (sec) Codes 1	<b>2</b>	3 🔁 🖪 🖪	1201	recalc2.run	🕨 🛅 ar	nother.mth 🕨	Chan	nel: 🗛 = A	A 💌	
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         Mo.       Name         (1)       (min)         (min)       (min)         (min)       (counts)         Code (sec)       Codes         1       1.6985       1.001       0.000         8       1.0724       1.11       0.000       31823         9       5.7639       2.735       0.000       27676       EB       4.0         7       20.7960       3.391       0.000       563050       VB       6.8       9       5.5257       4.450       0.000       26620       EB       8.0         10       17.0891       5.4520       0.000       266200       EB       8.0         10       17.0891       5.4520       0.000       266	Title Run Fi Method Sample	: le : c:\v: l File : c:\v: : ID : Test	arianws\chrom arianws\chrom Chromatogram	aexamples aexamples a	s\recalc2 s\another	.run .nth				
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         No.       Name ()         (min)       (min)         (min)       (min)         (a)       1.000         8       1.051         1       1.6985         1       1.6985         1       1.0510         2       1.3186         1.001       0.000         8       9.2735         3       1.0724         1       1.6893         1.001       0.000         3       1.0724         3       1.0724         4       0.6606         1.27096       3.391         2.735       0.000         2.747       2.07960         3.9263       1.977         4       1.6877         3.470       0.000	Inject	ion Date: 11,	/10/1989 1:05	AM (	Calculati	on Date: 6/	20/200	05 9:22	AM	
Ret.         Time         Width           Peak         Peak         Result         Time         Offset         Area         Sep.         1/2         Status           Mo.         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         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         266200         BB         5.0           9         5.5257         4.450         0.000         266200         BB         5.0           10         17.0891         5.452         0.000         823262         BV	Operat Workst Instru Channe ** MS Run Mc Peak M Calcul	For : Christ Station: LC_RE: Ment : Star : $1 : \lambda = \lambda$ Workstation de : H Reasurement: H ation Type: H	Kellogg SEARCH Integrator Ev (Demo) RC3 Ve Analysis Peak Area Percent	Det Bus Sar Rur Pur	tector Ty, 5 Address mple Rate 1 Time .5 ** 001	pe: ADCB (1 : 16 : 10.00 H : 8.002 m 01-24c1-e26	Volt) iz in -40a4	**		
Peak         Peak         Result         Time offset (min)         Area (counts)         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         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         266200         BB         5.0           10         17.0891         5.452         0.000         823262         BV         9.2           11         24.6013         5.69				Ret.	Time			Width		
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         43023         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         263050         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	Peak	Peak	Result	Time	Offset	Area	Sep.	1/2	Status	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	No.	Name	0	(min)	(min)	(counts)	Code	(sec)	Codes	
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       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	1		1.6985	1.001	0.000	81827	BV	1.5		
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       107676       BB       4.0         7       20.7960       3.91       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	2		1.3186	1.051	0.000	63521	vv	2.1		
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       2707676       BB       4.0         7       20.7960       3.911       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	3		1.0724	1.111	0.000	51664	vv	2.6		
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       3.91       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	4		0.6606	1.211	0.000	31823	VB	3.0		
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       1183163       VB       8.0	5		8.9263	1.977	0.000	430023	BB	3.5		
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	6		5.7639	2.735	0.000	277676	BB	4.0		
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	7		20.7960	3.391	0.000	1001846	BV	4.7		
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	8		11.6877	3.470	0.000	563050	VB	6.8		
10         17.0891         8.482         0.000         823262         BV         9.2           11         24.6013         5.698         0.000         1185163         VB         8.0	9		5.5257	4.450	0.000	266200	BB	5.0		
	10		24 6012	5.452	0.000	823262	BV	9.2		
1 1 2 0 8599 7 390 0 000 41426 BB 11 7	12		24.0013 N 8599	3.698 7 390	0.000	41426	VB BB	0.0		_

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 Peak Names Show Integration Baselines Show Cursor/Peak Information Show Run File Information Show Event Marks Show Blank Baseline Preview Blank Baseline Preview Blank Baseline Show Peak Area/Height Units (vs counts) Show Run File List Plot Type O Dutline O Point	Peak Events Show/Hide Peak Events Show All Events Show Active Events Hide All Events Active Peak Event Shape Triangle Line	
OK Cancel	Use Defaults Help	

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

📥 Intera	tive C	iraphics	- Method: an	other.mth					
File Edit	View	Results	Edit Method	Help					
🗃 🖬	✓ Locator Window								
	Mo	nitor Wind	low						
I 🔬 🤉	🗸 Me	thod Quid	k Link Button						
-9-9	🖌 Ma	in Toolbar							
	🖌 Sta	🗸 Status Bar							
	🗸 Qu	ick Actival	te Bar						
	Vis	ual Metho	d Edit Window						
200	🖌 Chi	romatogra	am Toolbar						
	🖌 Att	enuation	Control						
	Pre	ferences		Ctrl+F					

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.** 

📑 Char	nnel A = A - Re	sults							
File Se	arch Font Opt	ions Windows	Help						
<b>e</b>	3 <u>2</u> E		recalc2.run	🔸 🛅 ar	nother.mth 🕨	Chan	nel: 🗛 = A	۱. ۱	•
Title	:								<b>_</b>
Run Fil	le : c:\va	arianws\chrom	example:	s\recalc2	.run				
netnoa Semple	TD · Test	chromatogram	exampies	slanother	.mth				
Inject:	ion Date: 11,	/10/1989 1:05	AM (	Calculati	on Date: 6/	20/200	)5 9:55	AM	
Operato	or : Chris	Kellogg	Det	cector Ty	pe: ADCB (1	Volt)			
Workst	ation: LC_RES	SEARCH	Bus	s Address	: 16				
Instru	ment : Star I	Integrator Ev	' Sar	mple Rate	: 10.00 H	z			
Channe.	1 : A = A		Rui	n Time	: 8.002 m	in			
** MS 1	Workstation	(Demo) RC3 Ve	rsion 6.	.5 ** 001	01-24cl-e26	-40a4	**		
Run Mo	de : j	Analvsis							
Peak M	easurement: 1	Peak Area							
Calcula	ation Type: 1	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	вv	1.5		
2		1.3186	1.051	0.000	63521	vv	2.1		
3		1.0724	1.111	0.000	51664		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	Z.735	0.000	277676	BB	4.0		
7		20.1774	3.391	0.000	972044	BV	4.7	0	
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	вv	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 O Peak Area O Peak Height O Sq. Rt. Height Initial Peak Reject Value:
Monitor Noise ● Before every run ● Once at start of method ● Fixed value: 1 ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	Peak Result Calculation Report Unidentified Peaks Report Missing Peaks Normalize Results
Save	Cancel

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.



#### 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.

Ele       Edit       Yiew       Window       Help         Help       Help       Help       Help	x de X d <b>e ?</b>	
Another.mth ADC Board - Address 16 ADC Board - Address 16 Charnel A=A Data Handling Data Handling Peak Table Calibration Setup Time Events Table	Peak Detection  Subtract Blank Baseline  Initial S/N Ratio:  Initial Peakwidth:  Height %:  Monitor Noise  Before every run  Dnce at start of method  Fixed value:  pVolts	Peak Measurement         Measurement Type         © Peak Area         © Peak Height         © Sg. Rt. Height         Initial Peak         Peak Result Calculation         ✓ Report Unidentified Peaks         Report Missing Peaks         Normalize Results
Ready	<b>   </b>	

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.

te f	RecalcL	ist	practice.RCL								<u>_   ×</u>
		[	Data File	Sample Name	Sample Type		Cal. level	Inj.	Recalc Notes	AutoLini	Add
	1	C	c:\varianws		Analysis	- 1			none	none	Insert
	2	ł				-	_				Delete
	4	1			1	-					Fill Down
	5	ł				-					Defaults
	7					-					Browse
	8	ł			-	-					Report
	•	-			-	-				<u> </u>	Actions
	Begin	1	Suspend Resume								

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.

i R	RecalcList: practice.RCL										
	[	Data File	Sample Name	Sample Type	Cal. level	lnj.	Recalc Notes	AutoLinl	Add		
	1	c:\varianws\chromexamples\star012.run	Manual Sample	Analysis 🗸		1	none	none	Insert		
╟┝	2			Calibration					Delete		
ΙĿ	4			Baseline					Fill Down		
	5			Print Calib New Calib Block					Defaults		
	7							i	Browse		
	8			-					Benort		
	9			<u> </u>				╘───────────	óctions		
Ľ	<u> </u>								-solone		
	Begin	Suspend Resume									

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	
an and a start and a start and a start	861
Instrument: Varian Star #1	end
Operator:	
Max Errors: 0	
Prompt on Automation Start?	
	100
OK Cancel	an.

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 Browse	
🖲 Recalc 🔷 Print	
OK Cancel	

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.

Cha	annel A = UVs u\	olts - Results								_ 🗆 ×
File S	Search Font Op	tions Windows	Help							
<b>2</b>	<u>s 2 L</u>		star012.run	• 🖹 A	NOTHER.M 🕨	Chan	nel: 🗛 = l	JVs uVolts	•	
Title	:									<b>_</b>
Run F	ile : c:\v	arianws\chron	nexamples	s\star012	.run					
Metho	d File : U:\V	arianWS\Chron	ngxamblea	5 \ ANUTHEF	C MTH					
Sambr	e ID . Manu	ar sampre								
Injec	tion Date: 10	/23/1990 3:00	S PM (	Calculati	on Date: 6/	20/200	05 11:3	3 AM		
Opera	tor : LAM		Det	cector Ty	pe: ADCB (1	Volt)				
Works	tation: LC_GC	_RSRCH	Bus	address	: 16					
Instr	ument : Varia	n GC/MS #1	Sar	mple Rate	: 20.00 H	Iz				
Chann	el : A = U	Vs uVolts	Rur	n Time	: 10.001	min				
** MS	Workstation	(Demo) BC3 V	ersion 6	5 ** 001	01-24c1-e26	-40a4	**			
Run M	ode :	Analysis								
Peak 1	Measurement:	Peak Area								
Calcu	lation Type:	Percent								
			Ret.	Time			Width			
Peak	Peak	Result	Time	Offset	Area	Sep.	1/2	Status		
No.	Name	0	(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.

Open Multiple Data F	iles			<u>?</u> ×
Look in: 🔂 Chrom	Examples		* 🎟 -	
ANALYSI.RUN ANALYSI.RUN ANALYSI.RUN ABADMON.RUN ABASECORR.RUN ABASECORR.RUN File name: detec Files of type: Data	A CAL_1.RUN A CAL_1A.RUN A CAL_2.RUN A CAL_3.RUN A CAL_4.RUN A CAL_5.RUN B CAL_5.RUN	A detect.RUN A DH_SAM01.RUN A DH_SAM02.RUN A DH_STD01.RUN A DH_STD02.RUN A DIPA_IS.RUN	A DIPA3020. A DIPA3037. A DIPA3037. A HIBKGRND A LEVEL1.RL A LEVEL2.RL P Recent Files >	Run Information File: [C:\rianWS\ChromExamples\detect
File	Name	Channel	V V V V V V V V V V V V V V V V V V V	Inject Date: 11/10/1989 1:05 AM Run Mode: Analysis Instrument: Star Integrator Ev Workstation: LC_RESEARCH Channel: A = A_RESULTS << Add To List
Del Results	Clear All	<u>C</u> lear <u>Up</u>	Dow <u>n</u>	Open File(s) Cancel

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**.



# **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.

AIA Import/Export
Convert Raw Data and Results to AIA Format
Convert Raw Data and Results to ASCII
Print Standard Report
Produce System Suitability Report
View Chromatogram in MS Data Review
View Results Only
View Standard Report
Remove
Move To Front
Show Run File Info
Show Run File List

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.

📑 Cha	nnel A = A - R	esults								
File S	iearch Font O	ptions Windows	Help							
<b>≥</b> €	3 ° A		detect.run	🕨 🛅 Р	ractice.mth 🕨	Chan	nel: A = A	4	<b>•</b>	
Sample	e ID : Test	t Chromatogram	1						<u> </u>	
Inject	tion Date: 1.	1/10/1989 1:05	5 AM (	Calculati	ion Date: 6/	20/200	5 11:5	58 AM		
Operator : Chris Kellogg Detector Type: ADCB (1 Volt) Workstation: LC_RESEAR <mark>S</mark> H Bus Address : 16 Instrument : Star Integrator Ev Sample Rate : 10.00 Hz										
			Ku	n lime	: 8.002 m					
** MS	Workstation	(Demo) RC3 Ve	ersion 6.	.5 ** 001	101-24c1-e26	-40a4	**			
Run Mo Peak M Calcul	ode : Measurement: lation Type:	Analysis Peak Area Percent								
			Ret.	Time			Width			
Peak	Peak	Result	Time	Offset	Area	Sep.	1/2	Status		
No.	Name	0	(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	вv	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).

📥 Intera	tive G	iraphics	- Method: pr	actice.mth
File Edit	View	Results	Edit Method	Help
1	<ul> <li>✓ Loc</li> <li>Mo</li> <li>✓ Me</li> </ul>	ator Wind nitor Wind thod Quid	low low k Link Button	
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200	Visi Chi Att	ual Metho romatogra renuation	d Edit Window am Toolbar Control	
	Pre	ferences		Ctrl+F

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.

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<b>2</b>	3 🔒 🖻		detect.run	🕨 🛅 Pi	ractice.mth 🕨	Chan	nel: 🗛 = A	ł	•
Sampl	e ID : Test	Chromatogram	m						<b>_</b>
Injec	tion Date: 11,	/10/1989 1:0	5 AM (	Calculati	on Date: 6/	20/200	)5 12:1	7 PM	
Operat Workst Instru Channe	tor : Chris tation: LC_RES ument : Star : el : A = A	Kellogg SEARCH Integrator B	Det Bus v Sar Rur	tector Ty 5 Address nple Rate 1 Time	npe: ADCB (1 ; : 16 ; : 10.00 H ; 8.002 m	Volt) z in	**		
~~ ns	workstation	(Demo) RUS V	ersion 6.	.5 ^^ 001	.01-2401-026	-4024	• •		
Run Mo Peak J Calcu	ode : / Measurement: ) lation Type: )	Analysis Peak Area Percent							
			Ret.	Time			Width		
Peak	Peak	Result	Time	Offset	Area	Sep.	1/2	Status	
No.	Name	0	(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	вv	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					
Detect	ted Peaks: 10	Rej	jected Pe	eaks: 2	Identif	ied Pe	eaks: 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.

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File Search Font O	ptions Windows	Help						
<b>23</b> 8		detect.run	🔸 🖹 pra	actice.mth 🕨	Chan	nel: 🗛 = A	٩	•
Operator : Chris Workstation: LC_RI Instrument : Star Channel : A = J ** MS Workstation Run Mode : Peak Measurement: Calculation Type:	s Kellogg ESEARCH Integrator Ev A (Demo) RC3 Ve Analysis Peak Area Percent	Det Bus Sar Rur ersion 6.	tector Typ s Address mple Rate h Time .5 ** 0010	pe: ADCB (1 : 16 : 10.00 H : 8.002 m D1-24c1-e26	Volt) Z in -40a4	**		
Peak Peak No. Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	
1 2	1.6901 1.3210	1.001	0.000	81456 63667	PV VV	1.5		
3 4 5	0.6593 8.9266 5.7640	1.211 1.211 1.977	0.000	31776 430233 277009	VV VB VP	2.6 3.0 3.5 4.0		
	20.6858 11.7869 5.5286	3.391 3.471 4.450	0.000	277808 996991 568093 266459	VV VP VP	4.7 7.1 5.0		
10 11 12	17.0987 24.5761 0.8867	5.452 5.698 7.389	0.000 0.000 0.000	824106 1184493 42734	VV VP VV	9.2 8.0 0.0		
Totals:	100.0000		0.000	4819688				
Total Unidentified Detected Peaks: 20	1 Counts : D5 Rej	4819688 ected Pe	counts eaks: 193	Identif	ied Pe	aks: 0	I	-

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.

Interactive Graphics		×
Layout Trace Settings Offsets	Colors	_,
<ul> <li>Show X Axis</li> <li>Show Y Axis</li> <li>Show Y Axis</li> <li>Show Horizontal Scroll Bar</li> <li>Show Vertical Scroll Bar</li> <li>Show Attenuation Control</li> <li>Wide Attenuation Control</li> <li>Show Tool Bar</li> <li>Show Tool Bar</li> <li>Show Time Events Log</li> <li>Show Method Editing Panel</li> </ul>	<ul> <li>Show Offset Perspective (Overlay Mode)</li> <li>Locked Zooming (Tile Mode)</li> <li>Layout Mode</li> <li>Tiled Chromatograms</li> <li>Overlayed Chromatograms</li> <li>Peak Annotations Panel</li> <li>Show Peak Retention Time</li> <li>Show Peak Names</li> </ul>	
ОК	Cancel Use Defaults Help	

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.

Тіме В	VENTS TABL	E			
	e Events Prog	ram —			
	Time	Event	Value / End Time	Description	<u>^</u>
1	0.0100	🔹	1.8000	(End time:0.0-1440.00 min)	
2		-			<u>i</u> nsert
3		-			Delete
4		-			Delete
5		-			Read I
6		-			aon
7		-			
8		-			<b>•</b>
			<u>S</u> ave	<u>C</u> ancel	

Choose **Save** to close the Time Events Table window. This will display the Time Event Window below the chromatogram trace.





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.

- 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.

IME EVEN		E am -					
	Time	Eve	ent	Value / End Tim	e	Description	<u>A</u> dd
1	4.0000	WI	-	32 sec	-	(0.5-256 sec)	
2	5.0000	WI	-	64 sec	-	(0.5-256 sec)	Insert
3			•				Delete
4			•				Delete
5			-				
6			•				Sort
7			•				
		1					1
				<u>S</u> ave		<u>C</u> ancel	

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

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.



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.

Fill Pea	ak Table	;						-40°
	F	Retention Time	Peak Name	Ref	Std	RRT	Standard Peak	
1						1	•	Aga
2				1			-	l <u>n</u> sert
3							•	Delete
4							•	
5							<b>•</b>	-
•								Sprt
🔽 (Eut)	table from	selection			-	<u>B</u> ave	Cancel	

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 📥	
1	1.003	Peak 1.003				•	0	1	Add
2	1.979	Peak 1.978				-	0	1	Insert
3	2.737	Peak 2.737				-	0	1	Delete
4	4.453	Peak 4.453				-	0	1	Delete
5	5.455	Peak 5.455				-	0	1 🖵	1
								•	

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	Le\≜ Am	Add
4	2.735	Fourth Peak		1		-	0	2	
5	3.391	Fifth Peak				-	0	_	l <u>n</u> sert
6	3.470	Sixth Peak				-	0		Delete
7	4.450	Seventh Peak				-	0	100	
8	5.452	Eighth Peak	1 1			-	0		
9	5.698	Peak 5.698	1 🔳			-	0		Sort
10						-			
11						-		-	
								- F	

# **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.

Peak	Table										
	Retention Time	Peak Name	Ref	Std	BBT	Standard Peak Name	Group	Level 1 Amount	Level 2 Amount	Level 3 Amount	Add
1	1.001	First Peak		1	1	Internal Standard 📼	0	1	2	4	
2	1.211	Second Peak				Internal Standard 👻	0	1	2	4	l <u>n</u> sert
3	1.977	Internal Standard	1	V		-	0	1	2	4	Delete
4	2.735	Fourth Peak				Internal Standard 👻	0	1	2	4	
5	3.391	Fifth Peak		1	1	Internal Standard 👻	0	1	2	4	Fill Do <u>w</u> n
6	4.450	Sixth Peak		1		Internal Standard 👻	0	1	2	4	
7	5.452	Seventh Peak		1	1	Internal Standard 👻	0	1	2	4	
8	5.698	Eighth Peak				Internal Standard 👻	0	1	2	4	
9						-					
10						-					
•										Þ	
	Define <u>P</u> eak Wind	lows	Print		<u>S</u> ave	Cancel					

#### **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.

CHANNEL	A = A -	RESULTS						_ 🗆 🗙
FILE SEARCH	H FO <u>N</u> T	OPTIONS HELP						
Run Mode Peak Measu: Calculatio	: rement: n Type:	Analysis Peak Area Percent						1
Peak Po No. No	eak ame	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel. Ret. Time	Sep. Code	Width 1/2 (sec)
1 First 2 3 4 Secon 5 Inter 6 Fourt 7 Fifth 8 9 Sixth 10 Seven 11 Eight 12 Total	Peak nal Sta h Peak Peak Peak th Peak h Peak  s:	1.6985 1.3186 1.0724 0.6606 8.9263 5.7639 20.7960 11.6877 5.5257 17.0891 24.6013 0.8599 	1.001 1.051 1.111 1.211 1.977 2.735 3.391 3.470 4.450 5.452 5.698 7.390	-0.001 0.000 -0.001 -0.001 -0.000 0.000 0.000 -0.003 0.001 0.001 0.000 -0.004	81827 63521 51664 31823 430023 277676 1001846 563050 266200 823262 1185163 41426 ====== 4817481	1.000 1.050 1.110 1.210 1.975 2.732 3.388 3.467 4.446 5.447 5.692 7.383	BV VV VV VB BB BV VB BB BV VB BB	1.5 2.1 2.6 3.0 4.0 4.7 6.8 5.0 9.2 8.0 11.7
Status Code R - Referen	es: nce peal	٢						
Total Unide	entified	d Counts :	719661	counts				v A

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
nenu command <i>Edit Method &gt;Peak Table</i> and click on the Define Peak Nindows tab.	
Peak Table	

1.003					Name		aloup	Amount	Amount	Am	
1.000	First Peak			V	Internal Standard	-	0	1	2		Add
1.213	Second Peak		1	1	Internal Standard	•	0	1	2		Insert
1.978	Internal Standard	1	V	1		•	0	1	2		Delete
2.737	Fourth Peak				Internal Standard	•	0	1	2	• •	1/01010
3.393	Fifth Peak			1	Internal Standard	•	0	1	2	•	Fill Dowr
4.453	Sixth Peak	1	1	1	Internal Standard	•	0	1	2		Sort
5.455	Seventh Peak		1	1	Internal Standard	•	0	1	2		0.011
5.701	Eighth Peak	1	1	1	Internal Standard	•	0	1	2		
		1	1	1		•					
		1	1	1		-					
	1.213 1.978 2.737 3.393 4.453 5.455 5.701	1.978 Internal Standard 2.737 Fourth Peak 3.393 Fitth Peak 4.453 Sixth Peak 5.455 Seventh Peak 5.701 Eighth Peak	1.213     Debuild Feak       1.978     Internal Standard       2.737     Fourth Peak       3.393     Fifth Peak       4.453     Sixth Peak       5.455     Seventh Peak       5.701     Eighth Peak	1.213     Second reak       1.978     Internal Standard       2.737     Fouth Peak       3.333     Fifth Peak       4.453     Sixth Peak       5.455     Seventh Peak       5.701     Eighth Peak	1.978     Internal Standard       2.737     Fourth Peak       3.393     Fifth Peak       4.453     Sixth Peak       5.455     Seventh Peak       5.701     Eighth Peak	1.978     Internal Standard       1.978     Internal Standard       2.737     Fouth Peak       3.393     Fifth Peak       4.453     Sixth Peak       5.455     Seventh Peak       5.701     Eighth Peak       1     Internal Standard	1.213       3 Second Peak       Internal Standard         1.978       Internal Standard       Internal Standard         2.337       Fouth Peak       Internal Standard         3.393       Fith Peak       Internal Standard         4.453       Sixth Peak       Internal Standard         5.455       Seventh Peak       Internal Standard         5.701       Eighth Peak       Internal Standard         Internal Standard       Internal Standard	1.213       3 Bookin Peak       1       1 Internal Standard       0         1.978       Internal Standard       0       0         2.737       Fourth Peak       1       1 Internal Standard       0         3.393       Fifth Peak       1       1 Internal Standard       0         4.453       Sixth Peak       1       1 Internal Standard       0         5.455       Seventh Peak       1       1 Internal Standard       0         5.701       Eighth Peak       1       1 Internal Standard       0         1       1       1       1       1	1.213       3 become Peak       Internal Standard       0       1         1.978       Internal Standard       ✓       0       1         2.737       Fourth Peak       Internal Standard       ✓       0       1         3.393       Fifth Peak       Internal Standard       ✓       0       1         4.453       Sixth Peak       Internal Standard       ✓       0       1         5.455       Seventh Peak       Internal Standard       ✓       0       1         5.701       Eighth Peak       Internal Standard       ✓       0       1         Internal Standard       ✓       ✓       ✓       ✓       1	1.113       3 Second rear       1       1       2         1.978       Internal Standard       0       1       2         2.737       Fouth Peak       1       Internal Standard       0       1       2         3.393       Fifth Peak       1       Internal Standard       0       1       2         4.453       Sixth Peak       1       Internal Standard       0       1       2         5.455       Seventh Peak       1       Internal Standard       0       1       2         5.701       Eighth Peak       1       Internal Standard       0       1       2         1       1       1       1       1       1       1       1         1       1       1       1       1       1       1       1       1         5.701       Eighth Peak       1       1       1       1       1       1       1       1         1	1.213       2.500 ft Peak       1       2       1         1.978       Internal Standard       V       0       1       2         2.737       Fourth Peak       Internal Standard       V       0       1       2         3.393       Fifth Peak       Internal Standard       V       0       1       2       1         3.453       Sixth Peak       Internal Standard       V       0       1       2       1         5.455       Seventh Peak       Internal Standard       V       0       1       2       1         5.701       Eighth Peak       Internal Standard       V       0       1       2       1

Define Peak Windows									
CDefine 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									
Save Cancel									

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:



# **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.

CH	IANNEL A = A -	RESULTS							
FILE	SEARCH FONT	OPTIONS HELI	P						
Run M Peak Calcu	Mode Measurement: ulation Type:	Analysis Peak Area Percent							
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel. Ret. Time	Sep. Code	Width 1∕2 (sec)	Status Codes
1 2 3 4	First Peak Second Peak Internal Sta	1.7248 1.3389 1.0890 9.0641	1.001 1.051 1.111 1.977	-0.001 0.000 -0.101 0.000	81827 63521 51664 430023	1.000 1.050 1.110 1.975	BV VV VV BB	1.5 2.1 2.6 3.5	
5 6 7	Fourth Peak Fifth Peak	5.8529 21.1171 11.8681	2.735 3.391 3.470	-0.002 0.001 0.000	277676 1001846 563050	2.732 3.388 3.467	BB BV VB	4.0 4.7 6.8	R
8 9 10	Sixth Peak Seventh Peak Eighth Peak	5.6110 17.3529 24.9811	4.450 5.452 5.698	-0.003 0.001 0.001	266200 823262 1185163	4.446 5.447 5.692	BB BV VB	5.0 9.2 8.0	R
	Totals:	99.9999		-0.104	4744232				
Statı R - F	us Codes: Reference peal	2							
Total	l Unidentified	d Counts :	626573	2 counts					
Detec	cted Peaks: 12	2 I	Rejected 1	Peaks: 2	Iden	tified 1	Peaks	: 8	
•									F

## **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.

EUE	SEADON FONT								
Run Mc Peak M Calcul	de Measurement: lation Type:	Analysis Peak Area Percent							-
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel. Ret. Time	Sep. Code	Width 1/2 (sec)	Status Codes
1 H 2 S 3 J 4 H	First Peak Second Peak Internal Sta Fourth Peak	1.7248 1.0890 9.0641 5.8529	1.001 1.111 1.977 2.735	-0.001 -0.101 0.000 -0.002	81827 51664 430023 277676	1.000 1.110 1.975 2.732	BV VV BB BB	1.5 2.6 3.5 4.0	R
5 E 6 S 7 S 8 E	Fifth Peak Sixth Peak Seventh Peak Eighth Peak	21.1171 5.6110 17.3529 24.9811	3.391 4.450 5.452 5.698	0.001 -0.003 0.001 0.001	1001846 266200 823262 1185163	3.388 4.446 5.447 5.692	BV BB BV VB	4.7 5.0 9.2 8.0	R
i	Fotals:	86.7929		-0.104	4117661				
Status R - R∈	s Codes: eference peak	2		_					
Fotal	Unidentified	i Counts :	626572	2 counts					

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.





🛕 Interactive Graphics - Meth	od: prac	tice.mth*
File Edit View Results Edit Me	ethod H	lelp
New Chromatogram Add/Remove Chromatogram	Ctrl+N Ctrl+O	Practice.mth
New Method	chili M	
Open Method Open Original Method	Ctri+M	
Build Method from Datafile		©
Save Method As	Ctrl+S	
Print Print Method Print Preview Print Setup	Ctrl+P	3:128
Exit		

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 As			<u>? ×</u>
Save in: 🔄 ChromExam	ples	- 🗕 🖻	* <b>III</b> •
analys2-a.mth ANOTHER.MTH cal_1-b.mth cal_3-b.mth DEFAULT.MTH ES_EXAMP.MTH	EXT_STD.MTH IDENT.MTH INT_STD.MTH IS_EXAMP.MTH MINIMUM.MTH NP_EXAMP.MTH	ith th E.MTH th 5.mth 5.mth	
File name: star012-a.r	nth		▶ Save
Save as type: Method Fil	es (*.mth)		Cancel
ADCB	Bus Address	iel ID	

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.

Т	MEE	VENTS TABL	E			_ 🗆 X						
Г	Time	e Events Prog	am —									
		Time	Even	t Value / End Time	Description	<u>A</u> dd						
	1	3.5000		4.1000	(End time:0.0-1440.00 min)	land land						
	2			-		Insert						
	3			-		Delete						
	4			•		Delete						
	5			•		Port						
	6		-	•		a <u>u</u> n						
	7		-	·		*						
	чĒГ	•			Þ							
	<u>Save</u>											

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.

🖹 СНА	NNEL À = U	/suVolts-Res	SULTS						×
<u>E</u> ILE §	BEARCH FONT	OPTIONS HELP							
Run Mo Peak M Calcul	de easurement ation Type	: Analysis : Peak Area : Percent							
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	
 1 2 3 4 5 6 7		0.3556 2.7078 6.8927 36.4073 11.7829 38.7728 3.0808	1.426 1.756 2.387 3.129 4.260 5.840 7.008	0.000 0.000 0.000 0.000 0.000 0.000 0.000	17762 135259 344306 1818631 588585 1936794 153896	BV VV VP PB BB BB TS	16.1 11.6 15.1 15.9 12.3 20.5 0.0		
т	otals:	99.9999		0.000	4995233				
Fotal	Unidentifi	ed Counts :	499523	4 counts					
Detect	ed Peaks:	7 R	ejected H	Peaks: O	Iden	tified	Peaks:	: 0	_
<u>.</u>									

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.

Сн	ANNEL A = UVs		SULTS						IX
FILE	<u>S</u> earch Fo <u>n</u> t	OPTIONS HELP							
Run M	ode :	Analysis							-
Peak I	Measurement:	Peak Area							
carcu.	lation type:	rercent							
			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 177	11.6		
3		3.0392 22 2400	2.30/	0.000	302303 2212607	177	15.1		
5		23.3405	3.125	0.000	2512007	107	10.2		
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 :	9908321	) counts					
Detec	ted Peaks: 8	R	ejected H	Peaks: 0	Iden	tified	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.



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.



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.

Тім	ЕÈ	VENTS TABL	E				_ <b>D</b> ×							
$ _{\Box} \mathbf{I}$	Time Events Program													
		Time	Eve	nt	Value / End Time	Description	<u> </u>							
	1	3.5000	SR	•	4.1000	(End time:0.0-1440.00 min)								
	2			•			Insert							
	3			•			Delete							
	4			•			Delete							
	5			•			0-4							
	6			•			son							
	7			•			-							
•	ĨĽ			_		•								
	<u>Save</u>													

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.

Сн	IANNEL A = UV	SUVOLTS - R	ESULTS					_ [	] ×
FILE	<u>S</u> earch Fo <u>n</u> t	OPTIONS HEL	.P						
Run M Peak Calcu	fode : Measurement: lation Type:	Analysis Peak Area Percent							
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1⁄2 (sec)	Status Codes	
1 2 3 4 5 6 7		0.3392 2.4286 6.0259 36.4454 21.8082 30.5288 2.4239	1.426 1.756 2.387 3.129 4.260 5.840 7.008	0.000 0.000 0.000 0.000 0.000 0.000 0.000	21524 154113 382383 2312687 1383868 1937245 153809	BV VV VV VV VB BB TS	16.1 11.6 15.1 15.9 21.4 20.5 0.0		
 Total	Totals: Unidentifie	100.0000 d Counts :	634562	0.000 8 counts	6345629				
Detec	ted Peaks: 7:		Rejected	Peaks: O	Ider	tified	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.

Тіме Е	IME EVENTS TABLE						
_ <u></u>	e Events Progr	am —					
	Time	Event	Value / End Time	Description 📩	<u>A</u> d d		
1	0.0100	VB 🔹	9.00 <u>00</u>	(End time:0.0-1440.00 min)	Incost		
2		-			insen		
3		-			Delete		
4		-			Detete		
5		-			R		
6		-			son		
7		-					
-⊺			1	F			
			Save	<u>C</u> ancel			

#### 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.



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.



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.

Interactive Graphics	×
Layout Trace Settings Offsets Col	ors
Amplitude Offset Options Offset Type: O Value Percentage Offset by: 5.0 2 % Preset Percentage Offsets Ampli 5%, Time 3% Ampli 2%	Time Offset Options         Offset Type:         O Value         Percentage         Offset by:       3.0         %         Time 2%         Ampli 0%, Time 0%
ОК	Cancel Use Defaults Help

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	Replicate Treatment
ි <u>%</u> (No Calibration)	<u>K</u> eep Replicates Separate
C Internal Standard	C Average Calibration Replicates
	Averaging Weight
C <u>N</u> ormalized %	Apply this weight to new replicates (%): 50
Number of 4	Replicate Tolerance
Lali <u>b</u> ration Levels:	• Always a <u>d</u> d new replicates
Multi-Level Parameters	C Ne <u>v</u> er add new replicates
Curve Defaults	O Add replicates within
Origin: Lapore	this <u>t</u> olerance (%): 10
	O <u>u</u> t of Tolerance Action
<u>F</u> it: Linear	
	Calibration Range Tolerance
View <u>L</u> urves	Peaks outside the range + tolerance generate calibration range errors.
Weighted Regression	<u>R</u> ange Tolerance (%): 10.0
<u>s</u> cheme to each peak:	Out of To <u>l</u> erance Action
(None)	
	Edit/Lock Calibration Data
Save	Cancel

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.

	Retention Time	Peak Name	Bef	Std	BBT	Standard Pea Name	k Group	Level 1 Amount	Level 2 Amount	Level 3 Amount	Level 4 Amount	Add
	5.673	2-Octanone	II	10	I		• 0	12	40	80	120	
:	6.970	1-Octanol	11	11	10		• 0	12	40	80	120	l <u>n</u> ser
1	7.507	n Decane	11	10	1		• 0	20	40	80	140	Dele
	8.473	2,6-dimetphe	I		1		• 0	12	40	80	120	
	8.997	n Dodecane	11		1		• 0	12	40	80	120	Fill Do
1	9.323	2,4-dimetani	11		11		• 0	12	40	80	120	Sort
	10.855	n Tridecane	H				- 0	12	40	80	120	
			11	11	11		*					
			10	11	11		*					

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 Setup
Verification runs are performed by System Control or Interactive Graphics by specifying a sample type of Verification'.
Deviation Tolerance (%):
Out-of-Tolerance Action
No Action
C Increment Error Count
C Terminate Sample List
C 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 Cancel

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 Ty	pe	Cal. Level	Internal Sta
V	c:\star\tutorial\cal_1.run	20.0 NP	Analysis	•		Amount(s
V	c:\star\tutorial\cal_1a.run	12.0 NP	Analysis	•		Amount(s
V	c:\star\tutorial\cal_2.run	40.0 NP	Analysis	-		Amount(s
×.	c:\star\tutorial\cal_3.run	80.0 NP	Analysis	-		Amount(s
M	c:\star\tutorial\cal_4.run	120.0 NP	Analysis	•		Amount(s
V	c:\star\tutorial\analysis.run	40.0 NP	Analysis	-		Amount(s
<b>V</b>	c:\star\tutorial\verif_3.run	80.0 NP	Analysis	-		Amount(s
Ilibration     Incorpor     Class Ca	Coefficients ate New Calibrations into	Data Set	Analysis Calibration Verification Baseline	<b>C</b> = = = =	Ch	<u>C</u> alculate Re

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	Divi
V	c:\star\tutorial\cal_1.run	Calibration	•	1	Amount(s)			
V	c:\star\tutorial\cal_1a.run	Calibration	•	1	Amount(s)			
V	c:\star\tutorial\cal_2.run	Calibration	•	2	Amount(s)	1		
V	c:\star\tutorial\cal_3.run	Calibration	•	3	Amount(s)			
V	c:\star\tutorial\cal_4.run	Calibration	•	4	Amount(s)			
V	c:\star\tutorial\analysis.run	Analysis	•		Amount(s)	0	1	1
M	c:\star\tutorial\verif_3.run	Verification	•	3	Amount(s)	0	1	1
•			_					
Incorporate New Calibrations into Data Set								

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.

Coeff	icients				L		×
	Retention Time	Peak Name	Lock Coeffs.	X^3	X^2	×	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
					0 <u>K</u>	Cancel	

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 C	Curve - 2-0	ctanone			>
Print P	rint <u>A</u> ll	Export	<u>O</u> verlay.	Point <u>I</u> nfo	<u>C</u> oefficients
External Star Curve Type: Origin: Ignor y = +2.0609e Replicates P 25000- e 20000- k 15000- S 10000- i 2 5000- e 000-	idard Analy Linear re =+002x +3. 2	ext_std.mth: /sis 7776e+003 1	ADCB.16.B:	2-Octanone Resp. Fact. F Corr. Coef.(F	RSD: 40.84% R): 0.979546
F		25	<sup>1</sup> 50 Amount	75	<sup>'</sup> 100 <sup>'</sup>
Peak Name:	1. 2-Oc	tanone		<b>_</b>	Exact View
Origin —		- Curve Fit —		urve O <u>n</u> ly	Save
⊙ Include ⊙ Ianore		© Linear O Quadrati	c ∣⊡×	, <u>Y</u> Cursor	<u>R</u> evert
O Force		C Cubic		<u>X</u> <> Y	Cancel

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".

File	annel B = FID RAN Search Fo <u>n</u> t Option	I <mark>GE 11 - Result</mark> s ns <u>H</u> elp	\$					_ 0	×
Run h Peak Calcu Level Toles	Mode : Measurement: ulation Type: l : rance :	Verificatio Peak Area External St 3 15.0%	on andard						
Peak No.	Peak Name	Expected Result ()	Calculated Result ()	Dev. %	Ret. Time (min)	Time Offset (min)	Area (counts)	Status Codes	
1 2 3 4 5	2-Octanone 1-Octanol n-Decane 2,6-dimetphe n-Dodecane	80.000 80.000 80.000 80.000 80.000 80.000	90.905 88.944 103.199 90.284 91.367	13.6 11.2 29.0 12.9 14.2	5.671 6.970 7.505 8.472 8.995	-0.002 -0.000 -0.002 -0.001 -0.001	22512 6898 14949 14597 18526	Δ	
6 7 	2,4-dimetani n-Tridecane Totals:	80.000 80.000	91.422 111.576	14.3 39.5	9.322 10.854	-0.001 -0.001	14256 16740	V 	
Stati V - (	us Codes: Dut of verific	cation toler	rance			0.007	1004/0		
Total	l Unidentified	d Counts :	17104 d	counts					
Deteo	oted Peaks: 12	2 F	Rejected Pea	aks: 3	Ide	ntified H	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**.

📑 Cha	annel B = FID RA	NGE 11 - Result	s						
File S	Search Font Opt	ions Windows	Help						
<b>2</b>	9 <u>2</u> [		analysis.rur	n 🕨 🛅 e	xt_std.mth 🕨	Chan	nel: B = F	TD RANGE 11	•
Title	:								<b>_</b>
Run F:	ile : c:\va	arianws\chro	mexample:	s\analys:	is.run				
Metho:	d File : c:\va - TD - 40 0	arianws\chro wn	mexample:	s\ext_sto	1.mth				
Sampi	e ID . 40.0	MF							
Inject	tion Date: 12,	/25/1992 12:	40 AM	Calculat	ion Date: 6	/21/20	005 3:0	18 PM	
Operat	tor :		Det	tector Ty	/pe: ADCB (1	Volt)	)		
Workst	tation: PEGASU	JS	Bus	s Address	5 : 16				
Instru	ument : Varian	n Star #2	Sar	mple Rate	e : 5.00 Hz				
Channe	el : B = F1	ID RANGE 11	Ru	n Time	: 15.003	min			
** MS	Workstation	(Demo) RC3 V	ersion 6	.5 ** 001	L01-24cl-e26	-40a4	**		
Run M	ode : <i>i</i>	Analysis							
Peak 1	Measurement: 1	Peak Area							
Calcu	lation Type: H	External Sta	ndard						
			Ret.	Time			Width		
Peak	Peak	Result	Time	Offset	Area	Sep.	1/2	Status	
No.	Name	0	(min)	(min)	(counts)	Code	(sec)	Codes	
	2_0.at an an a	40 1625			12000	 DD			
2	1-Octanol	45.1023	6 965	-0.010	3792	BB	1.3		
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**.





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 © <u>%</u> (No Calibration)	Replicate Treatment
© Internal Standard © E <u>x</u> ternal Standard © Normalized %	C <u>Average Calibration Replicates</u> Averaging Weight Apply this weight to
Number of Cali <u>b</u> ration Levels:	new replicates (%):     50       Replicate Tolerance       © Always add new replicates
Multi-Level Parameters Curve Defaults Origin: Ignore	<ul> <li>○ Never add new replicates</li> <li>○ Add replicates within this tolerance (%): 10</li> <li>○ Out of Tolerance Action</li> </ul>
View <u>C</u> urves	Calibration Range Tolerance Peaks outside the range + tolerance generate calibration range errors.
Weighted Regression Apply this weighting <u>s</u> cheme to each peak: (None)	<u>Range Tolerance</u> (%):     10.0       Out of Tolerance Action
Save	Edit/Lock Calibration Data

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.

	Retention Time	Peak Name	Ref	Std	BBT	Standard Pe Name	ak	Group	Level 1 Amount	Level 2 Amount	Level 3 Amount	Level 4 Amount	Add
T	4.626	n Nonane	II	<i>¥</i>	11			0	100	100	100	100	NÃO
ľ	5.673	2-Octanone	11			n Nonane		0	12	40	80	120	l <u>n</u> ser
1	6.970	1-Octanol	11	11	11	n-Nonane	×	0	12	40	80	120	Delet
	7.507	n-Decane	Ⅲ	11	11	n-Nonane	×	0	20	40	80	140	
	0.473	2,6-dimetphe	Ⅲ	11	11	n-Nonane	×	0	12	40	80	120	Fill Do
	0.997	n-Dodecane	Ⅲ	11	11	n-Nonane	×	0	12	40	80	120	Sort
	9.323	2,4-dimetani	II	10	II	n-Hexbenzene	1	0	12	40	80	120	
	10.013	n-Hexbenzene	11	1	10			0	200	200	200	200	
	10.855	n-Tridecane	11	10	10	n-Hexbenzene	1	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 Ty	ре	Cal. Level	Internal Stan
V	c:\star\tutorial\cal_1.run	20.0 NP	Analysis	-		Amount(s)
M	c:\star\tutorial\cal_1a.run	12.0 NP	Analysis	-		Amount(s)
V	c:\star\tutorial\cal_2.run	40.0 NP	Analysis	-		Amount(s)
M	c:\star\tutorial\cal_3.run	80.0 NP	Analysis	-		Amount(s)
M	c:\star\tutorial\cal_4.run	120.0 NP	Analysis	-		Amount(s)
M	c:\star\tutorial\analysis.run	40.0 NP	Analysis	-		Amount(s)
M	c:\star\tutorial\verif_3.run	80.0 NP	Analysis	-		Amount(s)
alibration	Coefficients	Data Set	Analysis Calibration Verification Baseline			<u>C</u> alculate Res

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.

Internal Standard Amounts		×
Name of Internal Standard	Amount Standard	
n-Nonane	100.0000	
n-Hexbenzene	200.0000	
		Update Amounts
		Cancel

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	Diviso
V	c:\star\tutorial\cal_1.run	Calibration	-	1	Amount(s)			
V	c:\star\tutorial\cal_1a.run	Calibration	•	1	Amount(s)	ĺ		
V	c:\star\tutorial\cal_2.run	Calibration	-	2	Amount(s)			
V	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
V	c:\star\tutorial\verif_3.run	Verification	•	3	Amount(s)	0	1	1
•								
alibration	Coefficients ate New Calibrations into	Data Set				C Ck	<u>C</u> alcula	ate Resu

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	×	Intercept	
1	4.626	n-Nonane		0	0	1	0	1
2	5.673	2-Octanone	1	0	0	3.3156	0.71593	
3	6.970	1-Octanol		0	0	1.1885	0.085124	
4	7.507	n-Decane	1	0	0	2.1992	0.20482	1
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	-
					0 <u>K</u>	Cancel		

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.



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".

Ch	annel B = FID BAN	GF 11 - Besult	8						
<u>File</u>	<u>Search Font Option</u>	ns <u>H</u> elp							
Run 1 Peak Calcu Leve: Tole:	fode : Measurement: ulation Type: l : rance :	Verificatio Peak Area Internal St 3 15.0%	⊃n tandard						
Peak No.	Peak Name	Expected Result ()	Calculated Result ()	Dev. %	Standard Peak Name	Ret. Time (min)	Time Offset (min)	Area (counts)	Status Codes
12 34 56 78 9	n-Nonane 2-Octanone 1-Octanol n-Decane 2,6-dimetphe n-Dodecane 2,4-dimetani n-Hexbenzene n-Tridecane Totals:	N/A 80.000 80.000 80.000 80.000 80.000 80.000 80.000 N/A 80.000	INT STD 84.854 83.820 97.253 84.459 84.672 86.069 INT STD 105.772 626.899	0.0 6.1 4.8 21.6 5.6 5.8 7.6 0.0 32.2	n-Nonane n-Nonane n-Nonane n-Nonane n-Hexbenzene n-Hexbenzene	4.624 5.671 6.970 7.505 8.472 8.995 9.322 10.011 10.854	-0.002 -0.002 -0.000 -0.002 -0.001 -0.002 -0.001 -0.002 -0.001 -0.001	6379 22512 6898 14949 14597 18526 14256 10725 16740 ======= 125582	s v 1 s v
Statu V = ( S = : Tota:	us Codes: Dut of verific Internal Stand 1 Unidentified	ation tolen lard peak 1 Counts :	rance 0 (	counts					
Detec	oted Peaks: 12	: 1	Rejected Pe	aks: 3	Identifi	ed Peak	в: 9		
Stand n-Nor n-Her	lard Peak Amou nane Am xbenzene Am	ints: iount = 100 iount = 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**.

Eile G	annel B = FID RAN	IGE 11 - Result: ns Help	\$							_ 🗆 >
Run M Peak Calcu	ode : Measurement: Mation Type:	Analysis Peak Area Internal St	andard							<u> </u>
Peak No. 	Peak Name	Result ()	Standard Peak Name 	Ret. Time (min) 4 618	Time Offset (min) -0.008	Årea (counts) 	Sep. Code  BB	Vidth 1∕2 (sec) 1 5	Status Codes 	
12 34 56 7 89	2-Octanone 1-Octanol n-Decane 2,6-dimetphe n-Dodecane 2,4-dimetani n-Hexbenzene n-Tridecane	46.5474 44.6624 52.8470 46.3648 46.7705 45.0947 INT STD 45.1868	n-Nonane n-Nonane n-Nonane n-Nonane n-Hexbenzene n-Hexbenzene n-Hexbenzene	5.663 6.965 7.497 8.463 8.985 9.312 10.004 10.843	-0.010 -0.005 -0.010 -0.010 -0.012 -0.011 -0.009 -0.012	13909 3792 8416 8852 12087 9286 10767 9349	BB BB BB BB BB BB BB BB	1.5 1.8 1.6 1.7 1.8 1.8 1.8 1.8	s 	1
Statu S — I Total	Totals: us Codes: internal Stand . Unidentified	327.4736 lard peak l Counts :	0 cov	ints	-0.087	82615				
Detec	ted Peaks: 11	1 F	Rejected Peaks	s: 2	Identi	fied Peaks	: 9			
Stand n-Non n-Hex	lard Peak Amou lane Ar ibenzene Ar	unts: mount = 100 mount = 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.

C:\star\tutorial\cal_1.run 20.0 NP Calibration ▼ 1 Amount(s) C:\star\tutorial\cal_1.a.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_4.run 120.0 NP Calibration ▼ 3 Amount(s) C:\star\tutorial\cal_4.run 120.0 NP Calibration ▼ 4 Amount(s) C:\star\tutorial\cal_8.run 80.0 NP Calibration ▼ 3 Amount(s) C:\star\tutorial\cal_9.run 80.0 NP Calibration ▼ 3 Amount(s) C.\star\tutorial\cal_9.run 80.0 NP Calibration ▼ 8 Cali	Run DH	Data File	Sample Name	Sample Ty	ре	Cal. Level	Internal Standa	
□       c:\star\tutoria\cal_1a.run       12.0 NP       Calibration       ▼       1       Amount(s)         □       c:\star\tutoria\cal_2.run       40.0 NP       Calibration       ▼       2       Amount(s)         □       c:\star\tutoria\cal_3.run       80.0 NP       Calibration       ▼       3       Amount(s)         □       c:\star\tutoria\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         □       c:\star\tutoria\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         □       c:\star\tutoria\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         ☑       c:\star\tutoria\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         ☑       c:\star\tutoria\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         ☑       c:\star\tutoria\cal_4.run       80.0 NP       Calibration       ▼       3       Amount(s)         ☑       c:\star\tutoria\cal_4.run       80.0 NP       Calibration       ▼       3       Amount(s)         ☑       u       u       u       u       u       u       u       u	-	c:\star\tutorial\cal_1.run	20.0 NP	Calibration	-	1	Amount(s)	
C:\star\tutoria\cal_2.run 40.0 NP Calibration ▼ 2 Amount(s)     C:\star\tutoria\cal_3.run 80.0 NP Calibration ▼ 3 Amount(s)     C:\star\tutoria\cal_4.run 120.0 NP Calibration ▼ 4 Amount(s)     C:\star\tutoria\cal_4.run 120.0 NP Calibration ▼ 4 Amount(s)     C:\star\tutoria\cal_4.run 80.0 NP Calibration ▼ 3 Amount(s)     C:\star\tutoria\cal_4.run 80.0 NP Calibration ▼ 3 Amount(s)		c:\star\tutorial\cal_1a.run	12.0 NP	Calibration	-	1	Amount(s)	
□       c:\star\tutoria\cal_3.run       80.0 NP       Calibration       ▼       3       Amount(s)         □       c:\star\tutoria\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         □       c:\star\tutoria\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         □       c:\star\tutoria\cal_4.run       40.0 NP       Analysis       ▼       Amount(s)         ✓       c:\star\tutoria\cal_4.run       80.0 NP       Calibration       ▼       3       Amount(s)		c:\star\tutorial\cal_2.run	40.0 NP	Calibration	-	2	Amount(s)	
□       c:\star\tutorial\cal_4.run       120.0 NP       Calibration       ▼       4       Amount(s)         □       c:\star\tutorial\cal_4.run       40.0 NP       Analysis       ▼       Amount(s)         ☑       c:\star\tutorial\cal_4.run       80.0 NP       Calibration       ▼       3       Amount(s)         ✓       c:\star\tutorial\verif_3.run       80.0 NP       Calibration       ▼       3       Amount(s)		c:\star\tutorial\cal_3.run	80.0 NP	Calibration	-	3	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)         ✓           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)	
	V	c:\star\tutorial\verif_3.run	80.0 NP	Calibration	•	3	Amount(s)	
alibration L'optimiente	<b>∢</b> [	Castfinianta						

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.



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.



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


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

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*.

📆 X <> Y	×
Enter Amount	t or Peak Size
Amt. / Amt. Std. (X)	PS / PS Std. (Y)
0.300000	1.19921
Calculate	Cancel

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.



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.

Calibration Curve - n-Tridecane							
Print	Print All	Export	(	) verlay	Point Info.	Coefi	ficients
int_std.mth: ADCB.16.B: n-Tridecane Internal Standard Analysis Resp. Fact. RSD: 45.59% Curve Type: Quadratic (Edited) Coeff. Det.(r*): 0.974568 Origin: Ignore							
Replicate	s 4	- 4.04410	2	2.10200.00	3		2
Р е 1. а К 1.	75 50- 25-			_	0		
S 1. z e 0.	00- 75-		0				
/ o. 5 o.	50- 0 250 250						
S 0. d Previous	00 3	<sup>I</sup> 0.1 A	<sup>I</sup> 0.2 mount/	0.3 Amt. Std.	0.4	<sup>1</sup> 0.5	1
Peak Name	a: 🧧 9. n-Tri	decane			• 🗄	Exactly	View
Origin-		- Curve Fit-	Curve Fit		e Only	Sav	/e
<ul> <li>Inclu</li> <li>Inclu</li> <li>Inclu</li> </ul>	ide re	C Linear Quadratic	r ratic		Cursor	Rev	ert
C Force	е	C Cubic		X <	> Y	Cano	cel
Point In	fo					X	
-Select Poir Level: 3	nt Replic	ate: 2 📑	Next	Previous	3 reps. at	level 3	
-File: c:\varianws\chromexamples\cal_3.run Amount: 80.0000 Peak Size: 13674 Deviation: -6.77% 5							

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.

Coefficients			×
0.000000e+000×3	-2.333965e+000 X²	3.820979e+000×	2.290091e-001
0.00000e+000	-2.333965e+000	3.820979e+000	2.290091e-001
Restore	Overlay	Save	Cancel

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.

😹 Calibration Curve - n-Tridecane 🛛 🔀					×
Print Print All.	. Export	Overlay	Point Info	Coefficients	
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+000x² +3.8210e+000x +2.2901e-001					
Curve Type: Qua Origin: Ignore y = -2.3340e+000	dratic (Edited) )x <sup>2</sup> +3.8210e+000x +	·3.2901e-00	Coeff. Det.(rª )1 (Edited)	): 0.972938	
Replicates	4 2		3	2	1
B 1.75 1.50 R 1.25 S 1.00 Z 0.75 / 0.50 B 0.25	L.75 L.50 K 1.25 S 1.00 Z 0.75 / 0.50 B 0.25				
Previous	Previous 0.1 0.2 0.3 0.4 0.5				1
Peak Name: 9, n-	Tridecane		• 🗧	Exact View	-
Origin O Include	Curve Fit	🗌 🗆 Curve	• Only	Save	
Ignore	Quadratic	🗆 X,Y C	Cursor	Revert	
C Force	C Cubic	X <	> Y	Cancel	
Coefficients					
0.000000e+000 X3	-2.333965e+000 X²	3.820979	e+000×	2.290091e-001	
0.000000e+000	-2.333965e+000	3.82097	9e+000	3.290091e-001	
Restore	Overlay	Sa	/e	Cancel	

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
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6	New Generation Ion Trap GC/MS Technology Axial Modulation
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7	Polychlorinated Biphenyl Analysis and Complex Matrices
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8	Determination of Volatile Organic Compounds in Water with the 2000 GC/MS;
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9	Use of the 2000 GC/MS Data System to Separate and Identify Multiple Components in Reconstructed Total Ion Current Chromatographic Peaks
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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
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12	The Determination of Triazine Herbicides at Ultra Trace Levels by Chemical Ionization GC/MS
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13	The Determination of Base/Neutrals in Extracts from Environmental Matrices
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14	The Determination of Phenols in Extracts from Environmental Matrices
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15	The Determination of Acid/Base/Neutrals in an Industrial Effluent
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16	Determination of EPA Methods 524.2, 624 and 8260 Analytes with an Open Split Interface to the 2000 GC/MS
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17	Determination of Semivolatile Analytes by US EPA Method 8270 with the 2000 GC/MS;
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	CLP Forms
18	The Determination of Volatile Organic Compounds (VOCs) in Air by the TO-14 Method Using the 2000 II GC/MS;
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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;
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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
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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
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28	GC/MS/MS Analysis of Residual Pesticides in Vegetable Extracts
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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
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34	Simultaneous Automated Spectral Screening and Quantitation of Picogram Levels of Drugs
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35	Rapid Simultaneous Quantitation of Triazines, Organophosphates and PAHs
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36	GC/MS/MS Analysis for the Identification of Impurities in Pharmaceutical Products
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37	The Determination of Trace Level FAMES Using CI Mode GC/MS
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38	Analysis of Estrogens in Sheep Liver Extracts Using Selected Ion Storage(SIS)
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40	GC/MS/MS Analysis for Pesticide Residues in Agricultural Products
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41	Reformulated Fuel Analysis by GC/MS: Total Aromatic Hydrocarbons
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43	GC/MS/MS Analysis of Dinitroaniline Based Herbicides in Fish
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	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
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48	GC/MS Analysis for Unsaturated Fat Content in Animal Feed
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49	Analysis of Hydrolyzed Vegetable Protein for Chloropropandiols using Selected Ion Storage
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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
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52	GC/MS/MS Analysis of $\beta$ -Damascenone in Rose Oil
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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
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55	GC/MS/MS Analysis of Cytostatic Drugs in Urine
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56	Rapid Analysis of Soils for Hazardous Waste by Direct Sample Introduction;
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58	App Note 58: Identification of Essential Oil Components EI/MS and Mixed Reagent CI/MS
	Key Words: Essential Oils, Flavors & Fragrances, Acetonitrile, Cl
59	App Note 59: GC/MS Analysis for Morphine and Other Opiates in Urine
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	Key Words: MS/MS, 2000

## Advantage Note Index

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	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;
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5	GC/MS/MS Analysis as a Separations Technique;
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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;
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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
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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
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13	Adv. Note 13: Confirmatory Analysis of Melatonin by EI and CI;
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15	Enhanced Molecular Weight Confirmation with Deuterated Acetonitrile CI
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16	Confirmation of Saturated Hydrocarbon Molecular Weight with Acetonitrile and d3-Acetonitrile CI
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