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Varian, Inc. 2700 Mitchell Drive Walnut Creek, CA 94598-1675/USA

325-MS LC/MS Quadrupole Mass Spectrometer MS Workstation Version 6

Software Operation Manual



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Introduction

Overview

MS Workstation software controls the 325-MS, associated Varian chromatography modules and several modules from other vendors. Information in this manual is also on the Help menu.

Additional Manuals

325-MS LC/MS Hardware Manual

This manual, part number 9300017200, provides information for using, maintaining, and repairing the 325-MS. This information is also available in Help.

MS Workstation Software Reference Manual

This Manual, part number 391496300, explains the operation of the MS Workstation Software. These operations include displaying and reviewing chromatographic and mass spectral data, library searching, and quantitative analysis. Although these tutorials use Saturn 2000 ion trap files, they illustrate the principles of MS Workstation software.

MS Data Handling Users Guide

This Manual, part number 395403800, provides post acquisition data handling procedures and tasks. These procedures include identifying internal standards, adjusting integration parameters, identifying target peaks, and generating calibration curves.

Workstation Toolbar

Overview

The MS Workstation Toolbar has the following applications: Methods, Data Files, SampleLists, RecalcLists, SequenceLists, and Reports. Installing additional MS Workstation options, such as Star Finder and Star Custom Report Writer, may add other application buttons to the Workstation Toolbar. The following is a brief introduction to the applications. Later sections explain each in detail.

As the cursor moves over a button, the name of the application appears below the cursor. Click an icon to launch the application.



Name of Application	Large Icons	Description
System Control/ Automation		Monitor instrument status, and perform automated injections and batch recalculations.
View/Edit Methods	C.	View and edit instrument operation, data acquisition, and data handling methods.
Edit Automation Files		Do off-line editing of SampleLists, RecalcLists, and SequenceLists.
Review/Process MS Data		Review chromatograms and spectra; perform library searches, and review and process quantitation results.
Standard MS Reports	No. of Contraction of	Create, edit, and view standard MS reports.
Custom MS Reports	ALL DE	Create, edit, and view customized MS reports.
Security Administration	~	Set MS Workstation security options and passwords.
SMS/MS File Conversion		Convert data files between DOS and Windows formats.
Batch Reporting		Generate standard reports for a group of Data Files by dragging and dropping them on the Batch Report Window.
View/Edit Chromatograms		Review GC chromatograms, edit data handling parameters, and recalculate results.
Standard Chrom Reports	J	Preview standard chromatogram and results reports.
Compound Set Editor	ACS Ed	Create and edit sets of compounds in the MS Data Handling Method allowing activation or deactivation under automation.

Start

Run a sample without using a Sample List.

Quick Link Buttons

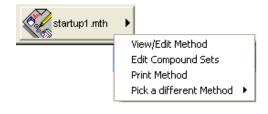
The QuickLink buttons are on the right of the application icons. They correspond to most recently used Data Files and Methods.



Most Recently Used Data File: Display options for the Most Recently Used Data File or select a different data file from the list of most recently used files.

OFN-EL:	ms 🕨	
	View/Edit MS Chromatogram	
	Print Custom MS Report	
	Print Standard MS Report	
	View Custom MS Report	
	View Standard MS Report	
	Pick a different Data File	۲

Most Recently Used Method: Display operations that can be performed on the Most Recently Used Method or a different method file selected from the list of most recently used method.



Workstation Toolbar Options

Moving the Workstation Toolbar

Move the Workstation Toolbar to any edge of the Windows screen. Click an area of the Workstation Toolbar that does not contain Application or QuickLink buttons and drag the toolbar to the preferred edge of the screen. The next time you open Workstation the toolbar will be at that spot.

Workstation Toolbar Menu

Put the cursor over an area of the Workstation Toolbar without an application or QuickLink button, and right-click to display the menu.



Move to Windows Taskbar

Display the Workstation Toolbar as a Windows Taskbar icon.

Click **Move to Windows Taskbar** from the **Workstation Toolbar** options menu and the Taskbar icons appear in the lower right of the Windows Taskbar. Click the **Workstation Toolbar** icon to display the options menu.

Show/Hide Applications on Toolbar

Select the applications for the Workstation Toolbar. The left side lists the applications shown on the toolbar and the right side list the applications that are installed but not shown on the toolbar.

- To remove an icon from the toolbar, select it from the top list and, then click **Remove**.
- To add an icon to the toolbar, select it from the bottom list, and then click **Add.**

Applications shown on Toolbar Select applications you wish to remove from the Toolbar and click on the Remove button. Batch Reporting		Applications not shown on the Toolbar Select applications you wish to add to the Toolbar and click on the Add button.
Compound Set Editor Dustom MS Reports Edit Automation Files Quick Startt Review / Process MS Data Security Administration SMS/MS File Conversion Standard Enrom Reports Standard MS Reports Standard MS Reports System Control / Automation View / Edit Chromatograms View / Edit Chromatograms	>>Remove>>	PolyView 2000
	< <add<<< td=""><td></td></add<<<>	

Enable/Disable Instrument Modules

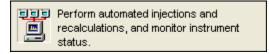
Enable instrument modules. When an instrument module is available, it appears in System Control. If the corresponding instrument is connected and on, you can create a Method section and format reports. The list on the left shows the instrument modules that are currently installed and enabled. The list on the right shows the instrument modules that are installed but not enabled.

- To disable an instrument module, select it from the list on the left, and then click **Disable**.
- To enable an instrument module, select it from the list on the right, and then click **Enable**.

istrüment Modules currently enabled elect instrument modules you wish to d	isable.	Instrument Modules currently disabled Select instrument modules you wish to enable.
2000 Mass Spec 200 MS Mass Spec 212-LC Solvert Delivery System 212-LG ranoL System 240-MS/4000 Mass Spec 3900 GC 3900 GC 450-GC 450-GC 450-GC 450-LG AutoSampler 500 MtoSampler 200 AutoSampler ADC Board CombiPAL AutoSampler Mistral Column Oven ProStar 230 Inter SDM ProStar 230 Inter SDM	ų	isable>> 2002 Micro-GC 2003 Micro-GC 3400 GC 3600 GC 3600 GC 3900 Micro-GC 3900 Micro-GC 3900 Micro-GC 9001 Solvent Delivery System 9001 Solvent Delivery System 9001 Solvent Delivery System 9010 Solvent Delivery System 9010 Solvent Delivery System 9011 Solvent Delivery System 9010 Solvent Delivery System 9012 Solvent Delivery System 9010 Solvent Delivery System 9010 LV-Vis Detector 9100 AutoSampler 4:200 AutoSampler 4:200 AutoSampler 4:200 AutoSampler ProStar 330 PDA Detector ProStar 520 Column Oven ProStar 520 Column Oven
ProStar 240 Inert SDM ProStar 240 SDM ProStar 310 UV-Vis Detector	~	

Application Descriptions

When checked, a description of an application is displayed when the cursor moves over the icons.



Small Toolbar Buttons

When Small Toolbar Buttons is enabled, a smaller version of the Toolbar opens. Notice that the graphics for some icons are slightly different.

🛒 🖿 🎫 📾 💊 🎎 🗱 🛋 📥 📽 🜌 🗖	ANALYS2.RUN
-------------------------	-------------

Run Application

List the applications in the Workstation Toolbar. Click to open an application.

Help on

Select an item from this list to display the online help.

Pick Data File for QuickLink

List of the eight most recently used data files in order of use. Select a data file, to display it in the QuickLink button.

Pick Method for QuickLink

List of the eight most recently used Methods in order of use. Select one to display it in the QuickLink button.

Help on Workstation Toolbar

Displays help for the open application.

Product Support Web Site

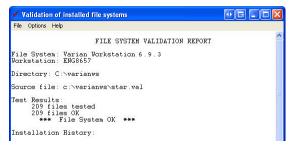
If you have Internet access and a web browser installed on your computer, click to open the MS Workstation Product Support Web Site. It has the latest software and documentation updates for the MS Workstation suite of products, and notes, tips, and answers to frequently asked questions. Visit this site periodically for new information.

About Workstation

About has information about the MS Workstation software version, installation history, and a list of the instrument modules installed. The following is an example; your software release may be different.

Enabled Module Drivers 800 Interface Box ADC Board 39%L GC 4900 Micro GC 8200 AutoSampler	<
Installation History MS Workstation S/N: Installed by Varian Inc Organization: Installation date: 11/3/2009 10:55 AM	<
Validate Installed Files	Close

Validate Installed Files: Uses checksums to test and document errors. Print a report documenting the file system tested, the results, the date printed and tested, and the ID of the person logged in (if login IDs are used). You can include a signature line. For more details, refer to online help. The following is an example



Quit

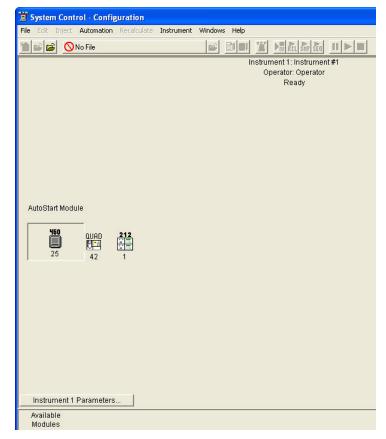
Close the Workstation Toolbar.

System Control

Overview

This section is an overview of System Control. Other functions are explained in detail in later sections.

Double-click anywhere on the Configuration screen to view the Instrument Status and Control window.



Instrument Window

Use the instrument window to monitor the status of the modules assigned to the instrument, monitor data acquisition, inject one or more samples, and do batch recalculations. From the top of the window, these features are System Control Menu, System Control Toolbar, Quad Status window, and various displays.

💆 System Control - Varian LC/MS - Waiting for I	nstrument Modules
File Edit Inject Automation Recalculate Instrument	Windows Help
🗎 🖻 🖆 startup1.mth	
👑 QUAD.42 - Not Ready	
File Method Configuration Tools Reports Help	▰▮◓◓◙◙◙

File Menu

File E	dit	Inject	Automation	Recalculate
Activ	vate	Method	ł	
Uplo	ad /	Active M	ethod from Ma	odules
New	Sar	npleList.		
Ope	n Sa	ampleList	t	
New	Re	alcList.		
Ope	n Re	ecalcList		
New	Sec	quenceLi	ist	
Ope	n Se	equence	List	
Print	ŝ			
Print	er S	Setup		
🗸 Rem	emb	er Last	Open Files	
Exit				

Activate Method: Select an existing method to set the conditions for the connected modules such as the LC, MS, autosampler, and data handling.

Upload Active Method from Modules: Save conditions from the existing modules in a new or existing method. This method becomes the active method.

New SampleList: Create a SampleList.

Open SampleList: Open an existing SampleList.

Look in: 🔀 VarianWS		- 🗲 🗈 💣 💷 -
500-MS Methods 500MSMethods 1200Service 1200sys 4000 4000Service	i 4000Sys Autotune Logs ChromExamples data data_xyz data_xyz debug	Examples Manuals methods MSGLOG MSTutorials SatSys
<		Open
Files of type: SampleL	ists (*.smp)	Cancel

New RecalcList: Create a recalculation list.

Open RecalcList: Select an existing recalculation list.

NOTE: Process a RecalcList in System Control or in MS Data Review. In MS Data Review, from the Quantitation menu, select Process/Review RecalcList. Although Processing a RecalcList takes longer from System Control, you can use AutoLink functions including automated reporting with templates such as EnviroPro, ToxProPlus, and Multicompound Reports.

New SequenceList: Create a sequence list.

Open SequenceList: Select an existing sequence list.

Open a System Co	ontrol Sequence F	ile	? 🛽
Look in: 🔂 Varian	WS	- - E) 💣 🔝 -
500-MS Methods 500MSMethods 1200Service 1200sys 4000 4000Service	4000Sys Autotune Logs ChromExamples data data_xyz debug	Examples Manuals Methods MSGLOG MSTutorials SatSys	Service SYSLOG System UserPML UserPMLSave WSDataFiles
Kile name: Sequ	ences (".seq)		Open Cancel Recent Files >

Print and **Printer Setup** are described in the MS Workstation Software Reference Manual.

Edit Menu

Edit Module Info

Click Edit and select Edit Module Info.

Edit	Inject	Automation	Recalculat
C	ut	Ctrl+X / Sh	ift+Del
Co	ру	Ctrl+C / C	trl+Ins
Pa	aste	Ctrl+V / Sh	ift+Ins
Cl	ear		Del
Ac	bb		
In	sert		Ins
Se	elect All	Ctrl+A / Ctrl	+Enter
Fil	Down		
Ec	lit Notes		
Edit Module Info			

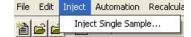
Each module has a message log and a documentation screen. Use the Module Information Editor to record performance, maintenance, hours used, or other comments.

Module Information Editor - C:WarianV	VS\MODULE01.MDF
File Help	
Add Edit Delete	Help Done
Module Description = ? Serial Number = ? Date Installed = ? Date Last Serviced = ? 	

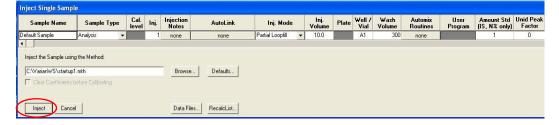
Use Add, Edit, and Delete to record comments.

Inject Single Sample Menu

Use **Inject Single Sample** to enter sample information before making an injection. The Module window must be in acquisition mode before making an injection.



Click **Inject** in the bottom left corner to begin either a manual or an autosampler injection.



Select a Sample Type from the list:

Sample Typ	e
Analysis	-
Analysis	
Calibration	
Verification	
Baseline	

Click Injection Notes to enter a description or comment.

	Notes		
Injection Notes	I		
none			
	ОК	Revert	Cancel

Use **AutoLink** to enter commands and parameters. Enter a command to execute a program after the data file is acquired.

For more information about other fields, see the "SampleList and RecalcList Fields" Section in the *MS Data Handling User's Guide*, part number 3954038.

For example, you can activate a custom MS Report to print the sample reports.

To run a Custom MS Report in automation:

- Type the directory path and the name of the Custom MS Report template (for example C:\VarianWS\EPA525.swt). Note that Custom MS Reports templates should always be stored in the VarianWS directory.
- 2. Click **Browse** to select the command executable file.
- 3. Click **OK** entering a selection.

AutoLink Parameters	
Command	Other parameters
Browse	OK Cancel

Automation Menu

The following shows the Automation menu and describes the options.

Automation	Recalculate	Instrument	W
Begin San	npleList		
Begin Seq	juence		
Begin At 9	Selected Samp	leList Line	
Begin At 9	Selected Seque	ence Line	
Suspend a	Automation		
Resume A	utomation		
Stop Auto	mation		
Reset Mo	dules		
🗸 Enable Au	utomated Print	ing	

Begin SampleList: Automation begins at line 1 and ends at the last line. Open SampleLists from the File menu.

Begin Sequence: Begin an open SequenceList. You can change methods and SampleLists during an automated run. Open the Sequence from the File menu.

Begin At: Start automation at a particular sequence or SampleList line and run until the last line is completed. A SampleList or SequenceList must be open to use this feature.

Suspend Automation: Stop automation after the last completed sample.

Resume Automation: Start automation after it was suspended. Automation starts from where it was suspended.

Stop Automation: Stop automation immediately and reset all modules.

Reset Modules: Bring the various modules to their ready states.

Enable Automated Printing: Allow printer functions to work during automation.

Recalculate Menu

The following shows the Automation menu and describes the options.

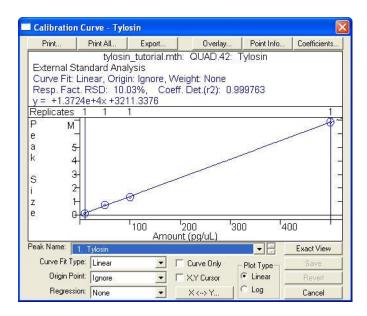
File Edit Inject Automation	Recalculate	Instrument	Windows	Help
🖀 🖻 📄 startup1.mth	Begin RecalcList Begin At Selected RecalcList Line View Calibration Curves			
🖉 QUAD.42 - Not Ready				

Begin RecalcList: Start an automated Recalculation. A Recalculation list must be opened under the File menu.

Begin at Selected RecalcList Line: Process a Recalculation list starting with a chosen line and proceeding with the rest of the list.

View Calibration Curves: View curves for the calibrated compounds in the active method. The Calibration Curve is saved as part of the Method.

NOTE: Recalculations can be done in System Control or in MS Data Review. From the command Quantitation, select Process/Review Recalc List. Processing a Recalc List takes more time when done from System Control than in MS Data Review but has the advantage of allowing AutoLink functions such as automated reporting with Custom MS Reports templates such as ToxProPlus or EnviroPro.



Instrument Menu

The following shows the Instrument menu and describes the options.



Varian LC/MS: The configured instrument. Enter the information in Instrument Parameters in the Configuration screen.

Configuration: Add new modules to the existing instrument.

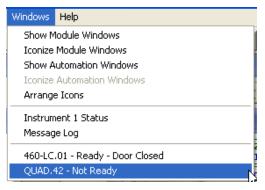
Remove Module Names: Remove associations between Module Names and Module Addresses. This lets you connect a Module with a different Module Name at that address. The next time the associated Module connects at that address, you are prompted to select the correct name for the Module. **Instrument 1 Faults**: View faults in a module of Instrument 1. Click Update to check for new faults.

Instrument 1 Faults	
Module - Address Faults	
Module QUAD.42: 0 Fault(s)	
 Recent Messages and Faults 	Update
C SelfTest Messages and Faults	View
Close	Print

Setup Ethernet Communication: Set up communication between the 212-LC, or other Ethernet modules, and System Control.

Setup COMM Ports: Set up communication between System Control and 212-LC, 460-LC, or CombiPal modules that communicate with the Workstation through the Serial ports on the PC. The first time you start System Control, the Star Communication Configuration Wizard starts automatically.

Windows Menu



Show Module Windows: Display the configured module windows. In this example, the modules are the 212-LC Pumps, the P460-LC, and the Quad.

Iconize Module Windows: Create icons of individual module windows at the bottom of the screen.

Show Automation Windows: View the open automation screens. The possibilities are SampleList, RecalcList, and SequenceList.

Iconize Automation Windows: Create an icon of any open automation screen windows to access windows behind the automation windows.

Arrange Icons: Arrange existing icons in a row at the bottom of the System Control window.

SequenceList: Display the active SequenceList.

SampleList: Display the active SampleList.

RecalcList: Display the active RecalcList.

Instrument 1 Status: View the status of the modules connected to the system.

Message Log: View the event log for the MS module. The log error messages for all configured modules.

Message L	og: MSGLOG1_10-26-2009_13_26_14.MLG 🗖 🗖 🔣
Oct 27 10:12:51	MS Report: No MS Report Method Section in File.
Oct 27 10:15:38	Data File AutomationTest2 10-27-2009 10-13-30 AM Default Sampl
Oct 27 10:15:39	MSDataHandling: No MS Data Handling Method!
Oct 27 10:15:41	MS Report: No MS Report Method Section in File.
Oct 27 10:18:24	Data File AutomationTest2 10-27-2009 10-16-16 AM Default Sampl
Oct 27 10:18:25	MSD ataHandling: No MS Data Handling Method!
Oct 27 10:18:27	MS Report: No MS Report Method Section in File.
Oct 27 10:21:10	Data File AutomationTest2 10-27-2009 10-19-02 AM Default Sampl
	MSDataHandling: No MS Data Handling Method!
	MS Report: No MS Report Method Section in File. 🛛 🗡

The last section of the Windows menu displays the attached modules and their status:

- 460-LC.01-Ready-DoorClosed
- Quad.42: Not Ready

Help Menu

The Help menu has the following options. Click Help to open the Help window.

Help	
He	lp Topics
Pro	oduct Support Web Site
Ab	out System Control
- Million	• • • • • • • • • • • • • • • • • • •

Help Topics

To find help on a topic, do one of the following:

• Click an item in Contents to open online help.

Ip Topics: MS Workstation System Control	?
Contents Index Find	
Click a book, and then click Open. Or click another tab, such as Inde	ex.
System Control Menus	~
System Control Toolbar	
System Control Windows and Dialog boxes	
Sector 2000 MS Module Control	
🔷 4000 MS Instrument Window	
S00-MS Instrument Control	<u> </u>
Superior (1200/1200L/310-MS/320-MS)	
🔷 3400/3600 GC System Control Window	
😻 3400/3600 GC Injecting a Single Sample	
😻 3400/3600 GC Injecting Multiple Samples	
😻 3400/3600 GC System Control Command Reference	
😻 3800 GC System Control Window	
😻 3800 GC Injecting a Single Sample	
😻 3800 GC Injecting Multiple Samples	
S800 GC System Control Command Reference	~
Open Print	Cancel

• Click the Index tab and enter the first few letters of a word. Click the desired selection and click Display.

lelp Topics: MS Workstation System Control	?
Contents Index Find	
1 Type the first few letters of the word you're looking for.	
Π	
2 Click the index entry you want, and then click Display.	
3800 GC Status and Control Window:	<u>~</u>
3800 Setup dialog box 39XL Operation Display 39XL Setup Dialog Box 4000 MS Icon 4900 Channel Display 4900 Channel Display 4900 Run Status 8200 AutoSampler	
8200 AutoSampler 8400/8410 Carrousel Display 8400/8410 SampleList Window Extensions 9100 SampleList Window Extensions 9200 Prospekt Command Table 9200 Prospekt Status and Control Window Acquisition Mode Active Sample Status	~
	1
Display Print	Cancel

• Enter a key word or phrase to list help topics. Click a topic and then click Display.

quad	Clear
Select some matching words to	narrow your search Options
quad Quad	Find Simil
quadrupole Quadrupole quadrupoles	Find No
quadrupoles	Rebuild
Click a topic, then click Display	

About System Control

About has information about the software version, installation information, and a list of the instrument modules installed. About System Control opens the same window as About Workstation Toolbar does. The following is an example.

Enabled Module Drivers 800 Interface Box ADC Board 39XL GC 4900 Micro GC 8200 AutoSampler	~
Installation History MS Workstation S/N: Installed by Varian Inc Organization: Installation date: 11/3/2009 10:55 AM	
Validate Installed Files	Close

Validate Installed Files: Uses checksums to test and document errors. Print a report documenting the file system tested, the success, or failure of the test, the date printed and tested, the ID of the person logged in (if login IDs are used). You can include a signature line. For more details, refer to online help. See "About Workstation" on page 9.

System Control Toolbar

The system control toolbar is near the top of the screen.

🏦 🖨 📴 startup 1.mth 🔰 🖻 🛍 📴 🖉 👪 🚮 🛄 🔲 🔲

The following describes the functions.

ltem	Description
1	Create a new automation file.
1	First button: open an existing automation file.
1	Second button: open the Message Log file.
🖺 startup1.mth 🕨	View/edit or print a Method.
1	Open a Method.
B	Edit notes for an automation file.
	Edit Module information for any online Module.
301	Open Instrument 1 Status
	Inject a single sample.
RCL	Start an open RecalcList.
SHP	Start an open SampleList.
SEQ	Start an open SequenceList
Ш	Suspend a running list.
4	Begin a list.
	Stop a running list.

Quad Status and Control Window

Check the status of the instrument components, monitor data acquisition, and process data using the Quad Status and Control window. Create your preferred window configuration.

File Edit Inject Automation Recalculate Instrument	Windows Help
🗎 🛋 🗈 startup1.mth	▶ 📾 📓 📲 亂 編 編 💷 🕨 Not Ready
🖉 QUAD.42 - Not Ready	
File Method Configuration Tools Reports Help	E

Quadrupole Module Menu

File: Open or print a file.

Method: Edit the current, active Method using Method Builder.

Configuration: Synchronization signal settings and MS Options settings.



Start Out Signal Enabled Wiring When the instrument starts an acquisition, it closes the contact between pins 4 and 5 on Row 2, Column C of the IO connector for 500 msec.	Ready Out Signal Wiring The instrument changess the contact between pins 4 and 5 on Row 1, Column C of the IO connecter when it is ready to acquire data, and reverses it when it is not ready.
Ready In Signal The instrument senses the readiness of another device through Pins 2 and 3 at Row 2, Column A of the ID Connector. When the state between these pins is sensed (open or closed depending upon which radio button is selected), the device is considered ready. Contact open = ready Contact closed = ready	Start In Signal ✓ Varian GC Cable 03-937179-01 GC/QuadMS ✓ Enabled ✓ Varian GC Cable 03-937413-01 Generic/QuadMS or Generic IO Board Assembly CUB06-00047 ✓ Looking for an up edge If the instrument is ready to acquire data, closing the contract between Pins 2 and 3 at Row 1, Column A of the IO connector will start an acquisition.

Sync Signal: Enable or disable the Start and Ready signals for external devices. To enable a Sync Signal operation, connect a cable from the device to the contact closure on the master instrument.

- Click Wiring to view a wiring diagram.
- Select the **Enabled** check box to activate the desired function.

MS Options: Hardware diagnostic tool. View and edit MS Options, and the current software settings.

- Check marks (\checkmark) indicate a Hardware match
- X indicates a Hardware conflict. If an X is present, call your Varian representative.

1	Mass-spec connection:	Automatic	Edit
1	System id :	030608141012	Edit
urrer	nt hardware configuration —		-
1	MS Model:	325(LC)	Override
1	System type:	MS/MS system (Triple quad)	Override
1	Mass range:	10 to 2000 amu	Override
1	Ion source type:	API Ion source	Override
urrer	nt software settings		
1	Scan Optimization:	Standard LC	Edit
1	MS ready:	Enabled	Edit
	Pressure units:	Torr and PSI	Edit
	Print mode:	Color	Edit

Tools: Access MS Tools such as MS/MS Breakdown, PmI Editor, Plot tic, readbacks, Bakeout system, and Overnight Standby.

Tools	Reports	Help
Quit	Macros	
User pml		
Pml	Editor	
Plot	tic,readba	acks,
Ana	Analog output of	
MSN	15 Breakdo	wn
Bake	eout syste	m
Ove	rnight Sta	ndby
Trou	ubleshootir	ng
Inst	setup/oth	iers

Quit Macros: Stop any MS function in progress.

User PML: Enter up to 10 PMLs (macros written in Paw Macro Language).

User PML	5	×
- Select a F	ML	
User 1	fm 10	
User 2	fm 20	
User 3		
User 4		

Run a PML by clicking the corresponding smiley face in the toolbar.



Plot tic, readbacks: Plot a graph of the following: the TIC, the area of 2 masses, 2 readbacks, or a PML to monitor instrument status. Select the **In use** check box and the check box next to the desired parameter, and then click **Apply**.

Plot in pict view	
🗖 In use	Clear Pict View
TIC	
🔲 Area of mass	100.0
Area of mass	200.0
🔲 Readback	1: +5V Digital
🔲 Readback	1: +5V Digital
F PML	3: -5V Analog 4: Power Board +5V Reference 5: +24V RF Current 6: -24V RF Current
	Apply Exit

Analog output: For service use only.

MSMS Breakdown: Optimize the collision energy for any given parent mass.

Bakeout System: Not recommended for the 325-MS

Overnight Standby: Turn off all gases and sets the MS to a low scan mass.

MS Standby	×
Put MS into standby now Exit standby mode	Put MS into standby while idle F Enable auto-standby The MS is idle if the sysem is not on and there is no keyboard input or data collection happening .After the system is idle for the given number of minutes, the system will go into standby and execute the following PML
[60 Minutes PML: OK Cancel

Troubleshooting: Run various troubleshooting procedures. See the hardware manual for more information.

Inst setup/others: Perform initial instrument setup operations.

- Coarse tune: Service use only
- Calibrate pressures: Calibrates the MS EFC/EPC pressures.

Reports: Select a print view setup.

Help: Online Help and the About window.

Quadrupole Toolbar





Choose File to Display: Open and display data files, and edit headers.

Start Collecting Data: Acquire data from only the MS and not other configured modules. Collect data using the current instrument conditions. Ensure that the source and detector are on before you use this feature.

File name:	APR 11 2007 12	2-14 🖉
	Browse	File naming methods
Operator:		
Sample Id:	Default Sample	
Notes:		
		dd 15.0 min. to run

Print: Print the window screen.

Turn Detector On and Off: Control electron multiplier. The color of the icon indicates the following:

- Gray: Off
- Green: On
- Yellow: System in process of turning on or is waiting, for example, a filament delay time. If it turns yellow and then turns gray, a fail-safe has prevented the filament and the multiplier from turning on.

Set Instrument Parameters: Set ion source and analyzer parameters, and trip points to protect the electronics and vacuum shut off. Tabbed views reflect either the configuration.

API Source: Select API on-off sequence, N2 filling time, and Gas pressure readbacks. Select Nebulizing gas type, and set the API Housing, Drying, and Vaporizer gas temperatures. For normal operation mode, the API on-off sequence is set to Automatic.

istrument Parameters	
API Source Analyzer Safety Syringe Pump	
API Analyzer voltage Ionization mode VESI ESI N2 nESI N2 nESI Sec. Equilibration	ce
Voltages on	
T Drying gas 18 psi	
└── Nebulizing gas 55.0 psi	
└── Vortex gas 25.0 psi	
Nebulizing gas type	
Heaters	
API Housing (0-65) 55 actua	l 0 °C (0% on)
Drying gas (50-400) 200 actua	l 0 °C (0% on)
Vortex gas 300 actua	l 0 °C (0% on)
Apply OK	Cancel

Analyzer: Vent the system and set the manifold temperature. Turn the CID gas on and off, set the electron multiplier (detector) voltage, and turn the Extended Dynamic Range on or off.

Pumps High Vacuum Vent	CID gas C On C Off CID gas pressure 2.00 mTorr	Турі	Deg. C to 65) cal: 40 temperature
	Turbo Speed 2.00 mTorr 99% 2.00 mTorr		Deq
Coff Extended Dyr Fixed Optimize Detector optimum = 1 Detector calibration is 30 days Detector gain last cor	namic Range 1500 Volts 890 Volts recommended every	temp 33 Manifol	mal Air erature Deg d Pressure -5 Torr

Safety: There are three sections and only Varian Representatives can change them.

- High voltage enable and Ion Gauge modes
- Trip points of the source pressure, manifold pressure and turbo speed
- Status of the PCB Protect Switch

PCB Protect Switch Trip Points Image: C The vacuum system is in automatic mode. Source Pressure Image: C The vacuum system is in manual mode. Torr	
C The vacuum system is in manual mode.	
HV Enable Manifold Pressu	
Auto Auto 1.00 mTorr	Torr
C On C On Turbo Speed :	ed :
C Off C Off 90 % full s	full spee

Refer to the Hardware manual for more information about the setting up the Syringe pump.

Instrument Parameters
API Source Analyzer Safety Syringe Pump
Syringe pump status: Stopped
StartStopStop
Speed 1.00 ul/Min. (0.01 to 79.41)
Purge cycle Speed 7.89 ul/Min. (0.01 to 79.41)
Syringe type Brand Volume
Add/Delete syringe to/from Standard size list Brand Volume Diameter
mm ID
Save Syringe Delete Syringe
C Other 1.46 mm ID
Apply OK Cancel

Define Scan Method: Set currently active scan parameters for Q1 and Q3, acquisition of centroid or profile data, and detection of positive or negative ions. Refer to Acquisition Method Window for more information.

Scan method: From Scan method: From	m Workstation	×
Method Specs.	Time segment 1 of 2	
Model 325(LC)	Add seg Remove seg Start at retention 0.00 Min.	
Ionization VESI	Scan Time (in Seconds)	
Method run time	Requested Time 13.004 Collect Data Load P Syringe On P CuD gas on Used Scan Time: Inter-Scan Time: Scan Cycle Time: Quad 1 1.0 V Quad 3 1.5 V Copy to all	
Data type C Centroid Profile	Mass List Add Insert Delete Clear All Cut Copy Paste Fill Down FD and I	
Collect delay	CAS Number Compound Name Polarity Q1 First Q1 Last Mass Mass Mass Capillary Collision Req. Dwell Act. Dwell Time	
Use delay	1 1232456 test compound Pos. 325.00 155.00 80.000 25.000 0.066	

AutoTune: Automatically tunes lenses, Q1 and Q3, and calibrates the mass scale. For information on the AutoTune process, refer to Autotune Functions and Options.

AutoTune		
Tune and Calibrate Calibrate Report	Analyzer C Quad 1 C Quad 3 C Both	Polarity C Positive C Negative C Both
Tune to target ratios Calibrate detector (EDR) Stop tuning	lon(s) C Single from list	¥59.000 <u>-</u>
Standard Compound(s)	Ed	lit Standard Compound(s)
Add high mass tune poin Use fixed detector value Comments: To be used for both positivity	ts Don't t	actory compound urn on Calibration Gas
		Cancel

325-MS Module Views



The five default views are:

View I: Displays Quad Instrument status, Profile, Centroid, and Readbacks windows.

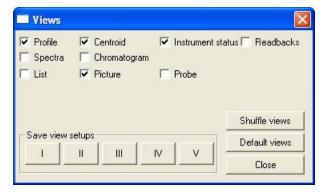
View II: Displays Quad Instrument status, Chromatogram, Spectra, and Centroid windows.

View III: Displays Quad Instrument status, Chromatogram, Spectra, and Profile windows.

View IV: Displays Quad Instrument status, Chromatogram, Spectra, and Library windows.

View V: Displays Quad Instrument status, Chromatogram, List, and Picture windows.

Select Views: Edit the five preset views. Select and display Views in the Quad window, shuffle them between different arrangements, and save them as I, II, III, IV, or V. Reset the settings with the Default button.



Profile: Real time plot of the digital signal acquired over the mass ranges scanned (profile data).

Spectra: Display acquisition of either profile or centroid spectra from the chromatograms.

List: Program for temporary storage of lists of numbers for manipulation or statistical analysis by Paw macro commands.

Centroid: Real time bar graph display of the mass peaks (centroid) of the digital signal acquired over the mass ranges scanned.

Chromatogram: Real time or post acquisition display of chromatograms.

Picture: Display program for graphing and drawing using Paw macro commands.

Instrument Status: Graphic of the analyzer, with temperatures and pressures.

Readbacks: Real time text display of either analog or digital parameters. Readbacks are a valuable resource for fast diagnostics and include voltages, temperatures, pressures, and analog and digital signal values. Monitor Readbacks are plotted in the Pict view, as a function of time other variables. You can include Readbacks a trace in a data file. Readbacks are updated about once every second.

Caution: Readback values are updated once every second whereas the RF and some DC voltages vary significantly faster during scanning or during pos/neg switching. As a result, the only meaningful readback values of these fast varying parameters are obtained when the scan is zoomed in on one mass and when there is no polarity switching.

Probe: GC/MS only and therefore not an option for the 325-MS. Real time text and plot display of either the DIP or DEP probe.

AutoTune

NOTE: The Hardware manual has a detailed description of AutoTune.

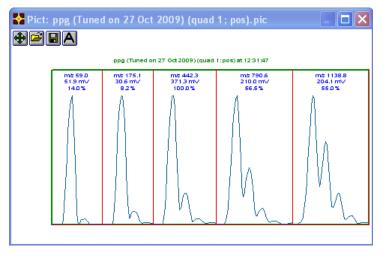
Overview

AutoTune optimizes the sensitivity, resolution, and mass calibration of the instrument.

A reference compound, such as polypropylene glycol (PPG) is used as a tuning solution. A custom tuning solution can also be used.

Autotune Examples

Each quadrupole can be tuned in both polarities (positive and negative). A report with a display of the peak shapes and intensities and a text report with tuning details is displayed and saved to c:\varianws\methods directory. See the following.



The following is a sample of the tune report.

Hardware diag	gnostics re	eport for V	/arian Quad	rupole Mass s	Spec		
Time: 12:20:	54						
No errors det	ected. Pr	roceeding w	vith autotu	ne.			
Tune report f	For Varian	Quadrupole	e Mass Spec				
Time: 12:32:3	31 Tune f	ile: ppg (T	runed on 27	Oct 2009)			
Ionization mo	de:vESI		Scan O	ptimization:	Standard LC	(Fixed det	ector)
Exact	Measured	Peak	Relative	Peak	Valley	Resolution	
	mass	neigni	nergnu	wiuun	~~ c · ``		
(amu)	(amu)	(mV)	(%)	(amu @ 50%)	(% OT 150)	(m/delta-m)	
(amu) 59.0 175.1	(amu) 59.0 175.1	(mV) 51.947 30.563	(%) 13.990 8.231	(amu @ 50%) 0.65 0.73	(% OT 150) 100 52	(m/delta-m) 91 240	
(amu) 59.0 175.1 442.3	(amu) 59.0 175.1 442.3	(mV) 51.947 30.563 371.323	(%) 13.990 8.231 100.000	(amu @ 50%) 0.65 0.73 0.74	(% OT 150) 100 52 53	(m/delta-m) 91 240 599	
(amu) 59.0 175.1 442.3 790.6 1138.8	(amu) 59.0 175.1 442.3 790.6 1138.8	(mV) 51.947 30.563 371.323 209.958 204.091	(%) 13.990 8.231 100.000 56.543 54.963	width (amu @ 50%) 0.65 0.73 0.74 0.75 0.74	(% OT 150) 100 52 53 51 54	(m/delta-m) 91 240 599 1053 1542	
(amu) 59.0 175.1 442.3 790.6 1138.8 (Detector = 1	1120.0	(mV) 51.947 30.563 371.323 209.958 204.091	(%) 13.990 8.231 100.000 56.543 54.963	(amu @ 50%) 0.65 0.73 0.74 0.75 0.74	(% OT 150) 100 52 53 51 54	(m/delta-m) 91 240 599 1053 1542	
(Detector = 1	1138.8 L300 V)	204.091	34.903	0.74	(% or iso) 100 52 53 51 54	(m/delta-m) 91 240 599 1053 1542	
(Detector = 1 Tuning of qua	1138.8 1300 V) ad 1 in pos	sitive mode	completed		34	1342	
(Detector = 1 Tuning of qua	1138.8 L300 V) ad 1 in po:	sitive mode	completed		(% of 150) 100 52 53 51 54	1342	
(Detector = 1 Tuning of qua Tune report 1	1138.8 1300 V) ad 1 in po: 	sitive mode Quadrupole	e completed		34	1342	
(Detector = 1 Tuning of qua Tune report 1 Time: 12:38:2	1138.8 L300 V) ad 1 in po: For Varian 27 Tune f [.]	sitive mode Quadrupole	e completed Mass Spec	0.74 oct 2009)	4L		
(Detector = 1 Tuning of qua Tune report 1 Time: 12:38:2 Ionization mo	1138.8 L300 V) ad 1 in po: For Varian 27 Tune f [.] ode:vESI	204.091 sitive mode Quadrupole ile: ppg (1	e completed Mass Spec Funed on 27 Scan O	0.74 oct 2009) ptimization:	J4 Standard LC	(Fixed det	ector)
(Detector = 1 Tuning of qua Tune report 1 Time: 12:38:2 Ionization ma	1138.8 L300 V) ad 1 in po: For Varian 27 Tune f [.] ode:vESI	204.091 sitive mode Quadrupole ile: ppg (1	e completed Mass Spec Funed on 27 Scan O	0.74 oct 2009) ptimization:	J4 Standard LC	(Fixed det	
(Detector = 1 Tuning of qua Tune report 1 Time: 12:38:2 Ionization mo	1138.8 L300 V) ad 1 in po: For Varian 27 Tune f [.] ode:vESI	204.091 sitive mode Quadrupole ile: ppg (1	e completed Mass Spec Funed on 27 Scan O	0.74 oct 2009) ptimization:	J4 Standard LC	(Fixed det	
(Detector = 1 Tuning of qua Tune report 1 Time: 12:38:2 Ionization mo	1138.8 L300 V) ad 1 in po: For Varian 27 Tune f [.] ode:vESI	204.091 sitive mode Quadrupole ile: ppg (1	e completed Mass Spec Funed on 27 Scan O	0.74 oct 2009) ptimization:	J4 Standard LC	(Fixed det	ector)
(Detector = 1 Tuning of qua Tune report 1 Time: 12:38:2 Ionization ma	1138.8 L300 V) ad 1 in po: For Varian 27 Tune f [.] ode:vESI	204.091 sitive mode Quadrupole ile: ppg (1	e completed Mass Spec Funed on 27 Scan O	0.74 oct 2009) ptimization:	J4 Standard LC	(Fixed det	ector)
(Detector = 1 Tuning of qua Tune report 1 Time: 12:38:2 Ionization mo	1138.8 1300 V) ad 1 in po: 	204.091 sitive mode Quadrupole ile: ppg (1 Peak height (mV) 40.831 23.542 280.980 146.413	e completed Mass Spec Funed on 27 Scan O	0.74 oct 2009) ptimization:	34 Standard LC valley (% of iso) 100 61 49 44	(Fixed det	ector)

Tune Stability

For optimum tune stability, the temperature of the mass analyzer must be stable and at the set point temperature of the manifold before you start AutoTune. This may take several hours if the instrument has not been under vacuum for a long time.

The tuning compound signal must be stable before starting AutoTune (Cal Gas on for at least 10 minutes, or PPG signal stable).

NOTE: It is not necessary to tune the instrument daily. If there is a significant loss of signal, the ion source maybe contaminated. Clean the ion source and AutoTune.

Tune the instrument after cleaning the ion source. This optimizes the instrument and ensures that the instrument is operating properly.

AutoTune Options

Click **AutoTune** on the Quad toolbar. Select the Analyzer and Polarity to tune and/or calibrate.

Tune and Calibrate	Analyzer	Polarity
Tune and Calibrate	C Quad 1	C Positive
Calibrate	C Quad 3	C Negative
Report	Both	Both
Tune to target ratios		
Calibrate detector (EDR)	C Single from list	+59.000 💌
Stop tuning	• All	
tandard Compound(s)	Edit	Standard Compound(s)
ppg		story compound
Add high mass tune poin	ts 🔽 Don't tur	n on Calibration Gas
	in report	
Use fixed detector value		
Comments:		
	ve and negative tuning, ov	er the entire mass range

Tune and Calibrate: Complete optimization of the mass analyzer for sensitivity, resolution, and mass calibration. Set the mass resolution with a peak width of 0.7 amu at half peak height.

A report is generated with a graphic of the peak shapes and intensities (saved with mode name and date) and a text report with detailed mass accuracy and mass abundance.

Calibrate: Update the loaded tune file by re-tuning mass resolution and recalibrating the mass. No changes are made to settings in the ion source or the non-analyzing quadrupole. A new file name and report are created and saved.

Report: Create and save a tune report. Compare with previous reports to determine if re-tuning is required. Creating a report does not change the tune file.

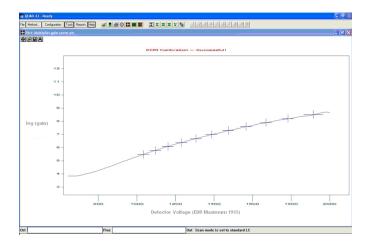
Tune to Target Ratios: Only for a GC/MS in El mode.

Calibrate Detector (EDR): Optimize and calibrate the detector voltages over the entire dynamic range. Run this routine periodically and after replacing the detector.

The detector calibration routine has two parts.

- The first determines the optimal detector voltage for the maximum gain (EDR Maximum).
- The second calibrates the detector gain as a function of voltage for a series of gain increments. This is used to run the detector in the automatic detector adjustment mode, or Extended Dynamic Range (EDR). EDR automatically adjusts the detector voltage to accommodate either small or large ion signals over the entire dynamic range of the mass spectrometer.

The following shows detector gain as a function of detector voltage.



Stop Tuning: Abort the Tuning procedures and restore the most recent Tune file.

Analyzer: Tune each quad individually or tune both. Use the default (Both) setting for triple quadrupole instruments. If using a triple quadrupole as a single quadrupole, select the quadrupole in use.

Polarity: Set AutoTune polarity mode. **vESI**: AutoTune either positive or negative (APCI uses vESI tune file)

lon(s): Select either a specific ion or all defined ions in the Compound list. If choosing Single from the list, select an ion from a list of ions in the Standard compound. Using a single ion adds a new data point to the loaded tune file and does not change existing points. If the same mass is present, it is tuned and changed accordingly. A report for the single mass is created and the tune file is named and saved.

Edit Standard Compound(s): Open Compound Editor and Modify a Standard compound or create a list of new compounds.

Standard Compound(s): List the Standard compounds available.

Cancel: Close AutoTune without executing a function.

Tune File Naming Conventions: AutoTune automatically names and saves each newly created tune file using the following format.

Compound Name/Mode (Date created).dac

If a file by that name already exists the file name increments.

First file:ppg (tuned on 27 Oct 2009 (quad 1: pos) .dacSecond file:ppg (tuned on 27 Oct 2009 (quad 3: pos) .dac

Standard Compound Editor

- 1. In the AutoTune window, select ppg from the Standard Compound(s) list.
- 2. Click Edit Standard Compound(s) to open the following.

ppg Factory cor Comments:			Factory comp	ound	🔽 Def	fault for this mode		New	
Fo be u		th positive a	nd negativi	e tuning, over t	he				
on Sou		-	1 🖂	et Capillary	* For vES	il please u:	se ESI ion s	ource	
Positive			- Cell6		- Negativ				
Jse in Tune	Use in Report	Exact m/z	No. of isotopes	Capillary (Volts)	Use in Tune	Use in Report	Exact m/z	No. of isotopes	Capillary (Volts)
M	$\overline{[v]}$	59.000	1	100.00	M	R	45.000	1	-40.00
M	$\overline{ \nabla }$	175.100	1	100.00	1	$\overline{\nabla}$	469,300	2	-40.00
$\overline{\mathbb{M}}$	$\overline{\mathbb{M}}$	442.300	2	30.00	M	<u>I</u> √	817.600	3	-60.00
$\overline{\mathbb{N}}$	$\overline{\mathbb{V}}$	790.600	3	80.00	M	V	1049.70	3	-80.00
$\overline{\nabla}$	$\overline{\mathbb{V}}$	1138.80	3	100.00		Г	<u> </u>	Γ.	<u></u>
Г			Ĺ			Γ		Ĺ	<u></u>
Г	Г		Ĺ			Г		Ĺ	<u> </u>
Г	Γ		Ē		Г	Γ	<u> </u>	Ĺ.	(
Г	Г		Γ.		Г		<u> </u>	Ĺ.	Í
Г	Г	<u> </u>	Ē	<u> </u>		Г	<u> </u>	Ē	(

Set Capillary: For standard compounds that you create, if the box is checked, set the capillary voltage for each ion to the value in Capillary (volts) field. If not checked or blank, the voltage from the capillary tune table is used.

Capillary (Volts): If the Set Capillary box is checked, set the capillary voltage for each tune ion, otherwise this option is not available.

If tuning with PPG, select Set Capillary and use the default capillary voltage values.

Method Builder

Using Method Builder

Click Method Builder in the MS WorkStation Toolbar.



Edit an Existing Method

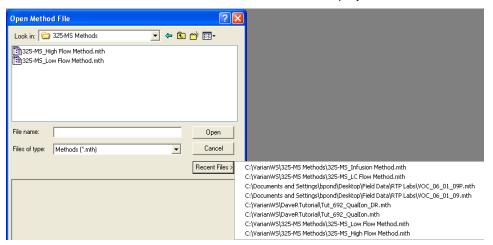
1. Click Open an Existing Method File and then click OK.

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

- 2. Select the method by doing one of the following:
 - Select the folder and then the file name.

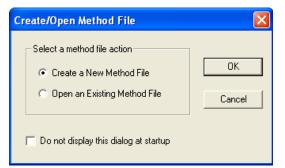
Open Metho	d File		? 🔀
Look in: 🔀	325-MS Methods	• 🗢 🔁	r 📰 🕈
-	gh Flow Method.mth w Flow Method.mth		
File name:	[Open
Files of type:	Methods (*.mth)	 •	Cancel
			Recent Files >

• Click Recent Files to display them and select one.



Build a New Method

1. Click **Create a New Method File** and click **OK** to open the Method Builder Wizard.



- 2. Select Instrument 1. The configured instrument is added to the method.
- 3. Click Next.

	select a custom configuratio continue. Select a Configuration C Custom Instrument 1	n for the method. 1	
--	---	---------------------	--

- 4. Select a detector. After the data is acquired, you can add data processing information to the method.
- 5. Click Next.

	Select the detector(s) for which you want to add post-run processing to the method. Then click Next to continue.
- All Burn	- Detector Modules
D	Quad Mass Spec at address 42
	Unselect All

- 6. Create sections for post-run processing. Add MS Data Handling to enter integration, peak names, and other parameters. Add Standard MS Reports to use the standard reports.
- 7. Click Next.

Select the channel(s) to process:	Select the Post-Run processes to perform:	
Channel 1=MS Data	Standard MS Reports MS Data Handling	
Unse	lect All	

- 8. Review the information on the Confirm configuration screen.
- 9. Click Next.

- 10. Review the configuration:
 - Click **Back** to make changes.
 - Click **Finish** to confirm.

To be added to method 460-LC Control - Address 1 212 Control - Address 2 QUAD - Address 42 QUAD 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.
---	--

11. The new method is displayed with a navigation tree on the left side and the method workspace on the right. Click a tree entry to open that method section.

Method Builder - [Method1*]	
File Edit View Window Help	
Method I Method Notes 460-LC AutoSampler Control - Address 1 Configuration 212-LC Solvent Delivery System Control - Addre Ump Program Quad Mass Spec - Address 42 Quad Mass Spec - Address 42 Quad Mass Spec - Address 42 Channel 1=MS Data Channel 1=MS Data Print Options Results Format Compound Reports Calibration Block Report Format Summary Report Format Summary Report Format Calulations Setup Compound Table Results Treatment	Location: [(Method File Not Saved) Created: Modified: Size: 21580 bytes Method File Attributes Read-only Hidden Archive Requires Password on Save Revision History: [No Revision History]

Add a Varian Instrument to a Method

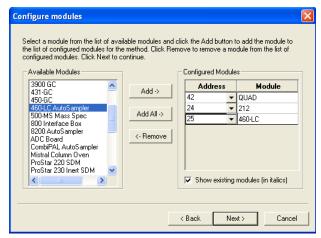
1. Select Create a New Method File and click OK.

elect a method file action	ОК
Open an Existing Method File	Cancel

2. Click Custom and then click Next.

Select a Configuration Configuration Custom Custom C Instrument 1	Iration for the method. Then click Next to
---	--

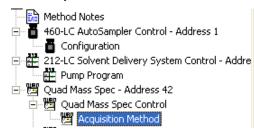
- 3. Select an available module from the list and click Add to make it one of the Configured Modules. Other options are Add, Add All, or Remove.
- 4. Click Next.



5. Continue with step 3 of "Build a New Method" on page 37.

Acquisition Method

Click Acquisition Method in the Method Builder tree.



Set scan segments and define MS experiments. The Mass List has a toolbar that makes completing the table like using a spreadsheet.

odel 325(LC) Inization ESI Use run time Use run time Data type Centroid		llect Data 1 Time (in Second 0.500	CID gas on				ime 0.00	Min. Copy to a FD and		nannel Valio	dation: Enabled
C Profile		CAS Number	Compound Name	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell 📥 Time
🔽 Use delay	1			Pos.	10.00	20.00					0.500
0.0 Min.	2			Pos.							
<u> </u>	3			Pos.							
 Display collected file in Chro 	4			Pos.							
Detector	5			Pos.							
C Use EDR	6			Pos.							
C EDB Maximum	7			Pos.							
1000.0 Volt	8			Pos.							
	10			Pos. Pos.							
Detector off at method end	11			Pos.							
metriod end	12			Pos.							
Scan width in SIM	13			Pos.							
and MRM mode	14			Pos.	1						
0.70 amu	15			Pos.							
	16			Pos.							
No overrides in effect	17			Pos.							
	18			Pos.							•
Advanced Options											
Schedule tMRM						1					

Method Specifications

The following describe the Method Specifications in the Acquisition Method.

Model

Select your instrument Model.

_ Metho	d Specs.
Model	325(LC) 💌
Ionizatio	300(GC) 310(LC) 320(GC&LC
_ Metho	320(GC&LC
	325(LC) 920(GC&LC 💙

Ionization

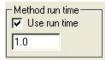
Select the ionization mode: Vortex Electrospray Ionization (vESI), or Atmospheric Pressure Chemical Ionization (APCI), Nano Electrospray Ionization (nESI), or Electrospray Ionization (ESI).

Ionization	vesi 💌
Method	ESI APCI nESI
	vESI

Method Run Time

There are two selections:

- Select **Use run time** to use the run time in the method.
- Type in the minutes to use a time other that in the method. If you do not enter a run time, the data is acquired until the end time is reached in all other modules.



Data Type

Set the scan type (Centroid or Profile).

NOTE: Quantitation can be done in ether Profile or Centroid mode.

Scan		
۲	Centroid	
0	Profile	

Collect Delay

This feature is useful for applications that have an initial clean up step.

To delay data collection after the run starts, do the following:

1. Check Use delay.

2. Enter a time in minutes. The delay time begins when the run starts.

Collect delay-	
0.0	Min.

Display File in Chro

Enable to display data files in the Chro view.

4	Display collected file in Chro
---	-----------------------------------

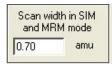
Detector

- Use EDR: (Extended dynamic range) the detector automatically adjusts the detector voltage over the dynamic range. EDR is calibrated during AutoTune.
- **EDR Maximum**: Use the optimum fixed voltage for maximum gain from the last EDR calibration (useful for low-intensity MS/MS experiments).
- **Fixed Voltage**: Enter a fixed voltage for the entire method or different ones for each segment using Advanced Options.
- The detector can be programmed to turn off when the method ends.

De	tector	
	Use EDR	
C	EDR Max	imum
C	1500.0	Volt
Γ	Detector method	

Scan Width

Set the scan window for an ion in single ion monitoring. The default value is 0.70. Increase this value to match the resolution on the scanning quad, see Peak Width. For example, if the resolution is 3.0 on scanning quadrupole peak width, set the scan Width between 0.7 and 3.0 for the largest possible signal.



Time Segment

Use the arrows to select time segments. Use **Add seg** to add a time segment and **Remove seg** to remove a time segment from the scan method. Start at Retention Time defines the retention time start for each segment.

Time segment 1 of 1		
Add seg Remove seg	Start at retention time 0.00	Min. Scan Channel Validation: Enabled

Scan Channel Validation

Indicates if Scan Channel Validation is enabled or not. Also See MS Data Review General Preferences.

Scan Channel Validation: ensures that the scan channel specifications for all compounds are correct after you add or delete transitions in the acquisition method.

- If you change segment acquisition times, scan channel validation automatically updates the compound retention times.
- If you select a compound that specifies a deleted transition, a warning message opens. The missing transition is referred to as "255" in the compound table.

 If you process a compound table that specifies a missing transition, the following message is logged, "Invalid scan function Cannot Quantitate".

The following show the part of the Method Builder window with Scan Channel Validation Enabled.

Γ	- i ime s	egme	ntiori					
	•	•	Add seg	Remove seg	Start at retention time 0.00	Min.	Scan Channel Validation:	Enabled

Scan Time

Set the scan speed of the MS. The scan speed is the amount of time the analyzing quadrupole does the scan. For full scan or MS/MS scan experiments, set the scan time to 0.5 seconds. More data points are acquired with shorter Scan Times. Increasing the scan time increases the signal to noise ratio since the quadrupole spends more time on the particular ion. For SIM, SRM, and MRM, decreasing the scan time allows more transitions to be scanned across a chromatographic peak.

0.500	-Scan Time	(in Seconds)
	0.50	0

Collision Cell Gas

Enable turn the CID gas (such as Argon) on for MS/MS operations.

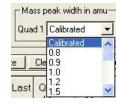
CID gas on

Peak Width

Set the resolution for Quadrupole 1 (Q1) or Quadrupole 3 (Q3).

Calibrated: the value from the most recent AutoTune file is the default setting. It is appropriate for most applications (Scan, SIM).

For SRM and MRM reactions in MS/MS mode, the peak width of Q1 can be increased while keeping Q3 calibrated or smaller. The signal increases by allowing Q1 to transmit a wider peak width while Q3 detects the product spectra in a narrower peak. Determine a balance between sensitivity and selectivity.



Mass List

Enter the desired mass range (Full Scan), single masses (SIM), and precursor and product masses (MS/MS).

Q3 is used for triple quadrupole instruments only.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	105.00	185.00					0.500

Ion Polarity

Click the Ion Polarity entry to switch between positive or negative ion detection.

	Polarity
1	Pos.
2	Neg.

Capillary

Enter the absolute value of the Capillary volts for each ion in the scan method. Method Builder enters the correct sign.

(apillary
	20.000

Collision Energy

Determine the Collision Energy for each reaction and enter it in the Mass List. It defines collision energies in electron volts for specific MS/MS reactions used in the collision cell.

7.5	llision nergy
	5.000

Requested Dwell Time

Specify the Requested dwell time of each scan channel. The shorter the dwell time the greater the number of data points for the transition. Specify either the scan time or the dwell time. If there are multiple full scan ranges in a single method segment, they must all scan at the same rate; despite the requested dwell time. The software gives each mass range a dwell time according to the overall Scan Time and the relative sizes of each full scan range.



Mass List Toolbar

Enter values in the Mass List in a similar manner as a spreadsheet. The options are Add, Insert, Delete, or Clear All lines; Cut, Copy, or Paste from a cell or a line. Other options are fill down a column, and fill down or increment the values in a column (FD and I). The Mass List can be copied to or from an Excel spreadsheet.

				10.00 AS				
Add	Insert	Delete	Clear All	Cut	Copy	Paste	Fill Down	FD and I

Advanced Options

Use Advanced Options to access parameters for other features. These vary by ionization mode, instrument, and the optional ion sources (APCI, ESI, and nESI). Use overrides when doing MS/MS breakdown curves. This provides a setpoint for the system to check.

The advanced options available depend on the ionization mode.

vESI: Overrides, Valve and Syringe Pump, Drying Gas, Vortex Gas, User Analogs In/Out, PMLs.

APCI: Overrides, Valve and Syringe Pump, Drying Gas, Vaporizer Gas, User Analogs In/Out, PMLs.

vESI Overrides

Enter values to override those specified in the method for specific time segments.

Advanced options [.mth] Overrides Valve and Syringe Pump Drying Gas V	ortev Gas I	llear àn:	alogs In/Dut PMLs		
	ontoin arao [and the set of the set		
▲		Copy	to all segments		
Disable all					
Use Re	eset to defa	aults	1		
Override	1000	v	Copy to all		
Needle voltage positive	4000	v	Copy to all		
Needle voltage negative	-4000	v	Copy to all		
Spray Shield voltage positive	600	v	Copy to all		
Spray Shield voltage negative	-600	v	Copy to all		
Spray Chamber temperature	55	с			
Drying gas temperature	200	с	Copy to all		
Vortex gas temperature	300	с	Copy to all		
CID gas pressure	1.50	mTorr	Copy to all		
Nebulizing gas pressure	55.0	psi	Copy to all		
Drying gas pressure	18.0	psi	Copy to all		
Vortex gas pressure	25.0	psi	Copy to all		
Display Pressure Unit	si C	Pa	C Torr,Pa		
ОК	Cancel	1			
	24100				

APCI Overrides

Enter values to override those specified in the method for specific time segments.

Advanced options [.mth]											
Overrides Valve and Syringe Pump Drying Gas Vaporizer	aas User Analo	ogs In/Out PMLs									
Time segment 1 of 1											
Disable all											
Use Reset to a	lefaults										
Detector 10	00 V C	Copy to all									
Corona Current Positive 2.	00 uA C	Copy to all									
Corona Current Negative -2.	00 uA 🖸	opy to all									
Spray Shield voltage positive	00 V 00	opy to all									
Spray Shield voltage negative	00 V C	Copy to all									
Spray Chamber temperature	65 C										
		opy to all									
Vaporizer gas temperature		opy to all									
		opy to all									
		opy to all									
		Copy to all									
Vaporizer gas pressure 18	.0 psi _C	Copy to all									
Display Pressure Unit 📀 Torr,Psi	C Pa C	Torr,Pa									
OK Can	el										

User Analog In/Outs

Use the Analog outputs to send a TIC signal to a device such as a LIMS system.

Use the User Input Traces to acquire data from another detector such as an Evaporative Light Scattering Detector.

Analog output #1 [User I/O conr	nector-> Signal = pin 14 , Gnd = pin 32 +/-5 Volts)
No output	
C Output TIC	
C Output TIC * 10	
C Output TIC x 100	
C Output TIC x 1000	
Analog output #2 (User I/O conr	nector-> Signal = pin 33 , Gind = pin 15 +/-5 Volts)
No output	
C Output TIC x 1	
C Output TIC x 10	
C Output TIC x 100	
C Output TIC x 1000	
Collect User Traces	
User Input 1	Chro label:
User Input 2	Chro label:
☐ User Input 3	Chro label:
Readback Detecto	Chro labet
IT PML	Chro labet

PMLs (Paw Macro Language)

MS Workstation provides PML macros you can use before or after a time segment. Modify and create PMLs through Tools in the Quad Module View.

Time segment 1 of 1	Copy to all
	Browse
PML to be executed after the file is collecte	d
	Browse

Valve and Syringe Pump

Use the six-port valve or the syringe pump or both. The six-port valve can be used in Manual, Diverter or Injector mode. Set the flow rate for the syringe pump.

vESI and APCI Drying Gas Temperature Ramp

Use the SelecTemp[™] feature to ensure the optimum drying gas temperature for the mobile phase composition of vESI, APCI, or ESI runs.

NOTE: If a multi-segment method with a temperature ramp is in progress and there is a segment with a temperature override, the temperature goes to the setpoint for that segment. The ramp continues after the segment is completed.

Drying Gas T	emperature Program in	use
	Temperatures (°C)	Times (minutes)
	Initial temp	Initial Time
	200.0	0.0
Rate (°C / min)	Final temp.	Hold Time

vESI Vortex Gas Temperature Ramp

Enter a vortex gas temperature program to ensure the optimum vortex temperature.

-Vortex Gas Tempera	ature	
volten alds i empere	store	
🗌 Vortex Gas Te	mperature Program ir	i use
	Temperatures (°C)	Times (minutes)
	Initial temp.	Initial hold time
	300.0	0.0
Rate (°C / min)	Final temp.	Hold time
То	tal run time: 0.0 Min.	

APCI Vaporizer Gas Temperature Ramp

Enter a vaporizer gas temperature program to ensure the optimum vaporizer temperature.

	Temperatures (*C)	Times (minutes)
	Initial temp	Initial Time
	100.0	0.0
Rate (°C / min)	Final temp.	Hold Time

Schedule tMRM

Use timed MRM transitions (tMRM[™]) transitions to automatically break up an acquisition method into multiple segments. Using many segments in an acquisition method allows for longer MRM dwell times and therefore increases the sensitivity. See theScheduling Timed MRM Transitions section on page 53 for more information.

Setting up MS and MS/MS Acquisitions

Overview

Please note that in the following figures the first two columns, CAS Number and Compound Number are omitted.

MS Operations

- Line 1: Single Ion Monitoring (SIM) of 250
- Line 2: Full Scan from 100 to 300

		Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
SIM	1	Pos.	250.00				80.000		0.500
Full	2	Pos.	100.00	300.00		()	100.000		0.100

MS/MS Operations

- Line 1: Selected Reaction Monitoring (SRM)
- Line 2: Precursor Scan
- Line 3: Neutral Loss Scan
- Line 4: Product Ion Scan

		Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
SRM	1	Pos.	260.00		187.00		80.000	5.000	0.500
Precursor	2	Pos.	50.00	400.00	95.00		100.000	10.000	0.100
Neutral Loss	3	Pos.	100.00	300.00	82.00	282.00	30.000	12.000	0.100
Product Ion	4	Pos.	219.00		50.00	250.00	30.000	5.000	0.100

MS Operations

Full Scan Acquisition: Scan for all ions over a defined mass range. Enter the start and end masses.

Example:

• Line 1: EI Full Scan, from mass 130 to 280

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	130.00	280.00					1.063

Single Ion Monitoring (SIM): Detect one or several ions. This mode is more sensitive than Full Scan.

Example:

- Line 1: Negative SIM 321
- Line 2: Negative SIM 414

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Neg.	321.00						0.500
2	Neg.	414.00						0.100

Mixed Scan Operations: Acquire data in Full Scan and SIM mode at the same time.

Example:

- Line 1: Positive SIM 120
- Line 2: Positive SIM 230
- Line 3: Positive Full Scan from 200 to 400

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	120.00						0.130
2	Pos.	230.00						0.130
3	Pos.	200.00	400.00					0.130

Mixed Polarity Operations: Acquire data in positive and negative ion modes for vESI, APCI, or ESI.

Example:

- Line 1: Positive SIM 120
- Line 2: Positive Scan from 200 to 400
- Line 3: Negative SIM 230
- Line 4: Negative SIM 290

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	120.00						0.130
2	Pos.	200.00	400.00					0.130
3	Neg.	230.00						0.130
4	Neg.	290.00						0.130

Multiple Time Windows: Several time windows (Segments) can be created for an acquisition. You can do any of the previous scan modes, or mixed scan modes in any time segment and select either positive or negative polarity.

Scan Channel Validation: Enable or disable in MS Data Review as follows.

- 1. From MS Data Review, click Preferences and then select General.
- 2. Open the Validation tab, and select the Validate Scan Functions check box or click to clear it.
 - Enabled: If you change the time for the start at retention time value, validation will shift the retention times of all peaks proportionally.
 - Disabled: Changes you make will not be in sync.

Time segment 1 of 1		
Add seg Remove se	Start at retention time 0.00	Min. Scan Channel Validation: Enabled

MS/MS Operations

Product Ion Scan : Scan for fragments of a particular ion. The precursor ion is selected in Q1. In Q3 the start and end masses set the scan range for the product ions.

Example: Precursor ion 219, Product scan 50 -250, and Collision energy of 5.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	219.00		50.00	250.00	ŝ	5.000	0.500

Precursor Ion Scan: The precursor ions of a selected product ion are scanned. A start and end mass is set in Q1 and Q3 is set to the desired product ion.

Example: Precursor scan 100-500, Product ion 195, and Collision energy of 10.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	100.00	500.00	195.00			10.000	0.500

Neutral Loss Scan: Precursor ions are scanned in Q1. Q3 scans the same range minus the neutral loss mass.

Example: For loss of m/z 18 (water); precursor scan 100-300, product scan 82-282. Scan displays product ions resulting from the precursor ions losing a neutral mass of 18.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	100.00	300.00	82.00	282.00		5.000	0.500

Selected Reaction Monitoring (SRM): This process monitors one or more MS/MS transition(s) of a precursor ion fragmenting with a specific energy into a product ion. The example shows a single transition although more can be monitored.

Example: Precursor ion 414, Product ion 219, and Collision energy -10.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	414.00	6	219.00			10.000	0.500

Multiple Reaction Monitoring (MRM): Multiple reaction monitoring is set up like selected reaction monitoring but allows many MS/MS transitions between Q1 and Q3 to be monitored. Set the Requested Dwell Time to allow for full monitoring.

Example:

Line 1: Positive SIM: Precursor 452, Product 225

Line 2: Positive SIM: Precursor 525, Product 315

Line 3: Positive SIM: Precursor 633, Product 380

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	452.00	6	225.00			5.000	0.500
2	Pos.	525.00		315.00		1	10.000	0.100
з	Pos.	633.00		380.00			15.000	0.100

Mixed Polarity MS/MS Operations: Acquisition of MRM in positive and negative ion mode.

Example:

Line 1: Positive SIM: Precursor 480, Product 225

Line 2: Negative SIM: Precursor 612, Product 375

Line 3: Positive SIM: Precursor 452, Product 225

Line 4: Negative SIM: Precursor 688, Product 315

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	480.00		225.00			5.000	0.500
2	Neg.	612.00		375.00			20.000	0.100
3	Pos.	452.00		225.00			5.000	0.100
4	Neg)	688.00		315.00			15.000	0.100

Multiple Time Windows: Several time windows can be set within one acquisition. Each time window allows Full Scan, SIM, MS/MS, or mixed operations. The Request Dwell time can be set.

Scan Channel Validation:

Enable or disable in MS Data Review as follows.

- 1. From MS Data Review, click Preferences and then select General.
- 2. Open the Validation tab, and select the Validate Scan Functions check box or click to clear it.
 - Enabled: If you change the time for the start at retention time value, validation will shift the retention times of all peaks proportionally.
 - Disabled: Changes you make will not be in sync.



Scheduling Timed MRM Transitions

Overview

Use timed MRM transitions (tMRM[™]) transitions to automatically break up an acquisition method into multiple segments. The use of many segments in an acquisition method allows for longer MRM dwell times and therefore increases the sensitivity.

Before you use tMRM, enable the Scan Channel Validation option in the Validation tab of the MS Data Review Preferences General dialog.

You can schedule timed MRM transitions by filling in a blank template or by exporting the MRM data from an existing method. The following describes both:

 To create timed MRM transitions from Method Builder or System Control: In Method Builder, select Acquisition Method, and then click Schedule tMRM to open the Schedule tMRM window. To create timed MRM transitions from the System Control Quad Module window:

Click the **Define Scan method** icon. When the window opens, click the **Schedule tMRM** button in the lower left.

The Schedule tMRM window has the following four areas:

- Create a tMRM spreadsheet template: Create a tab-delimited *.txt file to use as a template in Microsoft Excel® to enter the tMRM transitions.
- Export a Scan Method to a tMRM spreadsheet: Export an existing method (*.mth) to a tab-delimited *.txt file
- Import MRMs to Scan Method: Import a text file (*.txt) with MRM data from Microsoft Excel into Method Builder.
- MRM Import Statistics: Summary information about the last tMRM import action performed.

NOTE: After a method is created with tMRM, make additional changes to the *.txt file and import it again with tMRM.

Method Specs.	Schedule tMRM (TM)
Model 320(GC&LC Ionization ESI Method run time Use run time	Create a tMRM spreadsheet template Click 'Make template' to create a blank spreadsheet Make template
Data type	Export a Scan Method to a tMRM spreadsheet Select a Scan Method to export and click 'Export' C Current method
Collect delay ✓ Use delay	Method file Browse Import MRMs to Scan Method
0.0 Min.	Select a tMRM spreadsheet, define parameters, and click 'Import'
Display collected file in Chro	Spreadsheet file Browse
O Use EDR	Requested scan time 2.000 Seconds
 C EDR Maximum ● 1000.0 Volt 	C Requested dwell time 0.010 Seconds Minimum segment time width 0.500 Minutes
Detector off at method end	Maximum number of segments 60
Scan width in SIM and MRM mode 0.70 amu No overrides in effect Advanced Options	MRM Import Statistics Number of SIM or MRM's in file: 0 Number of time segments: 0 Total acquisition time: 0.00 Maximum scan time: 0.002 Seconds Maximum dwell time per MRM: 0.002 Seconds
Schedule tMRM	Help

Click **Help** to open a window that outlines how to create a tMRM.

Schedule tMRM (TM) Help
To use the tMRM feature, complete the following:
 Create a tMRM Spreadsheet To create a blank tMRM spreadsheet from scratch, click on the "Make Template" button. To extract transitions from an existing method, select a Method and click on the "Export" button.
2) Add MRMs to the Spreadsheet a. Open the tMRM Spreadsheet in Excel® and fill in the missing data for each transition. b. Save the spreadsheet database as a tab-delimited text file.
3) Import MRMs to Scan Method from Spreadsheet a. Select the input spreadsheet name in the "Import MRMs to Scan Method" File name box. b. Select either Scan Time or Dwell Time and fill in the relevant fields. c. Click on the "Import" button to load the MRMs into the Scan Method.
The columns that must be defined in the tMRM Spreadsheet are: CAS Number, Compound Name, Polarity (+/-), Q1 First Mass, Q1 Last Mass, Q3 First Mass, Q3 Last Mass, Capillary (V), Collision Energy (V), Dwell Factor (scalar), Retention time (min.), RT Window width (min.)
ОК

NOTE: The spreadsheet must have the CAS Number, Compound Name and Last Mass columns. You do not need to enter values.

Using tMRM with a New Method

To create tMRM transitions in a blank template, complete the following two-part procedure.

- 1. Create and complete a new spreadsheet template, using the Create a tMRM spreadsheet template area.
- 2. Import the spreadsheet into a new method, using the Import MRMs to Scan Method area.

Creating a tMRM spreadsheet template

To define tMRM transitions in a new method do the following:

- 1. Click Make template in the Create a tMRM spreadsheet template area.
- 2. Enter a file name and click **Save**. A tab-delimited template file is saved in the VarianWS\methods folder.
- 3. Open Excel®, and then open the file.
- 4. Select **Text Files** in the **Files of type** field in the lower part of the **Open** window.

File <u>n</u> ame:		
Files of <u>t</u> ype:	All Excel Files (*.xl*; *.xlsx; *.xlsm; *.xlsb; *.xlam; *.xltx; *.xltm; *.xls; *.xlt; *.htm; *.html; *.mht;	
	All Files (*.*) All Excel Files (*.xl*; *.xlsx; *.xlsm; *.xlsb; *.xlam; *.xltx; *.xltm; *.xls; *.xlt; *.htm; *.html; *.mht; Excel Files (*.xl*; *.xlsx; *.xlsm; *.xlsb; *.xlam; *.xltx; *.xltm; *.xls; *.xla; *.xlt; *.xlm; *.xlw) All Web Pages (*.htm; *.html; *.mht; *.mhtml)	Cancel
-	XML Files (*.xml)	
	Text Files (*.prn; *.txt; *.csv)	

- 5. The spreadsheet has the following fields. The CAS Number, Compound Name, and the Last Mass columns are required. You do not have to put data in these three fields: the others fields must be completed.
 - CAS Number: A string containing the Chemical Abstract Services number for the compound.
 - Compound Name: A string describing the compound
 - Polarity: The polarity for acquisition method:
 - Use '+' or '-' or
 - Use the number 0 for positive and 1 for negative.
 - Q1 First Mass: The mass for Q1, m/z.
 - Q1 Last Mass: Use this only if a mass range is to be filtered in Q1, m/z.
 - Q3 First Mass: The mass for Q3, m/z.
 - Q3 Last Mass: Used only if a mass range is to be passed in Q3, m/z.
 - Capillary: Volts.
 - Collision Energy: Volts.
 - Dwell Factor: Relative factor for setting dwell times. The default is 1.
 - Requested Dwell Time: the Dwell Time is multiplied by the Dwell Factor for each transition.
 - Requested Scan Time: the Dwell Time is based on the number of transitions in the segment and is multiplied by the Dwell Factor for each transition. The range is 0.006 to 14 seconds.
 - Retention time: Minutes in decimal minutes. For example 1 minute and 30 seconds is 1.50 minutes.

	А	В	С	D	E	F	G	Н	- I	J	К	L
												RT
	CAS	Compound		Q1 First	Q1 Last	Q3 First	Q3 Last		Collision	Dwell	Retention	Window
1	Number	Name	Polarity	Mass	Mass	Mass	Mass	Capillary	Energy	Factor	time	width
2	122-39-4	Diphenylami	+	170	0	93	0	68	25.5	9.666667	0.25	0.5
3	122-39-4	Diphenylami	+	170	0	170	0	68	5	9.666667	0.25	0.5
4	60168-88-	Fenarimol	+	331	0	189	0	105	38	9.666667	0.25	0.5

RT Window: Retention Time Window in decimal minutes

- 6. Complete the spreadsheet with the information for your compounds using the MRM parameters in the MRM database or another source, such as an application note, commercial or personal database. Be sure to complete the required fields.
- 7. Use the **Save As** command to save the spreadsheet with a new name. Keep the tab-delimited *.txt file format.

Importing MRMs to Scan Method

To import a completed MRMs spreadsheet to the scan method, do the following:

- 1. From the Schedule tMRM window, click Browse in the Import MRMs to Scan Method area. The Import file name window opens.
- 2. In the **Import file name** window, locate the spreadsheet, and then click **Open**. The file name appears in the **Schedule tMRM** window.
- 3. Select **Requested scan time** or **Requested dwell time**, and enter the time in seconds.
- 4. Enter the **Minimum segment time width** in minutes. This determines the shortest time width that tMRM uses to optimize the method. By using a smaller time width, tMRM breaks the method into more segments.
- 5. Enter the **Maximum number of segments**. This is the maximum number of segments tMRM uses to optimize the method. The maximum value is 60.

Creating tMRM transitions from an Existing Method

If you have an existing method and want to make tMRM transitions, complete the following two-part procedure.

- 1. To make a spreadsheet from an existing method and enter data to complete it, use the Exporting Scan Method to the MRM spreadsheet area.
- 2. Import the spreadsheet into a new method using the Importing MRMs to Scan Method area.

Exporting a Scan Method to a tMRM spreadsheet

- 1. From the Schedule tMRM window, click Browse in Export a Scan Method to a tMRM spreadsheet area.
- 2. Enter the file name of the existing method with the MRMs transitions, and then click **Open**.
- 3. Click **Export** to save the MRMs as a tab-delimited *.txt file in the VarianWS\methods directory.
- 4. Open Excel®, and then open the file.
- 5. Select **Text Files** in the **Files of type** field in the lower part of the **Open** window.

.....

File <u>n</u> ame:		*	
Files of <u>t</u> ype:	All Excel Files (*.xl*; *.xlsx; *.xlsm; *.xlsb; *.xlam; *.xltx; *.xltm; *.xls; *.xlt; *.htm; *.html; *.mht	; 🗸	
	All Files (*.*) All Excel Files (*.xl*; *.xlsx; *.xlsm; *.xlsb; *.xlam; *.xltx; *.xltm; *.xls; *.xlt; *.htm; *.html; *.mht Excel Files (*.xl*; *.xlsx; *.xlsm; *.xlsb; *.xlam; *.xltx; *.xltm; *.xls; *.xls; *.xlt; *.klm; *.xlw) All Web Pages (*.htm; *.html; *.mht; *.mhtml) XML Files (*.xml)	•	Cancel
	Text Files (*.prn; *.txt; *.csv)	~	

- 6. The spreadsheet has the following fields. The CAS Number, Compound Name, and the Last Mass columns are required. You do not have to put data in these three fields: the others fields must be completed.
 - CAS Number: A string containing the Chemical Abstract Services number for the compound.
 - Compound Name: A string describing the compound name.
 - Polarity: The polarity for acquisition method:
 - o Use '+' or '-' or
 - Use the number 0 for positive and 1 for negative.
 - Q1 First Mass: The mass for Q1, m/z.
 - Q1 Last Mass: Use this only if a mass range is to be filtered in Q1, m/z.
 - Q3 First Mass: The mass for Q3, m/z.
 - Q3 Last Mass: Used only if a mass range is to be passed in Q3, m/z.
 - Capillary: Volts.
 - Collision Energy: Volts.
 - Dwell Factor: Relative factor for setting dwell times. The default is 1.
 - Requested Dwell Time: the Dwell Time is multiplied by the Dwell Factor for each transition.
 - Requested Scan Time: the Dwell Time is based on the number of transitions in the segment and is multiplied by the Dwell Factor for each transition. The range is 0.006 to 14 seconds.
 - Retention time: Minutes in decimal minutes.
 - RT Window: Retention time window in decimal minutes

	А	В	С	D	E	F	G	Н	1	J	K	L
												RT
	CAS	Compound		Q1 First	Q1 Last	Q3 First	Q3 Last		Collision	Dwell	Retention	Window
1	Number	Name	Polarity	Mass	Mass	Mass	Mass	Capillary	Energy	Factor	time	width
2	122-39-4	Diphenylami	+	170	0	93	0	68	25.5	9.666667	0.25	0.5
3	122-39-4	Diphenylami	+	170	0	170	0	68	5	9.666667	0.25	0.5
4	60168-88-	Fenarimol	+	331	0	189	0	105	38	9.666667	0.25	0.5

- 7. Complete the spreadsheet with the information for your compounds using the MRM parameters in the MRM database or another source, such as an application note, commercial or personal database. Be sure to complete the required fields.
- 8. Click **Save As**, and then save the spreadsheet with a new name.

Importing MRMs to Scan Method

To import a completed tMRM spreadsheet to the Scan Method, do the following:

- 1. From the Schedule tMRM window, click Browse in the Import MRMs to Scan Method area.
- 2. In the **Import file name** window, locate the spreadsheet, and then click **Open**.
- 3. Select either **Requested scan time** or **Requested dwell time** and enter the value in seconds.
- 4. Enter the **Minimum segment time width** in minutes. This determines the shortest time width that tMRM uses to optimize the method. By using a smaller time width, tMRM breaks the method into more segments.
- 5. Enter the **Maximum number of segments**. This is the maximum number of segments tMRM uses to optimize the method. The maximum acceptable value is 60.

Statistics

The Statistics area displays summary information about the most recent tMRM that was imported.

MRM Import Statistics		
Number of SIM or MRM's in file:	0	
Number of time segments:	0	
Total acquisition time:	0.00	Minutes
Maximum scan time:	0.002	Seconds
Maximum dwell time per MRM:	0.002	Seconds
Minimum dwell time per MRM:	14.000	Seconds

Injecting Samples

This section explains doing a single manual injection and programming multiple injections using the autosampler

To make a single injection, use Inject Single Sample.

To program multiple injections, use SampleList.

Overview

Samples are injected using System Control. There are four steps in data acquisition:

- 1. Create or modify an existing method.
- 2. Set up a SampleList or enter sample information for a single sample.
- 3. Prepare and load the samples on the autosampler.
- 4. Start the run.

Startup / Shutdown

The startup and shutdown procedures and the injection methods are similar for vESI and APCI methods.

Startup

The instrument must be pumped down and running before you begin.

- 1. Click Turn Spray On in the Quad View to turn on the gasses and heaters.
- 2. After the Drying gas temperature reaches at least 75% of the set value, start the LC pumps.
- 3. Wait a few minutes for the temperature to stabilize and click **Analyzer On/Off** to turn on the Detector.



Shutdown

- 1. Turn off the LC pumps.
- 2. Allow the LC pump pressure to drop down and stabilize so the flow into the MS stops.
- 3. Click the Spray icon to turn off the gasses and heaters. The Analyzer turns off automatically when you turn off the source.

vESI: The gas stays on until the temperature drops below 250 °C and then chamber cannot be removed until then.

Syringe Pump and Valve

Use the six port valve to divert flow away from MS, or to make injections. Set the flow of the syringe pump and have the pump shut off at the end of the method.

The Hardware manual shows how to set up the syringe pump.

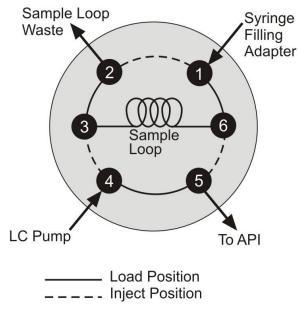
- 1. Click Advanced Options and select the Valve and Syringe Pump tab.
- 2. Select **Manual**, **Diverter**, or **Injector** for the valve. The system can turn the syringe pump off at the end of the run.
- 3. Enable **Use Syringe Pump**, type in the flow rate, and enable the check box **Turn off at end of method**.

Advanced	Options 🛛 🗙
Overrides	Valve and Syringe Pump Drying Gas User Analogs In/Out PMLs
	Use of the Six-Port Valve Manual Diverter Injector Load position required for ready state Syringe Pump Flow Rate 50 uL/min Vuse Syringe and of method
	OK Cancel

Manual Injections

Use the manual injection mode to collect data from the MS. Methods are not run on the configured modules.

Plumb the switching valve as shown.



To do manual injections:

- 1. Monitor the baseline using the **Plot Tic** tool in the **Tools** menu.
- 2. After the baseline stabilizes, click the Traffic light and the Collect data file window opens.

NOTE: The source and detector must be on to use the Traffic light to collect data.

File name:	APR 11 2007 12	2-14 💆
	Browse	File naming methods
Operator:		
Sample Id:	Default Sample	
Notes:		

- 3. Click File naming methods to open the Automatic File Naming window.
- 4. Click Auto-increment. Type in the name of the data file.
- 5. Enter 1 for Sequence #, and 2 for digits.

NOTE: Click **Browse** to review or select an existing file name that you may wish to use as a root for the file name of your sample.

🔲 Collect data f	ile on c:Wari		
File name:	1		
	Browse	File naming methods	
Operator:			🗖 🗖 Automatic File Naming 🛛 🛛 🔀
Sample Id:	Default Samp	ole	C Default name sample
Notes:			C Date and Time
	-	Add 5.0 min. to ru	C Month Day Year and sequence no. (Jan 27 1999 #001)
Start MS St	op MS	Apply OK Cancel	C Month Day and sequence no (Jan 27 #001)
			Sequence # 1
			Auto-increment
			Name Reserpine OK
			Sequence # 1 digits: 2 Cancel

6. Click OK and the Automatic File Naming window closes.

File name:	APR 11 2007 1	2-14
	Browse	File naming methods
Operator:		
Sample Id:	Default Sample	1
Notes:		
		dd 15.0 min. to ru

- 7. Complete the Collect data window.
- 8. Click Apply and if you are ready to collect data, click Start MS.
- 9. Check that the valve is in the load position.
- 10. Overfill the 5 µL switching valve loop with 3 or 4 times the volume. Push the button on the valve cover. Allow at least 1 minute between injections or click the valve icon in the Quad view.
- 11. Stop data collection by opening the Collect data window and clicking Stop MS.

Injecting Single Samples

To do one injection, that uses the configured modules, do one of the following:

- Click Inject Single Sample from the Inject menu
- Click the Inject Singe Sample icon on the System Control menu bar.



Clicking **Inject Single Sample** from the Inject menu opens the following window. The fields are dependent on the sampling device. For more information about these fields, see the "SampleList and RelcalcList Fields" section in the *MS Data Handling User's Guide*, part number 3954038.

Sample Name	Sample Type	Cal. level	lnj.	Injection Notes	AutoLink	Inj. Mode		Inj. Volume	Plate	Well Vial
Default Sample	Analysis 👻		1	none	none	Partial Loopfill	-	10.0		A1
Inject the Sample usi	ng the Method:									
Inject the Sample usi C:\VarianWS\startu				Browse	Defaults					
	o1.mth			Browse	Defaults					
C:\VarianWS\startu	o1.mth			Browse	Defaults					
C:\VarianWS\startu	o1.mth			Browse	Defaults					

Enter the Data File Name and Path

Data File names can have up to 255 characters. They can be a combination of Sample ID, injection date, module name, and injection number.

Click **Data Files** to open the Data Files Generation window.

Data File Generation	
	d by detector modules using this SampleList. the file already exists. Do not include the file 24 25 Example: Sample 1
40005ervice 40005iys 500-MS Methods Autotune Logs ChromExamples data data data vyz D bebug	Use the following symbols to enter the corresponding variable data to the file name. %s = Sample ID %i = Injection number
New Folder Drives:	Xai – Injectom Hainber Xai – Date Xm – Detector Nodule name Xai – Injection Time Xh – Method Name Xo = Operator Name Xn = Instrument Name
OK	Cancel

Select the drive letter and subdirectory (path) to store your files from the left side. The default directory is the data subdirectory of your VarianWS directory.

Create a filename format on the right side. You can combine a text entry with the "%" symbols to specify filenames with variable sample injection information. An example of the filename updates as you enter filename specification.

Select a RecalcList

You can store the files the SampleList creates in a RecalcList, which tracks the names of the created files and other parameters. You can reprocess the RecalcList later, for example, to reprint the reports or to process data with another method.

Click **RelcalcList** to open the RelcalcList Generation window. You can create a new RecalcList, append to an existing RecalcList, overwrite the RecalcList, or not create or update a RecalcList.

RecalcList Generation	
You can automatically create or update a RecalcList with files generated during automated Specify the RecalcList generation options for this SampleList below.	l injections.
C Do not automatically create and update a RecalcList.	
C Create and update a new RecalcList.	
RecalcList name:	Browse
C Append to an existing RecalcList.	
RecalcList name:	Browse
Overwrite the Recalc List each time the SampleList Begins. OK Cancel	

Click **Overwrite the RecalcList each time the SampleList Begins** to overwrite the RecalcList.

Click **Append to an existing RelcalcLis**t, to append a number to the new RecalcList filename.

QuickStart

QuickStart injects a single sample without using System Control directly.

Customize QuickStart to set up instruments for routine use. See online help for more information.

QuickStart starts System Control and waits for all modules to log in before opening.



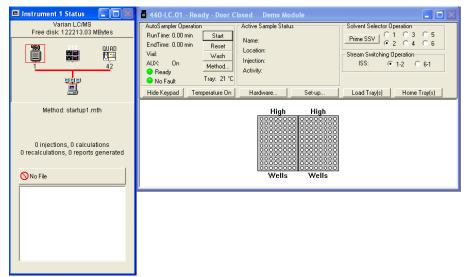
Enter information in the MS QuickStart window about your injection. For more information about the fields, see the "SampleList and RelcalcList Fields" section in the *MS Data Handling User's Guide*, part number 3954038.

MS QuickStart - 1					
File Screen Options					
Instrument Num <u>b</u> er	1				
Instrument Name	Instrument #1				
Operator Name	Operator				
<u>S</u> ample Name	Default Sample				
Sample Description <u>1</u>					
Sample Description 2					
Primary Met <u>h</u> od					
C:\VarianWS\startup1.mth					
Folder for Data File Storag	e				
C:\VarianWS					
Browse 430					
Sample Type	Well/Vial A1				
C Baseline	# Injects 1				
C Calibratio <u>n</u>	Wash Vol. 0				
Analysis	Volume 1				
○ <u>V</u> erification	Amount 1				
Clear Coefficients	Factor 1				
	Multiplier 1				
AutoLink	Divisor 1				
	Level 1				
In	j. Mode Full Loop 🛛 👻				
	Hardware				
Stagt Stop	Help E <u>x</u> it				

Autosampler Display

Each of the supported autosamplers has a graphic representation of the carrousel or tray. In System Control, click the autosampler icon in the Instrument Status Window to open it. Inject from a vial by double-clicking on the appropriate vial in the display.

After the Inject Single Sample dialog opens, enter the information, and make the injection.



Running Priority Samples Using Inject Single Sample

Use either the graphical vial selection or Inject Single Sample to make a priority injection during automated analyses.

- 1. While automation is running, click **Suspend** on the current active SampleList. Automation is suspended after the current run is completed.
- 2. Double-click the vial position of the priority sample or choose inject single sample.
- 3. After completing the priority run, click Resume to re-start the SampleList.

Autosamplers

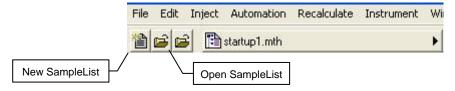
You can create SampleLists for different autosamplers, and use more than one method for your injections.

SampleLists in System Control

- To inject multiple samples from System Control, use a SampleList from the File menu or from System Control.
- To create a new SampleList: On the File menu, click **New SampleList** or click **Open SampleList** to select an existing one.
- For more information about the fields, see the "SampleList and RelcalcList Fields" section in the *MS Data Handling User's Guide*, part number 954038.

File	Edit	Inject	Automation	Recalculate
A	ctivate	e Method	ł	
U	pload	Active M	ethod from Ma	odules
N	ew Sa	mpleList.		
0	pen S	ampleLis	i	
N	ew Re	calcList.	ä	
0	pen R	ecalcList		
N	ew Se	quenceL	ist	
0	pen S	equence	List	
Pi	rint			
Pi	rinter :	Setup		
✔ R	ememl	ber Last	Open Files	
E	xit			

From the System Control toolbar, create a new SampleList or open an existing one



Generic SampleList

If you do not have a sampling device configured, a generic SampleList opens. This SampleList does not have fields for vial positions, sampling options or hardware configuration options.

The SampleList fields are like spreadsheet columns and can be sized by dragging the border. Right-click a column header to display formatting options.

eneric	SampleList: UNTITL	ED SMP	, L			_ / I			
	Sample Name	Sample Type	Cal. level	Inj.	Injection Notes	AutoLink	Amount Std (IS, N% only)	Unid I 🔺 Fac	Add
1		-						2	Insert
2		-							macri
3		-					1		Delete
4		-							Fill Down
5		-							THE DOWN
6		-							Add Lines
							1		Defaults

The Sample Name field remains fixed as you scroll to the right to help you enter information about your samples.

You can enter data handling parameters, such as, the amount for single internal standard calculations, the unidentified peak factor, a multiplier, and a divisor.

If your requirements are more complex (such as multiple internal standards or multiple detectors, which require different entries) use the MultiChannel MultiStandard field.

Each autosampler has a unique SampleList. A SampleList can have more than one autosampler section so it can be used with different autosamplers.

ProStar 420 and 430 SampleList

The ProStar 420 and ProStar 430 SampleLists have generic System Control SampleList fields and fields for operations specific for each AutoSampler.

To build a ProStar 420 SampleList or a ProStar 430 SampleList, click Add.

	Sample Name	Plate	Well / Vial	Wash Volume	Automix Routines	User Program	Amount Std (IS, N% only)	Unid Pe 📥 Facto	Add
1	Default Sample		A1	300	none		1	0	Insert
2	Default Sample		A1	300	none		1	0	mseit
3	Default Sample		A1	300	none		1	0	Delete
4	Default Sample		A1	300	none		1	0	Fill Down
5	Default Sample		A1	300	none		1	0	
6			1						Add Lines
	U U				10 Pi	r			Defaults.

You can enter information common for all samples. You can select the number of entries to be added sequentially to the SampleList. With the ProStar 420, you can specify the first and last vials to be added to the SampleList with the requirement that the first and last vial numbers start with the same Tray Type letter, A, B, C, or D.

With the ProStar 430, you can specify the first and last Row and Column to be added. You can also select to add by Rows or Columns, which determines if the plate is processed from left to right by row or from top to bottom by columns.

Select Partial Loop fill, Full Loop, or μ L Pickup from the Injection Mode field. Select the User Program from this field also. Several fields are disabled when User Program is selected.

For the ProStar 430, use the Plate column to specify the plate that has the well or vial with the sample. Enter a plate number between 1 and 7. This field is disabled unless the ProStar 430 is equipped with the Plate Feeder option.

The ProStar 430 Well/Vial field specifies the sample location as a combination of row letter and column number. The available rows and column numbers depend on the type of plate configured.

- 48 standard vials: A1 through F8
- 96 well plate: A1 through H12
- 384 well plate: A1 through P24

Use the Well/Vial field to randomly access any well or vial. For example, you can periodically recalibrate from a single standard in a fixed location.

For the ProStar 420, the Vial field specifies the sample vial as a combination of Tray type letter and Vial number. The available Tray types and Vial numbers depend on the Tray Segments configured. You can randomly access any vial.

In the Wash Volume field enter a wash volume in the range of 300 to 9999 $\mu\text{L},$ or enter 0 for no wash.

In the Automix Routines field, define a specific automixing program to be done before the sample is injected. When doing an Automix routine, you can program the AutoSampler to aspirate liquid from a well, vial, or a reagent vial, and transfer it to the sample well or vial from which an injection is to be made.

You can mix the sample by aspirating and dispensing specified amounts.

You can program the AutoSampler to wait for a specific time interval. Use the Automix Steps dialog to select Action, Position, Amount, Speed of the syringe, and Height of the needle. The following is an example of an Automix routine.

	Action		Positio	n	Amount	Speed	ł	Height	Comment	
1	Aspirate From	•	Sample	-	10.0 ul	3 (normal)	•	5 mm		Ado
2	Dispense To	•	Sample	-	10.0 ul	3 (normal)	•	5 mm		
3	Aspirate From	•	Sample	-	20.0 ul	3 (normal)	•	5 mm		Inse
4	Dispense To	•	Sample	+	20.0 ul	3 (normal)	•	5 mm		Dele
5	Repeat	•	é.		2 times		•	2 steps		
6	Wait	•		-	1.00 min		•			Impor
7	Rinse+Wash	•		-	100.0 ul		•			
8		•		*			•			
9		•	8	-			•			
10		•		-			•			
	Destination We	ell: [r	none			Destinal	ion	Plate: Sampl	e Plate 🔄	
	Reagent A Via	al: T	None		•	Reage	ent I	Vial: None	-	
	Reagent B Via	al: T	None		•	Reage	nt [) Vial: None	•	

A maximum of 240 steps can be programmed in one Automix routine. After entering all the steps, enter a name for this Automix Program in the box next to Cancel and click **OK**. The program is saved in the SampleList, and can be exported to other SampleLists. Alternatively, Automix Programs from other SampleLists can be imported to the active SampleList using Import.

The User Program column is enabled if selected in the Injection Mode field for the sample. The User Program can do sample handling and injection actions.

When the User Program is selected as the Injection Mode, the following fields are disabled; Injection Volume, Wash Volume and Automix Routines. The User Program steps are used instead of the method parameters. You must use the User Program if the 10mL syringe is selected. The following figure shows lists the User Program Actions. For each specified action, there could be several options such as Position, Amounts, Speed Setting, and Height. Start the run, by selecting **Start run on the INJECT Marker** or selecting **Start run at End of User Program**.

Action		Position		Position		Amount	Speed Setting		Height	Comment	
Aspirate From	1 🔻	Reagent A	•	10 ul	3 (normal)	•	5 mm		Γ		
Dispense To	•	Sample	•	10 ul	3 (normal)	•	5 mm		-		
Wait	•		•	1.00 min		•					
Repeat	-		•	1 times		Ŧ	3 steps				
Wash	•		•	100 ul		•			-		
Valve	-	Injector	•		Inject	•					
Syringe	-	Load	•	100 ul	3 (normal)	•					
Compressor	•		-		Off	Ŧ]				
Set Output	•	INJECT	-			•]				
	-		-	ľ		•					
Destination V											
Reagent A V				_	-		Vial: none				
Reagent B V	ial: r	none			Reage	nt E) Vial: none				
art Run on INJI	ЕСТ	Marker									

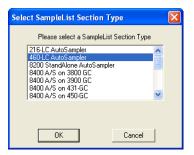
460-LC SampleList

To create a 460-LC SampleList, do the following:

- 1. Click the Edit Automation Files button to open the Automation File Editor.
- 2. Click the New Automation File button and click new SampleList.
- 3. Enter a file name and click Save.

Automation F	ile Editor		• 🖬 🗖	
File Edit Help				
ê 🖬 🖆	a 🕹 🕹 🔁 🖬			
h5	- Consolid int Eile			
Create a nev	v SampleList File			
Save in: 🗀	VarianWS	- + 🗈	💣 🎟 -	
0200sys 325-M5 Me 500-M5 Me 40005ervic 40005ys 40005ys Autotune L	thods 🔂 data_xyz e 🏠 Debug C Examples	methods MSGLOG MSTutorials QuadService quadsys QuadTest	Release SatSys Sorvice SySLOG System TutorialsQuar	
<			>	
File name:	Testsmp		Save	
Save as type:	SampleLists (*.smp)	•	Cancel	
			Recent Files >	

4. Select the 460-LC AutoSampler and click **OK**.



- 5. An empty SampleList with your file name opens.
 - Click **Add** to add one sample.

File	e 6	dit	nation File Editor - Help												•	
<u> </u>	Test.smp - 460-LC SampleList															
			Sample Name	Sample Type	Cal. level	Inj.	Injection Notes	AutoLink	Inj. Mode		lnj. Volume	Tray	Vial / Well	Washes	Aul 🔺 Rot	Add
	F	1	Default Sample	Analysis 👻	•	1	none	none		-	10	Left 🗸	A1	1		Insert
		3		-						•		-	1			Delete
	H	4 5			•				_	• •		-	-			Fill Down
	<u> </u>	6 7		-	-					-		•	1			Add Lines
	<u> </u>	/ 8		-	•					• •		•				Defa <u>u</u> lts
	•	ſ							1 7	-1			1		•	Hardware
	Data Files RecalcList															

• Click Add Lines to add several samples or a plate.

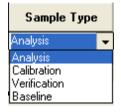
Add Li	ldd Lines to 460-LC AutoSampler SampleList 🛛 🔀																
Sa	mple Name	Sample Type	Cal. level	lnj.	Injection Notes	AutoLink	Inj. Mode	lnj. Volume	Tray	Vial / Well	Washes	Automix Routines	Amount Std (IS, N% only)	Unid Peak Factor	Multiplier	Divisor	MultiChann MultiStanda
Default S	Sample	Analysis 👻		1	none	none	Partial Loopfill 🛛 👻	10	Left 🔻	A1	1		1	0	1	1	none
4																	•
	Number o	f Lines to Add: 96				-	Tray During This Add	Lines Session	n								
~	Number Sampl	e Names from: 1				oer Columns fr umber Rows fr											
A	Add Inser	t Cancel					 By Columns By Rows 										

• Click **Hardware** to specify the tray type for each position, sample loop volume and indicate if the Syringe for Prep Mode is installed.

460-LC AutoSampl		
🔲 Prep Mode (250	ОК	
🔲 84+3 Adapter Ti	ay	Cancel
Left Tray Type:	96 High Wells 💌	
Right Tray Type:	96 High Wells 📃	
Loop Volume:	Not Installed 96 Low Wells 96 High Wells 384 Low Wells 48 Vials	
	48 Viais 12 Vials	

Sample Name: Enter the "root" name for all samples.

Sample Type: Select Analysis, Calibration, Verification, or Baseline from the menu.



Cal. Level: This numerical field is used with Calibration and Verification Sample Types.

Inj. Enter the number of injections in this numerical field.

Injection Notes: Enter a note about the sample or the injections in this text field.

Sar	npleLis				
е	Cal. level	lnj.	Injection Notes	AutoLink	In
•	6	1	none	none	Partial L
96 	Note Test n	s nethod. OK	A Rever	t Cancel	T Pm pm

	Injection Notes
1	Test method A

AutoLink: Add a Command or Other parameters for post run event.

ction Notes	AutoLink	Inj. Mode		lnj. Volume	Tray		Vial
st method A	none	Partial Loopfill	•	10	Right	•	
AutoLink Command Browse.	- 1		ner pa	arameters	ок с	anc	;el

Injection Mode: Select **No Injection**, **Partial Loopfill**, **Full Loop**, or **µL Pickup** from the menu.

Inj. Volume: The 460-LC comes with a 100 μL sample loop. Injections can be made with 1 μL increments.

Tray: Create a sample list for either the Left or Right tray.

Vial/Well: Enter a combination of a row letter and a column number. The available rows and column numbers depend on the type of plate configured.

- 96 well plate: A1 through H12
- 384 well plate: A1 through P24

Wash Volume: Enter 1 to 9 washes.

Automix Routines: Use these routines only with the 84+3 Adapter Tray.

Combi PAL SampleList

HTS PAL for LC/MS has settings for Tray selection, Vial position, and Injection Volume.

	Sample Name	Inj.	Injection Notes	AutoLink	Injection Mode	Tray	-	Add
1	Standard	1	none	none	Automatic 👻	Tray1 🚽		Insert
2		1			-	Tray1		
3					-	Tray2 Tray3		Delete
4					-	Tray4		Fill Down
5								Add Lines
•				1				Defaults

Default SampleList Entries

When you add a new row to a SampleList, default values are entered. To change them, click **Defaults** and the appropriate **SampleList Defaults** dialog box opens. Enter the desired default values and click **Save**.

Volume, rack, and vial number are not displayed, even if an AutoSampler is present.

Saving SampleLists for Later Use

When you make changes to the open SampleList, the changes are automatically saved to the SampleList file and are used for the automated runs in progress. If you want to edit a SampleList other than the open SampleList, use the offline Automation File Editor application described in the next section of this manual.

Using More Than One Method for Injections

Perform automated injections using more than one Method. Either change the active Method in the SampleList or use a SequenceList.

Changing the Method in the SampleList

Acti	ivate Method							\int	AutoLink	
test.	smp - 460-LC Samp	leList								
	Sample Name	Sample Ty	pe	Cal. level	lnj.	Injection Notes	1	utoLink	Inj. Mode	
1	Default Sample	Analysis	-		1	none		none	Partial Loopfill	-
2	Default Sample	Analysis	▼		1	none	7	none	Partial Loopfill	-
3	Default Sample	Analysis	-		1	none	17	none	Partial Loopfill	-
4		Activate Metho	d 🛒				1	460-wash		•
5		Verification	~	Ś						-
6		Baseline Print Calib								-
7		New Calib Bloc	k 🔳							-
8		Autolink								-
8 Q		Activate Metho	d 🔽				_			

Change the Method by activating a Method in a SampleList row.

Activate Method			
		Enter Me	thod Name
Method PathName			
C:WarianWS\420_Dynamax.mth	-		
Browse Select Method	OK	Cancel	

Using the SequenceList Window

On the File menu, select a New SequenceList or Open SequenceList.

File	Edit	Inject	Automation	Recalculate						
Activate Method										
Upload Active Method from Modules										
New SampleList										
0	pen Sa	ampleList								
N	ew Re	calcList								
0	pen Re	ecalcList.								
N	ew Sei	quenceLi	ist							
0	pen Se	equencel	List							
P	rint									
Pi	rinter S	Setup								
✔ Remember Last Open Files										
Exit										

In the SequenceList, select an **Action**. Enter the **Method** and the **SampleList** or click **Browse** to select from a list.

			S	elect Action	Æ	Enter the Method	and	SampleList
Seque	nceList	: test.seq						
		Action		Method		Sample/RecalcList		
1	Inject		▼ 0: ⁴	varianws\420_dynamax.mtl	h	c:\varianws\420_demo.smp		Add
2	Inject		-					Insert
3 4	Inject Reca		_					Delete
4	- Print	dessage Log						
6	Pump	(s) Off						
7	Lamp	(s) Off	-				-	
•							•	Browse
<u>B</u> egin	Susg	end Resum	ie					/
				Browse for a l	Meth	od or SampleList	7	

Monitor Run Status

After making an injection, monitor the run in **System Control.** The status and control window and the Toolbar display the module status.

Message Log

Automation actions and errors are recorded in the Message Log. The most recent entry is at the bottom. Double-click the status bar at the bottom of the instrument window to display the entire Message Log. The log is saved in the c:\VarianWS\MSGLOG directory. Each Message Log entry is stamped with the time and date.

Oct 27 10:12:51	MS Report: No MS Report Method Section in File.
Oct 27 10:15:38	Data File Automation Test2 10-27-2009 10-13-30 AM Default Sampl
	MSDataHandling: No MS Data Handling Method!
	MS Report: No MS Report Method Section in File.
Oct 27 10:18:24	Data File AutomationTest2 10-27-2009 10-16-16 AM Default Sampl
	MSDataHandling: No MS Data Handling Method!
	MS Report: No MS Report Method Section in File.
Oct 27 10:21:10	Data File AutomationTest2 10-27-2009 10-19-02 AM Default Sampl
Oct 27 10:21:11	MSDataHandling: No MS Data Handling Method!
	MS Report: No MS Report Method Section in File.

Automated MS Report Generation

Add Report sections to your Methods to generate automated reports from System Control after each injection, after Recalculations, or by using Print actions in the Sequence window. When Automated Printing is enabled, a checkmark appears in the front of the Enable Automated Printing menu listing. To disable automated Report printing during an automated sequence of injections or Recalculations, click **Enable Automated Printing** from the Automation menu to disable it and remove the check mark. A SCII file is generated, if selected in the Report Method section. When you disable automated printing, automation continues but no reports are printed.

Automation	Recalculate	Instrument	W
Begin San	npleList		
Begin Seq	uence		
Begin At 9	Selected Samp	leList Line	
Begin At 9	Selected Seque	ence Line	
Suspend a	Automation		
Resume A	utomation		
Stop Auto	omation		
Reset Mo	dules		
10/07	dules itomated Print	ing	_

Stopping an Acquisition

During automation and while the system is running you can suspend or stop data acquisition from the Automation menu in System Control.

Suspend Automation: Suspend automation after the current run is completed.

Stop Automation: Stop the current run, reset the modules, and suspends automation.

Resume Automation: Resume automation after suspension.

Reset Modules: Stop the current run, and reset all modules. Automation proceeds to the next injection after all the modules go to the Ready state.



If the Acquisition was started from Start collecting data **1** in the window Toolbar, click **Stop MS** to end data acquisition or in the Automation Menu bar click **Stop Automation**.

File name:	APR 11 2007 1	2-14
	Browse	File naming methods
Operator:		
Sample Id:	Default Sample	1
Notes:		
		dd 15.0 min. to ru

Set a Collect Delay

Use collect delay to start data acquisition after the sample is injected. A separation can be set up to remove buffer from the sample. The solvent or buffer can be diverted to waste and data collection can be started after the valve switches to bring the flow into the MS. In the first 30 seconds of a separation, data collection can be turned off while un-retained compounds that are not of interest are washed off the column. Data collection can start when the mobile phase composition changes and the sample begins to elute.

LC/MS

Use the 6 port valve to divert the LC effluent away from the API Source. Frequently, the solvent front contains un-retained non-volatile salts from the sample. If not diverted, these salts would deposit on the source and the source may require more frequent cleaning. The delay parameter switches the diverter valve to waste for a set time.

While the flow is diverted from the source, you can delay data collection in the Acquisition Method. The syringe pump can be programmed to provide make up flow to the API while the LC effluent is diverted.

Acquisition Method

In Method Builder, open the Acquisition Method. Enabled: Use delay and enter the delay time in minutes.

Collect delay-	
0.0	Min.

MS/MS Breakdown

The Breakdown Curve makes MS/MS method development easy. The sample is introduced into the mass spectrometer API source with a syringe pump. A precursor ion is selected for MS/MS breakdown curves.

In the **Sample Control** window, the precursor ion can be viewed in the profile or centroid mode by entering *SW xxx* in the Ctrl text field (at the bottom left of the window0, where *xxx* is the desired mass.

	miz ado edo	1 1060	, abo	200 600 Ouad 3 100	1400 200	600 1400 Guad 1 ¥
6	Ctrl	Proc		Out		
			Instrument 1 : Nov 09 08:51:22	CPAL: Injecting into LC VIv1		
	Start g. System Control - Vari	4 Automatic Updates				😧 🧐, 💟 🌉 🕅 8:52 AM

To create a Breakdown Curve, do the following:

- 1. On the Tools menu, click **MS/MS Breakdown**.
- 2. Select Q2 Collision Cell Breakdown, or API Capillary Breakdown.

User	Macros pml Editor				
	tic,readba og output	0.0000000000			
MSM	S Breakdo	wn	>		
	out syste might Star				
Trou	bleshootir	ıg			
Inst	setup/oth	ers			

The Create a breakdown curve window opens.

- 1. Enter the precursor ion in Parent mass.
- 2. Click Turn on CID gas. Wait about a minute for it to stabilize.
- 4. Select either **Normal** (0.5 V steps) or **Fast** (2V step) Collection speed. Either is adequate for most conditions.
- 5. To save the data to a method, select **Enable**, select **Save**, choose the number of products to save.
- 6. Click **Browse** to select the method in which to save the product ions with the highest intensities.
- 7. Select Apply, and then Start.

Create a breakdown c	urve		×
This function is used to op mass. It will generate and p Q2 collision energy. (in real	olot the relative intensity of	the product ion	
Parent mass	239		
🔲 Include pa	rent -> parent in breakdow	in curves	
C Auto find p	roduct masses from mass	50 to	230
Use these	product masses	1. I. I.	
Mass 1	197.0		
Mass 2	195.0		
Mass 3	175.0		
Mass 4	132.0		
Mass 5	130.0		
Collection speed	Graph scale	- Smooth curv	/es
C Fast	Absolute	Smooth	
Normal	C Scale each trace	6 Po	pints
Add ions to scan method	19	4	
	Enable Brow	vse	
C Save	est ions to method M6G_	gluc_recalc	•
Method folder:C:\VarianV	VS\Examples\1200 MS D	ata Files\	
Turn off syringe pump af	ter collecting breakdown (curves	
	top Turn on CID gas		Cancel

The breakdown curves are displayed.

To add additional ions to the method, repeat the process for the next precursor ion. For example, type **SW 790** in the Ctrl line (or SW and whatever precursor ion you choose).

For this additional precursor ion, select **Auto find product masses** or specify the product masses.

To add ions and collision voltages to an existing method, select **Append** and the appropriate method.

One option is to create a breakdown curve by scanning the API capillary.

To create a breakdown curve by scanning the API capillary, do the following:

- 1. Click Create a breakdown cure by scanning the API capillary.
- 2. Enter a range to auto find product masses or enter up to five masses.
- 3. Select either **Normal** (0.5 V steps) or **Fast** (2V step) **Collection speed**. Either is adequate for most conditions.
- 4. To save the product ions to a method, select **Enable**, select **Save**, and choose the number of products to save.
- 5. Click **Browse** to select the method in which to save the product ions with the highest intensities.
- 6. Select Apply, and then Start.

Create a breakdown curve by scanning the API capillary 🛛 🚺
This function is used to optimize the API capillary. It will generate and plot the relative intensity of the product ions vs. capillary voltage.
C Auto find product masses from mass 10.0 to 400.0
Use these masses
Mass 1
Mass 2
Mass 3
Mass 4
Mass 5
Collection speed Graph scale Smooth curves ○ Fast ○ Absolute ✓ Smooth ● Normal ○ Scale each trace 6
Add ions to scan method Add ions to scan method C Save Save Append Megliuc_recalc Method folder:C:\VarianWS\Examples\1200 MS Data Files\
Turn off syringe pump after collecting breakdown curves Start Redraw Stop Apply Cancel

The breakdown curves are displayed.

To add additional ions to the method, repeat the process for the next precursor ion. For example, type **SW 790** in the Ctrl line (or SW and whatever precursor ion you choose).

For this additional precursor ion, select **Auto find product masses** or specify the product masses.

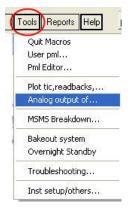
To add ions and collision voltages to an existing method, select **Append** and the appropriate method.

Analog Channels

Two analog channels can be configured in the 300 Series using Analog Out selections or by defining PML outputs.

To use Analog Out, do the following:

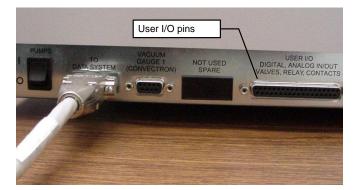
1. Select Analog output of from the Tools Menu.



2. The Analog Out widow opens.

In Use TIC (Total Ion current)	Gain for TIC or mass trace
C Area of mass 0.0	
Ariy FML expression (sno	ould return a value between -5 and 5)
	Example: readback(55) * 1000
Analog output #2 (User I/O conne	Example: readback(55) * 1000
	ctor-> Signal = pin 33 , Gnd = pin 15 +/-5 Volts }-

- 3. Connect the wires to the pins on the User I/O connector on the back of the instrument. For example, in the preceding screen Analog output #1, the signal wire, connects to pin 14 and the ground connects to pin 32.
- 4. Check In Use for the appropriate channel in the Analog Out dialog. The part number of the user I/O Board is CUB06-0047.



Automation File Editor

Overview

Use the Automation File Editor to create and edit SampleLists, RecalcLists, and SequenceLists outside of the MS Workstation System Control application. Access the off-line Automation File Editor and not stop automated runs.

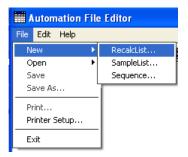
NOTE: SampleLists and Sequences that are active and running in System Control cannot be accessed in the off-line Automation File Editor.

Accessing the Automation File Editor

Click the **Automation File Editor** button **III** on the Workstation Toolbar.

Create or Edit a RecalcList

Select a New RecalcList, or Open an existing RecalcList from the File menu.



The RecalcList window opens. The RecalcList may contain autosampler and sample specific data handling information. The RecalcList contains the Data File name and data file specific data handling information. Fields, common to both the RecalcList and the SampleList, are described in the SampleList section.

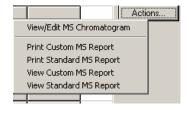
tylosin	_tutorial.rcl - Recalcl	ist									
	Data File	Sample Name	Sample Type	Ca leve		Recalc Notes	AutoLink	Amount Std (IS, N% only)	Unid Peak Factor	Multiplic	Add
1	c:\documents and settings	10_0	Calibration 🔹	·	1 3	none	none	1			Insert
2	c:\documents and settings	50_0	Calibration 🔹	•	23	none	none	1			
3	c:\documents and settings	100_0	Calibration	•	3 3	none	none	1			Delete
4	c:\documents and settings	500_0	Calibration	•	4 3	none	none	1			Fill Down
5				•							
6			-	•							Defaults
7				•							Browse
8				•							Derest
9				•							Report
10				•							Actions

Recalc Notes: Enter notes about the Recalculation of the Data File.

AutoLink: Enter post calculation operations.

Report: Generate a report for the selected data file. Note that if a data file is not highlighted in the Data File column, no report is generated.

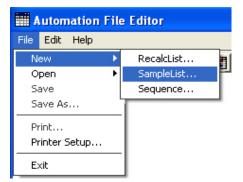
Actions: After generating a report using the Report button, click Actions to show the possible actions.



Create or Edit a SampleList

To create or edit a SampleList, do the following:

1. From the **File** menu, select **New** to create a new SampleList or select **Open** to open an existing SampleList to edit.



2. Select a folder for the SampleList, type a name, and click **Save**.

Create a New System Control SampleList File											
Save in: 🗀 Varian	WS	• 🖬 🍅 🖬 •									
C200sys 225-MS Methods 500-MS Methods 4000Service 4000Sys Autotune Logs	ChromExamples data data_xyz Debug Examples Library	imethods MSGLOG MSTutorials QuadService quadSys QuadTest	Callerate								
<			>								
File name: UNTI	TLED.SMP		Save								
Save as type: Samp	leLists (*.smp)	•	Cancel								

3. Size the Spreadsheet columns by dragging their borders with the left mouse button. Right-clicking a column header displays formatting options. The Sample Name column does not scroll.

T	Auton	nation File Editor - [Untitled.smp]													•	
File Edit Help																	
Untitled.smp - 460-LC SampleList																	
		Sample Name	Sample Type	Cal. level	lnj.	Injection Notes	AutoLink	Inj. Mode		lnj. Volume	Tray		Vial / Well	Washes	Automix Routines	Amount St (IS, N% on	Add
	1		-						-			-					Insert
L	2		•						-			-					
l	3		•						-			• •					Delete
	4		-						• •			-					Fill Down
	5		•	-					÷			÷					Add Lines
	7		-						÷			÷					
l	8		-						Ŧ			-					Defaylts
l	9		•						-		-	-					Hardware
L	10		-						-		-	-					
	•															Þ	
																Data Files	RecalcList

SampleList Fields

Sample Name: Enter the sample name. Click **Data Files** to enter sample names. If only '%s' is listed in the Data Files, the sample name is as you entered it.

Sample Type: Select from the menu.

Sample Type	;	Sample Type					
Analysis	-	Analysis	-				
Analysis	~	Baseline	~				
Calibration	\models	Print Calib	_				
Verification	≡	 New Calib Block					
Baseline		Autolink					
Print Calib		Activate Method	_				
New Calib Block	4	 Print Summary	*				

Cal. Level: Designate the calibration level for a Calibration sample. You can enter up to ten levels.

Inj: Specify the number of injections of the sample. You may make up to nine replicate injections of each sample.

Injection Notes: Enter notes about the sample.

	Note	es			X
Injection Notes	I.				
none					
	ſ	OK	Revert	Cancel	(

AutoLink: Refer to "Inject Single Sample Menu" on page 13.

- In the Vial, Injection Volume, and Injectors Used fields, enter the appropriate information.
- The field beginning with Amt. Std. is only for data files acquired with standard GC detectors.

Select the **Method** for the run. Click **Browse** to select a file.

Other Fields

Add: Adds a new line to the end of the list.

Insert: Inserts a new line before the currently active line.

Delete: Deletes the currently active line.

Fill Down: When sections of columns or entire lines are highlighted, the information in the top highlighted row is repeated to the lowest selected row.

Add Lines: Add several similar lines to the SampleList. Enter the common information in the dialog box. Fields in Add Lines may vary from one autosampler to another. Only the fields associated with the selected autosampler are displayed.

Add Lines to 460-LC	AutoSampler Sa	mpleLi	st															
Sample Name	Sample Type	Cal. level	lnj.	Injection Notes	AutoLink	lnj. Mode	\ \	lnj. /olume	Tra	y	Vial / Well	Washes	Automix Routines	Amount Std (IS, N% only)	Unid Peak Factor	Multiplier	Divisor	MultiChann MultiStanda
	Analysis 👻		1	none	none	Partial Loopfil	-	10	Left	۲	A1	1		1	0	1	1	none
•																		•
Number o	of Lines to Add: 96				Using Left	Tray During This Ar	dd Line	s Session										
Vumber Samp	le Names from: 1				iber Columns fr umber Rows fr		agh: H agh: 11											
Add Inse	rt Cancel					 By Column By Rows 												

For sequentially numbered Sample names, check the box and enter the starting number and the number of entries to add to the SampleList. The Sample Names will have these numbers appended to them.

Defaults: Change the default entries in the table, such as Injection Volume, Injectors Used, etc. After defining new defaults, click **Save** to activate the new defaults.

Data Files: Change the location and name of the Data Files.

RecalcList: Click and generate automatically a RecalcList associated with single sample acquisitions.

Specifying the Data File Name and Path

Data File names can be up to 256 characters long. Sample ID, injection date, module name, and injection number can be embedded in the file name making the Data File name correlate with each sample injection.

Click **Data File** to open the **Data Files Generation** window. Select the path and the file name for the data file.

Data File Generation	
	d by detector modules using this SampleList. the file already exists. Do not include the file Data File names
VARIANWS C 0200sys C 325-MS Methods C 4000Service V4000Sys S 500-MS Methods Autotune Logs C chromExamples data data_xyz Debug New Folder Drives:	Example: Sample 1 Use the following symbols to enter the corresponding variable data to the file name. %s = Sample ID %i = Injection number %d = Date %m = Detector Module name %t = Injection Time %h = Method Name %o = Operator Name %n = Instrument Name
	Cancel

Select the data file directory from the left side. Create a file name specification on the right. Combine text entry with the "%" variable symbols to specify file names with sample injection specific information. An example of the file name is updated as you enter the file name specification.

Specifying a RecalcList

To select the desired RecalcList option, click **RecalcList**. The RecalcList Generation window opens. You can create a new RecalcList, append to an existing RecalcList, or not create nor update a RecalcList. When you select create and update a new RecalcList, the automatically generated RecalcList does not overwrite an existing RecalcList. If a RecalcList with the same file name exists, the newly created RecalcList has a number appended to its file name to make it unique and to prevent the older RecalcList from being overwritten.

	create or update a RecalcList with files generated durin t generation options for this SampleList below.	y automateu injections.
C Do not automatic	ally create and update a RecalcList.	
Create and upda	te a new RecalcList.	
RecalcList name	c:\varianws\untitled.rcl	Browse.
Append to an exi	sting RecalcList.	
RecalcList name		Browse

Changing Default SampleList Entries

When you add a new row to a SampleList, default values are used for each entry.

To change the default values:

- 1. Click Default in the open SampleList window.
- 2. Enter the desired default values and click Save.

Set 460-LC AutoSam	ipler SampleList I	Default	s															
Sample Name	Sample Type	Cal. level	lnj.	Injection Notes	AutoLink	lnj. Mode		lnj. Volume	Tra	y	Vial / Well	Washes	Automix Routines	Amount Std (IS, N% only)	Unid Peak Factor	Multiplier	Divisor	MultiChann MultiStanda
Default Sample	Analysis 💌		1	none	none	Partial Loopfil	-	10	Left	٠	A1	1		1	0	1	1	none
•																		•
Save Canc	el																	

Using Several Methods for Injections

Change the active Method from the SampleList or use a Sequence to perform automated injections using more than one Method.

Changing the Method in the SampleList

Activate a Method in a SampleList row to change the Method.

	Sample Name	Sample Type	•	Cal. level	lnj.	Injection Notes	AutoLink	Inj. Mode		lnj. Volume	Тг	ay	Vial / Well	Add
1	sample 1	Analysis	•		1	none	none	Partial Loopfill	•	10	Left	•	A1	
2	sample 2	Analysis	•		1	none	none	Partial Loopfill	•	10	Left	-	A2	Insert
3	sample 3	Analysis	•		1	none	none	Partial Loopfill	•	10	Left	-	A3	Delețe
4		Activate Method	•				none		•			•		Fill Down
5	sample 4	Analysis	•		1	none	none	Partial Loopfill	•	10	Left	-	A4	Fill DOW
6			-						•			-		Add Lines
7			-						•			-		Defaults.
8			•						•			-		
9			-						•			-		Hardware
10			-					1	•			-		

- 1. Select Activate Method from the Sample Type cell.
- 2. Click AutoLink in the row that you are working. The Activate Method widow opens.
- 3. Enter the name of the Method or click Browse to select the Method from a list.

Activate Method	
Method PathName	
C:\VarianWS\325-MS Methods\325-f	MS_High Flow Method.mth
Browse	OK Cancel

Create or Edit a Sequence

To create or edit a sequence do the following:

1. From the **File** menu select **New** and then **Sequence** or select **Open** and then **Sequence** from the File menu.

e Edit He	alb	RecalcList
New Open		SampleList
Save		Sequence
Save As	1	
Print		
Printer Setu		

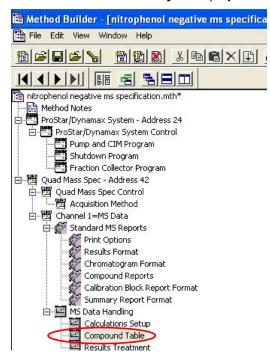
- 3. Click Add to add lines to the SequenceList.
- 4. Enter the required number of Sequence lines.
- 5. Select the **Action** for the selected step.
- 6. Click Browse to select the Method and SampleList files.

U	ntitl	ed.seq - SequenceLi	st			
		Action	Method	Sample/RecalcList		
	1	Inject -		Sample/HecalcList	-1	Add
	2	Recalc 🗸 🗸	c:\varianws\325-ms methods\325-ms			Insert
	3					Delete
	4				-	
	5				-	
	7					
	8	-			•	Browse
4						

Compound Table

Use Compound Table for automatic quantitation with external or internal standards. The Compound Table is part of the data handling method. In the Method Builder tree, click **Compound Table** under MS Data Handling.

Click Show/Hide Directory to display the method full screen.



Creating a Compound Table

- 1. Click Compound Table.
- 2. Click Select Data File, click the data file of your choice, and click Open File. This data file becomes the Reference Data File.
- 3. Click Build Compound List to open the data file in MS Data Review.
- 4. Click the apex of the desired peak in the chromatogram to generate a spectra and place information in the Spectrum List.
- 5. Close MS Data Review.
- 6. Click Import Compound List, and click the spectra from the table and then click Select.

The compound is added to the Compound List.

Double-clicking a cell displays more information.

	RT	IS	Compound ID	Quan Ion	Calculations	Integration	Identification	Ref. Spectrur
1 6	6.491	Г	Benzene, 1,3-dimel	133.9, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	133.9, 77.0, 78.5
2	7.385		Hexachlorocyclope	236.9, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	236.9, 203.0, 20
3 8	8.209		Benzene, 2-methyl-	165.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	165.0, 63.0, 147.
4 8	8.493	X	Acenaphthene-d10	162.2, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	162.2, 164.1, 16
5 8	8.209		Benzene, 1-methyl-	165.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	165.0, 118.9, 89.
6 9	9.447		Benzene, 2,4-dimel	120.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	120.0, 176.0, 16
7	10.650		Benzene, hexachlc	284.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	284.0, 286.0, 28
8	10.965		Simazine	201.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	201.0, 186.0, 17
9	11.054		Atrazine	200.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	200.0, 215.0, 20:
10	11.393		Lindane	183.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	183.0, 181.0, 21
11	11.756	X	Anthracene-D10-	188.1, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	188.1, 189.1, 18
12	13.087		Metribuzin	197.9, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	197.9, 199.0, 14
13	13.255		Alachlor	188.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	188.0, 160.1, 14
14	13.545		Heptachlor	272.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	272.0, 100.0, 27:
15	14.602		Metolachlor	162.1, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	162.1, 238.0, 24
16	14.946		Cyanazine	225.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	225.0, 198.0, 17:
17	14.947		Aldrin	212.2, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	212.2, 263.0, 66.
18	16.384		Heptachlor epoxide	352.9, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	352.9, 354.9, 35
19	17.382		cis-Chlordane	375.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	375.0, 373.1, 37
20	17.755		Butachlor	176.2, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	176.2, 160.2, 18
21	17.956		trans-Chlordane	375.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	375.0, 373.0, 371
22	18.069		trans-Nonachlor	409.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	409.0, 406.9, 411
23	19.208		Dieldrin	79.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20	0.20, Spec	79.0, 81.0, 276.5
								•
ferenc	ce Data f	File:						<u>S</u> elect Data Fil

The Compound table has the following fields; Retention time (Ret), Internal Standard (IS), Compound ID, Quanitation Ion (Quan Ion), Calculations, Integration, Identification, and Reference Spectrum (Ref Spectrum).

- Click Select Data File to open a data file.
- Sort, Add, Insert, Delete, Fill Down, or Restore compounds.
- Other functions are: Build Compound List, Import Compound List, Export Compound List, and Print.
- Before processing calibration files, review the compound table to check that the entries are correct.
- Double-click a cell to open the **Compound Tab** view. In this example, Ret 7.316 was selected.

Compound Attributes

The following shows the **Compound Attributes** tab view after the retention time 7.613 was double-clicked. The Chromatogram and Spectrum Displays are on top and the bottom part has fields. Add information, such as Compound Name and CAS Number, during the peak addition process.

Compound Attributes	Quan Ions Calculations	Integration	Identification	Ref. Spectrum
7.316 2,4-Dimethylphenol 107.	.0, C:1 Linear, Ignor, 1	0.25, W:4.0, S:2	0.20, Spec	107.0, 122.0, 12
	Mat 8.25 8.50 8.75 2 3 4 4 5 2 7 5 2 2 7 5 2 2 2 2 2 2 2 2 7 5 2 7 7 7 5 2 7 7 5 2 2 7 7 5 5 2 2 7 7 5 5 2 2 7 7 5 5 2 7 5 5 2 7 5 5 2 7 5 5 2 2 7 5 5 5 5	0% 5%	Scan: 542, Chai 77.0 107 61725 6 min. Scan: 54 107 77.0 9 394 107 77.0 9 394 107 77.0 9 394 107 77.0 107 107 107 107 107 107 107 107	7.0 219 2 lon: 220 us 7.0 19 - - - - - - - - - - - - -
Compound Attributes Retentign Time (min): 7.316 Compound Name:	с	<u>A</u> nalyte	✓ Active RRI Reference ✓ Identification Re	
2,4-Dimethylphenol CAS Num <u>b</u> er:	67-9 IS to <u>L</u>			<u> </u>
~	<u>G</u> roup Na	me:		_

Edit Name, CAS Number, Retention Time

Edit the Compound Name and CAS fields to describe your compound. These entries are independent of the sample list entries.

Click the chromatogram display to update the Retention Time. The spectrum of the selected scan is displayed. It becomes the new reference spectrum if you select it when you exit the tab dialog, or click **Next**, or **Previous** to move to a different entry.

Identify Internal Standards

By default, compounds are identified as Analytes. Identify Internal Standards by selecting the **Internal Standard** in the Compound Type section, as in previous screen shot. Click **Next**, or **Previous** to check the Compound ID information of the other analytes. If more than one internal standard is identified, select the internal standard for a particular target compound from the IS to Use list.

Identify Group Members

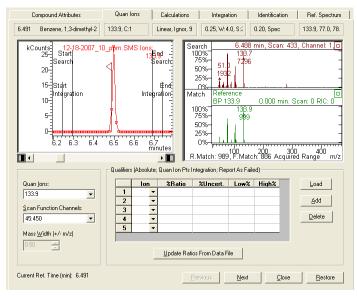
Sometimes target compounds are identified as a member of a group of compounds (for example, Trihalomethanes (THM), alkyl aromatics, etc.).

Enter the desired name in the Group Names field.

If more than one Group is designated in a method, select the Group Name from the list.

Quan lons

Click the **Quan lons** tab in the Compound Table editor. When peaks are added to the Compound Table, the software names the Base Peak (most intense ion) the Quantitation Ion. The plot shows the mass chromatogram for the chosen Quan Ion.



Edit Quan lons

Select a different Quan ion, a sum of ions, or a range of ions.

96.9	-
RIC	
lon(s)	
96.9	

If you selected Ion(s), Select Ions to Plot opens. Select Show Format and Examples to display the ways to specify ions. The Plot is redrawn automatically after you select the Quan Ions. You can determine if the chosen Quan ions integrate cleanly or if there are interferences due to co-eluting peaks or background contamination.

NOTE: The Scan Function Channels field is only relevant for MS/MS or SIM analysis, because quantitation is done only on individual Channels of information.

-Edit Ion Formula		
The valid ion range is from :	2 0 to 2000 0	STATUS: Valid Ion Formu
IONS: RIC	1.0 10 2000.0.	
RIC		
June		
Show Format and Examples	•	Help OK Cance
Format and Examples		
Specify an ion selection by co	ombining one or more	
of the following items, with the	e '+' and '-' operators.	
 Individual Ions (e.g., 40.0 	3)	
- Range of ions (e.g., 40.0:	.60.0)	
- All ions: RIC		
Examples: 28.0	(Plot ion 28.0 only.)	
40.0:450.0	(Plot ions 40.0 through 450.0.)	
RIC-128.0	(Plot all ions except ion 128.0.)	
91.0+128.0	(Plot ions 91.0 and 128.0.)	
60.0:450.0-128.0	(Plot ions 60.0 through 450.0 except ion 128.0.)	

Edit Retention Time

To change the retention time, click the scan in the Chromatogram display.

Look at Other Compounds

Click Next, or Previous to see Quan Ion information for other peaks.

Select Qualifier lons

Click **Load** to enter automatically the three most intense qualifier ion candidates present in the Reference Spectrum.

	lon	1	%Ratio	%Uncert.	Low%	High%	Lo	bac
1	77.0	•	105.5	20.0	85.5	125.5		_
2	78.9	•	97.7	20.0	77.7	117.7	A	dd
3	106.0	•	52.3	20.0	32.3	72.3		-
4		•					<u>D</u> e	le
5		•						

Click Add to add the next most intense reference spectrum ion.

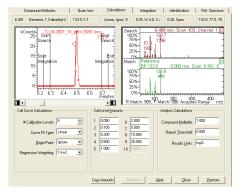
The Qualifiers Table has the following fields:

- **Ion**: Click in the Ion field to see the choices.
- **Ratio**: Shows the intensity of the qualifier ion as a percentage of the chosen Quan ion(s).
- **%Uncert.**: Allowed percentage deviation from the Ratio. You can change the default of 20%. The % Uncertainty allowable range dependents on the Ion Ratio Type setting in the Calculations Setup Dialog (Absolute % or Relative %).
- Low% and High%: For ion 63, the top line in the previous example and the allowed range for the ratio is $87.5.0 \pm 20\%$ (absolute). Therefore 67.5% is the Low% value and 107.5% is the High% value area.

As samples are processed (Calibration, Analysis, or Verification runs), the Qualifier Ion tests are done before the peak is integrated.

Calculations Tab

Click the Calculations tab to display the following.



Enter Number of Calibration Levels and Amounts

Enter the number of calibration levels, information about the calibration curve, the calibration amounts, and results units.

Cali Curve Calculations	Cali Level Amounts	Analysis Calculations
# Calibration Levels: 6 📩	1 10.000 6 160.000	Compound Multiplier: 1.000
	2 20.000 7 1.000	
Curve Fit Type: Linear 💌	3 40.000 8 1.000	Report Threshold: 0.000
Origin Point: Ignore 💌	4 80.000 9 1.000	Results Units: ng/mL
Begression Weighting	5 120.000 10 1.000	
Regression Weighting: 1/nx2 💌		

The Calculations Information is updated in the Compound Table. This information can be automatically entered for the other compounds in the table.

Quan Ion	Calculations	Integration
133.9, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20
236.9, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20
165.0, C:1	Linear, Ignor, 9	0.25, W:4.0, S:20

Click **Copy Amounts** to automatically enter the first value for the other levels. Repeat this process for all standards in the Compound Table.

Select Curve Fit

Select the desired curve fit from the list.

Cali Curve Calculations—		
# Cali <u>b</u> ration Levels:	1	÷
Curve Fit Type:	Linear	•
<u>O</u> rigin Point:	Linear Quadratic Cubic	

For most calibration curves, use either Linear or Quadratic.

Security Administration

Overview

The Security Administration window contains three categories of security parameters. These features are tools for compiling with 21CFR part 11.

- Passwords
- Application Locking
- File Revision Settings

Click the **Security** button on the Workstation Toolbar to open the Security Administration window.



Passwords

asswords A	pplication Locking File Revision Settings
Password fo	or this application
You may sel this applicati	t, change or remove the password required to enter ion.
	Change Password
Change Mai	Mark Decemends
	thod Passwords
- You may sel Methods. C	t or change passwords required to save changes to lick on the button below to select the Method, and the password.
You may sel Methods. C then modify	t or change passwords required to save changes to lick on the button below to select the Method, and the password.
You may sel Methods. C then modify Remove Me You may rer	t or change passwords required to save changes to lick on the button below to select the Method, and the password.

Area	Description
Password for this application	Specify a password required to open the Security Administration application.
	NOTE: There is no way to recover this password if it is lost, so document the password in a secure location.
Change Method Passwords	Method passwords can be added, changed, and removed from the Security Administration application. Click Select Method to browse for and select the desired Method. After the Method is selected, you are prompted either for a new password (if no previous password exists for the Method), or for the old and new password (if the Method already contains a password). NOTE: that if creation of new passwords is disabled in the File Revision tab, you cannot add a new password to a Method that does not already contain one. Change or add Method passwords in the Method Builder and Interactive Graphics applications.
Remove Method Passwords	Security Administrators can remove Method passwords without entering the existing password. This is useful if the password for a Method was lost. Click Select Method, select the desired Method, and the password is removed.

Application Locking

6 Workstation	
Passwords Application Locking File Revision Settings	
Unlocked Applications	1
 Select applications you wish to lock and click on the button. 	Lock
MS Data Review - [Plot Chromatogram MS Data Review - Plot Chromatograms MS Data Review - Plot Chromatograms Program Manager Star Toolbar System Control - MS - Not Ready	<u>*</u>
Locked Applications	
Select applications you wish to unlock and click on the Unlock button.	*
	ick
Done Help	

Area	Description
Unlocked Applications	Lists the top-level windows currently running and not locked. Select any number of them and click Lock.
	They move to the Locked Applications list and their windows are disabled (they will not respond to mouse or keyboard input).
	Note applications locked by the Security Administration stay locked after the Security Administration application closes.
Locked Applications	Lists the top-level windows currently running and locked. Select any number of them and click Unlock. They move to the Unlocked Applications list and their windows are enabled (they respond to mouse or keyboard input).

File Revision Settings

MS Workstation	
Passwords Application Locking	File Revision Settings
Require Revision Log ent are saved.	ry when changes to Methods
Update Revision Log in D date, time and Method na	ata Files with recalculation me.
Enable results deletion fro boxes.	m "Open Data File" dialog
Disable creation of new p	asswords in Method files.
Done	Help

Area	Description
Require Revision Log entry when changes to Methods are saved.	When checked, prompts you for a description of changes when a Method file is altered and saved. The Revision Log appears:
	 Listed in the Notes of the File Open dialog box.
	 Listed in the Method Builder application window when the Method is open.
	Included in the Method printout.
Update Revision Log in Data Files with Recalculation date, time, and Method name.	Data Files are updated with a time stamp and Method name when they are recalculated (either from System Control or from Interactive Graphics). The Log can be included in printed reports.

Area	Description	
Enable results deletion from "Open Data File" dialog boxes.	A button appears in the Open Data File dialog allowing results to be deleted from a specified channel of a Data File.	
	Results deletions are logged in the Data File's Revision Log.	
	This option only affects standard GC results. GC/MS results will not be deleted.	
Disable creation of new passwords in Method files.	When checked, new passwords cannot be added to Methods.	
	Methods with passwords prompt users for their password before saving changes.	

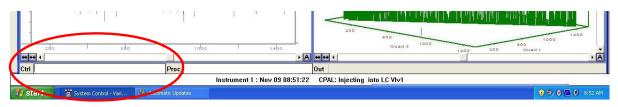
PML

Overview of PML

The procedures that generate information reporting results from the mass spectrometer are written in a language called Paw Macro Language (PML). It has commands that control the instrument or data processing. Users can write procedures to customize data processing.

Simple Commands

Enter simple commands in the **Ctrl** field, bottom left.



Typing "Hello" (quotes included) in the **CTRL** text box prints Hello in the **Out Status** display on the task bar.

CANCEL stops the Paw macro running at the top level.

QUIT stops all paw macros. If you use the *Quit* key word, type *scan* to restart the system.

PML Syntax

The macros are text files with the extension.pml. There are macros in the PML Directory (default: C:\VarianWS\1200sys\PML). If you create your own macros or make new versions of the existing ones, store them in the User PML Directory (default: C:\VarianWS\UserPML). Macros can be edited using the **Pml Editor** described later in this section.

The names can contain letters, numbers, or underlines, but they must begin with a letter.

Run a macro by typing the name of the command lines of the instrument window (CTRL) or including the name in another macro. You can send arguments to a macro and it will return a value.

Paw Macros Consist of Statements:	Examples:
Action or function keywords:	OFF DOZE(2.5)
Macro calls:	CGON SW(219)
Assignment statements:	DETECTOR = 1000 DETECTOR += 50 DETECTOR -= 50 x = COS(PI)
Conditional Statements:	IF (WIDTH > 10) ELSE WHILE (x < 100 & y <= 100) UNTIL (x = 100 y != 100) REPEAT 100 REPEAT
Completing Statements:	END terminates a conditional statement
	RETURN(n) returns from a subroutine with result n
	CANCEL stops the paw macro running "at the top level"
	QUIT stops all paw macros
Printing statements:	?DETECTOR prints the current detector value on the display.
	"Hello" prints Hello on the display.

The Statements Refer To:

numerical values	e.g.	20, -3.5, 1000H
variable keywords	e.g.	DETECTOR
function keywords	e.g.	COS
action keywords	e.g.	FISH
macros	e.g.	STANDBY
user variables	e.g.	X,Y
arguments	e.g.	%1,%2,%3,%4,%5

Notes

- 1. Statements are separated from each other by colons, semicolons, or line breaks.
- 2. Conditional statements must have a matching END statement:
 - if $(x < y) \& \sim (x = 2)$: fish : else : scanlens : end
 - if x = 3 : sw(502) : end
 - i = 1 : repeat 100 : sn = i: list(i) = tic : i + = 1 : end

END statements at the bottom of a PAW Macro can be deleted.

- 3. Printing statements (using ? and "") can be strung together. They are terminated with the keyword CR (carriage return).
- 4. Some keywords create text like the printing statements using ? and "."

GET_FILENAME LIB_FORMULA LIB_NAME LIST_TO_STRING SAMPLE_NAME STRING_RESTORE SUB_FILE_NAME VERSION

5. Some keywords re-route the text created by printing statements (using ? and ") to other places than the display.

: "file name" : CR
: "file name" : CR
: "message" : CR
: "compound name" : CR

LIST_RESTORE	: "file name" : CR
LIST_SAVE	: "file name" : CR
MSGBOX	: "label text" : CR : "default": CR:
"Macro": CR	
PICT_LABEL	: "label text" : CR
PICT_TITLE	: "title text" : CR
STRING_SAVE	: "text" : CR (ten user strings)
STRING_TO_LIST	: "text" : CR
SUB_NAME	: "text" : CR

- Statements may include comments. A # ends the statement and starts the comment, e.g., :DETECTOR += 10 # raise the detector to make the peak bigger.
- All numerical values referred to in PAW Macros are real numbers. Usual algebraic syntax is allowed:, e.g., x = (sin(y) + cos(sqrt(detector))) / (30 * month + day).
- 8. When parameters are passed to macros, they are referred to within the macro as %1, %2, %3, %4 and %5. e.g., if the macro TIMES is defined as ?%1 : "times" : ?%2 : "equals" : ?(%1 * %2) : CR and you type "TIMES 7 9" and the instrument will print out "7 times 9 equals 63". NOTE: %1 %5 can be used as local variables within any macro.
- 9. When passing parameters to a macro or keyword, you can leave out parentheses at the top level.
- 10. All these are valid statements:

FISH(3) FISH 3 FISH 3 2 FISH 3,2 FISH(3,2) When assigning a value to a variable or parameter you can leave out the = sign. DETECTOR = 1000 DETECTOR 1000

but: "lens(1) = 100" is ok while "lens 1 100" is ambiguous.

11. When using printing statements on the control line, or if the string you type starts with? or " or VERSION, you don't have to type the terminating CR.

System Paw Macros

- 1. When the Status Window is opened, a Ctrl: PML (default: INIT) and a Proc: PML (default: QUAN_INIT) are both run.
- 2. In the Collect menu (toolbar start button) a Ctrl: PML is defined which runs when the file being collected is opened.
- 3. In the queue menu (Collect Queue edit button) a Proc: PML is defined which runs when the file being collected is closed.
- 4. A Windows application outside the instrument can request a PML through the windows DDE mechanism.
- 5. Using the MSGBOX keyword you create and run PMLs.
- 6. Some PMLs run when you click on buttons or menu items:

File menu Reset: INIT

File menu Standby: STANDBY

Status control menu: CGON CGOFF CION CIOFF CIDON CIDOFF

AutoTune menu: ATUNE1 ATUNE2 ATUNE3 ATUNE4 ATUNE5 ATUNE6

Diagnostic menu: DIAGNOSTIC(test_number)

Tune Table menu optimize button: OPTIMIZE

Status probe button: PROBE_MAIN

Species Boiling Pt. Range menu compute button: SHELL

Clicking in Chro: CHRO_CLICK(sn,rt,type_rt + (10*type_sn) + (1000*make_substance))

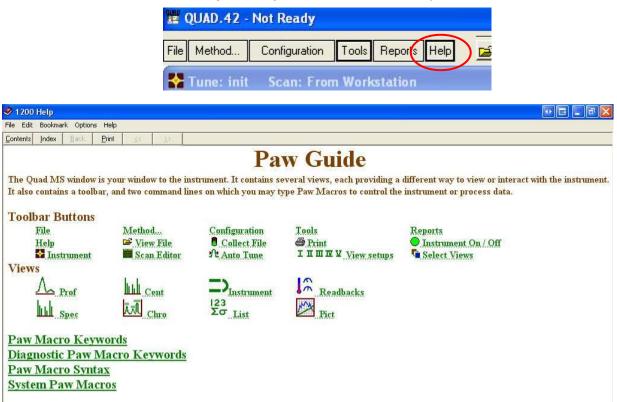
(click on the CHRO scales button to see where type_rt,type_sn, and make_substance are defined)

Other popular PMLs are:

BL	PAR
DAU	QIMS
FM	Q3MS
LM	SCAN
MIXED	SW
NL	

Help Files for PML

Click Help in the System Control window to open the Paw Guide.



Some PML Procedures

The Cioff.pml macro closes the CI gas valve, prints "Evacuating CI Gas (takes 5 seconds)" in the status area of the taskbar, opens and closes the vent valve, and prints "CI gas off" in the status area of the taskbar.

CIOFF.pml

valve 2 = 0	# close CI valve	
"Evacuating CI Gas (takes 5 seconds)";cr	#printing statement, terminated with the keyword CR (carriage return)	
doze 1	#wait one second	
valve 4 = 1	# open CI vent valve for 0.2 seconds	
doze .2		
valve 4 = 0	# close vent valve for one second	
doze 1		
valve 4 = 1	# open vent for 3 seconds	
doze 3		
valve 4 = 0	# finally close vent	
"CI Gas off!";cr	#printing statement, terminated with the keyword CR (carriage return)	

The LEAK.pml assists in finding vacuum system leaks. It sets a series of masses from a compound or mixture used for leak detection. First. the Pict view window is cleared, the title in the Pict window is set, centroid mode is set, mass range is set, the parameters are optimized to prevent saturation, and a series of mass intensities are plotted while the user sprays a gas at possible leak locations.

LEAK.pml

pict_clear	# clear the picture/graph window	
pict_title:"1->mass 18, 2->mass 28, 3->mass 33, 4->mass 45, 5- >tic":cr	#Sets the title in the pict view	
cent	#Go to centroid mode	
fm 10	#Set first mass (FM) to 10	
lm 50	#Set last mass (LM) to 50	
optdet	#Optdet runs opt_detector.pml to prevent detector saturation after each scan.	
repeat	#repeat items below	
fish	#go get one scan of data	
pict(,area(18),1)	#get area of mass 18 and plot point in pict view position 1	
pict(,area(28),2)	#get area of mass 28 and plot point in pict view position 2	
pict(,area(33),3)	#get area of mass 33 and plot point in pict view position 3	
pict(,area(45),4)	#get area of mass 45 and plot point in pict view position 4	
pict(,tic,5)	#get area of mass total ion current (tic) and plot point in pict view position 5	
end		

Mixed.pml gets one scan in centroid data and one scan of profile data.

MIXED.pml. When Prof and Cent views are selected in the window, both real time centroid and Profile data is observed.

Repeat	#repeat items below	
Prof	#set to scan of profile data	
Fish	#go get one scan of data	
Cent	#set to scan of centroid data	
Fish	#go get one scan of data	
End		

PML Editor

The **PML Editor** is used for creating and editing PMLs for the 300 Series software. The PML Editor allows access to the factory PML files; however, if a factory PML is modified it will then be saved as a user PML in the UserPML directory.

Accessing the PML Editor

Click Tools in the Quad module window to display the PML editor.



The PML editor window opens.

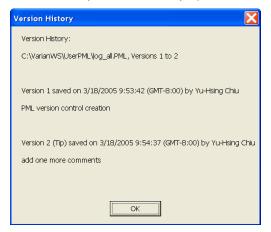
🖹 Untitled	PML Editor	
File Edit Vie	v Tool Help	
0 🛩 🖬	Version History PML Info PML Utility	5 ·

- Varian PMLs are stored in c:\varianws\1200sys\PML directory.
- User created PMLs or modified factory PMLs are stored in c:\varianws\UserPML directory.
- The Status Bar of the PML Editor displays the line number of the current cursor position.

E aporchum i touro orre		ک بے بے
<u>File E</u> dit <u>V</u> iew Tool <u>H</u> elp		
🗋 D 🚅 🖬 🖦 X 🖻 f	1 × #	
abort_request = 1.0 # The C Code will then i # It in turn will restore th or QUIT 2 to stop the pro # This is only used in th	ne appropriate state and the ocess	ů –
#must_quit = 0 if (bl_running) bl_running baseline	Selected line number	ır
average a∨mode scan_time	= bl_average = bl_avmode = bl_scan_time	
Ready		Ln: 7

• The Tool menu has three menu items:

Version History: If the 21CFR11 application is enabled, click this to display the version history of the currently open PML.



PML Info: Display the PML path name, release version number, and any descriptions that have been in the PML header section. (Lines begin with ##).



PML Utility: Perform syntax checking of the currently open PML and produce a call tree, PML keywords and User Variables defined in the PMLs. For the keywords listed, user can select and use F1 to get online help about the keywords.

In the right part of the utility dialog box, enter a specified keyword, and find out the PMLs contain this keyword.

Compile a macro or macros (wild cards ok)	Find all references to a keyword, macro or user variable
Compile abort	Find
🗸 Show user variables 🔽 Show keywords 🗖 Show 'all-in-on	e' 🔽 Show direct references only
ABORT FINISH_ABORT FINISH_ABORT FINISH_ABORT ARSTORE_PRE_AUTOTUNE_STATE MAKE_TUNE_NAME MAKE_TUNE_NAME MAKE_TIME_STRING MAKE_TIME_STRING MAKE_TIME_STRING AMAKE_TIME_STRING CDON CDOFF CDON CDOFF COFF COFF COFF COFF DE_ASSELINE OBL_AVRAGE DL_AVRAGE DL_SCAN_TIME OBL_CENT SU	The Macro abort is used directly by pmls:
Guccesses	
Compiled abort Compiled accomp Compiled alloff Compiled api_lid	<u>×</u>
Problems-	