Varian, Inc.
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325-MS LC/MS
Quadrupole Mass Spectrometer
MS Workstation
Version 6

Software Operation Manual

QUALITY SYSTEM ISO 9001 CERTIFIED

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Contents

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.

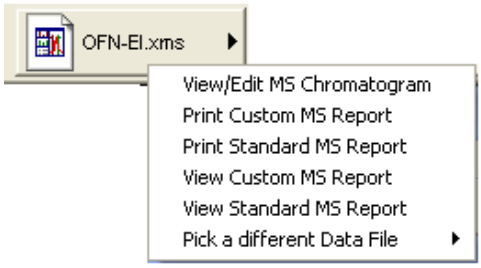
Name of Application	Large Icons	Description
System Control/ Automation		Monitor instrument status, and perform automated injections and batch recalculations.
View/Edit Methods		View and edit instrument operation, data acquisition, and data handling methods.
Edit Automation Files		Do off-line editing 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		Create, edit, and view standard MS reports.
Custom MS Reports		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		Preview standard chromatogram and results reports.
Compound Set Editor		Create and edit sets of compounds in the MS Data Handling Method allowing activation or deactivation under automation.

Quick Start		**Run a sample without using a Sample List.**

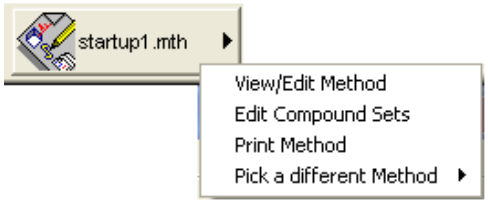
Quick Link Buttons

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.



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

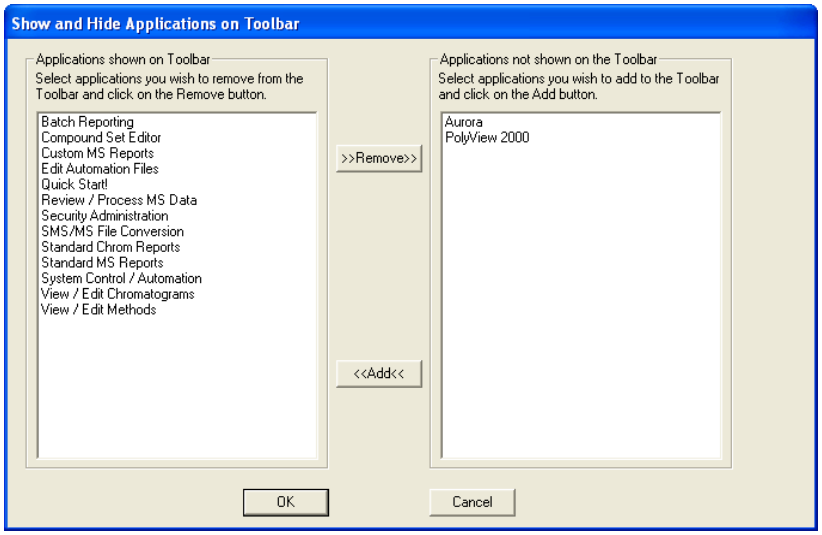
Display the Workstation Toolbar as a Windows Taskbar icon.

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.

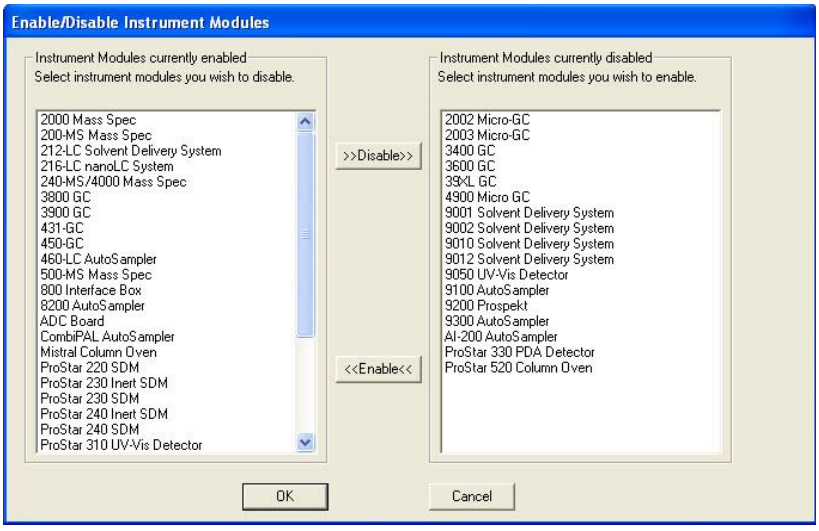
- To remove an icon from the toolbar, select it from the top list and, then click **Remove**.
- To add an icon to the toolbar, select it from the bottom list, and then click **Add.**



Enable/Disable Instrument Modules

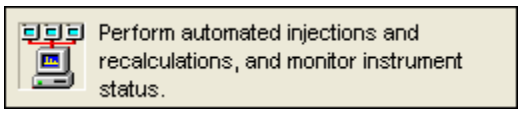
Enable instrument modules. When an instrument module is available, it appears in System Control. If the corresponding instrument is connected and on, you can create a Method section and format reports. The list on the left shows the instrument modules that are currently installed and enabled. The list 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**.



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.

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

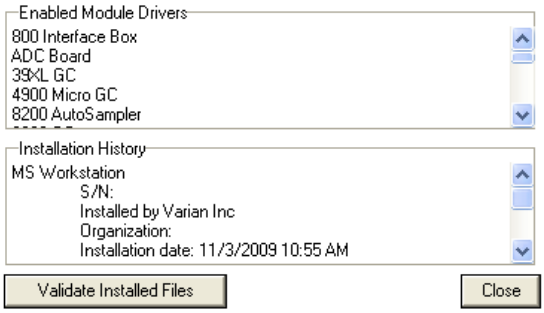
Displays help for the open application.

Product Support Web Site

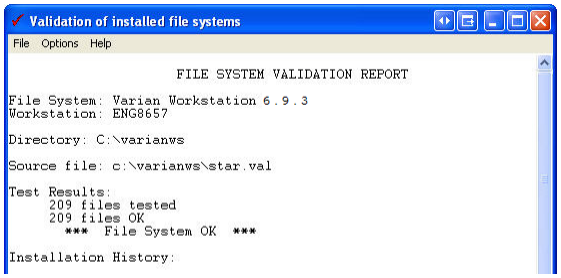
If you have Internet access and a web browser installed on your computer, click to open the MS Workstation Product Support Web Site. It has the latest software and documentation updates for the MS Workstation suite of products, and notes, tips, and answers to frequently asked questions. Visit this site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



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

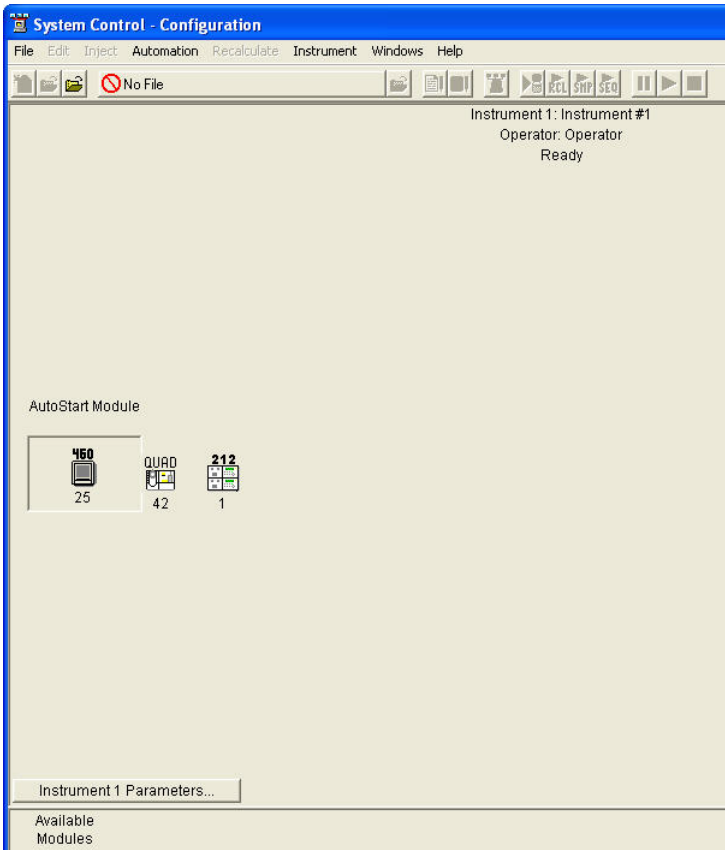
Close the Workstation Toolbar.

System Control

Overview

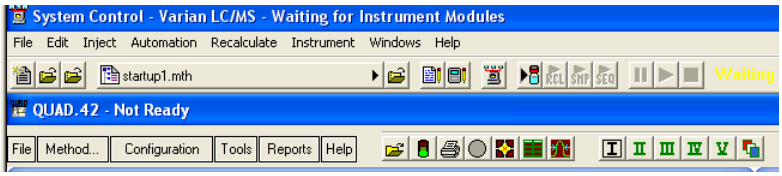
This section is an overview of System Control. Other functions are explained in detail in later sections.

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

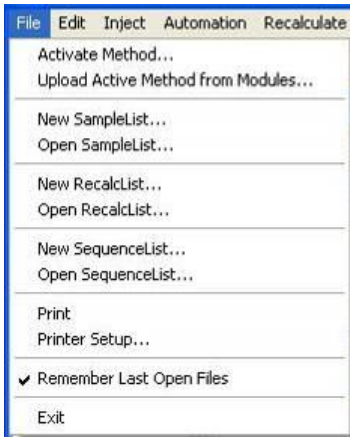


Instrument Window

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.



File Menu

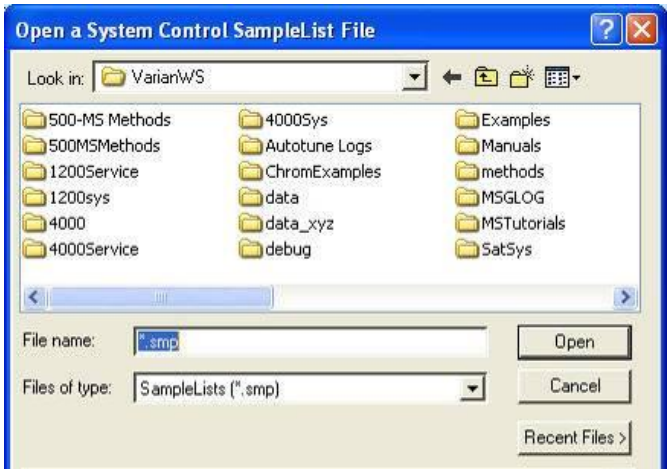


Activate Method: Select an existing method to set the conditions for the connected modules such as the LC, MS, autosampler, and data handling.

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.



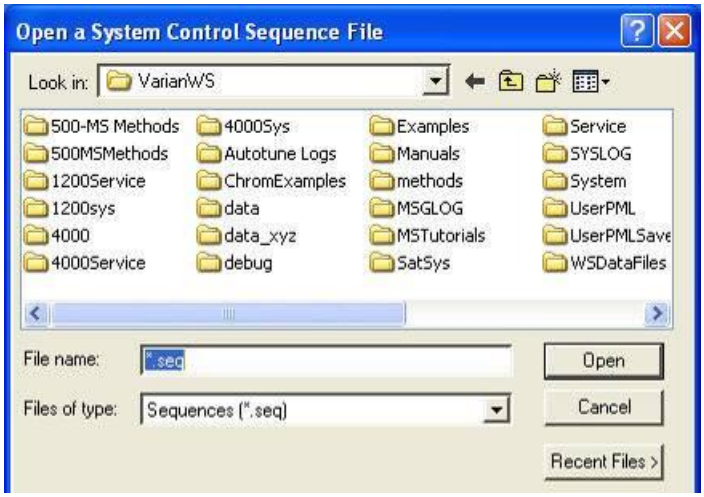
New RecalcList: Create a recalculation list.

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.

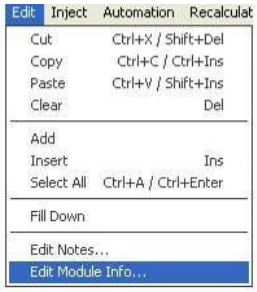


Print and **Printer Setup** are described in the MS Workstation Software Reference Manual.

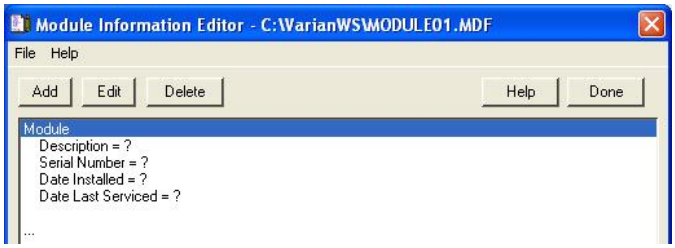
Edit Menu

Edit Module Info

Click **Edit** and select **Edit Module Info**.



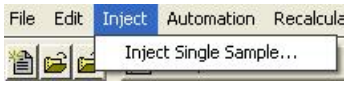
Each module has a message log and a documentation screen. Use the Module Information Editor to record performance, maintenance, hours used, or other comments.



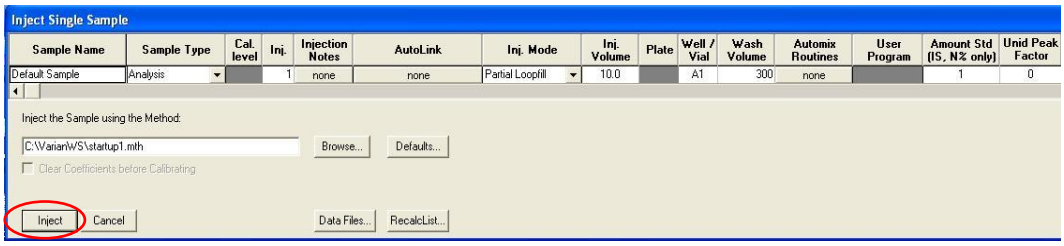
Use **Add**, **Edit**, and **Delete** to record comments.

Inject Single Sample Menu

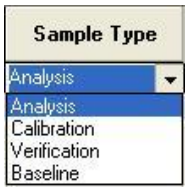
Use **Inject Single Sample** to enter sample information before making an injection. The Module window must be in acquisition mode before making an injection.



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



Select a **Sample Type** from the list:



Click **Injection Notes** to enter a description or comment.

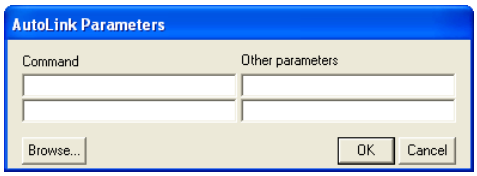
Use **AutoLink** to enter commands and parameters. Enter a command to execute a program after the data file is acquired.

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:

1. 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.



Automation Menu

The following shows the Automation menu and describes the options.

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

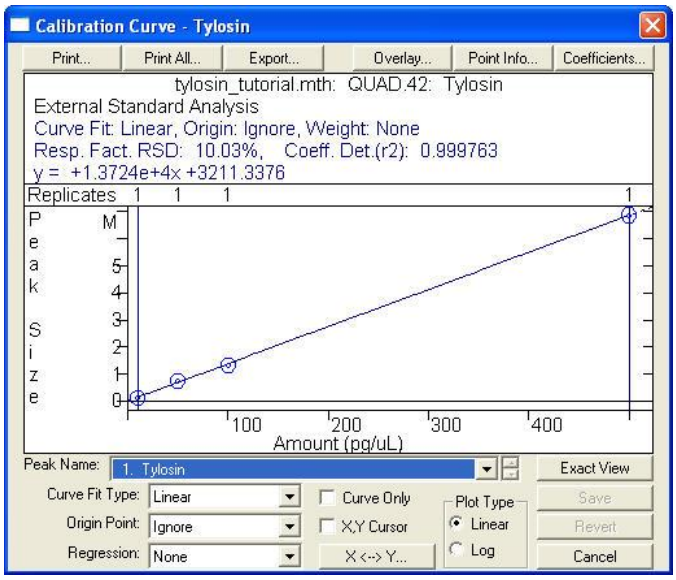
The following shows the Automation menu and describes the options.

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

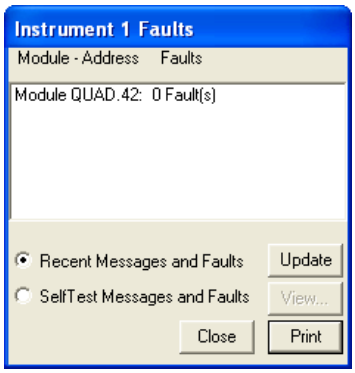
The following shows the Instrument menu and describes the options.

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.

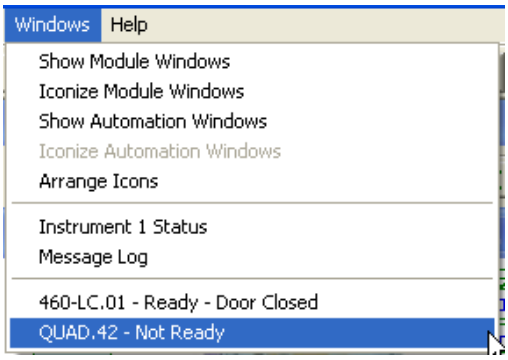
Instrument 1 Faults: View faults in a module of Instrument 1. Click Update to check for new faults.



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.

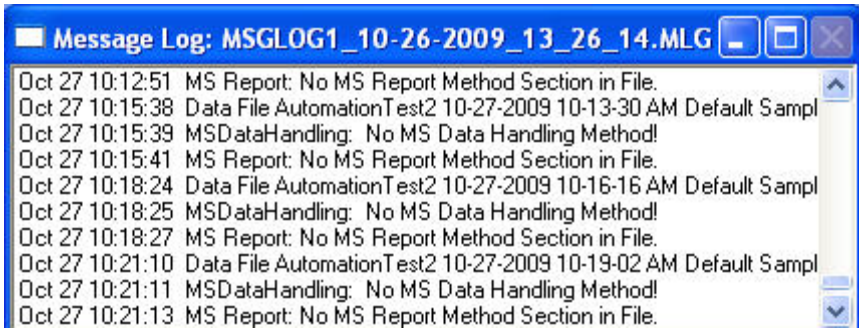
SequenceList: Display the active SequenceList.

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.



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

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

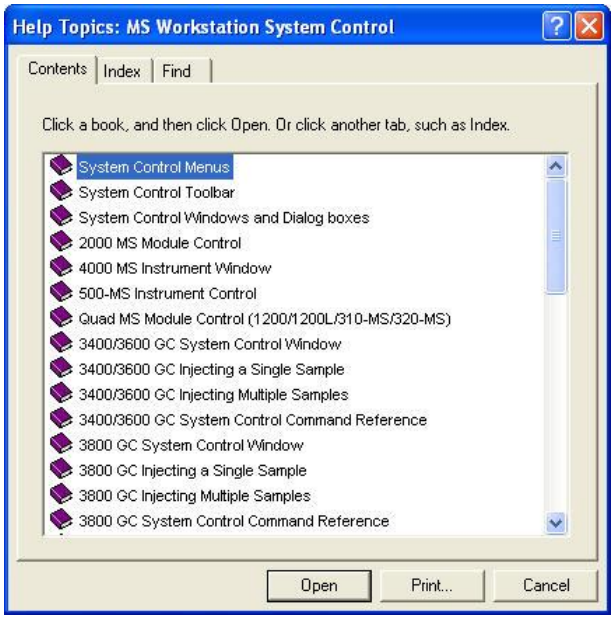
Help Menu

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

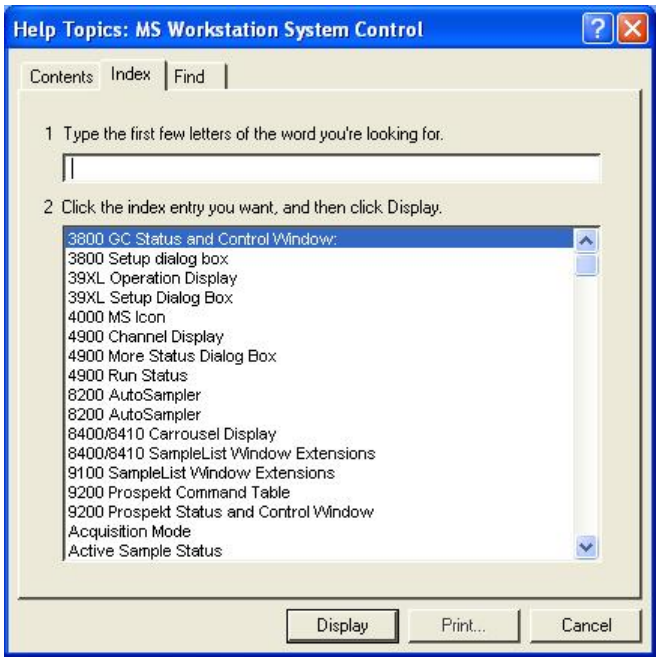
Help Topics

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

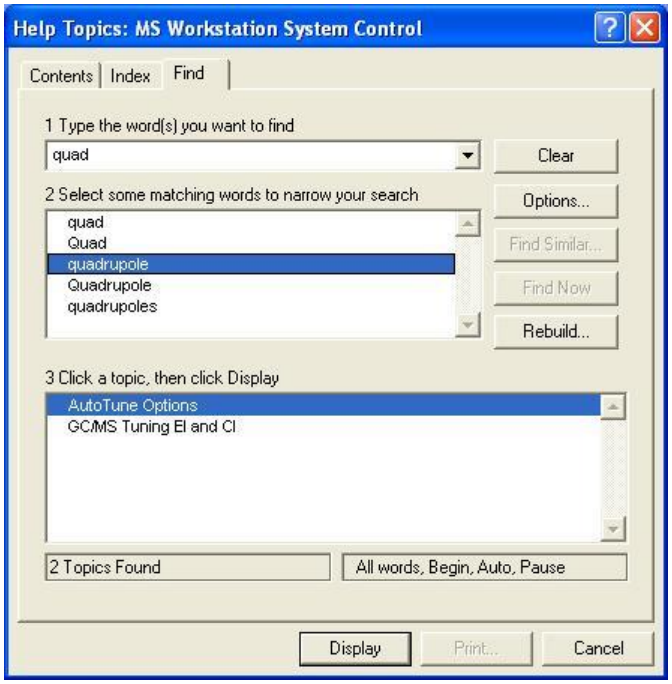
- Click an item in Contents to open online help.



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

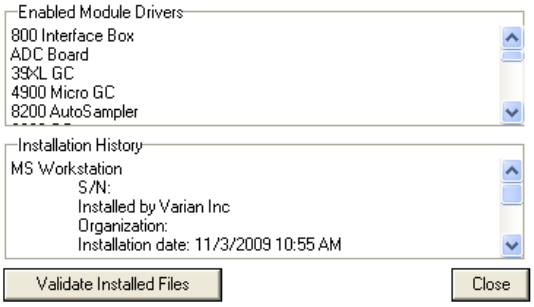


- Enter a key word or phrase to list help topics. Click a topic and then click Display.



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.



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.

The following describes the functions.

Item	Description
	Create a new automation file.
	First button: open an existing automation file.
	Second button: open the Message Log file.
	View/edit or print a Method.
	Open a Method.
	Edit notes for an automation file.
	Edit Module information for any online Module.
	Open Instrument 1 Status
	Inject a single sample.
	Start an open RecalcList.
	Start an open SampleList.
	Start an open SequenceList
	Suspend a running list.
	Begin a list.
	Stop a running list.

Quad Status and Control Window

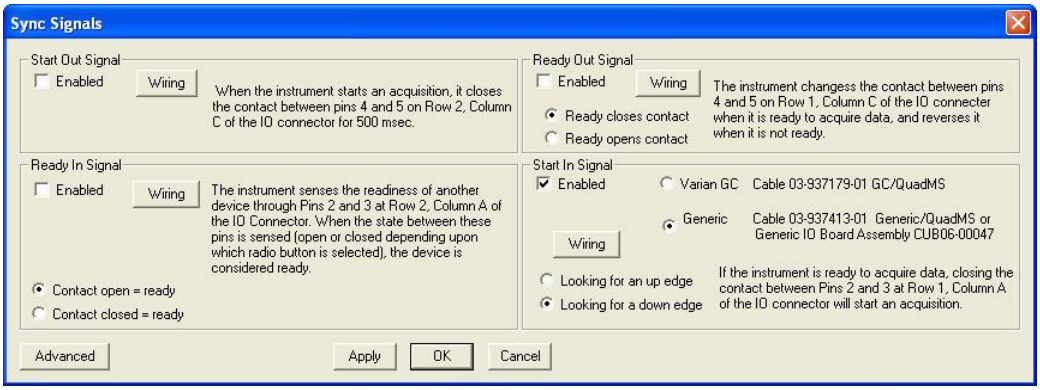
Check the status of the instrument components, monitor data acquisition, and process data using the Quad Status and Control window. Create your preferred window configuration.

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.

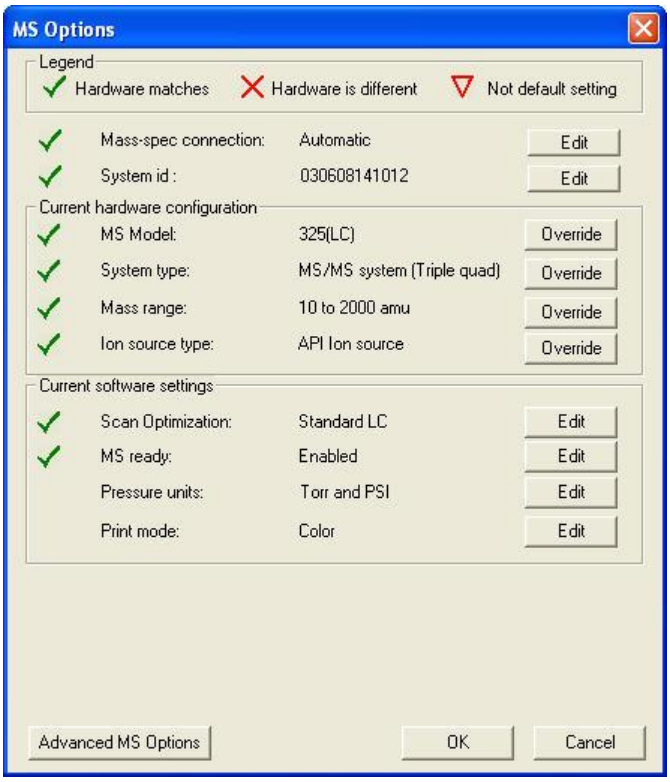


Sync Signal: Enable or disable the Start and Ready signals for external devices. To enable a Sync Signal operation, connect a cable from the device to the contact closure on the master instrument.

- 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.

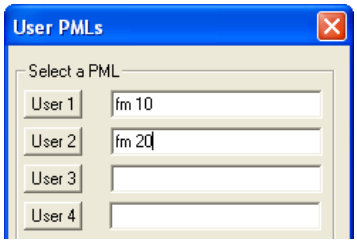
- Check marks (✓) indicate a Hardware match
- X indicates a Hardware conflict. If an X is present, call your Varian representative.



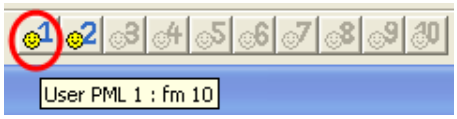
Tools: Access MS Tools such as MS/MS Breakdown, Pml Editor, Plot tic, readbacks, Bakeout system, and Overnight Standby.

Quit Macros: Stop any MS function in progress.

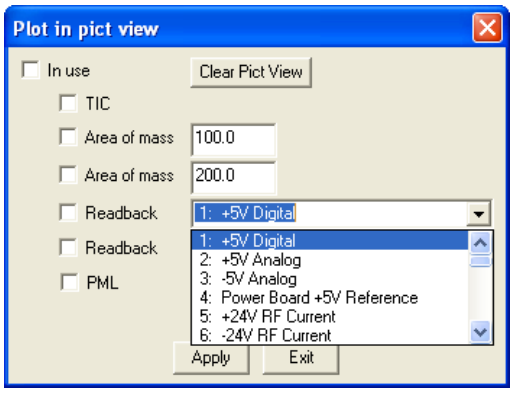
User PML: Enter up to 10 PMLs (macros written in Paw Macro Language).



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**.

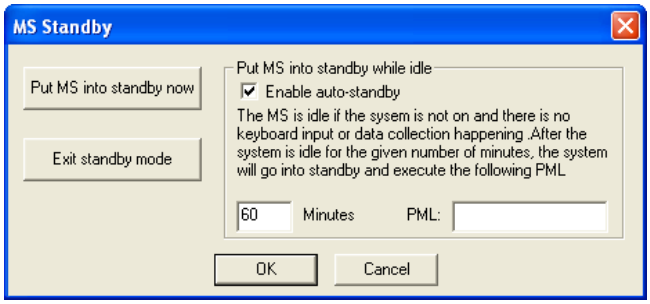


Analog output: For service use only.

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.



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.

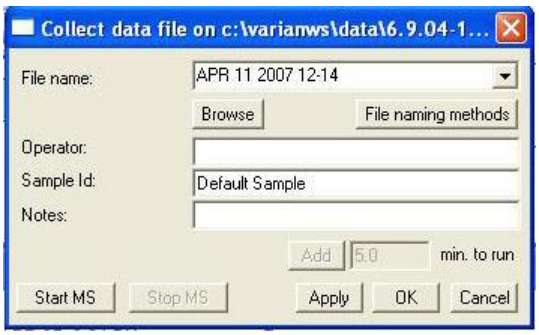
Reports: Select a print view setup.

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.



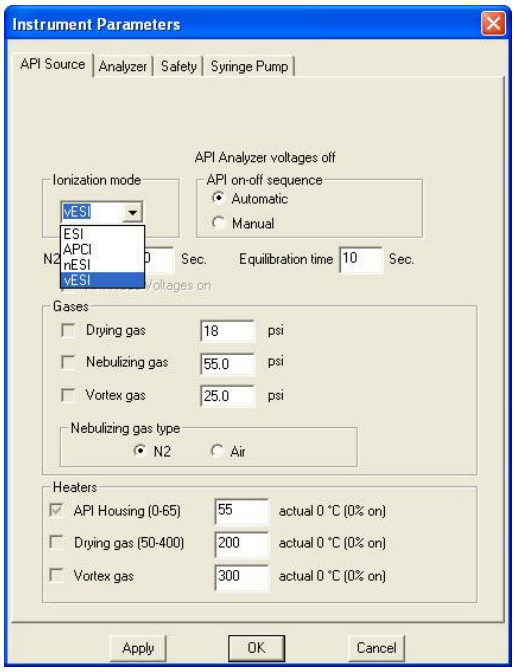
 **Print**: Print the window screen.

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

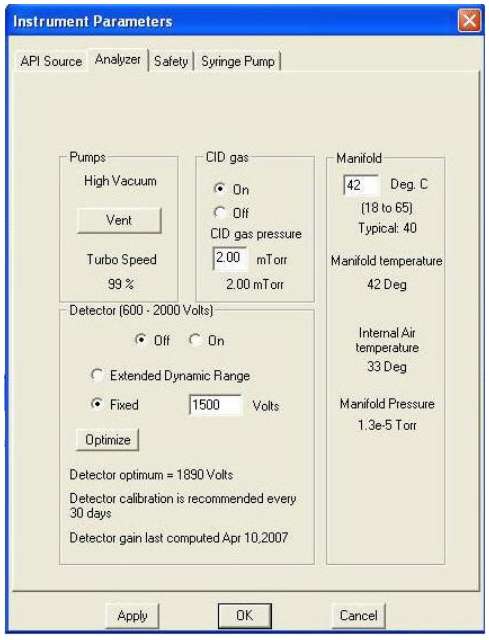
- Gray: Off
- Green: On
- Yellow: System in process of turning on or is waiting, for example, a filament delay time. If it turns yellow and then turns gray, a fail-safe has prevented the filament and the multiplier from turning on.

 **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.

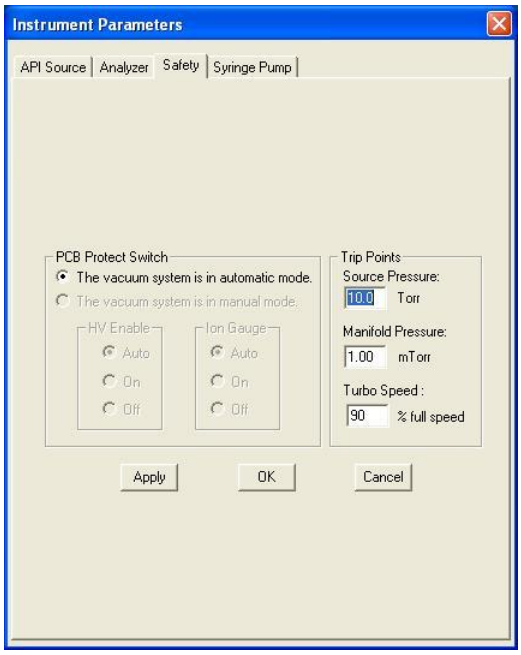


Analyzer: Vent the system and set the manifold temperature. Turn the CID gas on and off, set the electron multiplier (detector) voltage, and turn the Extended Dynamic Range on or off.

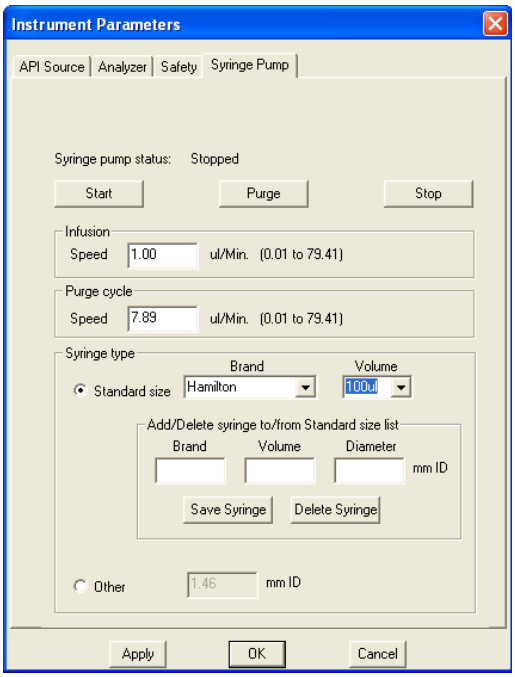


Safety: There are three sections and only Varian Representatives can change them.

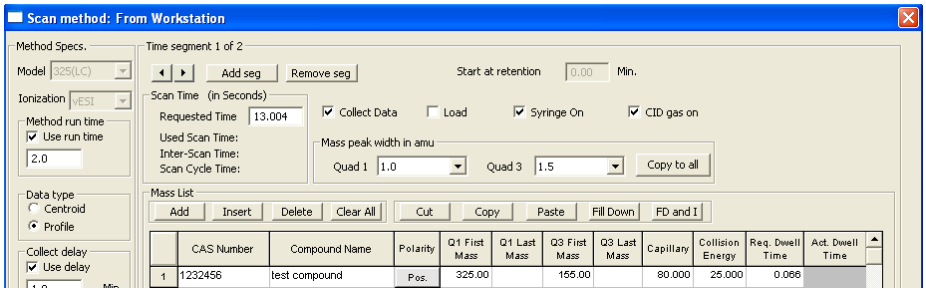
- High voltage enable and Ion Gauge modes
- Trip points of the source pressure, manifold pressure and turbo speed
- Status of the PCB Protect Switch



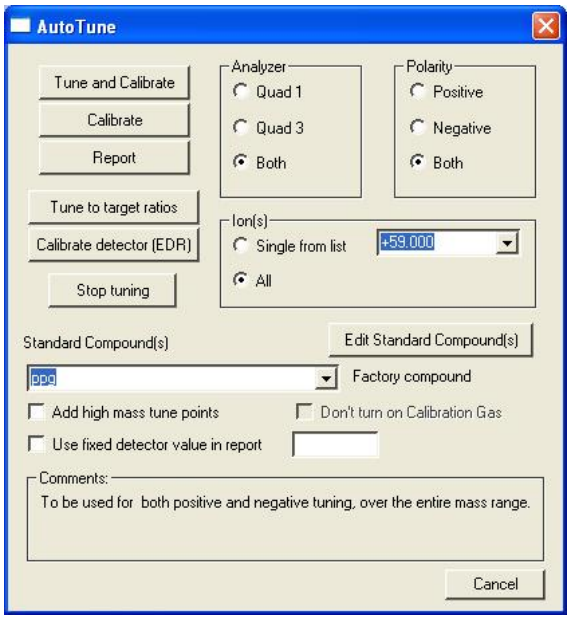
Refer to the Hardware manual for more information about the setting up the Syringe pump.



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.



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



325-MS Module Views

The five default views are:

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

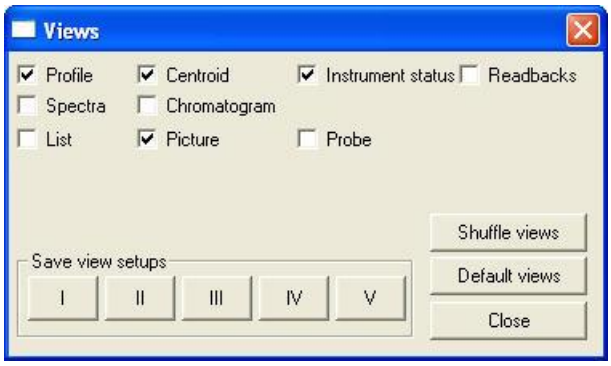
View II: Displays Quad Instrument status, Chromatogram, Spectra, and Centroid windows.

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.

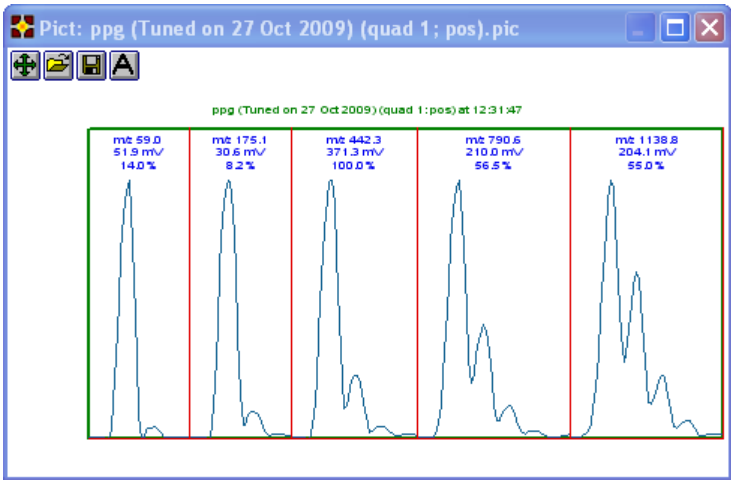
Overview

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

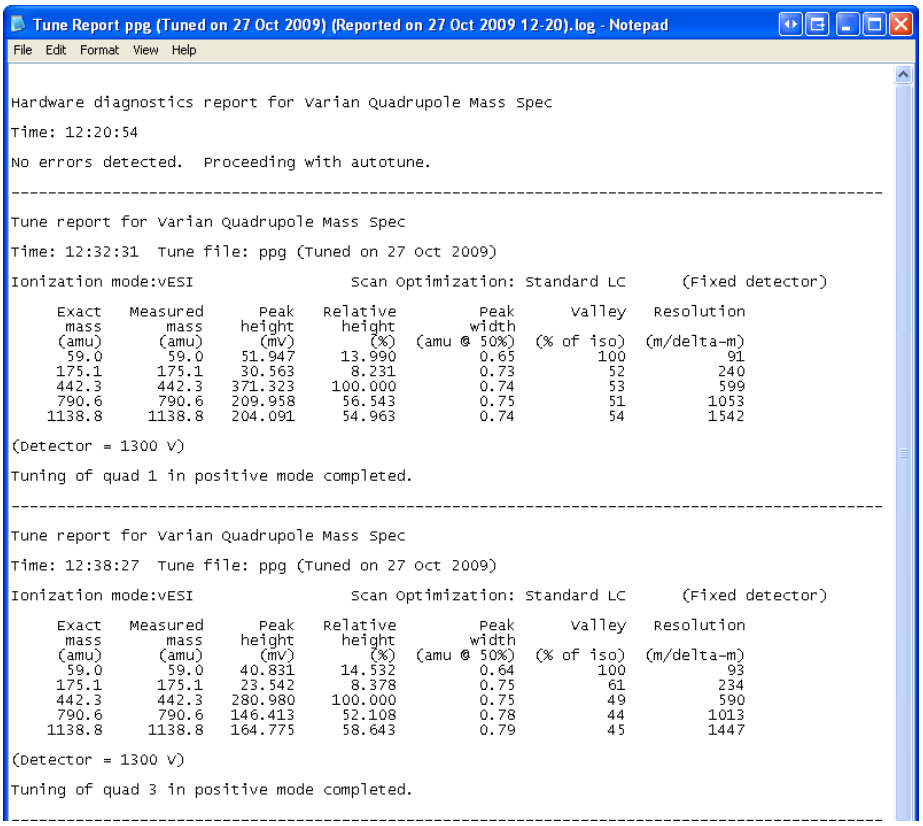
A reference compound, such as polypropylene glycol (PPG) is used as a tuning solution. A custom tuning solution can also be used.

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.



Tune Report ppg (Tuned on 27 Oct 2009) (Reported on 27 Oct 2009 12-20).log - Notepad

File Edit Format View Help

```
Hardware diagnostics report for Varian Quadrupole Mass Spec

Time: 12:20:54

No errors detected.  Proceeding with autotune.

-------------------------------------------------------------------------------------------------------

Tune report for Varian Quadrupole Mass Spec

Time: 12:32:31  Tune file: ppg (Tuned on 27 Oct 2009)

Ionization mode:vESI                Scan Optimization: Standard LC      (Fixed detector)

     Exact   Measured      Peak   Relative         Peak      Valley   Resolution
      mass       mass    height     height        width
     (amu)      (amu)      (mv)        (%)  (amu @ 50%)  (% of iso)  (m/delta-m)
      59.0       59.0    51.947     13.990         0.65         100           91
     175.1      175.1    30.563      8.231         0.73          52          240
     442.3      442.3   371.323    100.000         0.74          53          599
     790.6      790.6   209.958     56.543         0.75          51         1053
    1138.8     1138.8   204.091     54.963         0.74          54         1542

(Detector = 1300 V)

Tuning of quad 1 in positive mode completed.

-------------------------------------------------------------------------------------------------------

Tune report for Varian Quadrupole Mass Spec

Time: 12:38:27  Tune file: ppg (Tuned on 27 Oct 2009)

Ionization mode:vESI                Scan Optimization: Standard LC      (Fixed detector)

     Exact   Measured      Peak   Relative         Peak      Valley   Resolution
      mass       mass    height     height        width
     (amu)      (amu)      (mv)        (%)  (amu @ 50%)  (% of iso)  (m/delta-m)
      59.0       59.0    40.831     14.532         0.64         100           93
     175.1      175.1    23.542      8.378         0.75          61          234
     442.3      442.3   280.980    100.000         0.75          49          590
     790.6      790.6   146.413     52.108         0.78          44         1013
    1138.8     1138.8   164.775     58.643         0.79          45         1447

(Detector = 1300 V)

Tuning of quad 3 in positive mode completed.

-------------------------------------------------------------------------------------------------------
```

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.

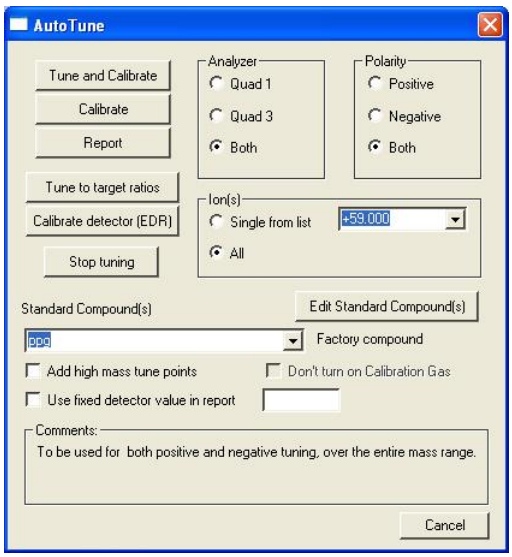
The tuning compound signal must be stable before starting AutoTune (Cal Gas on for at least 10 minutes, or PPG signal stable).

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: 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 re-calibrating 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.

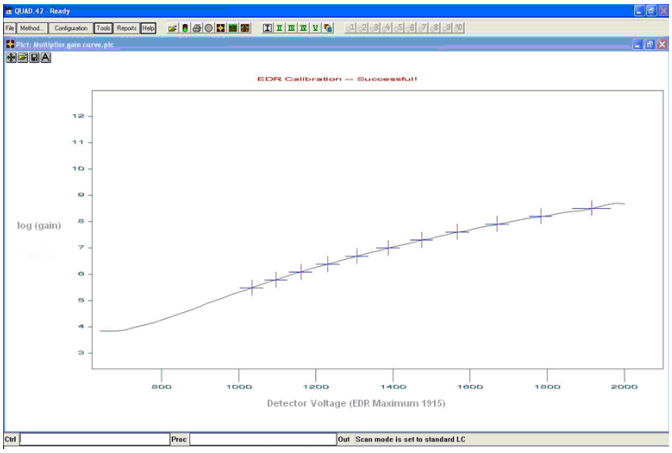
Tune to Target Ratios: Only for a GC/MS in EI mode.

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)

Ion(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.

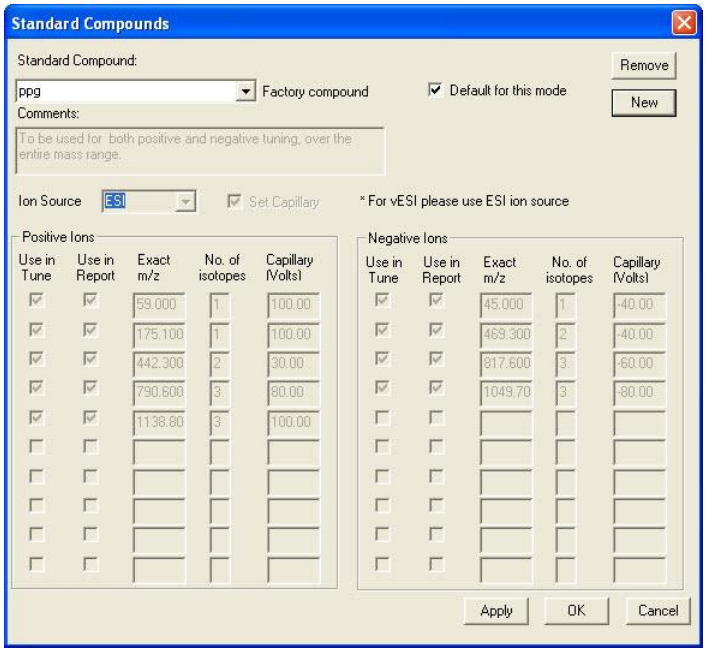
Compound Name/Mode (Date created).dac

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

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



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.

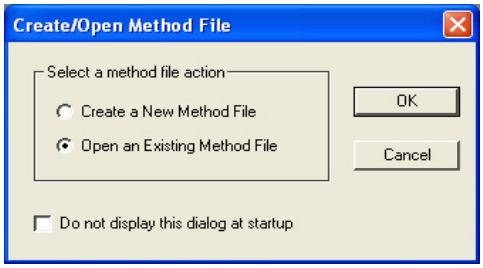
Method Builder

Using Method Builder

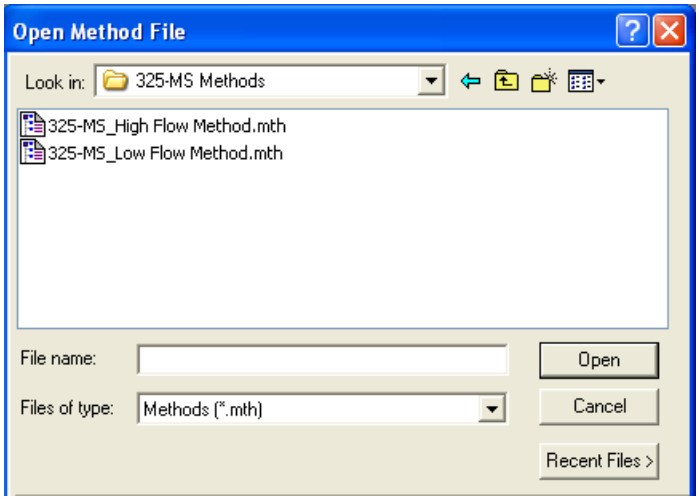
Click **Method Builder** in the MS WorkStation Toolbar.

Edit an Existing Method

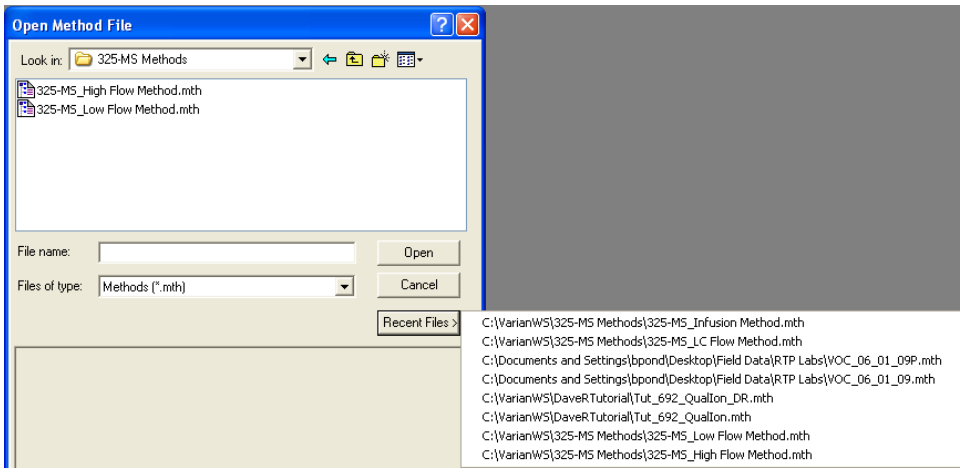
1. Click **Open an Existing Method File** and then click **OK**.



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

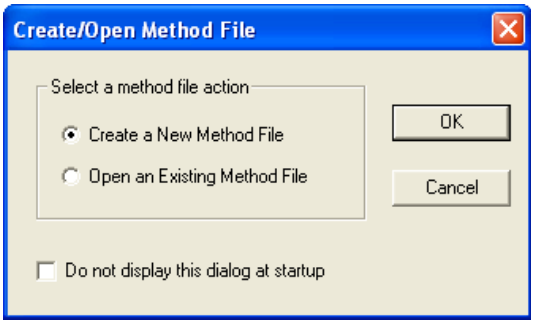


- Click Recent Files to display them and select one.

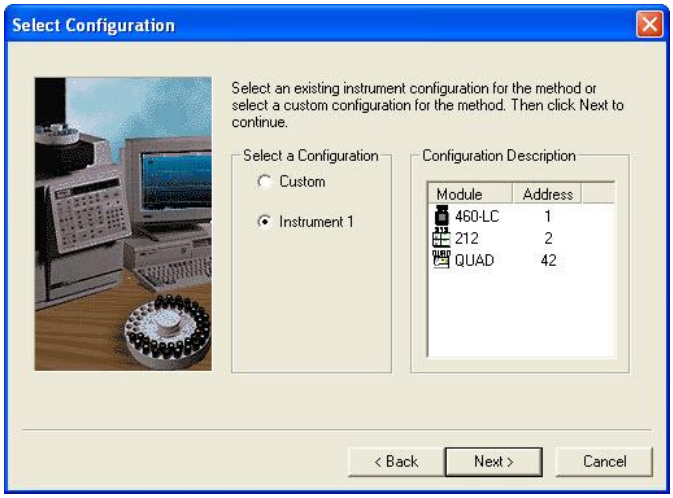


Build a New Method

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

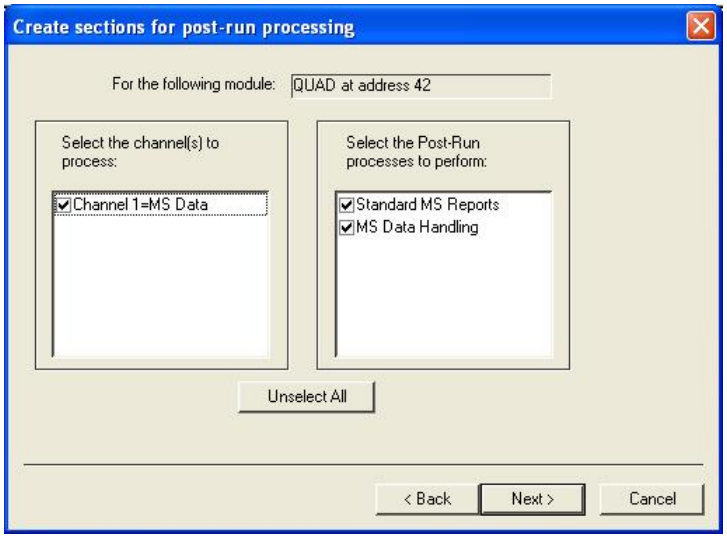


2. Select Instrument 1. The configured instrument is added to the method.
3. Click Next.



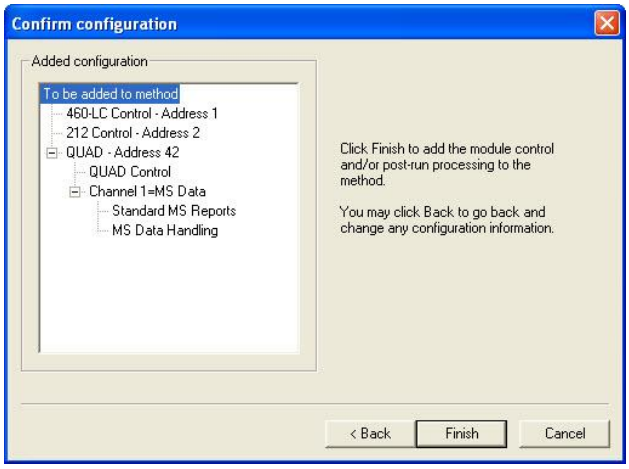
4. Select a detector. After the data is acquired, you can add data processing information to the method.
5. Click Next.

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.

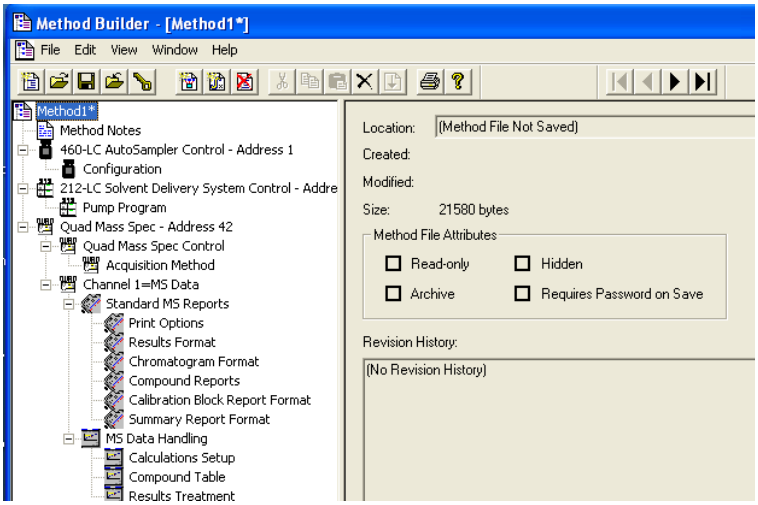


8. Review the information on the Confirm configuration screen.
9. Click Next.

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

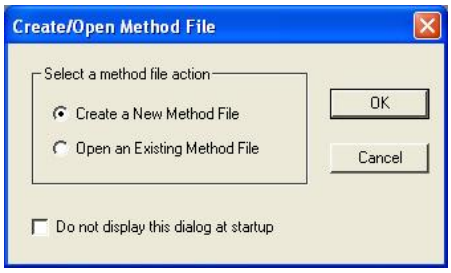


11. The new method is displayed with a navigation tree on the left side and the method workspace on the right. Click a tree entry to open that method section.

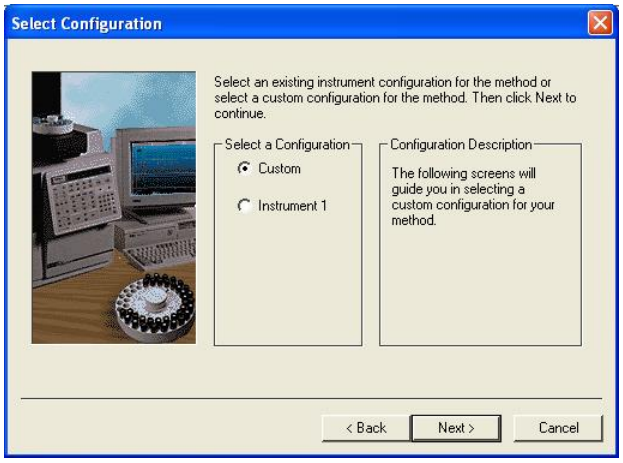


Add a Varian Instrument to a Method

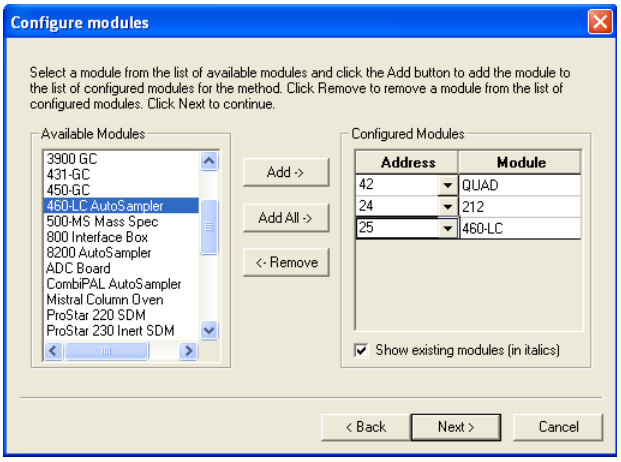
1. Select **Create a New Method File** and click **OK**.



2. Click Custom and then click Next.



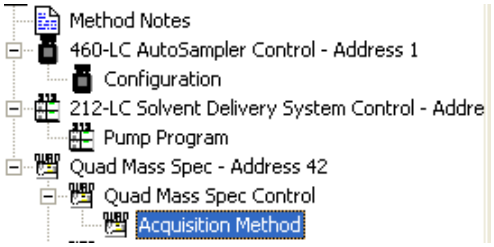
3. Select an available module from the list and click Add to make it one of the Configured Modules. Other options are Add, Add All, or Remove.
4. Click **Next**.



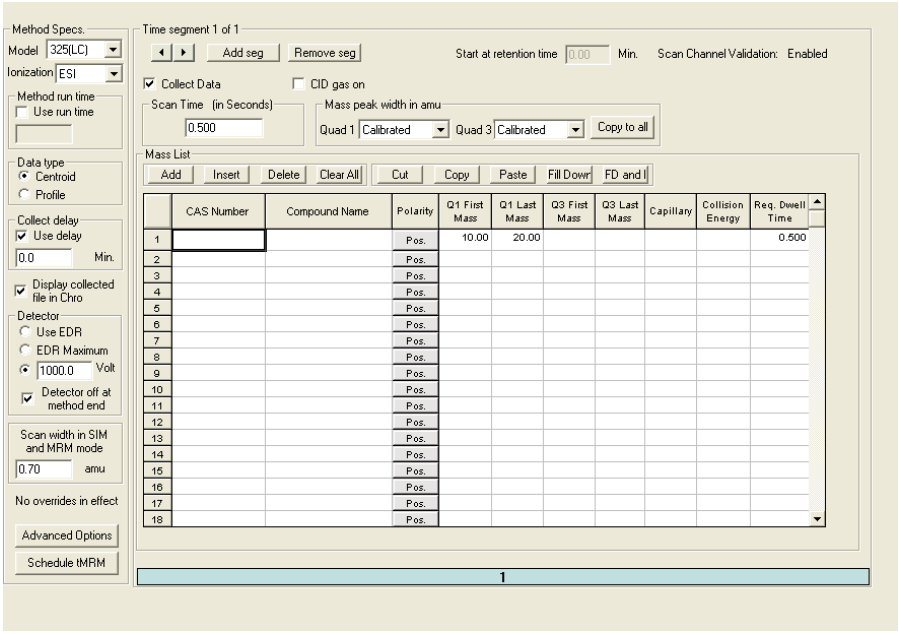
5. Continue with step 3 of “Build a New Method” on page 37.

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.

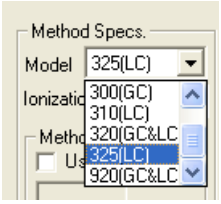


Method Specifications

The following describe the Method Specifications in the Acquisition Method.

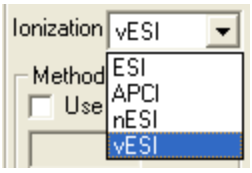
Model

Select your instrument Model.



Ionization

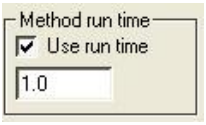
Select the ionization mode: Vortex Electrospray Ionization (vESI), or Atmospheric Pressure Chemical Ionization (APCI), Nano Electrospray Ionization (nESI), or Electrospray Ionization (ESI).



Method Run Time

There are two selections:

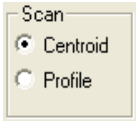
- Select **Use run time** to use the run time in the method.
- Type in the minutes to use a time other that in the method. If you do not enter a run time, the data is acquired until the end time is reached in all other modules.



Data Type

Set the scan type (Centroid or Profile).

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

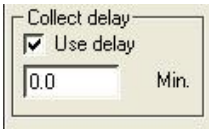


Collect Delay

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.

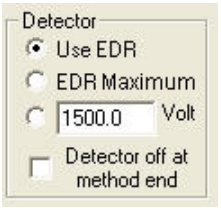


Display File in Chro

Enable to display data files in the Chro view.

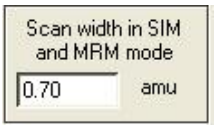
Detector

- **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.



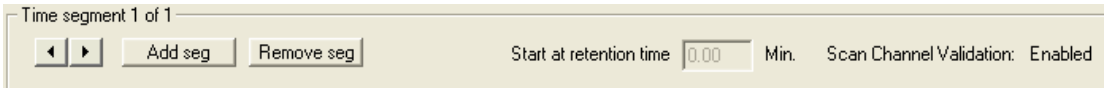
Scan Width

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.



Scan Channel Validation

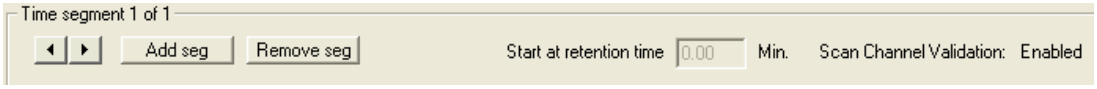
Indicates if Scan Channel Validation is enabled or not. Also See MS Data Review General Preferences.

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.

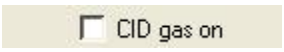


Scan Time

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.

Collision Cell Gas

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

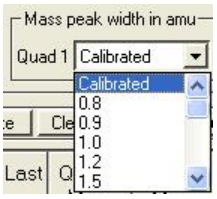


Peak Width

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.

Capillary
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.

Collision Energy
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.

Req. Dwell Time
0.500

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.

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.

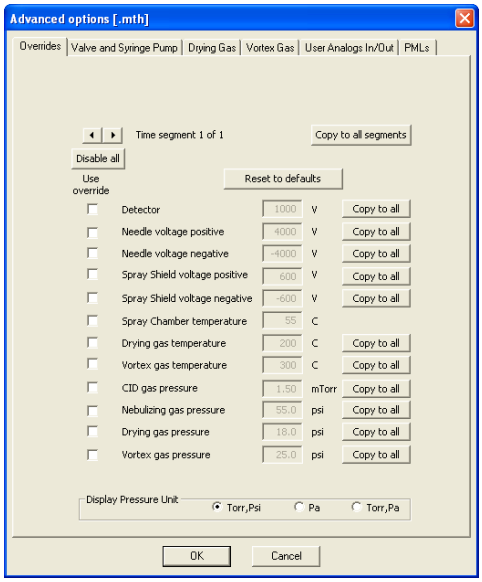
The advanced options available depend on the ionization mode.

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.

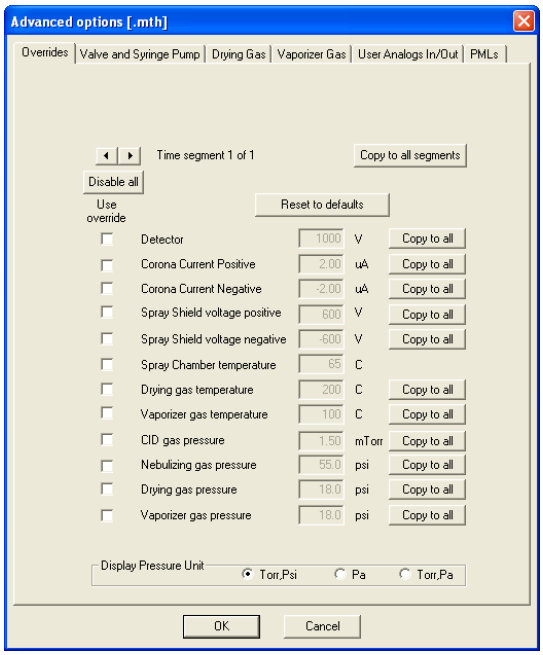
vESI Overrides

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



APCI Overrides

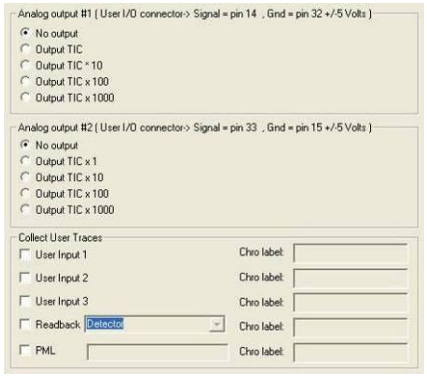
Enter values to override those specified in the method for specific time segments.



User Analog In/Outs

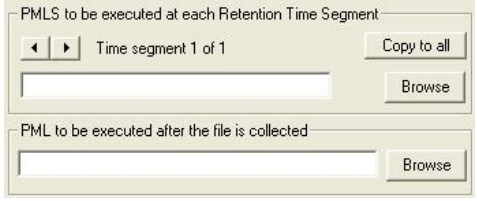
Use the Analog outputs to send a TIC signal to a device such as a LIMS system.

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



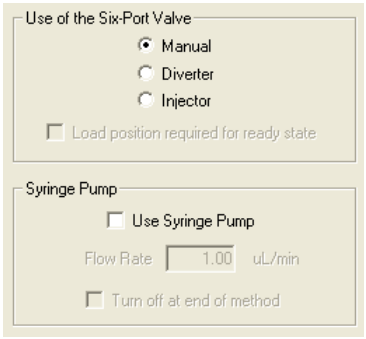
PMLs (Paw Macro Language)

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.



Valve and Syringe Pump

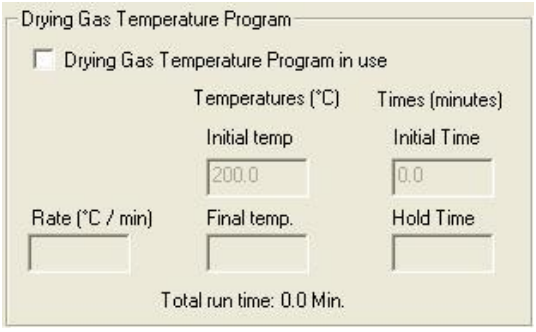
Use the six-port valve or the syringe pump or both. The six-port valve can be used in Manual, Diverter or Injector mode. Set the flow rate for the syringe pump.



vESI and APCI Drying Gas Temperature Ramp

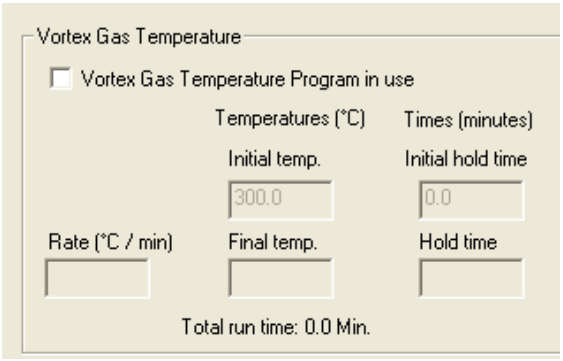
Use the SelecTemp™ feature to ensure the optimum drying gas temperature for the mobile phase composition of vESI, APCI, or ESI runs.

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.



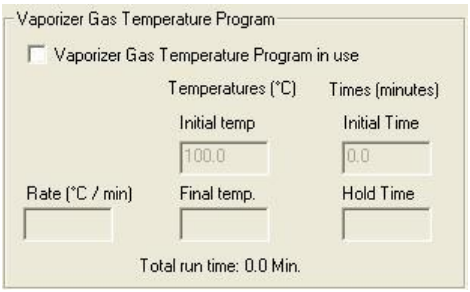
vESI Vortex Gas Temperature Ramp

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



APCI Vaporizer Gas Temperature Ramp

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



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:

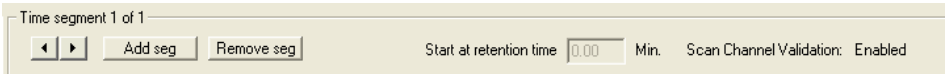
- Line 1: Positive SIM 120
- Line 2: Positive Scan from 200 to 400
- Line 3: Negative SIM 230
- Line 4: Negative SIM 290

	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.



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		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		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		225.00			5.000	0.500
2	Pos.	525.00		315.00			10.000	0.100
3	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

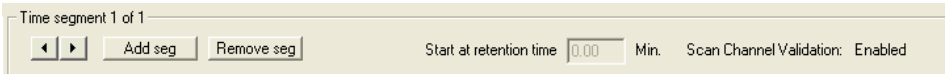
	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	480.00		225.00			5.000	0.500
2	Neg.	612.00		375.00			20.000	0.100
3	Pos.	452.00		225.00			5.000	0.100
4	Neg.	688.00		315.00			15.000	0.100

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:

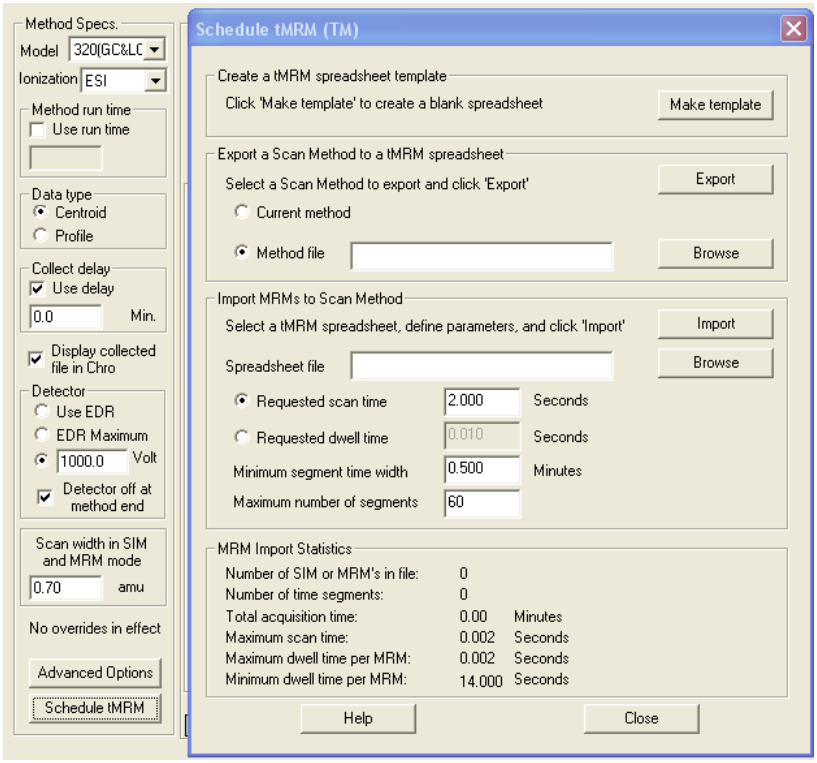
- To create timed MRM transitions from Method Builder or System Control: In **Method Builder**, select **Acquisition Method**, and then click **Schedule tMRM** to open the **Schedule tMRM** window.

- 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.

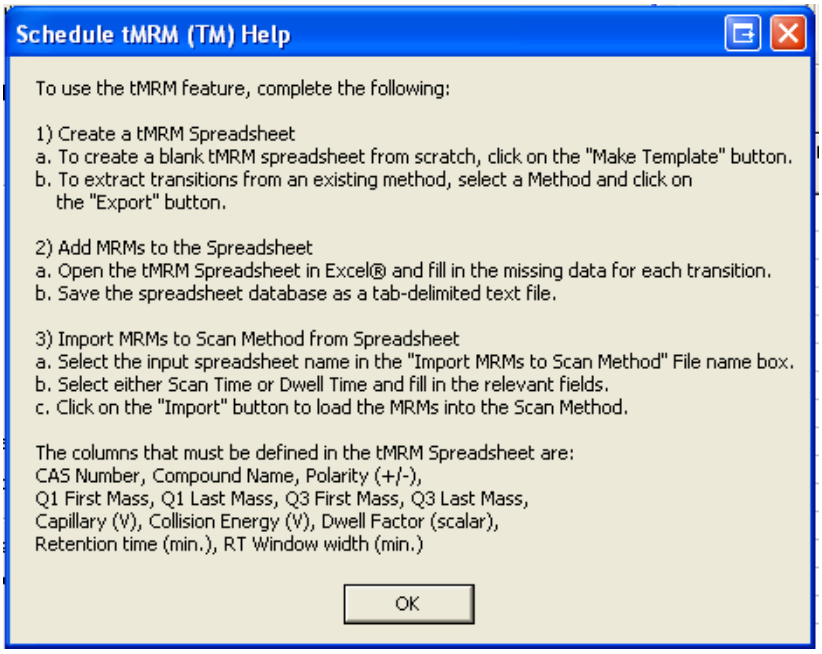
The Schedule tMRM window has the following four areas:

- 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.



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



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

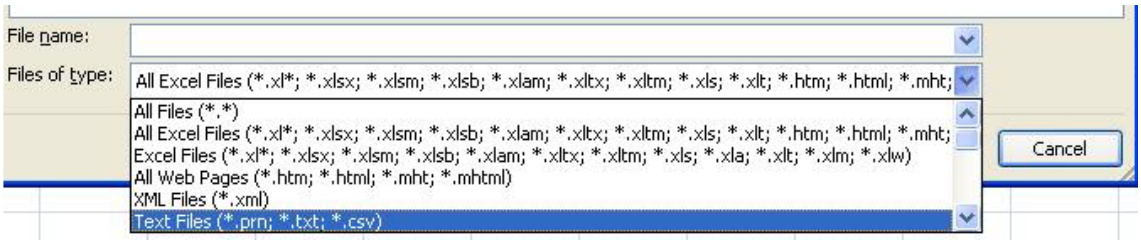
To create tMRM transitions in a blank template, complete the following two-part procedure.

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.



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.
    - RT Window: Retention Time Window in decimal minutes

	A	B	C	D	E	F	G	H	I	J	K	L
1	CAS Number	Compound Name	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Dwell Factor	Retention time	RT Window 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	60168-88-9	Fenarimol	+	331	0	189	0	105	38	9.666667	0.25	0.5

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.

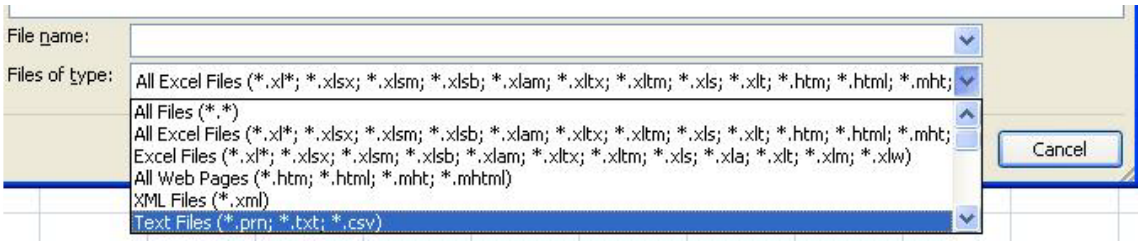
Creating tMRM transitions from an Existing Method

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.



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:
     - 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

	A	B	C	D	E	F	G	H	I	J	K	L
1	CAS Number	Compound Name	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Dwell Factor	Retention time	RT Window 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	60168-88-9	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.

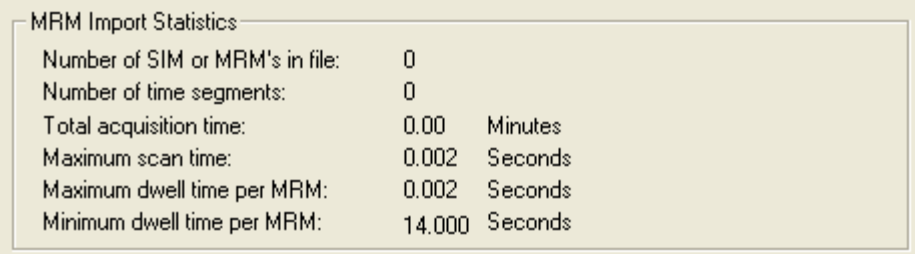
Importing MRMs to Scan Method

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.



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.

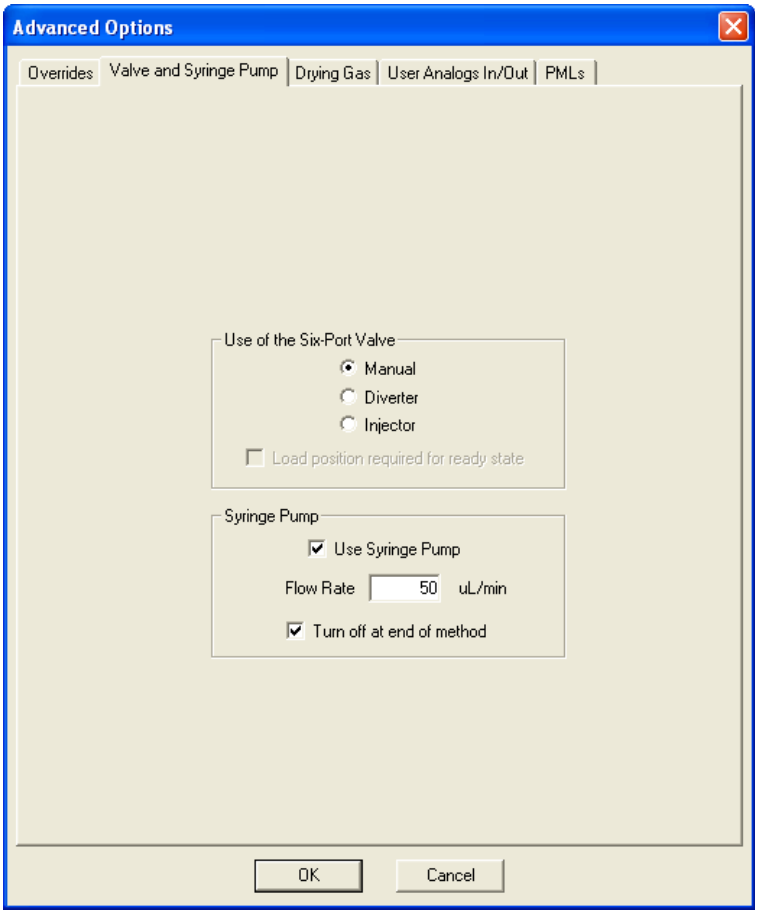
   vESI: The gas stays on until the temperature drops below 250 °C and then chamber cannot be removed until then.

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.

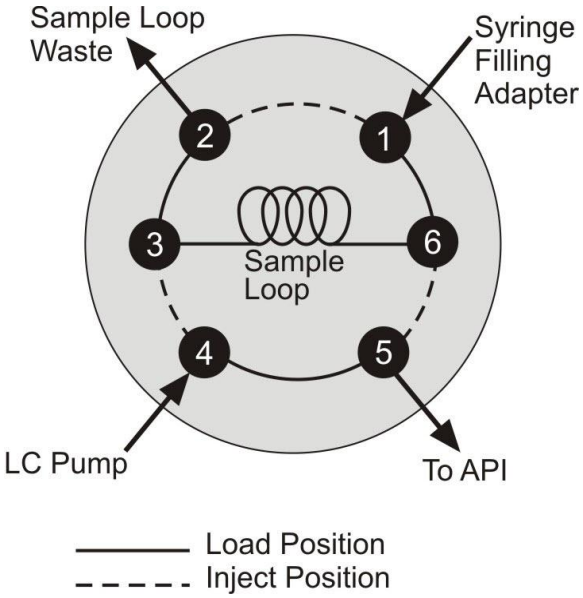
1. Click **Advanced Options** and select the **Valve and Syringe Pump** tab.
2. Select **Manual**, **Diverter**, or **Injector** for the valve. The system can turn the syringe pump off at the end of the run.
3. Enable **Use Syringe Pump**, type in the flow rate, and enable the check box **Turn off at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



Manual Injections

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

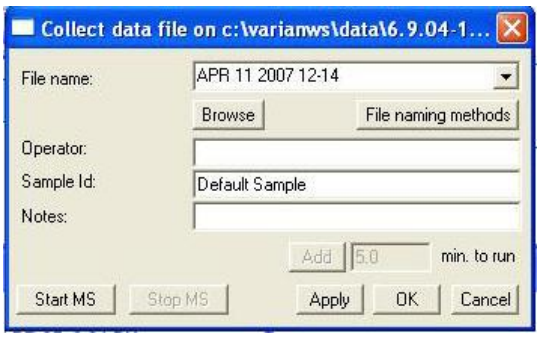
Plumb the switching valve as shown.



To do manual injections:

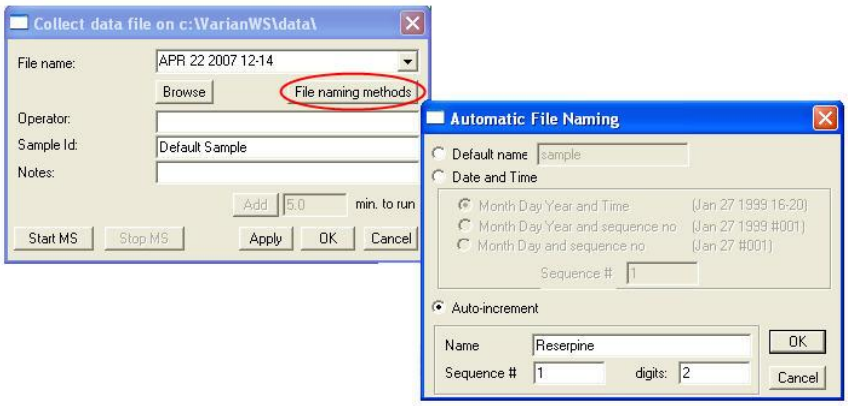
1. Monitor the baseline using the **Plot Tic** tool in the **Tools** menu.
2. After the baseline stabilizes, click the Traffic light and the Collect data file window opens.

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



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.



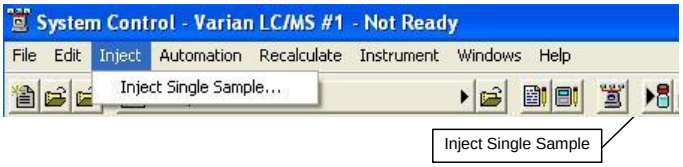
6. Click OK and the Automatic File Naming window closes.

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.

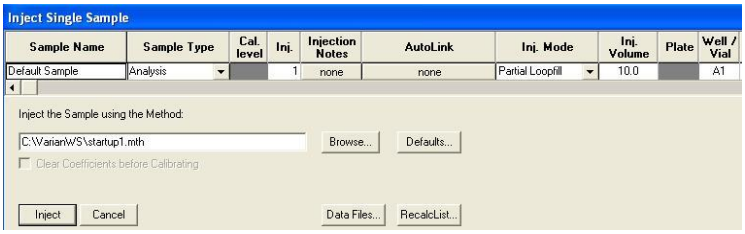
Injecting Single Samples

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.



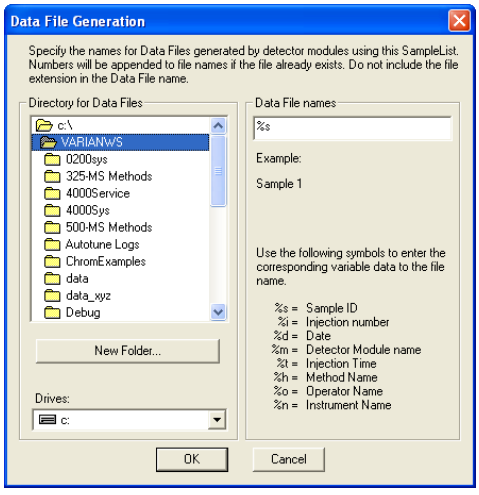
Clicking **Inject Single Sample** from the Inject menu opens the following window. The fields are dependent on the sampling device. For more information about these fields, see the “SampleList and RelcalcList Fields” section in the *MS Data Handling User’s Guide*, part number 3954038.



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.



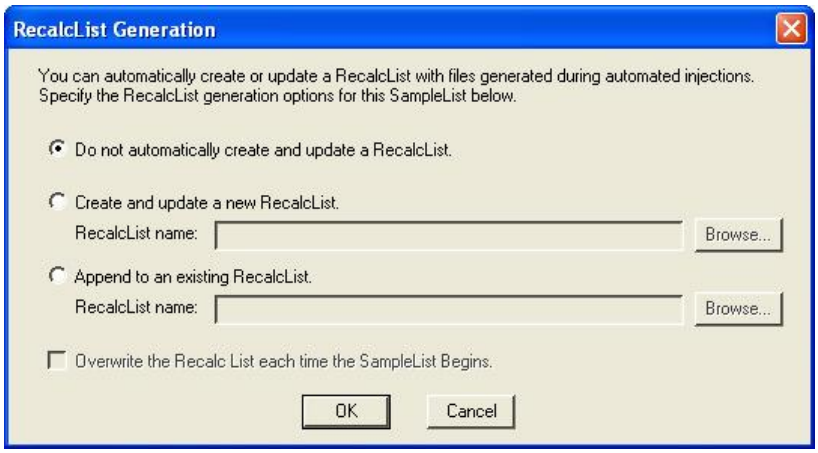
Select the drive letter and subdirectory (path) to store your files from the left side. The default directory is the data subdirectory of your VarianWS directory.

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.



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.

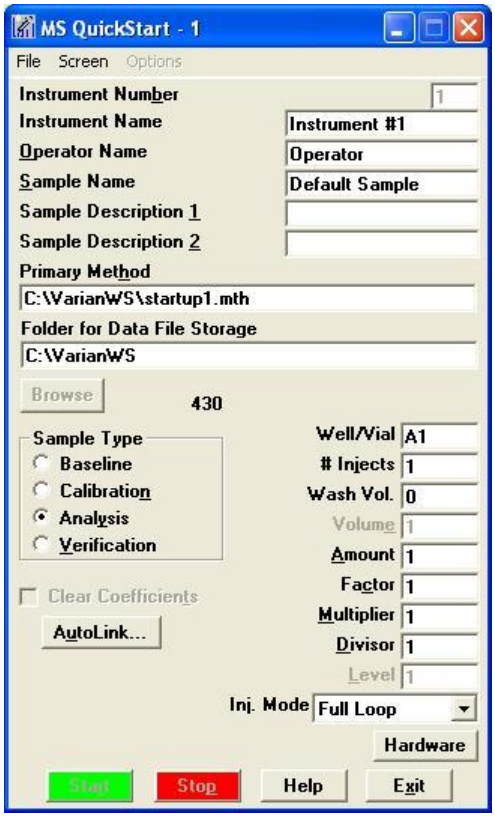
QuickStart

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.

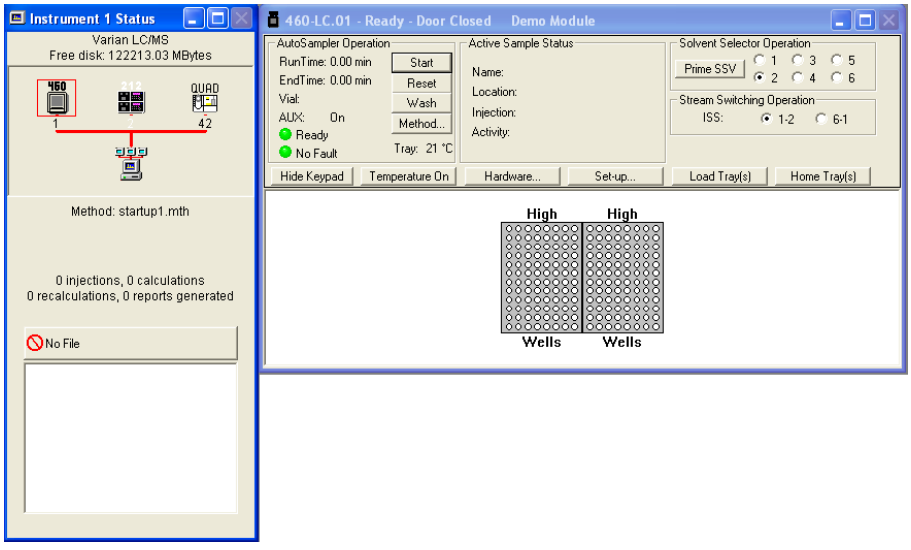
Enter information in the MS QuickStart window about your injection. For more information about the fields, see the "SampleList and RelcalcList Fields" section in the *MS Data Handling User's Guide*, part number 3954038.



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.

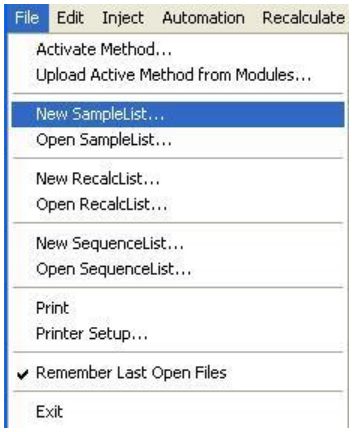
1. While automation is running, click **Suspend** on the current active SampleList. Automation is suspended after the current run is completed.
2. Double-click the vial position of the priority sample or choose inject single sample.
3. After completing the priority run, click Resume to re-start the SampleList.

Autosamplers

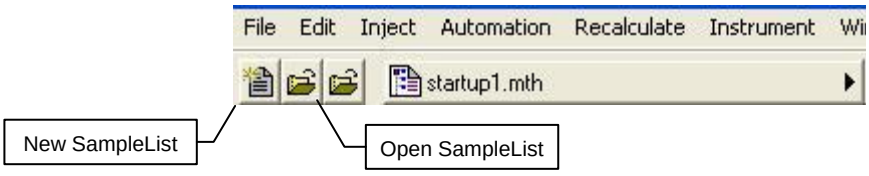
You can create SampleLists for different autosamplers, and use more than one method for your injections.

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.



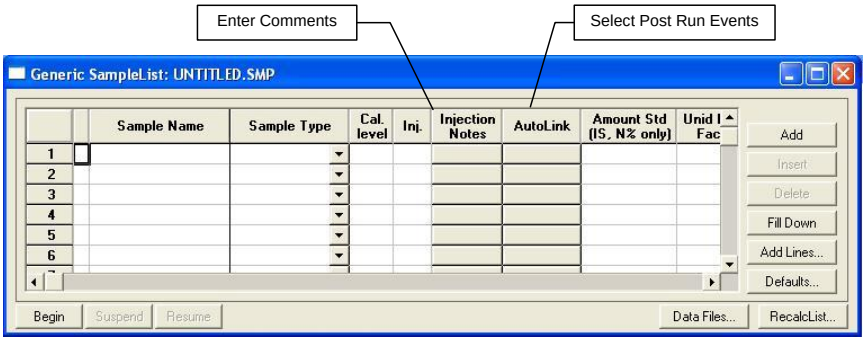
From the System Control toolbar, create a new SampleList or open an existing one



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.



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.

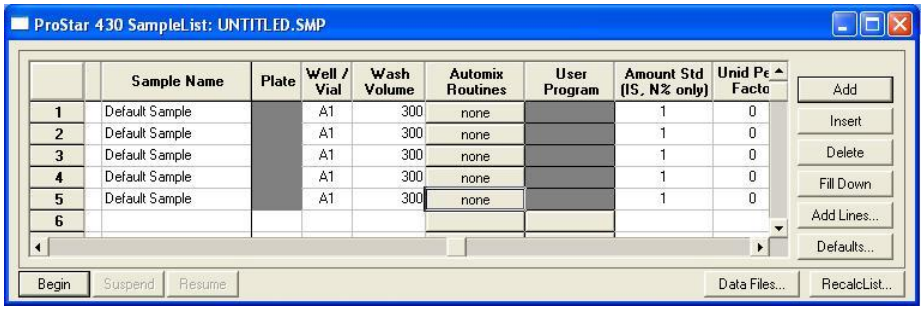
If your requirements are more complex (such as multiple internal standards or multiple detectors, which require different entries) use the MultiChannel MultiStandard field.

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**.



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 µL 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.

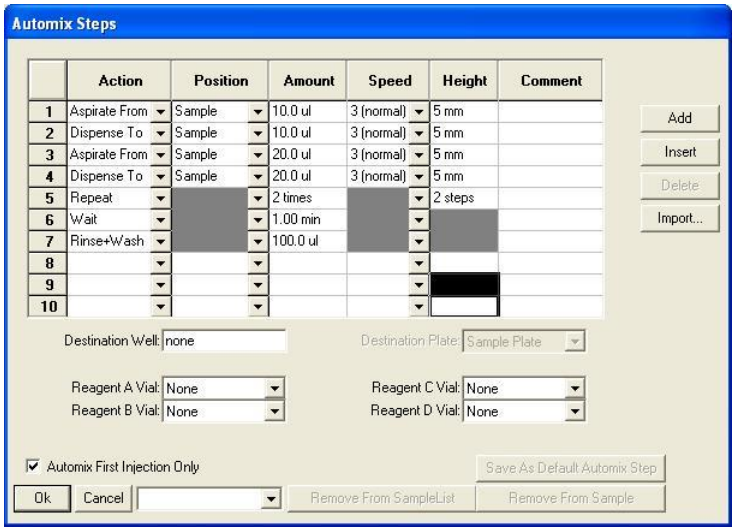
For the ProStar 420, the Vial field specifies the sample vial as a combination of Tray type letter and Vial number. The available Tray types and Vial numbers depend on the Tray Segments configured. You can randomly access any vial.

In the Wash Volume field enter a wash volume in the range of 300 to 9999 µL, 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.

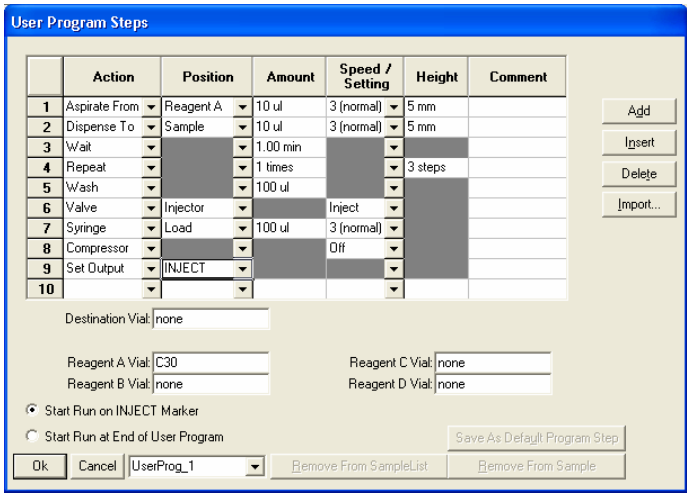
You can program the AutoSampler to wait for a specific time interval. Use the Automix Steps dialog to select Action, Position, Amount, Speed of the syringe, and Height of the needle. The following is an example of an Automix routine.



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.

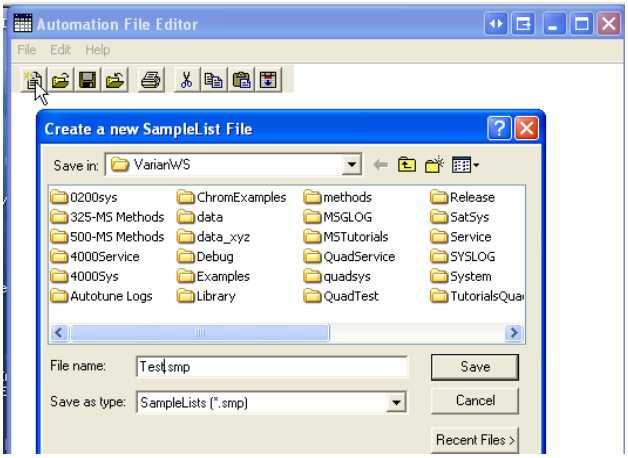
When the User Program is selected as the Injection Mode, the following fields are disabled; Injection Volume, Wash Volume and Automix Routines. The User Program steps are used instead of the method parameters. You must use the User Program if the 10mL syringe is selected. The following figure shows lists 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**.



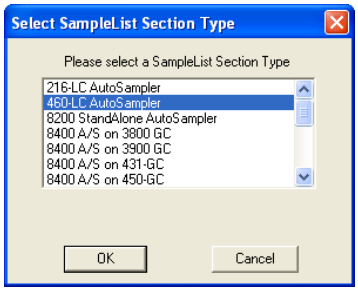
460-LC SampleList

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

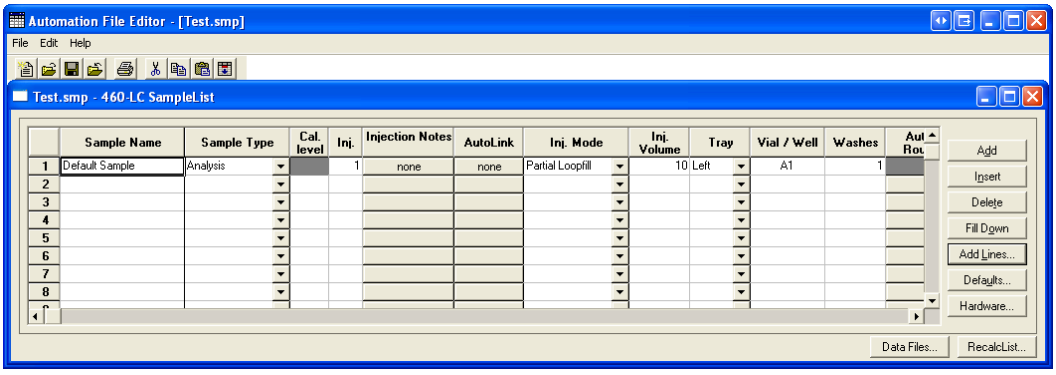
1. Click the Edit Automation Files button to open the Automation File Editor.
2. Click the New Automation File button and click new SampleList.
3. Enter a file name and click Save.



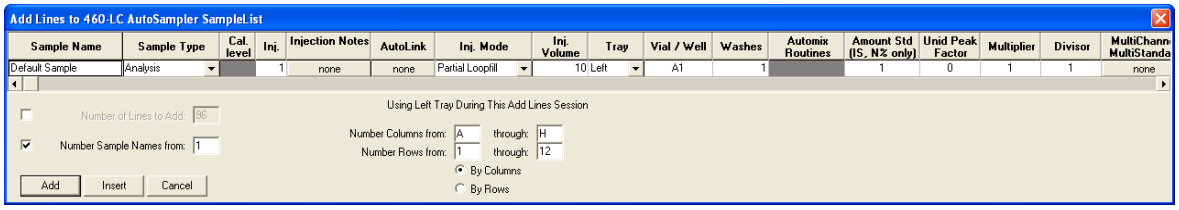
4. Select the 460-LC AutoSampler and click **OK**.



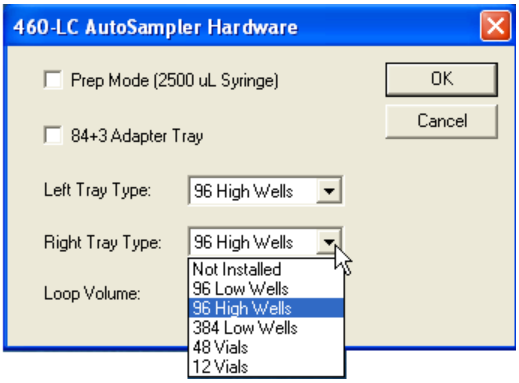
5. An empty SampleList with your file name opens.
    - Click **Add** to add one sample.



    - Click **Add Lines** to add several samples or a plate.

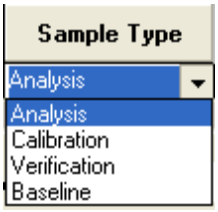


- Click **Hardware** to specify the tray type for each position, sample loop volume and indicate if the Syringe for Prep Mode is installed.



Sample Name: Enter the “root” name for all samples.

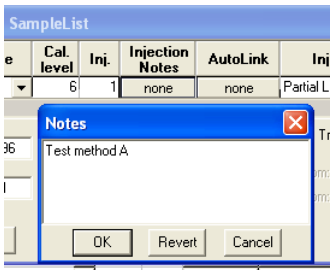
Sample Type: Select **Analysis**, **Calibration**, **Verification**, or **Baseline** from the menu.

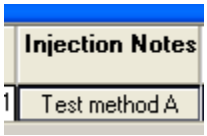


Cal. Level: This numerical field is used with Calibration and Verification Sample Types.

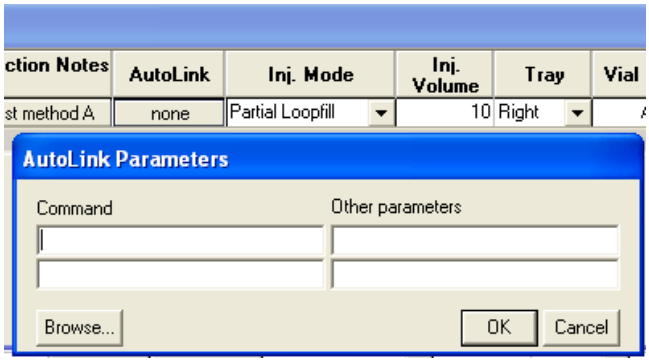
Inj. Enter the number of injections in this numerical field.

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





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



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.

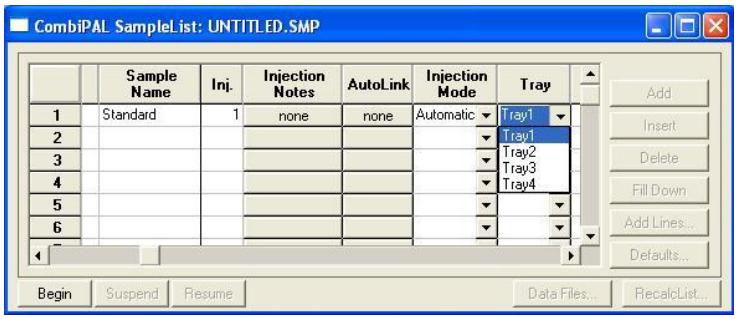
- 96 well plate: A1 through H12
- 384 well plate: A1 through P24

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.



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

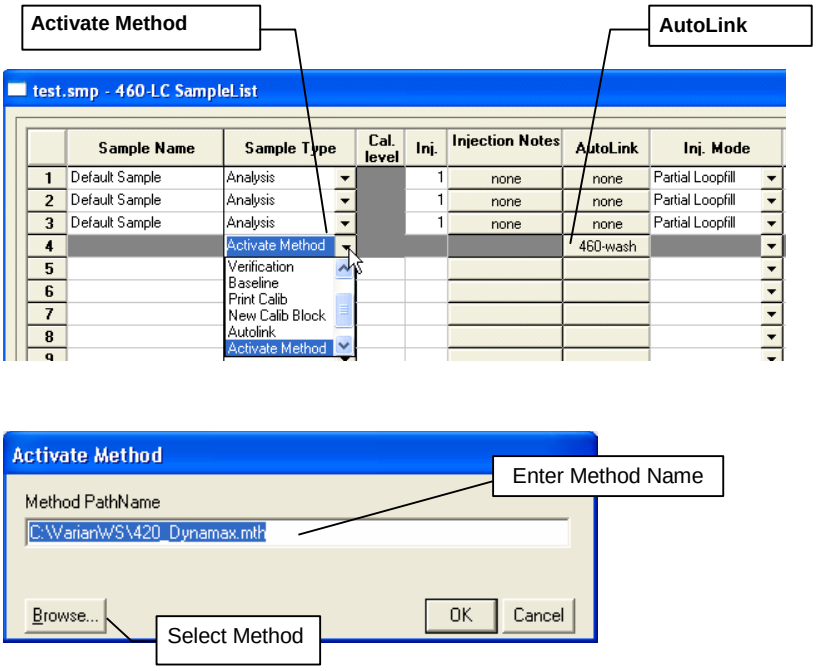
When you make changes to the open SampleList, the changes are automatically saved to the SampleList file and are used for the automated runs in progress. If you want to edit a SampleList other than the open SampleList, use the offline Automation File Editor application described in the next section of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

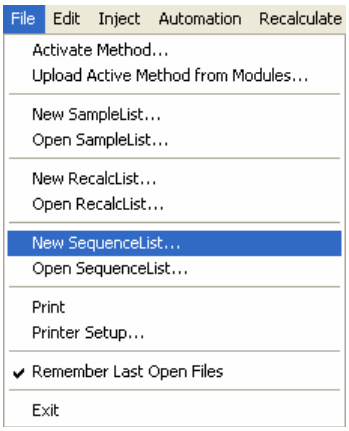
Changing the Method in the SampleList

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

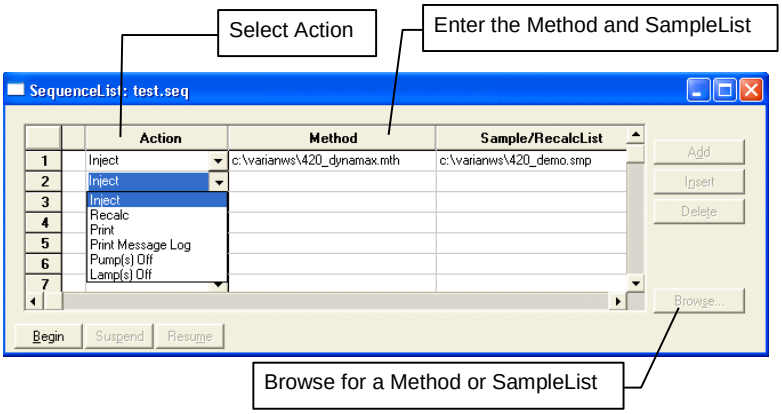


Using the SequenceList Window

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



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

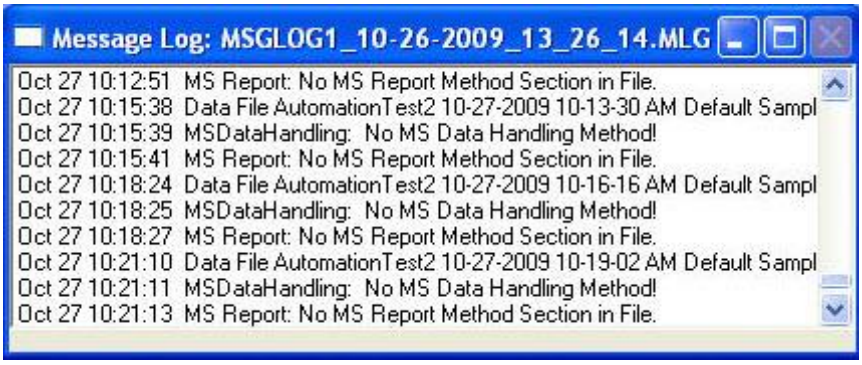


Monitor Run Status

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

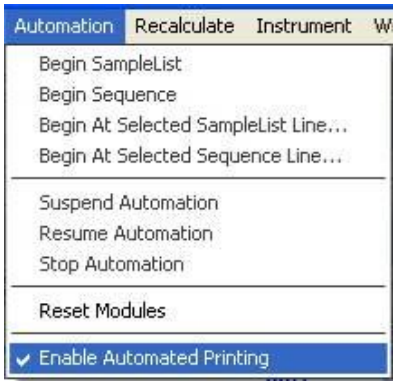
Message Log

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.



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.



Stopping an Acquisition

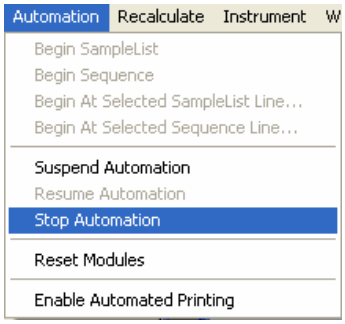
During automation and while the system is running you can suspend or stop data acquisition from the Automation menu in System Control.

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

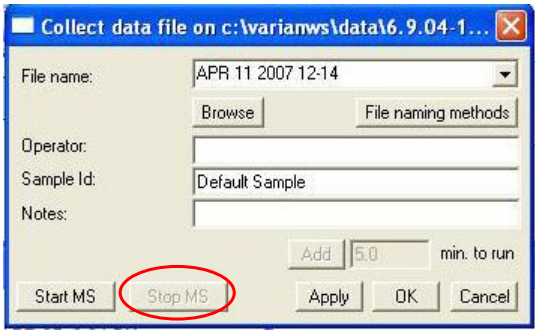
Stop Automation: Stop the current run, reset the modules, and suspends automation.

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  in the window Toolbar, click **Stop MS** to end data acquisition or in the Automation Menu bar click **Stop Automation**.



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.

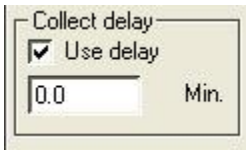
LC/MS

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

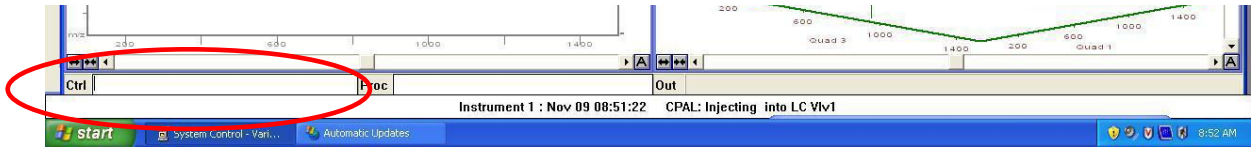
In Method Builder, open the Acquisition Method. Enabled: Use delay and enter the delay time in minutes.



MS/MS Breakdown

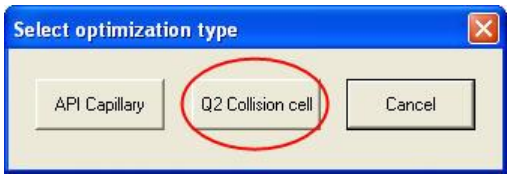
The Breakdown Curve makes MS/MS method development easy. The sample is introduced into the mass spectrometer API source with a syringe pump. A precursor ion is selected for MS/MS breakdown curves.

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.



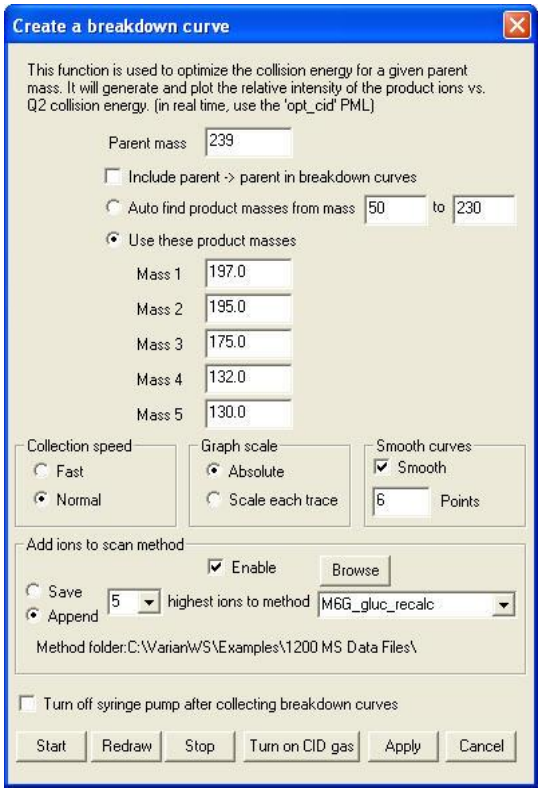
To create a Breakdown Curve, do the following:

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



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**.



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).

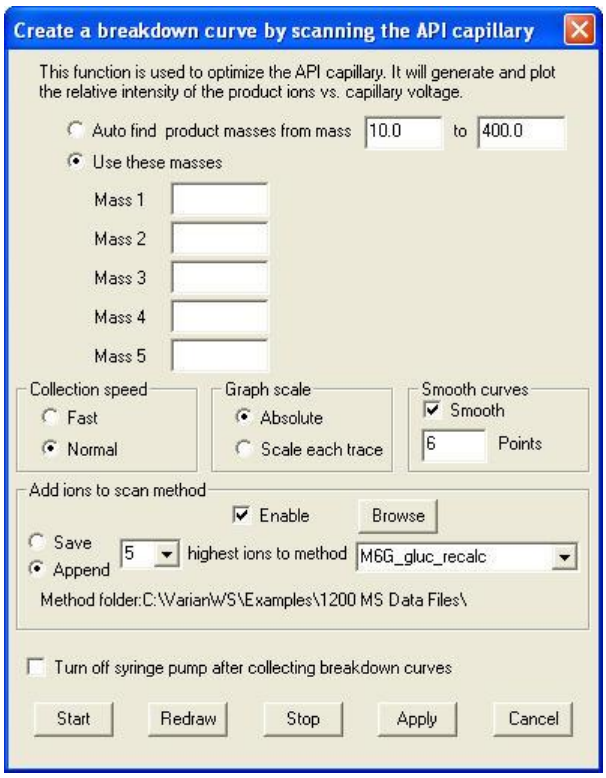
For this additional precursor ion, select **Auto find product masses** or specify the product masses.

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**.



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.

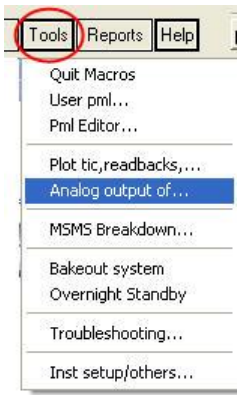
To add ions and collision voltages to an existing method, select **Append** and the appropriate method.

Analog Channels

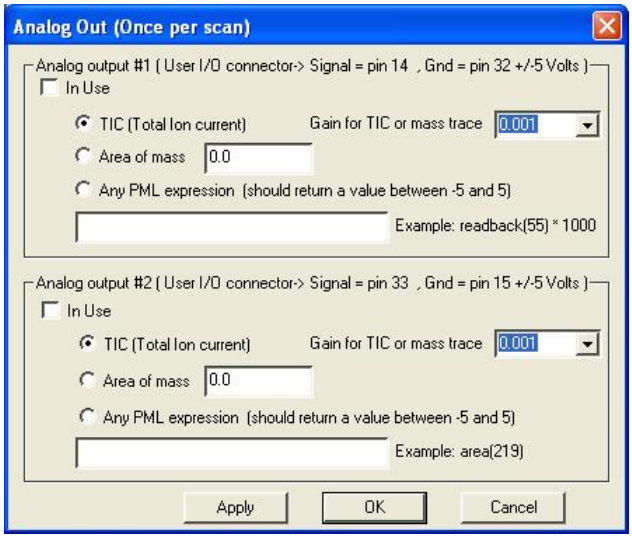
Two analog channels can be configured in the 300 Series using Analog Out selections or by defining PML outputs.

To use Analog Out, do the following:

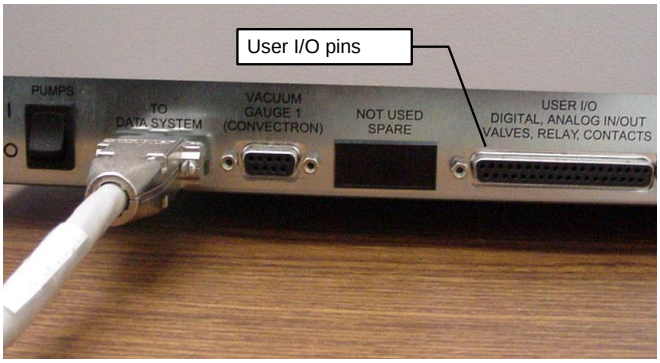
1. Select **Analog output of** from the Tools Menu.



2. The **Analog Out** widow opens.



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.

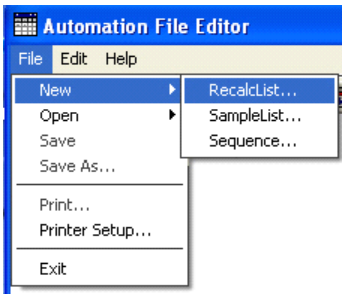
NOTE: SampleLists and Sequences that are active and running in System Control cannot be accessed in the off-line Automation File Editor.

Accessing the Automation File Editor

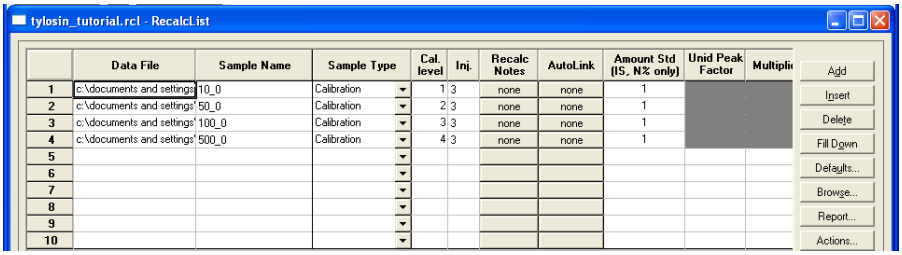
Click the **Automation File Editor** button  on the Workstation Toolbar.

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.

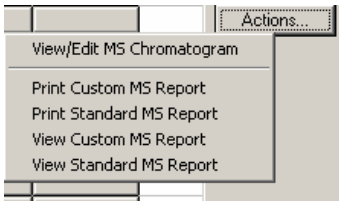


Recalc Notes: Enter notes about the Recalculation of the Data File.

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.

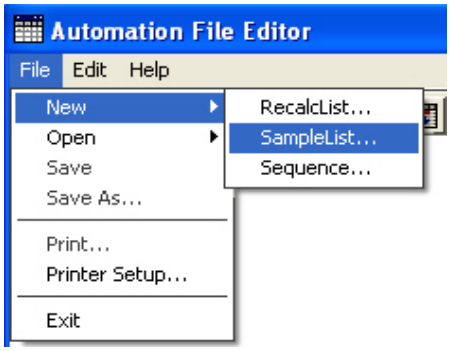
Actions: After generating a report using the Report button, click **Actions** to show the possible actions.



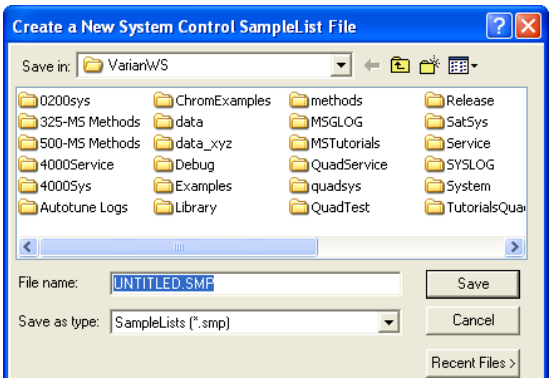
Create or Edit a SampleList

To create or edit a SampleList, do the following:

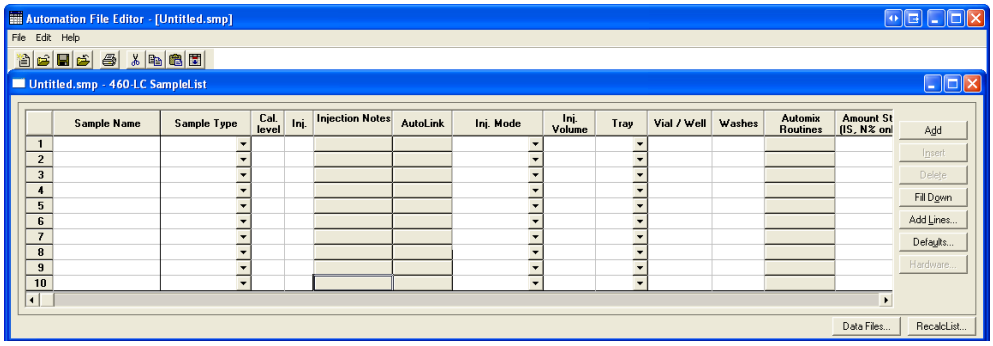
1. From the **File** menu, select **New** to create a new SampleList or select **Open** to open an existing SampleList to edit.



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



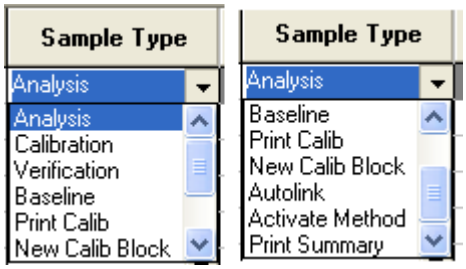
3. Size the Spreadsheet columns by dragging their borders with the left mouse button. Right-clicking a column header displays formatting options. The Sample Name column does not scroll.



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.



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.

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

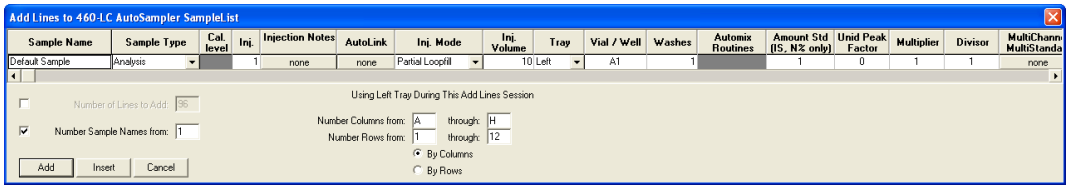
Add: Adds a new line to the end of the list.

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.



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.

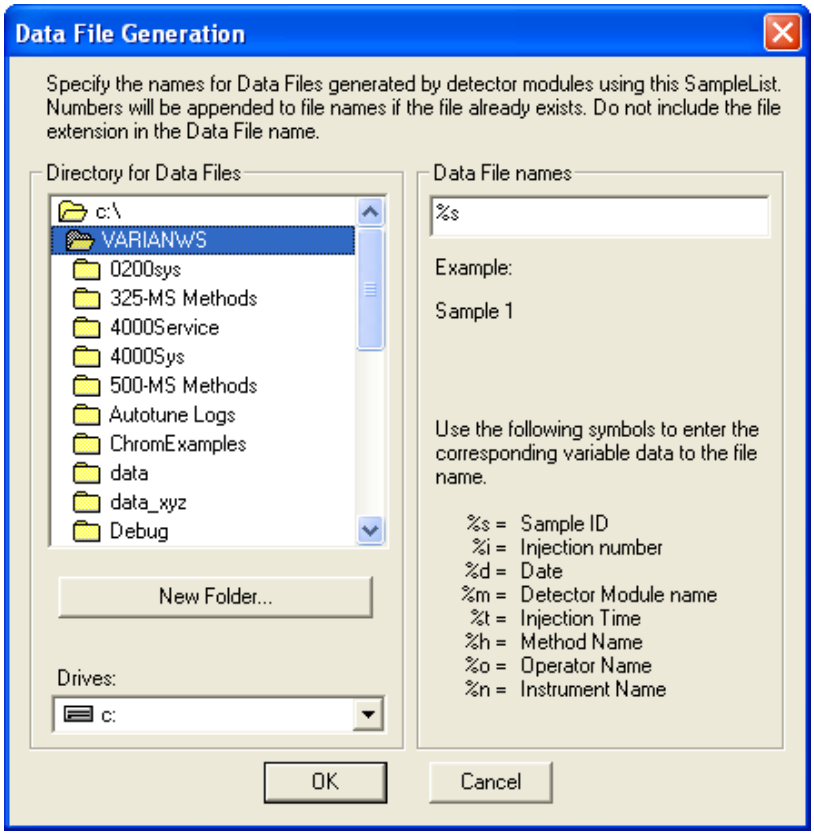
Data Files: Change the location and name of the Data Files.

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.

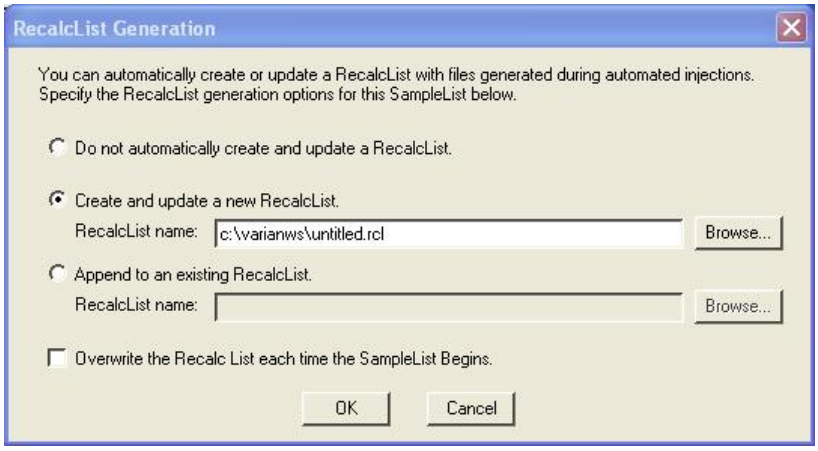
Click **Data File** to open the **Data Files Generation** window. Select the path and the file name for the data file.



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.



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:

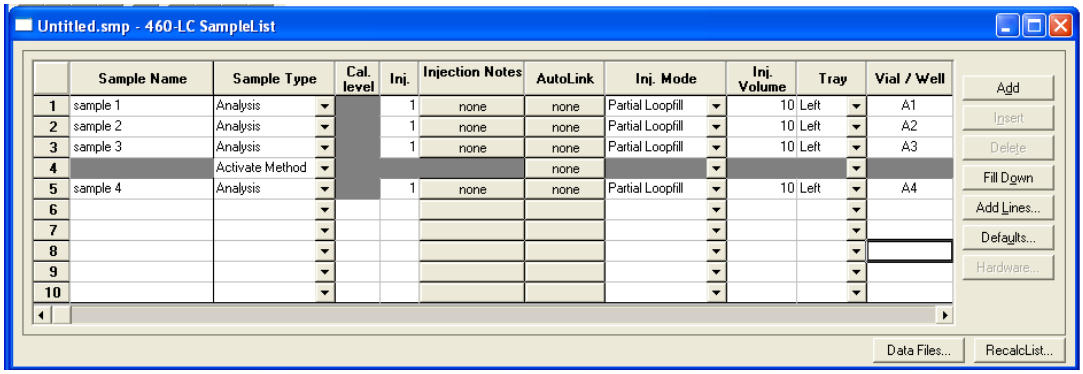
1. Click **Default** in the open SampleList window.
2. Enter the desired default values and click **Save**.

Using Several Methods for Injections

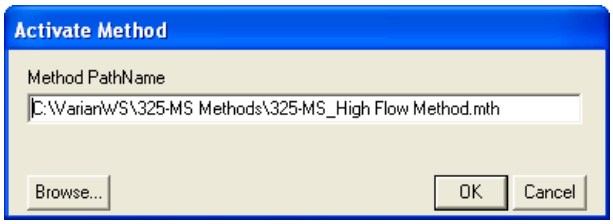
Change the active Method from the SampleList or use a Sequence to perform automated injections using more than one Method.

Changing the Method in the SampleList

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



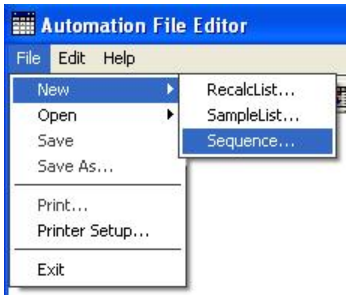
1. Select Activate Method from the Sample Type cell.
2. Click AutoLink in the row that you are working. The Activate Method widow opens.
3. Enter the name of the Method or click Browse to select the Method from a list.



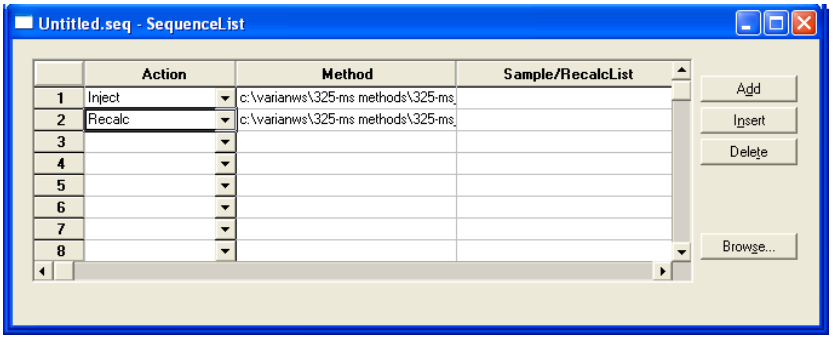
Create or Edit a Sequence

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.



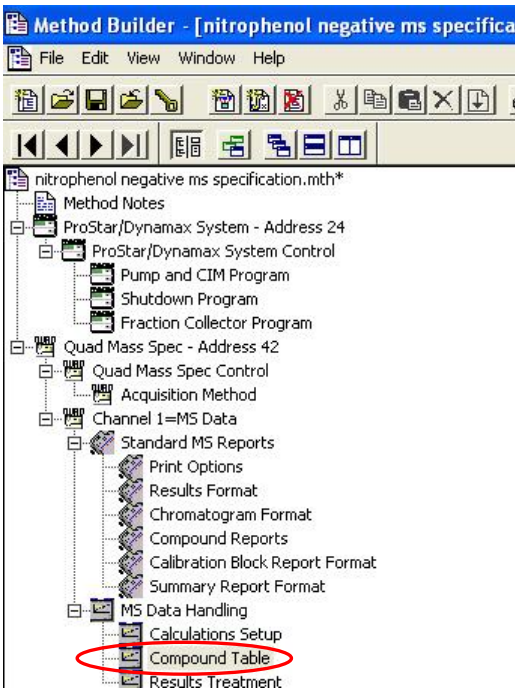
3. Click **Add** to add lines to the SequenceList.
4. Enter the required number of Sequence lines.
5. Select the **Action** for the selected step.
6. Click **Browse** to select the Method and SampleList files.



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.

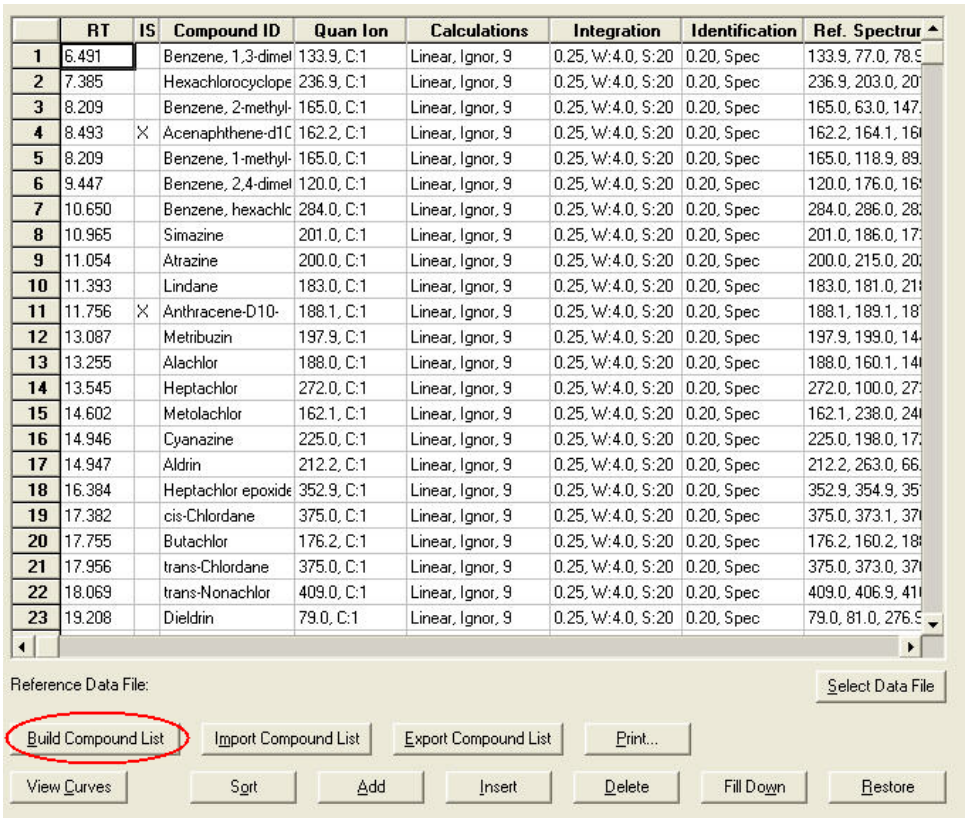


Creating a Compound Table

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.

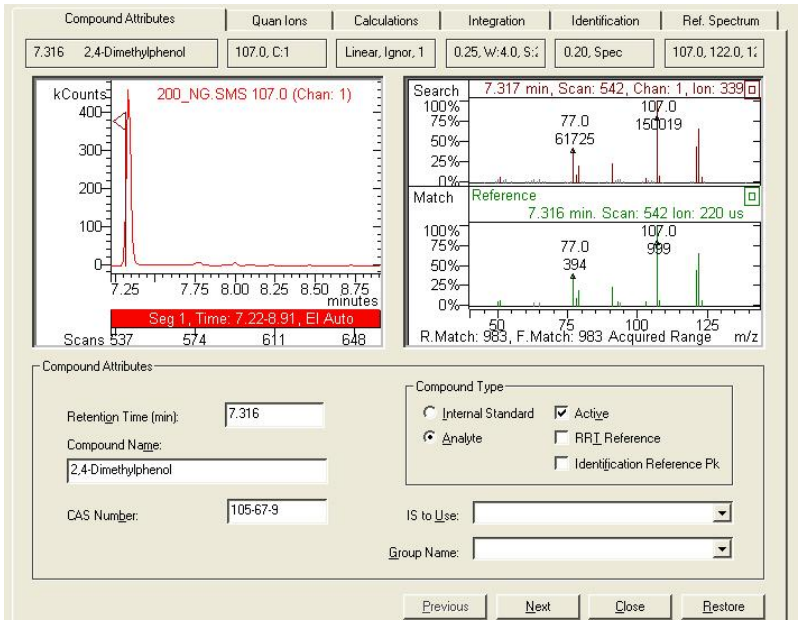


The Compound table has the following fields; Retention time (Ret), Internal Standard (IS), Compound ID, Quanitation Ion (Quan Ion), Calculations, Integration, Identification, and Reference Spectrum (Ref Spectrum).

- 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.



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

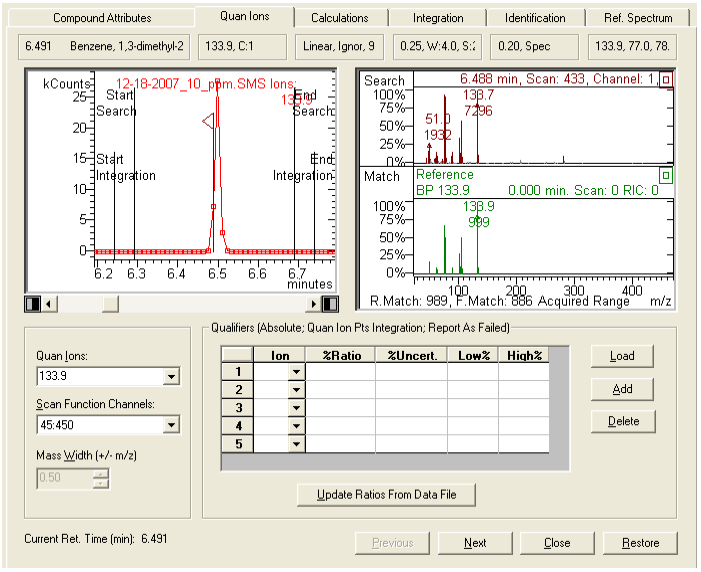
Sometimes target compounds are identified as a member of a group of compounds (for example, Trihalomethanes (THM), alkyl aromatics, etc.).

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.

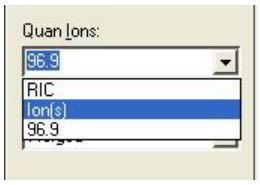
Quan Ions

Click the **Quan Ions** 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.



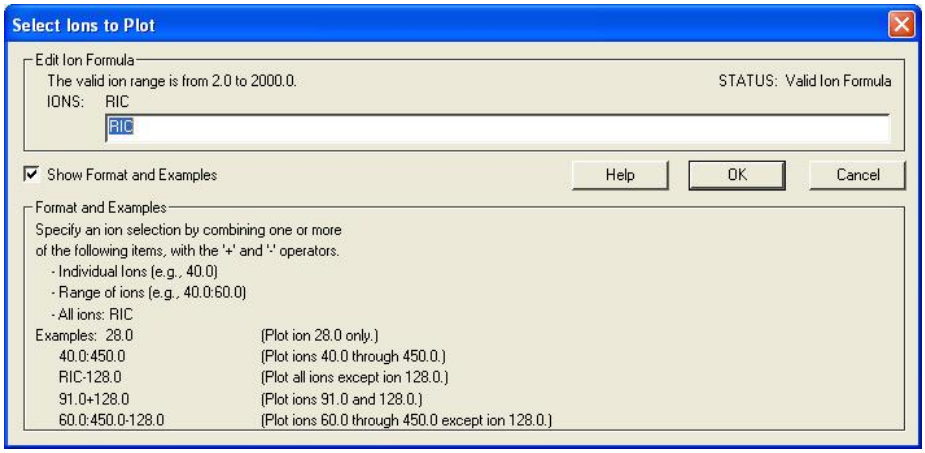
Edit Quan Ions

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



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 Retention Time

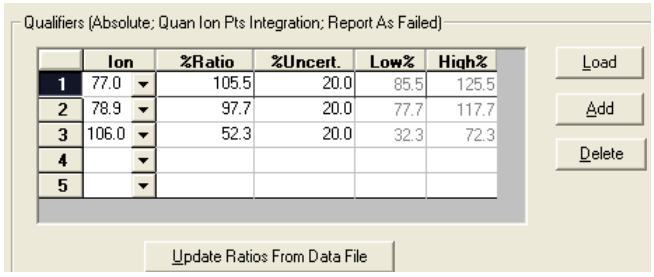
To change the retention time, click the scan in the Chromatogram display.

Look at Other Compounds

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

Select Qualifier Ions

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



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

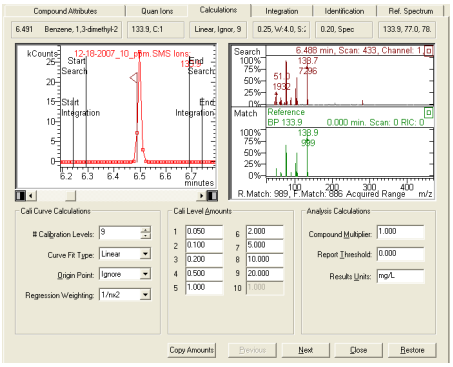
The Qualifiers Table has the following fields:

- **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 ± 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.

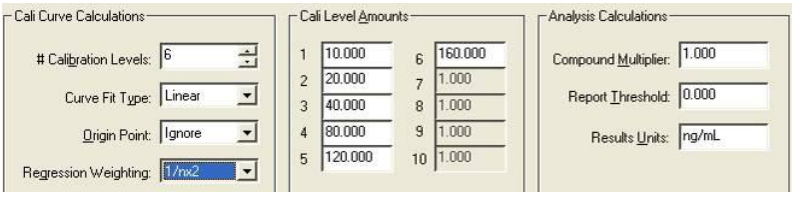
Calculations Tab

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.



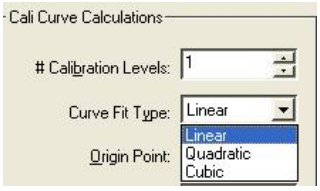
The Calculations Information is updated in the Compound Table. This information can be automatically entered for the other compounds in the table.

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.



For most calibration curves, use either Linear or Quadratic.

Security Administration

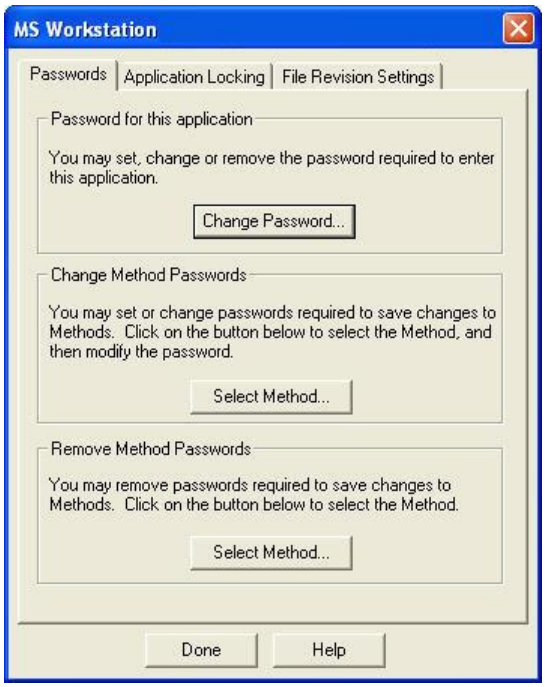
Overview

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

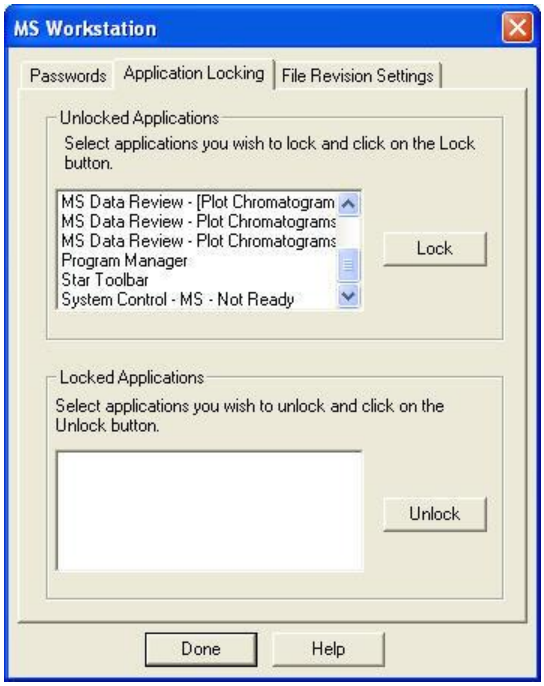
Click the **Security** button on the Workstation Toolbar to open the Security Administration window.

Passwords



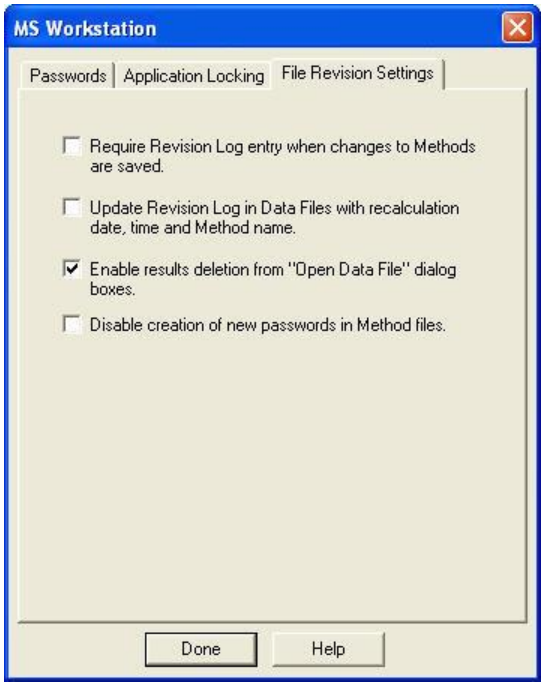
Area	Description
Password for this application	Specify a password required to open the Security Administration application. **NOTE:** There is no way to recover this password if it is lost, so document the password in a secure location.
Change Method Passwords	Method passwords can be added, changed, 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



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



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.

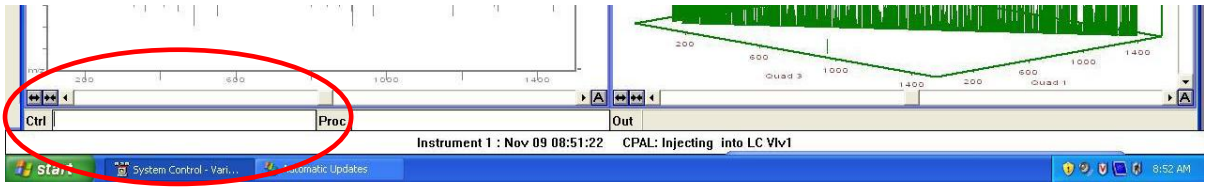
PML

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) & ~(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.

   DACS_RESTORE : "file name" : CR

   DACS_SAVE : "file name" : CR

   FILE_APPEND : "file name" : CR

   FILENAME : "file name" : CR

   FILE_OPEN : "file name" : CR

   IONS_RESTORE : "file name" : CR

   IONS_SAVE : "file name" : CR

   LCD : "message" : CR

   LIB_FIND : "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

6. Statements may include comments. A # ends the statement and starts the comment, e.g., :DETECTOR += 10 # raise the detector to make the peak bigger.
7. 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))

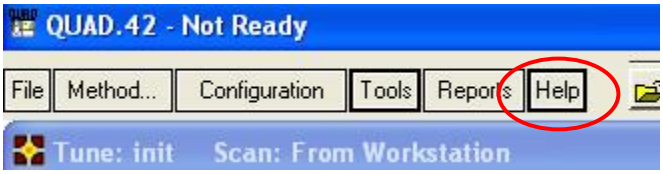
   (click on the CHRO scales button to see where type_rt,type_sn, and make_substance are defined)

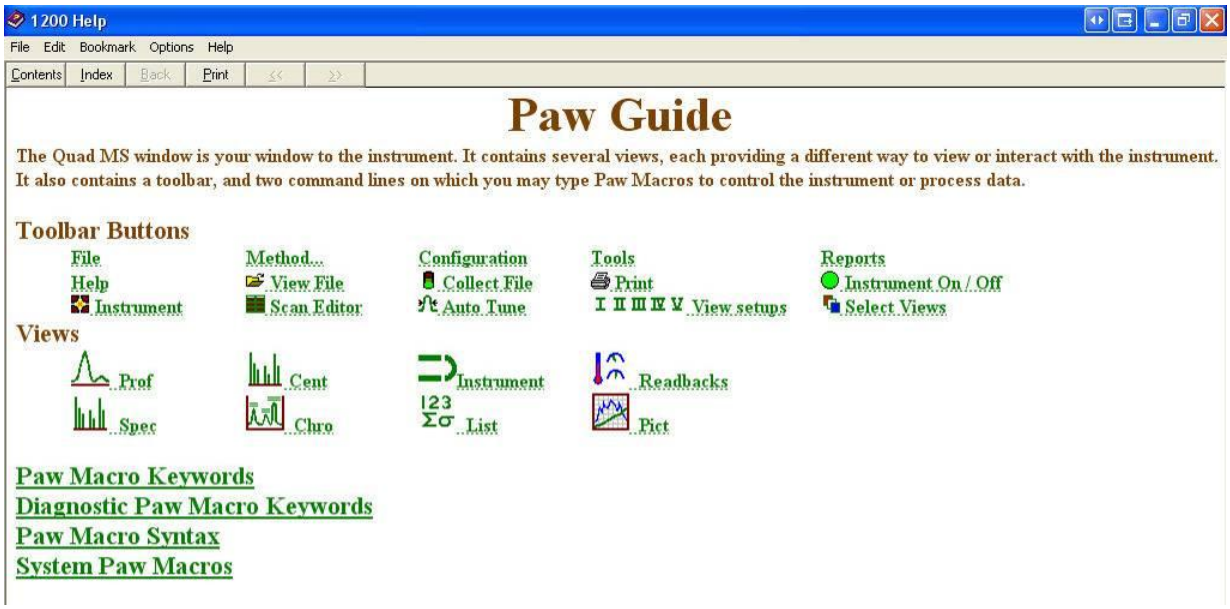
Other popular PMLs are:

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.

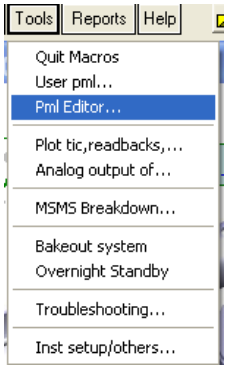
Repeat	**#repeat items below**
Prof	#set to scan of profile data
Fish	#go get one scan of data
Cent	#set to scan of centroid data
Fish	#go get one scan of data
End	

PML Editor

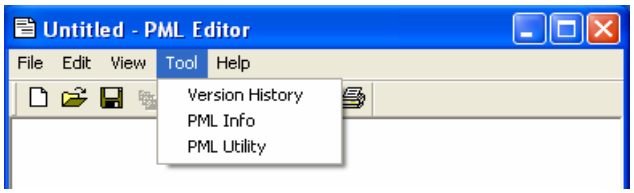
The **PML Editor** is used for creating and editing PMLs for the 300 Series software. The PML Editor allows access to the factory PML files; however, if a factory PML is modified it will then be saved as a user PML in the UserPML directory.

Accessing the PML Editor

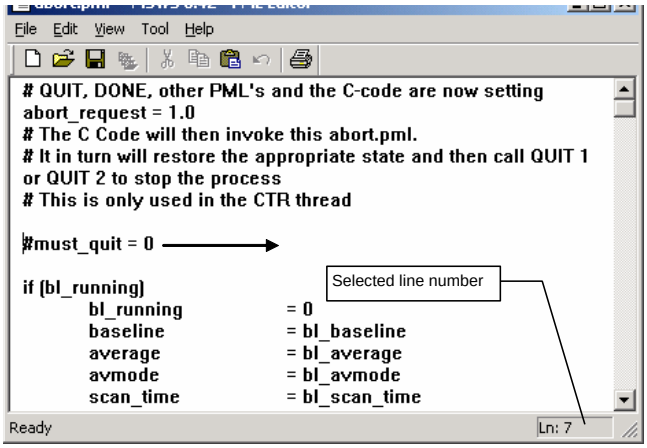
Click **Tools** in the Quad module window to display the PML editor.



The PML editor window opens.

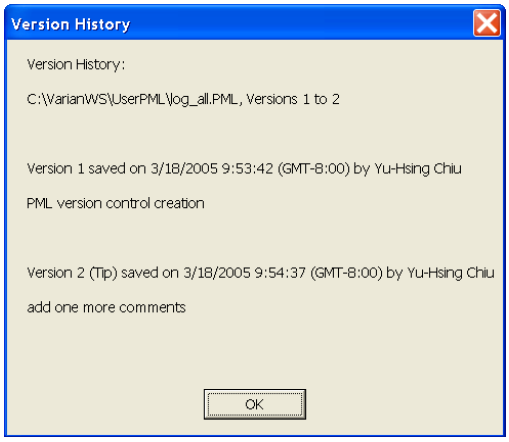


- Varian PMLs are stored in c:\varianws\1200sys\PML directory.
- User created PMLs or modified factory PMLs are stored in c:\varianws\UserPML directory.
- The Status Bar of the PML Editor displays the line number of the current cursor position.

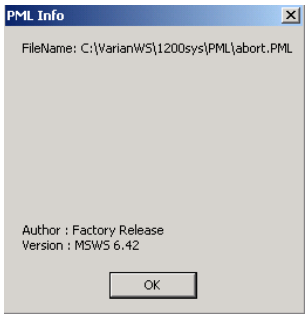


- The Tool menu has three menu items:

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.