Varian, Inc.
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300 Series GC/MS and LC/MS Quadrupole Mass Spectrometer MS Workstation

Version 6

Software Operation Manual

QUALITY SYSTEM ISO 9001 CERTIFIED

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Introduction

Overview

The Varian MS Workstation Version 6 software controls the 300 Series Quadrupole instruments, associated Varian chromatography modules and several modules from other vendors. Use this manual to maximize your application of the features.

Additional Manuals

300 Series Hardware Operation Manual

This manual**,** part number 3954912500, provides the necessary information for using, maintaining, and repairing your 300 Series System. This information is also available in Online Help.

MS Workstation Software Reference Manual

This manual, part number 391496300, explains using the MS Workstation Software with acquired data. 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 software.

Data Acquisition with 3800 GC Control

This manual, part number 391473100, explains how to build methods, acquire data, and operate the 3800 GC with your Varian MS Workstation. This manual also describes the configuration of the 3800 GC for standard Ethernet communication with the System Control application.

Data Acquisition with LC Control

This manual, part number 391473200, describes the operation of the MS Workstation with Varian HPLC modules. It covers configuring an instrument for data acquisition, building a method, and running samples.

DIP/DEP Solids Probe Manual

This manual, part number 395414900, which comes with the optional DIP/DEP probe for the 300-MS or the 320-MS GC/MS, describes the operation of the DIP/DEP solids probe.

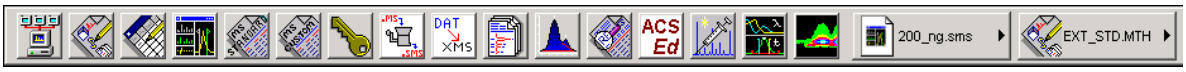
Chromatography Hardware Manuals

Refer to the manuals of your chromatography modules.

MS Workstation Applications

Overview

The applications of the Varian MS Workstation Toolbar are: Methods, Data Files, SampleLists, RecalcLists, SequenceLists, and Reports. They are on the Varian MS Workstation Toolbar and in the Varian MS Directory. When you install other MS Workstation options, such as Star Finder and Star Custom Report Writer you can add those application buttons. The followingtgr4 is a brief introduction to the applications. Each is explained in detail in later sections.



 **System Control -** Control Varian instruments including the 300 Series MS, HPLC, and GC modules. There are two main windows, the Configuration window, and the Data Acquisition and Control window.

 **Method Builder -** Edit or create methods for the 300 Series MS, Varian HPLC, and GC chromatography modules.

 **Automation File Editor -** Build SampleLists, RecalcLists, and SequenceLists for System Control automation. You may also build these Automation Files directly in System Control.

 **MS Data Review -** Review chromatograms and spectra, do library searches, and review and process quantitation results.

 **Standard MS Report -** Edit the standard MS report template.

 **Custom MS Report -** Create custom report templates.

 **Security Administration -** Select MS Workstation security options and passwords.

 **Data File Conversion -** Convert older data files collected with Varian Ion Trap Mass Spectrometers to the current data file formats. Keep an archive of existing data with current data files for quick and easy reference.

DAT to MS Conversion - Convert Kodiak DAT files to MS Workstation XMS format.

 **Batch Reporting -** Automatically print one or many reports by dragging data file names from the Explorer or My Computer window and dropping them onto the Batch Reports window. The reports are printed with the method last used to process the data files.

 **View/Edit Chromatograms -** View and edit chromatographic data gathered by conventional HPLC and GC detectors. You may load up to 7 chromatograms, manually or automatically assign or re-assign chromatogram events, and generate new results.

 **Standard Chrom Reports -** Display and edit the preferences for reports generated from traditional chromatography detectors such as UV/Vis or FID detectors. These reports may be viewed one-at-a-time.

Compound Set Editor - Create and edit sets of compounds in the MS Data Handling Method allowing activation or deactivation under automation.

 **Quick Start -** Make a single LC/MS or GC/MS injection quickly. Do not use this for multiple injections. Refer to the Injecting Samples and Automation section for more information.

 **PolyView™ 2000 -** View and process PDA data from your Varian HPLC Photo Diode Array detector.

 **Aurora PDA -** View HPLC Photo Diode Array data in a 3D presentation.

MS Workstation Toolbar Options

Moving the Workstation Toolbar

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

The Workstation Toolbar Options Menu

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

Move to Windows Taskbar

Display the Workstation Toolbar as a Windows Taskbar icon.

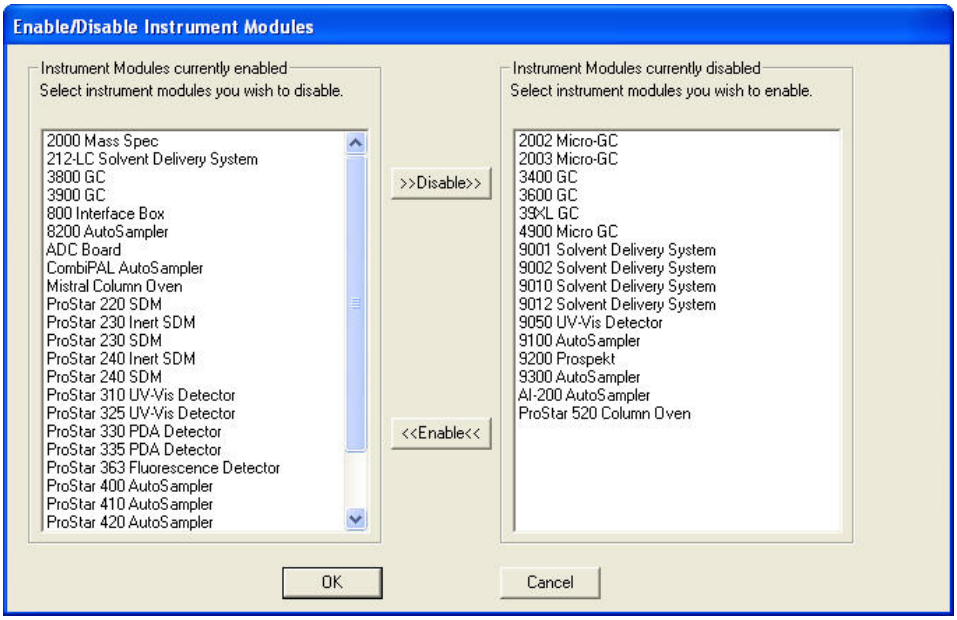
Select Move to Windows Taskbar from the Workstation Toolbar options menu. Taskbar icons appear in the lower right of the Windows Taskbar. Click the Workstation Toolbar icon to display the options menu.

Show/Hide Applications on Toolbar

Select which MS Workstation applications to represent with icons on the Workstation Toolbar. The top list box shows applications displayed in the toolbar. The bottom list shows all installed applications not displayed. To remove an icon from the toolbar, select it from the top list and click Remove. To add an icon to the toolbar, select it from the bottom list and click Add.

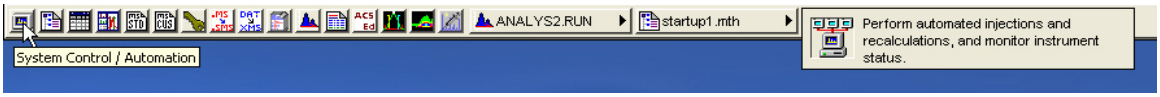
Enable/Disable Instrument Modules

Select which MS Workstation instrument modules are available. Available instrument modules are in System Control, if they are connected and turned on.



The list on the left has all the instrument modules currently installed and enabled in the MS Workstation. The list on the right shows all instrument modules that are installed but not enabled. To disable an instrument module, select it from the list on the left and click **Disable**. To enable an instrument module, select it from the list on the right and click **Enable**.

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.

Application Descriptions

Pause the cursor over an icon or a button to display a description.

Run Application

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

Pick Data File for QuickLink

Lists the eight most recently used Data Files in order of use. When you select a data file, it is displayed in the QuickLink button.

Pick Method for QuickLink

Lists the eight most recently used Methods in order of use. When you select one, the Method displayed in the QuickLink button changes to the selected file.

Help On

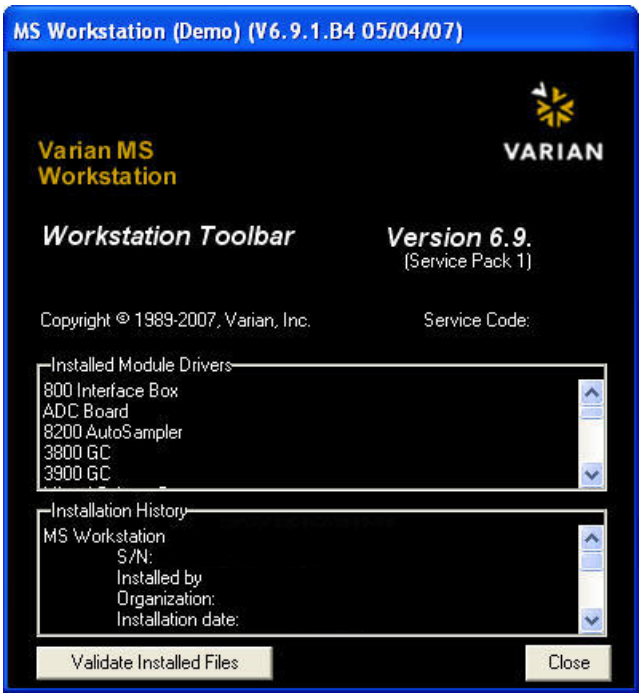
Displays all applications on the Workstation Toolbar.

Product Support Web Site

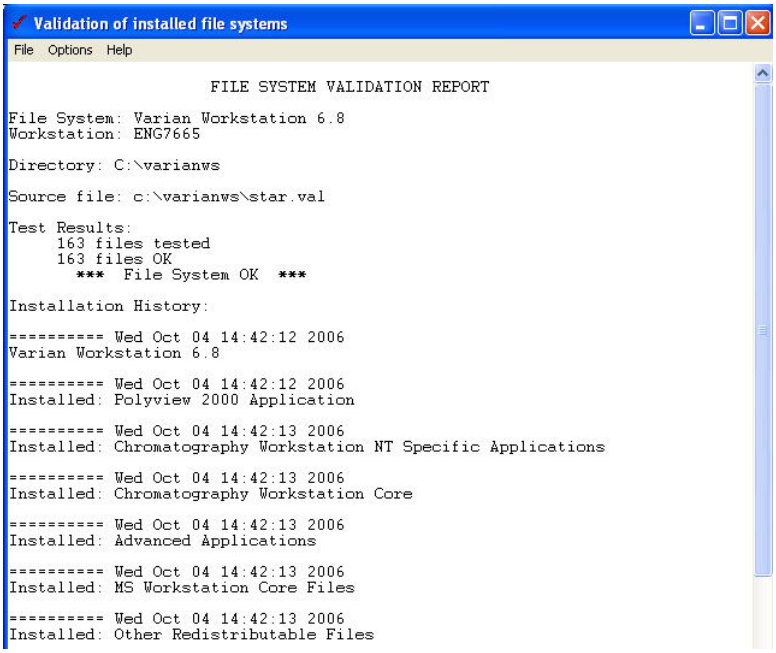
If you have Internet access and a web browser installed on your computer, click to open the Varian MS Workstation Product Support Web Site. It has the latest software and documentation updates for the MS Workstation suite of products, and notes, tips, and answers to frequently asked questions. Visit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

About Workstation Toolbar

Displays the About box for the Workstation Toolbar, which contains information about the MS Workstation software version, installation history, and a list of the instrument control modules installed. The following is an example.



Validate Installed Files - Uses checksums to test and document errors. Print a report that documents 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.



Quit

Click to close the Workstation Toolbar application. If you choose to run the Workstation Toolbar automatically when Windows starts, the Workstation Toolbar reappears the next time you start Windows.

Launching Applications from the Workstation Toolbar

Application Icons

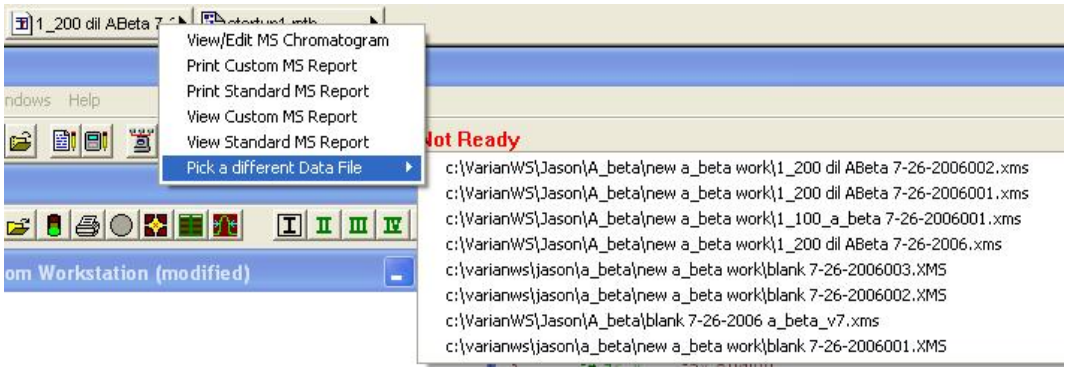
MS Workstation applications are icons on the Workstation Toolbar. As you move the cursor over them, the name of the application appears below the cursor.

Click an icon to launch that application.

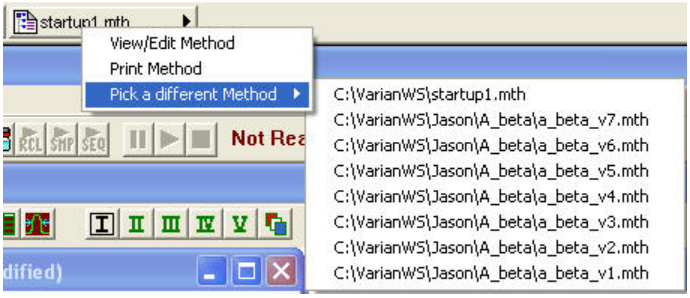
Data File and Method QuickLink Buttons

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 select a different method file from the list of most recently used method.

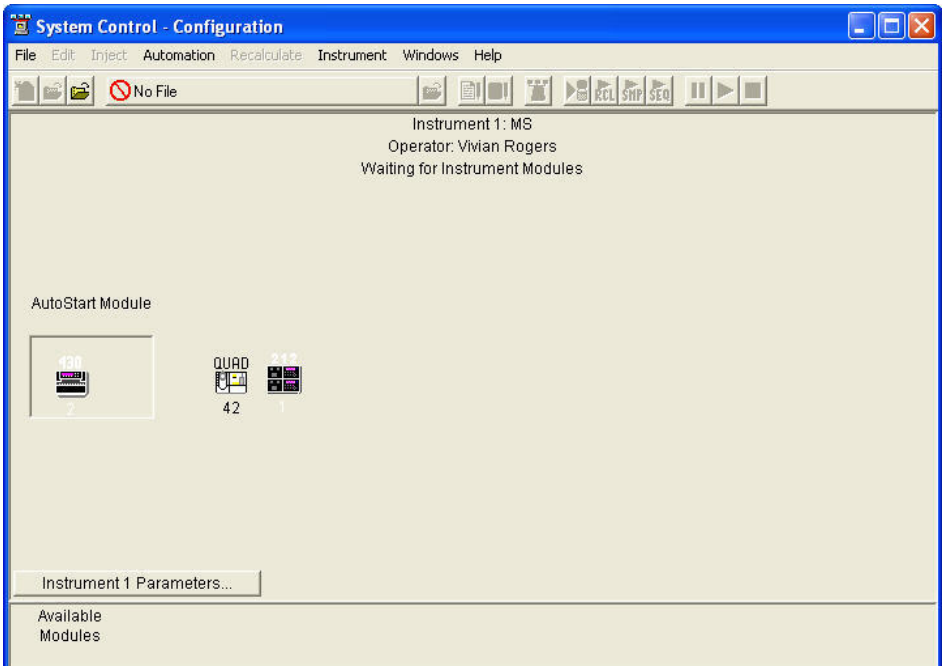


System Control

Overview

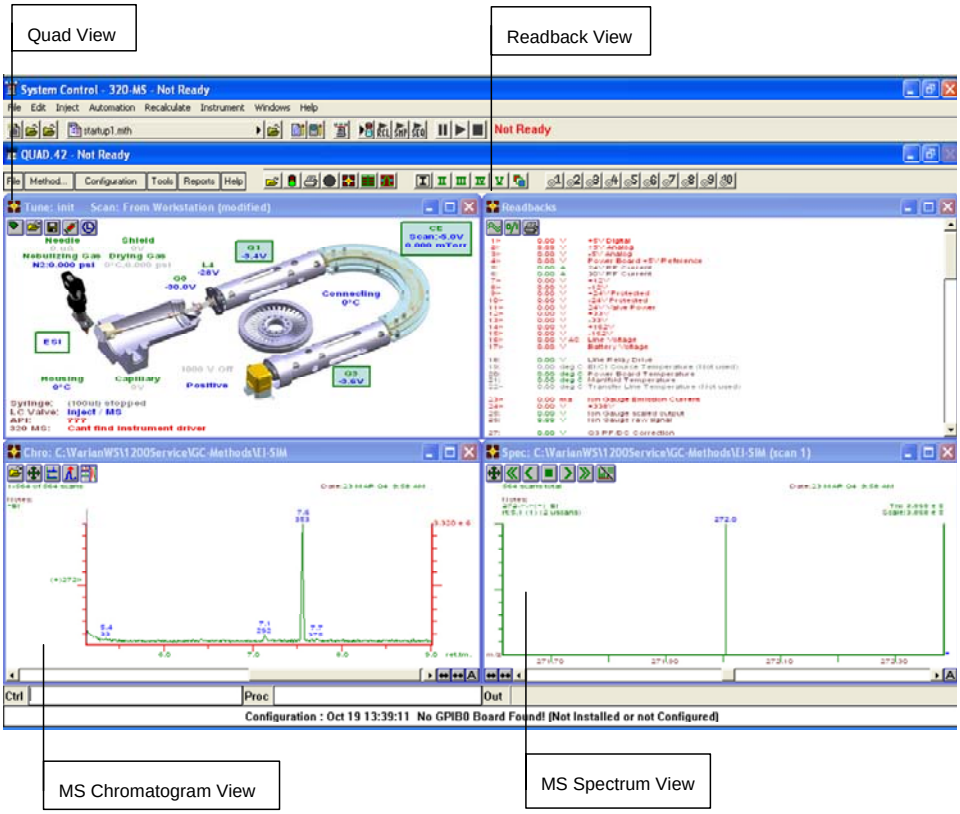
This section presents an overview of System Control functions. Other functions are explained in detail in later sections.

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

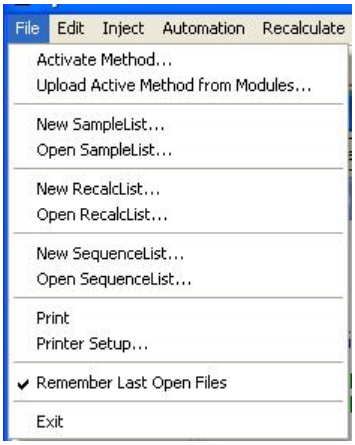


Instrument Window

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



System Control File Menu

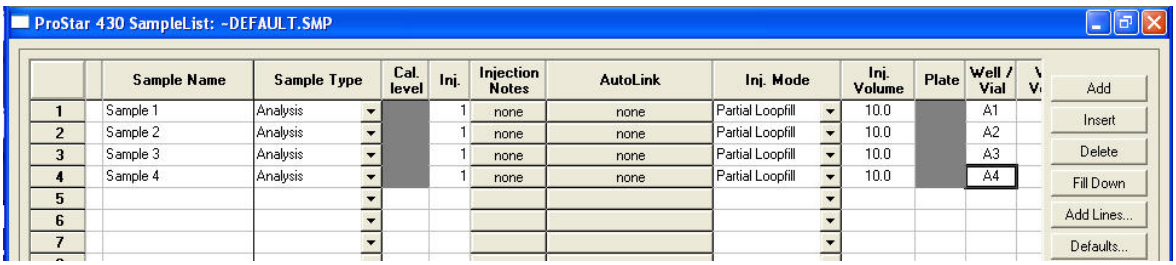


Activate Method - Select an existing method. This sets the conditions for the connected modules such as, LC, GC, MS, AutoSampler, and data handling.

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.



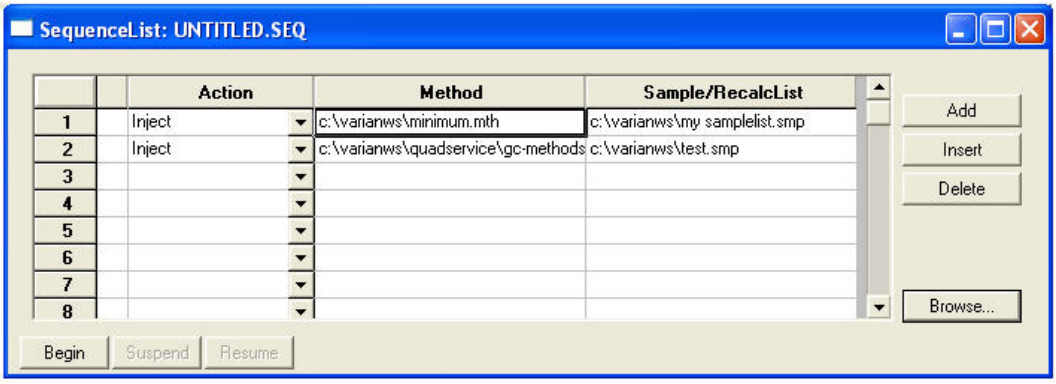
New RecalcList - Create a recalculation list.

Open RecalcList - Select an existing recalculation list.

NOTE: Process a RecalcList in System Control (click **Begin**) or in MS Data Review. In MS Data Review, select **Quantitation> 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.

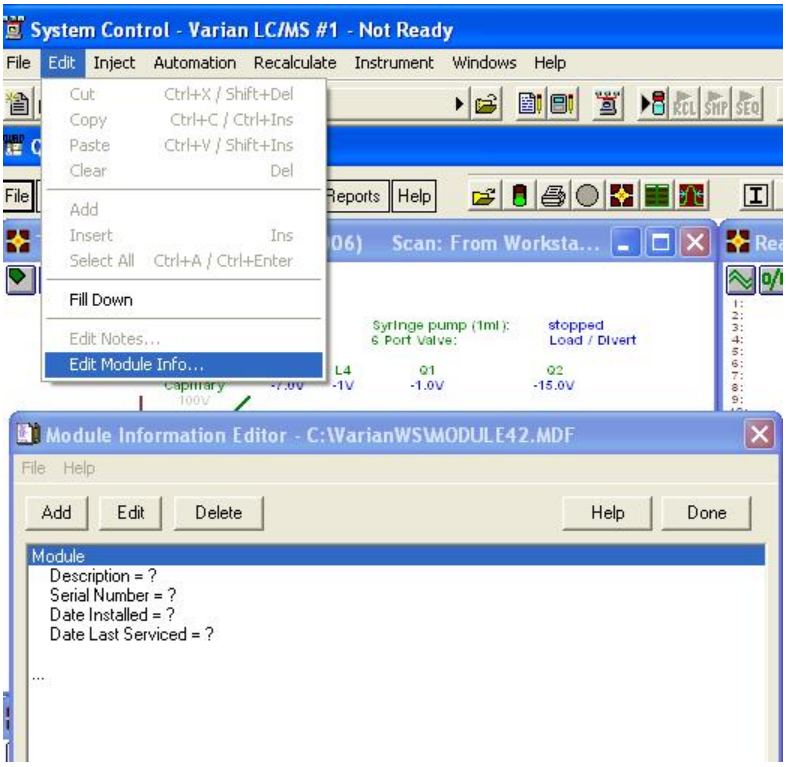


Print and **Printer Setup** are described in the MS Workstation Software Reference Manual.

Edit Menu

Edit Module Info

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

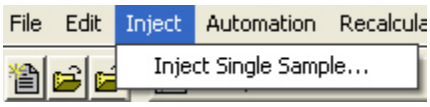


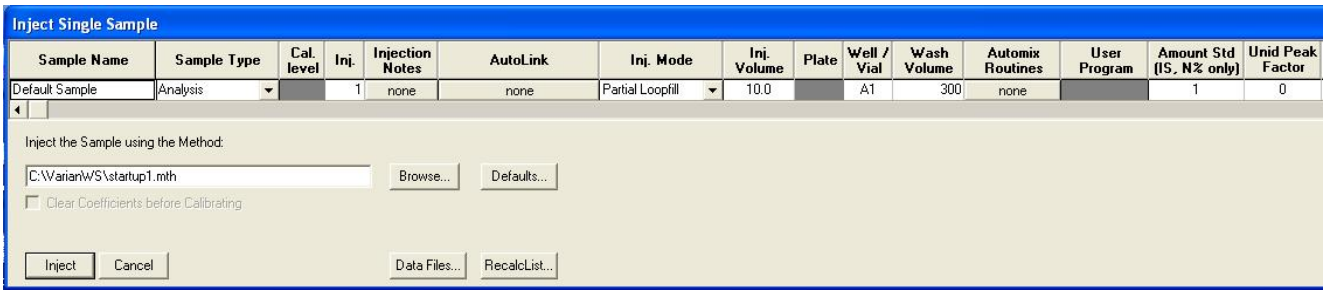
Documenting Module Information - Each module in your system (300 Series, 3800 GC, LC modules, Combi PAL, or HTS PAL AutoSampler) has a message log and screen. Use Module Information to keep a record of module

performance, maintenance, hours used, or other comments. From the System Control menu bar, select **Edit > Edit Module Info.**

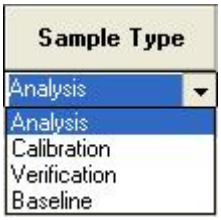
Inject Single Sample Menu

Click **Inject Single Sample** to open the Inject Single Sample window. Enter information about your sample. The 300 Series Module and all modules in the system must be in the Ready state before you make an injection. You can do a manual injection or use the autosampler.



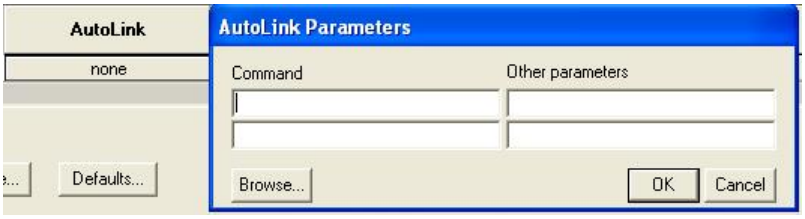


Select a **Sample Type** from the list:



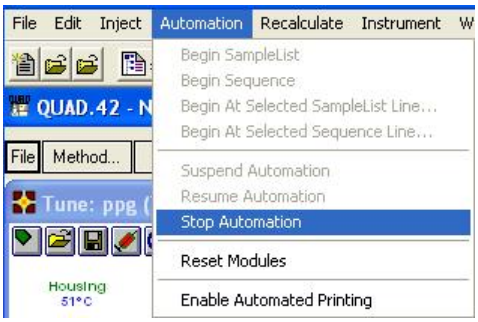
Click **Injection Notes** to enter a description or comment.

Use **AutoLink** to enter a command to execute a program after the data file is acquired. For example, you can create and print sample reports. To run a Custom MS Report in automation, enter the directory path and the name of the Custom MS Report template (for example C:\VarianWS\EPA525.swt) in the Command line. Note that Custom MS Reports templates are stored in the VarianWS directory. Use the browse command to select the command executable files.



Make a selection and click **OK**.

Automation Menu



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.

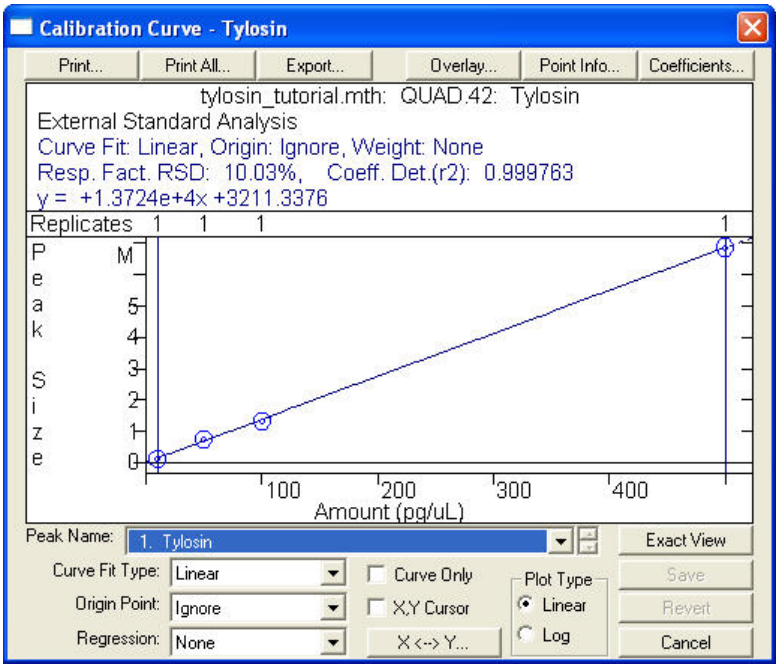
Enable Automated Printing - Allow printer functions to work during automation.

Recalculate Menu

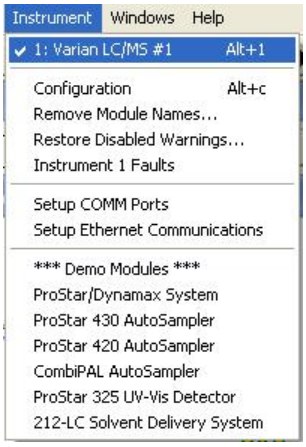
Begin RecalcList - Start an automated Recalculation. A Recalculation list must be opened under the File menu.

Begin at Selected RecalcList Line - Process a Recalculation list starting with a selected 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.



Instrument Menu

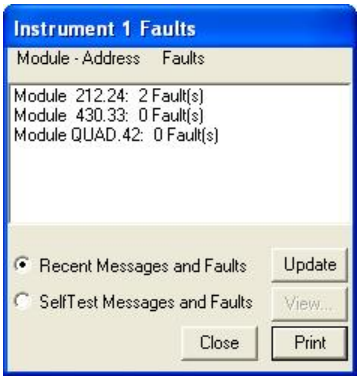


Varian LC/MS #1 - Configured instrument.

Configuration - Add new modules to the existing instrument, such as, a Combi PAL AutoSampler or LC pumps.

Remove Module Names - Remove associations between Module Names and Module Addresses. You can 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 - Determine if there are faults in any module of Instrument 1. See the following example. Click **Update** to check for new faults.



Setup Ethernet Communication - Set up communication between the 3800 GC or other Ethernet modules and System Control. Refer to the Ethernet Communication Setup section in one of the following manuals for more information: Data Acquisition with 3800 GC Control (part number 391473100) or Data Acquisition with LC Control (part number 91473200).

Setup COMM Ports - Set up communication between System Control and modules communicating with the Workstation through the Serial ports on the PC. The first time you start System Control, the Star Communication Configuration Wizard starts automatically. Refer to Data Acquisition with LC Control (part number 0391473200) for more information.

Windows Menu

Show Module Windows - Display the configured module windows. In this example, the modules are the 212 Pumps, the ProStar 430 Autosampler, 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.

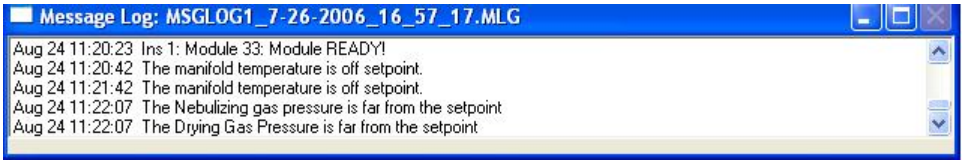
SequenceList - Display the active SequenceList.

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 - Tracks errors that occur during automation. Double-click the Status bar at the bottom of the Instrument window to view the message Log for all modules configured in the Instrument.



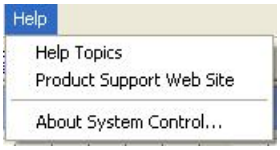
The last section of the Windows menu displays the attached modules and their status

212.24 - Stopped

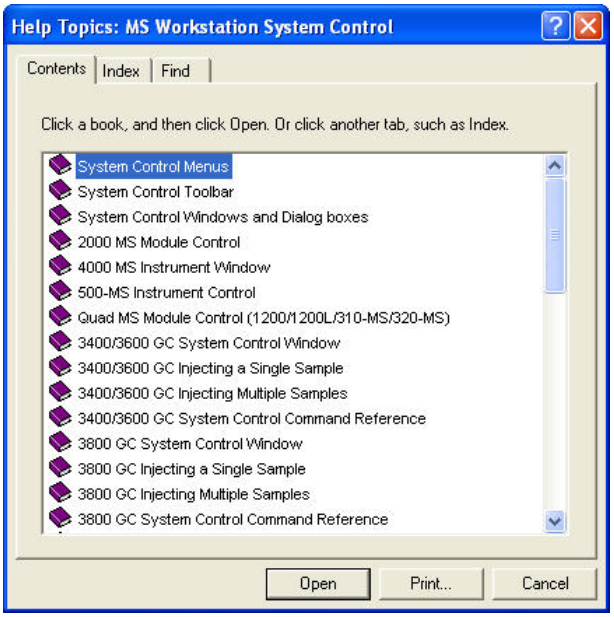
430.33 - Ready

Quad.42 - Not Ready

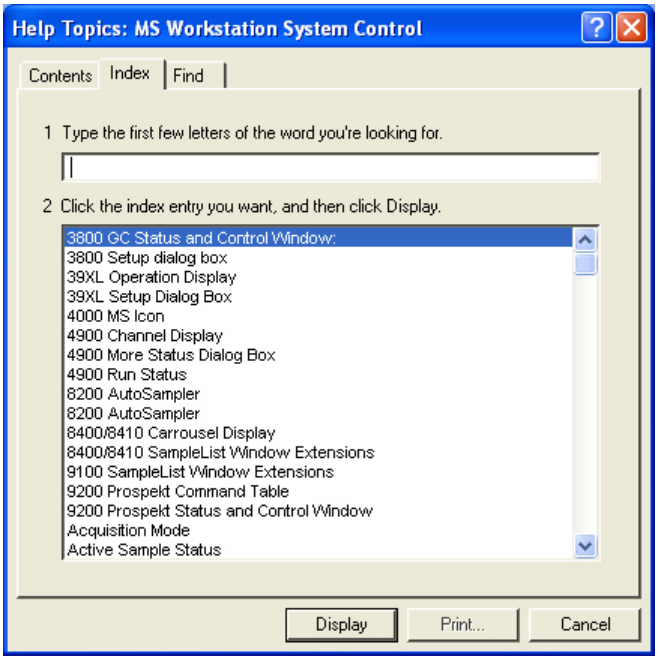
Help Menu



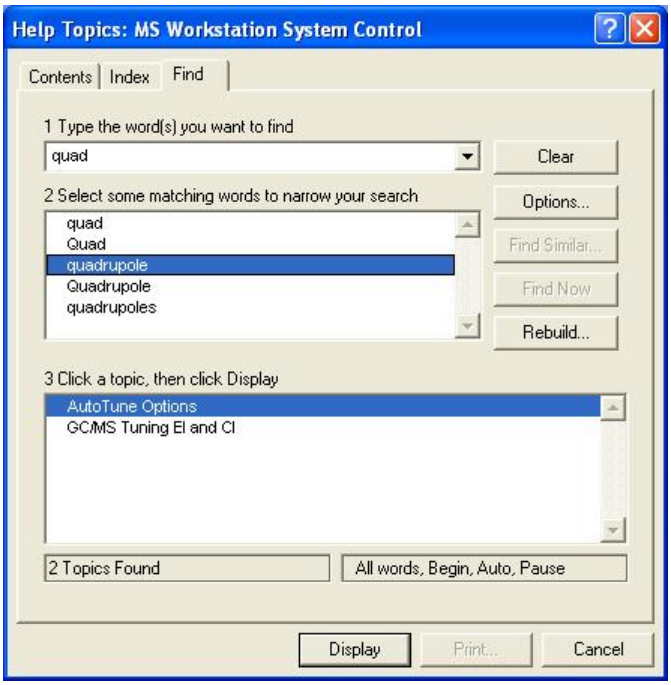
Select **Help Topics** to display the following. Click an item in Contents to see the online help.



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.



About System Control provides information on the MS Workstation software version, a list of the Installed Modules, and the Workstation Installation History.

Validate Installed Files tests the installed system files and documents errors using checksums produced at the factory. These reports can be printed.

System Control Toolbar

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

 Create a new automation file.

 First **-** open an existing automation file.

 Second **-** open the Message Log file.

View, edit, print, or reactivate a method.

 Open a method.

 Edit notes for an automation file.

 Edit module information for any online module.

 Display the Instrument Status window when it is hidden. From the Instrument Status Window, you can access module windows if they are hidden.

 Inject a single sample.

Start an active RecalcList.

Start an active SampleList.

Start an active SequenceList.

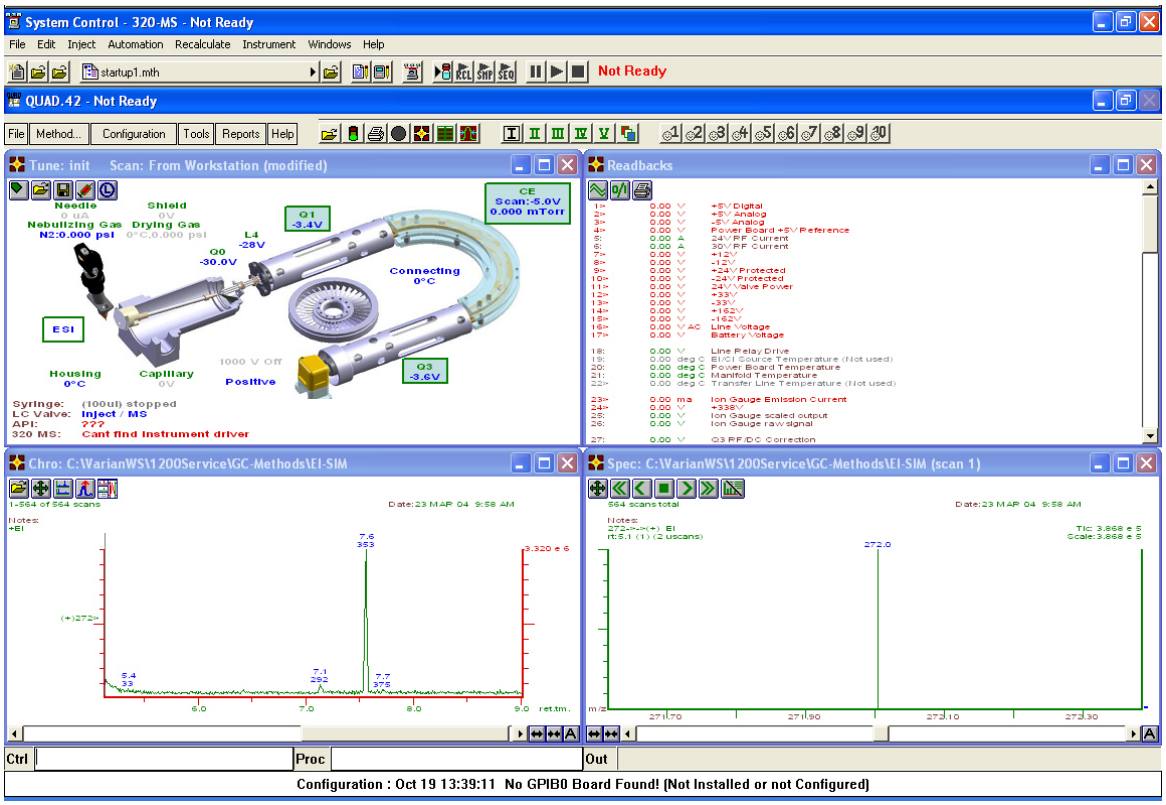
 Suspend Automation.

 Begin acquisition.

 Stop an automation run.

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.

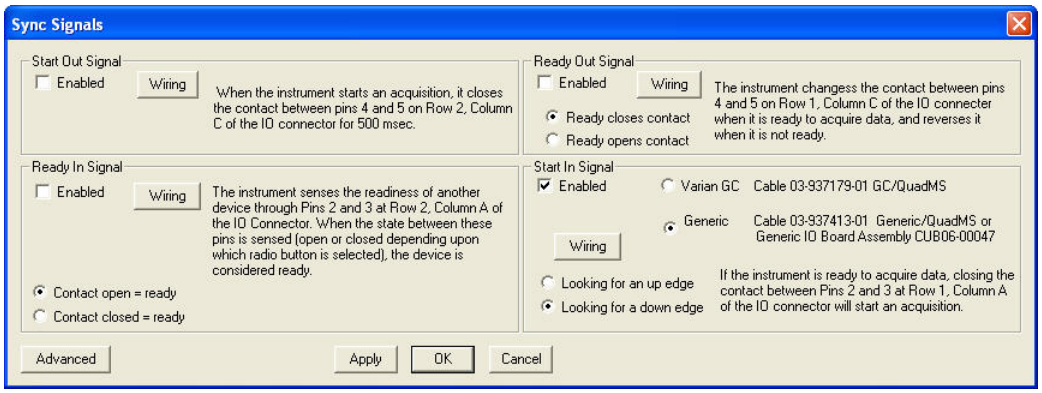


Quadrupole Module Menu

File - Open or print a file.

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

Configuration - Synchronization signal settings and MS Options settings.

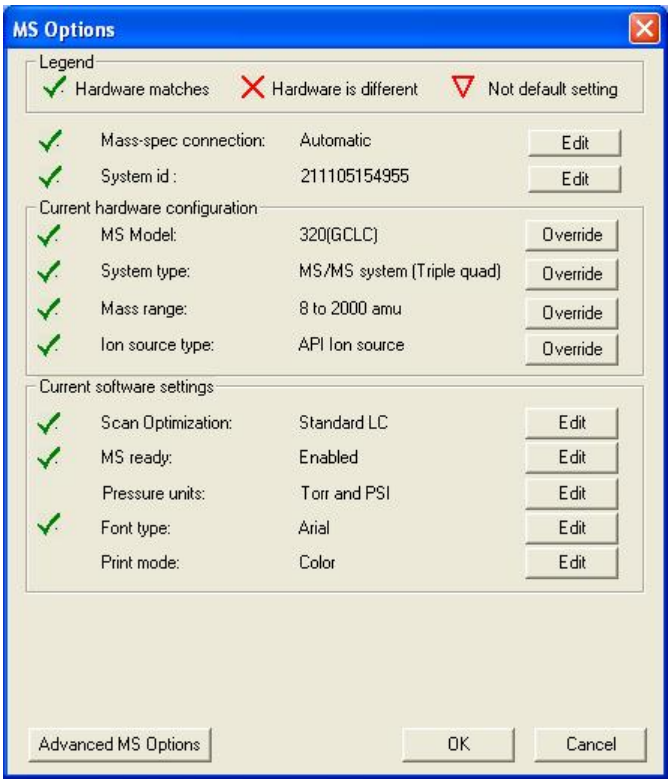


Sync Signal - Enable or disable the Start and Ready signals for external devices. To enable a Sync Signal operation, connect a cable from the device to the contact closure on the master instrument.

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

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

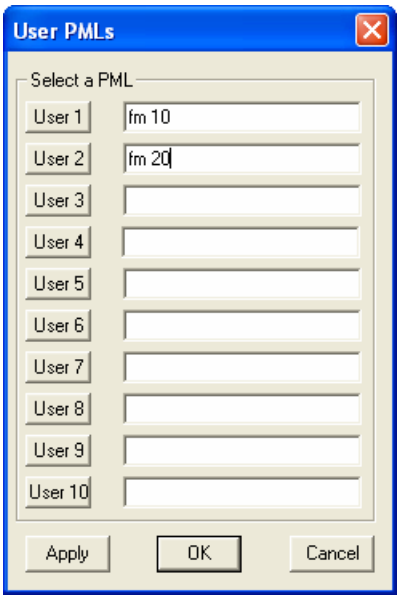
- Check marks indicate a Hardware match
- X indicates a Hardware conflict If an X is present, call your Varian representative.



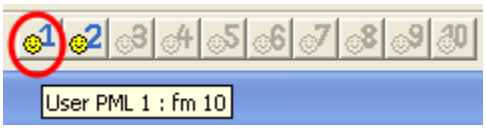
Tools - Access several MS Tools such as MS/MS Breakdown, Pml Editor, Plot tic, readbacks, in Bakeout system, and Overnight Standby.

Quit Macros: Stop any MS function in progress.

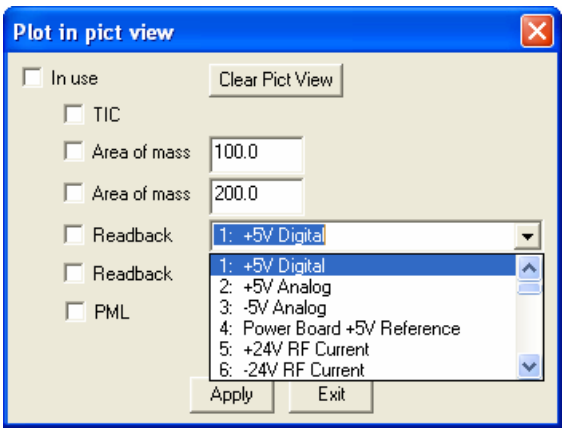
User pml: Enter up to 10 PMLs (macros written in "Paw Macro Language").



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



Plot tic, readbacks - Plot a graph of the TIC, up to 2 masses, up to 2 readbacks, or a PML to monitor instrument status. Check In use and the check box next to the desired parameter and then click Apply.



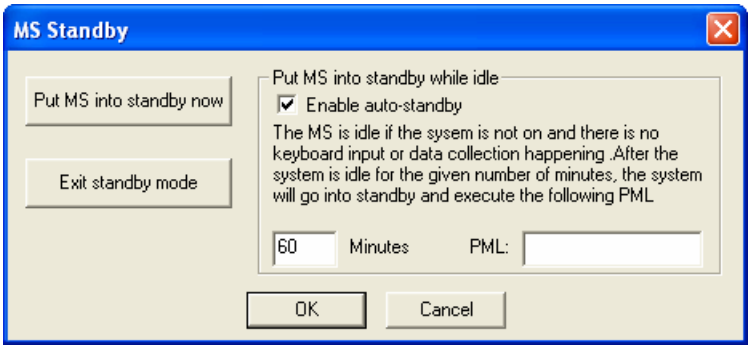
Analog output - For service use only.

MSMS Breakdown - Optimize the collision energy for any given parent mass. Used primarily in LC/MS.

Bakeout System

- GC/MS - Heats the source to 200 °C and the manifold at 50 °C for 3 hours, then cools the system to initial conditions. Also plots a graph of the source and manifold temperatures.
- LC/MS - Heats the API manifold to 65 °C and the MS manifold to 52 °C for 3 hours, and then cools the system to initial conditions. Also plots a graph of the source and manifold temperatures.

Overnight Standby - Turns 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.

Evacuate calgas bulb - Flush calgas into the source for several minutes. Do this procedure if the calgas bulb was removed and re-installed.

Cool down and vent - Cools the source to 100 °C; then turns off the foreline pump(s) and activates a solenoid allowing air to enter the source.

Reports - Select a print view setup.

Help - Online Help and the About window.

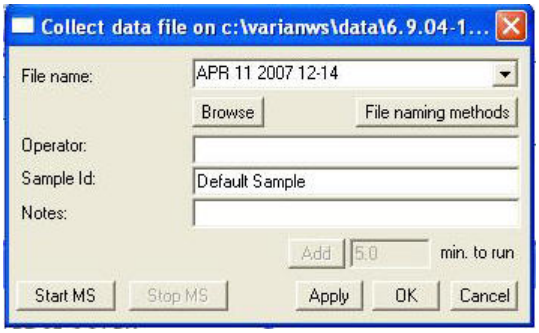
Reports - Select a print view setup.

Help - Online Help and the About window.

Quadrupole Toolbar

 **Choose File to Display -** Open and delete data files, and edit headers.

 **Start Collecting Data -** Acquire data from only the 300 Series and not other configured modules. Collect the current scan, which may not be the active method.



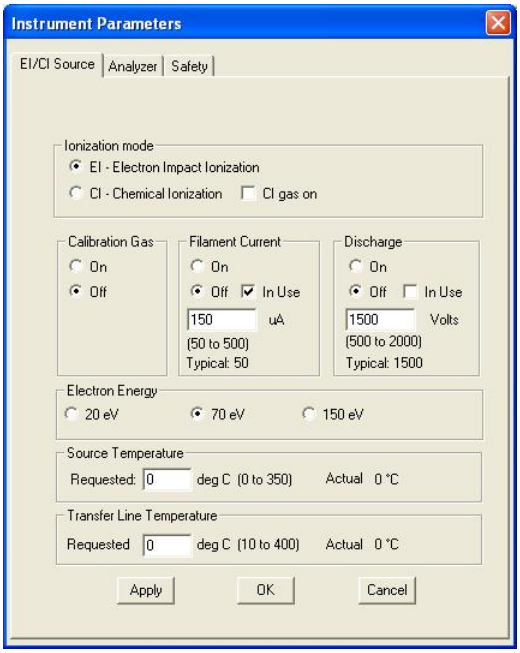
 **Print -** Print the window screen.

 **Turn Detector On and Off -** Control the filament or discharge voltage, accelerator and electron multiplier. The color of the icon indicates the following:

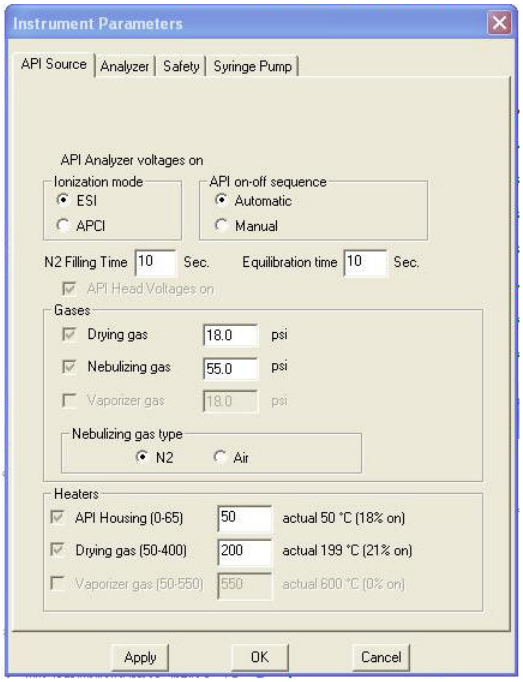
- Gray - Off
- Green - On
- Red - A filament is broken
- Yellow - System is in process of turning on or is waiting, for example, a filament delay time. If it turns yellow and then turns gray again, a fail-safe has prevented the filament and the multiplier from turning on.

 **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 GC or LC configuration.

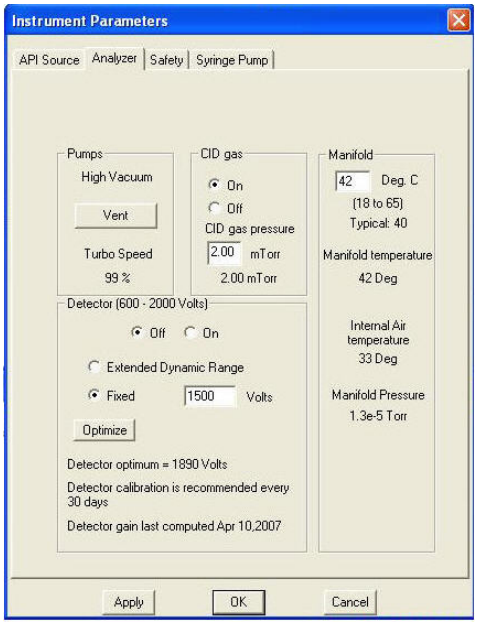
EI/CI Source - (GC only) Turn on and off; calibration and CI gases and the filament. Set filament current, discharge voltage, and electron energy and set the Source and Transfer Line temperature.



API Source - (LC Only). 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.

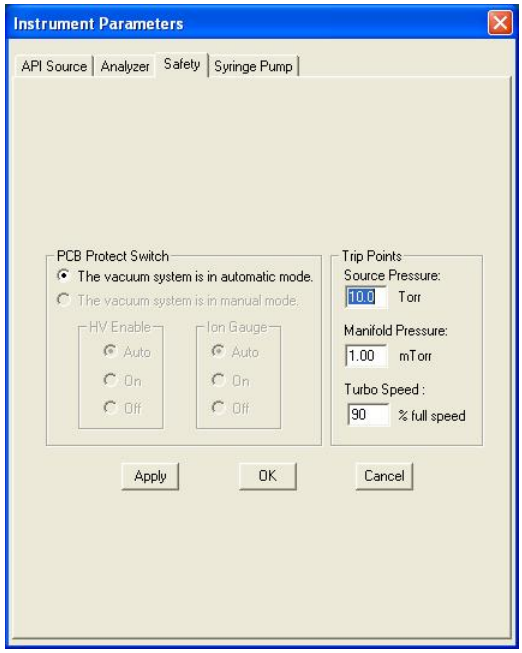


Analyzer - Vent the system and set the manifold temperature. Turn the CID gas on and off, set the electron multiplier (detector) voltage, and turn the Extended Dynamic Range on or off.

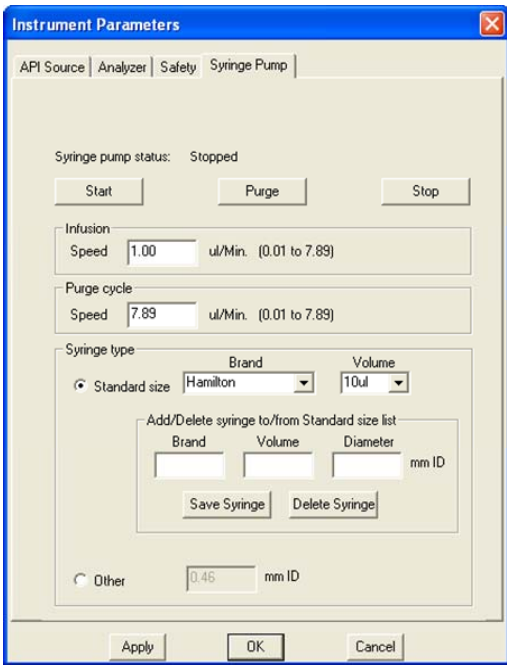


Safety - has three sections. Only Varian Representatives can make changes.

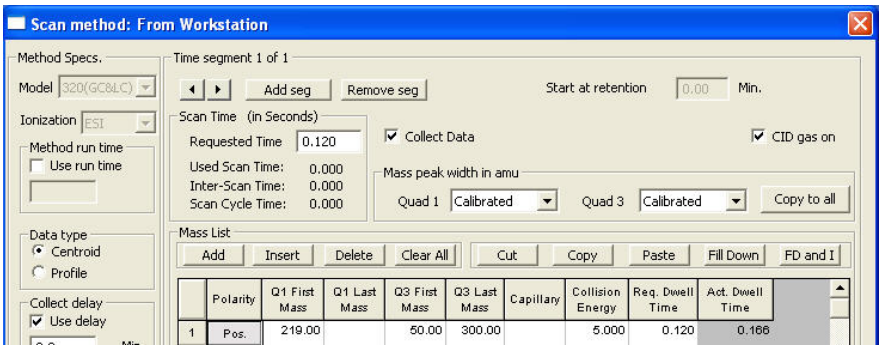
- High voltage enable and Ion Gauge modes
- Trip points of the source pressure, manifold pressure and turbo speed
- Status of the PCB Protect Switch



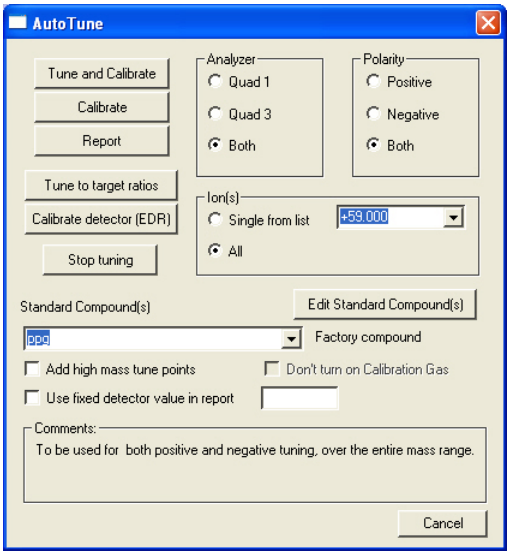
Syringe Pump- Only available for the 320-MS LC/MS or LC/GC/MS instruments. Refer to the Hardware manual for more information.



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.



300 Series Module Views

The five default views are:

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

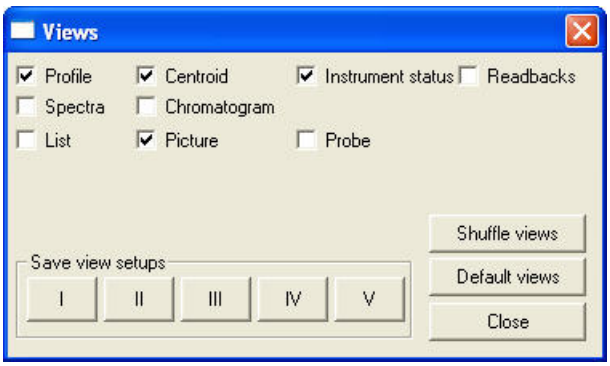
View II - Displays Quad Instrument status, Chromatogram, Spectra, and Centroid windows.

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, and 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 - Post acquisition display 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, showing temperature and pressure status.

Readbacks - Real time text display of either analog or digital instrument parameters.

Probe - Real time text and plot display of either the DIP or DEP probe. This is an option for either 320-MS or 300-MS systems.

300 Series Autotune Functions and Options

NOTE: For a detailed description of Autotune steps, refer to the LC/MS and GC/MS sections in the 300 Series Hardware Operation Manual.

Overview

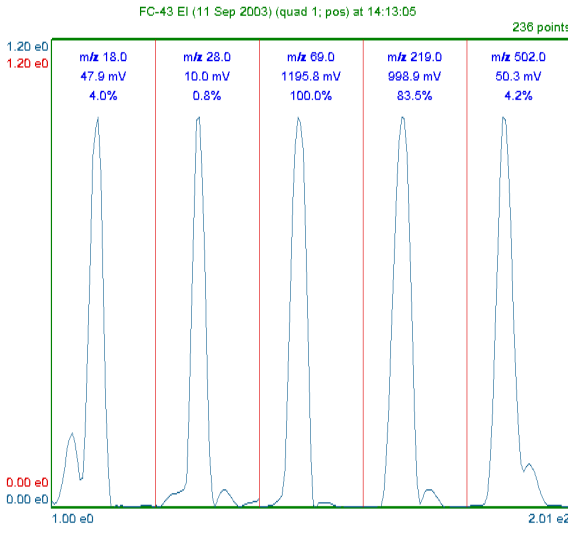
Autotune optimizes the instrument for sensitivity, resolution, and mass calibration.

For LC/MS, a reference compound such as polypropylene glycol (PPG) is used as a tuning solution. You may also use a custom tuning solution.

For GC/MS, FC-43 is used as a tuning solution. A supply bulb is inside the front panel.

Both quadrupoles 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.

```
Tune report for Varian 1200

Time: 14:13:05  Tune file: FC-43 EI (11 Sep 2003)

     Exact    Measured      Mass       Peak    Relative          Peak       Valley    Resolution
      mass        mass     error     height      height         width
     (amu)       (amu)     (amu)       (mV)         (%)  (amu @ 50%)  (% of iso)  (m/delta-m)
      18.0        18.2       0.2       47.9         4.0         0.58         100           32
      28.0        28.1       0.1       10.0         0.8         0.55         100           52
      69.0        69.0       0.0     1195.8       100.0         0.69         100          101
     219.0       219.0      -0.0      998.9        83.5         0.75          79          290
     502.0       501.9      -0.0       50.3         4.2         0.68          18          741

(Detector = 1440 V)

Tuning of quad 1 in positive mode successfully completed.

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

Tune report for Varian 1200

Time: 14:16:07  Tune file: FC-43 EI (11 Sep 2003)

     Exact    Measured      Mass       Peak    Relative          Peak       Valley    Resolution
      mass        mass     error     height      height         width
     (amu)       (amu)     (amu)       (mV)         (%)  (amu @ 50%)  (% of iso)  (m/delta-m)
      18.0        18.2       0.2      593.5        38.7         0.54         100           34
      28.0        28.1       0.1      162.6        10.6         0.62         100           45
      69.0        69.0      -0.0     1535.2       100.0         0.68         100          102
     219.0       219.0      -0.0     1010.1        65.8         0.71          86          307
     502.0       502.0      -0.0       57.3         3.7         0.66          29          755

(Detector = 1640 V)

Tuning of quad 3 in positive mode successfully completed.
```



Tune Stability - For optimum tune stability, the mass analyzer must be stabilized 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.

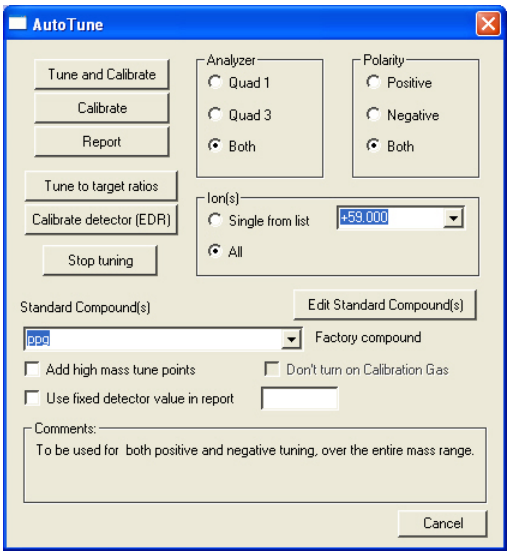
The tuning compound signal must be stable before starting the Autotune procedure (Cal Gas on for at least 10 minutes, and signal from PPG infusion stable).

NOTE: It is not necessary to tune the instrument daily. Tune if the ion source becomes contaminated and there is a significant loss of signal. If this happens, clean the ion source and AutoTune.

Tuning is not required after changing or replacing an ion volume; however, tuning the instrument after cleaning the ion source is recommended. 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 quadrupoles. A new file name and report are created and saved.

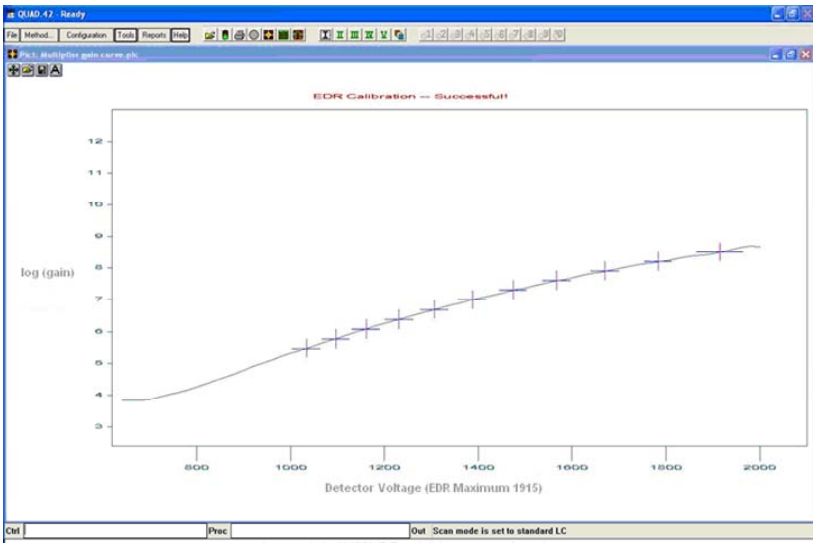
Report - Create and save a tune report. Verify tune stability by comparing with previous reports to determine if re-tuning is required. Creating a report does not change the tune file.

Tune to Target Ratios - Modify existing tune files. See 'Tune to Target Ion Ratios' Section.

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

The detector calibration routine has two components. 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 plot shows detector gain as a function of detector voltage.



Stop Tuning - Abort the Tuning procedures and restores the most recent Tune file.

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

Polarity - Set AutoTune polarity mode.

GC/MS

CI - AutoTune either positive or negative
EI - AutoTune positive only

LC/MS

ESI - AutoTune either positive or negative
(for APCI use ESI tune file)

Ion(s) - Select either a specific ion or all defined ions in the Compound list. If you choose 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 already present, it is tuned and changed accordingly. A report for this single mass is created and the tune file is named and saved.

Edit Standard Compound(s) - Open Compound Editor. You can modify a Standard compound or create a list of new compounds.

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

Cancel - Close the Autotune window 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.

Example: Second Negative CI Tuning on October 6th 2003

First file: FC-43 Neg CI (06 Oct 2003).dac
Second file: FC-43 Neg CI (06 Oct 2003) (2).dac

Tune to Target Ratios

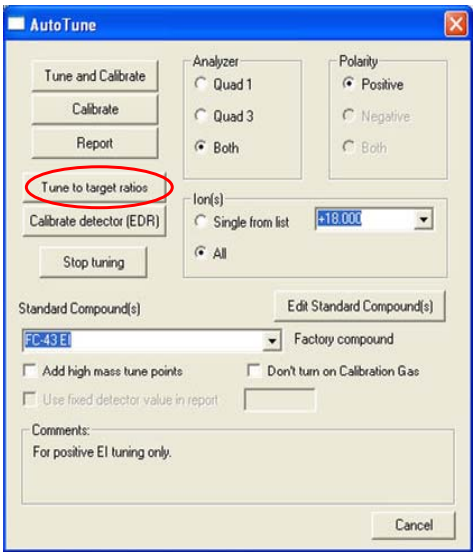
This is a GC/MS feature for EI ionization. Tune to Target Ratios modifies an existing mass spectrometer tune file so that relative ion ratios match user-defined target criteria. Examples are the ion ratio requirements for running EPA methods 524 (BFB) or 8270 (DFTPP) for GC/MS with an EI source.

The routine adjusts the ratios relative to the base peak of 3 to 5 calibration gas ions that are relatively far apart in the mass spectrum. These ions are selected for their proximity to ions of importance in the sample spectrum. Ordinarily, this brings the entire spectrum within target ion ratio requirements. Since it is not possible to make an ion peak larger, the ratio adjustments are made by reducing the sizes of ion peaks. This reduces some of the sensitivity. Therefore, the user must verify that the ion ratios in the compound of interest (usually BFB or DFTPP) are not within acceptable parameters with the existing autotune file before running this program.

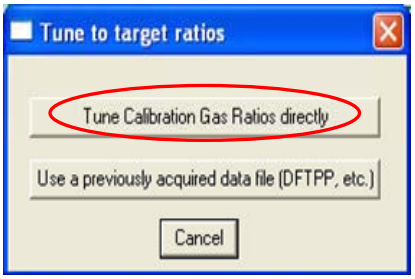
Click either **Tune the Calibration Gas Ratios directly**, or **Use a previously acquired data file**.

To tune the calibration gas ratios directly:

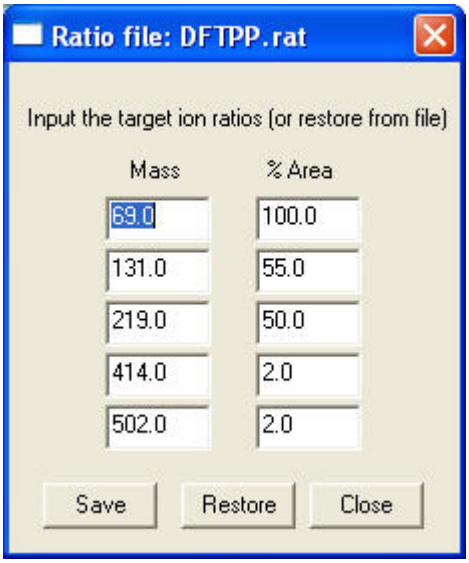
1. Open the **AutoTune** window.
2. Click **Tune to target ratios**.



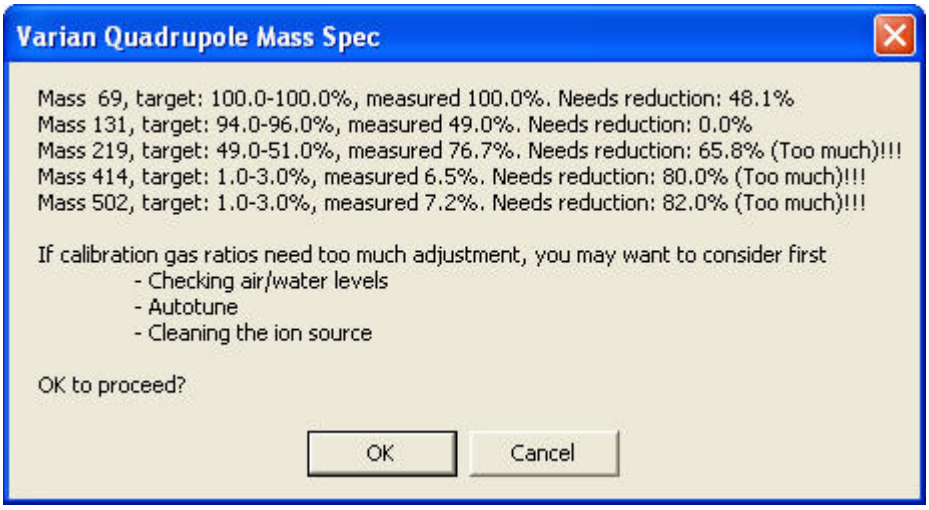
3. In the Tune to target ratios window, click **Tune Calibration Gas Ratios directly**.



4. Select the Tune Compound data file. Ion ratios that normally pass DFTPP and BFB EPA requirements can be selected from the drop down list. If other ion ratios are required, click **Edit** in the Tune to target ratios directly window.
5. If editing the ion ratio list, input the ion mass in the left column and the target ratio relative to the base peak in the right column. There must always be a base peak in the list with 100% as the % Area. Click **Save**.



6. In the **Tune to target ratios directly** window, click **Proceed with tune button**.
7. If the ion ratios selected require peak reductions over 50%, a warning window appears before the program begins tuning. Do not reduce the ion peaks this drastically because much sensitivity is lost. If the peak reductions are less than 50%, click **OK**.

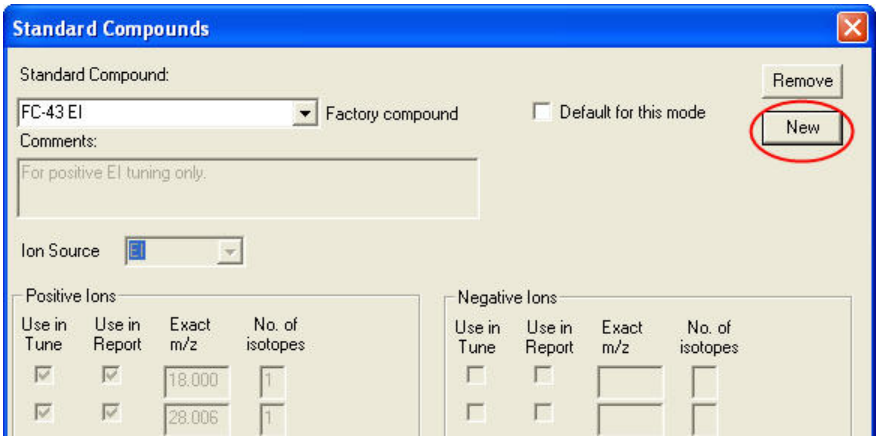


After the tune completes, save the new tune file. The system will not automatically implement the new tune. To implement the new tune open the tune file using the Restore tune tables in the Instrument Status window.

Standard Compound Editor

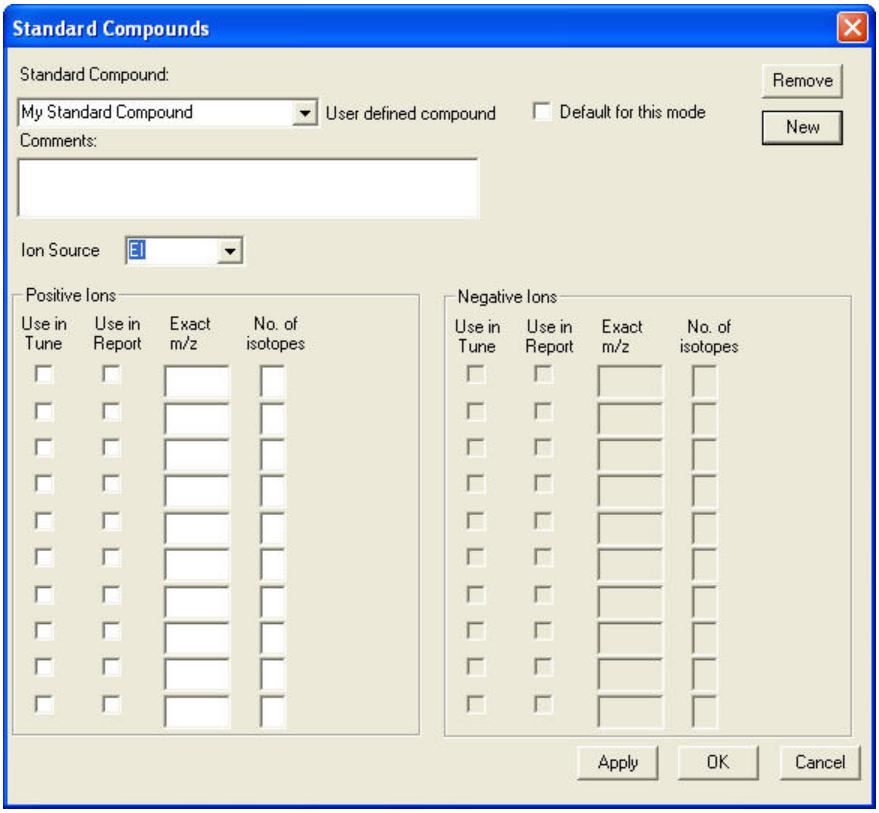
GC/MS EI Example

1. Click **Edit Standard Compound(s)** in the AutoTune widow and the following opens.
2. Click **New** to define a new standard compound.



3. Enter a *name* for the new standard compound.
4. Enable the **Clear all fields** box to clear entries in the **Standard Compound** dialog box.
5. Click **OK**.

6. In the **Standard Compounds** dialog box, enter information about your Standard Compound.



Standard Compound - List Standard Compounds for the selected mode.

Default for this Mode - Set displayed Standard Compound as the default for the selected mode.

New - Add new list of Standard Compounds.

Remove - Remove a Standard Compound.

Comments - Enter information about the Standard Compound.

Ion Source - Select the ion source (EI, CI, ESI, or APCI).

Use in Tune - Select which ions are used for Tune or Calibrate. The selected ions are optimized.

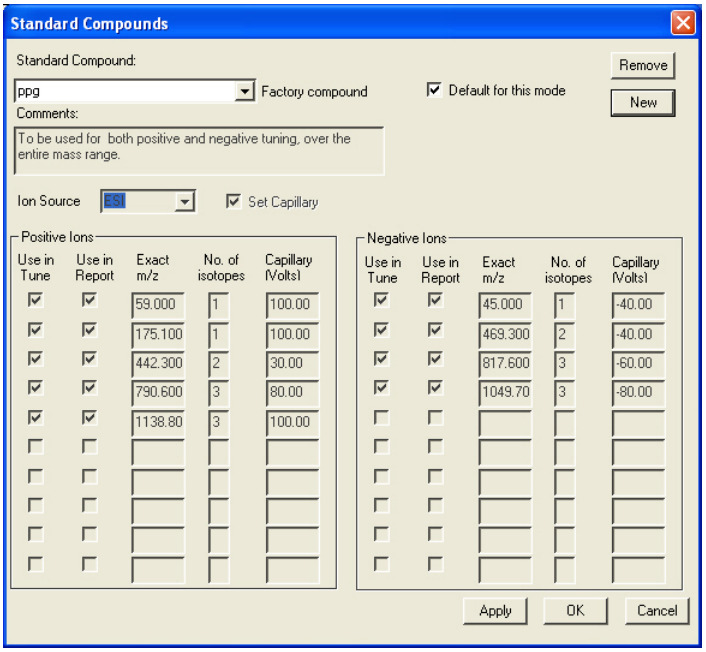
Use in Report - Select ions for the tune report. Only the selected ions are reported.

Exact m/z - Enter the exact m/z of each ion in the Standard compound.

No. of Isotopes - Enter the number of isotopes for the ion displayed in the tune report graphic.

LC/MS ESI Example

1. In the AutoTune window, select **ppg** from the Standard Compound(s) list.
2. Click **Edit Standard Compound(s)**



Set Capillary (LC/MS mode only) - 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) (LC/MS mode only) - If the Set Capillary box is checked, you can set the capillary voltage to be used for each tune ion, otherwise this option is not available.

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

300 Series Method Builder

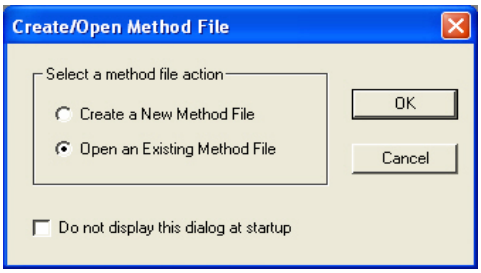
Quad Method Builder

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

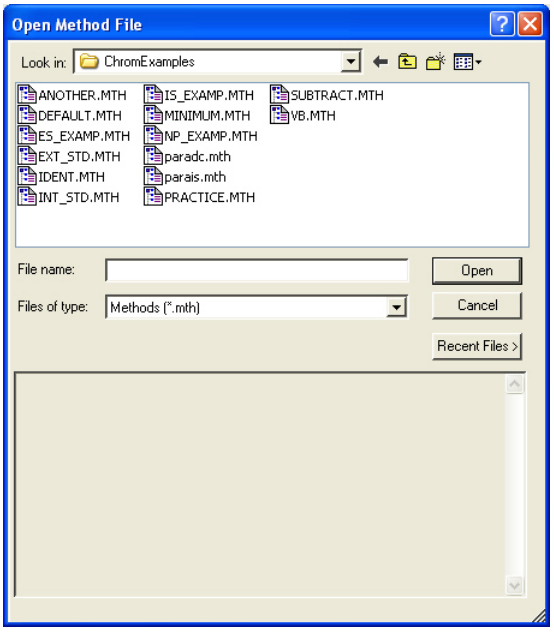
Edit an Existing Method

Sometimes it is easier to modify an existing method than building a new method.

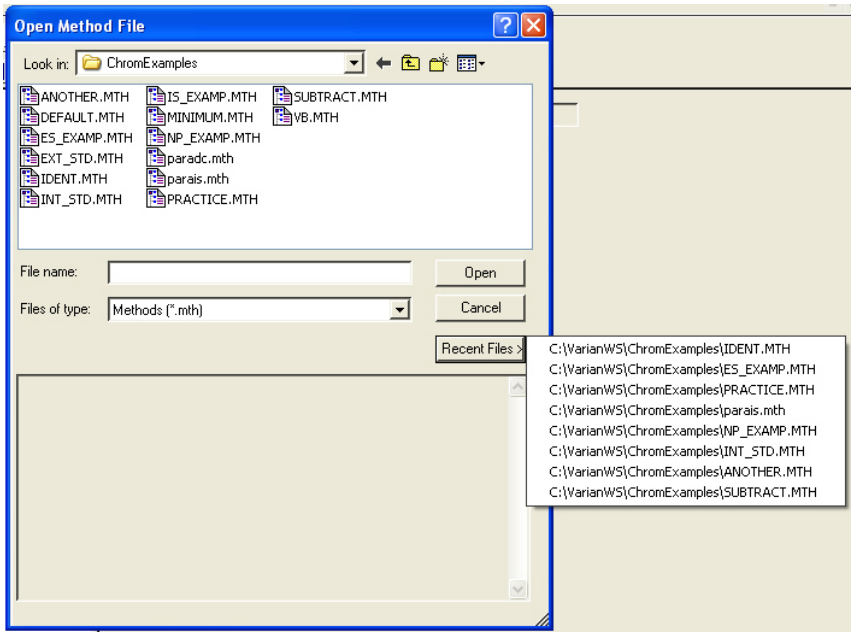
1. Select **Open** an Existing Method File and click **OK**.



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

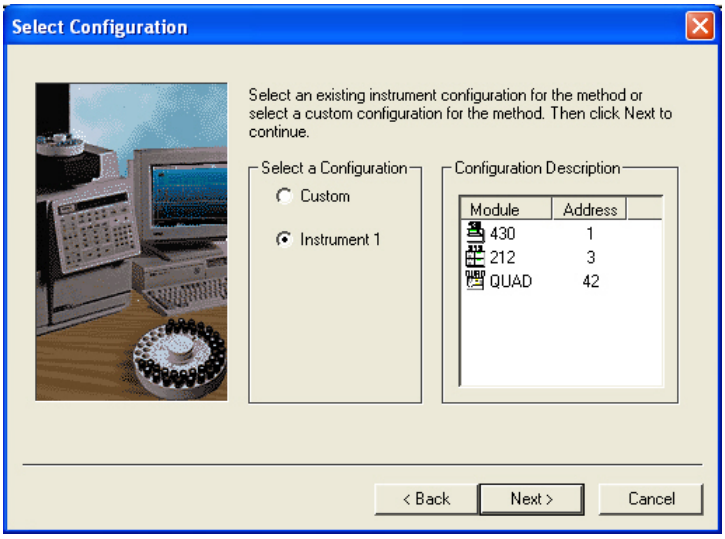


    - Click Recent Files to display them and select one.

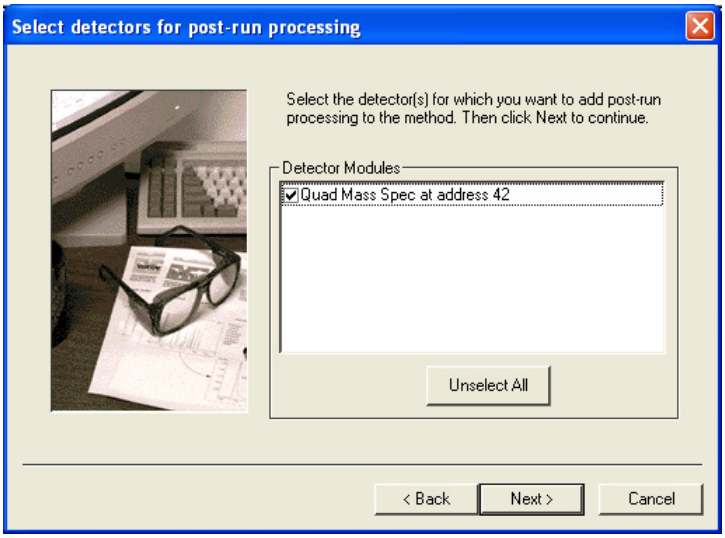


Build a New Method with the Configured Instrument

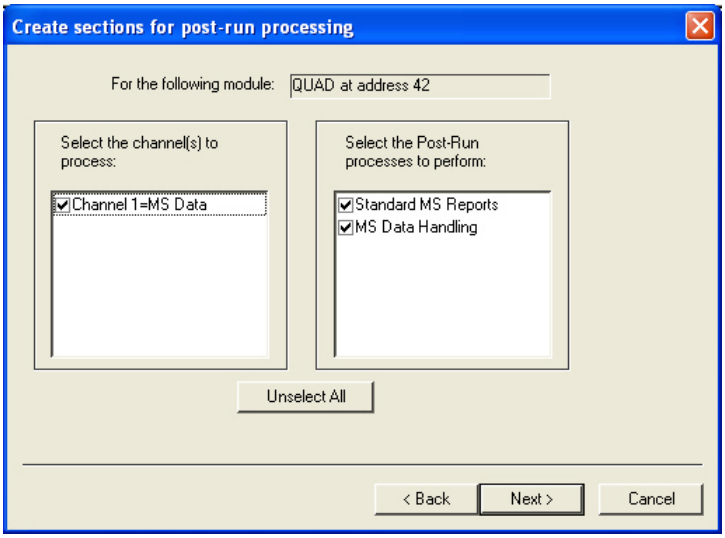
1. Select **Create a New Method File** and click **OK** to open the Method Builder Wizard.
2. Select **Instrument 1**. The configured instrument is added to the method.
3. Click **Next**.



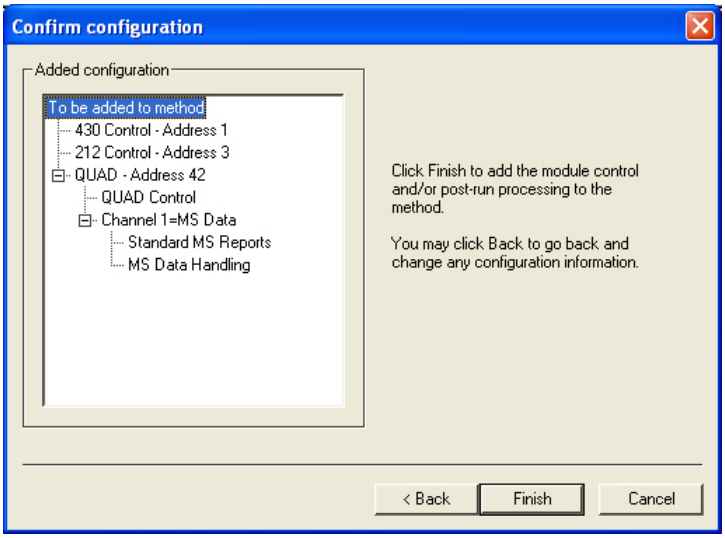
4. Select a detector. After the data is acquired you can add data processing information to the method.
5. Click **Next**.



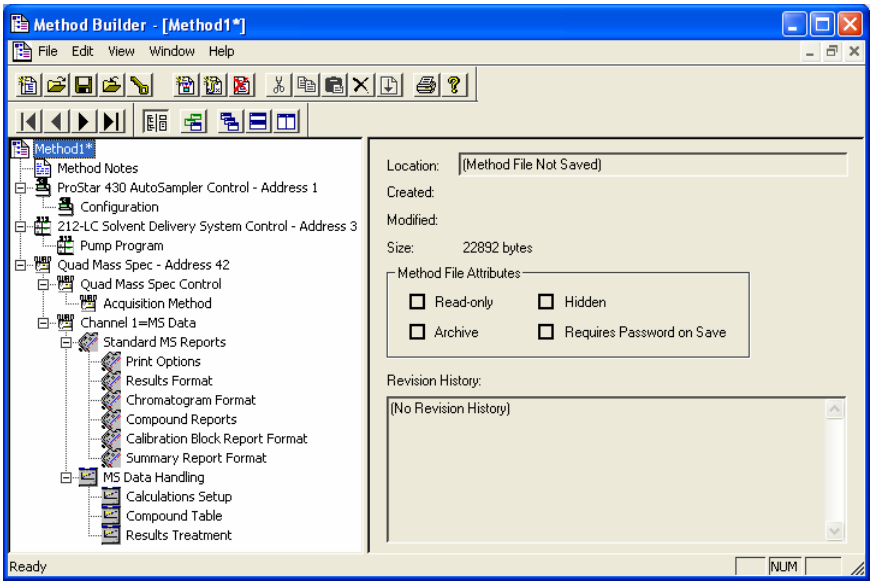
6. Create sections for **post-run processing**. Add MS Data Handling to enter integration, peak names, and other parameters. Add Standard MS Reports to use the standard reports.
7. Click **Next**.



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.



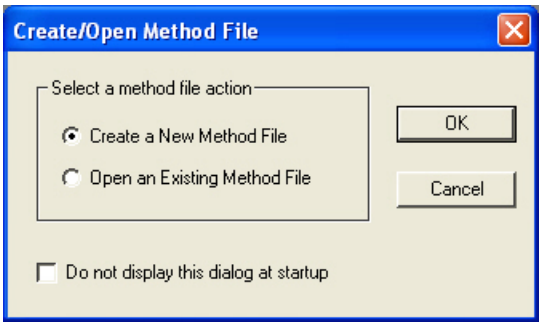
11. The new method is displayed with a navigation tree on the left side and the method workspace on the right. Click a tree entry to open that method section.



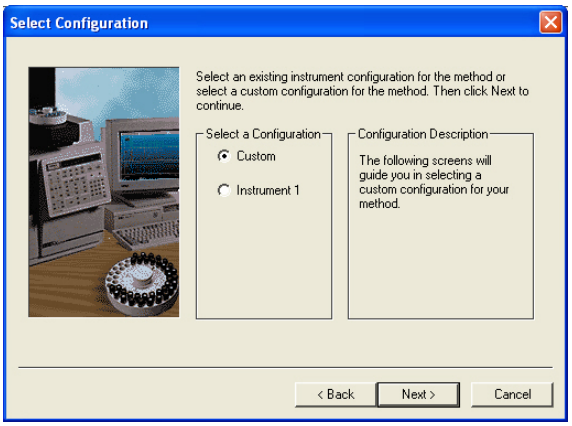
Build a New Method adding a Varian Instrument

You can build a method as follows:

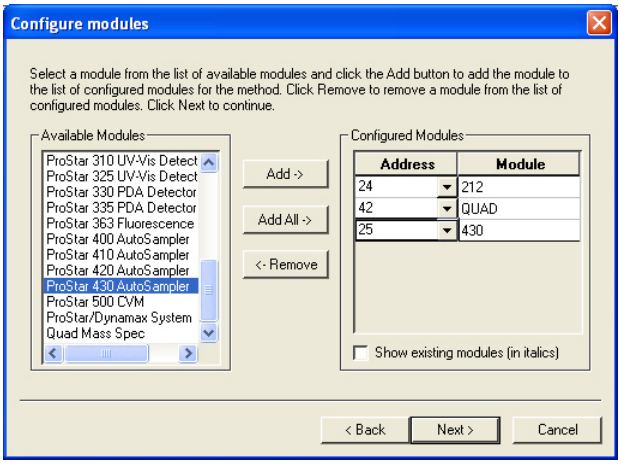
1. Select **Create a New Method File** and click **OK**.



2. Select **Custom** and 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**.

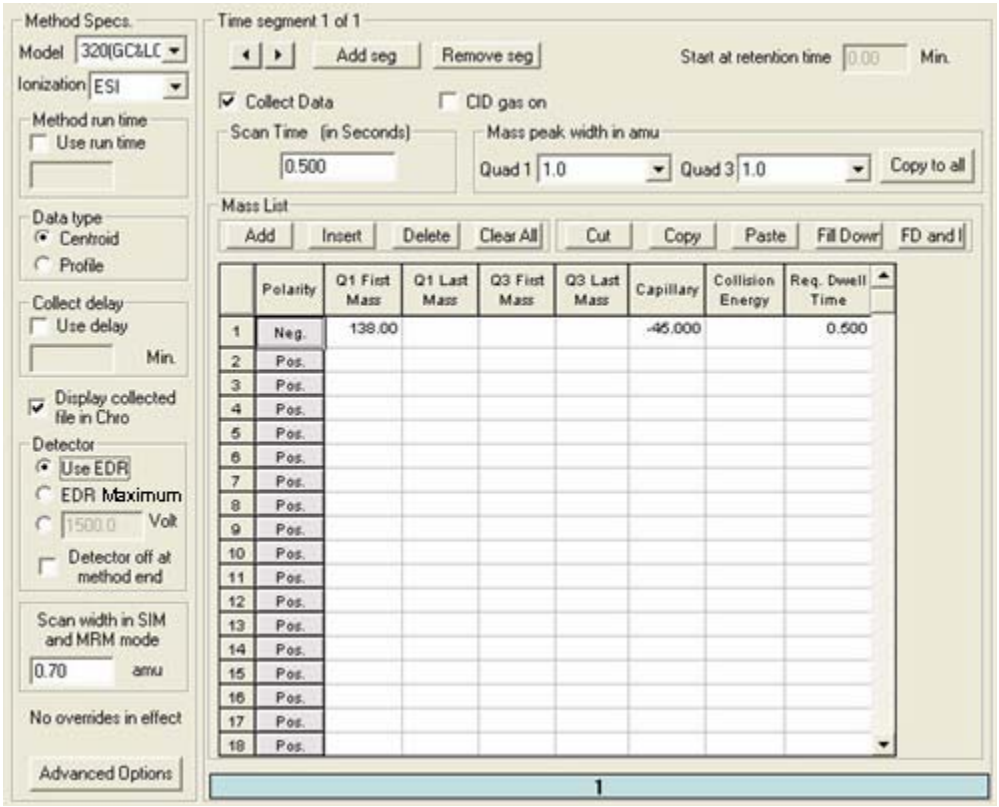


5. Continue with step 3 of “Build a New Method.”

300 Series Acquisition Method

Click **Acquisition Method** in the Method Builder tree.

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

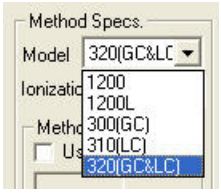


Method Specifications

The following explains the Method Specifications in the Acquisition Method.

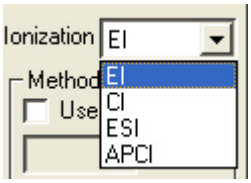
Model

Select your instrument Model.



Ionization

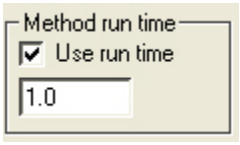
Select the ionization mode (EI, CI, ESI, APCI) - Electron Ionization, Chemical Ionization, Electrospray Ionization, or Atmospheric Pressure Chemical Ionization.



Method Run Time

You have two choices:

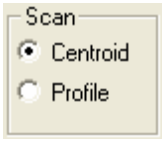
- Select **Use run time**.
- Enter a time in minutes. If you do not enter a run time, the data will be acquired until the end time is reached in all other modules.



Data Type

Set the scan type (Centroid, Profile).

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

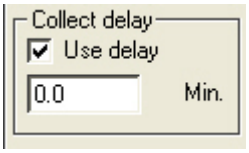


Collect Delay

This feature is useful for applications with 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.

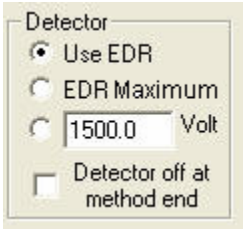


Display File in Chro

Enable to display data files in Chro.

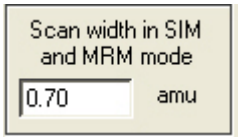
Detector

- **Use EDR** - (Extended dynamic range) the detector automatically adjusts the detector voltage over the dynamic range. EDR is calibrated during the Autotune procedure (see the '*Autotune Functions'* section).
- **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.
- You can have the detector turn off when the method ends.



Scan Width

Set the maximum peak scan window for an ion in single ion monitoring. The default value is 0.70. Increase this value if resolution on the scanning quad is opened (see parameter peak width). Adjust it according to the resolution. For example, if the resolution is 3.0 on scanning quadrupole SIM width, set the SIM Width between 0.7 and 3.0 for largest signal).



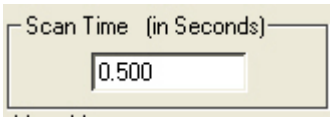
Time Segment

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

Scan Time

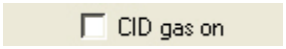
Set the scan speed of the MS. The scan speed is the amount of time the analyzing quadrupole goes through the scan. For full scan or MS/MS scan experiments set the scan time to 0.5 seconds. The shorter the Scan Time the more data points can be acquired. Increasing the scan time increases signal to noise 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. When 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.



Collision Cell Gas

Select to enable the CID gas (such as Argon) for MS/MS operations.

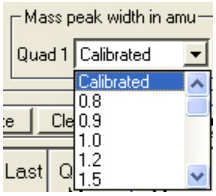


Peak Width

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

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

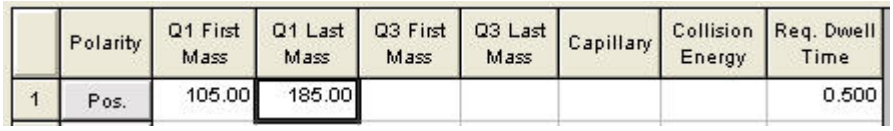
For SRM and MRM reactions in MS/MS mode, the peak width of Q1 can be increased while keeping Q3 calibrated or smaller. The signal increases by allowing Q1 to transmit a wider peak width while Q3 detects the product spectra in a narrower peak.



Mass List

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

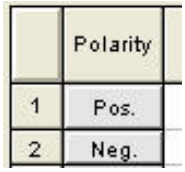
Q3 is used for triple quadrupole instruments only.



	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.

GC/MS
EI positive
CI positive and negative
LC/MS
ESI positive and negative
APCI positive and negative

Capillary

Only for LC/MS instruments. Enter the absolute value of the Capillary volts for each ion in the scan method. Method Builder enters the correct sign.

Collision Energy

For triple quadrupole instruments only.

The Collision Energy is experimentally determined for each reaction and entered into the Mass List. It defines collision energies in electron volts for specific MS/MS reactions used in the collision cell. (Refer to the 300 Series Hardware Operation Manual, *MS, and MS/MS*).

Requested Dwell Time

Specify the Requested dwell time of each scan channel. The shorter the dwell time allows more channels to be used. Specify either the scan time or the dwell time.

Mass List Toolbar

Enter values in the Mass List in a similar manner as a spreadsheet. You can Add, Insert, Delete or Clear all lines; Cut, Copy Paste from a cell or a line. You can also fill down a column, or fill down or increment the values in a column (FD and I). You can copy and paste from or to an Excel spreadsheet.

Advanced Options

Use Advanced Options to tailor your method. The Advanced Options of the 320-MS LC/GC/MS are displayed. These vary by ionization mode, instrument, and the optional ion sources (APCI and DIP/DEP probe).

The advanced options depend on the ionization mode.

EI - EI Overrides, User Analogs In/Out, PMLs, Probe

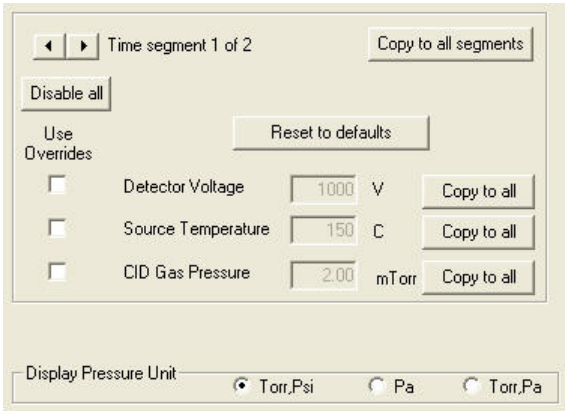
CI - CI Overrides, CI Gas Type, User Analogs In/Out, PMLs, Probe

ESI - ESI Overrides, Valve, and Syringe Pump, Drying Gas, User Analogs In/Out, PMLs

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

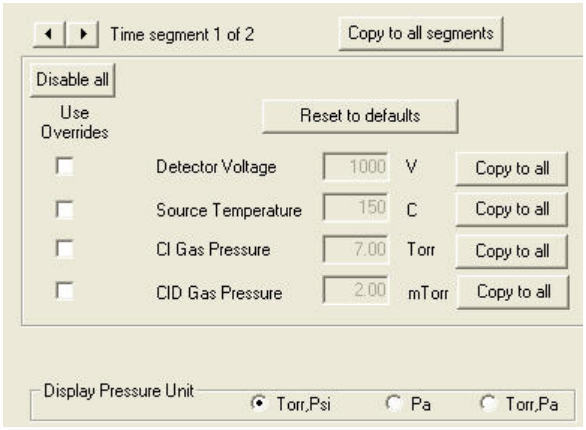
EI Overrides

Enter values to override those specified in the method for specific time segments. In the bottom section, select the units for the pressure displayed.



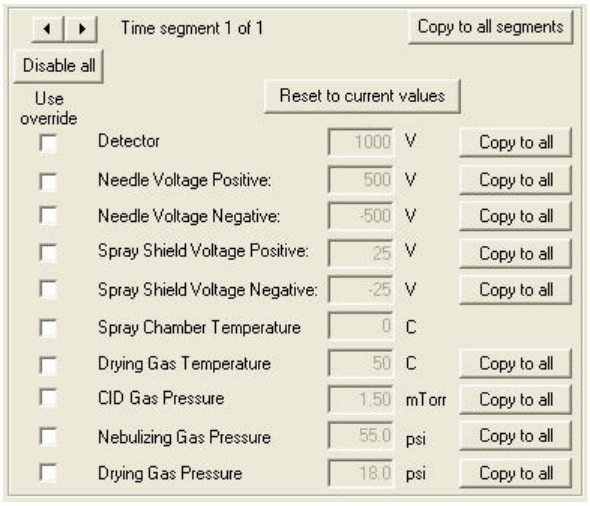
CI Overrides

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



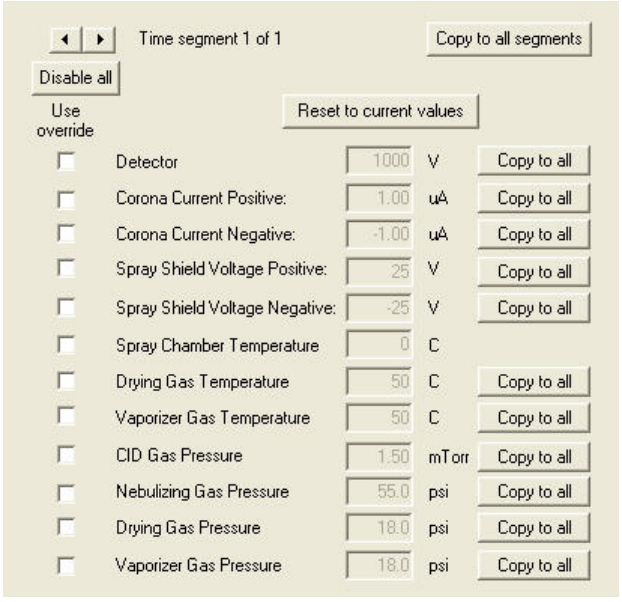
ESI Overrides

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



APCI Overrides

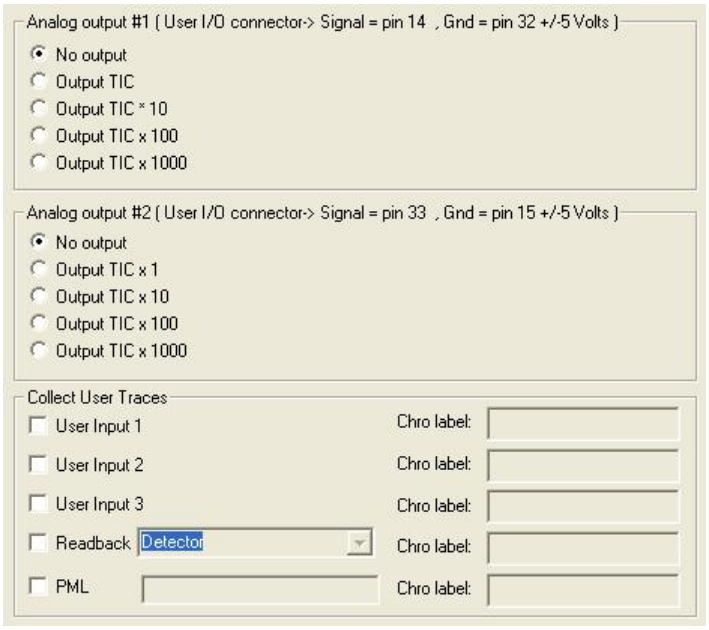
Enter values to override those specified in the method for specific time segments.



User Analog In/Outs

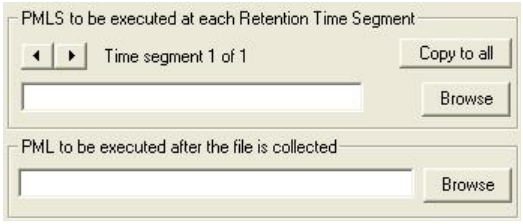
Use the Analog outputs to send the TIC signal to another device such as a LIMS system.

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



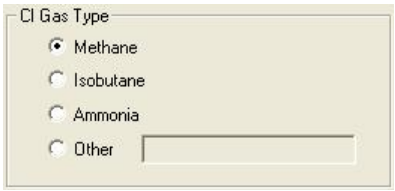
PMLs (Paw Macro Language)

Varian provides PML macros you can run either before or after a time segment. You can modify and create PMLs through Tools in the Quad Module View.



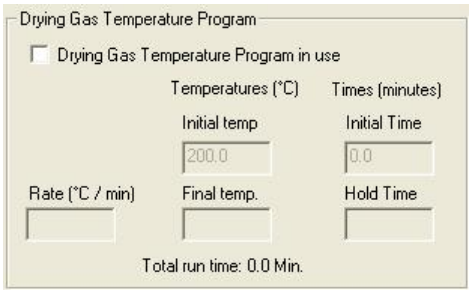
CI Gas Type

Select the Gas Type. You can also specify another gas that is configured with your instrument.



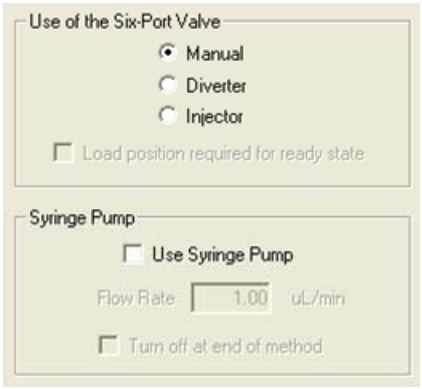
ESI and APCI Drying Gas

Use the SelectTemp™ feature to ensure the optimum drying gas temperature for the mobile phase composition for ESI or APCI runs.



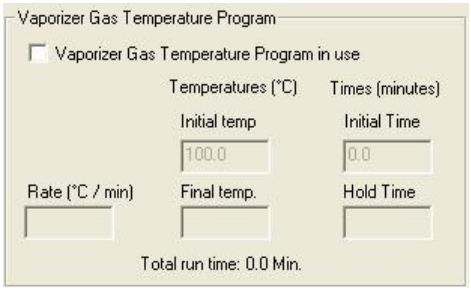
ESI and APCI: Valve and Syringe Pump

For the 320-MS, 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.



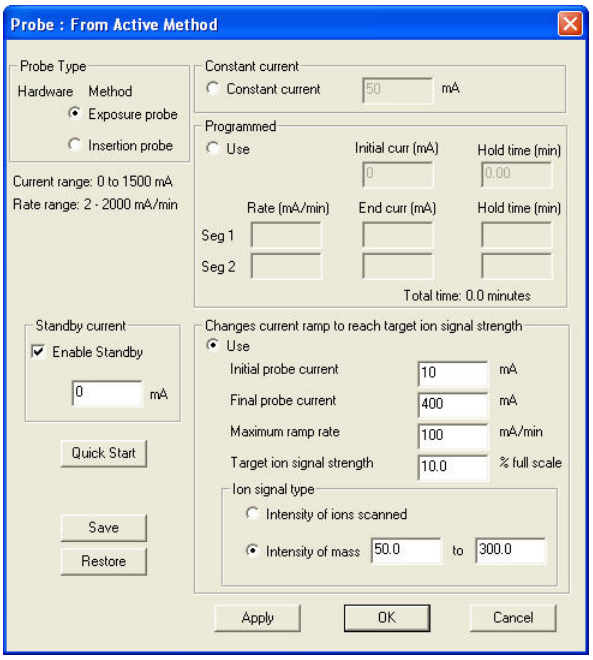
APCI Vaporizer Gas

Enter a vaporizer gas temperature program to ensure the optimum vaporizer temperature for APCI runs.



DIP/DEP Probe (Optional Ion Source)

Enter a vaporizer gas temperature program to ensure the optimum vaporizer temperature for DEP runs.



Setting up MS and MS/MS Acquisitions

Overview

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 ESI and APCI.

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 full scan, SIM, or mixed operations in each time segment and select either positive or negative polarity.

MS/MS Operations

Product Ion can - 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 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 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 loosing 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 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 can allows Full Scan, SIM, MS/MS, or mixed operations and the Request Dwell time can be set appropriately.

Injecting Samples

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 onto the AutoSampler.
4. Start the run.

Overview

To make a single injection, use Inject Single Sample.

To program multiple injections, use SampleList.

If System Control is not displayed, click this icon  on the large button Workstation Toolbar.

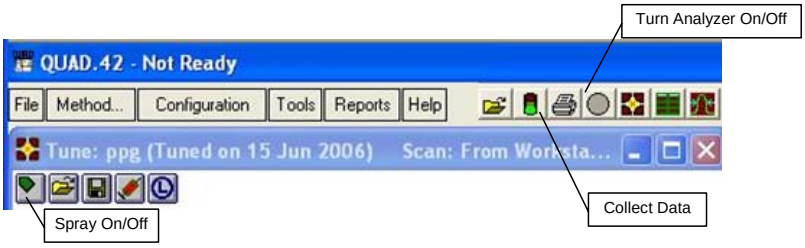
Startup / Shutdown of LC/MS and Analyzer

The startup and shutdown procedures and the injection methods are the same for both the Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) methods.

Startup

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

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



Shutdown

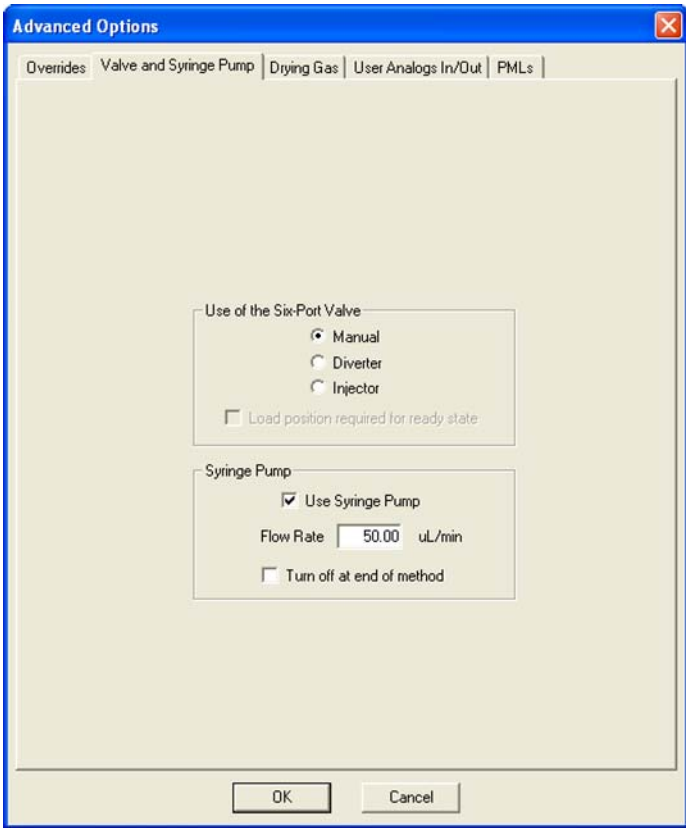
Turn off the LC pumps. Allow the LC pump pressure to drop down and stabilize so the flow into the MS stops. Click the **Spray icon** to turn off the gasses and heaters. The Analyzer turns off automatically when you turn off the source.

320-MS LC/MS Syringe Pump and Valve

You can use the six-port valve to divert flow away from the 320-MS, or to make injections. You can also set the flow of the syringe pump and have the pump shut off at the end of the method.

Refer to the 300 Series LC/MS and GC/MS Quadrupole Hardware Operation Manual to set up the syringe pump.

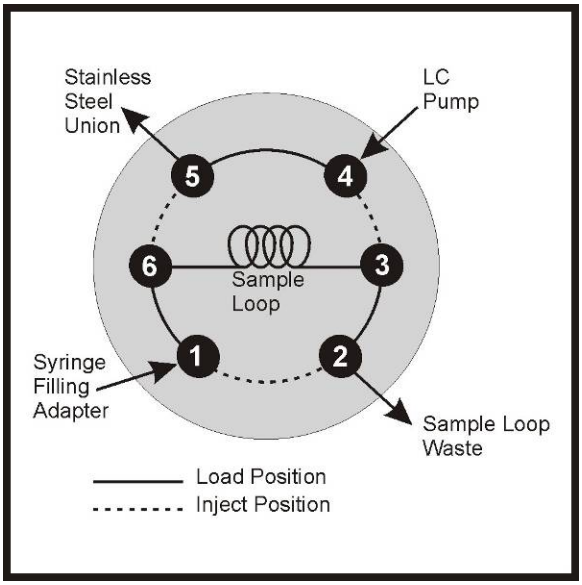
1. Click **Advanced Options** and select the **Valve and Syringe Pump** tab.
2. Select **Manual**, **Diverter**, or **Diverter** for the valve.
3. The system can turn the syringe pump off at the end of the run.
4. Enable the syringe pump, set the flow, and turn it off when the method is done.



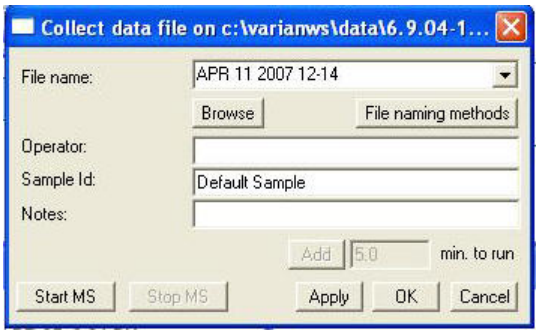
LC/MS Manual Injections

Manual Injection

Plumb the switching valve as shown.

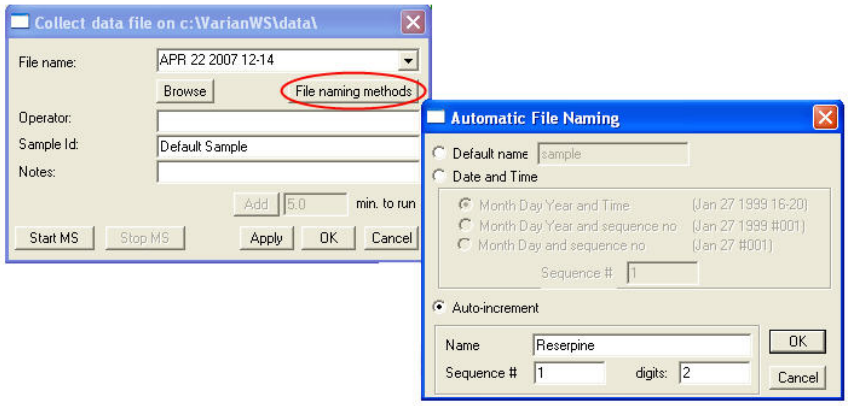


1. Monitor the baseline using the **Plot Tic** tool in the **Tools** menu.
2. After the baseline stabilizes, click the **Traffic light** and the **Collect data file** window opens.

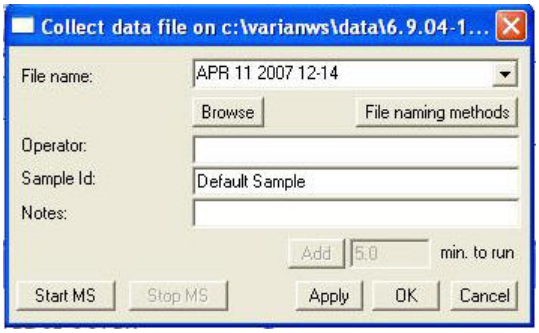


3. Click **File naming methods** and the **Automatic File Naming** window opens.
4. Click **Auto-increment**. Enter the *name* of the data file.
5. Enter **1** for Sequence #, and **2** for digits.

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.



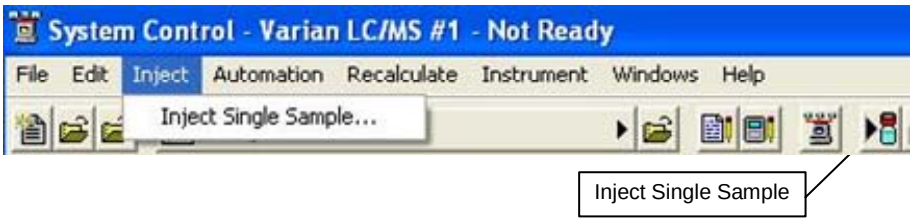
6. Click **OK** and the **Automatic File Naming** window closes.



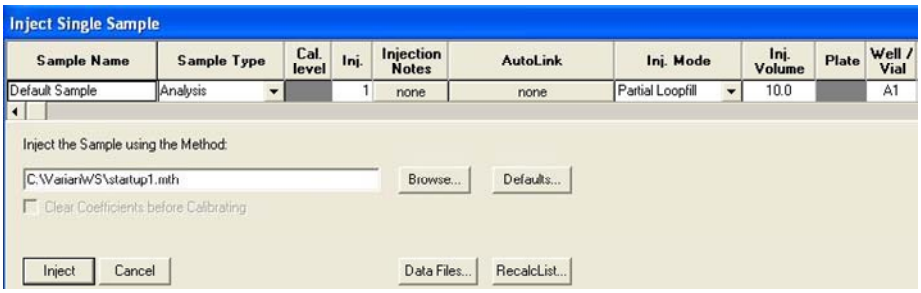
7. Complete the **Collect data** window.
8. Click **Apply** and if you are ready to collect data, click **Start MS**.
9. Check that the valve is in the load position.
10. Overfill the 5 µL switching valve loop with 3 or 4 times the volume. Push the button on the valve cover. Allow at least 1 minute between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

GC/MS or LC/MS Injecting Single Samples

To do one injection, either select **Inject Single Sample** from the **Inject** menu or click the **Inject Singe Sample** icon on the System Control menu bar.



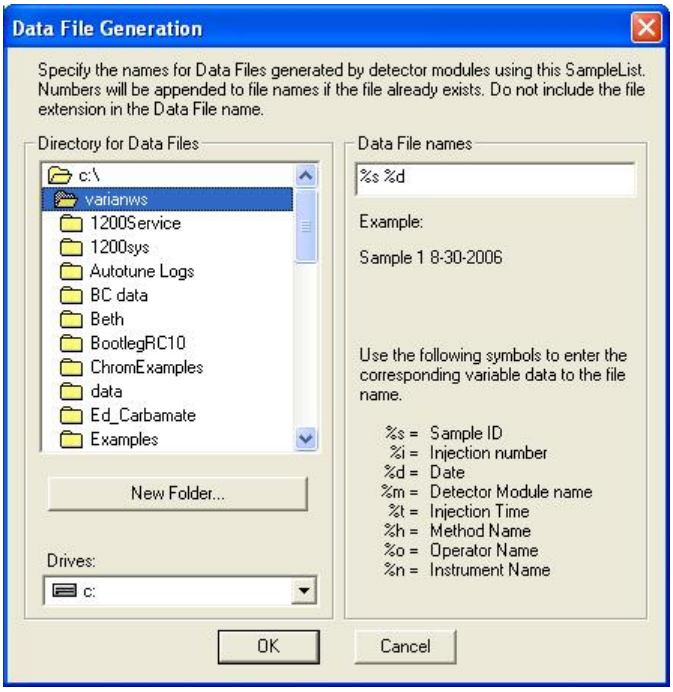
If you select **Inject Single Sample** from the Inject menu, the following window opens. The fields are dependent on the sampling device.



Enter the Data File Name and Path

Data File names can up to 255 characters. You can make them a combination of Sample ID, injection date, module name, and injection number.

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



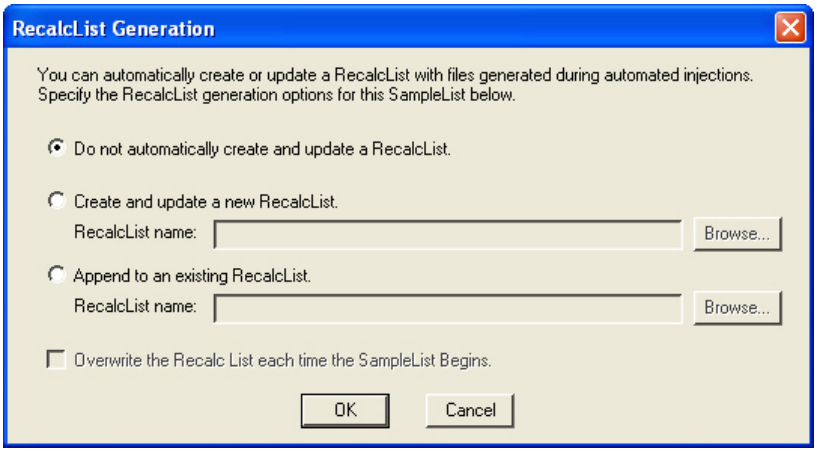
Select the drive letter and subdirectory (path) to store your files from the left side. The default directory is the data subdirectory of your VarianWS directory.

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

The files created by SampleList can be stored 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.



If you select **Overwrite the RecalcList each time the SampleList Begins** the RecalcList is overwritten.

If you select **Append to an existing RelcalcLis**t, the new RecalcList has a number appended to the filename

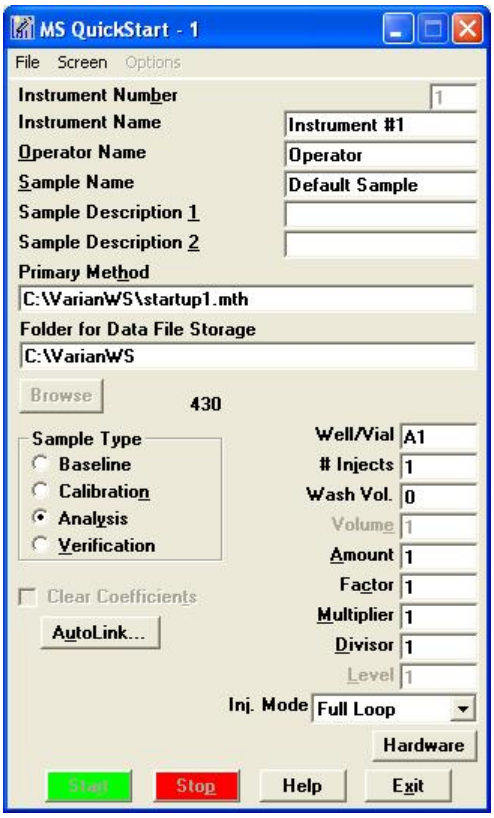
QuickStart

QuickStart injects a single sample without using System Control directly.

You can customize QuickStart and set up instruments for routine use. Refer to online help for further details.

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

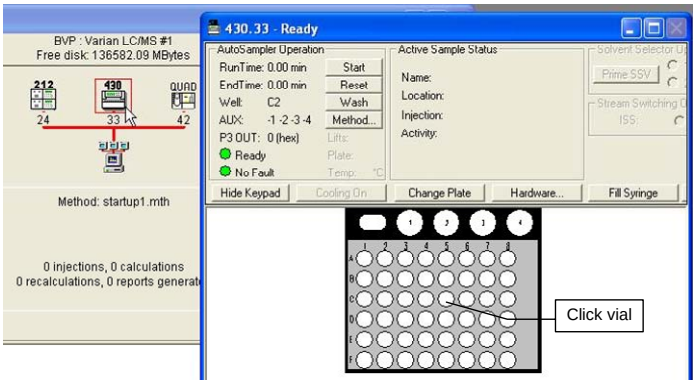
Enter information in the MS QuickStart window about your injection.



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 on the **vial position** of the priority sample or choose **inject single sample** and run your sample.
3. After completing the priority run, click **Resume** to re-start the SampleList.

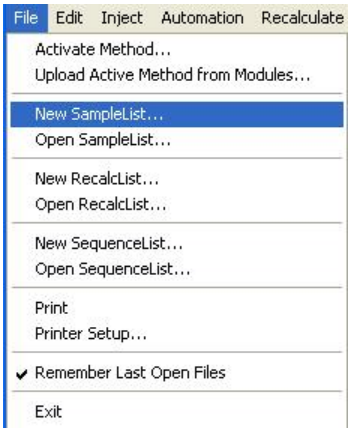
LC/MS or GC/MS Injecting Multiple Samples

Create SampleLists for different auto-samplers, and using more than one method for your injections.

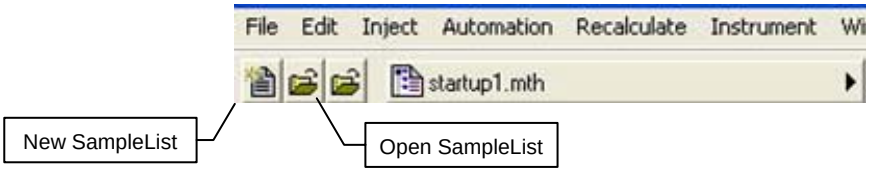
SampleLists in System Control

To inject multiple samples from System Control use a SampleList either 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.



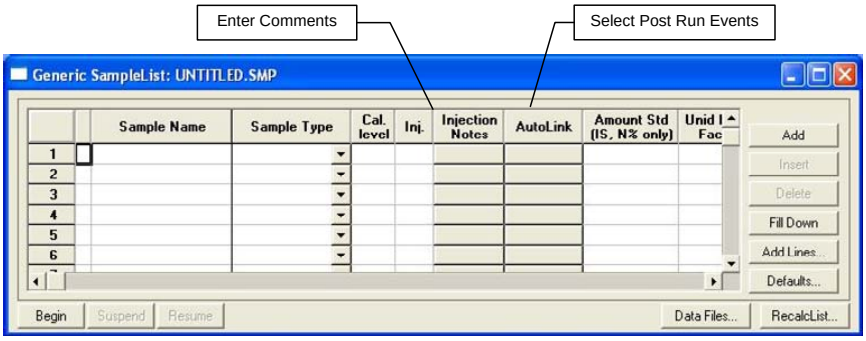
From the System Control toolbar, you can create a new SampleList or open an existing one



Generic SampleList

If no sampling device is 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.

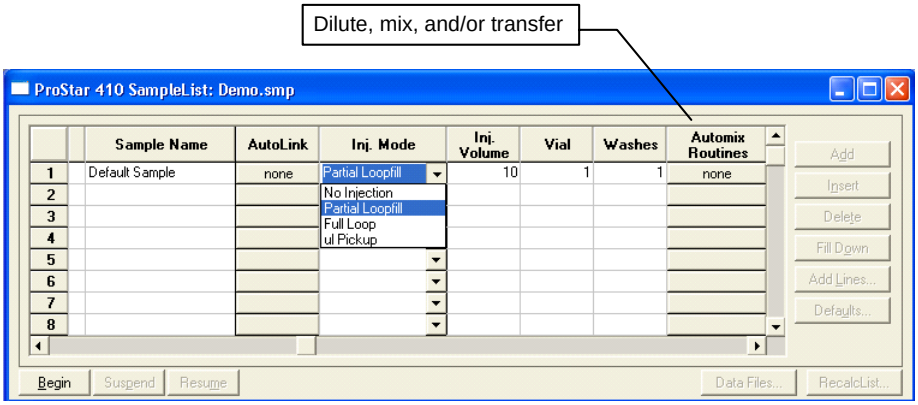
You can enter data handling parameters, such as, the amount for single internal standard calculations, the unidentified peak factor, a multiplier, and a divisor.

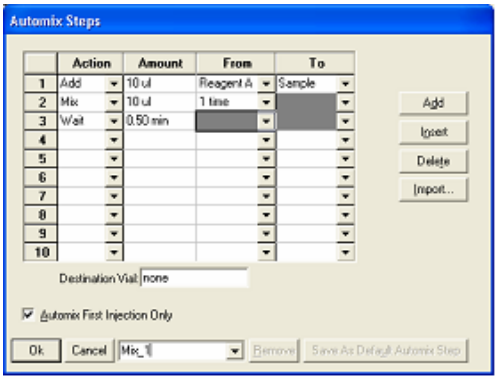
If your requirements are more complex (such as multiple internal standards or multiple detectors, which require different entries) use the MultiChannel MultiStandard field.

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

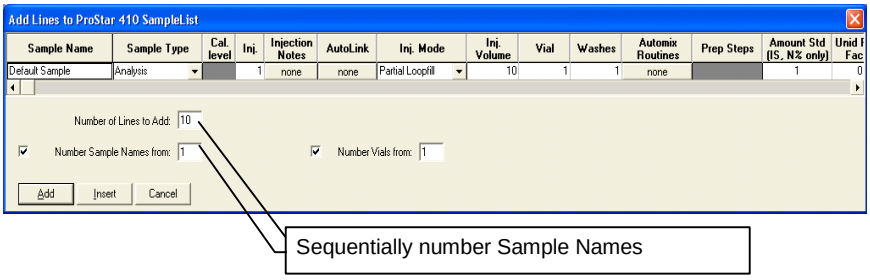
LC/MS - ProStar 410 SampleList

In addition to the Generic SampleList features, the ProStar 410 supports other features such as different Injection Modes, Vial specification, and AutoMix functions.



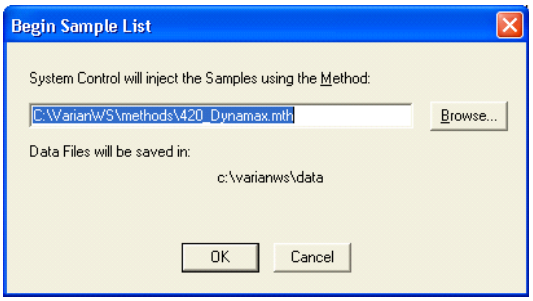


Click Add Lines to add several lines to the SampleList.



Press **Begin**, and you are prompted to select a Method. Use the active Method that was pre-selected, or click **Browse** to use another.

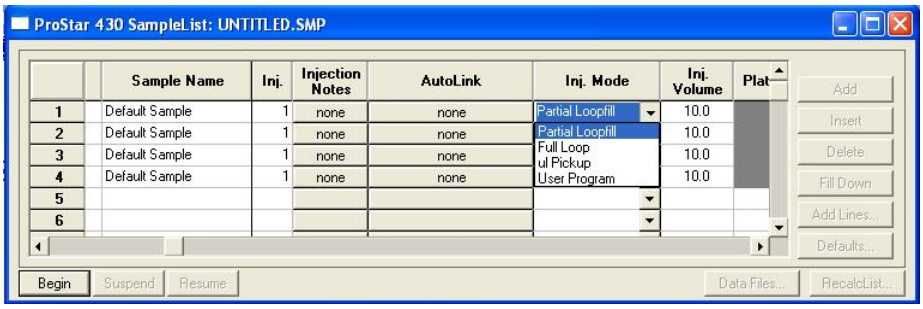
Click **OK**, to begin the run.



LC/MS - 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.

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 with the well/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.

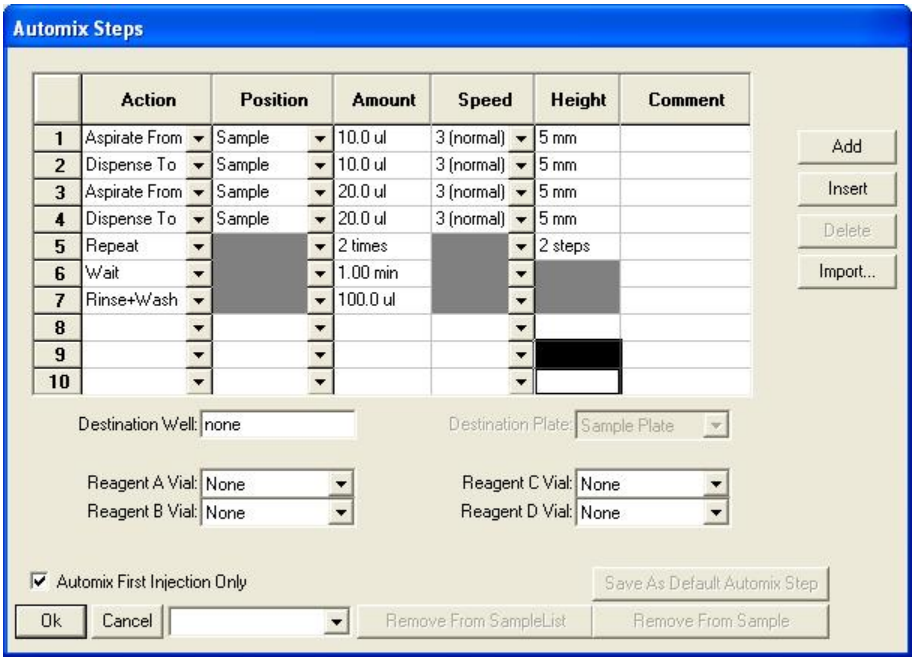
For the ProStar 420, the **Vial** field specifies the sample vial as a combination of Tray type letter and Vial number. The available Tray types and Vial numbers depend on the Tray Segments configured. You can randomly access any vial.

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.

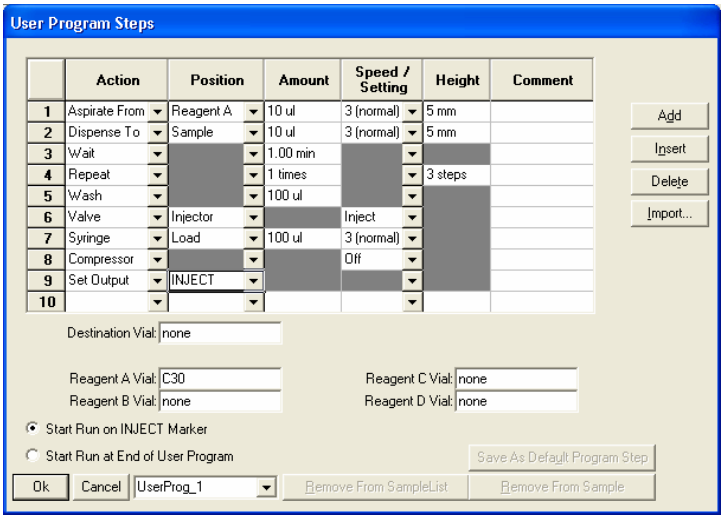
You can program the AutoSampler to wait for specific time interval. Use the **Automix Steps** dialog to select Action, Position, Amount, Speed of the syringe, and Height of the needle. The following is an example of an automix routine.



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 press **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.

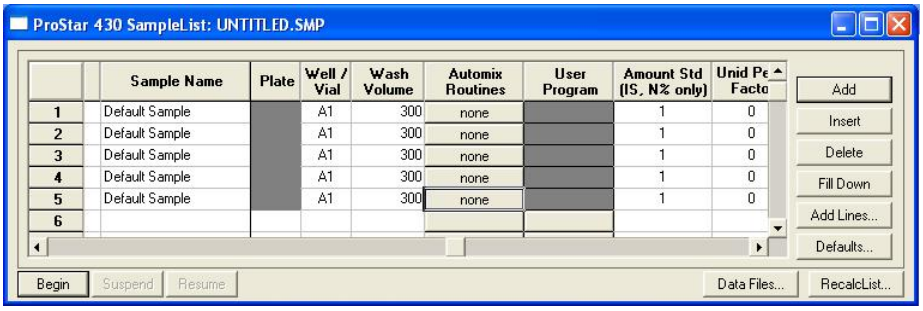
The User Program column is enabled if selected in the Injection Mode field for the sample. The User Program can do sample handling and injection actions.

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 10-ml 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.



You can start the run, either on the INJECT Marker (step 9 in the above example), or at the End of User Program. Up to 240 steps can be programmed in one User Program. After adding all steps, enter a name for this User Program in the box next to Cancel (UserProg_1 in the above example), and press **OK.** The User Program is saved in the SampleList, and can be exported to other SampleLists. Or, use Import to bring User Programs from other SampleLists into the active SampleList.

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

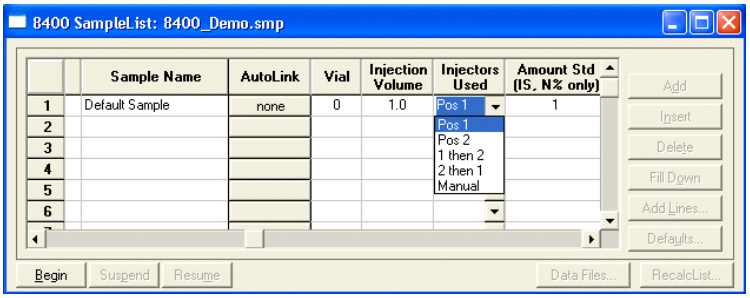


You can enter information that is common for all samples. You can select the number of entries to be added sequentially to the SampleList. With the ProStar 420, you can specify the first and last vials to be added to the SampleList with the requirement that the first and last vial numbers start with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

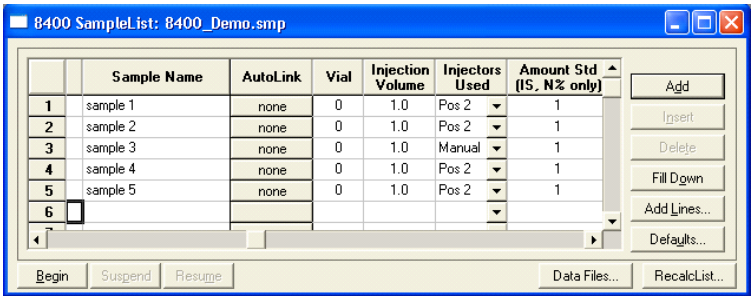
With the ProStar 430, you can specify the first and last Row and Column to be added. You can also select to add by Rows or Columns, which determines if the plate is processed from left to right by row or from top to bottom by columns.

GC/MS - CP-8400/8410 SampleList

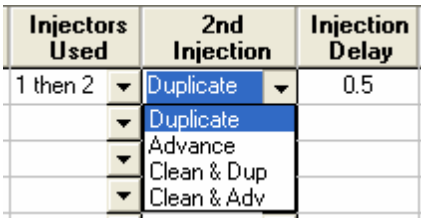
In addition to the Generic SampleList features, the CP-8400/8410 has settings for Vial position, Injection Volume and Injector Selection. If SPME hardware is installed, the Injection Volume field is disabled and the fiber Adsorption Time set in the GC control section of the Method is used for sample extraction.



Select Manual from the Injectors Used field to disable the CP-8400/8410 and make a manual injection. In the following example, the CP-8400/8410 injects samples 1 and 2, then waits for a manual injection/start from the GC for sample 3, and continues with samples 4 and 5 using the CP-8400/8410.

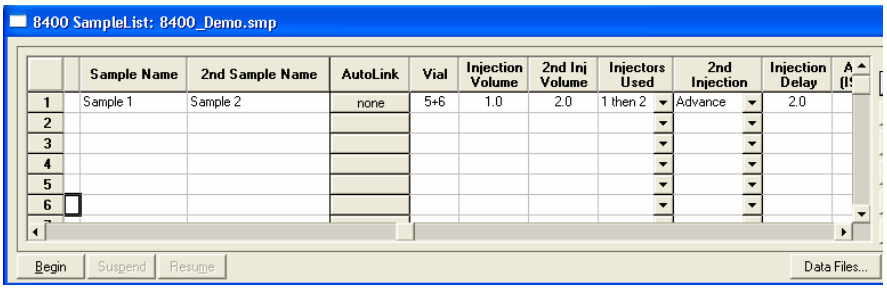


The CP-8400/8410 can also make two injections in the same GC method run. Select second injector from the Injectors Used field.



If two injectors are used, you can, for the second injector, inject the same sample as the first injection (Duplicate) or inject the sample from the next vial position (Advance). You can wash the syringe in between injections (Clean & Dup, or Clean & Adv).

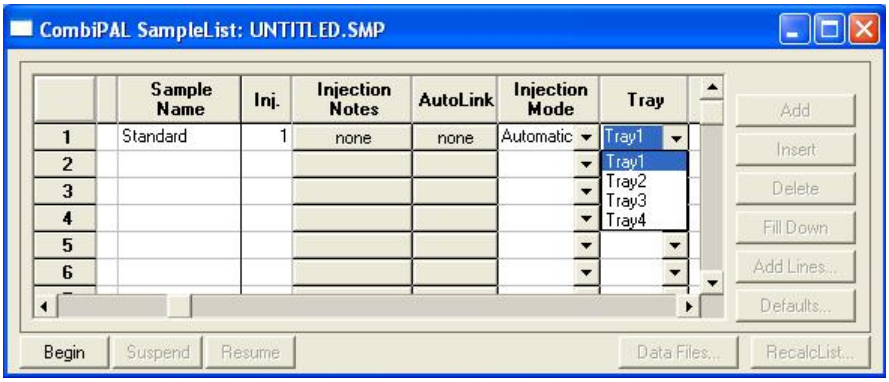
In the following example 1.0 µL of Sample 1 from vial 5 is injected by injector 1, there is a 2.0 seconds delay, and 2.0 µL of Sample 2 from vial 6 is injected by injector 2. A separate data file is created for each sample.



Combi PAL SampleList

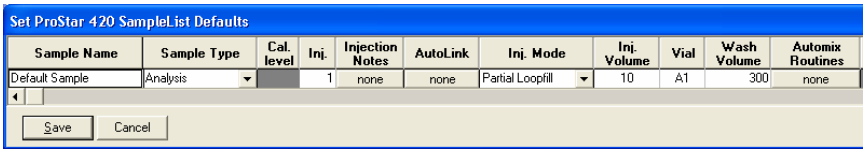
In addition to the Generic SampleList features, the Combi PAL for GC/MS and HTS PAL for LC/MS have settings for Tray selection, Vial position, and Injection Volume. If the Combi PAL is configured for SPME sampling, then the Injection Volume field is disabled and the fiber Extraction Time set in the Combi PAL control section of the Method is used for sample extraction.

All other sampling parameters are specified in the Combi PAL section of the Method.



Default SampleList Entries

When you add a new row to a SampleList, default values are entered. To change them, click **Defaults** and the **Set ProStar 420 SampleList Defaults** dialog box opens. Enter the desired default values and click **Save**.



Note that neither volume nor rack and vial number is displayed, even if an AutoSampler is present.

Saving SampleLists for Later Use

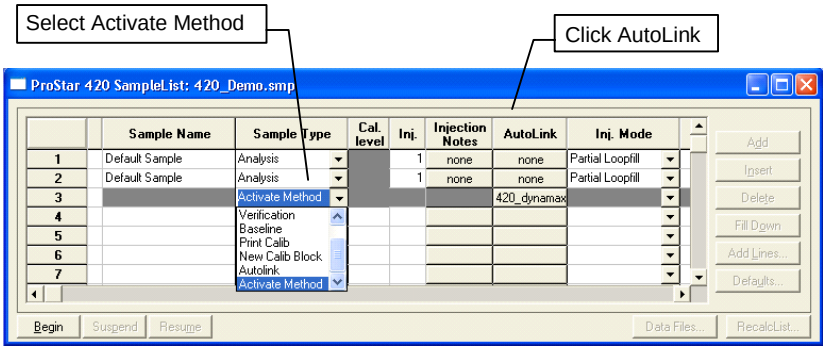
When you make changes to the open SampleList, the changes are automatically saved to the SampleList file and are used for the automated runs in progress. If you want to edit a SampleList other than the open SampleList, use the offline Automation File Editor application described in the next section of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

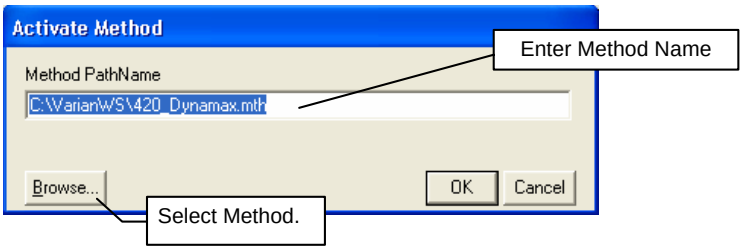
Using More Than One Method for Injections

You can do automated injections using more than one Method. There are two ways to do this: change the active Method in the SampleList or use a SequenceList.

Changing the Method in the SampleList

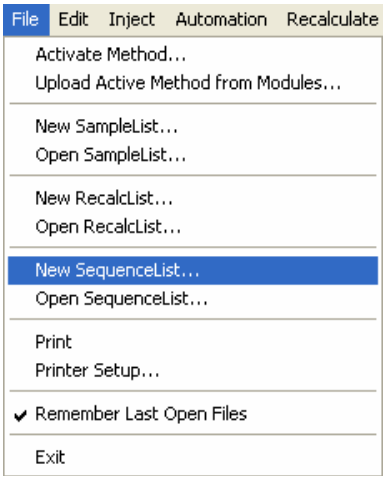
Change the Method by activating a Method in a SampleList row.



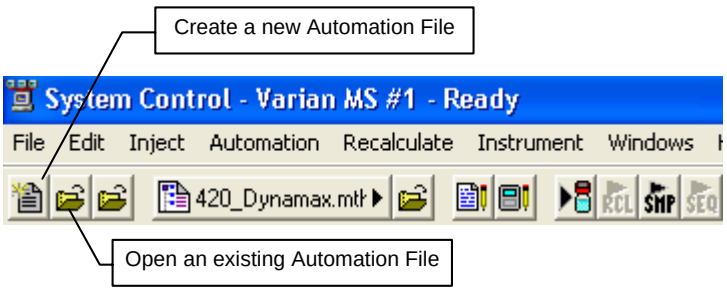


Using the SequenceList Window

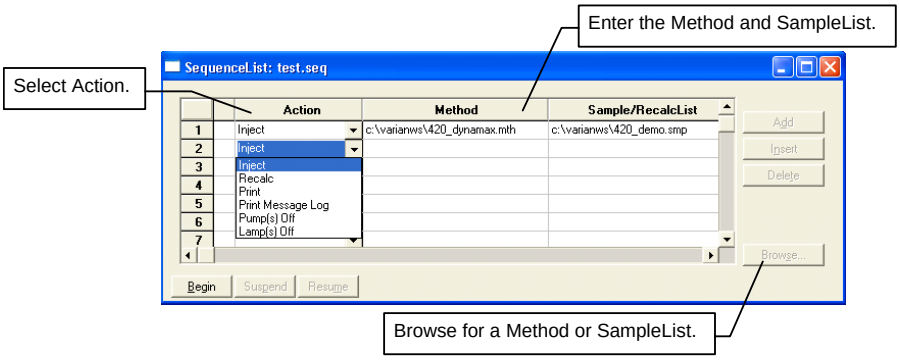
On the **File** menu, select a **New SequenceList** or **Open SequenceList**.



You can also create a new SequenceList or open one from the System Control toolbar.

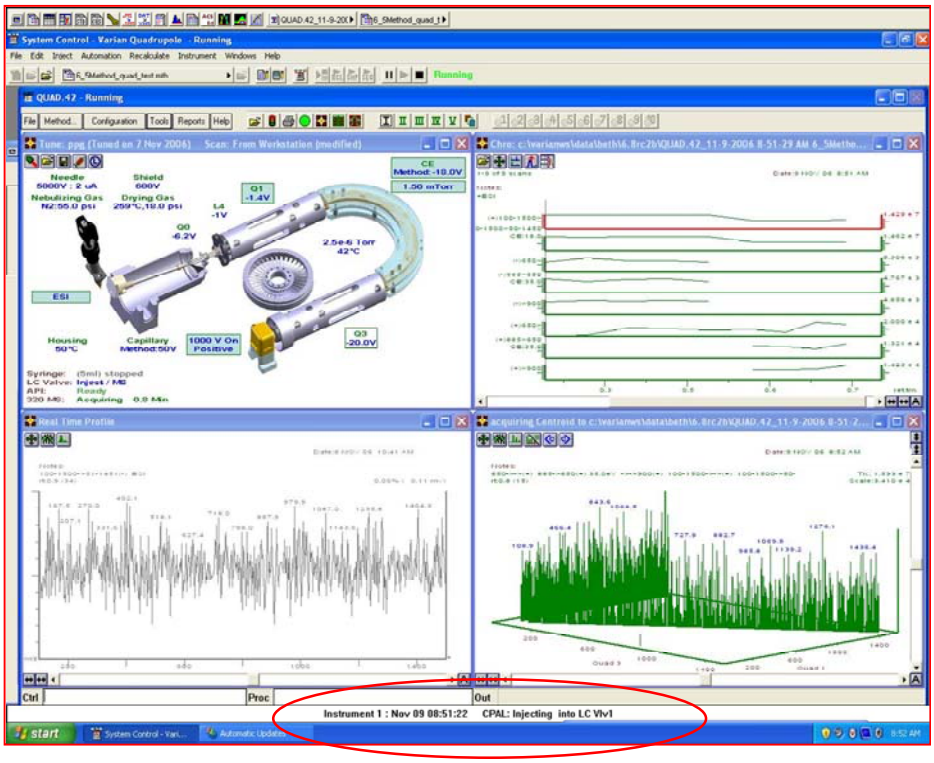


In the SequenceList, select an **Action**. Enter the **Method** and the **SampleList** or click **Browse** to select from a list.



Monitor Run Status

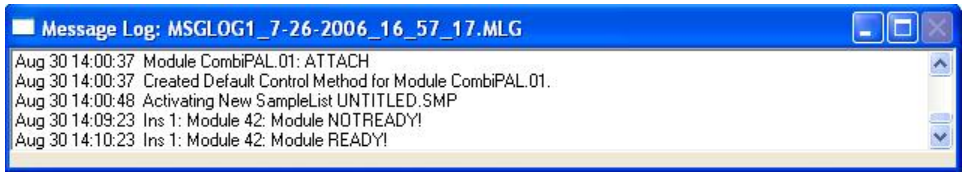
After making an injection, monitor the run in System Control.



The status and control window and the Toolbar display the module status.

Message Log

Automation actions and errors are logged 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.

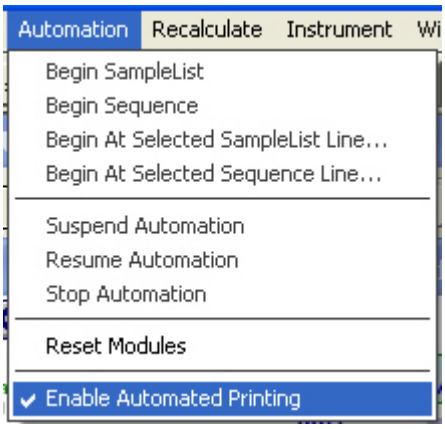


Message Log entries are stamped with the time and date.

Automated MS Report Generation

By adding Report sections to your Method, you can 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. SCII file generation is still performed, if selected in the Report Method section. When you disable automated printing, automation continues but no reports are printed.



Stopping an Acquisition

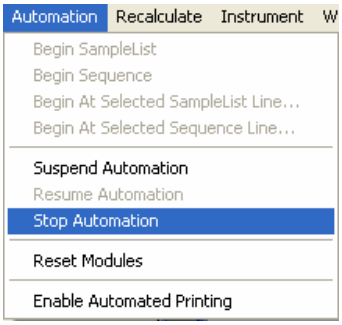
During automation and while the LC/MS or the GC/MS system is running you can suspend or stop data acquisition from the Automation menu in System Control as follows:

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

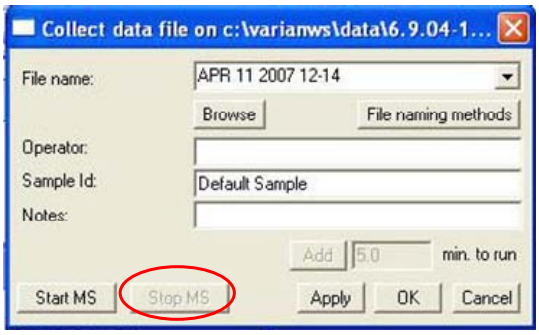
Stop Automation - Stop the current run, resets all modules and suspends automation.

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



Set a Collect Delay

LC/MS

The 320-MS 6 port valve can be used 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 interval.

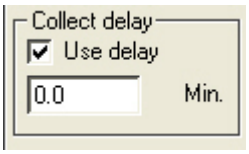
While the flow is diverted from the source, you can delay data collection in the Acquisition Method. The syringe pump can be connected and programmed to provide flow to the API while the LC effluent is diverted.

GC/MS

Use the Delay parameter to turn the filaments on after a time interval. This is used in GC/MS runs to prevent the filaments from being damaged or broken when the solvent peak elutes.

Acquisition Method

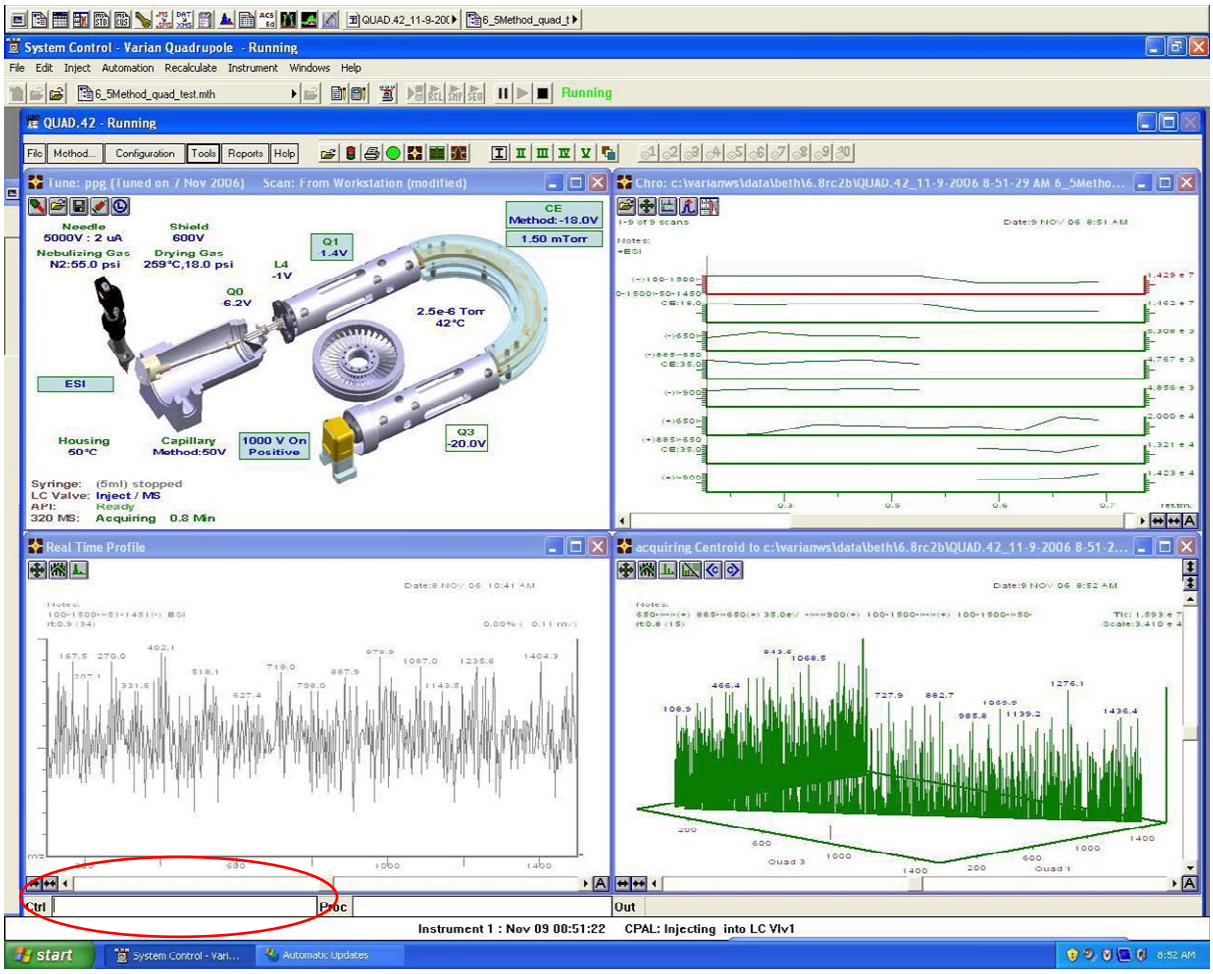
In **Method Builder**, open the **Acquisition Method.** Enable **Use delay** and enter the delay time in minutes.



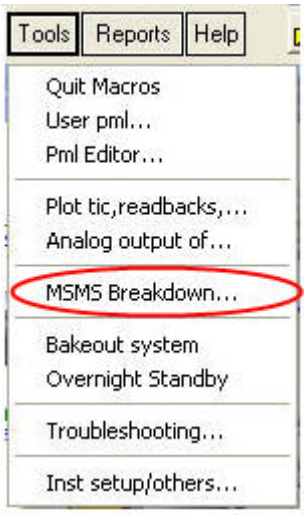
MS/MS Breakdown

The Breakdown Curve feature 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.

The precursor ion can be viewed in the profile or centroid mode by entering **SW xxx** in the Ctrl text field (see below).



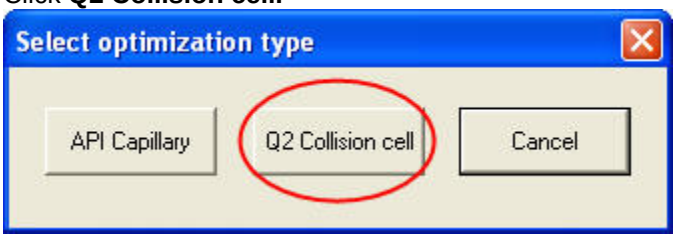
On the **Tools** menu, click **MS/MS Breakdown**.



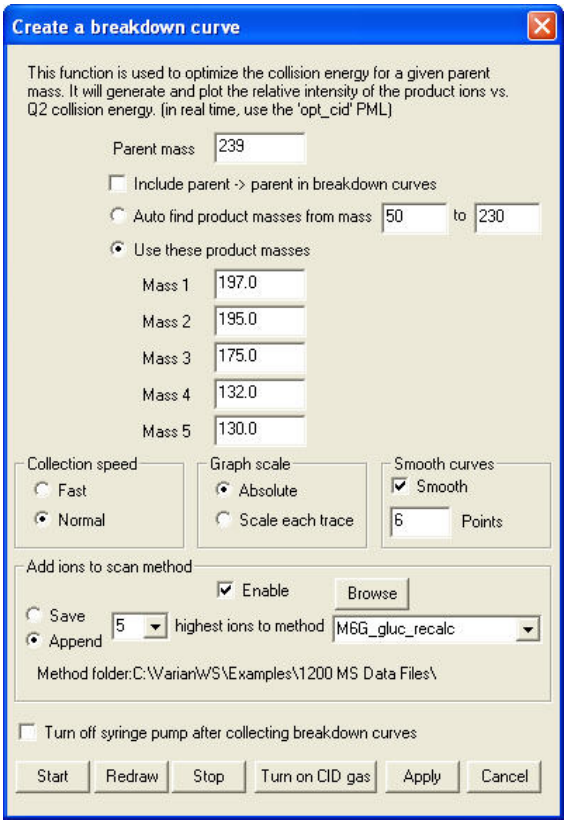
In GC/MS mode, your only choice is Q2 collision cell.

In LC/MS mode, you can select Q2 Collision Cell Breakdown, or API Capillary Breakdown.

Click **Q2 Collision cell.**



The **Create a breakdown curve** window.



1. Enter the precursor ion in Parent mass.
2. Click **Turn on CID gas**. Wait about a minute for it to stabilize.
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**, 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**.

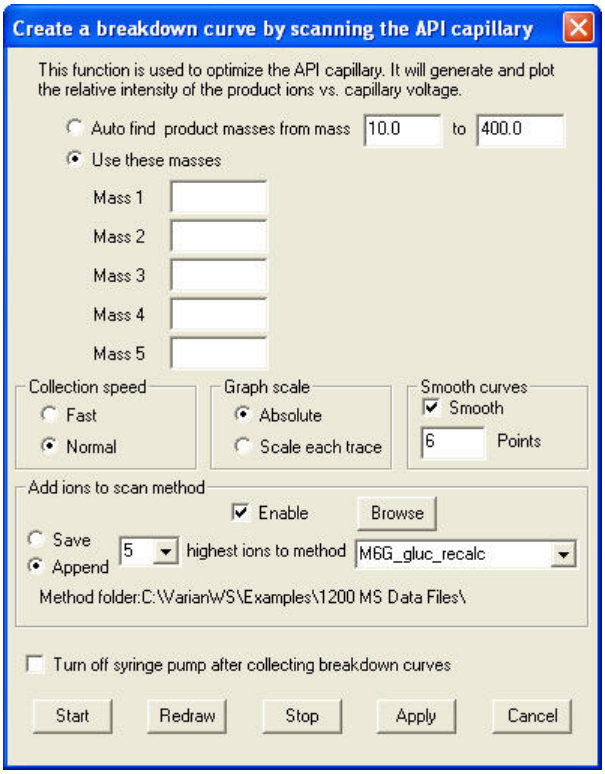
The breakdown curves are displayed.

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.

If you select API capillary, do the following after the **Create a breakdown curve by scanning the API capillary** window opens.



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

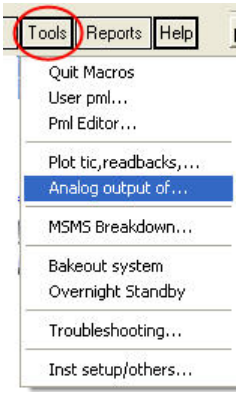
For this additional precursor ion, select **Auto find product masses** or specify the product masses.

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

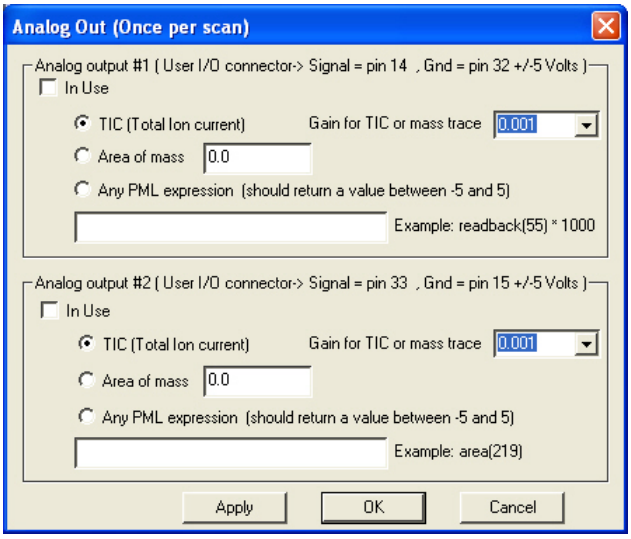
Analog Channels

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

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

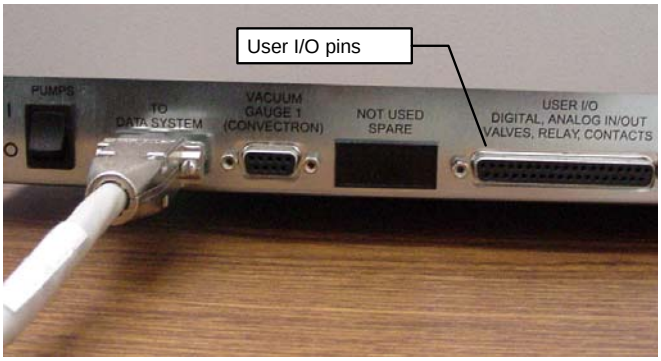


The **Analog Out** widow opens.



To create an Analog signal output:

1. 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.
2. Also check **In Use** for the appropriate channel in the Analog Out dialog. The part number of the user IO Board is CUB06-0047.



Automation File Editor

Overview

Use the Automation File Editor to create and edit SampleLists, RecalcLists, and SequenceLists outside of the Varian MS Workstation System Control application. Access the off-line Automation File Editor without disrupting automated runs.

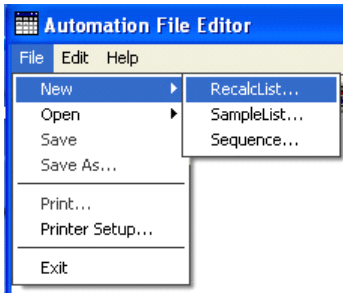
NOTE: SampleLists and Sequences that are active and running in System Control cannot be accessed in the off-line Automation File Editor.

Accessing the Automation File Editor

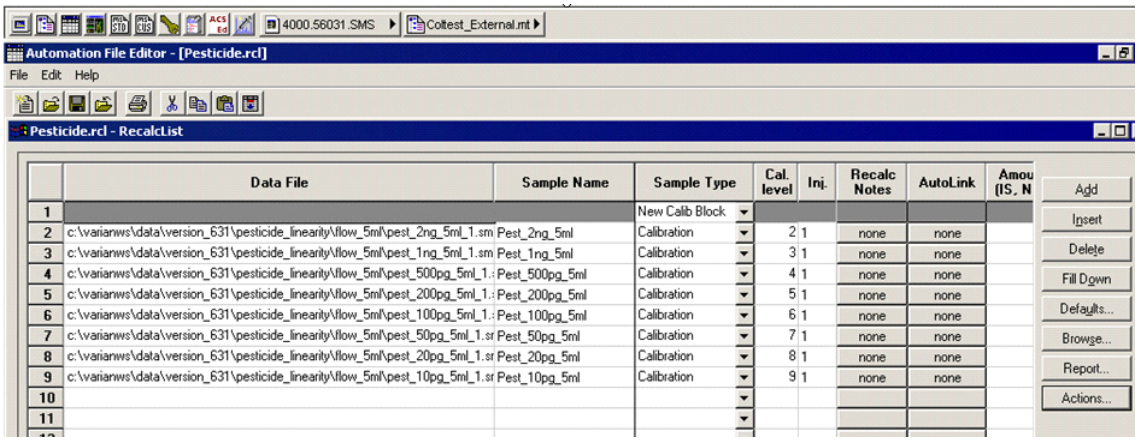
Click the **Automation File Editor** icon  on the Workstation Toolbar.

Create or Edit a RecalcList

Either select a New RecalcList or Open an existing RecalcList from the File menu.



The RecalcList window opens. The table is like a SampleList. 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.

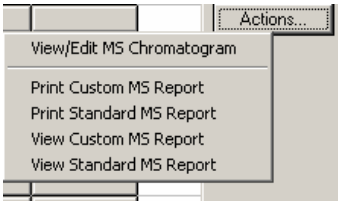


Recalc Notes - Enter notes about the Recalculation of the Data File.

AutoLink - Enter post calculation operations.

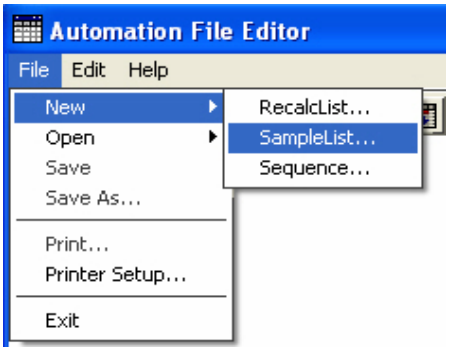
Report - Generate a report for the selected data file. Note that if a data file is not highlighted in the Data File column, no report is generated.

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

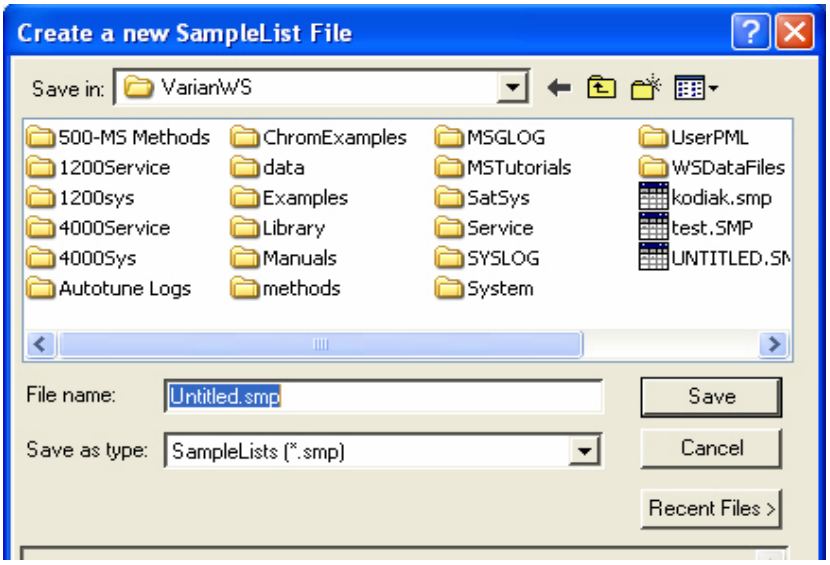


Create or Edit a SampleList

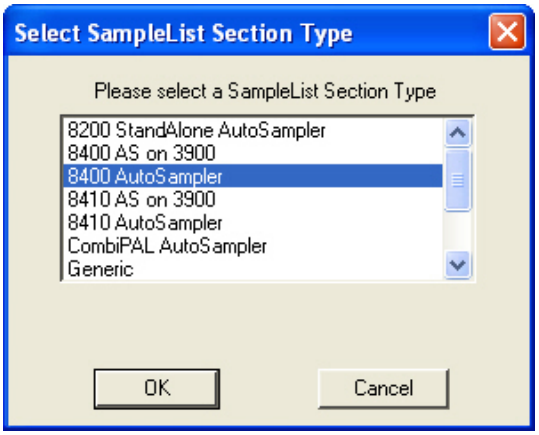
Either select a New SampleList or Open an existing SampleList from the File menu.



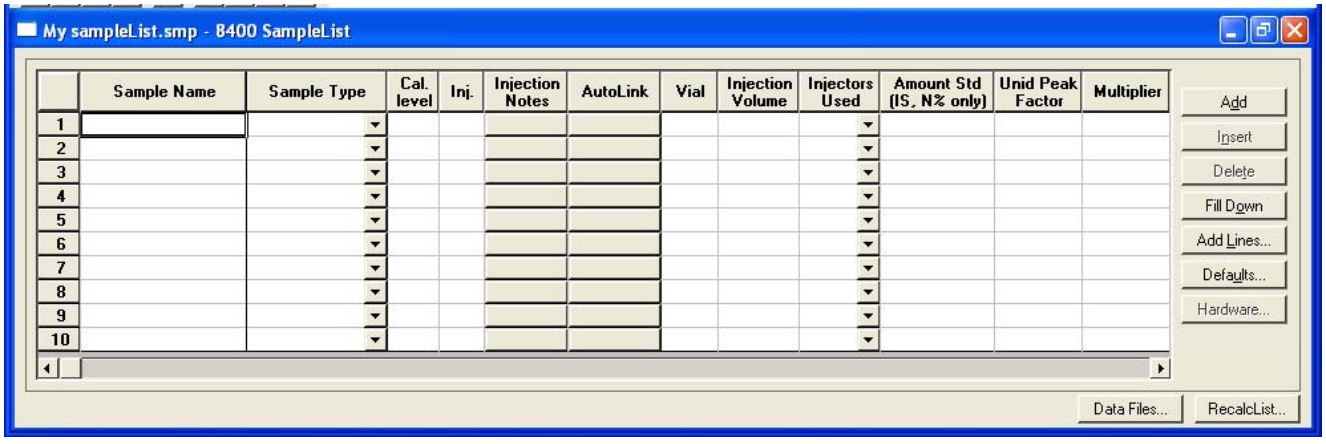
Select a folder for the SampleList, enter a name, and click **Save**.



Select the autosampler and click **OK**.



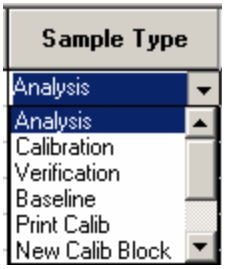
Size the Spreadsheet columns by dragging their borders with the left mouse button. Right-clicking a column header displays formatting options. The Sample Name column does not scroll.

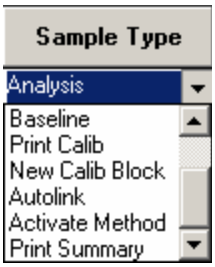


SampleList Fields

Sample Name - Enter the sample name. Create sample names by clicking **Data Files**. If only '%s' is listed in the Data Files the sample name is as you entered it.

Sample Type - Use the scroll bar to see all options.

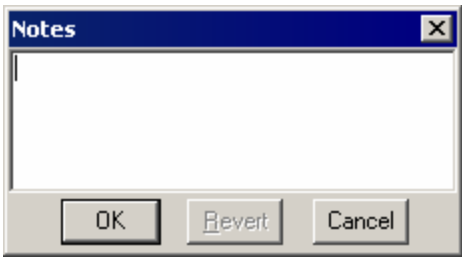




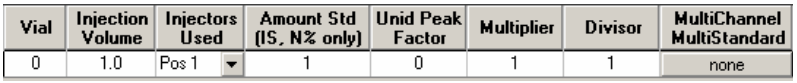
Cal. Level - Designate the calibration level for a Calibration sample. You can enter up to ten levels.

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.

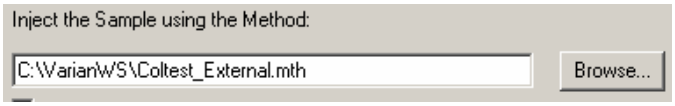


AutoLink - Refer to the 'Specifying Data Handling Parameters' section in this manual.



Vial	Injection Volume	Injectors Used	Amount Std (IS, N% only)	Unid Peak Factor	Multiplier	Divisor	MultiChannel MultiStandard
0	1.0	Pos 1	1	0	1	1	none

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

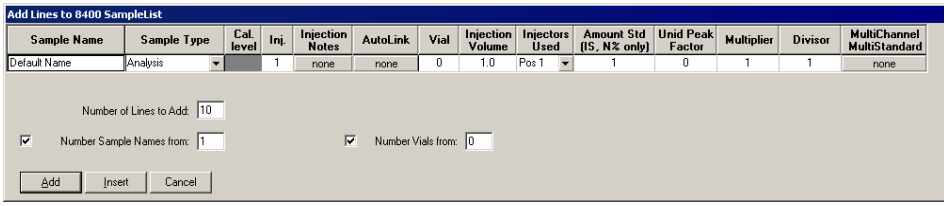
Add - Adds a new line to the end of the list.

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.

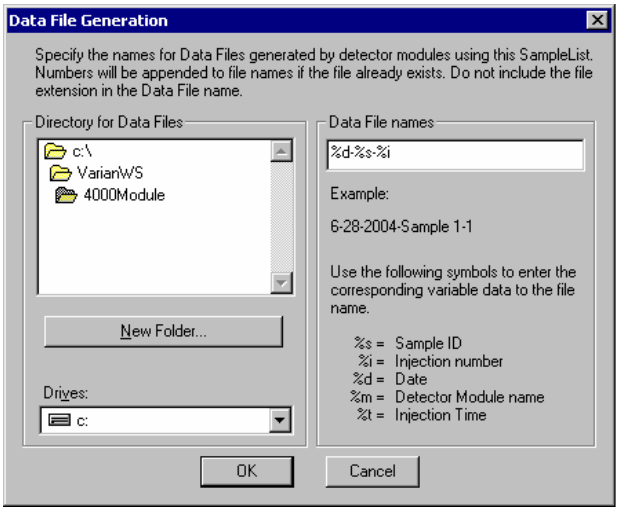
Data Files - Change the location and name of the Data Files.

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.

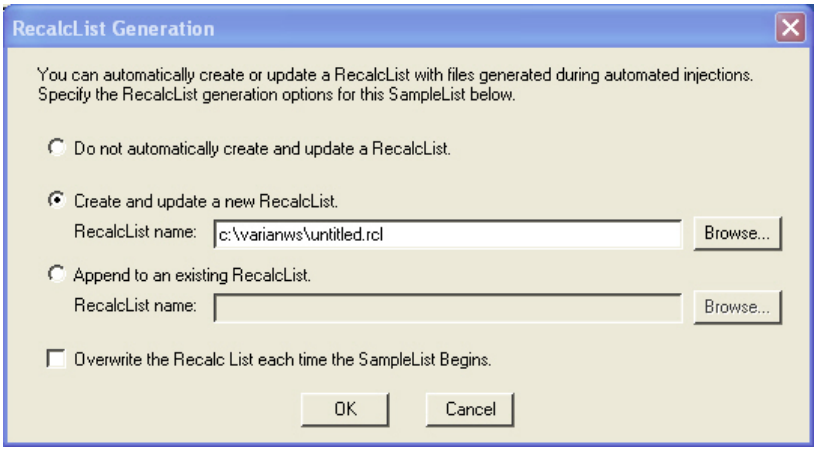


Select the data file directory from the left side. Create a file name specification on the right. You can combine text entry with the “%” variable symbols to specify file

names that contain 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.

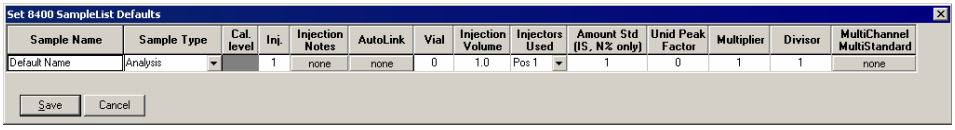


Changing Default SampleList Entries

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**.

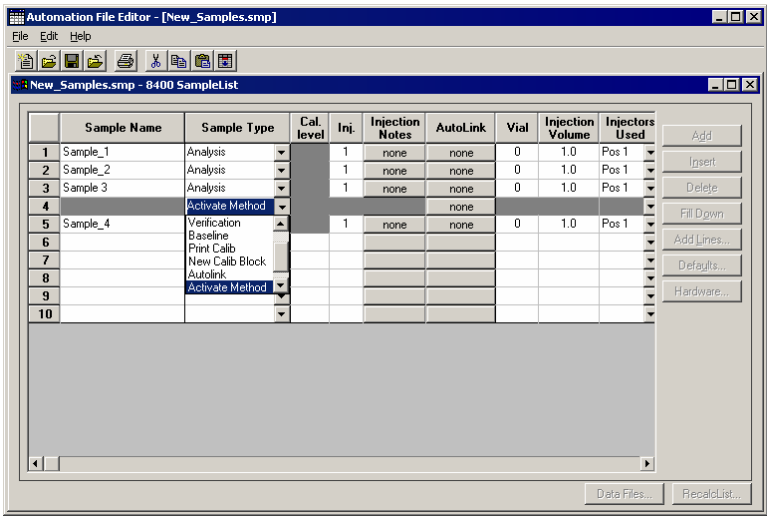


Using More Than One Method for Injections

You can do automated injections using more than one Method by changing the active Method from the SampleList or using a Sequence.

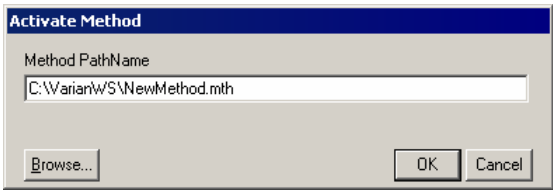
Changing the Method in the SampleList

Change the Method used during injections by activating a Method in a SampleList row.

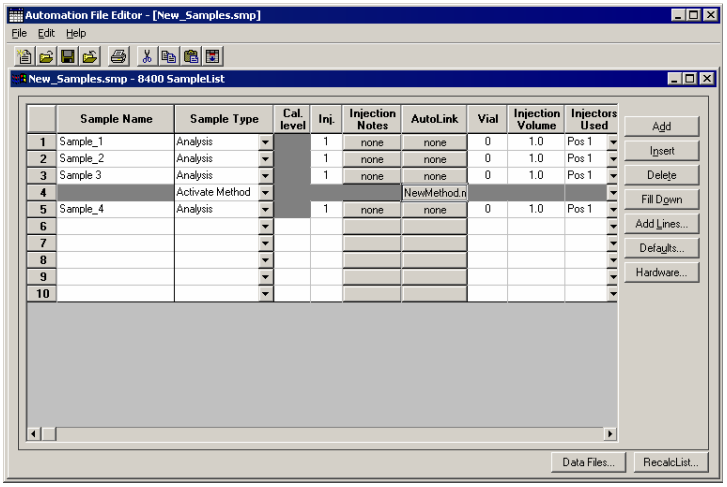


Select **Activate Method** from the Sample Type cell.

Click **AutoLink** in the row that you are working. The Activate Method widow opens.



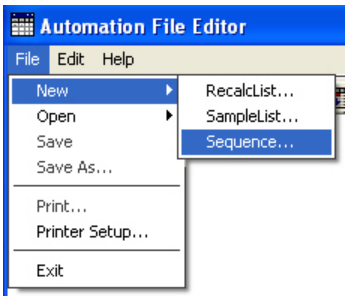
Enter the name of the Method or click **Browse** to select the Method from a list.



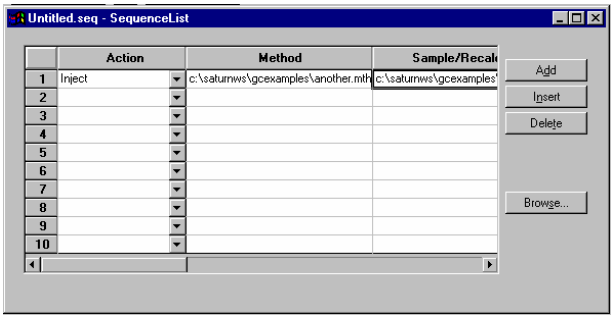
Specify any number of Methods in the SampleList.

Create or Edit a Sequence

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



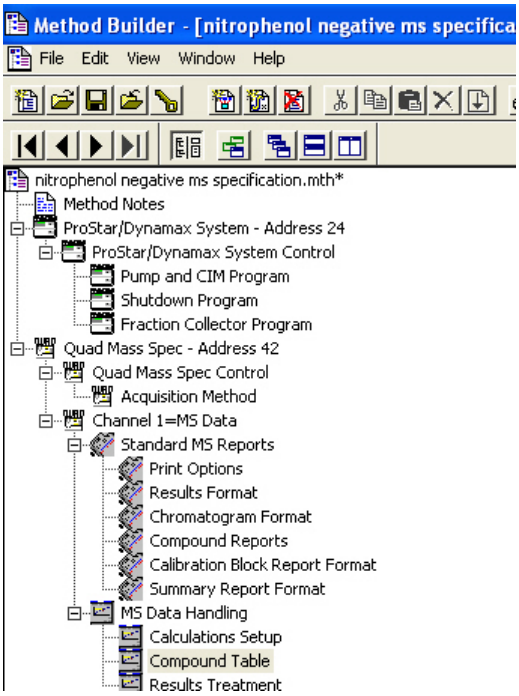
The Sequence window opens.



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

Compound Table

In the Method Builder tree, click **Compound Table** under MS Data Handling.



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).

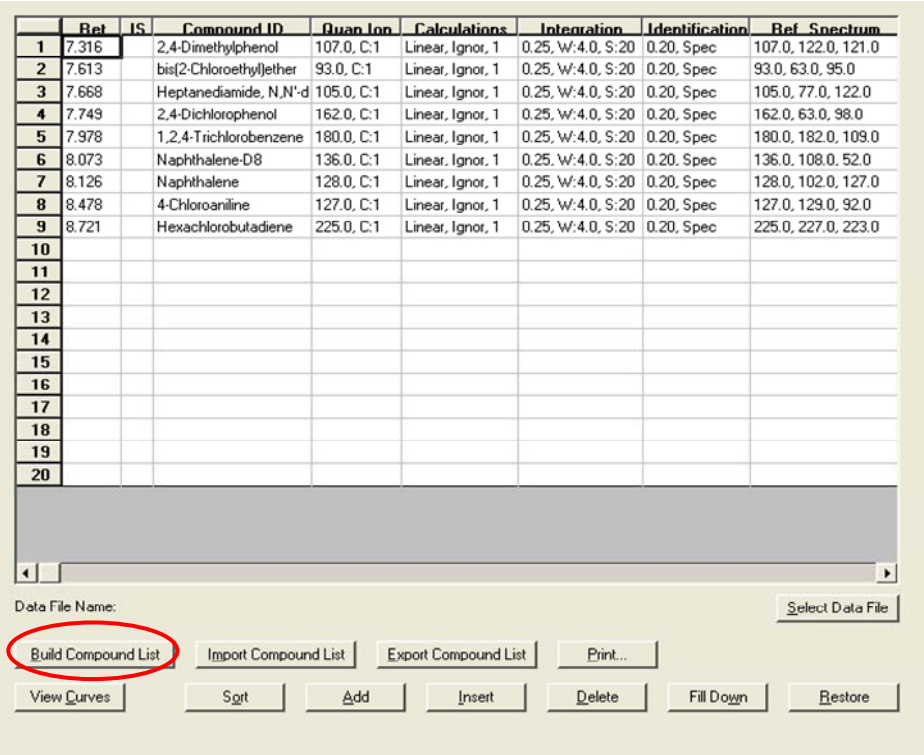
- Click **Select Data File** to open a different data file.
- Click to **Sort**, **Add**, **Insert**, **Delete**, **Fill Down**, or **Restore** compounds.
- Other functions are: **Build Compound List**, **Import Compound List**, **Export Compound List**, and **Print**.
- Before processing calibration 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.

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

Creating a Compound Table

1. Click **Build Compound List**.
2. Click each peak to create an entry.
3. In the Method Editor, click **Import Compound List,** and click **Select** to put your compound list into the method.

Note: You can double-click a cell to display more information.

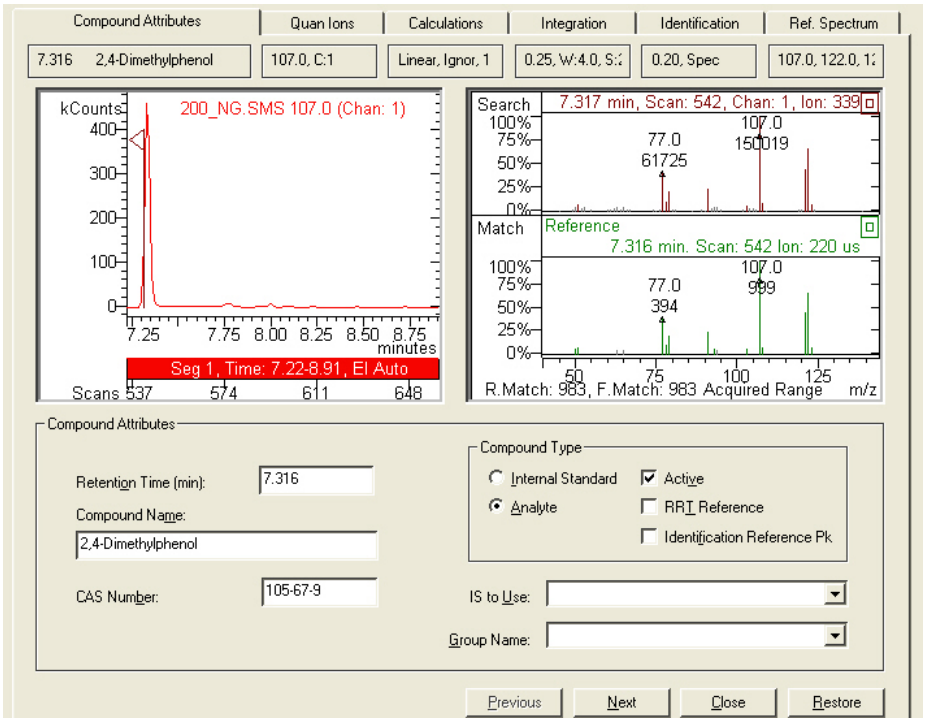


	Ret	IS	Compound ID	Quan Ion	Calculations	Integration	Identification	Ref. Spectrum
1	7.316		2,4-Dimethylphenol	107.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	107.0, 122.0, 121.0
2	7.613		bis(2-Chloroethyl)ether	93.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	93.0, 63.0, 95.0
3	7.668		Heptanediamide, N,N'-d	105.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	105.0, 77.0, 122.0
4	7.749		2,4-Dichlorophenol	162.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	162.0, 63.0, 98.0
5	7.978		1,2,4-Trichlorobenzene	180.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	180.0, 182.0, 109.0
6	8.073		Naphthalene-D8	136.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	136.0, 108.0, 52.0
7	8.126		Naphthalene	128.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	128.0, 102.0, 127.0
8	8.478		4-Chloroaniline	127.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	127.0, 129.0, 92.0
9	8.721		Hexachlorobutadiene	225.0, C:1	Linear, Ignor, 1	0.25, W:4.0, S:20	0.20, Spec	225.0, 227.0, 223.0
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

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. After you select **Quan Ions,** other than the RIC, (for example, in the Build Compound Table dialog in MS Data Review or in the Quan Ions Tab view) use the selected Quan Ions for Plot parameters.

Add information, such as Compound Name and CAS Number, during the peak addition process.



Edit Name, CAS Number, Retention Time

You can edit the **Compound Name** and **CAS** fields.

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 all Internal Standards by checking them, as in preceding 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

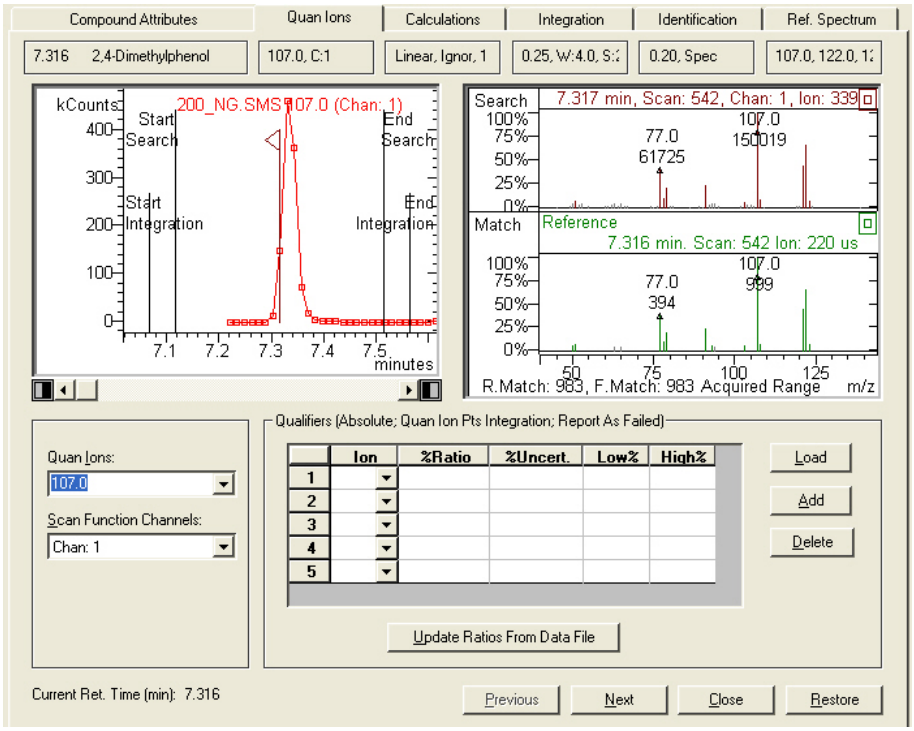
Sometimes target compounds are identified as a member of a group of compounds (for example, Trihalomethanes (THM), alkyl aromatics, etc.).

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.

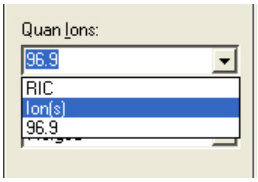
Quan Ions

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.



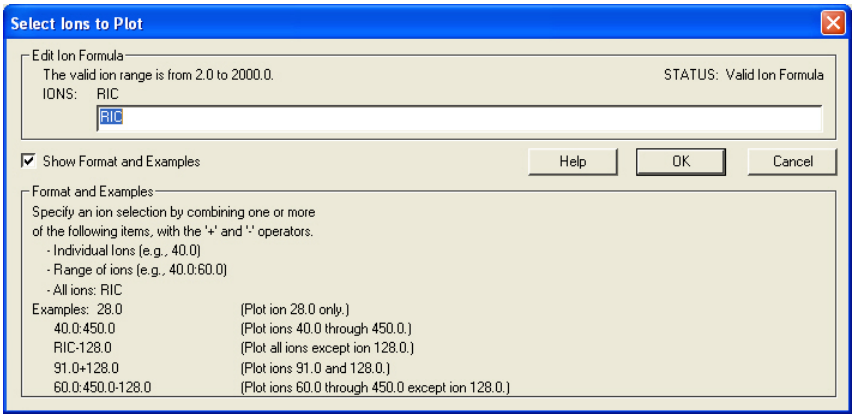
Edit Quan Ions

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 may be done on individual Channels of information.



Edit Retention Time

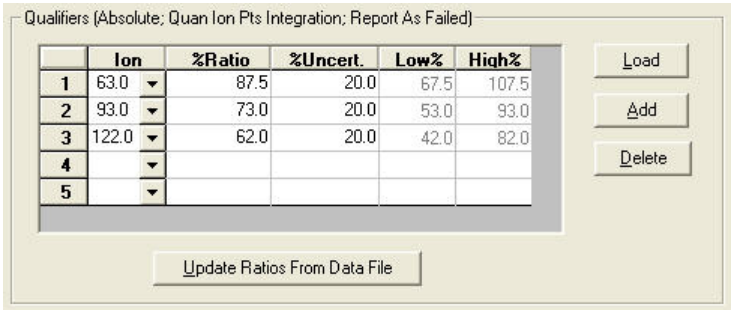
To change the retention time, click the desired scan in the Chromatogram display.

Look at Other Compounds

Click **Next**, or **Previous** to see the Quan Ion information for other peaks in the Compound Table.

Select Qualifier Ions

In addition to specifying quantitation ions, you can 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).

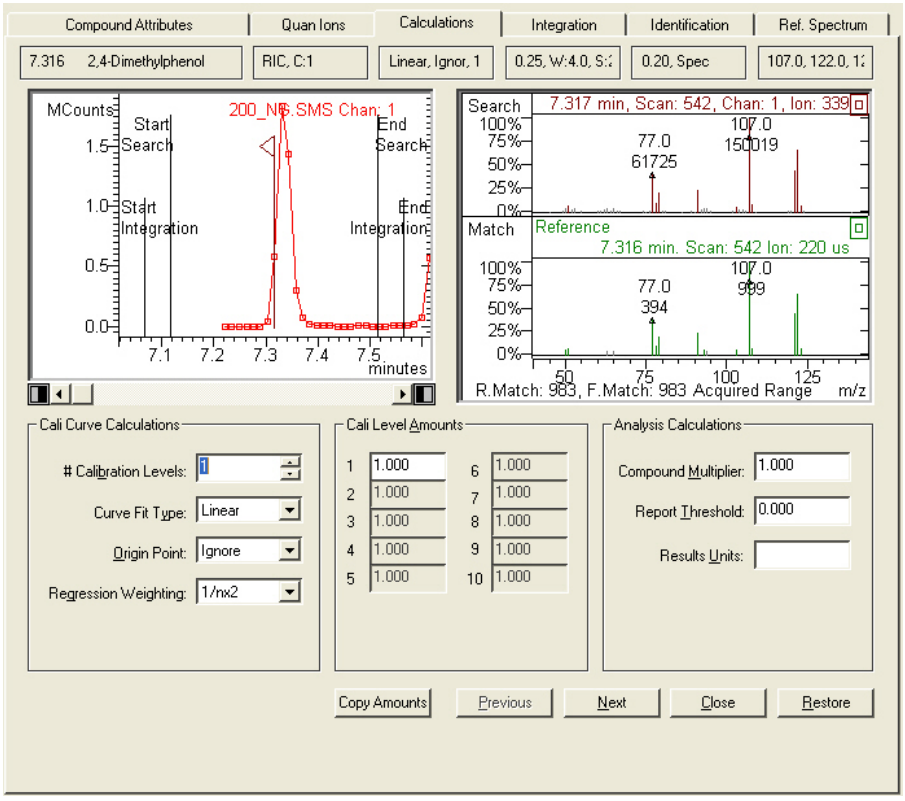
%Uncert. - Allowed percentage deviation from the Ratio. You can change the default of 20%. The % Uncertainty allowable range dependents on the Ion Ratio Type setting in the Calculations Setup Dialog (Absolute % or Relative %).

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 as Calibration, Analysis, or Verification runs, the Qualifier Ion tests are done before the peak is integrated.

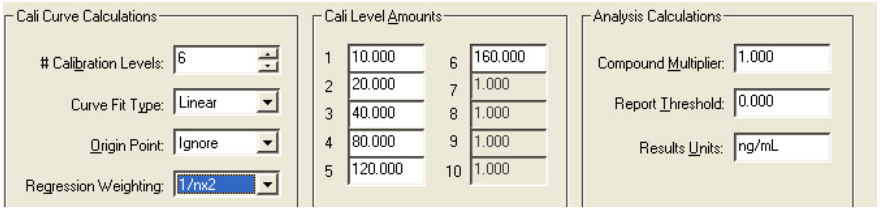
Calculations Tab

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.



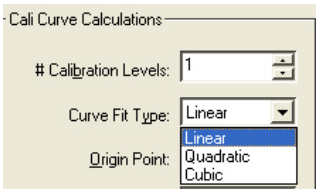
The Calculations Information is updated in the Compound Editor dialog. This information can be automatically entered for the other compounds in the table.

Quan Ion	Calculations	Integration
95.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20
61.9, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20
78.0, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20
82.9, C:M	Linear, Ignor, 1	0.25, W:4.0, S:20

Click **Copy Amounts** to enter 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**.

NOTE: For more information about the Integration, Identification, and Reference Spectrum Tabs, and other information about Quantitation and Data Handling: see the *MS Software Reference Manual* (part number 391496300).

Security Administration

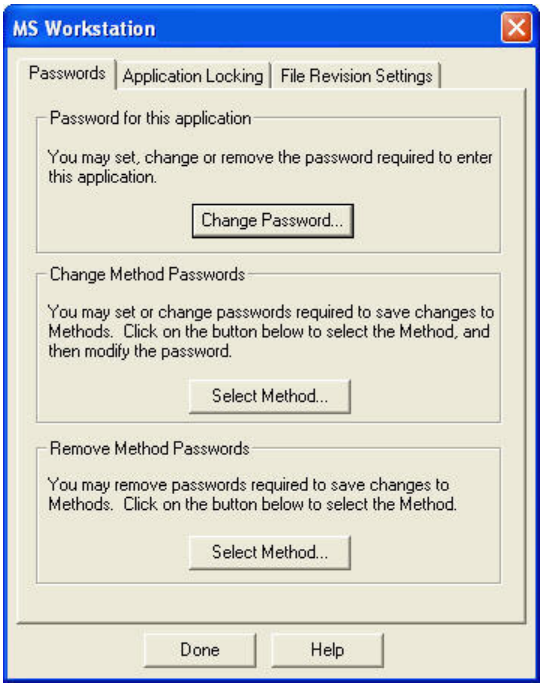
Overview

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

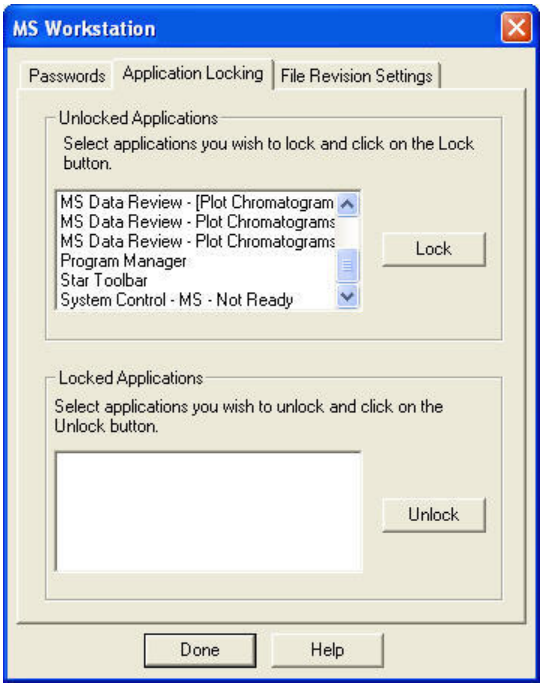
Click the Security button on the Workstation Toolbar to open the Security Administration window.

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.

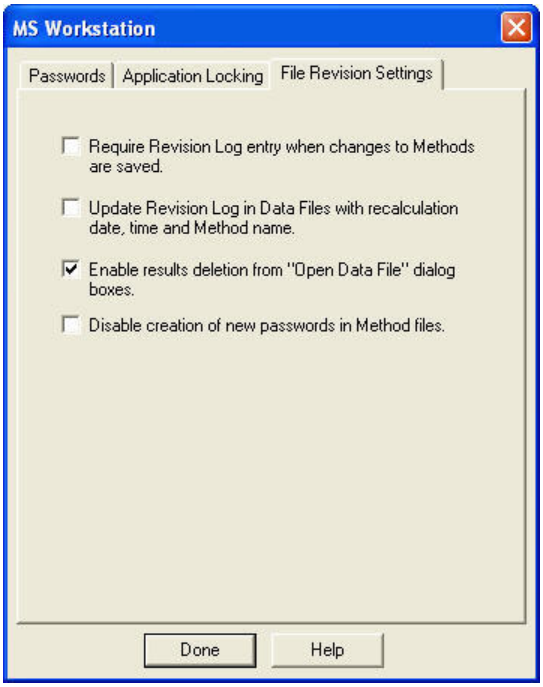
Application Locking



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.
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.


MS Workstation
Passwords
Application Locking
File Revision Settings
Require Revision Log entry when changes to Methods are saved.
Update Revision Log in Data Files with recalculation date, time and Method name.
Enable results deletion from "Open Data File" dialog boxes.
Disable creation of new passwords in Method files.
Done
Help

Configuring HPLC Modules

Overview

The Varian MS Workstation supports the Varian HPLC modules and the HTS PAL autosampler.

Refer to Data Acquisition with LC Control, part number 391473200 and 212-LC Operation Manual, part number, 395410300 for details.

Configuring GC Modules

Overview

Since the 3800 GC communicates with the Workstation over a standard Ethernet connection, your system can easily be expanded from a single 3800 GC/single Workstation configuration, to multiple 3800 GC/Workstations, to a fully networked lab with a virtually unlimited number of GCs and Workstations. The term “Ethernet” refers to the cables and interface cards that connect devices to the network. If you are connecting your 3800 GC to an existing Ethernet network, you need to know which type of cable to use. Refer to the *Communications* section of the 3800 GC Operator’s Manual.

The 3800 GC uses TCP/IP (Transmission Control Protocol / Internet Protocol) to communicate over the Ethernet network. Since the 3800 GC uses TCP/IP to communicate with the Varian MS Workstation, the Workstation PC and the 3800 GC need unique IP addresses—the addresses that are used to identify each networked TCP/IP device.

Refer to Data Acquisition with 3800 GC Control, part number 391473100, for detailed information about how to acquire data, build methods, and operate the 3800 GC with your Varian MS Workstation. This manual also describes the configuration of the 3800 GCs for standard Ethernet communication with System Control.

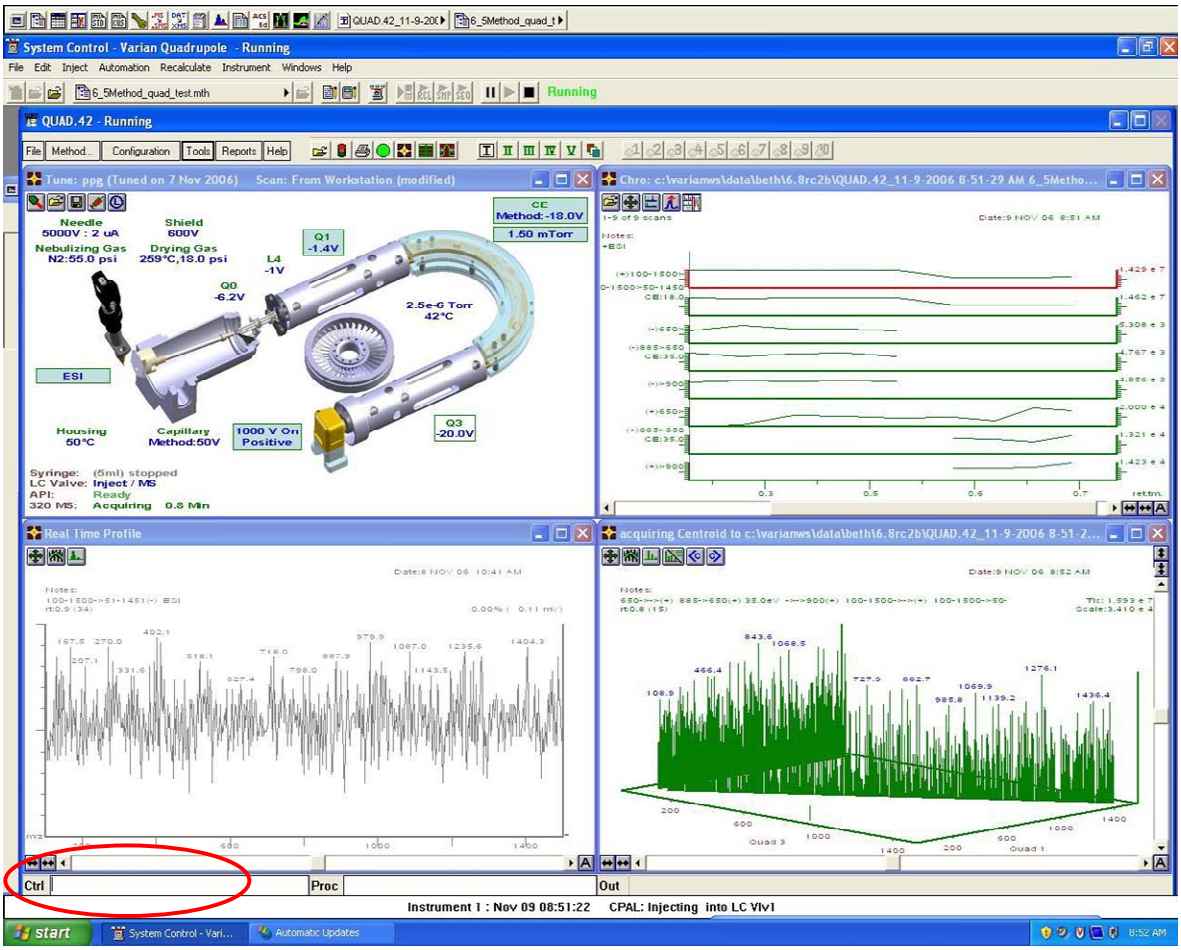
PML

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 single commands that control the instrument or data processing. Users can write procedures to customize data processing.

Simple Commands

Simple commands can be entered in the **CTRL** field.



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, e. g.:
   - 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

However, 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.

   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.
10. 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.

Paw Guide

The Quad MS window is your window to the instrument. It contains several views, each providing a different way to view or interact with the instrument. It also contains a toolbar, and two command lines on which you may type Paw Macros to control the instrument or process data.

Toolbar Buttons

File | Method... | Configuration | Tools | Reports

Help | View File | Collect File | Print | Instrument On / Off

Instrument | Scan Editor | Auto Tune | I II III IV V View setups | Select Views

Views

Prof | Cent | Instrument | Readbacks | Spec

Chro | Map | Library | 123 Σσ List | Pict

Paw Macro Keywords
Diagnostic Paw Macro Keywords
Paw Macro Syntax
System Paw Macros

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.

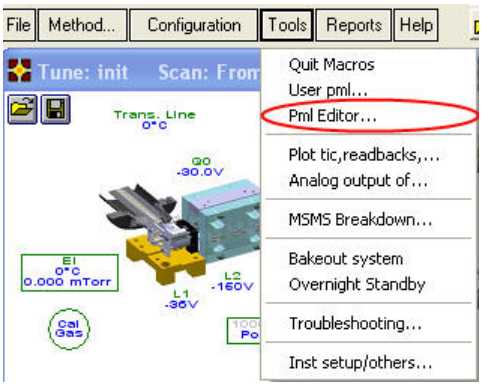
Repeat	**#repeat items below**
Prof	#set to scan of profile data
Fish	#go get one scan of data
Cent	#set to scan of centroid data
Fish	#go get one scan of data
End	

PML Editor

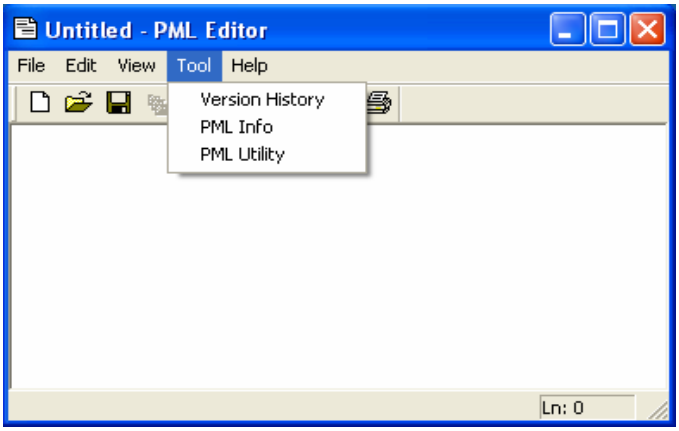
The **PML Editor** is used for creating and editing PMLs for the 300 Series software. The PML Editor allows access to the factory PML files; however, if a factory PML is modified it will then be saved as a user PML in the UserPML directory.

Accessing the PML Editor

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

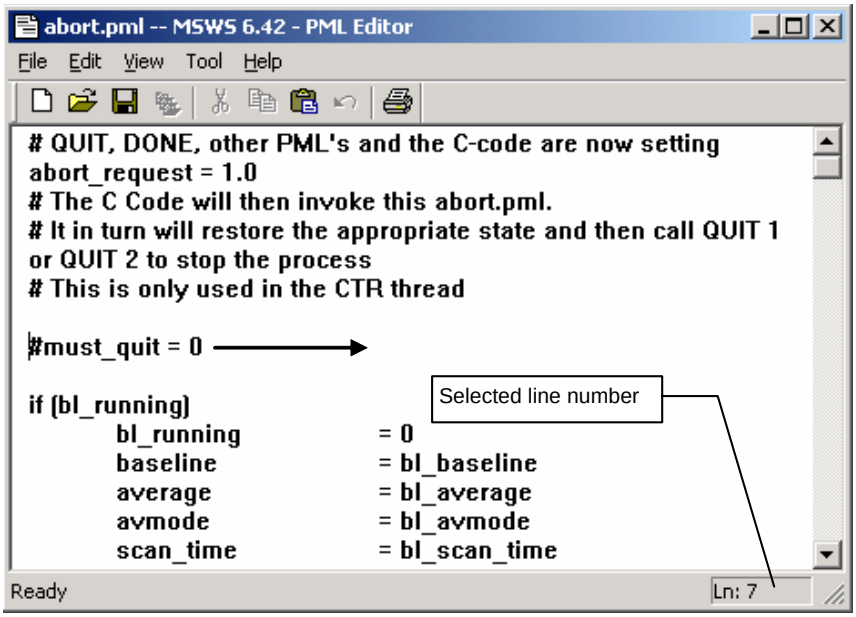


The PML editor window opens.



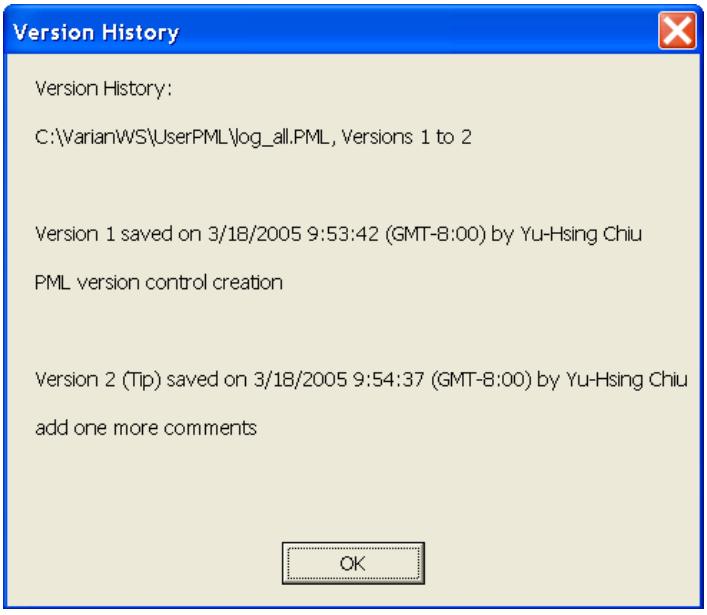
- Varian PMLs are stored in c:\varianws\1200sys\PML directory.
- User created PMLs or modified factory PMLs are stored in c:\varianws\UserPML directory.

- The Status Bar of the PML Editor displays the line number of the current cursor position.

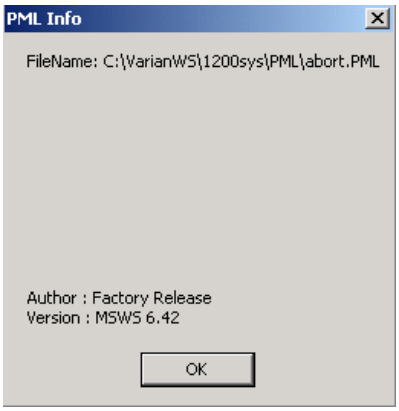


- The Tool menu has three menu items:

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: Use this to 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, user can enter a specified keyword, and find out the PMLs contain this keyword.