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

4000 MS

4000 MS Users Guide
External Ionization

QUALITY SYSTEM
ISO 9001
CERTIFIED

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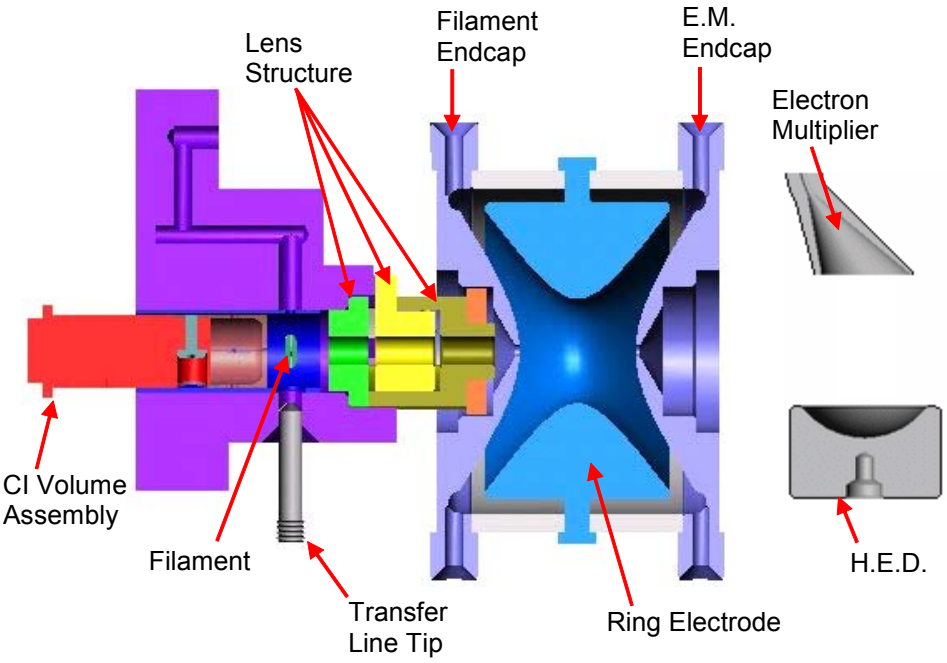
Contents

Overview of External Configuration

The External Configuration is one of the three operational configurations of the 4000 GC/MS system. In this configuration it is possible to collect electron ionization (EI) as well as either negative ion or positive chemical ionization (NCI or PCI) data. In common with the other configurations, it is possible to perform ion preparation techniques including Selected Ion Storage (SIS) and Tandem Mass Spectrometry (AMD, MS/MS, MS^n, and MRM).

The 4000 GC/MS analyzes the gas-phase ions formed from a sample in terms of their mass-to-charge (m/z) ratios and their relative abundances in the resulting spectra. The mass spectrum is a graphical representation of the ion intensities versus the mass-to-charge ratio.

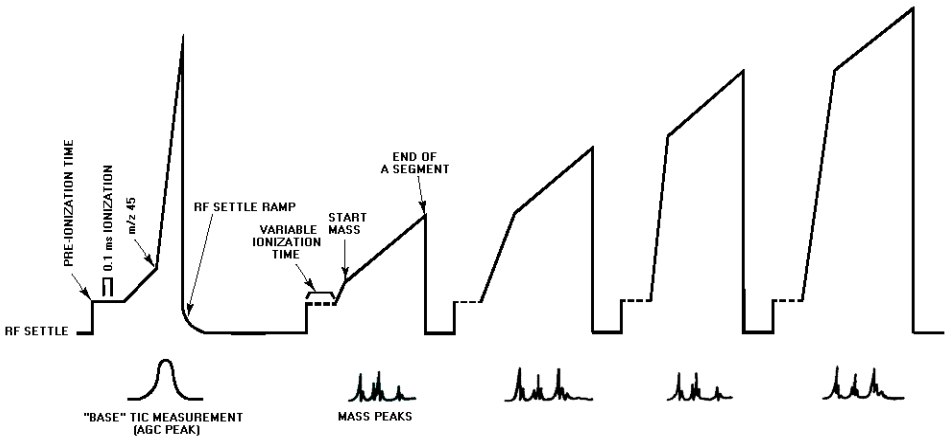
In External Configuration the 4000 GC/MS creates the mass spectrum with an external ion source and an ion-trap analyzer. Ions are formed in the external source and transferred to and stored in the ion trap before mass analysis. The ion trap confines the ions within a single region where they experience time-dependent electromagnetic fields.



Schematic Diagram of the External Ionization Configuration

Scan Functions Control MS Processes

Scan functions are programmable time sequences for applying voltages to the external source, lens, and ion trap electrodes. The scan function controls the variations in time of the RF potential applied to the ring electrode as well as any supplementary waveforms applied to the endcap electrodes. The 4000 MS module has two techniques for generating sample ions in the external source. Consequently, there are two basic scan functions to perform electron ionization and chemical ionization (either positive or negative). These scan functions are discussed in more detail in the 4000 MS Operations Manual.



The AGC Scan Function for Electron-impact Ionization

Steps to Create a Mass Spectrum

Sample analysis may be divided into several steps:

Sample Introduction

Compounds are introduced via a transfer line through a GC column to the external ion source.

Ionization

Analytes eluting from the column are ionized in the mass spectrometer by:

- Striking the molecules with energetic electrons to remove an electron from a molecular orbital, creating a molecular ion. This is called ***electron ionization*** (EI).
- Introducing a reagent gas (usually methane) to the external source and performing EI on that gas to form reagent ions. The reagent ions then undergo ion-molecule reactions with GC analytes to create various ions. This process is called ***positive chemical ionization*** (PCI).

- Introducing a buffer gas (usually methane) to the external source to thermalize electrons from the filament with that gas. These thermal (low-energy) electrons can then attach to GC analytes that have a high electron affinity. This process is called ***negative chemical ionization*** (NCI).

Fragmentation

Depending on the structure of the molecular ion and the excess internal energy remaining after electron impact, there may be further unimolecular decomposition of a portion of the molecular ions to form various fragment ions and neutrals. Unimolecular decomposition is on a picosecond time scale – within the time scale of a few molecular vibrations – so it is effectively occurring at the same time as ionization as far as we could tell with the mass spectrometer.

Ion Transfer and Storage

Both molecular and fragment ions are immediately drawn out of the ion source by a set of tuned lenses of the opposite polarity and directed into the ion trap. They are then stored and stabilized in the ion trap cavity by an RF field applied to the ring electrode of the ion trap. During ionization, the voltage of this RF field is relatively low so that ions of the entire desired mass range are stored. An auxiliary helium gas flow to the ion trap buffers the ion motion and focuses the ions more to the center of the trap. Helium is used as the buffer gas because heavier gases would give poor mass spectral resolution.

Ion Preparation

Once ions are stored in the trap they can be manipulated, if desired. Examples of ion preparation techniques are tandem mass spectrometry (MS/MS) and selected ion storage (SIS). Advantages associated with ion preparation methods are similar to those of other sample preparation methods, e.g., reduction of noise and increased selectivity.

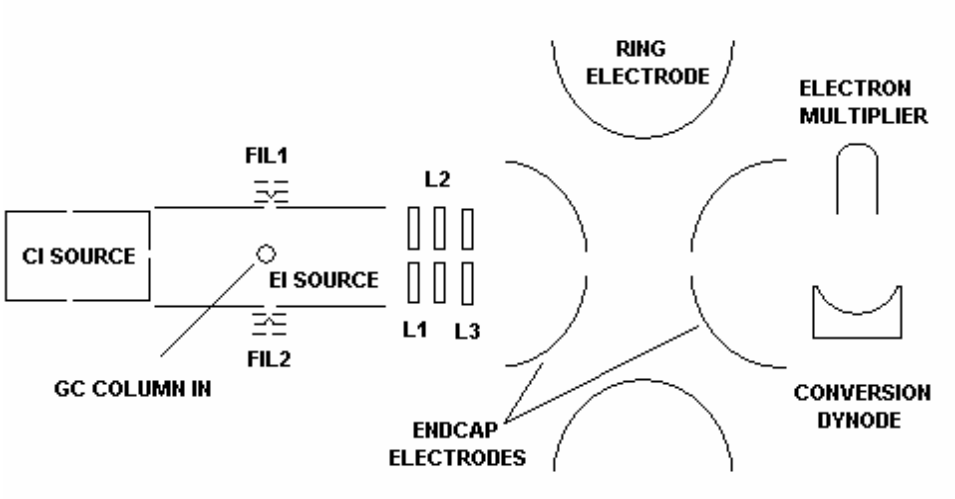
Ion Analysis

The stored ions are analyzed by ramping the RF voltage applied to the ring electrode to a high value, during which time ions from low to high mass are successively destabilized and ejected from the trap. Upon ejection the ions strike a conversion dynode, initiating a signal multiplication process at the electron multiplier. Supplemental dipole and quadrupole voltages applied to the endcap electrodes improve the mass resolution of the process.

Each step of the process to collect mass spectra is discussed in general in this user's guide, and in detail in the 4000 MS Operations Manual.

About External Electron Ionization

The configuration for collection of electron ionization data is shown here. The EI source is open and easily pumped. GC column flow enters the EI source perpendicular to the path of electrons from the filament.



Schematic Diagram for External EI

Forming Ions

Either the Filament 1 or Filament 2 electron lens is gated on during the ionization period to admit 70 eV electrons into the external source. The ionization time is determined by an automatic gain control (AGC) prescan. In EI Auto mode, the objective is to always fill the ion trap with an appropriate target number of ions.

Between ionization times, the filament lens is gated off. This gating process reduces source contamination and the frequency of source cleaning. The high-energy electrons strike a small percentage of analyte molecules A entering the EI source from the GC column, removing an electron from the molecule A and creating energetically excited molecular ions A^{+*}. Some of the excited molecular ions equilibrate through collisions with helium but others undergo unimolecular decomposition to create various fragment ions, f_i^+.

$A + e^- \rightarrow A^{+*} + 2e^-$	Ionization (A^{+*} is excited)
$A^{+*} \xrightarrow{He} A^+$	De-excitation
$A^{+*} \rightarrow f_1^+ + n_1$	Equilibration
$\rightarrow f_2^+ + n_2$	
$\rightarrow f_3^+ + n_3$	Fragmentation

The ion trap has a maximum storage capacity beyond which mass resolution and spectral quality deteriorate. The number of ions created is proportional to the ionization time; more ions are produced the longer the ionization time. Automatic Gain Control (AGC) controls the ionization time to always create an optimum number of ions in the trap.

The AGC scan function consists of a prescan and up to six analytical scan segments. The number of ions detected in the prescan is used to calculate the ionization time for the analytical scan.

Transferring and Trapping Ions

A set of three lenses between the ion source and the ion trap is used to draw analyte ions from the ion source into the ion trap. Like the electron lens, Lens 2 is gated on only during the ionization time. All positive ions being formed in the source are then directed toward the ion trap. All ions with masses above a chosen value set by the RF Storage Level are stored in the ion trap and ions higher than the selected high mass limit are eliminated by waveforms applied to the endcaps.

Ion Preparation Options

The 4000MS can use a combination of waveforms applied to the ion trap electrodes to isolate or remove specific ions once they have been formed and are stored in the trap.

Options like Selected Ion Storage (SIS) and Tandem Mass Spec (MS/MS) can be performed on the ions stored in the ion trap before mass analysis takes place. In MS/MS, a parent ion is isolated and then dissociated by energetic collisions with helium buffer gas to form product ions. In SIS, resonant waveforms are applied to eject unwanted ions within the stored mass range and fill the trap only with ions in the mass range(s) of interest.

The External configuration can have SIS, MS/MS, MS^n, and MRM as ion preparation options. SIS is included with all instruments, while MS/MS, MS^n, and MRM are available with the MS/MS option installed.

Scanning Ions to Collect Mass Spectra

After ionization, trapping, and ion preparation steps, ions are scanned out of the trap to the conversion dynode and electron multiplier. Mass scanning is implemented by increasing the RF voltage on the ring electrode; the mass spectrum is collected in order from low to high mass over the user-designated scan range. In positive mode, ions strike the conversion dynode, held at -10 KV, and then electrons are ejected from the conversion dynode and repelled to the electron multiplier. In negative mode, positive ions are ejected from the conversion dynode, held at +10 KV, and are and repelled toward the electron multiplier. The signal is enhanced by ~10^5 by the electron multiplier and sent through an integrator to collect an intensity for each m/z. MS data are stored as sets of ion-intensity pairs for each m/z over the acquired mass range. A complete mass spectrum is stored for each analytical scan. There are actually two types of mass scanning in external EI. First there is a prescan to count the number of ions formed in a short fixed ion time. After a calculation based on the prescan ion count, ions are formed for the ionization time recommended by the AGC prescan algorithm and the analytical scan is carried out. The analytical scan can be broken up into up to six segments and the relative ion times for the segments can be adjusted to meet tuning requirements for methods such as those of the US EPA for the compounds DFTPP and BFB.

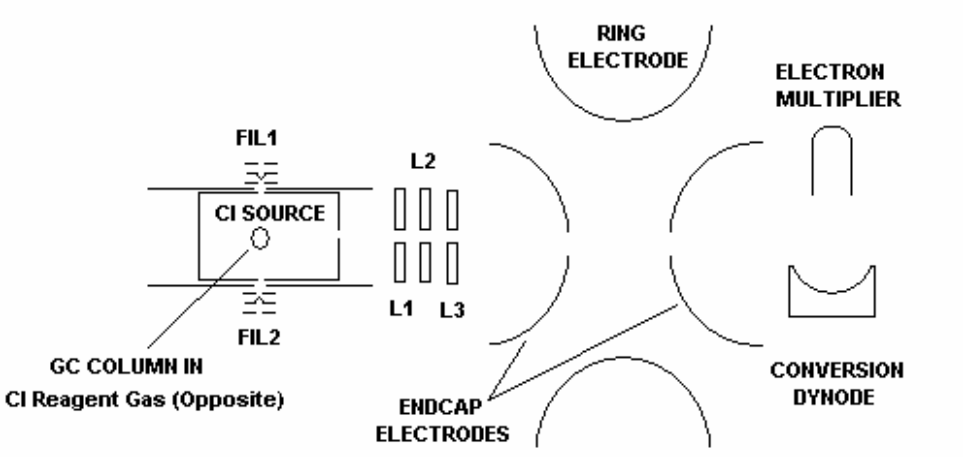
Library Searching

The mass spectra collected from the MS are analyzed through Varian MS Data Review (MSDR). The identity of most compounds is determined by the comparison of the collected spectrum with a reference library. The mass and intensity listing is compared to results collected on other instruments. Such listings include the NIST library, the Wiley MS library, and the PMW library. Each

library has a different focus, from pharmaceutical to environmental analysis. Custom libraries can also be generated from results collected on the 4000 MS system.

About External Chemical Ionization

The configuration for collection of chemical ionization data is shown here. The CI source is more enclosed than that for EI so that high-pressure CI reactions can take place rapidly. GC column flow enters the EI source perpendicular to the path of electrons from the filament and CI reagent gas enters opposite the column flow.



Schematic Diagram for External CI

Positive Chemical Ionization in the External Ion Source

CI ionization and reaction processes must occur within a few microseconds in the External CI source before the ions are accelerated through lenses into the ion trap. Methane is the regent of choice to perform chemical ionization in External configuration. Therefore a relatively high pressure (~100 μTorr) of methane is added to the CI source to allow rapid reactions. Methane is first ionized to create CH_4^+ molecular ions and fragment ions CH_3^+. These ions undergo further reactions to form three dominant stable ions: CH_5^+ ions at m/z 17, $C_2H_5^+$ ions at m/z 29, and $C_3H_5^+$ ions at m/z 41. These species are called CI reagent ions. The reagent ions undergo any of several types of CI reactions with analytes coming off the GC column, such as proton transfer and adduct ion formation. Go to the section PCI Reactions in the Ion Source for more details.

NOTE: Reagent ion formation can be a complex process. For example, when methane used as the reagent gas, reagent gas ions are formed as follows:

First, methane is ionized to form two primary ions:

$$CH_4 + e^- \rightarrow (CH_4^{\bullet})^+ + 2e^-$$

$$CH_4 + e^- \rightarrow CH_3^+ + e^- + H^-$$

These primary ions then react very rapidly to form predominantly the secondary ions, CH_5^+ and $C_2H_5^+$:

$$(CH_4^{\bullet})^+ + CH_4 \rightarrow CH_5^+ + CH_3^{\bullet}$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

CI is a softer ionization technique than EI. That is, CI imparts less energy to the sample molecules than does EI. Thus, the ionized sample molecule undergoes less fragmentation, and an ion indicative of the molecular weight is more likely to be observed. In addition to molecular weight confirmation, CI mass spectra often provide other significant structural information that may not be available from EI mass spectra.

NOTE: The electron lens is pulsed positive, or ON, only during the ionization period, which is a small percentage of the analysis time. Since electrons are brought into the ion source only when they are needed for ionization, the source stays clean much longer and requires little maintenance.

Chemical Ionization Reactions in PCI

In the second step, the reagent gas ions react with sample molecules in the external source to form sample ions. There are four principal reactions between reagent gas ions and sample molecules. They are:

(A) Proton transfer: $(RH)^+ + M \rightarrow (MH)^+ + R$

(B) Hydride abstraction: $R^+ + M \rightarrow (M-H)^+ + RH$

(C) Association: $R^+ + M \rightarrow (MR)^+$

(D) Charge transfer: $R^+ + M \rightarrow M^+ + R$

where R^+ is the secondary reagent gas ion and M is the neutral sample molecule.

For methane CI, proton transfer (A) is a major reaction, and association (C) is the next most often observed reaction. In both cases the resulting even-electron ions are often relatively stable, and the observation of strong (M+1) protonated molecule or (M+29) and (M+41) ***adduct ions*** is often observed even if the EI spectrum of the same component shows no molecular ion. Methane is recommended as the most useful PCI reagent gas in the External configuration.

Negative Chemical Ionization in the External Ion Source

Methane serves a different function in negative chemical ionization than it does in PCI. Besides ionizing methane in the source, electrons striking methane transfer much of their energy to the methane molecules and ions during the process. When the methane pressure in the source is high enough so that there are many collisions between methane molecules and electrons, this energy transfer eventually “thermalizes” the electron energy to levels of less than 1 eV. When electron energy is this low, attachment to molecules with high electron affinities is possible.

Transferring and Trapping Ions

Ions are transferred to the ion trap by applying voltages of the opposite polarity to the three lenses between the ion source and the ion trap. Lens voltages are negative for PCI and positive for NCI. The voltages on the lenses are tuned in Auto Tune to optimize focusing the ions toward the ion trap. The Trap DC offset voltage applied to the ion trap creates a potential well to trap all ions above a mass determined by the RF Storage Level. The default RF storage level is 35u, so only ions above this m/z are stored in the ion trap. Therefore the CI reagent ions at m/z 17 and 29 are not stored but that does not present a problem because the CI reactions with GC peaks have already taken place in the external CI source!

Ion Preparation Options

Ion preparation processes are the same after chemical ionization as they are after electron ionization. Selected Ion Storage and MS/MS can be performed on the ions stored in the ion trap before mass analysis takes place. In MS/MS a parent ion is isolated and then dissociated by energetic collisions with helium buffer gas. In SIS, resonant waveforms are applied to eject unwanted ions within the stored mass range.

Scanning Ions to Collect Mass Spectra

The scanning process for chemical ionization is the same as for electron ionization. After ionization, trapping, and ion preparation steps, ions are scanned out to the conversion dynode and electron multiplier. Mass scanning is implemented by increasing the RF voltage on the ring electrode; the mass spectrum is collected in order from low to high mass over the user-designated scan range. In positive modes, electrons are ejected from the conversion dynode held at –10000V and repelled to the electron multiplier. In negative mode, positive ions are ejected from the +10000V dynode and repelled toward the multiplier. The signal is enhanced by ~10^5 by the multiplier and sent through an integrator to collect an intensity for each m/z. MS data are stored as sets of ion-intensity pairs for each m/z over the acquired mass range. A complete mass spectrum is stored for each analytical scan. There are two types of mass scanning in external CI. First there is a prescan to count the number of ions formed in a short fixed ion time. After a calculation based on the prescan ion count, ions are formed for the ionization time recommended by the AGC prescan algorithm and the analytical scan is carried out.

Library Searching

There are no libraries of external PCI or NCI mass spectra included with 4000 MS software; however, user libraries of these spectra may be created. For details on how to create user libraries go to the section in the MS Workstation Manual.

Selectivity Considerations

Type of Sample Matrix

One of the advantages traditionally noted for chemical ionization is selectivity. In PCI, hydrocarbons have poor response in methane CI. It may therefore be much easier to locate target compounds in a hydrocarbon-contaminated sample using methane PCI than using EI. Similarly, negative CI gives a good response only for species with a high electron affinity such as halogenated compounds; chemical background from other types of species will not even show up in the chromatogram.

Because of these selectivity considerations, it is often worth the time during method development to analyze samples using the suite of different ionization and ion preparation options available on your MS system.

Using EI and PCI to Get More Information

For many species, there is so much unimolecular fragmentation of molecular ions that there is little or no intensity in the mass spectrum to identify the molecular mass. An examination of the NIST Mass Spectral Library confirms this statement. When one is attempting to identify unknown species it is often useful to acquire data in PCI as well as EI to get the needed molecular weight information.

Conversion from Internal to External Configuration

Converting the 4000 MS from internal to external configuration involves changing both the ion source and the column position. The Internal ion source assembly is removed from the trap assembly and replaced with the External source assembly.

The transfer line orientation is changed from the rear position to the front position, and the position switch changed to External. The polymeric internal transfer line tip is replaced with the external metallic tip and the column is cut to about 1 mm past the end of the tip.

For details on how to add/remove the source assemblies, adjust the transfer line, and column installation, go to the 4000 GC/MS Hardware Operation Manual.

Checklist

- Remove the analyzer assembly from the MS manifold
- Change the ion source to external
- Move the heat shield to the forward position
- Remove the filament adaptor and connect the flex cable

- Change the transfer line to the front position
- Change from the polymeric internal transfer line tip to the external metallic tip
- Cut the column 1 mm past the end of the transfer line
- Change the transfer line switch to External
- Replace the analyzer in the MS manifold

Conversion from Hybrid to External Configuration

Changing from Hybrid configuration to External configuration does not require changing the ion source assembly. However, the transfer line needs to be changed from front to rear position, and the transfer line tip must be changed to the External type.

Checklist

- Change the transfer line orientation from front to rear position.
- Take off the Hybrid source plug.
- Replace the internal transfer line tip with the external tip.
- Cut the column 1 mm past the transfer line tip.
- Flip the transfer line switch to the External position

Effects of Hardware Configuration Changes

When the configuration is changed, for example from External to Internal configuration, several things will happen when System Control is restarted. When the 4000 MS module connects, it compares the current configuration stored in the current Module Attributes with the configuration reported by the hardware. If these do not match, the Module Attributes are updated (preset) to the appropriate configuration. A similar process occurs for the default method (Default.mth).

As such, after making the hardware configuration change, any method newly built will have the appropriate instrument configuration by default.

NOTE: The presetting of Module Attributes requires that the user run all Auto Tune routines, as the prior Auto Tune results will be invalid.

Starting the Instrument

Initial Pump-down

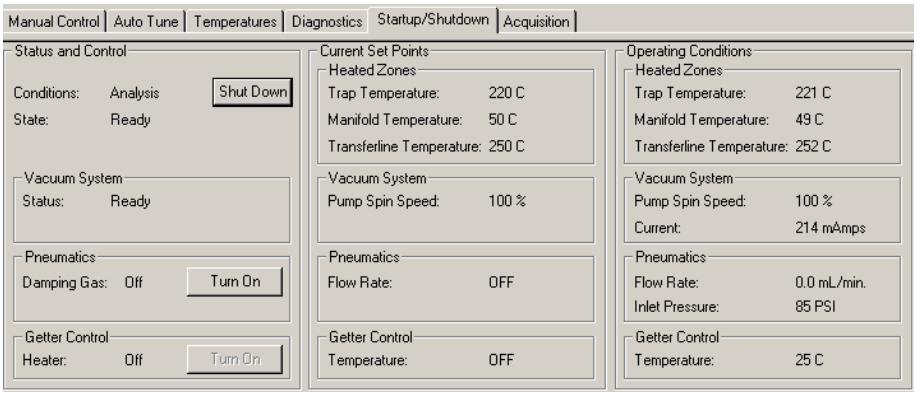
Things to check:

- Vacuum connections
- Make sure the transfer line is in
- Make sure the vent valve is closed fully clockwise
- Make sure the column is not broken

Turn on the power at the main power switch; the roughing pump should stop gurgling after about 10 to 20 seconds. If the pump continues to gurgle, then

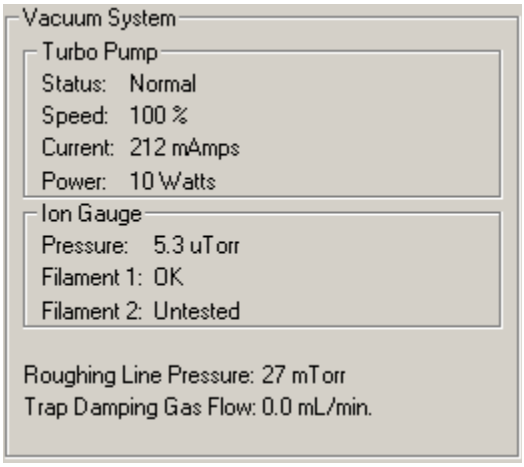
1. Check that the analyzer assembly is seated on the manifold properly (there should be no gaps)
2. Check that the transfer line is in, and
3. Make sure the vent valve is sealed.

Start System Control; it will open to the Startup/Shutdown page.



Startup/Shutdown Page

Check the Vacuum Status



Vacuum System Field

The vacuum readings tell a lot about the state of the MS after pump down (and during operation). Typical operating ranges for the 4000 MS in internal mode are:

Speed	100%
Current	200 – 300 mAmps
Power	9 – 13 Watts
Ion Gauge Pressure	< 20 μTorr
Roughing Line	< 50 mTorr

If the Pump Spin Speed does not steadily increase, there may be a leak in the system. Large leaks will be indicated by a turbo speed less than 100%. Small leaks will show up by an increase in the pump current once at 100% or in the ion gauge pressure (See Diagnostics section). Small leaks are diagnosed by changes in the ion gauge reading and can be pinpointed using the leak check section in the method EService.mth. For more detail on troubleshooting leaks, go to the Troubleshooting section in the 4000 GC/MS Hardware Operation Manual.

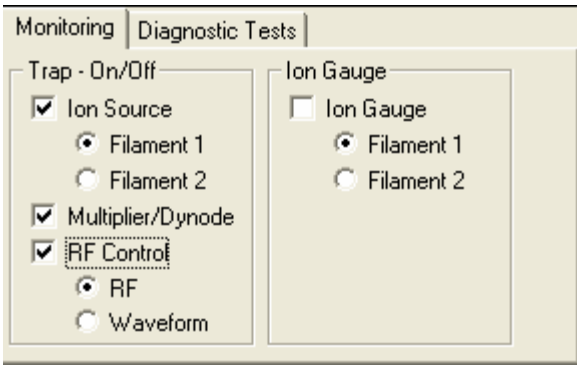
Start Auxiliary Gas Flow

When the turbomolecular pump speed reaches 100%, turn on the Damping Gas and Getter Heater using the buttons in the lower left of the Startup/Shutdown dialog. Once the flow has started you can check the rate in the Operating Conditions field on the right side of the dialog. The buffer flow is necessary to maintain mass spectral resolution; He flow also improves the trapping of ions entering the trap from the external source. Although trapping efficiency and therefore instrument sensitivity dependence on He flow rate is compound-dependent, a good choice of flow rate is 3-4 mL/min.

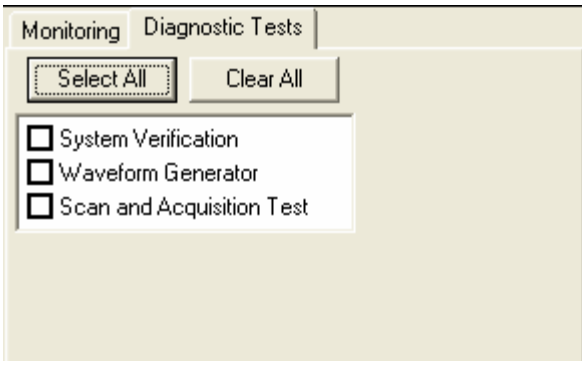
NOTE: He buffer gas flow rate is set in the Module Attributes tab dialog in Manual Control.

Diagnostic Tests

The Diagnostics tab is used to either monitor the current state of the instrument or to perform hardware checks on the 4000 MS. The diagnostics can monitor the vacuum system, the EM, the waveform system, temperatures, and the ion source.



Diagnostic Monitoring Options



Diagnostic Tests

For more details on the diagnostic tests, go to the *Diagnostics Mode* section in the 4000 GC/MS Software Operation Manual.

Set System Temperatures

Analysis Temperatures

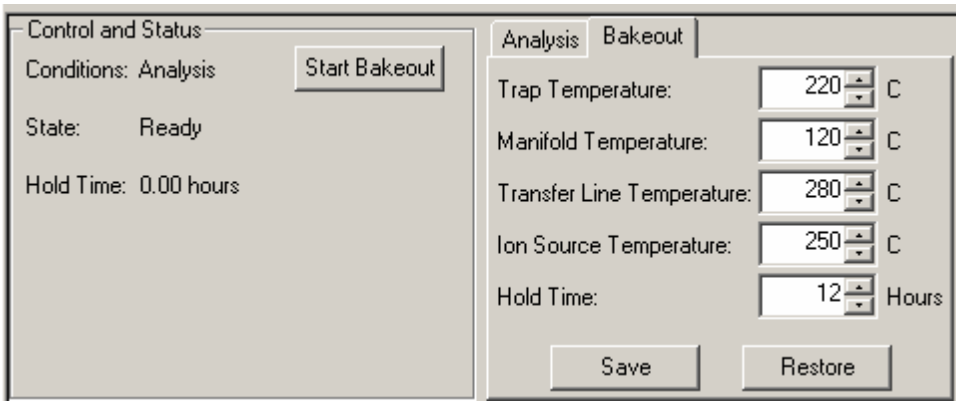
Whereas ion trap temperature is an important variable for analyses performed in Internal or Hybrid Configurations, the default analysis temperature of 100 °C should be acceptable for all External Configuration analyses. However, the external source temperature may need to be raised above the default 170 °C to avoid condensation of heavy semivolatile species. The symptom to look for with higher molecular weight species is peak tailing that cannot be reduced by raising transfer line, injector, and maximum column temperature. For example, in semivolatile environmental analyses, the temperature of the ion source should be set to about 250 °C so that the heavy PAHs (benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) do not tail.

Changing the source temperature takes only a few minutes and may affect lens tuning and mass calibration. We recommend that you perform Mass Calibration and Trap Frequency Calibration shortly after the desired source temperature is reached and then again several hours later or at the start of the next day.

The transfer line temperature should be set so that there is no cold spot between the GC column oven and the MS. A transfer line temperature 20 °C below the maximum column temperature of the active method should be adequate.

The default manifold temperature, typically 50 °C, is used to reduce any effects room temperature variation may have on the system.

System Bakeout



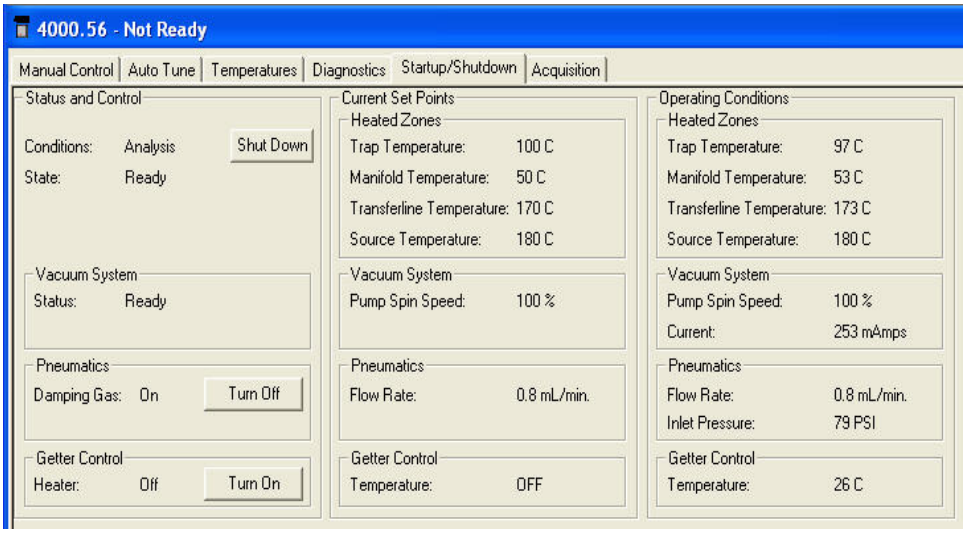
Bakeout Settings in the Temperatures Dialog

To remove water that has adsorbed on the manifold while the 4000 MS has been vented, the system should be baked out. Bakeout is done from the Temperatures dialog in System Control. Bakeout can also be used to remove chemical background from the MS after running heavy matrix samples such as environmental or biological extracts.

Typical bake-out settings are shown in the figure above. When the **Start Bakeout** button is clicked, the temperatures are raised to those set in the Bakeout tab dialog. The Hold Time in the Control and Status field is then decremented until bakeout is complete. System temperatures are then returned to those set in the Analysis tab dialog.

NOTE: The transfer line temperature should not exceed the maximum isothermal temperature of the column.

Startup and Shutdown

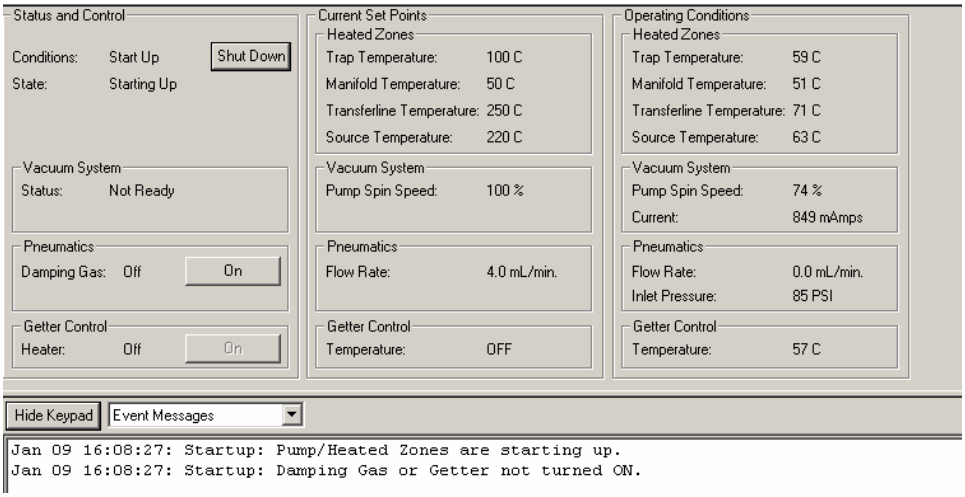


External Startup/Shutdown Tab

The Startup/Shutdown tab is used to start up or shut down the system in a safe and orderly fashion.

Starting the System

When the system is first turned on, System Control only operates in Startup/Shutdown mode.

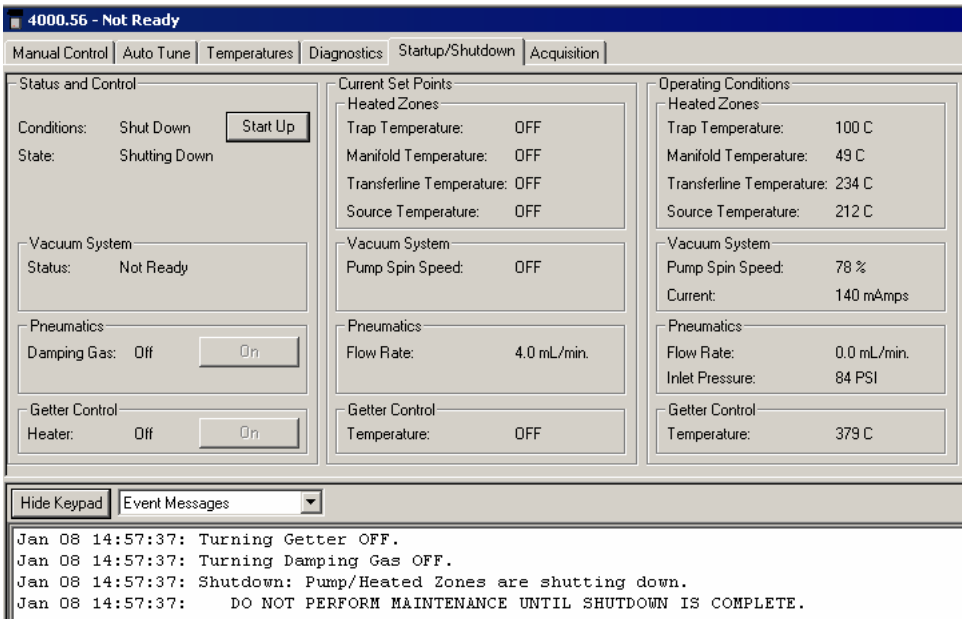


During system startup you will be able to observe the increase in Pump Spin Speed in the Operating Conditions field. The software will be locked in the Startup/Shutdown mode until the speed reaches 100%. You will also see the temperature readings for heated zones begin to increase in the Operating Conditions field. If the system is in External configuration, turn on the Damping Gas flow and the Getter Heater in the Status Control field after the Pump Spin Speed reaches 100%.

NOTE: Failure to reach 100% pump speed in a reasonable time indicates a vacuum leak and corrective action should be taken. Go to the appropriate *Troubleshooting* section in the 4000 GC/MS Hardware Operation Manual.

Shutting Down the System

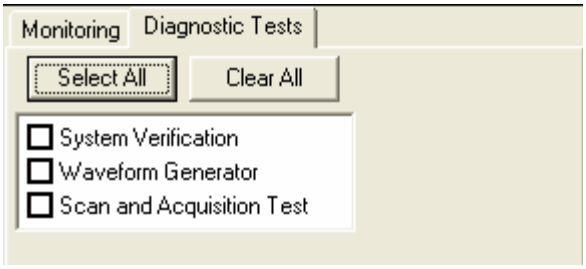
To shut down the 4000 MS, click the **Shut Down** button in the upper left corner of the screen. The heaters will be turned off and the speed of the turbo pump will be gradually reduced to 35% of full speed. In the screen shown below, the shutdown command has been activated. Notice that the turbo pump speed is being reduced as the temperature is decreasing.



After all temperature zones are below 80 °C, turn OFF the main power switch at the rear of the system. Manually vent the system for at least 5 minutes using the lever on the front panel.

To restart the system after you have activated the shutdown, click the **Start Up** button on the left side of the screen above. This will restart the pumps and turn on the heaters.

Diagnostic Checks



After the turbomolecular pump reaches 100% speed, you can begin normal operations with the MS. It is a good idea to check for instrument problems by running all of the routines in the Diagnostic Tests tab dialog of the Diagnostics mode. Click the **Select All** button and then click the **Start Diagnostic** button in the Control and Status field to the left. If a test fails, go to the relevant *Troubleshooting* section in the 4000 GC/MS Hardware Operation Manual.

Adjusting and Tuning the 4000 MS

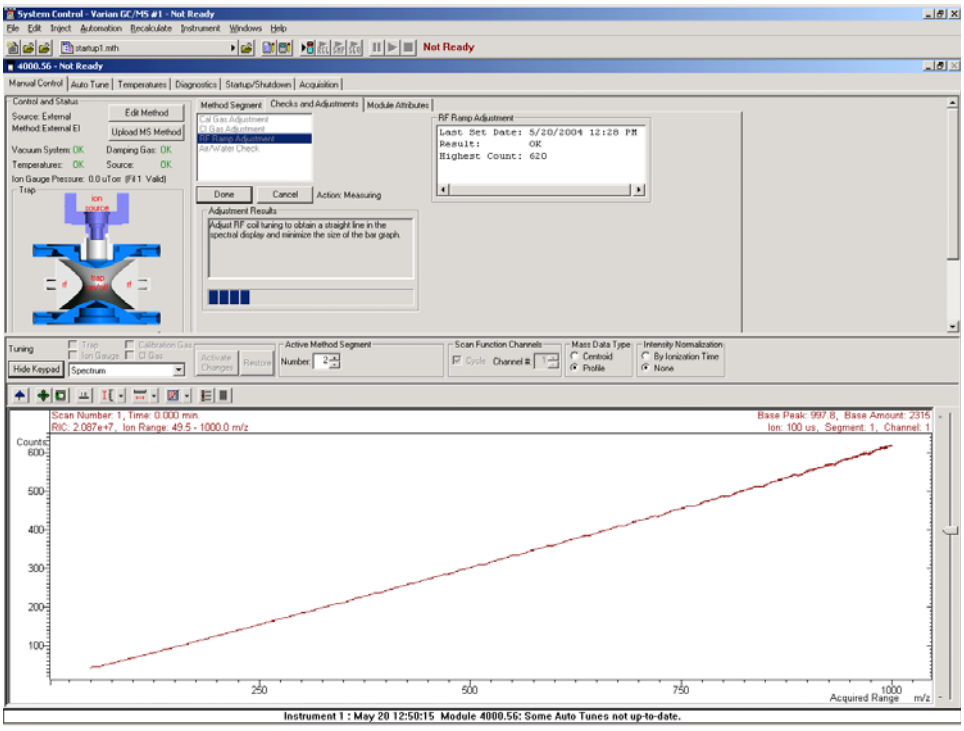
Checks and Adjustments

RF Tune

Whenever MS maintenance has been performed, the analyzer assembly has been changed, or the MS configuration is changed, RF tuning should be adjusted in the Checks and Adjustments tab dialog of Manual Control.

RF Ramp Adjustment

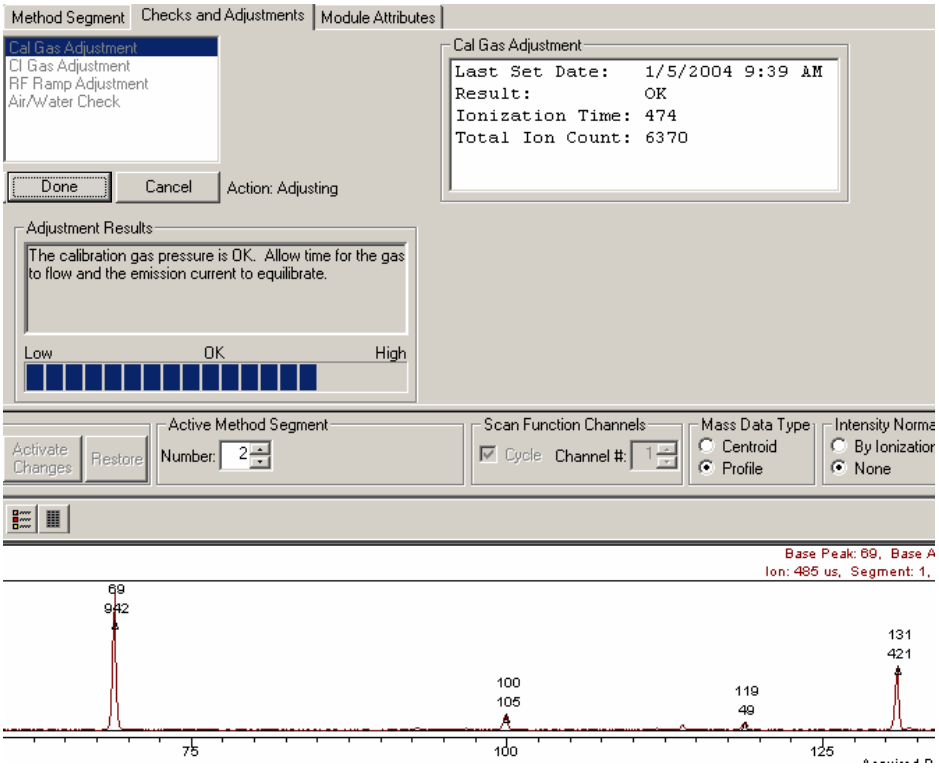
Highlight RF Ramp Adjustment in this dialog and click **Start**. Use a flathead screwdriver to turn the RF Adjustment screw inside the front door of the 4000 MS either clockwise or counterclockwise until the tuning display shows a straight line and the intensity is at a minimum. The Status Bar in the Adjustment Results field should read OK.



RF Tune Display

Calibration Gas Adjustment

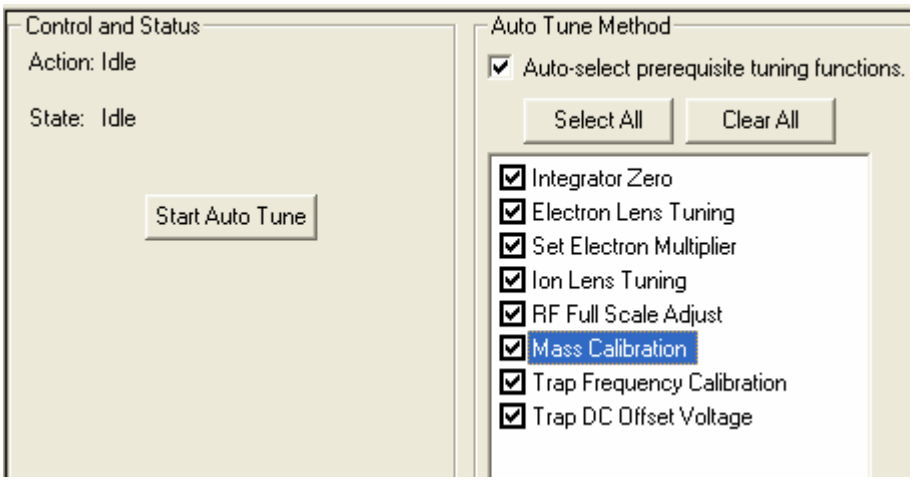
The flow of perfluorotributylamine (PFTBA or FC-43) calibration gas should be checked before Auto Tune procedures are performed. Highlight **Cal Gas Adjustment** in the Checks and Adjustments tab dialog in Manual Control. Turn the Cal Gas valve inside the front door of the 4000 MS either clockwise to decrease or counterclockwise to increase the flow. Adjust the flow so that the status bar in the Adjustment Results field reads OK.



Calibration Gas Adjust Display

CI Gas Adjustment

Before acquiring data in chemical ionization (CI) mode, adjust the CI reagent gas pressure. Details on how to set up methane CI gas are found in “Setting Up CI Reagents” on page 27.



Auto Tune Options

Depending on your configuration and settings, you may or may not see all the available Auto Tune routings. Perform auto tune when the instrument is first set up and whenever significant maintenance operations are performed. Also, perform Mass Calibration and Trap Frequency Calibration whenever the temperature or RF adjustment is changed.

Auto Tune works the same way in either EI or Hybrid CI modes; you do not need to run a different automatic setup, tuning, and calibration program for Hybrid CI.

Integrator Zero

The **Integrator Zero** function obtains the average value of the signal level coming from the integrator circuitry when the filament is off. When the filament is off, the major source of signal coming from this circuitry is electronic noise. The integrator zero is adjusted so that electronic noise does not create an artificial ion but that ions from the trap striking the multiplier create a measurable signal.

Set Electron Multiplier

The **Set Electron Multiplier** routine determines two proper settings: the multiplier voltage needed to achieve a multiplier gain of approximately 10^5 and the Electron Multiplier voltage boost for optimum peak intensity and resolution.

Electron Lens Tuning

Electron lens tuning involves measuring the transient behavior of the emission current immediately after the lenses have been switched on or off. If the lenses are unbalanced, the emission current will change in time and be proportional to the imbalance. If the balance is outside the range of 200 to 300 µA, the algorithm will search the optimal values by changing values of four variables one at a time. If it fails to find the best voltage setting for lens tuning, auto tune will generate an error message, and restore the last values in the instrument.

When the Electron Lens Tuning Box is clicked, an additional “Turn on CI gas flow during tune” option appears. For CI methods in Hybrid mode, the electron /repeller lens must be tuned with the CI plunger (CI volume) in place and the CI gas turned on. The user should adjust the CI gas flow in Manual Control before this tune function is done.

Ion Lens Tuning

The Ion Lens system consists of three lenses (Lens 1, 2 & 3). These lenses are tuned using Cal Gas ions at m/z 131 and 414. Optimum voltages are determined based on weighted intensities of the two ions. This is an iterative process in which transmission of both low and high mass ions is monitored as a function of lens voltages.

RF Full Scale Adjust

The **RF Full Scale Adjust** routine sets the full scale adjust potentiometer to give the correct mass assignment for high mass ions in the calibration gas spectrum. This routine should always be run before Mass Calibration and Trap Frequency Calibration.

Mass Calibration

The **Mass Calibration** function locates and correctly assigns the masses of the PFTBA calibration gas ions at m/z 69, 131, 264, 414, 464, and 614.

Ion trap temperature changes can shift the mass calibration axis, **so this procedure should not be run until the ion trap temperature has stabilized for at least two hours**. There could also be subtle effects on mass assignments after ion source temperature changes. Mass calibration does not have to be performed again after auxiliary He buffer gas flow rate is changed.

Trap Frequency Calibration

After the mass calibration has been completed, the **Trap Frequency Calibration** must be performed. This calibration determines parameters that are required for the operation of ion preparation methods such as MS/MS and SIS. These parameters also help to isolate the range of ions to be acquired in full scan acquisitions. The routine takes several minutes.

NOTE: Trap Frequency Calibration should always be run ***after*** Mass Calibration is done.

Trap DC Offset Voltage

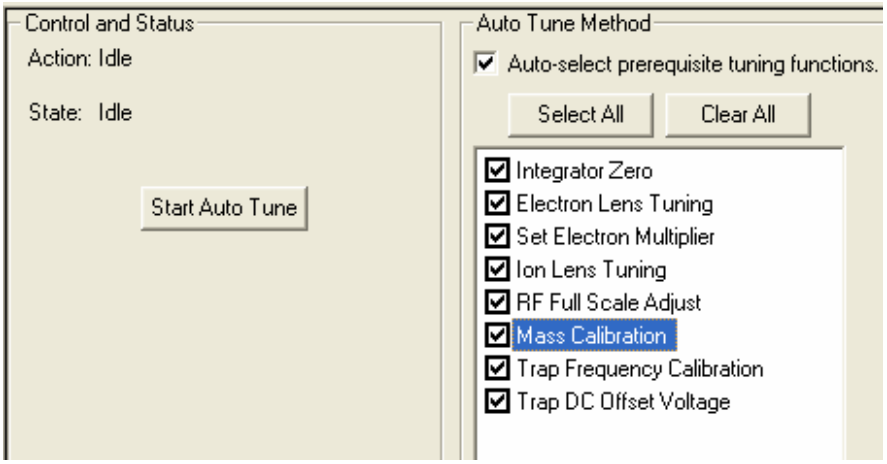
The trap DC offset is adjusted by this routine to optimize the ion signal for m/z 414 in the calibration gas. An optimal value for this parameter assures good high mass sensitivity for External configuration.

Air/Water Check

Too high a pressure of air or water in the system because of an air leak or a need to bake out the system will result in poor performance. This checking routine gives advice on the levels of air and water.

NOTE: The Air/Water Check uses the electron multiplier voltage giving a 10^5 gain and not the manual setting. If the electron multiplier is replaced, auto tune of the Electron Multiplier must be done *before* the Air/Water Check.

Auto Tune



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Trap DC Offset Voltage

The trap DC offset is adjusted by this routine to optimize the ion signal for m/z 414 in the calibration gas. An optimal value for this parameter assures good high mass sensitivity for External configuration.

Setting Up CI Reagents

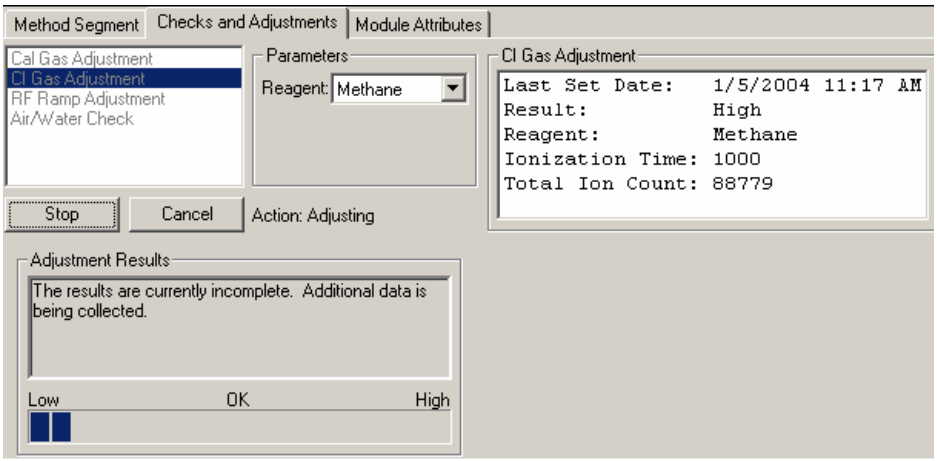
Although several liquid and gaseous reagents are useful in Internal and Hybrid configurations, methane appears to be the reagent of choice in External configuration. Liquid reagents like methanol and acetonitrile give weak responses for most analytes in External Positive Chemical Ionization, PCI.

Installing Methane CI

For full details on installing a CI gas, go to the *Installing a CI Reagent Gas* section in the 4000 GC/MS Hardware Operation Manual. Connect the two-stage regulator of the methane gas cylinder to the back of the instrument through a 50 mL/min restrictor connected to the CI inlet on the rear of the 4000 MS. Open the methane tank and set the second stage of the regulator to 20 psi.

Adjusting CI Gas Flow

Go the Checks and Adjustments tab dialog in Manual Control.



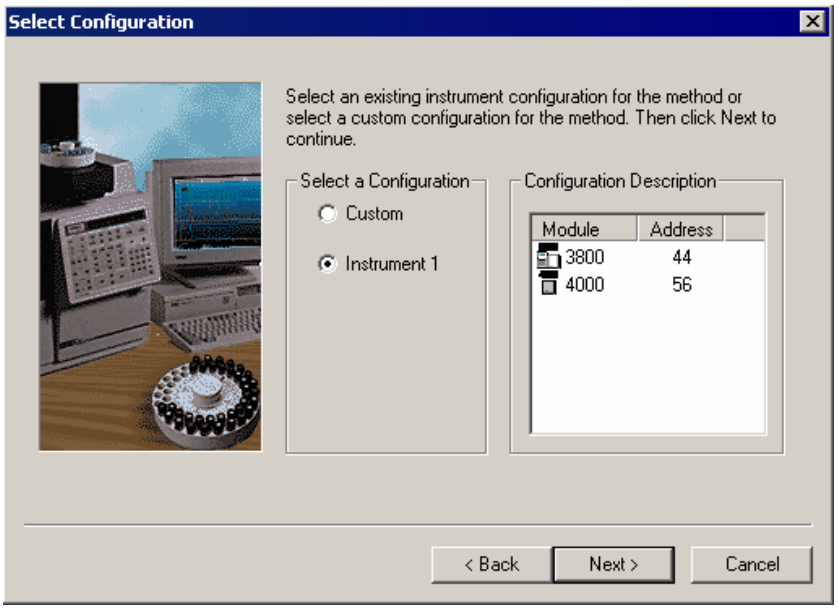
Highlight **CI Gas Adjustment** and click the **Start** button. Use the CI Gas Adjust Valve (#3) inside the front door of the 4000 MS. Turn the knob clockwise to increase the flow or counterclockwise to decrease the flow. The objective is to set the ion gauge pressure within the range of 70 – 100 μTorr.

Preparing a GC/MS Method for Data Acquisition

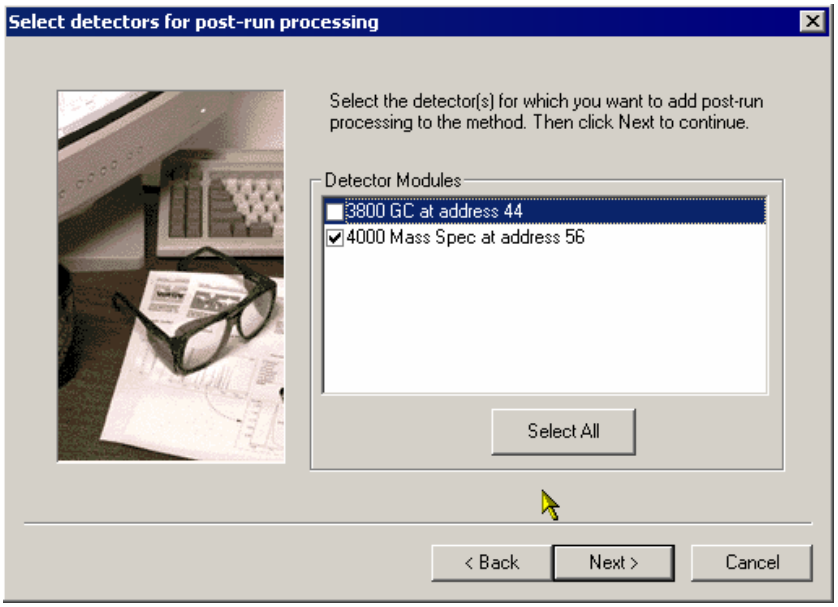
Building the MS Method

From the Star Toolbar, open the Method Builder. 

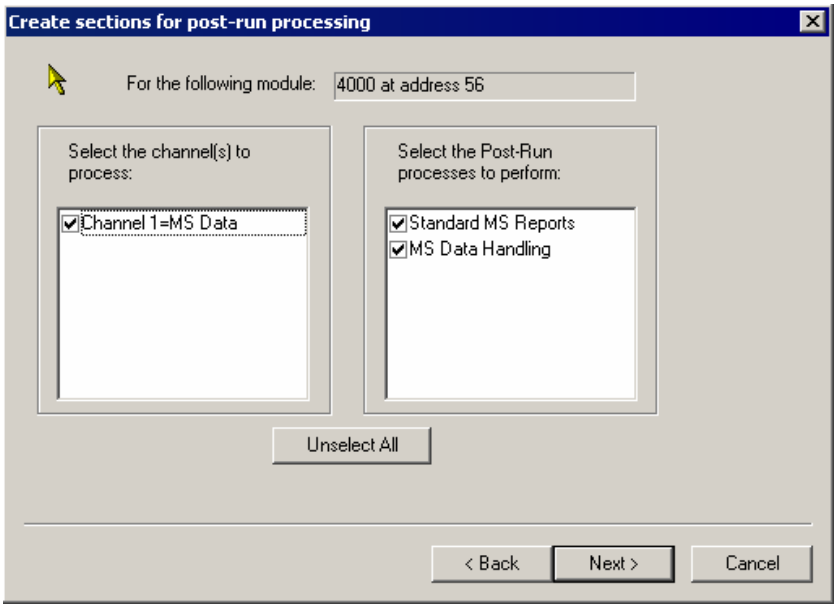
Select the instrument. You can build an offline method by selecting the Custom configuration and selecting the desired modules.



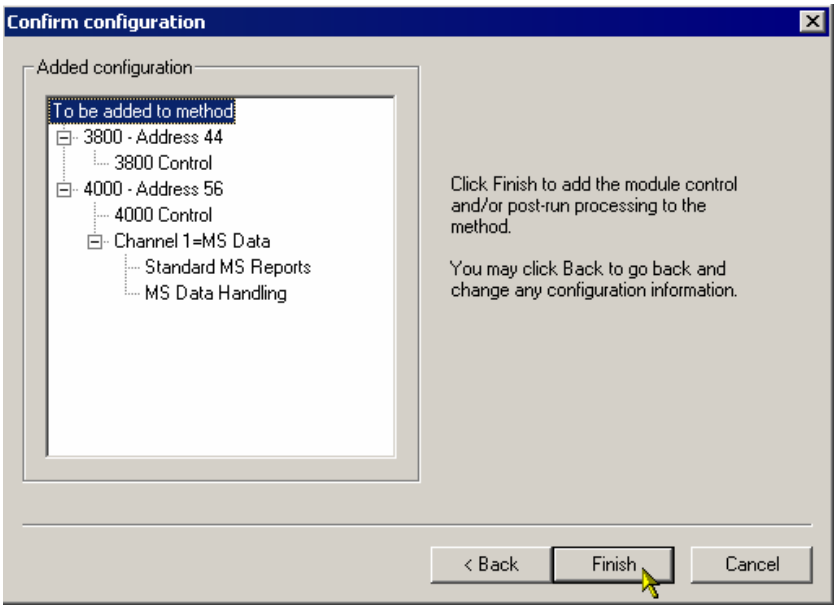
Select which detectors (MS and/or GC) will have data handling.



Select what data analysis sections are required; Standard MS Reports and/or MS Data Handling.

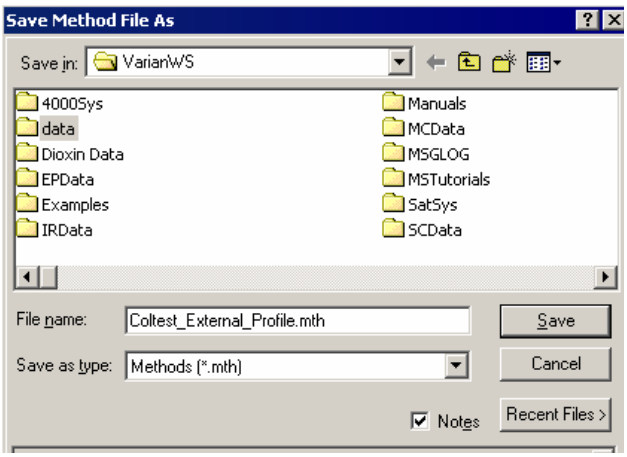


The method will contain the following sections: 3800 GC Control, 4000 MS Control, Standard MS Reports and MS Data Handling.



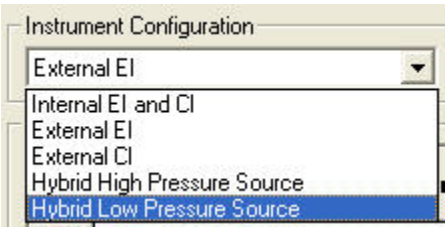
Name the Method

Use the command ***File… Save As***. Enter a name for the method and save it.



Set the 4000 MS Instrument Configuration

The GC and the MS will be set to the overall configuration of the instrument connected to Varian MS Workstation. For the 4000 MS External configuration, one may select either to do External EI or External CI. It is not possible to do both EI and CI segments in a single run, unlike Internal EI and CI methods.



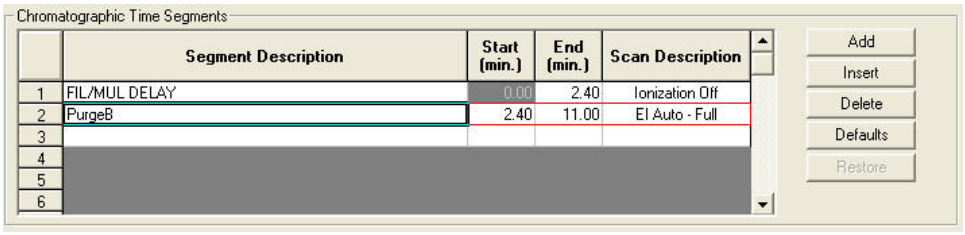
Select the Data Type

Centroid is the default acquisition type, as data handling, library searching, and spectral comparison can only be done from this type of data.

Profile is typically chosen only for diagnostic purposes. Profile data files are also approximately 10 times larger, but can be converted to centroid data after acquisition.

Edit Chromatographic Time Segments

The Chromatographic Time Segments table allows you to time-program analysis conditions to get the best results for each segment in the analysis. Up to 250 time segments can be created for runs up to 650 minutes in length. By default, there is a Filament/Multiplier Delay segment at the start of the run so that the system will not be stressed during the elution of the chromatographic solvent. Following this segment, one could just acquire the mass spectra in full-scan with a single analysis segment. However, one can tailor variables such as acquired mass range, insert MS/MS segments for individual analytes and otherwise set up the instrument to acquire the best data for each analyte.



Chromatographic Time Segments Table

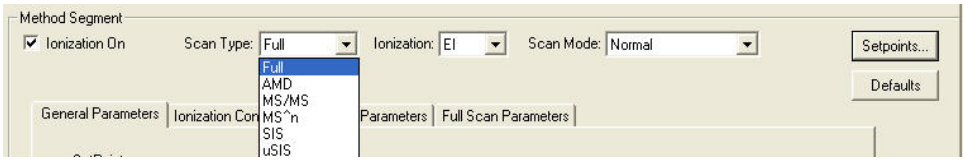
Adding or inserting a segment will copy all of the parameters from the previous segment to the newly created segment. Double-click on the required field to edit the Segment Description, Start Time, or End time of a segment.

Edit the 4000 MS Method Segments

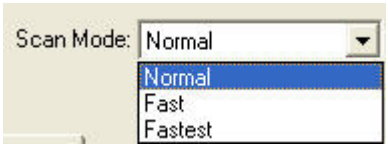
In this section we describe editing of parameters for External EI methods. For advice on performing External CI, go to the section Building GC/MS Methods… External PCI and NCI in the 4000 GC/MS Software Operation Manual.

Scan Function Settings

In Scan Type, one can choose from a variety of types including full scan and different MS/MS methods. In External configuration, it has already been noted that the Instrument Configuration governs whether one can acquire EI or CI data, so there is only one choice in the Ionization menu.



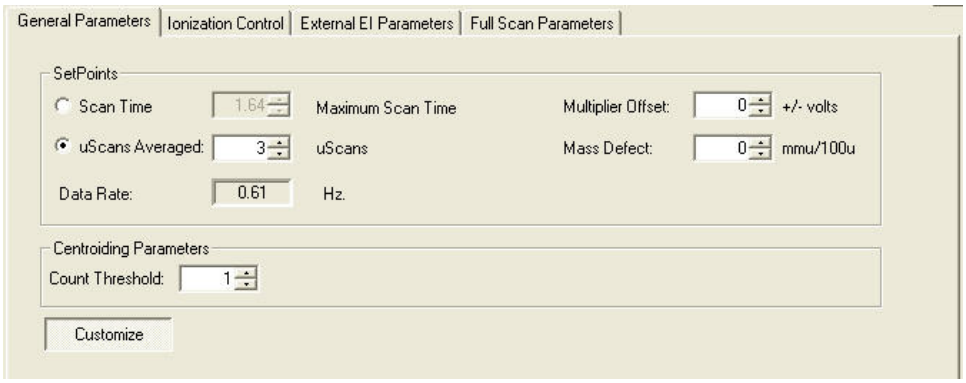
The 4000 MS has three Scan Modes: normal speed (5,000 u/sec), fast (10,000 u/sec), or fastest (10,000 u/sec without a prescan). Fastest only allows acquisition with single-segment mass ranges.



Mass Range	Tune	µScans	Normal	Fast	Fastest
50 – 1000	1 Segment	3	0.76 sec	0.47 sec	0.40 sec
50 – 1000	4 Segment	3	1.14 sec	0.85 sec	N/A
50 – 400	DFTPP	3	0.73 sec	0.61 sec	N/A

Seconds/Scan for Different Scan Rates

General Parameters Tab



General Parameters Tab

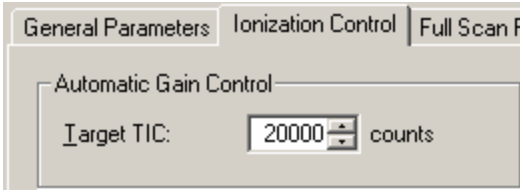
Scan Time, **Scans Averaged**, and **Data Rate** are all linked. The number of scans averaged is updated when the scan time is adjusted and vice versa. The best way to set the scan time is to set the mass range from the Scan Parameters tab and then change the scans averaged to three. Three scans averaged gives the best compromise between a high chromatographic data rate and good spectral averaging.

The **Mass Defect** allows for a systematic correction of the difference between the nominal mass of an atom (or ion) and its exact mass. Its importance arises from the fact that the NIST library reports molecular weights to the nearest integer mass unit only. The Varian MS Workstation software must decide to which mass to assign measured intensity. If the exact mass of an ion happens to fall close to the dividing line between integer masses, the software may make an incorrect mass assignment. This scenario is more likely for molecules with higher molecular weights, since the mass defects for several atoms may add together to produce a sizable mass defect. For example, the exact mass for the lightest isotope form of C_2Br_6 is 497.51002, which could easily be assigned as either 497 or 498.

The **Multiplier Offset** adjusts the EM voltage by as much as ±300V relative to the current multiplier setting in the Module Attributes tab dialog in Manual Control (this is usually the 10^5 gain value from Auto Tune). Sometimes better sensitivity is achieved, particularly in techniques such as MS/MS, when the multiplier voltage is increased. Note that this adjustment can be made on a segment-by-segment basis.

The **Count Threshold** is normally 1; a value of 2-3 counts will reduce the number of low-level ions reported in the mass spectrum. This approach may improve library searches and reduce data file size at the cost of somewhat less detailed information in the mass spectra. The count threshold is shown only if the **Customize** button is active.

Ionization Control

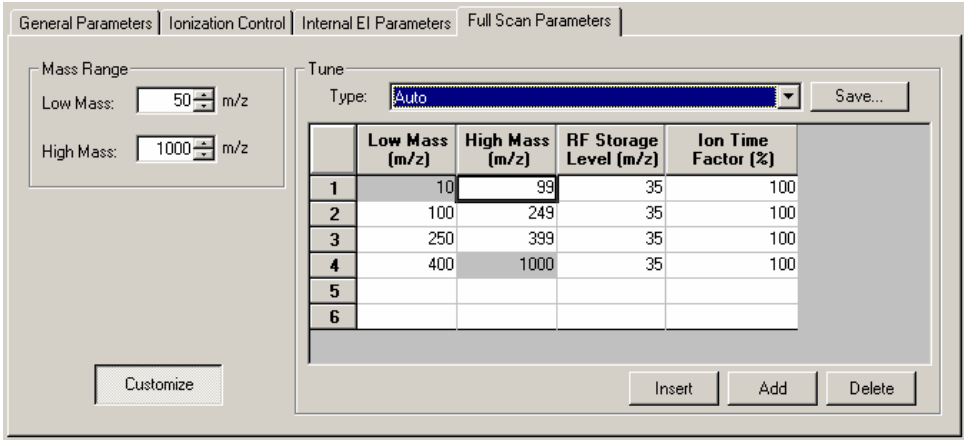


The **Target Total Ion Current**, or TIC is specified here. The Automatic Gain Control (AGC) algorithm uses the ion count from a prescan at fixed ion time, along with this target value, to calculate an ion time necessary to fill the ion trap with the target number of ions during the analytical scan. The objective is to fill the trap with an optimal number of ions during each analytical scan. The Target TIC is usually not set below 10,000 for full scan acquisitions, but it should also not be set too high or spectral distortions due to space charge may result (loss of MS resolution and/or shift in mass assignments for strong chromatographic peaks). Typically, a Target TIC between 20,000 and 40,000 counts gives the best results.

Scan Parameters Tab Dialogs

Each of the MS scan types has different parameters that need to be specified. Below are examples of the two most common scan types used in the Internal configuration, Full Scan and MS/MS. For detailed information on all scan types, go to the Building GC/MS Methods section in the 4000 GC/MS Software Operations Manual.

Setting Parameters for a Full Scan Method Segment



Full Scan data acquisition is used for general-purpose GC/MS analysis. In the Mass Range area (upper left), enter **Low Mass** and **High Mass** values to specify the full scan mass range. The entire accessible mass range of m/z 10 - 1000 is broken up by default into four segments if the Tune Type is Auto: 10 - 99, 100 - 249, 250 - 399 and 400 - 1000. The **RF Storage Level (m/z)** and the section can be adjusted on a mass segment basis.

When DFTPP and BFB tune types are selected, mass segments and ion time factors, which will be good starting points for meeting US EPA semivolatile and volatile tuning requirements, are displayed in the mass segment table.

Each mass segment has its own RF Storage Level. With AGC on, the default storage level is set to 35 m/z, causing all ions above 35 m/z to be stored. This value gives good storage efficiency for ions up to 650 m/z. For masses up to 1000 m/z, a storage level of 45 m/z may be required.

This parameter is particularly important for External configuration, because the kinetic energy of ions entering the trap increases proportionally with the RF Storage Level. Fragile molecular or fragment ions can dissociate by collisions with He buffer gas as they are being trapped and this can affect mass spectral quality. Using lower RF Storage levels of 25-30 for the first two segments of the

mass range can improve spectral quality in such cases. For more detail on this issue, go to the section *External Electron Ionization… Ion Transport Processes* in the 4000 GC/MS Software Operation Manual.

The **Ion Time Factor** is a number that is multiplied by the calculated ionization time (determined by the AGC pre-scan calculation) to give the actual ionization time for each segment of the mass range. The default value is 100%. Adjust this factor to increase or decrease the relative intensity of any segment in the acquisition mass range. For example, adjusting four or five segments appropriately allows the system to pass DFTPP or BFB tune requirements for US EPA environmental methods.

Setting Parameters for an MS/MS Method Segment

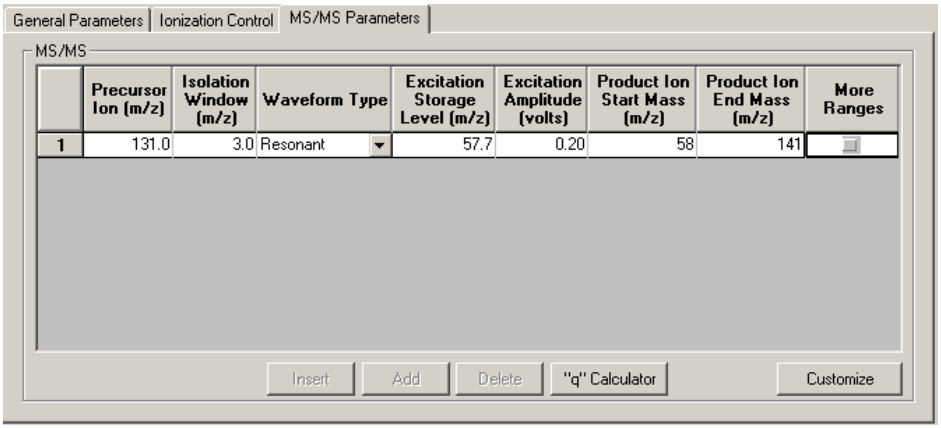
Tandem mass spectrometry, or MS/MS, uses ion preparation steps after the ionization step and before mass analysis. MS/MS may be performed after either electron or chemical ionization. Briefly, all ions are eliminated from the stored mass range except at the m/z of a precursor ion. The precursor ions are then excited by waveforms applied to the ion trap. When enough energy is deposited in this way, collisions of precursor ions with helium buffer gas cause dissociation of the precursor ions to lower mass product ions. The remaining ions are then scanned to collect an MS/MS spectrum.

When designed well, an MS/MS method will:

- Fill the ion trap with only the selected precursor ions, so that trap capacity is used so that in many cases, co-eluting interfering compounds are excluded from the trap.
- Create product ions via a unique dissociation pathway, eliminating chemical noise

NOTE: MS/MS is useful only when the target compounds of an analysis are known. It is not useful for general qualitative analysis except to the degree one is determining a set of isomers of a given class such as PCBs or Dioxins.

The MS/MS Parameters tab dialog is shown below.



The **Precursor Ion** is usually an intense ion in the full scan mass spectrum. Usually, the **Isolation Window** is the parent ion mass ±1.0, (3.0 mass units wide). **Waveform Type** is either Resonant or Non-resonant. The **Excitation Storage Level** is the lowest mass stored during collision-assisted dissociation. A good value can be calculated using the **"q" Calculator** at the bottom of the

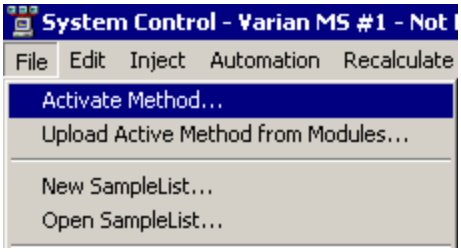
window. The “q” Calculator sets arbitrary limits to the Excitation Storage Level so you should be aware that it may calculate a value of 300 when the Precursor Ion m/z is large. The excitation amplitude needed to dissociate the precursor ion must be determined experimentally; e.g., using several runs with different ranges of excitation amplitudes. Using the AMD (Automated Method Development) mode is the easiest way to determine this voltage. The **Product Ion Mass** range during method development encompasses the range from **Excitation Storage Level** to the **Precursor Ion** mass. For more detail on MS/MS methods, go to the Tandem Mass Spectrometry section in the 4000 GC/MS Software Operation Manual.

Viewing Method parameters in Manual Control

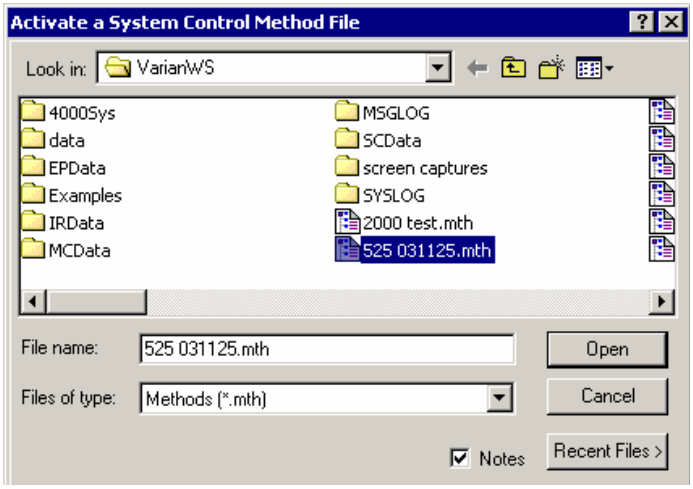
After a method has been created in the Method Builder section of the Varian MS Workstation, it can be previewed in Manual Control. All of the MS parameters can be edited and previewed before a run. However, the number of segments - or the start and end times of existing segments - cannot be changed unless one clicks the **Edit Method** button and goes directly to the Method Builder to make those changes.

Activating a Method

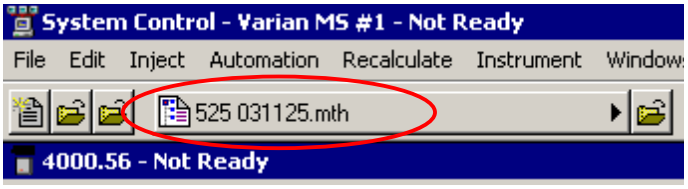
To activate a different method, use the ***File… Activate Method*** command to see a selection menu.



Choose a method and click **Open**. The new GC/MS method will be downloaded to the instrument. The eight most recent methods are available under the **Recent Files** button (see below).



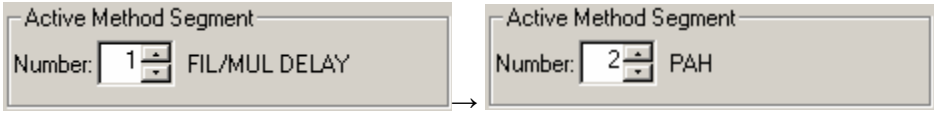
The active method is displayed in the tool bar.



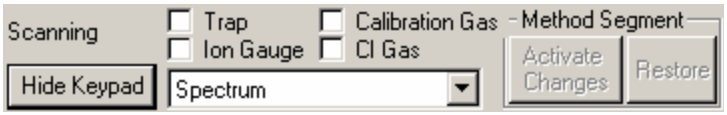
Displaying Ions

To view ions using the method, turn on the ion trap, and select a method segment to view. You may turn on the calibration gas or CI gas by selecting the appropriate box.

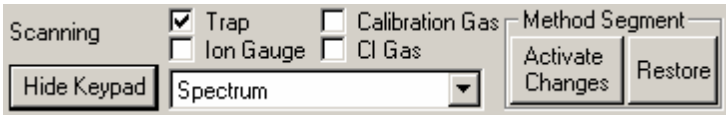
You cannot turn on the ion trap in a segment where ionization is OFF as in the Fil/Mul Delay segment #1. Change to an ionization segment:



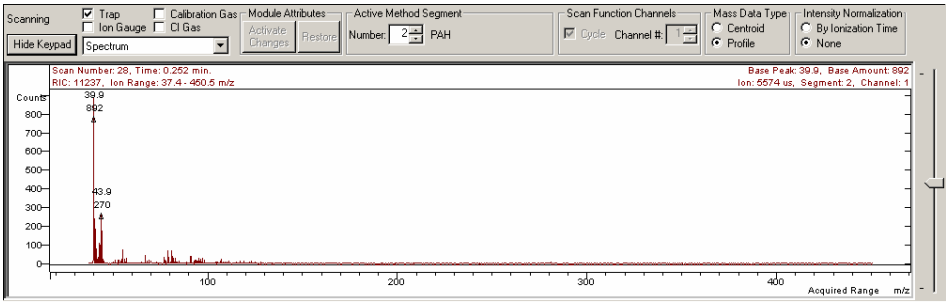
The ion trap controls will now be active:



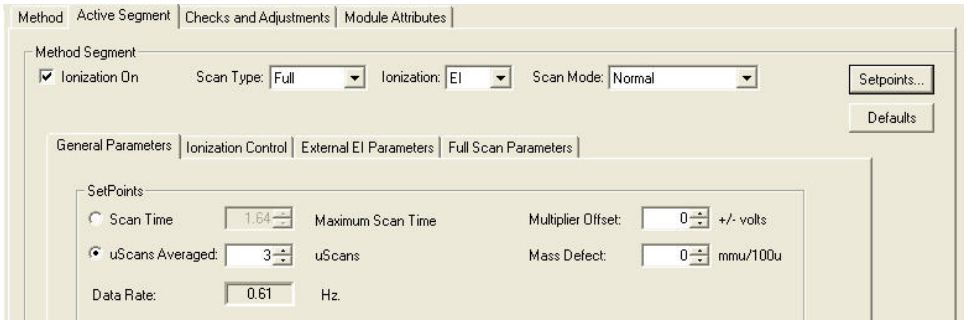
Turn on the Trap:



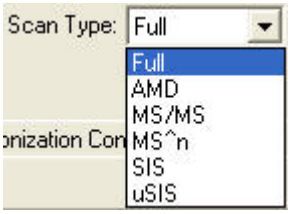
The current mass spectrum will be displayed:



Viewing Method Parameters

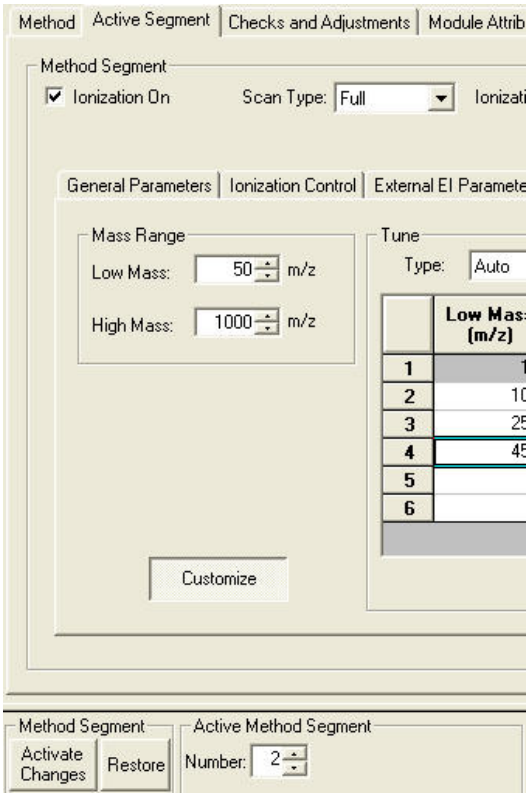


In this image, the Method Segment tab dialog is shown along with some method related controls in the lower pane. Examining the information in the top row of the Method Segment tab, one can identify whether ionization is on, the Scan Type, Ionization mode, and Scan Mode.



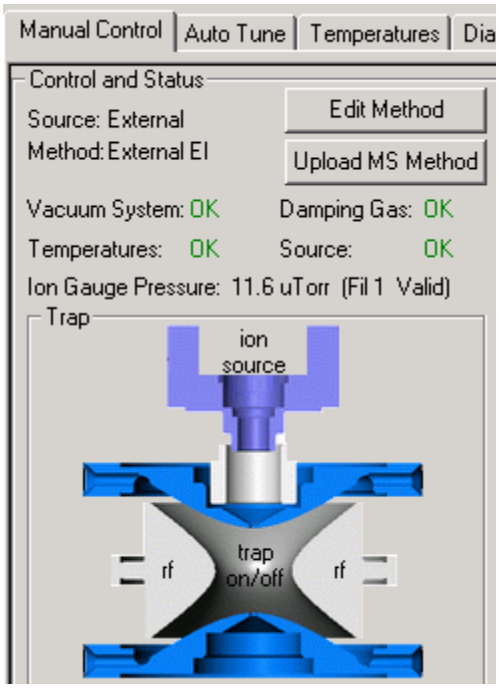
Editing a Method in Manual Control

In a subset of tab dialogs, one can examine and edit all the parameters in the active MS method and observe the effects of these changes on the mass spectra currently being acquired. The exact set of tab dialogs depends on the ionization and ion preparation modes in the current method segment. After editing a parameter, you can implement the change by clicking the **Activate Changes** button as shown in the lower left of the next image.

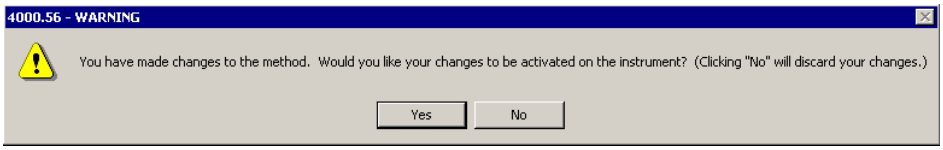


Saving a Method

To save changes to the method, click the **Upload MS Method** button above the Ion Trap icon. Note that you can also go directly to the Method Builder to edit the method by clicking the **Edit Method** button in this area.



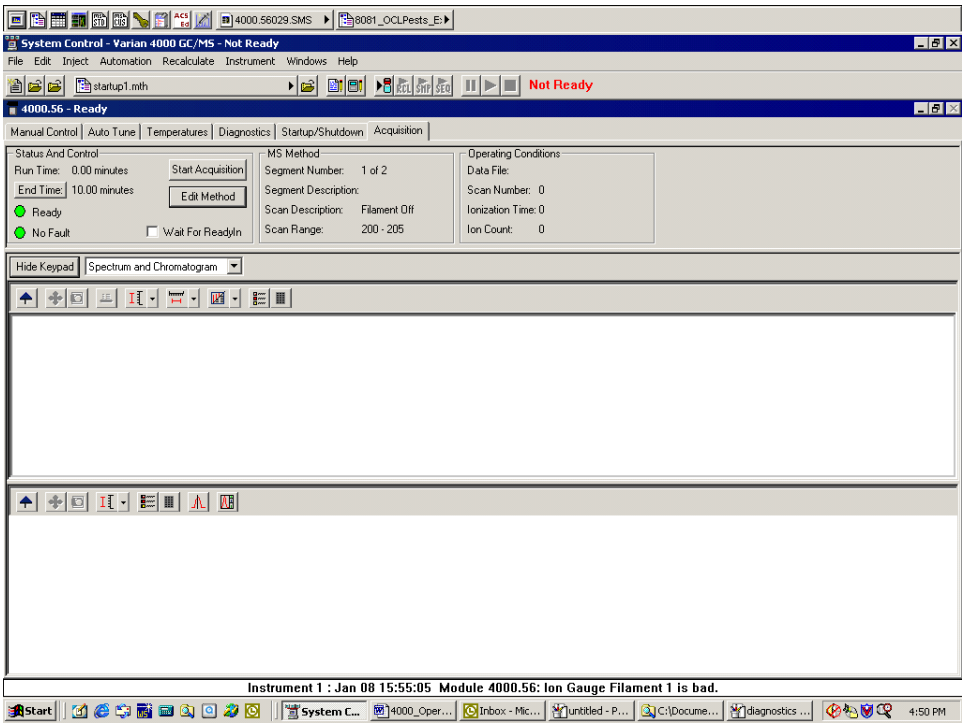
If you do not upload changes, the method is still checked to see if changes are made when the segment is changed or when you leave **Manual Control** or the **Method Segment**. If changes have been made then you will be given the option to save these changes or discard them.



Acquiring Data

Acquisition

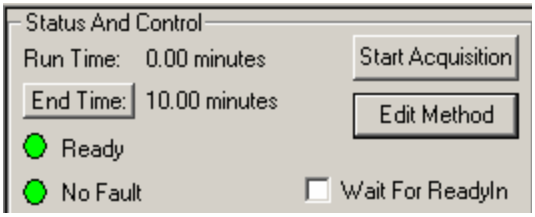
Selecting the **Acquisition** button activates the 4000 module for analysis. If you start an analysis while the instrument is in another mode, the software will automatically shift the MS module into Acquisition mode.



Within a few moments of entering Acquisition mode, you should see the yellow (Not Ready) light turn to green (Ready). This is an indication that the 4000 MS module is ready for analysis. If the GC is not ready you will see a Not Ready message at the top of the screen. Once the GC and AutoSampler come to a ready state, the Not Ready message will change to Ready. To determine the individual ready states of the components, you can go to the top pull down menu under Windows and see the states for the 4000, 3800, and Combi PAL modules. Once all components are ready, you can start an analysis.

An analysis can be run as a single sample or through an automated sequence. To run a single sample, go to the topic “Injecting a Single Sample” on page 40. If you want to run in automation mode, go to “Injecting Using a SampleList” on page 41. Additionally, both single samples and sample lists can be run from QuickStart. For more information on using QuickStart, go to the 4000 GC/MS Software Operation Manual.

Status and Control



Before an acquisition has been started, the Status and Control field on the acquisition page will look like the screen above. The Run Time will be 0.00 minutes because the run has not started. The End Time will be the run length specified for the 4000 MS module in the active method. Ready and No Fault lights should be green. The **Start Acquisition** button can be used to override automation and start a run even before the system comes to Ready. However, the file name of a run started in this way will be named as 4000.x.sms, not the file name specified for automation runs. Clicking the **Edit Method** button will allow you to open the Method Builder and modify the method. You will be prompted to re-activate the method after you save changes and return to System Control.

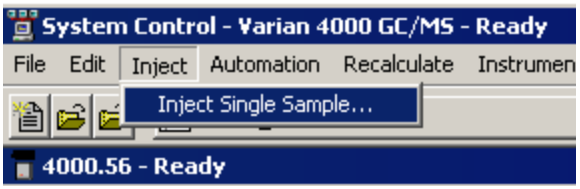
NOTE: A change in the End Time for the MS module does not change the GC End Time. You must access the GC module from the Windows pull-down menu and change the GC End Time separately or change the method on the keypad on the front panel of the GC.

Activating a Method

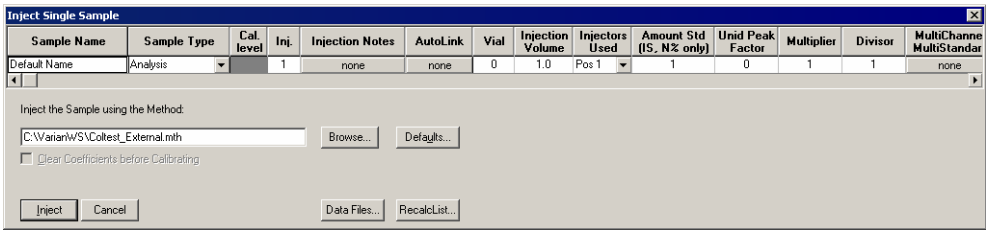
To activate a GC/MS method, use the ***File… Activate Method*** button to see a selection menu. Choose a method and click **Open**. This method will be downloaded to the instrument.

Injecting a Single Sample

You can inject a single sample from System Control by using the Inject Single Sample dialog.

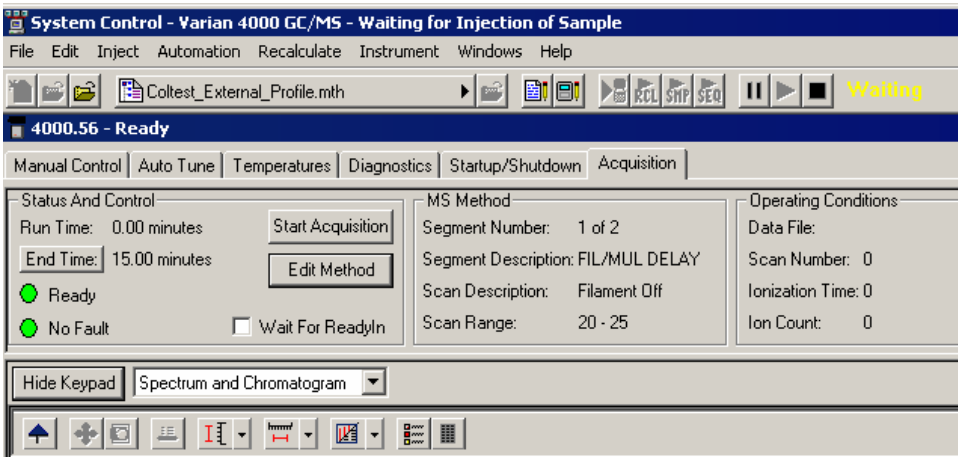


Display the Inject Single Sample dialog by selecting it from the Inject menu command ***Inject… Inject Single Sample***… or by clicking on the **Inject Single Sample** button on the toolbar  . If enabled, an Instrument Parameters dialog will be displayed. Otherwise, the Inject Single Sample dialog box is displayed.



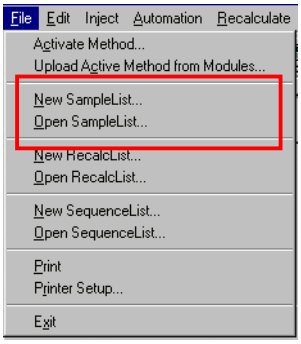
Enter a sample name in the upper left corner. If an AutoSampler is configured, enter the vial number where you have placed the sample. Check that the injection volume and injector used are correct. If you need to change the default values for any parameter, click the **Defaults** button. To create a name that includes more information such as date and time, or to change the directory for data file storage, click the **Data Files** button. When you are ready to acquire the sample, click the **Inject** button.

If the MS is not in Acquisition mode, it will be changed to that mode automatically. If an AutoSampler is doing the injection, it will proceed when instrument modules are all Ready. If you are doing a manual injection, wait until the System Control title bar reads “Waiting for Injection of Sample” and there is a blinking yellow Waiting light on the right of the System Control toolbar. Then inject the sample.

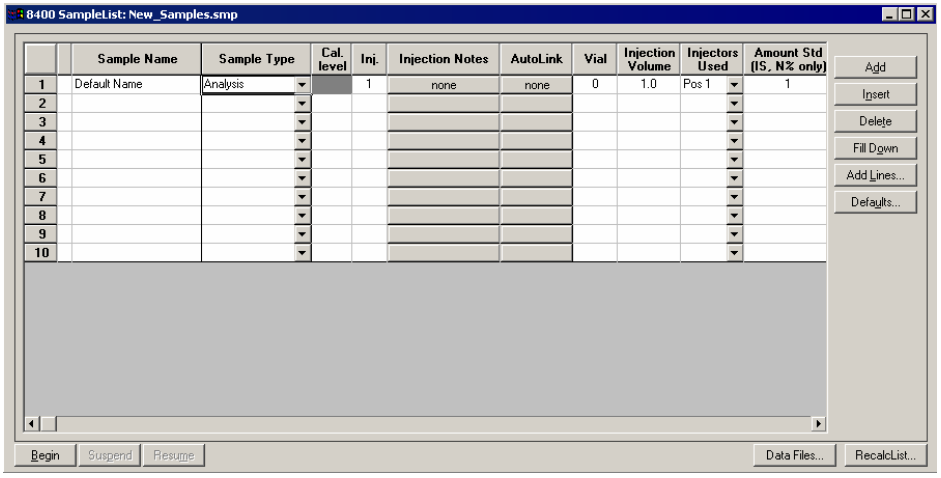


Injecting Using a SampleList

You can create and edit a SampleList in the Automation File Editor or in System Control. In this section, you will learn how to edit a SampleList and inject multiple samples from System Control.



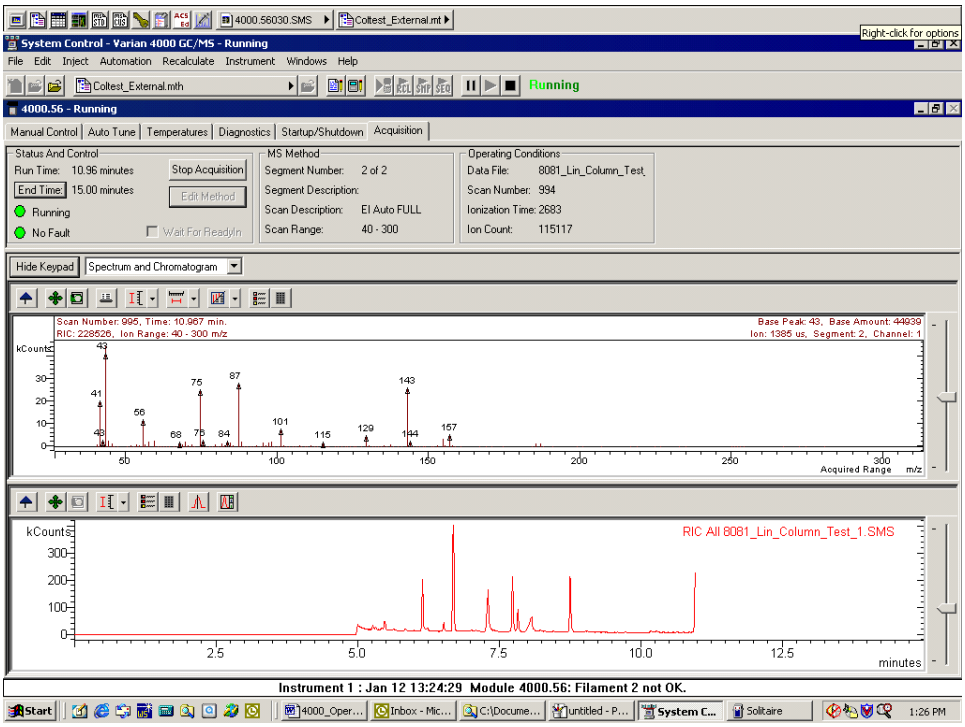
Choose to open either a New SampleList or an existing SampleList from the File menu. The SampleList window for the open SampleList is displayed. It contains fields that are specific to the sampling device configured in the instrument. If a sampling device such as the 8400 AutoSampler is configured, the corresponding SampleList is used, as depicted in the following image.



Spreadsheet columns can be sized by dragging their border using the left mouse button. Right click on the column headers for formatting options. When the table is scrolled to the right, the Sample Name column doesn't scroll so you can easily tell for which sample you are entering additional parameters. Click the **Add** button to add additional samples. Enter the name, sample type, and vial number for all samples. Then click the **Begin** button in the lower left corner to start the SampleList.

Monitoring the Status of Runs

After an injection is performed, the status of the run can be monitored in the instrument window.



The module status is shown in the status and control windows and on the Toolbar. You can monitor the chromatogram and spectra here in System Control, or you can click the far right button in the Chromatogram toolbar to transfer to MS Data Review, where you can perform operations like library searching while the data file is being acquired.

For more information of data acquisition features, go to the section *Acquiring GC/MS Data* in the 4000 GC/MS Software Operation Manual.