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4000 MS

4000 MS Users Guide
Hybrid Ionization

QUALITY SYSTEM ISO 9001 CERTIFIED

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Overview of Hybrid Configuration

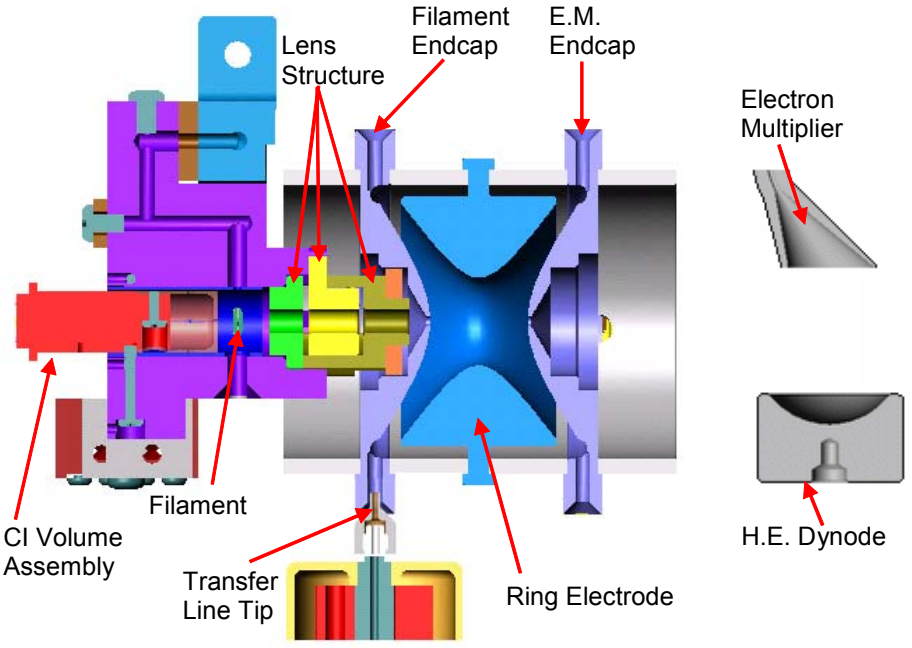
About Hybrid Chemical Ionization

The Hybrid configuration is one of the three operational configurations of the 4000 GC/MS system. In Hybrid configuration, reagent ions are generated in the external source through Electron Ionization of reagent gas. These reagent ions are then drawn into the ion trap to react with analytes eluting from the GC column. These ion-molecule reactions create analyte ions which are held in the ion trap.

This approach has a number of potential advantages, including avoiding ion-molecule reactions with the neutral reagent and avoiding losses of negative ions that occur when they move from the external source to the trap.

Hybrid CI is a softer ionization technique than EI. That is, Hybrid 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 analyte molecular weight is more likely to be observed. In addition to molecular weight confirmation, Hybrid CI mass spectra often provide other significant structural information that may not be available from EI mass spectra.

The Hybrid mode requires the external Ionization option, chemical ionization option, and a security chip to be present but does not involve any unique hardware. In hybrid mode, the external source must be in place and the transfer line must be positioned with the sample directly entering the ion trap. In common with the other configurations, it is possible to perform ion preparation techniques including Selected Ion Storage (SIS) or with optional software and equipment, Tandem Mass Spectrometry: Automated Methods Development (AMD), MS/MS, MS^n, and Multiple Reaction Monitoring (MRM). Refer to the 4000 GC/MS Software Operation Manual for help with setting up these experiments.



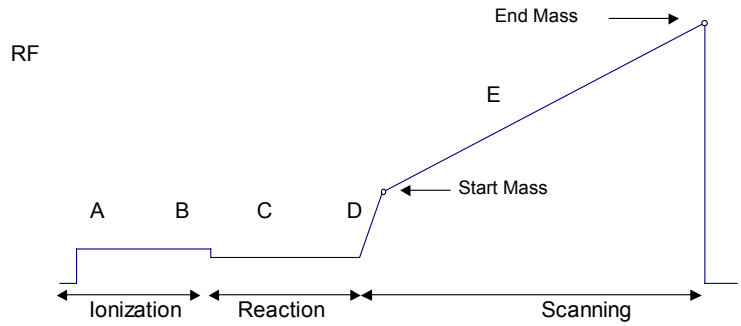
Schematic Diagram of the Hybrid Ionization Configuration

Hybrid CI Scan Function

In the Hybrid CI configuration, the external ion source is installed, but the transfer line directs the sample into the ion trap. CI reagent ions are generated in the external source and only those reagent ions which are selected are stored in the ion trap. These trapped reagent ions are allowed to react with sample molecules as they enter the ion trap, forming CI product ions by ion-molecule reactions. Hybrid CI may be used with either positively-charged or negatively-charged reagent ions.

The ion trap operates in a pulsed mode. Reagent ions are created only during the ionization pulse and are consumed during the reaction period to form analyte ions. The number of analyte ions depends on the concentration of the analyte, the initial reagent ion intensity, and the reaction time.

Space charge control in the Hybrid CI mode is achieved by using the results from an AGC prescan to calculate the ionization time and reaction time for the analytical scan. Because the spectral intensity is proportional to sample concentration and reaction time, linear calibration curves can be obtained.



Hybrid CI Scan Function (Analytical scan only)

During the Hybrid CI analytical scan, the following steps occur:

A The reagent gas is ionized for the length of time determined by the prescan.

B The selected reagent ions are stored in the ion trap. Ejection of ions above the Selected Reagent High Mass is accomplished by applying a broadband waveform between the ionization and reaction periods.

C Reagent gas ions react with sample molecules to form sample ions. (The reaction time is determined by the prescan.)

D Reagent ions are ejected.

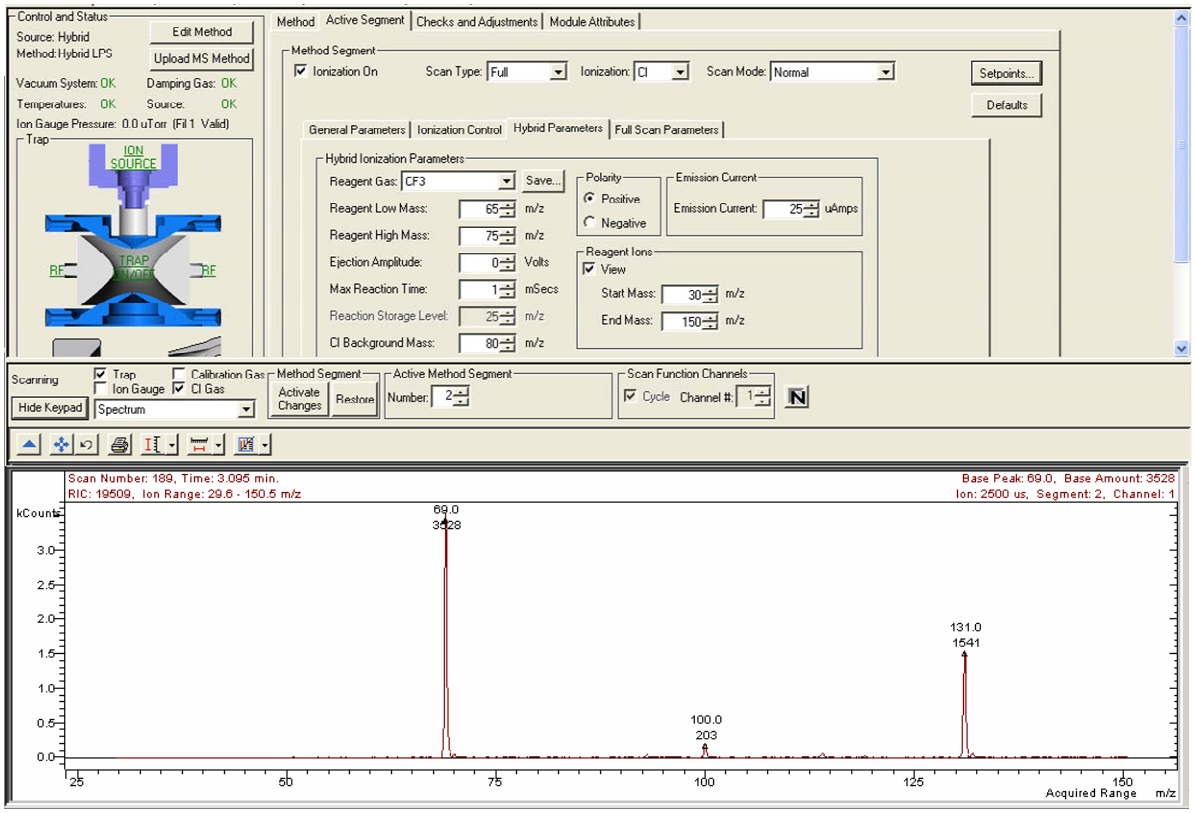
E The Hybrid CI mass spectrum is acquired for the sample ions.

The ionization and reaction storage RF can be set at the same level or different levels.

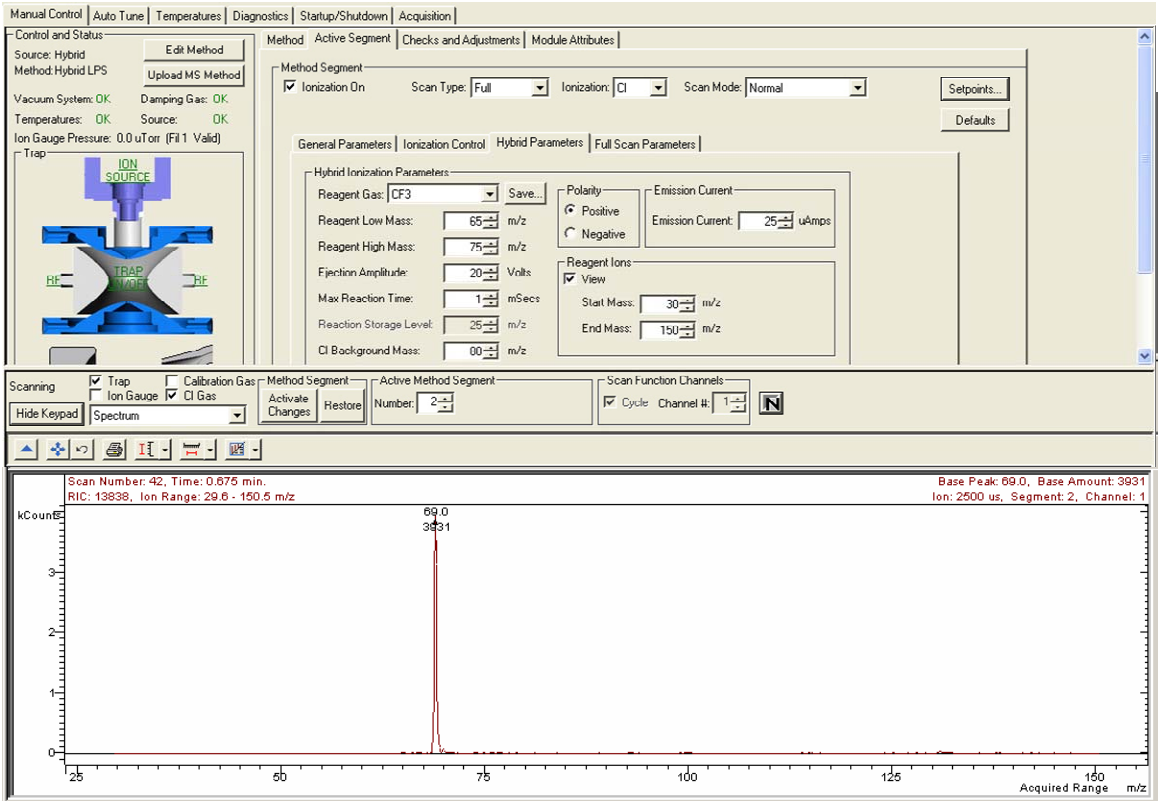
View Reagent Ions Function

The “View Reagent Ions” function can be used to tune the isolation of individual reagent ions (see the following figures). In the first figure, you can view all ions as set in the Reagent Ion Start and End Masses.

Because there is no Ejection Amplitude specified, all the ions in the range can be viewed:



In the next figure, the Ejection Amplitude is set at 20 V, thereby eliminating the ion at 100 m/z and 131 m/z.



Steps to Create a Mass Spectrum

Sample analysis may be divided into several steps:

Reagent Ion Formation

In the first step, reagent gas ions are formed as the reagent gas is ionized by interaction with electrons emitted by the filament. These reagent ions will then be used to react with GC analytes to create various ions in a process known as ***Positive Chemical Ionization*** (PCI).

Reagent ion formation can be a complex process. For example, when methane is 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 $C_2H_5^+$:

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

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

Stable negative ions are formed in the external ion source under electron ionization.

For example, methanol CI reagent forms a stable negative ion of m/z 31:

$$CH_3OH + e^- \rightarrow (CH_3OH)^{-\bullet}$$

$$(CH_3OH)^{+\bullet} + CH_3OH \rightarrow CH_3O^{\bullet} + CH_3O^- + H_2$$

Transferring and Trapping Reagent Ions

The reagent 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 Hybrid PCI and positive for Hybrid NCI. The voltages on the lenses are tuned in Auto Tune to optimize the 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, and only reagent ions above 35u are available to react with analyte molecules. This ability to select the reagent ion can add to the selectivity of the Hybrid mode of operation.

Sample Introduction

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

Sample Ionization

In the second step, sample molecules eluting from the GC column are ionized in the mass spectrometer via either Positive Chemical Ionization or Negative Chemical Ionization.

Positive Chemical Ionization: Ion-molecule reactions between the positively charged reagent ions and the GC analytes.

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 Hybrid CI using methane, 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 strong (M+1) protonated molecules or (M+29) and (M+41) ***adduct ions*** are 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 Hybrid configuration.

Negative Chemical Ionization in the Ion Trap: Negatively charged low-energy electrons attaching to GC sample molecules with high electron affinities.

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.

Ion Storage

Following reaction between the reagent ions and the analyte, analyte ions are 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 Options

Once ions are stored in the trap they can be manipulated, if desired. The 4000 MS 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 ion trap.

Options like Tandem Mass Spec (MS/MS) and Selected Ion Storage (SIS) 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. Advantages associated with ion preparation methods are similar to those of other sample preparation methods, e.g., reduction of noise and increased selectivity.

The Hybrid 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 Multiple Reaction Monitoring (MRM) are available with the MS/MS option installed.

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. Supplemental dipole and quadrupole voltages applied to the endcap electrodes improve the mass resolution of the process. Upon ejection, the ions strike a conversion dynode, initiating a signal multiplication process at the electron multiplier.

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.

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.

Scanning Ions to Collect Mass Spectra

The scanning process for Hybrid 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. Ions ejected from the ion trap are attracted to the conversion dynode. In positive modes, electrons are ejected from the conversion dynode, held at –10,000 V, and repelled to the electron multiplier. In negative mode, positive ions are ejected from the dynode, held at +10,000 V, and repelled toward the electron multiplier. The signal is amplified 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 Hybrid 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 Hybrid 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 Hybrid chemical ionization is selectivity. In Hybrid 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 Hybrid Mode to Obtain 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 the ability to select the reagent ion may allow highly selective reagent ion/analyte reactions to assist in identifying the analyte molecular weight and isomeric configuration.

Conversion from Internal to Hybrid Configuration

Converting the 4000 MS from internal to hybrid configuration involves changing only the ion source. The Internal ion source assembly is removed from the trap assembly and replaced with the External ion source assembly.

The transfer line orientation remains in the Internal position.

For details on how to add/remove the source assemblies, 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
- Replace the analyzer in the MS manifold

Conversion from External to Hybrid Configuration

Changing from External configuration to Hybrid 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 internal type.

Checklist

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

Effects of Hardware Configuration Changes

When the configuration is changed, for example from Internal to Hybrid 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 to the current configuration. A similar process occurs for the default method (Default.mth). Thus, after making the hardware configuration change, any method newly built will have the appropriate instrument configuration by default.

NOTE: The presetting of Module Attributes generated by the automatic sensing of a configuration change requires that the user run all Auto Tune routines, as the prior Auto Tune results will be invalid.

Starting the Instrument

Initial Pump-down

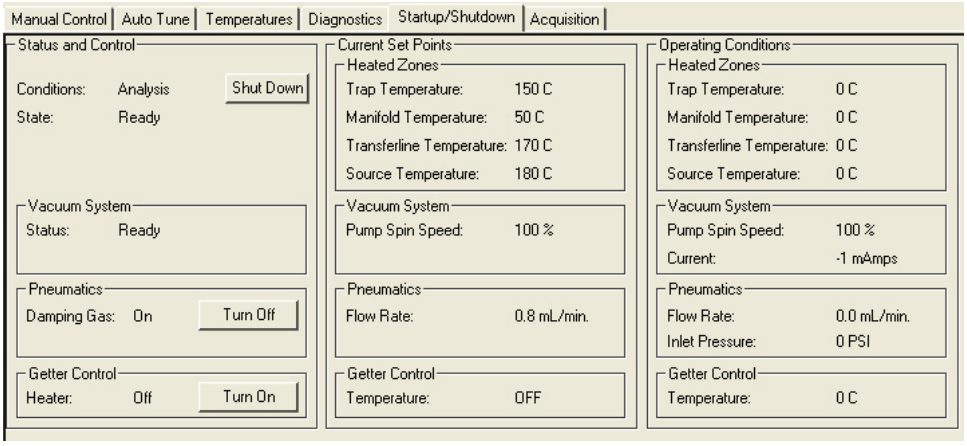
Things to check;

- 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

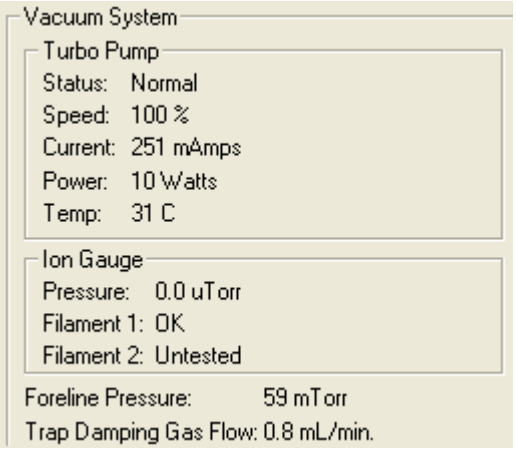
1. Check that the analyzer assembly is seated properly on the manifold (there should be no gaps)
2. Check that the transfer line is in, and
3. Make sure the vent valve is sealed.

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 as an increase in the pump current once at 100% or in the ion gauge pressure (See Diagnostics). Small leaks are diagnosed by changes in the ion gauge reading and can be pinpointed using the leak check section. For more detail on troubleshooting leaks, go to the Troubleshooting section in the 4000 GC/MS Hardware Operation Manual.

Start Damping Gas Flow

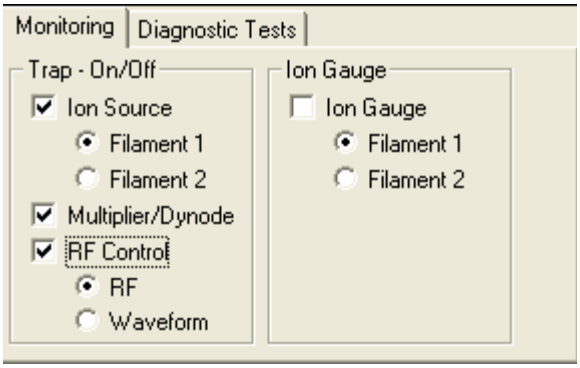
The addition of damping gas may or may not improve sensitivity. Start without damping gas and then increase to 0.5 mL/min to determine if sensitivity increases.

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 initial choice of flow is 3-4 mL/min.

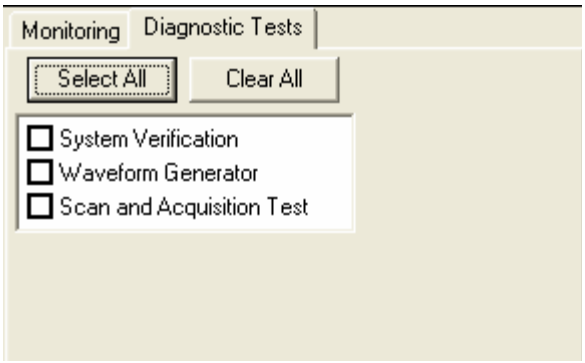
NOTE: He buffer gas flow rate is set in the Module Attributes tab dialog in Manual Control.

Diagnostic Tests

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



Diagnostic Monitoring Options



Diagnostic Tests

For more details on the diagnostic tests, go to the section on *Diagnostics Mode* in the 4000 MS Operations Manual.

Set System Temperatures

Analysis Temperatures

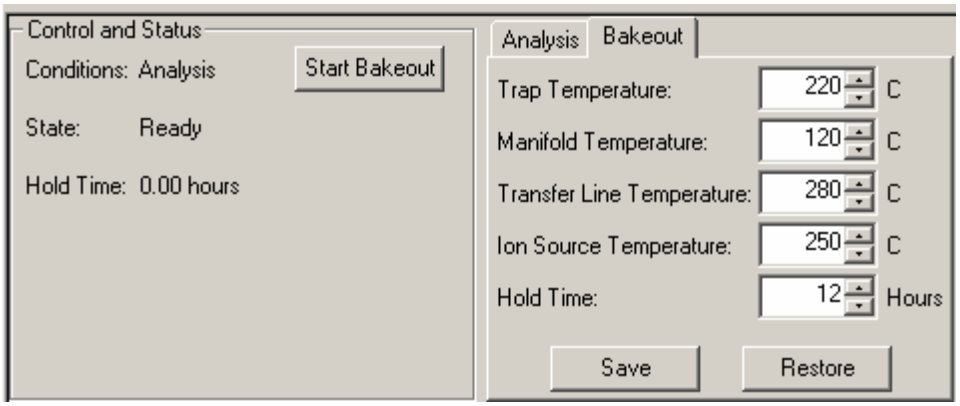
Ion trap temperature is an important variable for analyses performed in the Hybrid Configuration as it must be high enough to prevent condensation of analyte as it elutes from the GC column into the ion trap.

Changing the source temperature takes only a few minutes. However, there may be subtle effects on lens tuning and mass calibration. We recommend that you perform mass calibration and trap function calibration shortly after the desired source temperature is reached and then again several hours later or 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 of room temperature variation on the system.

System Bakeout



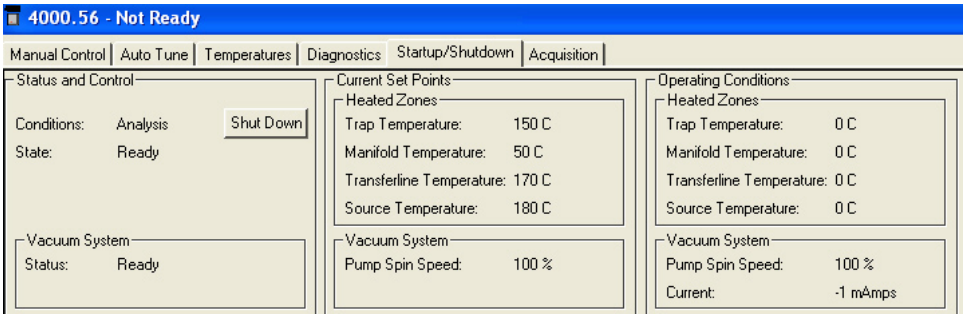
Bakeout Settings in the Temperatures Dialog

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 bakeout 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. We recommend that you wait at least two hours after bakeout is completed before attempting to AutoTune or run the 4000MS, to allow all temperature zones to equilibrate thoroughly.

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

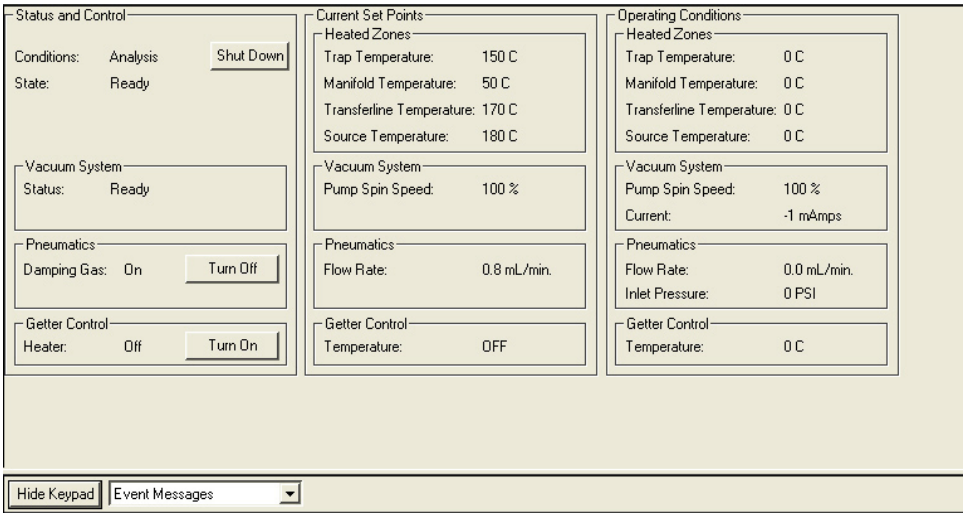
Startup and Shutdown



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.

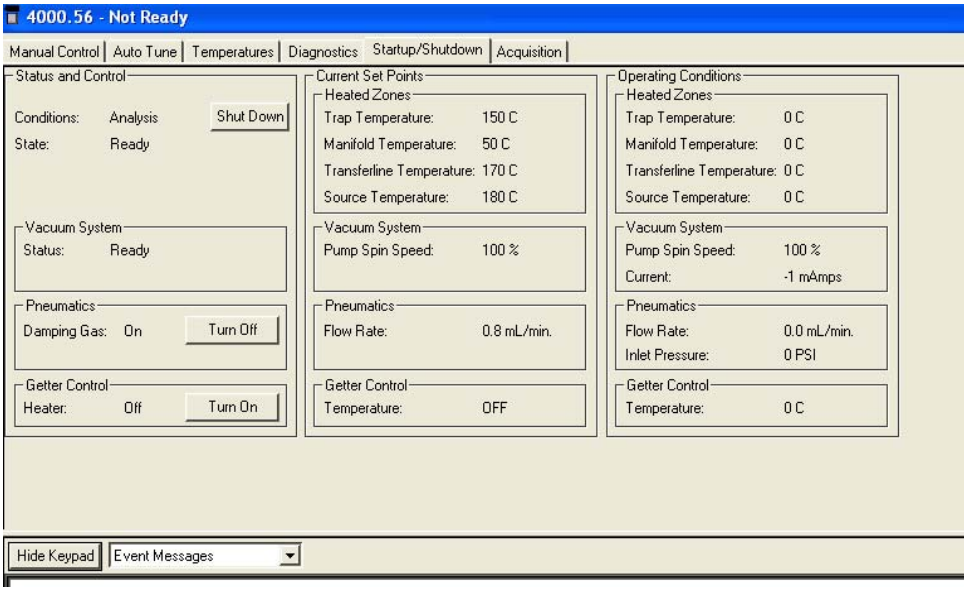


During system startup you will be able to observe the increase in Pump Spin Speed in the Operating Conditions field. The software will be locked in the Startup/Shutdown mode until the speed reaches 100%. You will also see the temperature readings for heated zones begin to increase in the Operating Conditions 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 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.

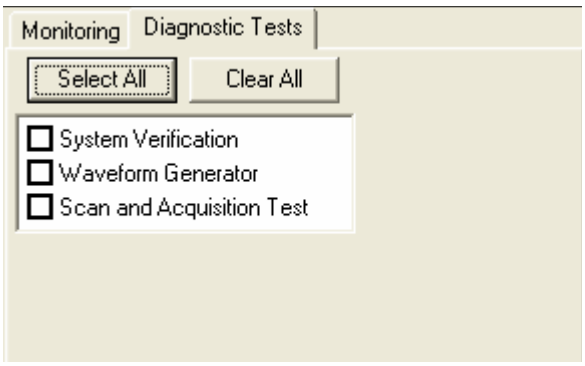


To restart the system after you have activated the shutdown, click the **Start Up** button on the left side of the screen above. This will restart the pumps and turn on the heaters.

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

NOTE: Retract the transfer line before lifting the analyzer assembly from the vacuum manifold. Failure to retract the transfer line can cause damage to the transfer line tip and to the trap assembly.

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, refer to the relevant section of the Troubleshooting Guide in the 4000 GC/MS Hardware Operation Manual.

Adjusting and Tuning the 4000 MS

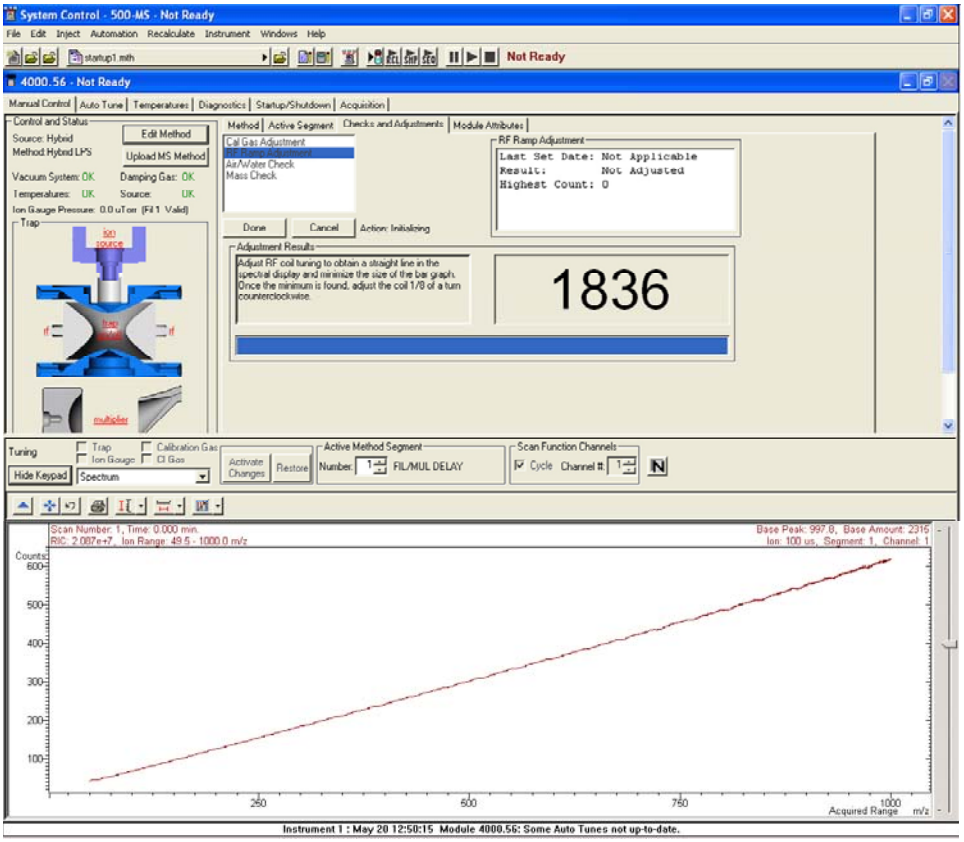
Checks and Adjustments

RF Tune

After doing MS maintenance, changing the analyzer assembly or changing the MS configuration, adjust RF tuning 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.

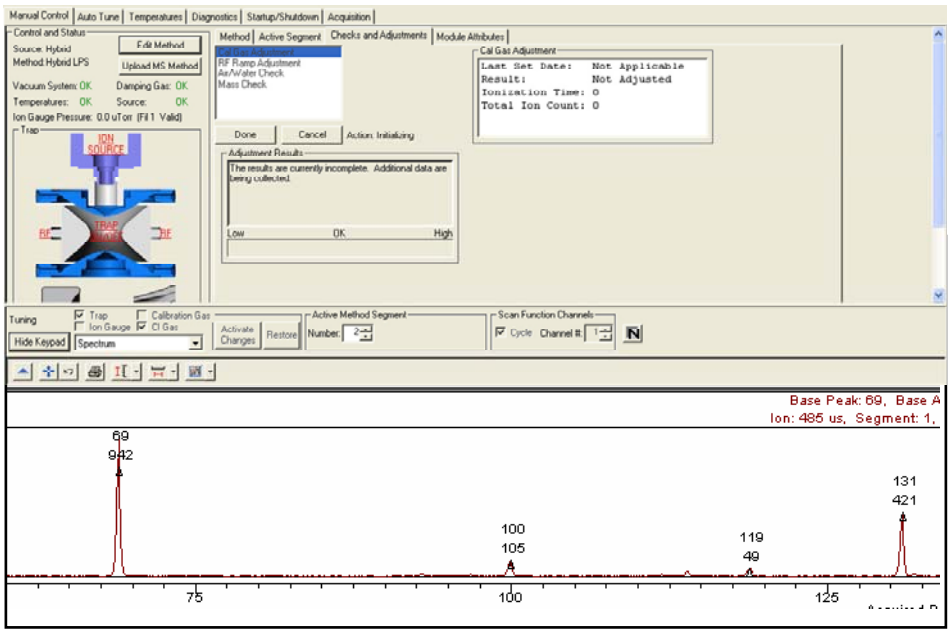


RF Tune Display

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

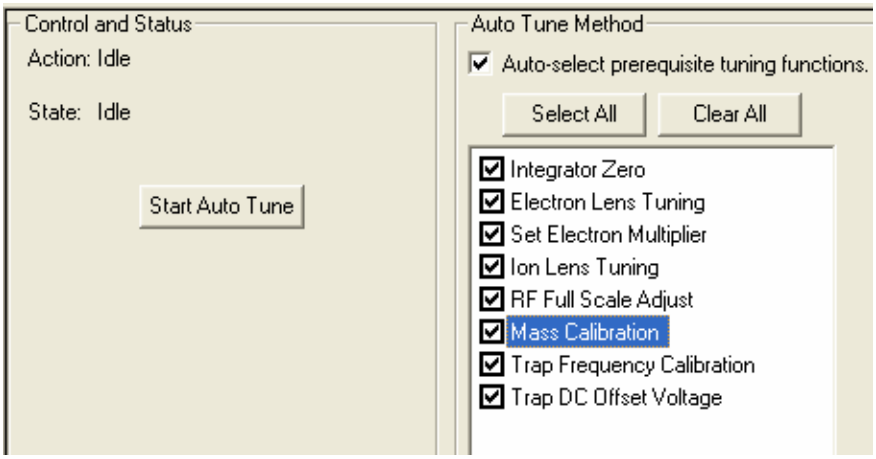
Before acquiring data in Hybrid chemical ionization (CI) mode, adjust the CI reagent gas pressure. Details on how to set up methane CI gas are found in the section “Setting Up CI Reagents” on page 22.

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 Electron Multiplier must be done *before* the Air/Water Check.

Auto Tune



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.

Setting Up CI Reagents

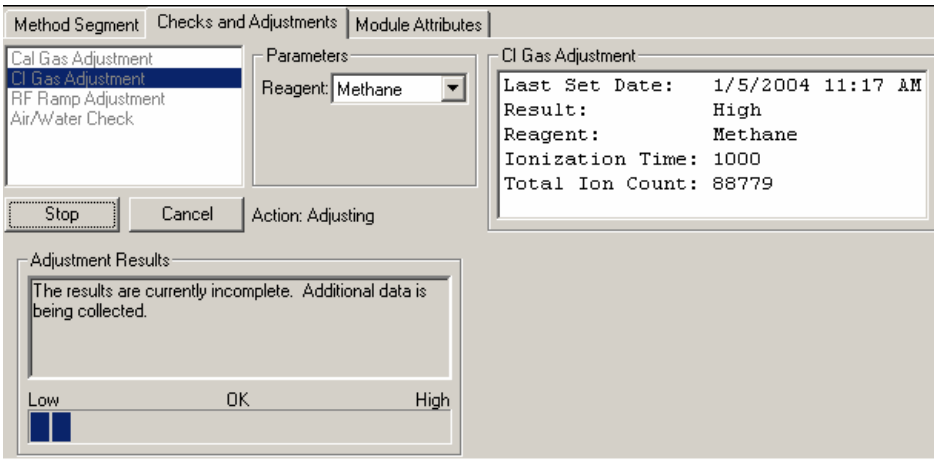
Although several liquid and gaseous reagents are useful in Hybrid configuration, methane appears to be the reagent of choice. Liquid reagents like methanol and acetonitrile give weak responses for most analytes in Hybrid positive chemical ionization, PCI.

Installing Methane CI

For full details on installing a CI gas, go to the section *Installing a CI Reagent Gas* 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 to 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.

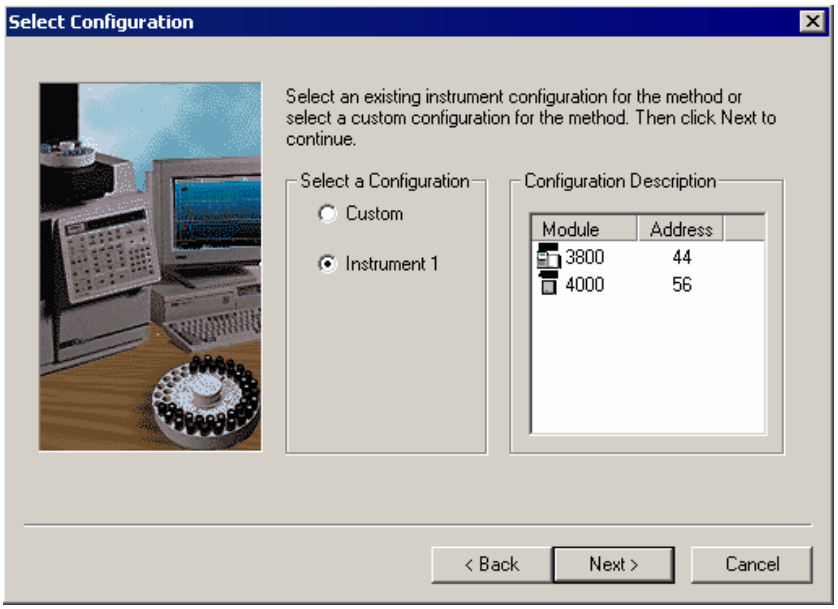
Preparing a GC/MS Method for Data Acquisition

Building the MS Method

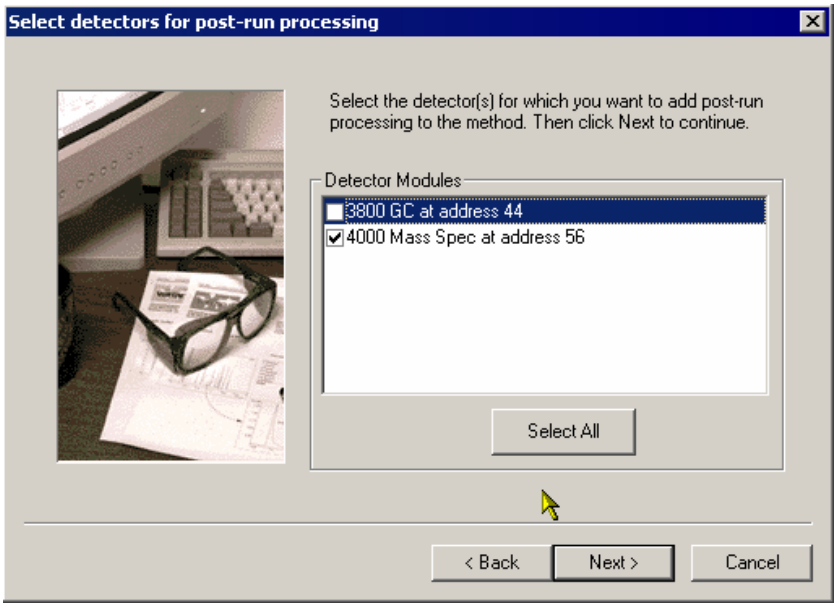
Building a New Method

From the Workstation Toolbar, open the Method Builder. 

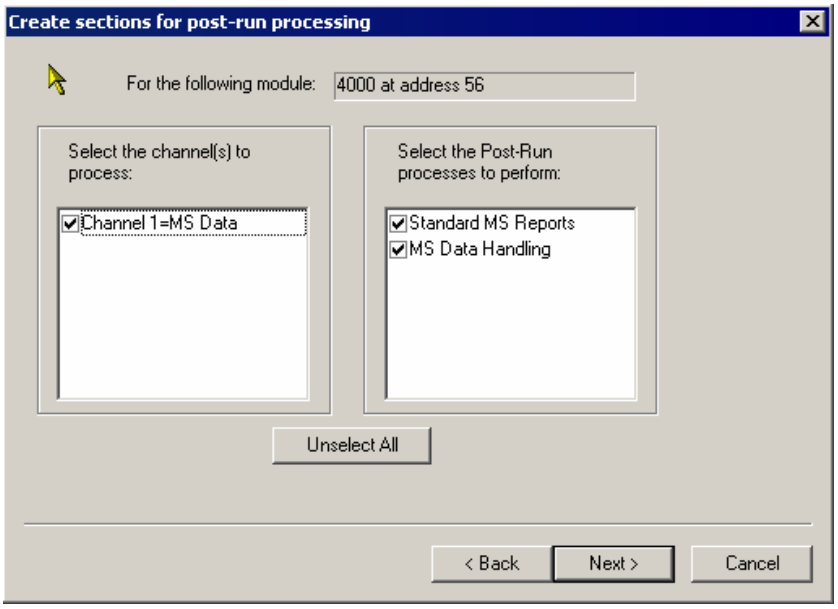
Select the instrument. You can build an offline method by selecting the Custom configuration and selecting the desired modules.



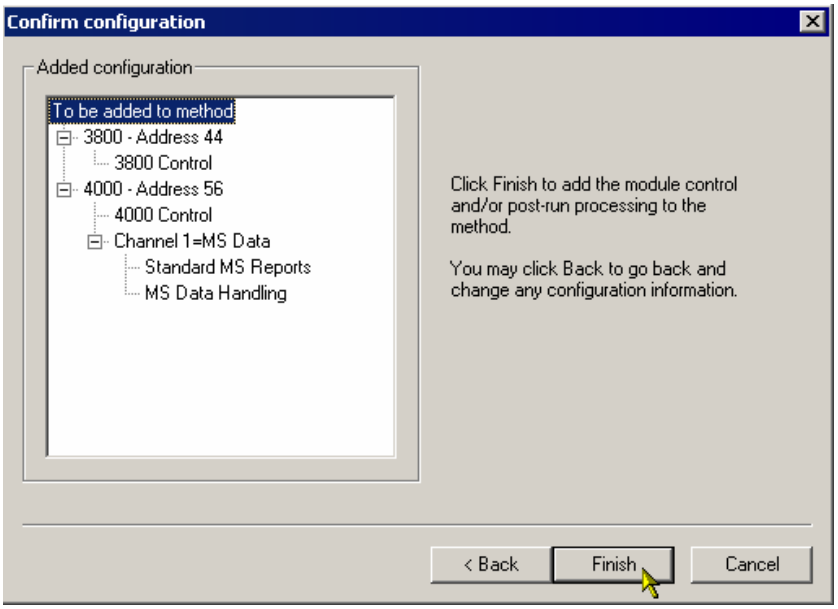
Select which detectors (MS and/or GC) will have data handling.



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

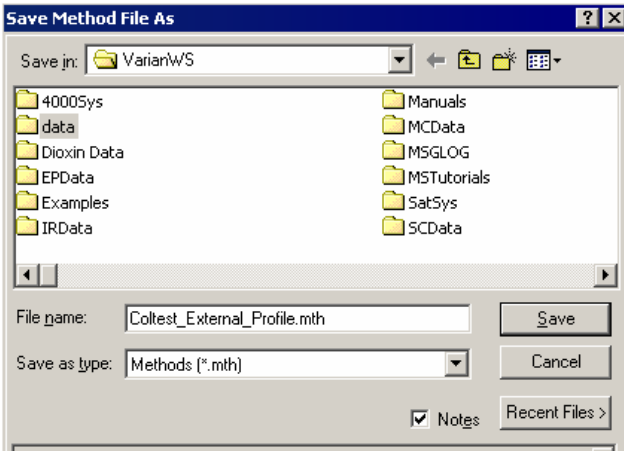


The method will contain the following sections: 3800 GC Control, 4000 MS Control, Standard MS Reports and MS Data Handling.



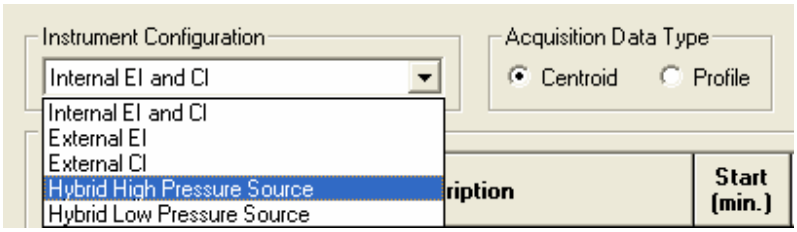
Name the Method

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



Setting the 4000 MS Instrument Configuration

The configuration defines what ionization modes can be used for data acquisition. In Hybrid Mode, the configuration is Chemical Ionization (CI). The instrument Configuration is set in the upper-left corner of the MS Method Editor by selecting from the drop-down list box.



Hybrid Configuration Options

Hybrid methods can be performed only in positive or negative ion CI modes (PCI or NCI) except when Auto Tune methods requiring EI mode are being run. For both these methods, CI reagent ions are formed in the external ion source and drawn into the ion trap to react with compounds eluting from the GC column. Note the Hybrid HPS and LPS options in the selection menu. Hybrid HPS (High Pressure Source) is performed with the CI volume inserted into the ion source whereas the LPS (Low Pressure Source) option occurs in the EI source.

Select the Acquisition Data Type

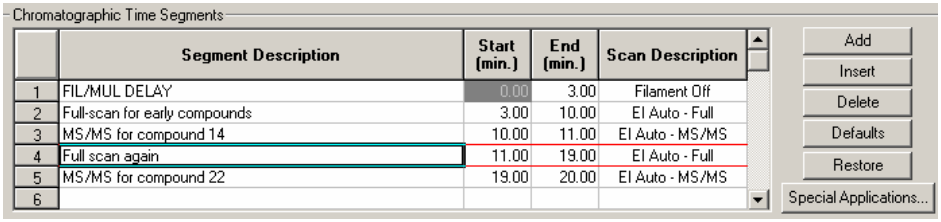
Centroid data is the default acquisition data type, as data handling, library searching, and spectral comparison can only be done from this type of data. Centroid data is reported as ion/intensity pairs to one described point.

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

Profile data is collected at 10 points per m/z and is displayed as peaks similar to a chromatogram. The display allows you to observe the true dispersion of the response and determine if adequate resolution has been obtained.

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

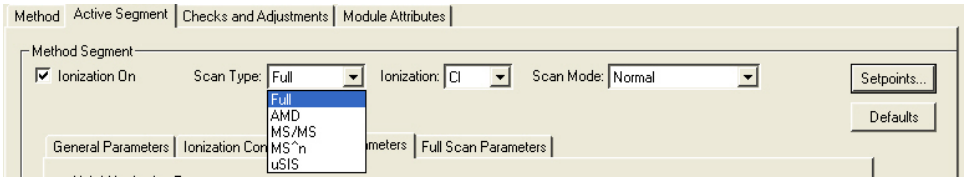
Adding or inserting a segment will copy all of the parameters from the previous segment to the newly created segment. Double-click on the required field to edit the Segment Description, Start Time, or End time of a segment.

Edit the 4000 MS Method Segments

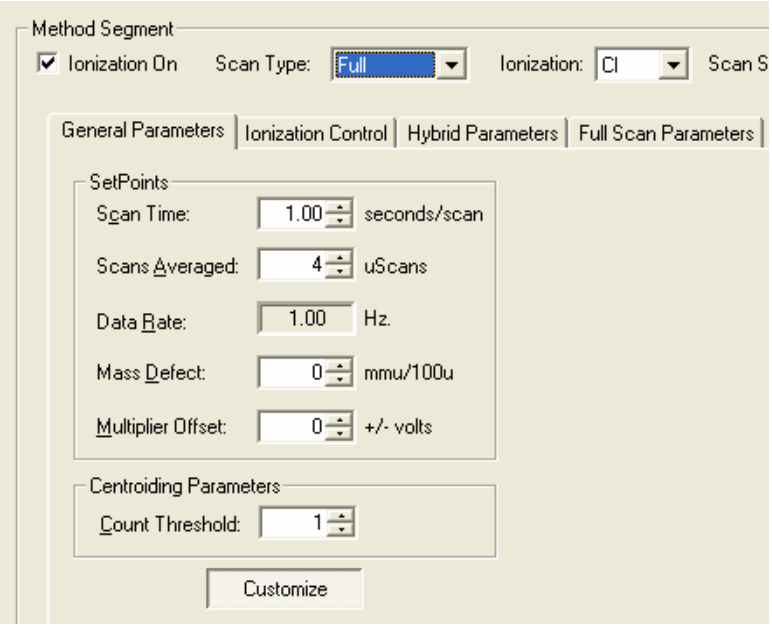
In this section we describe editing of parameters for Hybrid CI methods. For more advice on performing Hybrid CI, go to the section Building GC/MS Methods - Hybrid PCI and NCI in the 4000 GC/MS Software Operation Manual.

Scan Function Settings

Under Scan Type, one can choose from a variety of types including full scan and different MS/MS methods. In Hybrid 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.



General Parameters Tab



General Parameters Tab

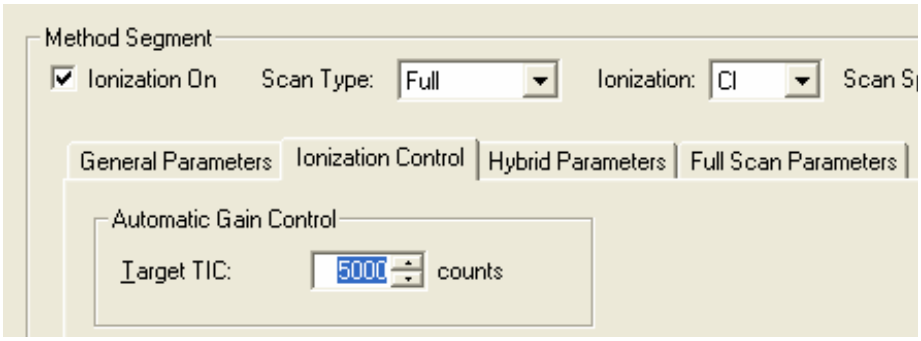
Scan Time, **Scans Averaged**, and **Data Rate** are all linked. The number of scans averaged is updated when the scan time is adjusted and vice versa. The best way to set the scan time is to set the mass range from the Scan Parameters tab and then change the scans averaged to three. Three scans averaged 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.

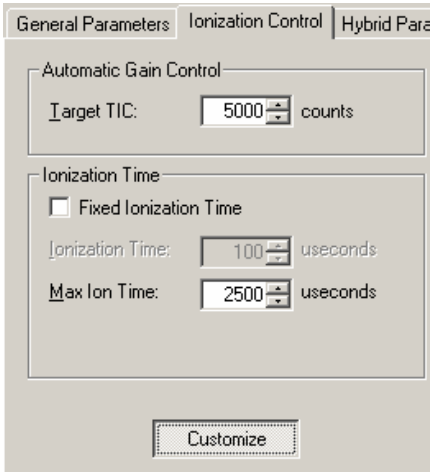
The **Count Threshold** is normally 1; a value of 2-3 counts will reduce the number of low-level ions reported in the mass spectrum. This approach may improve library searches and reduce data file size at the cost of somewhat less detailed information in the mass spectra. The count threshold is shown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

Ionization Control

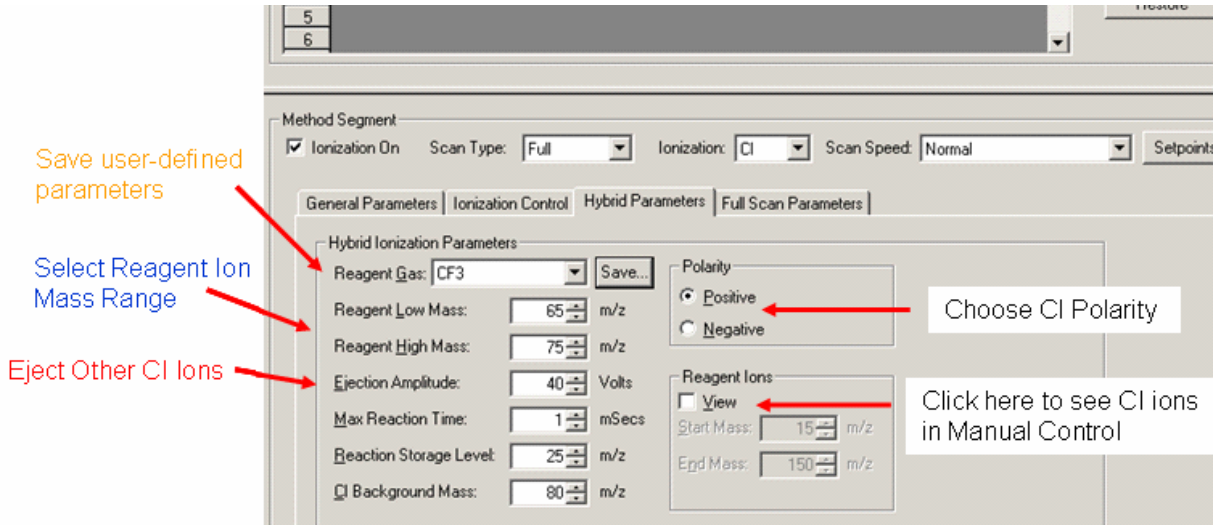


The **Target Total Ion Current**, or TIC is specified here. The Automatic Gain Control (AGC) algorithm uses the ion count from a prescan at fixed ion time, along with this target value, to calculate an ion time necessary to fill the ion trap with the target number of ions during the analytical scan. 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.

The default Target TIC for positive or negative chemical ionization is 5,000. The target can be set as high as 65,000. When the **Customize** button is clicked (see below), one has the option to run fixed ion time experiments with ion times as high as 65,000 μsec or to change the Maximum Ion Time for CI Auto experiments. One can turn on the CI Gas and the ion trap in Manual Control and check the ion time in CI Auto mode.



Hybrid Parameters



The **Reagent Low Mass** and **Reagent High Mass** values are set to bracket the CI reagent ion mass range of interest. The Reagent Low Mass must be set to at least 10u below the mass of the lowest reagent ion of interest without a loss of intensity for that reagent ion. It is helpful to adjust these parameters in Manual Control with the **View** box checked in the field to the right of this dialog. The Reagent Low Mass parameter sets the RF storage level to exclude ions below the selected m/z. This is not a precise way to perform isolation. By contrast, the Reagent High Mass isolation step occurs after the ionization time, when resonant waveforms are applied to the ion trap endcaps to eliminate ions with m/z above the selected Reagent High Mass.

Ejection Amplitude is the voltage of the waveforms for high mass isolation of CI reagent ions. The default value is 15V.

Max Reaction Time is the maximum time in μsec allowed for CI reaction. If the ion time is reduced below the maximum based on the results of the prescan, the ion time will be scaled back proportionately. The allowed range for this parameter is 1-2000 μsec.

The **Reaction Storage Level** is the RF storage level in the ion trap during CI reaction, following the ionization period. It should not be set above the m/z of the CI reagent ion with which you wish to perform the CI reaction, else these ions will be ejected from the ion trap.

The **CI Background Mass** is the lowest m/z counted during the CI prescan. It can be higher than the low mass of the acquisition range but is usually set to be at or below the Start Mass value.

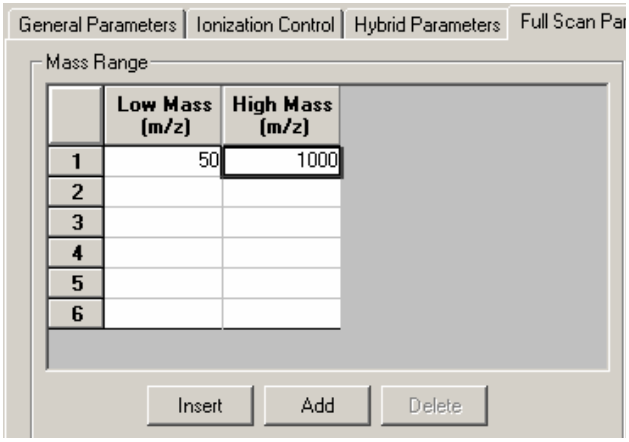
Polarity is selected for either positive or negative hybrid CI.

Set the **Start Mass** and **End Mass** ions to **View** in this dialog. Click the **View** box when the method is opened in Manual Control to observe the effects of Reagent Start Mass and Reagent End Mass isolation adjustments. Remember you must manually turn on the ion trap and CI gas icons to observe the CI reagent ions in this way.

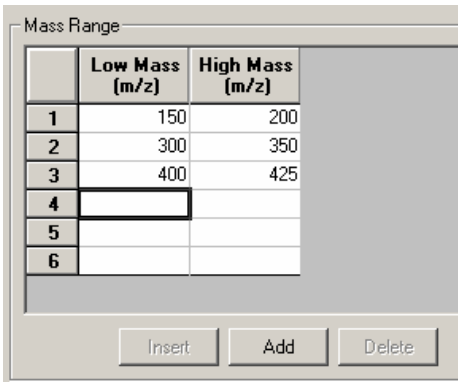
Scan Parameters Tab Dialogs

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 Hybrid configuration, Full Scan and MS/MS. For detailed information on all scan types, go to the section Building GC/MS Methods in the 4000 GC/MS Software Operation Manual.

Full Scan Parameters



It is typical (see above) to use only a single mass range segment in CI, in which case one simply enters the desired values for Low Mass and High Mass of the acquisition range. However, as shown below, one can enter up to six non-contiguous mass ranges (separated by at least 10u). This feature can also be time programmed on a chromatographic segment basis. It is then possible to tailor the CI acquisition ranges for CI to different target analytes depending on the mass spectrum of each compound. An example of a three-range acquisition is shown here:



Setting Parameters for an MS/MS Method Segment

Tandem mass spectrometry, or MS/MS, uses ion preparation steps after the analyte 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

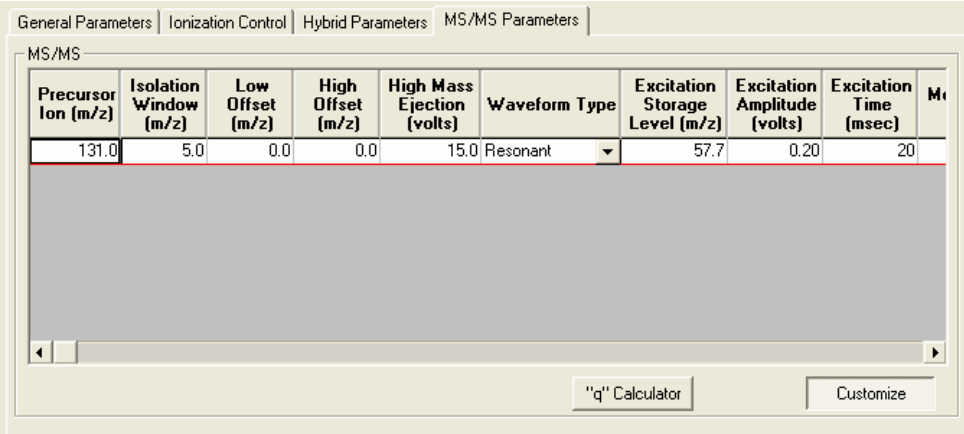
dissociation of the precursor ions to lower mass product ions. The remaining ions are then scanned to collect an MS/MS spectrum.

When well designed, 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.



Precursor Ion (m/z): The precursor ion is the desired ion m/z that will be isolated in the MS/MS isolation step. This Precursor Ion m/z value is used in both Resonant and Non-Resonant Methods of MS/MS.

Isolation Window (m/z): The full mass isolation window range is 1.0 to 14.0 m/z. The actual range is dependent on the precursor ion. The default value is 3.0 m/z. Integral and fractional mass isolation window values are both accepted. If an isolation window smaller than 1.5 m/z is used, then the exact mass of the precursor ion should be entered in the Precursor Ion Mass field.

If the Low or High Edge Offset range is not sufficient to completely isolate the desired ions, either increase (in the case of desired ions not being present) or decrease (in the case of unwanted ions being isolated) the Isolation Window value.

Low Edge Offset: The mass offset to optimize the ejection of the mass just below the precursor ion mass. The Low Edge Offset range is -0.5 m/z to 0.5 m/z. The default value is 0.

Low Edge Offset affects the isolation window on the low mass side of the precursor ion. Increasing the mass offset (increasing the default from 0 to 0.1 m/z) makes the isolation window on the low mass side of the precursor ion larger. Decreasing the offset (decreasing from the default 0 to -0.5 m/z) decreases the window on the low mass side. The offset should be adjusted to minimize the amplitude of the adjacent masses below the precursor ion. Initially, adjust in 0.2 m/z increments.

High Edge Offset: The mass offset to optimize the ejection of the mass just above the precursor ion mass. The High Edge Offset range is -0.5 m/z to 0.5 m/z. The default value is 0.

High Edge Offset affects the isolation window on the high mass side of the precursor ion. Increasing the mass offset (increasing the default from 0 to 0.1 m/z) makes the isolation window on the high mass side of the precursor ion larger. Decreasing the offset (decreasing from the default 0 to -0.1 m/z) decreases the window on the high mass side. The offset should be adjusted to minimize the amplitude of the adjacent masses below the precursor ion. Initially, adjust in 0.2 m/z increments.

If the Low or High Edge Offset range is not sufficient to completely isolate the desired ions, either increase (in the case of desired ions not being present) or decrease (in the case of unwanted ions being isolated) the isolation window.

High Mass Ejection: Amplitude of broadband waveform used to eject masses above the isolated precursor ion. Default is 35 volts. If precursor ions are lost due to dissociation, reducing this amplitude may help. However, some ions with a m/z higher than the precursor ion may not be ejected.

Waveform Type: The waveform type is either resonant or non-resonant. Resonant waveforms are harmonious with the frequencies of electrons held in the ion trap. Non-resonant waveforms are not harmonious with the frequencies of the electrons held in the ion trap.

Excitation Storage Level (m/z): The RF storage level in m/z when the dissociation waveform is applied following isolation. The excitation storage level range depends on the precursor mass, but the lowest product ion must be more than several mass units above the excitation storage level. A starting excitation storage level for a precursor ion can be calculated using the “q” calculator. The “q” calculator is accessed by right-clicking on any of the fields in the MS/MS parameters table.

The optimum excitation storage level is a tradeoff between a storage level high enough to allow fragmentation of the precursor ion and a storage level low enough to allow efficient trapping of the lowest m/z product ion. A higher excitation storage level allows more energy to be imparted to the precursor ions by allowing a higher excitation amplitude to be used.

Excitation Amplitude (volts): Voltage used to excite the precursor ion causing it to dissociate into product ions. The amplitude range for non-resonant excitation is 0 to 120 volts. For resonant excitation, the range is 0 to 60 volts. The default values are 0.2 volts for the resonant excitation method and 20 volts for the non-resonant excitation method.

If the excitation amplitude used is too large, the precursor ion and product ion spectra will be absent because both ions will be ejected from the trap. If the value is too small, the precursor ion spectrum will be dominant and the product ion spectrum will be weak or missing.

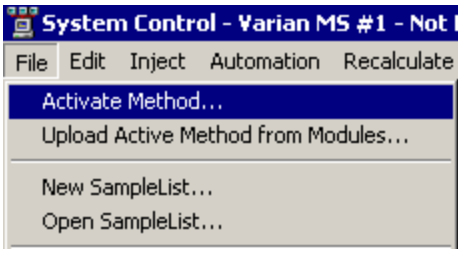
Excitation Time: The excitation time is the time required for collision-induced dissociation (CID) by ion excitation. The excitation time range is 0 to 650 msec. The default excitation time is 20 msec for both resonant and non-resonant excitation.

Viewing Method Parameters in Manual Control

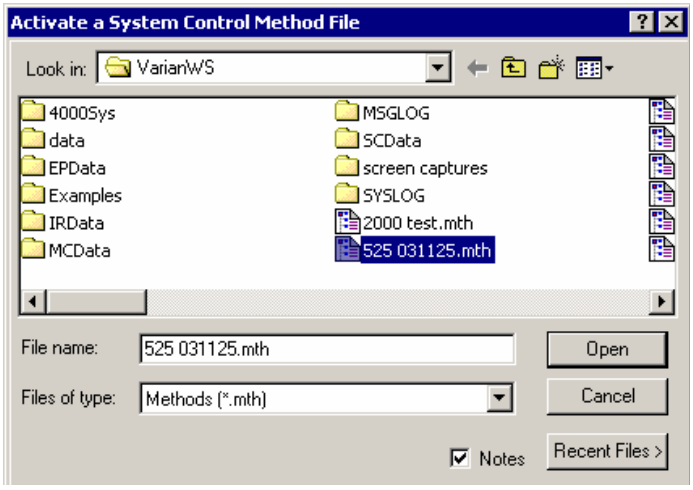
After a method has been created in the Method Builder section of the Varian MS Workstation, it can be previewed in Manual Control. All of the MS parameters can be edited and previewed before a run. However, the number of segments - or the start and end times of existing segments - cannot be changed unless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

Activating a Method

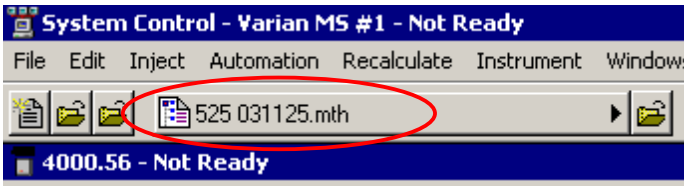
To activate a different method, use the ***File… Activate Method*** command to see a selection menu.



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).



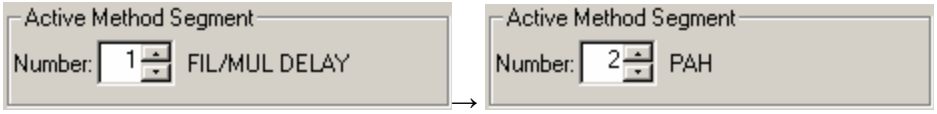
The active method is displayed in the tool bar.



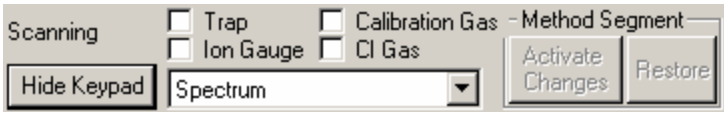
Displaying Ions

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.

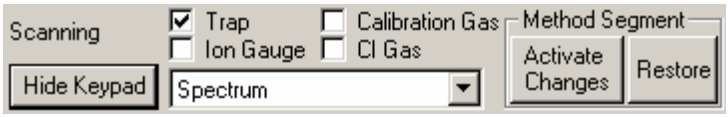
You cannot turn on the ion trap in a segment where ionization is OFF as in the Fil/Mul Delay segment #1. Change to an ionization segment:



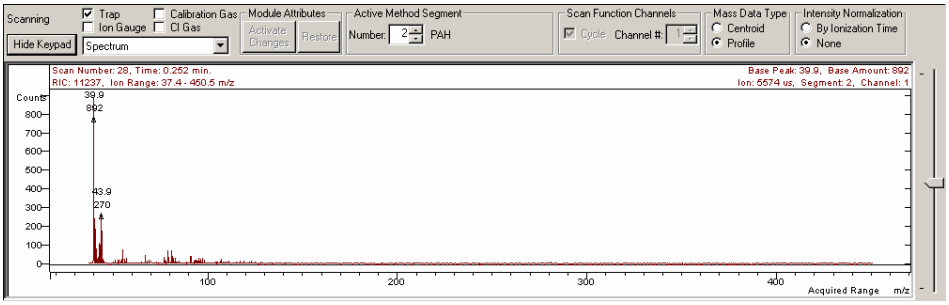
The ion trap controls will now be active:



Turn on the Trap:



The current mass spectrum will be displayed:

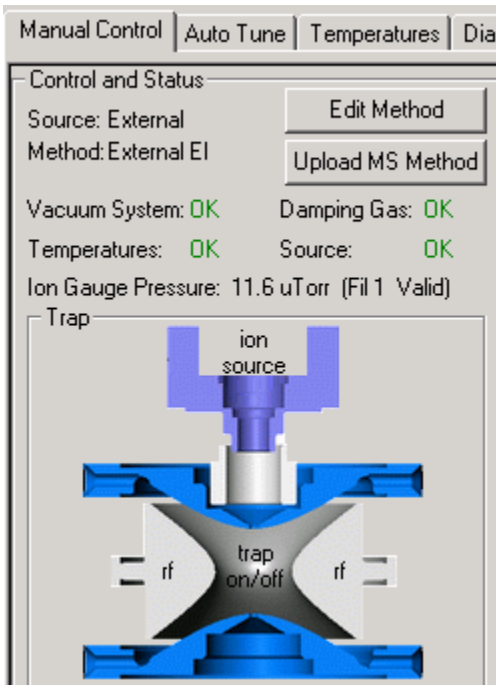


Editing a Method in Manual Control

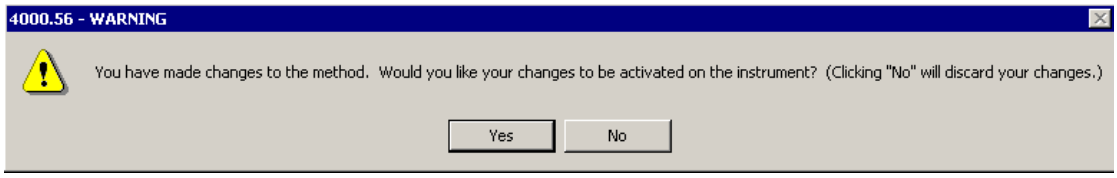
In a subset of tab dialogs, one can examine and edit all the parameters in the active MS method and observe the effects of these changes on the mass spectra currently being acquired. The exact set of tab dialogs depends on the ionization and ion preparation modes in the current method segment. After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

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.



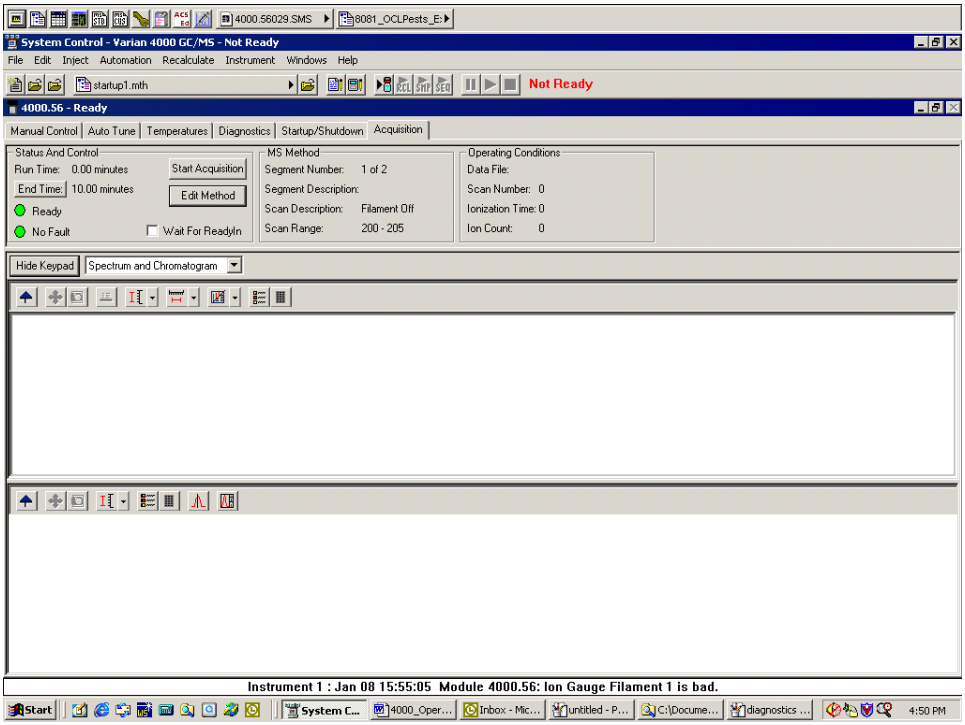
If you do not upload changes, the method is still checked to see if changes were made when another segment is selected or when you leave **Manual Control** or the **Method Segment**. If changes have been made then you will be given the option to save these changes or discard them.



Acquiring Data

Acquisition

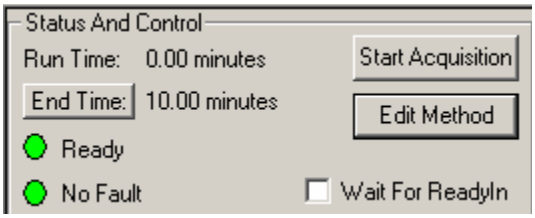
Selecting the **Acquisition** button activates the 4000 module for analysis. If you start an analysis while the instrument is in another mode, the software will automatically shift the MS module into Acquisition mode.



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. If you wish to run a single sample, go to “Injecting a Single Sample: on page 38. If you want to run in automation, go to “Injecting Using a SampleList” on page 39. 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.

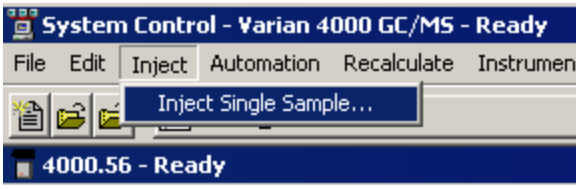
NOTE: A change in the End Time for the MS module does not change the GC End Time. You must access the GC module from the Windows pull-down menu and change the GC End Time separately or change the method on the keypad on the front panel of the GC.

Activating a Method

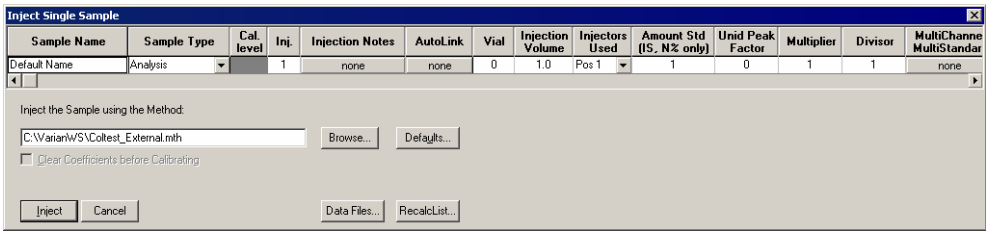
To activate a GC/MS method, use the ***File… Activate Method*** button to see a selection menu. Choose a method and click **Open**. This method will be downloaded to the instrument.

Injecting a Single Sample

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

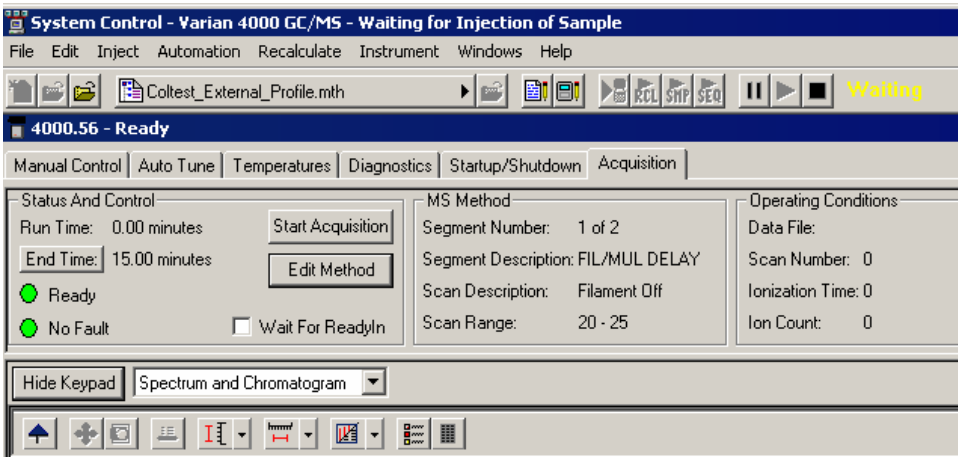


Display the Inject Single Sample dialog by selecting it from the Inject menu command ***Inject… Inject Single Sample***… or by clicking on the **Inject Single Sample** button on the toolbar  . If enabled, an Instrument Parameters dialog will be displayed. Otherwise, the Inject Single Sample dialog box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



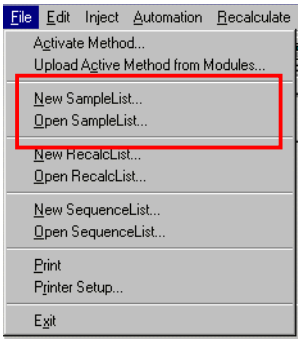
Enter a sample name in the upper left corner. If an AutoSampler is configured, enter the vial number where you have placed the sample. Check that the injection volume and injector used are correct. If you need to change the default values for any parameter, click the **Defaults** button. To create a 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.

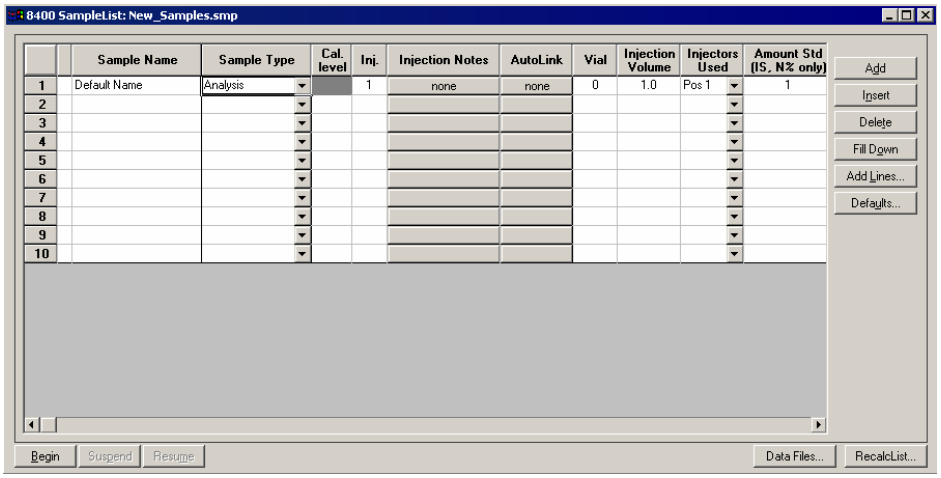


Injecting Using a SampleList

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.



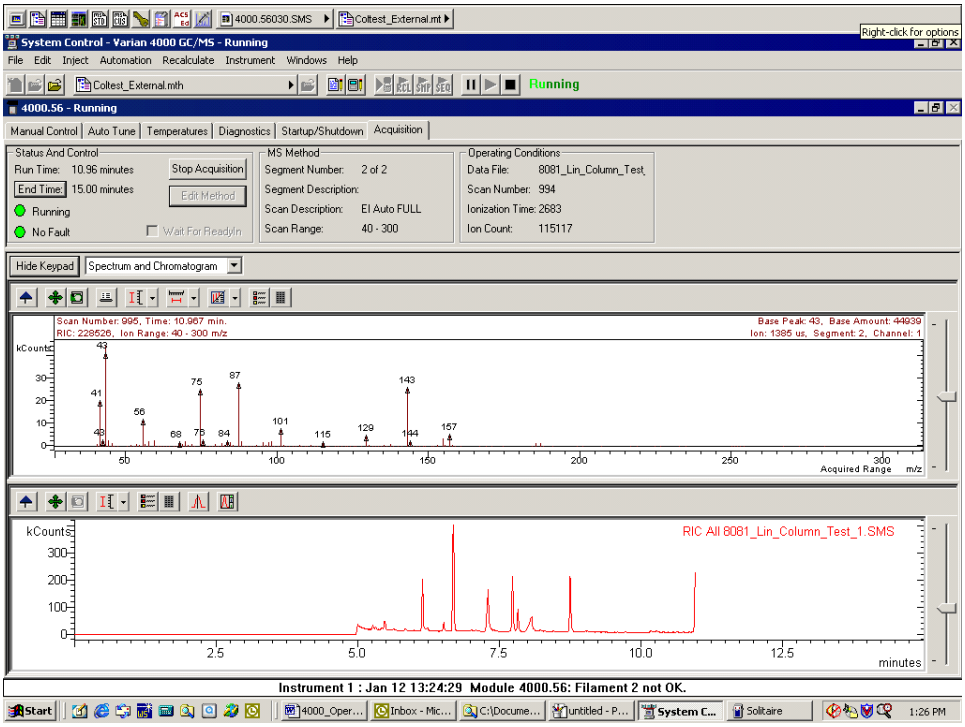
Choose to open either a New SampleList or an existing SampleList from the File menu. The SampleList window for the open SampleList is displayed. It contains fields that are specific to the sampling device configured in the instrument. If a sampling device such as the 8400 AutoSampler is configured, the 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 on data acquisition features, go to the section “Acquiring GC/MS Data” in the *4000 GC/MS Software Operation Manual*.