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4000 MS

4000 MS Users Guide
Internal Ionization

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Contents

Overview of Internal Configuration

The Internal configuration is one of the three operational configurations of the 4000 GC/MS system. In this configuration, it is possible to collect either electron ionization or positive chemical ionization (EI or PCI) data. Unlike the External Ionization configuration, it is possible to collect EI or PCI data during different time segments of a single chromatographic acquisition. However, it is not possible to collect Negative Chemical (NCI) data in Internal configuration. 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 on-line help information on setting up these experiments.

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.

The 4000 GC/MS creates the mass spectrum with an ion trap analyzer. The ion trap confines the ions within a single region where time-dependent electromagnetic fields control ion processes.
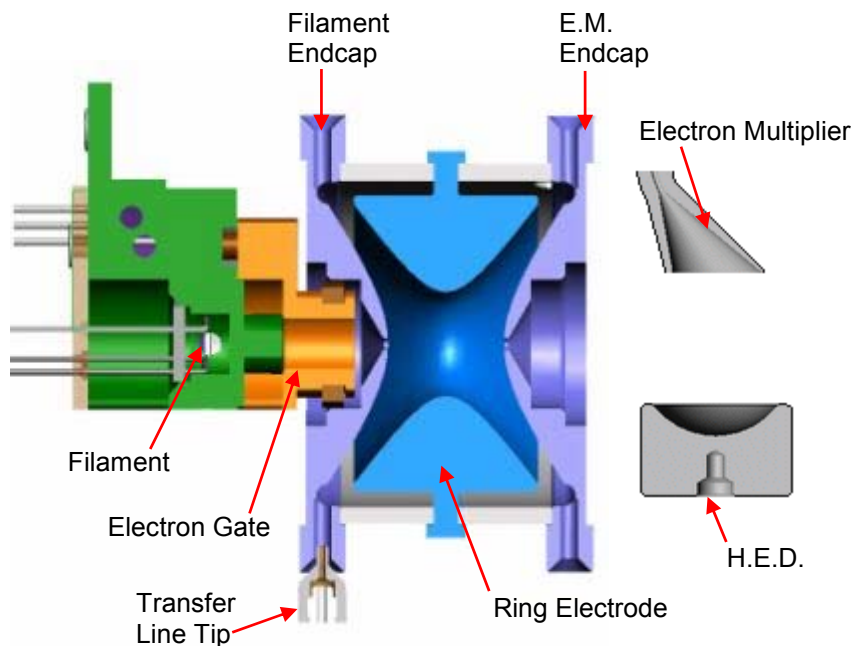
Scan Functions Control MS Processes

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, Electron Ionization (EI) and Chemical Ionization (CI), 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). The diagram below illustrates the RF voltage profile of a typical EI scan function. These scan functions are discussed in more detail in the 4000 GC/MS Software Operation Manual.
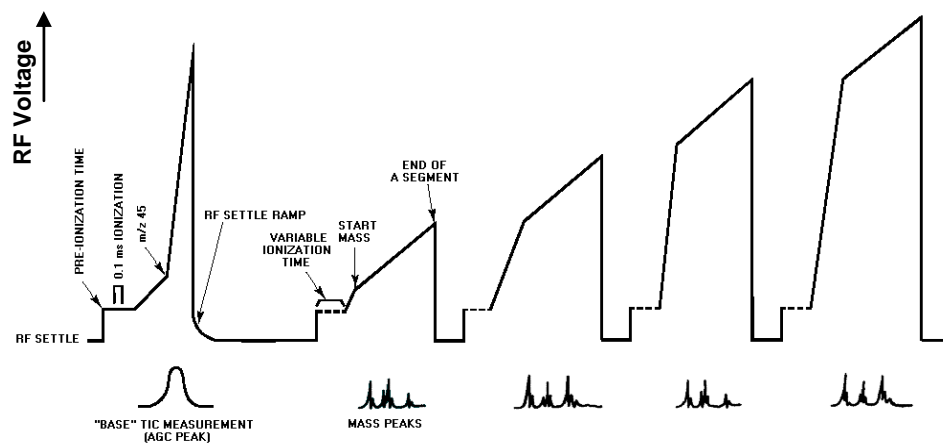
Steps to Create a Mass Spectrum

Sample analysis with an ion trap analyzer may be divided into several steps:

Sample Introduction

Sample components are introduced from the transfer line to the ion trap analyzer through the direct-coupled capillary column.

Ionization

The sample is ionized in the mass spectrometer by one of the following method:

- Electron Ionization (EI): The sample molecules are struck with energetic electrons removing an electron from a molecular orbital, to create molecular ion.

- Positive Chemical Ionization (PCI): A reagent gas is introduced in the ion trap and EI is performed on that gas to form reagent ions. The reagent ions then undergo ion-molecule reactions with the sample molecules to create ions of the sample molecules and their fragments.

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 some of the molecular ions to form various fragment ions and neutrals. Unimolecular decomposition happens in picoseconds, the time scale of a few molecular vibrations, effectively occurring at the same time as ionization.

Ion Storage

The molecular and fragment ions are stored and stabilized in the ion trap cavity, where they travel in defined orbits governed by voltages applied between the ring electrode and the endcap electrodes. Helium buffer gas is also present in the cavity. It buffers the ions into more compact orbits that produce well-resolved mass peaks as they are scanned out. Helium is used as a buffer gas because it is light and does not degrade MS resolution. While helium ions are created in the trap, they are ejected electronically as soon as they are formed.

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

Each of the areas above is discussed in general in this guide, and in detail in the *4000 GC/MS Software Operation Manual.*

About Internal Electron Ionization

Internal electron ionization is the most common mode of operation of the 4000 MS. All of the sample ionization, fragmentation, storage, and scanning steps occur within the confines of the ion trap. Electron ionization generates fragments that are specific to the compound, providing fingerprint spectra. It is, however, not selective and will show ions for all compounds present. EI is sensitive but the sensitivity can be improved by reducing the interfering ions using ion preparation techniques.

Forming Ions
In the EI mode, electrons are gated into the ion trap cavity during the ionization period. These electrons collide with the neutral sample (or Analyte) molecules A, removing an additional electron to create 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^+. This set of fragment ions, characteristic of the sample molecule, makes up the mass spectrum.

$$A + e^- \rightarrow A^{+*} + 2e^- \qquad \text{Ionization } (A^{+*} \text{ is excited})$$

$$A^{+*} \xrightarrow{\text{He}} A^+ \qquad \text{De-excitation}$$

$$A^{+*} \rightarrow f_1^+ + n_1 \qquad \text{Equilibration}$$
$$\rightarrow f_2^+ + n_2$$
$$\rightarrow f_3^+ + n_3 \qquad \text{Fragmentation}$$
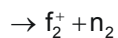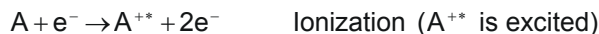
The ion trap has a maximum storage capacity beyond which mass resolution and spectral quality deteriorate. The number of ions created depends on the ionization time. As the ionization time increases, more ions are created. Automatic Gain Control (AGC) controls the ionization time to always create an 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.

Ion Preparation Options

The 4000 MS can use a combination of waveforms applied to 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 Spectrometry (MS/MS) can be performed on the ions stored in the ion trap before mass analysis takes place. 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. In MS/MS, a parent ion is isolated and then dissociated by energetic collisions with helium buffer gas to form product ions.

The Internal configuration can have SIS, MS/MS, MS^n, and Multiple Reaction Monitoring (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. The ions are analyzed by applying a radio frequency (RF) voltage to the ring electrode encircling the trap cavity. As the voltage increases on the ring electrode, ions are sequentially ejected from the trap according to their mass-to-charge ratio. Supplemental waveform voltages are applied to the endcap electrodes during the analysis to improve mass resolution and mass axis stability. Ions strike the conversion dynode and then electrons are ejected from the conversion dynode, held at -10 kV, and are repelled to the electron multiplier. The signal is amplified 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 internal EI. First there is a prescan to count the number of ions formed in a short fixed ionization 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 ionization 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 and Data Handling

The mass spectra collected from the MS are analyzed through Varian MS Data Review. 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 Internal Chemical Ionization

Chemical ionization (CI) provides mass spectral data that complement electron ionization (EI) data for the analysis of complex compounds. The generation of ions in chemical ionization is a two-step process. A CI reagent gas is introduced into the ion trap analyzer. The reagent gas is ionized by EI. Then, the sample molecules are ionized by ion-molecule reactions with the reagent gas ions.

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.

Forming Reagent Ions

In the first step, reagent gas ions are formed as the reagent gas is ionized by interaction with electrons emitted by the filament. The ion trap operates in a pulsed mode. The supply of reagent ions is created during the ionization pulse and consumed during the reaction period to form analyte ions.

In internal mode, the CI reagent can be either liquid or gas. The most common reagents are methane, methanol, acetonitrile, and isobutane.

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:

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

$$CH_4 + e^- \rightarrow CH_3^+ + e^- + H^-$$
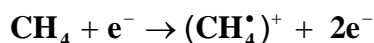
These primary ions then react very rapidly to form predominantly the secondary ions, CH_5^+ and $C_2H_5^+$:

$$(CH_4^{\bullet})^+ + CH_4 \rightarrow CH_5^+ + CH_3^{\bullet}$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$
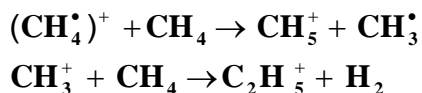
Chemical Ionization Reactions in PCI

In the second step, the reagent gas ions react with sample molecules in the ion trap to form sample ions. The four principal reactions between reagent gas ions and sample molecules are as follows:

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

(B) Hydride abstraction: $R^+ + M \rightarrow [M\text{-}H]^+ + RH$

(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 the major reaction, and hydride abstraction (B) 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) or (M-1) ions is possible even if the EI spectrum of the same component shows no molecular ion. The exothermicity of the reactions determines the amount of energy deposited; therefore the degree of fragmentation can be controlled by the choice of a suitable CI reagent gas. The proton affinities of some common reagent gases of this type, known as proton transfer agents or Bronsted acids, range from 130 kcal/mol to 200 kcal/mol in the following order: methane, water, isobutane, and ammonia (with ammonia resulting in the "softest" ionization). Among the common liquid CI reagents, methanol has a proton affinity of 180.3 kcal/mol, while acetonitrile is 1862 kcal/mol. By choosing a suitable reagent gas, you can obtain high specificity (i.e., less efficient detection of background or matrix interferences compared to the analyte) as well as molecular weight information for the compounds of interest.

Association reactions (C) typically have very low reaction rates, and the reaction products require rapid collisional stabilization. The products of these association reactions are called adduct ions because the reagent ion has been added to the analyte. They are typically seen on the 4000 GC/MS in Internal configuration at much lower abundance then the (M+1) ion, but when (M+29) and (M+41) adduct ions are observed using methane, they are useful for verifying the molecular weight.

The charge transfer reaction (D) produces a radical molecular ion (i.e., an ion with an odd number of electrons) that dissociates quickly, giving EI-like spectra. However, the energy deposited in the molecular ion and the resulting fragmentation pattern does not depend on the electron energy of the ionizing electrons.

Ion Preparation Options

The 4000 MS uses a combination of waveforms and RF to isolate, or remove, specific ions after they are formed and are stored in the trap. 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.

Scanning Ions to Collect Mass Spectra

The scanning process for chemical ionization is similar to that 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 -10,000 V, and repelled to the electron multiplier. In negative mode, positive ions are ejected from the conversion dynode, held at +10,000 V, and repelled toward the electron multiplier. The signal is amplified 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 is no prescan in internal PCI; the ionization time is calculated based on the base peak intensity of the previous scan. Ions are formed for this ionization time and the analytical scan is carried out.

Library Searching

The 4000 MS has small CI libraries generated using internal configuration ion trap GC/MS systems. The libraries are organized by the CI reagent, which include methane, methanol, and isobutane.

Selectivity Considerations

Type of Matrix

An advantage of chemical ionization is selectivity. In PCI, hydrocarbons have a poor response in methane CI. It is easier to locate target compounds in a sample contaminated with hydrocarbons using methane PCI than using EI. Because of these selectivity considerations, the time spent to develop a method using the different ionization and ion preparation options is well spent.

Using EI and PCI to Get More Information

Generating EI and CI data on a single sample gives both the ion-intensity fingerprint information allowing library searching as well as the molecular weight information to confirm species identification. Fatty acid methyl ester (FAME) analysis is an example. Under EI conditions, the FAME fragmentation is extensive and molecular ion intensity is weak. Using CI, the molecular weight is obvious and the most intense ion is M+1.

Because the 4000 MS is able to switch between EI and CI in a single run, the best analytical conditions can be used for a given compound.

NOTE: Wait several seconds to switch between EI and CI. The CI segment should not be less than 60 seconds wide and the CI peak should be at least 20 seconds into the segment to allow CI reagent stabilization.

Conversion from External to Internal Configuration

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

See the *4000 GC/MS Hardware Operation Manual* for details.

Checklist

- Remove the analyzer from the MS manifold.
- Change the ion source to internal.
- Move the heat shield to the forward position.
- Add the filament adapter and connect the flex cable.
- Change the transfer line orientation.
- Change the transfer line tip.
- Cut the column to 7 mm.
- Change the transfer line switch to Internal/Hybrid.
- Replace the analyzer in the MS manifold.

Conversion from Hybrid to Internal Configuration

Changing from the Hybrid to Internal configuration only requires changing the ion source. The column position and transfer line are already directed to the trap and do not need to be adjusted.

Outline

1. Change the ion source.
2. Move the heat shield to the forward position.
3. Add the filament adapter and connect the flex cable.

Effects of Hardware Configuration Changes

After changing the configuration, for example from External to Internal configuration, the following occurs when System Control is restarted.

1. System Control compares the current configuration stored in the current Module Attributes with the configuration reported by the hardware.
2. 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).
3. After making the hardware configuration change, new methods will have the appropriate instrument configuration by default.

NOTE: Presetting the Module Attributes requires performing all Auto Tune routines, because the prior Auto Tune results are invalid.

Starting the Instrument
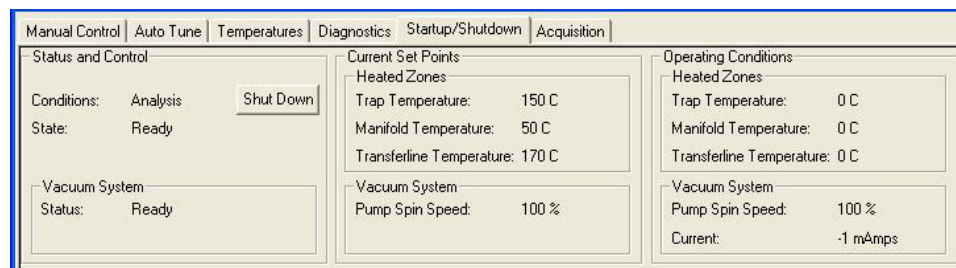
Initial Pump down

Check the following:

- Check the Vacuum connections.

- Make sure the transfer line is in.

- Make sure the vent valve is fully closed.

- 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 do the following.

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.

3. Check that the vent valve is sealed.

Open **System Control** and the **Startup/Shutdown** page opens.

Manual Control	Auto Tune	Temperatures	Diagnostics	Startup/Shutdown	Acquisition

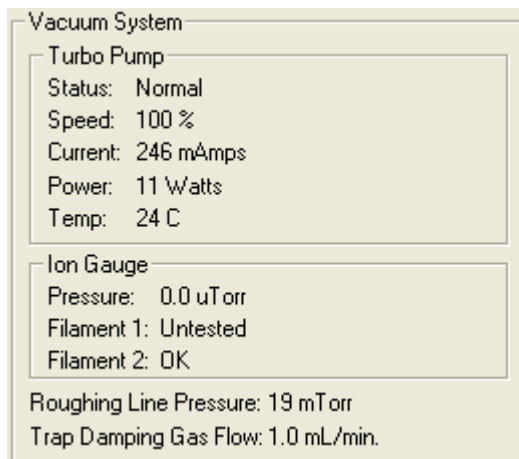| Status and Control		Current Set Points		Operating Conditions	
		Heated Zones		Heated Zones	
Conditions:	Analysis	Shut Down	Trap Temperature:	150 C	Trap Temperature:	0 C
State:	Ready		Manifold Temperature:	50 C	Manifold Temperature:	0 C
			Transferline Temperature:	170 C	Transferline Temperature: 0 C
Vacuum System		Vacuum System		Vacuum System	
Status:	Ready		Pump Spin Speed:	100 %	Pump Spin Speed:	100 %
					Current:	-1 mAmps

Check the Vacuum Status

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 are indicated by a turbo speed less than 100%. Small leaks are indicated by an increase in the pump current after 100% is reached, 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 in the method Service.mth. For more detail on troubleshooting leaks, see the Troubleshooting section in the 4000 GC/MS Hardware Operation Manual.

Vacuum System
Turbo Pump
Status: Normal
Speed: 100 %
Current: 246 mAmps
Power: 11 Watts
Temp: 24 C

Ion Gauge
Pressure: 0.0 uTorr
Filament 1: Untested
Filament 2: OK

Roughing Line Pressure: 19 mTorr
Trap Damping Gas Flow: 1.0 mL/min.

Diagnostic Tests

Monitor the current state of the instrument using the Monitoring tab. Monitor the vacuum system, the electron multiplier, the waveform system, temperatures, and the ion source.
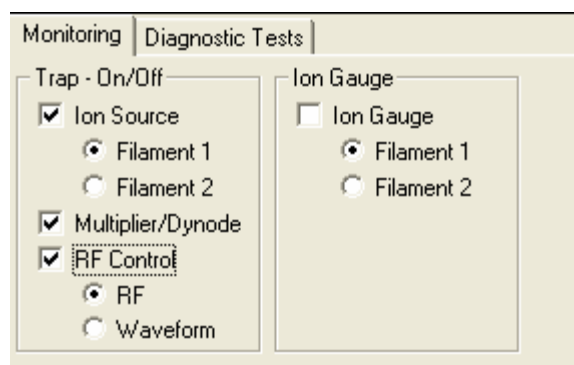
Perform hardware checks on the 4000 MS using the Diagnostics tab. For more details on the diagnostic tests, seethe *Diagnostics* section in the *4000 MS Ion Trap Software Operation Manual.*
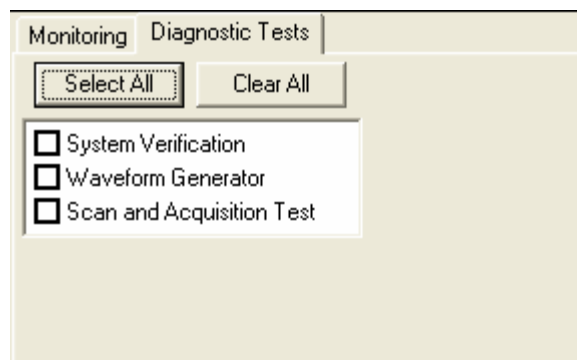
Setting System Temperatures

Analysis Temperatures

Analysis temperatures are sample dependant. The stability of the compounds and their volatility affect the temperature choice. For species that are more fragile, decrease the trap temperature. For example, to obtain molecular ion information on saturated hydrocarbons, which have unstable molecular ions, set the trap temperature to 80 °C. However for semi-volatile analysis set the trap temperature to 220 °C so the heavy PAHs (benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) do not tail.

After changing the temperature setting, allow about one hour for the trap to reach the new setting. After the setting is reached, allow about two hours for the trap to stabilize. The temperature of the trap affects mass calibration, and therefore the trap frequency calibration.

Set the transfer line temperature so there are no cold spot between the GC column oven and the MS. This is typically about the maximum column temperature (± 20 °C).

The manifold temperature (typically 50 °C) reduces the effect room temperature variation may have on the system.

System Bakeout

To remove water adsorbed on the manifold while the 4000 MS was vented, perform a Bakeout from the Temperatures tab in System Control.

Perform a system bakeout to remove chemical background after running samples. After doing a bakeout to remove contamination, decrease the temperature to the analytical temperature.
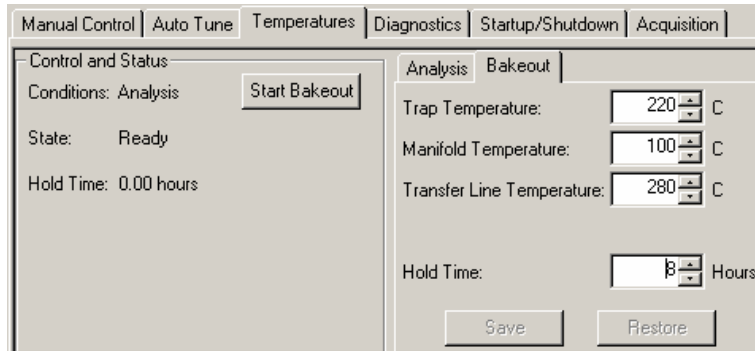
Typical bake-out settings are:

- Trap Temperature   230 °C
- Manifold Temperature   110 °C
- Transfer Line Temperature   280 °C
- Bakeout time   12 hr

NOTE: You can use shorter bakeout times.

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

Startup and Shutdown

Starting the System

When the system is first turned on, System Control only operates in Startup/Shutdown mode. During system startup, observe the increase in Pump Spin Speed in the Operating Conditions field. The software is 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.

NOTE: Failure to reach 100% pump speed in a reasonable time indicates a vacuum leak and corrective action should be taken. For details, see the appropriate Troubleshooting section in the 4*000 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 Startup/Shutdown dialog. The heaters will be turned off and the speed of the turbo pump will be gradually reduced to 35% of full speed.

After the temperature zones have cooled below 80 °C, turn off the main power by placing the switch at the rear of system into the OFF (down) position. Manually vent the system for at least 5 minutes by opening the vent valve on the front panel one full turn counterclockwise.

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.
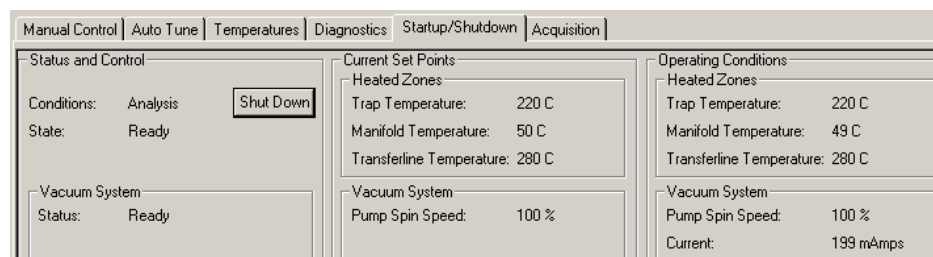
Adjustment and Tuning of the MS

Checks and Adjustments

RF Tune

Adjust the RF tuning in the Checks and Adjustments tab dialog of Manual Control after performing one of the following:

- Performing MS maintenance.

- Changing the analyzer assembly.
- Changing the MS configuration.

Adjusting the RF Ramp

Adjusting the RF Ramp

To adjust the RF ramp, do the following:

1. Click RF Ramp Adjustment in the Checks and Adjustments tab.

2. Click **Start**.

3. 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 be just below OK.

Adjusting the Calibration Gas

To adjust the calibration gas, do the following:

1. Check the flow of perfluorotributylamine (PFTBA or FC-43) calibration gas before doing Auto Tune procedures.

2. Click **Cal Gas Adjustment** in the **Checks and Adjustments** tab in Manual Control.

3. Turn the Cal Gas valve inside the front door of the 4000 MS either clockwise to decrease the flow or counterclockwise to increase the flow. Adjust the flow so that the status bar in the Adjustment Results field reads OK.
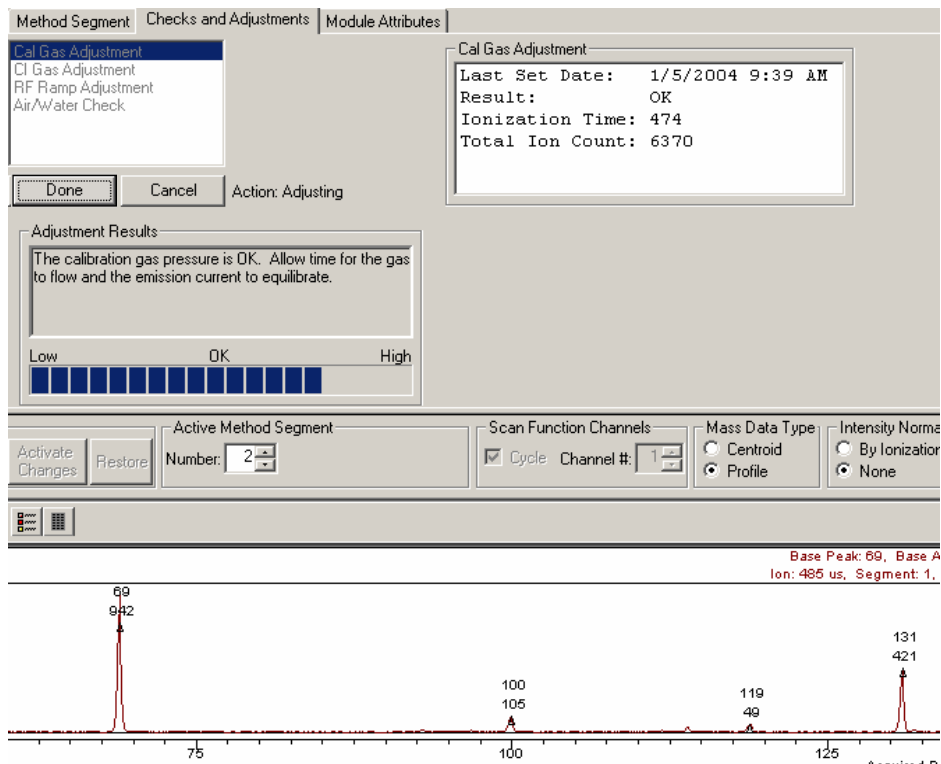
CI Gas Adjustment

Before performing chemical ionization, adjust the reagent gas pressure. The next section explains the set up of the most common reagent gases.

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, not the manual setting. After replacing the electron multiplier, auto tune of Electron Multiplier **before** performing the Air/Water Check.

Setting Up CI Reagents

Before running CI experiments, adjust the CI gas pressure.

To adjust the CI gas pressure, do the following:

1. Click the **Checks and Adjustments** tab of the Manual Control window.

2. Select **CI Gas Adjustment**. Select your CI reagent from the drop down box in the Parameters section.

3. Click **Start** below the adjustments selection.

4. The criteria for adjusting the flow of CI reagent into the instrument are shown.

5. After completing this, you must perform a mass calibration and trap function calibration before beginning your experiments.
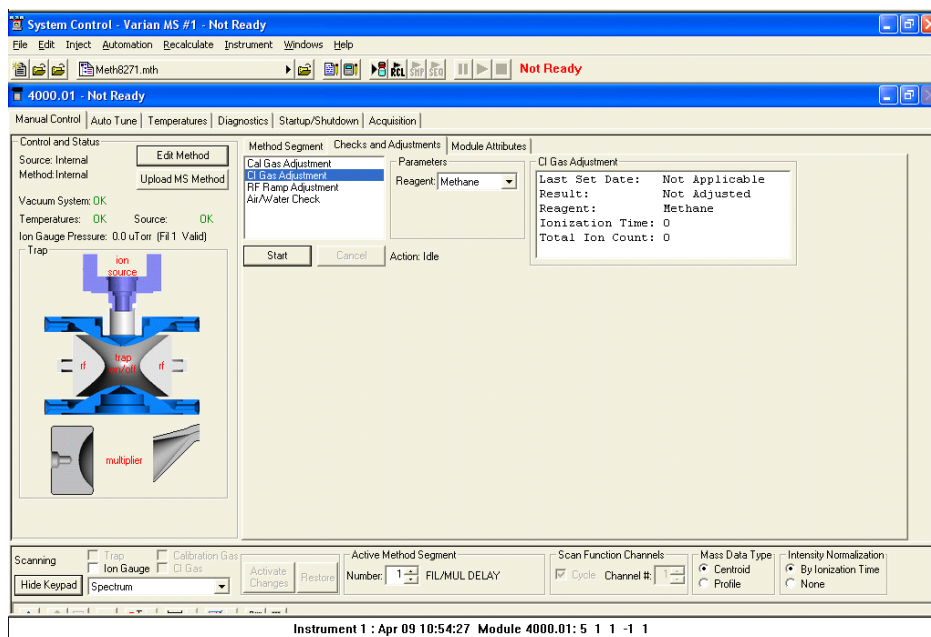
Installing a Liquid Reagent

To connect a liquid reagent reservoir to the liquid reagent inlet block, do the following:

1. Connect a liquid reagent reservoir containing the chosen liquid to the liquid reagent inlet block.

2. Open the CI needle valve 6-7 turns clockwise.

3. Click **CI Gas Adjustment** to open the CI gas valve solenoids.

4. Allow the vapor flow from the reservoir to equilibrate. If, after several minutes, there is not enough CI gas entering the trap, open the needle valve (clockwise).

5. While observing the spectrum, use the Adjust CI Gas, and turn the CI needle valve to increase or decrease the amount of reagent entering the trap until the resolution between M and M+1 just starts to degrade.

Details for the three most common reagents can be found under the following sections in the *4000 GC/MS Software Operation Manual*: PCI with CH_4, PCI with CH_3CN, and PCI with CH_3OH.
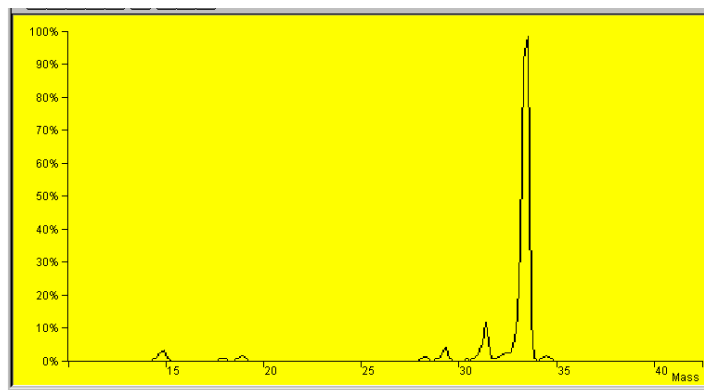
Properly Adjusted Methanol Reagent Spectrum
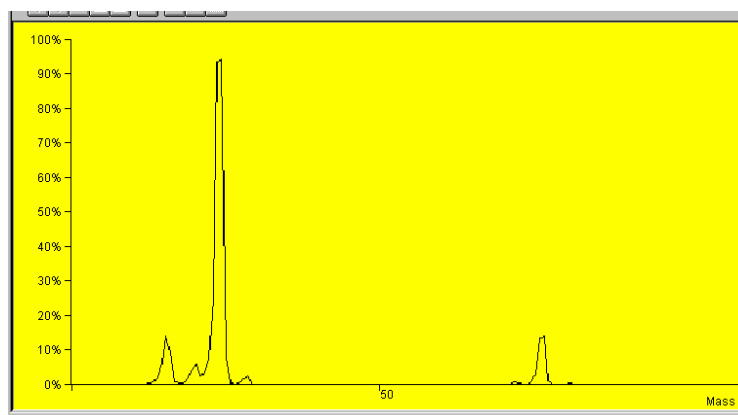
Properly Adjusted Acetonitrile Reagent Spectrum

NOTE: For best results with acetonitrile, use a filament emission current of at least 20 μA and maintain at least 50% valley between m/z 41 and m/z 42.

Installing a Gaseous Reagent

To install a gaseous reagent, do the following:

1. Connect the regulator of the gas cylinder to the back of the instrument using a 4 mL/min restrictor.

2. See the *4000 MS Ion Trap Hardware Operation Manual* for more details.

Auto Tune

Depending on your configuration and settings, you may not see all the available Auto Tune routings. Perform auto tune after 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.
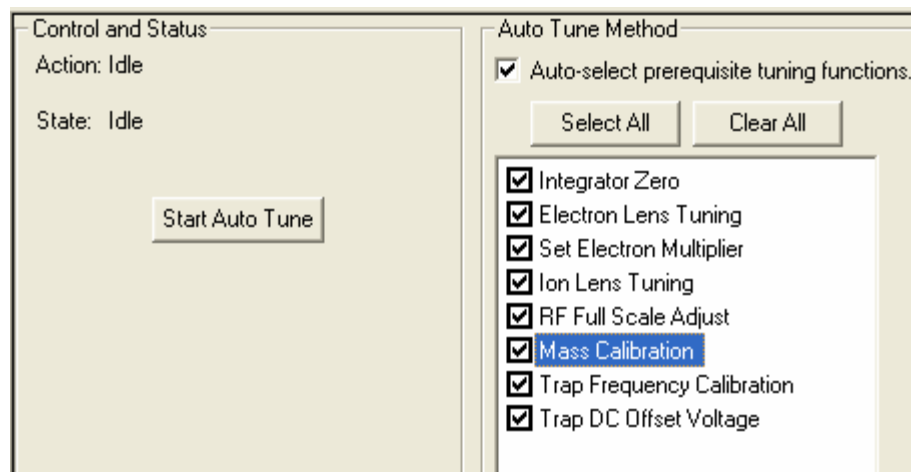
Integrator Zero

Integrator Zero obtains the average value of the signal level from the integrator circuitry when the filament is off. When the filament is off, the major source of signal coming is electronic noise. The integrator zero is adjusted so electronic noise does not create artificial ions, which may create a measurable signal.

Set Electron Multiplier

Set Electron Multiplier determines two 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

RF Full Scale Adjust 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

Mass Calibration 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; **do not run this procedure 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 are changed. Mass calibration does not have to be performed again after the auxiliary Helium 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.

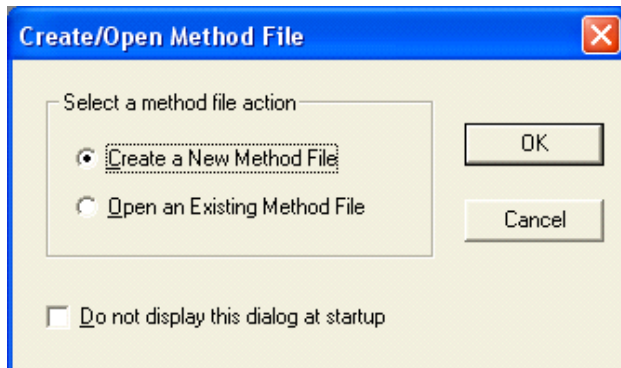### Trap DC Offset Voltage

The trap DC offset is adjusted by this routine to optimize the ion signal for m/z 414 in the calibration gas.

# Preparing an MS Method for Data Acquisition

Building a New MS Method

From the Workstation Toolbar, open the Method Builder .

The 'Create/Open Method File' dialog will be displayed. You may choose to create a new method or open an existing method. If you do not need to see this dialog again, check the box labeled 'Do not display this dialog at startup.'
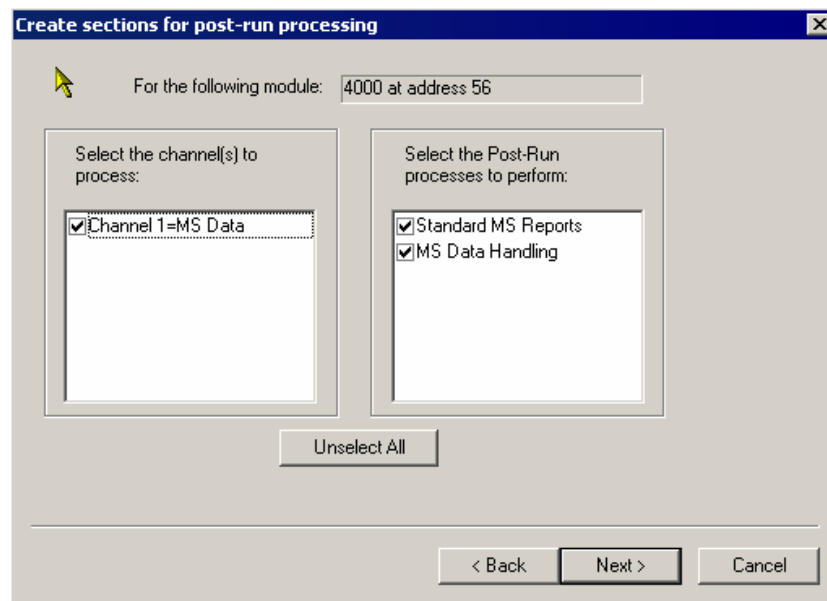
Select the instrument. You can build an offline method by selecting the Custom configuration and selecting the desired modules.
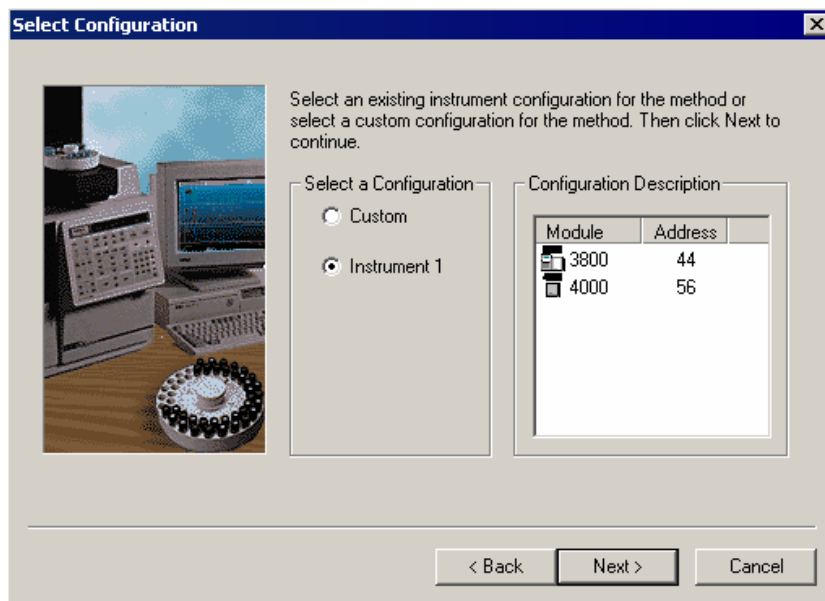
Select which detectors (MS and/or GC) will have data handling.
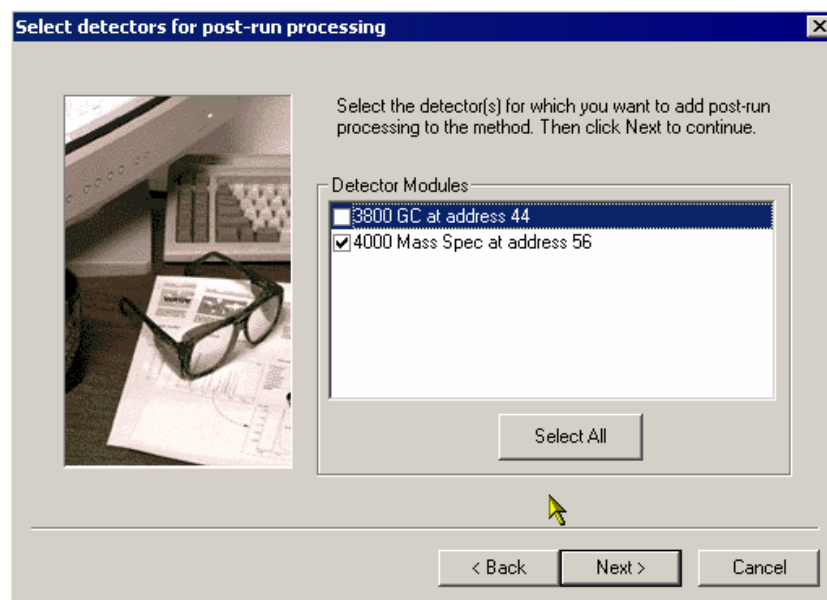
Select what data analysis sections are required; Standard MS Reports and/or MS Data Handling.
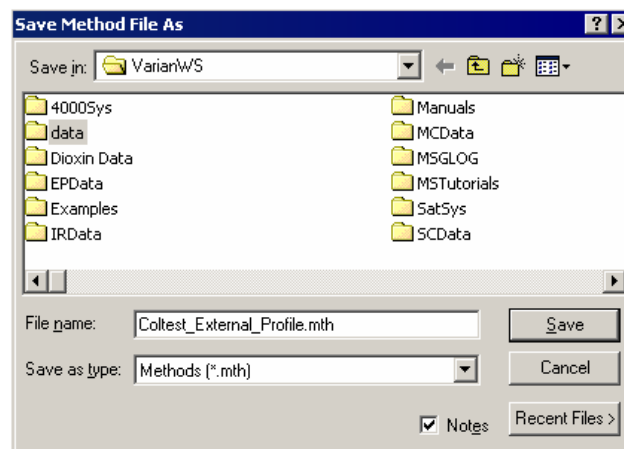
The method will contain the following sections: 3800 GC Control, 4000 MS Control, Standard MS Reports and MS Data Handling.
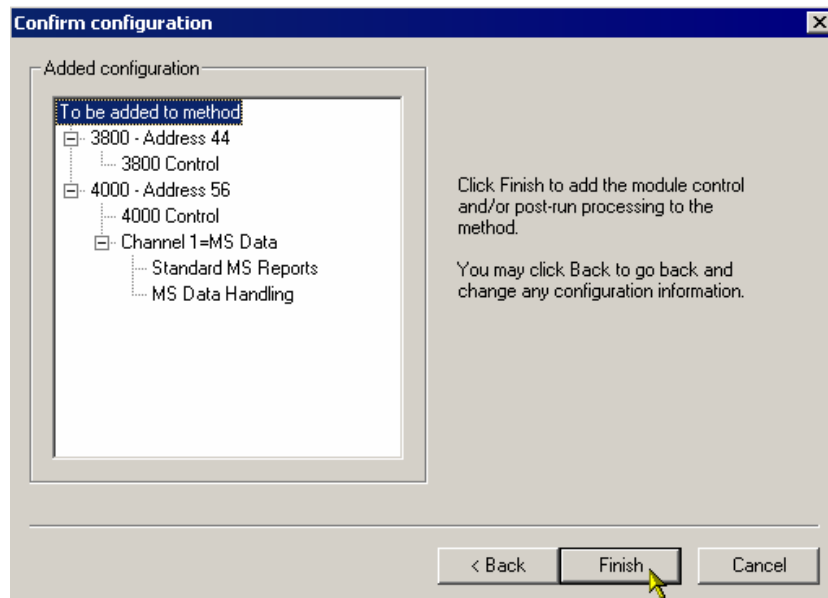
Name the Method

Use the command *File… Save As*. Enter a name for the method and save it, either in the default folder or another folder you select.

Set the 4000 MS Instrument Configuration

The GC and the MS will be set to the configuration of the instrument connected to the Varian MS Workstation. For the 4000 MS Internal configuration, both EI and CI can be performed during the same run and so there is only one internal setting.
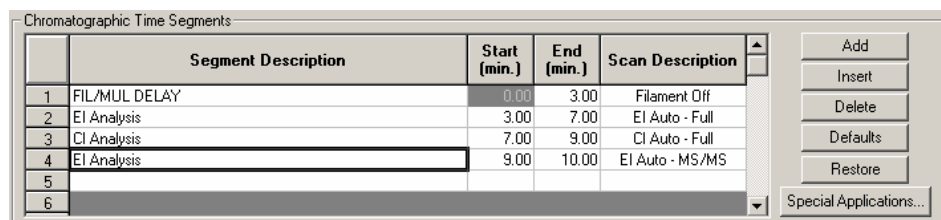
Select the Acquisition Data Type

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 pts/mz 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

When the 4000 MS is in Internal Configuration, it can do EI, CI, EI/MS/MS, and CI/MS/MS in a single run. 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, you could just acquire the mass spectra in full-scan with a single analysis segment. However, you 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.

	Segment Description	Start (min.)	End (min.)	Scan Description
1	FIL/MUL DELAY	0.00	3.00	Filament Off
2	EI Analysis	3.00	7.00	EI Auto - Full
3	CI Analysis	7.00	9.00	CI Auto - Full
4	EI Analysis	9.00	10.00	EI Auto - MS/MS
5				
6				

*Chromatographic Time Segments Table*

Adding or inserting a segment copies all 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

For advice on performing Internal CI, see the appropriate section in the *4000 GC/MS Software Operation Manual.*

Scan Function Settings

The 4000 MS method segments control what MS scan function is performed. The different scan functions in the Internal configuration are EI and CI, full scan, MS/MS, Multiple Reaction Monitoring (MRM), or Selected Ion Storage (SIS).
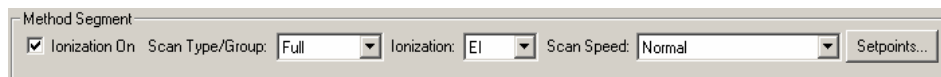

Scan Function selection

The 4000 MS has three scan modes - normal speed (5,000 u/sec), fast speed (10,000 u/sec), or fastest (10,000 u/sec without a prescan). The Fastest speed only allows acquisition with single-segment mass ranges.
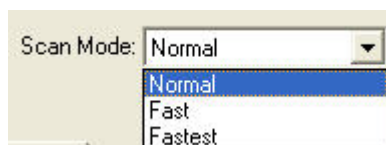
Mass Range	Tune	µScans	Normal	Fast	Fastest
50 – 1000	1 Segment	3	0.76 sec	0.47 sec	0.41 sec
50 – 1000	4 Segment	3	1.08 sec	0.79 sec	N/A
50 – 400	DFTPP	3	0.70 sec	0.59 sec	N/A

General Parameters Tab
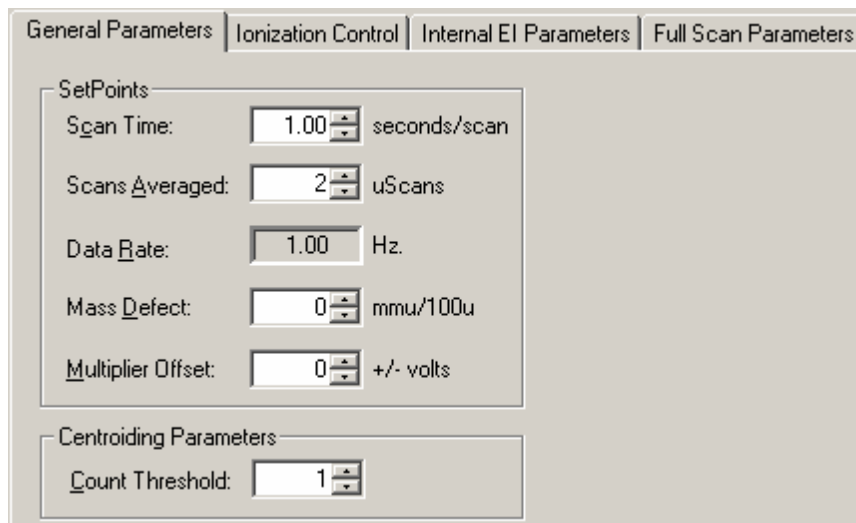
Scan Time, **Scans Averaged**, and **Data Rate** are all linked. The number of scans averaged is updated when the scan time is adjusted and vice versa. The best way to set the scan time is to set the mass range from the Scan Parameters tab and then change the scans averaged to three. Three scans averaged 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
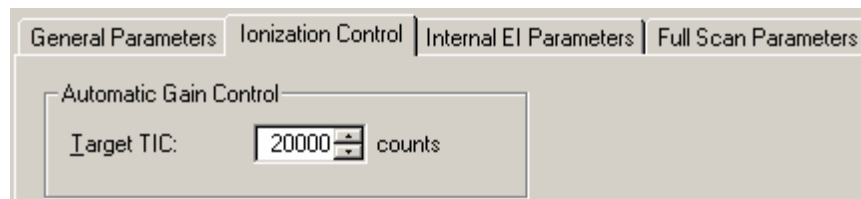
from the fact that the NIST library reports molecular weights to the nearest integer mass unit only. The Varian MS Workstation software must decide to which mass to assign measured intensity. If the exact mass of an ion happens to fall close to the dividing line between integer masses, the software may 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 be assigned as either 497 or 498.

The **Multiplier Offset** adjusts the EM voltage as by 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.

General Parameters	Ionization Control	Internal EI Parameters	Full Scan Parameters

Automatic Gain Control

Target TIC: 20000 counts

EI Ionization Parameters

The **Emission Current** is the current of electrons produced by the filament and it controls the number of electrons that enter the trap. By increasing the emission current so that the ionization time of the baseline is approximately the maximum ionization time, the maximum number of ions will be present. In certain cases, a high emission current can be used to increase the sensitivity of the analysis. An example of this is in EI/MS/MS where the MS/MS isolation step eliminates the higher background that would normally arise from a high emission current.
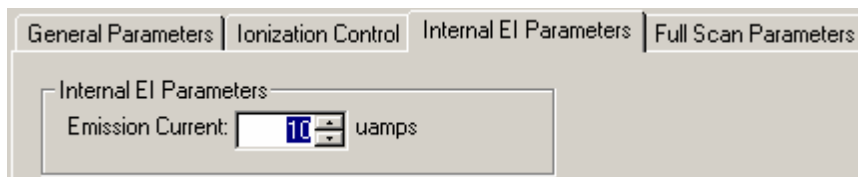
Setting Parameters for Chemical Ionization

After selecting a standard reagent (methane, isobutane, acetonitrile, or methanol), the CI parameters for the selected reagent are set automatically so you do not need to change the default values of the CI parameters. However, the remaining CI parameters (Reaction Storage Level, Ejection Amplitude (V), and Max Reaction Time) can be optimized for sensitivity. After adjusting these parameters, click **Save**. You will be prompted for a name under which to save the modified values.

Reagent Low Mass is the lowest mass stored in the trap during ionization.

Reagent High Mass is the highest mass stored in the trap during ionization. All masses above this mass are ejected during the ionization step. This reduces the number of EI generated ions that are in the trap.

The combination of **Reagent Low** and **Reagent High Mass** and **Reaction Storage Level** allows for the selection of a specific reagent ion in the CI process. For example, only mass 29 of methane could be used as the reagent ion by ejecting mass 41 during ionization and by reacting at a mass greater than 19 in the reaction step.

NOTE: When a reagent ion that is formed through a complex process, such as $\mathbf{C_2H_5^+}$ formed by the reaction of $\mathbf{CH_4^+}$ with neutral $\mathbf{CH_4}$, both ions must be within the High and Low mass range.
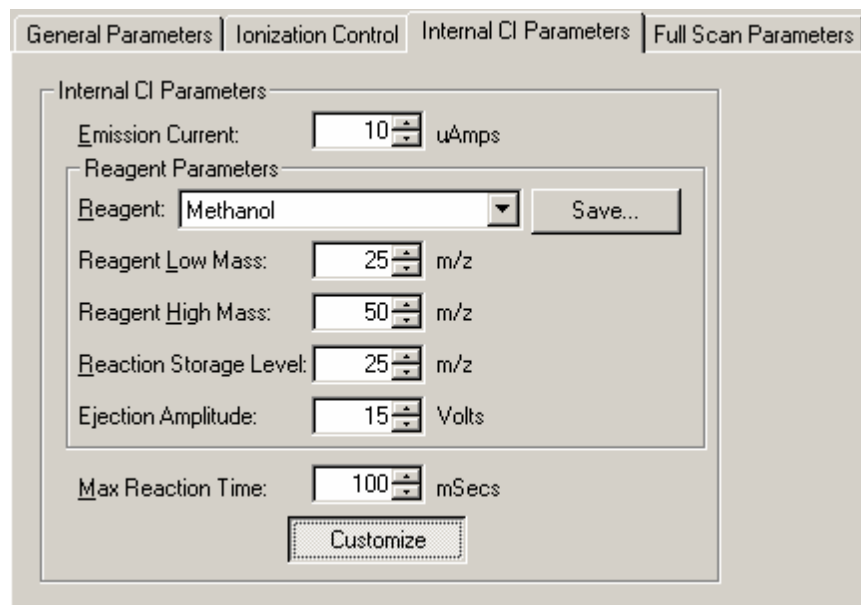
Reaction Storage Level is the value of the smallest mass stored in the ion trap during the reaction period. The optimum Reaction Storage Level will depend on

the molecular ion of the analyte. Generally, one should use higher Reaction Storage Levels for higher molecular ions, without raising the storage level to cause ejection of CI reagent ions. For example, the molecular ion of the analyte is 352 m/z. Using acetonitrile, raising the RF storage level to 25 m/z may give better sensitivity than the default of 19 m/z. If the CI storage level is raised, the Ejection Amplitude will have to be increased.

Ejection Amplitude (V) is a voltage that corresponds to a low mass ejection cutoff that is slightly higher than the Reagent High Mass. This voltage actively ejects unwanted ions (i.e., not reagent ions) that are produced during ionization. Generally, higher CI storage values require higher ejection voltages. The voltage should not be set so high as to cause the ejection of CI reagent ions.

Max Reaction Time is the maximum time that reagent gas ions are allowed to react with sample molecules to form ions. The maximum reaction time can be set to any value from 1 to 2000 milliseconds. The typical reaction time is 100 milliseconds

For details on how to optimize these parameters, or on how to set up for non-standard reagents, refer to the 4000 GC/MS Software Operation Manual.

NOTE: Be aware that if you do change a CI parameter and set it incorrectly, you may not be able to operate properly in CI.

Full Scan Parameters

Each MS scan type has different parameters that need to be specified. The following are examples of the two most common scan types used in the Internal configuration; Full Scan and MS/MS. Detailed information on these, and the other scan function parameters can be found in the *4000 GC/MS Software Operation Manual*.

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. This is the most common scan type for the 4000 MS. The mass range also determines the scan range of the AGC calculation.

The Tune field specifies how the mass range is scanned. There are three specified tune types; Auto, DFTPP, and BFB. Under the Auto tune type each EI scan is divided by default into four mass segments: 10 to 99 m/z, 100 to 249 m/z, 250 to 399 m/z and 400 to 1000 m/z. Under these conditions, the **RF Storage Level (m/z)** and the **Ionization Time Factor (%)** 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**. This is the RF voltage used to hold ions in the trap during the ionization period and is specified in mass units. It affects ion storage in two ways C the storage efficiency of higher mass ions increases as the level increases, while lower mass ions are not stored if their mass falls below the cutoff. 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.

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.
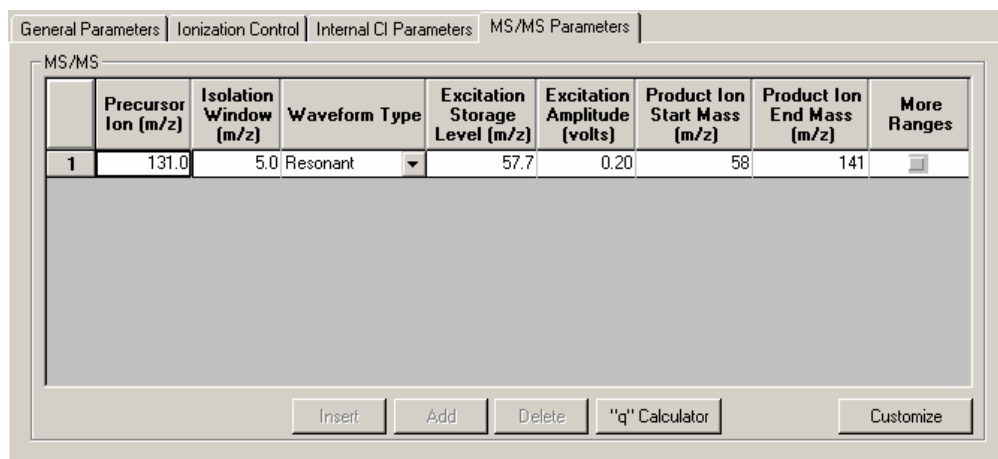
When properly designed, an MS/MS method will:

- 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 of unknowns except to the degree one is determining a set of isomers of a given class such as PCBs or Dioxins.

	Precursor Ion (m/z)	Isolation Window (m/z)	Waveform Type	Excitation Storage Level (m/z)	Excitation Amplitude (volts)	Product Ion Start Mass (m/z)	Product Ion End Mass (m/z)	More Ranges
1	131.0	5.0	Resonant ▼	57.7	0.20	58	141	☐

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, seethe Tandem Mass Spectrometry section in the 4000 GC/MS Software Operation Manual.

Viewing Method Parameters in Manual Control

After a method is created in Method Builder, it can be previewed in Manual Control. All MS parameters can be edited and previewed before a run. However, the number of segments, or the start and end times of existing segments, can only be changed in Method Builder.

Activating a Method

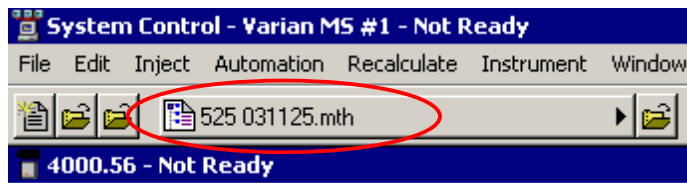To activate a different method, do the following:
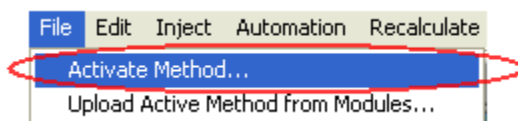
1. Click the **File** menu.

2. Click **Activate Method**.

3. Select a method by doing one of the following:

   - Click **Recent Files** to display the eight most recent methods.

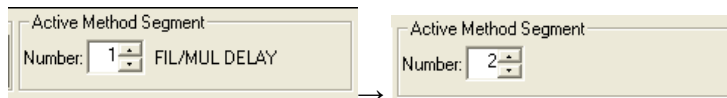   - Click **Open** after selecting a method from a folder.
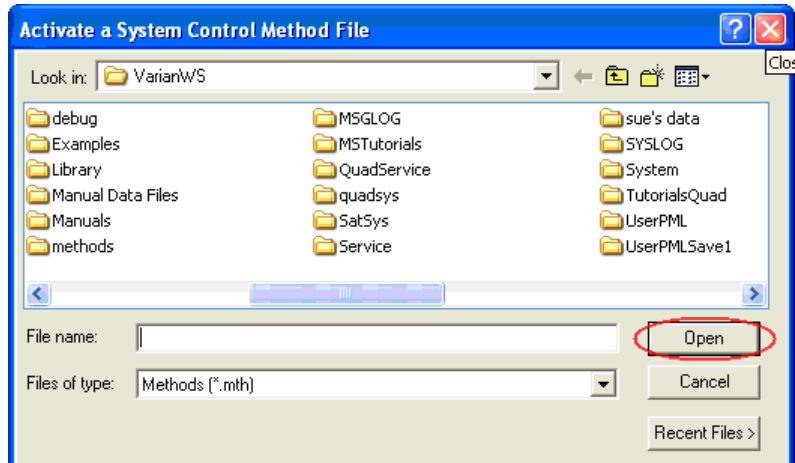
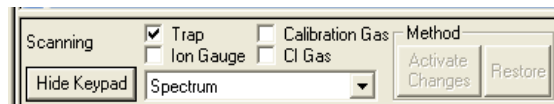4. The active method is displayed in the toolbar.

Displaying Ions

To view ions do the following:

1. Select an ionization segment in which the ionization is on. 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:
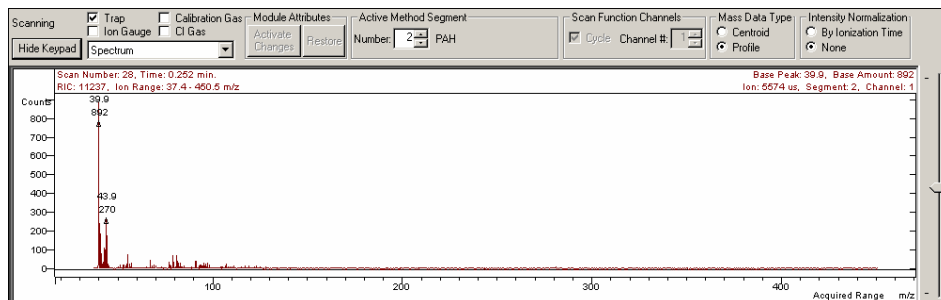

2. Click the Trap check box to turn on the ion trap.

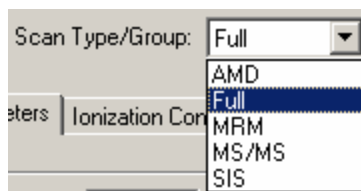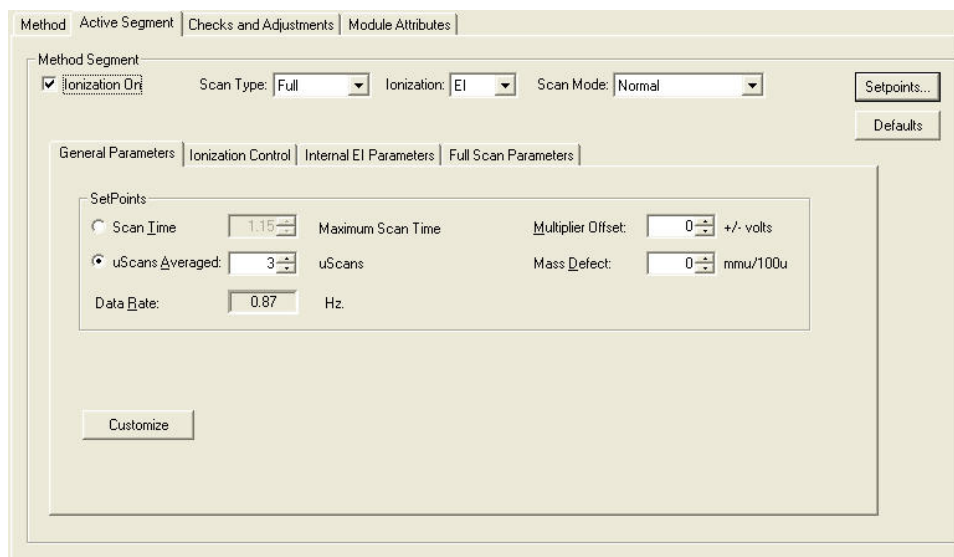3. Select the method segment to view. Turn on the calibration gas or CI gas by selecting the check box.

Viewing Method Parameters

In the following figure, the Method Segment tab dialog is shown with method related controls in the lower pane.

The information in the top row of the Method Segment tab indicates if the trap is on, the Scan Type, Ionization mode, and Scan Mode.

Editing a Method in Manual Control

Examine and edit all the parameters in the active MS method and observe the changes on the mass spectra 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, implement the change by clicking the **Activate Changes** button.
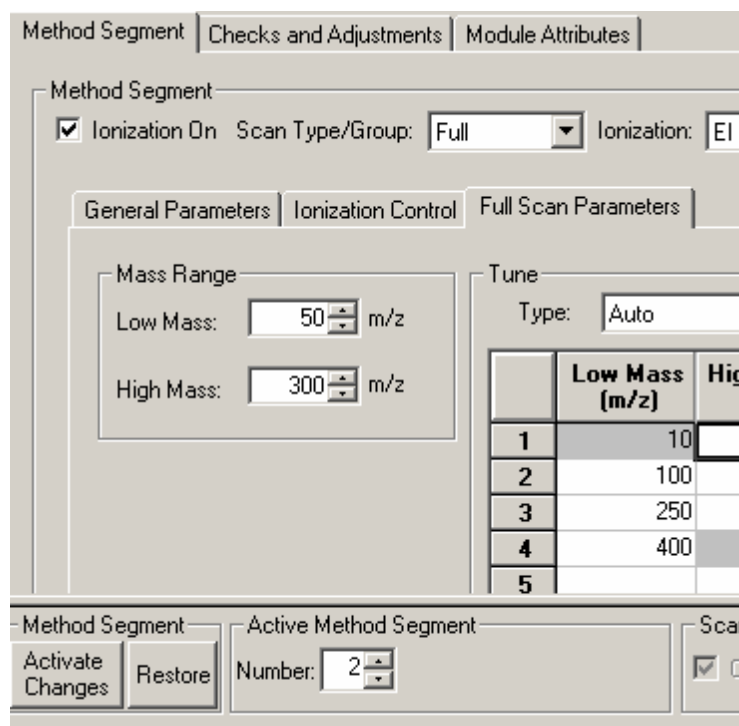
The changes are reflected in the spectrum. The example here is a change in the Start Mass from 50 m/z to 100 m/z:
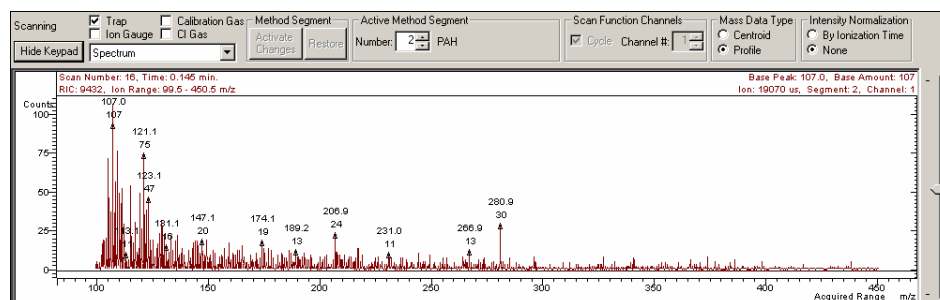
Saving a Method

To save changes to the method, click the **Upload MS Method** button above the Ion Trap icon. Click **Edit Method** to open the Method Builder and make other changes.
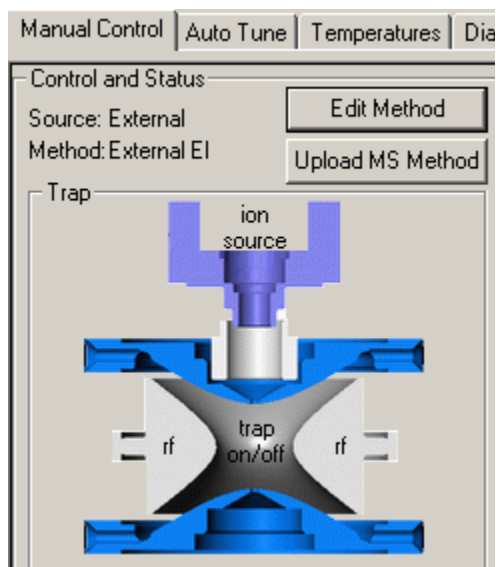
If you do not upload changes, the method is still checked to see if changes are made when the segment is changed or when you leave **Manual Control** or the **Method Segment**. If changes have been made, you will be given the option to save these changes or discard them.
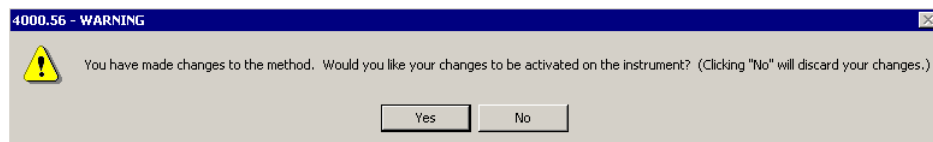
NOTE: If you leave System Control by starting automation or choosing Inject Single Sample, you are prompted to save the method.

Click **Yes**, the injection operation is cancelled, and you are returned to Manual Control so you can save the method.
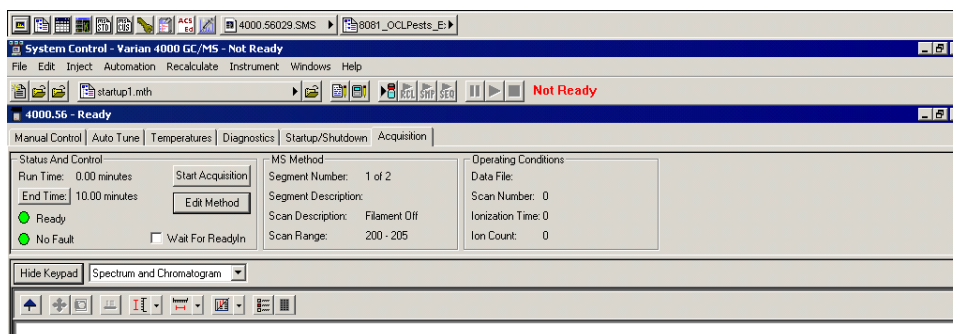
Click **No**, the last saved copy of the method is used to acquire the data file.

Acquiring Data

Acquisition

Click **Start Acquisition** to start a run. If you start an analysis while the instrument is in another mode, the software automatically shifts the MS module into Acquisition mode.

If the GC is not ready, you will see a Not Ready message at the top of the screen. After 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 see the top pull down menu under Windows and see the states for the 4000, 3800, and Combi PAL modules. After 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, see "Injecting a Single Sample" on page 36.

- To run in automation mode, see "Injecting Using a SampleList" on page 38.

Status and Control

Before an acquisition has been started, the Status and Control field is similar to the following figure.

- The Run Time is 0.00 minutes.

- The End Time is the run length specified in the active method.

- The Ready and No Fault lights are green.

Click the Start Acquisition button to override automation and start a run before the system becomes Ready. However, the file name of a run started in this way is named as 4000.x.sms, not the file name specified for automation runs.

Click the Edit Method button to open the Method Builder and modify the method. You are prompted to re-activate the method after saving changes and are returned 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.
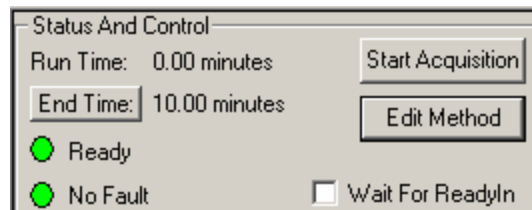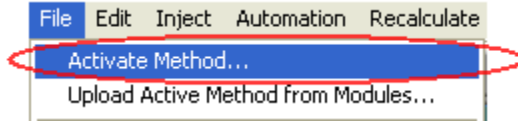
Activating a Method

To activate a GC/MS method, do the following:

1. Click the **File** menu.

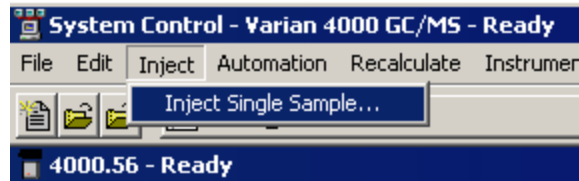2. Click **Activate Method**.

3. Select a method by doing one of the following:

   - Click **Recent Files** to display the eight most recent methods.

   - Click **Open**, after selecting a method from a folder.

Injecting a Single Sample

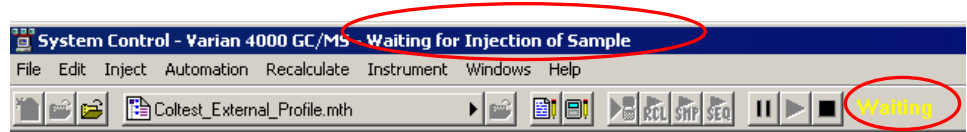To inject a single sample from System Control using Inject Single Sample do the following:

1. Click Inject Single Sample, from the Inject menu.

2. After the Inject Single Sample window opens, do the following:

   a. Type a sample name.

   b. Enter the vial number of the sample vial if an autosampler is configured.

   c. Check that the injection volume and injector used are correct.

   d. Click **Defaults**, to change the default values for any parameter.

   e. Click **Data Files** to create a name that includes more information such as date and time, or to change the directory for data file storage.

   f. Click **Inject** to acquire the data.
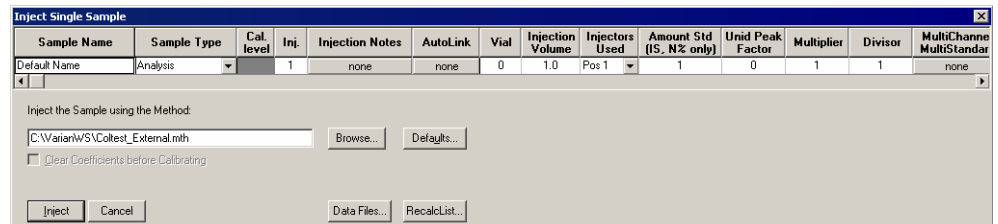
- If the MS is not in Acquisition mode, it changes to that mode automatically.

- If an AutoSampler is doing the injection, it begins after the instrument modules are 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.
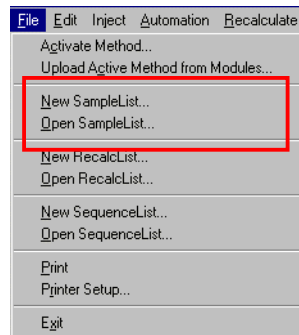

Injecting Using a SampleList

You can create and edit a SampleList in the Automation File Editor or in System Control.

To edit a SampleList and inject multiple samples from System Control do the following:

1. Click either **New SampleList** or **Open SampleList** from the File menu.

2. The SampleList window for the open SampleList opens. It contains fields that are specific to the configured autosampler. See the following figure.

   - Change the size of the spreadsheet columns by dragging their border with the left button of the mouse.

   - Right-click a column header for formatting options. When the table is scrolled to the right, the Sample Name column does not scroll so you can easily tell for which sample you are entering additional parameters.

   - Click **Add** to add additional samples. Enter the name, sample type, and vial number for all samples.

3. Click the **Begin** button in the lower left corner to start analyzing the samples on the SampleList.

Monitoring the Status of Runs

Monitor the status of the run in the instrument window. The Status and Control window and the Toolbar show the run status.

Monitor the chromatogram and spectra in System Control, or click the far right button in the Chromatogram toolbar to open 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, see the Acquiring GC/MS Data section in the *4000 GC/MS Software Operation Manual.*