Agilent 240 Ion Trap GC ⁄ MS

Internal Ionization User's Guide

Agilent Technologies

Notices

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Overview

Internal Ionization is one of three configurations of the 240 GC/MS system. It can be used with electron impact (EI) or positive chemical ionization (PCI). Unlike the External Ionization configuration, EI or PCI data can be collected during different time segments of a single sample run. It is not possible to collect negative chemical data (NCI) using internal ionization.

Ion preparation techniques can be performed after ionization but before ion analysis. These include:

- Selected Ion Storage (SIS)

and, with optional software and equipment:

- Tandem Mass Spectrometry
- Automatic Methods Development (AMD)
- MS/MS
- MS$^{\mathrm{n}}$
- Multiple Reaction Monitoring (MRM)

See the *240 GC/MS Software help* for more information.

The 240 GC/MS is an ion trap analyzer. The ion trap confines the ions within a single region and time-dependent electromagnetic fields control ion processes. The ion trap, which is the heart of the instrument, is shown in Figure 1 on page 7.

Filament
Endcap

E.M.
Endcap

Electron Multiplier

Filament

Electron Gate

Transfer
Line Tip

Ring Electrode

H.E.D.
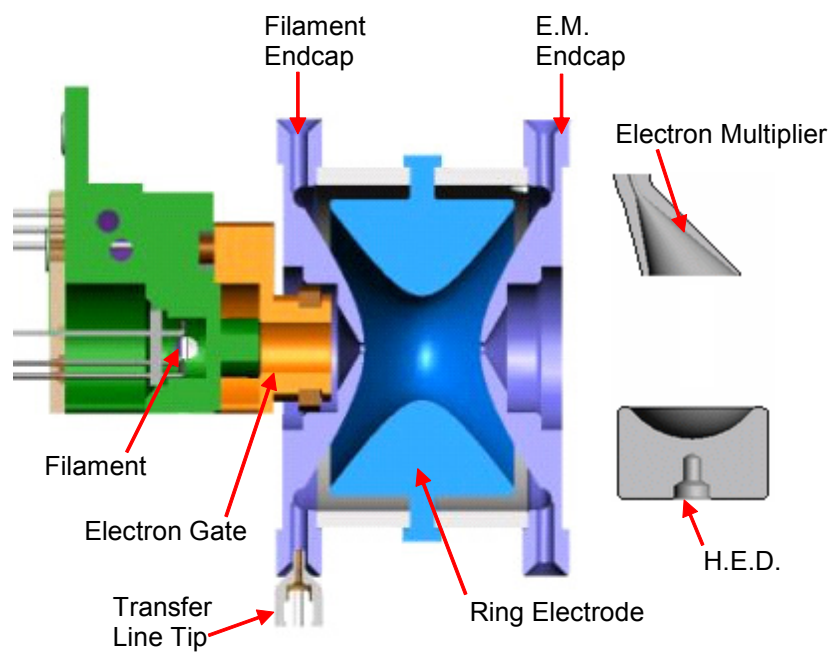
Figure 1   The ion trap

Sample Introduction and Ionization

Samples from the capillary column are introduced to the ion trap analyzer through the transfer line. The sample is ionized in the mass spectrometer by one of the following methods:

- **Electron Ionization (EI)**: The sample molecules are struck by energetic electrons, removing an electron from a molecular orbital to create a 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 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 transfer and storage

The molecular and fragment ions are stored and stabilized in the ion trap cavity. They travel in defined orbits governed by voltages applied between the ring electrode and the endcap electrodes. Helium buffer gas is in the cavity to buffer 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. Although helium ions are created in the trap, they are ejected electronically as soon as they are formed.

Ion preparation

After ions are stored in the trap, they can be manipulated. 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, such as reduction of background noise and increased selectivity.

Ion analysis and detection

The stored ions are ramped by the RF voltage applied to the ring electrode to a high value. 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. See the *240 Software Operation help* for more information.

Electron Impact Ionization (EI)

Internal electron ionization is the most common mode of the 240 MS. All of the sample ionization, fragmentation, storage, and scanning steps occur within the ion trap. Electron ionization generates fragments specific to the compound, providing fingerprint spectra. It is not selective and shows ions for all compounds present. EI is sensitive and the sensitivity can be improved by reducing the interfering ions using ion preparation techniques.

Ion formation

In EI, 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 and others undergo unimolecular decomposition to create various fragment ions, f_i^+ . The set of fragment ions, characteristic of the sample molecule, makes up the mass spectrum.

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

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

$$A^{+*} \rightarrow f_1^+ + n_1 \qquad \text{Equilibration}$$
$$\rightarrow f_2^+ + n_2$$
$$\rightarrow f_3^+ + n_3 \qquad \text{Fragmentation}$$
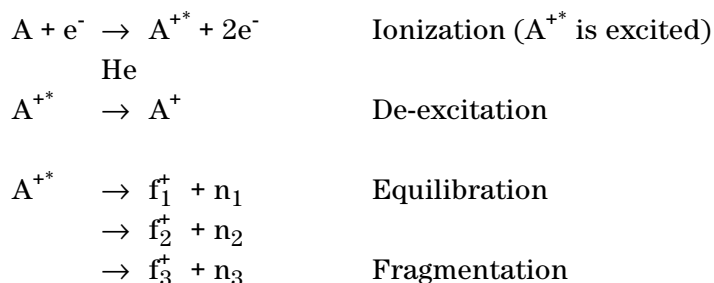
The ion trap has a maximum storage capacity beyond which mass resolution and spectral quality deteriorate. The number of ions created depends on the ionization time. As the ionization time increases, more ions are created. Automatic Gain Control (AGC) controls the ionization time to create the optimum 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 240 MS can apply a combination of waveforms to the ion trap electrodes to isolate or remove specific ions after they are formed and 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 precursor 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 240 MS instruments. MS/MS, MS^n, and MRM are available if the MS/MS option is installed.

Scanning ions to collect mass spectra

After the 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 about 10^5 by the electron multiplier and sent through an integrator to collect an intensity value for each *m/z*. MS data is 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.

Internal EI has two types of mass scanning.

• First, a prescan counts the number of ions formed in a short fixed ionization time.

- After a calculation based on the prescan ion count is done, ions are formed for the ionization time recommended by the AGC prescan algorithm and the analytical scan occurs. 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

Examine the mass spectra in MS Data Review. Determine the identity of most compounds by comparing 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. Create custom libraries from results collected on the 240 GC/MS system.

Chemical Ionization (CI)

Chemical ionization (CI) provides mass spectral data that complements electron ionization (EI) data for the analysis of complex compounds. Ions are generated in a two-step process.

1 A CI reagent gas is introduced into the ion trap analyzer. The reagent gas is ionized by EI.

2 The sample molecules are ionized by ion-molecule reactions with the reagent gas ions.

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

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. 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 complex. For example, when methane is used as the reagent gas, reagent gas ions are formed as follows:

First, methane is ionized forming two primary ions:

$$CH_4 + e^- \; \rightarrow \; (CH_4^{\bullet}\,)^+ \; + 2e^-$$
$$CH_4 + e^- \; \rightarrow \; CH_3^+ \; + e^- + H^-$$
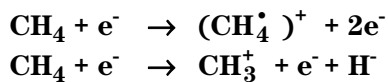
These primary ions then react very rapidly to form predominantly the secondary ions, CH_5^+ and $C_2CH_5^+$:

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

$$CH_3^+ \; + CH_4 \rightarrow C_2CH_5^+ + H_2$$
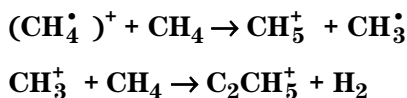
Positive CI reactions

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 $\qquad (RH)^+ + M \rightarrow (MH)^+ + R$

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

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

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

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. How exothermic the reactions are determines the amount of energy generated. Therefore, the degree of fragmentation can be controlled by choosing a suitable CI reagent gas. The proton affinities of some common reagent gases, 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 (that is, 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 association reactions are called adduct ions because the reagent ion is added to the analyte. They are typically seen on the 240 MS in internal ionization 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, that is, an ion with an odd number of electrons. It dissociates quickly, giving EI like spectra. However, the energy

placed in the molecular ion and the resulting fragmentation pattern does not depend on the electron energy of the ionizing electrons.

Ion preparation options

Ion preparation processes are the same after chemical ionization as after electron ionization. The 240 MS uses a combination of waveforms and RF to isolate, or remove, specific ions after formation and storage 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 electron ionization. After ionization, trapping, and ion preparation, ions are scanned out to the conversion dynode and electron multiplier. Mass scanning occurs by increasing the RF voltage on the ring electrode. The mass spectrum is collected from low to high mass over the user-designated scan range. In positive mode, electrons are ejected from the conversion dynode, held at −10,000 V, and repelled to the electron multiplier.

The signal is amplified by about 10^5 by the electron multiplier and sent through an integrator to collect the intensity of 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 occurs.

Library searching

The 240 GC/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

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 for 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 240 MS can switch between EI and CI in a single run, the best analytical conditions can be used for a given compound.

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.

Setting Up CI Reagents

Before running CI experiments, adjust the CI gas pressure.

1 Click the **Checks and Adjustments** tab on the **Manual Control** tab.

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 adjusting the flow of CI reagent, a mass calibration and trap function calibration must be performed before experiments are started.
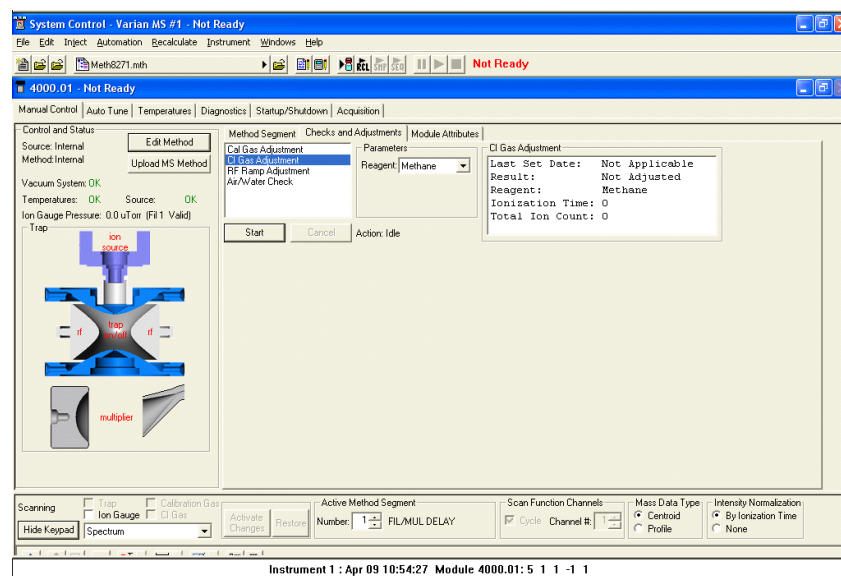
Installing a liquid reagent

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

2 Open the CI needle valve 6 or 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 **CI Gas Adjustment** 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 are in the *240 Software Operation help* sections, "PCI with CH_4", "PCI with CH_3CN", and "PCI with CH_3OH".
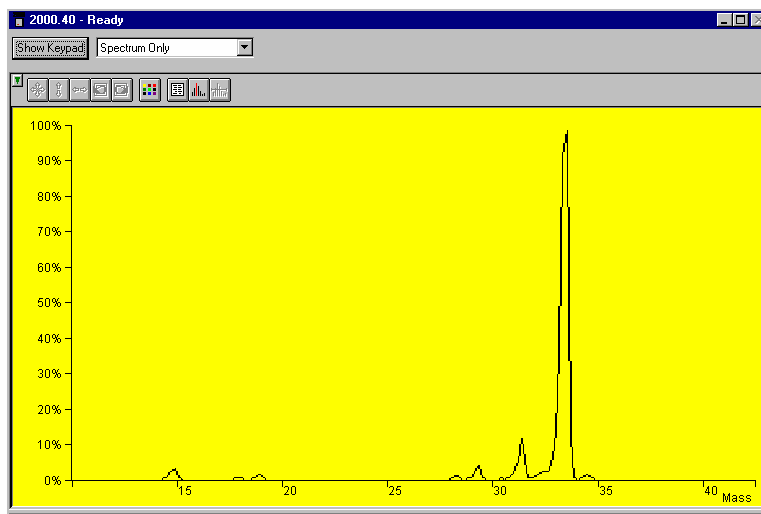

Figure 2   Methanol reagent spectrum properly adjusted
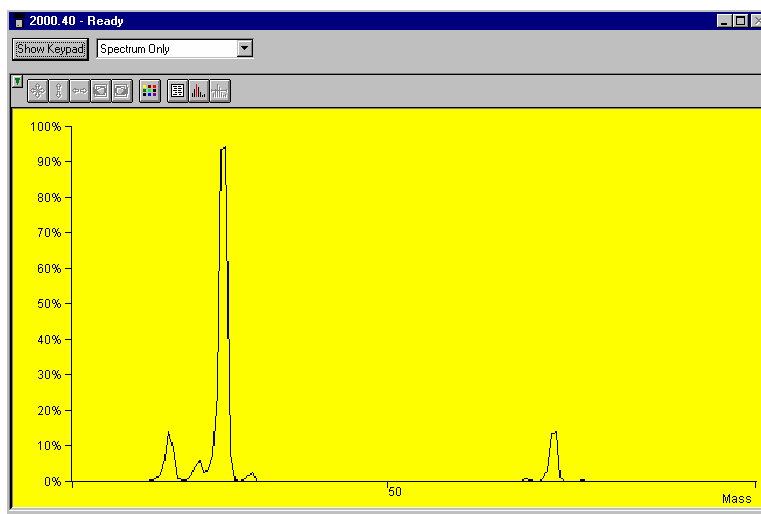

Figure 3   Acetonitrile reagent spectrum properly adjusted

For best results when using 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

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

2 See the *240 Ion Trap GC/MS 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.
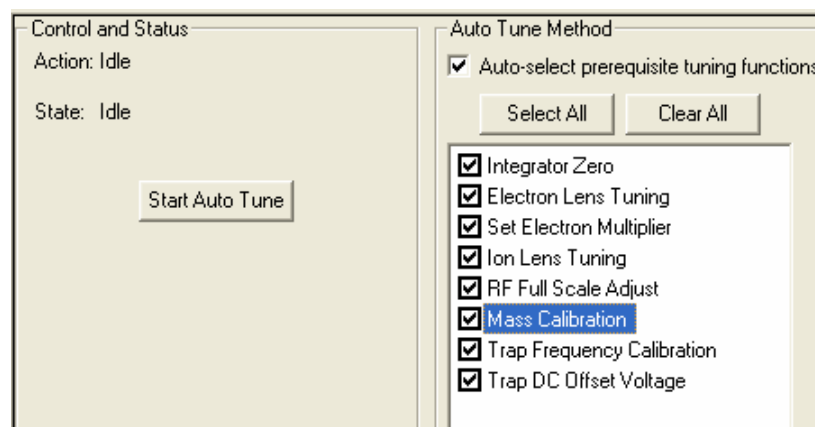
Integrator zero

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 signal 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 changes and is proportional to the imbalance. If the balance is outside the range of 200 to 300 µA, the algorithm searches the optimal values by changing the values of four variables one at a time. If the algorithm fails to find the best voltage setting for lens tuning, auto tune generates an error message and restores 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. 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), and is tuned using Cal Gas ions at m/z 131 and 414. Optimum voltages are determined based on weighted intensities of the two ions. Transmission of both low and high mass ions is monitored as a function of lens voltages in this iterative process.

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 is changed. Mass calibration does not have to be performed again after the auxiliary Helium buffer gas flow rate is changed.

Trap frequency calibration

After completing the mass calibration, the **Trap Frequency Calibration** must be performed. This calibration determines the parameters required for 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. **Trap Frequency Calibration** should always be run *after* **Mass Calibration** is done.

Trap DC offset voltage

This routine adjusts the trap DC offset to optimize the ion signal for m/z 414 in the calibration gas.

Acquiring Data

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.
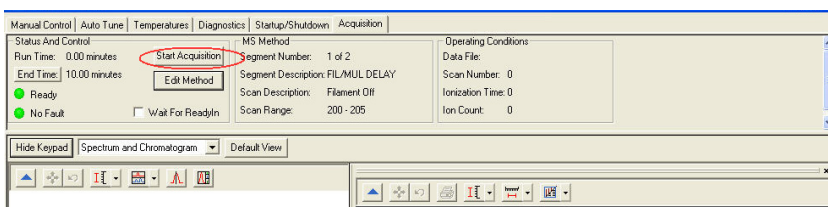
If the GC is not ready, a **Not Ready** message is displayed at the top of the screen. After the GC and AutoSampler come to a ready state, the Not Ready message changes to Ready. To determine the individual ready states of the components, you can go to the top pull down menu under Windows and see the states for the GC module. After 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 the topic "Injecting a single sample" on page 23.

- To use an automated sequence, see the topic "Injecting from a SampleList" on page 24.

Status and control

Before an acquisition is started, the Status and Control field is similar to the next 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.
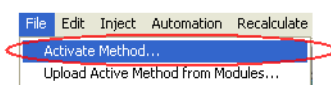
Click the **Edit Method** button to open the **Method Builder** and modify the method. You are prompted to re-activate the method after saving changes and are returned to System Control.

Changing the End Time for the MS module does not change the
GC End Time. You must access the GC module from the
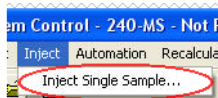Windows menu and change the GC End Time separately.
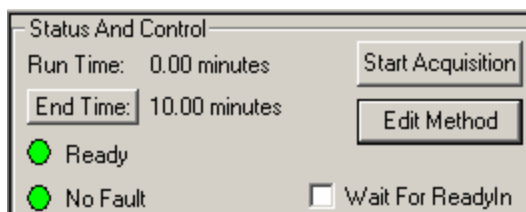
Activating a method

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

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

2 After the Inject Single Sample window opens:

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.
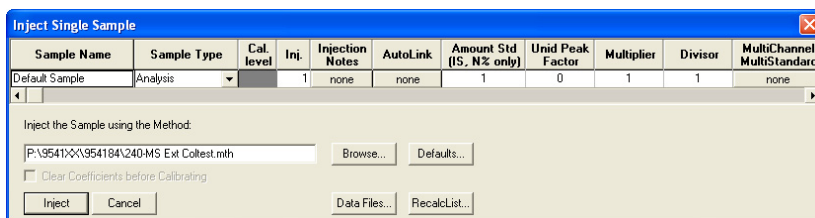
- If the MS is not in Acquisition mode, it changes to that mode automatically.

- 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.
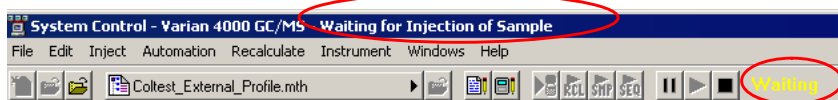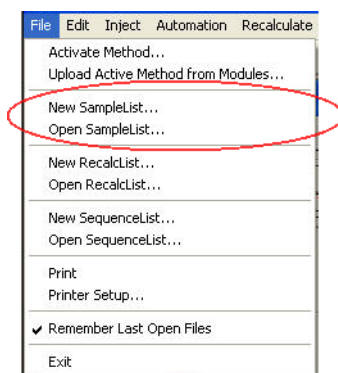

Injecting from a SampleList

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 figure below.

- 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 run status

Monitor the status of the run in the instrument window. The Status and Control windows 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, go to the "Acquiring GC/MS Data" section in the *240 Software Operation help*.
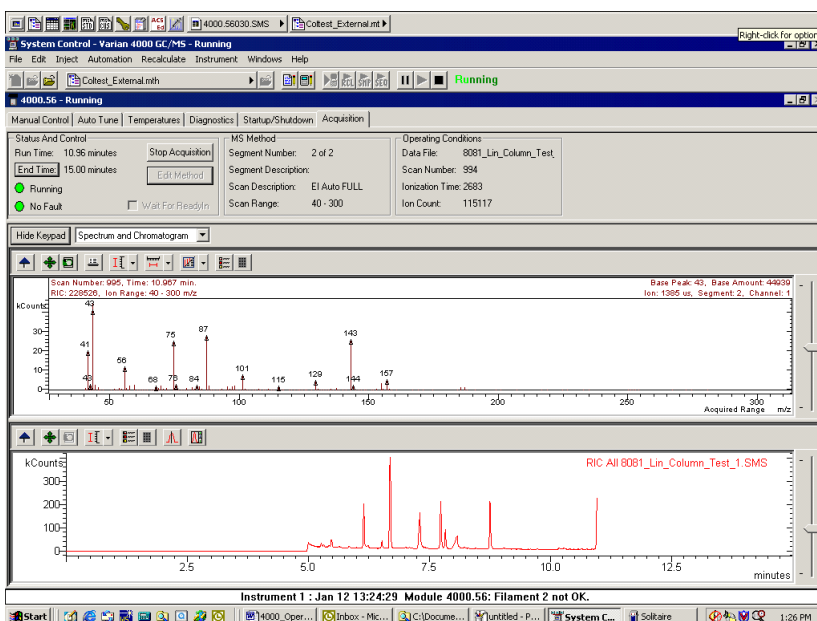
2

Starting the Instrument

Initial Pump Down

Check the following:

- Verify that the Vacuum connections are not leaking.
- Verify that the transfer line is inside the analyzer.
- Verify that the vent valve is closed clockwise.
- Verify that 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.
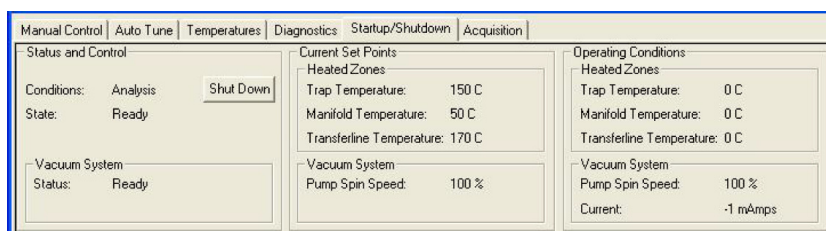
Check the vacuum status

The vacuum readings provide information about the MS after pump down (and during operation). Table 1 lists the typical operating ranges in internal mode.

Table 1   Typical operating ranges in internal mode

Speed	100%
Current	200 to 300 mAmps
Power	9 to 13 Watts
Ion gauge pressure	< 20 µTorr
Roughing line	< 50 mTorr

If the **Pump Spin Speed** does not increase steadily, 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 the "Diagnostics" section in the *240 Ion Trap GC/MS Hardware Operating Manual.* Diagnose small leaks by observing changes in the ion gauge reading and pinpoint them using the leak check section in the method Service.mth. For more detail on troubleshooting leaks, go to the "Troubleshooting" section in the *240 Ion Trap GC/MS Hardware Operating Manual.*

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 240 GC/MS using the **Diagnostics Tests** tab. For more details on the diagnostic tests, go to the "Diagnostics" section in the *240 Software Operation help*.
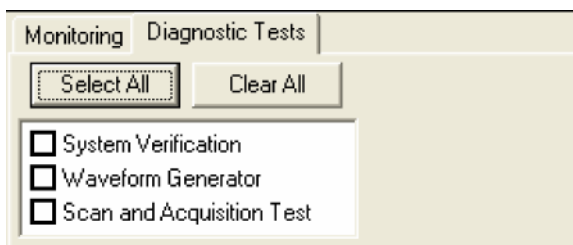
Setting system temperatures

Analysis temperatures

Analysis temperatures are sample dependent. 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[g,h,i]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 spots 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 that room temperature variation may have on the system.

System bakeout

To remove water adsorbed on the manifold while the 240 GC/MS was vented, perform a **Bakeout** from the **Temperatures** tab in **System Control**.

Perform a system bakeout to remove chemical background from the MS after running samples. After doing a bakeout to remove contamination, decrease the temperature to the analytical temperature.

Typical bakeout settings are:

- Trap temperature   230 °C
- Manifold temperature   110 °C
- Transfer line temperature   280 °C
- Bakeout time   12 hr

You can use shorter bakeout times.

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 **Turbo Pump Speed** in the **Operating Conditions** field. The software is locked in the **Startup/Shutdown** mode until the speed reaches 100%. You can also see the temperature readings for heated zones rise in the **Operating Conditions** field.

Failure to reach 100% pump speed in a reasonable time indicates a vacuum leak and corrective action should be taken. For details, go to the appropriate "Troubleshooting" section in the *240 Ion Trap GC/MS Hardware Operating Manual*.

Shutting down the system

To shut down the 240 GC/MS, click the **Shut Down** button in the upper left corner of the **Startup/Shutdown** dialog. The heaters are turned off and the speed of the turbo pump is 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.

To restart the system after starting shutdown, click the **Start Up** button on the left side of the screen. This restarts the pumps and turn on the heaters.
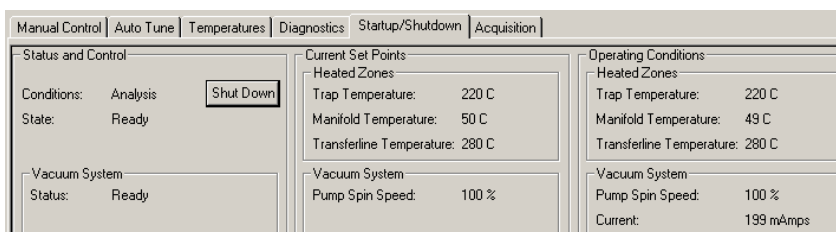

Adjusting and tuning

RF tune

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

- Performing MS maintenance
- Changing the analyzer assembly
- Changing the MS configuration

Adjusting the RF ramp

1 Click **Checks and Adjustments > RF Ramp Adjustment** in **Manual Control** tab.

2 Click **Start**.

3 Use a flathead screwdriver to turn the RF Adjustment screw, inside the front door of the 240 GC/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**.
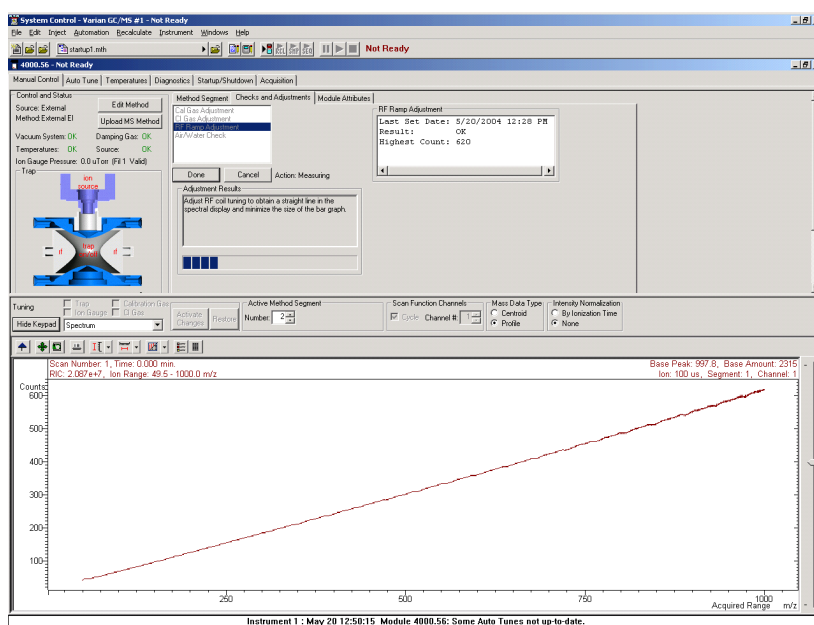
Adjusting the calibration gas

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

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

3 Turn the Cal Gas valve inside the front door of the 240 GC/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**.
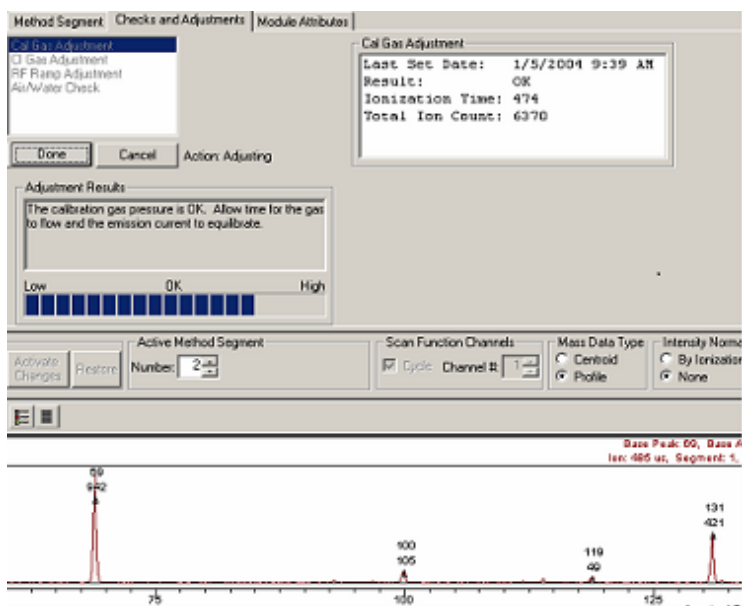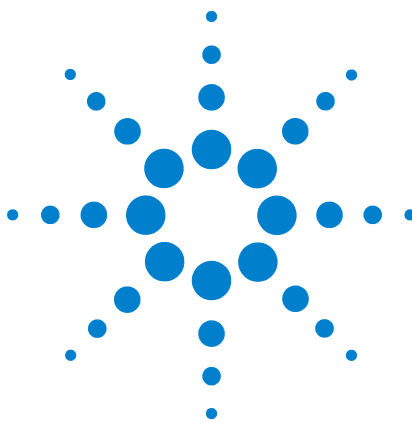
Air/water check

Two possible reasons for poor performance are air leaks and a need to bake out the system. These result in the pressure of air or water becoming too high. Checking Air/Water provides information about possible problems.

The Air/Water Check uses the electron multiplier voltage with a gain of 10^5 not the manual setting. After replacing the electron multiplier, auto tune the Electron Multiplier **before** performing the Air/Water check.

3

Creating Methods

A method is a complete description of what you want the MS to do. The **Method** Wizard, also called the **Method Builder**, is a series of screens that help you enter this information.

The method then creates a scan function that controls the voltages and times that actually operate the MS. A typical four-segment scan function for the 240 GC/MS looks like Figure 4.
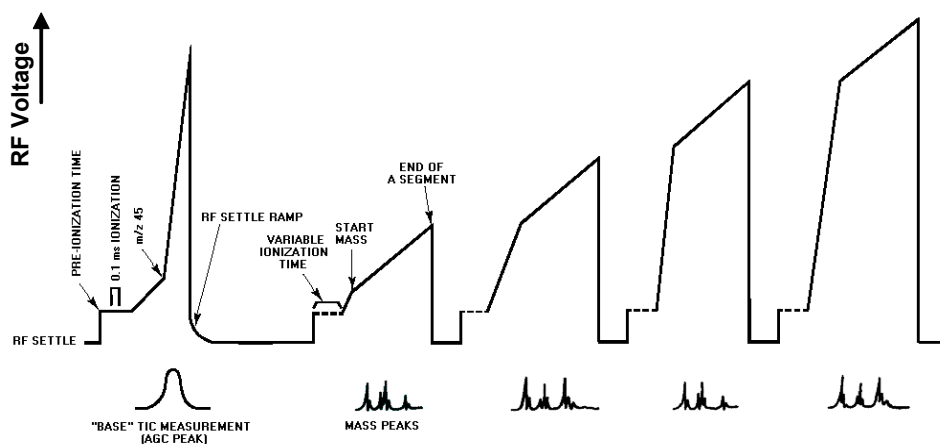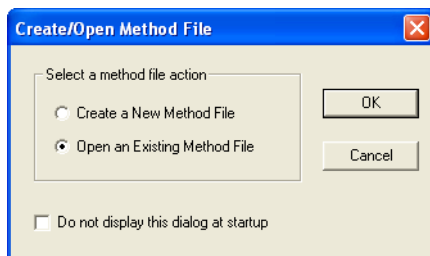
Figure 4   Scan function

Using the New Method Wizard

1 Click the **Method Builder** icon on the **Workstation** Toolbar.

2 Click **Create a New Method File**. The Wizard guides you in building this new method. If you do not want to see this message again, check the box **Do not display this dialog at startup**.

3 Select **Instrument 1** and click **Next**. Use **Custom** configuration to create methods on a PC remote from the instrument.
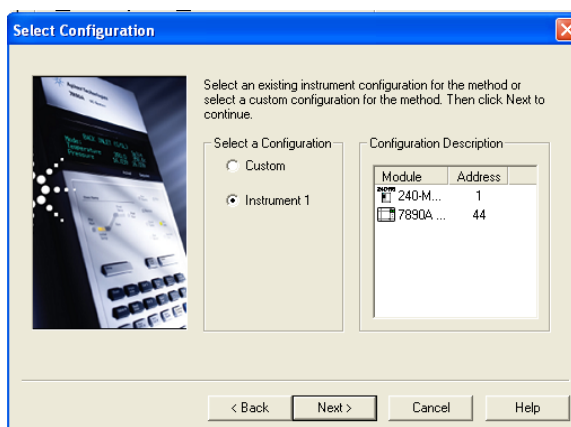
4 Select the detector(s) for post-run processing and click **Next**.
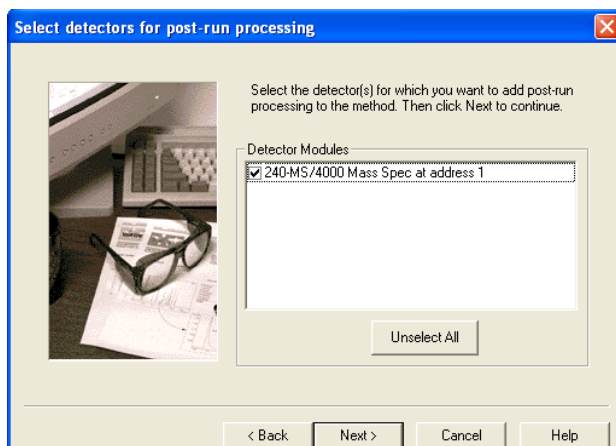
5 Select the data channels and type(s) of post-run processing for each detector and click **Next** to display the next detector if configured.
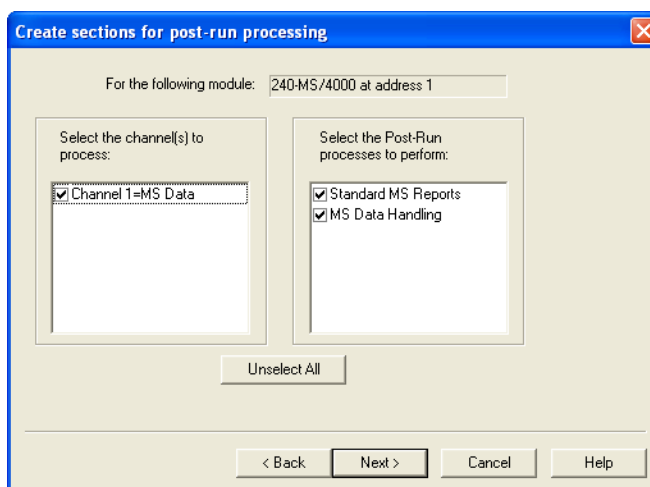
6 Click **Finish** to add the method. The wizard creates a method containing all the sections needed to control the hardware, collect data, and do the post-run processing specified. The method contains default values for all parameters. See the *240 Software Operation Manual* for information about data handling and reports.
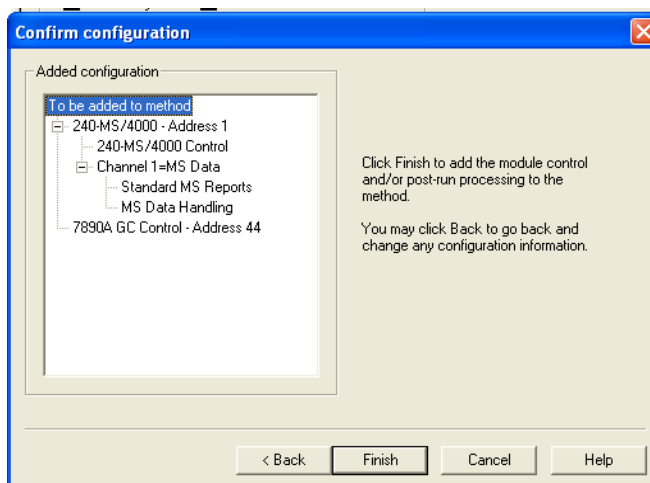
Naming the method

1 From the **File** menu, click **Save As**.

2 Type a name for the method.

3 Select the folder in which to keep the method.

4 Click **Save**.

240 GC/MS instrument configuration

The GC and the MS are set to the configuration of the instrument connected to the MS Workstation. For the 240 GC/MS Internal configuration, both EI and CI can be performed during the same run so there is only one internal setting.
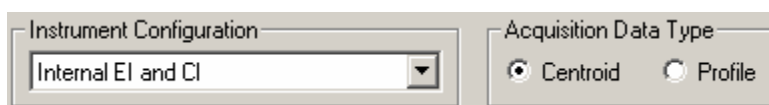

Acquisition data type

Centroid data is the default acquisition data type. Data handling, library searching, and spectral comparison can only be done using centroid data. The analog signal from the detector is sent to an analog-to-digital converter. The software determines the center of gravity of the digitized ion signal, the centroid. The software creates the *stick* spectrum from the digitized ion signals.
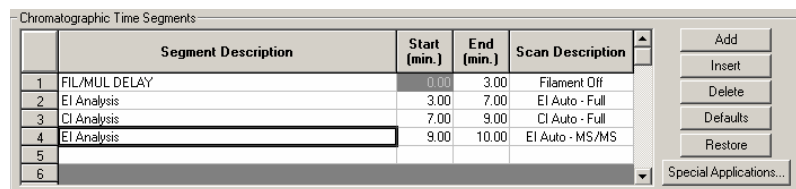
Profile data is used mainly for diagnostic purposes. Profile files are also approximately 10 times larger than centroid files, but they can be converted to centroid after acquisition.

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.

Chromatographic time segments

In Internal Configuration, the 240 GC/MS can do EI, CI, EI/MS/MS, and CI/MS/MS in a single run. Use the **Chromatographic Time Segments** table to program analysis conditions for 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 is not stressed during the elution of the chromatographic solvent. Following this segment, you could acquire the mass spectra in full-scan with a single analysis segment. However, you can tailor variables such as the mass range, insert MS/MS segments for individual analytes, and set up the instrument to acquire the best data for each analyte.

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

Edit the method segments

For more information on performing Internal CI, go to the appropriate section in the *240 Software Operation Manual*.

Scan function settings

The method segments control which 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). See Table 2 for detailed scan data.
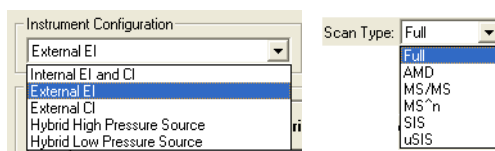
The 240 GC/MS has three scan modes. The default **Scan Mode** is normal.

• **Normal:** This scan mode uses a prescan in **Automatic Gain Control** mode to determine optimum ionization time, and then ions are scanned at 5000 u/sec to collect the mass spectrum.

- **Fast:** This scan mode also uses a prescan in **Automatic Gain Control** mode to determine optimum ionization time, but ions are scanned at 10000 u/sec to collect the mass spectrum.

- **Fastest:** This Scan mode uses **no prescan** and ions are scanned at 10000 u/sec to collect the mass spectrum. This mode is only available in **Full** scan type.
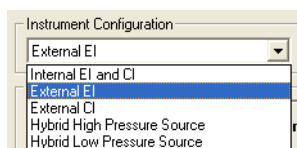

Table 2   Detailed scan data

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. 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 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 or 3 counts reduces 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.

Select Scan Time to adjust the seconds/scan. The minimum # of uScans to average the data rate will be automatically computed.

Select uScans Averaged to adjust the number of uScans to average. The Maximum Scan Time and data rate will be automatically computed.

Ionization control

Specify the Target Total Ion Current, or TIC. 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 or shift in mass assignments for strong chromatographic peaks). Typically, a **Target TIC** between 20,000 and 40,000 counts gives the best results.

EI ionization parameters

The **Emission Current** is the current of electrons produced by the filament and 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.
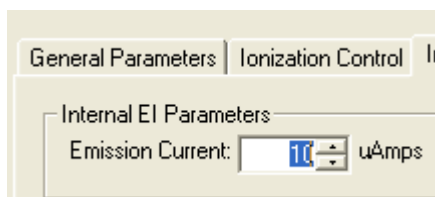
Setting parameters for CI

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

When a reagent ion that is formed through a complex process, such as $C_2H_5^+$, formed by the reaction of CH_4^+ with neutral CH_4, both ions will be in 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 (for example, 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, see the *240 Software Operation help*.

If you change a CI parameter and set it incorrectly, you will have problems operating in CI.

Scan parameters

Each MS scan type has different parameters. The following are examples of the two most common scan types used in the Internal configuration, **Full Scan** and **MS/MS**. For more information on all scan types, see the "Building GC/MS Methods" section in the *240 Software Operation help*.

Setting full scan parameters

Use **Full Scan** data acquisition 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 240 GC/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 semi volatile and volatile tuning requirements, are displayed in the mass segment table.

Each mass segment has its own **RF Storage Level (m/z)**. 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, 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 MS/MS parameters

Tandem mass spectrometry, or MS/MS, does an ion preparation step 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.

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)	Ionization Storage Level (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	35	3.0	Resonant	57.7	0.20	58	141	

General Parameters | Ionization Control | Internal EI Parameters | MS/MS Parameters

MRM

The **Precursor Ion (***m/z***)** is usually an intense ion in the full scan mass spectrum. Usually, the **Isolation Window (***m/z***)** is the parent ion mass ± 1.0 (3.0 mass units wide). **Waveform Type** is either **Resonant** or **Non-resonant**.

The **Excitation Storage Level (***m/z***)** 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 (***m/z***)** so you should be aware that it might 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. That is, using several runs with different ranges of excitation amplitudes. Using the **AMD** (Automated Method Development) mode is the easiest way to determine this voltage.

The **Product Ion Mass** range during method development encompasses the range from **Excitation Storage Level** to the **Precursor Ion Mass**. For more detail on MS/MS methods, go to the "Tandem Mass Spectrometry" section in the *240 Software Operation help*.

Viewing methods in manual control

After a method is created in **Method Builder**, preview it 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**.
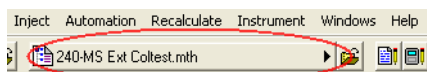
Activating a Method
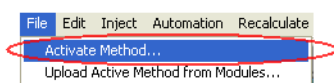
1 Click the **File** menu.

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
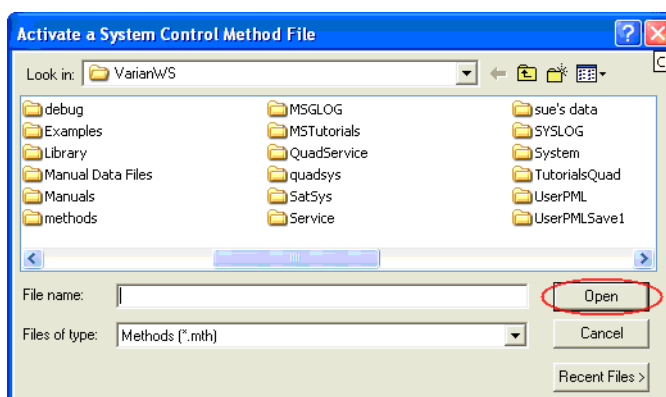
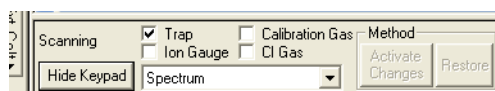**4** The active method is displayed in the toolbar.

Displaying ions

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.
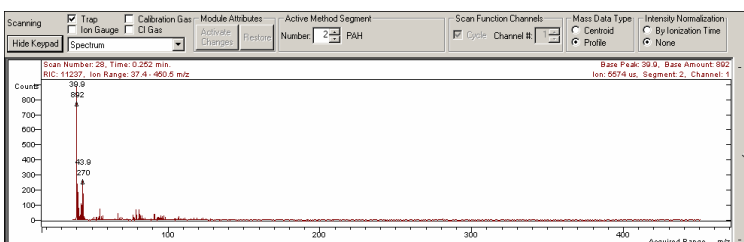
Viewing method parameters

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

The information in the top row of the **Active Segment** tab indicates if the trap is on, the **Scan Type**, **Ionization** mode, and **Scan Mode**.
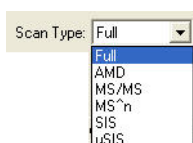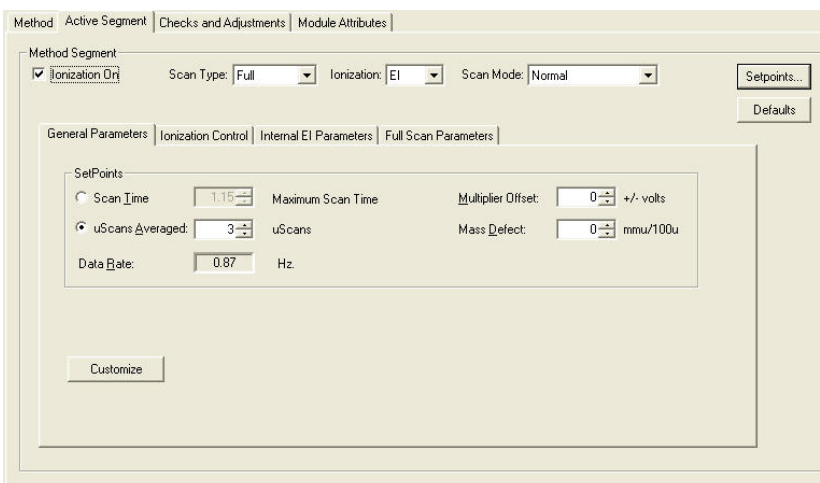
Editing a method in manual control

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.
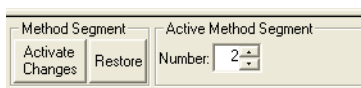
The changes are reflected in the spectrum. The example here is a change in the start mass from 50u to 100u:
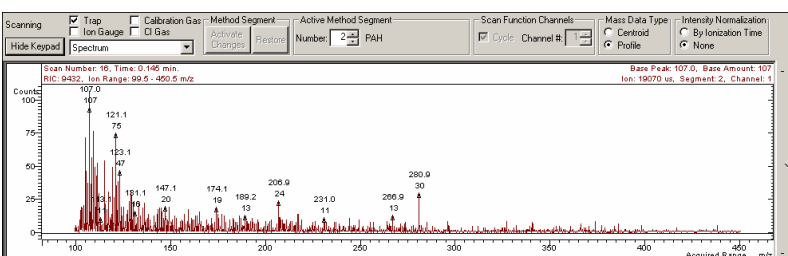
Saving a Method

To save changes to the method, do one of the following:

- Click the **Upload MS Method** button above the **Ion Trap** icon.
- Click the **Edit Method** button, open the **Method Builder**, and make and save the changes.
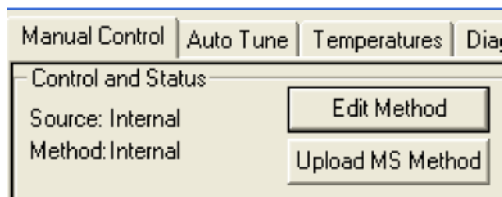

If you do not upload changes, the method is still checked to see if changes are made when the segment is changed or when you leave **Manual Control** or the **Method Segment**. If changes have been made, you will be given the option to save these changes or discard them.
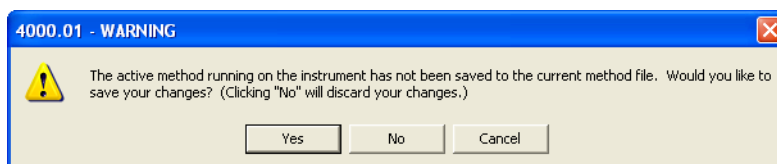
If you leave **System Control** by starting automation or choosing **Inject Single Sample**, you are prompted to save the method.

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.
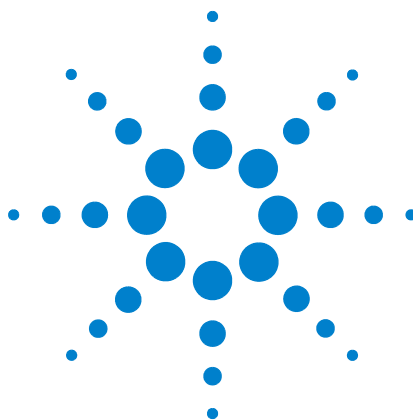
4

Mode Conversion

For more detailed information on either of the following topics, see the *240 Ion Trap GC/MS Hardware Operation Manual.*

External to Internal

Converting the 240 MS from External to Internal ionization mode involves changing both the ion source and the column position.

1 Remove the analyzer from the MS manifold.

2 Change the ion source to internal.

3 Move the heat shield to the forward position.

4 Add the filament adapter and connect the flex cable.

5 Change the transfer line entry to the ion trap.

6 Change the transfer line tip to internal.

7 Cut the column to 7 mm.

8 Change the transfer line switch to Internal/Hybrid.

9 Replace the analyzer in the MS manifold.

Hybrid to Internal

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.

1 Remove the analyzer from the MS manifold.

1 Change the ion source to internal.

2 Move the heat shield to the forward position.

3 Add the filament adapter and connect the flex cable.

4 Replace the analyzer in the MS manifold.

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Effects of hardware 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.

Presetting the Module Attributes requires running all of the Auto Tune routines, because the prior Auto Tune results are invalid.