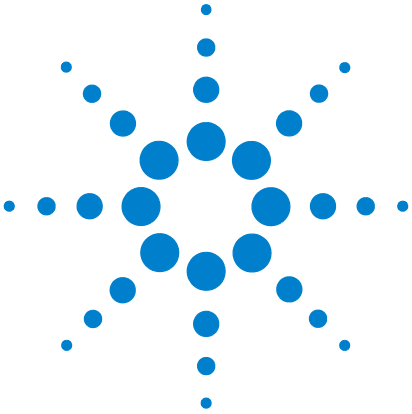

Agilent 240 Ion Trap GC/MS

External Ionization User's Guide

Agilent Technologies

Notices

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Contents

1 Sample Analysis

Overview

External Ionization is one of three configurations of the 240 GC/MS system. It can be used with electron impact ionization (EI), positive chemical ionization (PCI), and negative chemical ionization (NCI).

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^n
- Multiple Reaction Monitoring (MRM)

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

The ion trap, which is the heart of the instrument, is shown in Figure 1.

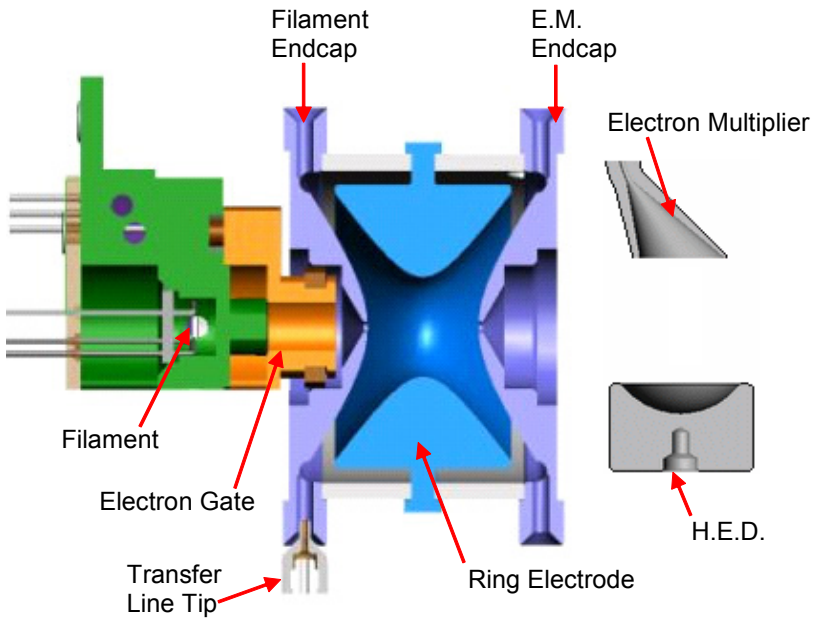


Figure 1 The ion trap

Sample Introduction and Ionization

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

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

- **Electron Ionization (EI)**: The sample molecules are struck by 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.
- **Negative Chemical Ionization (NCI)**: Introducing a buffer gas (usually methane) to the external source to thermalize electrons from the filament with that gas. These thermal (low-energy) electrons can then attach to GC analytes that have a high electron affinity.

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

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

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 noise and increased selectivity.

Ion analysis

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 Ion Trap GC/MS Software Operation Manual* for more information.

External Electron Impact Ionization (EI)

Figure 2 shows the instrumentation for collection of electron ionization data. The EI source is open and easily pumped down. The GC column flow enters the EI source perpendicular to the path of electrons from the filament.

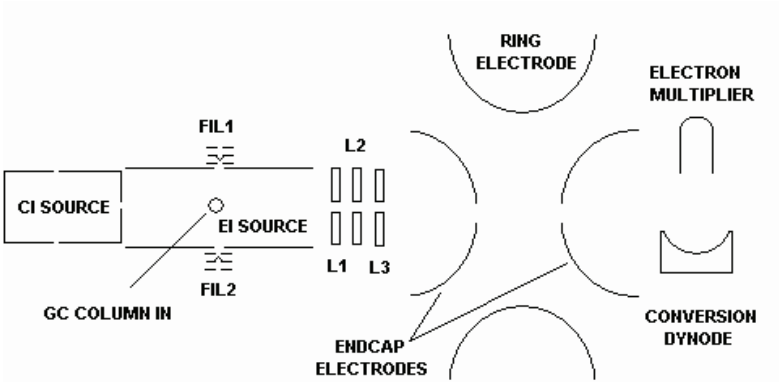


Figure 2 Instrumentation for collection of electron ionization data

Forming ions

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

Between ionization times, the filament lens is gated off. This gating process reduces source contamination and the frequency of source cleaning. The high-energy electrons strike a small percentage of analyte molecules A entering the EI source from the GC column, removing an electron from the molecule A and creating energetically excited molecular ions A^{+*}. Some of the excited molecular ions equilibrate through collisions with helium but others undergo unimolecular decomposition to create various fragment ions, f_1^+ .

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

The ion trap has a maximum storage capacity beyond which mass resolution and spectral quality deteriorate. The number of ions created 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 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.

Transferring and trapping ions

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

Ion preparation options

The 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 GC/MS instruments. MS/MS, MS^n, and MRM are available if the MS/MS option is installed.

Scanning ions to collect mass spectra

After ionization, trapping, and ion preparation steps, ions are scanned out of the trap to the conversion dynode and electron multiplier. Mass scanning is implemented by increasing the RF voltage on the ring electrode. The mass spectrum is collected in order from low to high mass over the user-designated scan range.

In positive mode, ions strike the conversion dynode held at –10 KV, and then electrons are ejected from the conversion dynode and repelled to the electron multiplier. In negative mode, positive ions are ejected from the conversion dynode, held at +10 KV, and are repelled toward the electron multiplier.

The signal is enhanced by approximately 10^5 by the electron multiplier and sent through an integrator to collect an intensity for each *m/z*. MS data are stored as sets of ion-intensity pairs for each *m/z* over the acquired mass range. A complete mass spectrum is stored for each analytical scan.

There are actually two types of mass scanning in external EI.

- First, 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 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 Agilent 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.

External Chemical Ionization (CI)

The configuration for collection of chemical ionization data is show in Figure 3. The CI source is more enclosed than the EI so that high pressure CI reactions can take place rapidly. GC column flow enters the EI source perpendicular to the path of electrons from the filament and CI reagent gas enters opposite the column flow.

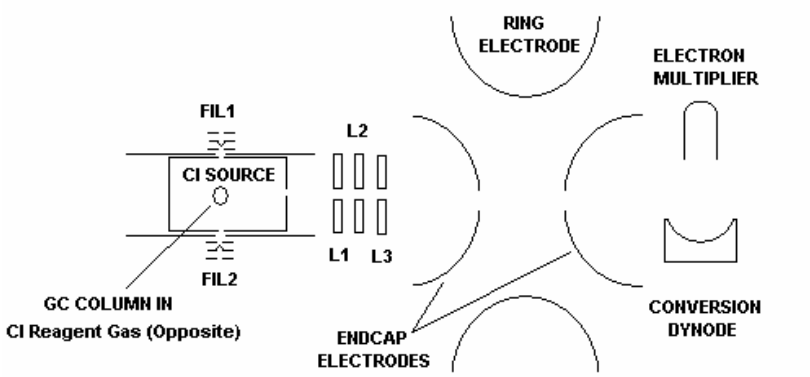


Figure 3 Schematic Diagram for External CI

Positive CI external

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

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

First, methane is ionized to form two primary ions:

$$CH_4 + e^- \rightarrow (CH_4^{\bullet})^+ + 2e^-$$
$$CH_4 + e^- \rightarrow CH_3^+ + e + H^-$$

These primary ions then react very rapidly to form predominantly the secondary ions, CH_5^+ and $C_2CH_5^+$:

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

$$CH_3^+ + CH_4 \rightarrow C_2CH_5^+ + H_2$$

CI is a softer ionization technique than EI. That is, CI imparts less energy to the sample molecules than does EI. 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.

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

Positive CI reactions

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

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

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

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

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

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

For methane CI, proton transfer (A) is a major reaction, and association (C) is the next most often observed reaction. In both cases the resulting even-electron ions are often relatively stable, and the observation of strong (M+1) protonated molecule or

(M+29) and (M+41) adduct ions is often observed even if the EI spectrum of the same component shows no molecular ion. Methane is the most useful PCI reagent gas in the External configuration.

Negative CI external

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

Transferring and trapping ions

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

Ion preparation options

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

Scanning ions to collect mass spectra

The scanning process for chemical ionization is similar to electron ionization. After ionization, trapping, and ion preparation, ions are scanned out to the conversion dynode and electron multiplier. Mass scanning is implemented by increasing the RF voltage on the ring electrode. The mass spectrum is collected in order from low to high mass over the user-designated scan range.

- In positive modes, electrons are ejected from the conversion dynode held at –10000V and repelled to the electron multiplier.
- In negative mode, positive ions are ejected from the +10000V dynode and repelled toward the multiplier.

The signal is enhanced by about 10^5 by the 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 are two types of mass scanning in external CI.

1. The first is a prescan to count the number of ions formed in a short fixed ion time.
2. After a calculation based on the prescan ion count, ions are formed for the ionization time recommended by the AGC prescan algorithm and the analytical scan is carried out.

Library searching

There are no libraries of external PCI or NCI mass spectra included with 240 GC/MS software. However, you can create a user library. For details on how to create user libraries, see the section in *MS Workstation help*.

Selectivity considerations

An advantage of chemical ionization is selectivity. In PCI, hydrocarbons have poor response in methane CI. It is easier to locate target compounds in a hydrocarbon-contaminated sample using methane PCI than using EI. Similarly, negative CI gives a good response only for species with a high electron affinity such as halogenated compounds. The chemical background of other species does not appear in the chromatogram.

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

For many species, so much unimolecular fragmentation occurs that is it difficult to determine the molecular mass of the precursor ion.

An examination of the NIST Mass Spectral Library confirms this. When attempting to identify unknown species acquire data in PCI as well as EI to obtain the needed molecular weight information.

Setting Up CI Reagents

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

Installing methane CI

For full details on installing a reagent gas, see the "Installing a CI Reagent Gas" section in the *Agilent 240 Ion Trap GC/MS Hardware Operation Manual*.

1 Connect the regulator of the gas cylinder to the back of the instrument through a 50 mL/min restrictor.

2 Open the methane tank and set the second stage of the regulator to 20 psi.

Adjusting CI gas flow

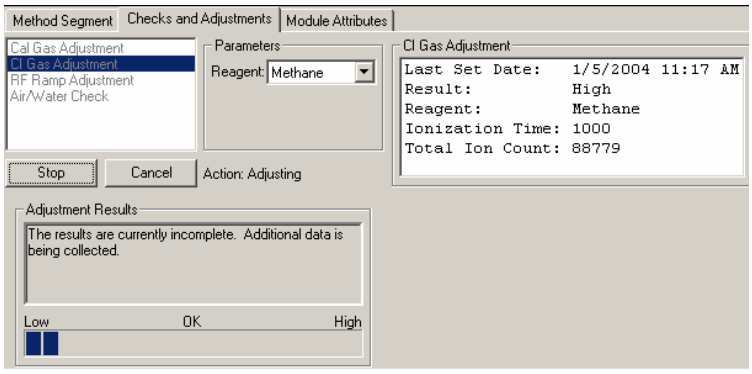
1 Open the **Checks and Adjustments** tab dialog in Manual Control.

2 Click **CI Gas Adjustment** and click the **Start** button.

3 Use the CI Gas Adjust Valve (#3) inside the front door of the 240 MS. Turn the knob clockwise to increase the flow or counterclockwise to decrease the flow.

The objective is to set the ion gauge pressure within the range of 70 to 100 µTorr.

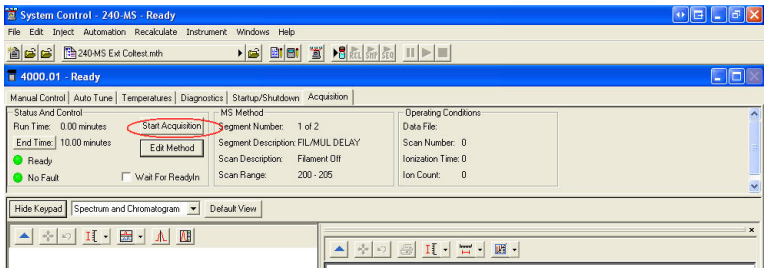
4 Adjust the gas until the adjustment result is **OK**.



Acquiring Data

Acquisition

Click **Start Acquisition** to start your 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 will change to Ready. To determine the individual ready states of the components, go to the top pull down menu under Windows and see the states for the 240 MS, 7890 GC, and Combi PAL modules. After components are ready, you can start an analysis.

An analysis can be run as a single sample or as an automated sequence.

To run a single sample, do the following:

- See "Injecting a single sample" on page 19.
- To run in automation mode, see "Injecting using a SampleList" on page 20.

You can run both single samples and sample lists using from QuickStart. For more information on using QuickStart, see the *240 Ion Trap MS Software Operation Manual*.

Status and control

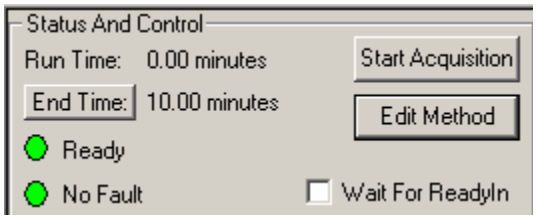
Before an acquisition starts, the **Status and Control** field will look like the following figure.

- The **Run Time** will be 0.00 minutes.
- The **End Time** will be the run length specified for the 240 MS module in the active method.
- The **Ready** and **No Fault** lights are green.

You can click the **Start Acquisition** button to override automation and start a run even before the system comes to **Ready**. However, the file name of a run started in this way will be named as 4000.x.sms, not the file name specified for automation runs.

Click **Edit Method** 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**.

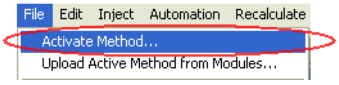
Changing the **End Time** for the MS module does not change the GC **End Time**. You must access the GC module from the **Windows** menu and change the GC **End Time** separately.



Activating a method

1 Click **File**.

2 Click **Activate Method**.

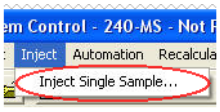


3 Select a method by either:

- Clicking **Recent Files** to display the eight most recent methods
- Clicking **Open**, after selecting a method from a folder

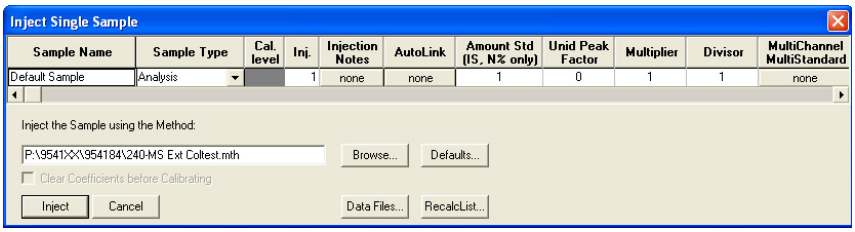
Injecting a single sample

1 Click **Inject > Inject Single Sample**.



2 After the **Inject Single Sample** window opens:

- Type a sample name.
- Enter the vial number of the sample vial if an autosampler is configured.
- Check that the injection volume and injector used are correct.
- Click **Defaults**, to change the default values for any parameter.
- 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.



3 Click **Inject** when you are ready to acquire the data.

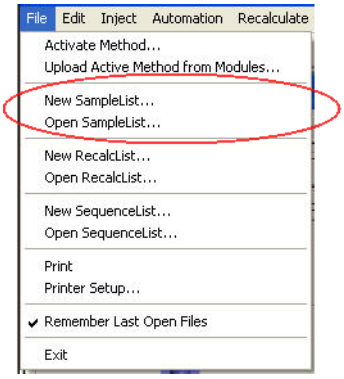
- If the MS is not in Acquisition mode, it changes to that mode automatically.
- If an AutoSampler is doing the injection, it begins after the instrument modules are Ready.
- If you are doing a manual injection, wait until the **System Control** title bar reads **Waiting for Injection of Sample** and 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**:

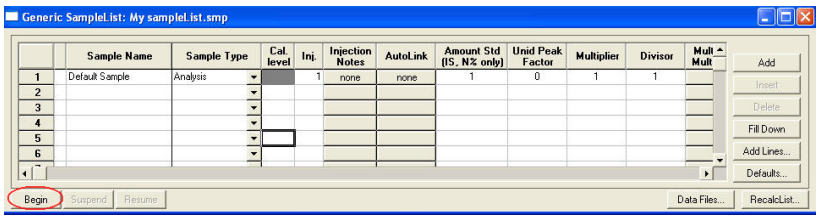
1 Click either **New SampleList...** or **Open SampleList...** from the **File** menu.



2 The **SampleList** window for the open SampleList opens. It contains fields that are specific to the autosampler configured, 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 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 **Begin**, in the lower left corner, to start the SampleList.

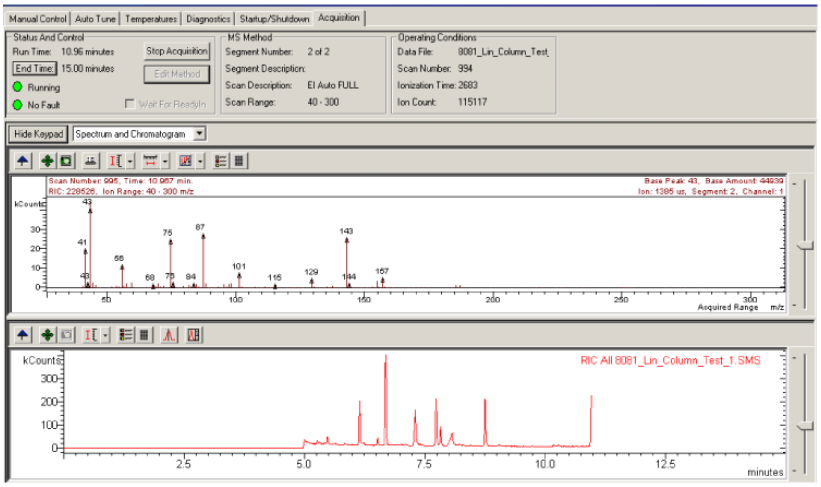


Monitoring run status

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 transfer to MS Data Review, where you can perform operations like library searching while the data file is being acquired.

For more information on data acquisition features, see the Acquiring GC/MS Data section in the *Agilent 240 Ion Trap GC/MS Software Operation Manual*.



2
Starting the Instrument

Initial Pump Down

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

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

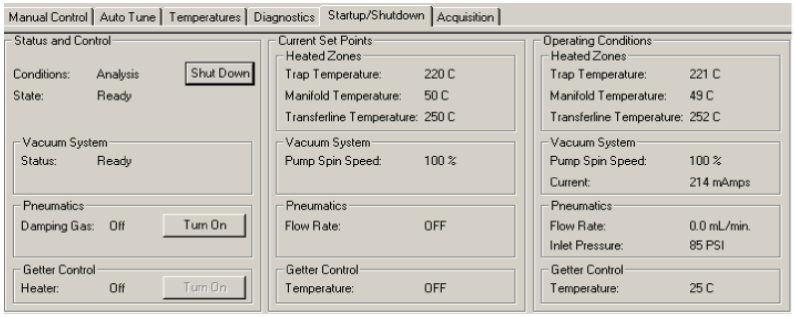
If the pump continues to gurgle:

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.

4 Open **System Control** and the **Startup/Shutdown** page appears.



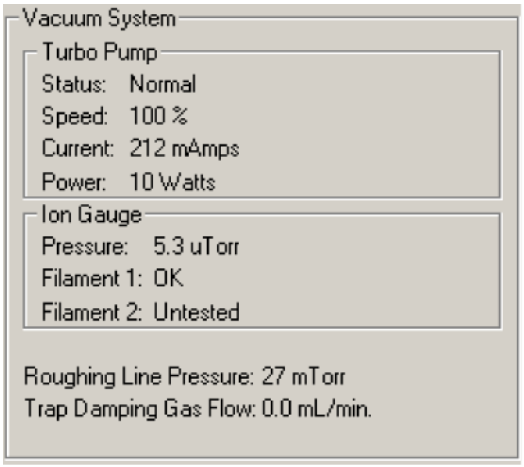
Check the vacuum status

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

Table 1 Typical operating ranges in external mode

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 increase steadily, there may be a leak in the system. Large leaks are indicated by a turbo speed less than 100%. Small leaks will show up as an increase in the pump current after 100% or in the ion gauge pressure diagnostics. 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, see the Troubleshooting section in the *Agilent 240 Ion Trap GC/MS Software Operation Manual.*



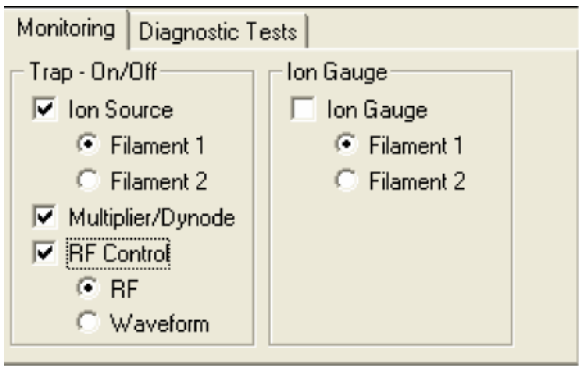
Start auxiliary gas flow

When the turbomolecular pump speed reaches 100%, turn on the **Damping Gas** and **Heater** using the buttons in the lower left of the **Startup/Shutdown** dialog. After the flow starts, check the rate in the **Operating Conditions** field on the right side of the dialog. The buffer flow is necessary to maintain mass spectral resolution. Helium flow also improves the trapping of ions entering the trap from the external source. Although trapping efficiency and instrument sensitivity dependence on helium flow rate is compound dependent, use 1 mL/min.

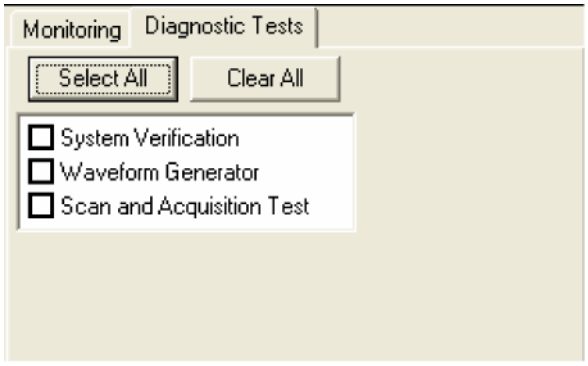
Helium buffer gas flow rate is set in the **Module Attributes** tab dialog in **Manual Control**.

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** tab. For more details on the diagnostic tests, see the Diagnostics section in the *240 Ion Trap GC/MS Software help*.



Setting system temperatures

Analysis temperatures

The default analysis temperature of 100 °C is appropriate for all external configuration analyses, despite the fact that ion trap temperature is an important variable for analyses performed in internal or hybrid configurations.

However, the external source temperature may need to be raised above the default value to avoid condensation of heavy semi-volatile species. The symptom to look for with higher

molecular weight species is peak tailing that cannot be reduced by raising transfer line, injector, and maximum column temperature. For example, in semi-volatile environmental analyses, the temperature of the ion source should be set to about 250 °C so heavy PAHs (benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) do not tail.

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

Set the transfer line temperature so there are no cold spots between the GC column oven and the MS. Setting the transfer line temperature 20 °C below the maximum column temperature of the active method should be adequate.

The manifold temperature, typically 50 °C, reduces the effects room temperature variation may have on the system.

System bakeout

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

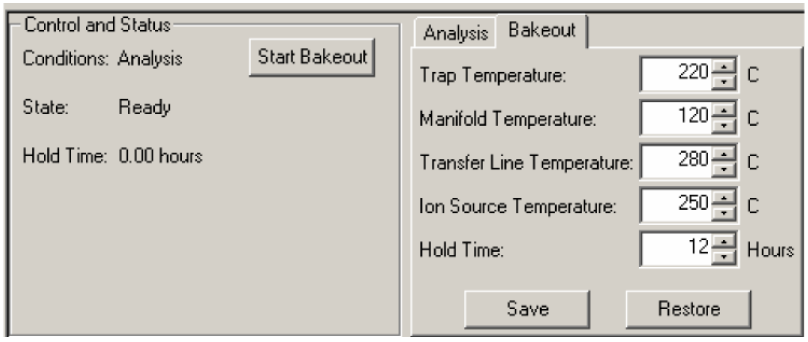
Bakeout can also remove chemical background from the MS after running heavy matrix samples such as environmental or biological extracts.

When bakeout begins, the temperatures are raised to those set in the **Bakeout** tab dialog. The **Hold Time** in the **Control and Status** field decreases until bakeout is complete. System temperatures are then returned to those set in the **Analysis** tab dialog.

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

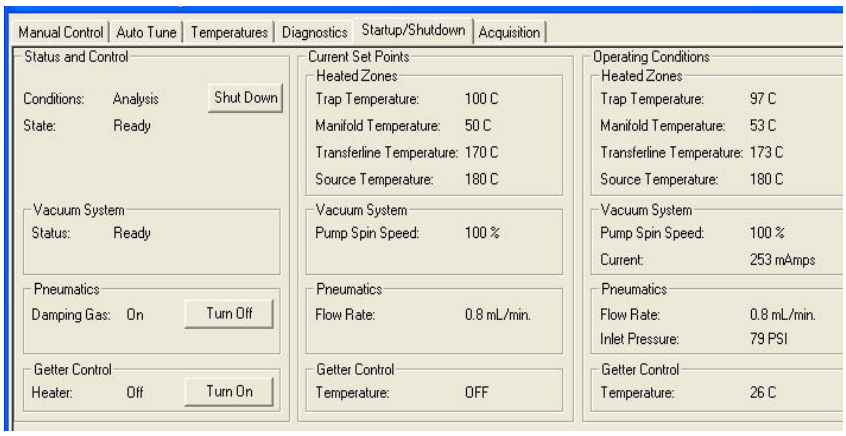
Typical bakeout settings are:

- Trap temperature 220 °C
- Manifold temperature 120 °C
- Transfer line temperature 280 °C
- Bakeout time 12 hr



Startup and shutdown

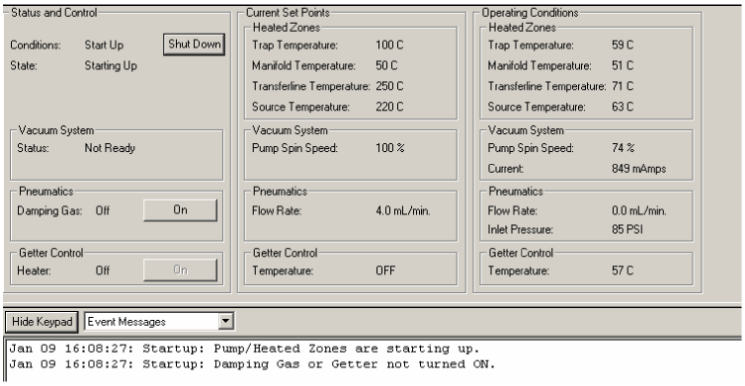
Use **Startup/Shutdown** to start up or shut down the system in a safe and orderly fashion.



Starting the system

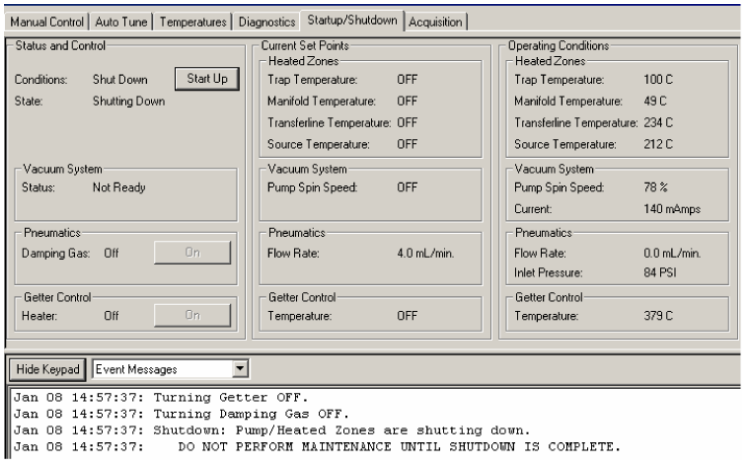
When the system is first turned on, **System Control** operates in **Startup/Shutdown** mode. During system startup, you can observe the increase in **Turbo Pump Speed** in the **Operating Conditions** field. The software is locked in the **Startup/Shutdown** mode until the speed reaches 100%. You can also observe the increase of the temperature readings for heated zones 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, see the appropriate Troubleshooting section in the *Agilent 240 Ion Trap GC/MS Hardware Operation Manual.*



Shutting down the system

To shut down the 240 MS, click the **Shut Down** button in the upper left corner of the screen. The heaters are turned off and the speed of the turbo pump gradually reduced to 35% of full speed. In the following figure, **Shut Down** was clicked. Note that the turbo pump speed decreases as the temperature decreases.



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

To restart the system after starting shut down, click the **Start Up** button on the left side of the screen. The pumps restart and the heaters turn on.

After the turbomolecular pump reaches 100% speed, you can perform normal operations. Check for instrument problems by running all of the routines in the **Diagnostic Tests** tab dialog of

the Diagnostics mode. Click the **Select All** button and then click the **Start Diagnostic** button in the **Control and Status** field to the left. If a test fails, see the relevant Troubleshooting section in the *Agilent 240 Ion Trap GC/MS Hardware Operation Manual*.

Adjusting and tuning the 240 MS

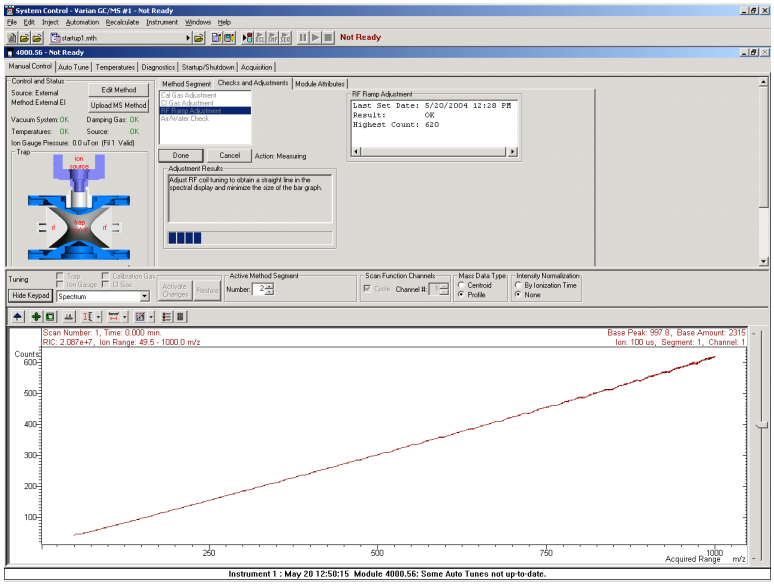
RF tune

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

RF ramp adjustment

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 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**.

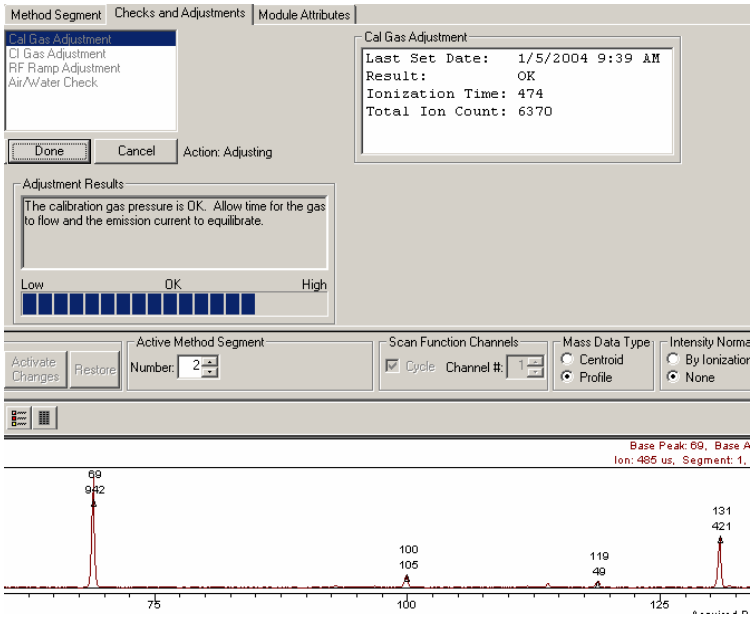


Calibration gas adjustment

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

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

2 Turn the Cal Gas valve inside the front door of the 240-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**.



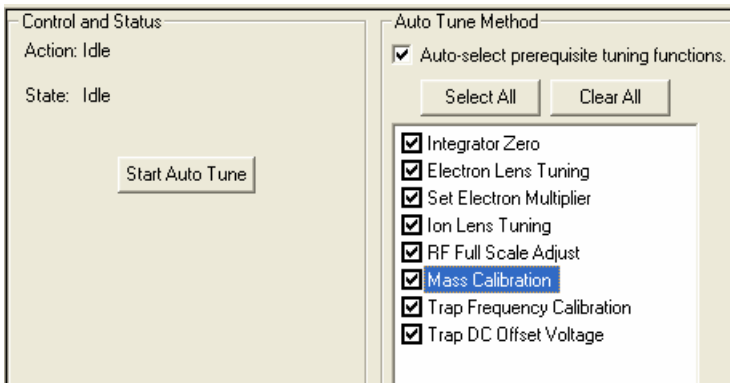
CI gas adjustment

Before performing chemical ionization, adjust the reagent gas pressure. Details of setting up methane CI gas are found in the *Agilent 240 Ion Trap GC/MS Hardware Operation Manual.*

Auto tune

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

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



Integrator Zero

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

Set Electron Multiplier

Set Electron Multiplier determines two settings, the multiplier voltage needed to achieve a multiplier gain of about 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 Tuning** system, consisting of three lenses (Lens 1, 2, and 3), 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. Set the **RF Full Scale Adjust** by doing **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 changes. Mass calibration does not have to be performed again after the auxiliary helium buffer gas flow rate is changed.

Trap Frequency Calibration

After completing mass calibration, perform **Trap Frequency Calibration**. 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.

Run Trap Frequency Calibration ***after*** completing Mass Calibration.

Trap DC Offset Voltage

Adjust the trap DC offset to optimize the ion signal for *m/z* 414 in the calibration gas. An optimal value for this parameter assures good high mass sensitivity.

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 MS is shown in Figure 4.

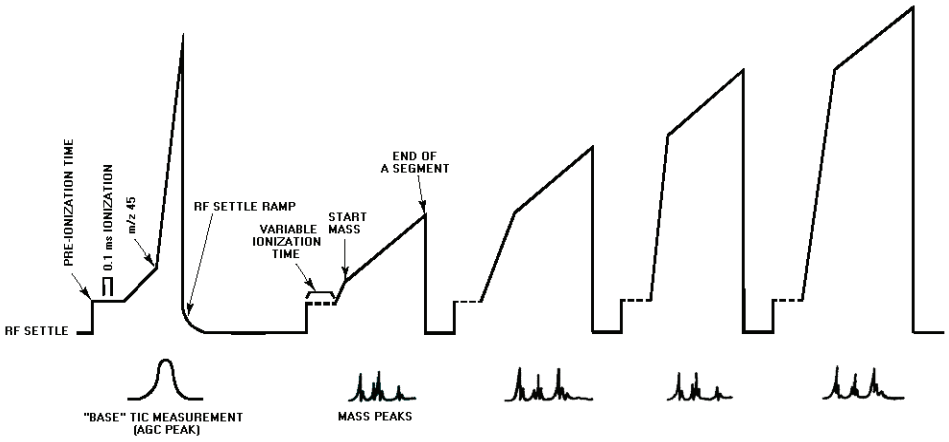
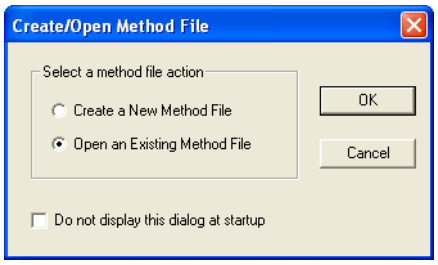


Figure 4 A typical scan function

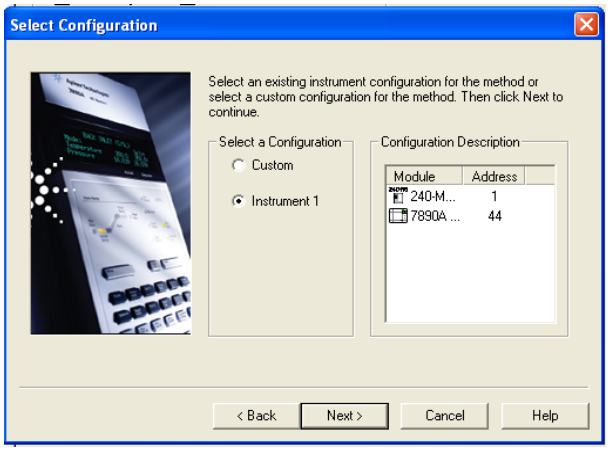
Using the Wizard for New Method

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

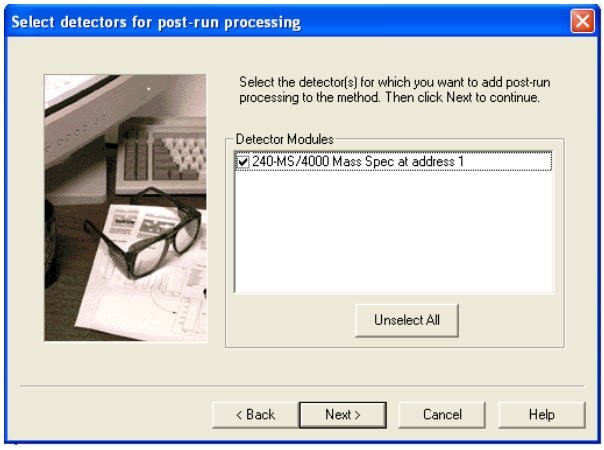
2 Click **Create a New Method File**. The wizard guides you in building this new method. If you do not want to see this message again, check the box **Do not display this dialog at startup**.



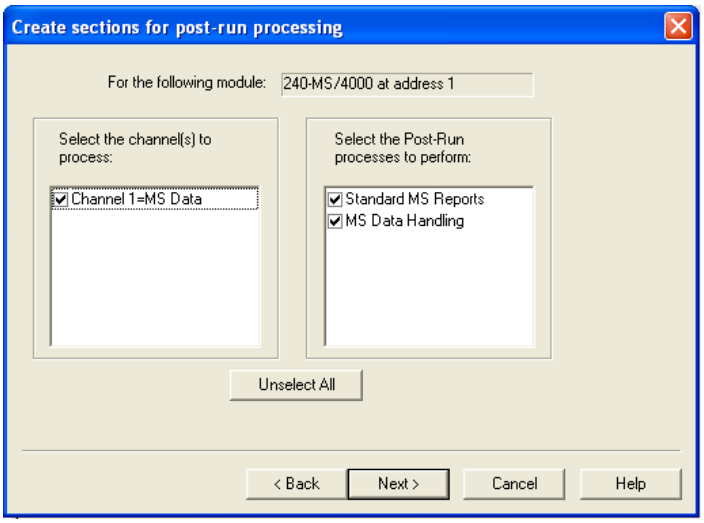
3 Select **Instrument 1** and click **Next**. Use **Custom** configuration to create methods on a PC remote from the instrument.



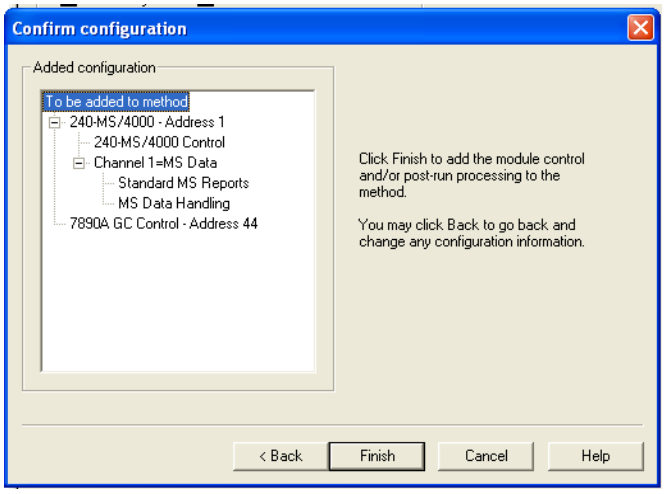
4 Select the detector(s) for post-run processing and click **Next**.



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. Refer to the *MS Workstation Software Reference Manual* for information about data handling and reports.



The method contains the following sections:

- 7890 GC Control
- 240 MS Control

- Standard MS Reports
- MS Data Handling.

Name the method

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

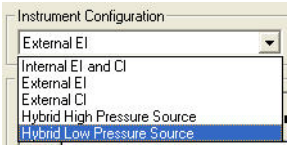
2 Type a name for the method.

3 Select the folder in which to keep the method.

4 Click **Save**.

Set the instrument configuration

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



Select the acquisition data type

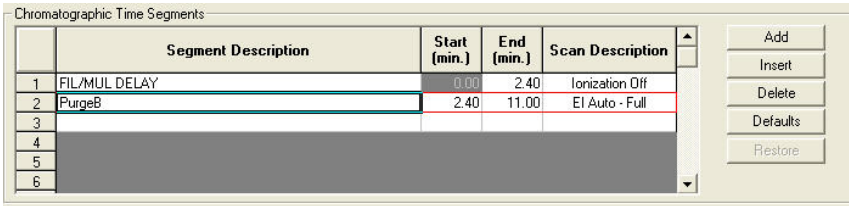
Centroid data is the default acquisition data type. Data handling, library searching, and spectral comparison can only be done using centroid data. The analog signal from the detector is sent to an analog to digital converter. The software determines the center of gravity of the digitized ion signal, 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.

Edit chromatographic time segments

The Chromatographic Time Segments table allows you to time-program analysis conditions to get the best results for each segment in the analysis. Up to 250 time segments can be created for runs up to 650 minutes in length. By default, there is a **Filament/Multiplier Delay** segment at the start of the run so that the system will not be stressed during the elution of the

chromatographic solvent. Following this segment, one could just acquire the mass spectra in full-scan with a single analysis segment. However, one can tailor variables such as acquired mass range, insert MS/MS segments for individual analytes, and set up the instrument to acquire the best data for each analyte.



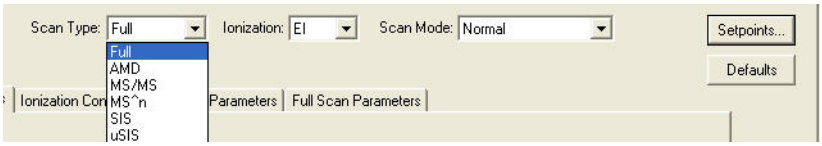
Adding or inserting a segment copies all of the parameters from the previous segment to the newly created segment. Double-click a field to edit the **Segment Description**, **Start** time, or **End** time of a segment.

Edit the 240 MS method segments

This section describes editing parameters for External EI methods. For information on External CI, see the section "Building GC/MS Methods External PCI and NCI" in the *240 Ion Trap GC/MS Software help*.

Scan function settings

Select a **Scan Type** from the menu. The **Ionization** menu has one choice for External ionization. See Table 2 on page 40 for detailed scan data.



The 240MS 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.

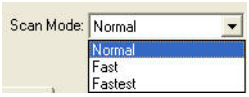


Table 2 Detailed scan data

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

General parameters tab

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

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 Agilent MS Workstation software must decide to which mass to assign measured intensity. If the exact mass of an ion happens to fall close to the dividing line between integer masses, the software may make an incorrect mass assignment. This scenario is more likely for molecules with higher molecular weights, since the mass defects for several atoms may add together to produce a sizable mass defect. For example, the exact mass for the lightest isotope form of C_2Br_6 is 497.51002, which could easily be assigned as either 497 or 498.

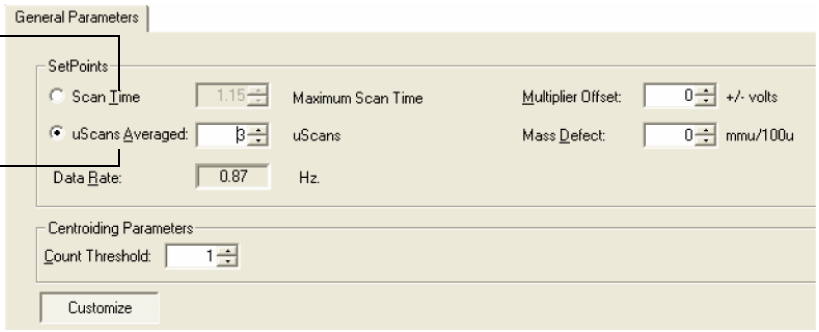
The **Multiplier Offset** adjusts the EM voltage by as much as ± 300V relative to the current multiplier setting in the **Module Attributes** tab dialog in **Manual Control** (this is usually the 10^5 gain value from **Auto Tune**). Sometimes better sensitivity is achieved,

particularly in techniques such as MS/MS, when the multiplier voltage is increased. Note that this adjustment can be made on a segment-by-segment basis.

The **Count Threshold** is normally 1. A value of 2 or 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.

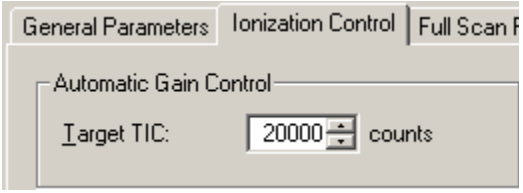
Select Scan Time to adjust the seconds/scan. The minimum # of uScans to average the data rate will be automatically computed.

Select uScans Averaged to adjust the # 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 and/or shift in mass assignments for strong chromatographic peaks). Typically, a **Target TIC** between 20,000 and 40,000 counts gives the best results.



Scan parameters

Each MS scan type has 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 "Building GC/MS Methods section" in the *240 Ion Trap MS Software help*.

Setting full scan parameters

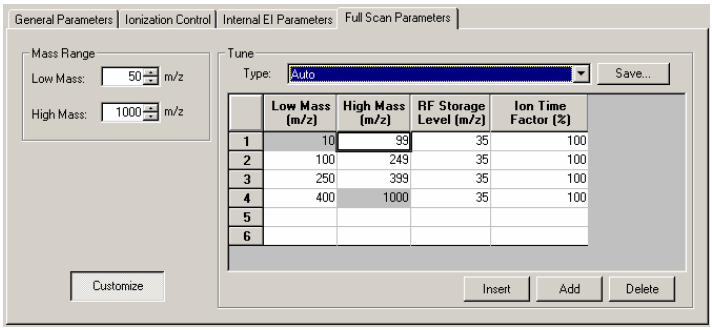
Use **Full Scan** data acquisition for general-purpose GC/MS analysis. In the **Mass Range** area (upper left), enter **Low Mass** and **High Mass** values to specify the full scan mass range. The entire accessible mass range of *m/z* 10 to 1000 is broken up into four segments if the **Tune Type** is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

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

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

This parameter is particularly important for External configuration, because the kinetic energy of ions entering the trap increases proportionally with the **RF Storage Level**. Fragile molecular or fragment ions can dissociate by collisions with helium buffer gas as they are being trapped and this can affect mass spectral quality. Using lower RF Storage levels of 25 to 30 for the first two segments of the mass range can improve spectral quality in such cases. For more detail on this issue, see the section "External Electron Ionization Ion Transport Processes" in the *240-MS GC/MS Software help*.

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

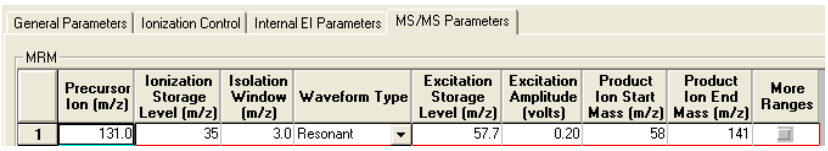
After the ionization step, tandem mass spectrometry, or MS/MS, does an ion preparation step before mass analysis. MS/MS may be performed after either electron or chemical ionization. Briefly, all ions are eliminated from the stored mass range except at the *m/z* of a precursor ion. The precursor ions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

When designed well, an MS/MS method will:

- Fill the ion trap with only the selected precursor ions, so that trap capacity is used so that in many cases, co-eluting interfering compounds are excluded from the trap.
- Create product ions using 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 except to the degree one is determining a set of isomers of a given class such as PCBs or Dioxins.

A figure of the **MS/MS Parameters** tab follows.



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 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, for example, using several runs with different ranges of excitation amplitudes. Using the **Automated Method Development (AMD)** 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, see the "Tandem Mass Spectrometry" section in the *240 GC/MS Software help*.

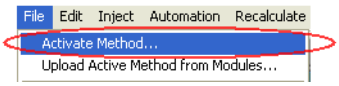
Viewing methods in manual control

After creating a method in the **Method Builder**, preview it in **Manual Control**. All MS parameters can be edited and previewed before a run. However, you cannot change the number of segments, or the start and end times of existing segments, unless you click **Edit Method** and the **Method Builder** to make those changes.

Activating a method

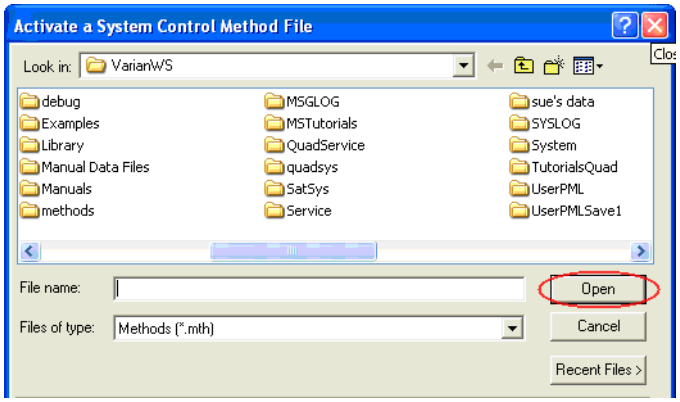
1 Click the **File** menu.

2 Click **Activate Method**.



3 Select a method by either

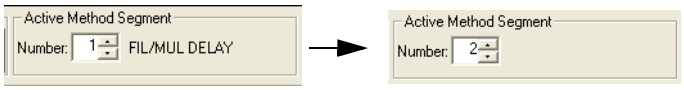
- Clicking **Recent Files** to display the eight most recent methods
- Clicking **Open** after selecting a method from a folder



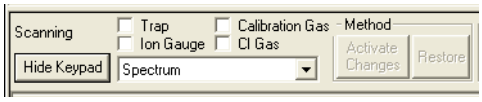
4 The active method is displayed in the toolbar.

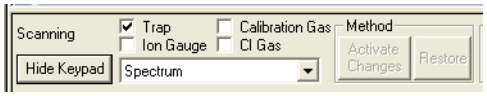
Displaying ions

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:

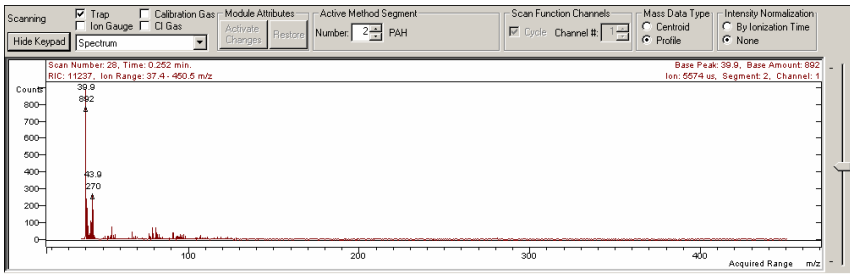


2 Click the **Trap** check box to turn on the ion trap.





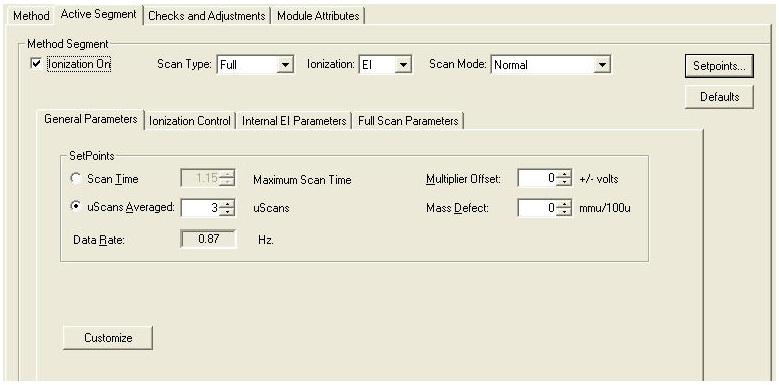
3 Select the method segment to view. Turn on the **Calibration Gas** or **CI Gas** by selecting the check box.

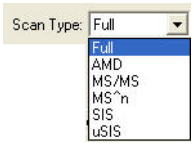


Viewing method parameters

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

Look at the information in the top row of the **Active Segment** tab, to identify if it is on, and its **Scan Type**, **Ionization** mode, and **Scan Mode**.

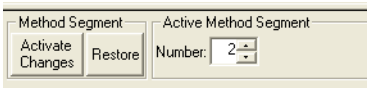




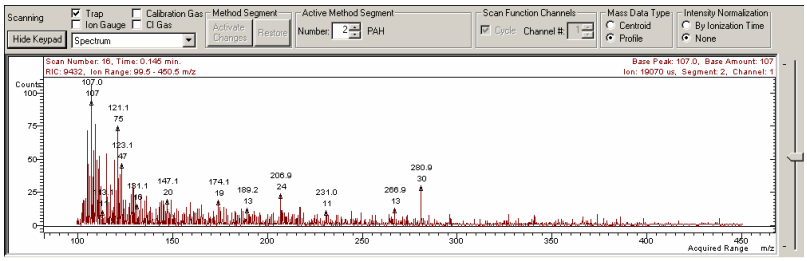
Editing a method in manual control

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 as shown in the next figure.



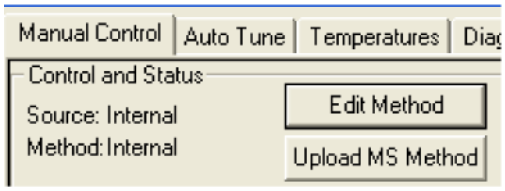
The changes are reflected in the spectrum. The example here is a change in the start mass from 50u to 100u:



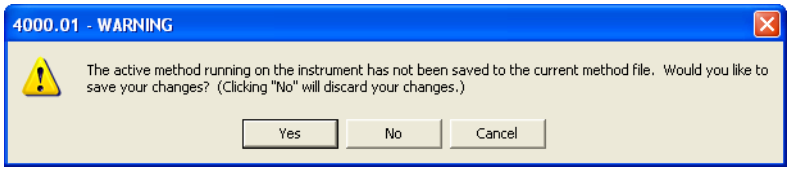
Saving a method

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 checks to see if changes are made when the segment was accessed. If changes were made, you have the option to save or discard these changes.



If you leave **System Control** by starting automation or choosing **Inject Single Sample**, you are prompted to save the method.

Click **Yes** to cancel the injection and return to **Manual Control**, to 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 the following topics, see the *Agilent 240 Ion Trap GC/MS Hardware Operation Manual.*

Internal to External

Converting the 240 MS from internal to external configuration involves changing both the ion source and the column position. The Internal ion source assembly is removed from the trap assembly and replaced with the External source assembly.

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

For details on how to add/remove the source assemblies, adjust the transfer line, and column installation, see the *Agilent 240 Ion Trap GC/MS Hardware Operation Manual* for detailed information.

1. Remove the analyzer assembly from the MS manifold.
2. Change the ion source to external.
3. Move the heat shield to the forward position.
4. Remove the filament adaptor and connect the flex cable.
5. Change the transfer line to the front position.
6. Change from the polymeric internal transfer line tip to the external metallic tip.
7. Cut the column 1 mm past the end of the transfer line.
8. Change the transfer line switch to External.

9 Replace the analyzer in the MS manifold.

Hybrid to External

Changing from Hybrid configuration to External configuration does not require changing the ion source assembly. Simply change the transfer line from internal to external position, and change the transfer line tip to the External type.

1 Change the transfer line trap entry location from internal to external.

2 Remove the Hybrid source plug.

3 Replace the internal transfer line tip with the external tip.

4 Cut the column 1 mm past the transfer line tip.

5 Flip the transfer line switch to the External position.

Effects of Hardware Changes

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

- System Control compares the current configuration stored in the current Module Attributes with the configuration reported by the hardware.
- If these do not match, the Module Attributes are updated (reset) to the appropriate configuration. A similar process occurs for the default method (Default.mth).
- After making the hardware configuration change, new methods will have the appropriate instrument configuration by default.

The resetting of **Module Attributes** requires you to run all **Auto Tune** routines, as the prior **Auto Tune** results are invalid.