

**Agilent InfinityLab  
LC/MSD Series and  
6100 Series LC/MS**

**Concepts Guide**



**Agilent Technologies**

# Notices

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## Software Revision

This guide is valid for the C.01.07 SR3 or later revision of the Agilent OpenLAB CDS ChemStation Edition software for the InfinityLab LC/MSD Series and 6100 Series LC/MS until superseded.

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## In This Guide...

The Concepts Guide presents an overview of the InfinityLab LC/MSD Series and 6100 Series LC/MS mass selective detector, to help you understand how the hardware and software work.

### **1 Overview of Hardware and Software**

Learn how the hardware works in the InfinityLab LC/MSD Series and 6100 Series LC/MS instrument, and get a brief introduction to the OpenLAB CDS ChemStation Edition program.

### **2 Instrument Preparation**

Learn the concepts you need to prepare the LC and column for an analysis, and to tune the MS.

### **3 Data Acquisition**

Learn about setting up methods and running samples.

### **4 Data Analysis**

Learn the concepts you need for qualitative and quantitative data analysis with the ChemStation Edition program.

### **5 Reports**

Learn about predefined results reports and about setting up custom reports.

### **6 Verification of Performance**

Learn the concepts for Operational Qualification/ Performance Verification (OQ/PV) and system verification with the ChemStation Edition program.

### **7 Maintenance and Troubleshooting**

Learn about tools that are provided in the ChemStation Edition program to help you maintain your system and diagnose and fix problems.

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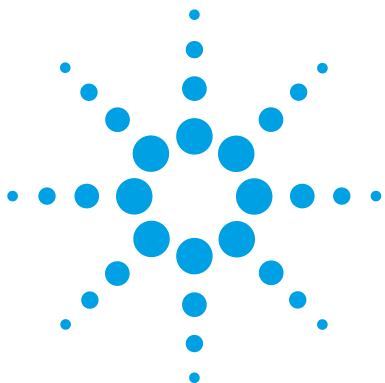
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This chapter provides an overview of the hardware and ChemStation Edition program that comprises the InfinityLab LC/MSD Series and 6100 Series LC/MS systems. The InfinityLab LC/MSD Series consists of two models: LC/MSD XT and LC/MSD. The 6100 Series LC/MS family consists of three models: 6120B, 6130B, and 6150B.



## How the Agilent mass selective detectors work

### Overview

Mass selective detectors (MSD) analyze ions moving through a vacuum to produce mass spectra. Mass spectra provide valuable information about the molecular weight, structure, identity, quantity, and purity of a sample. MSDs add specificity to both qualitative and quantitative analyses.

A quadrupole mass analyzer is sometimes called a quadrupole mass filter or a quadrupole.

Figure 1 shows a diagram of Agilent MSD instrument. The ionization of a sample occurs at atmospheric pressure in the ion source that is shown on the left. The Agilent MSDs are compatible with a number of Agilent atmospheric pressure ionization (API) sources.

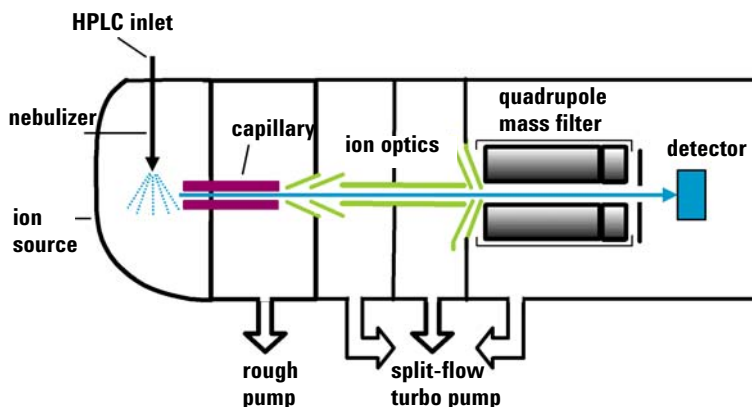


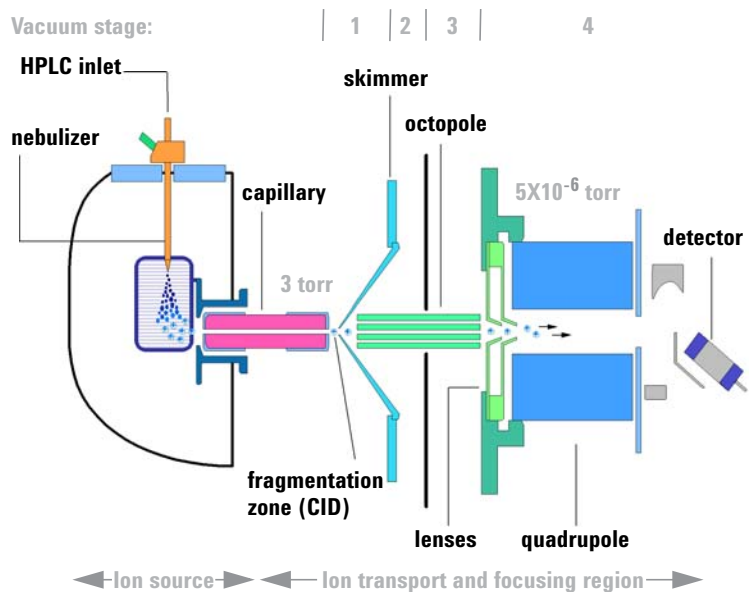
Figure 1 Block diagram for an Agilent MSD

API – atmospheric pressure ionization

A common atmospheric sampling interface introduces ions from these ionization sources into the vacuum system of the mass spectrometer. Various ion-optic elements focus and guide the ions through a series of vacuum stages until they reach the quadrupole mass analyzer, which separates the ions. The ions then travel to the detector, where they are recorded as signals.

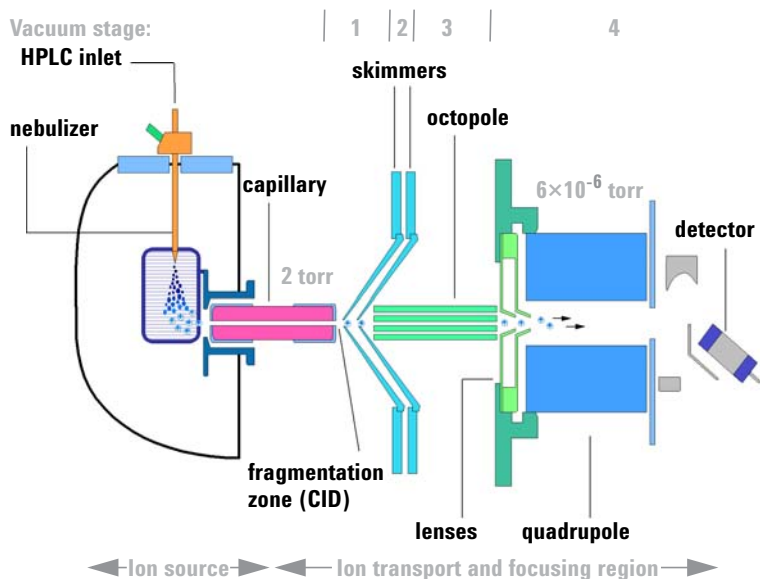
## Details

Figure 2 and Figure 3 show more detailed schematics of the ion paths in an Agilent MSD. After the API source forms ions, the ion-optic elements in the ion transport and focusing region of the system direct the ions toward the quadrupole and the detector. During transit, the ions move from atmospheric pressure (760 torr) at the source to a vacuum in the  $10^{-6}$  torr range at the quadrupole and detector.



**Figure 2** Ion path for LC/MSD, LC/MSD XT, 6130 and 6150

## 1 Overview of Hardware and Software Details



**Figure 3** Ion path for 6120

The ion transport and focusing region of the Agilent MSD is enclosed in a vacuum manifold. The function of the vacuum system is to evacuate regions of ion focusing and transport and keep the quadrupole at low pressure.

Because the nebulizer is at a right angle to the inlet capillary, most of the solvent is vented from the spray chamber and never reaches the capillary. Only ions, drying gas, and a small amount of solvent are transmitted through the capillary.

*By autotuning the instrument, you automatically set most of the voltages for the elements in the ion path. See “Preparation of the MS – tuning” on page 42.*

The following discussion of the ion optics is organized according to the stages of the ion path and the vacuum stages of the mass spectrometer.

### **Ion transport and fragmentation (first vacuum stage)**

Ions produced in the API source are electrostatically drawn through a drying gas and then through a heated sampling capillary into the first stage of the vacuum system. Near the exit of the capillary is a metal skimmer with a small hole. Heavier ions with greater momentum pass through the skimmer

aperture. Most of the lighter drying gas (nitrogen) molecules are deflected by the skimmer and pumped away by a rough pump. The ions that pass through the skimmer move into the second stage of the vacuum system.

The atmospheric pressure ionization techniques are all relatively “soft” techniques. They generate primarily:

- Molecular ions  $M^+$  or  $M^-$
- Protonated molecules  $[M + H]^+$
- Simple adduct ions  $[M + Na]^+$
- Ions representing simple losses, such as the loss of a water molecule  $[M + H - H_2O]^+$

#### CID – collision-induced dissociation

These types of ions give molecular weight information, but you often need complementary structural information. To gain structural information, you can fragment the analyte ions in the first vacuum stage. To do that, you give them extra energy and collide them with neutral molecules in a process known as collision-induced dissociation (CID). A voltage is applied at the end of the atmospheric sampling capillary to add energy to the collisions and create more fragmentation. For more information, see “[Generation of fragment ions: low versus high fragmentor](#)” on page 16.

#### Ion transport (second and third vacuum stages)

An octopole ion guide is a set of small parallel metal rods with a common open axis through which the ions can pass.

**LC/MSD, LC/MSD XT, 6130 and 6150** In the second vacuum stage, the ions are immediately focused by an octopole ion guide that traverses two vacuum stages. The ions pass through the octopole ion guide because of the momentum they received from being drawn from atmospheric pressure through the sampling capillary. Radio-frequency voltage applied to the octopole rods repels ions above a particular mass range to the open center of the rod set. The ions exit this ion guide and then pass through two focusing lenses into the fourth stage of the vacuum system.

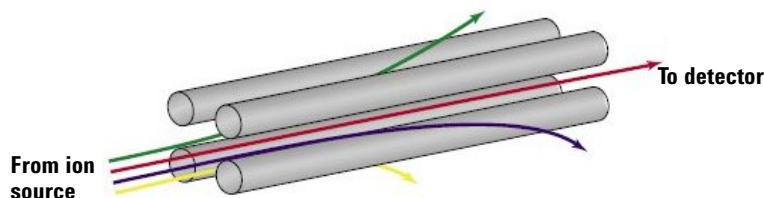
**6120** In the second vacuum stage, the ions are transported between skimmer 1 and skimmer 2. They then enter the third vacuum stage, where they pass through the octopole ion guide. The ions exit this ion guide and then pass through two focusing lenses into the fourth stage of the vacuum system.

### Ion separation and detection (fourth vacuum stage)

In the fourth vacuum stage, the quadrupole mass analyzer separates the ions by mass-to-charge ratio. An electron multiplier then detects the ions.

*m/z* – mass/charge ratio

The quadrupole mass analyzer (Figure 4) consists of four parallel rods to which specific direct-current (DC) and radio-frequency (RF) voltages are applied. The analyte ions are directed down the center of the rods. Voltages applied to the rods generate electromagnetic fields. These fields determine which mass-to-charge ratio of ions can pass through the filter at a given time. The ions that pass through are focused on the detector.



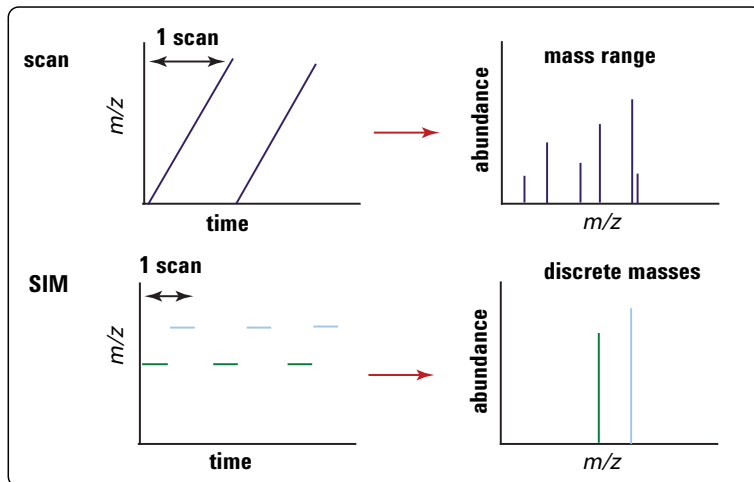
**Figure 4** Quadrupole mass analyzer

## Types of data you can acquire

### Scan versus selected ion monitoring (SIM)

You set up a scan or SIM analysis in the Method and Run Control view, described in [Chapter 3](#).

As shown in [Figure 5](#), quadrupole mass analyzers can operate in two modes. To get the most from your analysis, it is important to pick the appropriate mode. The discussion below will help you choose.



**Figure 5** A quadrupole mass analyzer can operate in either scan mode or selected ion monitoring (SIM) mode

#### Scan mode

In scan mode, a range of  $m/z$  values are analyzed, for example,  $m/z$  200 to 1000. The quadrupole sequentially filters one mass after another, with an entire scan typically taking about a second. (The exact time depends on mass range and scan speed.) The MS firmware steps the quadrupole through increasing DC and RF voltages, which sequentially filters the corresponding  $m/z$  values across a mass spectrum.

## 1 Overview of Hardware and Software

### Generation of fragment ions: low versus high fragmentor

A full scan analysis is useful because it shows all of the ions in a given mass range that are present in the ion source. Because it provides a complete picture of all the ionized compounds that occur above the detection limit in the chosen mass range, a full scan analysis is often used for sample characterization, structural elucidation, and impurity analysis. It is also the starting point for development of methods for SIM data acquisition (discussed next).

#### Selected ion monitoring (SIM) mode

To obtain the best sensitivity, the quadrupole is operated in SIM mode. In SIM mode, the quadrupole analyzes the signals of only a few specific  $m/z$  values. The required RF/DC voltages are set to filter one mass at a time. Rather than stepping through all the  $m/z$  values in a given mass range, the quadrupole steps only among the values that the analyst chooses. Because the quadrupole spends more time sampling each of these chosen  $m/z$  values, the system can detect lower levels of sample.

SIM mode is significantly more sensitive than scan mode but provides information about fewer ions. Scan mode is typically used for qualitative analyses or for quantitation when analyte masses are not known in advance. SIM mode is used for quantitation and monitoring of target compounds.

## Generation of fragment ions: low versus high fragmentor

*When you set up a method for data acquisition, you can control the amount of fragmentation with the fragmentor setting. You set up a method in the Method and Run Control view, described in Chapter 3.*

Fragment ions, also known as product ions, are formed by breaking apart precursor ions. On the InfinityLab LC/MSD Series and 6100 Series LC/MS instruments, the fragmentation region is between the capillary exit and the skimmer, where the gas pressure is about 2 to 3 torr. Depending on the voltage in this region, precursor ions may pass through unchanged or they may be fragmented.

When a lower voltage is applied across this region, the ions pass through unchanged. Even if these ions collide with the gas molecules in this region, they usually do not have enough energy to fragment. (See [Figure 6](#).)



Generation of fragment ions: low versus high fragmentor

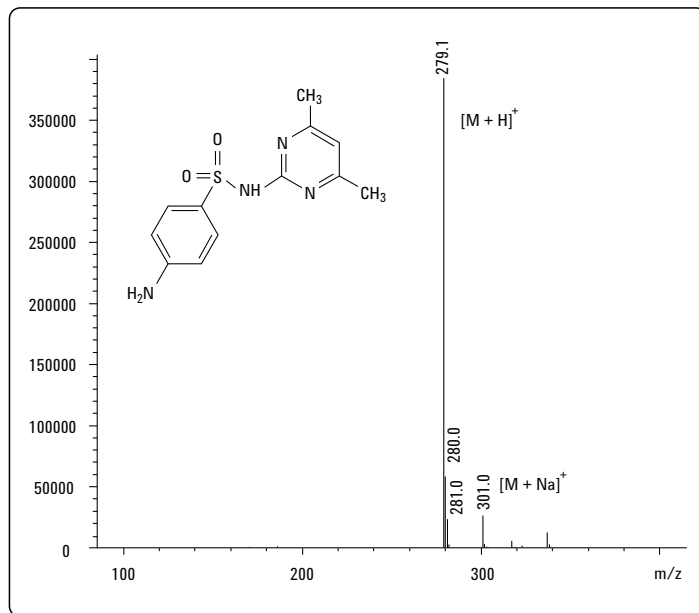


Figure 6 Mass spectrum of sulfamethazine – low fragmentor

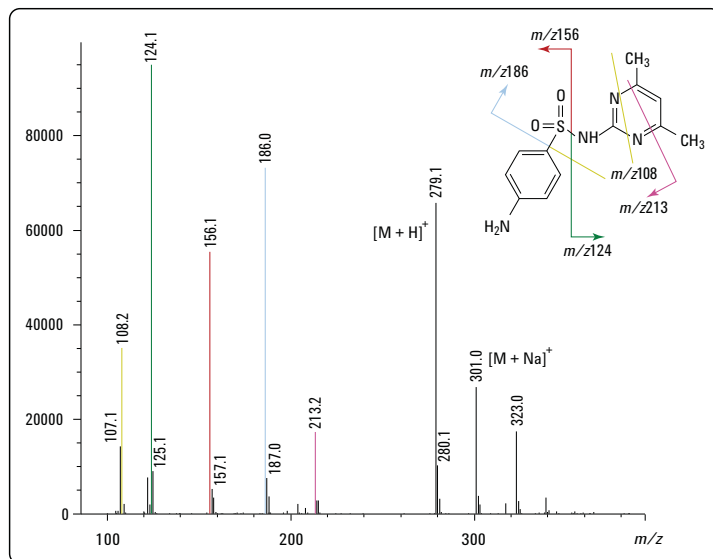


Figure 7 Mass spectrum of sulfamethazine – high fragmentor

## 1 Overview of Hardware and Software

### Positive versus negative ions

If the voltage is increased, the ions have more translational energy. Then if the ions collide with gas molecules, the collisions convert the translational energy into molecular vibrations that can cause the ions to fragment. This is called collision-induced dissociation (CID). [Figure 7](#) shows an example. Even though this fragmentation does not occur where the ions are formed at atmospheric pressure, it is a tradition to call this type of fragmentation “in-source CID.” The ions from molecular fragments are used for structural determination or confirmation of the presence of a particular chemical species.

It is possible to produce both molecular ions and fragment ions within the same spectrum by using an intermediate fragmentation voltage.

#### FIA – flow injection analysis

The ideal fragmentation voltage depends on the structure of the compound and the needs of the analysis. For target compound analysis, it is good practice to determine in advance the compound’s response to fragmentor setting. The fastest way to accomplish this is with a flow injection analysis (FIA) series. An FIA series allows you to inject the compound multiple times within the same run, and to vary the fragmentor setting in different time windows. From the resulting data, you can judge the best fragmentor setting. For more information on FIA, see “[Flow injection analysis](#)” on page 67.

## Positive versus negative ions

*You set the ion polarity when you set up a method in the Method and Run Control view, described in [Chapter 3](#).*

Atmospheric pressure ionization techniques can produce both positive and negative ions. For any given analysis, the predominant ion type depends on the chemical structure of the analyte and (particularly for electrospray ionization) the pH of the solution. While either or both ion types may be present in the ion source, the polarity of the ion optics in the ion transport and focusing region determines which ion type is detected.

Analyses of positive and negative ions require different settings for the ion optics. The software-controlled autotune process optimizes the settings for both positive and negative ions, and

stores them in a single tune file. During data acquisition, the ChemStation Edition program accesses the tune file for the appropriate settings.

## Multiple signal acquisition

*You establish the conditions for multiple signal acquisition in the Method and Run Control view, described in [Chapter 3](#).*

The InfinityLab LC/MSD Series and 6100 Series LC/MS models allow you to acquire multiple types of data during a single analysis. Within a single analytical run, you can choose alternating positive and negative ionization; alternating high and low fragmentor settings; and alternating scan and SIM modes. Because optimum MS conditions vary from compound to compound, this multisignal capability enables you to analyze more compounds, with greater sensitivity, within a single run.

### Polarity switching

The InfinityLab LC/MSD Series and 6100 Series LC/MS models allow you to switch from scan to scan between analysis of positive ions and analysis of negative ions. To switch polarities very quickly, these models incorporate fast-switching power supplies for the API source, the lens system, the quadrupole, and the detector. The ability to switch polarities on the chromatographic time scale is very useful for analysis of complete unknowns because it obviates the need to run the sample twice to detect both types of ions.

### Alternating high/low fragmentor

With the InfinityLab LC/MSD Series and 6100 Series LC/MS models, you can also alternate from scan to scan between high and low fragmentation voltages. This capability allows you to acquire scans at low fragmentor settings for molecular weight information, and high fragmentor settings for structural information.

#### Alternating SIM/scan

Many analyses require use of SIM mode to monitor and/or quantitate target compounds at very low levels. Sometimes it is also desirable to characterize the other sample components with a scan analysis. The InfinityLab LC/MSD Series and 6100 Series LC/MS models allow you to alternate between SIM and scan modes, so you can accomplish both goals in a single analysis.

#### Putting it all together

The InfinityLab LC/MSD Series and 6100 Series LC/MS can cycle through four different user-selected acquisition modes on a scan-by-scan basis within a single run. For example, you can set up a single run to do the following:

- Positive ion scan with low fragmentor voltage
- Positive ion scan with high fragmentor voltage
- Negative ion scan with low fragmentor voltage
- Negative ion scan with high fragmentor voltage

Such an analysis is ideal for a mixture of compounds where some respond better in positive mode and some respond better in negative mode, and where you need both molecular ions and fragment ions.

The time required for one cycle varies depending on the number of modes chosen, the scan range, and the interscan delay required for the switching. For separations with narrow chromatographic peaks, it is important to ensure that total cycle time is short enough that the instrument makes sufficient measurements across the peak.

The system issues a warning message when parameter settings result in a cycle time that is longer than recommended for optimum performance. You can fix the problem by selecting Fast Scan or Ultrafast scan or by increasing the Peakwidth.

## Ion sources

The InfinityLab LC/MSD Series and 6100 Series LC/MS instruments operate with the following interchangeable atmospheric pressure ionization (API) sources:

- ESI (electrospray ionization)
- Agilent Jet Stream (LC/MSD XT, 6130 and 6150 only)
- APCI (atmospheric pressure chemical ionization)
- APPI (atmospheric pressure photoionization)
- MMI (multimode ionization)

### NOTE

The sources that are used on the InfinityLab LC/MSD Series and 6100 Series LC/MS systems are the B-type sources. The InfinityLab LC/MSD Series and 6100 Series LC/MS systems are not compatible with the A-type sources that were used on previous Agilent LC/MS models.

## Electrospray ionization (ESI)

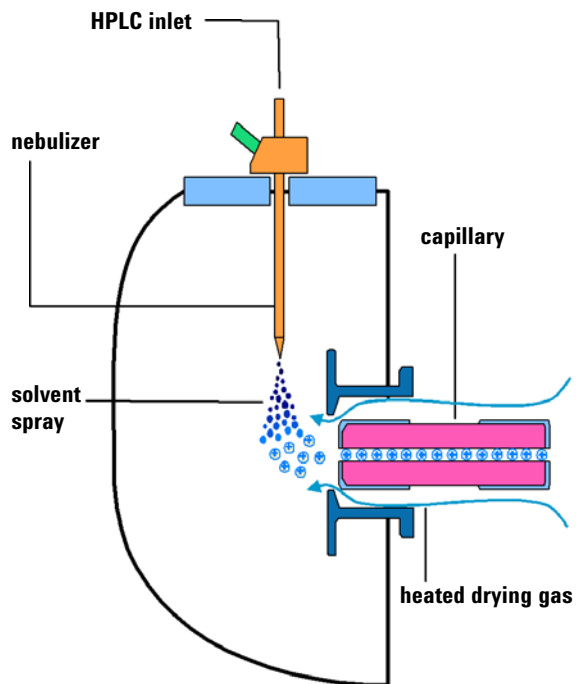
*You control the spray chamber parameters (nebulizer pressure, drying gas flow and temperature, and capillary voltage) when you set up a method in the Method and Run Control view, described in [Chapter 3](#).*

Electrospray ionization relies in part on chemistry to generate analyte ions in solution before the analyte reaches the mass spectrometer. As shown in [Figure 8](#), the LC eluent is sprayed (nebulized) into a spray chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The electrostatic field occurs between the nebulizer, which is at ground in the Agilent design, and the capillary, which is at high voltage.

The spray occurs at right angles to the capillary. This patented Agilent design reduces background noise from droplets, increases sensitivity, and keeps the capillary cleaner for a longer period of time.

## 1 Overview of Hardware and Software

### Electrospray ionization (ESI)



**Figure 8** Electrospray ion source

Electrospray ionization (ESI) consists of four steps:

- 1 Formation of ions
- 2 Nebulization
- 3 Desolvation
- 4 Ion evaporation

#### Formation of ions

Ion formation in API-electrospray occurs through more than one mechanism. If the chemistry of analyte, solvents, and buffers is correct, ions are generated in solution before nebulization. This results in high analyte ion concentration and good API-electrospray sensitivity.

Preformed ions are not always required for ESI. Some compounds that do not ionize in solution can still be analyzed. The process of nebulization, desolvation, and ion evaporation creates a strong electrical charge on the surface of the spray droplets. This can induce ionization in analyte molecules at the surface of the droplets.

### Nebulization

Nebulization (aerosol generation) takes the sample solution through these steps:

- a** Sample solution enters the spray chamber through a grounded needle called a nebulizer.
- b** For high-flow electrospray, nebulizing gas enters the spray chamber concentrically through a tube that surrounds the needle.
- c** The combination of strong shear forces generated by the nebulizing gas and the strong voltage (2–6 kV) in the spray chamber draws out the sample solution and breaks it into droplets.
- d** As the droplets disperse, ions of one polarity preferentially migrate to the droplet surface due to electrostatic forces.
- e** As a result, the sample is simultaneously charged and dispersed into a fine spray of charged droplets, hence the name *electrospray*.

Because the sample solution is not heated when the aerosol is created, ESI does not thermally decompose most analytes.

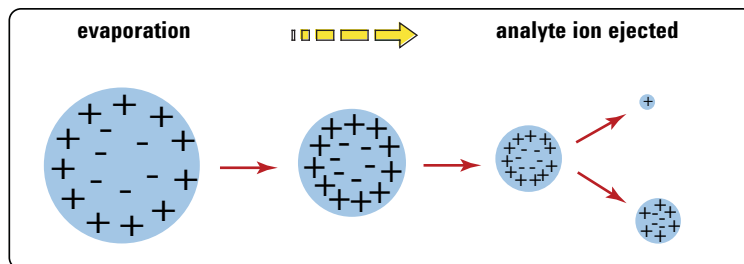
### Desolvation and ion evaporation

Before the ions can be mass analyzed, solvent must be removed to yield a bare ion.

A counter-current of neutral, heated drying gas, typically nitrogen, evaporates the solvent, decreasing the droplet diameter and forcing the predominantly like surface-charges closer together (see [Figure 9](#)).

## 1 Overview of Hardware and Software

### Electrospray ionization (ESI)



**Figure 9** Desorption of ions from solution

Coulomb repulsion – repulsion between charged species of the same sign

When the force of the Coulomb repulsion equals that of the surface tension of the droplet, the droplet explodes, producing smaller charged droplets that are subject to further evaporation. This process repeats itself, and droplets with a high density of surface-charges are formed. When charge density reaches approximately  $10^8$  V/cm<sup>3</sup>, ion evaporation occurs (direct ejection of bare ions from the droplet surface). These ions are attracted to and pass through a capillary sampling orifice into the ion optics and mass analyzer.

### The importance of solution chemistry

The choice of solvents and buffers is a key to successful ionization with electrospray. Solvents like methanol that have lower heat capacity, surface tension, and dielectric constant, promote nebulization and desolvation. For best results in electrospray mode:

- Adjust solvent pH according to the polarity of ions desired and the pH of the sample.
- To enhance ion desorption, use solvents that have low heats of vaporization and low surface tensions.
- Select solvents that do *not* neutralize ions through gas-phase reactions such as proton transfer or ion pair reactions.
- To reduce the buildup of salts in the ion source, select more volatile buffers.



## Multiple charging

Electrospray is especially useful for analyzing large biomolecules such as proteins, peptides, and oligonucleotides, but can also analyze smaller molecules like drugs and environmental contaminants. Large molecules often acquire more than one charge. Because of this multiple charging, you can use electrospray to analyze molecules as large as 150,000 u even though the mass range (or more accurately mass-to-charge range) for a typical quadrupole LC/MS instrument is around 3000  $m/z$ . For example:

$$100,000 \text{ u} / 10 \text{ z} = 1,000 \text{ m/z}$$

*The optional Agilent LC/MSD Deconvolution & Bioanalysis Software performs the calculations to accomplish deconvolution.*

When a large molecule acquires many charges, a mathematical process called deconvolution is used to determine the actual molecular weight of the analyte.

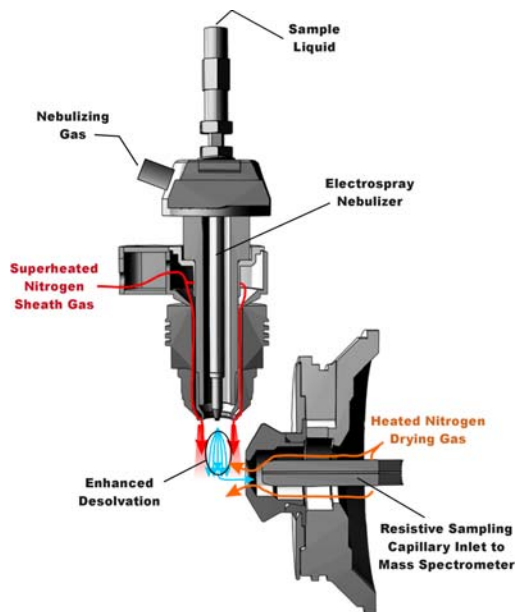
## Agilent Jet Stream

The Agilent Jet Stream source is supported on LC/MSD XT, 6130 and 6150 models only.

Agilent Jet Stream source enhances analyte desolvation by collimating the nebulizer spray and creating a dramatically “brighter signal.” The addition of a collinear, concentric, super-heated nitrogen sheath gas (Figure 10) to the inlet assembly significantly improves ion drying from the electrospray plume and leads to increased mass spectrometer signal to noise allowing the single quadrupole to surpass the femtogram limit of detection.

## 1 Overview of Hardware and Software

### Agilent Jet Stream



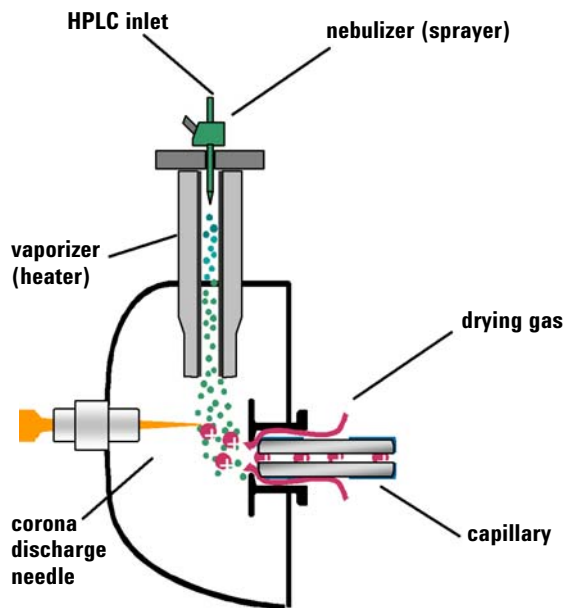
**Figure 10** Agilent Jet Stream source

Agilent Jet Stream thermal gradient focusing consists of a superheated nitrogen sheath gas that is introduced collinear and concentric to the pneumatically assisted electrospray. Thermal energy from the superheated nitrogen sheath gas is focused to the nebulizer spray producing the most efficient desolvation and ion generation possible. The enhanced molecular ion desolvation results in more ions entering the sampling capillary as shown in [Figure 10](#) and resulting improved signal to noise. Parameters for the Agilent Jet Stream source are the superheated nitrogen sheath gas temperature and flow rate and the nozzle voltage.

## Atmospheric pressure chemical ionization (APCI)

APCI is a gas-phase chemical ionization process. The APCI technique passes LC eluent through a nebulizing needle, which creates a fine spray. The spray is passed through a heated ceramic tube, where the droplets are fully vaporized (Figure 11).

The resulting gas/vapor mixture is then passed over a corona discharge needle, where the solvent vapor is ionized to create reagent gas ions. These ions in turn ionize the sample molecules via a chemical ionization process. The sample ions are then introduced into the capillary.



**Figure 11** Atmospheric pressure chemical ionization (APCI) source

APCI requires that the analyte be in the gas phase for ionization to occur. To vaporize the solvent and analyte, the APCI source is typically operated at vaporizer temperatures of 400 to 500 °C.

## 1 Overview of Hardware and Software

### Atmospheric pressure chemical ionization (APCI)

APCI is applicable across a wide range of molecular polarities. It rarely results in multiple charging, so it is typically used for molecules less than 1,500 u. Because of this molecular weight limitation and use of high-temperature vaporization, APCI is less well-suited than electrospray for analysis of large biomolecules that are non-volatile or thermally unstable. APCI is well suited for ionization of the less polar compounds that are typically analyzed by normal-phase chromatography.

## Atmospheric pressure photoionization (APPI)

With the APPI technique, LC eluent passes through a nebulizing needle to create a fine spray. This spray is passed through a heated ceramic tube, where the droplets are fully vaporized. The resulting gas/vapor mixture passes through the ultraviolet light of a krypton lamp to ionize the sample molecules (Figure 12). The sample ions are then introduced into the capillary.

APPI and APCI are similar, with APPI substituting a lamp for the corona needle for ionization. APPI often also uses an additional solvent or mobile phase modifier, called a “dopant”, to assist with the photoionization process.

APPI is applicable to many of the same compounds that are typically analyzed by APCI. APPI has proven particularly valuable for analysis of non-polar compounds.

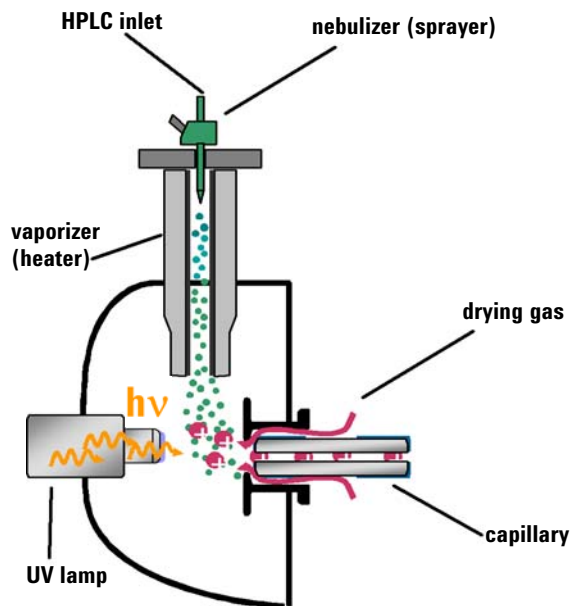
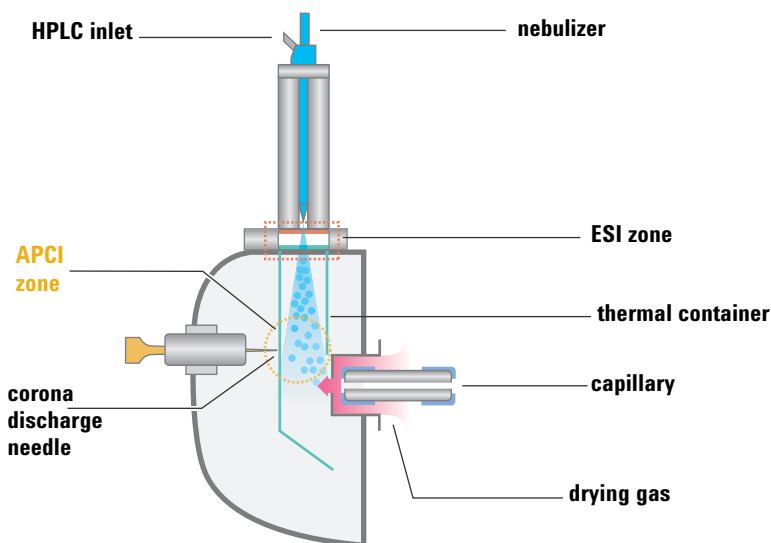


Figure 12 Atmospheric pressure photoionization (APPI) source

## Multimode ionization (MMI)

The multimode source is an ion source that can operate in three different modes—APCI, ESI or simultaneous APCI/ESI. The multimode source incorporates two electrically separated, optimized zones—one for ESI and one for APCI. During simultaneous APCI/ESI, ions from both ionization modes enter the capillary and are analyzed simultaneously by the mass spectrometer.



**Figure 13** Multimode source

Multimode ionization (MMI) is useful for screening of unknowns, or whenever samples contain a mixture of compounds where some respond by ESI and some respond by APCI. In these cases, the multimode source obviates the need to run the samples twice to accomplish a complete analysis.

Unlike the APCI and APPI sources where the temperature of the vaporizer is monitored, in the multimode source the actual vapor temperature is monitored. As a result, the vaporizer is typically set to between 200 and 250 °C.

# Introduction to the ChemStation Edition program

## Overview

The ChemStation Edition program is organized into views. Each view allows you to do a specific set of tasks. The menus and toolbars change with each view.



**Figure 14** These buttons allow you to switch among the six ChemStation Edition views

The following summarizes the ChemStation Edition views and their functionality:

### Method and Run Control

- Set up methods
- Change setpoints for the Agilent 1100/1200/1260/1290 Series LC modules, including the Chip Cube
- Change setpoints for the MSD
- Change setpoints for the Agilent API sources
- Run single samples
- Run automated sequences
- Run an FIA series
- View data in real time, as it is acquired

*For more information about the Method and Run Control view, see Chapter 3.*

## 1 Overview of Hardware and Software

### Overview

For more information about the Data Analysis view, see [Chapter 4](#).

#### Data Analysis

- View chromatograms and spectra from the MS and UV detectors
- Integrate chromatographic peaks
- Perform quantitation
- Check peak purity
- Deconvolute multiply charged spectra
- Generate reports
- Reprocess data from sequences

For more information about the Report Layout view, see [Chapter 5](#).

#### Report Layout

- Design custom report templates

For more information about the Verification view, see [Chapter 6](#).

#### Verification (OQ/PV)

- Verify system performance

For more information about the Diagnosis view, see [Chapter 7](#).

#### Diagnosis

- Learn possible causes of instrument problems
- Run tests to diagnose instrument problems
- Receive notification when it is time to perform system maintenance
- Pump down and vent the system

For more information about the MSD Tune view, see [Chapter 2](#).

#### MSD Tune

- Optimize and calibrate the MS



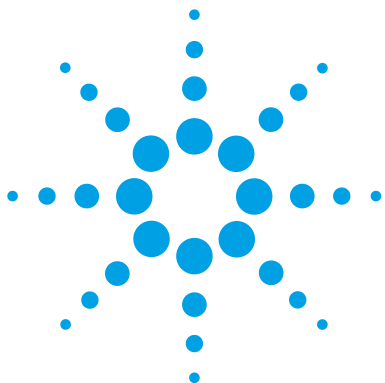
## Reviewing data remotely

There are two ways to set up a computer so you can review ChemStation Edition data remotely.

One way is to install a Data Analysis-only version of the ChemStation Edition program on the remote computer. This installation provides the same Data Analysis functionality that you have on the ChemStation Edition that controls your MSD. It is ideal if you need full features for in-depth data analysis.

Another way is to install the Analytical Studio Reviewer on the remote computer. Analytical Studio Reviewer lets you easily review ChemStation Edition LC and MSD data files, but the functionality is different than with the full ChemStation Edition Data Analysis. The Analytical Studio Reviewer program is ideal for synthetic chemists and others who use the MSD system for “walk-up” analysis.

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## 2 Instrument Preparation

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In this chapter, you learn the concepts that help you prepare the instrument for an analysis. This chapter assumes that the hardware and ChemStation Edition program are installed, the instrument is configured and the performance verified. If this has not been completed, see the *System Installation Guide*.



## Preparation of the LC system

### Purpose

To achieve good sensitivity, it is important to properly prepare the LC and column prior to an LC/MS analysis.

For best signal-to-noise, the entire LC system must be free of contamination from salts (such as nonvolatile buffers) and unwanted organic compounds. Some contaminants that are not bothersome for a UV detector can cause problems for the MS. Contaminants may cause ion suppression and/or high background, and these problems can seriously degrade sensitivity.

To achieve a smooth baseline with little noise, the LC flow must also be very stable.

### Summary of procedures

The exact LC preparation steps depend on how the LC was used previously and the type of analysis to be performed. The following provides guidelines:

#### Typical preparation

Before beginning an analysis, the entire LC path should be contaminant-free and the flow should be stable. Usually, you can accomplish these goals by doing the following:

- 1 Purge the pump to remove air bubbles. Purge each channel that you plan to use.

For instructions to purge the pump, search the online Help for the keyword “purge” and scroll down the list of topics until you see entries that begin with the word “purge.”

- 2 Condition the column to remove impurities or residual sample.

For more information, see “[Column conditioning and equilibration](#)” on page 39.

- 3 Equilibrate the column at the initial mobile phase composition.

For more information, see “[Column conditioning and equilibration](#)” on page 39.

- 4 Ensure that the system flow and pressure are stable.

For more information, see “[Monitoring the stability of flow and pressure](#)” on page 41.

### More extensive preparation

While the four-step procedure that is outlined above works well on a day-to-day basis, more extensive LC/column flushing may be necessary if any of the following are true:

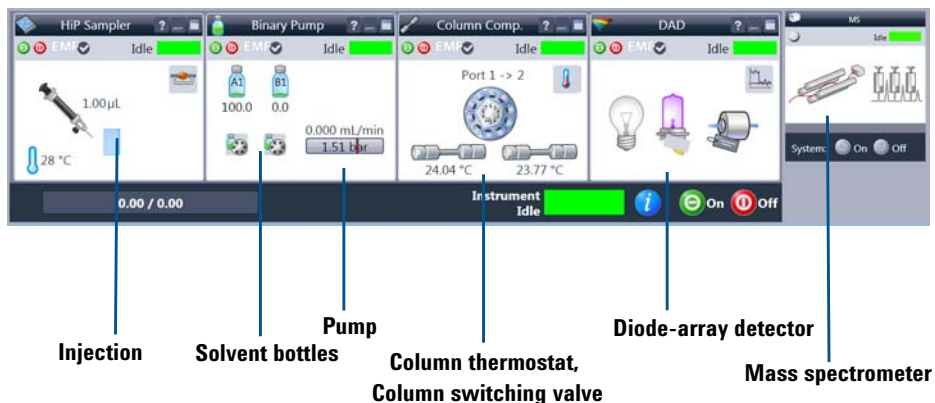
- You have not used this LC for MS.
- The column is new.
- You are changing to a different mobile phase composition.
- The LC was used to analyze dirty samples.
- The next analysis requires ultimate sensitivity.

A protocol for more thorough LC cleaning is given in the *System Installation Guide*. See the section on conditioning the LC in the chapter on system verification.

When you flush the LC, remember to flush all channels that you plan to use. Also, flush the injector by making several injections of the same solvent(s) that you use to flush the system.

## Setting parameters for LC modules

You set up the LC modules in the Method and Run Control view. Within the system diagram, click each module to set parameters.



**Figure 15** Example system diagram (yours may be different)

To access help for any system module, click **Help** on the module shortcut menu. To access help for a given dialog box, click the **Help** button on the dialog box.

### To set module control parameters

This procedure uses the pump module as an example.

- 1 Click **More Pump > Control HPLC Pump** on the Instrument menu to open the Pump Control dialog box.
- 2 Set desired control parameters and click **OK**.

#### Alternate method

Select the desired control parameter such as **Standby** from the Pump shortcut menu.

### To set module setpoint parameters

This procedure uses the binary pump module as an example.

- 1 Click **Set up Instrument Method** on the Instrument menu to open the Setup Method dialog box.
- 2 Click the **Binary Pump** tab.
- 3 Set desired setpoints and click **OK**.

### To access other instrument parameters

- 1 Click to open the **Instrument** menu.
- 2 Click the desired command such as **Select Injection Source**, **Columns**, or **Instrument Configuration**.

## Column conditioning and equilibration

There are several ways to set parameters to condition and equilibrate a column.

### Conditioning

Column conditioning eliminates any previously separated compounds or impurities from the column, particularly after runs with solvent of a single composition (isocratic runs).

There are a number of ways to condition a column before a sample run. One way is to pump the organic solvent that you intend to use (100% solvent B) through the column for a period of time. Another way is to run the gradient that you intend to use, then extend the time at the final composition until no further peaks elute.

When a column is new, “conditioning” may include injecting a few samples or high-level standards until peak area and retention time are stable.

### Equilibration

Column equilibration returns column characteristics to their initial state after a gradient run. To equilibrate a column before a sample run, you pass the solvent of initial composition through the column for a period of time.

### Column conditioning and equilibration

You can condition and equilibrate a column in one of three ways with the ChemStation Edition program.

- Interactively

You set the pump to the solvent composition for the end of the run and higher-than-normal flow rates. You can then immediately apply these setpoints to the pump. After you pump about three column-volumes of solvent, then set the pump to the solvent composition and flow rate for the beginning of the run. With this procedure, you do not store a data file.

If you use this procedure, you can tune the MS while you condition and equilibrate the column. When you tune the MS, the MS stream selection valve automatically diverts the LC effluent to waste. For information on tuning, see [“Preparation of the MS – tuning”](#) on page 42.

*You set up a method or sequence in the Method and Run Control view, described in [Chapter 3](#).*

- With a method in an interactive run

You set up a method for your analysis and then run a solvent blank. The run uses the method stop time. You can also use a post-run time within the method to equilibrate the column.

With this procedure, you store a data file.

- With a sequence

You set up a method for your analysis and then set up a solvent blank as the first run in a sequence. The method includes a post-run time to equilibrate the column.

With this procedure, you store a data file.



## Monitoring the stability of flow and pressure

The LC solvent flow and the system backpressure must be stable to ensure a quiet baseline and best results for API-MS. The best time to monitor the stability of flow and pressure is after you have equilibrated the column, and before you start the analysis.

*Chapter 3 provides more information about online signals, which are also called online plots.*

You can measure stability with the ChemStation Edition program. To do this, you set up an isocratic method with the same solvent composition as the initial composition you intend to use for your analysis. During the run, you monitor the online signals for flow and pressure.

## Preparation of the MS – tuning

### Overview

*Use the MSD Tune view for all tasks that relate to tuning.*

Tuning is the process of adjusting MS parameters to generate high quality, accurate mass spectra. During tuning, the MS is optimized to:

- Maximize sensitivity
- Maintain acceptable resolution
- Ensure accurate mass assignments

### Parameters that are adjusted

The InfinityLab LC/MSD Series and 6100 Series LC/MS instruments have two sets of parameters that can be adjusted. One set of parameters is associated with the formation of ions. These parameters control the spray chamber (for example, electrospray or APCI) and fragmentor. The other set of parameters is associated with the transmission, filtering, and detection of ions. These parameters control the skimmer, octopole, lenses, quadrupole mass filter, and high-energy dynode (HED) electron multiplier (detector).

Tuning is primarily concerned with finding the correct settings for the parameters that control the transmission, filtering, and detection of ions. It is accomplished by introducing a calibrant into the MS and generating ions. Using these ions, the tune parameters are then adjusted to achieve sensitivity, resolution, and mass assignment goals. With a few exceptions, the parameters that control ion formation are not adjusted. They are set to fixed values known to be good for generating ions from the calibrant solution.

### **Tune files and reports**

The product of tuning is a tune file (actually a folder, such as **c:\Chem32\6130btun**) that contains parameter settings for both positive and negative ionization. When data acquisition uses a tune file, the settings appropriate for the ion polarity specified by the data acquisition method are loaded automatically.

Autotune, the automated tuning program, also generates a report. See [page 51](#).

### **Use of tune files during data acquisition**

During data acquisition, the parameters associated with ion formation are controlled by the data acquisition method. The parameters associated with ion transmission are controlled by the tune file assigned to the data acquisition method.

## Ways to tune

*Access this functionality via the Tune menu in the MSD Tune view.*

The ChemStation Edition program provides the following two ways to tune the MS:

- Autotune is an automated tuning program that tunes the MS for good performance over the entire mass range. It uses known compounds in a standard calibration mixture that is introduced via the Calibrant Delivery System (CDS). This is the tuning method that you use in most cases.
- Manual Tune allows you to tune the MS by adjusting one parameter at a time until you achieve the desired performance. Manual tuning is most often used when you need maximum sensitivity, when your analysis targets a restricted mass range, or when you need a tuning compound other than the standard calibrants.

In addition, a Check Tune program allows you to determine whether you need to tune.

Check Tune, Autotune, and Manual Tune are discussed in more detail in the next sections.

### NOTE

Frequent tuning is not required for normal operation. Once tuned, the LC/MS is very stable. Tuning is generally not needed more often than monthly, or at most weekly. If you suspect problems related to tuning, use the Check Tune program to confirm that the MS is out of adjustment before you retune it.

### CAUTION

Wait at least **four** hours after pump-down before tuning or operating your MSD system. Ceramic components in the quadrupole mass filter can absorb moisture from the air. Tuning or acquiring data too soon may result in arcing. Further, it takes the analyzer at least **11** hours to reach thermal equilibrium. Tune files created or data acquired before the MS is at thermal equilibrium may have incorrect mass assignments and other inaccuracies.

## When to tune – Check Tune

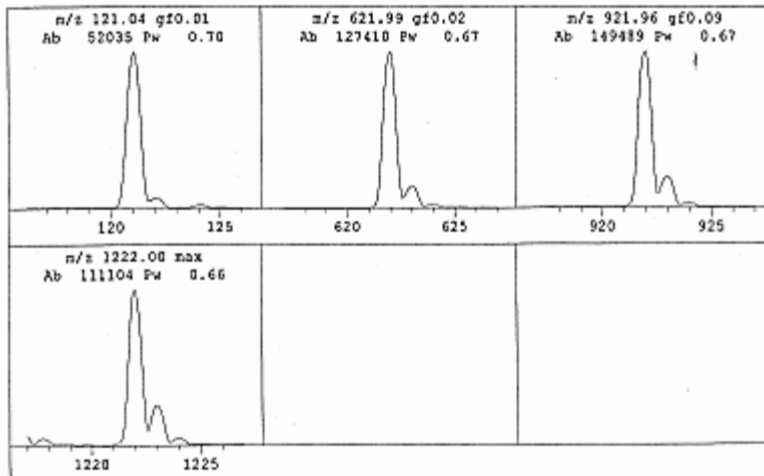
Check Tune allows you to quickly determine whether the MS is correctly tuned without performing a complete autotune. It performs a single profile scan of the tune masses and compares the peak widths and mass axes with target values. If the values obtained by Check Tune are within acceptable ranges, the tune report indicates that Check Tune passes. (See [Figure 16](#) on page 46.) If the values are outside of acceptable ranges, Check Tune suggests that you adjust peak widths or calibrate the mass axis.

A third parameter, gain, may be added to the parameters checked by Check Tune. If so, Check Tune compares the current gain value with the gain value from the most recent autotune. For a discussion of gain, see [page 52](#).

## 2 Instrument Preparation

### When to tune – Check Tune

MSD Check Tune G6100: MM-ES Positive mode - Standard Scan  
 Instrument : RRHT 6140 Rev. B.02.01-SP2 [260]  
 Mon Jan 22 13:18:22 2007 C:\Chen32\1\6140ATUN\ATUNES.TUN



Mass Axis Calibration			Resolution Setting			Signal Intensity
Target Mass	Tune Mass	P/F	Target Pk	Peak Width	P/F	Signal
121.05	121.05	pass	0.65	0.49	pass	54533
622.03	622.00	pass	0.65	0.67	pass	126658
922.01	921.97	pass	0.65	0.67	pass	149290
1221.99	1222.00	pass	0.65	0.66	pass	108229

#### Results Summary

	Mass Axis	Peak Width
Allowed Diff.	<=0.13 amu	<=0.1 amu
Actual Diff.		
Mass 1	pass	pass
Mass 2	pass	pass
Mass 3	pass	pass
Mass 4	pass	pass

All masses pass the mass axis and peak width tests

Figure 16 Check Tune report

## Autotune

Autotune is a program that adjusts the MS for good performance over the entire mass range.

### Autotune files

A tune file, ATUNES.TUN is provided as a starting point for autotune.

The results of autotune are saved to the current autotune file. This makes autotune faster because each autotune begins from the most recent good tune parameters. This means, however, that previous tune values are lost. You may want to occasionally save the autotune file to a different file name before using autotune.

A single autotune file contains the results of both positive ionization and negative ionization autotunes.

### Autotune polarity and scan speed

You have up to six choices for running an autotune, depending on MSD model:

- Dual Polarity
- Positive Polarity
- Negative Polarity
- Dual Polarity Fast Scan
- Positive Polarity Fast Scan
- Negative Polarity Fast Scan

The Dual Polarity modes perform an autotune for both positive ionization and negative ionization. Positive Polarity and Negative Polarity modes perform autotunes only for the specified polarity.

### Gain checking in autotune

Gain checking occurs in positive and negative polarity autotune, but only in Standard mode. For more information on gain checking, see “Gain calibration” on page 52.

### **Spray chamber parameters**

Autotune adjusts the parameters that affect ion transmission. The spray chamber parameters, which affect ion formation, are not adjusted by autotune. They are set to default values known to be good for generating ions of the calibrant solution.

Because there may be slight variations from spray chamber to spray chamber, the default parameters may not be optimum for a particular spray chamber. Changes such as adjustment of the nebulizer and normal aging of the LC/MS system components can also result in a system where the default values are not optimum. Therefore, it is possible to manually set the spray chamber parameters. Typically, this is necessary only when the instrument fails autotune or when the results of autotune show a significant decrease in system performance. Be aware that other factors can also decrease system performance.



## Manual tuning

The autotune program adjusts the MS for good performance over the entire mass range, and this tune program is sufficient for most applications. There are times, however, when it is advantageous to tune the MS manually. These include:

- When you want to achieve maximum sensitivity by sacrificing some resolution
- When you want to tune specifically for the very low end of the mass range (< 150 u)
- When you want to tune with a compound other than the standard calibrants.

### CAUTION

The calibrant delivery system (CDS) is designed specifically for precise delivery of the Agilent calibrants in a 90:10 acetonitrile:water solvent mix. Other solvents and mixes may have different flow rates, which may not be optimum for tuning. Further, the O-ring seals in the CDS are ethylene-propylene. They can be damaged by aliphatic, aromatic, or halogenated hydrocarbons. Never use these types of solvents in the CDS. We recommend delivering non-standard calibrants using a syringe pump or through the LC.

### Tips for manual tuning

#### Ionization modes

Separate tuning is *not* required for electrospray, APCI/APPI and multimode-ESI+APCI. Tuning involves adjusting the parameters that control the transmission of ions. It does not matter how those ions are generated. A tune file created in electrospray mode will generally provide good results for APCI/APPI samples. The reverse is also true.

#### Polarity

Separate tuning is required for positive and negative ionization. The MS must, at some time, be tuned twice – once with positive ions and once with negative ions. The two tunes can be stored under the same tune file name. Positive ion and negative ion settings can be updated separately.

**Mass range** Tuning is almost always done over the entire mass range. The exception is if the ions of interest are smaller than approximately 150 u. Correct manual tuning over a reduced mass range can improve transmission and detection of these very low mass ions.

Manual tuning generally improves results if any of the ions of interest are below approximately 150 u, because most of the ion optics parameters are optimized using the 2nd tune ion ( $m/z$  622 in Positive mode or  $m/z$  602 for Negative mode).

### **Steps to manual tune**

The online Help includes step-by-step instructions for manual tuning. The instructions assume the use of one of the standard calibrants and tuning over the entire mass range, but they can be altered for non-standard calibrants or narrower mass ranges.

To access the manual tuning instructions:

- 1** Open the online Help.
- 2** Click to expand “How to Work with Your ChemStation.”
- 3** Click to expand “Basic Tasks.”
- 4** Click to expand “Method and Run Control.”
- 5** Click to expand “MSD Tune.”
- 6** Click the link to “Manually Tuning the LC/MSD or CE/MSD.”
- 7** Scroll down until you see “Steps.”

## Tune reports

At the end of every autotune, the system prints a tune report. You can also manually print a tune report from the File menu.

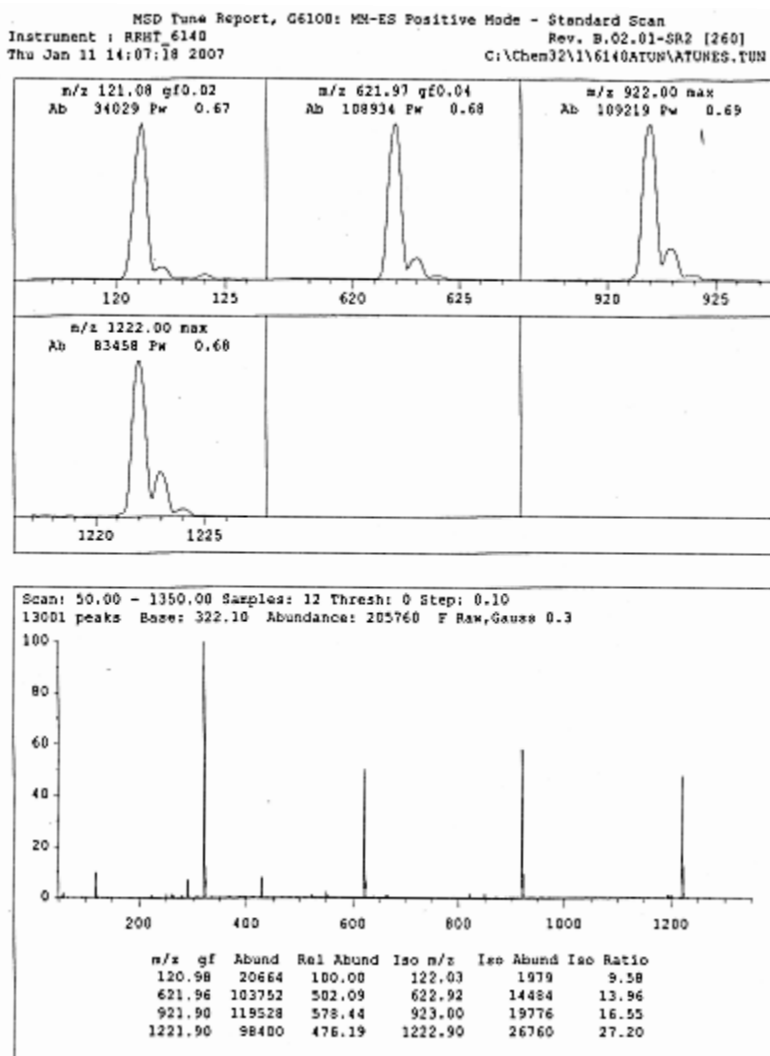


Figure 17 Tune report (page 1 of 4)

## Gain calibration

Gain calibration sets the electron multiplier voltage in a way that simplifies method development and portability. Gain calibration is performed automatically during autotune. You can also check and adjust the gain calibration during manual tune.

### What is gain?

The HED electron multiplier receives an input current that is generated by the ions that strike it, amplifies that current, and generates a proportional output current.

$$\textit{Gain} = \textit{Output current} / \textit{input current}$$

Gain is controlled by the electron multiplier voltage (EMV). The higher the EMV, the higher the gain. The relationship between EMV and gain is log linear. This linear relationship is common for all multipliers. Since the slope of this line is constant, to set the gain, the ChemStation Edition program needs only to adjust the intercept. The gain calibration routine simply adjusts the value of the intercept for the specific instrument. The instrument-specific gain curve coefficients are then stored in the MS.

### Gain calibration curve

A gain calibration curve is generated by taking readings of the ion current generated at discrete multiplier settings across the range of the multiplier. Separate gain calibration curves are generated for positive and negative polarities, since the ion current generated in these two modes varies to some extent. A gain of 1 is set to be a multiplier value of 1e6. That is, one electron striking the input side of the detector will generate 1e6 on the output side. A gain check can be generated on both polarities or on the current polarity.

### Gain and method portability

The use of gain enables the distribution of methods from instrument to instrument. In principle, using similar gain on two different instruments should result in the same signal

response, thereby easing method development and instrument portability. Because the relationship between EMV and gain is log linear, a gain of 2.0 should give twice the abundance of a gain of 1.0.

### More gain is not always better

In general, it is best to run the detector at the lowest gain that still produces adequate abundance. High gains increase noise as well as signal and often result in poorer signal-to-noise ratios. Increasing the gain increases the EMV, which shortens the life-span of the electron multiplier. The maximum EMV is 3000 eV no matter how high you set the gain. For a new EM horn, a gain of 70 or higher elicits the 3000 eV setting. For an old EM horn, 3000 eV may be reached with a gain of only 3 to 5.

As an electron multiplier ages, it slowly becomes less efficient. For a given ion current input, it generates a smaller and smaller output current (abundance). Low abundance caused by an aging electron multiplier is not easily distinguished from low abundance caused by poor ion generation or transmission (low ion current input). It may be tempting to compensate for poor ion generation or transmission by increasing the gain (thereby increasing the EMV) even though the electron multiplier is actually performing correctly at its previous gain. The increased gain will improve abundance, but may decrease the signal-to-noise ratio and will shorten the life-span of the electron multiplier.

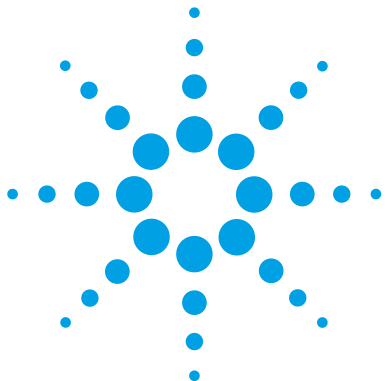
### Check Gain Calibration

*Access this functionality via the Tune menu in the MSD Tune view.*

During a Gain Calibration check, a reading of the ion current is taken and checked against the current gain calibration curve for the current polarity. If it is within acceptable limits, no change is made to the curve for that polarity. If the reading falls outside the limits, a new gain curve is generated. A gain of 1 is set to be a multiplier of  $1e6$ . That is, one electron striking the input side of the detector will generate  $1e6$  on the output side. If that multiplier setting exceeds 2700 volts, which indicates decreasing electron multiplier performance or instrument performance, you are warned that system maintenance may be required.

## **2 Instrument Preparation**

### **Gain calibration**



## 3 Data Acquisition

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In this chapter, you learn the concepts that help you run samples and acquire data. This chapter assumes that the hardware and ChemStation Edition program are installed, the instrument is configured and the performance verified. If this has not been completed, see the *Installation Guide* for your MSD.



## Working with methods

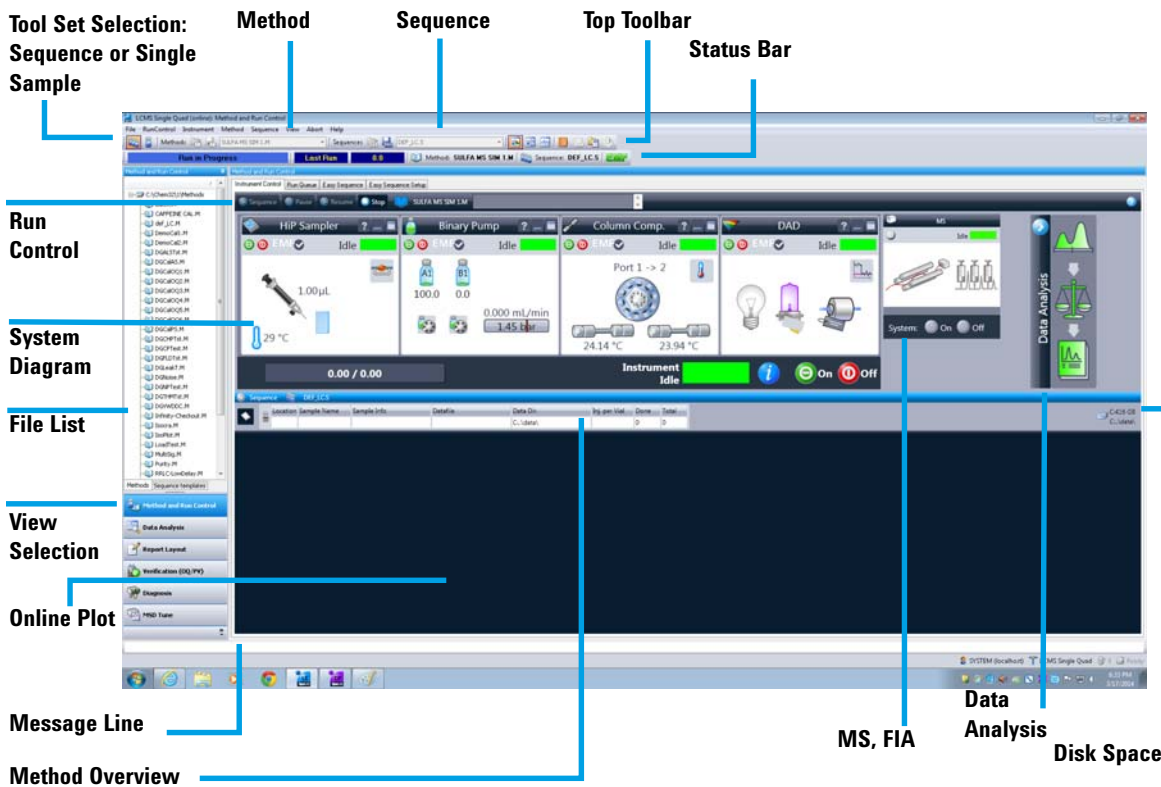
### Method and Run Control View

You set up methods and run analyses from the Method and Run Control view, shown in [Figure 18](#).

ChemStation Edition methods control the instrument during data acquisition. The easiest way to prepare a method is to load a similar method (or load **DEF\_LC.M**), save it with a new name, modify (edit) it, and save it again. You can then use the method to run a single sample or multiple samples via a sequence.

You can also set up a method for flow injection analysis (FIA), where you make multiple injections in a single run.

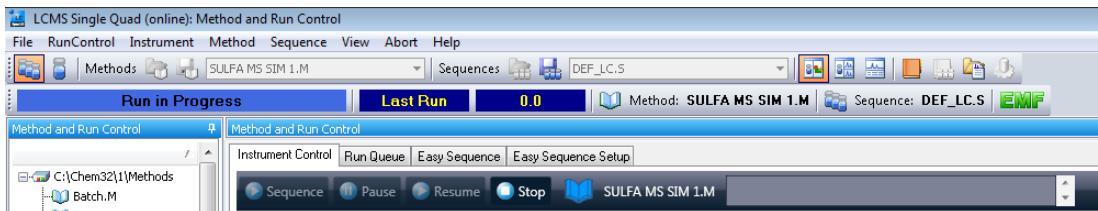




**Figure 18** In this view, you set up methods, establish instrument settings, and inject samples.


**Status and Run Bars: Main Tab**

Right-click the Method icon to see the following commands: Run Time Checklist, Method Information, Edit Entire Method, Method Audit Trail, Print Method, and Help.



## Loading, editing, saving and printing methods

When you need to work with methods, there are several ways to access the necessary functionality:

- Select from the **Method** menu, which allows you to load, edit, save, and print methods.
- Use the Method icon in the Run Control Bar. Right-click the icon to access the shortcut menu. 
- Use the toolbar or method drop-down list.

When you want to view all the tools for methods (or to run a single sample interactively), you first click the icon for the **Single Sample Toolset**, as shown in Figure 19.



Figure 19 Toolset selection



Figure 20 When you click the icon for the **Single Sample Toolset**, you access tools to work with methods.

- Use the ChemStation Explorer pane on the left side of the Method and Run Control view.

Click the **Method** tab at the bottom to display the method files.


Double-click a method to load it.

Right-click in the white area to view the shortcut menu, which contains options such as Load Method.

## More on editing methods

There are a number of ways to edit a method. If you need to create a new method for a new analysis, then it is best to review and edit the entire method, as described just below. If you want to make only a few changes to an existing method, then it is easiest to edit only the settings that need to change, as described in “Editing a portion of the method” on page 60.

### Editing the entire method

You can edit the entire method from the **Method** menu or the **Edit entire Method** icon in the Run Control Bar  or the **Method** icon in the Status Bar. If you elect to edit the entire method, the appropriate dialog boxes are displayed in succession. The first dialog box (Figure 21) lets you select the parts of the method to view and edit. For more information as you edit the method, click the **Help** button in each dialog box.



**Figure 21** Parts of the method that you can view and edit

The Run Time Checklist, the last item listed in Figure 21, controls which parts of the method the ChemStation Edition program executes when you run your analysis. As shown in Figure 22, a complete method can include both data acquisition and data analysis.

#### NOTE

Be sure to save the method after you edit it.

### 3 Data Acquisition

More on editing methods

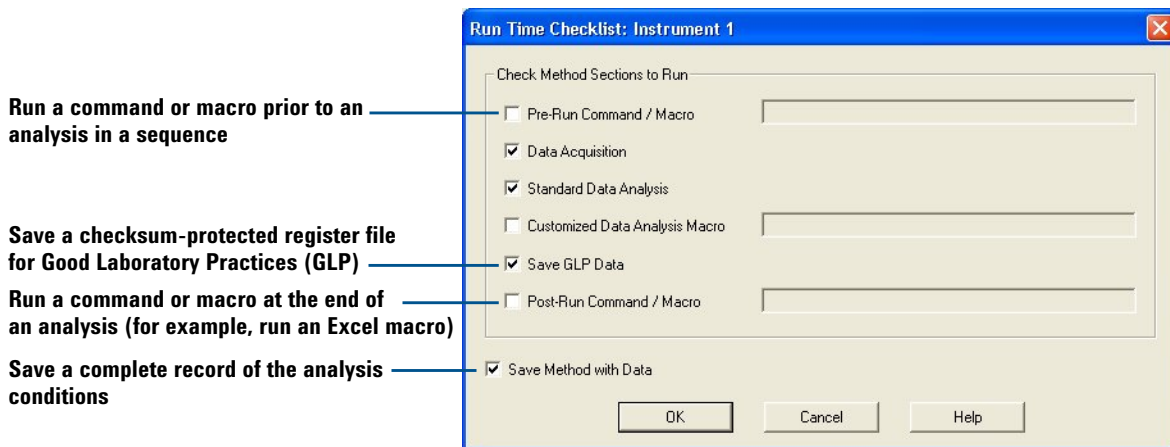


Figure 22 Run Time Checklist

#### Editing a portion of the method

If you need to change only a few settings in a data acquisition method, you can edit a subset of the method. You can do that either from the system diagram (Figure 23) or the **Instrument** menu.

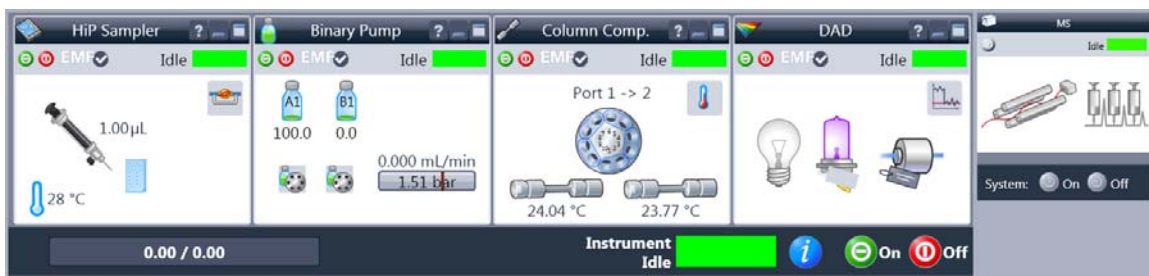


Figure 23 You can right-click a module in the system diagram to access context menus that allow you to change settings.

#### NOTE

Be sure to save the method after you change the instrument settings.

To access MSD parameters, refer to the table below.

**Table 1** Tasks for setting up the MSD

<b>If you want to:</b>	<b>Click the MS icon and select this menu item:</b>	<b>Or choose the following from the Instrument menu</b>
Set scan range or SIM ions	Set up MSD Signals	Set up MSD Signals
Set instrument polarity	Set up MSD Signals	Set up MSD Signals
Set drying gas flow or temperature or nebulizer pressure	Spray Chamber	More MSD > Spray Chamber
Set the capillary voltage	Spray Chamber	More MSD > Spray Chamber
Select MSD parameters to store during a run	Data Curves	More MSD > Data Curves

## Running samples

The ChemStation Edition program provides three ways to run samples:

- Run a single sample interactively and create a single data file.
- Run sample(s) via a sequence and create a data file for each sample.
- Run multiple injections within a single data file, via FIA.

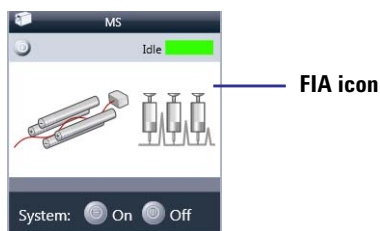
FIA = flow injection analysis

You can use the same method to run either a single sample or samples in a sequence.



Display Single Sample Toolset

Display Sequence Toolset

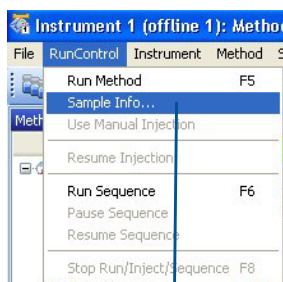


**Figure 24** To run samples, you first click the appropriate icons.

For FIA, you must modify the method to enable multiple injections in the same run.

## Running a single sample

After you have set up and saved a method, you are ready to run a single sample interactively as shown below. First you click the icon to display the **Single Sample Toolset**.



Click to enter sample information



Click to start the run

**Figure 25** How to enter sample information and start the run.

## Running a sequence

To run samples in an automated, unattended sequence, you first click the icon to display the **Sequence Toolset**. The Sequence icon on the left provides access to the Sequence Toolset and the sampling diagram that shows the sample tray.



Figure 26 Sequence Toolset selection icon.

### Loading, editing, saving and printing sequences

When you need to load, edit, save, and print sequences, there are several ways to access the necessary functionality:

- Select from the **Sequence** menu.

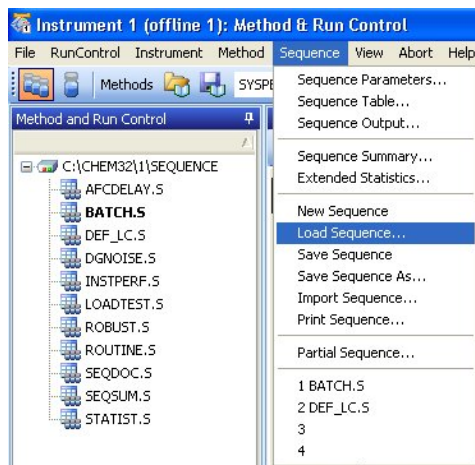
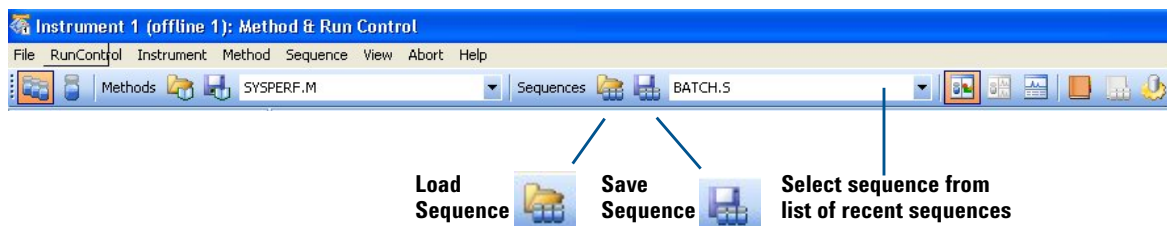


Figure 27 The **Sequence** menu allows you to load, edit, save, and print sequences.



- Use the toolbar or sequence drop-down list.



**Figure 28** Tools to work with sequences

- Use the Method and Run Control file list. If necessary, first click the **Sequence** tab at the bottom. You can right-click in the white area to view the menu, or double-click a sequence to load it.
- Click the sampling diagram to access the menu.

### More on editing sequences

When you edit a sequence, the following dialog boxes are applicable:

- Sequence Parameters – allows you to enter information like data file path, parts of the method to run, and a post-sequence command/macro to shut down the system
- Sequence Table – includes the sample list and any calibration information
- Sequence Output – controls printing of reports (sample results and the sequence summary)
- Import Sequence – allows you to import a sample table from a text (.txt) file or a delimited file, such as a comma-separated value (.csv) file created in Microsoft® Excel

**NOTE**

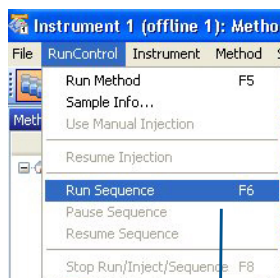
After you have edited your sequence, be sure to save it.

### 3 Data Acquisition

#### Running a sequence

#### Starting the sequence

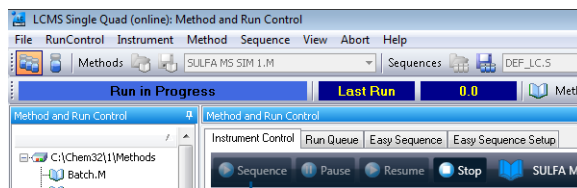
After you have set up and saved your sequence and any methods that it uses, you are ready to run the sequence. As shown in Figure 29, the ChemStation Edition program provides more than one way to start the sequence.



Click Run Sequence button in Sequence Table and Partial Sequence dialog boxes



Click to start the sequence



Click to start the sequence

**Figure 29** Multiple ways to start the sequence.

## Flow injection analysis

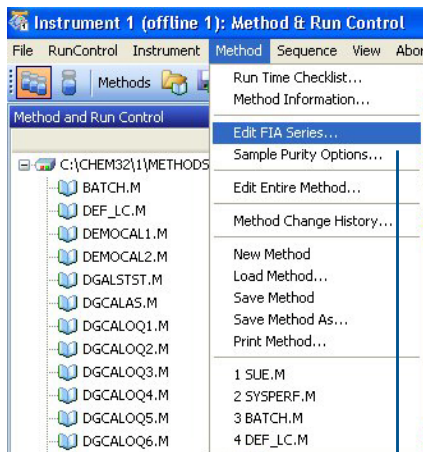
Flow injection analysis (FIA) is injection of multiple samples within the same run, and is typically performed without a column. FIA is very useful when you want to optimize MS parameters such as:

- Drying gas flow and temperature (which depend on the mobile phase and flow rate, as well as the ionization mode).
- Nebulizer pressure (which depends on the mobile phase and flow rate, as well as the ionization mode).
- Fragmentor voltage (which depends on the structure of the compound).

FIA is also useful to check quickly for compound sensitivity or linearity, or any time you want to perform an analysis without a separation.

### Setting up FIA within a method

You can add FIA to a method either through the **Method** menu or the **FIA** icon (see Figure 30).



Click to enable FIA or set up FIA series

Figure 30 Two ways to enable FIA within a method

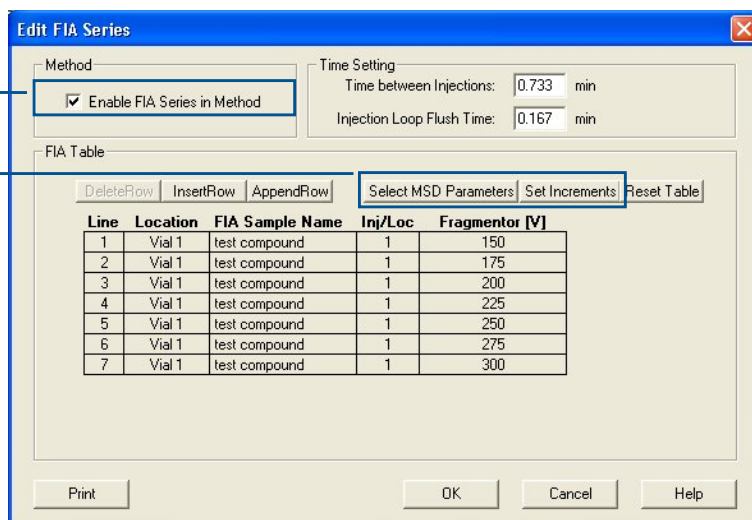
**To optimize settings with a single sample** The Edit FIA Series dialog box has an autofill function so you can automatically build an FIA table. You can select up to two MSD parameters, and then automatically increment their settings. This feature makes it easy to set up FIA to optimize settings, as shown with the example in Figure 31. If you decide later to add a setpoint, you can use the insert row/append row functionality.

**NOTE**

Some parameters, such as gas temperature, take time to reach their setpoints. In these cases, perform multiple injections at each setpoint and allow more time between injections.

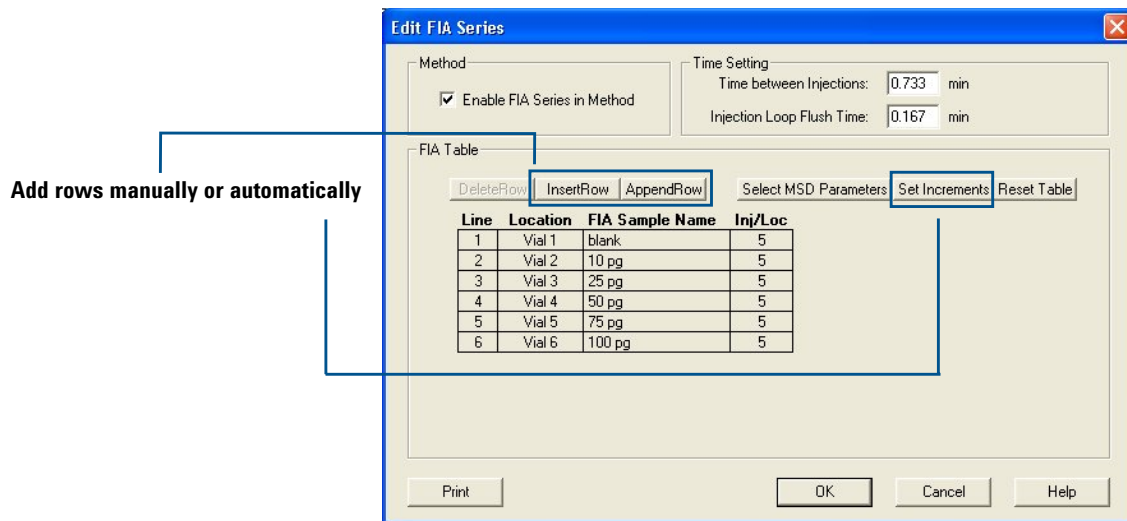
Mark check box first

Automatically build an FIA table



**Figure 31** The FIA series is a convenient way to optimize MS parameters. The example shows a test of fragmentor voltage.

**To inject multiple samples** If you need to inject multiple samples to check for sensitivity or linearity, you can also use the autofill functionality within the Edit FIA Series dialog box. In this situation, you keep the MS parameters constant and increment only the vial numbers. Alternatively, you can use the insert row/append row functionality to add samples. Figure 32 shows an example of FIA for a linearity check.



**Figure 32** For pure samples, the FIA series can be used to check sensitivity or linearity. The example shows a linearity check.

### Running multiple FIA methods

If you have multiple compounds to test or you need to perform more complex method development, you can run a succession of FIA methods, analogous to a sequence. You access that capability via the **RunControl** menu.

## Monitoring analyses

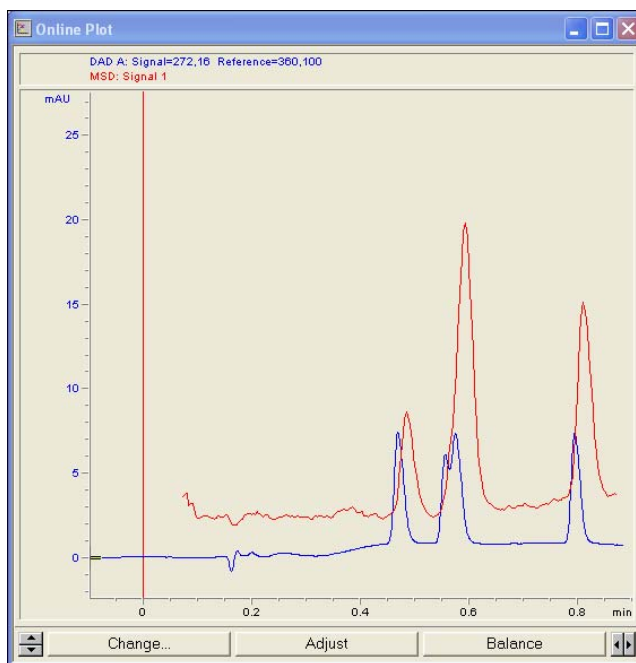
### Online signal plots

During an analysis, you can view online signals, as shown in [Figure 33](#). (Select **View > Online Signals**.)

You can click the little box with the arrows in the lower left of the signal display to display the online plot as a separate window. This feature is useful if you wish to make the window larger so you can view more signals.



For the online plots, you can choose which signals to display, and you can adjust the scales of the x and y axes.



**Figure 33** Online plot

## Logbooks

Several logbooks are important in the Method and Run Control view. You can view logbooks from the View menu or the logbook icons as show in [Figure 34](#).

### Current logbook

The current logbook stores all error, system, and event messages, and is helpful for general information and troubleshooting. It logs events such as ChemStation startup and shutdown, loading of sequences and methods, running of sequences and methods, etc.

The log file exists as **INSTRx.LOG** (Where **x** is the instrument number), in the temporary folder under the instrument folder in **CHEM32** (for example, **C:\CHEM32\1\TEMP**).

### Run logbook

**RUN.LOG** stores all messages related to analysis of a particular sample. It resides in the data file folder. You can use it to check that the sample was processed correctly.

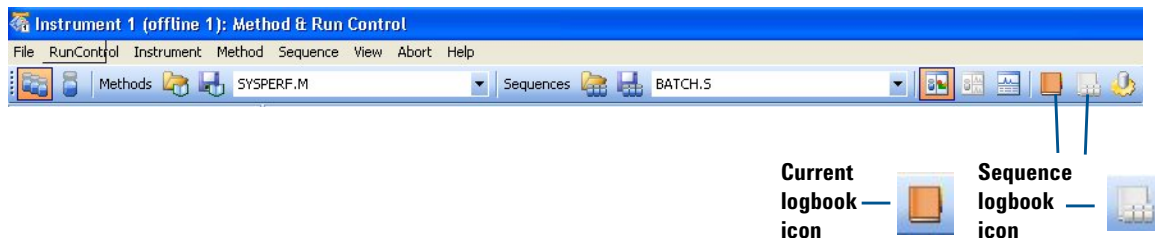
### Sequence logbook

The sequence logbook shows what happened as a sequence ran. You can use it to troubleshoot errors that occurred during unattended operation.

The log file exists as **sequencename.LOG** (where **sequencename** is the name of the ChemStation sequence). Each time a sequence runs, the ChemStation Edition program produces a log file, usually in the **DATA** folder within the instrument folder (for example, **C:\CHEM32\1\DATA**).

### 3 Data Acquisition

#### Instrument shutdown



**Figure 34** How to open logbooks with icons.

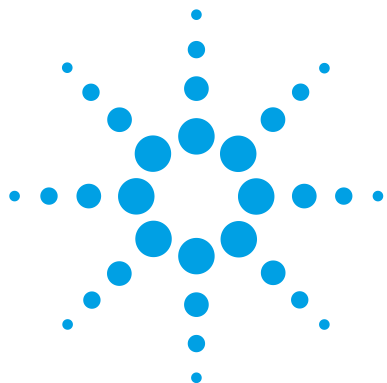
## Instrument shutdown

At the end of your analysis, if you do not plan to run more samples until the next day, it is important to:

- Flush the system with a mobile phase without buffers.
- Put the system into standby mode.

For details, see the Basic Operation section of the *Agilent OpenLAB CDS ChemStation Edition Quick Start Guide*.





## 4 Data Analysis

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In this chapter, you learn the concepts that help you analyze data. For more information on the concepts related to data analysis, see these two manuals:

- Agilent ChemStation: Understanding your ChemStation
- Agilent ChemStation for LC 3D Systems: Understanding Your Spectra Module

For more in-depth task-related information and specifics about dialog boxes and toolbars, see the online Help. For descriptions of all buttons on toolbars, double-click **User Interface Reference**, then double-click **Toolbars** in online Help.



## The Data Analysis View

The Data Analysis view, shown in [Figure 35](#), provides all the tools for qualitative and quantitative data analysis. In this view, you can:

- Evaluate chromatograms and spectra
- View and compare signals from both the MS and UV detectors
- Integrate peaks and perform quantification
- Check peak purity
- Perform deconvolution (with the optional Agilent Deconvolution & Bioanalysis Software)
- Generate reports from predefined report templates
- Reprocess data from sequences.

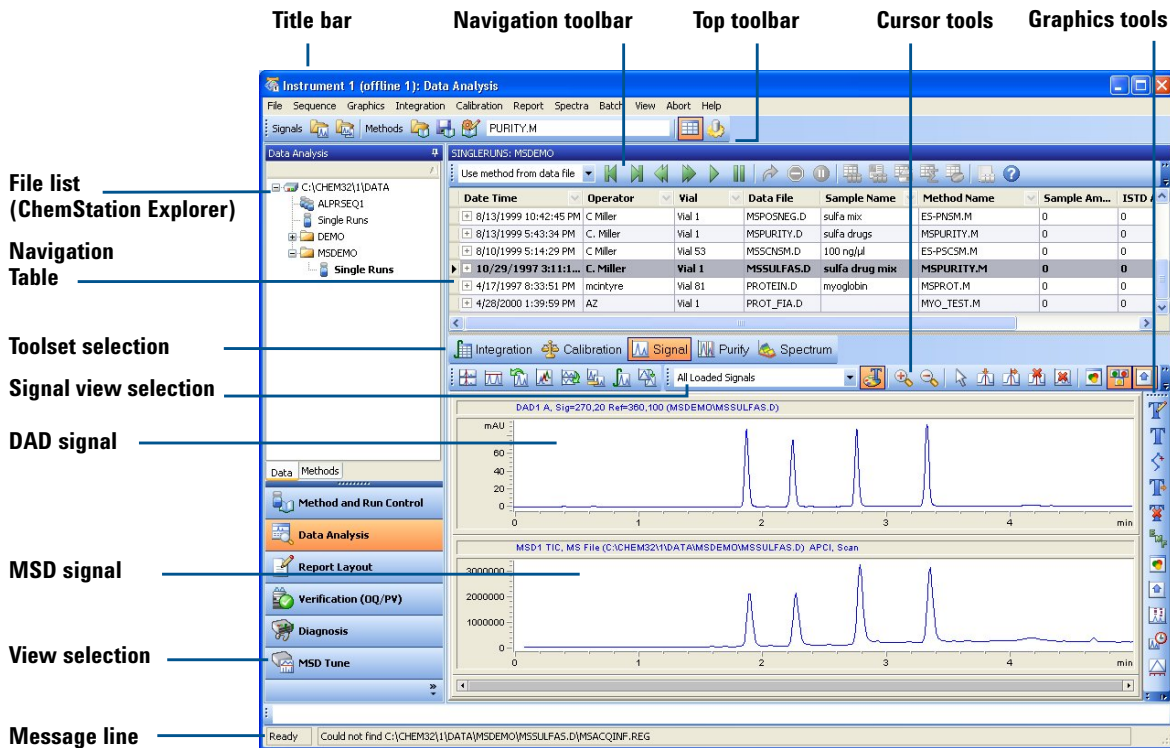


Figure 35 The Data Analysis view provides menus and tools for qualitative and quantitative analysis.

# Loading and manipulating chromatograms

*In the ChemStation Edition program, chromatograms are called signals.*

ChemStation Edition program stores multiple signals in the same data file. Examples of signals are MS chromatograms and UV chromatograms.

For many analyses, it is important to be able to compare results from different detectors or different instrument settings, or to compare results from various samples. ChemStation Edition program allows you to load and compare signals within a data file and between data files. For example, within a data file, you can simultaneously load and evaluate UV and MS data, or MS data at two different fragmentor settings or ion polarities. You can load data files from multiple samples to compare retention times and peak heights.

TIC – total ion chromatogram  
EIC – extracted ion chromatogram  
BPC – base peak chromatogram

Specialized chromatograms available for MS data include:

- Total ion chromatogram (TIC) – chromatogram that sums all the ions at each time point
- Extracted ion chromatogram (EIC) – chromatogram of a chosen  $m/z$  value or range of values
- Base peak chromatogram (BPC) – chromatogram that represents the abundance of the largest peak of each spectrum in the data file

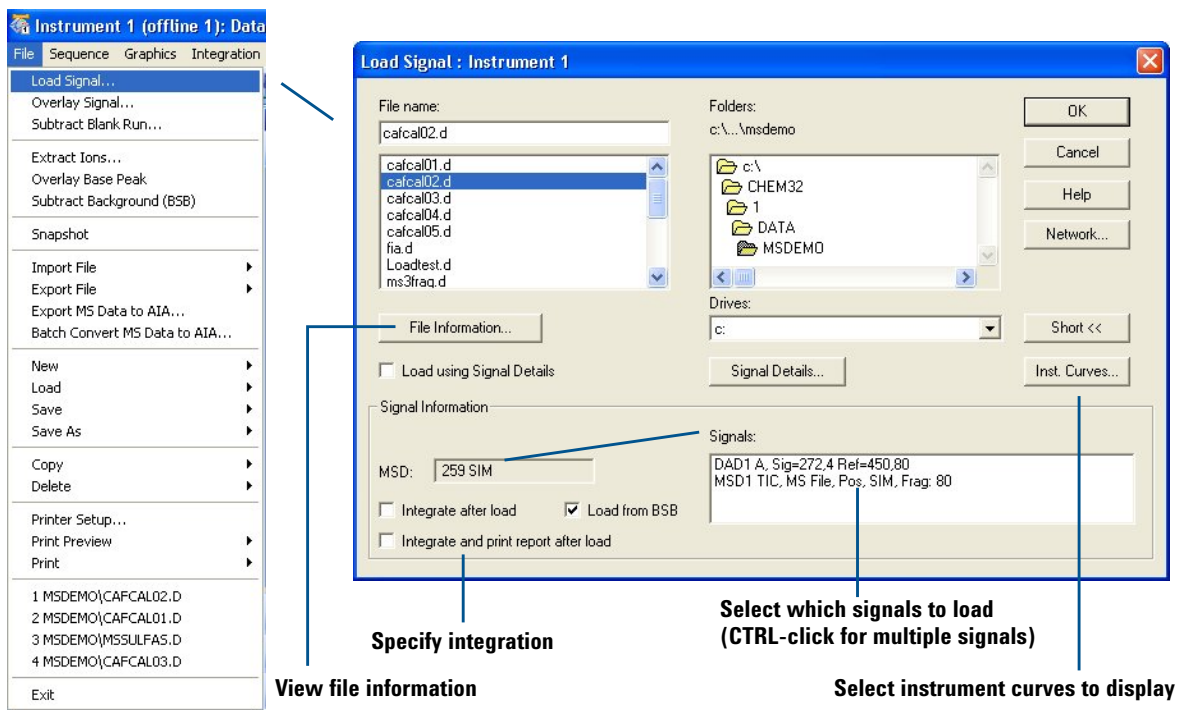
Total ion chromatograms and base peak chromatograms are useful to find all the chromatographic peaks in the sample. Because of their specificity, extracted ion chromatograms are ideal for quantification. You can also use them to check for purity of chromatographic peaks. When chromatographic peaks are composed of more than one component, the EICs often fail to align.

## Loading signals

### Ways to load signals

You can load chromatograms (signals) in the following ways:

- Select from the **File** menu.

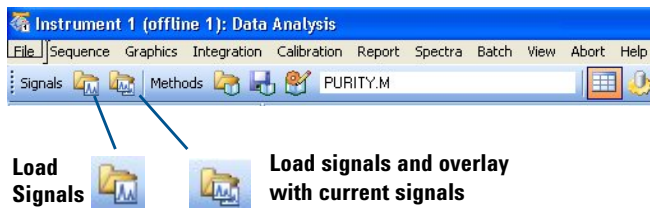


**Figure 36** The Load Signal dialog box lets you select which data files and signals to load.

- Use the top toolbar.

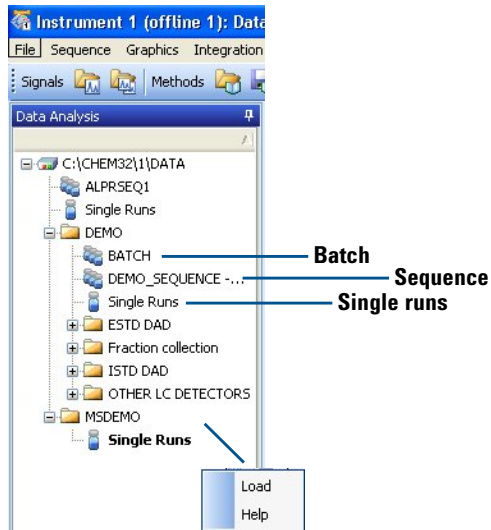
## 4 Data Analysis

### Loading signals



**Figure 37** Tools to load signals.

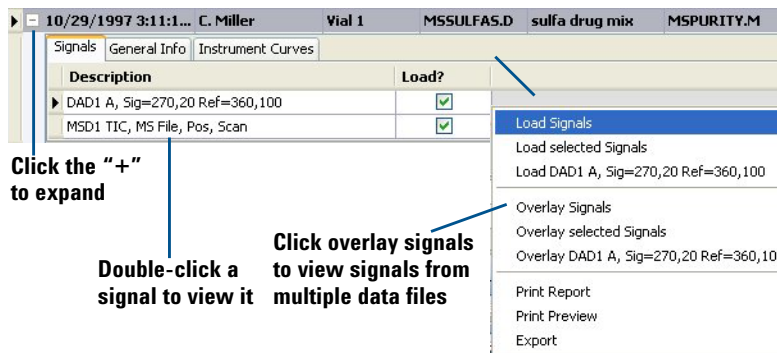
- Use the Data file list in the ChemStation Explorer. (If necessary, first click the **Data** tab at the bottom.)



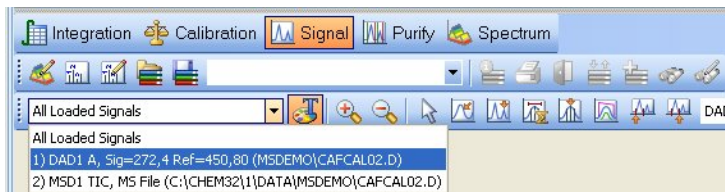
**Figure 38** You can right-click the vials to view the menu, or double-click the vials to load multiple data files.

### Refining your selection

Once you have loaded signals for a data file or files, you can refine your selection as shown in Figure 39 and Figure 40.



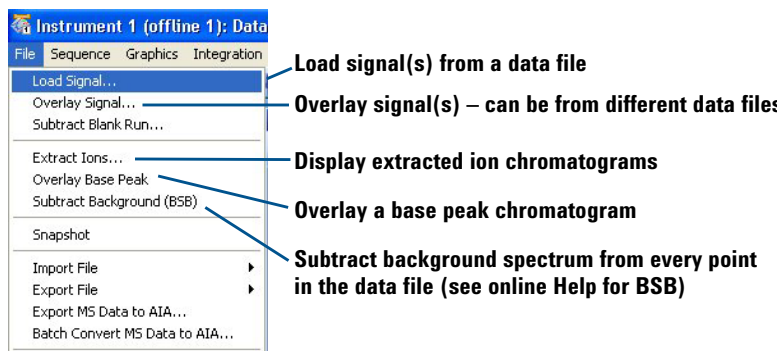
**Figure 39** You can view specific signals by double-clicking a signal (on left) or by right-clicking to display the menu at right.



**Figure 40** You can view specific signals by selecting from the signal list.

## More about signals

In addition to loading signals from a single data file, the **File** menu allows you to load signals from multiple files and to display extracted ion chromatograms and background-subtracted chromatograms.



**Figure 41** The **File** menu provides ways to load chromatograms and perform background subtraction.

*In ChemStation Edition program, “overlay” means to simultaneously load more than one signal.*

In the **File** menu, **Overlay Signal** allows you to compare signals from different samples or injections so you can examine fine differences. Note that while the term “overlay” may imply that you display the signals one on top the other in the same window, you actually control the display using buttons that you access from the Signal Toolset, the Purify Toolset, or the Graphics Toolset. These buttons are:



Display overlaid  
(In the Graphics Toolset, this button toggles between overlaid and separate.)



Display separate



## Removing signals from the chromatogram display

If you display too many signals at the same time, the individual displays can become too small or difficult to view. If so, you can remove signals from the display in either of the following ways:

- Select **View > Window Functions > Delete Window**.
- Use the tool within the Signal Toolset and the Purify Toolset that allows you to delete signals from your chromatogram display.



## Changing how chromatograms are displayed

When you analyze data, it is very helpful to have control over the data display. For example, you may want to display chromatograms in separate windows or overlaid in a single window. Separate windows are often the best choice if you wish to view UV and MS chromatograms from the same analysis. Overlaid windows may be better if you need to compare fine differences between two complex samples.

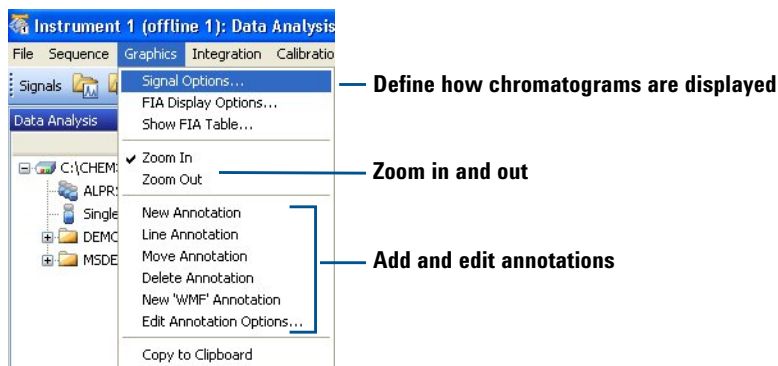
It is also helpful to be able to zoom in and out, to add annotations, to align chromatograms (for example, UV and MS chromatograms), and to control whether or not retention times and integration baselines are displayed.

## 4 Data Analysis

### Changing how chromatograms are displayed

The chromatogram display can be customized as follows:

- Select from the **Graphics** menu.



**Figure 42** The **Graphics** menu provides ways to change how the chromatograms are displayed.

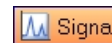
- Use the toolsets that are described below.
  - Graphics Toolset



The graphics tools provide ways to change your signal display. You can add annotations and change whether compound names, retention times, baselines, titles, and axes are displayed. You can print windows and copy them to the clipboard.

The icon to display the Graphics Toolset is located near the middle of the Data Analysis window.

- Signal Toolset



The signal tools let you change the chromatogram display. You can change from a separate display to overlaid, align x- and y-axes, display signals full-scale or on the same scale, smooth chromatograms, subtract chromatograms (for example, subtract a blank run) and perform other operations on chromatograms.

## Working with spectra

*In ChemStation Edition program, background spectra are called reference spectra.*

With the ChemStation Edition program, you can display both UV and mass spectra.

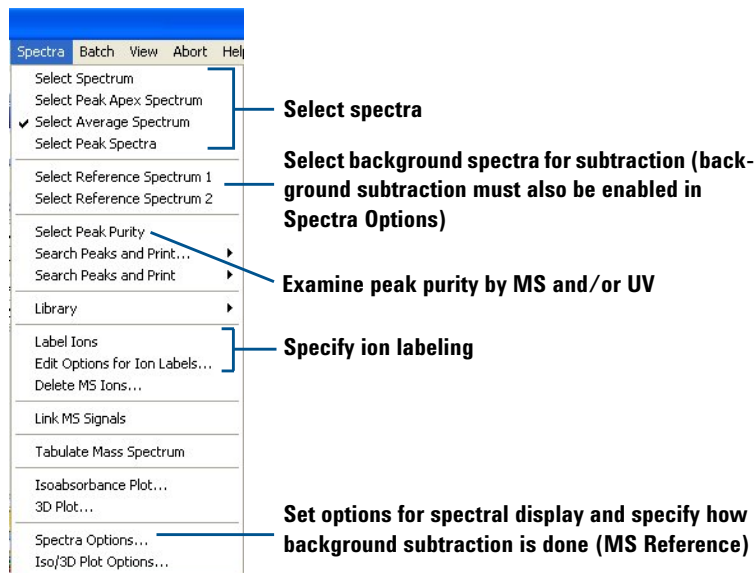
With MSD data, each point in a chromatogram has an associated mass spectrum. You can display single spectra, averaged spectra, and background-subtracted spectra. For MSD data, you generally subtract a background spectrum from each analyte spectrum. When you subtract a background spectrum, you remove the ions that are attributable to mobile phase and other background.

For UV signals, depending on the storage mode, there may be spectra only for the peak apexes or across the peaks. There is a spectrum for each point in the chromatogram only when you set **Store** to **All** when you set up the UV detector portion of the method.

## Displaying spectra

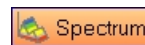
You can display spectra in the following different ways:

- Select from the **Spectra** menu.



**Figure 43** The **Spectra** menu provides ways to select and display spectra.

- Use the toolsets that are described below.
  - Spectrum Toolset



The spectrum tools help you to quickly display and evaluate spectra. You can select spectra (including background spectra), zoom in and out, save spectra to a library, search a spectral library, set spectral options, select a chromatographic peak for purity analysis, and perform other tasks related to spectra.

- Calculation Toolset



The calculation tools allow you to multiply, add, and subtract spectra. You access them from the icon shown here, which is part of the Spectrum Toolset.

## Peak purity

For many analyses, it is important to know whether a chromatographic peak represents a single component or multiple components. ChemStation Edition program allows you to evaluate peak purity using UV and/or MS data.

To start, you select **Spectra > Select Peak Purity**. For detailed concepts about peak purity, see *Agilent ChemStation for LC 3D Systems: Understanding Your Spectra Module*.

## Performing quantification

ESTD – external standard  
calibration

ISTD – internal standard  
calibration

Quantification is the process of determining how much of a compound is present in a sample. You perform quantification by comparing the peak areas or heights of analytes in your sample with those in standards of known concentration. To each standard and sample, you may add a constant amount of an internal standard that you can use to correct for slight variations in injection volume, detector sensitivity, etc.

A calibration curve is a plot of peak area or height on the y-axis versus concentration on the x-axis.

For quantification, it is best to use SIM mode for data acquisition because it generates more data points across a peak, which provides the best precision and accuracy. When you perform quantification, you typically analyze standards at concentrations that bracket those of your samples. You then generate a calibration curve for each analyte, and determine the amount of each analyte in your sample, using ChemStation Edition program.

## Integrating peaks

Integration – the process of  
measuring peak areas

Qualifier ions – ions that are used  
to verify that the ion used for  
quantification is attributable to the  
analyte rather than an interference

Determination of peak areas is a basic component of quantification. To avoid interferences, you use the peak areas from extracted ion chromatograms (EICs) rather than total ion chromatograms. You can use the EIC of the molecular ion for quantification and the EIC(s) of one or more fragment ions for confirmation. The latter are called qualifier ions, and they must be present in the proper ratio to confirm the presence of the analyte.

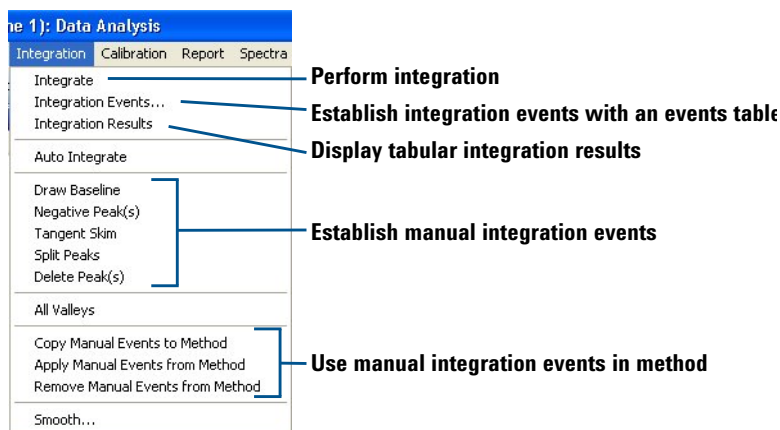
In the ChemStation Edition program, the Signal Details specify which extracted ion signals are used for quantification and which (if any) are used for qualifier ions. You can access the Signal Details under the **Calibration** menu, and you can save the Signal Details within the method.

To ensure that peaks are properly integrated, you need to establish the appropriate integration parameters. The standard of lowest concentration usually presents the greatest challenge for integration, so it is best to use it to refine your integration

settings. You can then save the integration parameters with the method and apply them to the higher-level standards and the samples.

You set integration parameters and perform peak integration in the following different ways:

- Select from the **Integration** menu.



**Figure 44** The **Integration** menu

- Use the Integration Toolset.



The integration tools help you perform integration and generate reports. You can define integration events, perform automatic and manual integration, draw baselines, zoom in and out, specify a report style, and view and print reports.

**NOTE**

When you are done, be sure to save the integration settings with your method.

For more information about integration concepts, see *Agilent ChemStation: Understanding your ChemStation*.

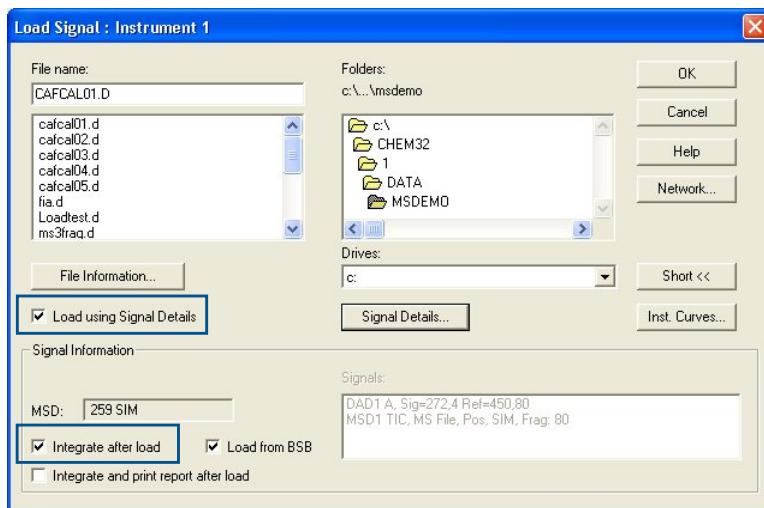
## Calibration

A calibration curve is a plot of peak area or height on the y-axis versus concentration on the x-axis.

When you perform calibration, you insert the peak integrations from standards into a calibration table. The area responses are used to generate a calibration curve.

In ChemStation Edition program, each concentration of standard is called a “level.” After you integrate the standard of lowest concentration, you add it to the calibration table as level 1. You then add the standard of second-lowest concentration as level 2, and so on, until you have added all standards.

**Tip:** If you have saved the Signal Details (see the **Calibration** menu) as part of the method, you can automatically load and integrate the extracted ion chromatograms from each standard. (See [Figure 45](#).)

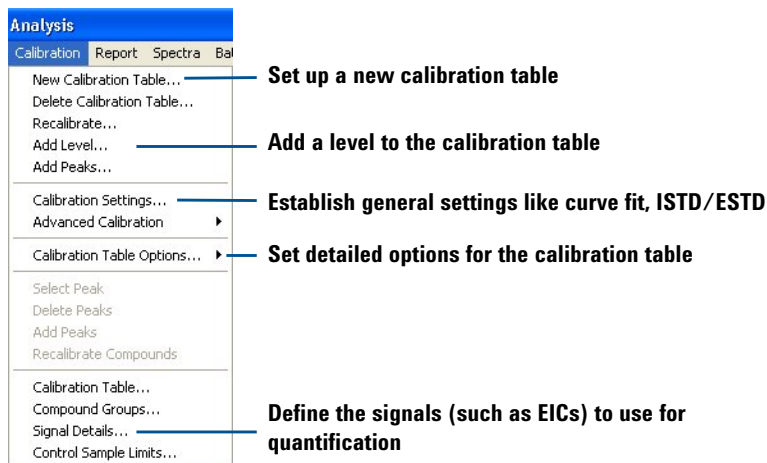


**Figure 45** The highlighted features make it faster to add levels to a calibration curve.



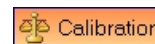
Access to calibration tools and commands are provided in the following ways in ChemStation Edition program:

- Select from the **Calibration** menu.



**Figure 46** The **Calibration** menu

- Use the Calibration Toolset.



The calibration tools provide a quick way to accomplish calibration tasks and generate reports. You can create a new calibration table, add levels and peaks to the table, recalibrate using the current chromatogram, and perform other calibration tasks. You can also specify a report style, and view and print reports.

#### NOTE

When you are done, be sure to save the calibration table with your method.

For more information about calibration concepts, see *Agilent ChemStation: Understanding your ChemStation*.

## 4 Data Analysis

### Data review and sequence reprocessing

# Data review and sequence reprocessing

ChemStation Edition program gives you two ways to quickly review multiple data files: the Navigation Table and Batch review described below.

## The Navigation Table

The Navigation Table shows the data files from a given folder and provides a convenient way to navigate between samples. You can use it for data review or to reprocess sequences.

Line	Inj	Vial	Sample Name	Method Name	Sample Type	Cal Level	Sample Info	Sample Am...	ISTD Amount	Multiplier	Dilution	Data File
+	1	Vial 1	isocratic standard ...	ISOCRA.M	Sample		DAD : slit 2n -	0	0	1	1	001-0101.D
▶	2	Vial 1	isocratic standa...	ISOCRA.M	Sample		DAD : slit 2n -	0	0	1	1	001-0201.D
+	3	Vial 1	isocratic standard ...	ISOCRA.M	Sample		DAD : slit 2n -	0	0	1	1	001-0301.D
+	4	Vial 1	isocratic standard ...	ISOCRA.M	Sample		DAD : slit 2n -	0	0	1	1	001-0401.D
+	5	Vial 1	isocratic standard ...	ISOCRA.M	Sample		DAD : slit 2n -	0	0	1	1	001-0501.D

Choose method for data review

Data review tools

Sequence reprocessing tools

Click for help with Navigation Table

Click "+" for sample details (signals, etc.)

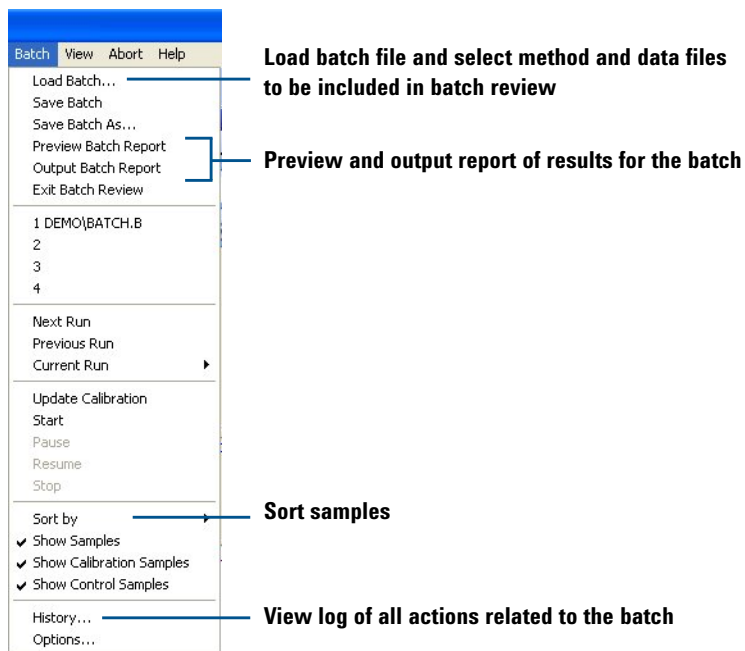
Figure 47 Navigation Table, shown with columns for sequence runs

## Batch review

Batch – a user-selected series of data files from a sequence that is processed using a single user-defined method

Batch review enables fast review of sequences or selections of analyses from a sequence. You can use batch review for either quantitative or qualitative analysis. Whenever you run a sequence, a batch file (with a **.b** extension) is generated and saved in the same folder as the data files. This batch file contains pointers to the data files in the batch review itself.

You load a batch through the **Batch** menu (Figure 48). When you load a batch, you have the opportunity to select the method to use for the batch, and to individually select the desired data files.



**Figure 48** The **Batch** menu

## 4 Data Analysis

### Batch review

When you load a batch, the Batch Review toolbar, the batch table, and the compound list are displayed automatically. For help, click the question mark on the toolbar.

The screenshot shows the Batch Review interface. At the top is a toolbar with various icons, including a question mark icon labeled "Click for help". Below the toolbar is a table with the following data:

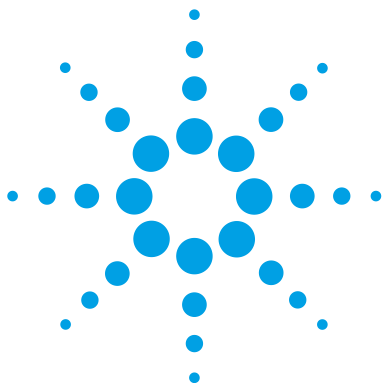
Sample	Location	SampleType	Run	DataFile
->	Isocratic Std	Vial 5	Calibration	1 005-0101.D
	Isocratic Std	Vial 5	Calibration	2 005-0102.D
	Isocratic Std	Vial 5	Control	3 005-0103.D
	Isocratic Std	Vial 6	Sample	4 006-0201.D

To the right of the table is a "Compound table" section with a "List of Compounds" list:

Compound table	List of Compounds
	Dimethylphthalate
	Diethylphthalate
	Biphenyl
	o-Terphenyl

Labels "Batch table" and "Compound table" are placed below their respective sections with arrows pointing to them.

**Figure 49** Batch Review toolbar, batch table, and compound list



## 5 Reports

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Defining custom reports	96
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In this chapter, you learn the concepts that help you generate reports of results.

For more concepts related to reports, see these two manuals:

- Agilent ChemStation: Understanding your ChemStation
- Agilent ChemStation for LC 3D Systems: Understanding Your Spectra Module

For in-depth task-related information and specifics about dialog boxes and toolbars, see the online Help. For descriptions of all buttons on toolbars, in the online Help double-click **User Interface Reference**, then double-click **Toolbars**.



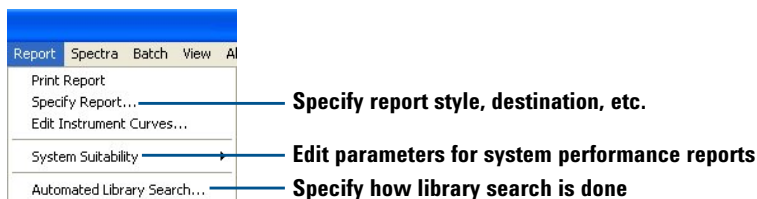
## Using predefined reports

A report provides qualitative or quantitative information about a sample you have analyzed. You can print a report, display it on your computer screen, or send it to a file. ChemStation Edition program includes a number of predefined report styles, and you can generate new styles for custom reports.

## Generating reports

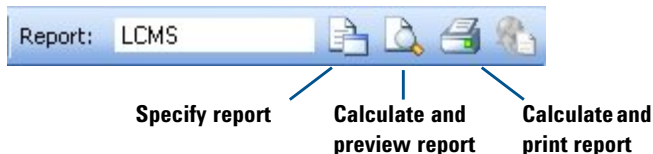
### Ways to generate reports in Data Analysis view

- Select from the **Report** menu.



**Figure 50** The **Report** menu provides ways to print reports and set properties of reports.

- Use the report tools in the Integration Toolset or the Calibration Toolset. You access these toolsets by clicking one of the buttons on the right.



**Figure 51** Tools to work with reports

## System suitability reports

In the **Report** menu, **System Suitability** allows you to check the performance of your system before and during your analyses. This report style allows you to set up high and low limits for retention time, peak height, peak width, theoretical plates, resolution, library match, peak purity, and other properties of calibrated peaks. When you choose one of the **Performance** report styles, the report indicates when any of the parameters are out of specification.

### NOTE

When you are done, be sure to save the report parameters with your method.

## Report styles

The ChemStation Edition program provides a wide variety of report styles. Most of them are described in one of these two manuals:

- Agilent ChemStation: Understanding your ChemStation
- Agilent ChemStation for LC 3D Systems: Understanding Your Spectra Module

Two report styles are specific to the MSD, and are not discussed in the above manuals.

- MSD – includes:
  - Header information from the data file
  - A total ion chromatogram (optional)
  - Tabulated and graphical spectra for each integrated peak
  - Deconvolution results (available with the optional Agilent Deconvolution & Bioanalysis Software).
- MSD Qualitative – includes:
  - Header information from the data file
  - A total ion chromatogram (optional)
  - Extracted ion chromatograms and spectra for each integrated peak.

## Defining custom reports

ChemStation Edition program provides the capability to set up custom report layouts that can include text, chromatograms, tables, and custom elements such as company logos. The text can be user-defined text or information stored within ChemStation Edition, such as sample name, operator, and data file name.

### Summary of process

The overall process to create and use a custom report is as follows:

- 1 Create a custom report layout using the Report Layout view, described on [page 97](#).
- 2 Save the custom layout as a report template.
- 3 Test the report template by loading a data file and generating a report.
- 4 Add the report template to the list of available report styles.
- 5 Add the new report style to a method and save the method.

For details, see the **Report Layout tutorial** within the **ChemStation Tutorial** section of the online Help.

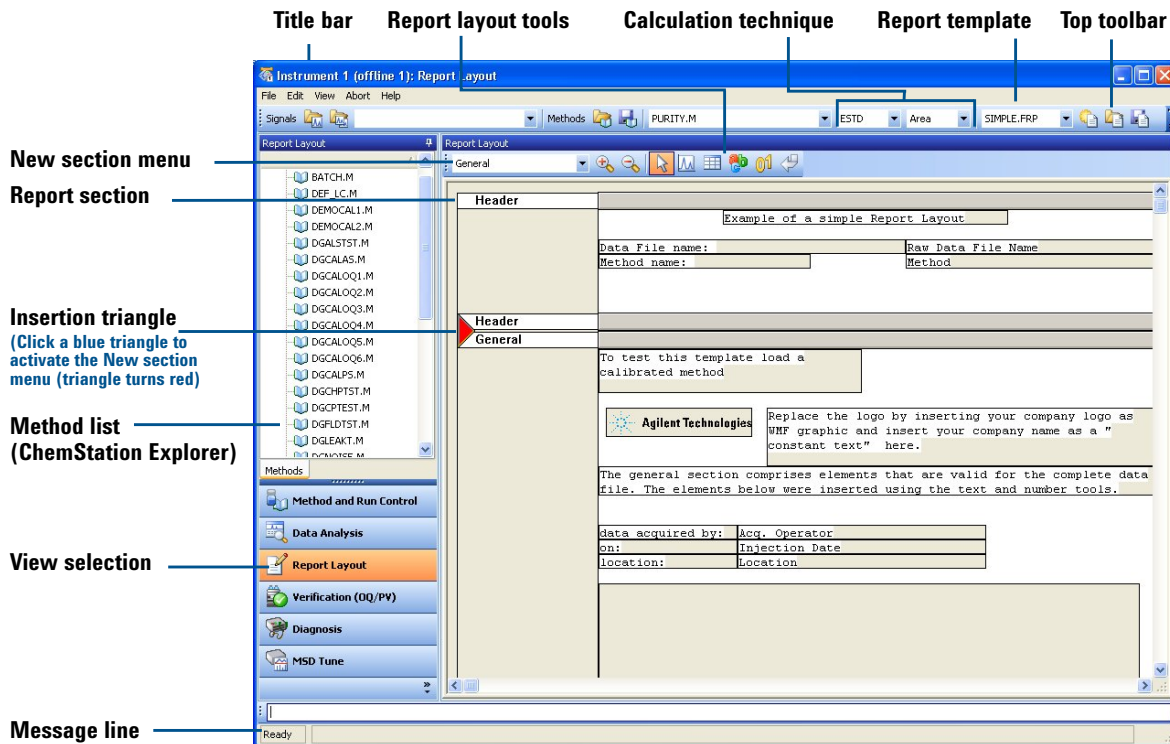
### Example report templates

ChemStation Edition program includes example report layouts. They are located in the **chem32\repstyle** folder. For descriptions of these layouts, see the *Quick Start Guide*.



## The Report Layout View

The Report Layout view, shown in [Figure 52](#), provides all the necessary menus and tools to create a template for a custom report.

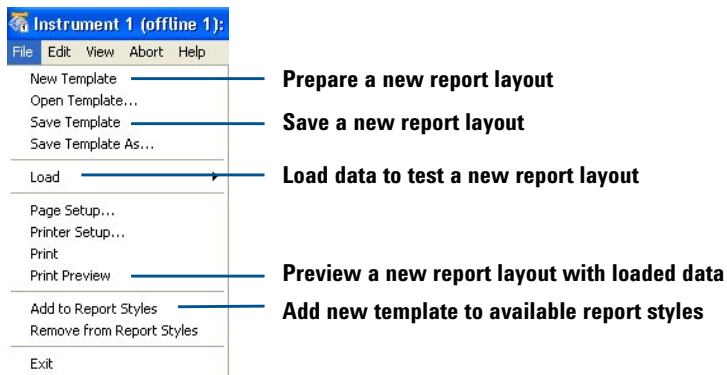


**Figure 52** The Report Layout view provides a graphical interface to set up custom reports.

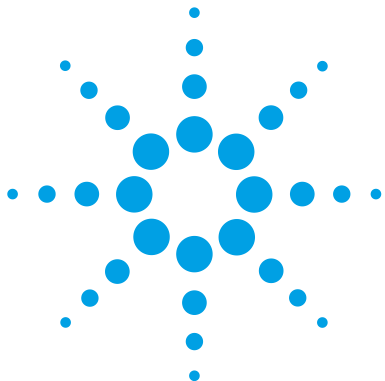
The file menu, shown in [Figure 53](#), provides the functionality to initiate, open, and save report templates. You can also access this functionality from the top toolbar. (See [Figure 52](#).) After you add a new section, you can use the report layout tools shown in [Figure 52](#) to add text, chromatograms, and tables.

## 5 Reports

### The Report Layout View



**Figure 53** The **File** menu within the Report Layout view



## 6 Verification of Performance

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In this chapter, you learn the concepts that help you verify the performance of the hardware and ChemStation Edition program in your system. ChemStation Edition program includes tests and analytical methods that help you verify that your system is working properly. This chapter provides an introduction to that functionality.



## The Verification (OQ/PV) View

If supported by the configured instrument, for example, the Agilent 1100/1200 Series modules for LC, the ChemStation Edition includes an additional view to do instrument verification and diagnosis tasks. For more information, see the online help system.

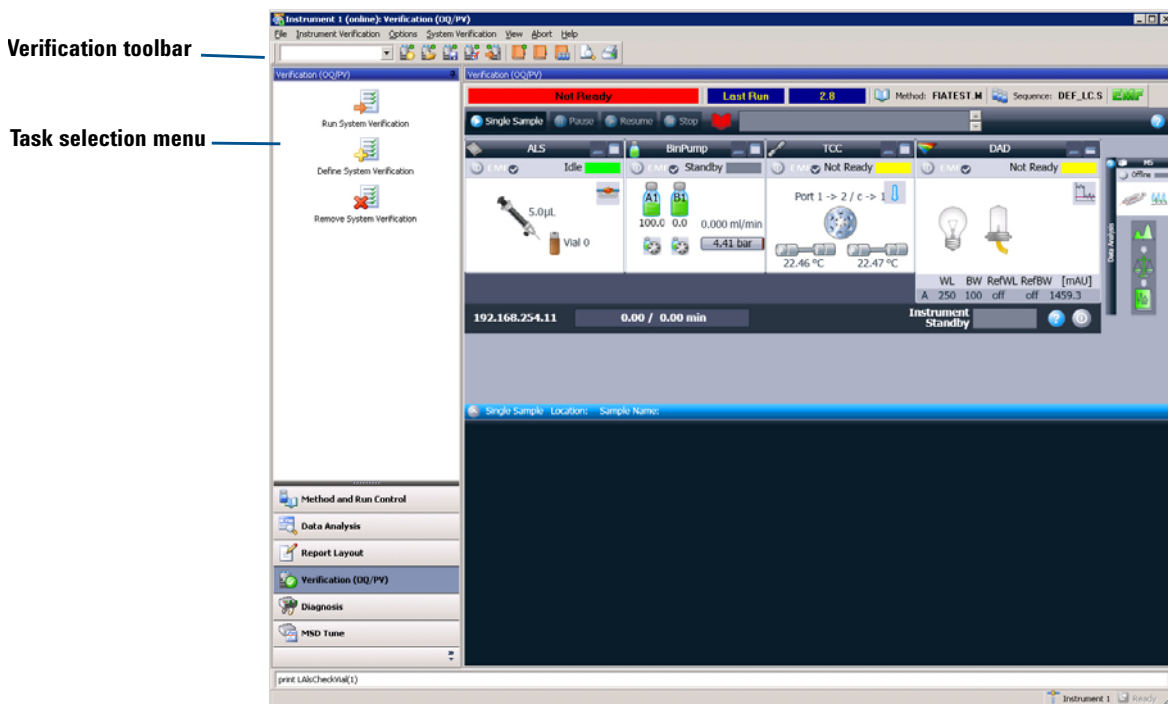


Figure 54 The Verification view lets you verify system performance.

## Instrument verification

Instrument verification tests are used to test the performance of your MSD system. To qualify a complete LC system, you must perform a series of tests. The specific tests depend on which LC modules you have configured. The tests may include:

- Carryover, linearity and reproducibility of the injector
- Gradient performance of the pump
- Temperature accuracy and precision of the column compartment. (Note that administration of this test requires a traceable digital thermometer. A digital thermometer is not included with the Agilent HPLC/UHPLC systems. Agilent can administer a complete OQ/PV as a paid service.
- Noise, drift, linearity and wavelength accuracy of the UV detector
- Others, as required.

You use the ChemStation Edition program to define which tests to run and the acceptance criteria for each. You obtain the appropriate standards from Agilent or your lab and load the autosampler with vials as specified by the ChemStation Edition program. The ChemStation Edition program then runs the tests and calculates and prints a report that compares the actual results with specified acceptance criteria. Each report includes a pass/fail designation.

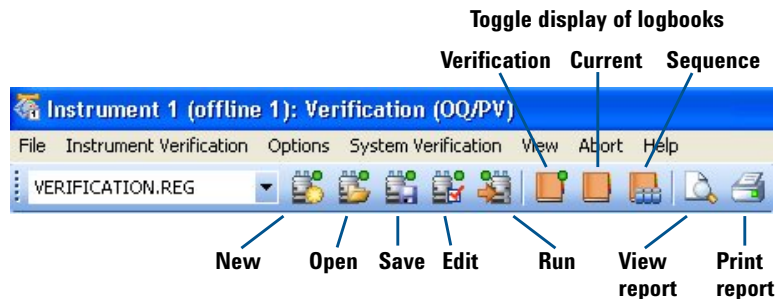
## Setting up and running instrument verification

You can set up and run instrument verification in the following ways:

- Select **File > New** or **File > Open** to display the dialog box shown in [Figure 56](#).

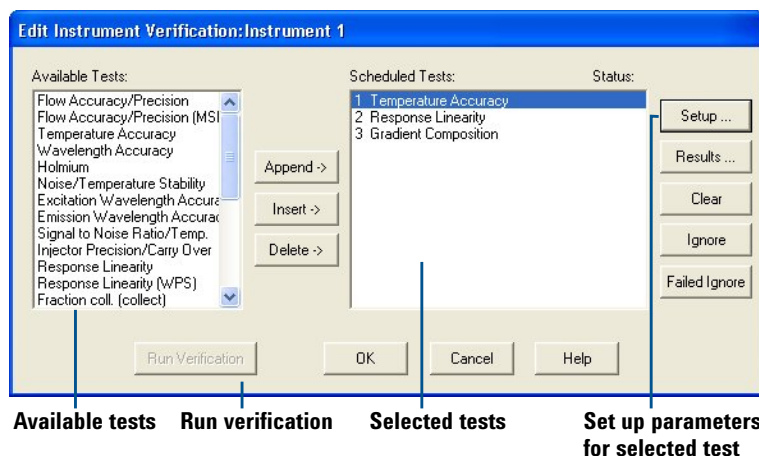
(Until you set up the first instrument verification with **File > New**, there are no files to open with **File > Open**.)

- Use the Verification Toolbar, shown in [Figure 55](#).



**Figure 55** Tools to work with instrument verification

When you set up a new instrument verification, open an existing instrument verification, or edit the current instrument verification, the Edit Instrument Verification dialog box shown in [Figure 56](#) is displayed.



**Figure 56** The Edit Instrument Verification dialog box lets you determine the specific tests and their order.

The **Setup** button in [Figure 56](#) is very important. When you click a scheduled test and click **Setup**, the dialog box that is specific to that test is displayed. Within the dialog box, you have the opportunity to:

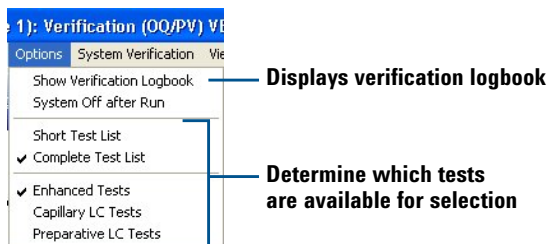
- Set up specific conditions for the test (for example, which detector or flow channel to test)
- Establish acceptance limits for the test
- Click the **Help** button to get:
  - General information about the test
  - Specific test conditions, such as the required standards and mobile phase.

## Available OQ/PV tests

ChemStation Edition program incorporates a wide variety of verification tests. Some of the tests are:

- Flow accuracy and precision. This test requires an external measurement device (a traceable digital flow meter).
- Injection precision and carryover
- Response linearity

The Options menu, shown in [Figure 57](#), determines the specific set of tests that are available at any given time.



**Figure 57** These options control the types of tests that you can select for instrument verification.

The online Help includes complete lists of available tests, along with a description of each one. To access this information:

- 1 Open the online Help.
- 2 In the Contents tab within the tree view on the left, double-click **User Interface Reference**.
- 3 Double-click **Menu Structure**.
- 4 Double-click **Verification (OQ/PV)**.
- 5 Click **Options**.
- 6 In the window on the right, click the link for **enhanced test suite**, **capillary test suite**, or **preparative test suite**.
- 7 Click any of the tests to view details.



## Verification logbook

The verification logbook records all events related to instrument verification. It contains a table that lists all the events, along with the date and time they occurred. The **Instrument Verification** menu gives you the opportunity to add notes to this logbook.

You can open the verification logbook from either the Verification Toolbar or the Options menu, as shown in [Figure 55](#) on page 102 and [Figure 57](#) on page 104.

## System verification

### Overview

The system verification tests enable you to verify the correct and consistent operation of your ChemStation Edition program. You can do this by reprocessing data files according to specific methods, and then comparing the results with a pre-defined standard. The verification tests are particularly important to prove the integrity of the integration and quantification results. You can use the standard Agilent verification test, or define your own tests using different method and data files. The Agilent verification test is a protected file and cannot be changed or deleted from outside ChemStation Edition program.

If your system is equipped with an Agilent 1100/1200 Series Diode-Array Detector, the system verification tests also allow you to test the detector electronics.

## Setting up and running system verification

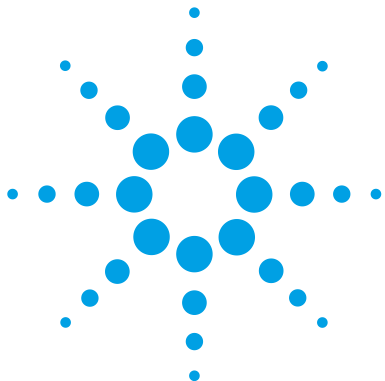
You can access the functionality to perform a system verification from either the System Verification menu or from the System verification task selection pane shown below.



**Figure 58** The system verification task selection pane.

You use the **Define Test** function to define your own verification test. You specify both a data file and a method file, which will both be stored in a protected register. When you run the test, the ChemStation Edition program processes the data with the test method, compares the results to the original results, and designates the results as either **Pass** or **Fail**.

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## 7 Maintenance and Troubleshooting

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In this chapter, you learn the concepts that help you maintain and troubleshoot your InfinityLab LC/MSD Series and 6100 Series LC/MS system. The Diagnosis view within ChemStation Edition program includes tools for both maintenance and troubleshooting.



## The Diagnosis View

### Overview

The Diagnosis view provides the functionality to:

- Learn possible causes of system problems
- Perform diagnostic tests
- Pump down and vent the MS
- Get early maintenance feedback so you can perform maintenance before problems occur
- Record maintenance in a maintenance logbook
- Get details about modules in your system, such as serial numbers, firmware revisions, and hours of operation.

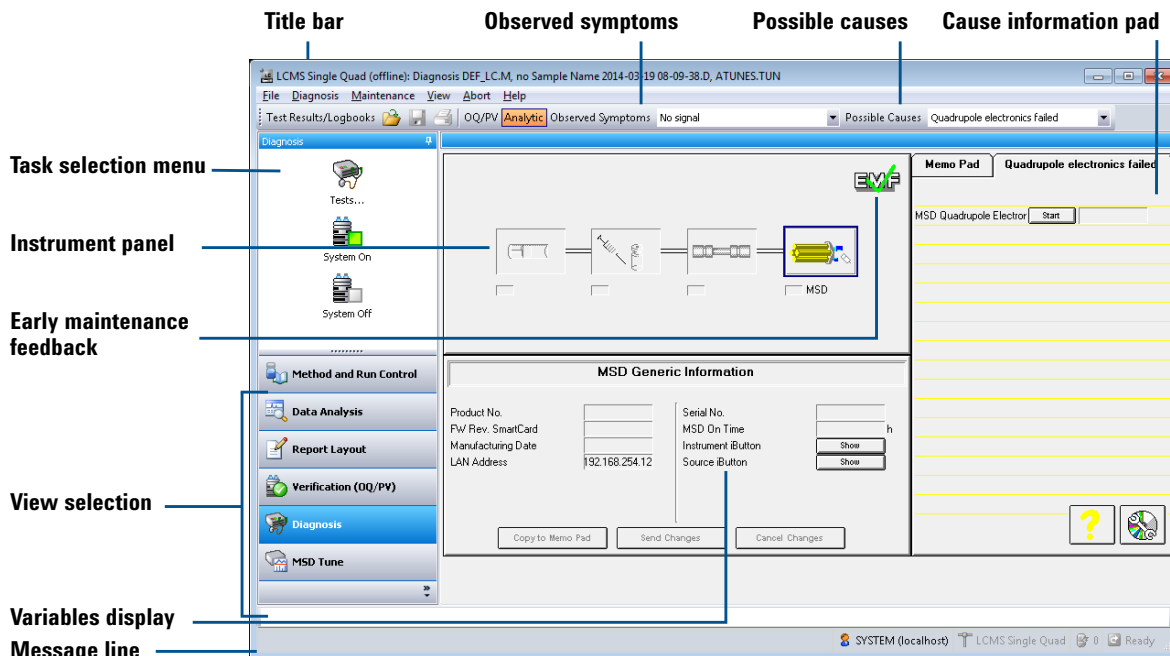


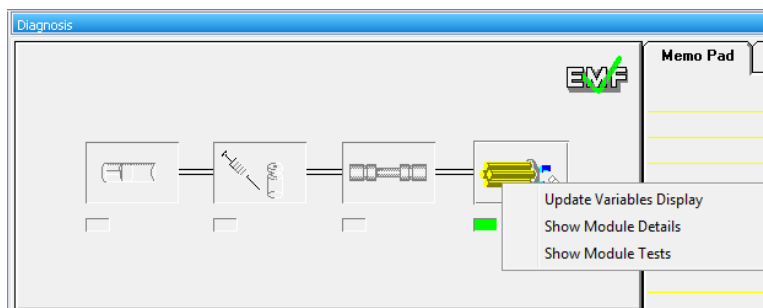
Figure 59 The Diagnosis view helps you to troubleshoot and maintain your system.

## Instrument panel

### Top-level display

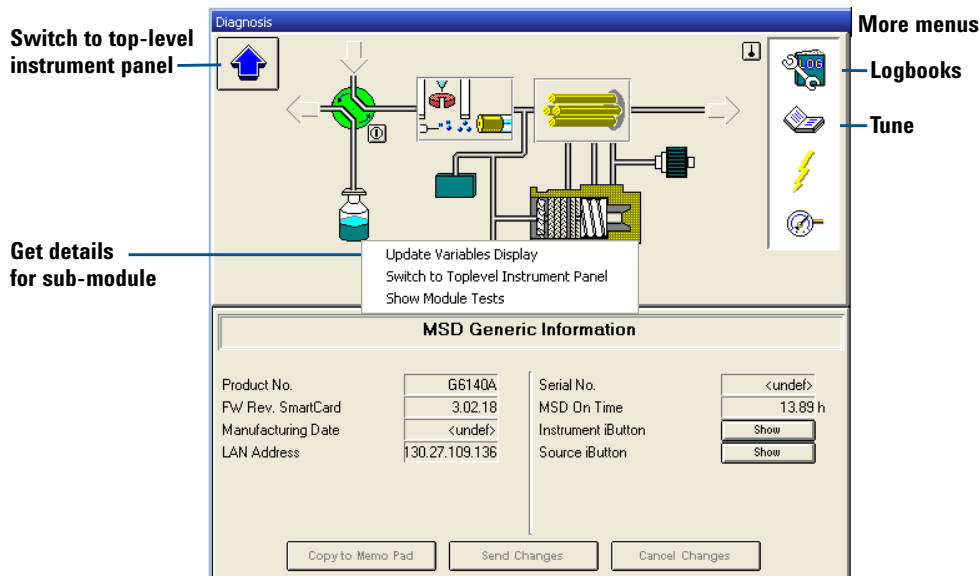
The instrument panel at the center of the Diagnosis view shows all the modules in your system. (See [Figure 60](#).) You can click each module to display a menu that enables you to do the following:

- **Update Variables Display** – Show module information, such as serial number, firmware revision, and LAN address, in the space just below the instrument panel.
- **Show Module Details** – Display a detailed schematic of the module, as shown in [Figure 61](#) on page 112.
- **Show Module Tests** – Display a list of diagnostic tests for that module, as shown in [Figure 65](#) on page 120.



**Figure 60** Instrument panel – top-level display

### Module details



**Figure 61** Instrument panel – module details for the MS.

As you move your mouse across the diagram shown in [Figure 61](#), whenever the cursor changes from a pointer to a hand, you can click to display a menu that gives access to details for a sub-module. For example, you can click the icon for the turbomolecular pump to view vacuum readings, or the icon for the quadrupole to view its temperature. You can also click the icons in the white area on the right to display their menus.



## Logbooks

The Diagnosis view provides access to a number of logbooks that help with troubleshooting and maintenance.

- Error logbook – view recent errors for the module
- Runs logbook – view start and stop times of the most recent runs
- Maintenance logbook – view the most recent maintenance events. For more about this logbook, see “[Maintenance logbook](#)” on page 115.
- Date change logbook – view date changes

You can access these logbooks in several ways:

- The **File** menu (**File > Open > Logbook**)
- The **Logbooks** icon, shown in [Figure 61](#) on page 112 (click the icon and select **Update Variables Display**)
- The **Open** icon on the top toolbar.



## Maintenance

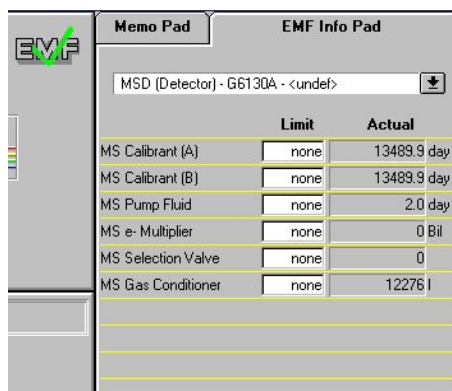
Proper maintenance of your LC/MS system helps to ensure the quality of your analyses and avoids expensive failures and unexpected downtime. ChemStation Edition program includes features to assist with a regular maintenance program.

### Early maintenance feedback

Early maintenance feedback (EMF) alerts you to necessary maintenance so you can prevent problems from occurring. The program allows you to use your past experience to predict when each type of maintenance will be needed. You can enter the limits for the maintenance intervals within the Diagnosis view.



Within both the Method and Run Control view and the Diagnosis view, when a maintenance limit is reached, the green EMF checkmark changes to a yellow question mark. At that point, you can click the EMF icon in the Diagnosis view to view the limits in the EMF Info Pad and to find out which module needs service. The limit for that module is highlighted in yellow.



	Limit	Actual
MS Calibrant (A)	none	13489.9 day
MS Calibrant (B)	none	13489.9 day
MS Pump Fluid	none	2.0 day
MS e- Multiplier	none	0 Bil
MS Selection Valve	none	0
MS Gas Conditioner	none	12276 l

Figure 62 EMF information pad

## Maintenance logbook

Maintenance records help to ensure that your system is kept in excellent condition, and are especially important when you have multiple operators. The ChemStation Edition maintenance logbook provides a convenient place to record any maintenance that you perform on your LC modules or your MS.

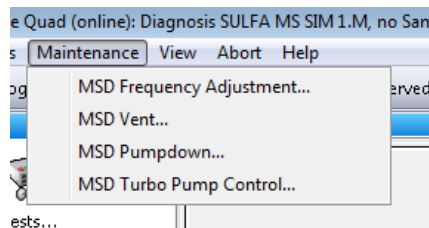
You access the maintenance logbook via the **Logbooks** icon, shown in [Figure 61](#) on page 112. (Click the icon and select **Maintenance Logbook Entry**.) You then manually enter the information via ChemStation Edition program.

Rather than being stored on your computer disk, the maintenance logbook is stored in the memory within the LC or MS module. That way, if you move the module to another system, the maintenance record moves with the module.

## Maintenance procedures

When you need to perform maintenance, you can find instructions for common procedures in the *Maintenance Guide*, or in the manuals for the LC modules.

You can conduct some maintenance procedures, such as wavelength calibration for a DAD detector, from the **Maintenance** menu in the Diagnosis view.



**Figure 63** The **Maintenance** menu contains software-driven maintenance tasks for the MS and the LC modules.

## Venting and pumping down the MS

### Venting

Sometimes you need to perform maintenance on MS components that lie within the vacuum system. Examples of such maintenance include changing the electron multiplier and cleaning the ion optics. Before you can perform maintenance on these components, you need to vent the MS. Venting releases the vacuum in a controlled fashion.

You use the **Maintenance** menu within the Diagnosis view to initiate the vent. (See [Figure 63](#) on page 115.) The ChemStation Edition program executes a sequence of steps that safely vents the instrument. A dialog box allows you to view progress of the vent, and to reverse the procedure if necessary.

### CAUTION

Do not vent the instrument by simply turning off the power button on the MS. Doing so will cause excessive wear on the turbomolecular pump.

### Pumping down

After you have completed maintenance, you pump down the system. When you pump down, the vacuum pumps turn on in the correct order to bring the system safely under vacuum. To pump down, you simply turn on the MS power switch, which initiates the correct sequence of events. You can monitor and reverse the pump-down process from a dialog box that you access from the **Maintenance** menu.

### Frequency of venting and pumping down

The MS should normally be left under vacuum, even when not in use. Repeated venting and pump-down causes unnecessary wear on the turbomolecular pump. When you keep the system under vacuum, you maintain the level of cleanliness that you need for sensitive analyses. If you do not plan to use the MS for a period of time, leave it in Standby Mode rather than venting it.

*For details on putting the MS into Standby Mode, see the Basic Operation section of the Quick Start Guide.*

## Diagnosing and fixing problems

### Symptoms and causes

Figure 64 shows components of the ChemStation Edition program that help you troubleshoot an instrument problem in a step-by-step fashion.

- 1 You click either **OQ/PV** for a failed OQ/PV test or **Analytic** for a symptom that you observed during an analysis.
- 2 You select the specific symptom.
- 3 A list of possible causes is displayed. You select one of the causes.
- 4 The memo pad displays instrument data and tests you can run to confirm or refute that cause of the symptom. You click **Start** to display the dialog box for a test.
- 5 You click **Start** to initiate the test, or **Explain** to view a detailed explanation of the test.

## 7 Maintenance and Troubleshooting

### Diagnostic tests for the MS

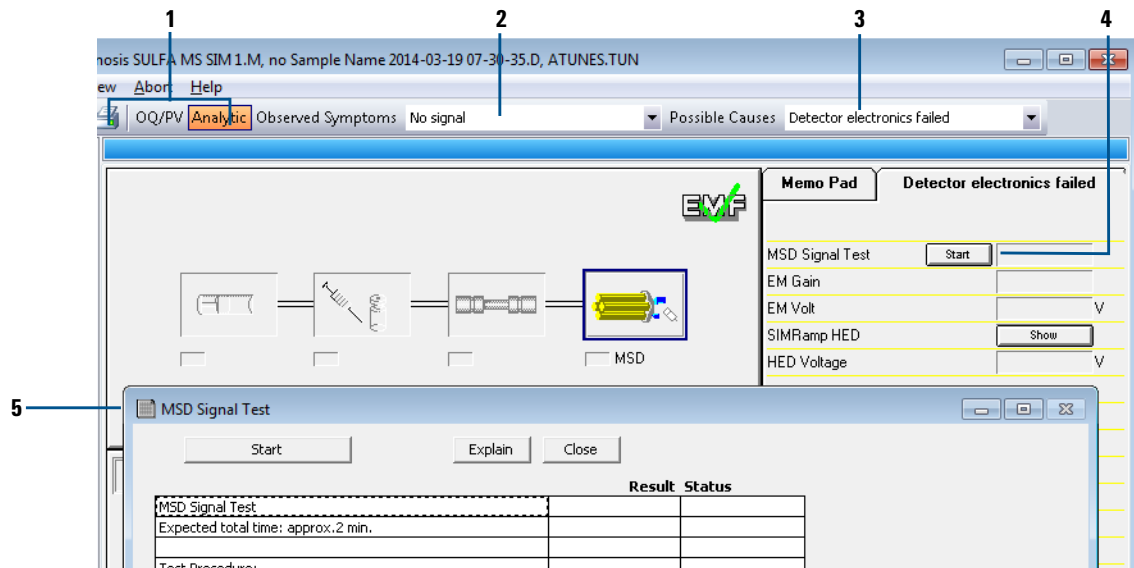


Figure 64 Overview of process to discover the root cause of instrument problems

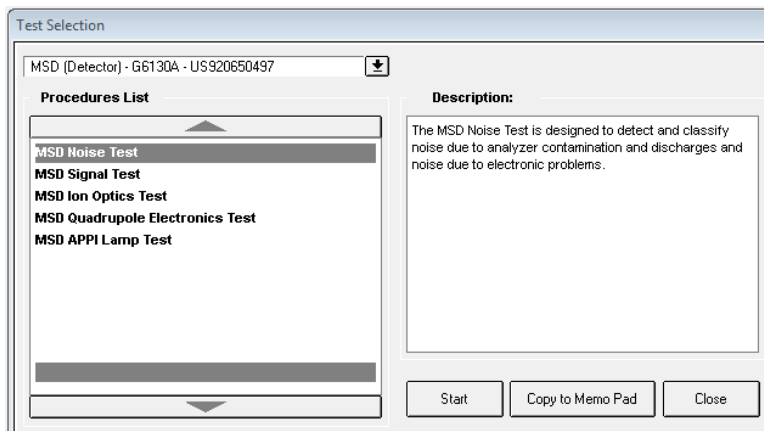
## Diagnostic tests for the MS

There are a number of preprogrammed diagnostic tests that you can access for each module in your system. You can display a complete list of tests for each module by clicking the module icon in the instrument panel and selecting **Show Module Tests**. Figure 65 shows the tests for the MS.



## 7 Maintenance and Troubleshooting

### Fixing problems



**Figure 65** Diagnostic tests for the MS

## Fixing problems

When you have diagnosed a problem and need to fix it, you can find instructions for common procedures in the *Maintenance Guide*. More serious problems may require that you call an Agilent customer engineer for service.





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## In This Book

*The Concepts Guide* presents an overview of the InfinityLab LC/MSD Series and 6100 Series LC/MS Quadrupole LC/MS systems, to help you to understand how the hardware and OpenLAB CDS ChemStation Edition program work.

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