Agilent 6100 Series Quad LC/MS System

Quick Start Guide

What's New in ChemStation B.02.01?

- New and improved user interface design
- New tree- and table-based navigation provides fast and flexible data handling within various ChemStation views
- Flexible storage locations for data, methods, and sequences
- Improved data review and reprocessing capabilities using the Data Analysis Navigation Table
- New packaging concept guarantees consistency for sequence data
- Additional signal options allow you to assign method-specific signal options to improve data review
- Option to save manual integration events in a Data Analysis method along with newly acquired data files
- ChemStation tutorial integrated with online help allows you to learn the software while working on your own methods and data
- Enhanced utilization of high-resolution monitors and available screen space
- Support for Agilent 6100 Series Quad LC/MS Systems

Agilent Technologies

Where to find more information

Use the following resources to learn how to use your Agilent 6100 Series LC/MS system.

Online Help

Use the online help for in-depth information not given in this *Quick Start Guide*. Display online help in any of the following ways:

• Click the **Help** button in the toolbar.

• Select **Help Topics** from the **Help** menu.

• Click the **Help** button on most dialog boxes to display task-specific help.
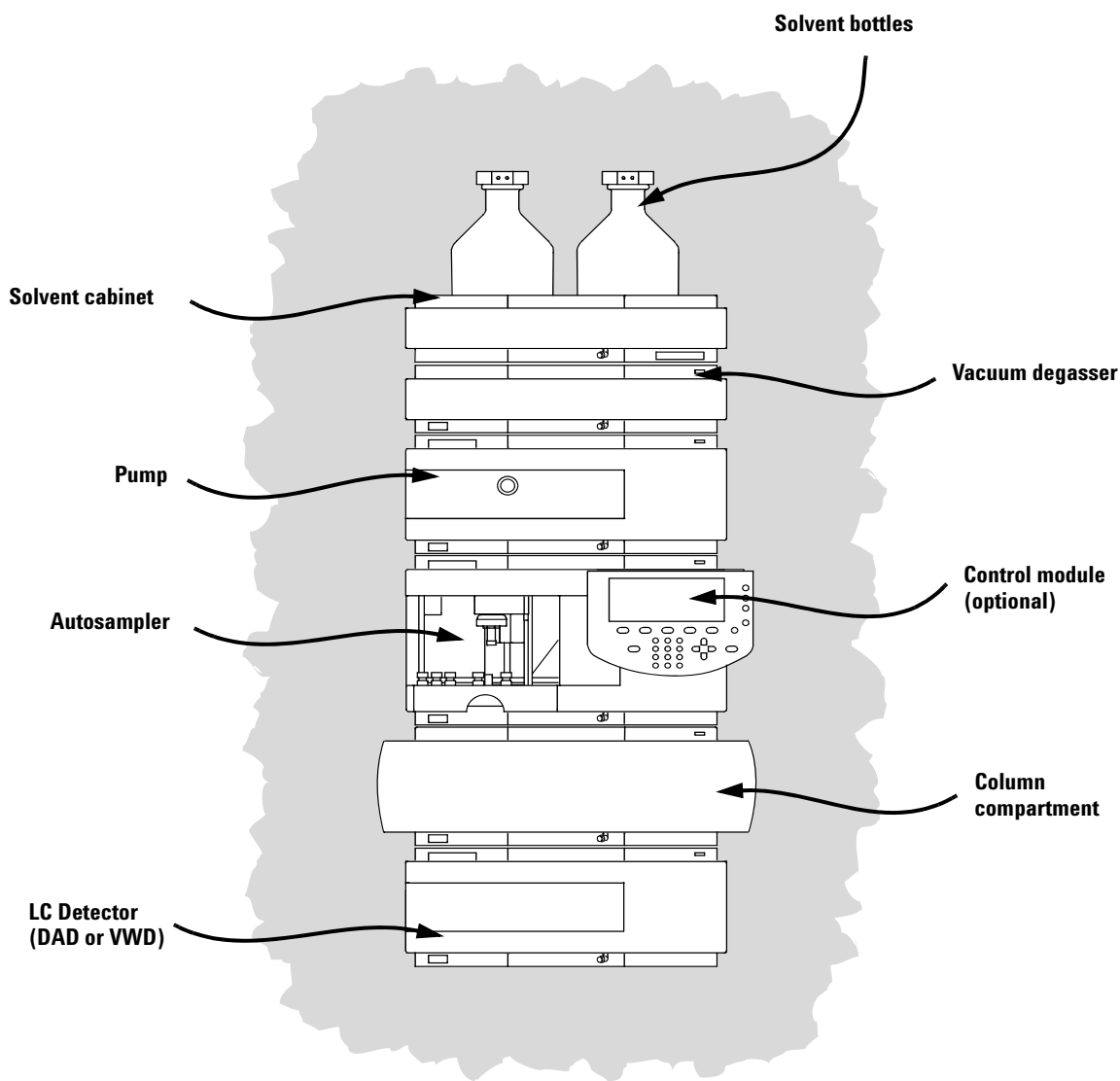
Manuals

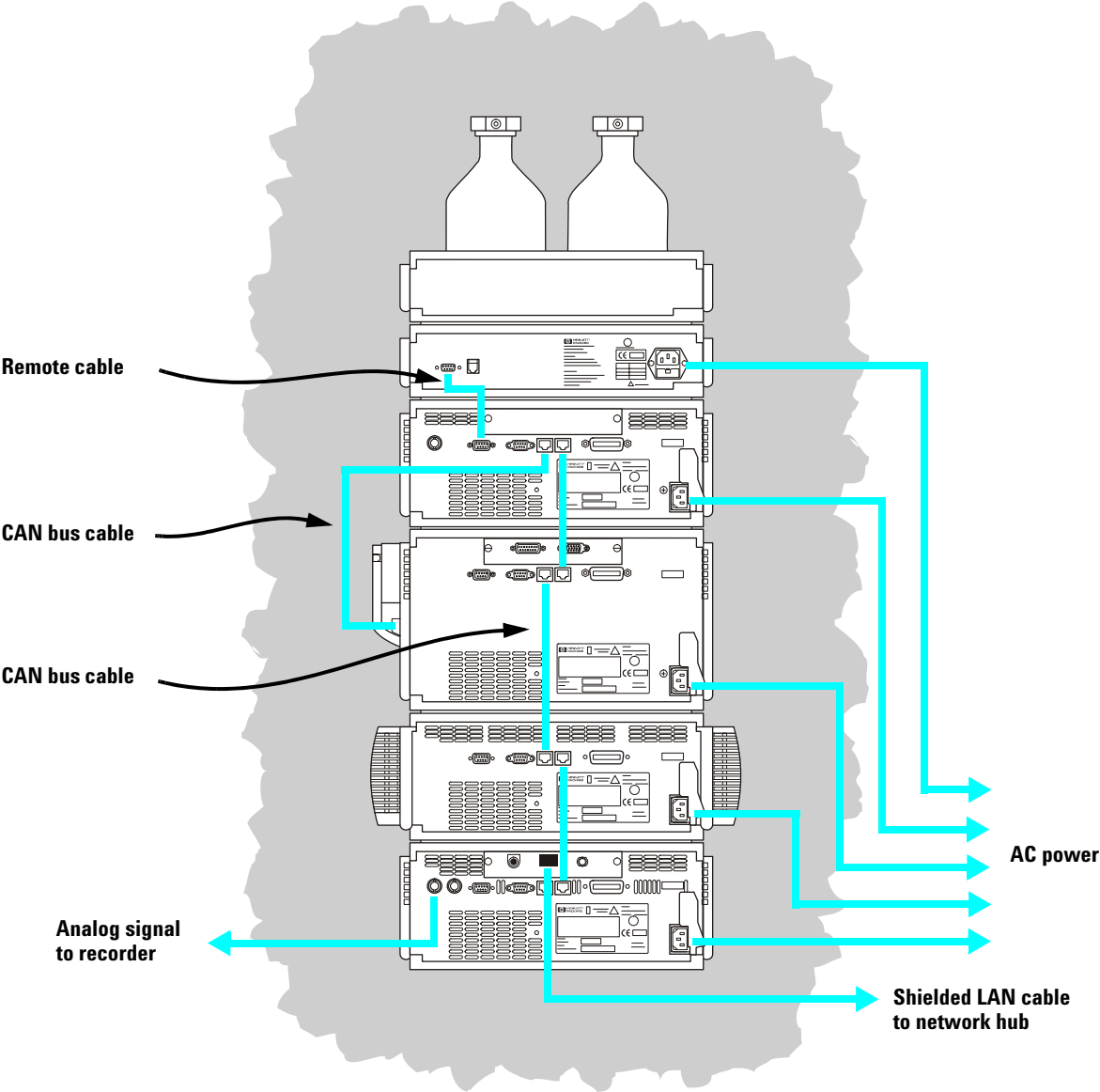The following manuals are shipped with your system and are also available in PDF format.

• *Agilent 6100 Series Quad LC/MS Systems Quick Start Guide* (G1960-90001)

• *Agilent 6100 Series Quad LC/MS Systems Installation Guide* (G1960-90002)

Configuration Diagrams

1100 or 1200 Series LC

Solvent bottles

Solvent cabinet

Vacuum degasser

Pump

Control module
(optional)

Autosampler

Column
compartment

LC Detector
(DAD or VWD)

LC Cabling

Remote cable

CAN bus cable

CAN bus cable

Analog signal
to recorder

AC power

Shielded LAN cable
to network hub

ChemStation Views

The LC/MS Quad ChemStation software consists of the following views: Method and Run Control, Data Analysis, Report Layout, Verification (OQ/PV), Diagnosis, and MSD Tune. The menu items and toolbar buttons depend on the current view. Each view allows you to perform a certain set of tasks.

Switch to a different view by clicking one of the view buttons in the lower left corner of the screen (shown below).
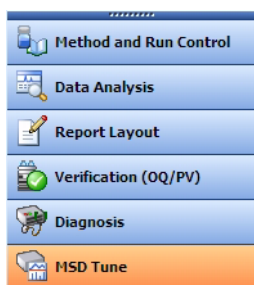
You can also select a different view from the **View** menu.

Method and Run Control View

In this view, you can set up methods and adjust instrument parameters to inject samples and acquire data one sample at a time or in automated sequences.

Data Analysis View

In this view, you can perform various data evaluation tasks on chromatograms and spectra. You can view both mass spectral and UV signals simultaneously. Common tasks include integration, quantitation, checking peak purity, and deconvolution. Once you have analyzed the data, you can select one of the predefined report types.

Report Layout View

In this view, you can design custom report templates to use with the data generated by your ChemStation.

Verification (OQ/PV) View

In this view, an Agilent service engineer can determine if your system is operating in a predictable manner. This is useful to show Good Laboratory Practice (GLP) compliance and may be required by some government agencies.
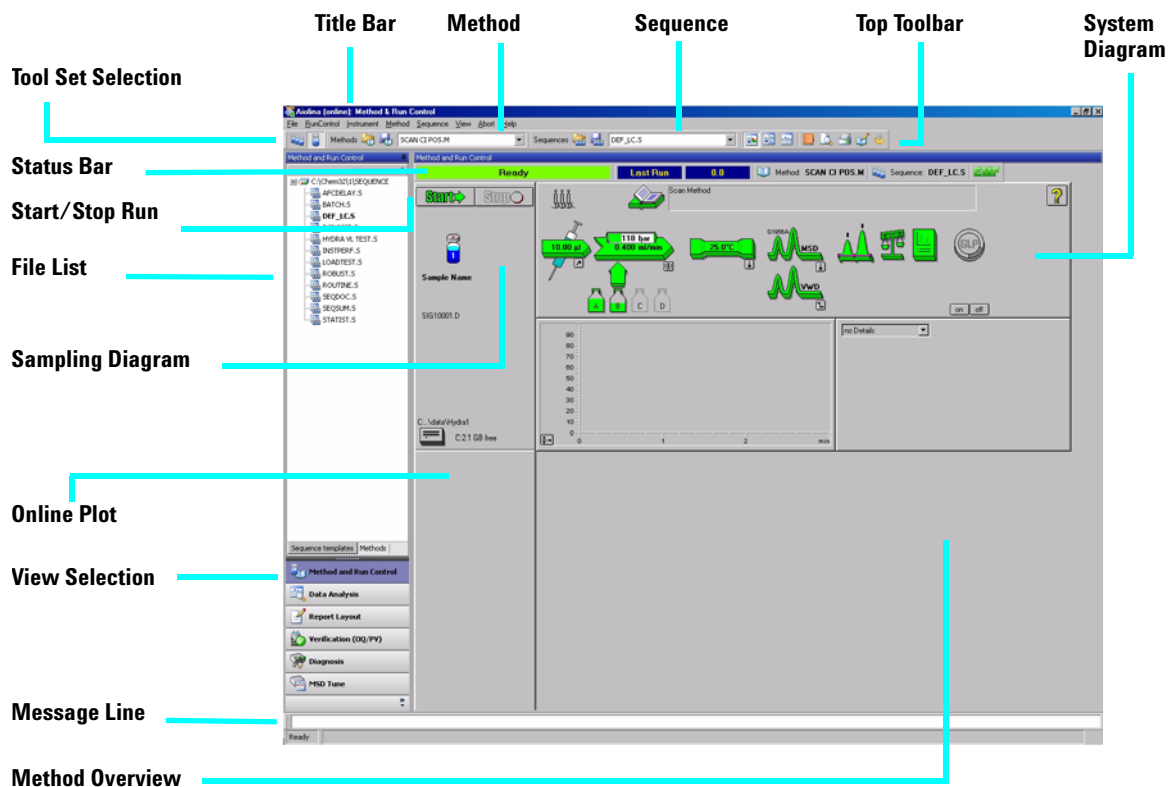
Diagnosis View

In this view, you can run tests to diagnose instrument problems and access information on how to resolve these problems. The early maintenance feedback (EMF) feature can be used to notify you when it is time to perform system maintenance before a problem occurs.
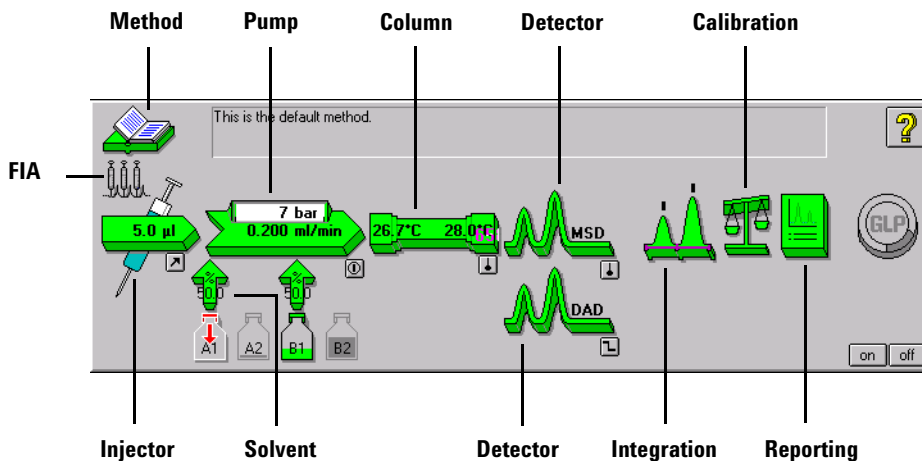
MSD Tune View

In this view, you can calibrate your LC/MS quad automatically. You can also set MS parameters manually for specific types of molecules.

Method and Run Control View

Title Bar   Method   Sequence   Top Toolbar   System Diagram

Tool Set Selection

Status Bar

Start/Stop Run

File List

Sampling Diagram

Online Plot

View Selection

Message Line

Method Overview

System Diagram   Each icon of the system diagram represents one component or module of your system. Click on an icon if you want to edit the method parameters or go to the online help for that particular component.

Method   Pump   Column   Detector   Calibration

FIA

Injector   Solvent   Detector   Integration   Reporting

Single Sample Tool Set   This toolset allows you to work on methods and run an analysis for a single sample. Move the cursor over a button to view a description of it.

Click the Single Sample icon to display this toolset.

Sequence Tool Set   This toolset allows you to work on sequences and run automated analyses of multiple samples. Move the cursor over a button to view a description of it.
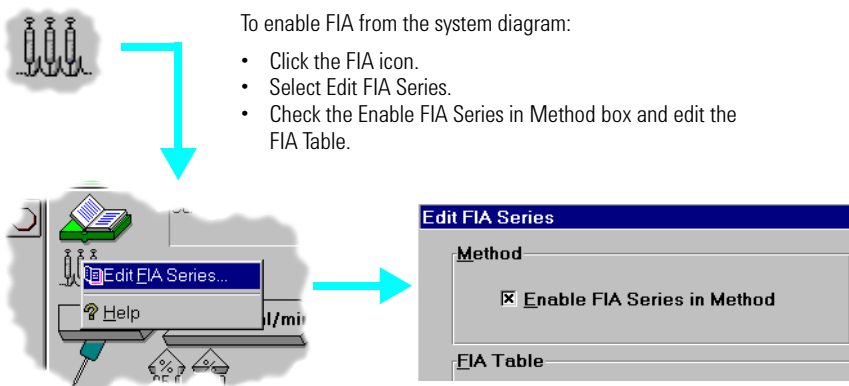
Click the Sequence icon to display this toolset. The sampling diagram now shows the sample tray.
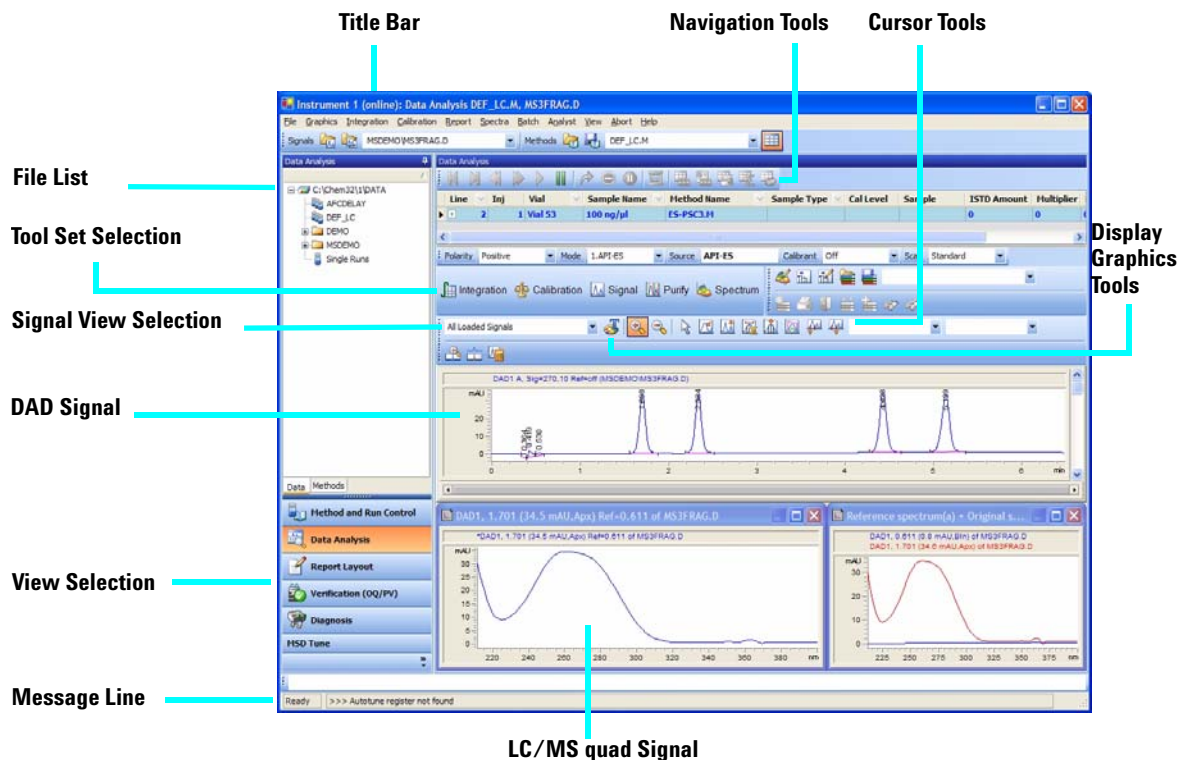
Flow Injection Analysis (FIA) This toolset allows you to inject multiple samples directly into the detector, bypassing the chromatographic column. The results are sent to a single data file. FIA can be used for method development or for applications that do not require chromatography.

To enable FIA from the system diagram:

- Click the FIA icon.
- Select Edit FIA Series.
- Check the Enable FIA Series in Method box and edit the FIA Table.

Data Analysis View

Title Bar   **Navigation Tools**   **Cursor Tools**

File List

Tool Set Selection

Display Graphics Tools

Signal View Selection

DAD Signal

View Selection

Message Line

LC/MS quad Signal

Common Tool Set

This toolset is always present in Data Analysis view. Move the cursor over a button to view a description of it.
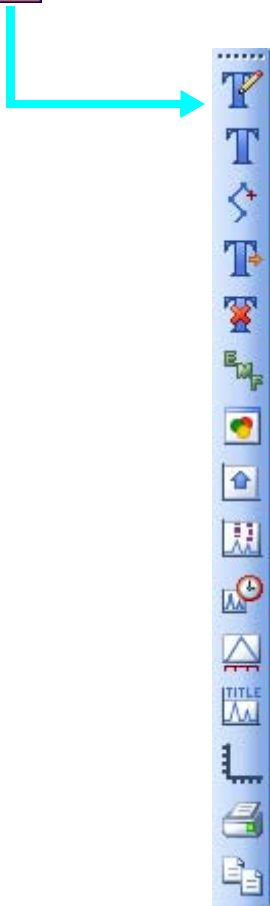
Graphics Tool Set

This toolset allows you to manipulate the graphic display. Move the cursor over a button to view a description of it.
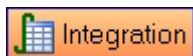
Click the Graphics icon to display this toolset, which appears on the right side of the plot window.
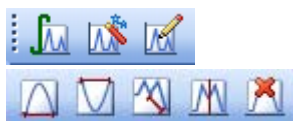
Integration Tool Set

This toolset allows you to perform integration and reporting tasks on a chromatogram. Move the cursor over a button to view a description of it.
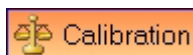
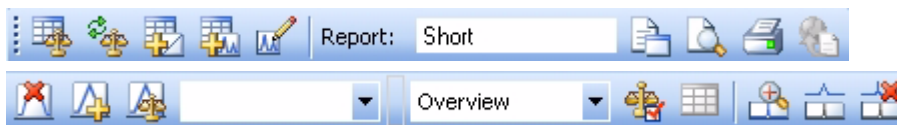**Click the Integration icon to display this toolset.**

Calibration Tool Set

This toolset allows you to perform calibration tasks for quantitation. Move the cursor over a button to view a description of it.
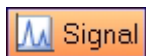
Click the Calibration icon to display this toolset.

Report: Short

Overview

Signal Tool Set

This toolset allows you to graphically work with the UV or MS signal. Move the cursor over a button to view a description of it.

Click the Signal icon to display this toolset.

Purify Tool Set

This toolset allows you to graphically work with your purification data.
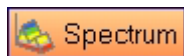
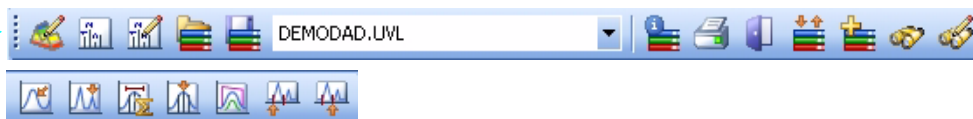**Click the Purify icon to display this toolset.**
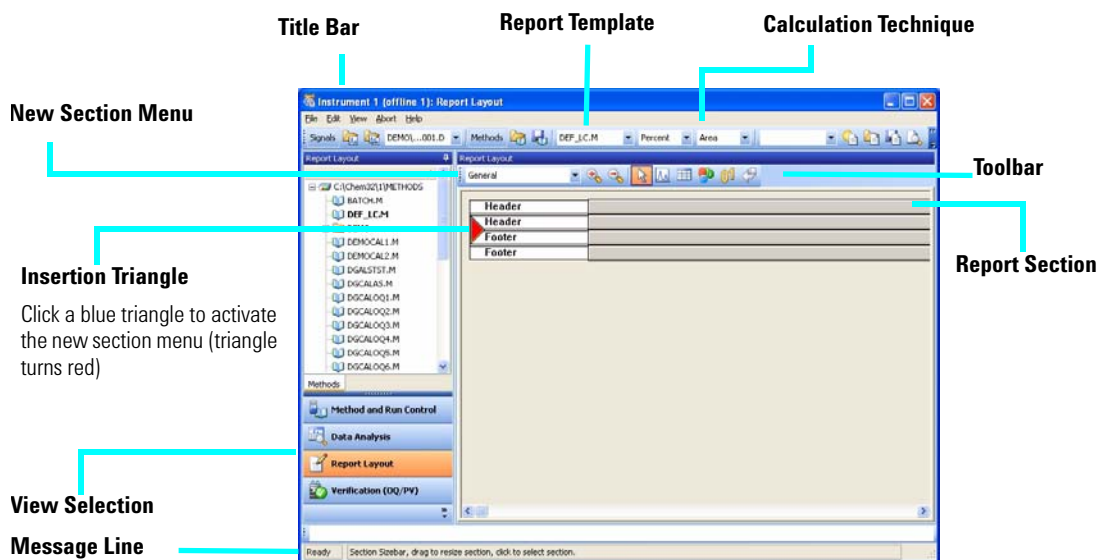

Spectral Tool Set

This toolset allows you to perform spectral evaluation tasks. Move the cursor over a button to view a description of it.

Click the Spectrum icon to display this toolset.

Report Layout View

Title Bar   Report Template   Calculation Technique

New Section Menu

Toolbar

Insertion Triangle

Click a blue triangle to activate the new section menu (triangle turns red)

Report Section

View Selection

Message Line

Report Layout Tool Set

This toolset is present in the Report Layout view. Move the cursor over a button to view a description of it.

Report Layout Tips

- Edit or create a report layout as described in the online help.
- To test your report layout, select a calculation technique from the drop-down list box to define how the results should be calculated.
- Load a data file. The results are loaded into the report template using the selected calculation.
- Select **File / Add to Report Styles** to add your completed report template to the list of available report styles. Now you can use your customized report within a method.

Sample Report Layouts (in \hpchem\repstyle)

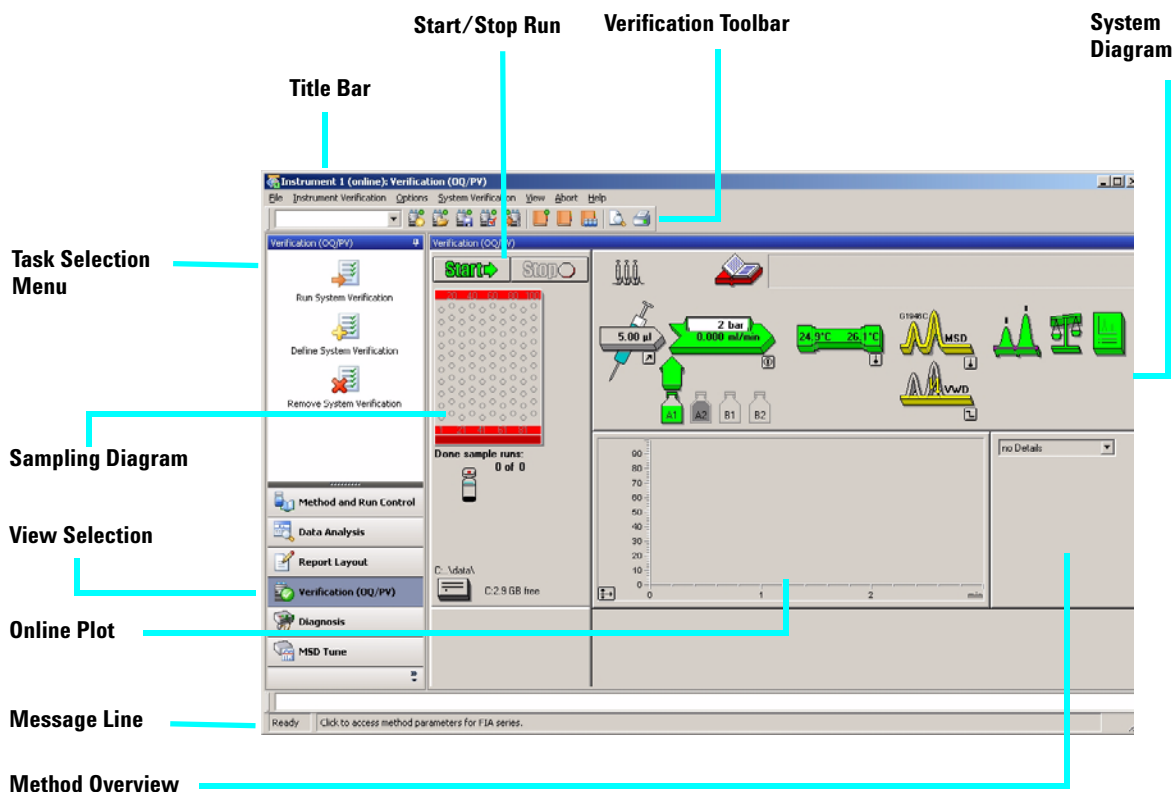| Template	Description
SIMPLE.FRP	Includes a chromatogram and calibration curves per calibrated compounds.
NESTED.FRP	Shows how to nest subsections.
SPECTRA.FRP	Shows how to include spectra.
PURITY.FRP	Shows how to include peak purity data.
ESTD.FRP	Uses the same elements as the standard ESTD reports so you can customize your external standard reports.
AREAPCT.FRP	Uses the same elements as the standard area percent report so you can customize your area percent reports.
LIBRARY.FRP	A customized report that uses library search results. You must have a method loaded that specifies the spectra library.

Print Preview button

Report — Example of a simple Report Layout

Data File name: C:\HPCHEM\1\DATA\MSDEMO\CAFCAL02.D
Method name: C:\HPCHEM\1\METHODS\CAFCALIB.M

To test this template load a
calibrated method

Agilent Technologies   Replace the logo by inserting your company logo as
                            WMF graphic and insert your company name as a "
                            constant text" here.

The general section comprises elements that are valid for the complete data
file. The elements below were inserted using the text and number tools.

data acquired by: mcintyre
on: 1/6/97
vial #   2.000

DAD1 A, Sig=272,4 Ref=460,80 (MSDEMO\CAFCAL02.D)
MSD1 TIC, MS File (MSDEMO\CAFCAL02.D)

HP LaserJet 4Si MX on LPT1:
Letter 8 1/2 x 11 in/Portrait

Close | normal | Page 1 of 2 | Prev | Next | Print | Help

Verification (OQ/PV) View

Start/Stop Run   Verification Toolbar   System Diagram

Title Bar

Task Selection Menu

Sampling Diagram

View Selection

Online Plot

Message Line

Method Overview

The Verification (OQ/PV) view allows an Agilent service engineer to test whether your analytical instruments and the ChemStation software are operating correctly according to predefined performance criteria.

• Operation Qualification (OQ)

Operation Qualification is the documented verification that the equipment-related system or subsystem performs as intended throughout representative or anticipated operating ranges.

• Performance Verification (PV)

Performance Verification is the documented verification that the process and or the total process-related system performs as intended throughout all anticipated operating ranges.

System Diagram   Each icon of the system diagram represents one component or module of your system.

Verification Toolbar   The Verification toolbar is displayed when you select **Show Top Toolbar** from the **View** menu. Move the cursor over a button to view a description of it.


Available Tests   The following verification tests are supplied with your ChemStation software for use by an Agilent service engineer. Refer to the online help for more information on these tests.

- VWD Wavelength Accuracy
- Intensity
- Holmium
- Temperature Accuracy
- Noise, Flow, Temperature
- DAD Wavelength Accuracy
- Injector Precision*
- Detector Linearity/Carry-over*
- Injector Linearity*
- Gradient Composition

_____

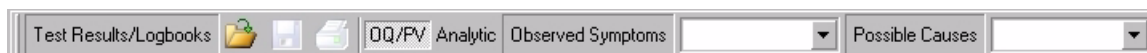   * These tests can be used to verify LC/MS quad performance.
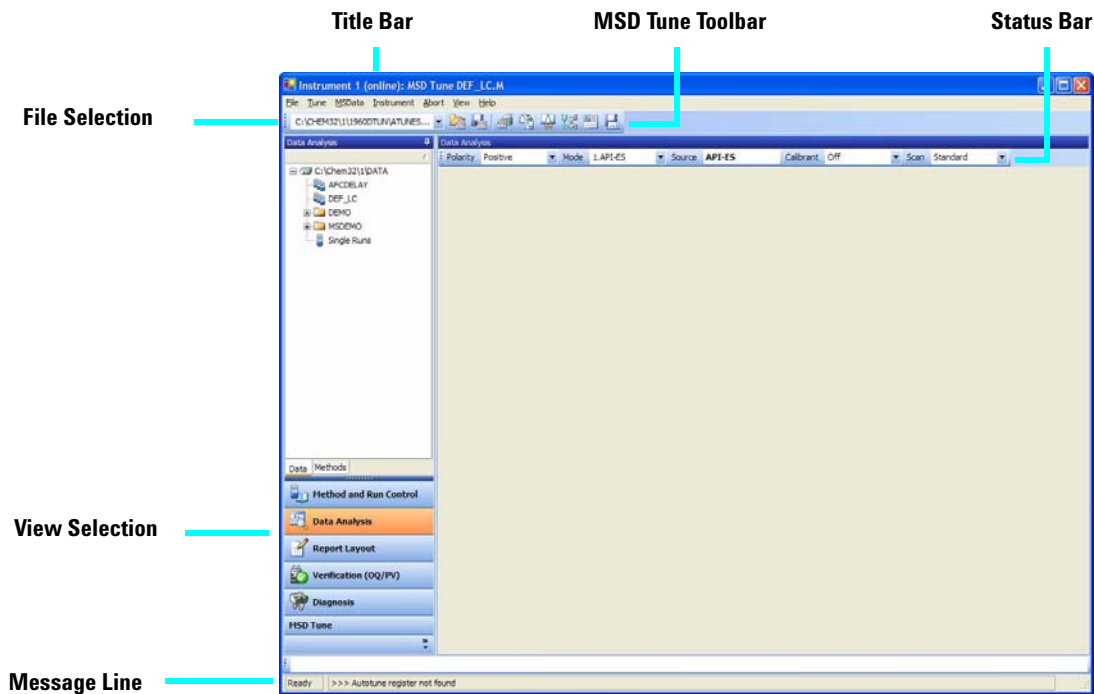
Diagnosis View
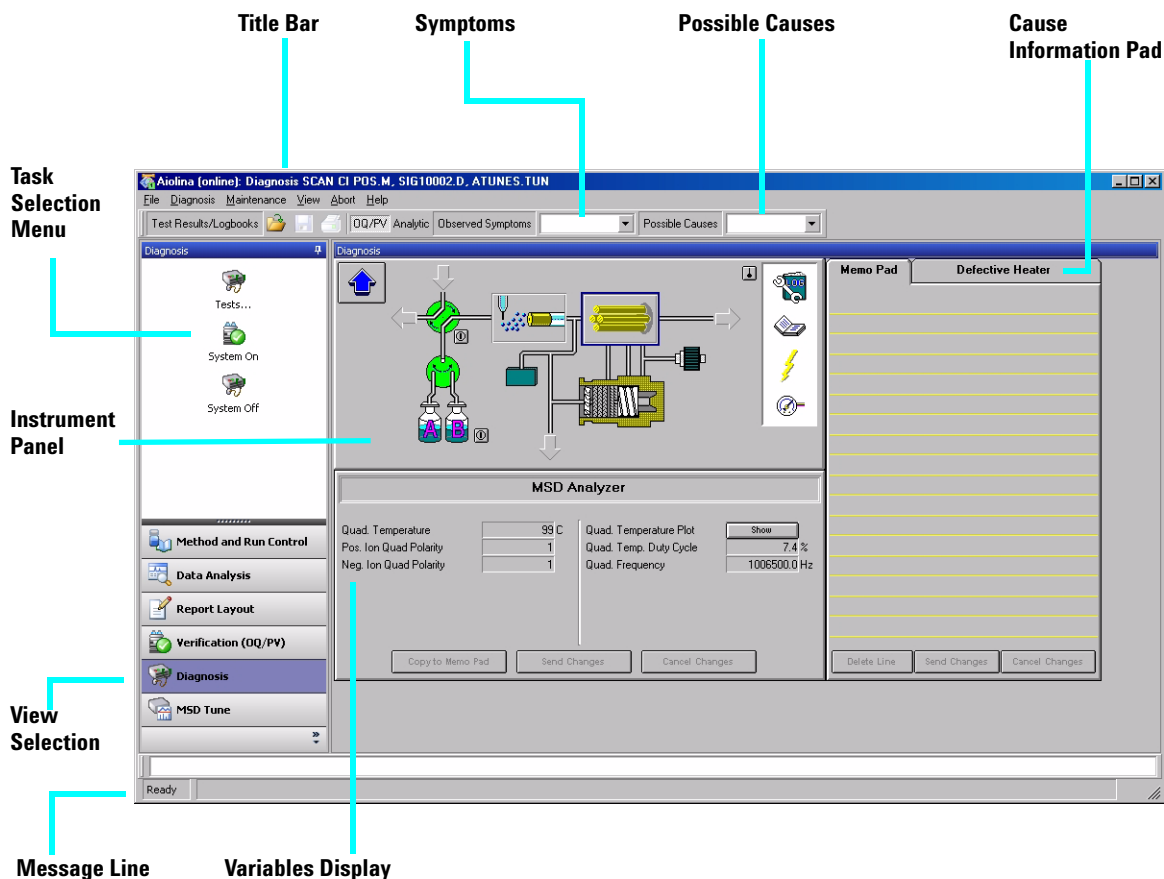
This view allows you to carry out diagnostic and maintenance activities on your system.

Diagnosis Toolbar   The Diagnosis toolbar is displayed when you select **Show Top Toolbar** from the **View** menu. Move the cursor over button to view a description of it.

MSD Tune View

Title Bar   **MSD Tune Toolbar**   **Status Bar**

File Selection

View Selection

Message Line

MSD Tune Toolset

This toolset is present in MSD Tune view.

Load an LC/MS quad tune file.

Save the current tune file.

Generate a profile and scan report.

Autotune the LC/MS quad and print a tune report.

Calibrate the mass axis.

Change spray chamber parameters.

Edit LC/MS quad parameters for manual tuning.

Acquire multiple scans and send results to data file.

Basic Operation

Start up and Shut Down the System

Task	Instruction	Comment
To start up the system	1 From the Method and Run Control view, click the **On** button in the system diagram. 2 Set up the LC conditions (pump, column heater, and detector). 3 Set up the LC/MS quad spray chamber conditions as described in the **MSD Spray Chamber dialog box** topic in the online help. Allow 15 minutes for the system to warm up.	This procedure assumes the system is under vacuum and the LC and LC/MS quad are properly connected. It also assumes the liquid flow path is properly set up.
To put the system in Standby Mode	1 Flush the system with pure mobile phase to ensure the flow path is clear of buffers. This avoids plugging the nebulizer. A plugged nebulizer can cause a high back pressure, which can damage the LC flow cell. It is good practice to flush the flow path (including the column) for 5-10 minutes with a mobile phase without buffers such as 50:50 water/acetonitrile. 2 Click the **Off** button in the system diagram. All modules are set to standby mode (indicated by the gray color in the system diagram). Standby for the LC/MS quad is 20 psi for the nebulizer, 3 L/min for drying gas, 300°C for drying gas temperature and, if present, 325°C for the APCI vaporizer. Also, the MS stream selection valve is set **LC to Waste**.	Put the system in standby mode overnight or whenever you won't be analyzing samples for an extended time. The standby state for the LC/MS quad leaves the nebulizer and drying gas on at low flow.

Tune the LC/MS quad

When the LC/MS quad is used as a detector for the LC, a mass spectrum is associated with each data point in the LC chromatogram. To obtain high quality, accurate mass spectra, the LC/MS quad must be optimized to:

*   Maximize sensitivity
*   Maintain acceptable resolution
*   Ensure accurate mass assignment

Tuning is the process of adjusting LC/MS quad parameters to achieve these goals. After the LC/MS quad parameters have been optimized, they must be saved in a tune file (.tun). This tune file is then specified in the method that is used to acquire data for your samples.

Frequent tuning, automated or manual, is not required. Once tuned, the LC/MS quad is very stable. Tuning should generally be needed no more often than monthly, weekly at most.

Wait ~12 hours after pumpdown before tuning or operating your LC/MS quad. The analyzer takes about 12 hours to reach thermal equilibrium. Tune files that are created or data that is acquired before the LC/MS quad is at thermal equilibrium may have incorrect mass assignments and other inaccuracies.

Task	Instructions	Comments
To use Autotune	1 From the MSD Tune view, select **Autotune** from the **Tune** menu or click the **Autotune** toolbar button. 2 Review the tune report that is printed automatically when tuning is completed.	Use autotune for automated adjustment of the LC/MS quad performance.

Basic Operation

Task	Instructions	Comments
To use Check Tune	1 Select **Check Tune** from the **Tune** menu. 2 Review the Check Tune report. Adjustments are suggested for values that are outside of acceptable ranges.	Use Check Tune to quickly determine whether the LC/MS quad is correctly tuned. It performs a single profile scan of the tune masses and compares the peak widths and mass axes with target values.
To use Manual Tune	1 Select **Manual Tune** from the **Tune** menu. 2 Set the desired mass resolution (adjusting width gain and width offset). 3 Calibrate the mass axis (adjusting mass gain and mass offset). 4 Optimize the ion transmission through the source ion optics (fragmentor, skimmer(s), lens 1, lens 2, octopole peak and octopole knee). 5 Adjust the signal strength (setting iris and adjusting the multiplier gain). Note that fragmentor and gain are method parameters. The fragmentor affects ion transmission and fragmentation. For more information, see the online help.	Use Manual Tune when you want to: • Achieve maximum sensitivity by sacrificing some resolution • Tune specifically for the very low end (<150 amu) of the mass range • Tune with a compound other than the standard calibrants

Acquire LC/MS Data

Modes of Acquisition

There are three modes of acquiring data:

- Running a method for a single sample
- Running a sequence for multiple samples
- Running an FIA series

Note the following about acquiring data:

- All three acquisition modes require an appropriate method.
- Samples may be injected either manually or with an ALS.
- A run must always be started from the software.
- An FIA method cannot be used in a sequence but multiple FIA methods can be run sequentially.

To edit a method and start a run

Once you know the acquisition mode you want to use, you need to set up an appropriate method. Methods are set up in the Method and Run Control view.

Instructions	Comments
1 Select **Method / Load Method** or click the **Open Method** button on the toolbar. Select a method from the list.	

2 Select **Method / Edit Entire Method**. This menu item is also available when you click the method icon on the system diagram.
3 Select the method sections you want to edit (select all sections to become familiar with the method parameters that are available).
4 Add any method comments you want to appear on your reports.
5 Set up the instrument parameters:
  • set up the pump parameters
  • set up the injector parameters
  • set up the DAD (or VWD) parameters
  • set up the column thermostat parameters
  • set up the MS quad signals
  • set up the MS quad spray chamber
6 Set up the Data Analysis parameters:
  • set up the signal details
  • edit integration events
  • specify report parameters
  • select the instrument curves
  • select the calibration curves
  • set up the calibration table
  • find the ion parameters
7 Complete the Run Time Checklist.
	A series of dialog boxes is displayed, which allows you to set up your method and instrument parameters. Click the **Help** button on any of the following dialog boxes for descriptive information on the items available.

8 Save the method using a different name. Select **Method / Save Method As** or click the **Save Method** button on the toolbar.	Once you are familiar with the options that are available, you can use the system diagram menus for quick access to particular method parameters, rather than using the edit entire method process.
9 When you are ready to begin a run, click the **Start** button.	

Use Deconvolution

Deconvolution is a process that transforms mass spectra from multiply-charged ions into a calculated molecular weight.

To deconvolute a mass spectrum

Instructions		Comments
1	In the Data Analysis view, load an electrospray MS data file. The TIC is displayed.	
2	Generate the MS spectrum that you want to deconvolute (select the Spectral task tool set and use the spectrum selection tools to pick the spectrum of interest).	Note that these operations are performed on data acquired in full scan mode.
3	Click the **Enter Deconvolution Tool** button to set up the deconvolution display area.	
4	When the spectrum is displayed, click the **Find Ions** button to locate masses to be used in deconvolution.	
5	Examine the ions that were found. Very noisy data or data with unresolved regions often need special settings to find ions.	You can use the **Find Ions Options** button to optimize the set of ions found.
6	Click the **Edit Deconvolution Parameters** button to change any of the parameters.	
7	Click the **Run Deconvolution** button to begin the deconvolution process.	Once the deconvolution is done, the components are displayed in the upper right window. The charge states are displayed in the lower right window.
8	Select specific components from the Component list to look at individual components or groups of components.	Optionally, click the **Preview Deconvolution Report** button or click the **Print Report** button.
9	Optionally, select components from the Component List box and then click the **Delete Components** button.	This action removes the peaks for the selected components from the original spectrum. You can then continue with step 4 above, using this new spectrum.
10	Repeat the process.	

Deconvolution Report

The Deconvolution report contains summary information about each component selected, along with detailed information on how each peak in a component contributes to the component's molecular weight. In the first part of the report, the components are ranked by percent relative abundance, which is useful for estimating the percent of impurities.

Note that the actual molecular weight may differ from the computed molecular weight even if the data fit a Gaussian curve perfectly, due to other errors such as errors in mass axis assignment or unresolved chemical impurities.

The Deconvolution software is optional and is part of the G2720AA Bioanalysis Software package.

Operating Tips

- Back up your data and methods **regularly** to avoid loss of data if the files are accidentally overwritten, deleted, or if a hardware problem develops with your disk drive.

- Put the system in standby mode overnight or whenever you won't be analyzing samples for an extended time.

- Verify that the tune file you are using is appropriate for your samples.

- Save Tune reports in an MS Logbook for future reference.

- Regular system maintenance can reduce problems. Keep a maintenance record.

- Use the Maintenance Logbook and EMF features in Diagnosis view to help you keep track of when maintenance is needed and to keep an online maintenance record.

- Flush the sample path and clean the spray chamber, capillary tip, and spray shield daily or at the end of each shift. Check the foreline pump fluid level every week.

- The spray chamber vent hose must be connected to a lab vent that is used **only** for the source (completely separate from the vent hose for the foreline pump). Otherwise, waste products can migrate into the spray chamber vent producing chemical noise.

- Samples need to be filtered. They should be salt and detergent-free if no chromatography is used.

- If a UV detector is available, use it in series with the LC/MS quad. Try to minimize chromatographic peak broadening by using low dispersion tubing.

- To avoid chromatographic band broadening, be sure that all tubing connections are free of dead volume. Use zero dead-volume (ZDV) fittings when possible.

- Use finger-tight fittings on the LC inlet union. Swaged fittings can compress the frit.

- If you are using APCI, the optimal flow rate is 1.0 ml/min. The range is 0.5 to 1.5 ml/min.

- Use the following table as a guide to using SIM, condensed scan, and full scan acquisition modes.

Task	Mode
Acquire electrospray data for samples containing large, multiply-charged analytes.	Full Scan
Analyze a mixture with unknown components (small molecules).	Scan
Analyze a mixture with known components in unknown amounts (quantitate).	Scan or SIM
Identify the presence of a few known compounds at low levels within a mixture.	SIM
Use fast chromatography on SL MS quads without missing peaks.	Fast Scan
Use fast chromatography on 6140A SL-HT MS quads without missing peaks.	Ultra Fast Scan

Troubleshooting Tips

No peaks

✔ Verify that there is spray from the nebulizer.

✔ Verify that the capillary voltage is set correctly.

✔ Verify that the LC/MS quad is tuned correctly.

✔ Verify that LC/MS quad pressures are within normal ranges.

✔ Check the drying gas flow and temperature.

✔ Verify that the fragmentor is set correctly.

Poor mass accuracy

✔ Recalibrate the mass axis.

✔ Verify that the ions used for tuning span the mass range of the sample ions and that they show strong, stable signals.

Low signal

✔ Check the solution chemistry. Verify that the solvent you're using is appropriate for your sample. Mixed samples can exhibit signal suppression of one or more components.

✔ Verify that the sample is fresh and has been stored correctly.

✔ Verify that the LC/MS quad is tuned correctly.

✔ Check the nebulizer condition.

✔ Clean the capillary entrance.

✔ Check the capillary for damage and contamination.

Unstable signal

✔ Verify that the drying gas flow and temperature are correct for the solvent flow you are using.

✔ Verify that the solvent is thoroughly degassed. Do **not** use ultrasonic degassing with protein samples.

✔ Verify that the LC backpressure is steady; this indicates a steady solvent flow.

High spectral noise

✔ Use appropriate mass filter values.

✔ Check the spray shape. Nebulizer may be damaged or incorrectly set.

✔ Verify that drying gas flow and temperature are correct for the solvent flow you are using.

✔ Verify that the solvent is thoroughly degassed. Do **not** use ultrasonic degassing with protein samples.

✔ Verify that the LC backpressure is steady; this indicates a steady solvent flow.

✔ If you are using water as part of the mobile phase, verify that it is de-ionized (>18MΩ).

Droplets, not spray, exiting the nebulizer

✔ Verify that the nebulizing gas pressure is set high enough for the LC flow being used.

✔ Check the position of the needle in the nebulizer.

✔ Stop the solvent flow and remove the nebulizer assembly. Use a magnifying glass to examine the end of the nebulizer for damage.

No flow

✔ Verify that the LC is on and there is sufficient solvent in the correct bottle.

✔ Check for LC error messages.

✔ Check for blockages. Repair or replace any blocked components.

✔ Check for leaks.

✔ Verify that the MS stream selector valve is set to **LC to MSD**.

Undesired fragmentation

✔ Fragmentor is set too high.

✔ Ionization is causing fragmentation (APCI vs. Electrospray).

✔ APCI temperature is too high.

Safety Warnings

Intended Purpose

Use of the LC/MS quad in a manner not intended by the manufacturer is prohibited.

Safety Class

The LC/MS quad is a Safety Class 1 instrument and has been designed and tested in accordance with IEC Publication 1010-1, Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use.

Stack Configuration

WARNING | **While it is possible to stack the LC modules on top of the LC/MS quad without damaging it, this arrangement is potentially unstable and dangerous and is not recommended.**

Placing the LC modules on top of the LC/MS quad is also inconvenient. It puts the solvent bottles and some LC controls out of easy reach and requires the LC stack to be disassembled and removed for LC/MS quad maintenance.

WARNING | **Use only Nitrogen as the drying and nebulizer gas. Air, oxygen, or other gases, when combined with solvents and high voltages in the spray chamber, can result in an explosion.**

WARNING | **Do not connect the LC/MS quad to a power source that is not equipped with a protective earth contact. You can create a shock hazard for the operator and can damage the instrument.**

WARNING | **Do not interrupt the protective conductor inside or outside the LC/MS quad or disconnect the protective earth terminal. You can create a shock hazard for the operator and can damage the instrument.**

WARNING   Do not adjust, maintain, or repair the opened instrument while it is connected to a power source. These operations should be performed only by trained persons who are aware of the hazards involved.

WARNING   Before you perform maintenance on the electronics or turbo controllers, turn off the main circuit breaker and disconnect the power cord. The power switch on the front of the LC/MS quad does not completely cut power to the instrument.

Safety Warnings

www.agilent.com

In this book

This book contains:

G1960-90000

∴ Agilent Technologies