Agilent 6400 Series Triple Quad LC/MS System

Quick Start Guide

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Use this guide for your first steps with the Agilent 6400 Series Triple Quad LC/MS, and as a road map for your user information.

What is the Agilent 6400 Series Triple Quad LC/MS system?

The Agilent Triple Quad LC/MS is a liquid chromatograph triple quadrupole mass spectrometer that performs MS/MS using three sets of parallel rods (in this case, quadrupole, hexapole, quadrupole). The first quadrupole separates ions into precursor ions that are fragmented in the hexapole into product ions, which are separated by the second quadrupole. Often, two or more precursor ions and their product ions are monitored in sequence in MRM (multiple reaction monitoring) mode. You can monitor up to 4000 MRM transitions by using Dynamic MRM.

The Agilent 6490 Triple Quadrupole contains the iFunnel Technology which provides more efficient sampling of ions into the mass spectrometer. The iFunnel Technology includes the Agilent Jet Stream Technology, shorter desolvation assembly with Hexabore Capillary, and Dual Offset Ion Funnel.

Agilent Technologies

The Agilent 6490 and 6460 Triple Quad LC/MS are shipped by default with the Agilent Jet Stream Technology that utilizes a super-heated sheath gas to collimate the nebulizer spray which dramatically increases the number of ions that enter the mass spectrometer.

You can set up an Agilent Triple Quad LC/MS with the Agilent 1100, 1200, 1260 or 1290 Infinity LC modules.

Also, the Agilent Triple Quad LC/MS comes with Agilent MassHunter Workstation Software that includes three major programs:

- Data Acquisition – From one screen you can tune the mass spectrometer, control and monitor instrument parameters, set up acquisition methods and worklists containing multiple samples and monitor real-time run plots.
- Quantitative Analysis – From one screen you can set up a batch of data files and quantify, evaluate and requantify the results. From this screen you have access to the Method Editor for setting up and editing the quantitation methods.
- Qualitative Analysis – From one screen you can extract and integrate chromatograms, subtract background, extract peak spectra, and compare data from different types of data files.

What's New in Data Acquisition

The Data Acquisition program for the 6400 Series Triple Quadrupole has many new features in this revision.

in B.04.01

- Supported models are 6410B, 6430A, 6460A, and 6490A.
- Windows 7 is supported.
- Excel 2010 is supported for Optimizer reports in Windows 7.
- Reports for the Acquisition Method, the Worklist and Tune are printed using a different tool called RDL.
- Positive/Negative polarity switching is available for Dynamic MRM.

in B.04.00

- Supported models are 6410B, 6430A, 6460A, and 6490A.
- The iFunnel technology is supported on the Agilent 6490A Triple Quadrupole.
- Optimizer B.04.00 is included.
- Enhanced resolution of 0.4 m/z FWHM is supported on the Agilent 6490A Triple Quadrupole.

- "Fast scan" mode with scan speed of 10000 m/z per second is supported on the Agilent 6490A Triple Quadrupole.
- Triggered MRM is supported on all supported Triple Quadrupoles.
- Generic ion source support is included.
- Collision cell accelerator voltage can be specified as a method parameter for each transition to optimize the signal.
- Dynamic MRM methods can be automatically split into 2 or more methods.
- Dynamic MRM methods can be combined into one method.
- The Study Manager program can create Drug Discovery Screening studies and Optimization Automation studies.
- The Bioanalysis Study, renamed from the PK LIMS Study, is supported.
- The Map File Generator program supports Worklists, Drug Discovery Screening and Optimization Automation.
- The Method Comparator is now available.
- Method Audit Trail in Compliance mode and comparison of method versions is available when compliance is enabled.
- The worklist contains the Sample Group and Sample Info. columns that are displayed in Quant.
- Barcodes can be specified for plates in a worklist.
- Locked mode runs are supported in Study Manager.
- A standby script can be specified in Study Manager.
- Checksum for studies is supported.

in B.03.01 with Service Pack 2

- Needle Seat Back Flush using G4227A Flexible Cube is supported.
- The Carryover Reduction feature for the 1260 Chip Cube is supported.
- The Agilent 1260 Isocratic Pump (G1310B) is supported.
- The Agilent 1260 Quaternary Pump (G1311B) is supported.
- The Agilent 1260 Quaternary Pump VL (G1311C) is supported.
- The Agilent 1260 Capillary Pump (G1376A) is supported.
- The Agilent 1260 Nano Pump (G2226A) is supported.
- The Agilent 1260 Binary Pump (G1312B) is supported.
- The Agilent 1260 Binary Pump VL (G1312C) is supported.
- The Agilent 1290 Binary Pump VL (G4220B) is supported.
- The Agilent 1260 Standard Autosampler (G1329B) is supported.

- The Agilent 1260 High Performance Autosampler (G1367E) is supported.
- The Agilent 1260 Micro High Performance Autosampler (G1377A) is supported.
- The Agilent 1290 LC Injector HTC/HTS (G4277A) is supported.
- The Agilent 1290 LC Injector HTC/HTS (G4278A) is supported.
- The Agilent 1260 Variable Wavelength Detector VL (G1314B) is supported.
- The Agilent 1260 Variable Wavelength Detector SL (G1314C) is supported.
- The Agilent 1260 Variable Wavelength Detector (G1314F) is supported.
- The Agilent 1290 Variable Wavelength Detector (G1314E) is supported.
- The Agilent 1260 Diode Array Detector SL (G1315C) is supported.
- The Agilent 1260 Diode Array Detector VL (G1315D) is supported.
- The Agilent 1260 Diode Array Detector (G4212B) is supported.
- The Agilent 1260 TCC (Thermostatted Column Compartment) (G1316A) is supported.

in B.03.01

- The Study Manager program is supported. You can create a study from a worklist or from a PK LIMS input file.
- Dynamic MRM now supports multiple time segments.
- You can update a dynamic MRM method using a data file or a Quantitative Analysis report results.
- You can view a dynamic MRM method in the Dynamic MRM Viewer window.
- The Map File Generator program is supported. This program allows you to modify a map file which is used to map columns in an import file to the column names in a worklist.
- The Agilent 1290 Infinity high performance Auto Sampler (G4226A) is supported.
- The Agilent 1290 Infinity Binary Pump (G4220A) is supported.
- The Agilent 1290 Infinity DAD (G4212A) is supported.
- The Agilent 1290 Infinity TCC (G1316C) is supported.
- Capillary voltage can be changed during a run for HPLC-Chip Cube systems.

in B.02.01 with Service Pack 1

- The Agilent 1290 Infinity high performance Auto Sampler (G4226A) is supported.
- The Agilent 1290 Infinity Binary Pump (G4220A) is supported.

- The Agilent 1290 Infinity DAD (G4212A) is supported.
- The Agilent 1290 Infinity TCC (G1316C) is supported.
- Peak width information is shown in Dynamic MRM user interface.
- The Dynamic MRM phase II database is supported.

in B.02.01

- The Agilent 6410A Triple Quadrupole LC/MS is supported.
- The Agilent 6410A 2K Triple Quadrupole LC/MS is supported.
- The Agilent 6410B Triple Quadrupole LC/MS is supported.
- The Agilent 6430A Triple Quadrupole LC/MS is supported.
- The Agilent 6460A Triple Quadrupole LC/MS is supported.
- The mass range is 5 to 2250 amu for the Agilent 6430 Triple Quadrupole LC/MS.
- The Agilent 6460A Triple Quadrupole LC/MS is still supported (see below).
- Dynamic MRM is supported. Dynamic MRM allows you to add up to 4000 transitions in a single time segment with up to 200 transitions overlapping at a time.
- Version B.02.00 with Patch 3 of the Agilent MassHunter Workstation Software - Qualitative Analysis program is supported.
- Version B.03.01 of the Agilent MassHunter Workstation Software - Quantitative Analysis program is supported.
- Fast polarity switching with 30 ms polarity switching time is supported for the 6430A and 6460A.
- Fast polarity switching with 500 ms polarity switching time is supported for the 6410B.
- When acquiring data on the 6410B, 6430A, or 6460A, no correction to the baseline for dark current or electronic noise is done. Thus, this noise is included in the data file.

in B.02.00

- Only the 6460A Triple Quadrupole is supported on this revision.
- The Agilent Jet Stream Technology is supported on the Agilent 6460 Triple Quadrupole LC/MS.
- The mass range is extended to 5 to 3000 amu for the Agilent 6460 Triple Quadrupole LC/MS. No tune masses are above 2600 amu.
- The mass range is 5 to 1650 for the Agilent 6410A Triple Quadrupole. The mass range is 5 to 2000 for the Agilent 6410B Triple Quadrupole.

- You can acquire up to 500 MRMs per time segment.
- You can acquire more than 150 MRMs per second.
- Fast positive/negative polarity switching is supported (100 ms switching delay).
- Agilent VWD Models D and E are supported.
- Gradient Delay Reduction is supported on the HPLC-Chip.
- The Agilent 1367D high performance Autosampler SL Plus is supported.
- Any language can be used to create scripts.
- A worklist can be inserted into the current worklist.
- A custom icon is used to indicate a MassHunter data file. The ".d Icon Changer" program is available to change the icon for data files that are already acquired.
- Logbook notifications can be displayed in the taskbar in the form of balloon messages.
- The amount of free disk space is displayed in the status bar of the Data Acquisition window.
- DA Reprocessing program is available to allow reprocessing of data files using the Qualitative Analysis program.

For information on what is new in the Qualitative Analysis and Quantitative Analysis programs, refer to the *Familiarization Guide* for each of these programs.

Where to find information

Help

Press F1 To get more information about a pane, window or dialog box, place the cursor on the part of the pane, window or dialog box of interest and press **F1**.

Help menu From the Help menu, access “How-to” help and reference help.

Documents

You can find these manuals delivered with the Triple Quad hardware or software. You can also find a PDF version on the installation disk, in the **Manuals** folder. Many of these manuals are also available on the www.chem.agilent.com web site.

Installation Guide This guide is used to install the hardware and software, configure the instrument, and verify performance.

Upgrade Guide This guide is used to upgrade your MassHunter Workstation software from a previous version.

Concepts Guide - The Big Picture Learn the background information to help you understand operation of the hardware and software.

Maintenance Guide Use this guide to help maintain and troubleshoot your Agilent Triple Quad LC/MS.

Data Acquisition for 6400 Series Triple Quad Familiarization Guide Do the exercises to learn to use the Triple Quad LC/MS hardware and Data Acquisition program for 6400 Series Triple Quad.

Study Manager Quick Start Guide Use this guide to learn to use the MassHunter Study Manager software.

MassHunter Optimizer Software Quick Start Guide Use this guide to learn about the MassHunter Optimizer program. The MassHunter Optimizer program provides a way to automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) on a Triple Quad instrument for each individual compound analyzed. *(This guide is on the MassHunter Optimizer disk.)*

MassHunter QQQ Compliance Quick Start Guide Use this guide to learn about the MassHunter QQQ Compliance program.

MassHunter Quant Compliance Quick Start Guide Use this guide to learn about the MassHunter Quant Compliance program. *(This guide is on the Quantitative Analysis disk.)*

Qualitative Analysis Familiarization Guide Do the exercises to learn to use the Qualitative Analysis program. *(This guide is on the Qualitative Analysis disk.)*

Quantitative Analysis Familiarization Guide Do the exercises to learn to use the Quantitative Analysis program. *(This guide is on the Quantitative Analysis disk.)*

Training

Familiarization Guide Use all three familiarization guides to get to know the software.

Quick Start Guide Use the quick start guides for Study Manager, Optimizer and Compliance to get to know these programs.

Report Training DVD Use the videos and familiarization exercises to learn how to print reports and customize reports using the Report Designer Add-in and Excel.

Training Courses Visit www.chem.agilent.com to view a listing of training courses for the Agilent Triple Quad LC/MS.

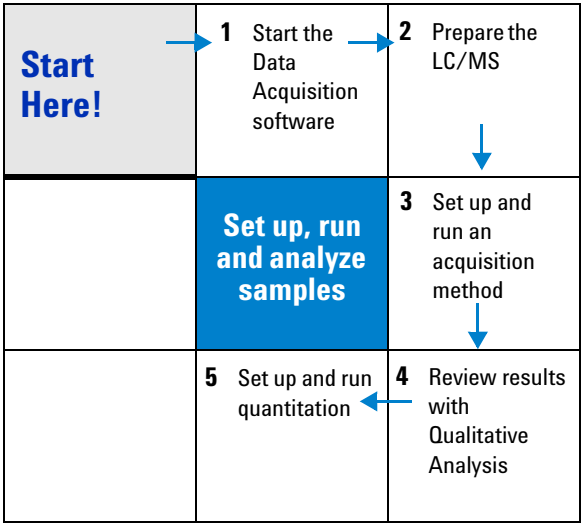
Getting Started

Install the Triple Quadrupole LC/MS hardware and software

Use the *Installation Guide* to install the hardware and software and verify performance.

Set up, run and analyze samples

The roadmap below shows you the steps to set up and run a batch of samples from start to finish. Follow the instructions on the next pages to get started and to learn where to find the information to help you with each step in this roadmap.



Step 1. Start the Data Acquisition software

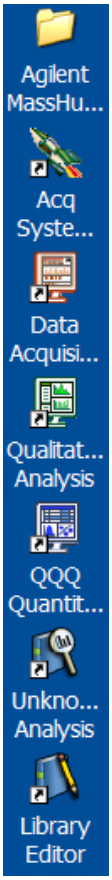
The instructions below include the following assumptions:

- The hardware and software are installed.
- The instrument is configured.

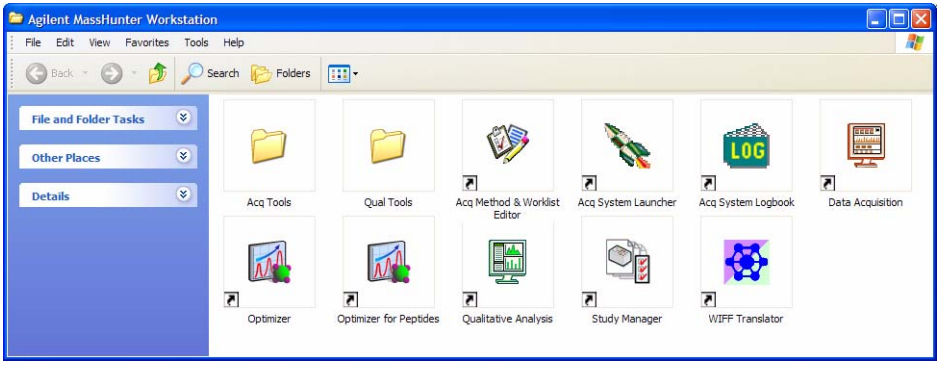
  Use instructions in the *Installation Guide* to configure the instrument for the first time and any time you change the LC configuration.

The LC modules and the Triple Quad MS are turned on, but the LC pump is not running.

After installation, you see all of the Agilent MassHunter Workstation Software icons on your Desktop.To start the Data Acquisition program, double-click the **Data Acquisition** icon.



The Data Acquisition window appears.



NOTE

When Data Acquisition opens, the software engines automatically start. If for any reason, they are stopped and you need to restart them, double-click the **Acq System Launcher** icon on your Desktop, and click **Start**.

If you have recently changed LC modules, remember to configure the instrument again. See the *Installation Guide* for instructions.

Four panes—where you do most of your work

When you first start the Data Acquisition program, the main window appears. You do almost all of your work within the five panes of this main window. These panes provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status, monitor runs and tune the instrument.

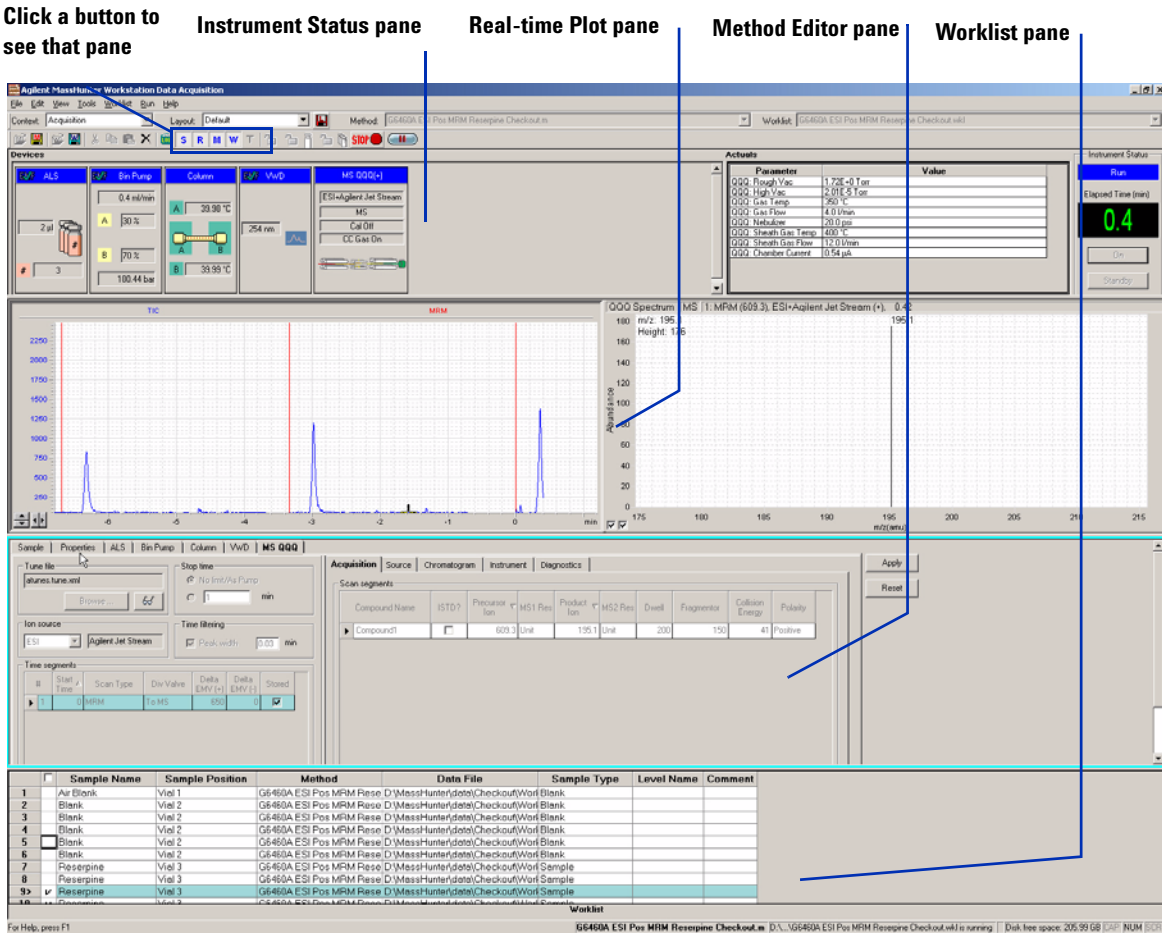


Figure 1 Main window of the Data Acquisition software program

Show/hide the panes You can show one pane at a time on the screen or up to four panes. You can never hide all four panes. To show or hide a pane, you click the icons in the main window toolbar or you use the commands in the **View** menu.

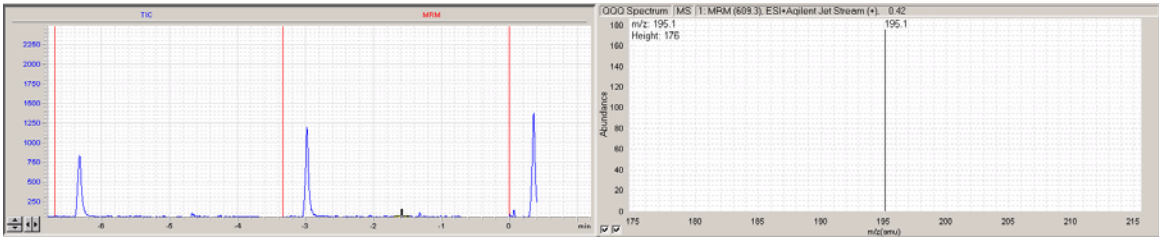
When you click on a pane, the active pane is outlined in blue. Press **F1** to obtain help on the active pane. You can also drag a pane border to resize the pane.

S–Instrument Status; R–Real-time Plot
M–Method Editor; W–Worklist; T-Tune

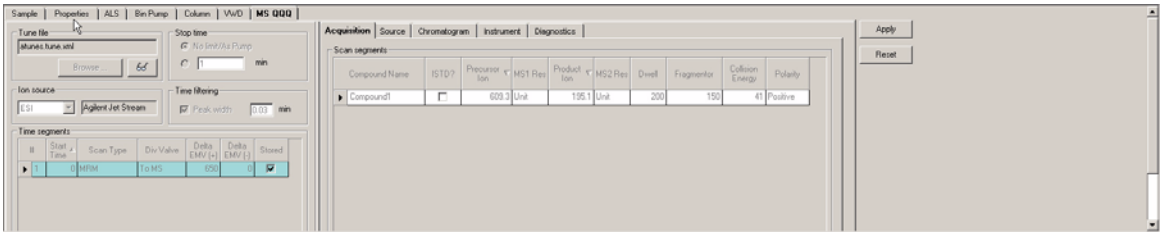
Instrument Status pane You may have several different LC modules in your LC stack, for example, both a well-plate sampler and micro well-plate sampler. With this pane you can make sure that the correct LC module is configured.

You also set non-method control and configuration parameters for the LC devices and MS device and monitor the status of the device parameters during a run.

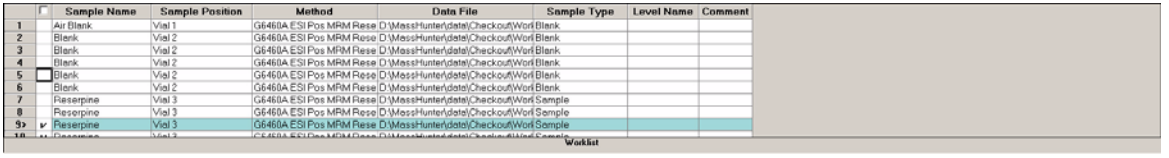
Real-time Plot pane With this pane you monitor the chromatogram and spectral plots in real time.



Method Editor pane With this pane you enter instrument settings for acquisition methods and sample information to run individual samples interactively.



Worklist pane With this pane you enter sample information for individual samples and information for worklist samples. When you run the worklist, the samples are automatically run in the order listed in the worklist.



Step 2. Prepare the LC modules

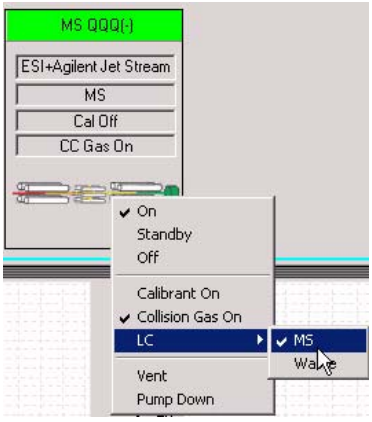
Read and follow the instructions in the online Help for each of the tasks in the checklist described on the following pages.

1 Switch **LC** stream to **Waste**.

While you condition or equilibrate the column, you can tune the Triple Quad MS. During this time you do not want pump effluent streaming into the Triple Quad MS, so you switch the direction of the LC stream away from the MS ion source and to waste.

If you have the LC connected to a VWD or DAD, you can still monitor the fluctuations of the VWD or DAD real-time chromatogram before a run.

a Right-click the **MS QQQ** device in the Instrument Status pane.



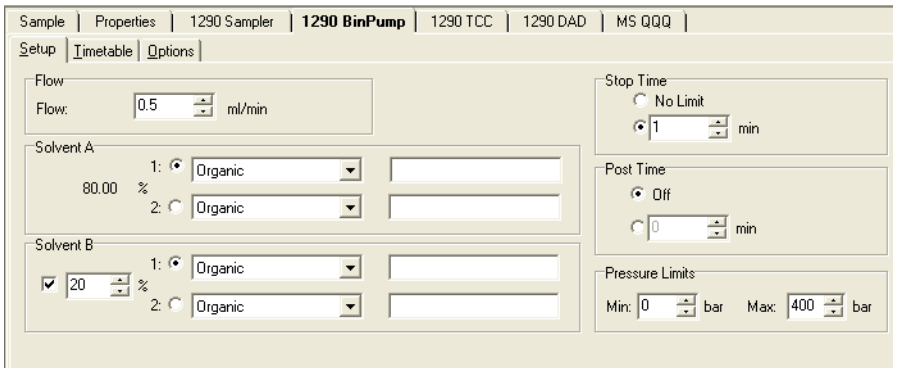
b Click **LC > Waste**.

2 Purge the LC pump.

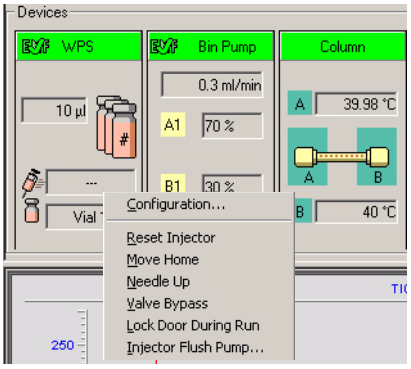
Follow the directions for purging the pump in the *User Guide* for your pump.

3 Set up to condition or equilibrate the column.

a Type LC parameters, and click **Apply** to download them to the LC.



b Right-click an LC module in the Instrument Status pane to change any non-method control parameters, if necessary.

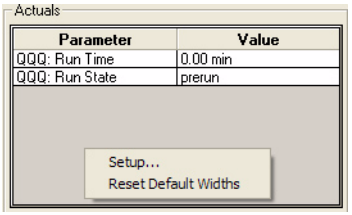


c Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See step 4 and step 5 on page 16.)

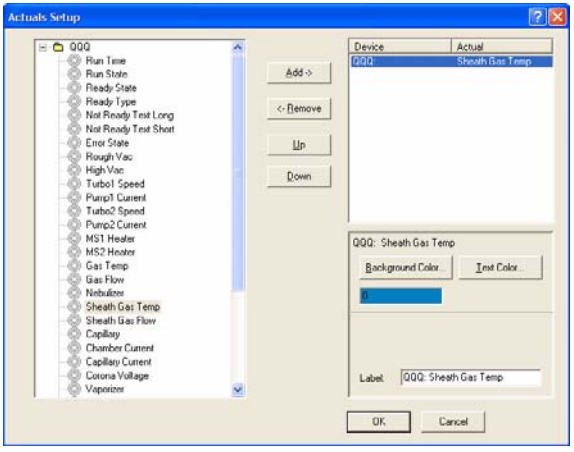
4 Set up to view real-time parameter values (actuals).

As you prepare for a run and during a run, you want to see the actual values of the instrument parameters. You can do this in the Instrument Status pane.

a Right-click the **Actuals** list to see the Setup command.



b Click **Setup** to bring up the list of Actuals available for monitoring.

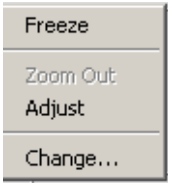


c Add all the parameter values you intend to monitor, and click **OK**.

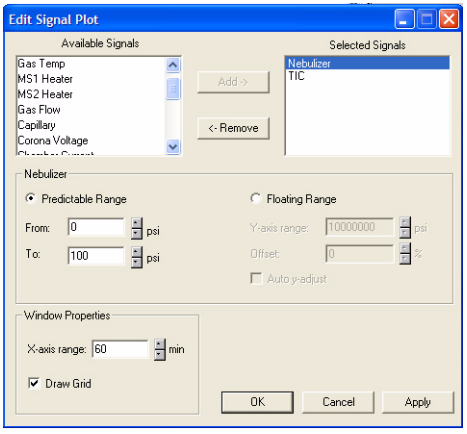
5 Set up real-time plot displays.

As you condition the column, you set up the displays to monitor the effluent.

- Right-click the chromatogram plot, and click **Change**.



In the Edit Signal Plot dialog box, you can select the type of display signal.

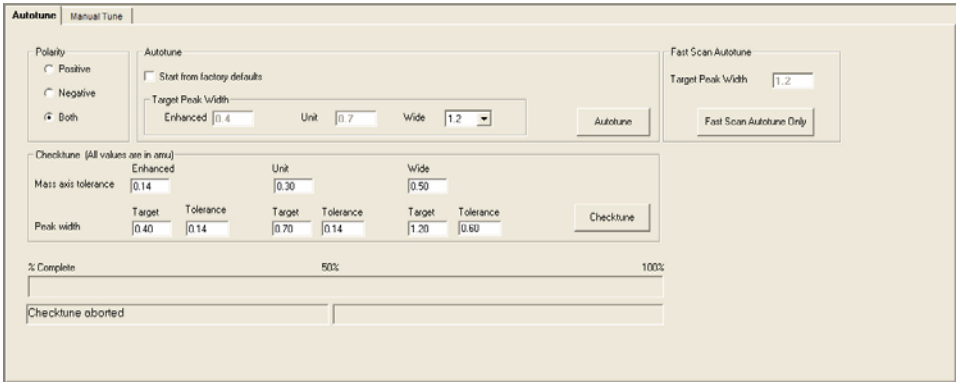


Step 3. Prepare the Triple Quad instrument

Do a Checktune, Autotune or Manual Tune

1 From the **Context** list, click **Tune**.

You can see the Instrument Status pane, the Real-time Plot pane and the Tune pane when you switch to the Tune context. Click the **Tune** icon in the toolbar if the Tune pane is not visible.



2 Click **Checktune** to evaluate if the MS parameters are within the limits to produce the specified mass accuracy and resolution. Checktune takes up to 15 minutes to run.

If the current tune file was last tuned with the Fast Scan Autotune, then the Checktune algorithm only checks MS2. If the current tune file was last tuned with Autotune, then the Checktune algorithm checks both MS1 and MS2.

If the current tune file was last tuned with the Fast Scan Autotune, then the **Fast Scan** check box is marked on the Manual Tune tab.

Do a **Checktune** regularly.

If **Checktune** passes, then skip to step 4.

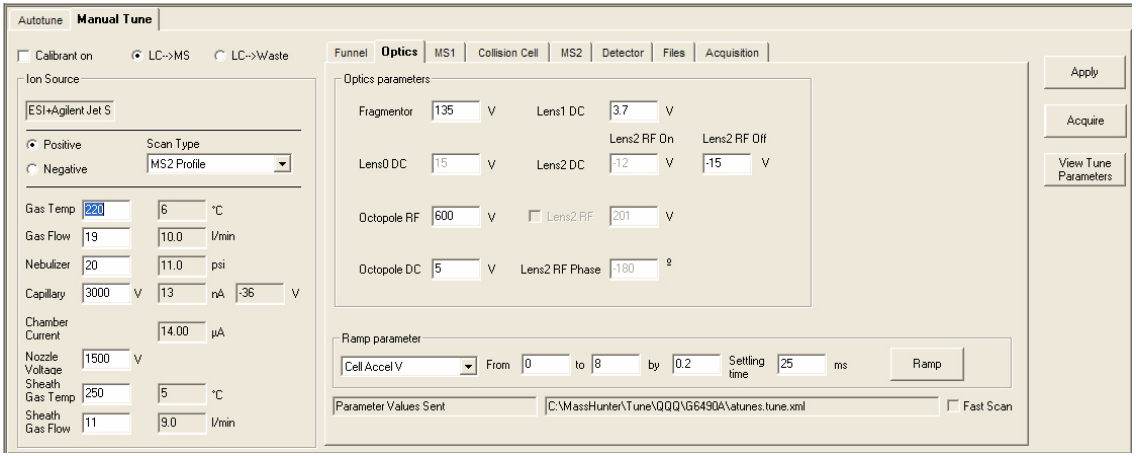
If **Checktune** fails, then you need to do an Autotune, which is described next.

3 Click **Autotune** to tune the MS automatically (15 to 20 minutes for all models except the 6490. The 6490 takes approximately 45 minutes per ionization mode.). The system automatically changes different tune parameters to tune the MS. You only do an Autotune when it is necessary.

You only mark the **Start from Factory Defaults** check box after an instrument shutdown and restart. Then, if you click **Autotune**, the instrument is tuned starting from the factory defaults (20 to 25 minutes for all models except the 6490. The 6490 takes approximately 45 minutes per ionization mode.).

A report is automatically generated after Autotune completes successfully. If Autotune fails, no report is printed.

If Autotune fails or you assess that the Triple Quadrupole MS needs custom values entered for its tune parameters, you can manually tune the instrument.



4 (optional) Click the **Fast Scan Autotune Only** button if you want to tune for Fast Scan. You only do this step if the Autotune results are acceptable.

Fast Scan acquisition is only supported on the 6490 with two of the scan types: **MS2 Scan** and **Product Ion** scan. Fast Scan autotune only tunes with the **Wide** (1.2) resolution, **Samples** set to 1 (on the Manual Tune > Acquisition Tab), and the step size must be 0.2. The scan time in the Acquisition tab is generated automatically based on the step size and the mass range.

5 From the **Context** list, click **Acquisition**.

Switch LC stream to MS

- After you condition the column and tune the Triple Quad MS, you switch the LC stream from Waste to MS. See "Switch LC stream to Waste." on page 14 for how to do this.

Monitor MS baseline and spectral displays

If you did not monitor the LC baseline with a VWD or DAD, make sure that the Triple Quadrupole baseline is stable and no spectra of interfering intensity appear in the display.

If you did monitor the LC baseline with a VWD or DAD, change back to the default Triple Quad displays.

1. Right-click the chromatogram plot, and click **Change**.
2. Select the MS signal, and click **OK**.

View the system logbook for events and errors

As you prepare the instrument, you may run into an error that you want to troubleshoot. You do this through the System Logbook Viewer.

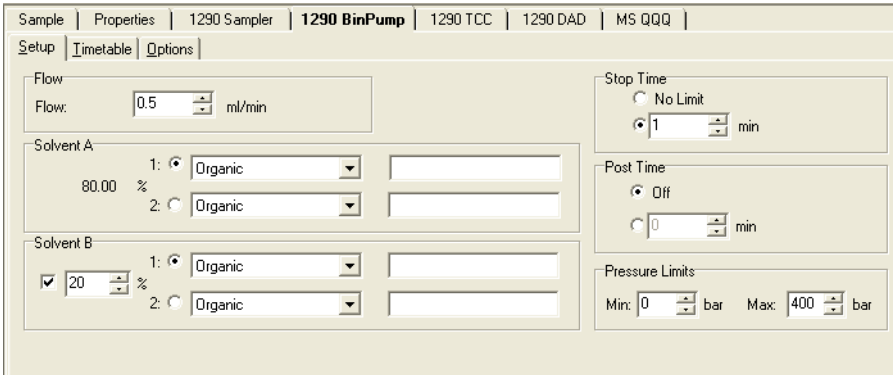
- Click **Tools > System Logbook Viewer** or click the button in the toolbar of the Data Acquisition window, and view the logged events. You can also enable automatic notification when an error or warning occurs. Right-click the button in the Quick Start tray and click **Configure**. If notification is not enabled, you also need to right-click the button and click **Enable Notification**.

Step 4. Set up and run an acquisition method

Read and follow the instructions in the online Help for each of the tasks described on the following pages.

Also, do Exercise 1 of the Data Acquisition *Familiarization Guide* to learn how to set up and run an acquisition method.

1 Set up the method:
   a Type the values and settings for each of the tabs below.
   b (optional) If you want to download the settings to the instrument, click **Apply**.
   c To save the method, click **File > Save As > Method**.
   d Name the method and click **OK**.
2 Enter values for all of the LC modules configured for the instrument.

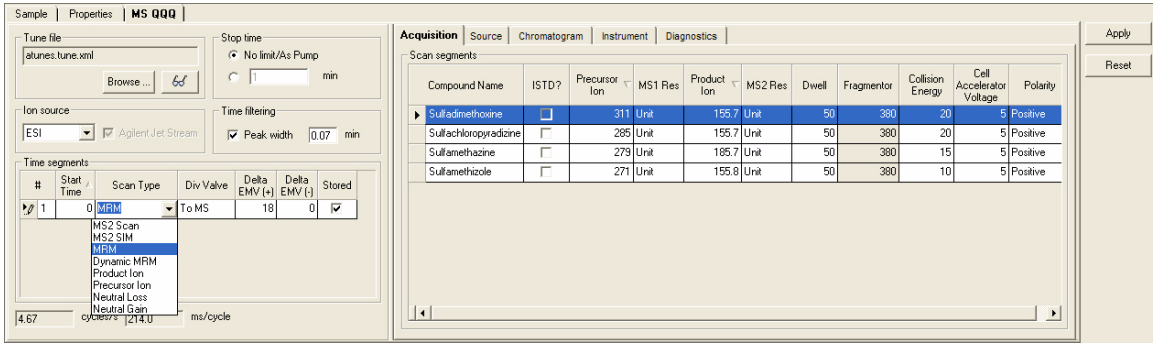


NOTE Make sure when you type the MS parameters on the next page that the tune file is the one that you want to use with the acquisition.

3 Enter the MS Triple Quad parameter values.
   a Select the **Scan Type** from the list.

      The parameters available on the right change depending on the **Scan Type**.
   b Type in any **Acquisition** values you want to change. You can enter multiple Scan segments.

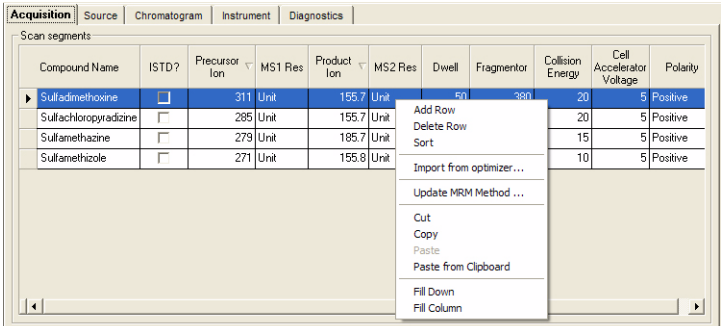
   You cannot set the fragmentor voltage in Acquisition if the instrument type is an Agilent 6490. It always uses the value in the tune file.



4 Set up to change Triple Quad MS parameters with segments and scans:

a To add a segment, right-click anywhere in the **Scan segments** section to bring up the Scan Segments menu, and click **Add Row**.

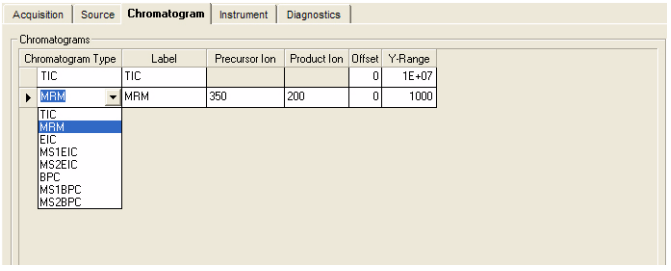
b Type the parameters for each Scan segment.



5 Set up signals for the real-time plot:

a Click the **Chromatogram** tab.

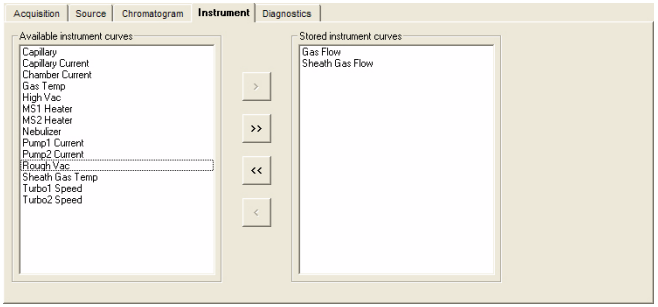
b Select the **Chromatogram Type**, and type other plot values.



6 Set up MS Actuals in the Instrument tab. In the Qualitative Analysis program, you can display values in the MS Actuals window for the selected spectrum. With the Triple Quadrupole, the values in the MS Actuals window are the values that you save in the Instrument tab.

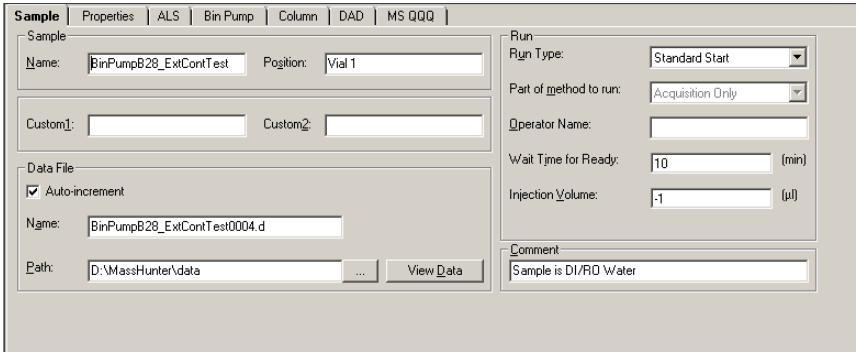
a Click the **Instrument** tab.

b Select the **Stored instrument curves**. These values will be shown in the MS Actuals window in the Qualitative Analysis program for each spectrum.



7 Set up and run interactive samples:

a Type the sample **Name**, data file **Name** and pathway, and method name.

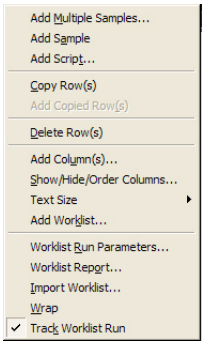


b To start the run, click the single sample button, .

You can run the single sample in either locked or unlocked mode. When the mode is locked, no one can change the method or sample parameters during the run.

8 Set up and run worklists

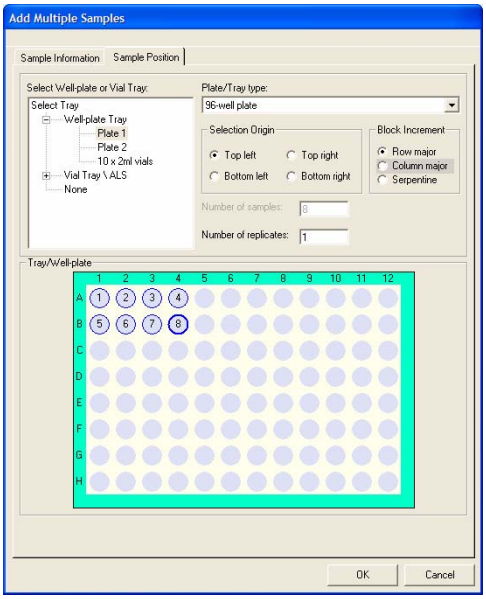
a Right-click the upper left corner of the worklist.



b Click **Add Multiple Samples.** The Add Multiple Samples dialog box opens.

c Enter all the information on the Sample Information tab.

d Click the **Sample Position** tab to specify the sample vial locations (make sure the specific sample tray type has been configured by right-clicking the autosampler device image).



e Specify the locations, and click **OK**.

f To set up the worklist run, right-click the upper left corner, and click **Worklist Run Parameters**.

g Click the **Page 1** tab.

h Type the paths for the method and data files.

i Click the **Page 2** tab.

j Review the information and click **OK**.

k To start the run, click the **Run Worklist** button, .

You can run the worklist in either locked or unlocked mode. When the mode is locked, no one can change the method or the worklist while the worklist is running.

Step 5. Review results with the Qualitative Analysis program

Use the Qualitative Analysis program to do these tasks and more:

- Review results for acquisition method development
- Select the most appropriate precursor and product ions for MRM analyses
- Find compounds
- Identify compounds
- Do molecular feature extraction

Do the exercises in the ***Qualitative Analysis Familiarization Guide*** to help you learn how to use the Qualitative Analysis program.

Do Exercise 1 of the Data Acquisition for Triple Quad ***Familiarization Guide*** to help you learn how to use the Qualitative Analysis program to develop acquisition methods.

Also, refer to online Help to learn how to do more operations to analyze your data.

Step 6. Set up and run quantitation

The primary tool for analyzing and reporting Triple Quad LC/MS results is the Quantitative Analysis program.

- Do the exercises in the ***Quantitative Analysis Familiarization Guide*** to learn how to do these operations to quantitate the acquired data files:
  - Set up a batch and a method to automatically quantitate a set of samples
  - Review results by learning how to view and use the Batch-at-a-Glance results screen
  - Identify and use outliers to change the method and requantitate the data using a better calibration curve fit or other more appropriate settings

Also, refer to online Help to learn how to do more operations to analyze your data.

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In this book

This book contains brief instructions to help you get started with your Agilent Triple Quad LC/MS. This book takes a quick look at using the Data Acquisition program to:

- Prepare the instrument for a run.
- Set up acquisition methods.
- Set up and run worklists.

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