Agilent 6400 Series Triple Quadrupole LC/MS System

Quick Start Guide

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 Quadrupole 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.06.00

- The supported models are 6410B, 6420A, 6430A, 6460A, and 6490A.
- Fast Scan is supported on all models.
- The Agilent G7100A Capillary Electrophoresis system is supported.
- The study types Worklist-Only and Worklist Import both support creating a Quantitative Analysis method.
- The Autotune program has the ability to tune only the unit mass.
- The Autotune program can report *m/z* below 100 for positive and negative autotunes.
- You can adjust the EMV separately in the Manual Tune > Detector tab.
- The Tune report contains the instrument serial number and the firmware revision.
- Triggered MRM databases are supported in Optimizer.
- Additional options for TMRM and compound naming are supported when searching, filtering and importing to an acquisition method.

- The Source and iFunnel Optimizer program is available to help optimize some source and ion funnel parameters.
- In Triggered MRM you can select two different triggers for each compound.
- In SIM, MRM, and DMRM methods you can specify a Compound Group.
- You can specify a trigger entrance delay, a trigger time delay and a trigger window to allow you to acquire triggered scans closer to the apex of the peak.
- Polarity switching is supported for TMRM. Different compounds can have different polarities.
- If the method is a Triggered MRM method, then you can enable or disable triggering for all compounds in a method for a specified sample type. For example, if the Sample Type is Calibration, all of the primary transitions are acquired, but the secondary transitions are never triggered.
- You can update the threshold value in a method using a Dynamic MRM or Triggered MRM data file.
- When you switch to Triggered Dynamic MRM, you can automatically set the default trigger to the transition with the highest m/z (the product ion).
- When you switch from a Triggered Dynamic MRM run to a Dynamic MRM run, the secondary MRMs are grayed out and are not acquired during the run.
- The Update MRM tool can also fill in the Trigger Window values from a data file or a Quantitation report. A variety of options are available.
- You can create an MS Indexed data file which supports faster operation in the Quantitative Analysis program.
- The user interface of the Instrument Status window has been updated.
- The user interface of the DMRM Viewer has been updated.
- The DMRM Viewer supports tMRM methods.
- The DMRM Viewer has added the ability to filter on primaries only or on all transitions.
- The DMRM Viewer has added Compound and Compound Group navigation.
- In the Study Manager, the Worklist-Only study and the Worklist Import study include a page to enable quantitation and report generation.
- You can select a post-analysis script that will run after every method in a worklist; it runs after the Data Analysis portion of the method is complete.
- The 1290 Quaternary Pump (G4204A) is supported.
- The FlexCube (G4227A) is supported in standalone mode.
- UIB 2 (G1390B) is supported.

For a list of updates made to previous versions, refer to the online Help.

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.

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

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

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.

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.)*

For information on what is new in the Qualitative Analysis program, refer to the *Qualitative Analysis Familiarization Guide*.

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For information on what is new in the Quantitative Analysis program, refer to the *Quantitative Analysis Familiarization Guide*.

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.

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

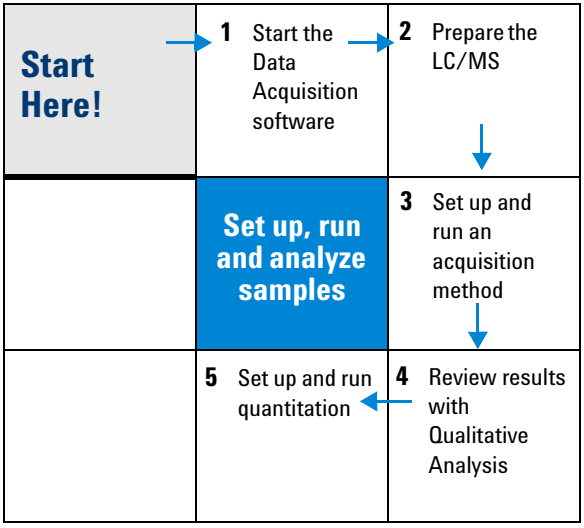
Getting Started

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:

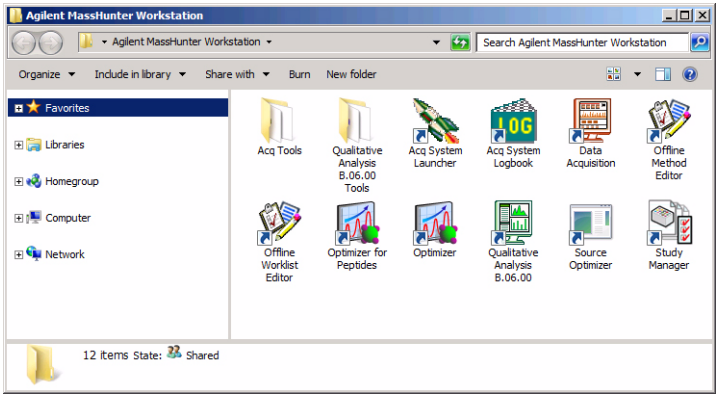
- The hardware and software are installed.
- The instrument is configured.

  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.



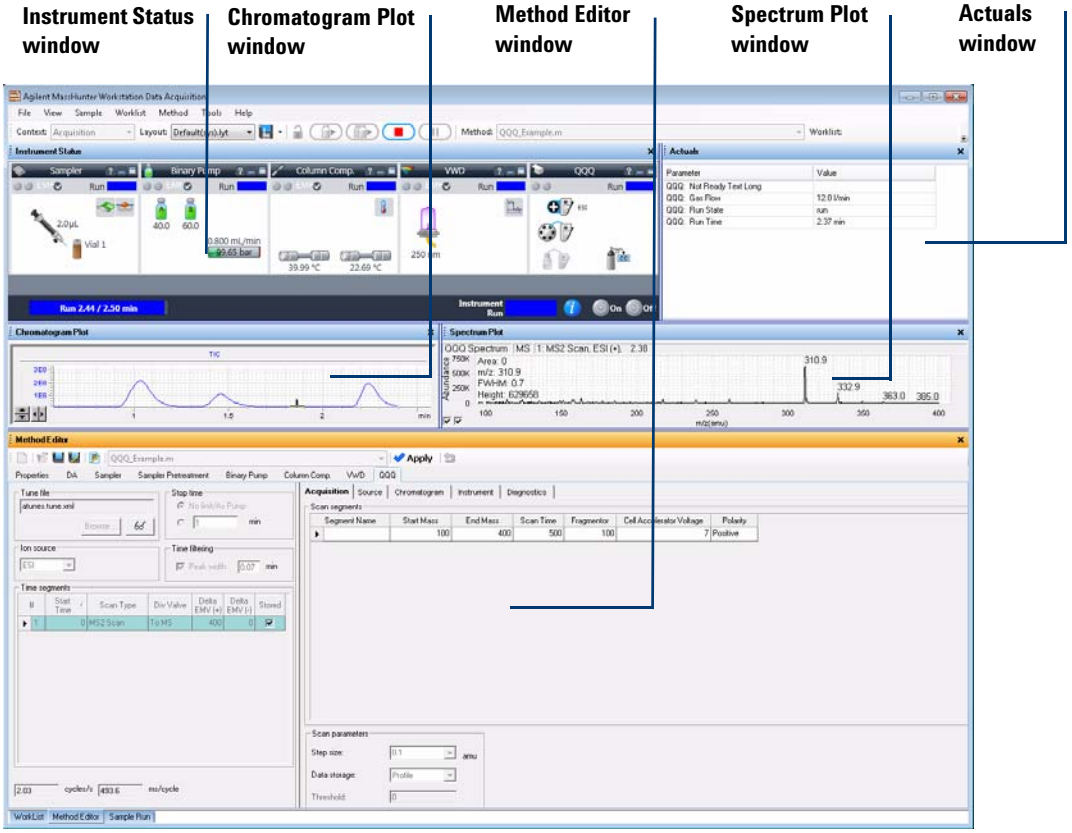
NOTE

When Data Acquisition opens, the software engines automatically start. If you need to restart them, right-click the **Acq System Launcher** icon in the system tray, and click **Start Engines**.

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

Windows—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 eight windows of this main window. These windows provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status, monitor runs and tune the instrument.



The Sample Run and Worklist windows are tabbed here. These three windows are "sharing" this space. You click the tab to switch to a different window.

Figure 1 Main window of the Data Acquisition software program

Show/hide the windows You can show one window at a time on the screen or up to seven windows. You can never hide all of the windows. To show or hide a window, you click the commands in the **View** menu. You can also hide a window by clicking the X icon in the upper right corner of the window.

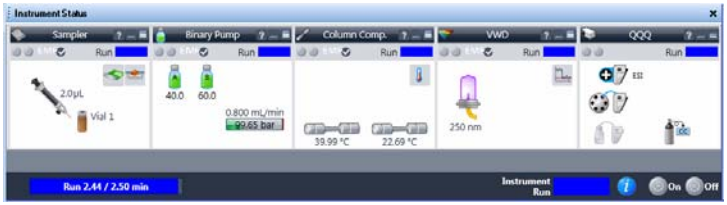
When you click a window, the title of the active window changes to a different color. Press **F1** to obtain help on the active window. You can also drag a window border to resize the window. If you double-click the title of the window, the window "floats" outside of the main window. You can double-click the title bar again to "dock" the window. You can also float and dock the window by right-clicking the title of the window and clicking **Floating**.

Instrument Status window With this window you view the status of each device configured with the instrument- **Error**, **Not ready**, **Pre-run**, **Post-run**, **Running**, **Injecting**, **Idle**, **Offline**, or **Standby**. You also set non-method control and configuration parameters for the LC devices and the MS instrument.

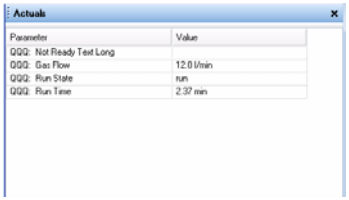
This window displays each device's current status both as text and by its color-coding:

Color Coding in the Instrument Status Window

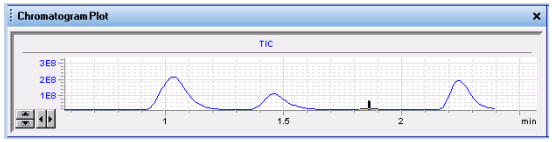
Color	Status
Red	Error
Yellow	Not ready
Purple	Pre-run, post-run, Waiting
Blue	Running, injecting
Green	Idle
Dark gray	Offline
Light gray	Standby (for example, lamps off)



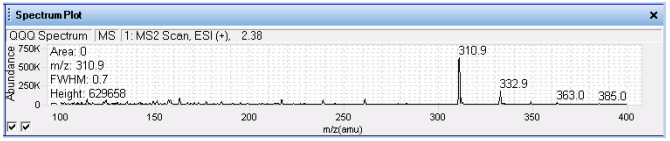
Actuals window With this window you view the current value of selected instrument parameters.



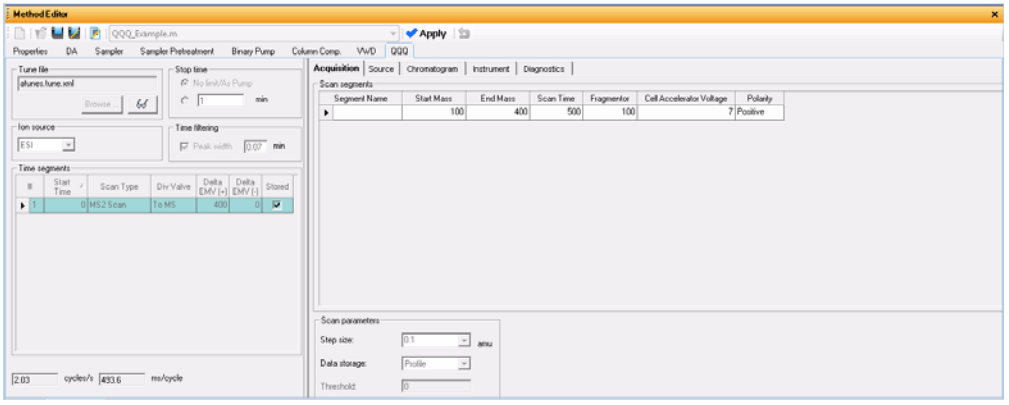
Chromatogram Plot window With this window you monitor the chromatogram plots in real time. These plots can be user-defined signals and/or instrument parameters.



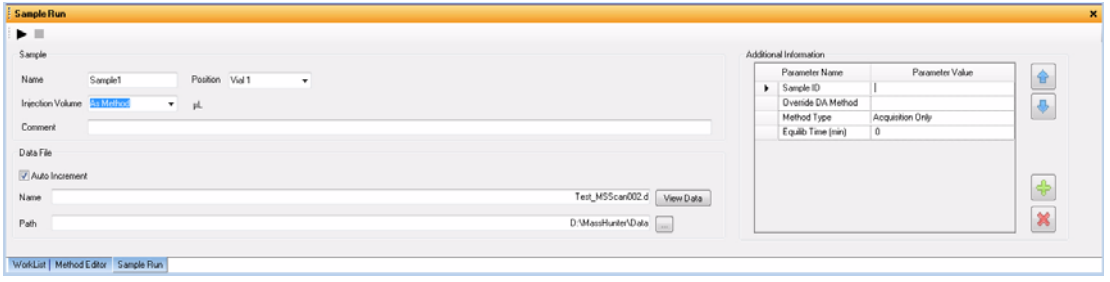
Spectrum Plot window With this window you monitor the spectral plot in real time.



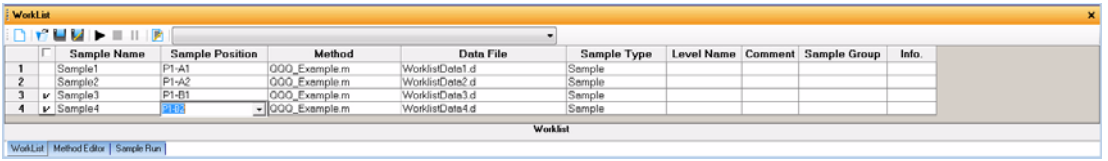
Method Editor window With this window you enter acquisition parameters for the method. If you click the button, then you can see the tune values in the Tune Parameters dialog box.



Sample Run window With this window you enter sample information to run individual samples interactively, and you can start a single sample run. You can also specify an Override DA Method and select either **Both Acquisition and DA** or **DA Only** for the **Method Type**, and then Data Analysis is run as part of the method.



Worklist window With this window you enter sample information for multiple samples. When you run the worklist, the samples are automatically run in the order listed in the worklist. You can select whether to run Acquisition Only, to run Both Acquisition and DA, or to run DA only by selecting one of these options for the Part of method to run in the Worklist Run Parameters dialog box.



Tune window With this window you tune the mass spectrometer. You can use the automatic tuning algorithms that are provided, or you can manually tune the instrument.

Step 2. Prepare the LC modules

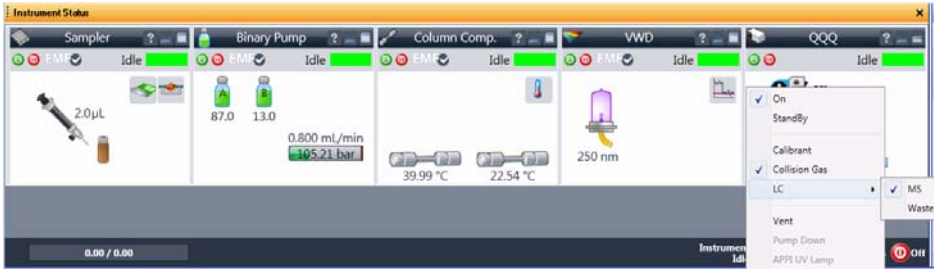
Read and follow the instructions in the online Help for each of the tasks in the checklist described on the following pages.

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 **QQQ** device in the Instrument Status window.



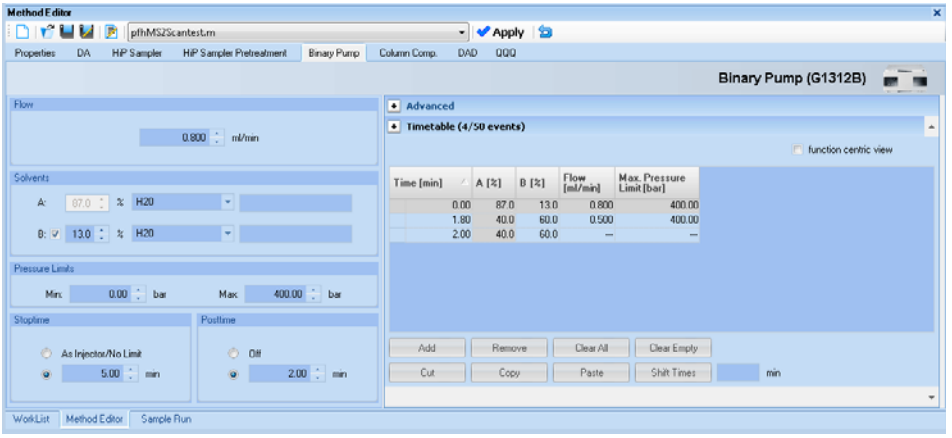
b Click **LC > Waste**.

2 Purge the LC pump.

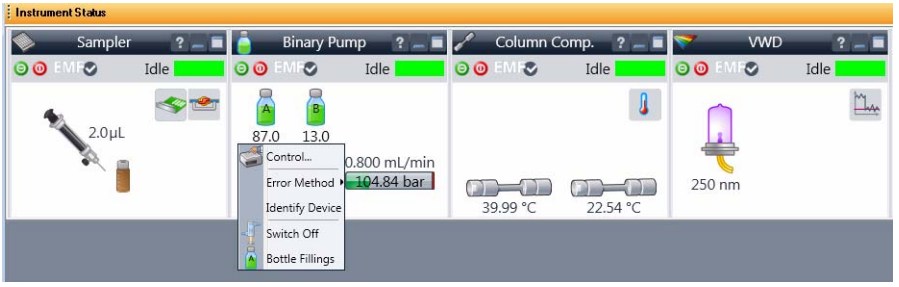
Follow the directions for purging the pump in the *User Guide* for your pump.

3 Set up to condition or equilibrate the column.

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



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

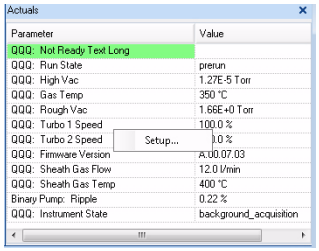


c Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See step 4 and step 5 on page 15.)

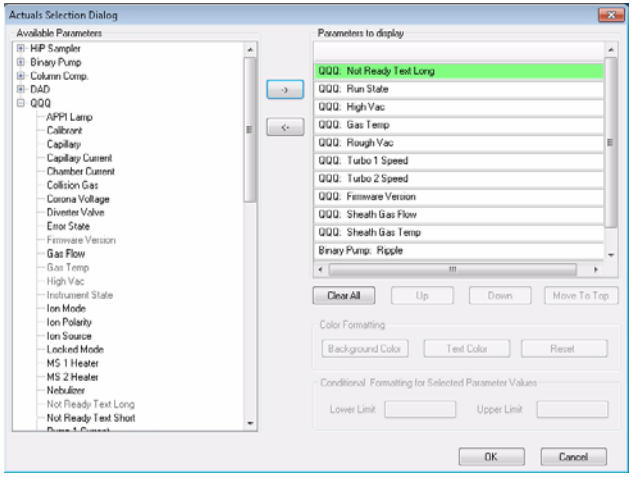
4 Set up to view real-time parameter values (actuals).

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 window.

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



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

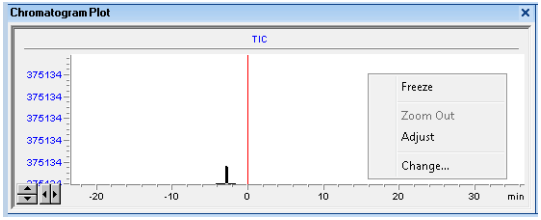


c Add all the parameter values you intend to monitor, and click the **OK** button. You can set the background and text color for each parameter. You can also set a range for the parameters which are numbers. If the value of the parameter is not within the limits which you entered, then the background of the parameter is set to red.

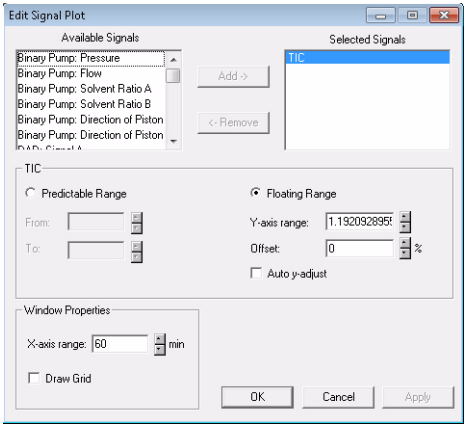
5 Set up real-time plot displays.

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

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



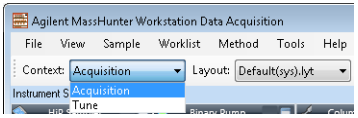
In the Edit Signal Plot dialog box, you can select the type of display signal.



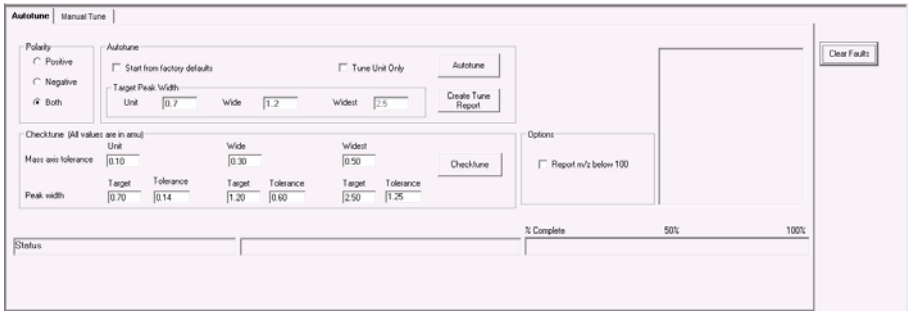
Step 3. Prepare the Triple Quad instrument

Do a Checktune, Autotune or Manual Tune

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



You can see the Instrument Status window, the Actuals window and the Tune window when you switch to the Tune context. Click **Tune** in the **View** menu if the Tune window 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.

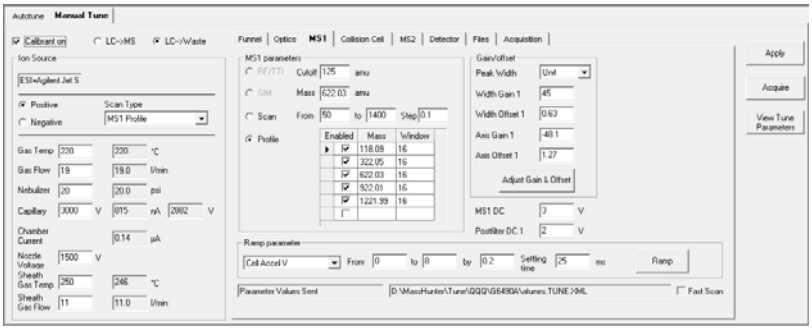
If **Checktune** passes, then skip to step 5.

If **Checktune** fails, then you can try using the Manual Tune tab to fix the problem.

3 Try the following quick changes to get Checktune to pass.

a Click the **Manual Tune** tab.

b Click the **MS1** tab and then click the **Adjust Gain & Offset** button.



c Click the **MS2** tab and then click the **Adjust Gain & Offset** button.

d Click the **Autotune** tab.

e Click the **Checktune** button.

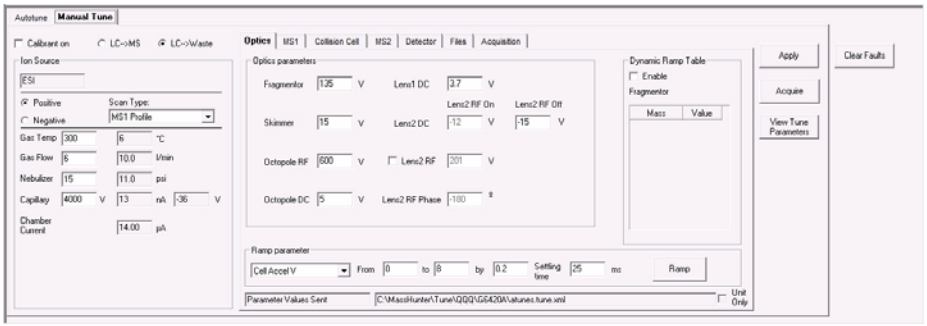
If **Checktune** fails again, you need to do an Autotune, which is described next.

4 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.).

Checktune and Autotune reports are automatically generated after Autotune completes successfully. If Autotune fails, no reports are 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.



5 (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.

6 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 13 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 the **Log** icon () in the toolbar of the Data Acquisition window, and view the logged events.
- Or click **Tools > System Logbook Viewer**.
- Or right-click the  icon in the system taskbar. First, click **Enable Notification.** Then, right-click the LOG icon and click **Configure**. The system can notify you of new errors and warning by showing messages from the taskbar.

When the System Logbook Viewer is open, you can select the time period to keep System Logbook entries. You can set the value from 1 week to 1 year. To do this, you click **Tools > Purge Settings**.

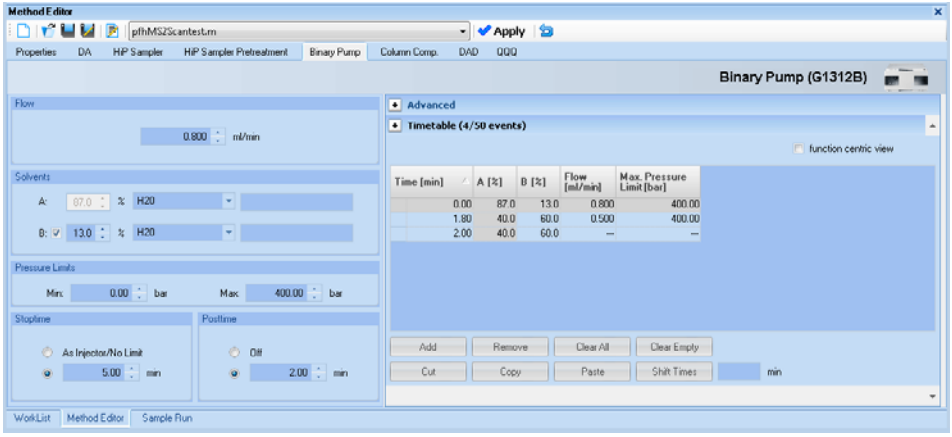
Step 4. Set up and run an acquisition method

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 **Method > Save As**.
   - **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.

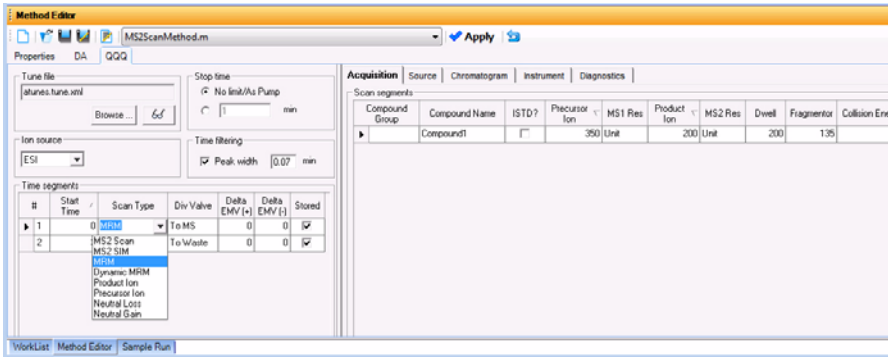
3 Enter the Triple Quadrupole parameter values.

a Select the **Scan Type** from the list in the **Time segments** table. The Scan segments table is cleared when you change the Scan Type. The parameters available on the right change depending on the Scan Type.

If you are changing the **Scan Type** from **MRM** to **Dynamic MRM** or to **Triggered MRM**, you can copy and paste the transitions from the Clipboard to the new **Scan segments** table. See the online Help for more information.

b Type in any **Acquisition** values you want to change. You can enter multiple Scan segments.

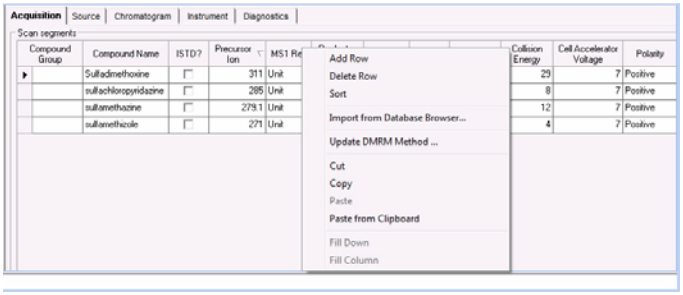
You cannot set the fragmentor voltage in Acquisition if the instrument type is an Agilent 6490. It always uses the value in the tune file.



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 shortcut menu, and click **Add Row**.

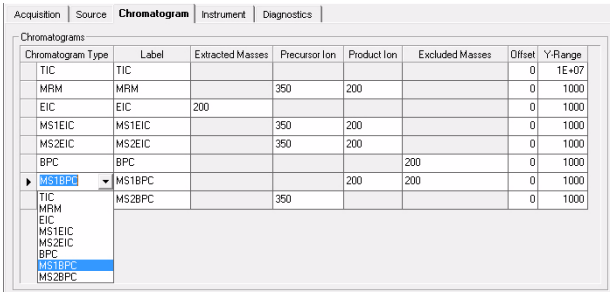
b Type the parameters for each Scan segment.



5 Set up signals for the Chromatogram plot:

a Click the **Chromatogram** tab.

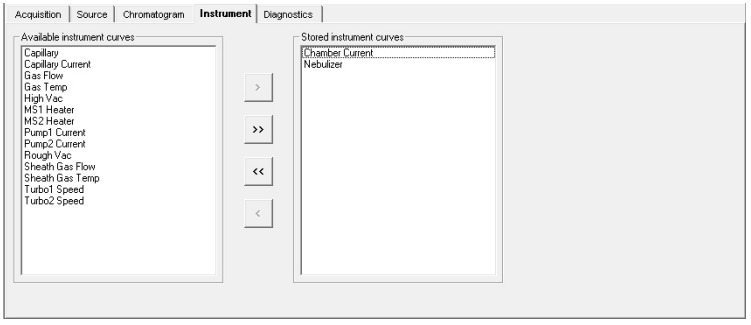
b Select the **Chromatogram Type**, and type other plot values.



6 Set up the **Stored instrument curves** in the Instrument tab. In the Qualitative Analysis program, you can display these values in the MS Actuals window for the current spectrum. With the Triple Quadrupole, the values in the MS Actuals window in the Qualitative Analysis program 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.

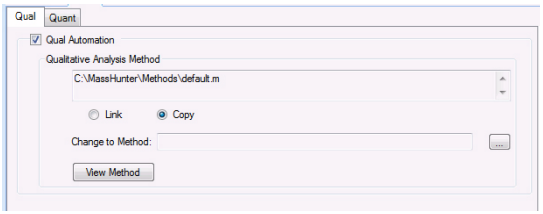


7 Set up the data analysis (**DA**) parameters.

A method can contain qualitative analysis parameters, quantitative analysis parameters or both. A Data Analysis method is a method that contains data acquisition parameters with either the **Qual Automation** check box marked on the Qual tab or the **Quant Automation** check box marked on the Quant tab.

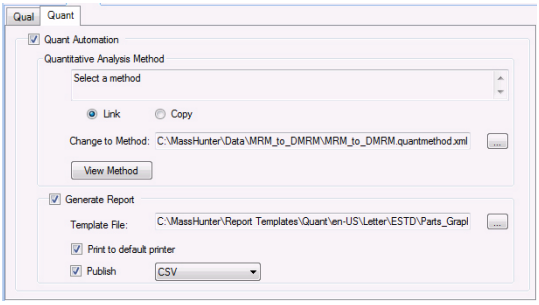
a Click the **DA** tab.

b (optional) Mark the **Qual Automation** check box. The name of the current Qualitative Analysis method is shown in the list. If you want to change the Qualitative Analysis method that is connected, click the "..." button to select a different method. When the Data Acquisition method is saved, the Qualitative Analysis method that you selected is copied or linked to the Data Acquisition method.



c (optional) Click the **Quant** tab. Mark the **Quant Automation** check box. The name of the current Quantitative Analysis method is shown in the list. If you want to change the Quantitative Analysis method that is connected, click the "..." button to select a different method. When the Data Acquisition method is saved, the Quantitative Analysis method that you selected is copied or linked to the Data Acquisition method.

d (optional) Mark the **Generate Report** check box on the Quant tab. Then, you select the **Template File** to use. If you want to print the report, mark the **Print to default printer** check box. You can also mark the **Publish** check box to create a CSV file, a TXT file, or a PDF file.



8 Save the method.

a Click **Method > Save As** or **Method > Save**.

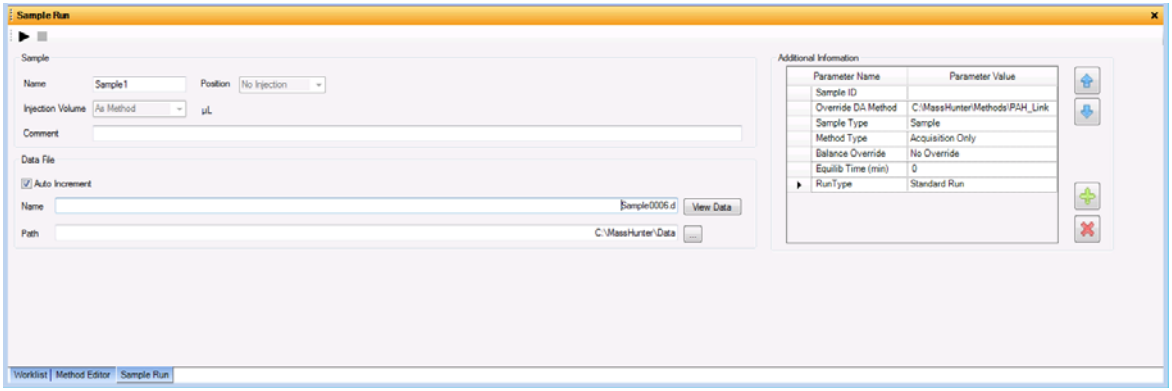
b If necessary, name the method and click **OK**.

9 Set up and run interactive samples:

a Click the **Sample Run** window. By default, it is tabbed with the **Worklist** and **Method Editor** windows.

b Enter the information such as the **Sample Name**, the **Data File Name** and the **Path**.

c Enter the **Additional Information**. You can change the value of the parameters in the **Additional Information** list.



You can run a Data Analysis method from this window by selecting **Both Acquisition and DA** or **DA Only** for the **Method Type**. In addition, you have to set **Override DA method** to indicate which Data Acquisition method contains the DA (Data Analysis) method to execute. You always have to do this.

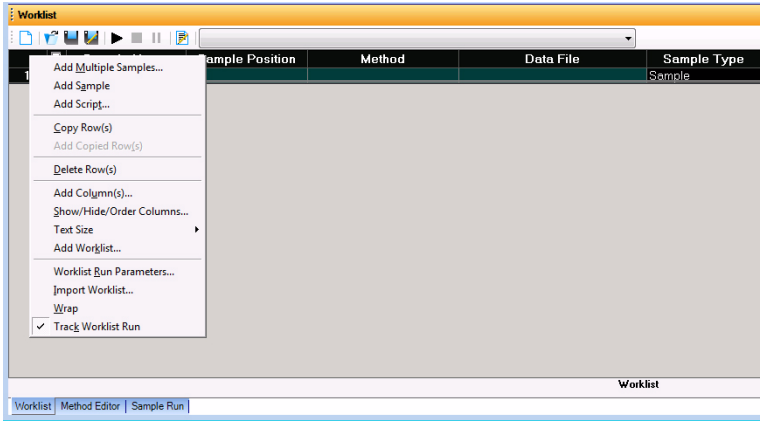
d To start the single sample run, click the Run button ( ) in the Sample Run window or the Run button ( ) in the main toolbar.

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 a run. You also cannot overwrite this data file in the Data Acquisition program. The Lock button ( ) in the main toolbar indicates that locked mode is on.

10 Set up and run worklists

a Click the **Worklist** tab to show the Worklist window. If the Worklist window is not visible, click **View > Worklist**.

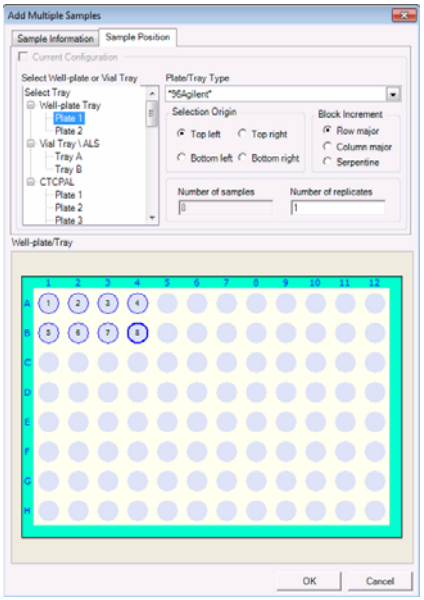
b Right-click the upper left corner of the worklist.



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

d Enter all the information on the Sample Information tab.

e 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).



f Specify the locations, and click the **OK** button.

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

h Click the **Page 1** tab.

i Type the paths for the method and data files.

j Click the **Page 2** tab.

k Review the information and click the **OK** button.

l To start the run, click the **Run Worklist** button ( ) in the main toolbar or the Run button ( ) in the Worklist window

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.

NOTE

To use an acquisition method that has a different DA method than the method entered in the worklist, show the column called **Override DA Method** in the worklist by using the **Show/Hide/Order Columns** dialog box. In this column, type the name of another method containing the DA parameters you want to use for the sample. The DA part of this method is used instead of the DA part of the current method.

You can also type the name of this method in the Add Multiple Samples dialog box.

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 the online Help for the Qualitative Analysis program to learn how to do more operations to analyze your data.

Step 6. Set up and run quantitation

Another 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 the online Help for the Quantitative Analysis program 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 Quadrupole 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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