

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 Triple Quad LC/MS, and as a road map for your user information.

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

The Agilent 6400 Series 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 and 6495 Triple Quad LC/MS includes the iFunnel Technology which provide more efficient sampling of ions into the mass spectrometer. The iFunnel Technology includes the Dual Offset Ion Funnel, the Agilent Jet Stream source, and a shorter desolvation assembly with Hexabore Capillary.

The Agilent 6495, 6490, 6470, and 6460 Triple Quad LC/MS are shipped by default with the Agilent Jet Stream source that uses a super-heated sheath gas to collimate the nebulizer spray, which dramatically increases the number of ions that enter the mass spectrometer.

The 6495B and 6495C models are equipped with a novel gate valve for replacing hexabore capillaries during routine maintenance without breaking vacuum.

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

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

- Data Acquisition – From one program 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 program you can set up a batch of data files and quantify, evaluate and requantify the results. From this program you have access to the Method Editor for setting up and editing the quantitation methods.
- Qualitative Analysis – From the Qualitative Analysis Navigator program you can extract and integrate chromatograms, subtract background, extract peak spectra, identify spectra, and compare data from different types of data files. From the Qualitative Analysis Workflows program, you can find compounds using a variety of algorithms and identify those compounds.

What's New in Data Acquisition

The Data Acquisition program for the Triple Quad LC/MS has a few new features in this revision.

in 10.0

- Supported models are 6410B, 6420A, 6430A, 6460A, 6460C, 6470A, 6490A, 6495A, 6495B, and 6495C.
- StreamSelect 8.0 is supported.
- Manual ramping for fragmentor voltage is disabled for all funnel models, including the 6495C.
- ECM 3.5 Update 5 with Microsoft Server 2012 is supported in Windows 10 only.
- Instrument configuration is now included in the data file for reference and traceability.
- In **Compliance (with ECM)** mode, user-initiated actions are properly attributed to a specific user, including Tune actions.
- You can specify session auto-lockout time in minutes in the System Configuration program if you enable either **Compliance (with ECM)** or **User Management and Audit Trail only**.
- Checksum validation is now extended to prevent usage of invalid methods if you enable either **Compliance (with ECM)** or **User Management and Audit Trail only**.
- If **Compliance (with ECM)** is enabled, the Qualitative Analysis part of the method is ignored.
- When you start a worklist, it is validated before it is run, and all errors are shown in red.
- Version A.02.19 SR2 for the LC drivers is supported.

in B.09.00

- Spectral plots for different devices are shown in different tabs in the Spectrum Pane window.
- Version A.02.19 for the LC drivers is supported.

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.

Installation and User Guides



You can access these guides from the **TQ LC/MS Resource App** or **LCMS Data Analysis Resource App**. Some of these guides are included with your system in printed format. They are also available at www.agilent.com.

MassHunter Workstation Software Installation Quick Start Guide This guide provides instructions to install or upgrade MassHunter Workstation software.

MassHunter Workstation Data Acquisition Familiarization Guide Do the exercises to learn to use the Triple Quad LC/MS and Data Acquisition program.

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

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 LC/MS for each individual compound analyzed.

MassHunter Workstation Qualitative Analysis Familiarization Guide Do the exercises to learn to use the Qualitative Analysis programs.

MassHunter Workstation Quantitative Analysis Familiarization Guide Do the exercises to learn to use the Quantitative Analysis program.

Where to find information

Training

MassHunter Data Acquisition Compliance Mode Quick Start Guide Use this guide to learn about the MassHunter Data Acquisition Compliance (with ECM) mode.

MassHunter Data Acquisition User Management and Audit Trail Quick Start Guide Use this guide to learn about the User Management and Audit Trail feature for the Data Acquisition program.

MassHunter Workstation Quantitative Analysis Audit Trail and Compliance Quick Start Guide Use this guide to learn about the Compliance (with ECM) and User Management and Audit Trail modes for the Quantitative Analysis program.

MassHunter Workstation Data Acquisition eFamiliarization Guide Use this interactive online guide to get to know the Data Acquisition program.

MassHunter Qualitative Analysis eFamiliarization for TQ Use this interactive online guide to learn to use the Qualitative Analysis program.

MassHunter Quantitative Analysis eFamiliarization Use this interactive online guide to learn to use the Quantitative Analysis program.

Agilent 6000 Series LC/MS Hardware eFamiliarization Use this interactive online guide to learn more about your Triple Quad LC/MS instrument.

Agilent 6200/6400/6500 Series Maintenance Guide (animated) Use this animated guide to help maintain and troubleshoot your Triple Quad LC/MS.

MassHunter Workstation Administration Guide This guide includes administration and troubleshooting tasks for your Triple Quad LC/MS.

Agilent 6000 Series LC/MS Safety Guide This guide contains safety and conformity information for your Triple Quad LC/MS.

Training

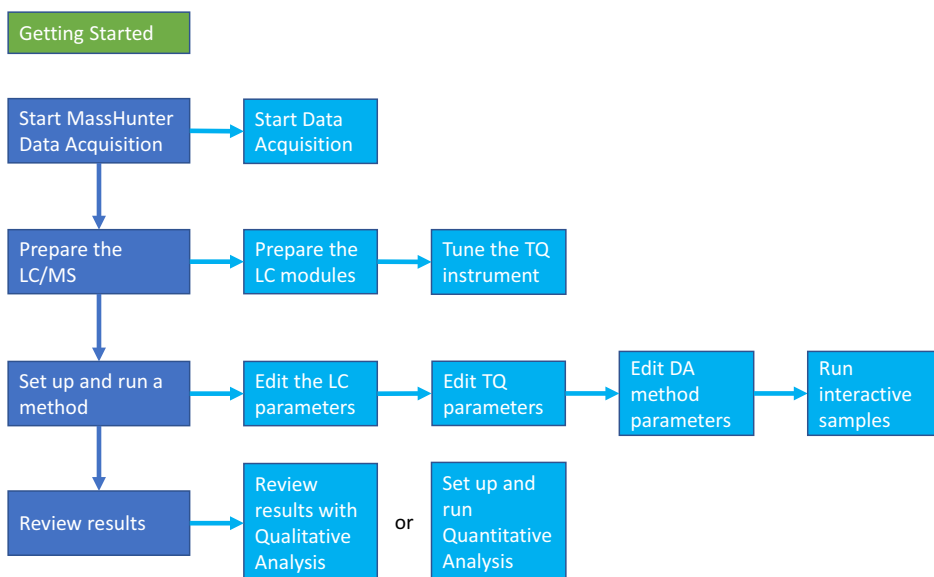
Resource Apps Use the material in the Resource Apps to learn to use your MassHunter Workstation software, to learn about the Triple Quad LC/MS instrument, and to maintain and troubleshoot your Triple Quad LC/MS.

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

Getting Started

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.



Getting Started

Step 1. Start the Data Acquisition software

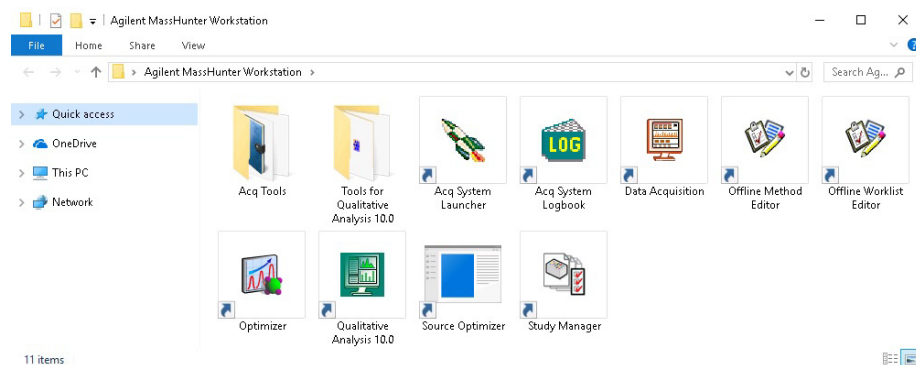
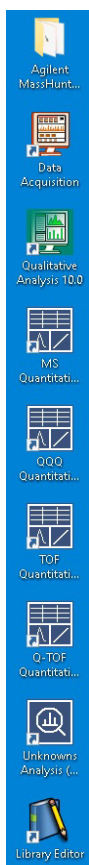
Step 1. Start the Data Acquisition software

The instructions below include the following assumptions:

- The hardware and software are installed.
- The instrument is configured.
- The LC modules and the Triple Quad LC/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. In Windows 7 you can instead click **Start > Agilent > MassHunter Workstation > Data Acquisition**. In Windows 10 you can instead click the Start button and then click **Agilent MassHunter LCMS Acq > Data Acquisition**.

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 *Administration Guide* for instructions.

Getting Started

Step 1. Start the Data Acquisition software

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 screenshot displays the Agilent MassHunter Workstation Data Acquisition software interface. The window is divided into several panes:

- Instrument Status:** Shows the status of various instrument components: HIP Sampler (On), Quat. Pump (Standby), Column Comp. (Not Ready), and QQQ (Standby). It also displays flow rates and temperatures.
- Actuals:** A table showing parameters and their values. Parameters include QQQ Ready Type, QQQ Ready State, QQQ Run Time, QQQ Run State, QQQ Rough Vac, QQQ Turbo 1 Speed, QQQ Turbo 2 Speed, QQQ High Vac, QQQ Instrument State, and QQQ Error State.
- Chromatogram Plot:** A Total Ion Chromatogram (TIC) showing a single sharp peak at approximately 125.5 minutes.
- Spectrum Pane:** A mass spectrum plot showing relative intensity versus m/z (mass-to-charge ratio) for the peak at 125.5 minutes.
- Method Editor:** A pane for configuring acquisition parameters, including Tune file, Stop time, Ion source, Time segments, and Scan parameters.

Blue arrows point from text labels on the right to the corresponding windows in the screenshot:

- Actuals window
- Instrument Status window
- Chromatogram Plot window
- Spectrum Pane window
- Method Editor window

Figure 1. Main window of the Data Acquisition software program. The Sample Run and Worklist windows are tabs grouped together here. These three windows are “sharing” this space. You click the tab to switch to a different window.

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.

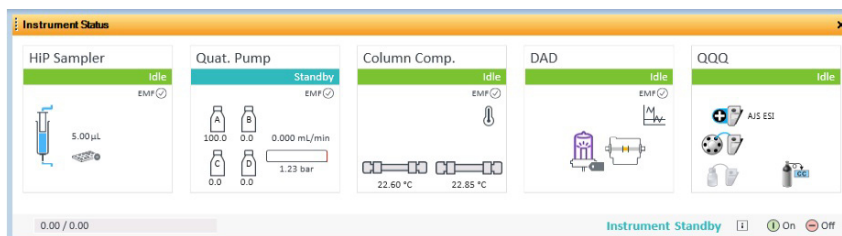
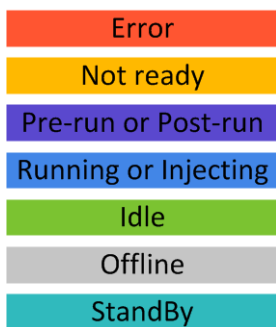
Getting Started

Step 1. Start the Data Acquisition software

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.

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.

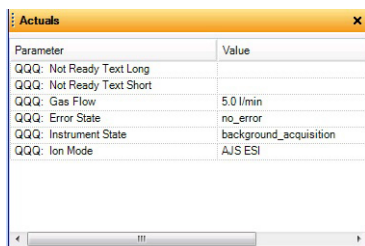
This window displays the current status of each device both as text and by its color-coding:



Getting Started

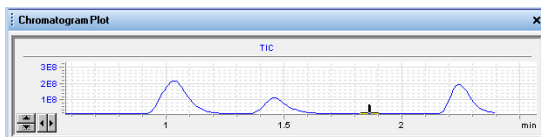
Step 1. Start the Data Acquisition software

Actuals window With this window you view the current value of selected instrument parameters. See “**Set up to view real-time parameter values (actuals).**” on page 14 for more information.

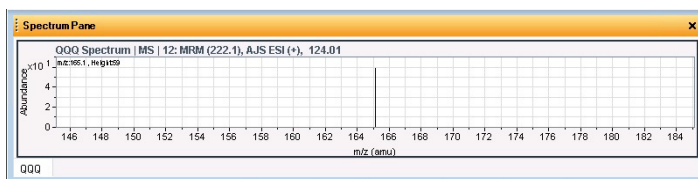



Parameter	Value
QQQ: Not Ready Text Long	
QQQ: Not Ready Text Short	
QQQ: Gas Flow	5.0 l/min
QQQ: Error State	no_error
QQQ: Instrument State	background_acquisition
QQQ: Ion Mode	AJS ESI

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. You select the plots in the Chromatogram tab in the **QQQ** tab in the Method Editor window.



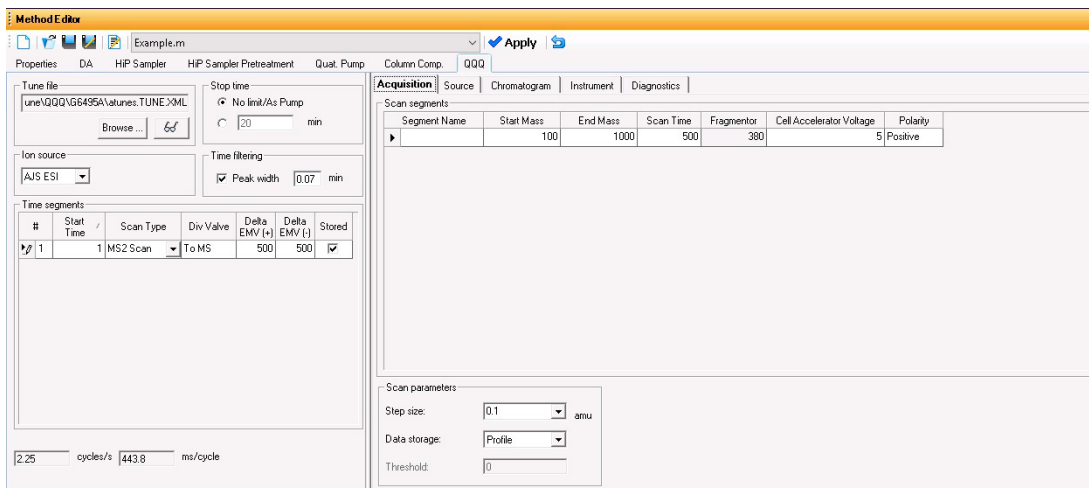
Spectrum Pane window With this window you monitor the spectral plot in real time. A different tab is available for different devices.



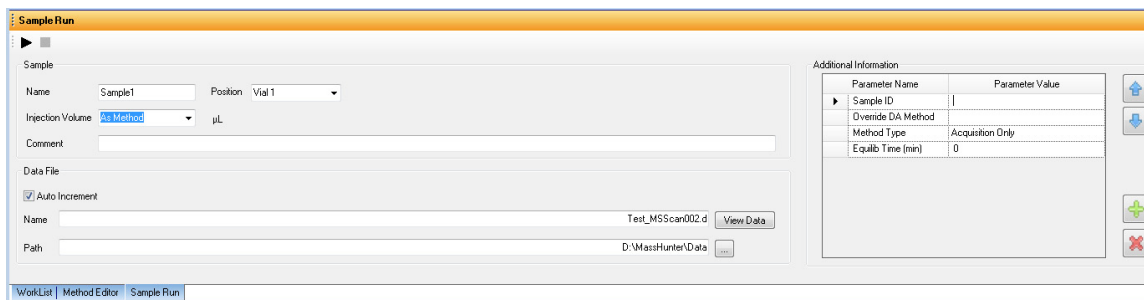
Method Editor window With this window you enter acquisition parameters for the method. If you click  in the **QQQ** tab, then you can see the tune values in the **Tune Parameters** dialog box.

Getting Started

Step 1. Start the Data Acquisition software



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.

Getting Started

Step 1. Start the Data Acquisition software

Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment	Sample Group	Info.
1 Sample1	P1-A1	QQQ_Example.m	WorklistData1.d	Sample				
2 Sample2	P1-A2	QQQ_Example.m	WorklistData2.d	Sample				
3 Sample3	P1-B1	QQQ_Example.m	WorklistData3.d	Sample				
4 Sample4	P1-B2	QQQ_Example.m	WorklistData4.d	Sample				

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. You have to switch to the Tune Context to see this window.

Agilent MassHunter Workstation Data Acquisition

Context: Tune Layout: Default(sys).lyt

Tune

During an Autotune or Checktune, some of the parameters are ramped to determine the best value.

Abundance vs. m/z (amu) plots showing peaks at 113.02, 2233.94, and 2233.91.

Autotune Manual Tune

Polarity: Positive, Negative, Both (Selected)

Autotune: Start from factory defaults, Tune Unit Only, Autotune, Create Tune Report

Checktune (All values are in amu): Mass axis tolerance, Peak width, Wide, Widest, Target, Tolerance, Checktune

Options: Report m/z below 100

Progress: % Complete 50% 100%

This section shows the current progress in the Autotune or Checktune.

If you make changes in the Manual Tune tab, you can print a Checktune and Autotune report when you click Create Tune Report.

Getting Started

Step 2. Prepare the LC modules

Step 2. Prepare the LC modules

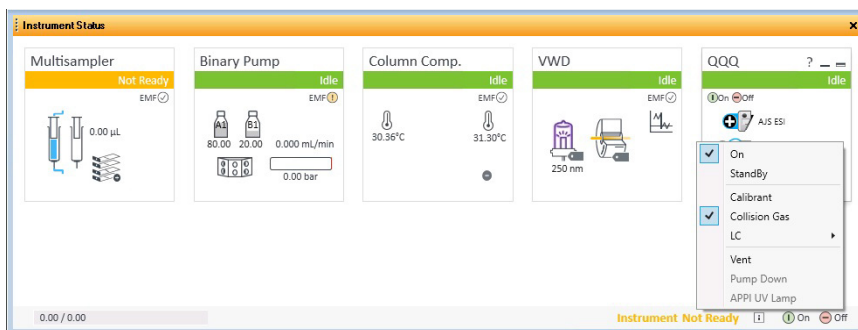
Read and follow the instructions in the *online Help* for each of the tasks in the checklist described on the following pages. While you condition or equilibrate the column, you can tune the Triple Quad LC/MS.

1 Switch LC stream to **Waste**.

When you are not acquiring data, 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.

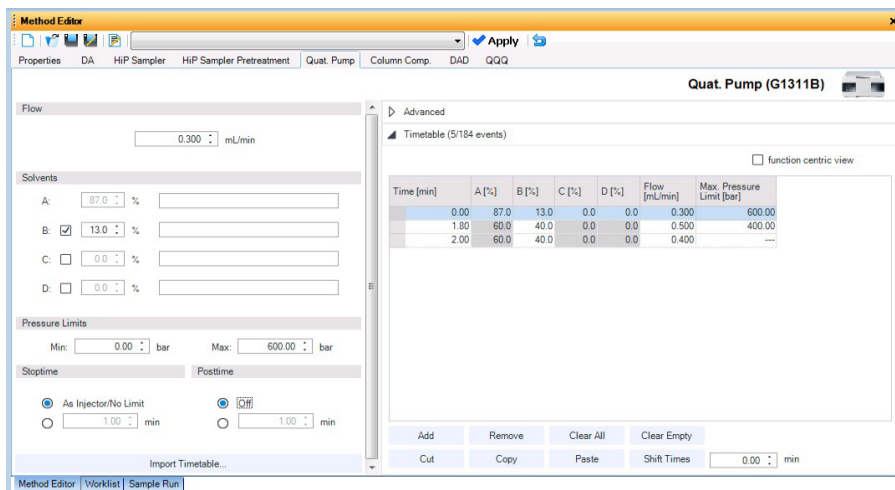
Follow the directions for purging the pump in the *User Guide* for your pump.

3 Set up to condition or equilibrate the column.

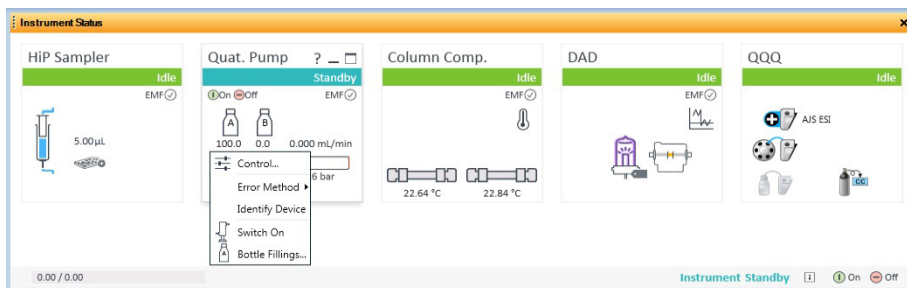
- a Type LC parameters, and click **Apply** in the toolbar to download them to the LC.

Getting Started

Step 2. Prepare the LC modules



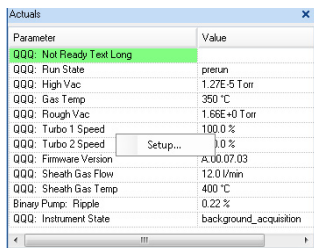
- b Right-click an LC module in the Instrument Status window and click one of the commands to change any non-method control parameters, if needed.



- 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 14**.)
- 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.

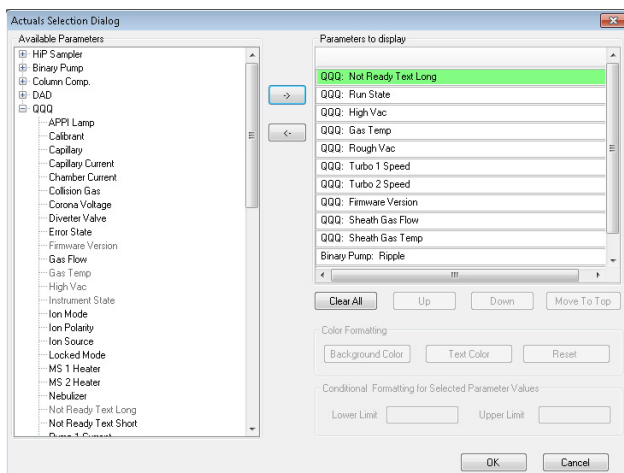
Getting Started

Step 2. Prepare the LC modules



Parameter	Value
QQQ: Not Ready Text Long	
QQQ: Run State	pretun
QQQ: High Vac	1.27E-5 Torr
QQQ: Gas Temp	350 °C
QQQ: Rough Vac	1.66E+0 Torr
QQQ: Turbo 1 Speed	100.0 %
QQQ: Turbo 2 Speed	10.0 %
QQQ: Firmware Version	A100.07.03
QQQ: Sheath Gas Flow	12.0 l/min
QQQ: Sheath Gas Temp	400 °C
Binary Pump: Ripple	0.22 %
QQQ: Instrument State	background_acquisition

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



c Add all the parameter values you intend to monitor, and click **OK**. Parameters that you may want to monitor include MS values (such as heater, and vacuum pressure) or LC values (such as binary pump, column, DAD, etc.) 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.

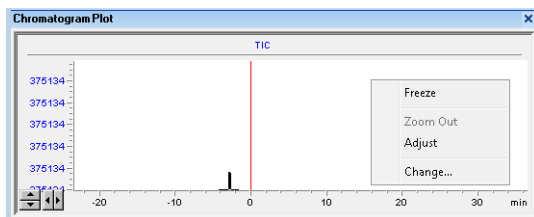
5 Set up real-time plot displays.

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

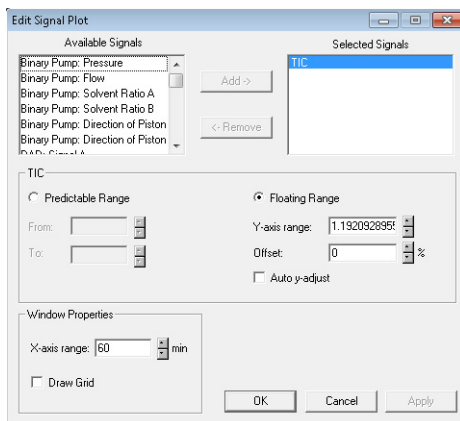
- Right-click the chromatogram plot, and click **Change**.

Getting Started

Step 2. Prepare the LC modules



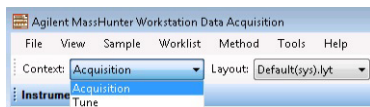
In the **Edit Signal Plot** dialog box, you can select the type of display signal.



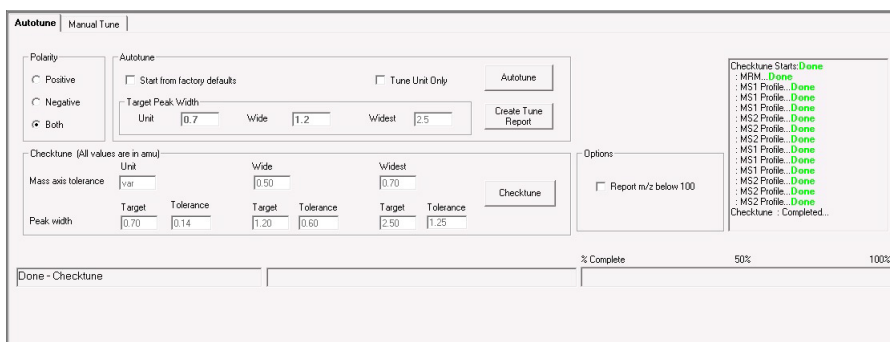
Step 3. Prepare the Triple Quad LC/MS

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 can take approximately 45 minutes.

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.

You can run a **Checktune** with the following sources: ESI, AJS ESI, and MMI.

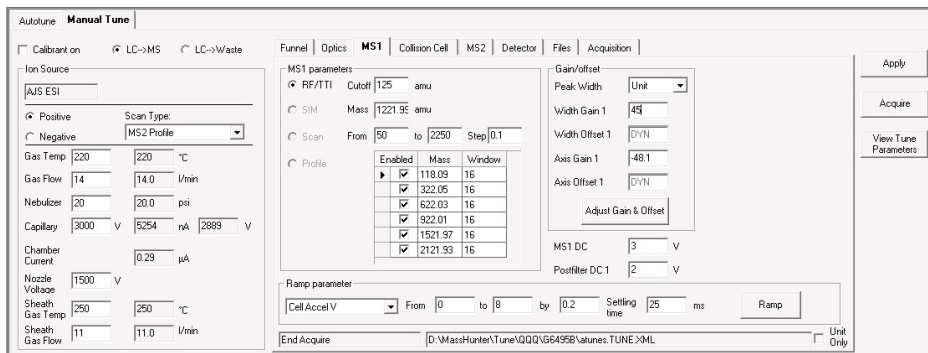
If **Checktune** passes, then skip to **step 6**.

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

Getting Started

Step 3. Prepare the Triple Quad LC/MS

- 3 Try the following quick changes to get Checktune to pass.
 - a Click **Manual Tune**.
 - b If the failure occurred in MS1, click **MS1**. Select the **Peak Width** based on which resolution failed in Checktune. You can select **Unit**, **Wide**, or **Widest**. Then, click **Adjust Gain & Offset**.



- c If the failure occurred in MS2, click the **MS2** tab. Select the **Peak Width** based on which resolution failed in Checktune. Then, click **Adjust Gain & Offset**.
 - d If the Adjust Gain and Offset passes successfully, then save the autotune file. Click **Manual Tune**. Then, click **Files**, and click **Save**.
 - e Click **Autotune**.
 - f Click **Checktune**.

If **Checktune** fails again, you must run an Autotune, which is described next.

- 4 Click **Autotune** to tune the MS automatically (approximately 30 minutes for each ionization mode for all models except the 6490 and the 6495; for 6490 and 6495, approximately 25 minutes per ionization mode). The system automatically changes different tune parameters to tune the MS. You only do an Autotune when needed.

For all instruments except the 6490 and the 6495, you can run an **Autotune** with the following sources: AJS ESI, and MMI. With the 6490 and 6495, you can run an Autotune with only the AJS source.

If **Autotune** fails, then you mark the **Start from Factory Defaults** check box. Then, if you click **Autotune**, the instrument is tuned starting from the factory defaults (approximately 30 minutes for each polarity for all models except the 6490 and the 6495. The 6490 and 6495 take approximately 25 minutes per ionization mode.).

Getting Started

Step 3. Prepare the Triple Quad LC/MS

Checktune and Autotune reports are automatically generated after **Autotune** completes successfully. If Autotune fails, no reports are printed. You can check the progress box in the lower right side of the Autotune tab to see the reasons why the tune failed. Then, you can either fix the problem, or call the Agilent service engineer and provide this information.

If Autotune fails or you assess that the Triple Quad LC/MS needs custom values entered for its tune parameters, you can manually tune the instrument. If you cannot get the instrument to tune successfully, then please call your Agilent service engineer.

The screenshot shows the 'Autotune Manual Tune' window. The 'Ion Source' section is set to 'AJS ESI' with 'Positive' polarity and 'MS2 Profile' scan type. Gas Temp is 300 °C, Gas Flow is 10 U/min, Nebulizer is 15 psi, Capillary is 4000 V, Chamber Current is 9998 nA, and Nozzle Voltage is 1500 V. Sheath Gas Temp is 250 °C and Sheath Gas Flow is 7 U/min. The 'Optics' section includes: Fragmentor (15 V), Skimmer (15 V), Octopole RF (600 V), Octopole DC (5 V), Lens1 DC (DYN V), Lens2 DC (DYN V), Lens2 RF On (DYN V), Lens2 RF Off (-12 V), Lens2 RF (DYN V), and Lens2 RF Phase (36 °). The 'Ramp parameter' section shows 'Cell Accel V' from 0 to 8 by 0.2 with a setting time of 25 ms. The 'Dynamic Ramp Table' is empty. The 'Parameter Values Sent' field contains 'D:\MassHunter\Tune\IQ02\IG6460\Values:TUNE.XML'.

- 5 *Optional.* Click **Fast Scan Autotune Only** 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.

For Fast Scan acquisition you use one of these two 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.

Fast Scan acquisition is not supported for the 6470 and 6495. Instead, you achieve the faster scan rate by increasing the step size to 0.3 *m/z*.

- 6 In the **Context** list, select Acquisition.

Switch LC stream to MS

- After you condition the column and tune the Triple Quad LC/MS, you switch the LC stream from **Waste** to **MS**. See "**Switch LC stream to Waste.**" on page 13 for how to do this.

Getting Started

Step 3. Prepare the Triple Quad LC/MS

Monitor MS baseline and spectral displays

If you did not monitor the LC baseline with a VWD or DAD, make sure that the Triple Quad LC/MS 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, you change back to the default Triple Quad LC/MS displays.

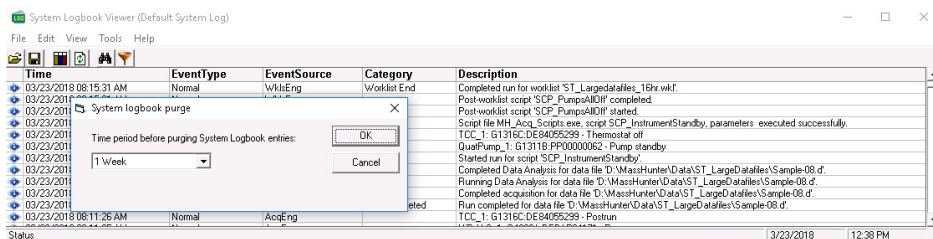
- 1 Right-click the chromatogram plot, and click **Change**.
- 2 Select the MS signal, and click **OK**.

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 **Log** (LOG) in the toolbar of the Data Acquisition window, and view the logged events.
- Or click **Tools > System Logbook Viewer**.
- Or right-click LOG in the system taskbar. First, click **Enable Notification**. Then, right-click **LOG** 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**. The **System logbook purge** dialog box opens.



Getting Started

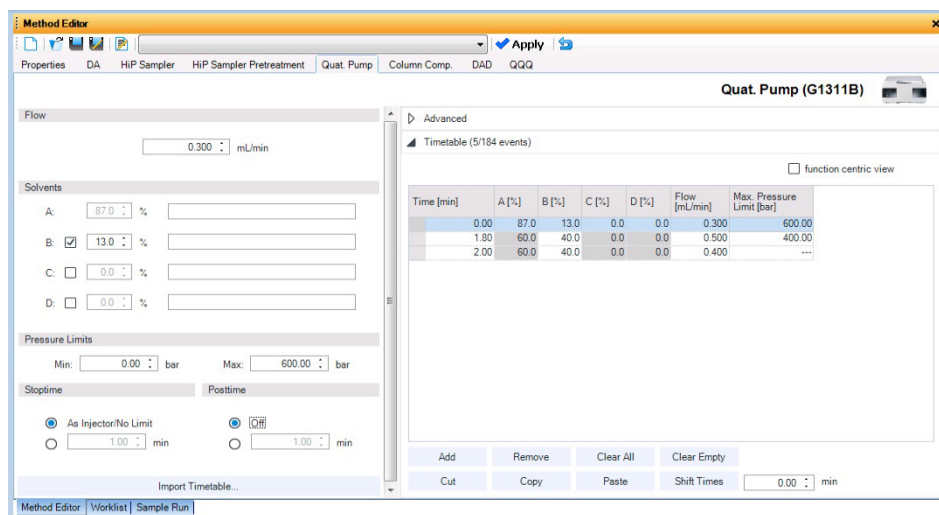
Step 4. Set up and run an acquisition method

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 QQQ 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

Getting Started

Step 4. Set up and run an acquisition method

parameters available on the right change depending on the **Scan Type** for the selected **Time segment**.

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 original **Scan segments** table to the Clipboard and then 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 or an Agilent 6495. It always uses the value in the tune file.

Segment Name	Start Mass	End Mass	Scan Time	Fragmentor	Cell Accelerator Voltage	Polarity
	100	1000	500	380		5 Positive

- 4** Set up to change Triple Quad LC/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.

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Sulfadimethoxime	<input type="checkbox"/>	311	Unit					29	5	Positive
	Sulfahloropyridazine	<input type="checkbox"/>	285	Unit					8	5	Positive
	Sulfamethazine	<input type="checkbox"/>	279.1	Unit					12	5	Positive
	Sulfamethizole	<input type="checkbox"/>	271	Unit					4	5	Positive

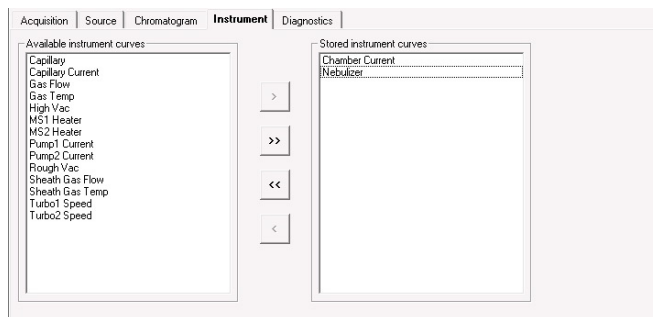
Getting Started

Step 4. Set up and run an acquisition method

- 5 Set up signals for the Chromatogram plot:
 - a Click **Chromatogram**.
 - b Select the Chromatogram Type, and type other plot values.

Chromatogram Type	Label	Extracted Masses	Precursor Ion	Product Ion	Excluded Masses	Offset	Y-Range
TIC	TIC					0	100000
MRM	MRM		350	200		0	1000
EIC	EIC	200				0	1000
MS1EIC	MS1EIC		350	200		0	1000
MS2EIC	MS2EIC		350	200		0	1000
BPC	BPC				200	0	1000
MS1BPC	MS1BPC			200	200	0	1000
MS2BPC	MS2BPC		350			0	1000

- 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 Quad LC/MS, the values in the MS Actuals window in the Qualitative Analysis program are the values that you save in the Instrument tab.
 - a Click **Instrument**.
 - b Select the Stored instrument curves. These curves can be shown in the Chromatogram Results window in the Qualitative Analysis program. The values can be seen in the MS Actuals window.



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

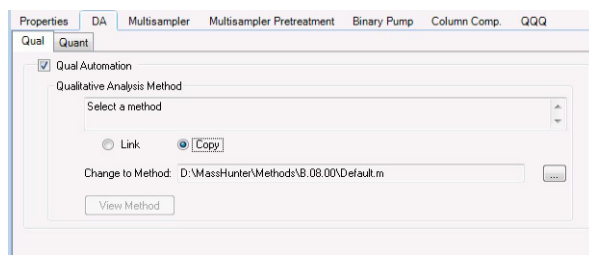
A method can contain BioConfirm parameters, Qualitative Analysis parameters, Quantitative Analysis parameters, or a combination. BioConfirm is a separate product that you can purchase. You can mark the **BioConfirm**

Getting Started

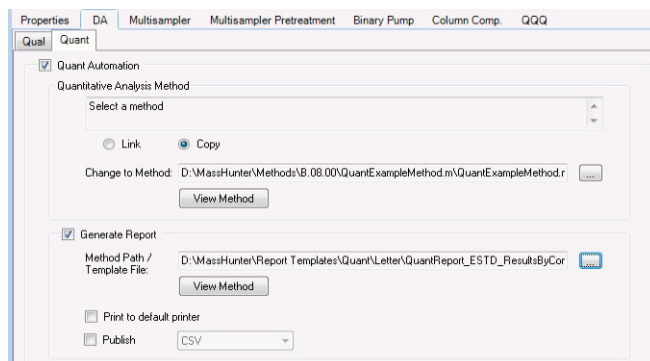
Step 4. Set up and run an acquisition method

Automation check box on the BioConfirm tab; the **Qual Automation** check box is marked on the Qual tab, and the **Quant Automation** check box is marked on the Quant tab.

- a Click **DA**.
- b *Optional.* Mark the **Qual Automation** check box. The name of the current Qualitative Analysis method is shown in the box. If you want to change the Qualitative Analysis method that is connected, click **...** 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.





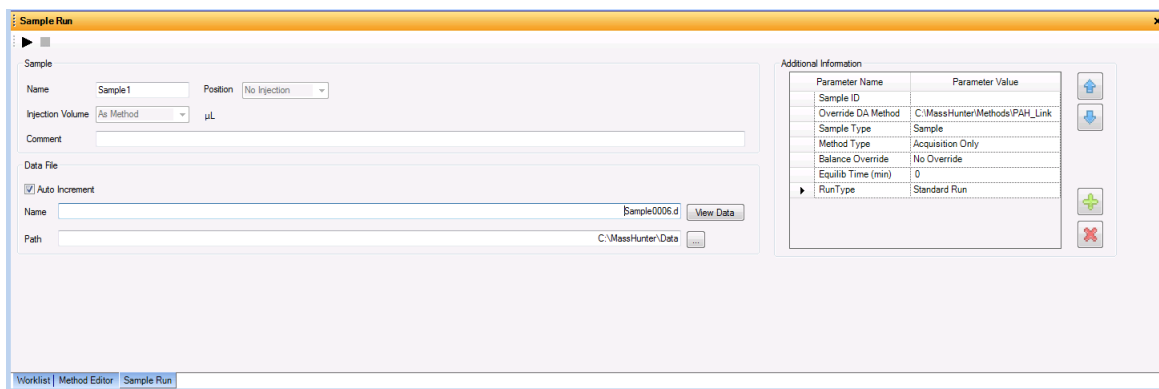
- c *Optional.* Click **Quant**. Mark the **Quant Automation** check box. The name of the current Quantitative Analysis method is shown in the list. If you want to change the Quantitative Analysis method that is connected, click **...** to select a different method. When the Data Acquisition method is 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 **Method Path / 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.





Getting Started

Step 4. Set up and run an acquisition method

- 8 Set up the Properties for this method.
 - a Click **Properties**.
 - b Click  to select the **Pre Run Script**.
 - c Click  to select the **Post Run Script**.
 - d Type the **Description** for this method.
- 9 Save the method.
 - a Click **Method > Save As** or **Method > Save**.
 - b If **User Management & Audit Trail** or **Compliance (with ECM)** is enabled, enter the Reason for creating a new version of this method. Click **OK**.
 - c If necessary, name the method and click **OK**.
- 10 Set up and run interactive samples:
 - a Click the **Sample Run** window. By default, the Sample Run window is grouped with the **Worklist** and **Method Editor** windows.
 - b Enter the **Sample Name**, the **Data File Name**, the **Path** and other values.
 - 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 **Run** () in the Sample Run window or **Run** () in the main toolbar. You can instead click **Sample > Run**.

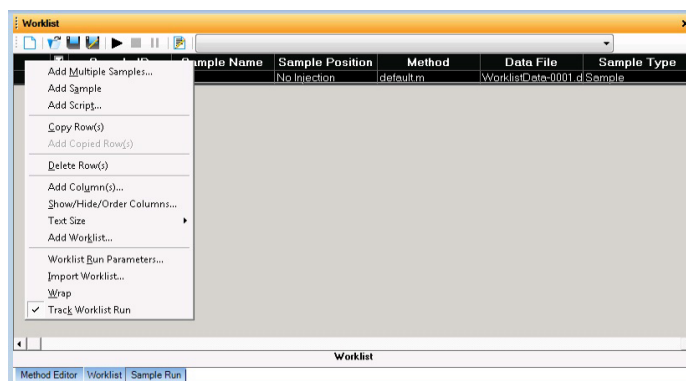
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Step 4. Set up and run an acquisition method

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.

11 Set up and run worklists

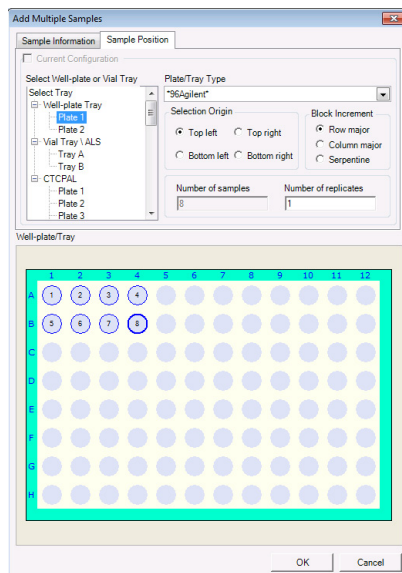
- a Click **Worklist** to show the Worklist window. If the Worklist window is not visible, click **View > Worklist**.
- b Right-click the upper left corner of the worklist.





- c Click **Add Multiple Samples**. The **Add Multiple Samples** dialog box opens.
- d Enter all the information on the Sample Information tab.
- e Click **Sample Position** to specify the sample vial locations (make sure the specific sample tray type has been configured by right-clicking the autosampler device image).

Getting Started

Step 4. Set up and run an acquisition method



- f Specify the locations, and click **OK**.
- g To set up the worklist run, right-click the upper left corner of the worklist, and click **Worklist Run Parameters**.
- h On the **Run Parameters** tab, type the paths for the method.
- i Click **Data File Settings**.
- j Type or select the folders for the data files. Select the **File Naming** options.
- k Click **Additional Parameters**.
- l Review the information and click **OK**.
- m To start the worklist, click **Run Worklist** () in the main toolbar or **Run** () in the Worklist window. You can also click **Worklist > Run**.

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

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Step 4. Set up and run an acquisition method

NOTE

To use an acquisition method that has a different data analysis (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 programs 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. View the Qualitative Analysis eFamiliarization Guide for TQ to get an overview of the software.

Do Exercise 1 of the Data Acquisition *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 software to learn how to do more operations to analyze your data.

Step 6. Analyze data with the Quantitative Analysis program

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 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.

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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