

Agilent 6400 Series Triple Quadrupole 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 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.



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 <u>www.chem.agilent.com</u> 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*.

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

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.

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.

Start Here!	1 Start the Data Acquisition software	2 Prepare the LC/MS
	Set up, run and analyze samples	3 Set up and run an acquisition method
	5 Set up and run quantitation	4 Review results with Qualitative Analysis

Step 1. Start the Data Acquisition software

The instructions below include the following assumptions:

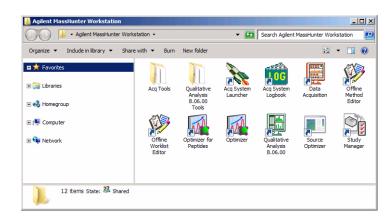
- The hardware and software are installed.
- The instrument is configured.

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.



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.

Instrument Status Chromatogram Plot window window		Method Editor window	Spectrum Plot window	Actuals window	
Instrument Status	99 0 Nun 99 0) Methods QQQ, Exemple m WDD 2 == 1 0000 2 = 2 Run 000 Run 0000 2 = 1 C 0 0 Run 0000 2 = 1 C 0 Run 00000 2 = 1 C 0 Run 00000 2 = 1 C 0 Run 00000	Worklin: Actual: Actual: Pownete: Vale 000 Run Tee Imp 120 Minin 000 Run Tee xn 000 Run Tee 237 een	s X	
Chemotogram Part	z nn z c cken Corp. Vv0 D00 Acquistion Source Crosstopan Scan aquesta n	Instrument Diagnostica End Mass Scan Time Fragmentor Cell Acc	500 500 500	X 305.0 400 X	
Ion source Time Being ESI	Stead - Scan parameter Step see 11	2) 400 500 100 	7 Postor		

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	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)

Color Coding in the Instrument Status Window



Step 1. Start the Data Acquisition software

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

Value				
12.0 l/min				
nun .				
2.37 min				
	12.0 Umin run			

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.

Chromatogram Plot			×
	TIC		
3E8 2E8 1E8	1.5	 2	min

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

Spect	rum Plot						>
	pectrum MS 1	: MS2 Scan, E	SI (+), 2.38				
250K	Area: 0				310	1.9	
500K	m/z: 310.9				3		
250K	FWHM: 0.7					332.9	
0	Height: 629658					<u> </u>	8.0 385.0
	100	150	200	250	300	350	400
· •				m/z(amu)			

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.

MethodEditor								×
: 📄 📽 📕 🛃 🖻 QQQ_bample.m		💙 Apply 🛛 🖄						
Properties DA Sampler Sampler Protocolment Binary Pump Colu	nn Comp. VWD G	90						
Tune file Stop time	Acquisition Source	Chromatogram	Instrument Di	agnostics				
atunes.tune.xml @ No limit/As Pump	Scan segments							
Browse 6d C 1 min	Segment Name	Start Mass	End Mass		Fragmentor	Cell Accelerator Voltage	Polarity	
	•	100	400	500	100	7	Positive	
Time filtering								
ESI V Peak width 0.07 min								
Time segments								
II Start / Scan Type Div Valve Delta Delta EMV (+) EMV (-) Stored								
1 0 MS2 Scan To MS 400 0 ₽								
· · · · · · · · · · · · · · · · · · ·								
	Scan parameters							-
	Step size:	0.1	anu					
	Data storage:	Profile						
2.03 cycles/s 433.6 ms/cycle	Threshold	0						

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.

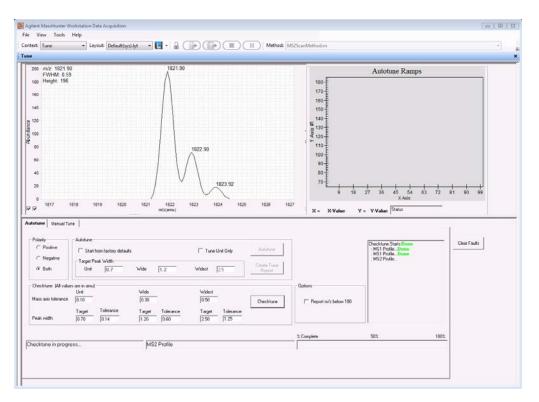
Sample Run				
> =				
Sample	Adde	ional Information		
Name Sample1 Position Vial 1		Parameter Name	Parameter Value	†
	1	 Sample ID Override DA Method 	1	
Injection Volume Building v pl.		Method Type	Acquisition Only	-
Comment		Equilb Time (min)	0	
Data File				
Auto Increment				
Name Test_MSScan002.d View Data				÷ ×
Pah D:MassHunter/Data and				×
WorkList Method Editor Sample Run				

Step 1. Start the Data Acquisition software

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.

VorkLis	e									
11	: 🖬 🖬 🕨 🖿 💷 🗌	P (-						
Г	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	000_Example.m	WorklistDate2.d	Sample					
3 1	 Sample3 	P1-B1	000_Example.m	WorklistData3.d	Sample					
4 1	Sample4	P1-02	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.



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.

Step 2. Prepare the LC modules

- **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.

Method E ditor						×
🗋 📄 💕 🖬 🛃 🍺 🗍 pfhMS2Scantest.m	-	💙 Appl	y 🔁			
Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump	Column Comp. DA	D QQQ				
						Binary Pump (G1312B)
Flow	Advanced					
	• Timetable (4	/S0 events)			
0.800 : m//min						function centric view
Solvents	Time (min)	A [%]	B [%]	Flow [ml/min]	Max. Pressure Limit [bar]	
A: 67.0 1 % H20 *	0.00		13.0	0.800		
8: 🗸 13.0 📜 🛠 H20 👻	1.80		60.0 60.0			
0. 0 100 . 4 100	2.00	40.0	00.0	_	_	
Pressure Limits						
Min: 0.00 🗧 bar Max 400.00 🗧 bar						
Stoptime Posttime						
🔿 As Injector/No Limit 📀 Off	Add	Remov	re (Clear All	Clear Emply	
😻 5.00 🗧 min 🔮 2.00 🗧 min	Cut	Сору		Paste	Shift Times	min
						*
WorkList Method Editor Sample Run						

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

Instrument Status							
left Sampler	? _	🧯 🛛 Binary P	ump 🛛 ? 👝 🗖	🦯 🛛 Column C	Comp. 🤉 🗕 🖬	💙 VWD	? 🗕 🗖
© @ EMF⊘	Idle 📃	🖸 🔘 EMF🔊	Idle 📃	🖸 🞯 EM 😎	Idle 📃	© @ EMF⊗	Idle 📃
2.0µL	**	A B 87.0 13.0 Control Error Method Identify Devic		39.99 °C	€ 22.54 °C	250 nm	
		Switch Off Bottle Fillings					

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.

Parameter		Value		
QQQ: Not Ready Text Lon	g			
QQQ: Run State		prerun		
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	Setup	0.0 %		
QQQ: Firmware Version		A.00.07.03		
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 bring up the list of Actuals available for monitoring.

Available Parameters		Parameters to display	
E-HiP Sampler			T
Binary Pump			
- Column Comp.		QQQ: Not Ready Text Long	
- DAD	-)	QQQ: Run State	1
- QQQ		QQQ: High Vac	
-APPI Lamp		Q00: Gas Temp	
- Calibrant	E <-		
Capillary		QQQ: Rough Vac	
- Capillary Current		QQQ: Turbo 1 Speed	
- Chamber Current		QQQ: Turbo 2 Speed	
- Collision Gas		000: Firmware Version	1
- Corona Voltage			
Diverter Valve Error State		QQQ: Sheath Gas Flow	
Error State		QQQ: Sheath Gas Temp	
Gas Flow		Binary Pump: Ripple	
- Gas Flow			ł
High Vac		< m >	
- Instrument State		Clear Al Up Down Move To To	
- Ion Mode		Clear Al Op Down Move To To	
Ion Polarity		Color Formatting	
- Ion Source		Color Formatting	
-Locked Mode		Background Color Text Color Reset	
MS 1 Heater			
MS 2 Heater		Configurate Francisco da Colonia di Branca da Malara	
- Nebulizer		Conditional Formatting for Selected Parameter Values	
- Not Ready Text Long		Lower Limit Upper Limit	
- Not Ready Text Short	-	cower clink	
Dume 1 Coment	•		

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.

Step 2. Prepare the LC modules

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**.

-1	TIC		
375134 375134		Γ	Freeze
375134			Zoom Out
375134- 375134-			Adjust Change

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

Edit Signal Plot	
Available Signals	Selected Signals
Binary Pump: Pressure Binary Pump: Flow Binary Pump: Solvent Ratio A Binary Pump: Solvent Ratio B Binary Pump: Direction of Piston Binary Pump: Direction of Piston	Add >
TIC	
C Predictable Range	Floating Range
From:	Y-axis range: 1.192092895!
To:	Offset: 0 ×
_	Auto y-adjust
Window Properties	
X-axis range: 60 🔭 min	
🗖 Draw Grid	OK Cancel Apply

Step 3. Prepare the Triple Quad instrument

Do a Checktune, Autotune or Manual Tune

1 From the Context list, click Tune.

📑 Agilent	: Mass	Hunter W	orkstati	on Da	ita A	cquisit	ion	
File V	íew 🛛	Sample	Work	list	Me	thod	Tools	Help
Context:			•	Lay	out:	Defau	lt(sys).lyt	•
Instrument S	<mark>Acqu</mark> Tune	isition		Rinar	v Dur	0.0		Colun

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.

Autotune Illanual Tu	ne						1	
Polarity C Positive C Nepative (R Both	Autobune Start from factory defaults Target Peak Width Unit 0.7	Wide 1.2	Tune Unit Only Widest 25	Autotune Create Tune Report			ClearFailtz	l
Checktune (All value Mass axis tolerance Peak width	es are in amu) Unit [0.10 Target Tolerance [0.70 [0.14	Wide 0.30 Taget Tolerance 1.20 0.60	Widest 0.50 Taget Tolerance 2.50 1.25	Checklune	Options Report m/z below 100			
Status					% Complete	50%	100%	

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.

Step 3. Prepare the Triple Quad instrument

- **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.

Autotune Manual Tune	Funnel Optice MS1 Collsion Cell MS2 Detector Files Acquisition	
Ion Souce E31-dgland, Jul 5 IP Training Scan Type IP Training Scan Type IP Training Scan Type Gase Temp (220) 1200 Gase Temp (200) 1200 Pailow 1200 Capaling 2000 V 1975 Provide V	MS1 parameters Gain/offiet C : ID/T1 C/doft [125] ansu C : Sim Mass [222:03] ansu C : Sim Mass [222:03] ansu C : Sim Finabled 160 Positie Positie 182:05 P / 202:05 16 P / 202:05 16 P / 202:05 16 P / 122:39 16 M31 DC 2	Apply Acquire View Tune Parameters
Damber Curret [0,14] μΔ Nocde Votage Sait Prov 1500 V Sheth Gas Tenp 250 246 °C Sheth Gas Flow 11 11.0 Unim	Reep pearate PointBit DC 1 2 V Coll Accel V From 0 to 0 55 ms Ramp Pleameter Values Sent (D:MasHurles/Ture/QQP/GS458A/values: TURE 204. Fail Scan	

- 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.

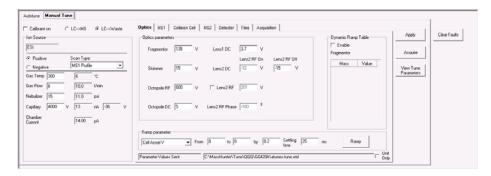
4 Click **Autotune** to tune the MS automatically (15 to 20 minutes for all models except the 6490. The 6490 takes approximately 45 minutes per ionization mode.). The system automatically changes different tune parameters to tune the MS. You only do an Autotune when it is necessary.

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.

Step 3. Prepare the Triple Quad 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.

Step 3. Prepare the Triple Quad instrument

- Click the **Log** icon (**100**) in the toolbar of the Data Acquisition window, and view the logged events.
- Or click Tools > System Logbook Viewer.
- Or right-click the time 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

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.

Step 4. Set up and run an acquisition method

- **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.

Method Editor					
🗋 💕 🖬 🚺 🍺 MS2ScanMethod.m	- 🗸 Apply 😒				
Properties DA QQQ					
Tune file Stop time	Acquisition Source Chromatogram	Instrument Diagnostics			
atunes.tune.xml No limit/As Pump	Scan segments				
Browse 66 C 1 min	Compound Compound Name IST	TD? Precursor T MS1 Res	Product T MS2 Res	Dwell Fragmentor	Collision Erv
- Ion source Time filtering	Compound1	350 Unit	200 Unit	200 135	
ESI ■ IP Peak widh 0.007 min Time segments I Start / Im Scan Type Div Valve Deta EMV (=) Deta Deta EMV (=) Deta EMV (=) Deta					

- **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.

Compound Group	Compound Name	ISTD?	Precursor ~	MS1 Re	Add Row	Collision Energy	Cell Accelerator Voltage	Polar
•	Sulfadmethoxine		311	Unit	Delete Row	29	7	Positive
	sulfachloropyridazine		285	Unit	Sort	8	7	Positive
	sultamethazine		279.1	Unit		12	7	Positive
	sulfamethizole		271	Unit	Import from Database Browser	4	7	Positive
					Update DMRM Method			
					Cut			
					Сору			
					Paste			
					Paste from Clipboard			
					Fill Down			
					Fill Column			

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

Cł	romatogram Type	Label	Extracted Masses	Precursor Ion	Product Ion	Excluded Masses	Offset	Y-Range
	TIC	TIC					0	1E+07
	MBM	MBM		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
	TIC MBM	MS2BPC		350			0	1000
	EIC MS1EIC MS2EIC BPC							

- **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.

Acquisition Source Chromatogram Instrument Diagnostics	
Available instrument curves Capilagy Current Gaplagy Current Bas Pow High Yoc MS1 Heater Pump1 Current Pump2 Current Sheash Gas Temp Turbo2 Speed	

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.

Quali	tative Analysis Method	
	C:\MassHunter\Methods\default.m	
	Copy	
	Change to 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.

/ Quant	Automation		
Quant	itative Analysis Meth	od	
	Select a method		
	Uink	🕞 Сору	
	Change to Method:	$\label{eq:c:MassHunter} C: \label{eq:MRM_to_DMRM_MRM_to_DMRM.quantmethod xml} C: \label{eq:MRM_to_DMRM_to_DMRM.quantmethod xml} \\$	
	View Method		
- 🔽 G	enerate Report		
	Template File:	$\label{eq:c:MassHunter} C:MassHunter\ensuremath{Report}\ensuremath{Templates}\ensuremath{Quant}\ensuremath{unter}\ensuremath{ESTD}\ensuremath{Parts}\ensuremath{Grapl}\ensurema$	
	Print to default p	rinter	
	Publish	CSV -	

Step 4. Set up and run an acquisition method

- **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.

Sample Run				
Sample	Addtio	nal Information		
Name Sample1 Position v		Parameter Name	Parameter Value	-
wawe Sauble1 Lorenu I/0 Lifector		Sample ID		
Injection Volume As Method - uL		Override DA Method	C:\MassHunter\Methods\PAH_Link	*
Comment		Sample Type	Sample	
Comment		Method Type	Acquisition Only	
Data File		Balance Override	No Override	
		Equilib Time (min)	0	
V Auto Increment	•	RunType	Standard Run	+
Name Bomple0006 d View Data				
Path C:\MassHurter\Data				*
ran u masmute u aa				
Worklist Method Editor Sample Run				

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 (

- **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.

Step 4. Set up and run an acquisition method

Add <u>M</u> ultiple Samples Add Sample	ample Position			*
Add Script		Method	Data File	Sample Type Sample
Add Copied Row(s) Delete Row(s) Add Colymn(s) Show/Hide/Order Columns Text Size	-			
Add Worklist Worklist <u>R</u> un Parameters Import Worklist <u>W</u> rap	-			
✓ Trac <u>k</u> Worklist Run			,	₩orklist

- c Click Add Multiple Samples. The Add Multiple Samples dialog box opens.
- \mathbf{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).

Step 4. Set up and run an acquisition method

Sample Information Sample F	Position
Current Configuration	
Select Well-plate or Vial Tray	Plate/Tray Type
Select Tray	 *96Agilent*
Well-plate Tray	E Selection Origin Block Increment
Plate 1 Plate 2	
Vial Tray \ALS	
Tray A	C Column majo
Tray B	C Serpentine
CTCPAL	Number of samples Number of replicates
Plate 1 Plate 2	B 1
Plate 2 Plate 3	+ lo li
A (1) (2) (3) (4 B (3) (6) (7) (4)	
6 (3) (5) (7) (6) 6 (3) (7) (7) (7) (7) (7) (7) (7) (7) (7) (7	

- **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.
- 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.

Step 4. Set up and run an acquisition method

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

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.

www.agilent.com

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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Printed in USA Revision A, November 2012



G3335-90137

