

Agilent Triple Quadrupole Mass Spectrometer (Model K6460 and K6420)

Quick Start Guide

For In Vitro Diagnostic Use

The K6460 and K6420 mass spectrometers are intended to be used to identify inorganic or organic compounds in human specimens by ionizing the compounds and separating the resulting ions by means of electrical field according to their mass.

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Use this guide for your first steps with the Agilent Triple Quadrupole Mass Spectrometer, and as a road map for your user information.

What is the Agilent Triple Quadrupole Mass Spectrometer?

The Agilent Triple Quadrupole Mass Spectrometers are liquid chromatograph mass spectrometers 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 multiple MRM transitions by using Dynamic MRM.

Model K6460 is shipped by default with the Agilent Jet Stream Technology (AJS) 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 K6460 and K6420 can be used with the Agilent K1260 Infinity LC system. Also, the K6460 and K6420 comes with Agilent MassHunter Workstation software that includes three major programs:

- LC/QQQ 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.

Recycling and disposal

This device is designed to accommodate recycling at the end of its useful life. Please dispose of this device in accordance with local regulations. Contact Agilent Technologies for more information on recycling and disposal.

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 Resource App if it is installed. Some of these guides are also included with your system in printed format.

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

MassHunter Workstation LC/QQQ Acquisition Familiarization Guide Do the exercises to learn to use the K6460 and K6420 instrument and LC/QQQ Acquisition program.

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

MassHunter Optimizer 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 Quadrupole instrument for each individual compound analyzed.

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

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

Where to find information

Training

MassHunter Workstation LC/QQQ Acquisition eFamiliarization Guide Use this online guide to learn how to use the LC/QQQ Acquisition program.

MassHunter Workstation Qualitative Analysis eFamiliarization Guide Use this online guide to learn how to use the Qualitative Analysis program.

MassHunter Workstation Quantitative Analysis eFamiliarization Guide Use this online guide to learn how to use the Quantitative Analysis program.

Agilent K6460 and K6420 Triple Quad Mass Spectrometer Hardware eFamiliarization Learn the background information to help you understand operation of the mass spectrometer.

Maintenance Guide (animated) Use this animated guide to maintain and troubleshoot your K6460 and K6420.

MassHunter Workstation Administration Guide This guide includes administration and troubleshooting tasks for your Triple Quadrupole Mass Spectrometer.

Safety Guide This guide contains safety and conformity information for your Triple Quadrupole Mass Spectrometer.

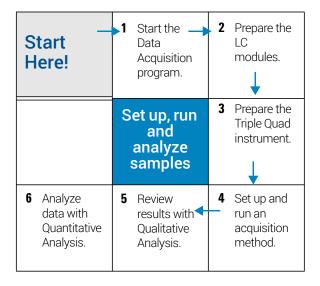
Training

Familiarization Guide Use the familiarization and eFamiliarization guides to get to know the MassHunter LC/QQQ Acquisition, Qualitative Analysis, and Quantitative Analysis programs.

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

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 LC/QQQ Acquisition program

This task assume that:

- The hardware and software are installed.
- The instrument is configured.
- The LC modules and the mass spectrometer are turned on, but the LC pump is not running.

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

The Data Acquisition window appears.

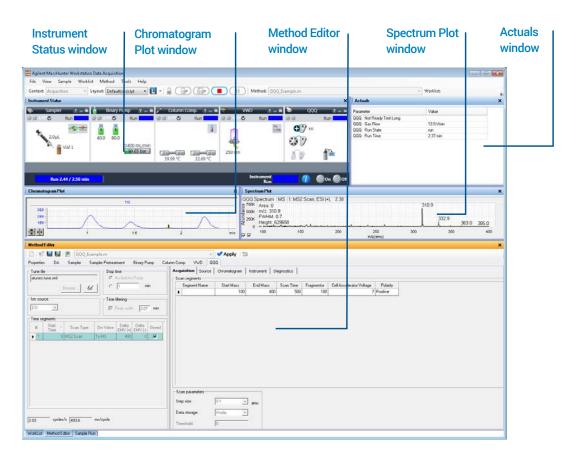
NOTE

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 *Administration 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 windows in 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 displayed as tabs 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 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 when you right-click the title of the window and click **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 the current status of each device both as text and by its color-coding:

Table 1 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. See "Set up to view real-time parameter values (actuals)." on page 13 for more information.

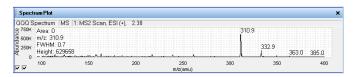


Step 1. Start the LC/QQQ Acquisition program

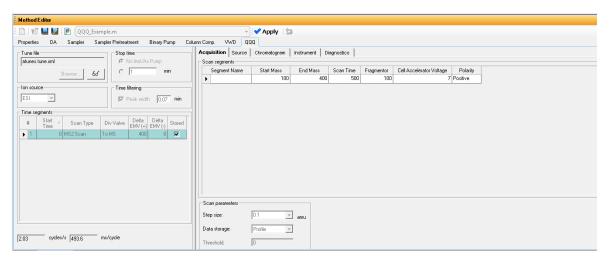
Chromatogram Plot window With this window you monitor the chromatogram plots in real time. These plots can be user-defined signals and/or instrument parameters. You select the plots in the Chromatogram tab in the QQQ tab in the Method Editor window.



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

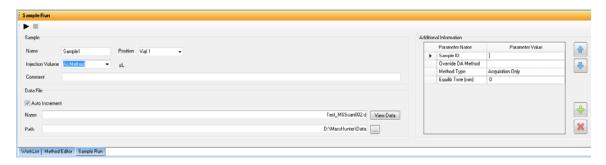


Method Editor window With this window you enter acquisition parameters for the method. If you click in the QQQ tab, then you can see the tune values in the Tune Parameters dialog box.

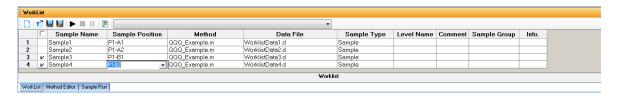


Sample Run window With this window you enter sample information to run individual samples interactively, and you can start a single sample run. You can also specify an **Override DA Method** and select either **Both Acquisition and DA** or **DA Only** for the **Method Type**, and then Data Analysis is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

Step 1. Start the LC/QQQ Acquisition program

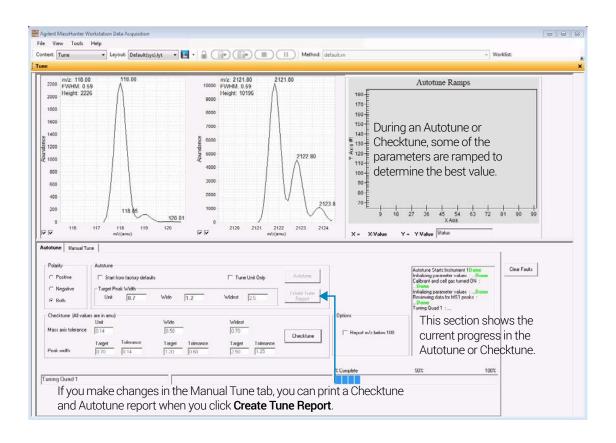


Worklist window With this window you enter sample information for multiple samples. When you run the worklist, the samples are automatically run in the order listed in the worklist. You can select whether to run **Acquisition Only**, to run **Both Acquisition and DA**, or to run **DA only** by selecting 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. You have to switch to the Tune Context to see this window.

Step 1. Start the LC/QQQ Acquisition program



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 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.

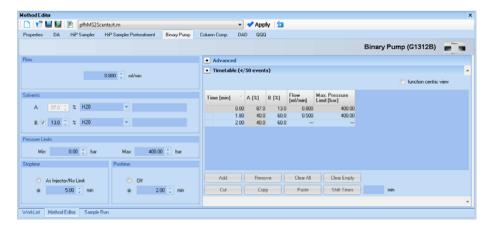
a Right-click the QQQ device in the Instrument Status window.



- b Click LC > Waste.
- 2 Purge the LC pump.

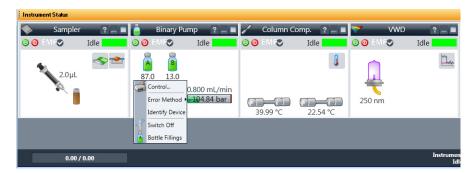
Follow the directions for purging the pump in the User Guide for your pump.

- **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.



Step 2. Prepare the LC modules

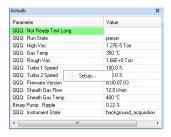
b Right-click an LC module in the Instrument Status window and click one of the commands to change any non-method control parameters, if necessary.

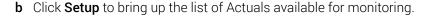


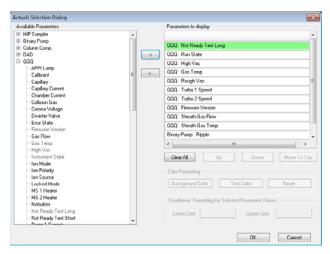
- **c** Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See **step 4** and **step 5** on **page 13**.)
- 4 Set up to view real-time parameter values (actuals).

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.



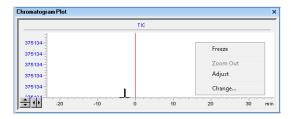




- c Add all the parameter values you intend to monitor, and click OK. Parameters that you may want to monitor include MS values (such as heater, and vacuum pressure) or LC values (such as binary pump, column, etc.) You can set the background and text color for each parameter. You can also set a range 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.

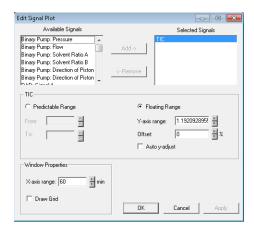
As you condition the column, you can set up the displays to monitor the effluent.

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



Step 2. Prepare the LC modules

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



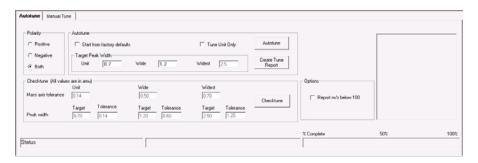
Step 3. Prepare the Triple Quad instrument

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 approximately 3 minutes to run for each polarity.

Do a **Checktune** regularly.

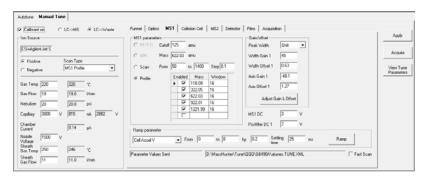
You can run a **Checktune** with the ESI or AJS.

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

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

- **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.

Step 3. Prepare the Triple Quad instrument



- 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). The system automatically changes different tune parameters to tune the MS. You only do an Autotune when it is necessary.

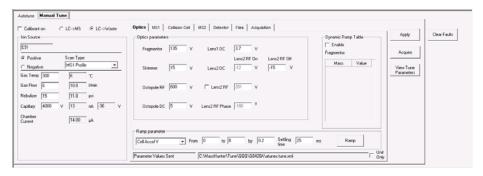
You can run an Autotune with only the AJS and ESI sources.

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).

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 Quadrupole 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.

Step 3. Prepare the Triple Quad instrument



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

Switch LC stream to MS

 After you condition the column and tune the Triple Quad MS, you switch the LC stream from Waste to MS. See "Switch LC stream to Waste." on page 12 for how to do this.

Monitor MS baseline and spectral displays

Make sure that the Triple Quadrupole baseline is stable, and no spectra of interfering intensity appear in the display.

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

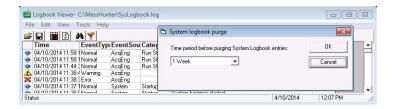
View the system logbook for events and errors

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 (in the toolbar of the Data Acquisition window, and view the logged events.
- Or click Tools > System Logbook Viewer.
- Or right-click 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.

Step 3. Prepare the Triple Quad instrument

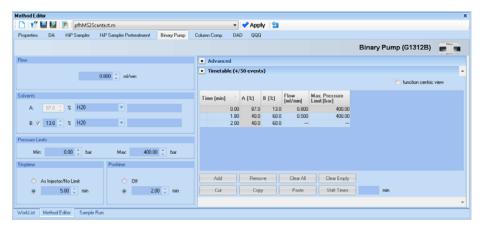


Step 4. Set up and run an acquisition method

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

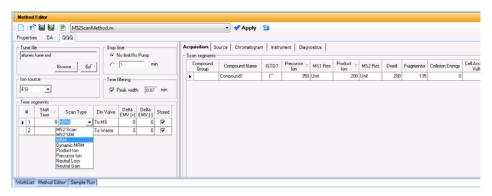
Make sure when you type the MS parameters on the next page that the tune file is the one that you want to use with the acquisition.

- 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**.

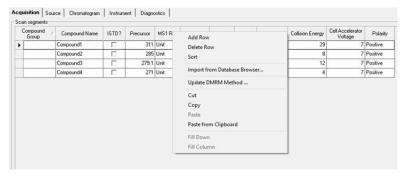
If you are changing the **Scan Type** from **MRM** to **Dynamic MRM** or to **Triggered MRM**, you can copy and paste the transitions from the original **Scan segments** table to the Clipboard and then to the new **Scan segments** table. See the *online Help* for more information.

Step 4. Set up and run an acquisition method

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

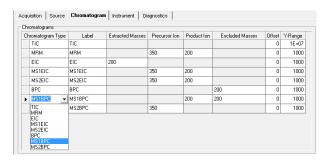


- **4** Set up to change Triple Quad MS parameters with segments and scans:
 - **a** To add a segment, right-click anywhere in the **Scan segments** section to bring up the Scan Segments shortcut menu, and click Add Row.
 - **b** Type the parameters for each Scan segment.

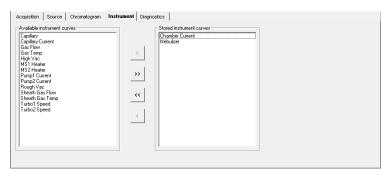


- **5** Set up signals for the Chromatogram plot:
 - a Click Chromatogram.
 - **b** Select the Chromatogram Type, and type other plot values.

Step 4. Set up and run an acquisition method



- 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 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 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.

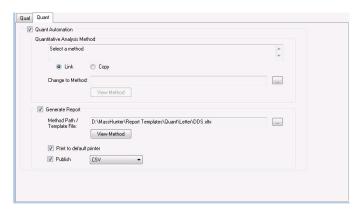
- a Click DA.
- **b** Optional. Mark the **Qual Automation** check box. The name of the current Qualitative Analysis method is shown in the box. If you want to change the Qualitative Analysis method that is connected, click to select a different method. When the Data Acquisition method is saved, the

Step 4. Set up and run an acquisition method

Qualitative Analysis method that you selected is copied or linked to the Data Acquisition method.



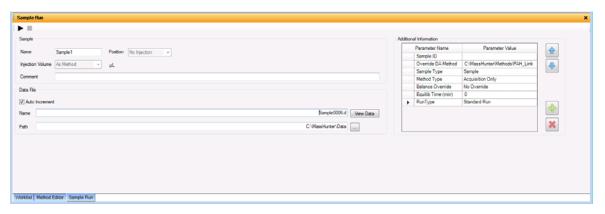
- **c** Optional. Click **Quant**. Mark the **Quant Automation** check box. The name of the current Quantitative Analysis method is shown in the list. If you want to change the Quantitative Analysis method that is connected, click to select a different method. When the Data Acquisition method is saved,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



- 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.

Step 4. Set up and run an acquisition method

- **9** Save the method.
 - a Click Method > Save As or Method > Save.
 - **b** If necessary, name the method and click **OK**.
- 10 Set up and run interactive samples:
 - **a** Click the **Sample Run** window. By default, it is a tab that 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.



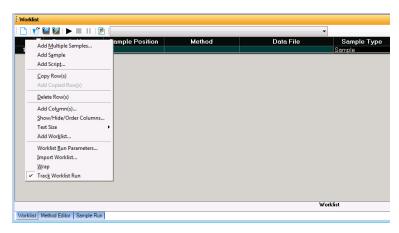
You can run a Data Analysis method from this window by selecting **Both Acquisition and DA** or **DA Only** for the **Method Type**. In addition, you have to set **Override DA method** to indicate which Data Acquisition method contains the DA (Data Analysis) method to execute. You always have to do this.

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

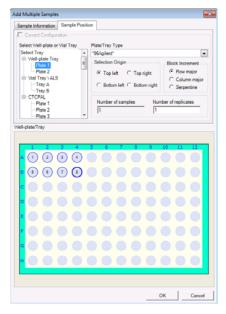
You can run the single sample in either locked or unlocked mode. When the mode is locked, no one can change the method or sample parameters during a run. You also cannot overwrite this data file in the Data Acquisition program. The Lock button () in the main toolbar indicates that locked mode is on.

- 11 Set up and run worklists
 - a Click 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



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

Step 4. Set up and run an acquisition method

- 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 **OK**.
- To start the worklist, click Run Worklist (→) in the main toolbar or Run (→) in the Worklist window

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.

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 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. Analyze data with the Quantitative Analysis program

Another primary tool for analyzing and reporting K6460 and K6420 results is the Quantitative Analysis program.

- Do the exercises in the Quantitative Analysis Familiarization Guide to learn how to guantitate 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 takes a quick look at using the MassHunter LC/QQQ Acquisition program to:

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

This guide supports these MassHunter Workstation revisions:

- LC/QQQ B.08.03
- Qualitative Analysis B.08.01
- Quantitative Analysis B.08.01

For In Vitro Diagnostic Use

Manufactured for:
Agilent Technologies Inc.
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www.agilent.com

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May 2018



