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 Quad Mass Spectrometer (Model K6460 and K6420), and as a road map for your user information.
What is the Agilent Triple Quad Mass Spectrometer (Model K6460 and K6420)?

The K6460 and K6420 MS are liquid chromatograph triple quadrupole 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 up to 4000 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 MS can be used with the Agilent K1260 Infinity LC system. Also, the K6460 and K6420 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.

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. They are also available at www.agilent.com.

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.

MassHunter Offline Installation Guide  This guide provides instructions to install Data Analysis software on a computer other than the one used to acquire data. It also supplements the Upgrade Guide and Installation Guide.

Animated Maintenance Guide  Use this animated guide to help maintain and troubleshoot your K6460 and K6420 MS.

Mass Spectrometer eFamiliarization Guide  Learn the background information to help you understand operation of the mass spectrometer.
Where to find information

Training

Data Acquisition for 6400 Series Triple Quadrupole LC/MS Familiarization Guide
Do the exercises to learn to use the K6460 and K6420 MS hardware and Data Acquisition program for K6460 and K6420 MS.

MassHunter 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.

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 MassHunter Data 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.
Getting Started

Install the K6460 and K6420 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.

<table>
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<th>Start Here!</th>
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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.

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

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

Windows—where you do most of your work

When you first start the Data Acquisition program, the main window appears. You do almost all of your work within the 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.
Getting Started
Step 1. Start the Data Acquisition software

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.
**Getting Started**

Step 1. Start the Data Acquisition software

**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>
<thead>
<tr>
<th>Color</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>Error</td>
</tr>
<tr>
<td>Yellow</td>
<td>Not ready</td>
</tr>
<tr>
<td>Purple</td>
<td>Pre-run, Post-run, Waiting</td>
</tr>
<tr>
<td>Blue</td>
<td>Running, Injecting</td>
</tr>
<tr>
<td>Green</td>
<td>Idle</td>
</tr>
<tr>
<td>Dark gray</td>
<td>Offline</td>
</tr>
<tr>
<td>Light gray</td>
<td>Standby (for example, lamps off)</td>
</tr>
</tbody>
</table>

**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.
Getting Started
Step 1. Start the Data Acquisition software

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.

![Chromatogram Plot Window]

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

![Spectrum Plot Window]

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

![Method Editor Window]

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 Window]
Getting Started
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.

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.
Getting Started
Step 1. Start the Data Acquisition software

This section shows the current progress in the Autotune or Checktune. During an Autotune or Checktune, some of the parameters are ramped to determine the best value.

If you make changes in the Manual Tune tab, you can print a Checktune and Autotune report when you click Create Tune Report.
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 the Apply button 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 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.
Getting Started
Step 2. Prepare the LC modules

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

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

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 ESI.

   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 the Manual Tune tab.
   b. If the failure occurred in MS1, click the MS1 tab. Select the Peak Width based on which resolution failed in Checktune. You can select Unit, Wide, or Widest. Then, click the Adjust Gain & Offset button.
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 the **Adjust Gain & Offset** button.

**d** If the Adjust Gain and Offset passes successfully, then save the autotune file. Click **Manual Tune**. Then, click the **Files** tab, and click **Save**.

**e** Click the **Autotune** tab.

**f** Click the Checktune button.

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.
Getting Started
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 the Log icon ( ) in the toolbar of the Data Acquisition window, and view the logged events.
- Or click Tools > System Logbook Viewer.
- Or right-click the LOG 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. The System logbook purge dialog box opens.
Getting Started

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.

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.
Getting Started
Step 4. Set up and run an acquisition method

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.

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

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

   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 the DA tab.

   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 the button to select a different method. When the Data Acquisition method is saved, the
Getting Started
Step 4. Set up and run an acquisition method

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

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.

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.

Set up the Properties for this method.

Click the Properties tab.

Click the button to select the Pre Run Script.

Click the button to select the Post Run Script.

Type the Description for this 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 tabbed 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 the Run button (▶) in the Sample
   Run window or the Run button (▶) in the main toolbar.

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

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

d Enter all the information on the Sample Information tab.

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

f Specify the locations, and click the OK button.

g To set up the worklist run, right-click the upper left corner of the worklist, and click Worklist Run Parameters.
Getting Started
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 the OK button.
l To start the worklist, click the Run Worklist button ( ) in the main toolbar or the Run button ( ) 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 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 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 takes a quick look at using the MassHunter Data 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:

• Data Acquisition B.08.00 SP1
• Qualitative Analysis B.07.00 SP2
• Quantitative Analysis B.07.01 SP1

For In Vitro Diagnostic Use

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