Agilent Ultivo Triple Quadrupole LC/MS System

Quick Start Guide

Use this guide for your first steps with the Agilent Agilent Ultivo Triple Quadrupole LC/MS system, and as a road map for your user information.

What is the Agilent Ultivo Triple Quadrupole LC/MS system?

The Agilent Ultivo 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 selects 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 Ultivo LC/TQ instrument is shipped by default with the Agilent Jet Stream source that uses a super-heated sheath gas to collimate the nebulizer spray, which dramatically increases the number of ions that enter the mass spectrometer.

You can set up an Ultivo LC/TQ with the Agilent 1100, 1200, 1260 or 1290 Infinity, 1260 Infinity II, or 1260 Infinity II Prime LC modules

Also, the Ultivo LC/TQ comes with Agilent MassHunter Workstation Software that includes three major parts:

- Data Acquisition – From one program you can tune the mass spectrometer, control and monitor instrument parameters, set up acquisition methods and worklists containing multiple samples and monitor real-time run plots.

- Quantitative Analysis – From one program you can set up a batch of data files and quantify, evaluate and requantify the results. From this program you have access to the Method Editor for setting up and editing the quantitation methods. You can run this program in Classic or configurable Quant-My-Way mode.

- Qualitative Analysis – From the Qualitative Analysis Navigator program you can extract and integrate chromatograms, subtract background, extract peak spectra, identify spectra, and compare data from different types of data files. From the Qualitative Analysis Workflows program, you can find compounds using a variety of algorithms and identify those compounds.

What's New in Data Acquisition

The Data Acquisition program for the Ultivo LC/TQ has many new features in this revision.

in C.01.00

- Only the Ultivo LC/TQ is supported. Other models are not supported.
- The user interface in the Method Editor has been updated.
- Autotune and Checktune are run from the Autotune section in the Method Editor.
- You can add a timetable to the method to change the diverter valve.
- The diverter valve position is not changed when you download a method.

Where to find information

Help

Press F1   To get more information about a pane, window or dialog box, select 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 **Ultivo LC/TQ Resource App**.

Some of these guides 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.

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 *Installation Guide*.

Maintenance Guide (animated)   Use this animated guide to help maintain and troubleshoot your Ultivo LC/TQ.

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

MassHunter Acquisition Familiarization Guide   Do the exercises to learn to use the Ultivo LC/TQ and the MassHunter Acquisition program.

MassHunter Acquisition eFamiliarization Guide   Follow the eFamiliarization guide to get to know the MassHunter Acquisition program.

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

MassHunter Optimizer Software Quick Start Guide   Use this guide to learn about the MassHunter Optimizer program. The MassHunter Optimizer program provides a way to automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) on an Ultivo LC/TQ instrument for each individual compound analyzed.

User Management and Audit Trail Quick Start Guide   Use this guide to learn how to use the User Management and Audit Trail features of the MassHunter Acquisition program.

You can access these guides from the **LC/MS Data Analysis Resource App**.

Qualitative Analysis for Triple Quad LC/MS Familiarization Guide   Do the exercises to learn to use the Qualitative Analysis programs.

Qualitative Analysis eFamiliarization   Use the interactive guide to learn to use the Qualitative Analysis program.

Quantitative Analysis Familiarization Guide   Do the exercises to learn to use the Quantitative Analysis programs.

Quantitative Analysis Audit Trail and Compliance Quick Start Guide   Use this guide to set up and use Quantitative Analysis compliance features, including audit trail and user management.

Quantitative Analysis eFamiliarization   Use the interactive guide to learn to use the Quantitative Analysis program in Classic mode.

Quant-My-Way Flavors Setup Familiarization Guide   Follow the guide to learn how to set up different Quant-My-Way flavors.

Quant-My-Way eFamiliarization   Follow the guide to learn to use the Quantitative Analysis program in Quant-My-Way mode.

Training

Familiarization Guides   Use the exercises in the Familiarization Guides with the MassHunter programs to learn the MassHunter Workstation.

eFamiliarization Guides   Use all of the interactive guides to get to know the software.

Quick Start Guides   Use the Quick Start guides for Study Manager, Optimizer and Audit Trail to get to know these programs or features.

Report Training DVD   Use the videos and familiarization exercises to learn how to print reports and customize reports using the Report Designer Add-in and Excel.

Training Courses   Visit **www.agilent.com** to view a listing of training courses for the Ultivo LC/TQ.

Getting Started

Install the Ultivo LC/TQ hardware and software

Use the *Installation Guide* to install the hardware and software and verify performance.

Set up, run, and analyze samples

The roadmap below shows you the steps to set up and run a batch of samples from start to finish. Follow the instructions on the next pages to get started and to learn where to find the information to help you with each step in this roadmap.
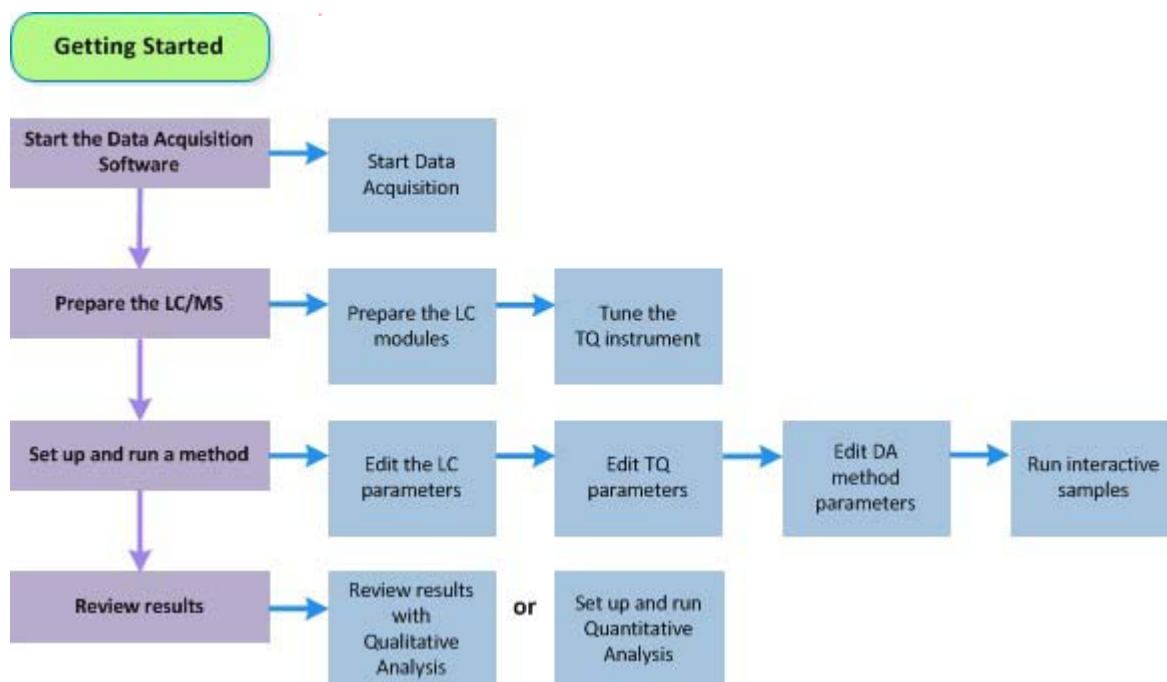
Step 1. Start the Data Acquisition software

The instructions below include the following assumptions:

• The hardware and software are installed.

• The instrument is configured.

Use instructions in the *Installation Guide* to configure the instrument for the first time and any time you change the LC configuration.

The LC modules and the Ultivo LC/TQ are turned on, but the LC pump is not running.

After installation, you see all of the MassHunter Workstation Software icons on your Desktop. To start the Data Acquisition program, double-click the Data Acquisition icon. In Windows 7 you can instead click **Start > Agilent > MassHunter Workstation > Data Acquisition**. In Windows 10 you can instead click the Windows icon and then click **Agilent > Data Acquisition**.

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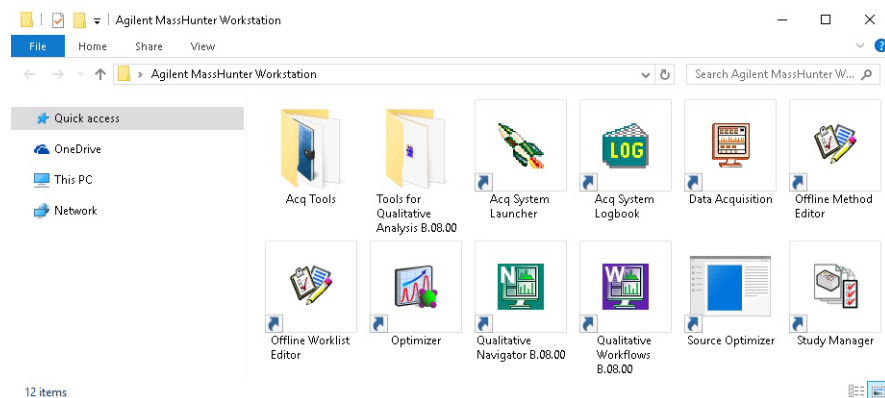
NOTE	When Data Acquisition opens, the software engines automatically start. If you need to restart them, right-click the Acq System Launcher icon in the system tray, and click Start Engines.

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

Windows—where you do most of your work

When you first start the Data Acquisition program, the main window appears. You do almost all of your work within the eight windows of this main window. These windows provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status, monitor runs and tune the instrument.

Instrument Status window   Chromatogram Plot window   Method Editor window   Spectrum Pane window   Actuals window
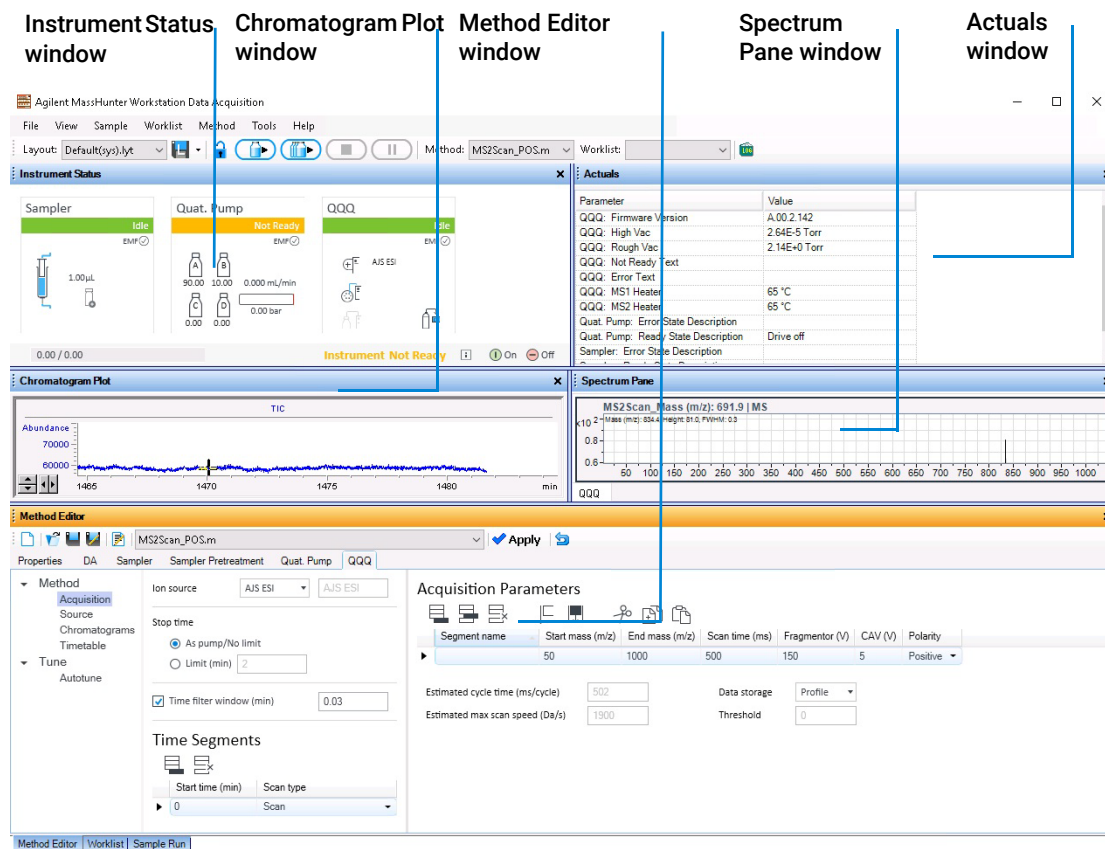
The Sample Run and Worklist windows are tabbed here. These three windows are "sharing" this space. You click the tab to switch to a different window.

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.

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.
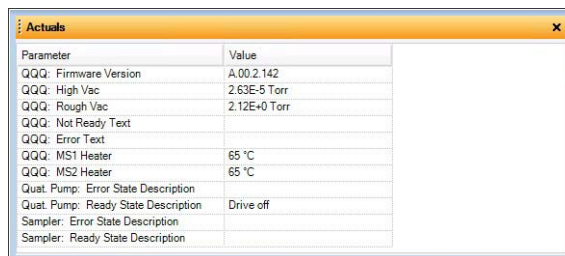
This window displays the current status of each device both as text and by its color-coding:
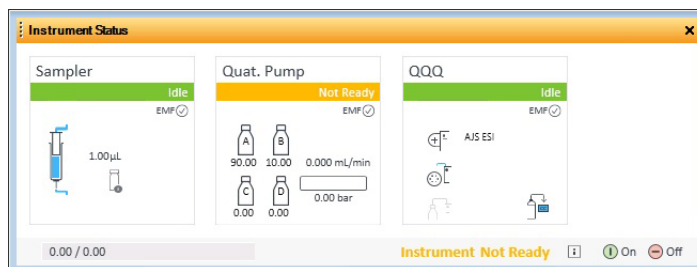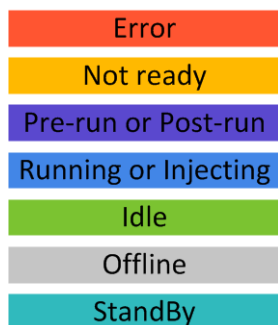
Actuals window   With this window you view the current value of selected instrument parameters. See **"Set up to view real-time parameter values (actuals)."** on page 14 for more information.

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 Chromatograms section in the QQQ tab in the Method Editor window.
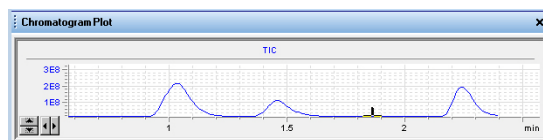

Spectrum Pane window   With this window you monitor the spectral plot in real time. This window shows spectra when you are acquiring data and when you are tuning the instrument. A separate tab is added for each detector that you have on the instrument. For example, if you have a Diode Array Detector, then a DAD tab is available in this window.
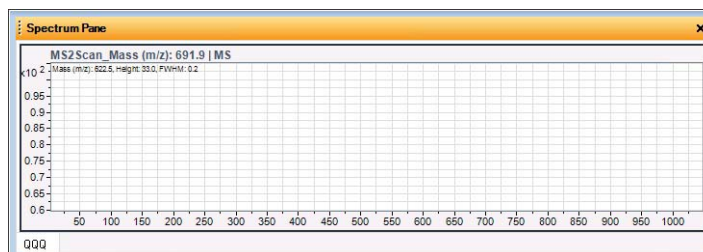
Method Editor window   With this window you enter acquisition parameters for the method. In the Autotune section, you can run either Checktune or Autotune.
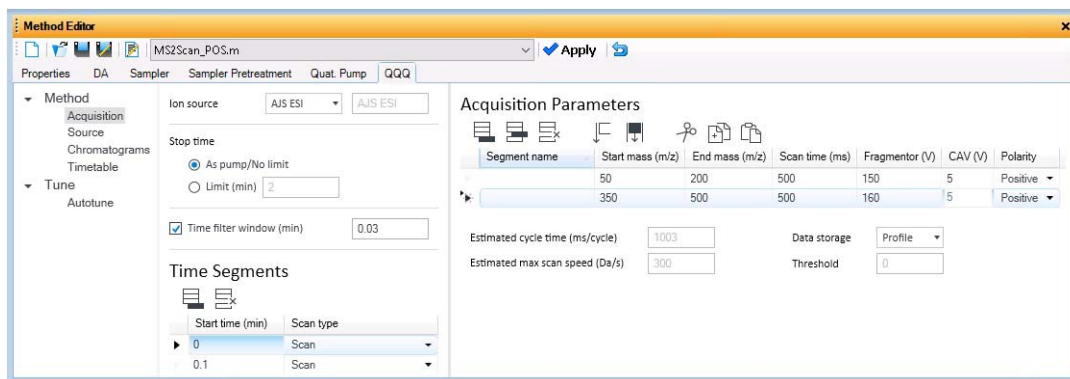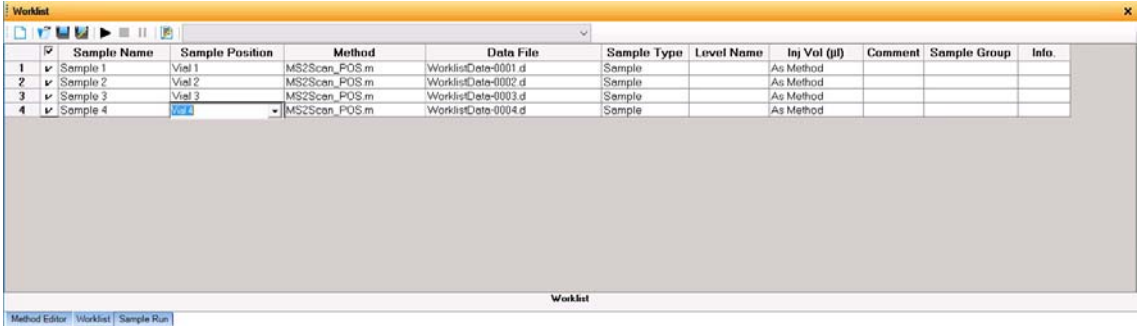
Sample Run window   With this window you enter sample information to run individual samples interactively, and you can start a single sample run. You can also specify an **Override DA Method,** and you can select **Both Acquisition and DA**, **Acquisition Only**, or **DA Only** for the **Method Type**. If you select either **Both Acquisition and DA** or **DA Only**, and then Data Analysis is run as part of the method.
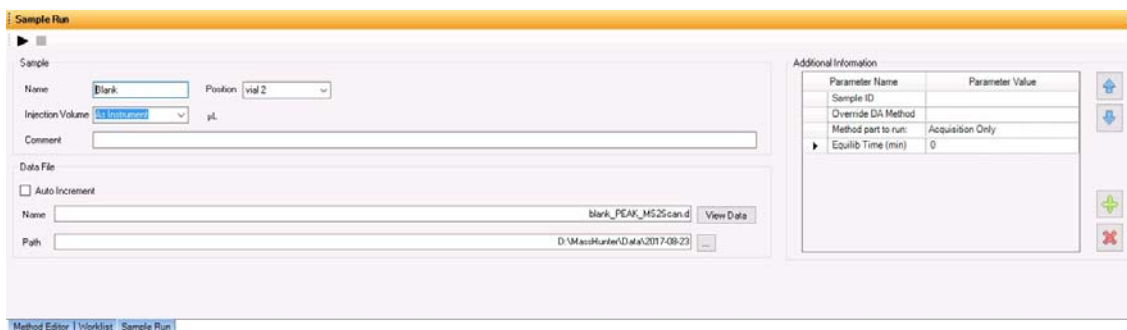
Worklist window   With this window you enter sample information for multiple samples. When you run the worklist, the samples are automatically run in the order listed in the worklist. You can select whether to run **Acquisition Only**, to run **Both Acquisition and DA**, or to run **DA only** by selecting one of these options for the **Part of method** to run in the Worklist Run Parameters dialog box.

Getting Started

Step 1. Start the Data Acquisition software

Agilent Ultivo Triple Quadrupole LC/MS Quick Start Guide

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 Ultivo LC/TQ.

1 Switch LC stream to **Waste**.

When you are not acquiring data, you switch the direction of the LC stream away from the MS ion source and to waste.

If you have the LC connected to a VWD or DAD, you can still monitor the fluctuations of the VWD or DAD real-time chromatogram before a run.

a Right-click the QQQ device in the Instrument Status window.
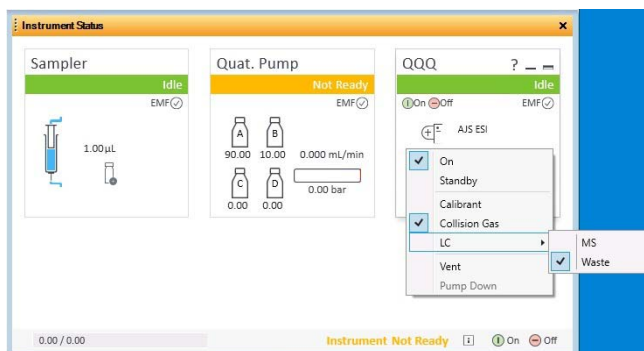
b Click **LC > Waste**.
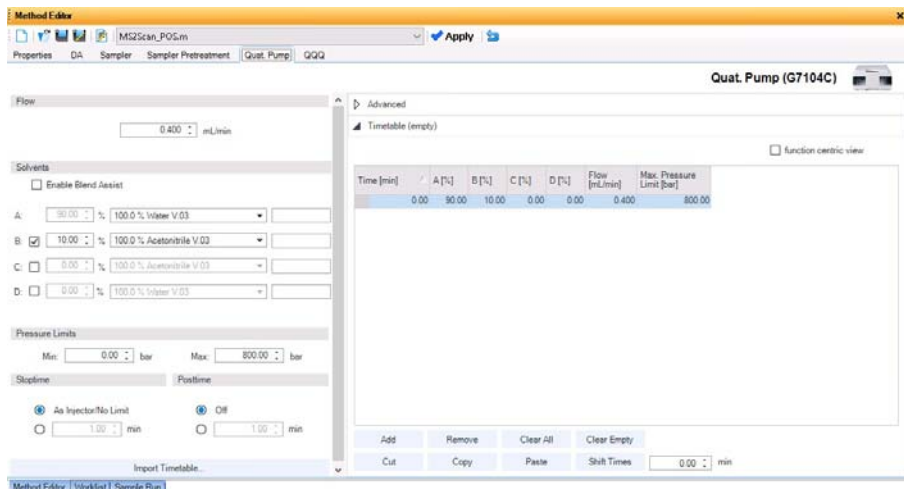
2 Purge the LC pump.

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

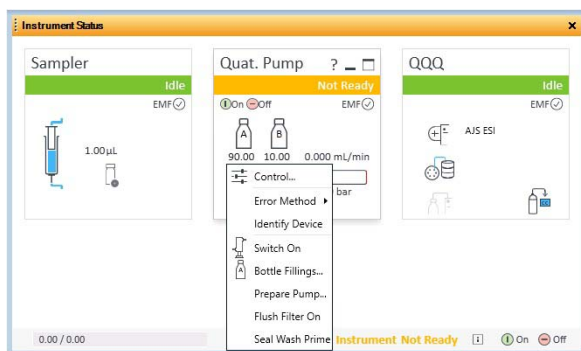**3** Set up to condition or equilibrate the column.

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

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 14**.)

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

As you prepare for a run and during a run, you want to see the actual values of the instrument parameters. You can do this in the Actuals window.

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

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

c Add all the parameter values you intend to monitor, and click the **OK** button. Parameters that you may want to monitor include MS values (such as heater, and vacuum pressure) or LC values (such as binary pump, column, DAD, etc.) You can set the background and text color for each parameter. You can also set a range for the parameters which are numbers. If the value of the parameter is not within the limits which you entered, then the background of the parameter is set to red.
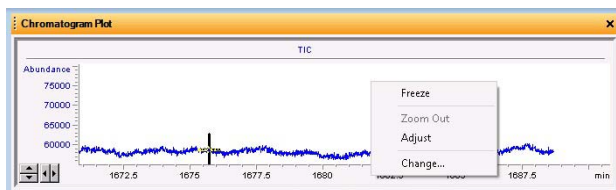
5 Set up real-time plot displays.

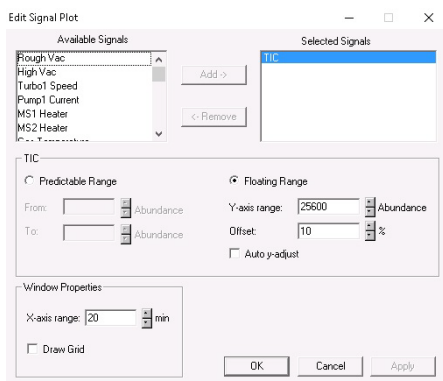As you condition the column, you can set up the displays to monitor the effluent.

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

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

Step 3. Prepare the Ultivo LC/TQ instrument

Do a Checktune or Autotune

1 Click the **QQQ** tab in the Method Editor window.

2 Click the **Autotune** section in the left pane of the QQQ tab.

3 Click 🔧 in the Autotune toolbar which locks the instrument to only allow Tune operations. You cannot start a run when the instrument is locked in Tune.
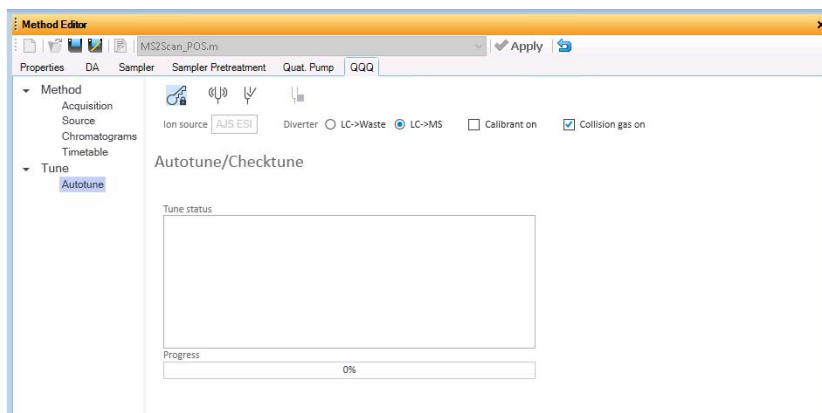
4 Click 🜋 to evaluate if the MS parameters are within the limits to produce the specified mass accuracy and resolution. Checktune takes approximately 3 minutes to run for each polarity.

Do a **Checktune** regularly.

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

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

If **Checktune** fails, then you can try using Autotune to fix the problem.

5 Click 📡 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 the following sources: ESI and AJS ESI.

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 Tune status list 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 you cannot get the instrument to tune successfully, then please call your Agilent service engineer.

6 Click ⛓ to unlock the instrument from Tune control.

Switch LC stream to MS

- After you condition the column and tune the Ultivo LC/TQ, 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 Ultivo LC/TQ baseline is stable, and no spectra of interfering intensity appear in the display.

If you did monitor the LC baseline with a VWD or DAD, you change back to the default Ultivo LC/TQ displays.

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

2 Select the MS signal, and click **OK**.

View the system logbook for events and errors

As you prepare the instrument, you may run into an error that you want to troubleshoot. You do this through the System Logbook Viewer.

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

When the System Logbook Viewer is open, you can select the time period to keep System Logbook entries. You can set the value from 1 week to 1 year. To do this, you click **Tools > Purge Settings**. The **System logbook purge** dialog box opens.
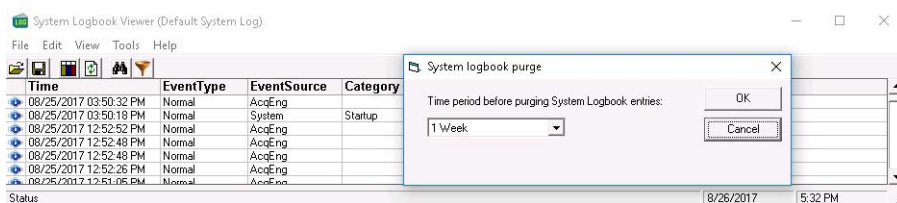
Step 4. Set up and run an acquisition method

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

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.
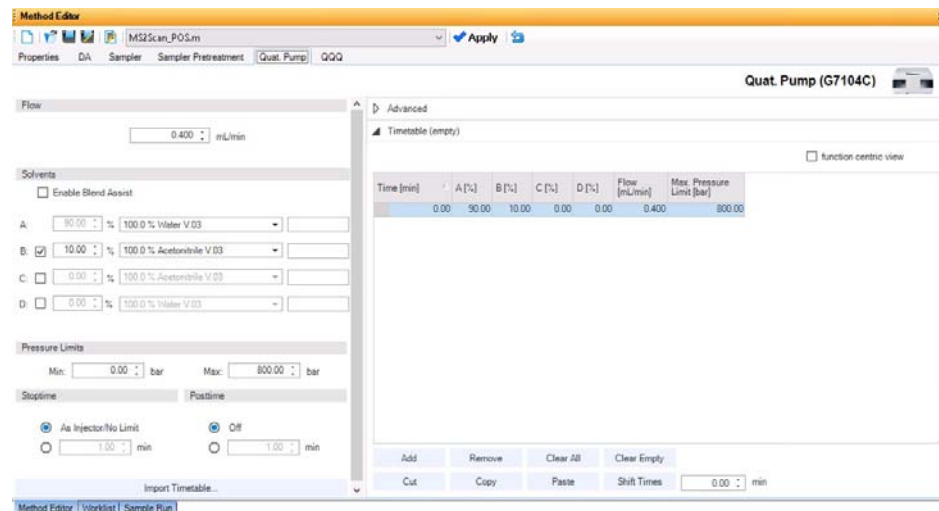
3 Enter the QQQ parameter values.

a Select the **Scan type** from the list in the **Time Segments** table. The Acquisition Parameters table is cleared when you change the **Scan type**. The parameters available on the right pane change depending on the **Scan type**.

If you are changing the Scan type from **MRM** to **dMRM** or to **tMRM**, you can copy and paste the transitions from the original **Acquisition Parameters** table to the Clipboard and then to the new **Acquisition Parameters** table. See the *online Help* for more information.

b Type in any Acquisition values you want to change. You can enter multiple rows in the **Acquisition Parameters**.
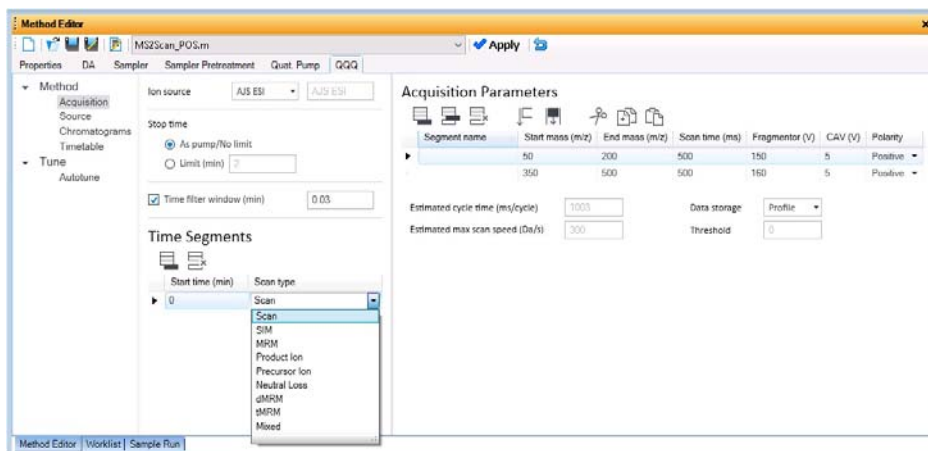
4   Set up to change Ultivo LC/TQ parameters with segments and scans:

a To add a segment, click 🔲 in the Acquisition Parameters toolbar.

b Type the parameters for each segment.
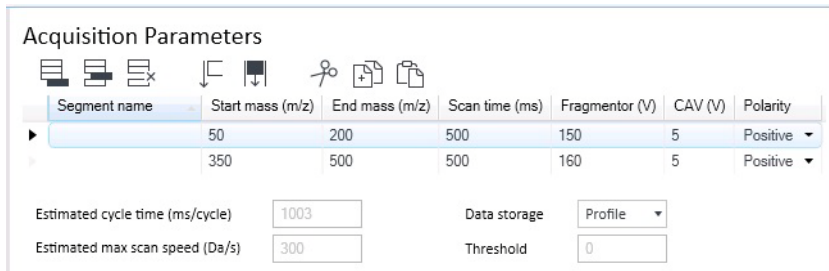
5   Set up signals for the Chromatogram plot:

a Click the **Chromatograms** section.

b Select the **Chrom type**, and type other plot values.

6 Set up the **Timetable** in the Timetable section.

   a Click the **Timetable** section.

   b Enter the **Start time (min)** and select **To MS**. You can select to start
      acquiring data after the start of the run.

   c (optional) Add another row to the Timetable. You can click ⊟ in the
      Timetable toolbar to add a row. You can then set the Diverter value to **To
      Waste** before the end of the run.

   d Mark or clear the **Post-run diverter position** check box. If you mark this
      check box, then you select either **To MS** or **To waste**.

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

   A method can contain Qualitative Analysis parameters, Quantitative Analysis
   parameters, or both. You can mark the **Qual Automation** check box on the
   Qual tab, and the **Quant Automation** check box 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
Qualitative Analysis method that you selected is copied or linked to the
Data Acquisition method.
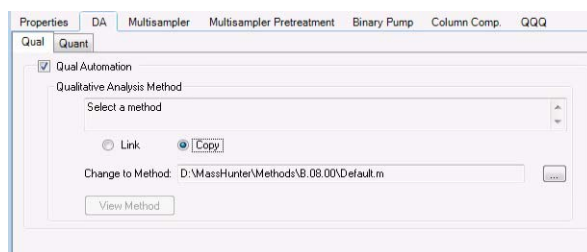
c *Optional.* Click the **Quant** tab. Mark the **Quant Automation** check box. The
name of the current Quantitative Analysis method is shown in the list. If you
want to change the Quantitative Analysis method that is connected, click
the ⎕ button to select a different method. When the Data Acquisition
method is saved, the Quantitative Analysis method that you selected is
copied or linked to the Data Acquisition method.

d *Optional.* Mark the **Generate Report** check box on the Quant tab. Then, you
select the **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.
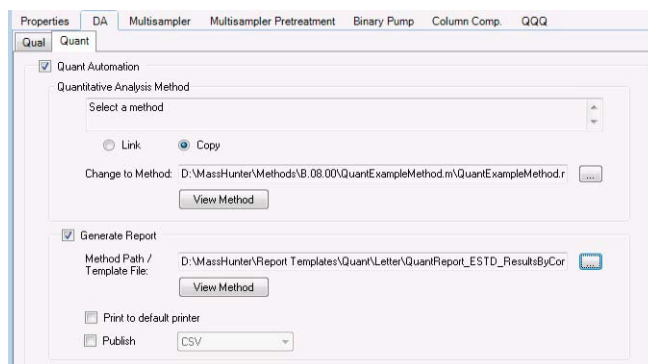

8 Set up the Properties for this method.

a Click the **Properties** tab.

b Click the ⎕ button to select the **Pre Run Script**.

c Click the ⎕ button to select the **Post Run Script**.

d Type the **Description** for this method.

9 Save the method.

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

   b If **User Management & Audit Trail** is enabled, enter the **Reason** for creating a new version of this method. Click **OK**.

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

10 Set up and run interactive samples:

   a Click the **Sample Run** window. By default, 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.
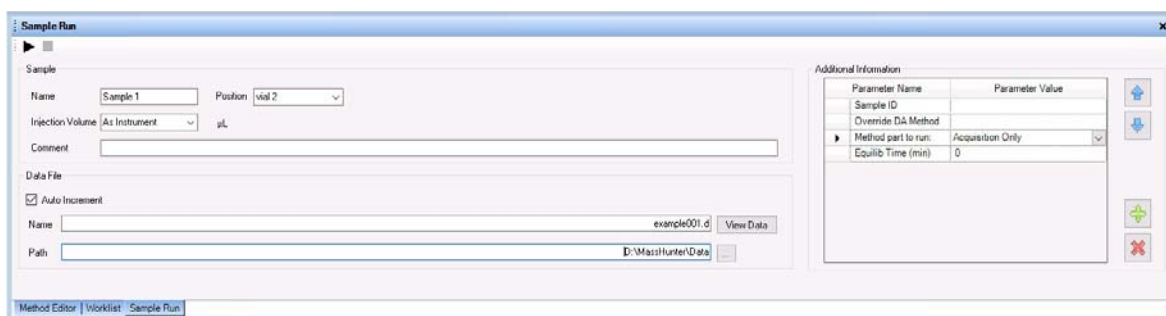

You can run a Data Analysis method from this window by selecting **Both Acquisition and DA** or **DA Only** for the **Method Type**. In addition, you have to set **Override DA method** to indicate which Data Acquisition method contains the DA (Data Analysis) method to execute. You always have to do this.

   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 instead click **Sample > Run**.

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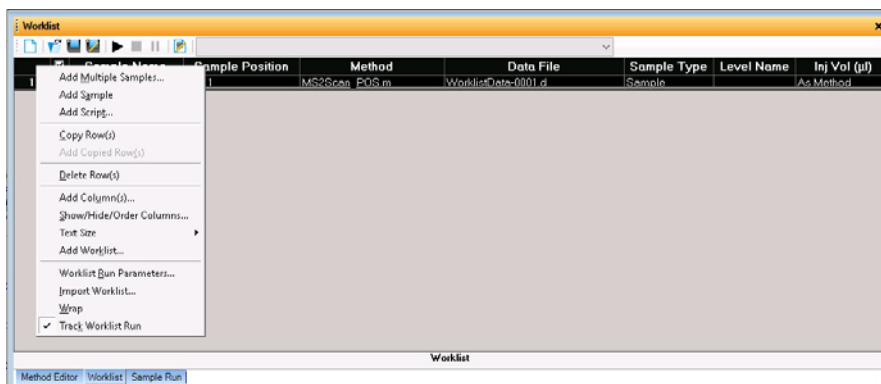
c Click **Add Multiple Samples**. The **Add Multiple Samples** dialog box opens.

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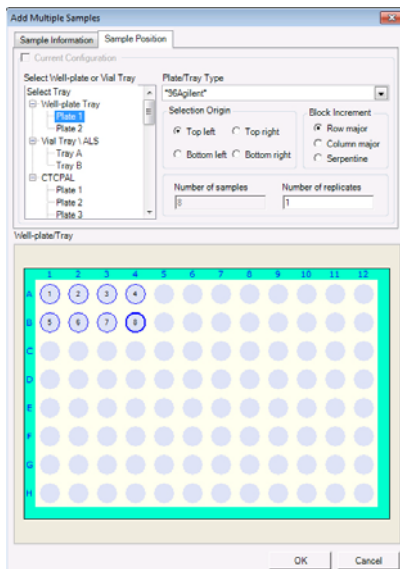
f Specify the locations, and click the **OK** button.

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

h On the **Run Parameters** tab, type the paths for the method.

i Click the **Data File Settings** tab.

j Type or select the folders for the data files. Select the File Naming options.

k Click the **Additional Parameters** tab.

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

m To start the worklist, click the Run Worklist button ( ) in the main toolbar or the Run button ( ) in the Worklist window. You can also click **Worklist > Run**.

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

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 Navigator and Qualitative Analysis Workflows programs to do these tasks and more:

- Review results for acquisition method development
- Select the most appropriate precursor and product ions for MRM analyses
- Find compounds
- Identify compounds
- Do molecular feature extraction

Do the exercises in the *Qualitative Analysis Familiarization Guide* to help you learn how to use the Qualitative Analysis program. View the *Qualitative Analysis eFamiliarization Guide for TQ* to get an overview of the software.

Do Exercise 1 of the Data Acquisition for Triple Quadrupole *Familiarization Guide* to help you learn how to use the Qualitative Analysis program to develop acquisition methods.

Also, refer to the *online Help* for the Qualitative Analysis software to learn how to do more operations to analyze your data.

Step 6. Analyze data with the Quantitative Analysis program

Another primary tool for analyzing and reporting Ultivo LC/TQ 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

Follow the instructions in the *Quantitative Analysis eFamiliarization Guide* to get an overview of the software.

In this book

This book contains brief instructions to help you get started with your Agilent Ultivo Triple Quadrupole LC/MS. This book takes a quick look at using the MassHunter Acquisition program to:

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