Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS

Quick Start Guide

Use this guide for your first steps with the Agilent 6200 Series Time-of-Flight and 6500 Series Quadrupole Time-of-Flight LC/MS system, and as a road map for your user information.

What is the Agilent 6200 Series TOF LC/MS system?

The Agilent TOF is an orthogonal acceleration time-of-flight mass spectrometer (oa-TOF). The ions reaching the time-of-flight chamber are impelled in a direction perpendicular to their original path, meaning that the acceleration pulse applied to send the ions down the flight tube is orthogonal to the direction that ions are entering the mass analyzer. This geometry minimizes the effect of the entrance velocity on the flight time, leading to higher resolution.

You can set up a TOF LC/MS with Agilent 1200, 1260, 1260 Infinity II, 1290 Infinity, or 1290 Infinity II LC modules and with one of several ion interfaces:

- ESI
- Dual ESI
- AJS ESI (Agilent Jet Stream ESI)
- Dual AJS ESI (Dual Agilent Jet Stream ESI)
- APCI
- APPI
- Multimode
- Nanospray (nanoESI)
- Dual nanospray (Dual nanoESI)
- MALDI
- GC-APCI

The 6230 Accurate-Mass TOF is the only TOF instrument that supports the Agilent Jet Stream ESI and the Dual Agilent Jet Stream ESI. The AJS ESI and Dual AJS ESI sources use a super-heated sheath gas to collimate the nebulizer spray which dramatically increases the number of ions that enter the mass spectrometer.

Each Agilent system has advantages for drug discovery, metabolomics, and environmental analysis — high throughput sample screening with highly sensitive detection and accurate mass assignment.

What is the Agilent 6500 Series Q-TOF LC/MS system?

The Agilent Q-TOF LC/MS is a liquid chromatograph-quadrupole time-of-flight mass spectrometer that performs MS/MS using a quadrupole, a hexapole collision cell and a time-of-flight analyzer to produce spectra. The quadrupole selects precursor ions that are fragmented in the collision cell into product ions, which are then impelled to the detector, at an angle perpendicular to the original path.

You can set up an Agilent Q-TOF LC/MS with Agilent 1200, 1260, 1260 Infinity II, 1290 Infinity, or 1290 Infinity II LC modules, and with one of several ion interfaces: ESI, Dual ESI, AJS ESI, Dual AJS ESI, APCI, APPI, Multimode, nanospray, dual nanospray, MALDI and GC-APCI.

The 6530, 6540 UHD Accurate-Mass Q-TOF, the 6545, 6546 Q-TOF, the 6545XT AdvanceBio Q-TOF, the 6550 iFunnel Q-TOF, and the 6560 Ion Mobility Q-TOF are the Q-TOF instruments that can be set up with the Agilent Jet Stream ESI source (AJS ESI) and the Dual AJS ESI. These sources use a super-heated sheath gas to collimate the nebulizer spray which dramatically increases the number of ions that enter the mass spectrometer.

What is the Agilent MassHunter Workstation Software?

The Agilent TOF and Q-TOF LC/MS comes with MassHunter Workstation Software that includes these programs:

*   Data Acquisition – From one application you can tune the mass spectrometer, control and monitor instrument parameters, set up methods containing acquisition parameters and worklists containing multiple samples and monitor real-time run plots.
*   Qualitative Analysis – From Qualitative Analysis, you can set up methods to extract and integrate chromatograms, extract peak spectra and compare data from different types of data files. More importantly, you can find compounds and generate formulas for those compounds. For more information on the Qualitative Analysis software, please refer to the *Qualitative Analysis Familiarization Guide for LC/MS, the Qualitative Analysis eFamiliarization Guide,* or the online Help for the Qualitative Analysis program.

- IM-MS Browser - From one application you can interactively browse and visualize data from a single LC-IM-MS data file, simultaneously examining the separation of complex mixtures in retention time, drift time, and m/z dimensions. You can visualize various 2D and 3D subsets of that data, export data in a variety of formats, and calculate collision cross sections.

- Quantitative Analysis - From one application you can set up a batch of data files and quantify, evaluate and requantify the results should you want to do this. For more information on the Quantitative Analysis program, please refer to the *Quantitative Analysis Familiarization Guide, the Quantitative Analysis eFamiliarization Guide,* or the online Help for the Quantitative Analysis program.

What's New

in 10.0

- The Agilent 6546 only is supported. No other TOF or Q-TOF mass spectrometers are supported in this release.
- Automated SureMass conversion is supported.
- Instrument configuration is now included in the data file for reference and traceability.
- You can specify session auto-lockout time in minutes in the System Configuration program if you enable **User Management and Audit Trail only**.
- Checksum validation is now extended to prevent usage of invalid methods, invalid worklists, and invalid studies if you enable **User Management and Audit Trail only**.
- When you start a worklist, it is validated before it is run, and all errors are shown in red.
- Version A.02.19 SR2 for the LC drivers is supported.

in B.09.00

- The Agilent 6545XT AdvanceBio Q-TOF mass spectrometer and the Agilent 6560 Ion Mobility Q-TOF mass spectrometer are supported.
- The Agilent 6550 iFunnel, 6545B, 6545A, 6540B, 6540A, 6538A, 6530C, 6530B, 6530A, and 6520B Q-TOF mass spectrometers are supported in this release.
- The Agilent 6230B, 6230A, and 6224A Accurate-Mass TOF mass spectrometers are supported in this release.
- Version A.02.19 for the LC drivers is supported.
- SWARM Autotune is available for the 6224 and 6230 TOF instruments.
- Spectral plots for different devices are shown in different tabs in the Spectrum Pane window.
- Instrument Configuration has been updated.
- Windows 7 and Windows 10 are supported.

For a list of updates made to previous versions, refer to the online Help.

Where to find information

Online Help

Press F1   To get more information about a window or dialog box, place the cursor on the window or dialog box of interest and press **F1**. In the Agilent MassHunter IM-MS Browser program, you instead click **Help > Contents**.

Help menu   From the Help menu, access "How-to" help and reference help.

Installation and User Guides

These guides are available from the **TOF and Q-TOF Resources** app. Some of these guides are included with your system in printed format. They are also available at **www.agilent.com**.

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

MassHunter Workstation Data Acquisition Familiarization Guide   Do the exercises to learn to use the 6200 Series TOF and 6500 Series Q-TOF LC/MS and Data Acquisition program.

MassHunter IM-MS Browser Quick Start Guide   (*For IM-QTOF only.*) Use this guide to learn to use the MassHunter IM-MS Browser software.

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

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.

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

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

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

MassHunter Qualitative Analysis eFamiliarization   Use this interactive online guide to learn to use the Qualitative Analysis programs.

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

Agilent 6000 Series LC/MS Hardware eFamiliarization   Use this interactive online guide to learn more about your 6200 Series TOF and 6500 Series Q-TOF LC/MS instrument.

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

MassHunter Workstation Administration Guide   This guide includes administration and troubleshooting tasks for your 6200 Series TOF and 6500 Series Q-TOF LC/MS.

Agilent 6000 Series LC/MS Safety Guide   This guide contains safety and conformity information for your 6200 Series TOF and 6500 Series Q-TOF LC/MS.

MassHunter BioConfirm Software Quick Start Guide   Learn how to use the BioConfirm software *(The Agilent MassHunter BioConfirm software can be purchased separately.)*

MassHunter BioConfirm Familiarization Guide   Do the exercises to learn how to use the BioConfirm software. *(The Agilent MassHunter BioConfirm software can be purchased separately.)*

MassHunter BioConfirm eFamiliarization Guide   Use this interactive online guide to learn how to use the BioConfirm software. *(The Agilent MassHunter BioConfirm software can be purchased separately.)*

MassHunter Mass Profiler Quick Start Guide   Learn how to use the Mass Profiler software *(The Agilent MassHunter Mass Profiler software can be purchased separately.) (This guide is in the Mass Profiler Resource app.)*

MassHunter Mass Profiler Familiarization Guide   Do the exercises to learn how to use the Mass Profiler software. *(The Agilent MassHunter Mass Profiler software can be purchased separately.) (This guide is in the Mass Profiler Resource app.)*

Training

Resource Apps   Use the material in the Resource Apps to learn to use your MassHunter Workstation software, to learn about the 6200 Series TOF and 6500 Series Q-TOF LC/MS instrument, and to maintain and troubleshoot your 6200 Series TOF and 6500 Series Q-TOF LC/MS.

Training Courses   Visit **www.agilent.com** to view a listing of training courses for the Series 6200 TOF and the Series 6500 Q-TOF LC/MS.

Getting Started

Install the TOF or Q-TOF LC/MS hardware and software

Use the *Installation Guide* for your instrument 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.
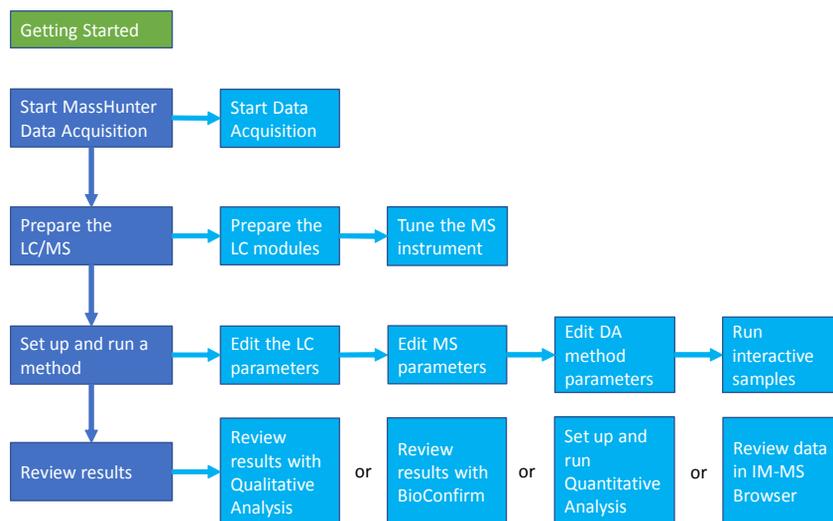
Step 1. Start the Data Acquisition software

The instructions below assume that:

- 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 LC/MS instrument are turned on, but the LC pump is not running.

After installation, you see all of the MassHunter Workstation program icons on your Desktop. (For B.09.00, Qualitative Analysis comprises two separate programs, so you will see two Qualitative icons.)

- To start Data Acquisition, double-click the **Data Acquisition** icon.

- In Windows 7, you can click **Start > Agilent > MassHunter Workstation > Data Acquisition**.

- In Windows 10, you can click **Start > Agilent > Data Acquisition**.
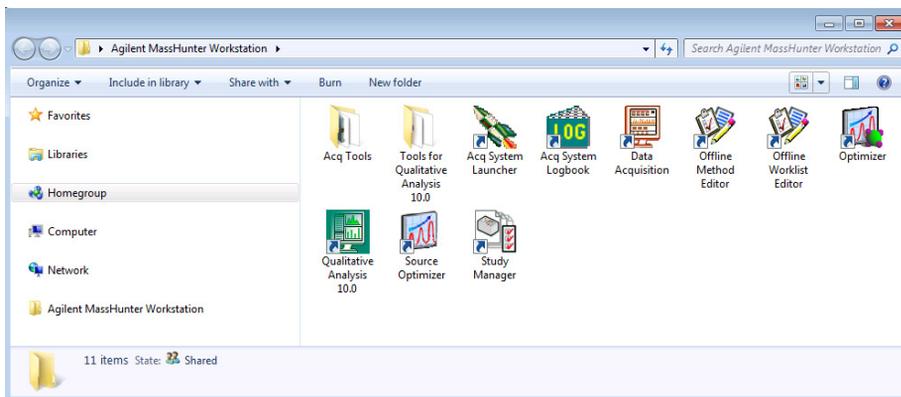
NOTE	When Data Acquisition opens, the software engines automatically start. If you need to restart them, right-click the **Acq System Launcher** icon in the system tray and click **Start Engines**.
	If you have recently changed LC modules, remember to configure the instrument again. See the *Installation Guide* for instructions.

The windows where you do most of your work

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

Figure 1. Main window of the Data Acquisition software. Method Editor, 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.

Show/hide the windows   You can show one window at a time on the screen or all 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 **X** 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. The possible values for Status are shown below. You also set non-method control and configuration parameters for the LC devices and the MS instrument. A shortcut menu is available for each device.

This window displays each device's current status both as text and by its color-coding:
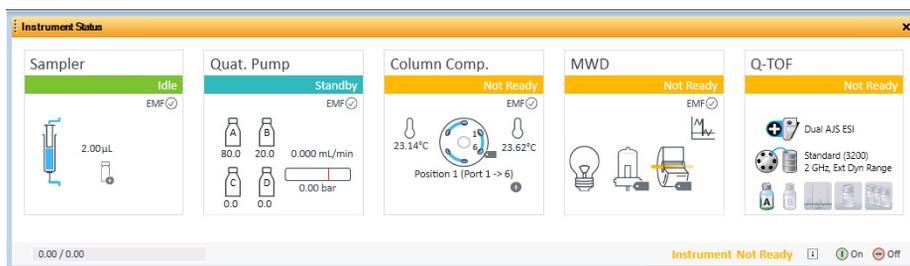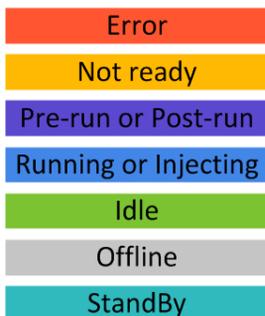
You can click **?** in any device pane to get help on that device. This button is visible when you hover over that device.

Actuals window   With this window you view the current value of selected instrument parameters. You can change the color choice and add conditional formatting in the **Actuals Selection** dialog box. See **"Set up to view real-time parameter values (actuals)."** on page 18.
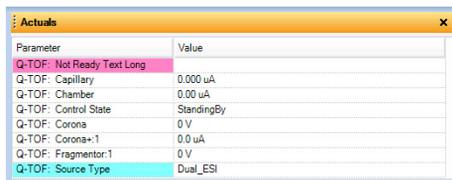
Chromatogram Plot window   With this window you monitor the chromatogram plots in real time. These plots can be user-defined signals and/or instrument parameters.
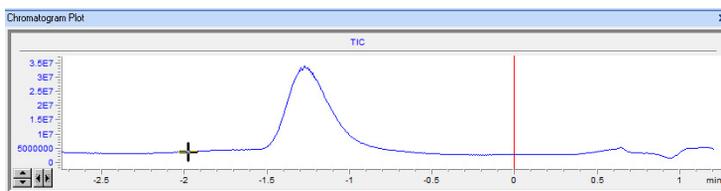
Spectrum Pane window   With this window you monitor the spectral plots in real time. Spectra from different devices are shown on different tabs.
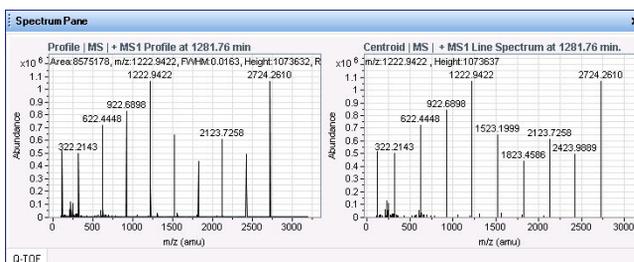
Method Editor window   With this window you enter acquisition parameters for the method. If you have an 6560 Ion Mobility Q-TOF, then the **Acquisition Mode** section is available under the **Stop Time** section.

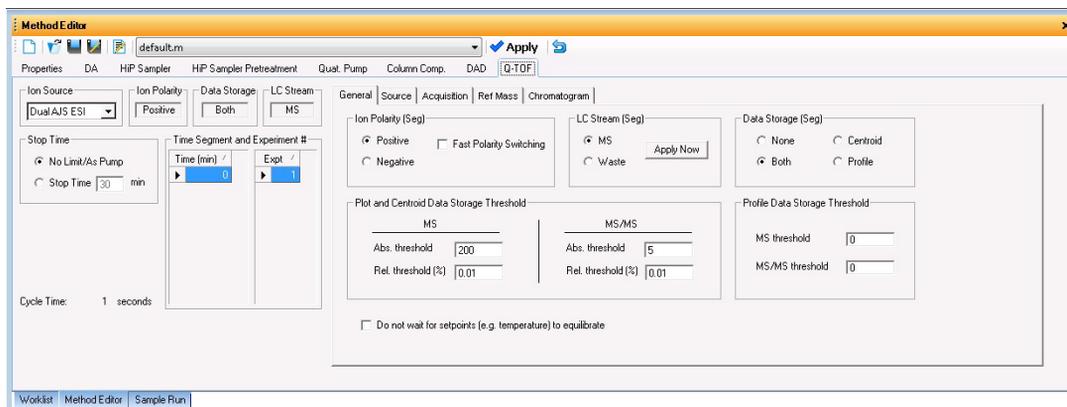**Sample Run window** With this window you enter sample information to run individual samples interactively, and you can start a single sample run. You can also specify an Override DA Method and select either **Both Acquisition and DA** or **DA Only** for the **Method Type**, and then Data Analysis is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

Worklist window With this window you enter sample information for multiple samples. When you run the worklist, the samples are automatically run in the order listed in the worklist. You can select whether to run **Acquisition Only**, to run **Both Acquisition and DA**, or to run **DA only** by selecting one of these options for the **Part of method to run** in the Worklist Run Parameters dialog box.

You can add one or more tune actions to the Worklist when you add a factory script to the worklist.
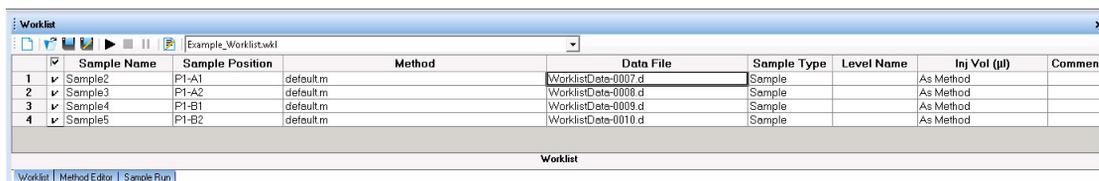
Tune window   With this window, you tune the mass spectrometer. You can use one of the automated tuning algorithms, or you can manually tune the instrument. Manual tuning can result in a less than optimal tune; however, if you perform a manual tune, Agilent recommends that you only manually tune the front part of the instrument: ion source and optics 1. Agilent does not recommend that you manually tune parameters that are after the collision cell.

You also select the instrument mode to use when acquiring data, and calibrate the instrument.

If you have a 6560 Ion Mobility Q-TOF, then the Tune window also contains the Acquisition Mode section where you click either **IM-QTOF** or **QTOF-Only**. If you have a different TOF or Q-TOF instrument, this section is not needed.

If you have a 6545, 6545XT, 6550, or 6560 Q-TOF, then additional options are available for doing a small molecule autotune. If you have a 6545XT, then you can also do a large molecule autotune.

NOTE   You cannot do a small molecule tune after you do a large molecule autotune. If you need to do one, upload an older system tune file first, or do a system tune.
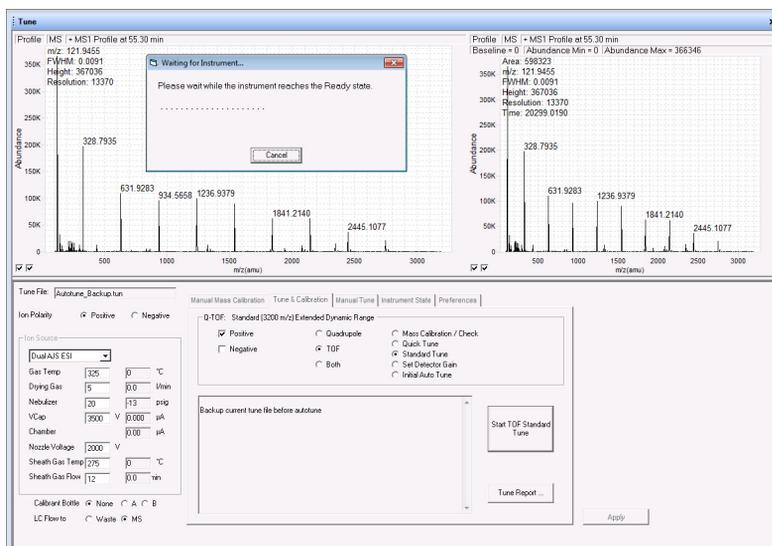

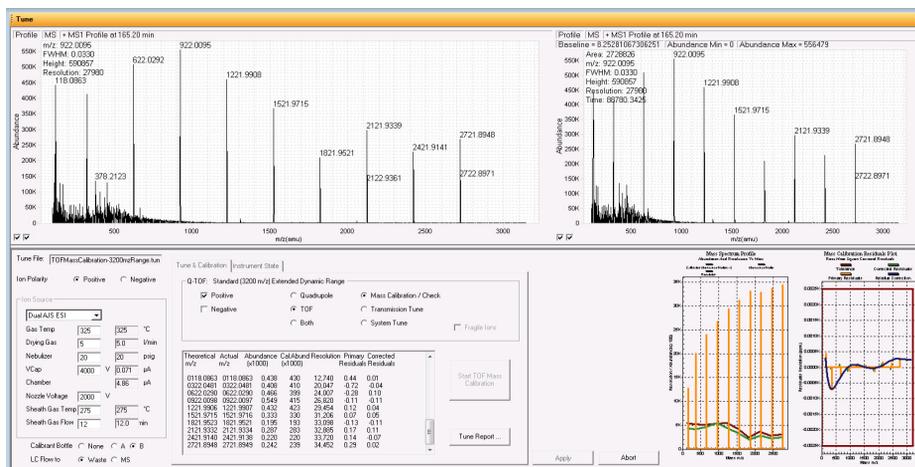**Figure 2.** Tune window for a 6530 Q-TOF instrument performing a classic autotune

Figure 3. Tune window for a 6545XT Q-TOF instrument showing two additional graphs for the SWARM autotune

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 TOF or Q-TOF MS. During this time you do not want pump effluent going into the TOF or Q-TOF 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 Click the **General** tab in the **TOF** or **Q-TOF** tab in the Method Editor window.

b In the **LC Stream (Seg)** group box, click **Waste**.

c Click **Apply Now**. This button only sends the current value of the LC eluent to the instrument. If you click **Apply** on the toolbar, then all of the method parameters are sent to the instrument.

2 Purge the LC pump.

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

3 Condition or equilibrate the column.

After you purge the pump, you set up to condition or equilibrate the column.

a Enter LC parameters, and click **Apply** to download them to the LC.
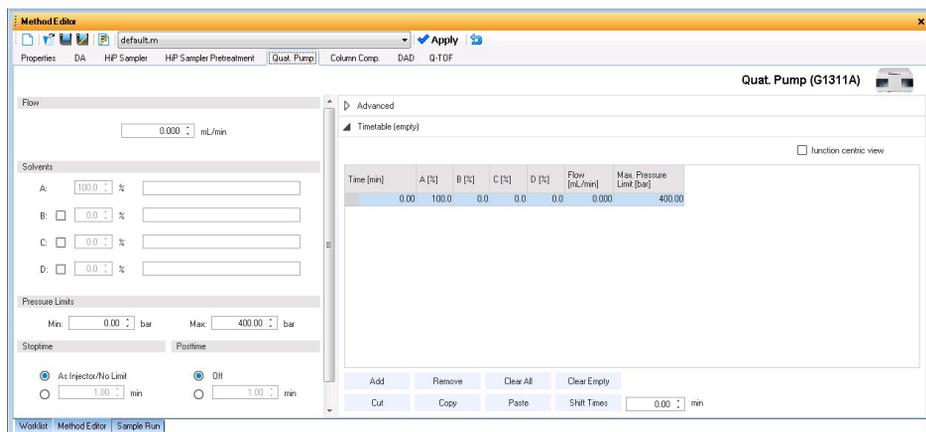
OR, to select an LC conditioning method, select one from the **Method** list at the top of the Data Acquisition window or from the **Method Editor** toolbar, or you select one from the list that appears when you click **Method > Open**.

b Right-click an LC module in the Instrument Status window to change any non-method control parameters, if necessary.
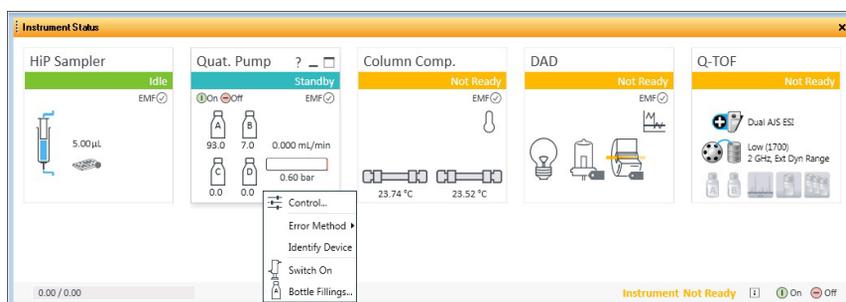
c Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See **step 4** and **step 5** on **page 18**.)

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 window to see the Setup command.
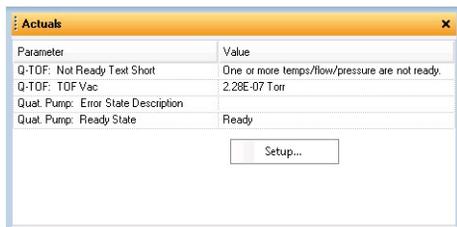
b Click Setup to bring up the list of Actuals available for monitoring. If you have configured a TOF instrument, the actuals for the TOF instrument are displayed instead.
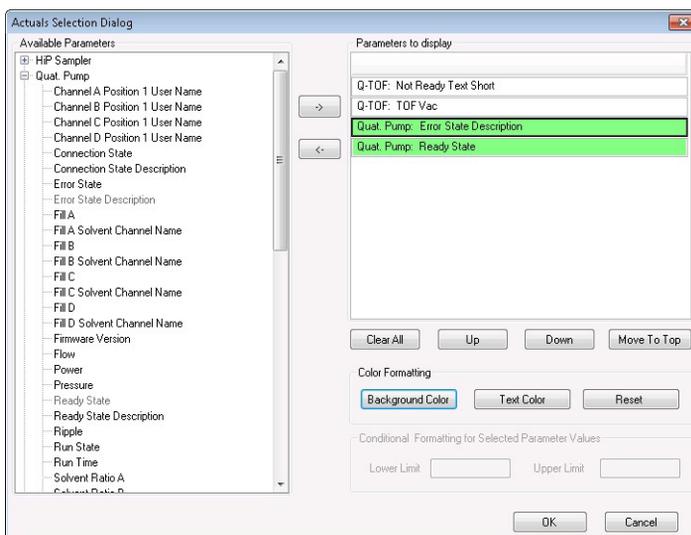
c Add all the parameter values you intend to monitor. you can customize the color of the background and the text. You can also add minimum and maximum values to use; if the value is not within the given range, then the background of the value is set to red in the Actuals window.

d Click **OK**.

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 window, and click **Change**.
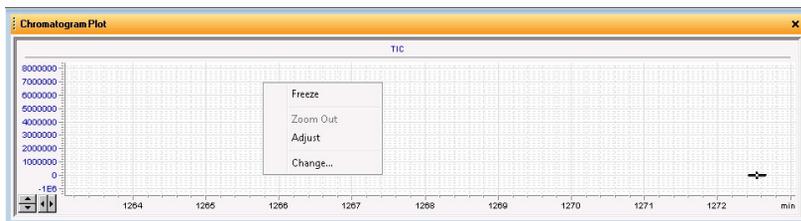
In the **Edit Signal Plot** dialog box, you can select multiple display signals and change the display range.
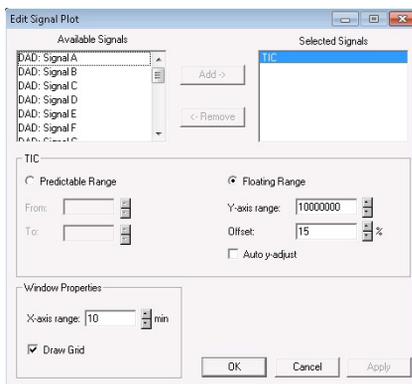
Step 3. Prepare the TOF and Q-TOF instrument

You need to tune the instrument the first time you use it or after maintenance, service or pump-down and restart. You do not need to tune often with standard use. Agilent recommends that you calibrate the mass-axis regularly.

The user interface in the Tune window changes based on the options that you select in the Instrument State tab and the Preferences tab and based on the type of TOF or Q-TOF instrument that you have. In the Preferences Tab, you can mark whether or not to show the Manual TOF Mass Calibration tab and the Diagnostics tab. For a 6530 and 6550 Q-TOF, you can also mark whether or not to use the Classic Autotune interface.

In the Instrument State tab, you can select the Mass Range and mode. The options in the Tune & Calibration tab change depending on the **Mass Range** and the mode.

If you have a 6224 or 6230 TOF LC/MS, or a 6530, 6545, 6545XT, 6546, 6550, or 6560 Q-TOF, then the SWARM autotune is available. The user interface shows the options available for the SWARM autotune. If you have a 6530, 6545, 6545XT, 6550, or 6560 Q-TOF and you select **Low (1700 m/z)** for the **Mass Range**, then you also have small molecule autotune options. If you have a 6545XT and you select **High (10K - 30K m/z)** for the **Mass Range**, then a large molecule transmission tune is done when you run a transmission tune.

If you are installing the instrument, please refer to the Installation Guide for instructions on how to tune the instrument for the first time. The instructions below describe the entire tuning process for an instrument that has previously been tuned, starting with the **Calibration / Check** algorithm.

SWARM Autotune When you tune a 6530, 6545, 6545XT, 6546, 6550, or 6560 Q-TOF instrument or a 6224 or 6230 TOF instrument, a tune algorithm called SWARM is (or can be) used. One of the key features to SWARM is the ability to find a global optimum value for some of the parameters because more than one parameter is tuned at the same time.

The new auto tune uses both Particle Swarm Optimization (PSO) and a simplex algorithm within the physical constraints of the mass spectrometer.

- For a well-known parameter, the auto tune uses the simplex algorithm to maximize speed.
- For unknown behavior, PSO is used.
- PSO and simplex are interchanged between coarse and fine adjustment.

- All the parameters are bound within the knowledge of ion physics, to limit the search space.

The theory of mass spectrometry is built into the algorithm so that no ion storage or ion blocking occurs during optimization.

You can only run **System Tune**, and **Set Detector Gain** with a source that is supported for all Autotunes and if the **Instrument Mode** is **Extended Dynamic Range**. These buttons are grayed out or missing if a different **Instrument Mode** is selected, or if a different source is installed.

If you are given the option, select **High Resolution** for the **Slicer Mode** to get the best results from tuning. You must select **High Resolution** for the **Slicer Mode** before you do a **System Tune**.

Also, you can only run **Set Detector Gain** or **System Tune (**for the TOF or Quadrupole), with the mass range set to 3200 *m/z*.

Classic Autotune
Before you run a **Set Detector Gain, Standard Tune** or **Initial Tune**, the **Instrument Mode** must be set to **Extended Dynamic Range**, and the **Mass Range** must be set to **Standard (3200 m/z)**. After you run one of these autotunes, if you want to acquire data with a different **Mass Range** or **Instrument Mode**, change these values to the appropriate values for your analysis. If you change the values in the Instrument State tab after you finish the autotune, you must recalibrate the TOF or Q-TOF. If you change the mass range, you must recalibrate the TOF or Q-TOF.

You can only run **Initial Tune**, **Standard Tune**, or **Set Detector Gain** with a source that is supported for all Autotunes and if the **Instrument Mode** is **Extended Dynamic Range**. These buttons are grayed out if a different **Instrument Mode** is selected, or if a different source is installed.

If you are given the option, select **High Resolution** for the **Slicer Mode** to get the best results from tuning. You must select **High Resolution** for the **Slicer Mode** before you do an **Initial Auto Tune**.

Also, you can only run **Set Detector Gain** or an **Initial Auto Tune (**for the TOF or Quadrupole), with the **Mass Range** set to **Standard (3200 *m/z)***. If the software is version B.05.01 SP1 or later, after you run an Initial Tune on the Quadrupole with the mass range set to 3200 *m/z*, then for small molecule applications you also perform an additional Quad Tune in the 1700 *m/z* range which leads to a performance increase for molecules < 300 *m/z*.

Supported Sources

Table 1   Supported sources for all Autotunes

Instrument Model Number	Supported Sources for All Autotunes
6224 TOF	ESI, Dual ESI
6230 TOF	ESI, Dual ESI, AJS ESI, Dual AJS ESI
6520B Q-TOF	ESI, Dual ESI
6530A, 6530B Q-TOF	ESI, Dual ESI, AJS ESI, Dual AJS ESI
6538 Q-TOF	ESI, Dual ESI
6540A, 6540B, Q-TOF	ESI, Dual ESI, AJS ESI, Dual AJS ESI
6545, 6545XT, 6546 Q-TOF	ESI, Dual ESI, AJS ESI, Dual AJS ESI
6550	ESI, Dual ESI, AJS ESI, Dual AJS ESI
6560	Dual ESI, Dual AJS ESI

For 6200 Series TOF and 6500 Series Q-TOF

You can do a **Mass Calibration / Check**, and **Quick Tune**, if available, with all instrument states and the following sources:

- ESI
- AJS ESI (Agilent Jet Stream ESI)
- Dual ESI
- Dual AJS ESI (Dual Agilent Jet Stream ESI)
- Multimode
- APPI
- APCI

You cannot do any of the automated tunes if the source is a nanoESI, a Dual nanoESI, a MALDI, or a GC-QTOF source.

Fast Polarity Switching   For the 6224 and 6230 TOF instruments and 6530, 6545, 6545XT[*], 6546, 6550, and 6560 Q-TOF instruments running SWARM tune, if **Fast Polarity Switching** is **Enabled**, you can only run the **Mass Calibration / Check** autotune or the **FPS System Tune**. For TOF and other Q-TOF instruments running Classic Tune, you can only run **Mass Calibration / Check** or **FPS Initial Auto Tune**.

[*]   The 6545XT is shipped with the dielectric capillary. To use Fast Polarity Switching, order the resistive capillary from Agilent.

Diluting the ESI-L Tune Mix

If your instrument is a 6530A, 6540A, 6545A, 6545XT, 6546, 6550A, or 6560A, with either an Agilent Jet Stream ESI source or a Dual AJS ESI source, autotune can fail unless you dilute the tuning mix.

For other models, you may need to dilute the tuning mix under certain conditions. When tuning in negative ion polarity, dilute the tuning mix to calibrate properly. When tuning in positive ion polarity, dilute the tuning mix if you are not getting proper calibration. The instructions for diluting the tuning mix are included in the Installation Guide and in the online Help.

Q-TOF

1:10 diluted tune mix using SWARM autotune

For the 6545, 6545XT, 6550A or 6560A with the Dual Agilent Jet Stream ESI, you tune with a 1:10 diluted tune mix in both polarities.

The 6530 with Dual Agilent Jet Stream ESI uses the 1:10 diluted tune mix in Positive Mode and a 1:40 diluted tune mix in Negative Mode. See the online Help or the Installation Guide for more information.

Table 2 Tune mix dilution for each type of tune using Classic Tune

Tune Type	6530A with Dual AJS ESI or 6540	6520B or 6530 with Dual ESI
Positive Ion TOF Tune	1:10 diluted tune mix	Undiluted tune mix
Negative Ion TOF Tune	1:40 diluted tune mix	Undiluted tune mix
Positive Ion Polarity Switching Tune	Undiluted tune mix	Undiluted tune mix
Negative Ion Polarity Switching Tune	Undiluted tune mix	Undiluted tune mix
Positive Ion Quad Tune	Undiluted tune mix	Undiluted tune mix
Negative Ion Quad Tune	Undiluted tune mix	Undiluted tune mix

If your instrument is a 6538A, then you use the undiluted tune mix.

TOF

If your instrument is a 6230 TOF, the tuning mix must be diluted with the AJS ESI or Dual AJS ESI source for both positive and negative ion. You use the 1:10 dilution for positive ion tuning and 1:40 for negative ion tuning. You do not dilute the tune mix for the Dual ESI source on the 6224 TOF; problems occur if you do dilute the tune mix and tune for positive ion.

Tune the 6530, 6545, 6546, 6545XT, 6546, 6550, or 6560 Q-TOF or 6224 or 6230 TOF LC/MS

SWARM Auto Tune

The 6530, 6545, 6545XT, 6546, 6550, and 6560 Q-TOF and 6224 and 6230 TOF LC/MS use the SWARM autotune. The options available in the Tune & Calibration tab are different for these instruments.

The System Tune is the SWARM Initial Auto Tune which starts with all default values and includes the detector tune. You can perform a System Tune when you select **Standard (3200 m/z)** as the **Mass Range**. System tunes are appropriate for initial system installations, after a vent cycle, after removal/replacement of ion optics or mass analyzer components, or if **Mass Calibration / Check** or **Transmission Tune** cannot complete successfully.

If you select **Low (1700 m/z)** as the **Mass Range**, then you cannot perform a **System Tune**. You can perform a **Transmission Tune** which does not include the detector tune and is more comparable with the **Standard Tune** in Classic Auto tune. The Transmission Tune should be done as frequently as needed (for example, when a different sample class is analyzed.) Start the Transmission Tune either from the last Q-TOF system tune, or the last detector tune, as detector and resolution parameters are not included in these tunes.

If you have a 6545XT and you select **High (10K -30K m/z)** as the **Mass Range**, then you also select the specific mass range in the Instrument State tab.

1 In the **Context** list, select Tune.

The Tune window appears. The Instrument Status window, the Actuals window, and the Tune window are available in the Tune context. Note that you can select to tune the Quadrupole, the TOF, or both parts of the mass spectrometer.

If the software was just installed, and an auto tune was completed as described in the *Installation Guide*, skip to **"Switch LC stream to MS"** on page 36.
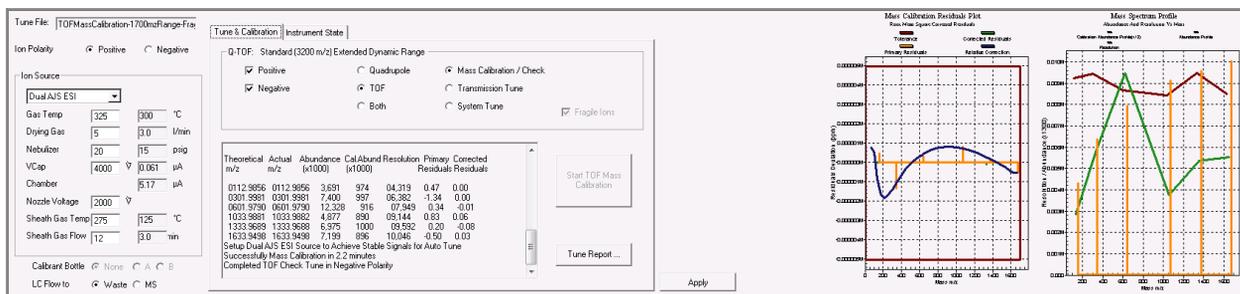
Figure 4. Tune & Calibration tab for a 6545 Series Q-TOF

2 Clear the **Pause Between Autotunes (for Calibration Solution Swapping)** check box.

3 Click the **Instrument State** tab.

4 Select the **Mass Range**. If you have a 6530, 6545, 6545XT, 6546, 6550, or 6560 Q-TOF and select **Low (1700 m/z)**, then additional Low Mass tune options are available in the Tune & Calibration tab.

5 Select either **Disabled** or **Enabled** for **Fast Polarity Switching**.

6 If available, select the **Slicer Mode**. You can click either **High Resolution** or **High Sensitivity**. You can run the **Mass Calibration / Check** if you select either option. Agilent recommends that you select **High Resolution** for the **Slicer Mode** for other Auto Tune algorithms. You must select **High Resolution** for the **Slicer Mode** before you do a **System Tune**.

7 Select the mode. If you want to have the most tune options available to use, click **Extended Dynamic Range (2 GHz)**. (For 6546, the Mode is set automatically and is not visible.)

8 Click **Apply**. You need to click **Apply** for these changes to be sent to the instrument. The options in the **Tune & Calibration** tab are not updated until you click **Apply**.
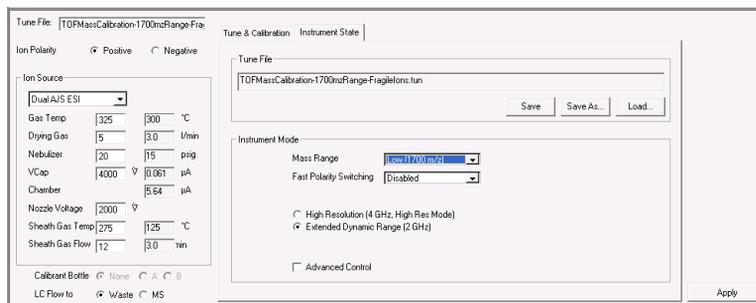

Figure 5. Instrument State tab

9 If you have a 6545XT and selected **High (10K - 30K m/z)** as the **Mass Range**, then you need to move the slider to select the specific mass range to use when acquiring a sample.

10 Click the **Tune & Calibration** tab.

11 Mark the polarity. You can mark **Positive**, **Negative**, or both check boxes. If **Fast Polarity Switching** is **Enabled**, these check boxes are not available.

12 Click the part of the Q-TOF to tune. You can click **Quadrupole**, **TOF**, or **Both**. If **Fast Polarity Switching** is **Enabled**, these options are not available. If you have a 6545XT Q-TOF and the **Mass Range** is **High (10K - 30K m/z)**, then these options are not available. For 6546, if the **Mass Range** is **High (10000)**, then these options are not available.

13 If you selected **Low (1700 m/z)** as the **Mass Range**, then select the type of low mass autotune to perform. If you click **Quadrupole** or **Both**, the **50 - 1700 m/z** low mass autotune is automatically run, and you cannot click one of the other options.

14 If available, mark or clear the **Fragile Ions** check box. Marking this check box is advisable when fragile molecule are expected to be present. Examples of fragile molecules include molecules that exhibit labile characteristics such as many metabolites and glycans.

The options in the right column on this tab are available depending on the settings for **Mass Range** and **Mode**. Both of these options are on the Instrument State tab. Different Low Mass tunes are available depending on the instrument. The dialog box shown is for the 6530.

15 Click **Start TOF Mass Calibration** or **Start Check Quadrupole & TOF Mass Calibration**.

The duration for each Check tune is approximately 30 seconds.

If the **Check tune** results are acceptable, then you can skip to **"Switch LC stream to MS"** on page 36.

If the **Check tune** results are not acceptable, then continue with **step 16**.

16 If available, click **Transmission Tune**. Click **Start TOF Transmission Tune** or **Start Q-TOF Transmission Tune**.

The duration of each **Transmission Tune** is approximately six to ten minutes. Due to this fast process, Agilent recommends to perform transmission tunes on a regular basis.

The small molecule tunes are designed for increased sensitivity and can result in small resolution losses for masses outside the specified window. If the obtained resolution is insufficient for the application, perform a tune in a higher *m/z* range. Ions at higher *m/z* values than the specified mass window are reduced compared to transmission tunes with larger window sizes.

If the **Fragile Ions** check box is marked, the Fragmentor Voltage is reduced to minimize fragmentation. This can lead to lower signal of calibrant ions compared to the non-fragile tune option, but increased transmission of fragile ions should be observed in the application.

If the **Transmission** tune results are acceptable, then you can skip to **"Switch LC stream to MS"** on page 36.

If the **Transmission** tune results are not acceptable, then continue with **step 17**.

17 Click **System Tune**. If this option is not available, do the following:

   a Click the **Instrument State** tab.

   b Select **Standard (3200 m/z)** for the **Mass Range**.

   c Click **Apply**.

   d Click the **Tune & Calibration** tab.

   e Click **System Tune**.

18 Click **Start TOF System Tune** or **Start Q-TOF System Tune**.

If the System Tune results are not acceptable, then contact your local field service engineer.

Calibrate the mass-axis

During calibration, a sample that contains known masses is infused into the source, and the actual flight times for ions of known masses are measured. These times and exact masses are used to calculate updated calibration coefficients. This process ensures accurate mass assignments for unknowns. Agilent recommends that you do this regularly.
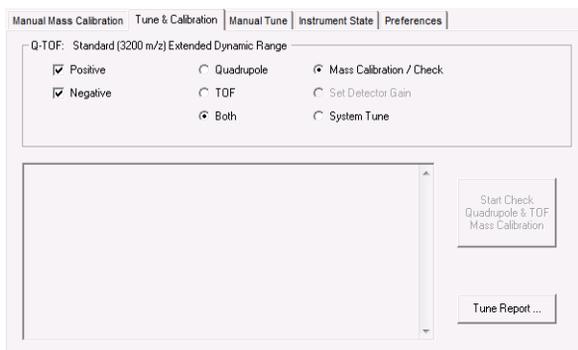
Mass Calibration is done when you click the **Mass Calibration / Check** button, the **Quick Tune** button, the **Standard Tune** button, or the **Initial Auto Tune** button. If you use the SWARM autotune, Mass Calibration is done when you click **Mass Calibration / Check** button, **Transmission Tune** button, or **System Tune** button. You only need to recalibrate and check the tune if:

- • You change the **Instrument Mode** in the Instrument State tab.
- • You change the **Mass Range** in the Instrument State tab.
- • You change the **Slicer Mode**.

1 On the **Toolbar**, select **Tune** in the **Context** combo box.

2 Click the **Tune & Calibration** tab.

3 Mark **Positive** or **Negative** or both.

4 Click the **Mass Calibration / Check** option.

5 Click the **Start Check Quadrupole & TOF Mass Calibration** button or the **Start TOF Mass Calibration button**.

Please note that if you have a 6545, and you click the **50 - 250 m/z** option, calibrant ions below 100 *m/z* are used.

Tune the TOF and Q-TOF MS except for the 6545, 6546, 6545XT, 6546, and 6560 Q-TOF

Classic Auto Tune

1 In the **Context** list, select Tune.

The Tune window appears. The Instrument Status window, the Actuals window, and the Tune window are available in the Tune context. You can select to tune the Quadrupole, the TOF, or both parts of the MS.

Initial autotunes are appropriate for initial system installations, after removal/replacement of ion optics or mass analyzer components, or if standard tunes cannot complete successfully. If the software was just installed and an autotune was completed as described in the *Installation Guide*, skip to **"Switch LC stream to MS"** on page 36.
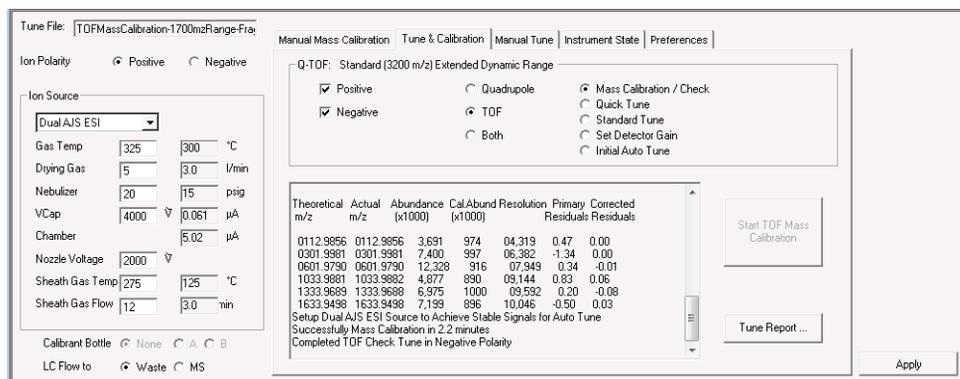

Figure 6.   Tune & Calibration tab for a 6530 Series Q-TOF

The **Quadrupole**, **TOF**, and **Both** options are only available if you have configured a Q-TOF instrument.

2   (optional) If the instrument is a 6560A Ion Mobility Q-TOF, click the Acquisition Mode. You can click either **IM-QTOF** or **QTOF-Only**.

3   (optional) Click the **Preferences** tab.

4   (optional) Mark whether or not you want the system to automatically adjust the abundance of the calibrant ions. This feature is for the 6530A/B models only.

This is an advanced feature, and you probably do not need to change this setting.

If you mark the **Adjust abundances for optimal calibration** check box, the system automatically adjusts the fragmentor voltage to reduce the abundance for calibration masses if the calibration masses are detected to be out of the 50 to 650K range. If the fragmentor voltage cannot be adjusted low enough to cause the abundance level to fall below 650K, the system tells you to dilute the calibrant and then to try the calibration or autotune again.

5   (optional) If the instrument is a 6550A iFunnel Q-TOF, clear the **Always perform only Quick Calibration** check box. No additional dilution for negative mode is necessary if you clear this check box.

6 (optional) Mark the **Pause Between Auto Tune (Swapping Calibrant)** check box if you use a different calibration solution for positive and negative autotunes.

7 If you have a 6550 Q-TOF instrument and you want to run small molecule applications, you must set the **Quad AMU** value before running any of the autotune procedures. If you are running small molecule applications, you also select **Low (1700 m/z)** for the **Mass Range** on the Instrument State tab.

 a Click the **Manual Tune** tab.

 b Click the **Quad** tab in the Manual Tune tab.

 c If you are going to tune in **Positive** polarity, do the following: Click the **Positive** polarity button. Type 100 in the **Quad AMU** box. Click the **Apply** button. You must click **Apply** before switching to the Negative polarity to save the new value.

 d If you are going to tune in Negative polarity, do the following: Click the **Negative** polarity button. Type 90 in the **Quad AMU** box. Click the **Apply** button.

8 Click the **Instrument State** tab.

9 Select the **Mass Range**.

10 Select either **Disabled** or **Enabled** for **Fast Polarity Switching**.

11 If available, select the **Slicer Mode**. You can click either **High Resolution** or **High Sensitivity**. You can run the **Mass Calibration / Check** if you select either option. Agilent recommends **High Resolution** for the **Slicer Mode** for other Auto Tune algorithms. You must select **High Resolution** for the **Slicer Mode** before you do an **Initial Auto Tune**.

12 Select the mode. If you want to have the most tune options available to use, click **Extended Dynamic Range (2 GHz)**.

13 Click **Apply**. You need to click **Apply** for these changes to be sent to the instrument. The options in the **Tune & Calibration** tab are not updated until you click **Apply**.
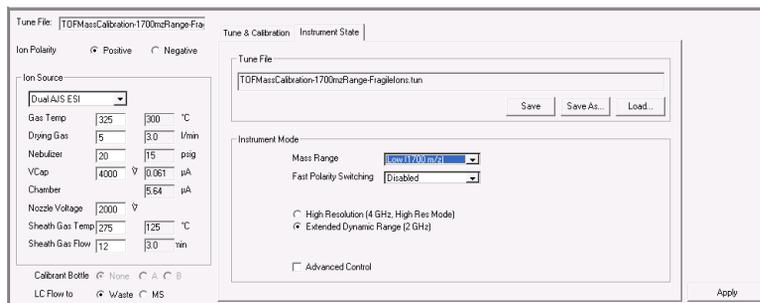

Figure 7. Instrument State tab

14 Click the **Tune & Calibration** tab.

15 Mark the polarity to use when tuning on the Tune & Calibration tab. You can mark **Positive**, **Negative** or both. If you selected **Enabled** for **Fast Polarity Switching**, then you do not need to select a polarity.
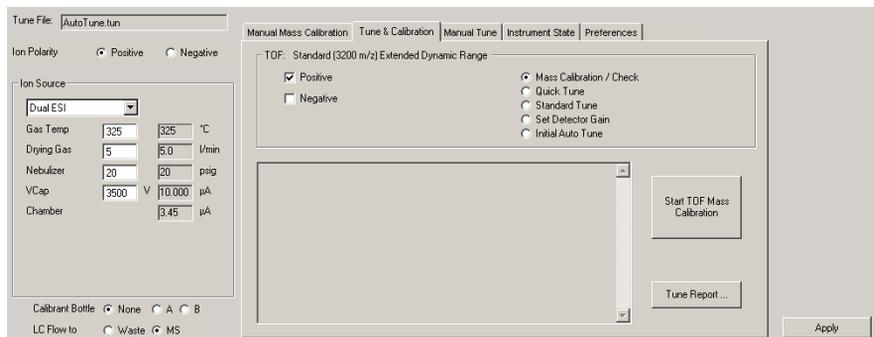
Figure 8. Autotune tab for a 6200 Series Time-of-Flight if you mark **Use Classic Auto Tune** on the Preferences tab

16 If you have a Q-TOF instrument, then click whether you want to tune the **Quadrupole**, the **TOF**, or **Both**. The software automatically tunes the parts of the instrument in the correct order, so click **Both** if you want to tune both parts.

For classic autotune if you are tuning a 6530 with Dual AJS ESI or a 6538 or a 6540, then do not click **Both**. You need to change the tuning mix between doing the TOF and the Quadrupole, so you do first tune the TOF and then tune the Quadrupole.

17 If available, click **Mass Calibration / Check** to calibrate and check the mass calibration and optimization. Then, click **Start TOF Mass Calibration** or **Start Check Quadrupole & TOF Mass Calibration** (**Mass Calibration/Check** tune takes a few minutes to complete for TOF tune. **Mass Calibration / Check** tune also takes 2 to 5 minutes for the Quadrupole.

If the system pauses between autotune to allow you to change calibration solutions, change the tuning mix to one diluted for the next tune as described in the online Help and in the Installation Guide.

You repeat **Mass Calibration / Check** after you dilute the calibration solution if the abundances are greater than 480,000 for any of the calibrating ions for the 6538/6540 Q-TOF or above approximately 650,000 for other Q-TOF and TOF instruments. The Agilent 6550 Q-TOF instrument always use the 1:10 dilution.If the **Check** tune results are acceptable, then you can skip to **"Switch LC stream to MS"** on page 36.

If the **Check** tune results are not acceptable, then continue with **step 18**.

18 If you selected **Enabled** for **Fast Polarity Switching** on the Instrument State tab, click **FPS Initial Auto Tune**. This option and **Mass Calibration /Check** are the only options available if **Fast Polarity Switching** is Enabled.

If the results from FPS Initial Auto Tune are acceptable, then you can skip to **"Switch LC stream to MS"** on page 36. If your tune results are still not acceptable, please contact Agilent Field Support.

19 If available, click **Quick Tune** to use a limited set of parameters to tune the MS automatically. Then, click **Start TOF Quick Tune** (Quick Tune takes 7 to 10 minutes to complete). You cannot run a Quick Tune if you clicked either **Quadrupole** or **Both** as the part of the instrument to tune.

If the system pauses between autotune to allow you to change calibration solutions, change the tuning mix to one diluted for the next tune as described in the online Help and in the Installation Guide.

You repeat **Quick Tune** after you dilute the calibration solution if the abundances are greater than 480,000 for any of the calibrating ions for the 6538/6540 Q-TOF or above approximately 650,000 for other Q-TOF and TOF instruments. For the 6550, the calibration solution is always diluted, so you do not repeat the **Quick une**.

If Quick Tune results are acceptable, then you can skip to **"Switch LC stream to MS"** on page 36.

If Quick Tune results are not acceptable, then continue with **step 20**.

20 Run a **Standard Tune** to use even more parameters. This tune takes 10 to 15 minutes for the TOF part of the instrument, and 10 to 15 minutes for the quadrupole part of the instrument.

The source that is used must be supported for all Autotunes when running a **Standard Tune**. The Dual ESI source is supported for all Autotunes for all instruments. Refer to **"Supported sources for all Autotunes"** on page 23 for a complete list of sources that are supported for all Autotunes.

You can only run a Standard Tune if you selected **Extended Dynamic Range** as the **Instrument mode** on the Instrument State tab.

a Click the **Instrument State** tab.

b For the **Instrument Mode**, click **Extended Dynamic Range**.

c Select **Disabled** for **Fast Polarity Switching**.

d Click **Apply**.

e Click the **Tune & Calibration** tab.

f Mark the polarity (polarities) to use when tuning.

g Click **Standard Tune**.

h Click **Start TOF Standard Tune** or **Start QTOF Standard Tune**.

You must use a source that is supported for all Autotunes when running a **Standard Tune**. The Dual ESI source is supported for all Autotunes for all instruments. Refer to **"Supported sources for all Autotunes"** on page 23 for a complete list of sources that are supported for all Auto Tunes.

If the system pauses between Auto Tunes to allow you to change calibration solutions, change the tuning mix to one diluted for the next tune as described in the online Help and in the Installation Guide. If Standard Tune results are acceptable, continue with **step 21**.

If **Standard Tune** produces unacceptable results, you can run an **Initial Auto Tune**. After performing an Initial Auto Tune, you need to adjust the Collision Cell Gas Pressure. See the online Help for more information.

If this also fails to give acceptable results, please contact Agilent Field Support.

Times for Initial Tune with Classic Tune

Instrument	Time for Initial Tune (TOF)
6538A, 6540A, 6550A Q-TOF	up to 60 minutes for each polarity
Other Q-TOF instruments and TOF instruments	up to 30 minutes for each polarity

It takes approximately 50 to 60 minutes to run an Initial Tune on the Quadrupole part of the instrument.

If you want to use custom tune parameter values, you can do a Manual Tune.
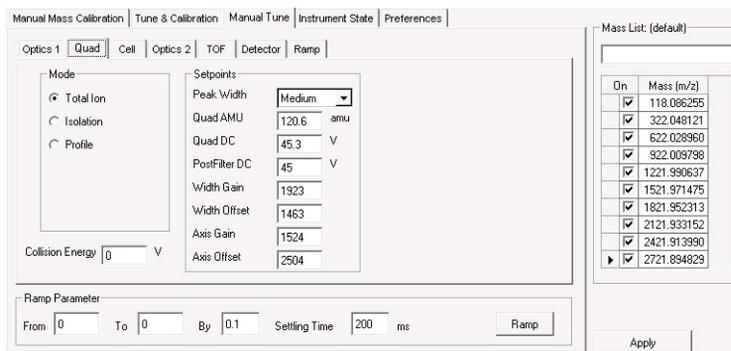
21 Set the **Mass Range** and the **Instrument Mode** that you want to use to acquire data.

   a Click the **Instrument State** tab.

   b Select the appropriate **Mass Range.**

   c Click the appropriate **Instrument Mode**.

   d Click **Apply**.

   e If you select **Low (1700 m/z)** and you ran the **Quad Tune** or **Initial Quad Tune** with **Standard (3200 m/z)** selected for the **Mass Range**, then you now run a **Quad Tune** to optimize for small molecules.

22 Recalibrate and check the tune parameters, if you changed the Mass Range or Instrument Mode.

Calibration is done when you click the **Mass Calibration / Check** button, the **Quick Tune** button, the **Standard Tune** button and the **Initial Tune** button. If you just completed one of these tasks, and if any of the following are true, you only need to recalibrate and check:

- You change the **Instrument Mode** in the Instrument State tab.
- You change the **Mass Range** in the Instrument State tab.
- The peak abundances are above approximately 480,000 for the 6538/6540 Q-TOF or above approximately 650,000 for other Q-TOF (except for the 6550) and TOF instruments. You always use the 1:10 dilution for the 6550. You have to dilute the tune calibrant before you recalibrate. You recalibrate to get optimal mass accuracy.

   a Click the **Tune & Calibration** tab.

b Mark the desired polarity or polarities in this tab.

c Click the **Mass Calibration / Check** option.

Tune reports are automatically generated at the end of a tune.

See the *Concepts Guide* for more information on the TOF and Q-TOF MS instruments.

Switch LC stream to MS

After tuning the instrument

After you condition the column and tune the TOF or Q-TOF MS, you switch the LC stream from Waste to MS.

a In the **Context** list, click **Acquisition**. You need to answer several questions when switching from the Tune context to the Acquisition context.

b Make sure that the General tab in the TOF or Q-TOF tab is selected in the Method Editor window.

c In the **LC Stream (Seg)** group box, click **MS**.

d Click **Apply Now** in the General tab.

Monitor MS baseline and spectral displays

• If you did not monitor the LC baseline with a VWD or DAD, make sure that the TOF or Q-TOF baseline is stable and no spectra of interfering intensity appear.

• If you did monitor the LC baseline with a VWD or DAD, change back to the default TOF or Q-TOF displays.

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

b 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 on the toolbar of the Data Acquisition window, and view the logged events.

• Or right-click on the system task bar. 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 task bar.

Step 4. Set up and run a method

A MassHunter method for TOF and Q-TOF can contain acquisition parameters, qualitative analysis parameters, quantitative analysis parameters, or all three.

In the Data Acquisition method, you can specify whether or not to run a Qualitative Analysis method and whether or not to run a Quantitative Analysis automation. You specify whether to copy or link the Qualitative Analysis method and the Quantitative Analysis method to the Data Acquisition method.

When you run multiple samples in a worklist with this *.m* method, you can specify whether to run both data acquisition and data analysis or to only run either data acquisition or data analysis. If you run a single sample in the Data Acquisition program, then both acquisition and data analysis are done.

If you select to run both data acquisition and data analysis in a worklist, then the data analysis method automatically follows acquisition if you mark either the **Qual Automation** check box or the **Quant Automation** check box or the **BioConfirm Automation** check box in the DA tab in the Method Editor window. You can also run a method to produce only raw data (acquisition only) or reprocess the data with a method containing only qualitative analysis parameters, BioConfirm parameters, or quantitative analysis parameters (data analysis only).

In this step you learn how to set up the method with acquisition parameters only, with qualitative analysis parameters only and with a combination of acquisition parameters and qualitative analysis parameters.

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

Set up a method with acquisition parameters

Do Exercise 1 and Exercise 2 of the TOF/Q-TOF Familiarization Guide to learn how to set up and run a method with only acquisition parameters.

Do Exercise 3 of the TOF/Q-TOF Familiarization Guide to learn how to set up and run a method in IM-QTOF mode.

Do Exercise 4 of the TOF/Q-TOF Familiarization Guide to learn how to optimize IM-MS methods for labile compounds, small molecules, and intact proteins.

Do Exercise 5 of the TOF/Q-TOF Familiarization Guide to learn how to set up a method for collision cross section (CCS) calculations.

Before you edit or run a method, set the options in the Instrument State tab in the Tune window.

- Type the values and settings for each of the tabs below.
- (optional) If you want to download the settings to the instrument, click **Apply** on the toolbar.
- To save the method after entering parameters, click either **Method > Save** or **Method > Save As**.
- Type the name for the method in the **Method** box, and click **OK**.

1 In the **Context** list, click **Acquisition**.

2 Enter LC parameter values.

Type the values for all of the LC modules configured for the instrument.
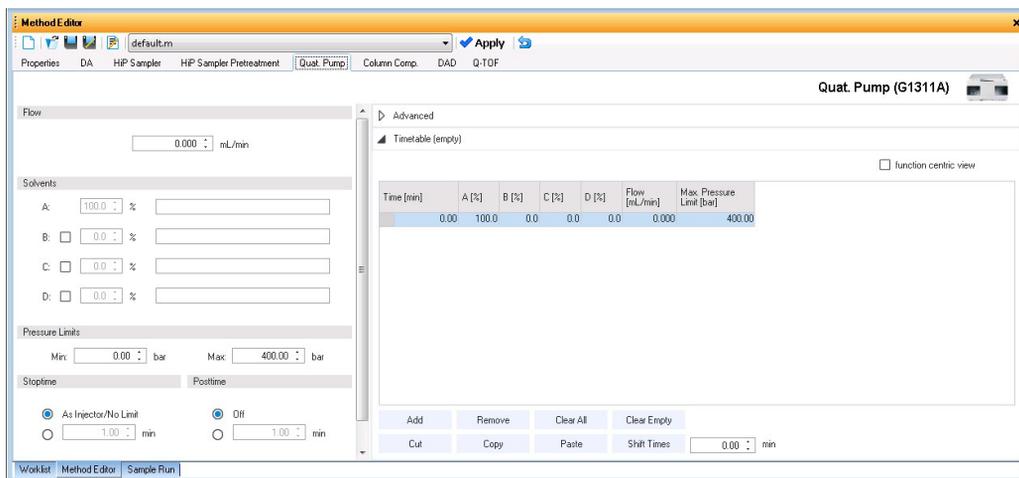
3 Set up to change TOF and Q-TOF parameters with segments and experiments:

   a Click the **TOF** or **Q-TOF** tab in the Method Editor window.

   b To add a segment, right-click the **Time Segment** section and click **Add Time Segment**. The time segment uses those parameter entries with (seg) next to their names. Those parameters can be changed for each time segment.

   c To add an experiment, right-click the **Experiment #** section, and click **Add Experiment**. The experiment will use those parameter entries with (Expt)

next to their names. Those parameters can be changed for each experiment.

d Enter the parameters for each segment and experiment. When you add a new time segment, the parameters for the time segment that is selected are used as the default values for the new time segment. When you add a new experiment, the parameters for the last experiment in the list are used as the default values for the new experiment. The Advanced Parameters tab is only available for the 6550 and 6560.

Please note that if you have an IM-QTOF instrument, some options for Collision Energy in the Acquisition tab allow you to have only one time segment and one experiment.

4 Enter TOF or Q-TOF parameter values:

a Click the **General** tab, and enter any general parameters that you want to change.

b Click the **Source** tab, and enter any source values you want to change.

c Click the **Acquisition** tab.

d Select the mode of operation for the Q-TOF LC/MS: MS mode, Auto MS/MS mode or Targeted MS/MS mode. If you have a TOF configured, you can only use **MS mode**. If you have a 6560 Ion Mobility Q-TOF, then **Auto MS/MS** is not enabled in IM mode.

Different parameters are made available depending on the mode selected.

To learn more about these parameters and how they affect results, refer to the *online Help*.

**e** Enter any values you want to change in the Acquisition tab.
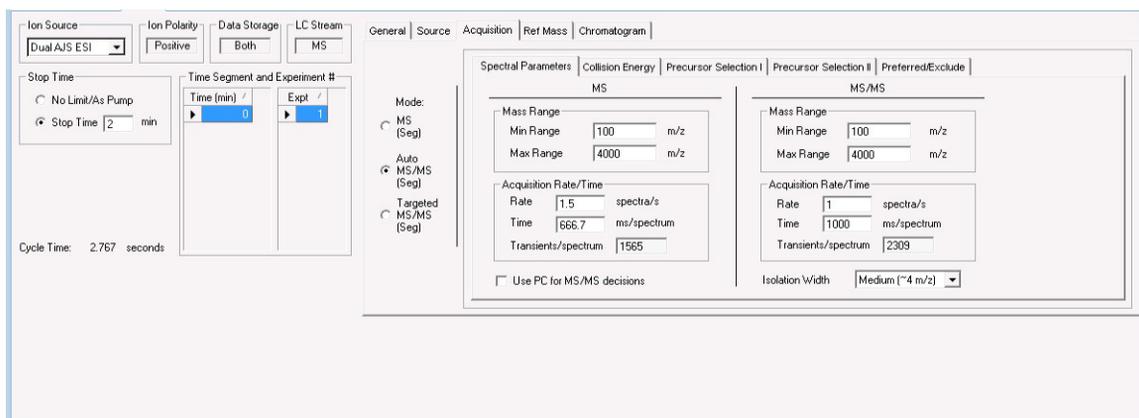
f Click the **Ref Mass** tab to set up the reference mass correction.

g Click the **Chromatogram** tab to set up the chromatograms to plot during a run.

h If you have an IM-QTOF instrument, then click the **Advanced Parameters** tab to set the parameters for Ion Mobility.

5 Set up the data analysis parameters. In the DA tab, you can specify the Qualitative Analysis parameters and Quantitative Analysis parameters. To set up the Qualitative Analysis parameters, do the following:

a Click the **DA** tab in the Method Editor.

b On the **Qual** tab, mark the **Qual Automation** check box.

c Click the **Link** or **Copy** button, depending on whether you want to always use the most recent version of the Qualitative Analysis method or copy the current Qualitative Analysis method and save it to the Data Acquisition method for future use. If you click **Copy**, the method entered in the **Change to Method** box is copied to the Data Acquisition method when the Data Acquisition method is saved.
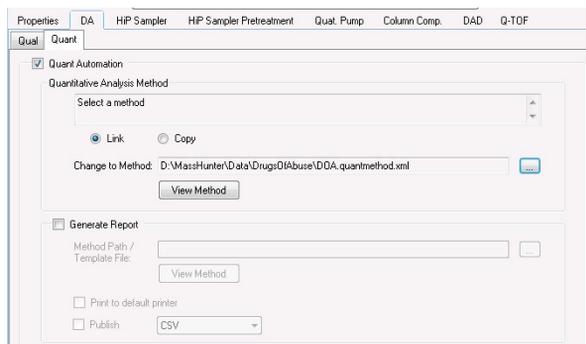
d Click the ⬚ button to select the Qualitative Analysis method.
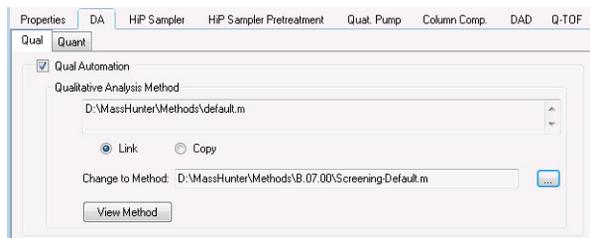
See the online Help for the MassHunter Qualitative Analysis software to learn how to set up automation parameters for the MassHunter Qualitative Analysis software.

To set up the Quantitative Analysis parameters, do the following:

a Click the **DA** tab in the Method Editor.

a Click the **Quant** tab.

b On the **Quant** tab, mark the **Quant Automation** check box.

c Click the **Link** or **Copy** button, depending on whether you want to always use the most recent version of the Quantitative Analysis method or copy the current Quantitative Analysis method and save it to the Data Acquisition method for future use. If you click **Copy**, the method entered in the **Change to Method** box is copied to the Data Acquisition method when the Data Acquisition method is saved.

d Click the ⬚ button to select the Quantitative Analysis method.

e (optional) Mark the **Generate Report** check box. If you mark this check box, then you also select the **Method Path** or the **Template File** to use and the locations to send the report. The **Method Path** is a path to a **Quantitative Analysis Report Method**.

See the online Help for the Quantitative Analysis program to learn how to set up automation parameters for the Quantitative Analysis program.

Set up and run interactive samples

1 Click the **Sample Run** window.

2 Enter the information such as the Sample Name, the Data File Name, and the Path.

3 Enter information in the **Additional Information** list. 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 the DA (Data Analysis) method to execute.

A method can contain data acquisition parameters, data 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, the **Quant Automation** check box marked on the Quant tab, or the **BioConfirm Automation** check box marked on the BioConfirm tab.

4 To start a single sample run, click the Run button (▶) on the **Sample Run** toolbar or **Run** on the main toolbar. You can also click **Sample > Run** or press **F5** on your keyboard.

You can run the single sample in either locked or unlocked mode. When the mode is locked, you cannot overwrite this data file in the Data Acquisition program. The **Lock** button on the main toolbar indicates that locked mode is on.
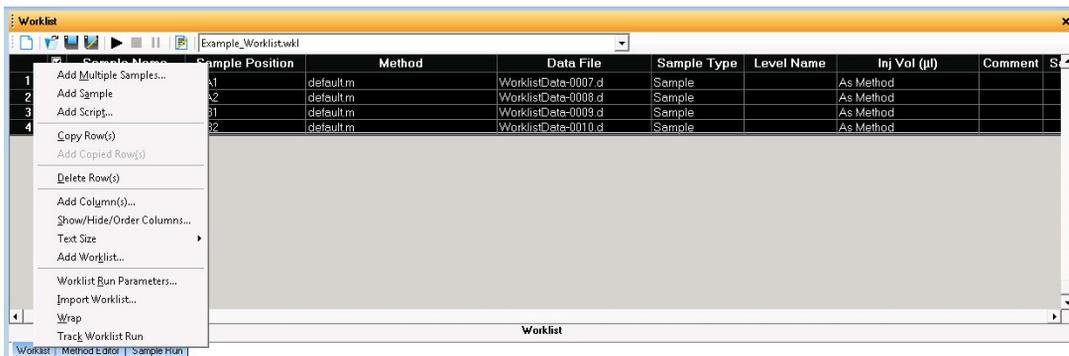
You can also specify an **Override DA Method** and select either **Both Acquisition and DA** or **DA Only** for the **Method Type**, and then Data Analysis is run as part of the method.

Set up and run worklists

1 Right-click the upper left corner of the worklist to display the following menu.

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

3 Enter all relevant information, and 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).

4 Specify the locations, and click **OK**.

5 To set up the worklist run, right-click the upper left corner, and click **Worklist Run Parameters**. The **Worklist Run Parameters** dialog box appears.

6 Type the paths for the method, the Override DA method, and the data files, and click **OK**.

7 To start the run, click **Run Worklist** ⬛▶ on the main toolbar, or click **Start Worklist Run** (▶)on the **Worklist** toolbar. You can also click **Worklist > Run**, or press **F4** on your keyboard.

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. The **Lock** button 🔒 on the main toolbar indicates that locked mode is on.

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 and find compounds with Qualitative Analysis

Use the Qualitative Analysis software to do these steps and more:

- Review results for acquisition method development
- Find compounds
- Identify compounds
- Do molecular feature extraction
- Export results
- Print reports

Refer to the online Help and do the exercises in the Qualitative Analysis Familiarization Guide to help you learn how to use the Qualitative Analysis program to do these tasks.

Step 6. Set up and run quantitation

If you want to quantitate your data, use the Quantitative Analysis program.

Refer to the online Help and to the *Quantitative Analysis Familiarization Guide* to learn how to do more steps to analyze your data.

Step 7. Review data in IM-MS Browser

If you have an IM-QTOF data file, use the MassHunter IM-MS Browser program to analyze the data. You can examine abundance maps of the data. You can also do collision cross section calculations. In addition, you can use the Ion Mobility Feature Extractor (IMFE) to find compounds.

Refer to the online Help for IM-MS Browser for more information.

If needed, install the IM-MS Browser program. From the MassHunter Data Acquisition installation disc, open the **IM-MS Browser** folder and run **setup.exe**.

In this Book

This book contains brief instructions to help you get started with your Agilent TOF or Q-TOF LC/MS system. This book takes a quick look at using the Agilent MassHunter Workstation software to:

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

This guide supports both MassHunter Workstation Data Acquisition B.09.00 and 10.0.

The Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS instruments are intended to generate high resolution accurate mass spectral information for the chemical samples introduced to the instrument. Analytes may include pesticides, environmental contaminants, metabolites, forensic toxicological compounds, and biomolecules.