

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

Quick Start Guide

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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 an Agilent TOF LC/MS with the Agilent 1200 LC or 1260 and 1290 Infinity modules and one of several ion interfaces:



Agilent Technologies

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

- ESI
- AJS ESI (Agilent Jet Stream ESI)
- Dual ESI
- Dual AJS ESI (Dual Agilent Jet Stream ESI)
- MMI
- APPI
- APCI
- MALDI
- Nanospray
- Dual nanospray
- HPLC-Chip interface.

The Agilent 6230 Accurate-Mass TOF also supports the Agilent Jet Stream ESI. The AJS ESI source utilizes 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 – 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 the Agilent 1200 LC or 1260 and 1290 Infinity modules and one of several ion interfaces: ESI, Dual ESI, APCI, APPI, multimode, MALDI, nanospray, dual nanospray or HPLC-Chip interface.

The Agilent 6538 and 6540 UHD Accurate-Mass Q-TOF also can be set up with the Agilent Jet Stream ESI source. This source utilizes 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 Agilent MassHunter Workstation Software that includes these programs:

- Data Acquisition – From one screen 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 one screen 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 program, please refer to either the *Qualitative Analysis Familiarization Guide* or the online Help for the Qualitative Analysis program.
- Quantitative Analysis - From one screen 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 either the *Quantitative Analysis Familiarization Guide* or the online Help for the Quantitative Analysis program.
- Report Designer Add-in - You can customize many of the reports that are used in the Qualitative Analysis program and the Quantitative Analysis program by using the Report Designer Add-in. Reports are created by using templates, Microsoft Excel and the Report Designer Add-in. For more information on the Report Designer Add-in, please refer to either the Reporting Training DVD or the online Help for the Report Designer Add-in. You cannot customize many of the reports in the Data Acquisition program.

What's New in B.04.00

- The Agilent 6220, 6224 and 6230 Accurate-Mass TOF mass spectrometers are supported.
- The Agilent 6210A TOF mass spectrometer, if it is upgraded to a 6220A, is supported.
- The Agilent 6520A, 6520B and 6530A Accurate-Mass Q-TOF mass spectrometers are supported in this release.
- The Agilent 6538 and 6540 UHD Accurate-Mass Q-TOF mass spectrometers are supported.
- The Dual AJS ESI source is supported on the Agilent 6538 and Agilent 6540 UHD Accurate-Mass Q-TOF mass spectrometers.
- The Dual AJS ESI source is supported on the Agilent 6230 UHD Accurate-Mass TOF mass spectrometer.
- Windows 7 is supported.
- Excel 2010 on Windows 7 is supported for printing Q-TOF and TOF tune reports.
- Reports for the Acquisition Method and the Worklist are printed using a different tool called RDL.
- When acquiring data in Auto MS/MS mode, you can specify a minimum purity.
- When acquiring data in Auto MS/MS mode, you can specify a target abundance and reject precursor ions that do not meet the target abundance.
- When acquiring data in Auto MS/MS mode, you can specify the isotope model.
- You can specify to use fixed collision energies, a Collision Energy table or a formula to determine the collision energy.
- Compliance for Data Acquisition is supported.
- The Study Manager program is supported. You can create a study from an existing worklist.
- The Map File Generator program supports Worklists, Drug Discovery Screening and Optimization Automation map files.
- The Method Comparator is now available.
- Method Audit Trail in Compliance mode and comparison of method versions is available when compliance is enabled.
- The worklist contains the Sample Group and Sample Info. columns that are displayed in Quant.
- Barcodes can be specified for plates in a worklist.
- Locked mode runs are supported in Study Manager.

- A standby script can be specified in Study Manager.
- Checksum for studies is supported.
- Needle Seat Back Flush using G4227A Flexible Cube is supported.
- The Carryover Reduction feature for the HPLC-Chip is supported.
- The Agilent 1260 Isocratic Pump (G1310B) is supported.
- The Agilent 1260 Quaternary Pump (G1311B) is supported.
- The Agilent 1260 Quaternary Pump VL (G1311C) is supported.
- The Agilent 1260 Capillary Pump (G1376A) is supported.
- The Agilent 1260 Nano Pump (G2226A) is supported.
- The Agilent 1260 Binary Pump (G1312B) is supported.
- The Agilent 1260 Binary Pump VL (G1312C) is supported.
- The Agilent 1290 Binary Pump VL (G4220B) is supported.
- The Agilent 1260 Standard Autosampler (G1329B) is supported.
- The Agilent 1260 High Performance Autosampler (G1367E) is supported.
- The Agilent 1260 Micro High Performance Autosampler (G1377A) is supported.
- The Agilent 1290 LC Injector HTC/HTS (G4277A) is supported.
- The Agilent 1290 LC Injector HTC/HTS (G4278A) is supported.
- The Agilent 1260 Variable Wavelength Detector VL (G1314B) is supported.
- The Agilent 1260 Variable Wavelength Detector SL (G1314C) is supported.
- The Agilent 1260 Variable Wavelength Detector (G1314F) is supported.
- The Agilent 1290 Variable Wavelength Detector (G1314E) is supported.
- The Agilent 1260 Diode Array Detector SL (G1315C) is supported.
- The Agilent 1260 Diode Array Detector VL (G1315D) is supported.
- The Agilent 1260 Diode Array Detector (G4212B) is supported.
- The Agilent 1260 TCC (Thermostatted Column Compartment) (G1316A) is supported.

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

Where to find information

Online Help

Press F1 To get more information about a pane or dialog box, place the cursor on the pane or dialog box of interest and press **F1**.

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

Documents

Some of these manuals are delivered with the TOF and Q-TOF LC/MS hardware or software. You can also find a PDF version of each manual on the installation disk, in the **Manuals** folder, and you can display several of these manuals from the online Help for the corresponding program.

You can find a PDF version of manuals for the offline or related MassHunter products on the installation disks for those programs. You can also access these manuals from the Getting Started section in the Online Help.

Installation Guide This guide is used by the Agilent customer engineer to install the hardware and software, configure the instrument, and verify performance. A different installation guide is available for the Q-TOF, the 6210/6220 TOF and the 6224/6230 TOF.

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

TOF/Q-TOF Familiarization Guide Do the exercises to learn to use the TOF/Q-TOF LC/MS hardware and acquisition software.

Maintenance Guide Use this guide to help maintain and troubleshoot your Agilent TOF LC/MS or your Agilent Q-TOF LC/MS.

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

MassHunter QQQ Compliance Quick Start Guide Use this guide to learn about the MassHunter QQQ Compliance program.

MassHunter Quant Compliance Quick Start Guide Use this guide to learn about the MassHunter Quant Compliance program. *(This guide is on the Quantitative Analysis disk.)*

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

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

Report Designer User Information DVD Learn how to customize the Excel templates that are used to create reports. *The User Information DVD contains movies to show you how to customize a template and familiarization exercises to learn to do to learn to customize a template.*

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

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

Training

Familiarization Guide Use all three familiarization guides (Data Acquisition, Qualitative Analysis and Quantitative Analysis) to get to know the software.

Quick Start Guide Use the quick start guides for Study Manager, Acquisition Compliance and Quantitative Analysis Compliance to get to know these programs.

Report Designer User Information DVD Watch the movies and use the familiarization guide to learn how to customize a report.

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

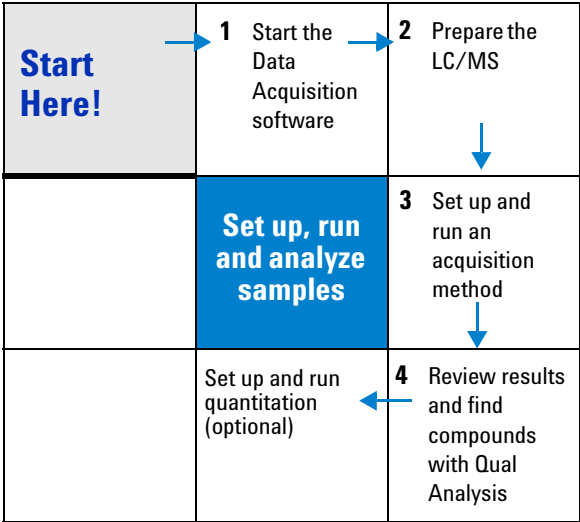
Getting Started

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.



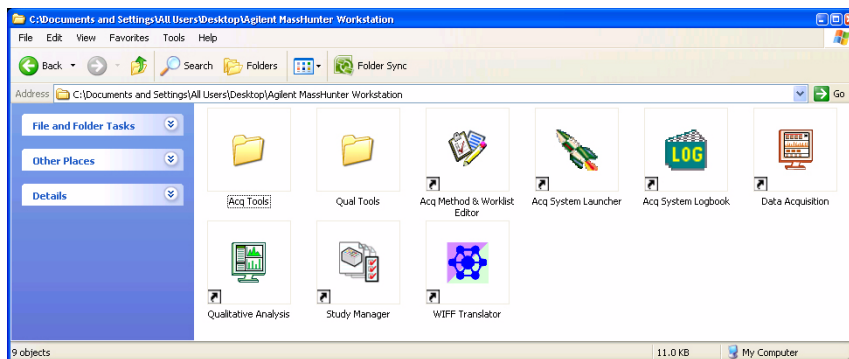
Step 1. Start the Data Acquisition software

The instructions below 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 LC/MS instrument are turned on, but the LC pump is not running.

After installation, you see all of the Agilent MassHunter Workstation software icons on your Desktop.

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



NOTE

When Data Acquisition opens, the software engines automatically start. If you need to restart them, double-click the **Acq System Launcher** icon on your Desktop, and click **Start**.

Do not run start any MassHunter program as administrator.

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

Getting Started

Step 1. Start the Data Acquisition software

Four panes—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 four panes of this main window. These panes provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status and monitor runs.

Click a button to see the pane you want to use.

Instrument Status pane

Real-time Plot pane

Method Editor pane

Worklist pane

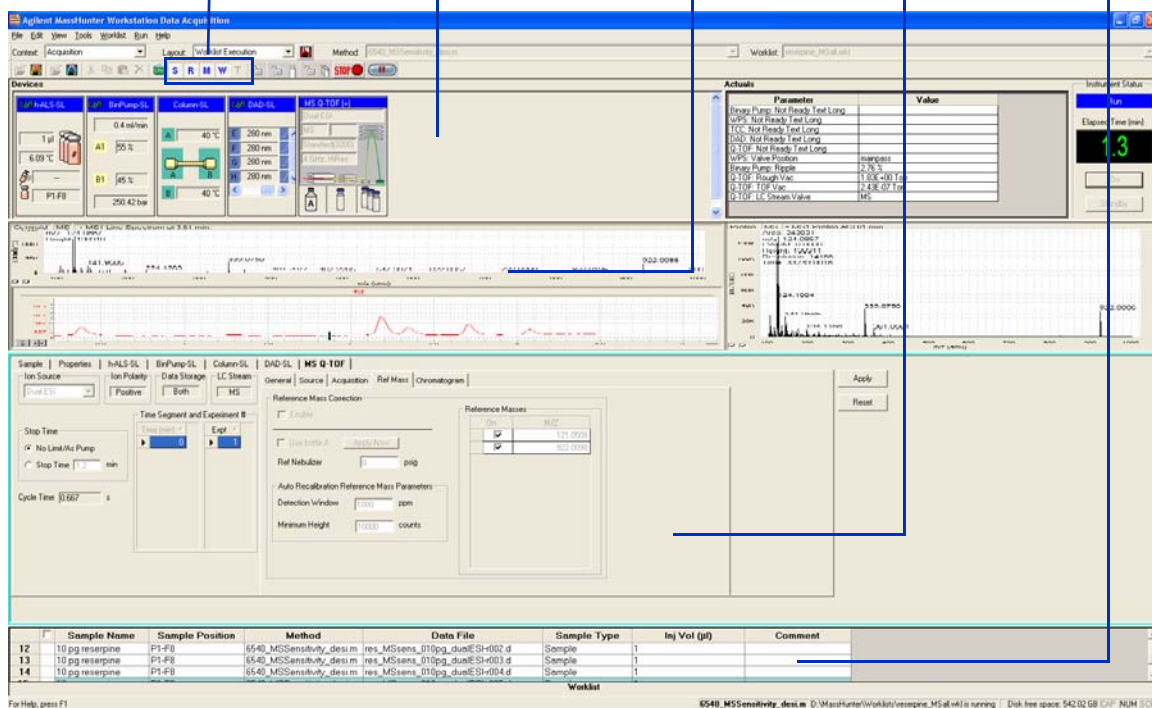


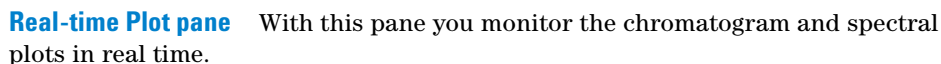
Figure 1 Main window of the Data Acquisition software

Show/hide the panes You can show one pane at a time on the screen or up to four panes. You can never hide all four panes. To show or hide a pane, you click the icons in the main window toolbar or you can use the menu items in the **View** menu.

When you click a pane, the active pane is outlined in blue. Press **F1** to obtain help on the active pane. You can also drag a pane border to resize the pane.



You also set non-method control and configuration parameters for the LC devices and MS and monitor the status of the device parameters during a run.



Getting Started

Step 1. Start the Data Acquisition software

Method Editor pane With this pane you enter acquisition parameters for the method and sample information to run individual samples interactively.

Sample

Properties

h-ALS-SL

BirPump-SL

Column-SL

DAD-SL

MS Q-TOF

Ion Source

Ion Polarity

Data Storage

LC Stream

Dual ESI

Positive

Both

MS

Stop Time

No Limit/As Pump

Stop Time 1.2 min

Cycle Time 0.667 s

Time Segment and Experiment #

Time (min) 0

Expt 1

Reference Mass Correction

Enable

Use bottle A

Ref Nebulizer 0 psig

Auto Recalibration Reference Mass Parameters

Detection Window 1000 ppm

Minimum Height 10000 counts

Reference Masses

On

M/Z

121.0509

322.0098

Apply

Reset

Worklist pane With this pane you enter sample information for multiple samples. When you run the worklist, the samples are automatically run in the order listed in the worklist.

	✓	Sample Name	Sample Position	Method	Data File
1	✓	Sulfa1	P1-A1	pfhms-only.m	Sulfa1.d
2	✓	Sulfa2	P1-A1	pfhms-only.m	Sulfa2.d
3	✓	Sulfa3	P1-A1	pfhms-only.m	Sulfa3.d

Worklist

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

The screenshot shows the 'General' tab of the Method Editor. The 'LC Stream (Seg)' group box has two radio buttons: 'MS' (selected) and 'Waste' (highlighted with a blue rectangle). Below the 'Waste' button is an 'Apply Now' button. Other visible controls include 'Ion Polarity (Seg)' with 'Positive' and 'Negative' radio buttons, 'Data Storage (Seg)' with 'None', 'Centroid', 'Both' (selected), and 'Profile' radio buttons, and a checkbox for 'Do not wait for setpoints (e.g. temperature) to equilibrate'. The 'Plot and Centroid Data Storage Threshold' section contains two columns: 'MS' and 'MS/MS', each with 'Abs. threshold' and 'Rel. threshold (%)' input fields.

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

- c Click **Apply**.

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.

Getting Started

Step 2. Prepare the LC modules

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

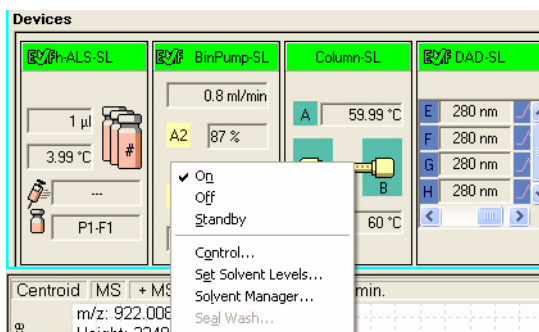
- a Enter LC parameters, and click **Apply** to download them to the LC.

The screenshot shows the 'BinPump-SL' configuration window. The 'Flow' section has a 'Flow' rate of 0.8 ml/min. The 'Solvent A' section shows a gradient from 87.00% H2O to 0.2% AcOH 1 mM NH4OAc. The 'Solvent B' section shows a gradient from 13% MeOH to 5 mM NH4 formate 90:10. The 'Stop Time' is set to 2 min, and the 'Post Time' is set to 2 min. The 'Pressure Limits' are set to Min: 0 bar and Max: 600 bar.

OR, to select an LC conditioning method, select one from the **Method** list at the top of the Data Acquisition window, or select one from the list that appears when you select **File > Open > Method**.

The screenshot shows a dropdown menu for the 'Method' field, with 'NewB28_1200Pump Test.m' selected.

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

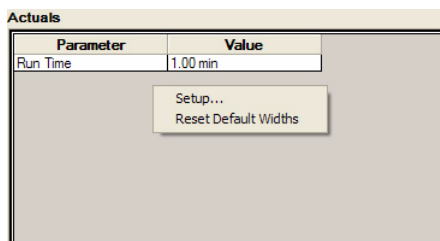


- c Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See [step 4](#) and [step 5](#) on [page 15](#).)

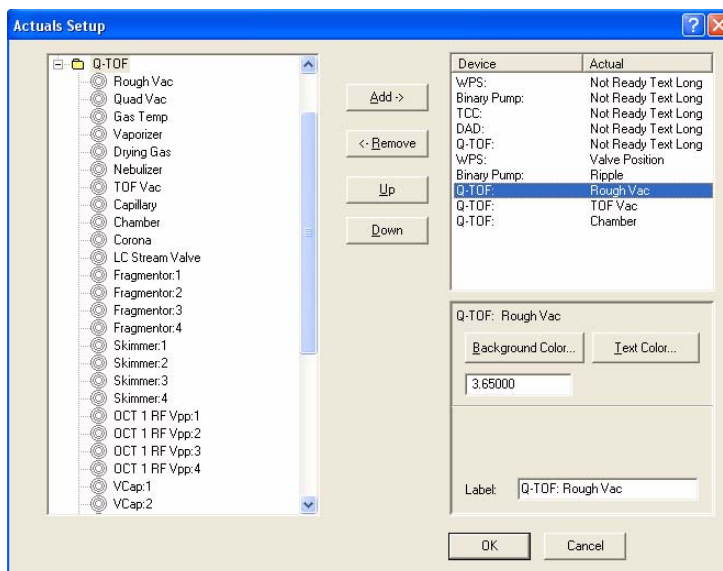
4 Set up to view real-time parameter values (actuals).

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

- a Right-click the Actuals section in the Instrument Status pane to see the Setup menu item.



- 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, and click **OK**.

Getting Started

Step 2. Prepare the LC modules

5 Set up real-time plot displays.

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

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



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

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 does recommend that you calibrate the mass-axis regularly.

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 Check Tune algorithm.

Before you run a **Set Detector Gain**, **Standard Tune** or **Initial Tune**, the **Instrument Mode** must be set to **Extended Dynamic Range**. 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 instrument.

You can only run **Initial Tune**, **Standard Tune**, **Set Detector Gain**, **Quad Tune** or **Initial Quad Tune** with a source 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.

Table 1 Supported sources for all Autotunes

Instrument Model Number	Supported Sources for All Autotunes
6220 TOF	ESI, Dual ESI
6230/6224 TOF	Dual ESI, AJS ESI, Dual AJS ESI
6520	Dual ESI
6530	Dual ESI, AJS ESI, Dual AJS ESI
6540/38	Dual ESI

For All Agilent 6200 Series TOF and 6500 Series Q-TOF

You can do a **Check Tune**, **Quick Tune** and **Check Quad Tune** 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)
- MMI
- APPI
- APCI

You cannot do any of the automated tunes if the source is a nanoESI, a Dual nanoESI, an HPLC-Chip or a MALDI source.

If **Fast Polarity Switching** is **Enabled**, you cannot run any of the Quad Autotune algorithms.

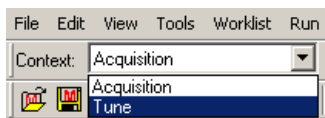
Diluting the ESI-L Tune Mix

If your instrument is an Agilent 6540A or 6530A 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.

Tune the TOF and Q-TOF MS

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



The Tune pane appears in place of the Method pane and the Worklist pane in the Data Acquisition window. Note that you tune the TOF separately from the quadrupole.

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 26.

Figure 2 Autotune tab for an Agilent 6538/6540 Series Q-TOF

The Quadrupole section is only available if you have configured a Q-TOF instrument.

- 2 (optional) Click the **Preferences** tab.
- 3 (optional) Mark whether or not you want the system to automatically adjust the abundance of the calibrant ions.

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

If you mark the **Adjust the abundance 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.

- 4 Click the **Autotune** tab.
- 5 Mark the polarity to use when tuning under TOF on the Autotune tab. You can mark **Positive**, **Negative** or both. For some instruments, you can also mark **Fast Polarity Switching**. If you mark the Fast Polarity Switching check box, then four different autotunes are done.
 - Positive

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

- Negative
- Fast Polarity Switching Positive
- Fast Polarity Switching Negative

The screenshot shows the 'Autotune' tab of the instrument's software interface. It is divided into several functional areas:

- Source Parameters:** Includes fields for Gas Temp (325 °C), Drying Gas (5 l/min), Nebulizer (30 psig), VCap (3500 V), and Chamber (0.00 uA).
- Ion Source:** Set to 'Dual ESI'.
- Ion Polarity:** Radio buttons for 'Positive' and 'Negative'.
- Mass List (default):** A table with columns 'On' and 'Mass [m/z]'. It contains a list of mass values with checkboxes in the 'On' column. Buttons for 'Add', 'Delete', 'Clear', 'Load', 'Save', 'All', and 'None' are present.
- TOF Mass Calibration:** Includes checkboxes for 'Pause Between Autotunes (for Calibration Solution Swapping)', 'Set CDS to None after Autotune', and 'Tune Report...'. Below this, there are radio buttons for 'Check Tune', 'Quick Tune', 'Standard Tune', 'Set Detector Gain', and 'Initial Tune'. A 'Start Autotune' button is also present.
- Calibrant Bottle:** Radio buttons for 'None', 'A', and 'B'.
- LC Flow to:** Radio buttons for 'Waste' and 'MS'.
- Checkboxes:** 'Disable CDS' and 'Enable CalibB in Acq'.

Figure 3 Autotune tab for an Agilent 6200 Series Time-of-Flight

- 6 (optional) Mark the **Pause Between Autotunes (for Calibration Solution Swapping)** check box if you use a different calibration solution for positive and negative autotunes.
- 7 Click **Check Tune** to check the TOF mass calibration and optimization. Then, click **Start Autotune** (Check Tune takes 3 to 5 minutes to complete).

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 **Check 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 750,000 for other Q-TOF and TOF instruments.

If Check Tune results are acceptable, then you can skip to [step 12](#) if you have a Q-TOF instrument or to [“Switch LC stream to MS”](#) on page 26 if you have a TOF instrument.

If Checktune results are not acceptable, then continue with [step 8](#).

- 8 Click **Quick Tune** to use a limited set of parameters to tune the MS automatically. Then, click **Start Autotune** (Quick Tune takes 7 to 10 minutes to complete).

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 750,000 for other Q-TOF and TOF instruments.

If Quick Tune results are acceptable, then you can skip to [step 12](#) if you have a Q-TOF instrument or to [“Switch LC stream to MS”](#) on page 26 if you have a TOF instrument.

If Quick Tune results are not acceptable, then continue with [step 9](#).

- 9** Run a **Standard Tune** to use even more parameters (10 to 15 minutes):
 - a** Click the **Instrument State** tab.
 - b** For the **Instrument Mode**, click **Extended Dynamic Range**.
 - c** (optional) Mark or clear the **Fast Polarity Switching** check box.
 - d** Click **Apply**.
 - e** Click the **Autotune** tab.
 - f** Mark the polarity (polarities) to use when tuning under **TOF** on the Autotune tab.
 - g** Click **Standard Tune**.
 - h** Click **Start Autotune**.

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 17 for a complete list of sources that are supported for all Autotunes.

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. If Standard Tune results are acceptable, continue with [step 10](#).

If **Standard Tune** produces unacceptable results you can do an **Initial Tune**. If this also fails to give acceptable results, please contact Agilent Field Support.

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

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

If you want use custom tune parameter values, you can also do a Manual Tune.

The screenshot shows the 'Manual Tune' tab in the TOF Mass Calibration software. The interface includes several tabs at the top: 'TOF Mass Calibration', 'Autotune', 'Manual Tune' (selected), 'Diagnostics', and 'Instrument State'. Below these are sub-tabs: 'Optics 1', 'Quad', 'Cell', 'Optics 2', 'TOF', 'Detector', and 'Ramp'. The 'TOF' sub-tab is active, displaying 'Setpoints' for 'Peak Width' (Medium), 'Quad AMU' (130 amu), 'Quad DC' (41 V), and 'PostFilter DC' (41 V). Below these are 'Width Gain' (2639), 'Width Offset' (1531), 'Axis Gain' (2774), and 'Axis Offset' (2349). At the bottom, the 'Ramp Parameter' section shows 'From' (10), 'To' (200), 'By' (10), 'Settling Time' (200 ms), and a 'Ramp' button.

10 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**.

11 Recalibrate, if necessary.

Calibration is done when you click the **Check Tune** 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:

- 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 750,000 for other Q-TOF and TOF instruments. You have to dilute the tune calibrant before you recalibrate. You recalibrate to get optimal mass accuracy.
- a** Click the **TOF Mass Calibration** tab.
 - b** Click the desired polarity in the top left corner of the Tune pane.
 - c** To select a different set of masses, click **Load** or select or clear individual masses in the list on the left side of the Tune pane.
 - d** Click the **Calibrate** button.
 - e** If you want to calibrate the TOF analyzer in the opposite polarity, repeat [step b](#) through [step d](#).

If you have a TOF instrument, skip to [“Calibrate the mass-axis”](#) on page 25.

- 12** If you have a Q-TOF instrument, check the options on the Instrument State tab before you tune the quadrupole.

If **Fast Polarity Switching** is **Enabled**, you disable it before you run any of the Quad Autotune algorithms. After the Quad tune results are acceptable, you will enable **Fast Polarity Switching** again and then recalibrate the TOF analyzer.

- a** Click the **Instrument State** tab.
- b** Select the appropriate **Mass Range**.
- c** Click the appropriate **Instrument Mode**.
- d** Select **Disabled** for **Fast Polarity Switching**.
- e** If you did not make any changes, skip to [step 13](#).
- f** Click **Apply**.
- g** Click the **TOF Mass Calibration** tab.
- h** Click the desired polarity in the top left corner of the Tune pane.
- i** To select a different set of masses, click **Load** or select or clear individual masses in the list on the left side of the Tune pane.
- j** Click the **Calibrate** button.
- k** If you want to calibrate the TOF analyzer in the opposite polarity, repeat [step h](#) through [step j](#).
- l** Click the **Autotune** tab.

- 13** Run the **Check Quad Tune** algorithm to check quadrupole optimization (2 to 5 minutes).

- a** Click the **Autotune** tab.
- b** Click the **Check Quad Tune** button.

If the Check Quad Tune results are acceptable, then skip to [step 16](#).

If Checktune results are not acceptable, then continue with [step 14](#).

- 14** Run the **Quad Tune** algorithm to optimize the quadrupole using all its parameters (10 to 15 minutes).

The source that is used must be supported for all Autotunes when running a **Quad Tune**. The Dual ESI source is supported for all Autotunes for all instruments. Refer to [“Supported sources for all Autotunes”](#) on page 17 for a complete list of sources that are supported for all Autotunes.

Also, you cannot run a Quad Tune if Fast Polarity Switching is **Enabled**.

- a** Click the **Instrument State** tab.
- b** For the **Instrument Mode**, click **Extended Dynamic Range**.
- c** Click **Apply**.
- d** Click the **Autotune** tab.
- e** Clear the **Fast Polarity Switching** check box.
- f** Click **Quad Tune**.

If **Quad Tune** produces unacceptable results you can do an **Initial Quad Tune** (50 to 60 minutes). If this also fails to give acceptable results, please contact Agilent Field Support.

If you would like to use custom tune parameter values, you can also do a Manual Tune.

- 15** 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**.

- 16** If you want to use Fast Polarity Switching, enable **Fast Polarity Switching** on the Instrument State tab and recalibrate.

If you have a Q-TOF instrument, **Fast Polarity Switching** was disabled before tuning the quadrupole. Follow these steps to enable Fast Polarity Switching:

- a** Click the **Instrument State** tab.
- b** Select **Enabled** for the **Fast Polarity Switching**.
- c** Click the appropriate **Instrument Mode**.
- d** Click **Apply**.
- e** Wait 20 minutes for the instrument to equilibrate.
- f** Click the **TOF Mass Calibration** tab.
- g** Click the desired polarity in the top left corner of the Tune pane.
- h** To select a different set of masses, click **Load** or select or clear individual masses in the list on the left side of the Tune pane.
- i** Click the **Calibrate** button.
- j** If you want to calibrate the TOF analyzer in the opposite polarity, repeat [step g](#) through [step i](#).

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.

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.

Calibration is done when you click the **Check Tune** 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:

- 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 750,000 for other Q-TOF and TOF instruments. You have to dilute the tune calibrant before you recalibrate. You recalibrate to get optimal mass accuracy.

1 In the **Combo Bar**, select **Tune** in the **Context** combo box.

2 Click the **TOF Mass Calibration** tab.

3 Select the **Ion Source** and **Polarity** on the left side of the Tune pane.

4 To select a different set of masses, click **Load** or select or clear individual masses in the list on the left side of the Tune pane.

5 Click **Calibrate**.

The TOF or Q-TOF Calibration Results dialog box opens.

6 (optional) Repeat [step 3](#) to [step 5](#) for the other polarity.

7 Click **Apply** to apply the updated calibration coefficients.

Switch LC stream to MS

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**.
- b** Make sure that the General tab in the MS TOF or MS Q-TOF tab is selected in the Method Editor pane.
- c** In the **LC Stream (Seg)** group box, click **MS**.
- d** Click **Apply**.

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 the **Log** icon in the toolbar of the Data Acquisition window, and view the logged events. 
- 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.

Step 4. Set up and run a method

An Agilent MassHunter Workstation software method for TOF and Q-TOF can contain acquisition parameters, qualitative analysis parameters or both.

When you run a single sample or 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 select to run both data acquisition and data analysis, then data analysis will automatically follow acquisition if qualitative analysis parameters are also contained in the method. 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 (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.

Before editing or running a method, set the options in the Instrument State tab in the Tune pane.

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

Getting Started

Step 4. Set up and run a method

- 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.

The screenshot shows the 'BinPump-SL' tab in the Agilent Method Editor. The 'Setup' section is active, displaying various LC parameters. The 'Flow' is set to 0.8 ml/min. 'Solvent A' is configured with 87.00% H2O and 13% MeOH. 'Solvent B' is configured with 13% MeOH and 5 mM NH4 formate 90:10. The 'Stop Time' is set to 2 minutes, and the 'Post Time' is also set to 2 minutes. The 'Pressure Limits' are set to a minimum of 0 bar and a maximum of 600 bar.

- 3 Set up to change TOF and Q-TOF MS parameters with segments and experiments:
 - a Click the **MS TOF** or **MS Q-TOF** tab in the Method Editor.
 - b To add a segment, right-click the Time Segment section and click **Add Row**.
The time segment will use 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 Row**. The experiment will use those parameter entries with (Expt) next to their names. Those parameters can be changed for each experiment.

Sample | Properties | ALS | Quat Pump | TCC | DAD | **MS Q-TOF**

Ion Source: Dual ESI | Ion Polarity: Negative | Data Storage: Both | LC Stream: MS

Stop Time: ☒ No Limit/As Pump ☐ Stop Time 30 min

Time (min)	Expt
0	1
1.4	
1.9	

Cycle Time: s

- 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.

DAD | **MS Q-TOF** | General | Source | Acquisition | Ref Mass | Chromatogram

Dual ESI (Seg)

Gas Temp: 325 °C | 325 °C

Drying Gas: 8 l/min | 7.0 l/min

Nebulizer: 40 psig | 25 psig

Dual ESI (Expt)

VCap: 4000 V | Capillary: 9.035 uA

Chamber: 3.61 uA

MS TOF (Expt)

Fragmentor: 175 V

Skimmer: 65 V

OCT 1 RF Vpp: 750 V

See Chapter 3 of the *Concepts Guide* for an explanation of how and why you use time segments and experiments.

- 4** Enter TOF or Q-TOF MS parameter values:
- Click the **General** tab, and enter any General parameters that you want to change.
 - Click the **Source** tab, and enter any Source values you want to change.
 - Click the **Acquisition** tab.
 - 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**.

Getting Started

Step 4. Set up and run a method

Different parameters are made available depending on the mode selected.

To learn more about these parameters and how they affect results, refer to Chapter 3 of the *Concepts Guide* or the online Help.

- e Enter any values you want to change in the Acquisition tab.

The screenshot shows the 'MS Q-TOF' software interface with the 'Acquisition' tab selected. The interface includes several configuration sections:

- General:** Ion Source (Dual ESI), Ion Polarity (Positive), Data Storage (Both), LC Stream (MS).
- Stop Time:** Radio buttons for 'No Limit/As Pump' (selected) and 'Stop Time' (30 min).
- Time Segment and Experiment #:** Two columns with 'Time (min)' and 'Expt #' headers. The first segment shows '0' and '1'.
- Mode:** Radio buttons for 'MS (Seg)' (selected), 'Auto MS/MS (Seg)', and 'Targeted MS/MS (Seg)'.
- TOF Spectra:**
 - Mass Range:** Min Range (100 m/z), Max Range (1000 m/z).
 - Acquisition Rate/Time:** Rate (1 spectra/s), Time (1000 ms/spectrum), Transients/spectrum (9897).
- Cycle Time:** 1 s.

- f Click the **Ref Mass** tab to set up the mass calibration.

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

5 Set up a method with both acquisition and qualitative analysis parameters.

You can create either part of the method first.

If you want to add acquisition parameters to a method with Qualitative Analysis parameters:

- a Set up a method with acquisition parameters.
- b Click **File > Save As > Method**.
- c Type the name of an existing method that contains Qualitative Analysis parameters.
- d Click **OK**.

The method now contains both acquisition and Qualitative Analysis parameters.

NOTE

The name of the method that contains only qualitative analysis parameters will not appear in the Name list in the Save Method As dialog box. Type the method name in the Method field and select the directory that contains that method in the Directory list.

If you want to add qualitative analysis parameters to a method with acquisition parameters:


- a** Set up and save a method with acquisition parameters.
- b** Open the **Qualitative Analysis** program.
- c** Open a data file that was created with the acquisition method you set up and saved in [step a](#).
- d** Enter the qualitative analysis parameters and change settings to meet your needs.
- e** Save the method using the same name that you used to save the method in [step a](#).

The method now contains both acquisition and qualitative analysis parameters.

See the online Help to learn how to enter or change parameters and set up tasks for this method.

Set up and run interactive samples

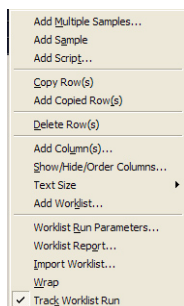
- 1** Type the sample name, data file name and path.


- 2** To start the run, click the single sample icon, .

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.

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**.
- 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**.
- 6 Type the paths for the method, the Override DA method, and the data files, and click **OK**.
- 7 To start the run, click the **Start Worklist run** icon, .

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.

NOTE

To use qualitative analysis parameters that are different than those contained in 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 qualitative analysis parameters you want to use for the sample.

You can also type this method in the Add Multiple Samples dialog box.

Step 5. Review results and find compounds with Qualitative Analysis

- Use the Qualitative Analysis program 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.

In this book

This book contains brief instructions to help you get started with your Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS system. This book takes a quick look at using the Agilent MassHunter Workstation software for:

- Preparing the instrument for a run
- Setting up acquisition methods
- Setting up and running worklists

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