

MassHunter Workstation for LC/TOF and LC/Q-TOF

Quick Start Guide

| | |
|---|----|
| What is the Agilent LC/TOF system? | 2 |
| What is the Agilent LC/Q-TOF system? | 3 |
| What is the MassHunter Workstation Software? | 4 |
| What's New in MassHunter 11.0 | 5 |
| Where to find information | 7 |
| Online Help | 7 |
| Installation and User Guides | 7 |
| Training | 7 |
| Agilent Community | 8 |
| Getting Started | 9 |
| Step 1. Start the Data Acquisition software | 10 |
| Step 2. Prepare the LC modules | 17 |
| Step 3. Prepare the TOF and Q-TOF instrument | 21 |
| Step 4. Set up and run a method | 37 |
| Step 5. Review results and find compounds with Qualitative Analysis | 45 |
| Step 6. Set up and run quantitation | 45 |
| Step 7. Review data in IM-MS Browser | 45 |

Use this guide for your first steps with the MassHunter Workstation for LC/TOF and LC/Q-TOF, and as a road map for your user information.

What is the Agilent LC/TOF system?

The Agilent LC/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 LC/TOF 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)
- HPLC-Chip
- APCI
- APPI
- Multimode
- Nanospray (nanoESI)
- Dual nanospray (Dual nanoESI)
- MALDI
- GC-APCI
- DESI

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 LC/Q-TOF system?

The Agilent LC/Q-TOF 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.

All 6500 series Q-TOF instruments support Agilent Jet Stream and 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.

You can set up an Agilent LC/Q-TOF 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, HPLC-Chip, APCI, APPI, Multimode, nanospray, dual nanospray, MALDI, GC-APCI, and DESI.

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 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 MassHunter Workstation Software?

The Agilent LC/TOF and LC/Q-TOF 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. For more information, please refer to the online Help for the IM-MS Browser.
- 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 MassHunter 11.0

- The following Agilent Q-TOF mass spectrometers are supported: 6560A, 6560B, 6550 iFunnel, 6546, 6545XT AdvanceBio, 6545B, 6545A, 6540B, 6540A, 6538A, 6530C, 6530B, 6530A, and 6520B.
- The Agilent 6230B, 6230A, and 6224A Accurate-Mass TOF mass spectrometers are supported in this release.
- OpenLab Control Panel is supported.
- Data Acquisition now has two configurations:
 - MassHunter Data Acquisition Workstation
 - MassHunter Data Acquisition Networked Workstation
- In **Workstation** configuration:
 - Data storage is always local, with no content management. You do not have an option to change this in the Control Panel.
 - Authentication Mode can be turned on or off in the Control Panel. Authentication Mode means that you have to log in to perform any action. You can set authentication to "internal" or "domain".
- In **Networked Workstation** configuration:
 - Data is always stored and managed in the Content Management component of OpenLab Server or ECM XT.
 - Authentication Mode is always on.
 - You can import existing Data Files, Methods, and Worklists into a project in the Content Management component.
 - Data Acquisition Networked Workstation includes features for the regulatory compliant environments.
- Data Acquisition has different user roles and privileges in this release. You may not be able to use some features if you do not have the correct role.
- All changes in the method are recorded in the Audit Trail except parameters in the Chromatogram tab (which are not data related parameters) and parameters in the 2D-LC File Splitter section in the DA > Data Conversions tab.
- You can review changes shown in the Audit Trail window and add a reason for the changes.
- 2D-LC acquisition is supported in content management mode, but only without file splitting.
- The Tune file is saved in the SmartCard hard drive.

What's New in MassHunter 11.0

- Electron-Capture Dissociation (ECD) (third party hardware controlled by e-MSion software and firmware add-on) is supported. Agilent software shares Polarity, Scan type (MS1,MS2), Acquisition rate, and collision energy. (CE)
- Optional SLIM hardware which is controlled by MOBILion software and firmware add-on is supported. SLIM stands for Structures for Lossless Ion Manipulation.
- RapidFire software is supported.
- Third party high resolution demultiplexing is supported in addition to the Agilent Demultiplexing tool.
- The Q-TOF driver can down-sample from 10 GHz to 4 GHz for the G6546A.
- The MS data file supports a new header schema which includes isolation windows width.
- Sure Mass optimization is supported for the 6546, the 6545, and the 6545XT AdvanceBio instruments.
- The 6546 Q-TOF supports using the PC for MS/MS decisions.
- Iterative MS/MS is introduced for classic MS/MS decisions.
- The Ribbon replaces the menu and the toolbar in the Data Acquisition program.

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**.



On the ribbon, click this button to access all help.



In the Instrument Status window, click this button in the title bar for any device to access help for that device.

Installation and User Guides

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

Training

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

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

Where to find information
Agilent Community

Agilent Community



To get answers to your questions, join over 10,000 users in the Agilent Community. Review curated support materials organized by platform technology. Ask questions to industry colleagues and collaborators. Get notifications on new videos, documents, tools, and webinars relevant to your work.

<https://community.agilent.com>

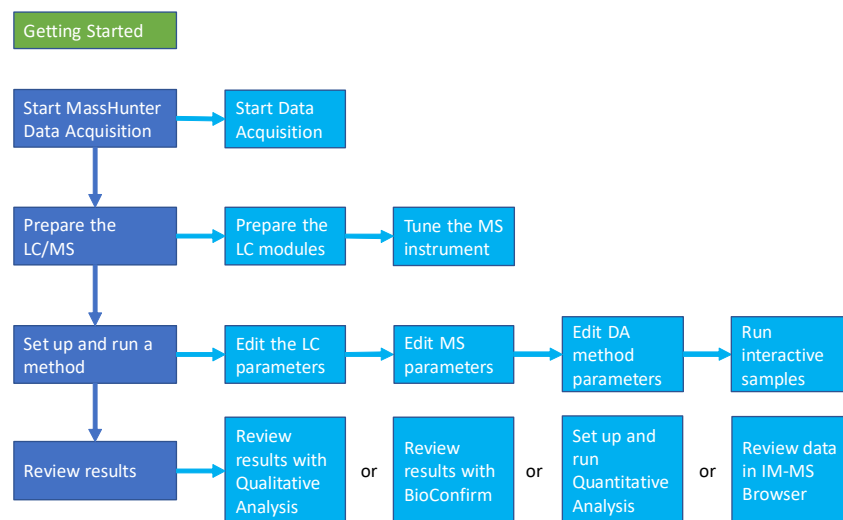
Getting Started

Install the LC/TOF or LC/Q-TOF 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.



Getting Started

Step 1. Start the Data Acquisition software

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.

To start Control Panel

- Double click the Control Panel icon on the desktop ().

- Click **Start > Agilent Technologies > Control Panel**.

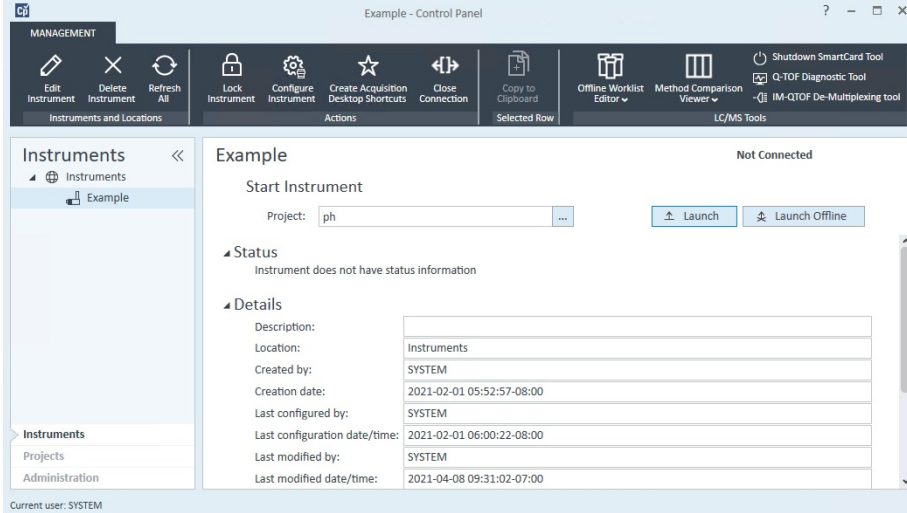
Create Desktop Shortcuts

- (optional) In the Control Panel on the ribbon in the **Actions** group, select an instrument and a project; then, click **Create Acquisition Desktop Shortcuts**.

To start Data Acquisition

- In the Control Panel, select an instrument and project, and click .

- If you created a desktop shortcut, click the online icon, .



| Property | Value |
|-------------------------------|---------------------------|
| Description: | |
| Location: | Instruments |
| Created by: | SYSTEM |
| Creation date: | 2021-02-01 05:52:57-08:00 |
| Last configured by: | SYSTEM |
| Last configuration date/time: | 2021-02-01 06:00:22-08:00 |
| Last modified by: | SYSTEM |
| Last modified date/time: | 2021-04-08 09:31:02-07:00 |

Getting Started

Step 1. Start the Data Acquisition software

NOTE

When Data Acquisition opens, the software engines automatically start. If you need to restart them, right-click the **LC/MS 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 *MassHunter Help & Learning* for instructions.


The ribbon

The ribbon contains the **Home** tab, which includes six groups.

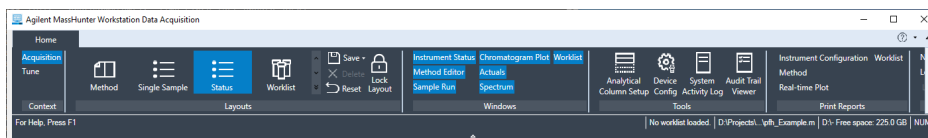
- **Context** - You can switch from Acquisition to Tune in this group.
- **Layouts** - You can select a layout. You can also create new layouts and save them.
- **Windows** - You can select which windows are visible in this group.
- **Tools** - You can start four acquisition tools.
- **Print Reports** - You can print 4 different types of reports.
- **Settings** - You can set **No Event Messages** and **Locked Mode**.

If all groups are not visible, a scroll arrow appears at the end of the ribbon. The scroll arrow can appear at either end, depending on where it is needed.

You can minimize the ribbon when you click  in the tab bar of the ribbon.

If the ribbon is minimized, you can click  to restore it.

You can also minimize and maximize the status area of the ribbon when you click the button under the status area.



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.

Getting Started

Step 1. Start the Data Acquisition software

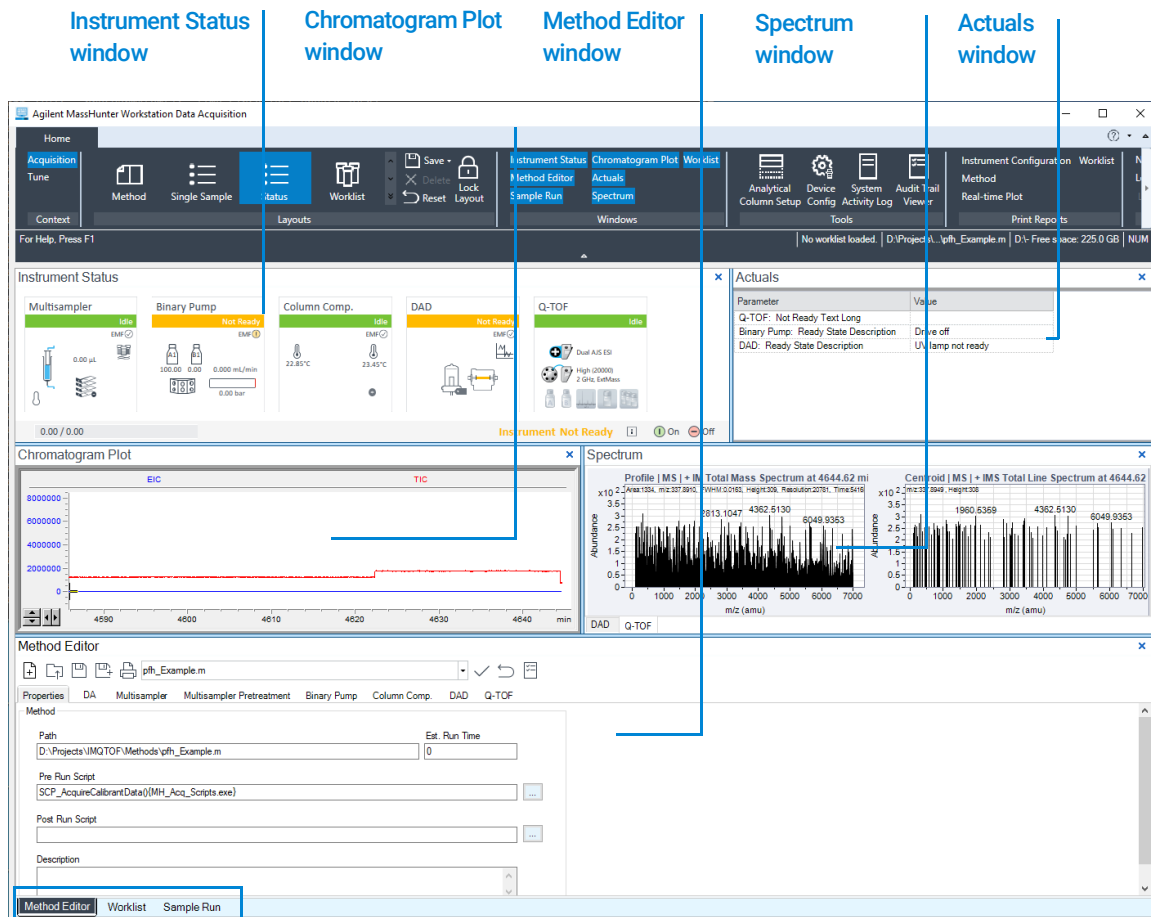


Figure 1. Main window of the Data Acquisition software. Method Editor, Sample Run and Worklist windows are tabbed here. These three windows are “sharing” this space. You click the tab to switch to a different window.

Show/hide the windows You can show one window at a time on the screen or all seven windows. On the ribbon in the **Windows** group, you click the buttons to show or hide a window. You can also hide a window by clicking **X** in the upper right corner of the window.

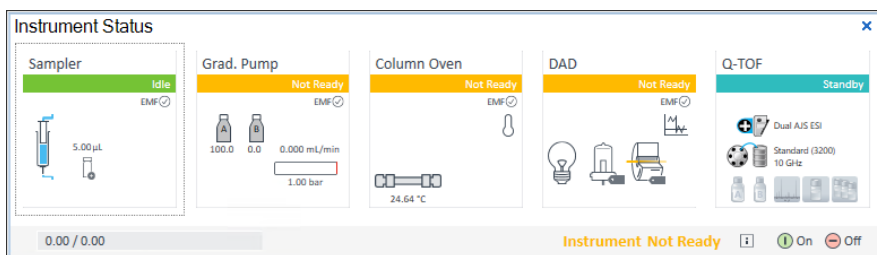
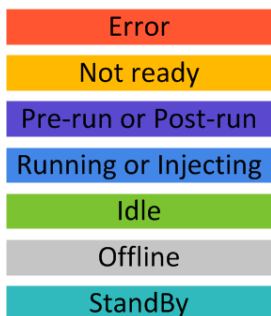
When you click a window, you can press **F1** to obtain help on the active window. You can also drag a window border to resize the window. If you double-click the title of the window, the window “floats” outside of the main window. You can double-click the title bar again to “dock” the window.

Getting Started

Step 1. Start the Data Acquisition software

Instrument Status window With this window you view the status of each device configured with the instrument. 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.

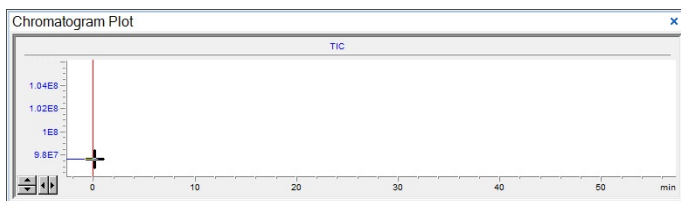
Actuals window With this window you view the current value of selected instrument parameters. You can change the color choice and add conditional formatting in the **Actuals Selection** dialog box. See **“Set up to view real-time parameter values (actuals).”** on page 18.

| Parameter | Value |
|-----------------------------|--|
| Q-TOF: Not Ready Text Long | One or more temps/flow/pressure are not ready. |
| Q-TOF: Control State | StandingBy |
| Grad. Pump: Solvent Ratio A | 100 % |

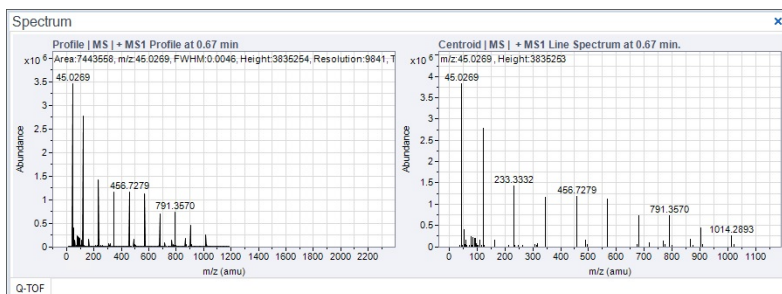
Getting Started

Step 1. Start the Data Acquisition software

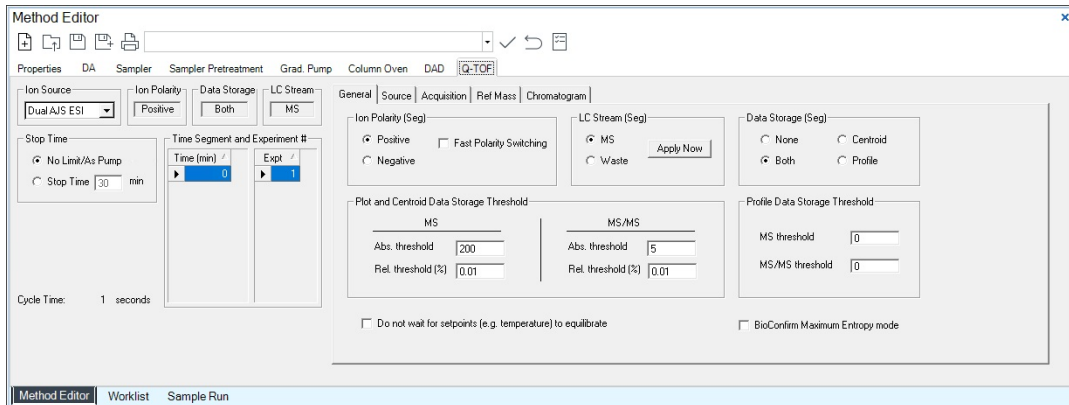
Chromatogram Plot window With this window you monitor the chromatogram plots in real time. These plots can be user-defined signals and/or instrument parameters.



Spectrum 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 a 6560 Ion Mobility Q-TOF, then the **Acquisition Mode** section is available under the **Stop Time** section.



Getting Started

Step 1. Start the Data Acquisition software

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 **Both Acquisition and DA** for the **Method Type**, and then Data Analysis is run as part of the method.

The Sample Run dialog box is divided into several sections:

- Sample:** Fields for Name (containing 'Sample'), Position (No Injection), Injection Volume (As Method), and a Comment field.
- Data File:** A checkbox for 'Auto Increment', a Name field (Sample0005.d), a 'View Data' button, and a Path field (C:\Projects\ph\Data).
- Additional Information:** A table with columns 'Parameter Name' and 'Parameter Value'.

| Parameter Name | Parameter Value |
|---------------------|------------------|
| Sample ID | |
| Override DA Method | |
| Method part to run: | Acquisition Only |
| Balance Override | No Override |
| Equilib Time (min) | 0 |
| OperatorName | SYSTEM (SYSTEM) |

At the bottom, there are tabs for 'Method Editor', 'Worklist', and 'Sample Run'.

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** or to run **Both Acquisition and DA** 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.

The Worklist window displays a table with the following data:

| | Status | Sample Name | Sample Position | Method | Data File | Sample Type | Level Name | Inj Vol (µl) | Comment | Sample Group |
|---|-------------------------------------|-------------|-----------------|----------------|---------------------|-------------|------------|--------------|-----------|--------------|
| 1 | <input checked="" type="checkbox"/> | | No Injection | ph_iterative.m | WorklistData-0001.d | Sample | | | As Method | |

At the bottom, there are tabs for 'Method Editor', 'Worklist', and 'Sample Run'.

Getting Started

Step 1. Start the Data Acquisition software

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.

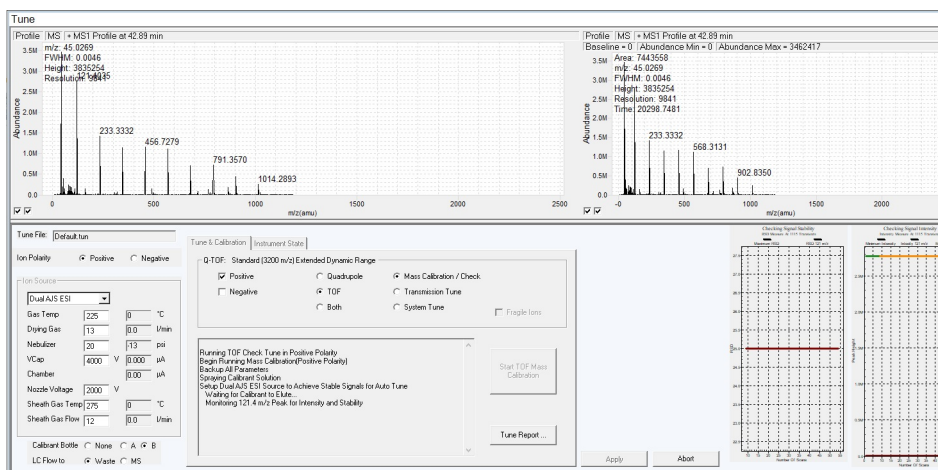


Figure 2. Tune window for a 6550A Q-TOF instrument showing two additional graphs for the SWARM autotune

Step 2. Prepare the LC modules

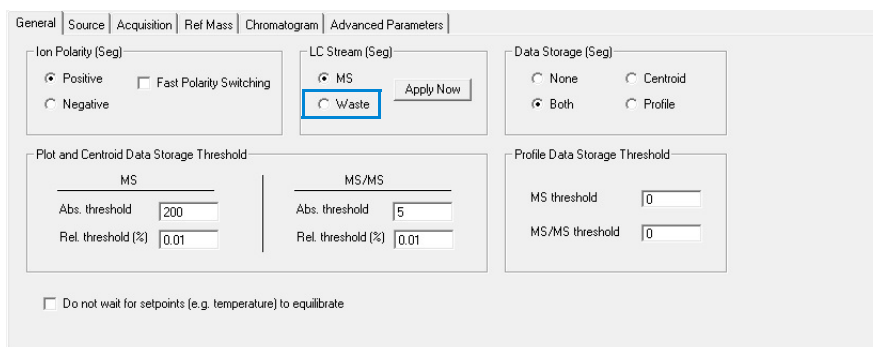
Read and follow the instructions in the online Help for each of the tasks in the checklist described on the following pages.

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.



The screenshot shows the 'Advanced Parameters' tab in the Method Editor. The 'LC Stream (Seg)' section is highlighted with a blue box, showing the 'Waste' radio button selected. The 'Apply Now' button is visible next to it. Other sections include 'Ion Polarity (Seg)' with 'Positive' selected, 'Data Storage (Seg)' with 'Both' selected, and 'Plot and Centroid Data Storage Threshold' and 'Profile Data Storage Threshold' sections with various threshold values.

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 ✓ 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.

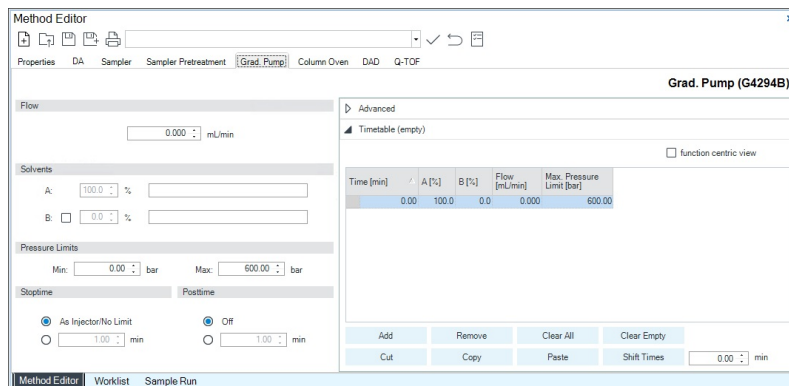
3 Condition or equilibrate the column.


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

a Enter LC parameters, and click ✓ on the toolbar to download them to the LC.

Getting Started

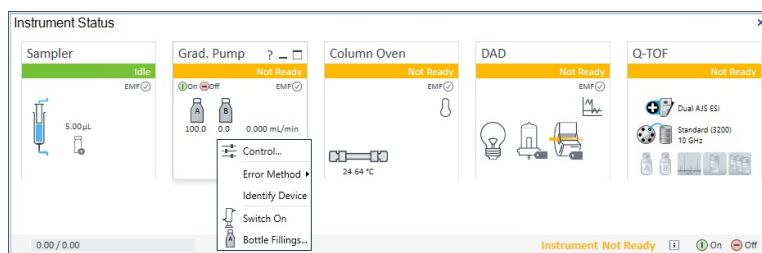
Step 2. Prepare the LC modules



You may be able to select an LC conditioning method. If so, you click  in the Method Editor toolbar to open the **Open Method** dialog box, or you select one from the **Method Editor** toolbar.

Default_With_DualAJS_ESI.m

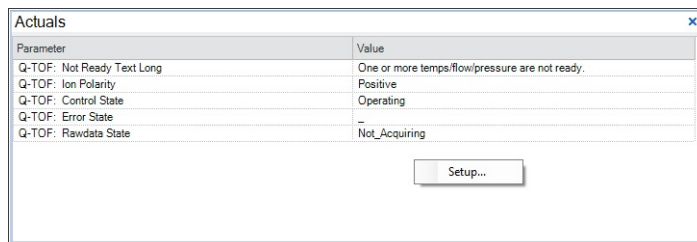
- b Right-click an LC module in the Instrument Status window to change any non-method control parameters, if necessary.



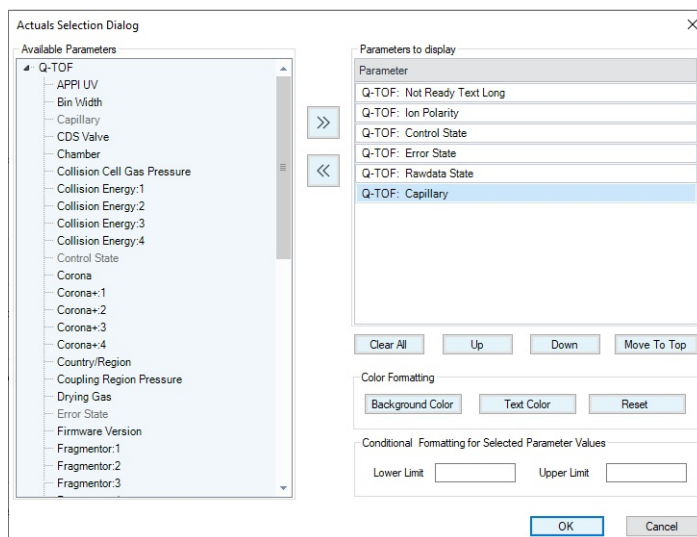
- c Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See [step 4](#) and [step 5](#) on [page 18](#).)
- 4 Set up to view real-time parameter values (actuals).
- As you prepare for a run and during a run, you want to see the actual values of the instrument parameters. You can do this in the Actuals window.
- a Right-click the **Actuals** window to see the Setup command.

Getting Started

Step 2. Prepare the LC modules



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

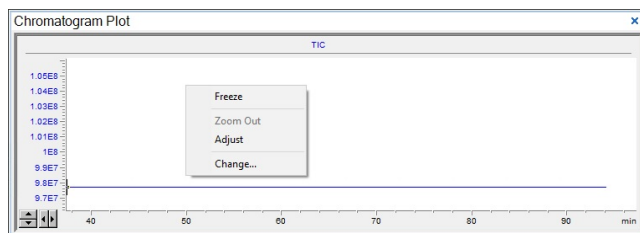
Getting Started

Step 2. Prepare the LC modules

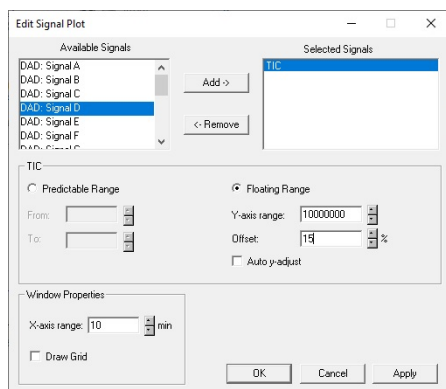
5 Set up real-time plot displays.

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

- Right-click the **Chromatogram Plot** 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

For additional information on tuning a Q-TOF LC/MS instrument, see the *MassHunter Q-TOF LC/MS Tuning Quick Start Guide* on the Resource App.

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 Diagnostics tab.

You can use either the SWARM autotune or the Classic Autotune depending on the type of instrument that you have. For some instruments, you can select which type of tune to use:

- SWARM Autotune only - 6560, 6550, 6546, 6545XT, 6545, and 6530 Q-TOF instruments, 6230 and 6224 TOF instrument
- Classic Auto Tune only - 6540, 6538, and 6520 Q-TOF instruments

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

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

- 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 Q-TOF. If you change the mass range, you must recalibrate the 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 .

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

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 Q-TOF | ESI, Dual ESI, AJS ESI, Dual AJS ESI |
| 6560 Q-TOF | 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, a GC-QTOF, or a DESI 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 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.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

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.

Make up fresh tuning solution at least once every month to ensure best performance.

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.

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.

**Dilution for
Classic
autotune**

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

| Tune Type | 6540 | 6520B or 6538 |
|--------------------------------------|-----------------------|--------------------|
| 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 |

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

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

SWARM Auto Tune

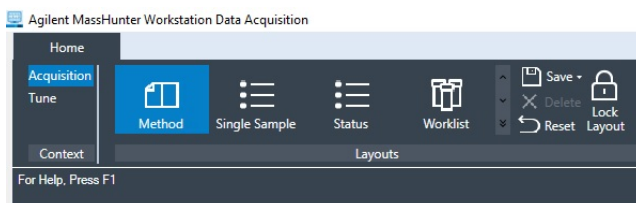
The 6530, 6545, 6545XT, 6546, 6550, and 6560 Q-TOF and 6224 and 6230 TOF LC/MS use the SWARM autotune. The options available in the Tune & Calibration tab are different for these instruments.

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 On the ribbon in the **Context** group, click **Tune**.



The Tune window appears. The Instrument Status window, the Actuals window, the Chromatogram Plot 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.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

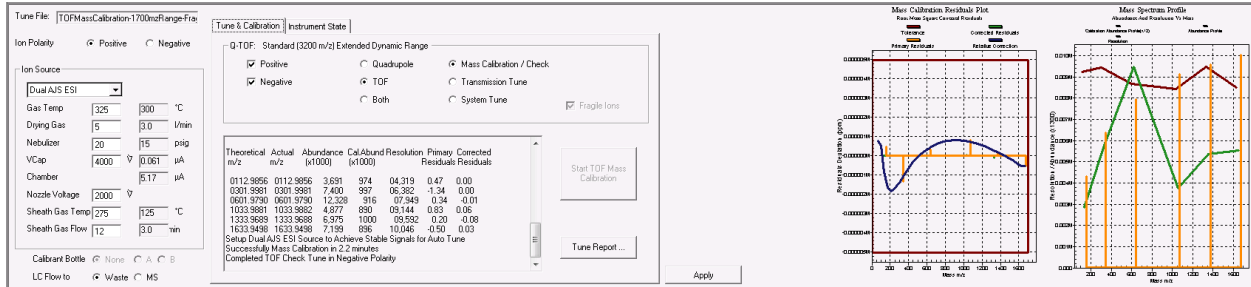


Figure 3. Tune & Calibration tab for a 6545 Series Q-TOF

- 2 Click the **Instrument State** tab.
- 3 Mark **Advanced Control**.
- 4 Click the **Preferences** tab.
- 5 Clear the **Pause Between Autotunes (for Calibration Solution Swapping)** check box on the Preferences tab.
- 6 Click the **Instrument State** tab.
- 7 Clear the **Advanced Control** check box.
- 8 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.
- 9 Select either **Disabled** or **Enabled** for **Fast Polarity Switching**.
- 10 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**.
- 11 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.)
- 12 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**.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

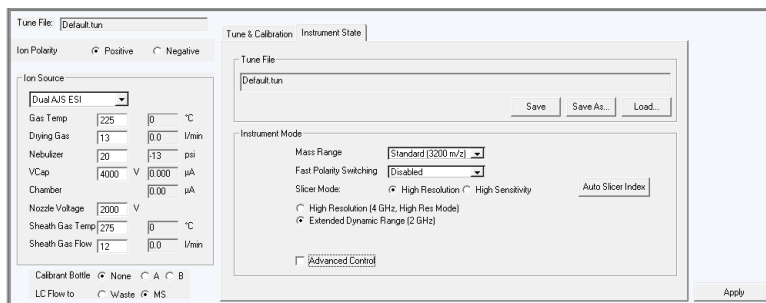
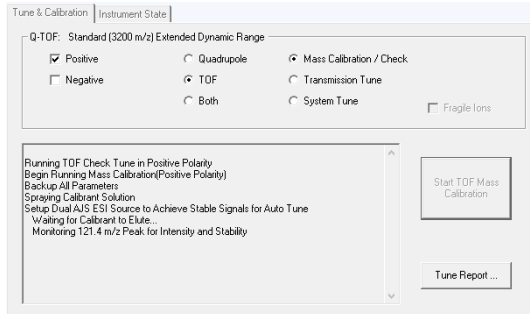


Figure 4. Instrument State tab

- 13 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.
- 14 Click the **Tune & Calibration** tab.
- 15 Mark the polarity. You can mark **Positive**, **Negative**, or both check boxes. If **Fast Polarity Switching** is **Enabled**, these check boxes are not available.
- 16 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 m/z)**, then these options are not available.
- 17 Click **Mass Calibration / Check**.
- 18 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.
- 19 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.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument



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.

20 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 21](#).

21 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 22](#).

22 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**.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

- c Click **Apply**.
- d Click the **Tune & Calibration** tab.
- e Click **System Tune**.

23 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.

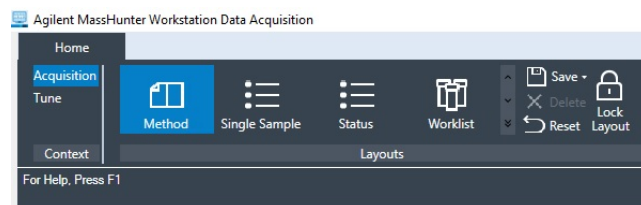
Calibrate the mass-axis

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**.

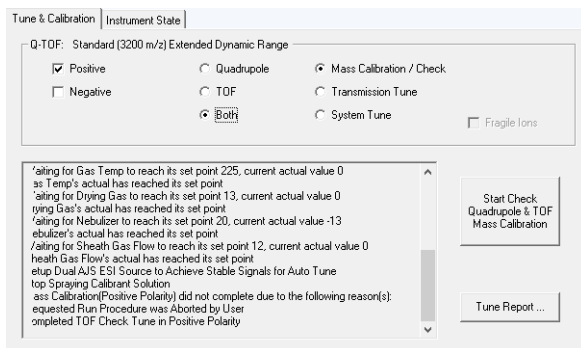
1 On the ribbon in the **Context** group, click **Tune**.



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

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument



- 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.

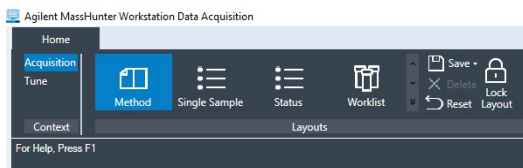
Please note that if you have a 6545, and you click the **50 - 250 m/z** option, calibrant ions below 100 *m/z* are used.

Tune the TOF and Q-TOF MS using Classic Auto Tune

You tune the following instruments using Classic Auto Tune: 6540, 6538, and 6520. You can select to tune the following instruments using Classic Auto Tune: 6530, 6550, 6230, and 6224.

Classic Auto Tune

- 1 On the ribbon in the **Context** group, click **Tune**.



The Tune window appears. The Instrument Status window, the Actuals window, the Chromatogram Plot 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.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

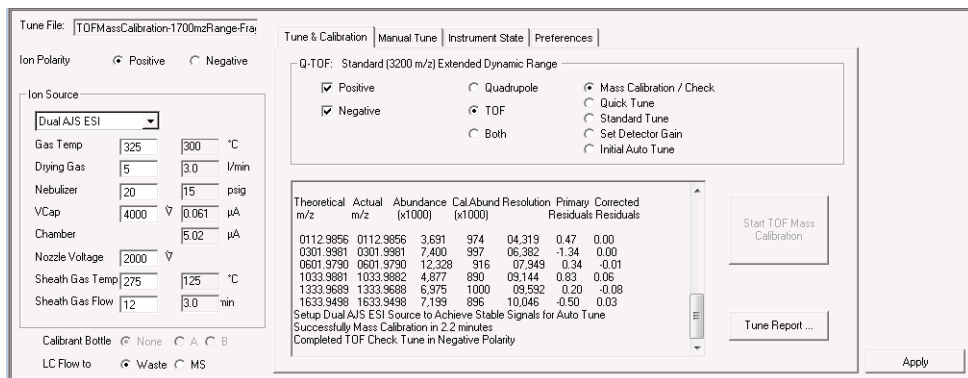


Figure 5. Tune & Calibration tab for a Q-TOF instrument using Classic Auto Tune

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

- 2 Click the **Instrument State** tab.
- 3 Click **Use Advanced Control**.
- 4 Click the **Preferences** tab.
- 5 (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.

- 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 Click the **Instrument State** tab.
- 8 Select the **Mass Range**.
- 9 Select either **Disabled** or **Enabled** for **Fast Polarity Switching**.
- 10 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

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

Auto Tune algorithms. You must select **High Resolution** for the **Slicer Mode** before you do an **Initial Auto Tune**.

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

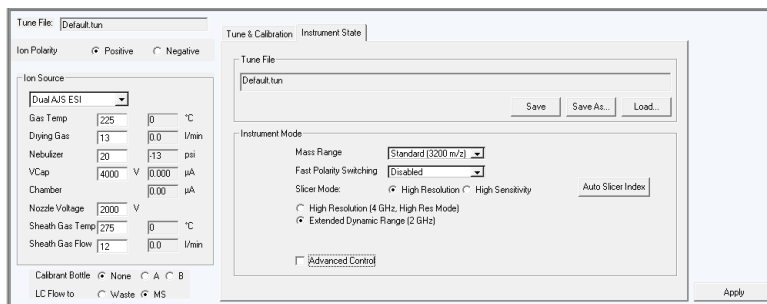


Figure 6. Instrument State tab

- 13 Click the **Tune & Calibration** tab.
- 14 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.
- 15 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 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 first tune the TOF and then tune the Quadrupole.
- 16 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 auto tune 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.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

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 instruments. 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 17**.

- 17** 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.

- 18** 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 auto tune 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 the 6520 Q-TOF instrument.

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 19**.

- 19** Run a **Standard Tune** to update 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**.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

- 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 20**.

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

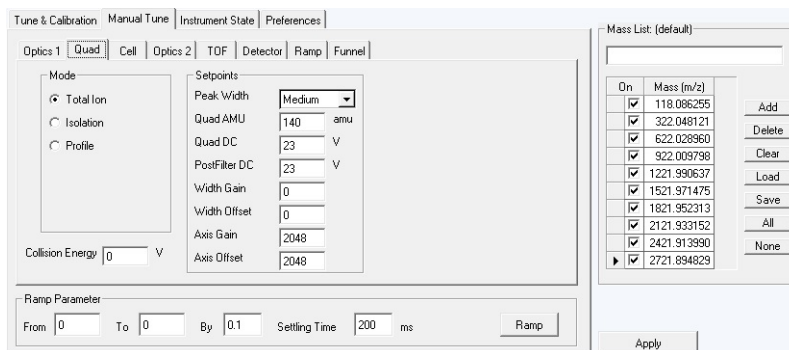
| Instrument | Time for Initial Tune (TOF) |
|-----------------------|------------------------------------|
| 6538A, 6540A | up to 60 minutes for each polarity |
| 6520 Q-TOF instrument | up to 30 minutes for each polarity |

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.

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument



20 Set the **Mass Range** and the **Instrument Mode** that you want to use to acquire data.

- Click the **Instrument State** tab.
- Select the appropriate **Mass Range**.
- Click the appropriate **Instrument Mode**.
- Click **Apply**.
- 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.

21 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 the 6520 Q-TOF. You have to dilute the tune calibrant before you recalibrate. You recalibrate to get optimal mass accuracy.
- Click the **Tune & Calibration** tab.
 - Mark the desired polarity or polarities in this tab.
 - Click the **Mass Calibration / Check** option.

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

Getting Started

Step 3. Prepare the TOF and Q-TOF instrument

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 On the ribbon in the **Context** group, click **Acquisition**. You need to answer several questions when switching from the Tune context to the Acquisition context.
- b In the Method Editor Window, make sure that the General tab in the TOF or Q-TOF tab is selected.
- 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 Activity Log.

- On the ribbon of the Data Acquisition program in the **Tools** group, click **System Activity Log**, and view the logged events. 
- Or right-click  on the system task bar. First, click **Enable Notification**. Then, right-click this icon 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 and whether or not to run a BioConfirm method. You specify whether to copy or link the Qualitative Analysis method, the Quantitative Analysis method, and the BioConfirm 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 data acquisition. If you run a single sample in the Data Acquisition program, you can select to run **Acquisition Only** or **Both Acquisition and DA**.

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 the **Qual Automation** check box or the **Quant Automation** check box or the **BioConfirm Automation** check box or any of the check boxes on the Data Conversions tab in the DA tab in the Method Editor window. You can also run a method to produce only raw data (acquisition only). If you use the **DA Reprocessor** program, you can 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.

Getting Started

Step 4. Set up and run a method

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 ✓ on the toolbar in the Method Editor window.
- To save the method after entering parameters, click either  or  in the toolbar.
- Type the name for the method in the **Save Method As** box, and click **OK**.

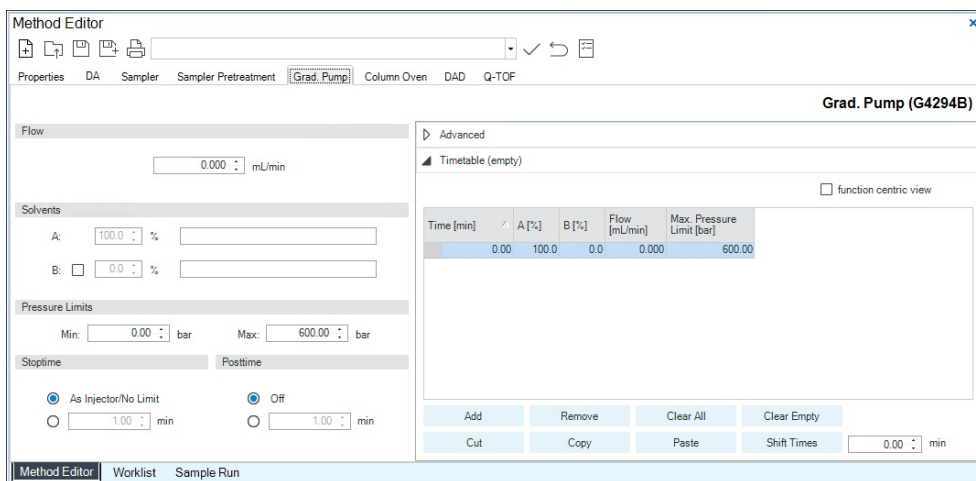
1 On the ribbon in the **Context** group, click **Acquisition**.

2 If needed, open the Method Editor window.

- On the ribbon in the **Layouts** group, click **Method**.
- On the ribbon in the **Windows** group, click **Method Editor** to add the Method Editor window to the current layout.

3 Enter LC parameter values.

Type the values for all of the LC modules configured for the instrument.



4 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.

Getting Started

Step 4. Set up and run a method

- 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.

The screenshot shows a software interface with several control panels. At the top, there are dropdown menus for 'Ion Source' (Dual AJS ESI), 'Ion Polarity' (Positive), 'Data Storage' (Both), and 'LC Stream' (MS). Below these, there are two main sections: 'Stop Time' and 'Time Segment and Experiment #'. The 'Stop Time' section has radio buttons for 'No Limit/As Pump' (selected) and 'Stop Time' (30 min). The 'Time Segment and Experiment #' section contains a table with columns for 'Time (min)' and 'Expt #'. The table has three rows: (0, 1), (1.4, 2), and (1.9, 2). Below the table, the 'Cycle Time' is displayed as 1.818 seconds.

| Time (min) | Expt # |
|------------|--------|
| 0 | 1 |
| 1.4 | 2 |
| 1.9 | 2 |

- 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.

The screenshot shows the 'Acquisition' tab of the software interface. It is divided into several sections: 'Dual AJS ESI (Seg)', 'MS TOF (Expt)', 'Dual AJS ESI (Expt)', and 'Chamber'. The 'Dual AJS ESI (Seg)' section has two columns of parameters. The 'MS TOF (Expt)' section has three parameters. The 'Dual AJS ESI (Expt)' section has three parameters. The 'Chamber' section has one parameter.

| Parameter | Value | Unit |
|-----------------------|-------|-------|
| Gas Temp | 200 | °C |
| Drying Gas | 13 | l/min |
| Nebulizer | 35 | psi |
| Sheath Gas Temp | 350 | °C |
| Sheath Gas Flow | 11 | l/min |
| Fragmentor | 380 | V |
| Skimmer | 65 | V |
| Oct 1 RF Vpp | 750 | V |
| VCap | 3500 | V |
| Capillary | 0.000 | µA |
| Nozzle Voltage (Expt) | 1000 | V |
| Chamber | 0.00 | µA |

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.

- 5** 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.

Getting Started

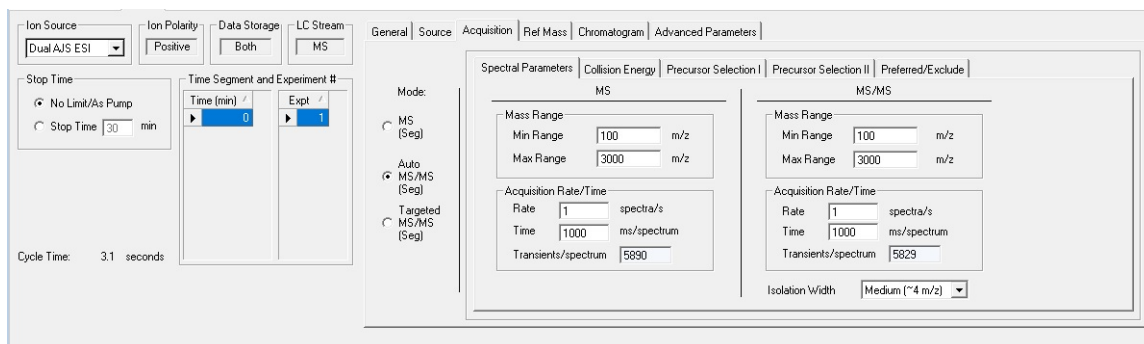
Step 4. Set up and run a method


- 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.
- 6 Set up the data analysis parameters. In the DA tab, you can specify the Qualitative Analysis parameters and Quantitative Analysis parameters and the BioConfirm parameters (if BioConfirm is installed). You can also set up data conversions. 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.
 - In Networked Workstation mode, **Copy** is not available.
 - d Click the  button to select the Qualitative Analysis method.


Getting Started

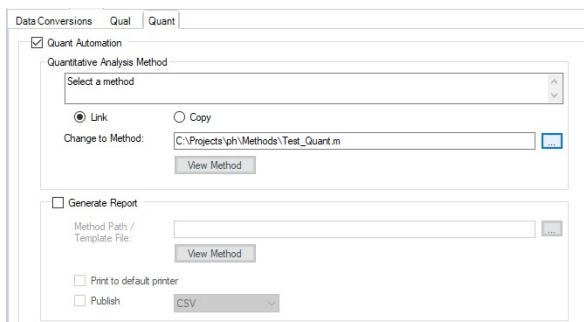
Step 4. Set up and run a method



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.
 - In Networked Workstation mode, **Copy** is not available.
- 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**.



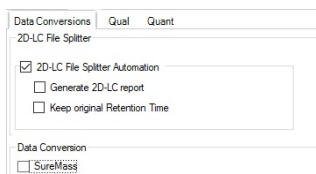
See the online Help for the Quantitative Analysis program to learn how to set up automation parameters for the Quantitative Analysis program.

Getting Started

Step 4. Set up and run a method

To set up any Data Conversions, do the following:

- a Click the **DA** tab in the Method Editor.
- a Click the **Data Conversions** tab.
- b (optional) Mark the **2D-LC File Splitter** check box. Mark or clear the **Generate 2D-LC report** check box. Mark or clear the **Keep original Retention Time** check box. See the online Help for more information.
- c (optional) Mark the **SureMass** check box. You can only mark this check box if you have installed the Quantitative Analysis program.



Set up and run interactive samples

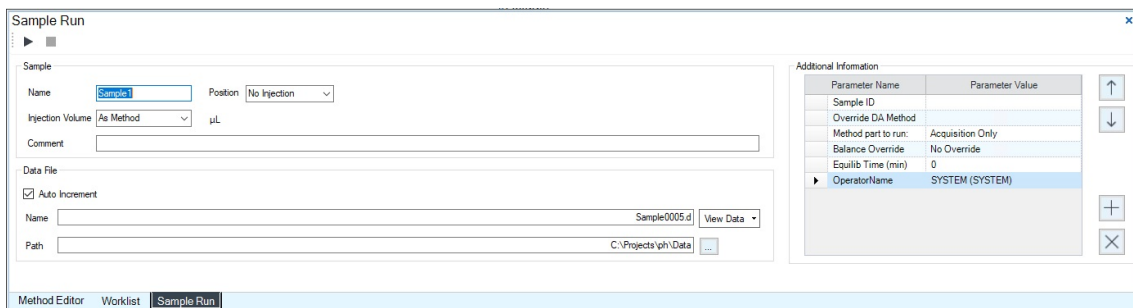
- 1 Click the **Sample Run** window. If the **Sample Run** window is not visible, either you click **Single Sample** in the **Layouts** group on the ribbon, or you click **Sample Run** in the **Windows** group on the ribbon.
- 2 Enter the information such as the Sample Name, the Data File Name, and the 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** 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.

Getting Started

Step 4. Set up and run a method



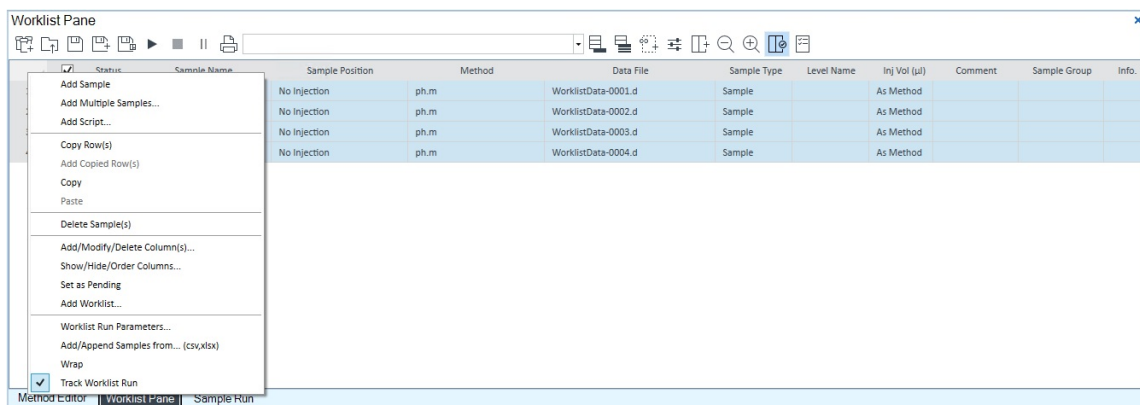
- 4 To start a single sample run, click the Run button (▶) on the **Sample Run** toolbar.


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. On the ribbon in the **Settings** group, if **Locked Mode** is highlighted, that indicates that Locked Mode is on.

You can also specify an **Override DA Method** and select **Both Acquisition and DA** 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**. You can instead click  in the toolbar in the Worklist window. 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).

Getting Started

Step 4. Set up and run a method

- 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**. You can instead click  in the toolbar in the Worklist window. 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  on the **Worklist** toolbar.

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. On the ribbon in the **Settings** group, if **Locked Mode** is highlighted, that 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**. Check SubscribeNet to make sure that you have the most recent version.

In this Book

This book contains brief instructions to help you get started with your Agilent LC/TOF or LC/Q-TOF system. This book takes a quick look at using the Agilent MassHunter Workstation 11.0 software to:

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

www.agilent.com

© Agilent Technologies, Inc. 2021

July 2021



M5960-90000 Revision A

