



6545XT AdvanceBio LC/Q-TOF System

User Guide



Notices

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Software Revision

This guide is valid for MassHunter 12.1, until superseded.

Instrument Manufacturing



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In This Guide

This guide provides information on the Agilent 6545XT LC/Q-TOF system running MassHunter Data Acquisition 12.1 or higher.

Additional Resources

User Documentation



Data analysis and library management documentation can be found by scanning the code or navigating to <https://aglt.co/DALibMgmtDocs>.



Instrument documentation, step by step videos, and more can be found by scanning the code or navigating to <https://aglt.co/LCMSUserDocs>.

Agilent Q-TOF LC/MS Supplies



Make sure that you don't run out of essential columns and supplies. Use this quick reference list to keep your shelves stocked by navigating to <https://aglt.co/LCQTOFSupplies>.

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6545XT AdvanceBio LC/Q-TOF

Front view

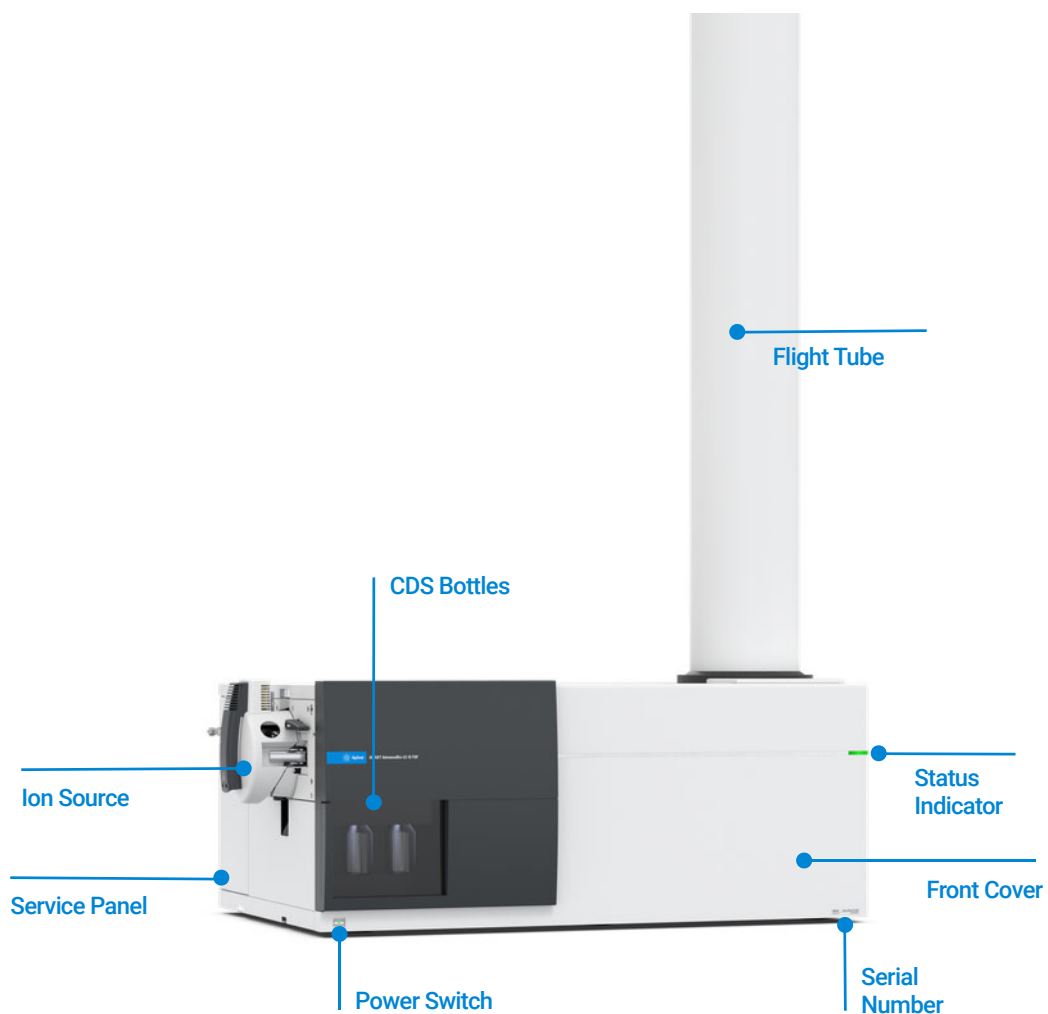


Figure 1. Front view of 6545XT LC/Q-TOF

Calibrant Delivery System

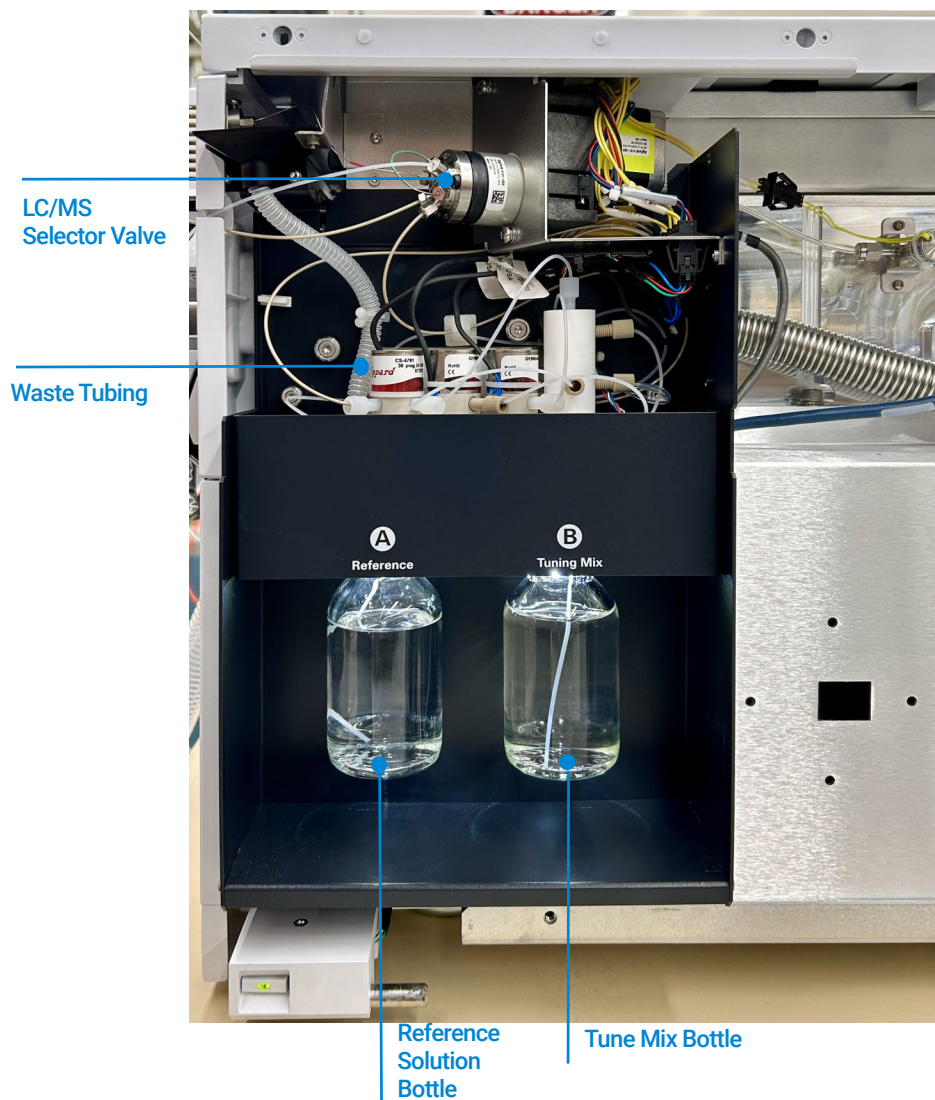


Figure 2. Calibrant delivery system

Side view

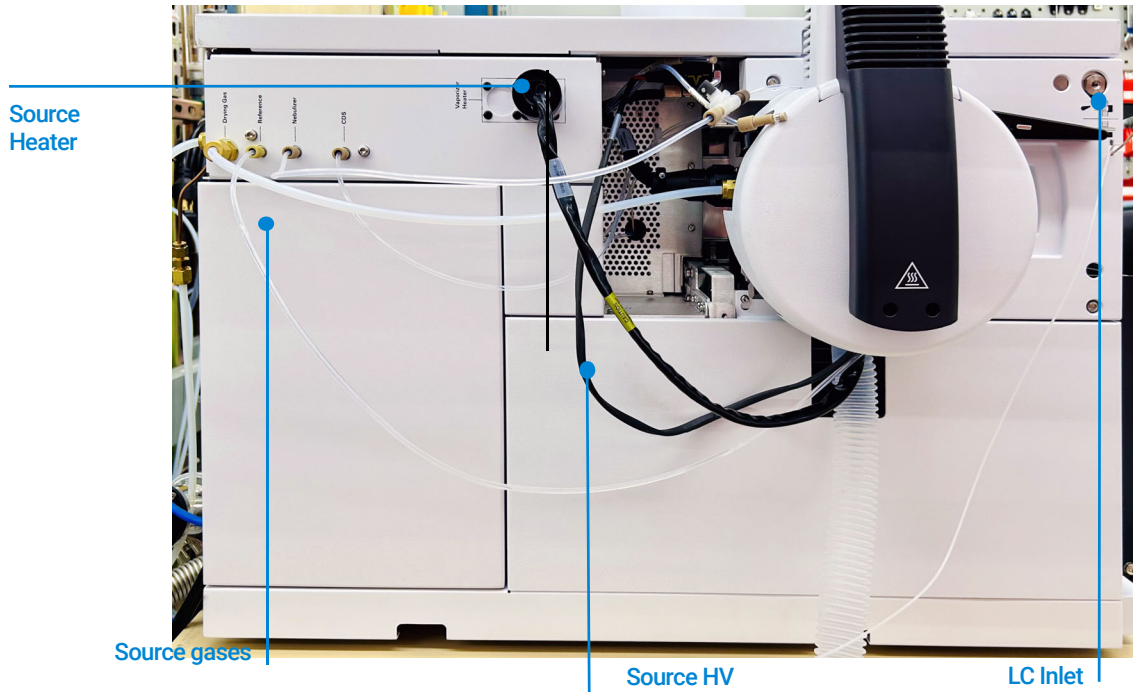
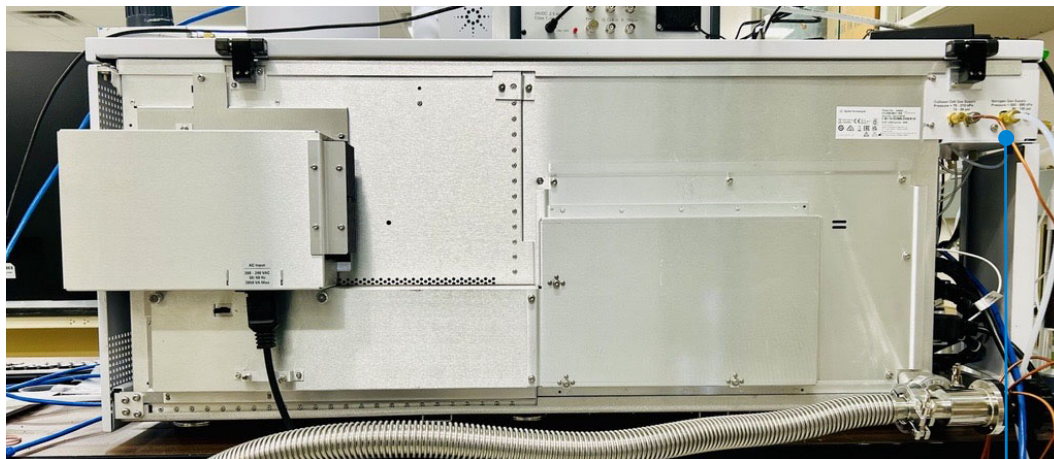


Figure 3. Side view of 6545XT LC/Q-TOF

Back view



Nitrogen

Figure 4. Back view of 6545XT LC/Q-TOF

Instrument Status Indicator Lights

Table 1 Status Indicator Lights on Instrument

 <p>Instrument State = BOOTING (FPGA Loaded) Status LED = Blinking Yellow Power Switch LED = Yellow</p>	 <p>Instrument State = ACQUISITION Status LED = Blinking Green Power Switch LED = Green</p>
 <p>Instrument State = BOOTING (LINUX Loaded) Status LED = Yellow Power Switch LED = Green</p>	 <p>Instrument State = FAULT Status LED = Red Power Switch LED = Green</p>
 <p>Instrument State = PUMPING DOWN Status LED = Blinking Yellow Power Switch LED = Green</p>	 <p>Instrument State = VENTING Status LED = Blinking Red Power Switch LED = Yellow</p>
 <p>Instrument State = STANDBY Status LED = Yellow Power Switch LED = Green</p>	 <p>Instrument State = Vented Status LED = Red Power Switch LED = Yellow</p>
 <p>Instrument State = IDLE Status LED = Yellow Power Switch LED = Green</p>	 <p>Instrument State = POWERING DOWN Status LED = Off Power Switch LED = Blinking Yellow</p>
 <p>Instrument State = BACKGROUND SCAN Status LED = Green Power Switch LED = Green</p>	 <p>Instrument State = Off Status LED = Off Power Switch LED = Off</p>

Instrument Status Indicators in Acquisition

In the Acquisition program, the Instrument Status window indicates the state of the instrument.

- Gray
 - Offline: The instrument is configured with the system and available to use, but the AIC/workstation is not currently running. The amount of time for an instrument to reach the Idle state depends on each instrument.
 - Disconnected: The connection to the instrument has been closed.
- Yellow - Unknown: The device is in an unknown run state.
- Light Orange - Not Ready: The instrument is connected but is not ready to run (due to not reaching the correct temperature or pressure required by the method, for example).
- Green - Idle: The instrument is on and ready to process samples.
- Teal - Standby: The instrument is in a standby/sleep state. Put the instrument in Standby mode when it is not in use or to change the ion source. When the instrument is in Standby mode:
 - The source interface is on, the drying gas remains heated, and nebulizer flows are maintained.
 - The source and ion optics voltages are turned off, and the mass spectrometer is not sending spectra to the computer.
 - For APCI and multimode sources: Vaporizer gas heaters are turned down.
 - For the Agilent Jet Stream (AJS) source: The sheath gas temperature, drying gas flow, and drying gas temperature are reduced.
 - The spray chamber high voltages are turned off.
 - The mass spectrometer stops generating spectra.
- Magenta - Prerun/Injecting: The instrument is on and is preparing to start acquisition.
- Blue - Running, postrun: The instrument is currently collecting data.
- Orange - Tuning: The instrument is in the process of adjusting MS parameters.
- Red - Instrument Error: The instrument has an error and cannot process samples.

Ion Sources

Electrospray Ionization (Dual ESI) source

Electrospray relies in part on chemistry to generate analyte ions in solution before the analyte reaches the mass spectrometer. The LC eluent is sprayed (nebulized) into a chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas.

The electrostatic field causes further dissociation of the analyte molecules. The heated drying gas causes the solvent in the droplets to evaporate. As the droplets shrink, the charge concentration in the droplets increases. Eventually, the repulsive force between ions with like charges exceeds the cohesive forces and the ions are ejected (desorbed) into the gas phase. These ions are attracted to and pass through a capillary sampling orifice into the mass analyzer.



Figure 5. ESI source with cover

Agilent Jet Stream (Dual AJS ESI) source

The Agilent Jet Stream (Dual AJS ESI) source:

- Uses the same ionization technique as the ESI source.
- Uses thermal gradient focusing technology, which is a process in which superheated nitrogen (N_2) is used to improve ion generation and desolvation.
- Improves sensitivity 5x or more for many small-molecule compounds that undergo electrospray ionization.

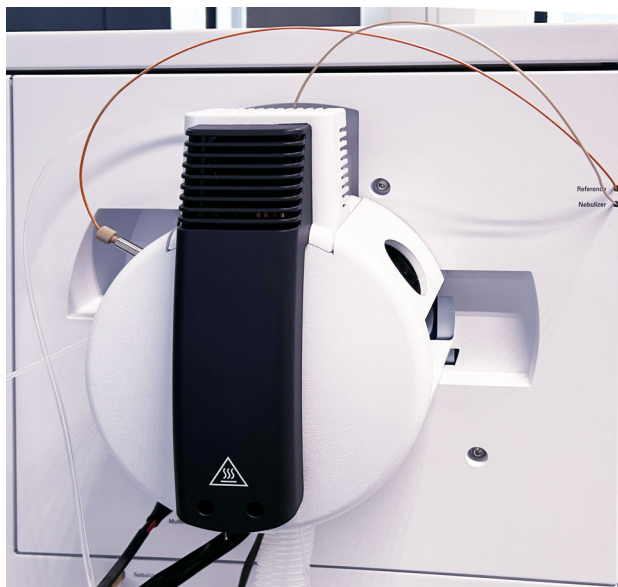


Figure 6. Dual AJS ESI with cover

Atmospheric Pressure Chemical Ionization (APCI) source

In Atmospheric Pressure Chemical Ionization (APCI), the LC mobile phase is sprayed through a heated vaporizer (typically 250 to 400 °C) at atmospheric pressure. The heat vaporizes the liquid. The resulting gas-phase solvent molecules are ionized by electrons discharged from a corona needle.

The solvent ions then transfer charge to the analyte molecules through chemical reactions (chemical ionization). The analyte ions pass through a capillary sampling orifice into the mass analyzer. APCI is applicable to a wide range of polar and nonpolar molecules.

APCI rarely results in multiple charging, so APCI is typically used for molecules smaller than 1,500 u.

Because high temperatures are also involved, APCI is not appropriate for analysis of large biomolecules. APCI is used with normal-phase chromatography more often than electrospray is because the analytes are usually nonpolar.



Figure 7. APCI source with cover

Multimode Ionization (MMI) source

The Multimode source for LC/MS can simultaneously do ESI and APCI ionization. The Multimode source can operate in ESI mode only, in APCI mode only, or in mixed ESI/APCI mode. This technology significantly improves the speed, accuracy, and productivity of high-throughput screening in drug discovery and other research applications.

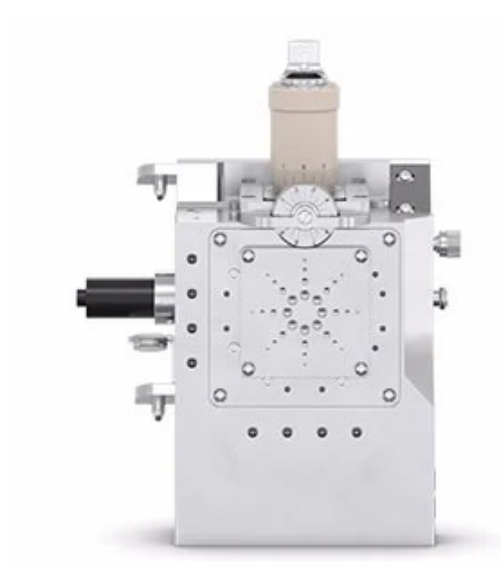


Figure 8. MMI cover, ion source, and spray shield

Simultaneous ESI and APCI capability and high LC flow rate compatibility (up to 2 mL/min) means more compounds can be detected in less time with the Agilent Multimode source.

Nanospray ESI source

The Agilent nanospray ESI source is a flexible solution for laboratories running low-flow LC/MS applications. The nanospray source allows both commercially available and custom nanoflow columns to be used with Agilent LC/MS systems.



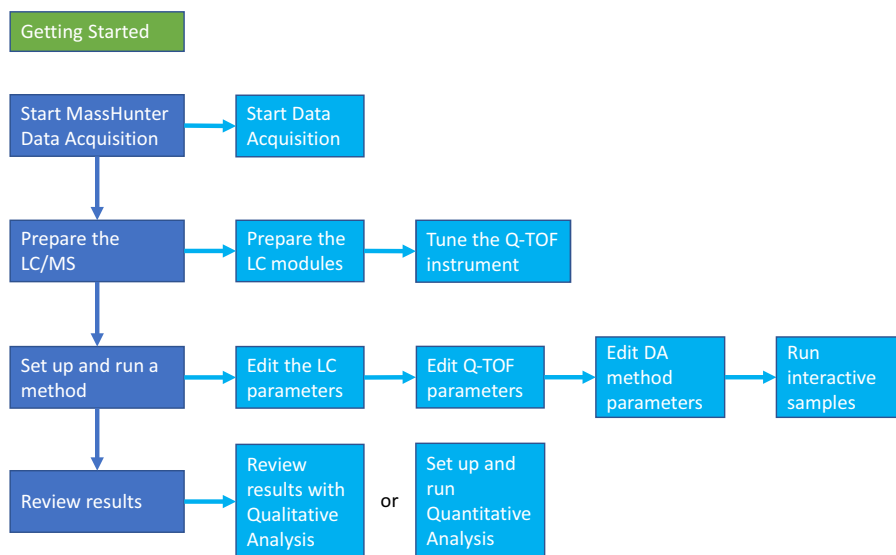
Nanospray ESI source

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Overview

The roadmap below shows the steps to set up and run a batch of samples from start to finish. Follow the instructions in this section to get started and review guiding information for each step.



Starting Up and Shutting Down the Instrument

Starting in Standby mode

- 1 Check that the nitrogen gas for the drying gas and for the collision cell are turned on.
- 2 Turn on the power for the computer and monitor.
- 3 Check that the instrument and rough pump are plugged into a power outlet.
- 4 Press the front power switch on the instrument.

The vacuum system automatically starts to pump down the instrument, and the electronics are turned on. The system card boots up and the firmware starts.

Turning off the instrument

Before turning off the instrument, complete any acquisition steps and save any data.

CAUTION

To prevent instrument damage, always vent the system before pressing the power button. Only the Lab Manager and Everything role has access to venting.

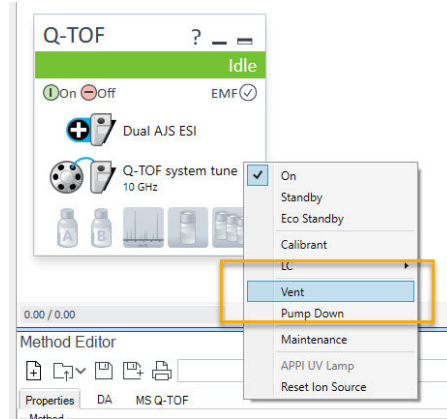
WARNING

Do not touch the spray shield and related spray chamber components. They are likely to be hot.

- 1 Turn off the solvent flow.
If there is any analyte in any of the components in the sample delivery system, flush the delivery system with pure solvent before continuing.
- 2 In Acquisition, right-click the instrument icon in the Instrument Status window and select **Vent** to vent the system.

Basic Operation

Turning off the instrument



3 Click **Yes**.

When venting the system, the following occurs:

- The spray chamber high voltages, the drying gas heater, the nebulizer flow, the detector, and other lens voltages are turned off.
 - The drying gas flow is set to Standby.
 - If the APCI source is installed, the vaporizer heater is also turned off.
 - If the installed source is an Agilent Jet Stream source, the sheath gas heater is also turned off.
 - The Rough Pump turns off when the turbo speed is below 20 percent.
- 4 Wait until the instrument completely vents (ensure that the Rough Vacuum reads approximately 760 Torr).
 - 5 Manually close the foreline valve by twisting clockwise until hand tight.
 - 6 Close the Acquisition program and shut down the computer.
 - 7 Press the power switch located in the lower left front corner of the instrument.



- 8 Unplug the main power cable behind the service panel. This action prevents the turbo pump components from overheating.

WARNING

When the LC/MS instrument is plugged into a power source, even if the power switch is off, dangerous voltages can exist:

- In the wiring between the LC/MS instrument power cord and the AC power supply.
 - In the AC power supply
 - In the wiring from the AC power supply to the power switch.
-

Starting the MassHunter Data Acquisition Software

The instructions below include the following assumptions:

- The hardware and software are installed.
- The instrument is configured. For information on configuring the instrument, refer to the MassHunter Control Panel help.
- The LC modules and the LC/Q-TOF are turned on, but the LC pump is not running.

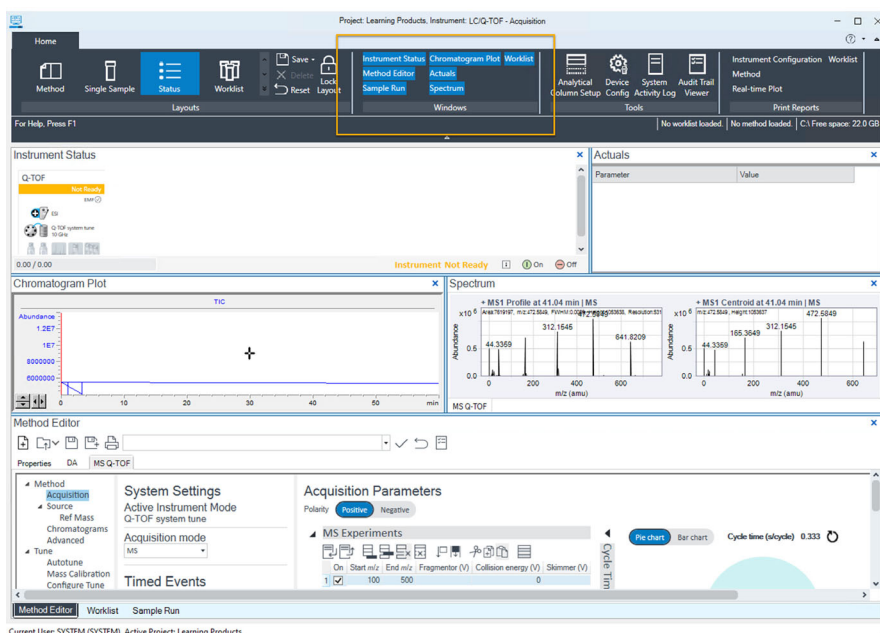
After installation, all the Agilent MassHunter Workstation Software icons appear on the desktop.

To start the MassHunter Data Acquisition program,

- 1** Double-click the **Control Panel** icon on the desktop, or select **Windows Start > Agilent Technologies > Control Panel**.
- 2** In Control Panel, select an instrument and click **Launch** to open the project in Acquisition. For more information, refer to the MassHunter Control Panel help or the Introduction Workbook.

MassHunter Data Acquisition Windows

When the MassHunter Data Acquisition program opens, the main window appears. These windows provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status, monitor runs, and tune the instrument.



Showing and hiding the windows

- To show or hide a window, click the window name in the Windows group of the ribbon.
- Hide a window by clicking the **X** icon in the upper right corner of the window.

At least one window must be shown.

NOTE

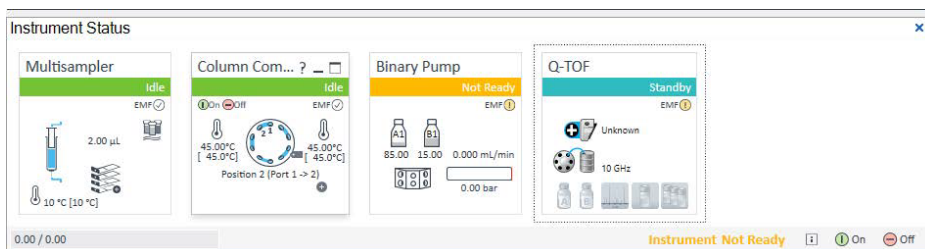
- To obtain help on the active window, click in a window and Press **F1**.
- To resize the window, drag a window border.

- To “float” the window outside of the main window, double-click the title of the window. Double-click the title bar again to “dock” the window.

Instrument Status window

This window displays the status of each device configured with the instrument: **Error, Not ready, Pre-run, Post run, Running, Injecting, Idle, Offline, or Standby.** Also set non-method control and configuration parameters for the LC devices and the MS instrument.

This window displays the status of each device both as text and by its color-coding. See **“Instrument Status Indicators in Acquisition”** on page 15.



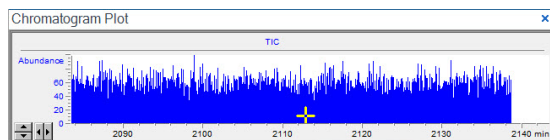
Actuals window

This window displays the current value of selected instrument parameters. See **“Set up to view real-time parameter values (actuals).”** on page 33 for more information.

Parameter	Value
MS Q-TOF: Run State	Idle
MS Q-TOF: TOF Vac	3.20E-07 Torr
MS Q-TOF: Drying Gas	.6 l/min
MS Q-TOF: Gas Temp	0 °C
MS Q-TOF: Not Ready Text Long	Not Ready
MS Q-TOF: Run Time	0 min
MS Q-TOF: Error State	No error
MS Q-TOF: Rough Vac	2.31E+00 Torr
MS Q-TOF: Quad Vac	3.89E-05 Torr
MS Q-TOF: LC Stream Valve	To Waste
MS Q-TOF: Ion Polarity	Positive
MS Q-TOF: Source Type	DualAIS

Chromatogram Plot window

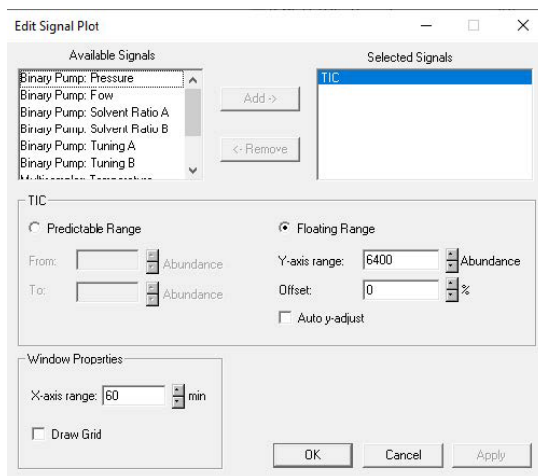
This window displays the chromatogram plots in real time. These plots can be user-defined signals and/or instrument parameters.



Monitoring baseline and adjusting plot

The **Edit Signal Plot** dialog box allows the selection of various instrument parameters and signals for display in the Chromatogram Plot window. The signals and their display characteristics can be adjusted as described in this section. The customizations made to the plot remain, even when the Acquisition software is closed and reopened.

- 1 In the Chromatogram Plot window, right-click the Chromatogram Plot window, and select **Change**. The **Edit Signal Plot** dialog box opens.



- 2 Select the signal to monitor and set the X and Y range for the plot.

NOTE

To add other MS signals, such as EICs, setpoints, and actuals, select these on the **Q-TOF** tab in the Method Editor window.

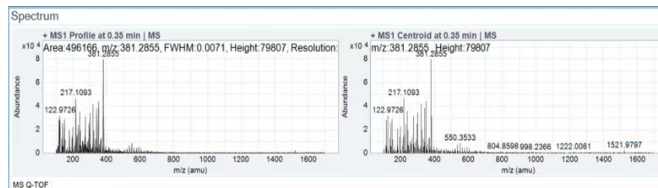
- 3 Click **OK** to close the **Edit Signal Plot** dialog box.

Basic Operation

MassHunter Data Acquisition Windows

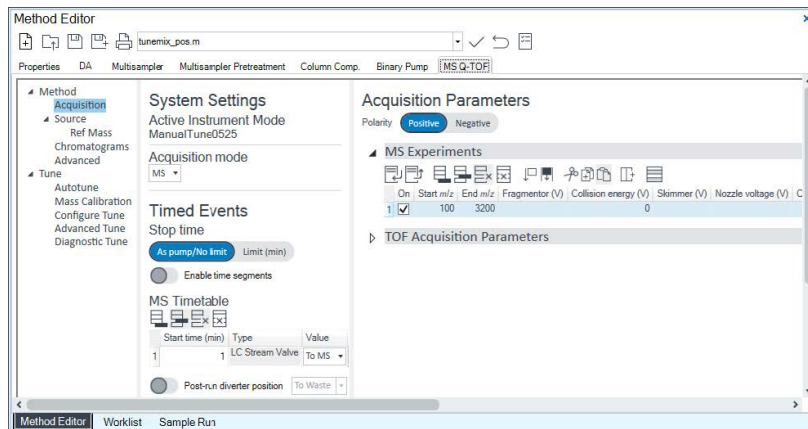
Spectrum window

This window displays the spectral plot in real time. Additional tabs are displayed for other modules.



Method Editor window

In this window, enter the acquisition parameters for the method. Tune values are displayed on the Autotune tab.



Sample Run window

In this window, enter sample information to run individual samples interactively, and start a single sample run. Specify an **Override DA Method** and select either **Both Acquisition and DA** (to run Data Analysis as part of the method) or **Acquisition Only** for the **Part of Method to run**.

Sample Run

Sample

Name: Position:

Injection Volume: μ L

Comment:

Data File

Auto Increment

Name:

Path:

Additional Information

Parameter Name	Parameter Value
Sample ID	
Override DA Method	
Part of Method to run	Acquisition Only
Equilib Time (min)	0

Method Editor | **Sample Run** | Worklist

Worklist window

In this window, enter sample information for multiple samples. When running the worklist, the samples are automatically run in the order listed. Select whether to run **Acquisition Only** or **Both Acquisition and DA** for the **Part of method to run** option in the Worklist Run Parameters dialog box.

Worklist

Method Editor | Sample Run | **Worklist**

	Status	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Inj Vol (ul)	Comment	Sample Group	Info.
1	<input checked="" type="checkbox"/> Pending	Sample1	PA-A1	default.m	WorklistData-0001.d	Sample		As Method			
2	<input checked="" type="checkbox"/> Pending	Sample2	PA-A2	default.m	WorklistData-0002.d	Sample		As Method			
3	<input checked="" type="checkbox"/> Pending	Sample3	PA-B1	default.m	WorklistData-0003.d	Sample		As Method			
4	<input checked="" type="checkbox"/> Pending	Sample4	PA-B2	default.m	WorklistData-0004.d	Sample		As Method			

Preparing the LC Modules

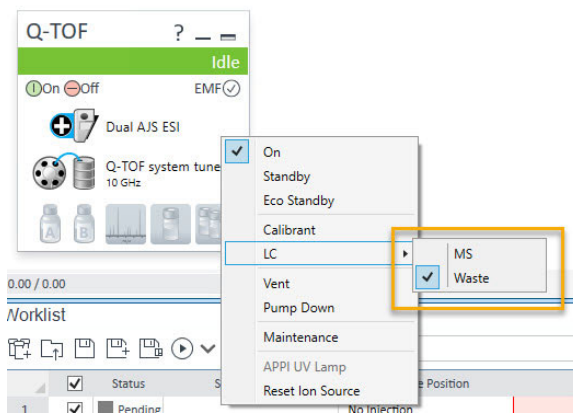
While conditioning or equilibrating the column, it is possible to tune the instrument.

1 Switch LC stream to **Waste**.

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

If the LC is connected to a VWD or DAD, the ability to monitor the fluctuations of the VWD or DAD real-time chromatogram before a run is available.

- a Right-click the **Q-TOF** device in the Instrument Status window.
- b Select **LC > Waste**.



2 Purge the LC pump.

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

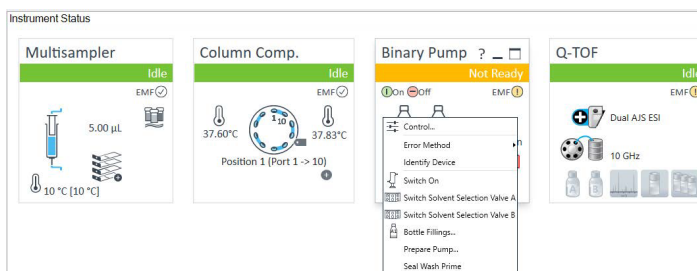
3 Set up to condition or equilibrate the column.

- a In the Method Editor window, click **Apply** in the toolbar to download the LC parameters to the LC.

Basic Operation

Preparing the LC Modules

- b Right-click an LC module in the Instrument Status window and select one of the commands to change any non-method control parameters, if needed.



- c Monitor the baseline and adjust the plot to make sure that the column is equilibrated and the baseline stable. (See [step 4](#) and [step 5](#) in this procedure.)
- 4 Set up to view real-time parameter values (actuals).

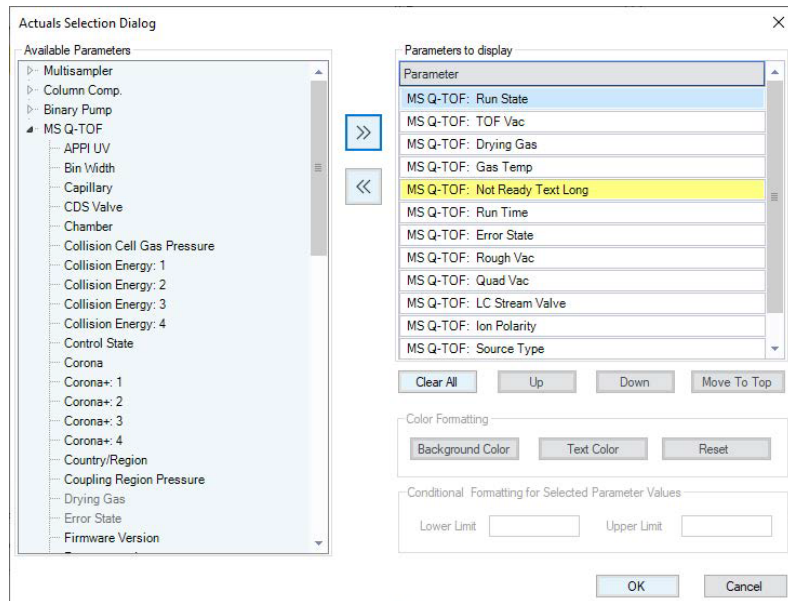
The Actuals window can be customized to display instrument conditions that you may want to view.

- a Click **Actuals** in the ribbon to display the Actuals window.
- b Right-click the **Actuals** window and select **Setup**.

Parameter	Value
MS Q-TOF: Run State	Idle
MS Q-TOF: TOF Vac	3.18E-07 Torr
MS Q-TOF: Drying Gas	5 l/min
MS Q-TOF: Gas Temp	325 °C
MS Q-TOF: Not Ready Text Long	Ready
MS Q-TOF: Run Time	0 min
MS Q-TOF: Error State	N
MS Q-TOF: Rough Vac	1
MS Q-TOF: Quad Vac	2.01E-05 Torr
MS Q-TOF: LC Stream Valve	To Waste
MS Q-TOF: Ion Polarity	Positive
MS Q-TOF: Source Type	DualAJS

- c Select parameters to appear in the Actuals window.

In the **Available Parameters** list, expand a device to see a list of the instrument parameters for that device. Only configured devices will be shown in the list.

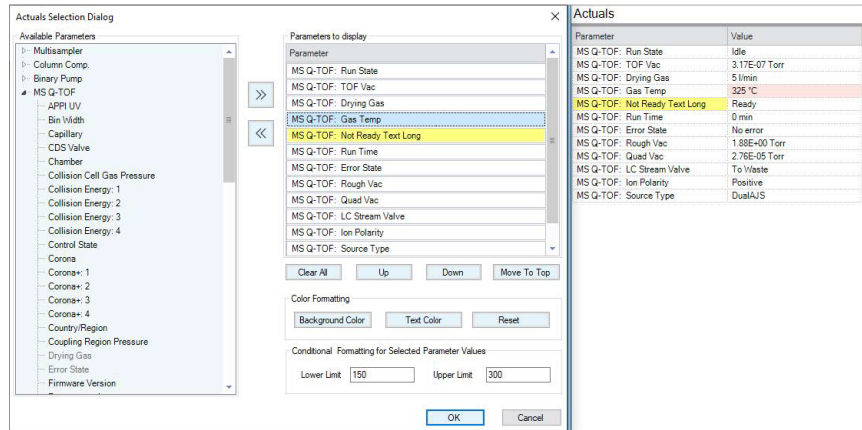


- d Select the parameters of interest, and click **>> Add**. Repeat this step for all the parameters that you want to view.
- e Select any parameter in the **Parameters to display** list that you do not want included, and click **<< Remove**.
- f Customize the background color and the text color for any parameters in the **Parameters to display** list.

- g Set a conditional formatting range for the parameters that are numbers.

NOTE

If the value of the parameter is not within the limits you entered, then the background of the parameter is set to red.



- h Click **OK** when you finish selecting parameters.

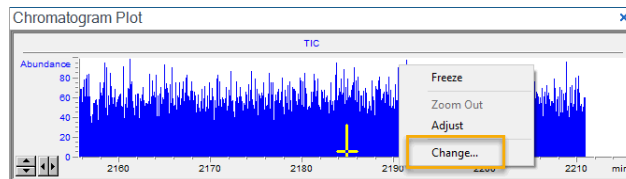
NOTE

The parameters you selected appear in the Actuals window. Note that the customizations you have made will remain, even if you close the Acquisition software and reopen it.

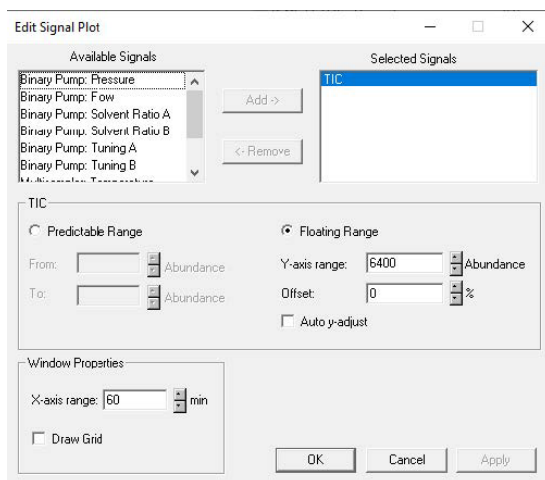
- 5 Set up real-time plot displays.

As the column is conditioning, set up the displays to monitor the effluent.

- a In the Chromatogram Plots window, right-click the chromatogram plot, and select **Change**.



- b In the **Edit Signal Plot** dialog box, select the type of display signal, and click **OK**.



Preparing the Q-TOF LC/MS

Performing a checktune or autotune

See [Chapter 3](#), “Tuning”.

Switching LC stream to MS

After you condition the column and tune the instrument, switch the LC stream from **Waste** to **MS**. See “[Switch LC stream to Waste.](#)” on page 32 for how to do this.

Monitoring MS baseline and spectral displays

If you do not monitor the LC baseline with a VWD or DAD, make sure that the instrument baseline is stable and no spectra of interfering intensity appear in the display.

If you do monitor the LC baseline with a VWD or DAD, change back to the default instrument displays.

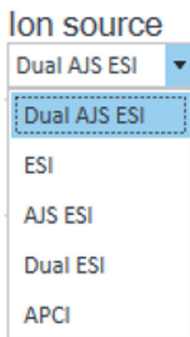
- 1 Right-click the chromatogram plot, and click **Change**.
- 2 Select the MS signal, and click **OK**.

Select the Q-TOF/TOF ion source

The Q-TOF/TOF ion source is automatically detected and displayed in the System Settings pane of the Acquisition Section of the Method Editor and in the Q-TOF dashboard tile in the Instrument Status pane.

If you are using the Offline Method Editor, select the desired ion source from the Method Editor window. To open the Offline Method Editor, select an LC/MS instrument in Agilent Control Panel, and click Launch Offline, or double-click in the leftmost column of a row in the Worklist window. An acquisition method must be specified to open the Offline Method Editor from this window.

- 1 To display the Method Editor window, click **Method Editor** in the windows section on the Ribbon. Or, click the **Method** layout in the Ribbon.
- 2 Select the **MS Q-TOF** tab.
- 3 Select the **Method > Source** section.
- 4 From the Ion Source drop-down list, select the ion source.



- 5 Click **Apply Method** in the Method Editor toolbar.

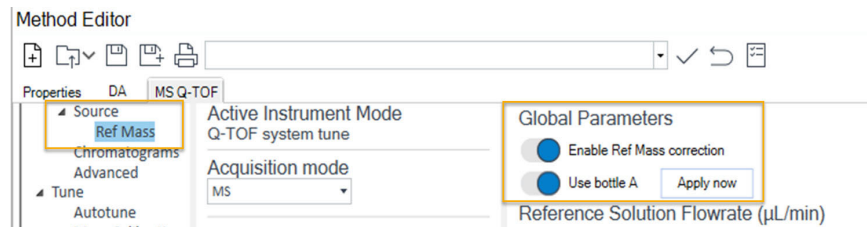
Internal Reference Mass Correction

In addition to manual calibration and calibration during autotune, the mass can also be automatically corrected during a run, referred to as using an Internal Reference Mass (IRM).

This procedure consists of introducing a mass reference standard into the source during a run via a reference sprayer valve available on dual AJS and dual ESI sources. The reference masses contained in the reference standard are used to automatically correct each acquired spectrum. The mass correction affects the current sample only and is not applied to future samples run.

For APCI ion sources, the UIRM-hardware kit (Universal Internal Reference Mass) should be used (part number G1969-67400).

- 1 To display the Method Editor window, click **Method Editor** in the windows section on the Ribbon. Or, click the **Method** layout in the Ribbon.
- 2 Select the **MS Q-TOF** tab.
- 3 Select the **Method > Source > Ref Mass** section, and set the following parameters:
 - Select Enable Ref Mass correction.
 - Select Use Bottle A.



Basic Operation

Internal Reference Mass Correction

NOTE

For those sources without a reference sprayer (APCI), there are two options: (1) Use the UIRM-hardware kit (p/n G1969-67400.) (2) For other sources that do not have a second sprayer, connect a tee into the LC tubing just before the sprayer and admit a reference mass solution into the tee using a syringe pump or other pumping device.

- 4 Enter the desired Reference parameters for Flowrate, Nebulizer (psi), and Reference Mass.

Reference Solution Flowrate ($\mu\text{L}/\text{min}$)

Setpoint

Reference Nebulizer (psi)

Setpoint Actual
 0.002

Reference Mass Parameters

Detection window (ppm)


Minimum height (counts)

- 5 In the Reference Masses table, select up to two reference standard masses for automatic mass correction.

Reference Masses

Polarity: Positive (+)		Polarity: Negative (-)	
On	Mass (m/z)	On	Mass (m/z)
<input type="checkbox"/>	64.01577	<input type="checkbox"/>	68.995758
<input checked="" type="checkbox"/>	121.050873	<input type="checkbox"/>	112.985587
<input type="checkbox"/>	149.02332	<input checked="" type="checkbox"/>	119.03632
<input type="checkbox"/>	322.048121	<input type="checkbox"/>	301.998139
<input checked="" type="checkbox"/>	922.009798	<input checked="" type="checkbox"/>	966.000725
<input type="checkbox"/>	1221.990637	<input type="checkbox"/>	980.016375
<input type="checkbox"/>	1521.971475	<input type="checkbox"/>	1033.988109
<input type="checkbox"/>	2421.91399	<input type="checkbox"/>	1633.948689
		<input type="checkbox"/>	1933.930624
		<input type="checkbox"/>	2533.892301

NOTE

Use default values or choose two masses spread across the application mass range. To add new values to the Reference Masses table, click  **Add row at the end** and enter the exact mass values.

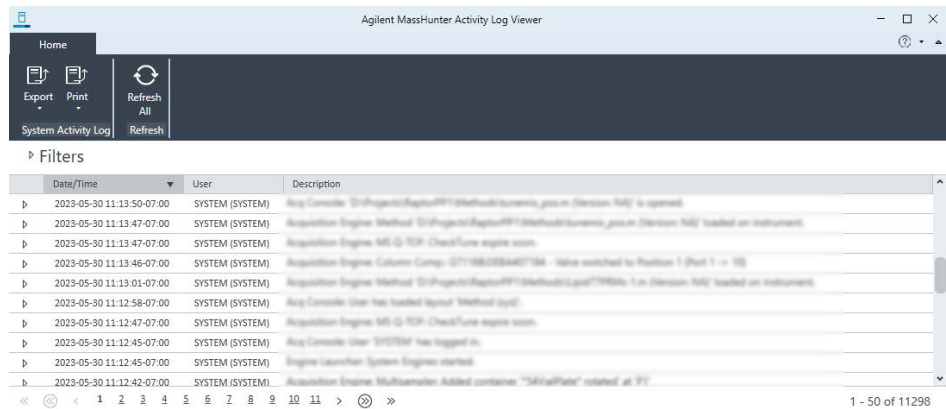
- 6 When finished, save the acquisition method.

- 7 Create a worklist of the samples to be analyzed and specify the acquisition method created in Steps 2 to 4. For a single sample, click **Apply** before starting the interactive run.


Viewing the System Activity Log for events and errors

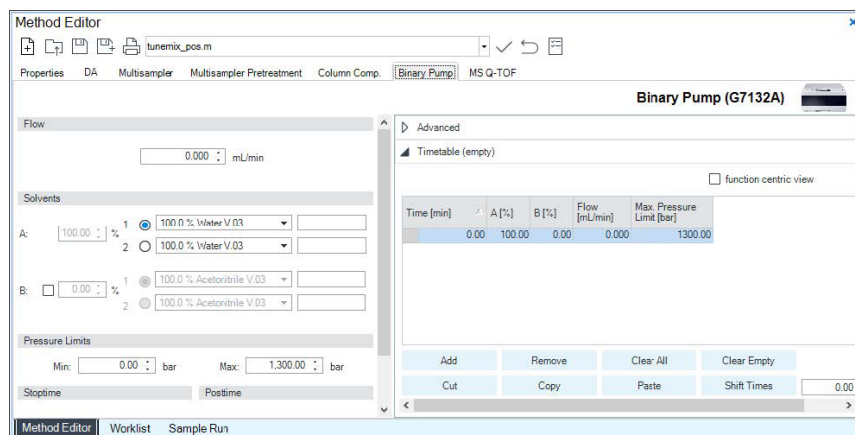
The System Activity Log provides information regarding errors to assist with troubleshooting.

To view the log, click **System Activity Log** in the ribbon of the Acquisition program, and view the logged events.



Setting Up an Acquisition Method

- 1 Set up the method in the Method Editor window:
 - a Enter or select the values and settings for each of the tabs below.
 - b *Optional.* To apply the settings to the instrument, click ✓ **Apply**.
 - c To save the method, click  **Save As**.
 - d Name the method and click **OK**.
- 2 Enter values for all of the LC modules configured for the instrument.

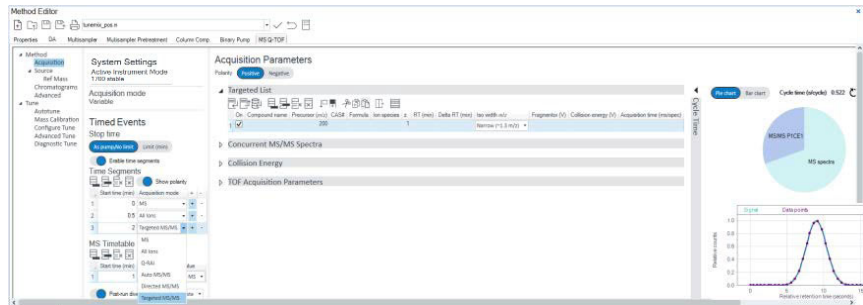


- 3 Enter the MS Q-TOF parameter values.
 - a On the Acquisition tab, select the acquisition mode from the list in the **System Settings** table. The Acquisition Parameters table is changed accordingly when you change the **acquisition mode**. The parameters available on the right change depending on the **acquisition mode**.

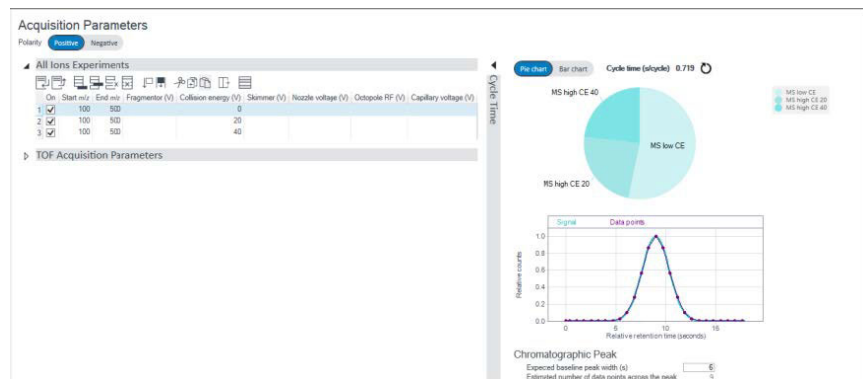
Basic Operation

Setting Up an Acquisition Method

- b Enter any acquisition parameters you want to change. Multiple acquisition modes are allowed when the time segments are enabled. Each acquisition parameter can be set on the acquisition parameter table in the corresponding time segment.



- 4 Set up to change instrument parameters with segments:
- To add a segment, click **Add Row** or **Insert Row**.
 - Enter the parameters for each Scan segment.

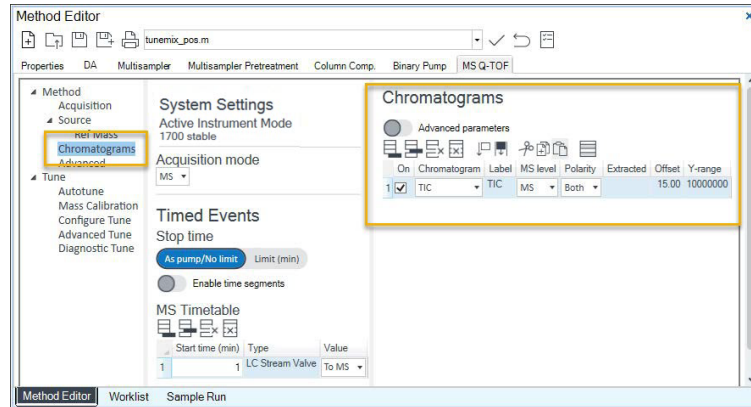


- 5 *Optional.* Set up signals for the Chromatogram plot:
- Click **Chromatograms**.

Basic Operation

Setting Up an Acquisition Method

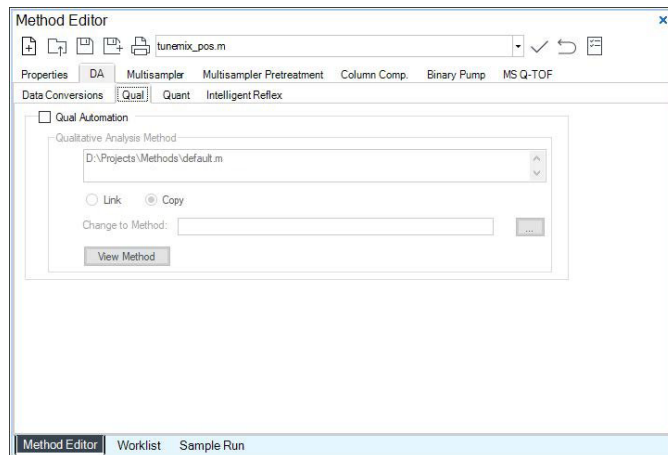
- b Select TIC or EIC under the Chromatograph column and the MS or MS/MS under the MS level column, and type other plot values.



- 6 Set up the data analysis (DA) parameters.

A method can contain Qualitative Analysis parameters, Quantitative Analysis parameters, or a combination. The **Qual Automation** check box is marked on the Qual tab and the **Quant Automation** check box is marked on the Quant tab.

- a Select the **DA** tab.

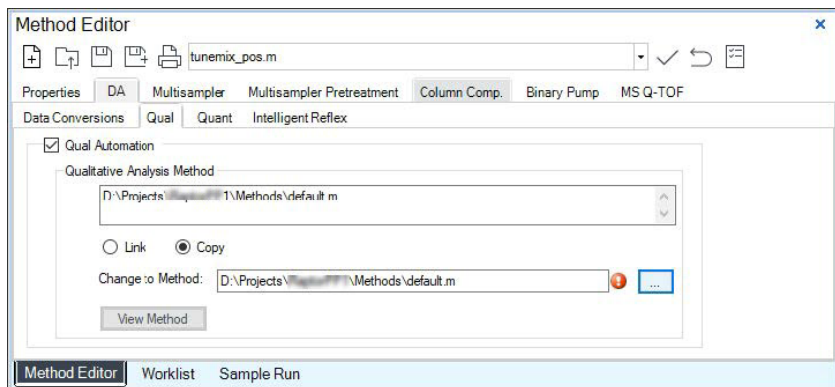


- b *Optional.* For information on the **Data Conversions** tab, refer to the online Help.

Basic Operation

Setting Up an Acquisition Method

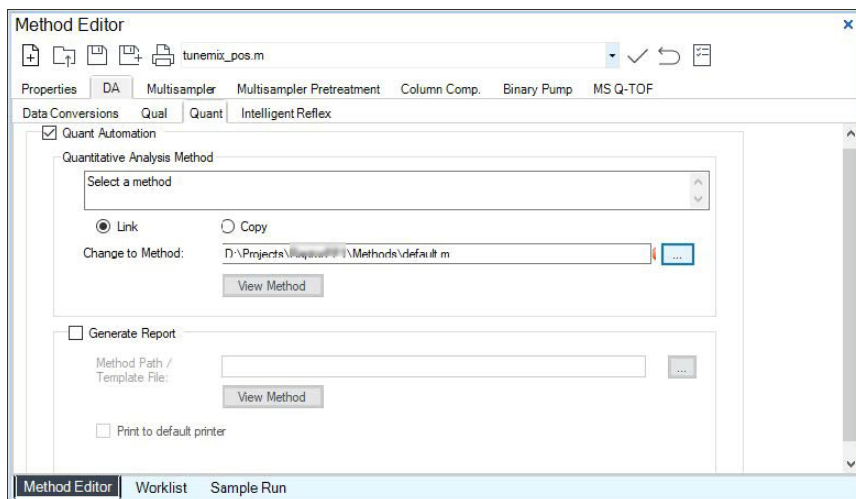
- c *Optional.* On the **Qual** tab, mark the **Qual Automation** check box. The name of the current Qualitative Analysis method is shown in the box. If you want to change the Qualitative Analysis method that is connected, click **browse** to select a different method. When the acquisition method is saved, the Qualitative Analysis method that you selected is copied or linked to the acquisition method.



- d *Optional.* On the **Quant** tab, mark the **Quant Automation** check box. The name of the current Quantitative Analysis method is shown in the list. If you want to change the Quantitative Analysis method that is connected, click **...** **Browse** to select a different method. When the acquisition method is saved, the Quantitative Analysis method that was selected is copied or linked to the acquisition method.
- e *Optional.* Mark the **Generate Report** check box on the Quant tab. Then, you select the **Method Path/Template File** to use. If you want to print the report, mark the **Print to default printer** check box. Mark the **Publish** check box to create a CSV file, a TXT file, or a PDF file.

Basic Operation

Setting Up an Acquisition Method



7 *Optional.* On the **Properties** tab, set up the properties for this method.

8 Save the method.

a Click  **Save** or  **Save As**.

b If the Audit Trail Settings in Control Panel are set to prompt for a reason when the method is saved, enter the **Reason for creating a new version of this method**. Refer to the MassHunter Control Panel online Help for more information. Click **OK**.

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

Setting Up and Running a Single Sample

- 1 Click the **Sample Run** window.
- 2 Enter the **Sample Name**, the **Data File Name** and **Path**, and other values.
- 3 *Optional.* Enter the **Additional Information**. If needed, change the value of the parameters in the **Additional Information** list.


Run a Data Analysis method from this window by selecting **Both Acquisition and DA** or **Acquisition Only** for the **Part of Method to run**.

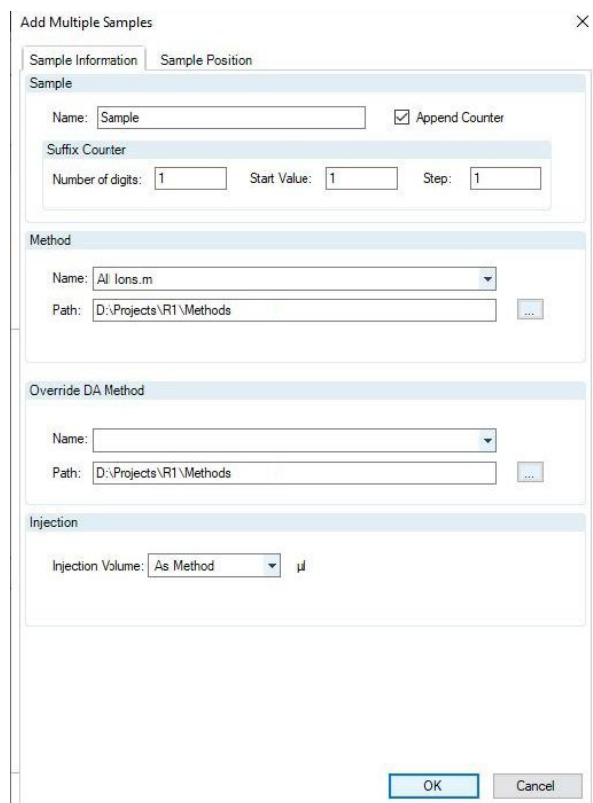
NOTE

If using Both Acquisition and DA Part of Method to run, the Override DA method is required.

- 4 To start the single sample run, click ► **Run** in the Sample Run window toolbar.

Setting Up and Running a Worklist

- 1 Click **Worklist** to show the Worklist window.
- 2 Click **Add Multiple Samples** . The **Add Multiple Samples** dialog box opens.
- 3 Enter all the information on the **Sample Information** tab.



The screenshot shows the 'Add Multiple Samples' dialog box with the 'Sample Information' tab selected. The dialog has two tabs: 'Sample Information' and 'Sample Position'. The 'Sample Information' tab contains the following sections:

- Sample**: A text field for 'Name' containing 'Sample', a checked 'Append Counter' checkbox, and a 'Suffix Counter' section with 'Number of digits' (1), 'Start Value' (1), and 'Step' (1) fields.
- Method**: A dropdown menu for 'Name' set to 'All Ions.m' and a 'Path' field containing 'D:\Projects\RT1\Methods' with a browse button.
- Override DA Method**: A dropdown menu for 'Name' and a 'Path' field containing 'D:\Projects\RT1\Methods' with a browse button.
- Injection**: A dropdown menu for 'Injection Volume' set to 'As Method' and a unit field containing 'µl'.


At the bottom right of the dialog are 'OK' and 'Cancel' buttons.

- 4 On the **Sample Position** tab, specify the sample vial locations (make sure the specific sample tray type has been configured by right-clicking the autosampler device image).

Basic Operation

Setting Up and Running a Worklist


The screenshot shows the 'Add Multiple Samples' dialog box. The 'Sample Position' tab is selected. The 'Current Configuration' checkbox is unchecked. Under 'Select Well-plate or Vial Tray', the tree view shows 'Well-plate Tray' expanded to 'Plate 1'. The 'Plate/Tray Type' dropdown is set to '*96Agilent*'. The 'Selection Origin' section has 'Top left' selected. The 'Block Increment' section has 'Row major' selected. The 'Number of samples' field contains '0' and the 'Number of replicates' field contains '1'. The 'Well-plate/Tray' grid is visible with columns 1-12 and rows A-H. The 'OK' and 'Cancel' buttons are at the bottom right.

- 5 Specify the locations, and click **OK**.
- 6 To set up the worklist run, click **Worklist Run Parameters** .
- 7 On the Worklist **Run Parameters** tab, type the paths for the method.

Basic Operation

Setting Up and Running a Worklist

8 *Optional.* On the **Additional Parameters** tab, enter a comment, and click **OK**.

9 To start the worklist, click  **Run Worklist**.

NOTE

To use an acquisition method that has a different data analysis (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, browse for and select the 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.

Or, select this method in the **Add Multiple Samples** dialog box.

Reviewing Results with Qualitative Analysis

Use the Qualitative Analysis program to do these tasks and more:

- Review results for acquisition method development
- Select the most appropriate precursor and product ions for MS/MS analyses
- Find compounds
- Identify compounds
- Molecular feature extraction

Refer to the online Help for the Qualitative Analysis software to learn more.

Analyzing Data

Another primary tool for analyzing and reporting instrument results is the Quantitative Analysis program.

Do the exercises in the *Quantitative Analysis Familiarization Guide* to learn how to quantitate the acquired data files:

- Set up a batch and a method to automatically quantitate a set of samples
- Review results by learning how to view and use the Batch-at-a-Glance results screen
- Identify and use outliers to change the method and requantitate the data using a better calibration curve fit or other more appropriate settings

Refer to the online Help for the Quantitative Analysis program to learn how to do more operations to analyze your data.f

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3**Tuning**

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About Tuning

When the mass spectrometer (MS) is used as a detector for a liquid chromatograph, a mass spectrum is associated with each data point in the chromatogram. To obtain high quality, accurate mass spectra, the MS must be optimized to:

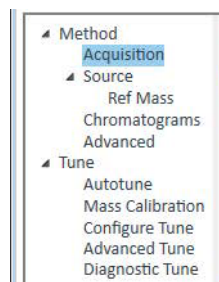
- Maximize sensitivity
- Maintain an acceptable resolution
- Ensure accurate mass assignments.

Tuning is the process of adjusting MS parameters to achieve these goals.

Tuning is primarily concerned with finding the correct settings for the parameters that control the transmission, filtering, and detection of ions. The Q-TOF/TOF Tune section within MassHunter acquisition software allows the tuning and mass calibration of the instrument. It includes the following general information and settings, plus tabs for setting extra parameters and performing various actions such as autotuning and manual tuning.

Tuning is accomplished by introducing a calibrant into the LC/MS and generating ions. Using these ions, the tune parameters are then adjusted to achieve sensitivity, resolution, and mass assignment goals. With a few exceptions, the parameters that control ion formation are not adjusted. They are set to fixed values known to be good for generating ions from the calibrant solution.

There are several different types of tunes (autotune, mass calibration/checktune, advanced, and diagnostic) that can be performed on the instrument. All tunes except for the Diagnostic tune can be scheduled to maximize instrument uptime.



With MassHunter Acquisition 12.0 for LC/Q-TOF software, the access to different tune functionalities is controlled via roles and privileges.

Roles

MassHunter Control Panel provides multiple roles available in the configuration when Authentication is enabled. Roles are collections of privileges that can be assigned to users or groups. You can create a new role or use roles available in your system. The Control Panel includes the following roles:

- Everything
- System Administrator
- Instrument Administrator
- Project Administrator
- Instrument User

Additional roles are available depending on your MassHunter configuration.

The roles are described in the Control Panel on the Roles page.

Name	Description
Everything	All privileges
System Administrator	Manage users and security settings
Instrument Administrator	Manage instruments and locations
Project Administrator	Manage projects and project groups
Project Content Deletion	Delete content from projects
Instrument User	View and run instruments
Lab Manager	Lab Manager Role for MassHunter Workstation
Scientist	Scientist Role for MassHunter Workstation
Analyst	Analyst Role for MassHunter Workstation
Operator	Operator Role for MassHunter Workstation
Reviewer	Reviewer Role for MassHunter Workstation
Activity Log Access	Activity Log access privileges


Current user: admin (admin)

NOTE

Only the Everything role has access to all roles and privileges. By default, only the Lab Manager role has the privilege to configure tunes. Other roles do not have access to this configuration and are only able to perform tunes from a predefined list configured by the Lab Manager. See MassHunter Control Panel help for more information on authentication, roles, and privileges.

Configure Tune

Accessible only to the Lab Manager or Everything role, configure tune is used to select from a broad range of tunes to make them available to other roles.

- 1 In MassHunter Acquisition, click **Method Editor** in the Windows section of the Ribbon to display the Method Editor window.
- 2 In the Method Editor window, click the **MS Q-TOF** tab.
- 3 Click **Tune > Configure Tune** in the left pane.
A list of instrument modes and tunes is displayed.
- 4 Click  **Tune control**.

This button locks control of the LC/Q-TOF instrument and changes the status of the instrument to Tune, as shown in **Figure 9**. A single sample run or a worklist cannot be started when Tune has control of the LC/Q-TOF instrument.



Figure 9. Tune Status

When clicked, tune setpoints are applied. If an action is not selected within the timeout period of three minutes, the system will go back to the method setpoints.

Tuning

Configure Tune

- Toggle the **Selected** switch to select specific instrument modes and tunes.

Binary Pump Column Comp. MS Q-TOF

Configure Tune

Select instrument modes to show in Tune and Method windows

Tune control Auto slicer index Restore default descriptions

Selected	Polarity	Instrument mode	Slicer	Spectrum mode	Description
<input checked="" type="checkbox"/>	+/-	Q-TOF system tune	Resolution	High Res.	This mode is added to autotune by default (3200 Stable). This mode will tune the intensity up to m/z 3,200, where mass resolution is most critical. A popular choice for Peptide mapping, Intact/subunit proteins, Oligos and Glycan analysis.
<input type="checkbox"/>	+/-	3200 fragile	Resolution	High Res.	This mode will tune the intensity up to m/z 3,200. The settings are optimized for transmission of labile large molecules, for example released Glycans.
<input type="checkbox"/>	+/-	1700 stable	Sensitivity	High Res.	This mode will tune the intensity up to m/z 1,700. This mode is an excellent choice when sensitivity is critical. A popular choice for Host Cell Protein (HCP) analysis.
<input type="checkbox"/>	+/-	1700 fragile	Resolution	High Res.	This mode will tune the intensity up to m/z 1700. The settings are optimized for transmission of labile small molecules.
<input type="checkbox"/>	+/-	750 stable	Resolution	High Res.	This mode will tune the intensity up to m/z 750, while acquiring data up to 1700 m/z. A popular choice for Metabolomics applications and DMPK studies. This mode is an excellent choice when sensitivity is critical.
<input type="checkbox"/>	+/-	750 fragile	Resolution	High Res.	This mode will tune the intensity up to m/z 750, while acquiring data up to 1700 m/z. A popular choice for Metabolomics applications, DMPK studies and other labile molecules. The settings are optimized for transmission of labile small molecules.
<input type="checkbox"/>	+/-	250 stable	Resolution	High Res.	This mode will tune the intensity up to m/z 250, while acquiring data up to 1700 m/z.
<input type="checkbox"/>	+/-	250 fragile	Resolution	High Res.	This mode will tune the intensity up to m/z 250, while acquiring data up to 1700 m/z. The settings are optimized for transmission of labile small molecules. A popular choice for Metabolomics applications.
<input type="checkbox"/>	+/-	High mass stable	Resolution		This mode will tune the intensity for large molecule analysis and has an m/z range of up to 30,000. This mode is an excellent choice when resolution is critical.
<input type="checkbox"/>	+/-	High mass large molecule	Resolution		This mode will tune the intensity for large molecule analysis and has an m/z range of up to 30,000. This mode is an excellent choice when sensitivity is critical and is popular choice for BioPharma applications including Intact Protein analysis.

Select manual instrument modes to show in Tune and Method windows + Add manual tune

- Click the **+/-** button to select specific Polarity for the Instrument mode. There are three states to choose from, **+**, **-**, and **+/-**.
- Click the Slicer drop down to select a mode. The Slicer controls the optics aperture, either narrow for resolution or wide for sensitivity
- (Optional) Double-click in the **Description** field to modify the default description of the predefined instrument modes.

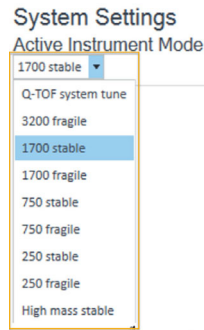
NOTE


The Q-TOF system tune is always selected as this tune uses the default values as the starting point and has the all elements involved in mass resolution tuning included.

Tuning

Configure Tune

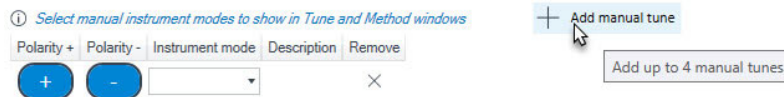
- 9 Select the System Settings Active Instrument Mode drop-down list to view the changes.



- 10 Click  **Tune control** to release control of the LC/Q-TOF instrument.

Settings are selected using instrument mode and polarity; either only positive or negative, or both. All tunes have a combined TOF tune and Quad tune included, ensuring that the right quadrupole settings are used based on the TOF tuning parameters.

In addition to these predefined instrument modes/tunes, the lab manager role allows the creation of up to 4 manual tunes that are made visible to operator and lab technician, if needed.



Calibration and Tuning Options

The mass calibration process is performed by infusing a solution with known masses into the source and measuring the actual flight times for ions of the masses. For optimum performance, run a mass calibration before analysis each day. Run mass calibration after changing any high-voltage parameters in manual tuning or changing the mass range. See **“Mass Calibration”** on page 63.

A checktune is used to determine if the tuning mix ion masses are properly assigned and if the response or sensitivity of these ions is within expectations. The checktune also ensures that the quadrupole filtering is within the set tolerances. In other words, a checktune verifies peak width and mass axis to make sure they are correct before starting an acquisition. The checktune concludes with a mass calibration (TOF). See **“Checktune”** on page 64.

Perform an autotune on monthly intervals to ensure that TOF resolution and sensitivity are optimized. Everything is automatic since the tuning mix is delivered by the calibrant delivery system, which is switched on automatically during the tune. See **“Autotune”** on page 66.

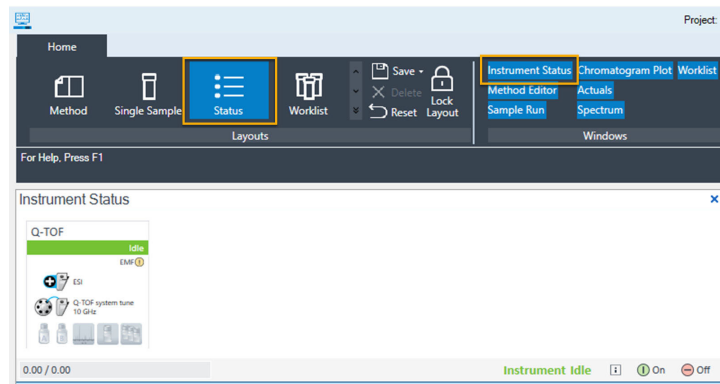
Use **Table 2** to select the correct tuning mix for the source installed.

Table 2 Tuning Mixes

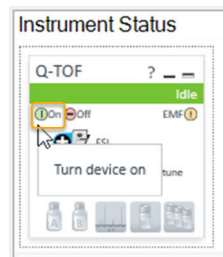
Source	Tuning mix	Part Number
ESI/AJS	ESI-X Tuning Mix	5191-6449
APCI	APCI-L Low Concentration Tuning Mix	G1969-85010

Starting the Instrument

- 1 Follow the procedures in **“Starting Up and Shutting Down the Instrument”** on page 23 to begin operation of the instrument.
- 2 Display the Instrument Status window if it is not visible in the Acquisition program, in one of the following ways:
 - Open the Status layout.
 - On the ribbon, click **Instrument Status** in the **Windows** section.



- 3 Hover over the Q-TOF device pane and click **On**.




- 4 Monitor baseline and adjust the plot if necessary. See **“Monitoring baseline and adjusting plot”** on page 29.

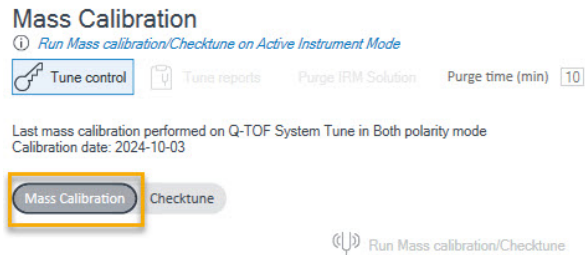
Mass Calibration



Mass Calibration performs a TOF mass calibration on the Active Instrument Mode, which is displayed in the Systems Settings panel. It assigns masses based on known masses of standard compounds. Agilent recommends running mass calibration daily or weekly, at a minimum for optimal precision and accuracy. During normal operation, mass calibration lasts approximately 5 minutes.

NOTE

Only the active instrument mode can be calibrated.

- 1 In the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Mass Calibration** in the left pane.
- 3 Click  **Tune control**.
- 4 Select **Mass Calibration**.




- 5 Click  **Mass calibration/Checktune**. When the tune completes, review the report.
- 6 Click  **Tune control** to release control of the LC/Q-TOF instrument.

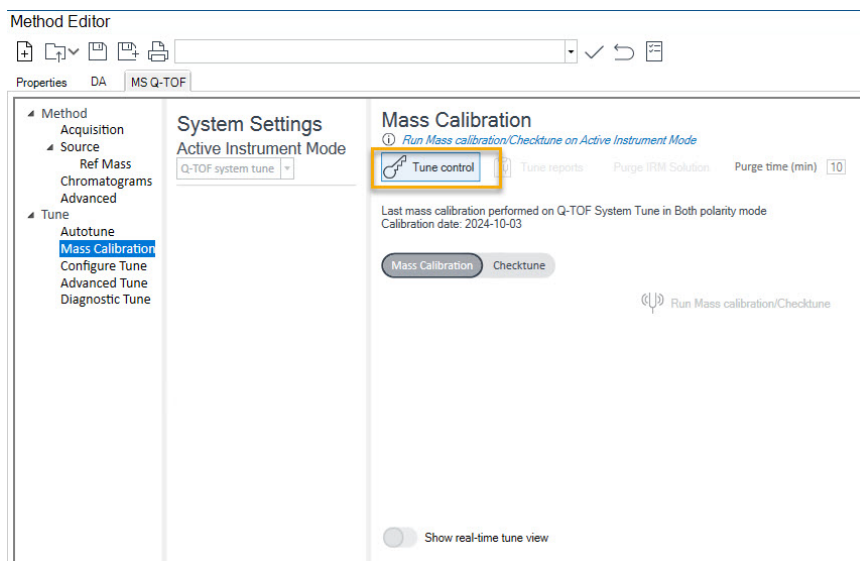
Checktune

A checktune is used to determine whether the instrument is correctly tuned by applying the current tune parameters to the source, quadrupole, and detector to confirm the signal intensity and resolution is within expectation. A checktune can be run with the following ion sources: ESI, AJS ESI, and APCI.

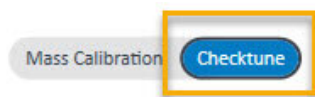
NOTE

Only the active instrument mode can be calibrated.

- 1 In the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Mass Calibration** in the left pane.
- 3 Click  **Tune control**.

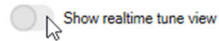


- 4 Select **Checktune**.

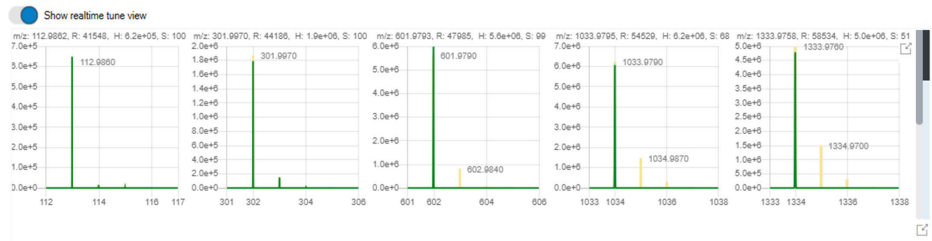



Tuning Checktune

- 5 Activate **Show realtime tune view** to view the real time data acquisition.




- 6 Click . View the progress of the tune in the Real Time tune results.

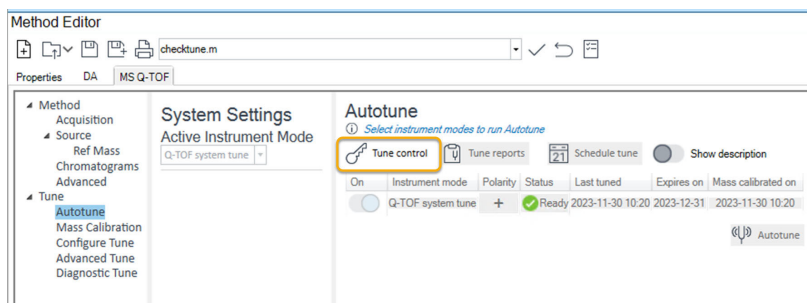


- 7 When the tune completes, review the report.
- 8 Click  **Tune control** to release control of the LC/Q-TOF instrument.

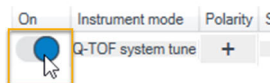
Autotune



Autotune is an automated tuning program that adjusts the MS for good performance over the entire mass range. An autotune can be run with the following ion sources: ESI, AJS ESI, and APCI.

- 1 In the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Autotune** in the left pane.
- 3 Click  **Tune control**.





- 4 Activate one or more specific tunes using the switch to select. More than one tune can be selected.

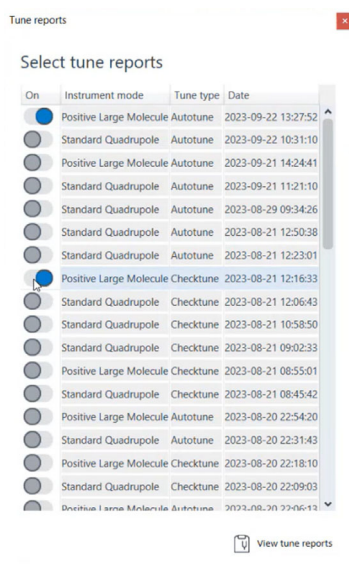




- 5 (Optional) Activate **Show realtime tune view** to view the real time data acquisition.
- 6 Click  **Autotune**.
- 7 When the tune completes, review the report.
- 8 Click  **Tune control** to release control of the LC/Q-TOF instrument.

Generating a Detailed Tune Report

Generate a detailed tune report after running an autotune or checktune.

- 1 In the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Autotune** in the left pane.
- 3 Click  **Tune control**.
- 4 Click  **Tune Reports**. If this button is not available, run either a checktune or an autotune first.
- 5 Use the toggles to select reports for viewing.





- 6 Click  **View tune reports** generate PDFs for viewing in a PDF viewer.
- 7 Click  **Tune control** in the toolbar to release control of the Q-TOF instrument.

Advanced Tune

In the Advanced Tune section, launch the advanced tune view to perform a manual tune.

NOTE

This application can only be accessed with specified user privileges. Access to the Manual Tune privilege must be enabled in MassHunter Control Panel to access both Configure Tune and Advanced Tune. See the MassHunter Control Panel Help for more information on setting privileges.

- 1 In Acquisition, in the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Advanced Tune** in the left pane.
- 3 Click  **Tune control**.
- 4 Click  **Launch advanced tune view**. The Advanced Tune View opens.

Advanced Tune View

MS SWARM Tune Calibration Diagnostics | Tune | Vacuum | G6575A/US12345 | @MassHunter/PC Users Manual Tune

Method Manual Tune Tools

Method: [Dropdown]

Ion Polarity: Positive

Scan Masses: Auto

Scan Rate (Hz): 1

Scan Type: MS

Isolation Width: Wide

Isolation Mass: 322

Number Quad Profile: 2

Collision Energy: 0

Refresh Apply

Standby Acquire

Actuals Console


Device	Name	Setpoint	Actual
AJS Source	Chamber Current (µA)	0.00	0.00
	Capillary Current (µA)	0.00	0.00
	Gas Temp (°C)	300	305
	Gas Flow (l/min)	5.0	5.2
	Nebulizer (psi)	20.0	20.2
Quadrupole	Capillary Voltage (V)	-4000	-4120
	Sheath Gas Temp (°C)	250	262
	Sheath Gas Flow (l/min)	12.0	12.0
Vacuum	M51 Heater (°C)	0	0
	Vacuum State	Pumped	Pumped
	Rough Vac (Torr)	2.34e-00	2.34e-00
	Quad Vac (Torr)	4.03e-05	4.03e-05
	TOF Vac (Torr)	8.98e-07	8.98e-07

Close

- 5 Click the **Tune** tab.
- 6 Click the **Manual Tune** tab.

Tuning

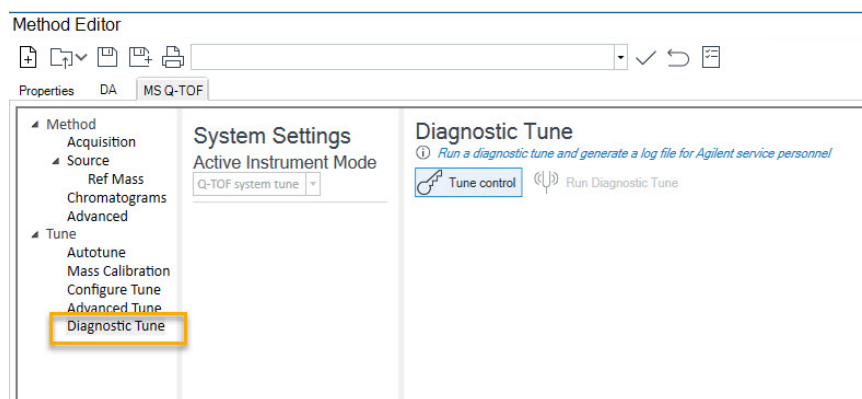
Advanced Tune



- 7 Select a tune file and make changes.
- 8 Click  to **Save** the tune file.

Diagnostic Tune

Diagnostic tunes should only be performed at the request of Agilent service personnel. During normal operation, a diagnostic tune will last approximately 3 hours and creates a log file.

- 1 In the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Diagnostic tune** in the left pane.

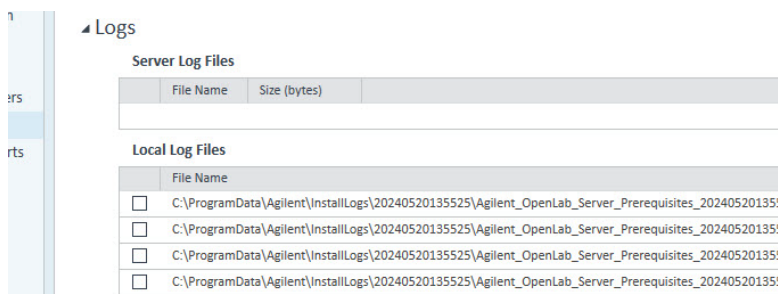


- 3 Click  **Tune control** in the toolbar to lock control of the instrument.
- 4 Click  **Run Diagnostic Tune** to start a diagnostic tune.

The Progress bar and Tune status window displays the progress and status of the tune.

- 5 In MassHunter Control Panel, navigate to the **Control Panel > Administration > Diagnostics** page.



The log is named: YYYY-MM-DD-h-m-sec-MSR.zip.

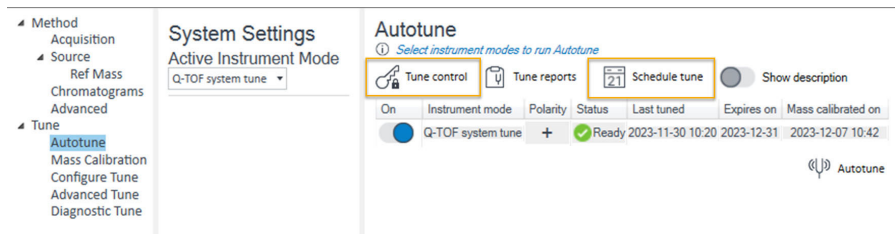


Scheduling Tunes

Scheduling a Mass calibration or Checktune

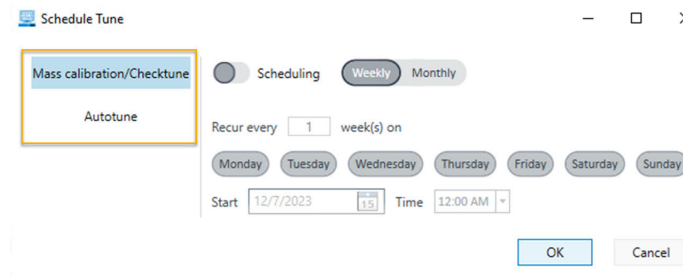
Schedule a mass calibration or checktune.

- 1 In the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Autotune** in the left pane.
- 3 Click  **Tune control**.
- 4 Click  **Schedule tune**.



The **Schedule Tune** dialog box opens.

- 5 Select **Mass calibration/Checktune** in the left pane.
The right pane shows the information for scheduling a tune.





- 6 Click **Scheduling** to toggle on **Scheduling**.
- 7 Select either **Weekly** or **Monthly**.

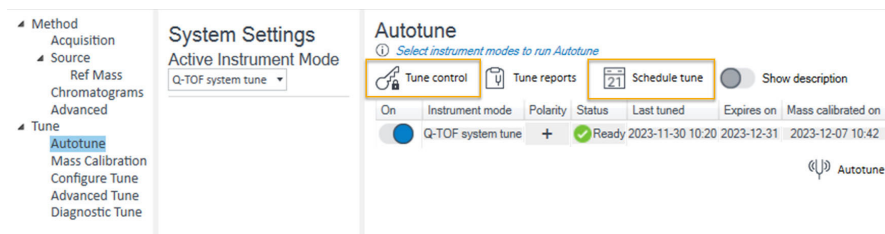
Tuning

Scheduling a Mass calibration or Checktune

- 8 Select other options to indicate how often to schedule the mass calibration or checktune.
- 9 Click **OK**.

Scheduling an Autotune

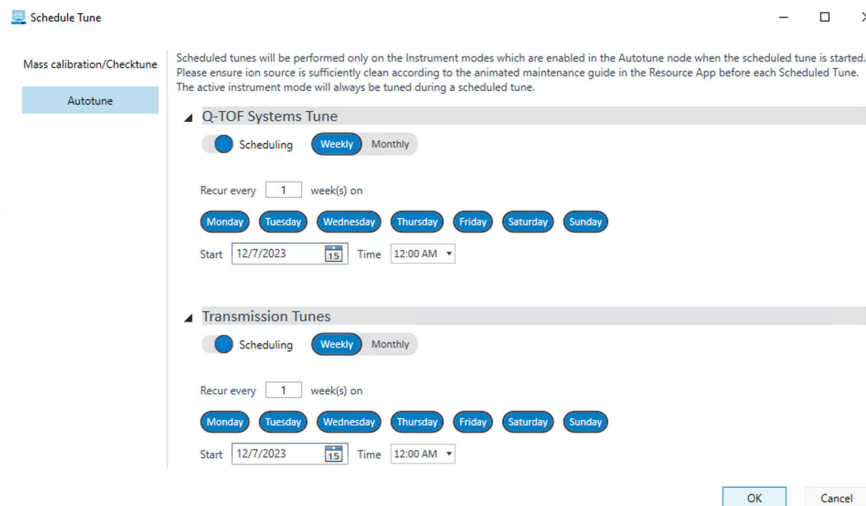
- 1 In the Method Editor window, click the **MS Q-TOF** tab.
- 2 Click **Tune > Autotune** in the left pane.
- 3 Click  **Tune control**.
- 4 Click  **Schedule tune**.



The **Schedule Tune** dialog box opens.

- 5 Select **Autotune** in the left pane.


The right pane shows the information for scheduling a tune.



- 6 Click **Scheduling** to toggle **Scheduling** on.
- 7 Select either **Weekly** or **Monthly**.

Tuning

Scheduling an Autotune

- 8 Select other options to indicate how often to schedule the Autotune or Transmission Tune.
- 9 Click **OK**.
- 10 Click  **Tune control** in the toolbar to release control of the Q-TOF instrument.

4

Maintenance

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Maintenance Overview

Your instrument needs servicing and maintenance to avoid contamination, which prevents signal suppression, reduces background noise, minimizes adduct formation, and enables the instrument to operate at its peak performance. To achieve the most accurate results, routine and periodic maintenance is crucial.


The difference is that routine maintenance is done regularly to keep the equipment in good working condition, while periodic maintenance is done at specific intervals to prevent breakdowns or failures.

MassHunter acquisition software for LC/MS systems tracks the number of injections and the number of days since a maintenance activity was performed for your LC/Q-TOF instrument using the Early Maintenance Feedback (EMF) dashboard. EMF allows you to plan for scheduled maintenance when it is convenient and increase the instrument uptime. If a limit is exceeded for an EMF item that is enabled, then the EMF icon changes to yellow, and the tooltip displays the cause in the device pane. Set the limits for each EMF item in the Maintenance dialog box following instructions detailed in online help.

This Maintenance chapter is broken down into maintenance procedures listed by routine, then periodic maintenance tasks with their recommended schedule of performance, with the EMF procedures cross referenced in the final section. Consult the online help or contact your Agilent consultant for further information.

LC/MS Maintenance Video Series



Procedures notated with the  Video Support icon are linked to videos intended to support written content. These links are also found in the online help and/or the instrument user guides. Refer to these videos for supplemental instructions. View the full series at <https://aglt.co/LCMSMaintenance>.

Routine Tasks

Cleaning the spray chamber - daily

Perform this maintenance daily or at the end of each shift or anytime you suspect carryover contamination from one sample or analysis to another.

NOTE

Use the weekly cleaning procedure if symptoms of contamination persist or if the spray shield or capillary cap show significant discoloration that is not removed by the regular, daily cleaning.

Tools needed:

- Clean, powder-free nitrile gloves
- Clean lint-free cloth
- LC/MS-grade isopropanol
- LC/MS-grade water
- Clean wash bottle

To perform daily cleaning of the spray chamber,

- 1 Put on clean powder-free nitrile gloves.
- 2 Lower the drying gas temperature to the minimum level.
- 3 For APCI/multimode, lower the vaporizer heater temperature to the minimum level.

WARNING

Make sure that the source is cooled before you clean the spray shield.

- 4 In a clean wash bottle, prepare a 50:50 mix of LC/MS-grade isopropanol and LC/MS-grade water.
- 5 Open the spray chamber.
- 6 Dampen a clean lint-free cloth with a mixture of LC/MS-grade isopropanol and LC/MS-grade water.
- 7 Wipe the spray shield and the area around the spray shield.
- 8 Close the spray chamber.

Checking and filling the calibrant bottle - weekly

Schedule

Perform this procedure monthly or weekly if you tune the instrument frequently, or if the calibrant has expired.

Equipment List

- APCI calibrant
- APCI-L tuning mix
- ESI-X tuning mix
- MMI-L tuning mix
- Reference mix
- Clean powder-free nitrile gloves

Steps for checking calibrant levels

- 1 Check to make sure that enough tuning mix is present to immerse the end of the intake tube.
- 2 If the tuning mix level is within a few millimeters of the end of the intake tube, refill the calibrant bottle.

Steps for refilling the calibrant bottle

- 1 Rinse the calibrant bottle once with water, then twice with ACN before refilling.

CAUTION

Dispose of rinsing agents appropriately.

- 2 Label the calibrant bottle with the tuning mix expiration date.
- 3 Refill the calibrant bottle with the appropriate tuning mix.
- 4 Put the intake tube into the calibrant bottle as you lift the calibrant bottle into position.

CAUTION

Do not touch the intake tube unless you are wearing clean gloves.

Maintenance

Checking and filling the calibrant bottle - weekly

- 5 Attach the calibrant bottle onto the fixed bottle cap. Turn the calibrant bottle counterclockwise to tighten.

CAUTION

The bottle only needs to be snug. Do not over-tighten the bottle. A leaky bottle cap can prevent the bottle from pressurizing and can lead to the evaporation of the contents.

CAUTION

The tuning mixes are not interchangeable. Failure to change the tuning mix when the source or inlet assembly is changed can result in miscalibration of the instrument and errors in mass assignments.



Video Support

Cleaning the spray chamber - weekly

<https://ggl.tcd/00156899>

Perform this maintenance:

- Weekly.
- Whenever symptoms indicate that contamination exists in the spray chamber.
- If the spray shield or capillary cap shows significant discoloration not removed by daily cleaning.

Tools needed:

- Clean powder-free nitrile gloves
- Clean lint-free cloth
- LC/MS-grade isopropanol
- LC/MS-grade water
- Clean wash bottle
- 4000 grit sandpaper

- 1 Put on clean powder-free nitrile gloves.
- 2 Disconnect the nebulizer sample line and sample nebulizer gas tubing from the nebulizer.
- 3 Disconnect all ion source connections to the instrument.
- 4 Open the latch of spray chamber.
- 5 Open the spray chamber.
- 6 Remove source and put the source under a fume hood.
- 7 Dampen a clean lint-free cloth with a mixture of LC/MS-grade isopropanol and LC/MS-grade water. For Multimode, be careful not to touch the thermocouple probe.
- 8 Wipe the interior of the spray chamber with a clean lint-free cloth.

Maintenance

Cleaning the spray chamber - weekly



- 9 Make sure you wear clean gloves. Use a tech wipe to hold the parts as you remove them. They will be warm.



- 10 Clean the spray shield, capillary cap, end plates, and contact ring under fume hood. Carefully remove the canted coil spring from the capillary cap before cleaning.
- 11 (Optional) Abrasive cleaning for persistent contaminants: Use 4000-grit abrasive paper to clean contaminants from the parts.



- 12 Put the spray shield, capillary cap, end plates, and contact ring in the beaker.
- 13 Add a 50:50 mix of LC/MS-grade isopropanol and LC/MS-grade water in a beaker.
- 14 Put the beaker in sonication for 5 minutes.
- 15 Drain the solvent and remove the parts from the beaker.
- 16 Dry the parts by using nitrogen gas.
- 17 Carefully insert the canted coil back into the capillary cap.
- 18 Reinstall the spray chamber parts and source on the instrument.
- 19 Reinstall the spray chamber on the instrument.
- 20 Connect all cables and tubing to the instrument.
- 21 Connect the nebulizer sample line and sample nebulizer gas tubing to the nebulizer.



Video Support

<https://aglt.co/D0115677>

Cleaning the nebulizer - weekly

Passing the vapors of solvents like IPA, methanol, acetonitrile, or water through the LC/MS system is called steam cleaning and is used to clean possible contamination in the LC/MS system. The usual conditions are:

- LC pump flow to 0.5 ml/min
- Nebulizer pressure to 60 psi
- Drying gas to 13 L/min
- Drying gas temp to 350 °C

Make sure that the MS stream selection valve is set to go to the MS. Steam Cleaning overnight has been shown to be one of the most important factors in improving the signal-to-noise level by reducing the contaminations.

To clean an MS, there are two options:

- Plain water/MeOH at high temp – 50:50 proportion
- IPA/MeOH/Water at high temperature

The IPA/MeOH/Water combo can be anything. IPA, maybe 50 (IPA):25:25. IPA cleans both aqueous and organic.

Schedule

Check the nebulizer weekly. If needed, flush traces of samples and buffers out of the tubing, valves, and nebulizer.

Equipment List

- LC/MS-grade methanol
- LC/MS-grade water

Steps

- 1 Install a mix of 30 to 70% methanol in water into a LC solvent bottle.
- 2 Remove the column from the LC column compartment.

Maintenance

Cleaning the nebulizer - weekly



- 3 Remove nebulizer from spray chamber and support the nebulizer so that it sprays into an appropriate waste container (beaker, bottle, etc.).



- 4 Control the LC flow through the acquisition software to pull from the solvent bottles.
- 5 Set flow rate to not more than 1 ml per minute.
- 6 Flush for 3 to 5 minutes. For heavy build up, additional flushing may be required.

Periodic Tasks

Checking the rough pump oil level

Schedule

Once every three months/once per quarter (recommended).

Equipment List

- Clean chemical-resistant gloves
- Safety glasses
- Funnel
- 10 mm hex key
- Paper towel or shop rag
- Small plastic tub
- AFV 60 Gold Oil

Steps

- 1 The oil level should be between the marks for Max and Min.
- 2 Check that the pump oil is clear and the color is lighter than amber.
- 3 If the pump oil is dark or full of suspended particles, replace it.



Video Support

Replacing the MS40+ pump oil and filter

<https://aglt.co/D0115665>

Schedule

Replace the pump oil every six months. Replace it sooner if the oil appears dark or cloudy.

Equipment List

- Clean chemical-resistant gloves
- Safety glasses
- Funnel
- Pair of pliers
- Filter
- Filter cap O-ring
- Small cap O-ring
- MS40 exhaust filter cap removal tool
- 10-mm hex key
- Paper towel or shop rag
- Small plastic tub
- AFV 60 Gold Oil

Draining the MS40+ pump oil

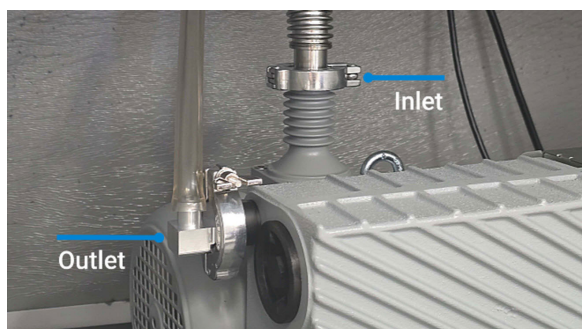
- 1 Put on clean, chemical-resistant gloves.
- 2 Place the instrument into 'Standby,' then 'Vent'.
- 3 Allow the rough pump to turn off automatically, waiting for approximately 15 minutes.
- 4 Unplug all power cords.

Maintenance

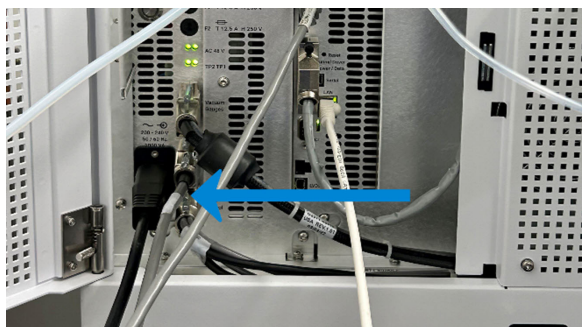
Replacing the MS40+ pump oil and filter



- 5 Disconnect the inlet and outlet pipes.



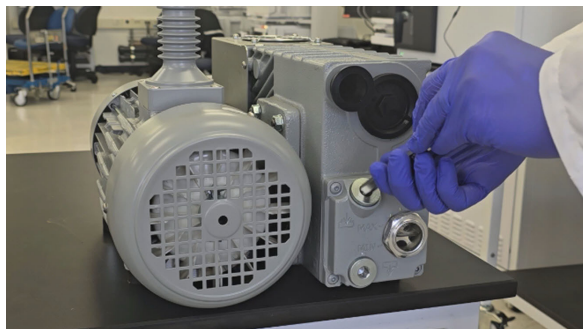
- 6 Unplug all power cords from the LC/MS instrument.



- 7 Unscrew the oil fill cap, rotating it counterclockwise using a 10-mm hex key. Remove it from the instrument and place it in a secure location.

Maintenance

Replacing the MS40+ pump oil and filter



- 8 Place a tank for waste oil, for example, a small plastic tub under the oil drain plug.
- 9 Unscrew slowly the oil drain cap by rotating it counterclockwise using the 10-mm hex key.



- 10 Let the oil flow out. In the meantime, clean the inside of the drain plug.



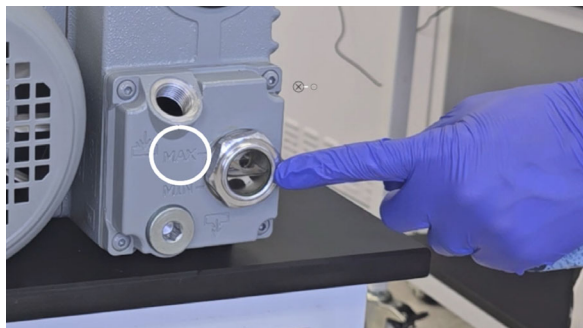
Maintenance

Replacing the MS40+ pump oil and filter

- 11 After the oil has been drained, close the drain plug tight. Take the tank of waste oil away and clean thoroughly, using rags of cotton or other suitable material to dry.

Replacing the MS40+ pump oil

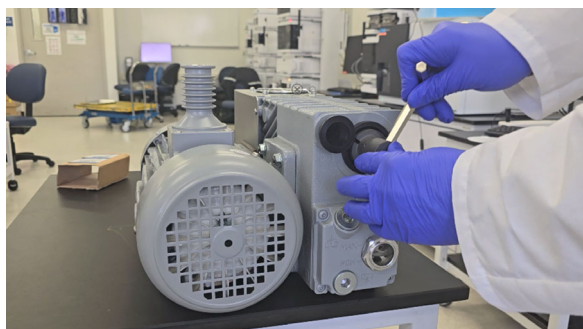
- 1 Add AVF 60 Gold oil until reaching the maximum level on the sight glass.



- 2 Close the oil fill cap tight using the 10-mm hex key.

Replacing the MS40+ oil mist filter

- 1 Unscrew the filter cap using the MS40 exhaust filter cap removal tool.



- 2 Remove the O-ring from the filter cap.

Maintenance

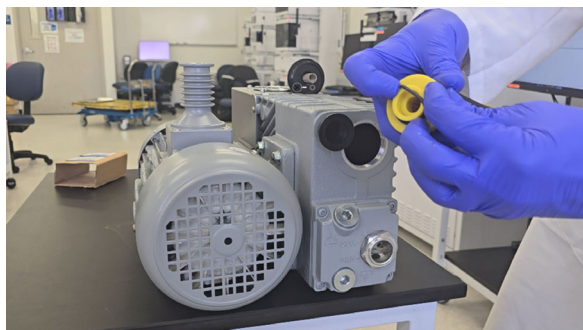
Replacing the MS40+ pump oil and filter



- 3 Remove the old filter from the instrument.
- 4 Remove the O-ring from the filter.



- 5 Install the new, small O-ring on the new filter, then install the filter into the pump in the proper orientation.



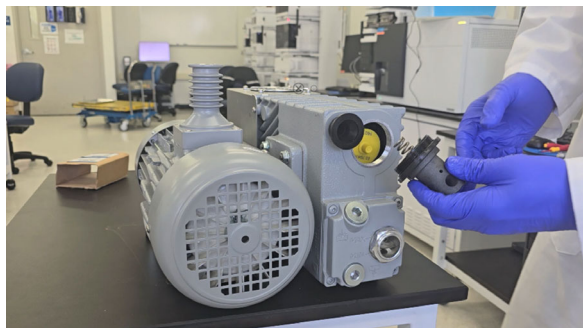
- 6 The distance of the filter should not be >2 cm from outside the cap threads.

Maintenance

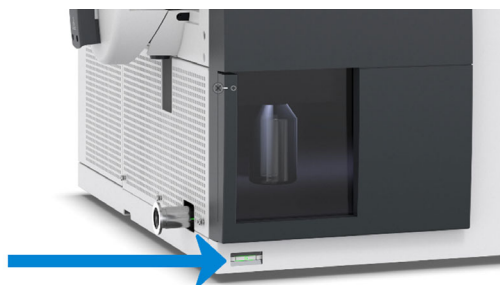
Replacing the MS40+ pump oil and filter



- 7 Install the new, large O-ring and filter cap.



- 8 Connect the inlet and outlet pipes.
9 Plug in the rough pump and all power cords from the LC/MS instrument.
10 Press the front power switch to initiate pump down.



HEPA Filter

Schedule

Equipment List

- Clean chemical-resistant gloves
 - HEPA Filter
- 1 Release the clamps of the HEPA filter canister.
 - 2 Open the lid.
 - 3 Remove the filter.
 - 4 Insert the new filter into the filter canister.
 - 5 Close the lid.
 - 6 Fasten the clamps.

Replacing the nitrogen gas filters

Schedule

Yearly, or when flagged by EMF.

Equipment List

- 2 1/2-inch OD Mounting Clip for Big Universal Traps (2/pk) - Part number UMC-5-2
- RMSN-4 1/4-inch Nitrogen

Steps

NOTE

Before installing the trap, make sure that the system is free of leaks and the system is generally in good working order.

- Maximum operating pressure is 250 PSIG.
- Maximum operating temperature is 100 °C.
- Maximum flow rate is 8 liters per minute.

CAUTION

If you are using stainless steel tubing, please order the purifier with stainless steel fittings RMSH-2-SS or RMSH-4-SS. Using brass fittings with stainless steel tubing increases the risk of cross-threading.

- 1 Shut down your LC/MC.
- 2 Set the gas source supply pressure to 10–15 psi and maintain flow in the gas source line before disconnecting it from the inlet of the old trap.
- 3 Remove the protective nut and plug from the INLET end of the new trap. DO NOT open the plug on the OUTLET end.
- 4 Immediately attach the new trap to the gas source tubing using one of the included ferrule sets.

CAUTION

Failure to connect the trap immediately may cause contamination of the adsorbents inside. Reduced adsorption capacity will contribute to elevated levels of contaminants observed by the detector. This may result in the need for additional purge time.

Maintenance**Replacing the nitrogen gas filters**

- 5 Insert the tubing through the nut and ferrule set until the tubing rests firmly against the shoulder in the fitting.
- 6 Finger-tighten the nut, then use a wrench to tighten the nut $3/4$ turn for $1/8$ -inch tubing and $1-1/4$ turn for $1/4$ -inch tubing.
- 7 Wait 3 minutes for the gas pressure inside the new trap to stabilize.
- 8 Increase the gas source pressure to 60 psi.
- 9 Open the outlet fitting and purge the trap for 3 minutes.
- 10 Adjust the gas source pressure to a normal working level. Connect the outlet fitting of the trap to the instrument tubing as described in step 5 and step 6.
- 11 Secure the trap in a vertical orientation. The length of the trap should stand perpendicular to the floor.

Wait for at least 3 minutes to purge out any air that may have entered the instrument tubing line. The trap is now ready for use.

General Maintenance Tasks



<https://aglt.co/D0115676>

Replacing the nebulizer needle

Schedule

Perform this procedure when you need to access the nebulizer for maintenance.

Equipment List

- Clean, powder-free nitrile gloves
- Replacement needle kit
- 1/4" Nebulizer adjustment fixture (GT430-20470)-inch x 5/16-inch open-end wrench (Quantity: 2)

Removing the nebulizer

- 1 Put on clean, powder-free nitrile gloves.
- 2 In MassHunter Data Acquisition, shut off the LC solvent flow and nebulizing gas.
- 3 Shut off the reference nebulizer gas valve.
- 4 Disconnect the nebulizer sample line from the nebulizer.
- 5 Disconnect the sample nebulizer gas tubing from the nebulizer.

WARNING

The tip of the nebulizer can be very hot. Do not touch the nebulizer until it is cool.

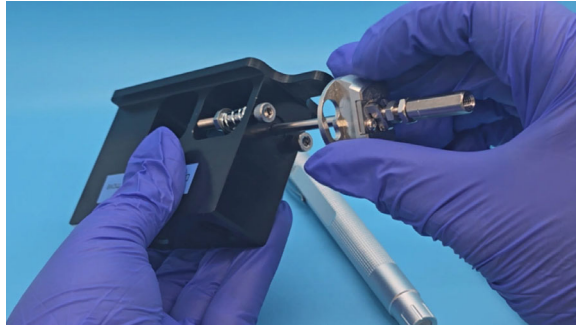
- 6 Turn the nebulizer counterclockwise until it disengages from the retaining screws.
- 7 Gently lift the nebulizer out of the spray chamber and place it in a safe location.

Maintenance

Replacing the nebulizer needle

Replacing the nebulizer needle

- 1 Determine your nebulizer type per the user guide or the document that comes with the kit.
- 2 Install the nebulizer in the Nebulizer Adjustment Fixture, turning clockwise to secure the nebulizer to the adjustment fixture.



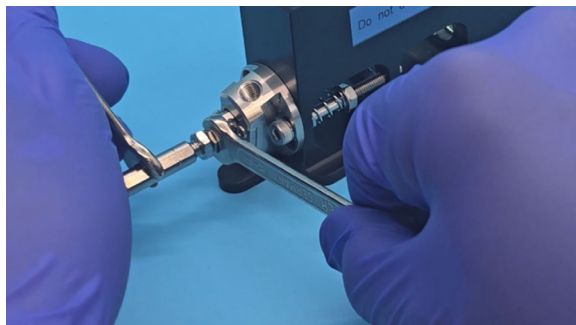
- 3 Loosen the fastener (locknut, 1.5-mm hex screw, or T6 Torx screw) that secures the needle holder in place.



- 4 Loosen the locknut next to the zero-dead-volume (ZDV) union by placing the 5/16-inch wrench on the nut, and the 1/4-inch wrench on the union, and turning the 5/16-inch wrench clockwise.

Maintenance

Replacing the nebulizer needle



- 5 Remove the union from the nebulizer needle holder.
- 6 Remove the union lock nut from the nebulizer.



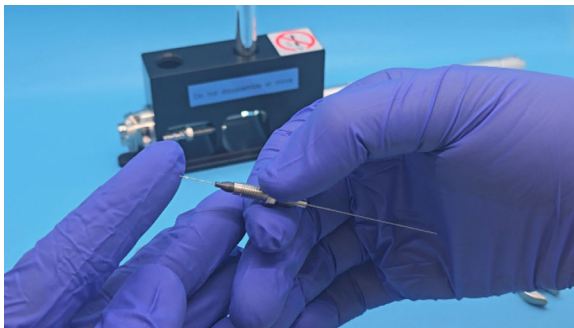
- 7 Loosen the needle holder and pull it out of the nebulizer body. Dispose of the used needle properly.
- 8 For more thorough cleaning, sonicate the nebulizer body. Pour isopropanol or methanol through the inside of the shaft.
- 9 Carefully remove the new needle from the shipping tube and adhesive strip. Identify the sharp end of the needle.
- 10 Carefully slide the sharp end of the needle through the ferrule until 1 cm of the needle remains at the ferrule.

Maintenance

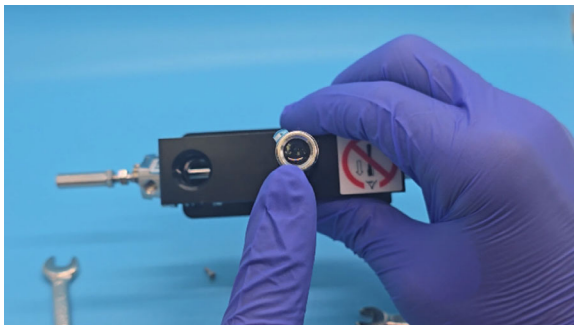
Replacing the nebulizer needle

CAUTION

Do not bend the needle as you slide it into the ferrule. The needle is fragile. A bent needle results in poor alignment with the nebulizer body.



- 11 Push the needle into the ferrule until the needle is slightly visible flush with the ferrule.
- 12 Reinstall the locknut and the union. Hand-tighten the union.
- 13 Hold the needle holder steady with a 1/4-inch x 5/16-inch open-end wrench, depending on your nebulizer holder style.
- 14 Tighten the union one-half to three quarters of a turn, or until an audible squeak is heard. The ferrule is now compressed.
- 15 Adjust the electro-spray needle position before reinstalling the nebulizer in the spray chamber.



- 16 Tighten the fastener (T6 Torx screw) that secures the needle holder in place.



Video Support

<https://aglt.co/D0115675>

Inspecting and adjusting the nebulizer needle

Schedule

Perform this project if there is increased LC back pressure, off-axis spraying, dripping from the nebulizer, or when the reference nebulizer needle is not spraying.

Equipment List

- Nebulizer Adjustment Fixture (GT430-20470) or Nebulizer adjustment kit (G1960-67470)
- Clean powder-free nitrile gloves
- T6 Torx driver
- 1/4-inch x 5/16-inch open-end wrench (Quantity: 2)

- 1 Put on clean, powder-free nitrile gloves.
- 2 Insert the tip of the nebulizer into the end of the Nebulizer Adjustment Fixture.
- 3 Slide the heads of the two spring loaded socket head cap screws through the mounting holes on the body of the nebulizer.
- 4 Turn the nebulizer body 1/8 turn counter-clockwise.

NOTE

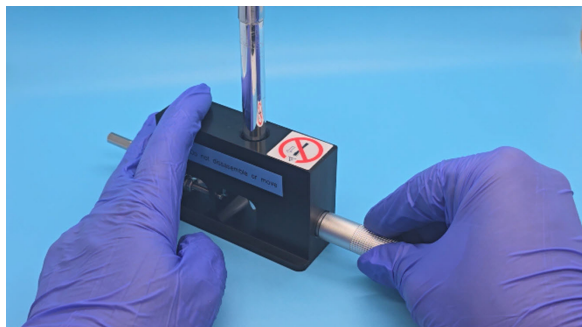
If the nebulizer is too tight or too loose, loosen or tighten the nut on the captured screws to adjust the tension on the spring. Turn clock wise to tighten, counter clock wise to loosen.



Maintenance

Inspecting and adjusting the nebulizer needle

- 5 Push the 40 x Magnifier into the vertical end position of the Nebulizer Adjustment Fixture.
- 6 Align the tapered tip of the magnifier to the tip of the nebulizer needle.
- 7 Push the LED Flashlight into the Nebulizer Adjustment Fixture.



- 8 Turn on the flashlight and, while looking down the magnifier, adjust the height of the magnifier until the tip of the nebulizer is clearly in focus.

Table 1 Correct needle position

Nebulizer	Distance
Microflow Nebulizer Assembly	0.003 inch
ES Tested Nebulizer	0.000 (flush)
Tested ESI Nebulizer Assembly (older style)	0.003 inch
Tested APCI Nebulizer Assembly	0.000 (flush)

The distance shown is the distance that the needle tip protrudes from the tip of the nebulizer assembly in inches.

Note the scale shown in the magnifying lens is also in inches, with one division equaling 0.001 inches.

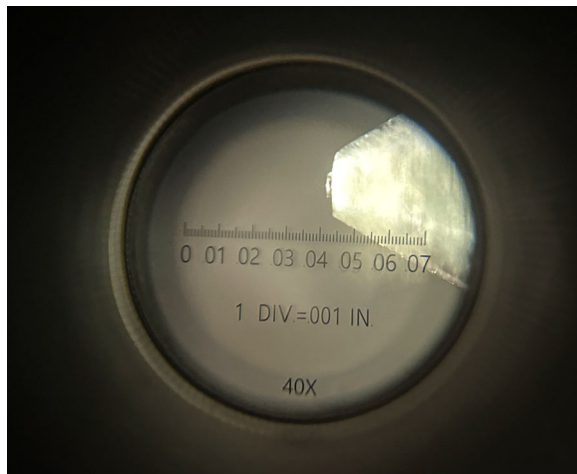


Figure 1. Correctly adjusted nebulizer needle.

NOTE

If the needle tip looks pitted, clogged with salt, corroded, or is not square and has jagged edges then the needle will need to be replaced.

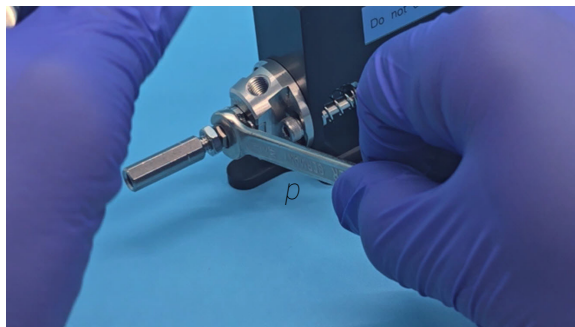
- 9 Using either a T6 Torx driver or 1.5 mm hex wrench, loosen the lock screw on the head of the nebulizer assembly. The nebulizer lock screw is either a T6 Torx or 1.5 mm hex bolt



- 10 Using the 1/4" end of the open-end wrench, rotate the needle assembly while looking down the magnifier. Turning the nut with either push the needle out or draw it into the nebulizer tip. Adjust the needle so that the distance matches the specified distance for your nebulizer type.

Maintenance

Inspecting and adjusting the nebulizer needle



- 11** If the needle distance looks correct, reverse the position of the magnifier and the flashlight so the flashlight is in the top port and the magnifier is in the side port of the fixture. Check that the needle tip is also centered (concentric) with the tip of the nebulizer assembly. If it is a long way off center after optimizing the distance, then the needle may need to be replaced.
- 12** Once the position of the needle has been optimized, carefully tighten the lock screw loosened earlier, while checking that the position of the needle has not moved during tightening.
- 13** The nebulizer needle adjustment is now complete.

Installing the capillary

Schedule

After cleaning the capillary or when installing a new capillary.

Equipment List

- Clean powder-free nitrile gloves
- LC/MS-grade isopropanol

Steps

- 1 Put on clean, powder-free nitrile gloves.
- 2 Lubricate the capillary entrance end with LC/MS-grade isopropanol.
- 3 Carefully insert the capillary straight into the desolvation assembly. The capillary must be aligned correctly so that its end fits into a fixed capillary cap inside the desolvation assembly.

CAUTION

Putting vertical and horizontal pressure on the capillary can break it. Spray the capillary with isopropanol to lubricate the capillary for easier removal.

- 4 When 2 to 3 cm of the capillary remains extended from the desolvation assembly, the capillary rests against the rear contact spring, which slightly restricts further insertion of the capillary. Slightly increase the insertion force to push the capillary through the rear contact spring to fully insert the capillary.
- 5 Continue to apply pressure until approximately 1 cm remains extended from the desolvation assembly.

CAUTION

Do not twist or turn the capillary cap when you install it or you can damage the metal plating.

- 6 Install the contact ring and end plate.
- 7 Install the two T10 Torx screws to hold the end plate.
- 8 Lubricate the capillary tip with LC/MS-grade isopropanol.
- 9 Install the capillary cap over the outer end of the capillary.
- 10 Install the spray shield.

11 Close the spray chamber.

Removing the capillary

Schedule

When you need to clean or replace the capillary on a system.

Equipment List

- Clean powder-free nitrile gloves
- Capillary Puller tool

Steps

WARNING

The spray chamber operates at very high temperatures. Do not continue until the spray chamber is cool.

- 1 Put on clean powder-free nitrile gloves.
- 2 Remove the capillary cap from the end of the capillary.
- 3 Screw the capillary puller tool fully into the spray shield mount.
- 4 Push the collet assembly over the capillary and then tighten by holding the puller handle. Turn the collet clamping knob clockwise.
- 5 Pull on the knob until the collet assembly stops moving.
- 6 Visually check that the capillary has been pulled out by the collet by looking through the slots on the tool.
- 7 Rotate the knob assembly counterclockwise slightly, and pull out the assembly to unlock the collet assembly from the puller.
- 8 Pull the tool out.
- 9 Carefully pull the capillary out along its long axis.

CAUTION

The capillary is glass or of similar material, and you can break it by putting vertical or horizontal pressure on it.

- 10 Loosen the collet clamping knob slightly to loosen the capillary from the capillary puller tool.
- 11 Pull out the capillary from the collet.
- 12 Unscrew the tool from the shield mount.



<https://aglt.co/D0115670>

Cleaning the capillary

Schedule

When you observe decreased sensitivity and decreased signal stability.

Equipment List

- Clean powder-free nitrile gloves
- LC/MS-grade isopropanol
- Alconox powdered precision cleaner
- Deionized (18 MΩ/cm) water
- 100-mL polypropylene graduated cylinder
- 10-mL polypropylene graduated cylinder
- Two 1-mL pipette tips

Steps

- 1 Put on clean, powder-free nitrile gloves.

CAUTION

Ensure the source heated zones are cool before working on the instrument.

- 2 Dissolve 1 gram of Alconox Powdered Precision Cleaner in 100 mL of deionized (18 MΩ-cm) water. This concentration is the recommended concentration for both manual or ultrasonic cleaning.

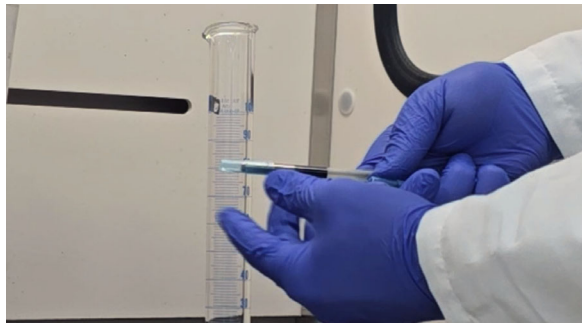
CAUTION

All cleaning and sonication should be done under a fume hood.

- 3 If you use a glass graduated cylinder, cover the end of the ion transport capillary with a 1-mL pipette tip to protect the metalized plating.

Maintenance

Cleaning the capillary



- 4 Trim the pipette tip to approximately 4 cm so that the capillary can be immersed in the cleaning solution.



- 5 Place the ion transport capillary upright in a graduated cylinder and fill with Alconox Powdered Precision Cleaner solution.
- 6 Sonicate the graduated cylinder that contains the ion transport capillary in an ultrasonic cleaner for 10 to 15 minutes. Do not clean longer than 15 minutes.

CAUTION

Sonication should be done under a fume hood.

Maintenance

Cleaning the capillary



- 7 Rinse the capillary and graduated cylinder several times with deionized water.
- 8 Fill the graduated cylinder with deionized water and sonicate for 10 to 15 minutes.
- 9 Remove the ion transport capillary from the graduated cylinder and remove the pipette tips (if any were used).
- 10 Blow out excess water from the ion transport capillary bore with nitrogen.

Cleaning a clogged capillary

This EMF item relies on the rough vacuum reading to determine a clog in the capillary/capillary. If the rough vacuum reading falls below a value of 1.4 Torr, the EMF item will be flagged.

Schedule

When the rough vacuum reading flags an error.

Equipment List

- Clean powder-free nitrile gloves
- LC/MS-grade isopropanol
- Capillary (new, if replacing)

Steps

- 1 Put on clean, powder-free nitrile gloves.
- 2 Remove and replace the capillary with a new unit, or re-insert the capillary after cleaning and drying.
- 3 Monitor the rough vacuum level to ensure that it does not fall below 1.4 Torr.

Flushing the Calibrant Delivery System (CDS)

Schedule

When there is suspected contamination of the CDS, low tune response, or possible blockage in the CDS, flushing the Calibrant system may be required.

WARNING

This procedure risks exposure to hazardous, toxic, or flammable solvents and reagents. Wear appropriate Personal Protective Equipment as described in the material handling and safety data sheet supplied by the chemical vendor, and always follow good laboratory practice.

Equipment List

- Clean, powder-free gloves
- Ultrapure LC/MS Grade Acetonitrile
- Ultrapure LC/MS Grade Water
- Tuning solution

Steps

- 1 Stop the LC/calibrant flow to the MS.
- 2 Disconnect the sample tubing from the top of the nebulizer and place into a clean beaker or waste bottle.
- 3 Remove the CDS bottle and discard any old tune solution.
- 4 Rinse the CDS bottle with LC/MS grade acetonitrile and then LC/MS grade water.

NOTE

In the case of a highly contaminated bottle, the bottle can be cleaned with Alconox and hot water (60 to 70 °C), then rinsed as per step 4.

- 5 Flush the CDS with 50/50 (v/v) LC/MS grade acetonitrile and LC/MS grade water:
 - a Fill the rinsed CDS bottle with approximately 20 to 30 mL of the acetonitrile/water mixture.
 - b Install the bottle and turn on the calibrant flow.
 - c Run the calibrant flush for at least 10 min.
- 6 Re-attach the sample tubing to the top of the nebulizer.

Maintenance

Flushing the Calibrant Delivery System (CDS)

- 7 Repeat step 5 with just LC/MS grade acetonitrile and flush for at least 10 min.

NOTE

To confirm cleaning procedures, monitor the MS spectrum and watch the contamination decrease over time.

- 8 Discard any remaining solvent and add fresh tune solution to the bottle.
- 9 Flush the fresh tune solution through the CDS for 5 min. Monitor tune ions to ensure that that response is adequate.



<https://aglt.co/D0115686>

Replacing the valve rotor seal

Schedule

After 10,000 diverter valve switches.

Equipment List

- 9/64-inch hex key
- MS selection valve rotor seal

Steps

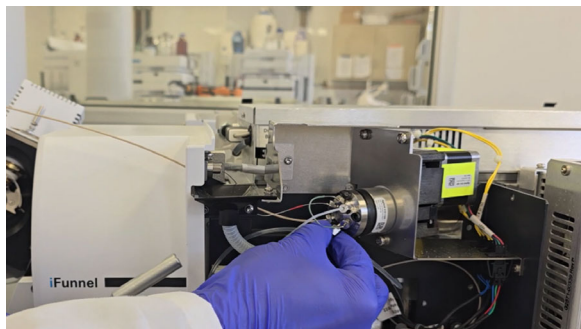
- 1 Stop the flow of LC solvent to the instrument via the acquisition software.
- 2 Disconnect the nebulizer sample line and sample nebulizer gas tubing.
- 3 Open the spray chamber.
- 4 Remove the front and top covers. Some instrument covers require disconnection of the LED ribbon cable located on the front cover.



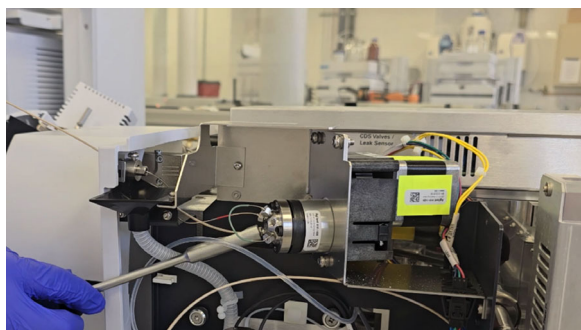
Maintenance

Replacing the valve rotor seal

- 5 Make a note of which port is plumbed to which tubing & unplug all the connections of MS selection valve.
- 6 Remove the LC PEEK (polyether ether ketone) tubing from the MS inlet, near the spray chamber.



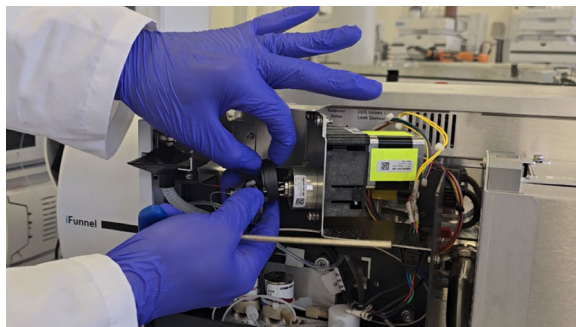
- 7 Unplug the connections of waste and nebulizer sample line.
- 8 Use the 9/64-inch hex key to remove the three hex head screws from the stator face of the selection valve.



- 9 Remove the rotor seal and replace it with a new one. Be sure to install it in the proper orientation.

Maintenance

Replacing the valve rotor seal



- 10** Use the 9/64-inch hex key to fix the three hex head screws from the stator face of the selection valve.
- 11** Plug in all the connections of the MS selection valve.
- 12** Install the upper left front cover or ion funnel cover.
- 13** Fix all the screws that hold the upper left front cover or ion funnel cover.
- 14** Plug in the connection of CDS liquid line.
- 15** Plug in the connections of waste line and PEEK tubing.
- 16** Attach the top and front covers.
- 17** Close the latch of the spray chamber.
- 18** Connect the sample nebulizer gas tubing and nebulizer sample line.

Cleaning the corona needle

Schedule

When you observe decreased sensitivity, decreased signal stability and increased corona voltage during APCI operation.

Equipment List

- Clean, powder-free nitrile gloves
- Clean lint-free cloth
- LC/MS-grade isopropanol
- 4000 grid abrasive paper

Steps

- 1 Before beginning any steps in this maintenance procedure, put on clean, powder-free nitrile gloves.

WARNING

The needle and related parts can be very hot. Do not touch the needle or related parts until they are cool.

- 2 Pull the corona needle assembly out of the spray chamber.

CAUTION

Do not bend or blunt the tip of the needle, it will decrease system performance. Sharpening the needle is not needed.

- 3 Fold a piece of 4000 grit abrasive paper over the base of the needle.
- 4 Pull and twist the 4000 grit abrasive paper along the needle and off the tip of the needle.

CAUTION

Do not hit the tip of the corona needle. The tip can bend, which can decrease system performance.

- 5 Starting at the base of the needle, wipe the needle with a clean lint-free cloth. The cloth can be dry or dampened with LC/MS-grade isopropanol.
- 6 Reinstall the corona needle assembly in the spray chamber.

Replacing the corona needle

Schedule

Perform this procedure when symptoms indicate poor corona needle performance and cleaning the needle does not restore performance.

Equipment List

- Clean, powder-free nitrile gloves
- Corona needle

Steps

- 1 Before beginning any steps in this maintenance procedure, put on clean, powder-free nitrile gloves.
- 2 Pull the corona needle assembly out of the spray chamber.
- 3 Remove the needle collar from the corona needle shaft.
- 4 Remove the old corona needle from the collar.
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- 5 Install a new corona needle, with its integral ferrule, in the collar.
- 6 Turn the collar onto the needle holder and tighten it by hand.

CAUTION

Do not hit the tip of the corona needle. The tip can bend, which can decrease the system.

- 7 Reinstall the corona needle assembly in the spray chamber.

Disinfection

Schedule

To prevent the spread of respiratory illness, disinfect your instrument as needed.

Equipment List

- Clean, powder-free nitrile gloves
- Clean lint-free cloth
- Gown
- Isopropanol
- Mask

Steps

- 1 Put on personal protective gear (gloves, mask, gown).
- 2 Make sure that your instrument is cooled to room temperature.
- 3 Dampen a clean lint-free cloth with the 70:30 isopropyl alcohol:water mix.
- 4 Gently wipe external surfaces to be cleaned using moistened cloth. Do not allow any liquid to drip into the instrument.
- 5 Wipe the outer surface of cables and their connectors but do not touch the electrical connections (for example pins and plugs inside the cable or connection port).
- 6 Use one cloth per instrument to prevent cross contamination.
- 7 Dispose of the cloth appropriately.
- 8 Allow all surfaces to completely air-dry. No moisture should be present on the instrument surfaces.
- 9 Discard the gloves and other personal protective equipment or clean them in an approved process, then wash your hands.

Source Maintenance

Changing to a source that uses a different tuning mix

Schedule

Every time you change a source that uses a different tuning mix.

Equipment List

None

Steps

- 1 Right-click the Q-TOF device pane and select **Calibrant**.
- 2 Run the calibrant for 5 minutes.
- 3 Perform a checktune. See **"Mass Calibration"** on page 63.
- 4 If the checktune results are not acceptable, run autotune on a supported tuning source. See **"Mass Calibration"** on page 63.
- 5 When the instrument is ready, load or create the method to use with the source.
- 6 Change the method settings for the new source.

Installing and removing sources

Schedule

Every time you change a source that uses a different tuning mix.

Before you begin:

- Do a complete autotune (see **"Mass Calibration"** on page 63). If an autotune is not available, do a checktune (see **"Mass Calibration"** on page 63).
- If the tune report does not show good results, tune the instrument. If needed, change the source to one that supports autotune.

- Put the system in Standby mode.

WARNING

In the tune view or context, turn down the gas temperature as low as possible. Leave the gas flow on for 30 minutes to cool the source.

Once complete, proceed to the desired source removal and installation instructions.

Electrospray Ionization (ESI) source

Removing

- 1 Disconnect the nebulizer sample line.
- 2 Disconnect the heating cable.
- 3 Disconnect the sheath gas line at the connector and unplug it.
- 4 Disconnect the AJS HV cable delivering AJS nozzle voltage.
- 5 Disconnect the Multimode cable delivering AJS nozzle voltage.
- 6 Open the latch of spray chamber.
- 7 Open the latch of spray chamber.

WARNING

The spray chamber operates at very high temperature. Do not continue until the spray chamber is cool.

- 1 Open the spray chamber.
- 2 Remove the ion source by lifting it along the axis of its hinges.

WARNING

The spray shield can be hot. Be careful not to burn yourself when you remove the spray shield.

- 3 If you are changing to a different source type, remove the spray shield

Installing

- 1 Install the spray shield, making sure the small hole is at the top of the "12 o'clock position". If needed, use a T10 Torx screwdriver to loosen the two screws in the end plate.
- 2 Rotate the ESI spray shield clockwise until the hole is in the correct position.
- 3 Gently tighten the Torx screws again.
- 4 Install the source.
- 5 Close the source.
- 6 Connect the MultiMode cable delivering AJS nozzle voltage.
- 7 Connect the heater cable.
- 8 Connect the sample nebulizer gas tubing.
- 9 Connect the nebulizer sample line.

10 Connect the reference nebulizer sample line and sample nebulizer gas tubing.

In the MassHunter Data Acquisition program:

- 1** Turn on the LC/MS instrument.
- 2** While you wait for the set points to equilibrate, prepare and install the calibrant for the new ion source.
 - a** Rinse a clean calibrant bottle with LC/MS-grade acetonitrile.
 - b** Pour the appropriate ESI Calibrant into the calibrant bottle for your instrument.
 - c** Install the calibrant bottle into the calibrant delivery system.

When all set points are equilibrated:

- 1** In the tune view or context, turn on the calibrant.
- 2** Purge the calibrant line for 5 minutes.
- 3** Do a complete autotune. If an autotune is not available, do a checktune. If the tune report does not show good results, tune the instrument. If needed, change the source to one that supports autotune.
- 4** If the checktune results are not acceptable, run autotune on a supported tuning source.
- 5** When the instrument is ready, load or create the method to use with the ion source.
- 6** Change the method settings for the new ion source.

Agilent Jet Stream (AJS ESI) source

Removing

- 1 Disconnect the nebulizer sample line and sample nebulizer gas tubing.
- 2 Disconnect the heating cable.
- 3 Disconnect the sheath gas line at the connector and unplug it.
- 4 Disconnect the AJS HV cable delivering AJS nozzle voltage.
- 5 Disconnect the Multimode cable delivering AJS nozzle voltage.
- 6 Open the latch of the spray chamber.
- 7 Open the latch of the spray chamber.

WARNING

The spray chamber operates at very high temperatures. Do not continue until the spray chamber is cool.

- 8 Open the spray chamber.
- 9 Remove the ion source by lifting it along the axis of its hinges.

WARNING

The spray shield can be hot. Be careful not to burn yourself when you remove the spray shield.

- 10 If you are changing to a different source type, remove the spray shield

Installing

- 1 Install the spray chamber.
- 2 Close the spray chamber.
- 3 Connect the MultiMode cable delivering AJS nozzle voltage.
- 4 Connect the heater cable.
- 5 Connect the sample nebulizer gas tubing.
- 6 Connect the nebulizer sample line.
- 7 Connect the reference nebulizer sample line and sample nebulizer gas tubing.

Maintenance

Agilent Jet Stream (AJS ESI) source

In the MassHunter Data Acquisition program

- 1 Turn on the LC/MS instrument.
- 2 While you wait for the set points to equilibrate, prepare and install calibrant for the new ion source.
 - a Rinse a clean calibrant bottle with LC/MS-grade acetonitrile.
 - b Pour the appropriate ESI Calibrant into the calibrant bottle for your instrument.
 - c Install the calibrant bottle into the calibrant delivery system.

When all set points are equilibrated

- 1 In the tune view or context, turn on the calibrant.
- 2 Purge the calibrant line for 5 minutes.
- 3 Do a complete autotune. If an autotune is not available, do a checktune. If the tune report does not show good results, tune the instrument. If needed, change the source to one that supports autotune.

Atmospheric Pressure Chemical Ionization (APCI) source

Removing

- 1 Disconnect the nebulizer sample line.
- 2 Disconnect the sample nebulizer gas tubing.
- 3 Disconnect the heater cable.
- 4 Disconnect the corona needle voltage cable.
- 5 For APPI, disconnect the heater cable.
- 6 Disconnect the serial output cable.
- 7 Disconnect the external power supply cable.
- 8 Open the latch of the spray chamber.

WARNING

The spray chamber operates at very high temperature. Do not continue until the spray chamber is cool.

- 9 Open the spray chamber.
- 10 Remove the ion source by lifting it along the axis of its hinges.

WARNING

The spray shield can be hot. Be careful not to burn yourself when you remove the spray shield.

- 11 If you are changing to a different source type, remove the spray shield.

Installing

- 1 Make sure the small hole is at the top ("12 o'clock position").
- 2 If needed, use a T10 Torx screwdriver to loosen the two screws in the end plate.
- 3 Rotate the ESI spray shield clockwise until the hole is in the correct position.
- 4 Gently tighten the Torx screws again.
- 5 Install the ion source, sliding it along the axis of its hinges.
- 6 Close the spray chamber.
- 7 Close the spray chamber latch.

Maintenance

Atmospheric Pressure Chemical Ionization (APCI) source

- 8 Connect the heater cable.
- 9 Connect the corona needle voltage cable.
- 10 Connect the sample nebulizer gas tubing.
- 11 Connect the nebulizer sample line.

In the MassHunter Data Acquisition program

- 1 Select the new source.
- 2 Turn on the LC/MS instrument. While you wait for the set points to equilibrate, prepare and install the calibrant for the new source.
- 3 Rinse a clean calibrant bottle with LC/MS-grade acetonitrile.
- 4 Pour the APCI Calibrant into the calibrant bottle.
- 5 Install the calibrant bottle into the calibrant delivery system.

When all set points are equilibrated

- 1 In the tune view or context, turn on the calibrant.
- 2 Purge the calibrant line for 5 minutes.
- 3 Do a complete autotune. If an autotune is not available, do a checktune. If the tune report does not show good results, tune the instrument. If needed, change the source to one that supports autotune.

5

Compliance

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Sustainability Information

Packaging End-of-Life

The Agilent 6545XT Q-TOF LC/MS system is packaged using cardboard, polyethylene foam, a polyethylene bag, and a wood pallet. In the US, cardboard is readily recyclable, and while plastic bags are recyclable, the infrastructure is not available in the majority of the market. In the US, Agilent is enrolled in the How2Recycle program (<https://how2recycle.info/>) to facilitate the communication of material recycling instructions.

In select countries, Agilent participates in the EU/UK Green DOT Package Recycling Program to support the take-back and responsible management of the packaging materials at their end-of-life. In the remaining EU countries, the cardboard is readily recyclable and the plastic bag, foam and wood pallets are recycled, landfilled, or incinerated depending on the country and its available infrastructure.

Product End-of-Life

Agilent offers a take-back program for its 6545XT LC/Q-TOF LC/MS system customers in US, EU, and UK markets. See <https://www.agilent.com/environment/product/index.shtml> for more information.

Agilent Regulatory Compliance Statement

CE Compliance



Your Agilent instrument has been designed to comply with the requirements of the applicable directives of the European Union, such as Electromagnetic Compatibility (EMC) Directive, Low Voltage Directive (LVD), Machinery Directive (MD), RoHS Directive, etc.

Agilent has confirmed that each product complies with the relevant Directives by testing samples against the harmonized EN (European Norm) standards published on the Official Journal of the European Union (OJEU).

Proof that a product complies with these directives is indicated by:

- the CE Marking appearing on the rear of the product, and
- the documentation package that accompanies the product containing a copy of the Declaration of Conformity. The Declaration of Conformity is the legal declaration by Agilent that the product complies with the relevant directives listed above, and shows the EN standards to which the product was tested to demonstrate compliance.

UK Compliance



Your Agilent instrument has been designed to comply with the requirements of the applicable regulations of the United Kingdom, such as The Electromagnetic Compatibility Regulations 2016, The Electrical Equipment (Safety) Regulations 2016, The Supply of Machinery (Safety) Regulations 2008, The Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment Regulations 2012, etc.


Agilent has confirmed that each product complies with the relevant Regulations by testing samples against the designated standards published on GOV.UK.

Proof that a product complies with these regulations is indicated by:

- the UKCA Marking appearing on the rear of the product, and
- the documentation package that accompanies the product containing a copy of the Declaration of Conformity. The Declaration of Conformity is the legal declaration by Agilent that the product complies with the relevant regulations listed above, and shows the designated standards to which the product was tested to demonstrate compliance.

Electromagnetic Compatibility

This product conforms to the following regulations on Electromagnetic Compatibility (EMC) and radio frequency interference (RFI):

- CISPR 11/EN 55011: Group 1, Class A
- EC/EN 61326-1
- AUS/NZ 
- Canada ICES-001 (This Industrial, Scientific and Medical (ISM) device complies with Canadian ICES-001. Cet appareil ISM est conforme à la norme NMB-001 du Canada).

Group 1 ISM equipment Group 1 contains all Industrial, Scientific and Medical (ISM) equipment in which there is intentionally generated and/or used conductively coupled radio- frequency energy which is necessary for the internal functioning of the equipment itself.

Class A equipment This equipment is suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This device complies with the requirements of CISPR11, Group 1, Class A as radiation professional equipment. Therefore, there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

- 1 Relocate the radio or antenna.
- 2 Move the device away from the radio or television.
- 3 Plug the device into a different electrical outlet, so that the device and the radio or television are on separate electrical circuits.
- 4 Make sure that all peripheral devices are also certified.
- 5 Make sure that appropriate cables are used to connect the device to peripheral equipment.
- 6 Consult your equipment dealer, Agilent Technologies, or an experienced technician for assistance.

Changes or modifications not expressly approved by Agilent Technologies could void the user's authority to operate the equipment.

EMC Declaration for South Korea

사용자안내문

This equipment has been evaluated for its suitability for use in a commercial environment. When used in a domestic environment, there is a risk of radio interference.

이 기기는 업무용 환경에서 사용할 목적으로 적합성평가를 받은 기기로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습니다 .

※ 사용자 안내문은 " 업무용 방송통신기자재 " 에만 적용한다 .

Detachable Power Cord Declaration for Japan

電源コードセットの取扱いについて（日本国内向け）

製品には、同梱された電源コードセットをお使いください。同梱された電源コードセット

は、他の製品では使用できません。

Notice - The power cords for Japanese market

Your product must only use the power cord that was shipped with this product. Do not use this power cord with any other product.

Sound Emission Certification for Federal Republic of Germany

Sound pressure

Sound pressure $L_p < 70$ dB(A) according to DIN EN ISO 7779.

Schalldruckpegel

Schalldruckpegel $L_P < 70$ dB(A) nach DIN EN ISO 7779.

Waste Electrical and Electronic Equipment (WEEE) Directive

This product complies with the European WEEE Directive marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.



NOTE

Do not dispose of in domestic household waste.

To return unwanted products, contact your local Agilent office or see <https://www.agilent.com/environment/product/index.shtml> for more information.

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