Agilent Q-ToF 6550 tune and run SOP v1.0

Start the Data Acquisition software
Note:
• The LC modules and the LC/MS instrument are turned on, but the LC pump is not running.
• To start the Data Acquisition program, double-click the Data Acquisition icon.
When Data Acquisition opens, the software engines automatically start. If you need to restart them, right-click the Acq System Launcher icon in the system tray and click Start Engines.
If you have recently changed LC modules, remember to configure the instrument again.

Prepare the LC modules
1 Switch LC Stream to Waste.
While you condition or equilibrate the column, you can tune the TOF or Q-TOF MS. During this time you do not want pump effluent going into the TOF or Q-TOF MS, so you switch the direction of the LC stream away from the MS ion source and to waste.
If you have the LC connected to the DAD, you can still monitor the fluctuations of the VWD or DAD real-time chromatogram before a run.
a Click the **General** tab in the **TOF** or **Q-TOF** tab in the Method Editor window.
b In the **LC Stream (Seg)** group box, click **Waste**.
c Click **Apply Now**. This button only sends to the current value of the LC eluent to the instrument. If you click **Apply**, then all of the method parameters are sent to the instrument.
2 Purge the LC pump.

3 Condition or equilibrate the column.
After you purge the pump, you set up to condition or equilibrate the column.
a Enter LC parameters, and click **Apply** to download them to the LC.
OR, to select an LC conditioning method, select one from the **Method** list at the top of the Data Acquisition window or from the Method Editor toolbar, or you select one from the list that appears when you click **Method > Open**.
b Right-click an LC module in the Instrument Status window to change any non-method control parameters, if necessary.
c Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable.
4 Set up to view real-time parameter values (actuals).
As you prepare for a run and during a run, you want to see the actual values of the instrument parameters. You can do this in the Instrument Status window.
a Right-click the Actuals window to see the Setup command.
b Click **Setup** to bring up the list of Actuals available for monitoring. If you

have configured a TOF instrument, the actuals for the TOF instrument are displayed instead.

c Add all the parameter values you intend to monitor. you can customize the color of the background and the text. You can also add minimum and maximum values to use; if the value is not within the given range, then the background of the value is set to red in the Actuals window.

d Click **OK**.

Set up real-time plot displays.

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

• Right-click the Chromatogram Plot window, and click **Change**.

In the Edit Signal Plot dialog box, you can select multiple display signals and change the display range.

Prepare the TOF and Q-TOF instrument

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

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

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

You can perform a **Check Tune**, **Quick Tune** and **Check Quad Tune** with all instrument
states and the following sources:

• ESI
• AJS ESI (Agilent Jet Stream ESI)
• Dual ESI
• Dual AJS ESI (Dual Agilent Jet Stream ESI)
• MMI
• APPI
• APCI

You cannot do any of the automated tunes if the source is a nanoESI, a Dual nanoESI, an HPLC-Chip or a MALDI source. We have the dual AJS ESI source connected most of the time.

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

Diluting the ESI-L Tune Mix

With the Dual AJS ESI source, autotune can fail unless you dilute the tuning mix.

When tuning in negative ion polarity, dilute the tuning mix to calibrate properly. When tuning in positive ion polarity, dilute the tuning mix if you are not getting proper calibration.

Note: Quick Calibration takes approximately 1.5 minutes rather than the longer calibration (approximately 15 minutes).

Tune the TOF and Q-TOF MS

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

The Tune window appears. Only the Instrument Status window, the Actuals window, and the Tune window are available in the Tune context. Note that you tune the TOF separately from the quadrupole.

Initial autotunes are appropriate for initial system installations, after removal/replacement of ion optics or mass analyzer components, or if standard tunes cannot complete successfully.

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

2 (optional) For the G6550A iFunnel Q-TOF, clear the **Always perform only Quick Calibration** check box. No additional dilution for negative mode is necessary if you clear this check box.

3 Click the **Autotune** tab.

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

• Positive
• Negative
• Fast Polarity Switching Positive
• Fast Polarity Switching Negative

5 Click **Check Tune** to check the TOF mass calibration and optimization. Then click **Start Autotune** (Check Tune takes 3 to 5 minutes to complete).

You repeat **Check Tune** after you dilute the calibration solution if the abundances are greater than 480,000 for any of the calibrating ions for the 6550 Q-TOF or above approximately 650,000 for other Q-TOF and TOF instruments.

If Check Tune results are acceptable, then you can skip to step 10

If Checktune results are not acceptable, then continue with step 6.

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

You repeat **Quick Tune** after you dilute the calibration solution if the abundances are greater than 480,000 for any of the calibrating ions for the 6550 Q-TOF or above approximately 650,000 for other Q-TOF and TOF instruments.

If Quick Tune results are acceptable, then you can skip to step 11
If Quick Tune results are not acceptable, then continue with step 7.
7 Run a **Standard Tune** to use even more parameters (10 to 15 minutes):
a Click the **Instrument State** tab.
b For the **Instrument Mode**, click **Extended Dynamic Range**.
c (optional) Mark or clear the **Fast Polarity Switching** check box.
d Click **Apply**.
e Click the **Autotune** tab.
f Mark the polarity (polarities) to use when tuning under **TOF** on the
Autotune tab.
g Click **Standard Tune**.
h Click **Start Autotune**.
I f results are acceptable, continue with step 8.
If **Standard Tune** produces unacceptable results you can do an **Initial Tune**. If
this also fails to give acceptable results, please contact Agilent Field Support.
If you want use custom tune parameter values, you can also do a Manual Tune.
8 Set the **Mass Range** and the **Instrument Mode** that you want to use to acquire
data.
a Click the **Instrument State** tab.
b Select the appropriate **Mass Range.**
c Click the appropriate **Instrument Mode**.
d Click **Apply**.
 Recalibrate, if necessary.
9 Calibration is done when you click the **Check Tune** button, the **Quick Tune**
button, the **Standard Tune** button and the **Initial Tune** button. If you just
completed one of these tasks, and if any of the following are true, you only need
to recalibrate:
• You change the **Instrument Mode** in the Instrument State tab.
• You change the **Mass Range** in the Instrument State tab.
• The peak abundances are above approximately 480,000 for the
6550 Q-TOF or above approximately 650,000 for other Q-TOF and
TOF instruments. You have to dilute the tune calibrant before you
recalibrate. You recalibrate to get optimal mass accuracy.
a Click the **TOF Mass Calibration** tab.
b Click the desired polarity in the top left corner of the Tune window.
c To select a different set of masses, click **Load** or select or clear individual
masses in the list on the left side of the Tune window.
d Click the **Calibrate** button.
e If you want to calibrate the TOF analyzer in the opposite polarity, repeat
step b through step d.
10 If you have a Q-TOF instrument, check the options on the Instrument State tab
before you tune the quadrupole.
If **Fast Polarity Switching** is **Enabled**, you disable it before you run any of the
Quad Autotune algorithms. After the Quad tune results are acceptable, you will
enable **Fast Polarity Switching** again and then recalibrate the TOF analyzer.
a Click the **Instrument State** tab.

b Select the appropriate **Mass Range.**
c Click the appropriate **Instrument Mode**.
d Select **Disabled** for **Fast Polarity Switching.**
e If you did not make any changes, skip to step 14.
f Click **Apply**.
g Click the **TOF Mass Calibration** tab.
h Click the desired polarity in the top left corner of the Tune window.
i To select a different set of masses, click **Load** or select or clear individual masses in the list on the left side of the Tune window.
j Click the **Calibrate** button.
k If you want to calibrate the TOF analyzer in the opposite polarity, repeat step h through step j.
l Click the **Autotune** tab.
11 Run the **Check Quad Tune** algorithm to check quadrupole optimization (2 to 5 minutes).
a Click the **Autotune** tab.
b Click the **Check Quad Tune** button.
If the Check Quad Tune results are acceptable, then skip to step 14.
If Checktune results are not acceptable, then continue with step 12.
12 Run the **Quad Tune** algorithm to optimize the quadrupole using all its parameters (10 to 15 minutes).
The source that is used must be supported for all Autotunes when running a **Quad Tune**. The Dual ESI source is supported for all Autotunes for all instruments. Refer to "Supported sources for all Autotunes" on page 19 for a complete list of sources that are supported for all Autotunes.
Also, you cannot run a Quad Tune if Fast Polarity Switching is **Enabled**.
a Click the **Instrument State** tab.
b For the **Instrument Mode**, click **Extended Dynamic Range**.
c Click **Apply**.
d Click the **Autotune** tab.
e Clear the **Fast Polarity Switching** check box.
f Click **Quad Tune**.
If **Quad Tune** produces unacceptable results you can do an **Initial Quad Tune** (50 to 60 minutes). If this also fails to give acceptable results, please contact Terence.
If you would like to use custom tune parameter values, you can also do a Manual Tune.
13 Set the **Mass Range** and the **Instrument Mode** that you want to use to acquire data.
a Click the **Instrument State** tab.
b Select the appropriate **Mass Range.**
c Click the appropriate **Instrument Mode**.
d Click **Apply**.
14 If you want to use Fast Polarity Switching, enable **Fast Polarity Switching** on the Instrument State tab and recalibrate
If you have a Q-TOF instrument, **Fast Polarity Switching** was disabled before

tuning the quadrupole. Follow these steps to enable Fast Polarity Switching:

a Click the **Instrument State** tab.

b Select **Enabled** for the **Fast Polarity Switching.**

c Click the appropriate **Instrument Mode**.

d Click **Apply**.

e Wait 20 minutes for the instrument to equilibrate.

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

g Click the desired polarity in the top left corner of the Tune window.

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

i Click the **Calibrate** button.

j If you want to calibrate the TOF analyzer in the opposite polarity, repeat step g through step i.

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

Calibrate the mass-axis

During calibration, a sample that contains known masses is infused into the source, and the actual flight times for ions of known masses are measured. These times and exact masses are used to calculate updated calibration coefficients. This process ensures accurate mass assignments for unknowns. Agilent recommends that you do this regularly.

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

• You change the **Instrument Mode** in the Instrument State tab.

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

• The peak abundances are above approximately 480,000 for the 6550 Q-TOF. You have to dilute the tune calibrant before you recalibrate. You recalibrate to get optimal mass accuracy. Typical tune mass abundances are in the range of 50,000 to 650,000 counts.

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

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

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

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

5 Click **Calibrate**.

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

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

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

Switch LC stream to MS

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

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

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

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

d Click **Apply**.

Monitor MS baseline and spectral displays

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

Prepare the TOF and Q-TOF instrument

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

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

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

View the system logbook for events and errors

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

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

• Or right-click the icon in the system taskbar. First, click **Enable Notification.** Then, right-click the LOG icon and click **Configure**. The system can notify you of new errors and warning by showing messages from the taskbar.

Set up and run a method

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

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

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

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

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

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

Set up a method with acquisition parameters

• (optional) If you want to download the settings to the instrument, click **Apply**.

• To save the method after entering parameters, click either **Method > Save** or **Method > Save As**.

• Type the name for the method in the Method field, and click the **OK** button.

Set up and run a method
1 In the Context list, click **Acquisition**.
2 Enter LC parameter values.
Type the values for all of the LC modules configured for the instrument.
3 Set up to change TOF and Q-TOF MS parameters with segments and
experiments:
a Click the **TOF** or **Q-TOF** tab in the Method Editor.
b To add a segment, right-click the **Time Segment** section and click **Add Time
Segment**. The time segment uses those parameter entries with (seg) next to
their names. Those parameters can be changed for each time segment.
c To add an experiment, right-click the **Experiment #** section, and click **Add
Experiment**. The experiment will use those parameter entries with (Expt)
next to their names. Those parameters can be changed for each experiment.
d Enter the parameters for each segment and experiment. When you add a
new time segment, the parameters for the time segment that is selected are
used as the default values for the new time segment. When you add a new
experiment, the parameters for the last experiment in the list are used as
the default values for the new experiment.
See Chapter 3 of the ***Concepts Guide*** for an explanation of how and why you
use time segments and experiments.
4 Enter TOF or Q-TOF MS parameter values:
a Click the **General** tab, and enter any General parameters that you want to
change.
b Click the **Source** tab, and enter any Source values you want to change.
c Click the **Acquisition** tab.
d Select the mode of operation for the Q-TOF LC/MS: **MS mode**, **Auto MS/MS**
mode or **Targeted MS/MS** mode. If you have a TOF configured, you can
only use **MS mode**.
Different parameters are made available depending on the mode selected.
e Enter any values you want to change in the Acquisition tab.
f Click the **Ref Mass** tab to set up the mass calibration.
g Click the **Chromatogram** tab to set up the chromatograms to plot during a
run.
5 Set up the data analysis parameters. In the DA tab, you can specify the
Qualitative Analysis parameters and Quantitative Analysis parameters. To set
up the Qualitative Analysis parameters, do the following:
a Click the **DA** tab in the Method Editor.
b In the Qual tab, mark the **Qual Automation** check box.
c Click the **Link** or **Copy** button, depending on whether you want to always
use the most recent version of the Qualitative Analysis method or copy the
current Qualitative Analysis method and save it to the Data Acquisition
method for future use. If you click **Copy**, the method entered in the **Change
to Method** box is copied to the Data Acquisition method when the Data
Acquisition method is saved.
d Click the button to select the Qualitative Analysis method.
To set up the Quantitative Analysis parameters, do the following:

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

a Click the **Quant** tab.

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

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

d Click the button to select the Quantitative Analysis method.

Set up and run interactive samples

1 Click the **Sample Run** window.

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

3 Enter the Additional Information. You can change the value of the parameters in the Additional Information list.

It is now possible to run a Data Analysis method from this window, by selecting **Both Acquisition and DA** or **DA Only** for the **Method Type**. In addition, you have to set **Override DA method** to indicate the DA (Data Analysis) method to execute.

A method can contain data acquisition parameters, qualitative analysis parameters or both. A Data Analysis method is a method that contains data acquisition parameters with either the **Qual Automation** check box marked on the Qual tab or the **Quant Automation** check box marked on the Quant tab.

4 To start a single sample run, click the Run button, , in the Sample Run toolbar or the Run button, , in the main toolbar.

You can run the single sample in either locked or unlocked mode. When the mode is locked, no one can change the method or sample parameters during a run. You also cannot overwrite this data file in the Data Acquisition program. The button, , in the main toolbar indicates that locked mode is on.

You can also specify an Override DA Method and select either **Both Acquisition and DA** or **DA Only** for the **Method Type**, and then Data Analysis is run as part of the method.

Set up and run worklists

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

2 Click **Add Multiple Samples.**

3 Enter all relevant information, and click the **Sample Position** tab to specify the sample vial locations (make sure the specific sample tray type has been configured by right-clicking the autosampler device image).

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

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

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

7 To start the run, click the **Start Worklist Run** icon, , in the main toolbar or click the **Start Worklist run** icon, , in the Worklist toolbar.

You can run the worklist in either locked or unlocked mode. When the mode is locked, no one can change the method or the worklist while the worklist is running. The button, in the main toolbar indicates that locked mode is on.
NOTE: To use an acquisition method that has a different DA method than the method entered in
the worklist, show the column called **Override DA Method** in the worklist by using the
Show/Hide/Order Columns dialog box.
In this column, type the name of another method containing the DA parameters you want to
use for the sample. The DA part of this method is used instead of the DA part of the current
method.
You can also type the name of this method in the Add Multiple Samples dialog box.

Review results and find compounds with Qualitative Analysis
• Use the Qualitative Analysis program to:
• Review results for acquisition method development
• Find compounds
• Identify compounds
• Do molecular feature extraction
• Export results
• Print reports