Agilent

Agilent 6500 Series Q-TOF and IM-QTOF LC/MS 6530, 6545, 6546, 6545XT, 6550, and 6560

Setup and Verification Guide

This guide contains the steps needed to complete the system setup and verify the performance specifications of the installed system.

Before you can use your system, you need to complete the installation and verify the system.

For 6545XT, 6546, and 6560, you can do a System Tune on the Dual AJS source only. For all other Q-TOF and IM-QTOF LC/MS, you can verify the system with the Dual ESI source, the Dual AJS source, or both.

CAUTION

The installation cannot be completed unless the high voltage electronics have been conditioned. Refer to the appropriate instrument *Installation Guide*.

Installation Completion

The steps in this section assumes that the instrument and all MassHunter Workstation software are properly installed.

Step 1. Install user documentation

- Install any user information apps that are included with the system, such as the Agilent Information Center (for LC) or Resource Apps (for LC/MS and MassHunter software).

  The **Resource Apps** and Agilent Information Center gives you access to all MassHunter user guides, including eFamiliarization guides and the animated instrument Maintenance Guide.

NOTE

The training material on these user documentation apps can greatly facilitate user learning of MassHunter programs and instrument maintenance. Make sure to install all that are included with the system.

Step 2. Update the LC firmware

- Follow the instruction that are provided with the LC instrument to update the LC firmware.

Step 3. Update the MS firmware

1. Insert the Firmware installation media into the media drive.
2. Click **Update MS Firmware** in the welcome page.
3. Follow the instructions to update the firmware.

Step 4. Condition the Agilent LC instrument

Parts Needed

- LCMS-grade deionized water, 18 MΩ (p/n 5190-6897, included)
- HPLC-grade isopropanol
- HPLC Flushing Solvent (p/n G1969-85026)
- LCMS-grade acetonitrile (p/n G2453-85050)
- LCMS-grade methanol (p/n 5190-6896, included)
- high-purity formic acid (p/n G2453-85060)
- Zorbax Extend C18 column (p/n 727700-902)

1 Flush the HPLC stack.

   Flush each solvent channel to remove contaminants from separate degasser and pumping channels. The flushing procedure effectively reduces background contaminants from the LC.

2 Remove any column(s), install a restriction capillary, and bypass the flow cell for the UV detector, unless it is rated for high-pressure (400 bar) operation. Route the outlet capillary to a waste container.

3 If required by your degasser model, connect A1/A2 degasser channels in series and B1/B2 degasser channels in series. Use the c-tubes included with the degasser.

   Do not re-use solvents used for the flushing procedure.

4 Use a hand-held controller or the Data Acquisition program to set up the pump module to deliver the solvents for the amount of time and rate listed in **Table 1**, followed by 5 injections of the same solvent to clean the injector path and sample loop.

CAUTION

Make sure that the HPLC Flushing Solvent does not stay in the LC for an extended period of time (for example, several days) because the cyclohexane in the HPLC Flushing Solvent can be harmful to the pump seals.

Table 1 Flushing solvents

Solvent	For	At	Then do 5 injections of
LCMS-grade deionized water	15 minutes	3 mL/minute	LCMS-grade deionized water
HPLC-grade isopropanol	5 minutes	3 mL/minute	HPLC-grade isopropanol
HPLC Flushing Solvent	15 minutes*	3 mL/minute	HPLC Flushing Solvent
HPLC-grade isopropanol	5 minutes	3 mL/minute	HPLC-grade isopropanol

* Alternatively, while the Q-TOF LC/MS pumps down, the flushing solvent can be pumped at a low flow rate (e.g. 0.1 mL/minute) overnight.

5 If applicable, remove the c-tubes from the degasser channels plumbed in series and reconnect the degasser tubing to the original configuration.

6 Install the **Zorbax Extend C18 column** after the autosampler. Wet the column with 100% **LCMS-grade methanol**.

All columns shipped with the Q-TOF LC/MS are shipped dry. Upon installation, these columns must be primed or wetted with Methanol to properly condition the column.

7 Set up the pump module again to deliver **LCMS-grade methanol** for at least **30 minutes** at **0.5 mL/minute**. Make sure that the solvent stream selector valve on the Q-TOF LC/MS is set to waste position or the outlet tubing from the column is *not* connected to the instrument.

If the HPLC stack has a Thermostatted Column Compartment (TCC), set the temperature of the column compartment to 60°C to reduce the back pressure and to help remove any contaminants on the column.

8 Mix the solvents in **Table 2** to create the checkout mobile phase.

Table 2 Checkout mobile phase

Solvent	Percentage
LCMS-grade deionized water	70%
LCMS-grade methanol	30%

9 For 6546 negative mode checkout:
   a For Channel A, install 1 liter of 100% **LCMS-grade deionized water**.
   b For Channel B, install 1 liter of 100% **LCMS-grade methanol**.
   c Make sure flow rate is set to **0.5 mL/minute** with **Checkout mobile phase** to condition column.
   d Do 5 injections with the **Checkout mobile phase** to clean the injector path and sample loop.
   e Continue at **"Step 1. Prepare the performance evaluation samples"** on page 20 to prepare the Negative Ion Mode checkout sample.

10 For 6546 positive mode checkout after negative mode checkout is completed, and for 6545, 6550, and 6560:
   a For Channel A, install 1 liter of **LCMS-grade deionized water** with 0.1% **high-purity formic acid**.
   b For Channel B, install 1 liter of 100% **LCMS-grade acetonitrile**.
   c Make sure the flow rate is set to 0.4 mL/minute.
   d Do 5 injections with the **Checkout mobile phase** to clean the injector path and sample loop.

11 For 6530, 6545XT, and 6560::
   a Install 1 liter of 70:30 **LCMS-grade acetonitrile**:**LCMS-grade deionized water** with 0.1% **high-purity formic acid**.
   b Make sure the flow rate is set to 0.4 mL/minute.
   c Do 5 injections with the **Checkout mobile phase** to clean the injector path and sample loop.

12 For 6545, 6550, and 6560:
   a For Channel A, install 1 liter of **LCMS-grade deionized water** with 0.1% **high-purity formic acid**.
   b For Channel B, install 1 liter of 100% **LCMS-grade acetonitrile**.
   c Make sure the flow rate is set to 0.4 mL/minute.
   d Do 5 injections with the **Checkout mobile phase** to clean the injector path and sample loop.

NOTE

Up to 0.1 percent **high-purity formic acid** can be added to the solvent for Channel A that you just prepared to help ionize the reserpine checkout sample. Do not add the **high-purity formic acid** until after you prepare the Reserpine checkout sample.

NOTE

Use solvents that are at a minimum HPLC grade. Solvents that are acceptable for most LC applications may contain high levels of background that are detectable by the more sensitive LC/MS instrument. LC solvents used with the LC/MS instrument should be rated for both HPLC and pesticide, environmental, or GC/MS analyses. Use the highest purity solvents you can obtain. Acceptability of solvents must be empirically determined.

13 For 6546, autotune the LC/MS in both Positive and Negative Ion Polarities after the system has reached equilibration (approximately 11 hours after initial pumpdown) and the Quadrupole Frequencies have been adjusted.

Step 5. Prepare the tuning mix

Dilute the tuning mix if you are tuning the TOF with the Dual AJS source in static polarity. Do not dilute the tuning mix for Quad tuning and for tuning in Fast Polarity Switching mode.

If no dilution is needed

- If no dilution is needed, fill calibrant bottle "B" with undiluted ESI-L Low Concentration Tuning Mix (G1969-85000) and install.

If dilution is needed

Parts needed

Part	Part number
ESI-L Low Concentration Tuning Mix	G1969-85000
Biopolymer Reference Mass Kit	G1969-85003
LCMS-grade acetonitrile	G2453-85050
18-Ω, HPLC grade deionized water	8500-2236
glass CDS bottle	9300-2576
glass CDS bottle cap	9300-2575

The materials shipped with the instrument are of sufficient purity for this purpose.

These instructions will make 100 mL of diluted tuning mix.

1 Add the components in **Table 1**, *in the order listed*, to a clean CDS bottle.

Make sure you add the components in the order listed below to avoid precipitation of any components of the tuning mix.

Table 1 Dual AJS

Component	Volume
ESI-L Low Concentration Tuning Mix	10 mL
LCMS-grade acetonitrile	85.5 mL
18-Ω, HPLC grade deionized water	4.5 mL
0.1 mM HP-0321 (included in the Biopolymer Reference Mass Kit)	3 µL

2 Mix the contents thoroughly and install the bottle on the instrument.

3 Fill bottle “B” in the Calibrant Delivery System with the diluted tuning mix.

Store the custom tuning mix in the refrigerator when it is not being used for extended periods of time.

Step 6. Prepare the reference mass solution

The reference solution provides internal reference masses for reference mass correction in positive and negative ion modes of operation.

1 Prepare the reference mass solution according to “Prepare the ES-TOF reference mass solution” in the online Help. For AJS source, make sure to follow the dilution instructions under “Diluting the ES-TOF Reference Mass Solution for the Agilent Jet Stream Technology” towards the bottom of the Help topic.

2 Fill calibrant bottle “A” with prepared reference mass solution and install.

Use these guidelines to adjust the amounts of IRM compounds to add to this preparation. Refer to the online Help for detailed description for the AJS source.

- IRM abundances less than 1,000 counts generally do not yield acceptable ion statistics for good correction. Ideally, abundances will be at the level of 50,000 (for 6530B) or 150,000 (for 6550) counts or greater at any point in the analysis where correction is desired. Abundances for all instruments are measured at 1 Hz acquisition rate, and expectation of abundances should be reduced in proportion to the rate of acquisition (half for 2 Hz, one-third for 3 Hz and so forth).
- The abundances of the reference mass compounds change during an HPLC gradient, with lesser abundances occurring at higher organic compositions. This is especially true when using acetonitrile as the organic component. Make sure the abundances are high enough during the entire gradient.
- Interference at one of the reference masses can cause problem with mass accuracy, most likely the reference mass 121 *m/z* at the low end of the scan range, where the background response is the highest. This causes an error in determining that reference mass value, leading to an error in assignment of other mass peaks in that scan. Sometimes, the problem can be lessened by increasing the amount of that reference mass component. Sometimes a different compound can be chosen to serve as the reference mass compound (e.g., a phthalate response at 391.284286 m/z). More often, the problem is remedied by altering the sample cleanup procedures to remove the interfering component(s).
- The values chosen for the reference mass correction depend on the adducts present during the analysis. Refer to the instruction sheet included with the internal reference mass kit for the accurate m/z values of the most common adducts.

- For instruments with Dual AJS only: Higher sheath gas temperatures and sheath gas flows will increase the response of the 922 m/z reference mass compound.

The internal reference mass solution allows you to get accurate mass time-of-flight data. A minimum reference mass signal abundance of several thousand counts and maximum abundance of several hundred thousand counts will provide accurate reference mass corrections. If LC mobile phase modifiers are present (e.g. Na+, K+, acetate, formate), competition may cause multiple molecular species to attenuate the reference mass response. The actual concentrations of the mass reference compounds in the solution you prepare will depend upon several instrument operating parameters:

- LC gradient or isocratic operation
- LC flow rate, mobile phases and modifiers
- MS source settings including fragmentor and octopole RF voltages

The data acquisition mass range should be set wide enough to include all of the reference masses. For small molecule analysis, this range is typically m/z 50 to 1000 for positive mode and m/z 50 to 1100 for negative mode. Note that m/z 1034 is the TFA adduct of HP-0921.

Table 2 ES-TOF Reference Masses (shaded cells indicate principal ions)

Species	Positive Ion (m/z)	Negative Ion (m/z)	Formula Wt.	Molecular Formula
CF3 (TFA fragment)		68.995758		C F3
TFA anion		112.985587	131.06	C2 O2 F3 (N H4)
Purine	121.050873	119.036320	120.11	C5 H4 N4
HP-0921	922.009798	1033.988109	921.24	C18 H18 O6 N3 P3 F24
HP-0921 (+ formate)		966.000725		
HP-0921 (+ acetate)		980.016375		

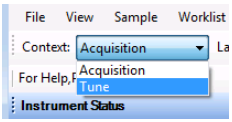
Step 7. Prepare to do a System Tune

For the 6545XT and 6560, you can do a System Tune on the Dual AJS source only.

1 Install the Dual ESI source or the Dual AJS source.

Signal-to-noise qualification specifications and mass accuracy qualification specifications are for the Dual ESI or the Dual AJS source only.

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



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

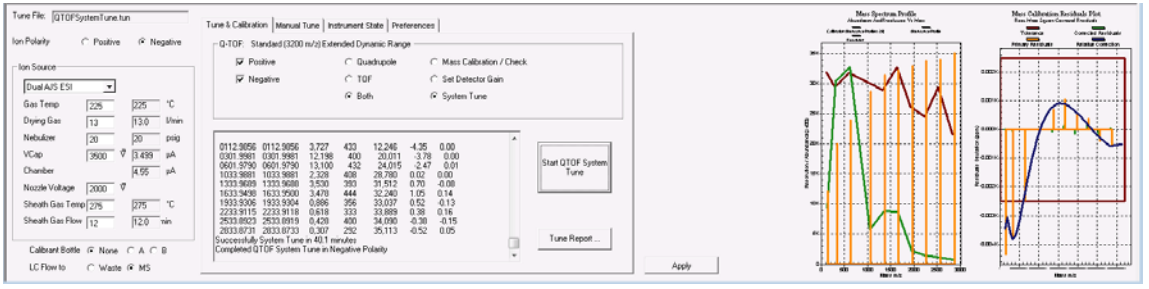


Figure 1. Tune & Calibration tab

3 Right-click the **Q-TOF** icon in the **Instrument Status** window and turn on the LC/MS instrument.

For 6560 IM-QTOF only

The remaining steps are for the 6560 IM-QTOF only. For all other Q-TOF LC/MS, continue at **"Step 8. Do a System Tune"** on page 14.

4 Adjust vacuum levels:

   a Change the **Context** to **Tune**.

   b Click **Manual Tune > IM > Pressure & Actuals**.

   c Make sure that the source temperature is stable at the temperature indicated in the method.

   d Locate the pressure valves on the front of the instrument, next to the ion source.

5 For 6560 *without* G2582A Drift Gas Kit:

   a Turn the **High Pressure Funnel** flow regulator until the pressure in the HPF chamber is approximately **4.8 Torr**.

   b Turn the **Drift Tube** regulator valve until the **Drift Tube Pressure** shows **3.95 ± 0.03 Torr**.

   c Adjust the **High Pressure Funnel** flow regulator until the **Drift Tube Pressure** is *higher* than the **Trap Funnel Pressure** by **150 mTorr**.

6 For 6560 with G2582A Drift Gas Kit:

   a Turn the **High Pressure Funnel** flow regulator until the pressure in the HPF chamber is approximately **4.3 Torr**.

   b Adjust the **Drift Tube** regulator valve until the **Drift Tube Pressure** shows **3.95 ± 0.03 Torr**.

   c Continue to adjust both valves until the **Drift Tube Pressure** is *higher* than **Trap Funnel Pressure** by **0.15 Torr** while the **Trap Funnel Pressure** remains close to **3.95 Torr**.

7 Load the Factory Autotune file:

   - Insert the factory checkout reports disc in to the disc drive.
   - In the Instrument State tab, under **Tune File**, click **Load**.
   - From the factory checkout reports disc, select **FactoryAutotune.tun**.
   - Click **Open**.

Step 8. Do a System Tune

The Q-TOF LC/MS use the SWARM auto tune. If the System Tune option is not available in the **Tune & Calibration** tab, change the option in the **Preference** tab.

The System Tune is the SWARM Initial Auto Tune which starts with all default values and includes the detector tune. You can perform a System Tune when you select **Standard (3200 m/z)** as the **Mass Range**.

If needed, you can do an Initial Fast Polarity Switching (FPS) tune after System Tune. You can do an FPS Initial Tune only after you run System Tune in both polarities. If you do not see the FPS Initial Tune option in the Tune & Calibration tab, then in the **Instrument State** tab, set **Fast Polarity Switching** to **Enabled**.

NOTE

In the next step, a state-change warning message appears each time you make a change to the Instrument State tab. Click **OK** each time.

1 In the **Instrument State** tab,

   a Set **Mass Range** to **Standard (3200 m/z)**.

   For 6560 IM-QTOF, continue at **step 2**.

   b Set **Fast Polarity Switching** to **Disabled**.

   c Set **Slicer Mode** to **High Resolution**.

   d **For all Q-TOF except 6546**: Select **Extended Dynamic Range (2 GHz)**.

   e Click **Apply**.

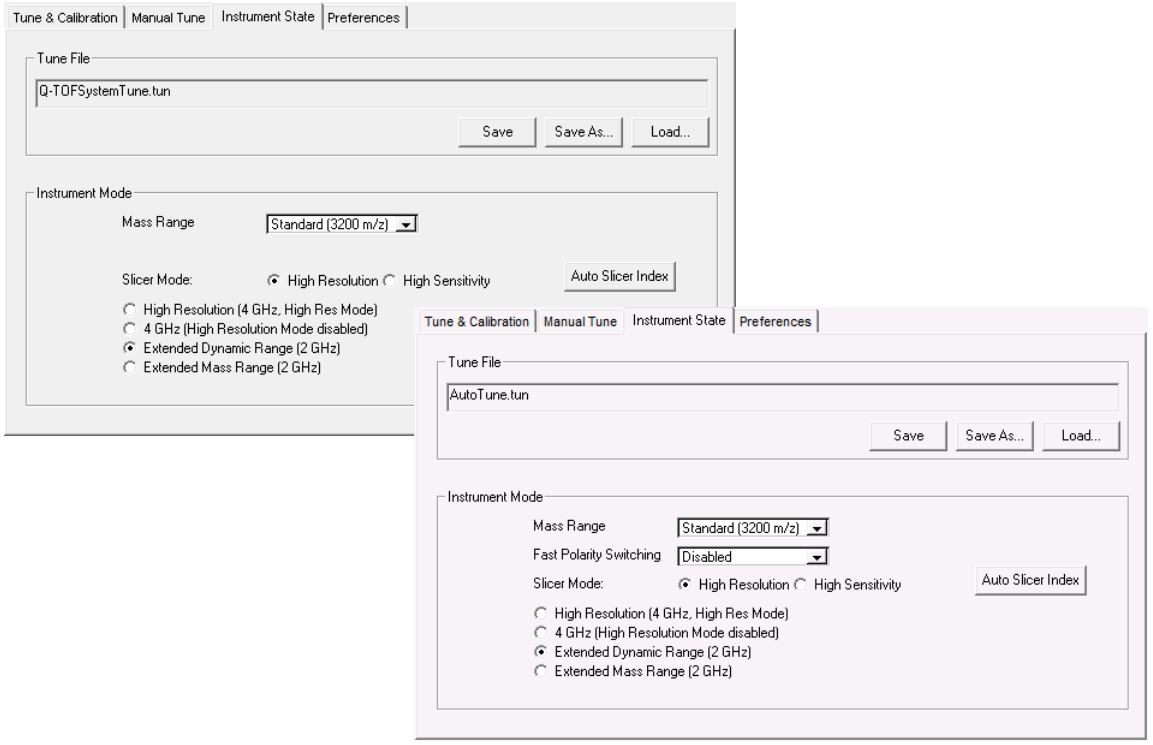


Figure 2. Instrument State tab for 6545XT (left) and all other Q-TOF and IM-QTOF LC/MS (right). (For 6546, the Mode is automatically set and is not visible.)

CAUTION

You must let the system equilibrate fully before you continue with the instrument verification. Otherwise, the signal-to-noise and mass accuracy tests will fail.

2 Run a System Tune:

- **a** Click the **Tune & Calibration** tab.
- **b** Install the 1:10 diluted tune mix.
- **c** Mark both the **Positive** and **Negative** check box.
- **d** Click **Both** to tune both the TOF and Quadrupole in one step.
- **e** Click **System Tune.**
- **f** Click **Start QTOF System Tune.**

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

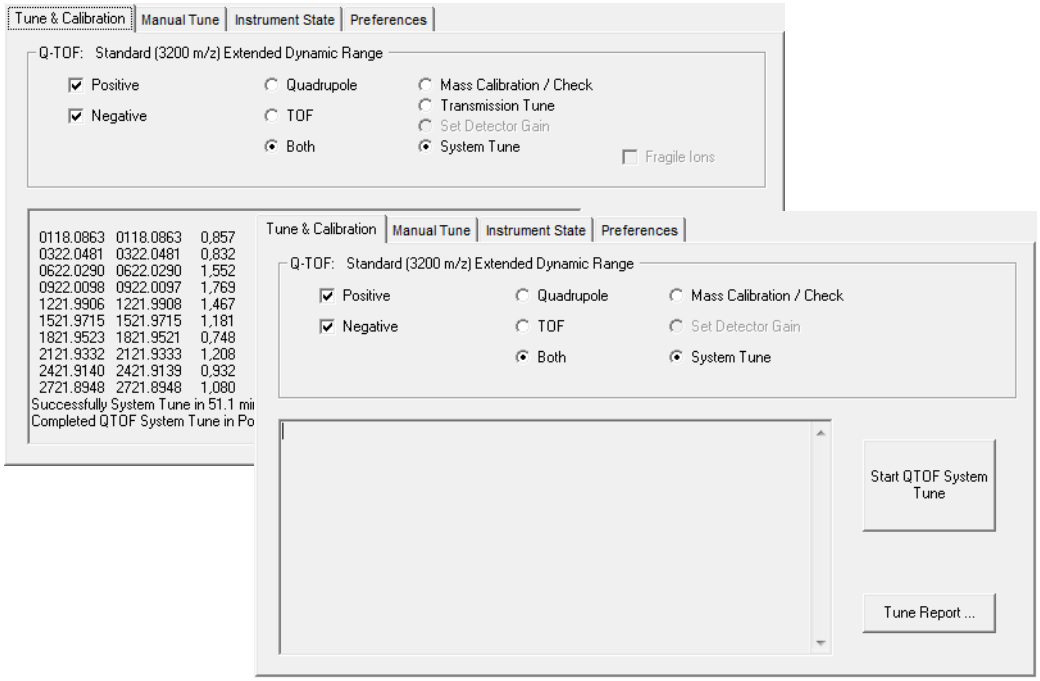


Figure 3. Tune & Calibration tab for 6545XT (left) and all other Q-TOF and IM-QTOF LC/MS (right).

Step 9. Set up the instrument for verification

Do this step to set up the instrument to verify the signal-to-noise ratio and mass accuracy performance.

1 Install the Dual AJS or Dual ESI source.

For the 6545XT and 6560, signal-to-noise qualification specifications and mass accuracy qualification specifications can be done only on a Dual AJS source.

2 Install the tuning mix.

For the Dual ESI source, use undiluted ESL-L tuning mix. For the Dual AJS source, install the 1:10 diluted tuning mix. See **"Step 17. Dilute the tuning mix (for Dual AJS only)"** on page 71.

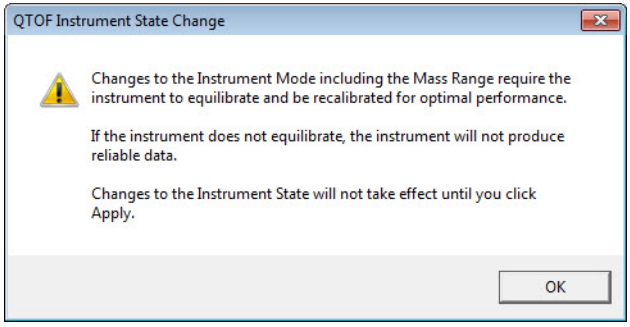
3 For **Calibrant Bottle**, click **B** to turn on the calibrant B bottle.

4 In the **Instrument State** tab:

a For all Q-TOF *except* 6560 IM-QTOF: Set **Fast Polarity Switching** to **Disabled**.

b For all Q-TOF *except* 6546: Select **High Resolution (4 GHz, High Res Mode)**. (For 6546, the Mode is set automatically and is not visible.)

If a message box appears to warn you that a change in instrument mode will require a recalibration of the TOF mass axis, click **OK**.



CAUTION **If you are warned to wait for the system to equilibrate fully, make sure you do so before you continue with the instrument verification.**

5 Click **Apply**.

Always click **Apply** after you make any changes to the **Instrument State** tab. Otherwise, you may not get the results that you expect.

6 In the **Tune & Calibration** tab:

a Mark only the **Positive** check box.

b Click **TOF**.

c Click **Mass Calibration / Check**.

d Click **Start TOF Mass Calibration**. A TOF Tune report is generated.

7 Compare the Tune report values to the values in **Table 3** to qualify the instrument for mass accuracy and mass resolution based on the tune compounds.

Table 3 Expected Tune Values

Model	Mass Resolution	Mass Accuracy
6530	> 10,000 on the 118 *m/z and* > 20,000 on the 1522 *m/z*	< 2 ppm on all masses in high resolution mode
6545/6550*	> 43,000 on the 2722 *m/z* tune ion	< 1 ppm on all masses in high resolution mode
6545XT	> 48,000 on the 2722 *m/z* tune ion	< 1 ppm on all masses in high resolution mode
6546	> 30,000 on the 118 *m/z* tune ion > 60,000 on the 2722 *m/z* tune ion	< 1 ppm on all masses in standard resolution mode
6550†	> 40,000 on the 2722 *m/z* tune ion	< 1 ppm on all masses in high resolution mode
6560	> 40,000 on the 2122 *m/z* tune ion	< 1 ppm on all masses in high resolution mode

* 6550 with serial number SGxxxx**B1**xx or higher.

† 6550 with serial number SGxxxx**B0**xx.

8 Click the **Instrument State** tab, and then:

a Change the **Mass Range** to **Low (1,700)**.

b Click **Apply**.

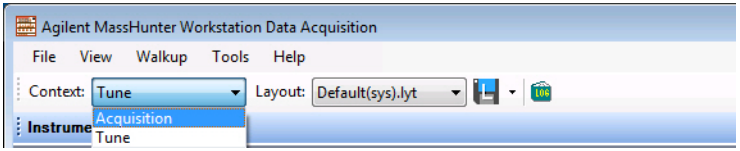
c Let the system equilibrate for 20 minutes.

d For 6545, 6545XT, and 6546: Click **50-1700 m/z**.

e Run **Mass Calibration / Check** again to recalibrate the system and generate a tune report.

The instrument is now tuned and calibrated for acquisition in Standard Mode (6546) or High Resolution mode (all other Q-TOF). You are now ready to check sensitivity and mass accuracy performance.

9 In the **Context** list, select **Acquisition**.

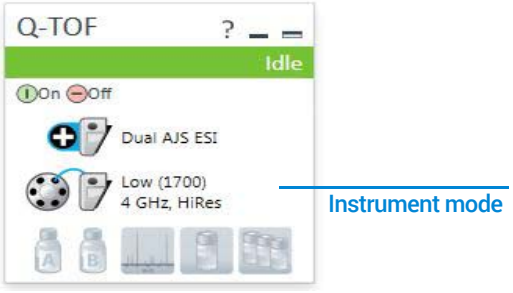


10 If you are asked whether you want to continue in the selected instrument mode, and your instrument is *not* a 6546, verify that the mode is **High Resolution (4 GHz, High Res Mode)**. Click **Yes**.

11 If you are asked whether to save the tune file, click **Yes**.

12 When you are told that the Mass Range and acquisition rate are changed, click **OK**.

13 To verify that the correct instrument mode is set, look at the Q-TOF device panel. Note that the source type displayed depends on the source that is used.



Checkout

Step 1. Prepare the performance evaluation samples

Parts needed (all Q-TOF LC/MS)

Component	Agilent part number
1-mL graduated pipette	9301-1423
50-mL volumetric flask	9301-1424
100-mL volumetric flask	9301-1344
30-mL polypropylene bottle	9301-1347
18-Ω, HPLC grade deionized water	8500-2236
LCMS-grade methanol	5190-6896
LCMS-grade acetonitrile	G2453-85050
reserpine (ES/APCI Positive Ion Performance Standard)	G2423A
reagent-grade formic acid	G2453-85060
plastic bottle	9301-1344 and 9301-1347

Additional parts needed (for 6546 only)

Table 4 Additional parts needed for 6546 Q-TOF LC/MS only

Component	Agilent part number
chloramphenicol (ESI Negative Ion Performance Standard)	5190-0591

Additional parts needed (for 6545XT only)

Table 5 Additoinal parts needed for 6545XT Q-TOF LC/MS only

Component	Agilent part number
15-mL conical centrifuge tube	
[Glu1]-Fibrinopeptide B standard	5190-6901
Trypsinogen Standard	5190-6902

6546 only Chloramphenicol (for Negative Mode verification)

1 Prepare 1 liter **water:methanol** solution:

Reagent	Ratio
18-Ω, HPLC grade deionized water	70%
LCMS-grade methanol	30%

2 Prepare the first dilution (100 pg/µL) **chloramphenicol**:

- **a** Use a clean **1-mL graduated pipette** to transfer 2.0 mL of the 5 ng/µL **chloramphenicol** to a pre-rinsed **100-mL volumetric flask**.
- **b** Dilute to the 100 mL mark with **water:methanol** solution. Invert the flask at least 10 times to ensure proper mixing of the checkout sample.
- **c** Use 5 to 10 mL of this solution to rinse a **plastic bottle** and fill it with the remaining solution.
- **d** Label this bottle "100 pg/µL chloramphenicol" along with the preparation date.

3 Prepare the second dilution (1 pg/µL) **chloramphenicol**:

- **a** Use a clean **1-mL graduated pipette** to transfer 1 mL of the 100 pg/µL **chloramphenicol** dilution to a pre-rinsed **100-mL volumetric flask**.
- **b** Dilute to the 100 mL mark with **water:methanol** solution.
- **c** Use 5 to 10 mL of this solution to rinse one of the plastic storage bottles and fill it with the remaining solution.
- **d** Label this bottle "1 pg/µL chloramphenicol" along with the preparation date.

This provides 1 pg/µL at 1 µL injection volume, which equals the final 1 pg chloramphenicol amount that is needed for performance verification.

Reserpine

1 Prepare 1 liter of **water:acetonitrile** solution:

Reagent	Ratio
18-Ω, HPLC grade deionized water	30%
LCMS-grade acetonitrile	70%

2 Prepare the first dilution (100 pg/µL) reserpine:

- **a** Use a clean **1-mL graduated pipette** to transfer 1.0 mL of the 5 ng/µL **reserpine** to a pre-rinsed **50-mL volumetric flask**.
- **b** Dilute to the 50 mL mark with the **water:acetonitrile** solution that was prepared in **step 1**. Invert the flask at least 10 times to ensure proper mixing of the checkout sample.
- **c** Use 5 to 10 mL of this solution to rinse **100-mL volumetric flask** or **30-mL polypropylene bottle** and fill it with the remaining solution.
- **d** Label this bottle "100 pg/µL reserpine" along with the preparation date.

This solution is stable for several weeks if kept refrigerated.

3 Prepare the second dilution (10 pg/µL) reserpine:

- **a** Use a clean **1-mL graduated pipette** to transfer 5.0 mL of the **100 pg/µL reserpine** dilution to a pre-rinsed **50-mL volumetric flask**.
- **b** Dilute to the 50 mL mark with the **water:acetonitrile** solution that was prepared in **step 1**. Invert the flask at least 10 times to ensure proper mixing of the checkout sample.
- **c** Use 5 to 10 mL of this solution to rinse one of the plastic storage bottles and fill it with the remaining solution.
- **d** Label this bottle "10 pg/µL reserpine" along with the preparation date.

Refrigerate this solution and use on the day it is prepared.

4 Prepare the third dilution (1 pg/µL) reserpine:

- **a** Use a clean **1-mL graduated pipette** to transfer 5.0 mL of the 10 pg/µL reserpine dilution to a clean 50 mL volumetric flask.
- **b** Dilute to the 50 mL mark with the **water:acetonitrile** solution . Invert the flask at least 10 times to ensure proper mixing of the checkout sample.
- **c** Use 5 to 10 mL of this solution to rinse one of the plastic storage bottles and fill it with the remaining solution.
- **d** Label this bottle "1 pg/µL reserpine" along with the preparation date.

6545XT Only

Acetonitrile Solution

1. Use a clean **1-mL graduated pipette** to transfer 5.0 mL of **18-Ω, HPLC grade deionized water** to a **15-mL conical centrifuge tube**.
2. Create Diluent A:
   a. Use a clean **1-mL graduated pipette** to transfer 5.0 mL of **LCMS-grade acetonitrile** to a **15-mL conical centrifuge tube**.
   b. Spin the **15-mL conical centrifuge tube** on vortex mixer.
   c. Label the solution "50:50 ACN:H_20 (Diluent A)".
3. Create Diluent B:
   a. Use a clean **1-mL graduated pipette** to transfer 5.0 mL of **Diluent A** to a fresh **15-mL conical centrifuge tube**.
   b. Add 5.0 µL of **reagent-grade formic acid** to the **15-mL conical centrifuge tube**.
   c. Spin the **15-mL conical centrifuge tube** on vortex mixer.
   d. Label the solution "50:50:0.1 ACN:H_20:FA (Diluent B)".

6545XT Only

Glu-Fib Standard

1 Prepare 100 pmol/µL **[Glu1]-Fibrinopeptide B standard**:
   - **a** Remove the seal and cap from the **[Glu1]-Fibrinopeptide B standard** bottle.
   - **b** Add 637 µL of Diluent A to the **[Glu1]-Fibrinopeptide B standard** bottle.
   - **c** Use a clean **1-mL graduated pipette** to dissolve the **[Glu1]-Fibrinopeptide B standard**.
   - **d** Transfer all solution to an empty 2-mL vial and cover with a cap.
   - **e** Label vial "100 pmol/µL Glu-Fib".

2 Prepare 3.16 pmol/µL **[Glu1]-Fibrinopeptide B standard**:
   - **a** Add 968.4 µL of Diluent A to a 2-mL vial.
   - **b** Add 31.6 µL of 100 pmol/µL and cover with a cap.
   - **c** Mix on a vortex mixer to homogenize.
   - **d** Label vial "3.16 pmol/µL Glu-Fib".

3 Prepare 100 fmol/µL **[Glu1]-Fibrinopeptide B standard**:
   - **a** Add 968.4 µL of **Diluent B** to a 2-mL vial.
   - **b** Add 31.6 µL of **3.16 pmol/µL** and cover with a cap.
   - **c** Mix on a vortex mixer to homogenize.
   - **d** Label vial "100 fmol/µL Glu-Fib".

6545XT Only

Trypsinogen Standard

1 Prepare 1 µg/µL **Trypsinogen Standard**:
   - **a** Remove the seal and cap from the **Trypsinogen Standard** bottle.
   - **b** Add 1,000 µL Diluent A to the **Trypsinogen Standard** bottle.
   - **c** Use a clean **1-mL graduated pipette** to dissolve the **Trypsinogen Standard**. *Do not use a vortex mixer.*
   - **d** Label vial "1 µg/µL Trypsinogen".
2 Prepare 100 ng/µL **Trypsinogen Standard**:
   - **a** Add 180 µL of Diluent B to a 200-µL vial.
   - **b** Add 20 µL 1 µg/µL **Trypsinogen Standard** and cover with a cap.
   - **c** Use a clean **1-mL graduated pipette** to dissolve the **Trypsinogen Standard**. *Do not use a vortex mixer.*
   - **d** Label vial "100 ng/µL Trypsinogen".

NOTE

Use the subsequent steps if the data analysis methods are not supplied or are not available.

Step 2. Create the performance verification methods

1 On the Data Acquisition installation disc, find the folder **Support\Installation\QTOF*model***. Copy the appropriate methods to the **D:\MassHunter\Methods** folder:

6530

- 6530_Sensitivity_ms.m
- 6530_Sensitivity_msms.m
- 6530_Accuracy_ms.m
- 6530_Accuracy_msms.m

6545

- 6545_Sensitivity_ms.m
- 6545_Sensitivity_msms.m
- 6545_Accuracy_ms.m
- 6545_Accuracy_msms.m

6545XT

- 6545XT_Res_Accuracy_ms.m
- 6545XT_Res_Accuracy_msms.m
- 6545XT_Res_Sensitivity_ms.m
- 6545XT_Res_Sensitivity_msms.m
- 6545XT_GluFib_Sensitivity_ms.m
- 6545XT_GluFib_Sensitivity_msms.m
- 6545XT_Trypsinogen_Accuracy_ms.m

6546

- 6546_CAP_MS_sensitivity.m
- 6546_CAP_MSMS_sensitivity.m
- 6546_CAP_MS_accuracy.m
- 6546_CAP_MSMS_accuracy.m
- 6546_Res_Sensitivity_ms.m
- 6546_Res_Sensitivity_msms.m
- 6546_Res_Accuracy_ms.m
- 6546_Res_Accuracy_msms.m

6550

- 6550_Sensitivity_ms.m
- 6550_Sensitivity_msms.m
- 6550_Accuracy_ms.m
- 6550_Accuracy_msms.m

6560 IM-QTOF

- 6560_Sensitivity_QTOF_ms.m
- 6560_Sensitivity_QTOF_msms.m
- 6560_Sensitivity_IM_ms.m
- 6560_Accuracy_QTOF_ms.m
- 6560_Accuracy_QTOF_msms.m
- 6560_Accuracy_IM_ms.m
- 6560_TuneMix_CCS_IM.m
- 6560_TuneMix_Resolution_IM.m

See the appropriate *Method Reference Guide* for method details.

The acquisition portion of these methods are listed in the *Method Reference Guides*. The methods on the Data Acquisition installation media contain qualitative analysis methods in addition to the acquisition methods. The qualitative analysis methods contain the steps required to generate the reports required for installation verification.

2 Remove the write-protection from these files:

a In File Manager, select the method files that you just copied.

b Right-click the selection and click **Properties**.

c Clear the **Read-only** check box.

d Click **Apply changes to this folder, subfolders and files**.

e Click **OK**.

3 For each of the methods that you copied:

a Open the method.

b If you are warned that default parameters will be used for the LC modules, check that the LC and TOF parameters for that method match those listed in **Chapter 7**, “Reference”.

c Save and close the method.

Step 3. Verify Q-TOF LC/MS sensitivity and mass accuracy

1 In the Autosampler tray, put the following vials in these locations:

Table 6 Vial Position - 6530, 6545, 6546, 6550, and 6560 LC/MS

Vial Position	Content
1	Solvent Blank
2	1 mL × 1 pg/µL Reserpine sample
3	1 mL × 100 pg/µL Reserpine sample

Table 7 Vial Position - 6545XT LC/MS

Vial Position	Content
1	Solvent Blank
2	1 mL × 1 pg/µL Reserpine sample
3	1 mL × 100 pg/µL Reserpine sample
4	1 mL × 100 fmol/µL GluFib sample
5	1 mL × 100 ng/µL Trypsinogen sample

Table 8 Vial Position - 6546 LC/MS in Negative and Positive Modes*

Vial Position	Content (Negative Mode)	Content (Positive Mode)
1	Solvent Blank (**water:methanol**)	Solvent Blank (**water:acetonitrile**)
2	1 mL × 1 pg/µL Chloramphenicol sample	1 mL × 1 pg/µL Reserpine sample
3	1 mL × 100 pg/µL Chloramphenicol sample	1 mL × 100 pg/µL Reserpine sample

* Start with Negative Mode vials. After running the Negative mode worklist, replace the Negative Mode vials with Positive Mode vials.

2 On the Data Acquisition installation disc, open the folder **Support\Installation\QTOF**. From the instrument-specific folder, copy the Worklist files named with the specific source type to the **D:\MassHunter\Worklists** folder.

CAUTION

Make sure that you copy the worklist that is appropriate for the instrument and source type. If the wrong worklist is copied and used for the instrument or source type, the installation verification will fail.

3 Remove the write-protection from these files:

   a In File Manager, select the worklist files that you just copied.

   b Right-click the selection and click **Properties**.

   c Clear the **Read-only** check box.

   d Click **OK**.

   Please note that the vial locations may need to be updated depending on the autosampler installed.

4 Open the Q-TOF mode checkout worklist and edit the vial locations to match the autosampler type that you are using.

5 For 6546 only:

   a Run the negative checkout (chloramphenicol) worklist.

   b Change solvents (to A = water w/0.1% formic acid and B = acetonitrile for Positive Ion Polarity).

   c Clean the injectors. See **step 11** on **page 6**.

   d Equilibrate the column for 1 hour at 30:70 A:B.

6 Run the Q-TOF positive mode checkout worklist.

   Turn on the Ref Nebulizer gas supply during the worklist for the Mass Accuracy runs for the Dual AJS source. Otherwise, the Mass Accuracy tests will fail due to the missing reference mass ions.

For 6560 IM-QTOF only

The rest of these steps apply to only the 6560 IM-QTOF. For all other Q-TOF LC/MS, continue at **"Step 4. Process the acquired data"** on page 31.

7 Prepare to run the IM mode checkout worklist:

- **a** Change to the **Tune** context.
- **b** In the **Instrument State** tab, under **Instrument Mode**, click **Extended Dynamic Range (2 GHz)**.
- **c** In the **Tune & Calibration** tab, mark the **Positive** and **Negative** check boxes.
- **d** Click **Start TOF Mass Calibration**.
- **e** Change to the **Acquisition** context.
- **f** Repeat **step 4** and **step 6** but use the IM mode checkout worklist.
- **g** Repeat **step 7** but in **step b**, change the **Mass Range** to **Standard (3200 m/z)** to run the TuneMix methods in the IM mode checkout worklist.

Step 4. Process the acquired data

If you use the preconfigured methods, reports are automatically generated.

Follow these steps only if the Data Acquisition program did not generate a report when the worklist was run.

1 After the Worklist completes, start the Qualitative Analysis program.

2 Create reports for MS sensitivity:

   a From the **Method** menu, open the MS sensitivity method for the instrument model.

   b Open all of the blank injections and the MS samples for the instrument model, until all of the MS data files are opened.

   c Click **Method > Run Method Automation (Workflow + Reports)**.

   d Select all data files and click **Run**.

   e Save the printed reports.

3 Create reports for MS/MS sensitivity:

   a From the **Method** menu, open the MS/MS sensitivity method for the instrument model.

   b Open all five MS/MS data files for the instrument model.

   c Click **Method > Run Method Automation (Workflow + Reports)**.

   d Select all MS/MS data files and click Run.

   e Save the printed reports.

4 Create reports for MS mass accuracy:

   a Open the MS mass accuracy method for the instrument model.

   b Open all five mass accuracy data files for the instrument model.

   c Click **Method > Run Method Automation (Workflow + Reports)**.

   d Select all mass accuracy data files and click **Run**.

   e Save the printed reports.

5 Attach the reports to the installation and familiarization checklist.

   The Q-TOF LC/MS must pass the following specifications:

Table 9 Specifications for 6530 with Dual ESI source

Conditions	Passing specs
Signal to Noise, 10 pg Reserpine, MS Mode	>10 to 1
Signal to Noise, 5 pg Reserpine, MS/MS Mode (summed EIC of 174, 195, 397 and 448 product ions of 609.2807 precursor ion)	> 50 to 1
Mass Accuracy at 609.2807 *m/z*, 100 pg Reserpine MS Mode	< 2 ppm
Mass Accuracy 100 pg Reserpine MS/MS Mode (397.2122 product ion)	< 5 ppm

Table 10 Specifications for 6530 with Dual AJS source

Conditions	Passing specs
Signal to Noise, 1pg Reserpine, MS Mode	>10 to 1
Signal to Noise, 1 pg Reserpine, MS/MS Mode (summed EIC of 174, 195, 397 and 448 product ions of 609.2807 precursor ion)	> 50 to 1
Mass Accuracy at 609.2807 *m/z*, 20 pg Reserpine MS Mode	< 2 ppm
Mass Accuracy 20 pg Reserpine MS/MS Mode (397.2122 product ion)	< 5 ppm

Table 11 Specifications for 6545 with Dual AJS source

Conditions	Passing specs
Signal to Noise, 1 pg Reserpine, MS Mode	>250 to 1
Signal to Noise, 1 pg Reserpine, MS/MS Mode (summed EIC of 174, 195, 397 and 448 product ions of 609.2807 precursor ion)	>750 to 1
Mass Accuracy at 609.2807 *m/z*, 40 pg Reserpine MS Mode	< 0.8 ppm
Mass Accuracy 40 pg Reserpine MS/MS Mode (397.2122 product ion)	< 2 ppm
Mass Resolution, FWHM	> 43,000

Table 12 Specifications for 6546 with Dual AJS source (Negative mode)

Conditions	Passing specs
Signal to Noise, 1 pg Chloramphenicol, MS Mode	>250 to 1
Signal to Noise, 5 pg Chloramphenicol, MS/MS Mode (summed EIC of 152.0353, 176.0353, 194.0459, and 257.0335 product ions of 321.0051 precursor ion)	>750 to 1
Mass Accuracy at 321.0051 *m/z*, 40 pg Chloramphenicol MS Mode	< 0.8 ppm
Mass Accuracy 40 pg Chloramphenicol MS/MS Mode (152.0353 product ion)	< 2 ppm
Mass Resolution, FWHM	> 60,000

Table 13 Specifications for 6546 with Dual AJS source (Positive mode)

Conditions	Passing specs
Signal to Noise, 1 pg Reserpine, MS Mode	>250 to 1
Signal to Noise, 1 pg Reserpine, MS/MS Mode (summed EIC of 174, 195, 397 and 448 product ions of 609.2807 precursor ion)	>750 to 1
Mass Accuracy at 609.2807 *m/z*, 40 pg Reserpine MS Mode	< 0.8 ppm
Mass Accuracy 40 pg Reserpine MS/MS Mode (397.2122 product ion)	< 2 ppm
Mass Resolution, FWHM	> 60,000

Table 14 Specifications for 6545XT with Dual AJS source

Conditions	Passing specs
Signal to Noise, 1 pg Reserpine, MS Mode	>250 to 1
Signal to Noise, 1 pg Reserpine, MS/MS Mode (summed EIC of 174, 195, 397 and 448 product ions of 609.2807 precursor ion)	>750 to 1
Mass Accuracy at 609.2807 *m/z*, 40 pg Reserpine MS Mode	<0.8 ppm
Mass Accuracy 40 pg Reserpine MS/MS Mode (397.2122 product ion)	<2 ppm
Mass Resolution, FWHM	>48,000
Fragmentation Efficiency MS/MSMS Glu-Fib 100 fmol	>35%
Mass Accuracy Trypsinogen 100 ng	<10 ppm

Table 15 Specifications for 6550 with Dual AJS source

Conditions	Passing specs
Signal to Noise, 1 pg Reserpine, MS Mode	>100 to 1
Spectra Abundance of 609.2807 ion, MS mode	>8K of average spectrum in peak >10% of peak height
Signal to Noise, 1 pg Reserpine, MS/MS Mode (summed EIC of 174, 195, 397 and 448 product ions of 609.2807 precursor ion)	≥ 500 to 1
Mass Accuracy at 609.2807 *m/z*, 50 pg Reserpine MS Mode	≤ 0.8 ppm (for s/n SGxxxx**B1**xx or higher) or ≤ 1.0 ppm (for s/n SGxxxx**B0**xx.)
Mass Accuracy 50 pg Reserpine MS/MS Mode (397.2122 product ion)	≤ 2 ppm

Table 16 Specifications for 6560 in Q-TOF mode

Conditions	Passing specs
Signal to Noise, 1 pg Reserpine, MS Q-TOF Mode	≥50 to 1
Signal to Noise, 1 pg Reserpine, MS/MS Q-TOF Mode (summed EIC of 174, 195, 397 and 448 product ions of 609.2807 precursor ion)	≥250 to 1
Mass Accuracy at 609.2807 *m/z*, 40 pg Reserpine MS Q-TOF Mode	≤ 1 ppm
Mass Accuracy 100 pg Reserpine MS/MS Q-TOF Mode (397.2122 product ion)	≤ 2 ppm

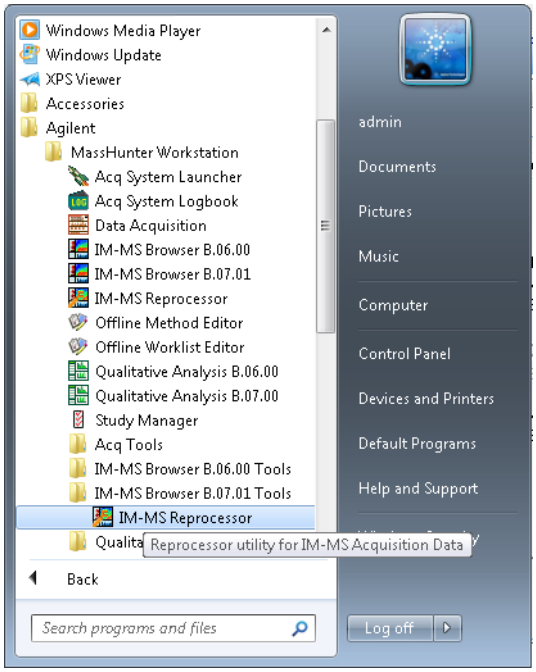
Table 17 Specifications for 6560 in Ion Mobility mode

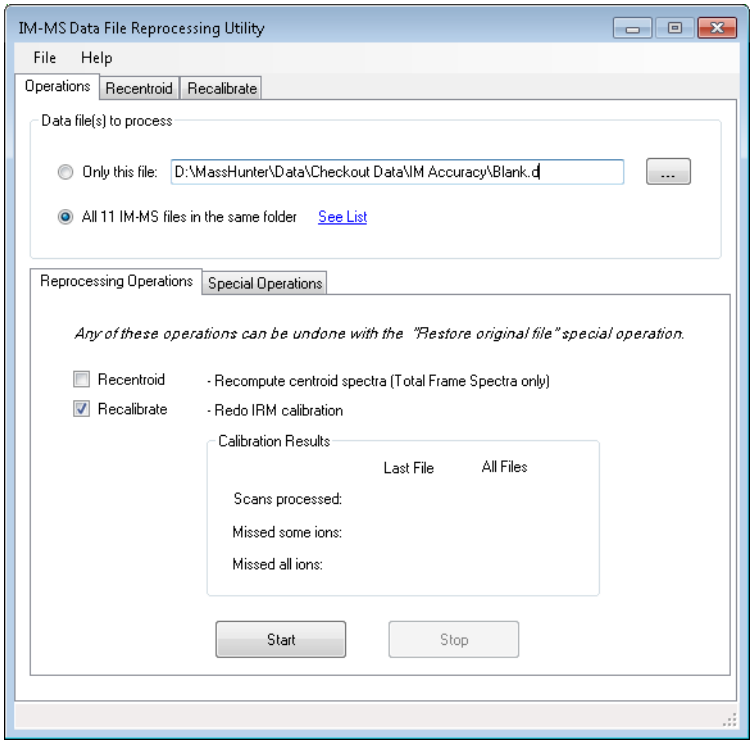
Conditions	Passing specs
Extracted Ion Chromatogram RSD, 1 pg Reserpine, MS, IM mode.	≤20% RSD
Mass Accuracy 40 pg Reserpine, MS, IM mode at 609.2807 m/z.	≤5 ppm
CCS Accuracy on 622 tune ion, using 922 for pressure correction.	≤2% deviation from expected
IM Resolution on 2722 ion at 1700 Volts on Drift Tube Entrance	≥50

Step 5. Reprocess IM mode data (6560 IM-QTOF only)

For the Q-TOF mode data and the IM mode sensitivity data, you do not need to manually process the acquired data.

- For the IM mode Accuracy data, reprocess the data for optimal mass assignments if needed. Use the **IM-MS Reprocessor** utility (installed with IM-MS Browser).





- For the IM CCS calculation performed on tune mix, manually process the data with the IM-MS Browser program.
- For the IM Resolution data, use the value from the Tune Report, if possible. If the Tune Report displays insufficient signal for 2722 *m/z*, acquire the IM Resolution data file in the IM checkout worklist. For the IM Resolution data collected through the worklist, you need to manually process the data with the IM-MS Browser program.

Step 6. Calculate Cross Section using tune ions (6560 IM-QTOF only)

1 Open the IM-MS Browser program.

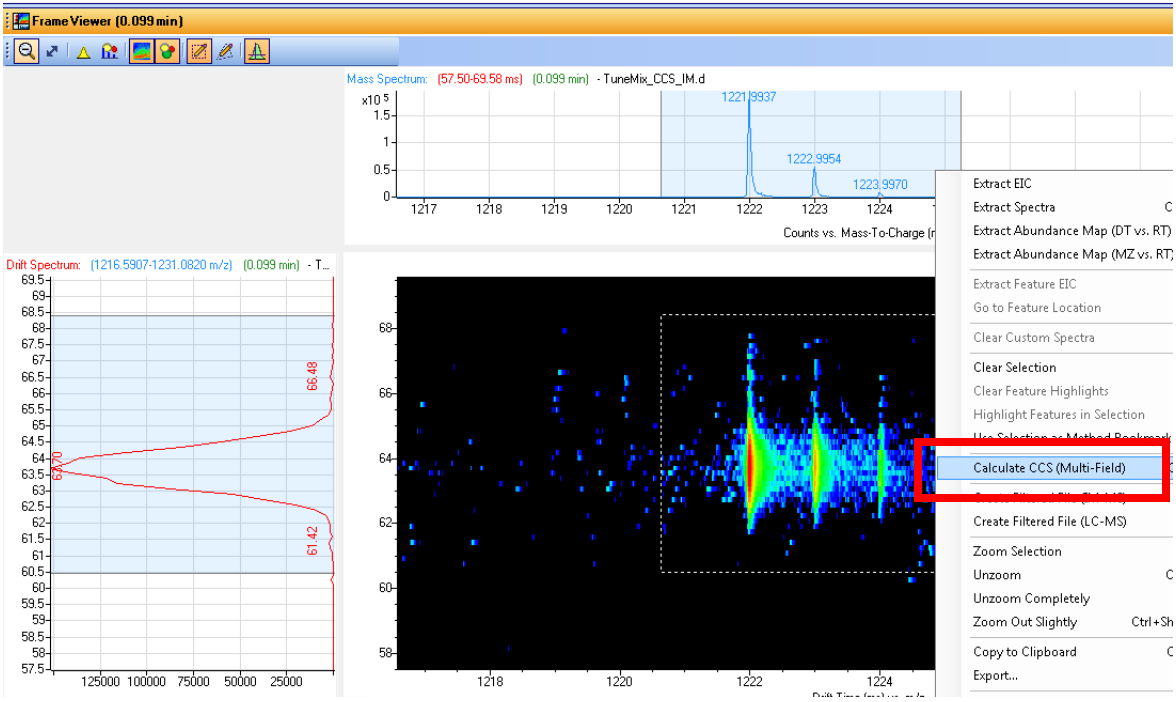
2 In the IM-MS Browser program, open the TuneMix data file that you acquired in IM mode.

The data file contains several time segments with different drift voltages.

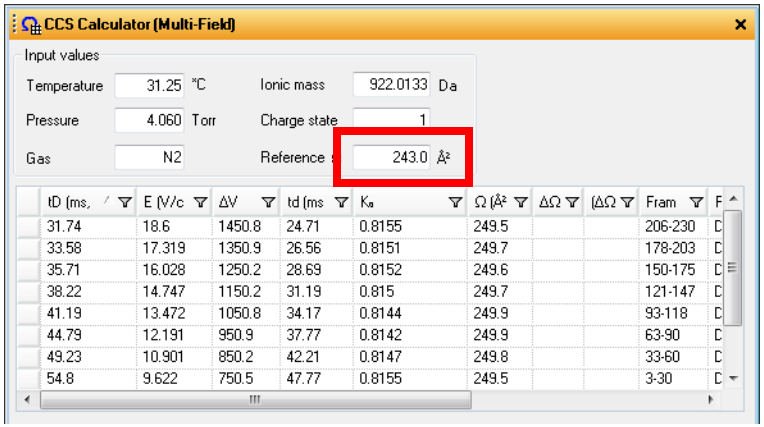
3 In the Frame Viewer, zoom in on the *m/z* 922 isotopic cluster.

4 Select the entire *m/z* 922 cluster with the left mouse button.

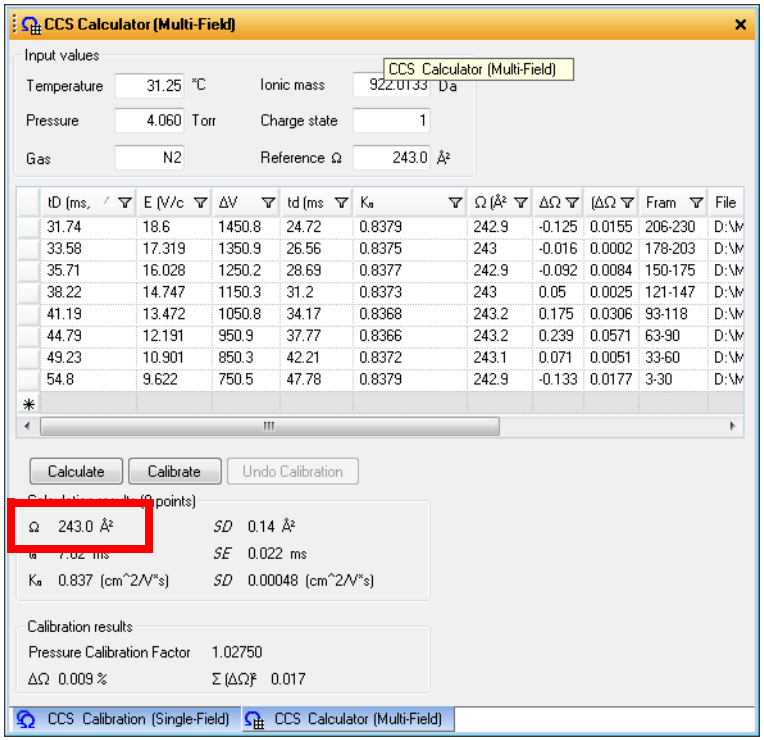
5 Right-click the selected box and click **Calculate Cross Section**.



6 In the Cross Section Calculator, set **Reference Ω** to **243.0** to calibrate the pressure correction factor. Click **Calibrate**.



7 Make sure that under **Results**, Ω shows a value of **243.0**.

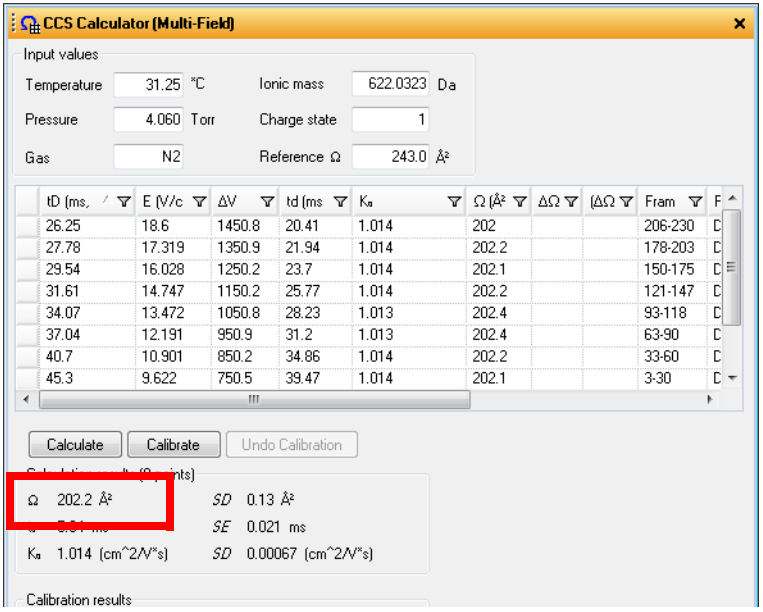


8 Check the cross section accuracy with the *m/z* 622 ion now that the calculation is calibrated.

a Zoom in on the *m/z* 622 isotopic cluster using the right mouse button, and select a window around the entire cluster with the left mouse button.

b Right-click the selected box and click **Calculate Cross Section**.

c Under **Results**, find the Ω value calculated for the *m/z* 622 ion. The expected value is 202.4.



d Calculate the error of the observed measurement.

Table 18 Passing specs for 6560 in Ion Mobility mode

Conditions	Passing specs
CCS Accuracy on 622 tune ion, using 922 for pressure correction.	≤2% deviation from expected

9 Print a screen capture of the results and attach to the installation checklist documentation.

Step 7. Verify IM Resolution (6560 IM-QTOF only)

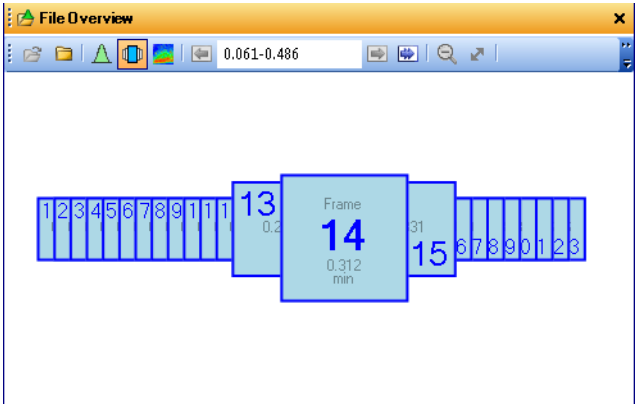
Do this step if in the Tune Report, the IM Resolution of the 2722 *m/z* tune ion fails. If the tune passes, keep the Tune Report.

1 Open the IM-MS Browser program.

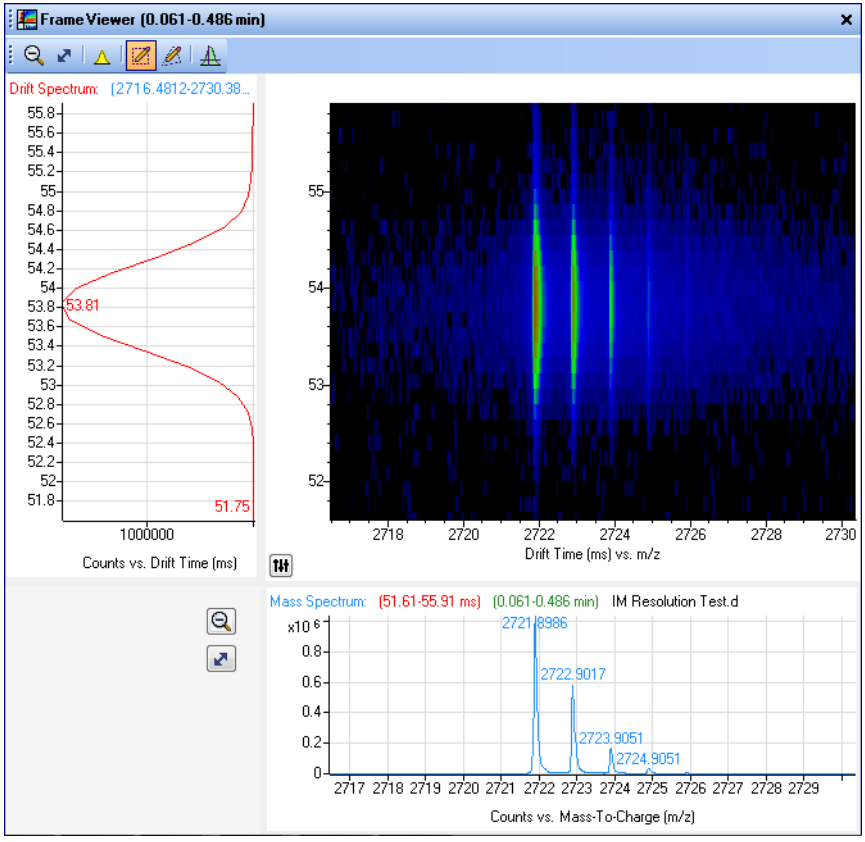
2 In the IM-MS Browser program, open the **IM Resolution Test TuneMix** data file that you acquired in IM mode.

The data file contains a single time segment with extended trap fill times.

3 In **File Overview**, select all frames for display.

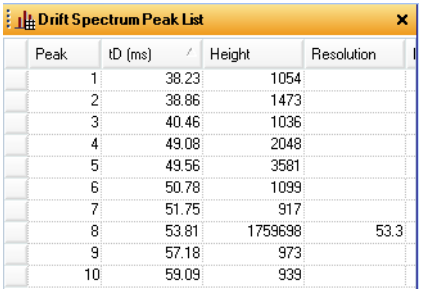


4 In the **Frame Viewer**, zoom in on the ***m/z* 2722 isotopic cluster**. Make sure that the **Drift Spectrum** displays the **Drift Time** associated with the *m/z* **2722 isotopic cluster**.



5 In the **Drift Spectrum Peak List**, locate the ***m/z* 2722 isotopic cluster** (by its corresponding **Drift Time**).

6 Confirm the **IM Resolution** of the ***m/z* 2722 isotopic cluster** in the **Drift Spectrum Peak List.**



Peak	tD (ms)	Height	Resolution
1	38.23	1054	
2	38.86	1473	
3	40.46	1036	
4	49.08	2048	
5	49.56	3581	
6	50.78	1099	
7	51.75	917	
8	53.81	1759698	53.3
9	57.18	973	
10	59.09	939	

Table 19 Passing specs for 6560 in Ion Mobility mode

Conditions	Passing specs
IM Resolution on 2722 ion at 1700 Volts on Drift Tube Entrance	≥50

Print a screen capture of the results and attach to the installation checklist documentation.

Installation and Verification of Other Sources

This section contains the steps needed to install and verify the operation of the APCI, multimode, and APPI sources.

If the APCI, multimode or APPI source was purchased with the Q-TOF LC/MS, you will need to install the source and verify its operation. Do this after you have done the system sensitivity checkout with the ESI source that came with your instrument.

There is no sensitivity checkout with the G1978B Multimode source on the Q-TOF LC/MS. To verify its proper operation, run a manual tune with the G2432A APCI/APPI Calibration Solution.

You can complete only a Mass Calibration / Check or a Quick Tune with the different source types: G1948B, G1947B, G1971C and G1978B. Make sure you use the correct tune calibrant in Bottle B.

To install the G1947B APCI source

1 Install these parts from the APCI enablement kits that ship with the G1947B APCI source into the Aux module on the Q-TOF LC/MS:
   - APCI High Voltage Power Supply (p/n G1946-80058)
   - Valve board—APCI HV PS cable (p/n G1960-60802)
   - Valve board—APCI Needle Interlock cable (p/n G1960-60856)

2 Pour the Electrospray calibrant back into its original bottle or another suitable container, rinse the calibrant bottle with acetonitrile, pour the APCI calibrant into the calibrant bottle, and attach the calibrant bottle back onto the CDS.

3 Remove the electrospray source and install the APCI source:
   a Put the nebulizer into the nebulizer adjustment fixture that is supplied in the shipping kit. Check that the nebulizer needle is properly adjusted. Make sure that the nebulizer needle is even with the end of the nebulizer nozzle.
   b Install the nebulizer in the spray chamber.

WARNING **Surfaces can be extremely hot. Let the instrument surfaces cool before you touch them.**

   c Remove the previously installed spray chamber. Install the standard spray shield if not already installed.
   d Orient the standard spray shield so the off-axis hole is at the 12 o'clock position by loosening the two T10 screws that secure the end plate, orienting the spray shield, and re-tightening the two T10 screws (do not over-tighten).
   e Install the APCI spray chamber on the spray chamber mount, close the spray chamber, and fasten the latch. If needed, adjust the latch to ensure that the O-ring seals completely. Use a ¼-inch × 5/16-inch wrench to loosen the lock nut, adjust the latch to the proper fit, and then tighten the lock nut so that the latch maintains its adjustment.
   f Connect the APCI corona and vaporizer heater cables to the connector on the instrument.
   g Connect the 1/8-inch nebulizing gas tubing from the LC/MS mainframe to the nebulizer gas fitting.
   h Connect the PEEK tubing from the selection valve (inside the front cover) to the top of the nebulizer.

To install the G1978B multimode source

If the multimode source is not already installed on your Q-TOF LC/MS, do these steps.

1 Install these parts from the multimode and APCI enablement kits that ship with the G1978B multimode source into the Aux module on the Q-TOF LC/MS:
   - APCI High Voltage Power Supply (p/n G1946-80058)
   - Valve board—APCI HV PS cable (p/n G1960-60802)
   - Valve board—APCI Needle Interlock cable (p/n G1960-60856)
   - Multimode HV board cable (p/n G1960-60858)

CAUTION

Do not install the G1960-65015 Multimode HV board on the Q-TOF LC/MS or you can damage the AJS. The G1960-65115 Multimode HV board is already correctly installed on the Q-TOF LC/MS and is backward-compatible with the G1978B multimode source.

The Multimode Power Data cable is factory-installed on the 6460 and 6495.

2 Pour the electrospray tuning mix back into its original bottle or another suitable container, rinse the calibrant bottle with acetonitrile, pour the MMI-L tuning mix into the calibrant bottle, and attach the calibrant bottle back onto the CDS.

3 Remove the electrospray source and install the multimode source:
   a Put the nebulizer into the nebulizer adjustment fixture that is supplied in the shipping kit. Check that the nebulizer needle is properly adjusted. Make sure that the nebulizer needle is even with the end of the nebulizer nozzle.
   b Install the nebulizer in the spray chamber.

WARNING

Surfaces can be extremely hot.

   c Remove the previously installed spray chamber. Install the multimode spray shield if not already installed.
   d Orient the standard spray shield so the field-shaping electrode is at the 6 o'clock and 9 o'clock positions by loosening the two T10 screws that secure the end plate, orienting the spray shield, and re-tightening the two T10 screws (do not over-tighten).

e Install the multimode spray chamber on the spray chamber mount, close the spray chamber, and fasten the latch. If needed, adjust the latch to ensure that the O-ring seals completely. Use a ¼-inch × 5/16-inch wrench to loosen the lock nut, adjust the latch to the proper fit, and then tighten the lock nut so that the latch maintains its adjustment.

f Connect the multimode high voltage, APCI corona, and vaporizer heater cables to the connector on the instrument.

g Connect the 1/8-inch nebulizing gas tubing from the LC/MS mainframe to the nebulizer gas fitting.

h Connect the PEEK tubing from the selection valve (inside the front cover) to the top of the nebulizer.

To install the G1971C APPI source

1 Pour the Electrospray calibrant back into its original bottle or another suitable container, rinse the calibrant bottle with acetonitrile, pour the APCI/APPI calibrant into the calibrant bottle, and attach the calibrant bottle back onto the CDS.

2 Install the APPI USB to Serial Converter Cable (p/n 8121-1013) to one of the available USB ports on the SmartCard on the left side of the Q-TOF instrument.

3 Remove the currently installed source and install the APPI source:

   a Put the nebulizer into the nebulizer adjustment fixture that is supplied in the shipping kit. Check that the nebulizer needle is properly adjusted. Make sure that the nebulizer needle is even with the end of the nebulizer nozzle.

   b Install the nebulizer in the spray chamber.

WARNING

Surfaces can be extremely hot.

   c Remove the previously installed spray chamber. Install the standard spray shield if not already installed.

   d Orient the standard spray shield so the off-axis hole is at the 12 o'clock position by loosening the two T10 screws securing the end plate, orienting the spray shield, and re-tightening the two T10 screws (do not over tighten).

   e Install the APPI spray chamber on the spray chamber mount, close the spray chamber, and fasten the latch. If needed, adjust the latch to ensure that the O-ring seals completely. Use a ¼-inch × 5/16-inch wrench to loosen the lock nut, adjust the latch to the proper fit, and then tighten the lock nut so that the latch maintains its adjustment.

   f Connect the vaporizer heater cable to the connector on the instrument.

   g Connect the 1/8-inch nebulizing gas tubing from the LC/MS mainframe to the nebulizer gas fitting.

   h Connect the PEEK tubing from the selection valve (inside the front cover) to the top of the nebulizer.

   Do *not* use the APPI USB to Serial Converter Cable (p/n 8121- 1013).

4 Connect the APPI power supply cable to the APPI DB9 power connector and screw it in. This connector contains both serial and power interfaces.

5 Connect the other end of the serial cable to the Serial connector on the smart card interface, which is located on the left side of the instrument chassis.

6 Plug the APPI power supply into an AC outlet using the power cord supplied with the APPI interface kit.

To verify the operation of the APCI, multimode or APPI source

1 Install the calibrant for your source.

Source	Calibrant
G1947B APCI	G1969-85010 APCI-L Low Concentration Tuning Mix
G1978B multimode	G1969-85020 MMI-L Low Concentration Tuning Mix
G1971C APPI	G1969-85010 APCI-L Low Concentration Tuning Mix

2 Check the tuning of the installed APCI, multimode, or APPI source:

- **a** In Data Acquisition program, set **Context** to **Tune**.
- **b** Open the auto tune file that was generated with the electrospray or multimode source.
- **c** Set the **Source type** to match your source.
- **d** In the **Tune & Calibration** tab, mark the **Positive** check box only.
- **e** Click **TOF**.
- **f** Click **Mass Calibration / Check**.
- **g** Click **Start TOF Mass Calibration**.

h Check the tune peak values as follows:

Table 1 Typical tune peak values for Q-TOF and IM-QTOF (in Q-TOF mode) LC/MS

Model	Mass Resolution	Mass Accuracy
6530	> 10,000 on the 118 *m/z and* > 20,000 on the 1522 *m/z*	< 2 ppm on all masses in high resolution mode
6545/6550*	> 43,000 on the 2122 *m/z* tune ion	< 1 ppm on all masses in high resolution mode
6545XT	> 48,000 on the 2722 m/z tune ion	< 2 ppm on all masses in high resolution mode
6546	> 30,000 on the 118 *m/z* tune ion > 60,000 on the 2722 *m/z* tune ion	< 1 ppm on all masses in standard resolution mode
6550†/6560	> 40,000 on the 2122 *m/z* tune ion	< 1 ppm on all masses in high resolution mode

* 6550 with serial number SGxxxx**B1**xx or higher.

† 6550 with serial number SGxxxx**B0**xx.

3 Save the tune file.

In This Book

This guide contains steps to complete the installation and checkout of the Agilent 6500 Series Q-TOF and IM-QTOF LC/MS.

This guide is intended to be used by trained Agilent personnel.

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