Agilent

6500 Series Q-TOF LC/MS

Tuning Guide

Use this guide for an introduction to tuning an Agilent Q-TOF LC/MS, using the MassHunter Workstation software. For additional information, see the *Quick Start Guide* for your MassHunter Workstation.

This guide covers the basics of setting up and doing a SWARM autotune and calibration for all Agilent Q-TOF systems, except 6520, 6538, and 6540. It also presents additional steps to the basic tune and calibration sequence for different Q-TOF models and molecule types.

The standard tune sources are the Agilent Jet Stream and the Agilent dual-nebulizer electrospray ionization (ESI) sources. These are the only sources for tuning. Some older sources support Check Tune only. Some newer sources support Mass Calibration/Check.

These mass ranges are discussed in this document:

- Standard 3200 *m/z* range
- 1700 *m/z* range (with transmission tune options)
- Extended mass range (10,000 *m/z,* 20,000 *m/z,* 30,000 *m/z*)

Images shown are for MassHunter Data Acquisition 10.1 and 11.0 and may be different for older versions of MassHunter.

Basic Tune and Calibration

This section shows you how to set up tuning and then do a basic tune for all models. The one exception will be called out. You will also learn when to select and run additional mass calibrations since automatic mass calibration is always part of an Agilent tune.

Before you begin

To effectively tune the instrument, the calibration tuning solution must be properly diluted. See the online Help for more information on the proper dilution factors.

- In positive and negative ion mode, for all Agilent Jet Stream (AJS) Q-TOF systems, use a 1:10 dilution of tune mix.

To prepare an LC/Q-TOF Jet Stream tuning/calibration mix of 1:10 dilution, follow these steps:

1. Add the following components in the sequence listed below to a clean glass CDS (Calibration Delivery System) bottle (9300-2576).
   - 85.5 mL LC/MS-grade acetonitrile
   - 4.5 mL water, 18 MΩ-cm resistivity or better
   - 10 mL ESI-L Low Concentration Tuning Mix (G1969-85000)
   - 3 µL HP-0321 from Biopolymer Reference Mass Kit (G1969-85003); for the 6530 use 5 µL.
2. Make sure you have a good/tight seal when placing the bottle on the LC/MS system.

   If you see waves when tuning with the tuning solution, the bottle may have a poor seal, which may cause problems with tuning.

   If you have a poor seal, replace the bottle with a new clean bottle.

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

Step 1. Open the Tune Window and tune file

You prepare for and do a tune (including automatic mass calibration) in the Tune Window.

1 In the Data Acquisition program, load **Default.m**.

Load the method before you open the Tune Window.

2 In the **Context** list, select **Tune** (version 10.1 or earlier), or

In the **Context** group, click **Tune** (version 11.0).

The Tune Window appears.

3 Click the **Instrument State** tab if not selected (**Figure 1**).

The Agilent model **6546** does not display the resolution options but does display the **Slicer Mode**, which is always kept in the **High Resolution** position.

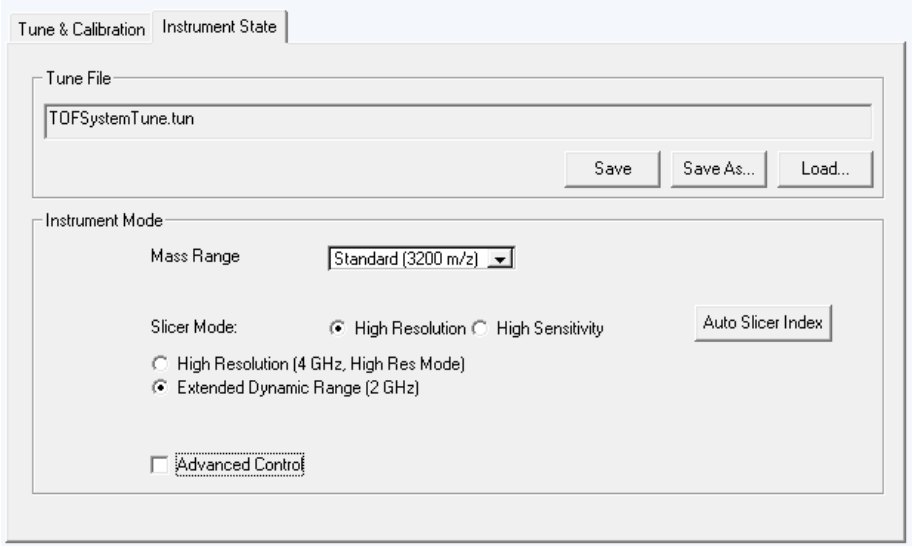

Figure 1. Tune window with Instrument State tab selected and Advanced Control clear

4 Click **Load**, and select the tune file you intend to use or change.

Do not load a prior tune for which you are unfamiliar with the tune details.

Step 2. Show additional tabs

You can show additional tabs within the Tune Window using options in the Instrument State tab and in the Preferences tab.

1 To see the **Manual Tune** and **Preferences** tabs, mark the **Advanced Control** check box in the **Instrument State** tab (**Figure 2**).

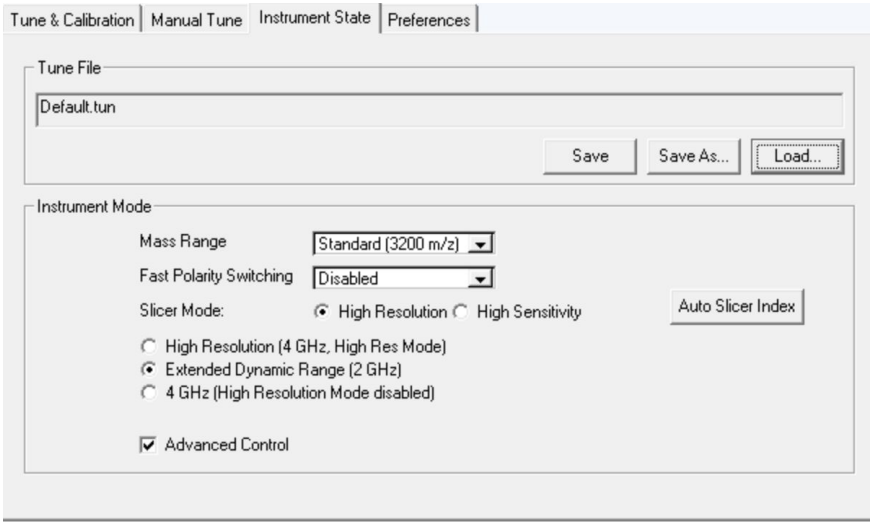

Figure 2. Instrument State tab with Advanced Control marked

A third resolution option also appears in the **Instrument State** tab when the **Advanced Control** check box is marked in any model, except 6546.

2 To view the **Diagnostics** tab, click the **Preferences** tab.

The Preferences dialog box appears (**Figure 3**).

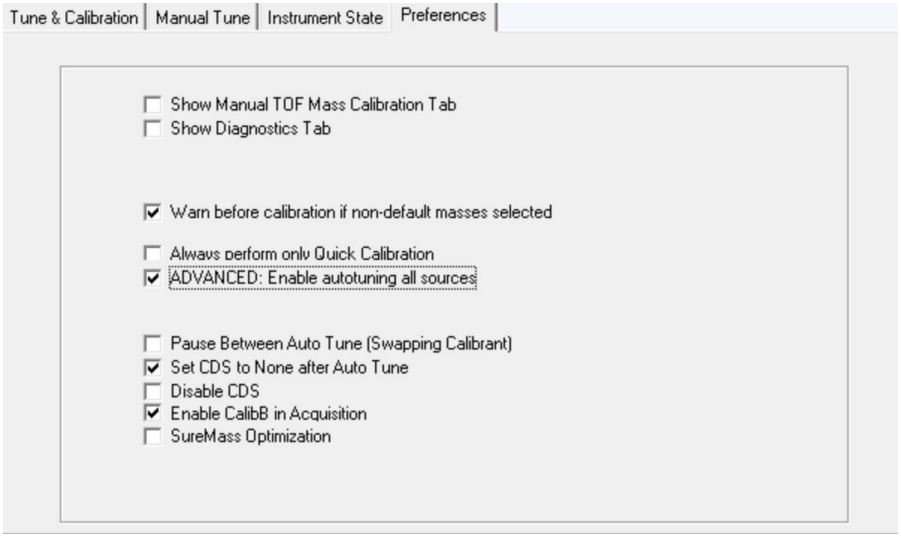

Figure 3. Preferences tab for Q-TOF models other than the 6546

3 Mark **Show Diagnostics Tab**, and click **Apply.**

4 Click **Diagnostics** tab.

The Diagnostics tab displays the MS vacuum pressures, as well as speed and power values (**Figure 4**).

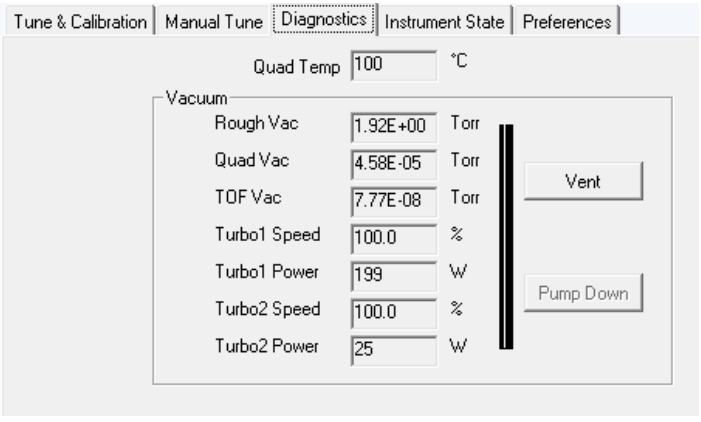

Figure 4. Diagnostics tab

Step 3. Select more preferences for tune and calibration

1 If needed, mark other preferences for tune and calibration in the **Preferences** tab. (See **Figure 3** on page 6.)

- Show Manual TOF Mass Calibration Tab - shows the current mass calibration constants and the exact mass of the ions used in calibration - **not available in the Preferences tab for the 6546**
- Warn before calibration if non-default masses selected - **usually marked**
- Always perform only Quick Calibration - **never used for SWARM tune**
- ADVANCED: Enable auto tuning all sources - **usually marked**
- Pause Between Auto Tune (Swapping Calibrant) - used only for older instruments that require different dilutions of calibrant for positive and negative mode.
- Set CDS to None after Auto Tune - **usually marked**
- Disable CDS
- Enable CalibB in Acquisition - **usually marked**
- SureMass Optimization. - used for MassHunter Quantitative Analysis workflows to optimize the performance of the SureMass algorithm

2 Click **Apply**.

Step 4. Select options for the Tune & Calibration tab

1 Return to the **Instrument State** tab. (See **Figure 1** on page 4 or **Figure 2** on page 5).

2 Make sure the **Slicer Mode** is in the **High Resolution** position.

For all tuning, the Slicer Mode must be in the High Resolution position.

The Slicer Mode controls the optics aperture, either narrow for resolution or wide for sensitivity.

Model **6546** only runs with the Slicer Mode in the **High Resolution** position.

3 Click **Auto Slicer Index** to make sure the selection is **High Resolution** (**Figure 5**).

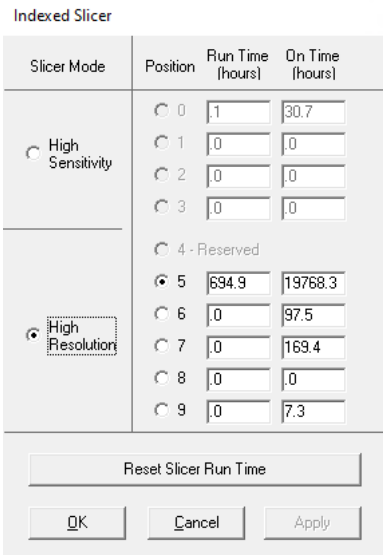

Figure 5. Indexed Slicer dialog box

The Indexed Slicer dialog box shows the number of hours samples have been run in a High Resolution position or in a High Sensitivity position. Each has five positions. If the resolution seems low after a number of sample runs, the slicer may be contaminated.

4 If you assess the slicer is contaminated, select a **Position** with no or low run-time hours, and click **Apply**.

Movement of the Slicer position can take several minutes.

5 Wait until the dialog box or the real time display shows that the Slicer position has been updated before you proceed to other steps.

After your next sample run, check the resolution. If it has not improved, call Agilent support.

6 Click **OK**.

7 For normal resolution click **Extended Dynamic Range (2GHz)** for the tuning process.

You must always use this option for all instruments except 6546, where this option is not available. The **High Resolution (4GHz. High Res Mode)** option is also not available in the 6546. (10GHz resolution is built into the system.)

8 Select a **Mass Range**.

Mass Ranges are typically 1700, 3200, and 10,000 *m/z*.

Upper Mass Range is 20,000 *m/z* for the 6530 and some 6560 instruments, and 30,000 *m/z* for the 6545XT.

NOTE

For the 6560 if you are using MassHunter Data Acquisition B.08 software, only a maximum mass range of 10,000 *m/z* is available for both the Q-TOF Only mode and the IM Q-TOF mode. If you have, or are intending to upgrade to, the MassHunter Data Acquisition B.09 or later software, only the IM Q-TOF mode allows a maximum mass range of 20,000 *m/z* and only if the acquisition board has the correct logic.

If you can select **High (10K-20K)** for your mass range in IM Q-TOF mode but see no results from 10,000 to 20,000 *m/z*, you need a logic board upgrade. Call Agilent support.

9 Click **Apply**.

Click **Apply** after every change or after the last change to the Instrument State tab before you move to the Tune & Calibration tab.

10 Save changes either to the loaded tune file or a new one. See **"To save a tune file"** on page 20.

NOTE

For instructions to make changes to the Instrument State Tab selections to optimize the tune resolution or sensitivity, see **"To optimize for resolution (except 6546)"** on page 13 or **"To optimize for sensitivity (except 6546)"** on page 15.

Step 5. Tune and calibrate

Do a system tune the first time you use the instrument or after maintenance, service, or pump-down, and restart. Also do a system tune before a 1700 *m/z* transmission tune.

Mass calibration takes place automatically as the last step of every tune. You calibrate again after changes in the Manual Tune tab, except for entries in the Optics 1, Quad, and IM tabs. After making changes to optimize for high resolution or high sensitivity, you must also recalibrate.

To do a Basic Tune

If you have made changes to the Instrument State tab, you usually need to wait 20 minutes before moving to the Tune & Calibration tab to do a tune.

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

If you have not waited the recommended equilibration time, **Figure 6** pops up in MassHunter Data Acquisition 11.0.

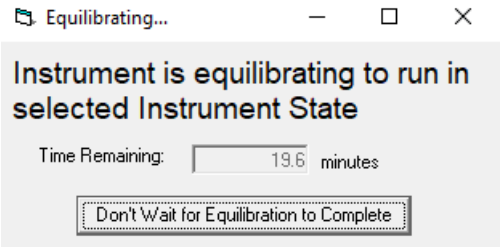

Figure 6. Equilibration time message (version 11.0)

2 Wait the recommended time, or

Click **Don't Wait for Equilibration to Complete**.

The Tune & Calibration dialog box appears with the options for the Mass Range and Resolution that you selected in the **Instrument State** tab.

Figure 7 appears if, in the Instrument State tab, you selected **Standard (3200 *m/z)*** as the Mass Range, and **Extended Dynamic Range (2 GHz)**.

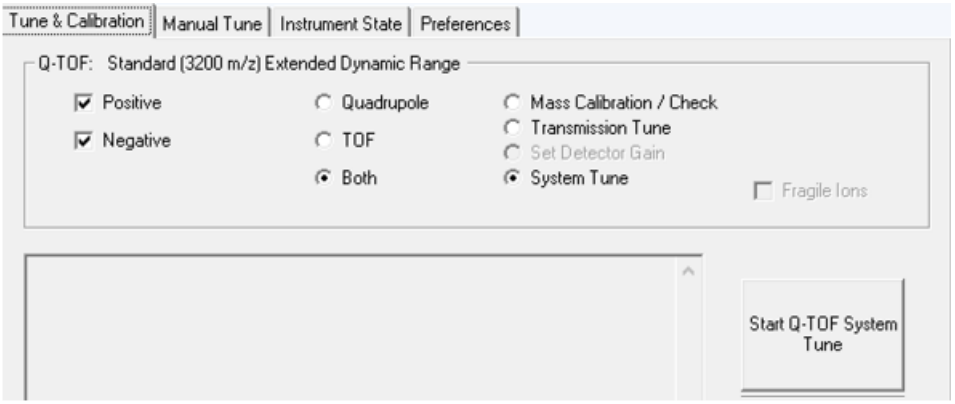

Figure 7. Tune & Calibration tab for 3200 *m/z* and Extended Dynamic Range

3 In the **Tune & Calibration** tab, do a **System Tune** (both TOF and Quadrupole) at 3200 *m/z* (**Figure 7**).

   a Mark the **Positive** and **Negative** check boxes.

   b Click **Both** (TOF and Quadrupole).

   c Click **System Tune**.

   d Click **Start Q-TOF System Tune**.

   e Review the tune reports. See **"To review the tune and calibration report"** on page 17.

   f Save changes either to the loaded tune file or a new one. See **"To save a tune file"** on page 20.

Now you are ready to do a 1700 m/z transmission tune.

Figure 8 appears if, in the Instrument State tab, you selected **Low (1700 *m/z*)** as the Mass Range, and **Extended Dynamic Range (2 GHz)**.

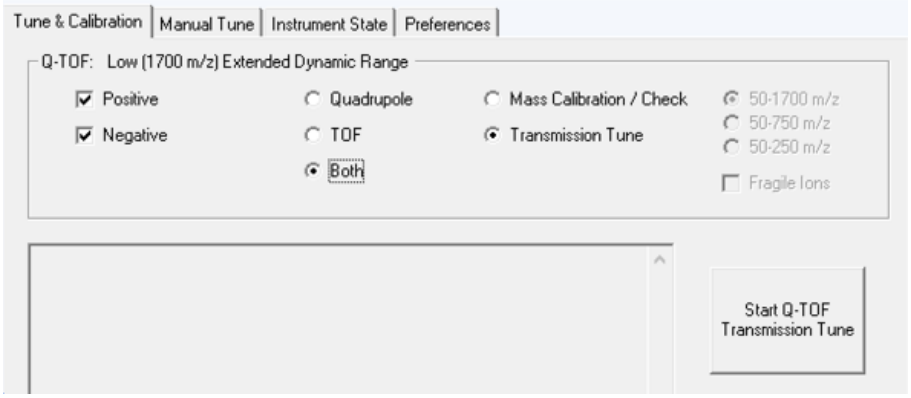

Figure 8. Tune & Calibration tab for 1700 *m/z* and Extended Dynamic Range

4 Do a 1700 *m/z* **Transmission Tune** (Figure 8).

Be sure you wait 20 minutes after you change from 3200 *m/z* to 1700 *m/z* in the Instrument State tab before you start this transmission tune in the Tune & Calibration tab.

a Mark the **Positive** and **Negative** check boxes.

b Click **Both** (TOF and Quadrupole).

c Click **Transmission Tune** for the 1700 *m/z* option.

d Click **Start Q-TOF Transmission Tune**.

e Review the tune reports. See **"To review the tune and calibration report"** on page 17.

f Save changes either to the loaded tune file or a new one. See **"To save a tune file"** on page 20.

Notice in Figure 8 that the low mass and fragile options are grayed out. This is because you have selected a quad transmission tune (**Both**). Yet, you can still do a low-mass or fragile quad transmission tune. For an explanation, see the low-mass and fragile tune and calibration sequences on **page 24** and on **page 26**.

NOTE

Relative to the 3200 *m/z* system tune, the 1700 *m/z* transmission tune gives minimal resolution loss and some sensitivity gain. The lower-mass tunes (250 and 750 *m/z*) provide a two- to three-fold sensitivity gain for low-mass ions, at the expense of some resolution loss.

Repeated transmission tunes may cause continual loss in resolution. The best practice is to do a system tune prior to a 1700 *m/z* transmission tune. Only a system tune provides best resolution to gain the best sensitivity increase in subsequent transmission tunes.

To optimize for resolution (except 6546)

1 Do a **Basic Tune**. (See **"To do a Basic Tune"** on page 10.)

2 Click the **Instrument State** tab.

3 Click **High Resolution (4GHz. High Res Mode).** (See **Figure 9**.)

The Advanced Control check box can either be marked or cleared.

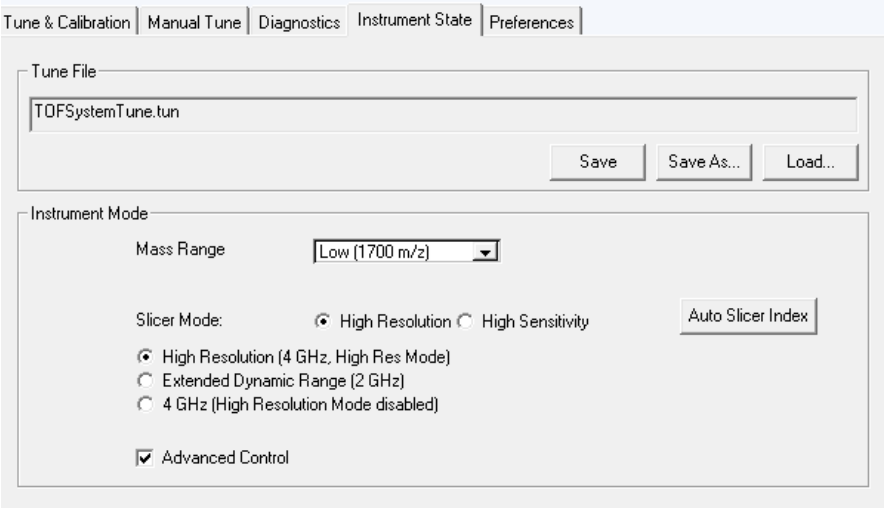

Figure 9. Instrument State tab - High Resolution selected

4 Click **Auto Slicer Index** to make sure the selection is **High Resolution**, and click **OK**.

For more information on the Slicer Index, see **"Step 4. Select options for the Tune & Calibration tab"** on page 8.

5 Click **Apply**.

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

7 Click **Mass Calibration/Check** (Figure 10).

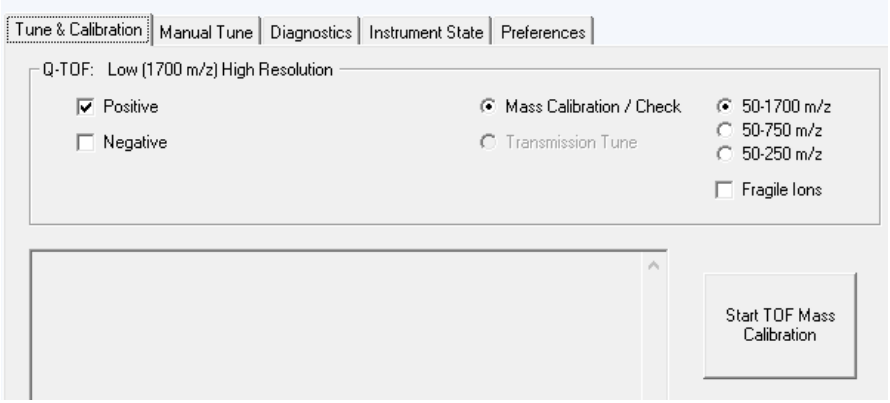

Figure 10. Tune & Calibration tab - Mass Calibration/Check selected

Notice that Transmission Tune is unavailable when the **High Resolution (4GHz. High Res Mode)** is selected in the Instrument State tab because you never do a tune after a change in resolution or sensitivity, only a mass calibration.

8 Click **Start TOF Mass Calibration**.

9 Review the calibration report. See **"To review the tune and calibration report"** on page 17.

10 Save changes either to the loaded tune file or a new one. See **"To save a tune file"** on page 20.

To optimize for sensitivity (except 6546)

1 Do a **Basic Tune**. (See **"To do a Basic Tune"** on page 10).

2 Unless you have a **6546**, for the **Slicer Mode**, click **High Sensitivity**.

Never change the **Slicer Mode** to **High Sensitivity** for the **6546** Q-TOF.

The Advanced Control option can either be marked or not.

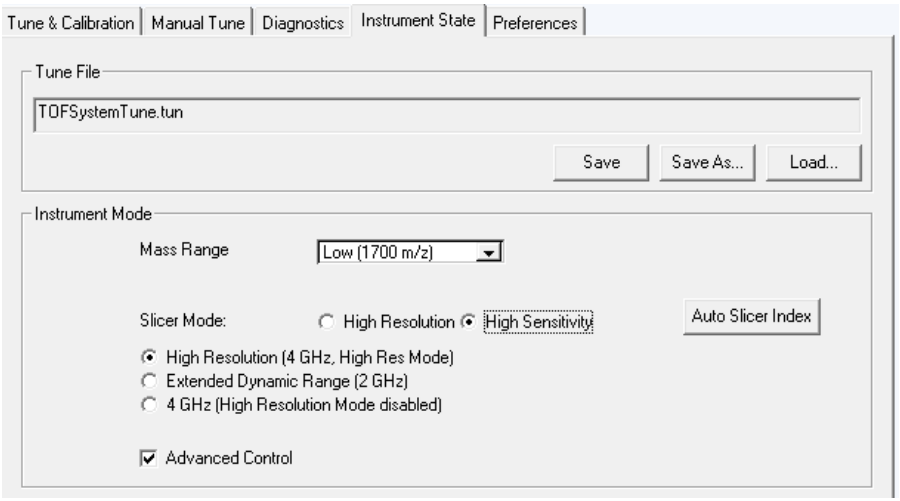

Figure 11. Instrument State tab with Slicer Mode - High Sensitivity selected

3 Click **Auto Slicer Index** to make sure the selection is **High Sensitivity**.

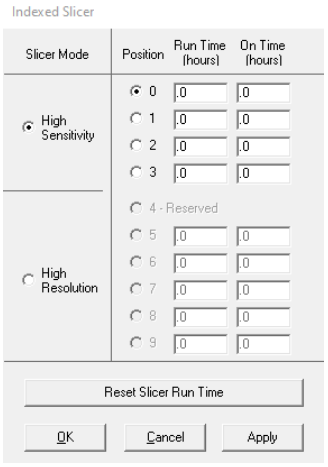

Figure 12. Auto Slicer Index - High Sensitivity

4 Click a different **Position**, if needed, and click **Apply**.

5 Confirm that the slicer position has been updated to the selected position before continuing.

6 Click **OK**.

For more information on the Auto Slicer Index, see **"Step 4. Select options for the Tune & Calibration tab"** on page 8.

7 Click **Extended Dynamic Range (2 GHz).** (See **Figure 13**.)

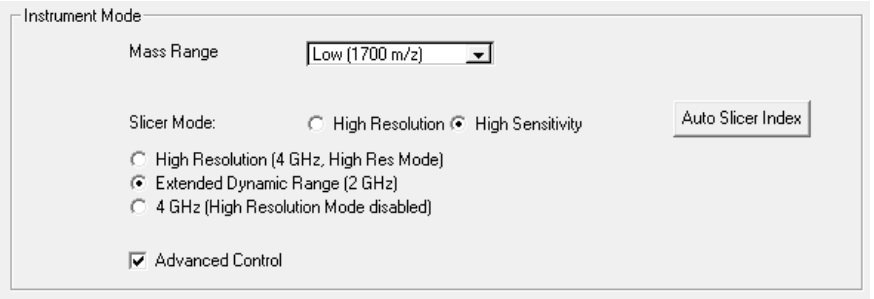

Figure 13. Instrument State tab - Extended Dynamic Range (2 GHz) selected

8 Click **Apply**.

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

10 Click and run the **Mass Calibration/Check** (**Figure 14**).

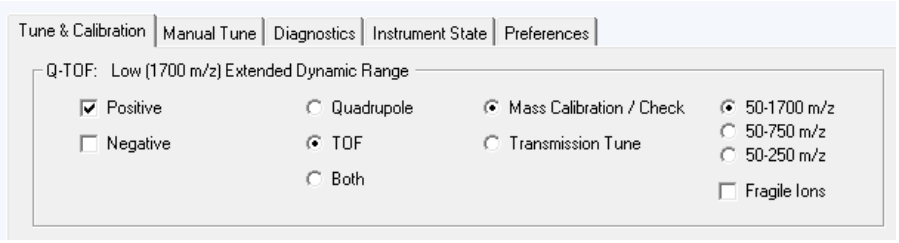

Figure 14. Tune & Calibration tab - Mass Calibration/Check selected

11 Review the calibration report. See **"To review the tune and calibration report"** on page 17.

12 Save changes either to the loaded tune file or a new one. See **"To save a tune file"** on page 20.

13 After saving the file, load a tune file that is a resolution file with either **Extended Dynamic Range (2 GHz)** or **High Resolution (4 GHz. High Res Mode)** selected in the Instrument State tab. Never tune with the **Slicer Mode** in the **High Sensitivity** position.

To review the tune and calibration report

After every tune, the report is saved automatically to the D drive folder as a PDF file, **D:\MassHunter\Tune\QTOF\Reports**. For MassHunter Data Acquisition 11.0 or later, tune reports are saved in the project folder. Tune reports are also saved with the data file during acquisition.

In addition, the Tune Report button becomes available in the Tune & Calibration tab (**Figure 15**).

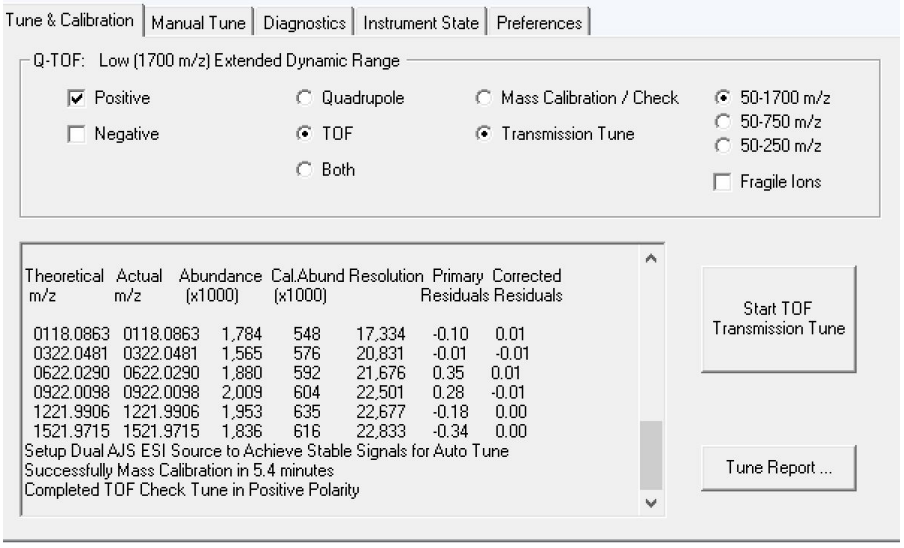

Figure 15. Tune & Calibration tab with Tune Report button

- To access a tune report:
  - Click **Tune Report**.

    The most current Tune Report appears (**Figure 16** on page 18).

Tune reports include results, a mass calibration table and MS parameters.

Q-TOF System Tune

Agilent Technologies

Instrument Information

MS Model	G6545XT	**Run Date**	04:12:21 16:36:42
Serial Number	SG1909M001	**Firmware Rev**	21.809
Instrument Mode	Extended Dynamic Range (2GHz)	**Slicer Mode**	High Resolution (Position: 5)
Mass Range	Standard (3200 m/z)	**SureMass Optimization**	Enabled
Source Type	Dual AJS ESI	**Ion Polarity**	Positive

Data Path D:\MassHunter\Tune\QTOF\Reports\Q-TOFSystemTune_Positive_20210412_163642\Q-TOFSystemTune_Positive_20210412_163642.tun

Positive Polarity Results

Quad Results

Wide

Width Target	9.00	**Mass Tolerance**	1.00	**Width Tolerance**	1.00

m/z Target	Mass Delta	Width Delta	Efficiency (%)	Score	Result
322.05	-0.07	-0.01	93	99	Passed
2121.93	-0.10	-0.03	76	99	Passed

Width Gain	1904	**Width Offset**	752
Mass Gain	1636	**Mass Offset**	2802
Isolation Efficiency	85	**Score**	99
Result	Passed		

Medium

Width Target	4.00	**Mass Tolerance**	.50	**Width Tolerance**	.50

m/z Target	Mass Delta	Width Delta	Efficiency (%)	Score	Result
322.05	0.02	-0.03	87	99	Passed
2121.93	0.03	0.06	68	98	Passed

Width Gain	1918	**Width Offset**	1516
Mass Gain	1635	**Mass Offset**	2481
Isolation Efficiency	77	**Score**	98
Result	Passed		

Narrow

Width Target	1.30	**Mass Tolerance**	.15	**Width Tolerance**	.15

m/z Target	Mass Delta	Width Delta	Efficiency (%)	Score	Result
322.05	0.00	0.00	67	100	Passed
2121.93	0.09	-0.05	38	57	Passed

Width Gain	1929	**Width Offset**	1970
Mass Gain	1634	**Mass Offset**	2296
Isolation Efficiency	53	**Score**	70
Result	Passed		

Lens 2

Lens 2 Phase	180.0

Mass	118.1	322.0	922.0	1522.0	2121.9	2721.9
RF Voltage	16.6	76.3	144.4	197.7	245.8	300.3
DC Voltage	9.0	19.2	12.1	6.0	1.0	1.1

Total Ion

Quad AMU	127.1

Quad Overall

Overall Score	88	**Overall Result**	Passed

Figure 16. Tune report (partial)

- Or open the **Reports** folder (**Figure 17**) and double-click the report you intend to view.

  Tune report names automatically include type of tune or calibration and the date and time.

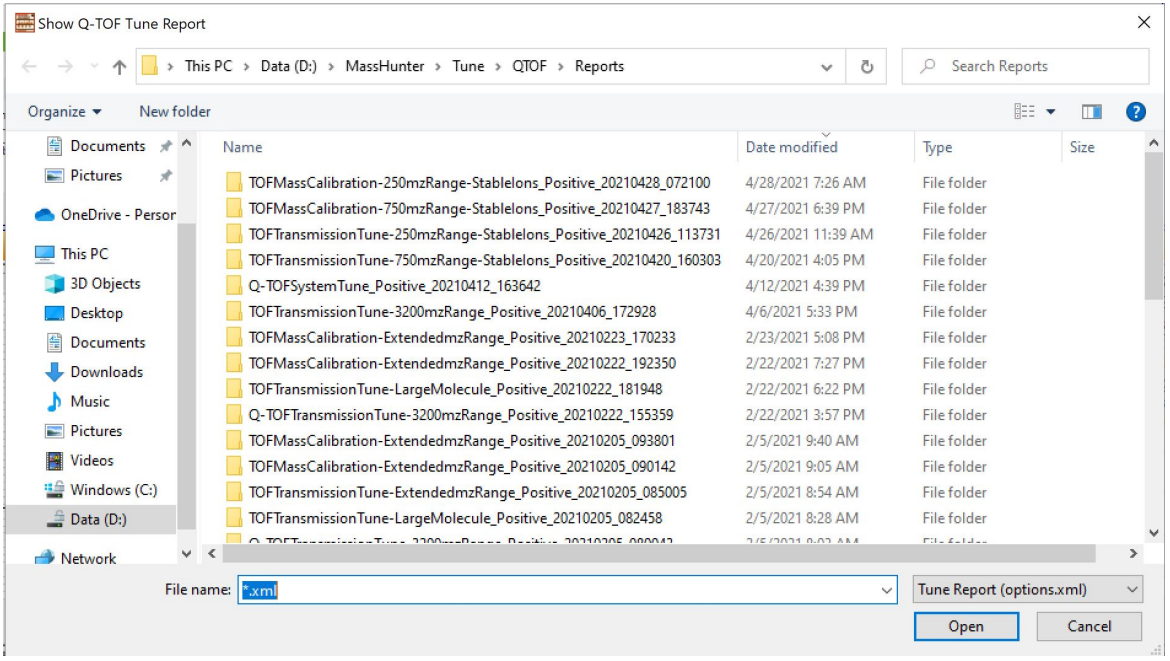

Figure 17. Location of tune reports

To check the Quad AMU in the tune report

No matter the kind of tune, check the **Quad AMU** in the tune report to make sure the number is consistent with the mass and stability of molecule you are looking at (**Figure 16** on page 18). The Quad AMU will be lower the lower the mass and even lower the more fragile the molecule is.

If the Quad AMU is not consistent or you see a lower abundance of certain calibrant ions, change the **Quad AMU** in the **Manual Tune > Quad** tab and click **Apply**.

If the Quad AMU value after the quad tune is higher than its value after the TOF tune, change the value to the TOF tune value and click **Apply**.

To save a tune file

1 In the **Tune Window**, click the **Instrument State** tab.

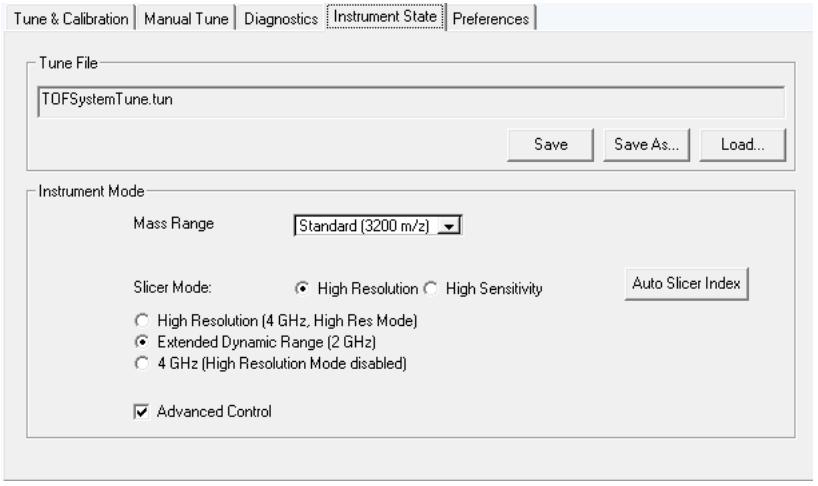

Figure 18. Instrument State tab

2 To save changes to the current loaded file, click **Save**, or

To save changes to a new file:

a Click **Save As**.

b Enter a name for the tune file that is descriptive. These are useful descriptors from the Tune & Calibration tab and the Instrument State Tab:

- Polarity
- Type of action (tune or calibration)
- Tune type (Q-TOF System or TOF transmission, for examplze)
- Slicer mode
- Extended dynamic range or High Res
- Mass Range
- Fragile Ions selected
- Date and time
- Comment

For example, to save the selected options in **Figure 14** on page 16, you might save the file as TOFMassCalibration-1700mzRange-EDR-polarity-Date and Time.tun.

c Click **Save**.

3 When confirmation messages appear, click **Yes**, unless the message asks to save the layout. In that case, click **No**.

Tune and Calibration Sequences

Basic tune and calibration (except 6546)

Figure 19 shows the basic tune and calibration sequence that applies to Q-TOF instruments other than the Agilent 6546 LC/Q-TOF. For 6546 instructions see **"Basic tune and calibration (6546)"** on page 23.

For detailed instructions on the basic sequence for all models, see **"Basic Tune and Calibration"** on page 3.

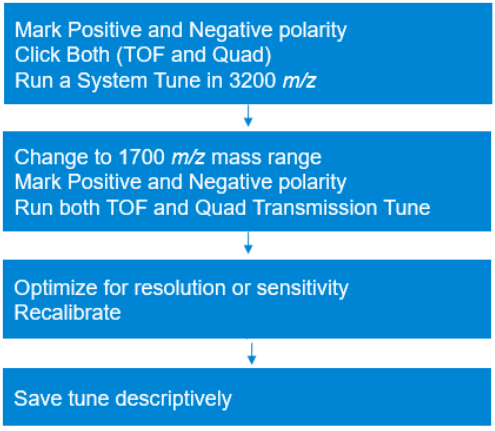

Figure 19. Basic tune and calibration sequence for a Q-TOF LC/MS other than the 6546

While most applications use the 1700 *m/z* range, only a system tune in 3200 *m/z* yields the best performance and begins the basic tune sequence.

See **"To check the Quad AMU in the tune report"** on page 19 to learn how to check the Quad AMU after each tune.

Calibration occurs automatically as the last step of every preprogrammed tune.

Also do a mass calibration step after:

- **Manual Tune** tab changes, except those in the Optics 1, Quad, and IM tabs
- Change in the **Slicer Mode** (**High Resolution** or **High Sensitivity**)
- Change in **Slicer Position** in the **Indexed Slicer** dialog box

Specific recommendations for various tune and calibration sequences follow.

Basic tune and calibration (6546)

Figure 20 shows the basic tune and calibration sequence for the Agilent 6546 LC/Q-TOF.

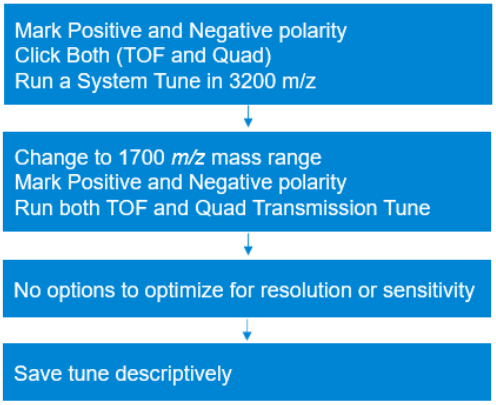

Figure 20. Basic tune and calibration sequence for an Agilent 6546 LC/Q-TOF

Here are some considerations for the Agilent 6546 basic tune and calibration:

- Never use the **High Sensitivity** slicer position.
- **10 GHz** is the only operating mode.
- Calibrate after every manual tune change, except for Optics 1, Quad, and IM changes. When you do a preprogrammed tune, the system automatically does a calibration.
- See **"To check the Quad AMU in the tune report"** on page 19 to learn how to check the Quad AMU after each tune.
- Confirm that you still see calibrant ions in your application range. Depending on the cut-off, you might need to manually increase or decrease the **Quad AMU** to see higher/lower *m/z* ions in sufficient intensities.
- Check the optics and resolution after the **Positive** tune and then after the **Negative** tune.

Low-mass tune and calibration (6530, 6545, 6545XT, 6546)

Figure 21 shows the tune and calibration sequence for low-mass analyses on the 6530, 6545, 6545XT, and 6546. The two options are a 250 *m/z* mass range and a 750 *m/z* mass range.

In MassHunter Acquisition 10 and later, you should observe a gain in abundance going to the low-mass ranges, when compared with the 1700 *m/z* acquisition mode.

These sequences assume that you have performed the basic tune sequence immediately prior to each sequence, or that the current resolution and ion abundances show you do not need to repeat the basic tune sequence.

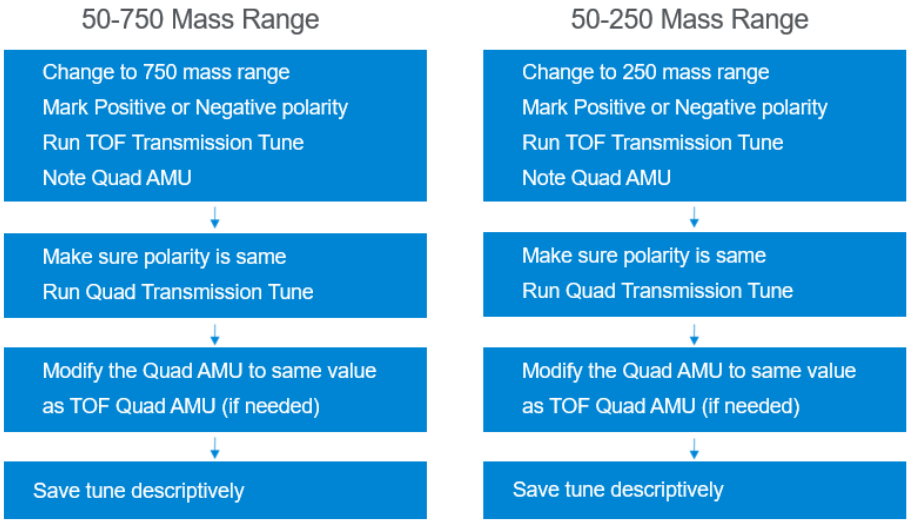

Figure 21. Low-mass tune and calibration (6530, 6545, 6545XT, 6546)

NOTE

The low-mass options only apply to the TOF tune. Once the TOF tune is completed using a low-mass setting, a quad tune can be done. The system is already set up with the lower voltages necessary for low-mass molecules even though the low-mass settings are unavailable for the quad tune. This also applies to the low-mass tune for the 6550 and 6560.

Here are other considerations for low-mass tune and calibration:

- For the **6546**, never select **High Sensitivity** as the **Slicer Mode.**
- If you have a **6546**, **10 GHz** is the only operating mode.
- The **Quad Tune** is required for the lower mass options because the ion optics voltages change when going from the system tune to the lower mass and fragile ion tunes, making the tuned values from the Quad Tune at 3200 *m/z* invalid for the low-mass and fragile ion tunes.
- Always check the **Quad AMU** value before and after doing a TOF or Quad tune. You can find the **Quad AMU** value under the **Manual Tune > Quad** tab or in the most recent tune report. Write down the Quad AMU after the TOF tune. If this value increases after the Quad tune, enter the TOF value instead, and click **Apply**.
- Confirm that you still see calibrant ions in your application range. Depending on the cut-off, you might need to manually increase or decrease the **Quad AMU** to see higher/lower *m/z* ions in sufficient intensities.
- Check the optics and resolution after the **Positive** tune and then after the **Negative** tune.

Fragile tune and calibration (6530, 6545, 6545XT, 6546)

Figure 22 shows the tune and calibration sequence for fragile mass analyses on the 6530, 6545, 6545XT, and 6546. In MassHunter Acquisition 10 and 10.1 you should observe a gain in fragile ion abundance (with minimized fragmentation) with the **Fragile Ions** setting when compared with the normal *m/z* 1700 acquisition mode.

This sequence assumes that you have performed the basic tune sequence immediately prior to the sequence, or that the current resolution and ion abundances show you do not need to repeat the basic tune sequence.

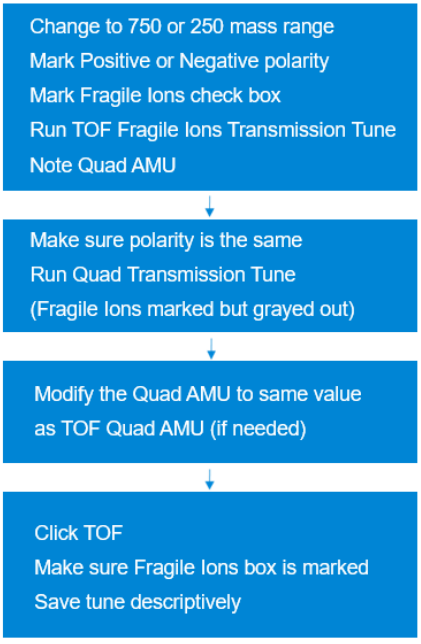

Figure 22. Fragile ions tune and calibration (6530, 6545, 6545XT, 6546)

NOTE

The Fragile Ions option only applies to the TOF tune. Once the TOF tune is completed using the fragile ions setting, a quad tune can be done. The system is already set up with the lower voltages necessary for fragile molecules even though the fragile ions setting is unavailable for the quad tune. This also applies for the fragile ions tune for the 6550 and 6560.

If you reload the tune and want to calibrate, having the fragile box marked ensures that low-mass calibrant ions applicable to the fragile setting are used during the calibration. You can only mark the Fragile Ions check box if you have selected TOF before saving the tune.

Here are other considerations specific for fragile molecule tune and calibration:

- For the **6546**, never select **High Sensitivity** as the **Slicer Mode.**
- Tune in **Extended Dynamic Range** mode. If you have a 6546, **10 GHz** is the only calibration mode. For models other than the 6546, you can change to the **4 GHz High Resolution** calibration mode to optimize resolution.
- The **Quad Tune** is required for the Fragile Ions option because the ion optics voltages change when going from the system tune to the lower mass and fragile ion tunes, making the tuned values from the Quad Tune at 3200 *m/z* invalid for the low-mass and fragile ion tunes.
- Always check the **Quad AMU** value before and after doing a TOF or Quad tune. You can find the **Quad AMU** value under the **Manual Tune > Quad** tab or in the most recent tune report. Write down the Quad AMU after the TOF tune. If this value increases after the Quad tune, enter the TOF value instead, and click **Apply**.
- Confirm that you still see calibrant ions in your application range. Depending on the cut-off, you might need to manually increase or decrease the **Quad AMU** to see higher/lower *m/z* ions in sufficient intensities.
- Check the optics and resolution after the **Positive** tune and then after the **Negative** tune.

Low-mass and fragile tune and calibration (6550)

Figure 23 shows the tune and calibration sequences for low-mass and fragile analyses on the 6550.

These sequences assume that you have performed the basic tune sequence immediately prior to each sequence, or that the current resolution and ion abundances show you do not need to repeat the basic tune sequence.

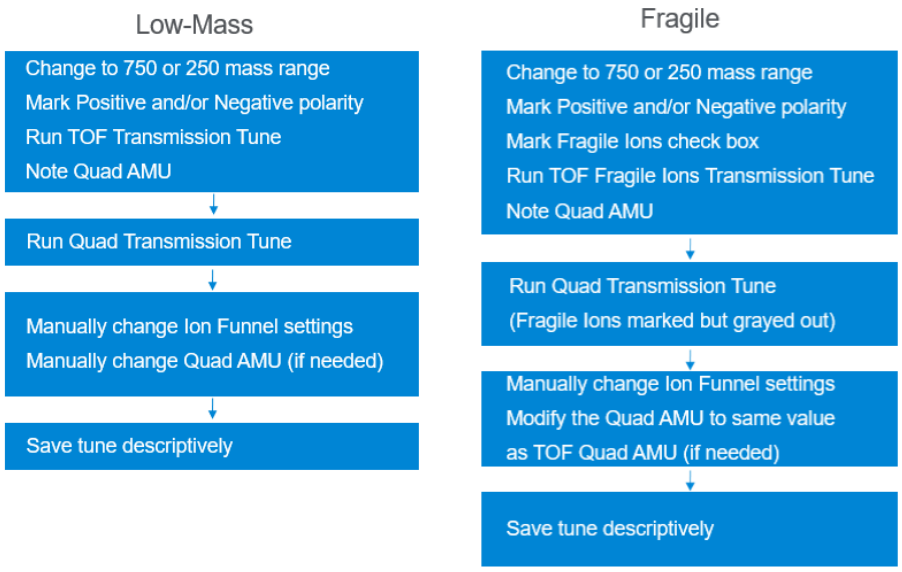

Figure 23. Low-mass and fragile tune and calibration sequences for the 6550

For the 6550 ion funnel instrument, the sensitivity drops in the higher masses going from a 1700 *m/z* tune to a low-mass tune (750 or 250 *m/z*) or a fragile tune because the default RF voltages for the 1700 *m/z* tune are too high for both tunes. See **Table 1** on page 29 for recommended RF Voltage settings for the 6550 low-mass and fragile tunes.

For the 6550 ion funnel instrument the default settings for RF voltages on the high-pressure (HP) and low-pressure (LP) funnels are set for optimum transmission of stable ions over a wide mass range.

For low-mass applications and for fragile ions, **Table 1** lists the recommended Manual Tune RF voltages to improve ion transmission while also reducing fragmentation of ions in the funnels.

Table 1. Recommended settings for low-mass and fragile analyses on the 6550

	6550 Tune (default)	6550 Generic small molecule	6550 Very small molecule (*m/z* < 120) and fragile adduct
Funnel DC	50	50	30
Funnel Voltage Drop (High Pressure Funnel)	150	150	100
RF Voltage (High Pressure Funnel)	200	150	90
Funnel Voltage Drop (Low Pressure Funnel)	100	100	50
RF Voltage (Low Pressure Funnel)	100	60	40
			Fragmentor to 175
		Quad AMU 120	Quad AMU 100

Other considerations for the Agilent 6550 low-mass and fragile tune and calibration:

- The **Quad Tune** is required for the lower mass and Fragile Ions option because the ion optics voltages change when going from the system tune to the lower mass fragile ion tunes, making the tuned values from the Quad Tune at 3200 *m/z* invalid for the low-mass and fragile ion tunes.
- Always check the **Quad AMU** value before and after doing a TOF or Quad tune. You can find the **Quad AMU** value under the **Manual Tune > Quad** tab or in the most recent tune report. Write down the Quad AMU after the TOF tune. If this value increases after the Quad tune, enter the TOF value instead, and click **Apply**.
- These are generic values for low-mass and fragile ions. Your system may need different values, which *must* be applied in the Advanced tab of the method in the MS acquisition display before acquisition.

Low-mass and fragile tune and calibration (6560)

Figure 24 shows the tune and calibration sequences for low-mass and fragile molecule analyses on the 6560.

These sequences assume that you have performed the basic tune sequence immediately prior to each sequence, or that the current resolution and ion abundances show you do not need to repeat the basic tune sequence.

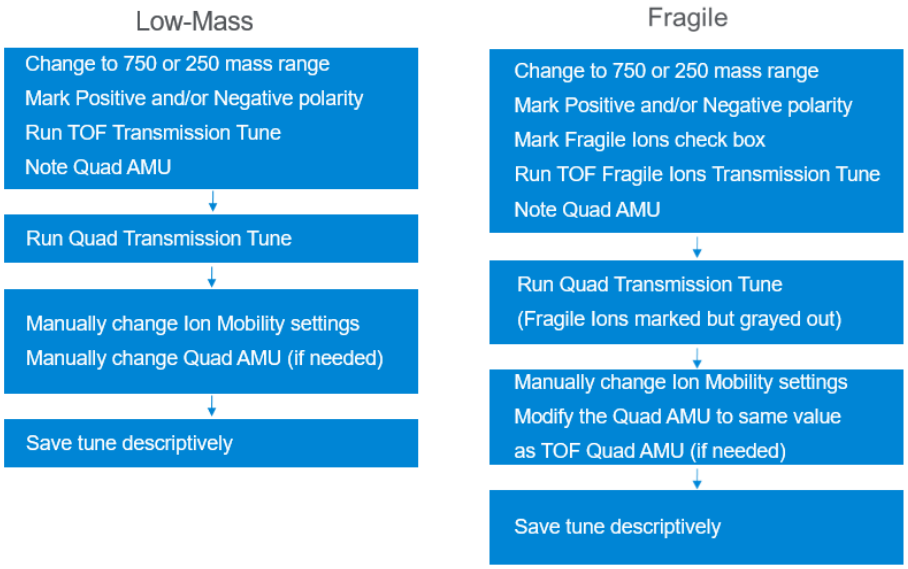

Figure 24. Low-mass and fragile molecule tune sequences for the 6560

For the 6560 ion funnel instrument the situation is more complex than with the 6550 because the 6560 is also an ion mobility instrument. For low-mass and fragile tunes in Q-TOF mode, its high-pressure RF voltage would be the same as that of the 6550. But the 6560 has additional Manual Tune IM parameter values that affect sensitivity for both low-mass and fragile ion tunes.

In IM Q-TOF mode, both the high-pressure and trap funnel RF values are too low, as is the trap release time. These low values decrease the sensitivity in both the low and high masses in the spectrum. **Table 2** lists the recommended Manual Tune values in IM Q-TOF mode.

Table 2. Recommended settings for low-mass and fragile analyses on the 6560

Mass Range	Fragmentor (V)	High Pressure and Trap Funnel RF (V)	Rear Funnel Exit (V)	Ion Mobility Hexapole Entrance (V)	Trap Fill Time (ms)	Trap Release Time (us)
1700 (Default)	400	150	43	41	20	150
50 - 750	400	120	43	41	15	300
50 - 750 Fragile Ion	380	130	36	34	15	300
50 - 250	400	100	42	41	15	300
50-250 Fragile Ion	380	100	36	34	15	300

Other considerations for the Agilent 6560 IM LC/Q-TOF:

- The **Quad Tune** is required for the lower mass and Fragile Ions option because the ion optics voltages change when going from the system tune to the lower mass fragile ion tunes, making the tuned values from the Quad Tune at 3200 *m/z* invalid for the low-mass and fragile ion tunes.
- Always check the **Quad AMU** value before and after doing a TOF or Quad tune. You can find the **Quad AMU** value under the **Manual Tune > Quad** tab or in the most recent tune report. Write down the Quad AMU after the TOF tune. If this value increases after the Quad tune, enter the TOF value instead, and click **Apply**.
- These are generic values for low-mass and fragile ions. Your system may need different values, which *must* be applied in the Advanced tab of the method in the MS acquisition display before acquisition.

High-mass tune and calibration (6545XT)

Figure 25 shows the tune and calibration sequence for high-mass analyses on the 6545XT.

This sequence assumes that you have performed the basic tune sequence immediately prior to this sequence, or that the current resolution and ion abundances show you do not need to repeat the basic tune sequence.

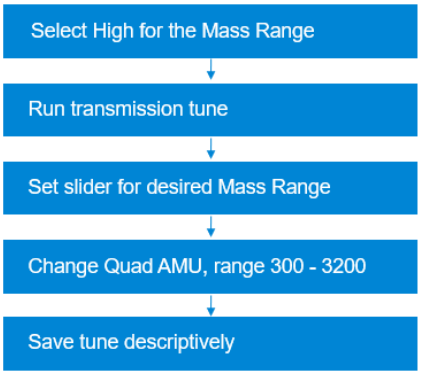

Figure 25. High-mass tune and calibration (6545XT)

The transmission tune in the 10,000 *m/z* range on the 6545XT (default range is 100-10,000 *m/z* when **High** is selected) consists of two parts:

- The actual tune, which occurs in the 3200 *m/z* range and generates a high-mass tune report after an automatic calibration

  The tune and calibration is occurring on the calibrant Agilent Tune Mix ions, whose masses do not exceed 3200 m/z.
- The high-mass calibration, which happens after the program completes the 3200 *m/z* tune and calibration, shifts to the 10,000 *m/z* extended mass range, and waits a 20-minute equilibration time.

Two reports are generated: a tune and calibration report after the tune has finished in the 3200 *m/z* range and a final calibration report in the 10,000 *m/z* range. Because most calibrant signals are saturated in the 10,000 *m/z* range, only the 3200 *m/z* tune and calibration in extended dynamic range mode gives calibrant abundance values that are reliable.

The slider allows you to change to higher masses, to a maximum of 3400 - 30,000. (Because the system cannot do 100 - 30,000 *m/z*, the lower end is adjusted to 3400.) The value of the **Quad AMU** depends on the mass to which you set the slider.

You are ready for acquisition after you:

- Set the slider **Mass Range** in the **Instrument State** tab (**Figure 26**) and click **Apply**.
- Enter a new **Quad AMU** in the **Manual Tune > Quad** tab, if necessary, and click **Apply**.

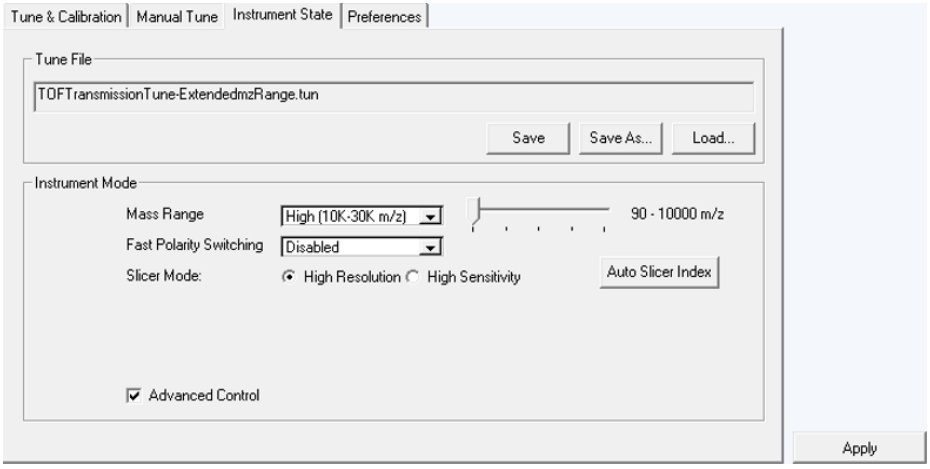

Figure 26. Slider for changing mass in the Instrument State tab

These are some high-mass tune considerations:

- Check the vacuum. Vacuum is critical for high-mass transmission and should be less than $10X10^{-8}$ Torr.
- When finished with high-mass work, reload the most recent tune.
- **Quad AMU** must be increased with an increase in protein size.

High-mass tune and calibration (except 6545XT)

Figure 27 shows the tune and calibration sequence for high-mass analyses on instruments other than the 6545XT.

This sequence assumes that you have performed the basic tune sequence immediately prior to this sequence, or that the current resolution and ion abundances show you do not need to repeat the basic tune sequence.

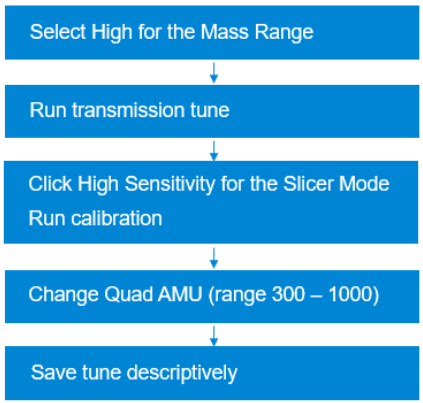

Figure 27. High-mass tune and calibration (not 6545XT)

This sequence is the same as that for the 6545XT with three exceptions:

- **High Sensitivity** is selected for the **Slicer Mode**.
- Depending on the instrument, the high mass range limit can be 10000 m/z or 20,000 m/z. None of these instruments go to 30,000 m/z.
- The slider does not appear for the high mass ranges for these models.

The following are some high-mass tune considerations:

- Vacuum is critical for high-mass transmission.
- When finished with the sample measurements, change the **Slicer Mode** back to **High Resolution**.
- When finished, reload the most recent 3200 *m/z* system tune file.

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In this Book

This book contains an overview and instructions to tune your Agilent Q-TOF LC/MS.

This guide supports all Agilent 6500 Series Q-TOF LC/MS systems except 6520, 6538, and 6540.

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