

Faculty/ Department or Institute/Centre	Cumming School of Medicine, Department of Pediatrics, Neurocritical Care Program, ACH BioCORE	Date Created:	December 3 2018
Location	<i>ACH B0-103</i>	Created By:	Matthew Rosin
Supervisor	Dr. Michael Esser & Dr. David Sinasac	Revision #:	1.0
		Revision Date:	December 3 2018
		Revised By:	Matthew Rosin

Purpose

This document provides instructions for tuning and calibrating the Agilent 6550 QTOF instrument.

Applicability & Authorization

Tuning and calibration is essential for accurate mass measurements on the QTOF. All users of the QTOF must receive training in BioCORE to perform the steps in this SOP. This SOP is critical for the proper tuning and calibration of the instrument to achieve accurate mass measurement.

Instrumentation

The Agilent 1290 UHPLC multi-sampler liquid chromatography system linked to the Agilent 6550 Quadrupole Time-Of-Flight (QTOF) mass spectrometer is in B0-110.

Background

Tuning is the process of adjusting both the quadrupole (for Q-TOF) and TOF parameters to maximize signal intensity and maintain acceptable resolution, or to maximize resolution and maintain acceptable signal intensity. Calibration is the process of assigning accurate masses based on the known masses of standard compounds. TOF mass calibration is performed by passing a calibrant with known masses from the calibrant bottle through the mass spectrometer. This can be done automatically (preferred) or manually. Automatic mass calibrations occur any time you perform any kind of tune (Mass Calibration/Check Tune, Quick Tune, Standard Tune, System Tune, and Initial Tune).

For the Agilent 6550 QTOF, always select the ion source called "Dual AJS ESI". All tunes can be performed when the instrument Mass Range is 3200 m/z, whereas limited options are available in 1700 m/z. Make sure Fast Polarity Switching is always disabled as this mode is harmful over time. Always select the 2-GHz Extended Dynamic Range instrument mode because this will enable up to 5 orders of magnitude of signal collection.

Initial Auto Tune only needs to be performed after installation or major service (venting), if the previous tune files were accidentally deleted, or if Standard Autotune does not work. It automatically adjusts all the tunable parameters to optimize signal, resolution, and mass axis calibration. Select both positive and negative polarities and "Both" Quad + TOF. Components of Initial Tune include optimization of the detector voltages, TOF transmission voltages, and quadrupole filtering parameters and voltages. Note that

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you must adjust the Collision Cell Gas pressure after running Initial Auto Tune using a procedure in the Help menu.

Materials & PPE

Reagents	Supplies	Personal Protective Equipment (PPE)
<ul style="list-style-type: none"> Tuning Mix Reference Calibration Mix MS-grade H2O MS-grade Formic Acid MS-grade Acetonitrile 	<ul style="list-style-type: none"> Dedicated glassware for MS-grade solutions Glass autosampler vials and caps 	<ul style="list-style-type: none"> Lab coat Safety goggles Nitrile gloves

Recipes for Tuning & Reference Mixes

Bottle A: Reference Mix (every 4 weeks)	Bottle B: Tuning Mix: ESI-L #G1969-85000
<ul style="list-style-type: none"> 15 mL water (previously 30 mL) 270 mL ACN (MS-grade, previously Methanol) [Optional: 150 uL TFA-NH4 (-ve mode only)] 115 uL 5mM purine (previously 100 uL) 30 uL HP-0921 (previously 100 uL) <p>(+) <350 m/z: 121.050875 (purine: need >5000 counts) (+) >500 m/z: 922.009798 (hexakis phosphazine) (-) <350 m/z: 112.985587 (TFA: need >5000 counts) (-) >500 m/z: 1033.988109 (hexakis phosphazine)</p>	<ul style="list-style-type: none"> 10 mL ESI-L 85.5 mL Acetonitrile (MS-grade) 4.5 mL H2O (MS-grade) 3 uL 0.1 mM HP-0321 (previously 5 uL) <p>Expected abundances (+) and (-) polarity: 50K – 450K</p> <p>Can dilute further if necessary.</p>

1. Q-TOF Standard Tune

What: Highest level of regular tune. Does not use SWARM algorithm (fails negative mass calibration).

When: Every 1-3 months, when transmission tune fails, after venting system for maintenance, after power outages, after software or hardware upgrades, and when performance issues arise that cannot be solved..

Description: Performs many of the same optimization operations as the Initial Tune but uses the current tune settings as starting values and does a limited set of ion optic ramps. Standard Tune on the TOF takes about 10-15 minutes and on the Quad takes about 10-15 minutes for each polarity.

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Preferences Tab:

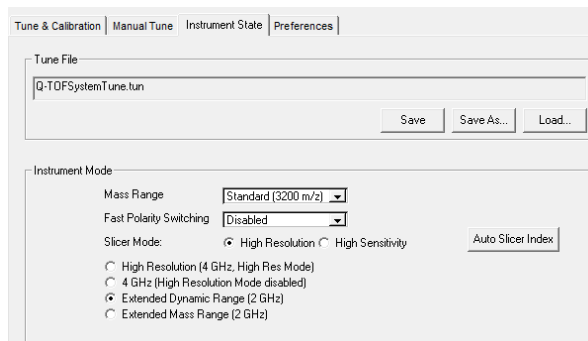
-Make sure the "Classic Autotune" check box is checked and "Disable CDS" is unchecked. Note that SWARM tune is optional on this model (6550 QTOF) but will fail System Tune due to issues with negative mass calibration.

Instrument State Tab:

- Mass Range: Standard (3200 m/z)
- Fast Polarity Switching: Disabled
- Slicer Mode: High Resolution (optional: Can select high sensitivity for a ~20% boost in sensitivity)
 - Auto Slicer Index: can select slicer position within High Res or High Sensitivity setting
- Click Apply
- A pop-up message will indicate that ~20 minutes is needed for equilibration (stabilization) of the TOF pulser (if mass range was 1700 m/z)

Tune & Calibration tab:

- Select Positive and Negative polarities
- Select Both (Quad + TOF)
- Select Standard Tune
- Ensure LC is going to Waste
- Click Start Standard Tune



For small molecule applications, proceed to **2. Quick Tune.**

For large molecule applications, proceed to **3. Check Tune.**

2. Quick Tune (TOF only)

What: Second level of tune. Used to refine tune for specific applications. Increases sensitivity in the low mass range by ~20 – 30%.

When: Every week, and any time the mass range is changed (between 1700 and 3200 m/z). A change to mass range will require ~20 min equilibration time for pulser to stabilize.



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Description: A TOF Quick Tune can be done in one or both polarities. The purpose of this tune is to optimize the electric voltages involved in TOF transmission and resolution (not detector). Tune in positive polarity takes ~5 minutes.

Instrument State Tab:

- Mass Range: Select low mass 1700 m/z (or can select 3200 m/z if switching from 1700 m/z)
- Fast Polarity Switching: Disabled
- Slicer Mode: High Resolution
- Click Apply
- A pop-up message will indicate that ~20 minutes is needed for equilibration

Manual Mass Calibration tab:

- Optional: if masses <100 m/z are of interested, add the low mass (+) ion 64.01577 (ACN + Na) and the (-) ion 68.9958 (CF3)

Tune & Calibration tab:

- Select Positive and Negative polarities
- Select TOF
- Select Quick Tune
- Start TOF Quick Tune

*For daily use of the Q-TOF, or if certain modifications are made to the instrument state, proceed to **3. Check Tune.***

3. Mass Calibration/Check Tune (TOF only)

What: Third level of tune. Performs mass calibration only, makes no changes to voltages.

When: Daily, before every run, if the room temperature has changed, any time the slicer is changed (type or position), if the instrument mode is changed from 2 GHz to 4 GHz (not advised as this sacrifices extended dynamic range), after changing mass range (depends on application), after changing polarity (only if Standard Tune was done in both polarities).

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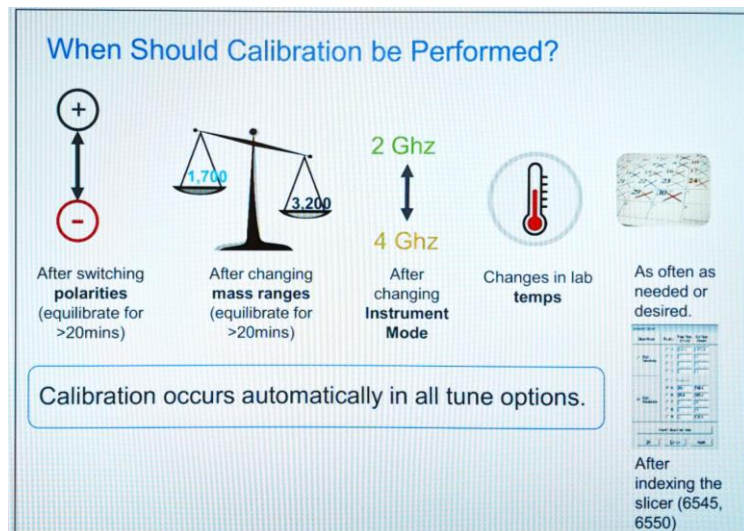
Description: A TOF Check Tune simply performs a mass calibration to ensure accurate mass detection. This is important to perform each day, and before every run if any of the above changes have been made.

Instrument State Tab:

- Mass Range: select Low (1700 m/z) or Standard (3200 m/z)
- Fast Polarity Switching: Disabled
- Slicer Mode: High Resolution (optional: Can select high sensitivity for a ~20% boost in sensitivity)
- Click Apply
- If changes have been made, a pop-up message will indicate that ~20 minutes is needed for equilibration

Tune & Calibration tab:

- Select Positive and/or Negative polarity
- Select TOF
- Select Check Tune/ Mass Calibration
- Start Check Tune/Mass Calibration



When Should Calibration be Performed?

- After switching polarities (equilibrate for >20mins)
- After changing mass ranges (equilibrate for >20mins)
- After changing Instrument Mode
- Changes in lab temps
- As often as needed or desired.
- After indexing the slicer (6545, 6550)

Calibration occurs automatically in all tune options.

4. Set Detector Gain

What: Optional, rare tune. This is a subset of Initial Auto Tune.

When: Only if a problem is suspected that cannot be fixed by Mass Calibration/Check or Quick Tune, specifically related to issues with apparently low dynamic range (unexpected signal saturation). After Set Detector Gain, must repeat Quick Tune.

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Description: TOF only. Adjusts the PMT voltage to obtain consistent gain (amplification) of the ion current into electrical current. If mode is set to Extended Dynamic Range (2 GHz), it also adjusts the preamp offset values and time delay between gain channels.

5. Evaluating Tune Performance

a. Quadrupole Tune: narrow, medium, and wide mass window

- i. Point 1: Any ions present in the MS1 scan that are within the isolation width of the Quad can appear in the MS2 scan.
- ii. Point 2: The narrower the isolation width, the lower the isolation efficiency (signal).
- iii. Narrow: ~1.3 Da. Use for building or searching MS/MS spectral libraries, to improve quality of unidentified MS/MS spectra, and to increase the score of identified compounds. Disadvantage is lower signal.
- iv. Medium: ~4 Da. Good for general MS/MS work. Higher signal, risk of chimeric spectra.
- v. Wide: ~9 Da. Not advised for general use.
- vi. Inspect Quad Tune Report scores at low and high m/z ions
- vii. For small molecule work, it is more important to have a higher score in each window for the low mass (322 m/z)
- viii. Score >80 is desired for 322 m/z ion in all windows
- ix. Score >50 is desired for 2122 m/z ion in all windows
- x. Repeat Quad Tune if the scores are out of these ranges

b. Calibration: Resolution

- i. Inspect the resolution column at each calibrant mass
- ii. Resolution will inherently be lower at lower mass
- iii. Ensure that all calibrants show at minimum resolution of 10,000

c. Primary Residuals

- i. Inspect the primary residuals column at each calibrant mass
- ii. For the 6550 QTOF, primary residuals should be <0.5 ppm, and are commonly ~0.4 ppm or lower
- iii. If primary residuals are >0.5 ppm, then Q-TOF system tune needs to be repeated

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