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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 the BioCORE to understand the steps in this SOP, however only BioCORE technicians are allowed to perform tuning on the QTOF instrument. 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 and associated data acquisition PC is in B0-110.

Background

Tuning is the process of adjusting both the quadrupole and TOF parameters to optimize mass accuracy (resolution) and monitor signal abundance (sensitivity) of tune ions. 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 solution with metabolites of known masses from the calibrant bottle into the primary nebulizer for ionization and into the QTOF. This can be done automatically (preferred) or manually. Automatic mass calibrations occur any time you perform any kind of tune (Mass Calibration/Check Tune, Standard 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 to the instrument hardware over time. Always select the 2-GHz Extended Dynamic Range instrument mode because this will enable up to 5 orders of magnitude of signal to be collected.

Initial Auto Tune only needs to be performed after installation or major service (venting for preventive maintenance or capillary cleaning, for example), if the previous tune files were accidentally deleted, or if Standard Tune 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 Autotune include optimization of the detector voltages, TOF transmission voltages,

and quadrupole filtering parameters and voltages. Note that 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)
• Tuning Mix • Reference Mix • MS-grade H2O • MS-grade Acetonitrile	• Dedicated glassware for MS-grade solutions • Glass autosampler vials and caps	• Lab coat • Safety goggles • Nitrile gloves

Recipes for Tuning & Reference Mixes

Bottle A: Reference Mix (every 4 weeks/as needed)	Bottle B: Tuning Mix: ESI-L #G1969-85000
• 270 mL ACN (MS-grade) • 15 mL H2O (MS-grade) • 145 uL 5mM purine (positive mode only) • 50 uL HP-0921 • Optional: 150 uL TFA-NH4 (negative mode only) (+) 121.050875 (purine) (+) 922.009798 (hexakis phosphazine) (-) 112.985587 (TFA) (-) 1033.988109 (hexakis phosphazine)	• 85.5 mL Acetonitrile (MS-grade) • 10 mL ESI-L (Tune Stock Solution) • 4.5 mL H20 (MS-grade) • 3 uL 0.1 mM HP-0321 (previously 5 uL) Expected abundances (+) and (-) polarity: 100K – 1M

1. Initial Autotune

What: Highest level of tune. [Do not use SWARM algorithm as it fails negative mass calibration.]

When: Initial QTOF setup, after venting instrument for any kind of maintenance, if Standard Tune fails, after power outages, after hardware upgrades, and when performance issues arise that cannot be solved..

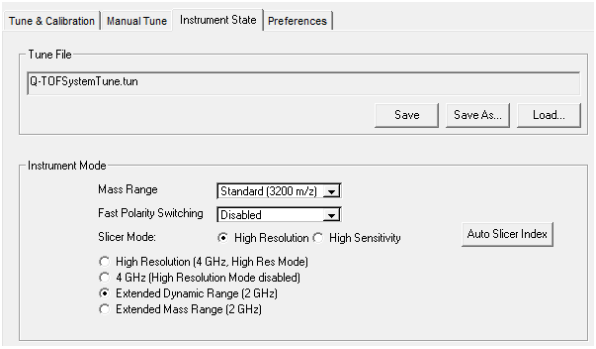
Description: Performs total system optimization of Q+TOF without assuming any previous tune values. Performs ion optic ramps, optimizes voltages, optimizes mass resolution using tune ions. Initial Autotune takes a total of ~2 hours (30 min per Quad/TOF per polarity).

Preferences Tab:

-Make sure the "Classic Autotune" check box is checked and "Disable CDS" is unchecked. Note that SWARM tune option is present 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 Resolution 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. Standard Tune.***
*For large molecule applications, proceed to **3. Check Tune.***

2. Standard Tune (Quad and TOF)

What: Second level of tune. If mass range is changed to 1700 m/z, then it can be used to increase sensitivity in the low mass range by ~20 – 30% for small molecule applications.

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 the pulser to stabilize.

Description: Performs many of the same optimization operations as the Initial Autotune but uses the current tune settings as starting values and does a limited set of ion optic ramps. No changes will be made

to the detector voltages. Standard Tune on the TOF takes about 10-15 minutes and on the Quad takes about 10-15 minutes for each polarity.

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 Quad + TOF
-Select Standard Tune
-Start Q+TOF Standard 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 a check of the 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).

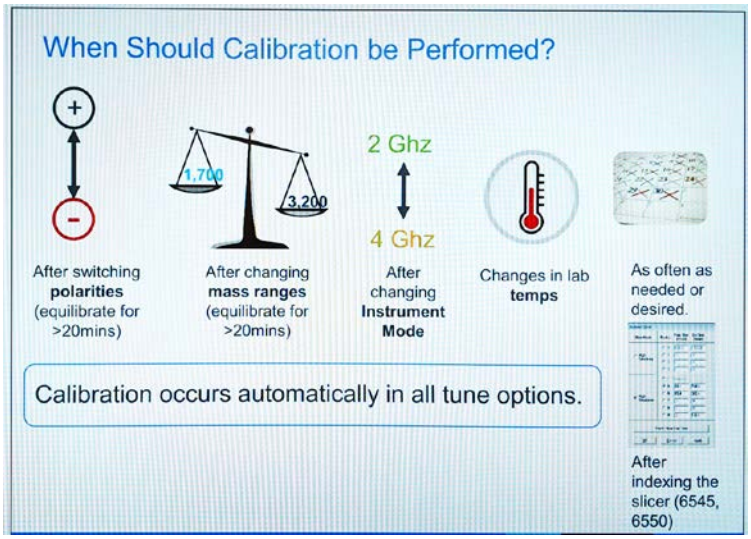
Description: A TOF Check Tune simply performs a mass calibration to ensure accurate mass detection. This is important to perform each day, and before every run if any of the above changes have been made.

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



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) or overall low signal abundance of all tune ions (low sensitivity, likely a dirty capillary problem). After Set Detector Gain, must repeat Standard Tune.

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

d. Graphing Tune Ion Abundances & Primary Residuals

i. Record regular tune report ion abundances, resolution, and primary residual values from TOF tune reports to monitor systemic issues such as sensitivity loss (due to dirty capillary), mass accuracy/resolution loss (hardware issue), and residuals/mass drift (indicates if higher level tune is needed). Use an ongoing excel sheet for this.

Personnel Name: _____ Date Printed: _____