

Faculty/ Department or Institute/Centre	Cumming School of Medicine, Department of Pediatrics, Neurocritical Care Program, ACH BioCORE	Date Created:	March 12 2019
Location	<i>ACH B0-103</i>	Created By:	Matthew Rosin
Supervisor	Dr. Michael Esser & Dr. David Sinasac	Revision #:	1.0
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		Revised By:	Matthew Rosin

Purpose

This document provides basic guidance for troubleshooting various aspects of running the Agilent LC modules and QTOF 6550 Instrument.

Applicability & Authorization

Only trained BioCORE staff and trained researchers are allowed to use the LC-QTOF system. Training is provided by BioCORE staff.

Instrumentation & Software

The Agilent 1290 UHPLC multi-sampler liquid chromatography system linked to the Agilent 6550 Quadrupole Time-Of-Flight (QTOF) mass spectrometer. MassHunter Data Acquisition software is used to control settings for both the LC and the QTOF, and will be used in this SOP.

Reference SOPs

Users should be familiar with the following SOPs and refer to them when needed:

- QTOF Tuning & Calibration_ACHBC00008_v1.0
- LC-QTOF General maintenance_ACHBC00010_v1.1
- Data Acquisition & QTOF Methods_ACHBC00012_v1.0

Background

The LC-QTOF needs to be carefully operated and maintained on a regular basis to function properly. Carelessness in monitoring solvent levels, preparing new solvent, sample preparation, tuning the instrument, cleaning the source, etc. can result in various negative outcomes. Less frequently, sensor malfunctions or other instrument faults independent of user error can cause the QTOF to error out, halting a running worklist immediately.

This document aims to keep an up-to-date list of known problems and solutions. If you encounter an error while operating the LC-QTOF, please let a BioCORE Technician know right away. If relevant to other users, these troubleshooting cases will be added to future versions of this document.

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Case 1: Wash Solvent S1 Bottle Empty

Symptoms:

- Successive runs show increasing abundance of 449 m/z ion in MS1 mode
- Running blank samples continue to show increasing abundance of 449 m/z ion

Cause:

- Wash Solvent S1 bottle is empty and resulting in air being pulled 'in-line' during the seat back flush step of multi-wash.
- The identity of the 449 m/z ion is unknown

Solution:

- Refill Wash Solvent S1 with fresh, MS-grade solvent
- Create a method for running blanks and in the multi-wash step, add 20 seconds to each step of the multi-wash and select seat back flush and needle wash. Run 5x blank samples and manually check that solvent fills the tubing. Monitor the 449 ion, and continue to run blanks if necessary to remove the ion from the spectrum (or to background levels).

Case 2: Contaminant Ions in Solvent Blanks

Symptoms:

- Blank injections show high levels of contaminant ions in MS1 mode
- Common ion contaminants may include Erucamide [(M+H)⁺ 338 m/z and (M+Na)⁺ 360 m/z], Phthalates [209 m/z]

Cause:

- Contamination arises very easily in glassware, including MS-designated glassware
- For example, Methanol was poured into a 250 mL Nalgene glass bottle before pipetting MeOH into a vial as a blank, this blank will very likely have contaminant ions for Erucamide and others.
- Phthalate contamination can arise from tubing, MeOH solvents, and N₂ generators.

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Solutions:

- Follow a strict cleaning SOP for MS glassware
- Use new glassware for pouring a one-time working volume of solvent (for compound resuspension, blanks, etc.)
- Identifying the source of Phthalate contamination may be difficult or impossible, so users may instead have to remove this ion from data analysis later.

Case 3: TOF Vacuum Error

Symptoms:

- Instrument State tab indicates the TOF vacuum is failing ($>10^{-7}$)
- Instrument State tab indicates an abnormally low QUAD vacuum ($<10^{-5}$)
- QTOF Indicator light is **red** indicating a problem

Cause:

- Anytime the QTOF instrument sees a hardware issue, it turns off the High Vacuum Sensor.
- The most common cause is a Nitrogen supply issue. Nitrogen pressure rising above or falling below the MS set points will cause this error.
- Nitrogen pressure exceeding 105 psi will trigger the Excess Pressure Relief Valve that bleeds off excess nitrogen gas to protect the instrument. This will dump excess N₂ directly to the atmosphere, which will sound like a hissing sound at the left rear of the instrument. When this happens, the Nitrogen pressure may drop too far (<80 psi) for a period of time, causing a hardware fault.
- The High Vacuum Sensor is interlocked with the High Voltage Power Supply, and when the sensor turns off, it turns off the high voltage power supply to protect the flight tube from possibly catastrophic failures.
- Anything, such as Nebulizer Pressure failing to maintain the desired set point will cause a hardware fault, the High Vacuum Sensor turns off, and the High Voltage power supply shuts off.
- Electrical power to the Nitrogen generator temporarily turning off also may cause a nitrogen pressure drop, causing the MS being unable to maintain the Nebulizer (and other gas flows) pressure, resulting in shutting down the High Vacuum Sensor.
- Nebulizer pressure momentarily dropping is the most common issue for this sort of shutdown.

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- In the system logbook, filtering by 'error', you may see a message indicating "Unable to maintain nebulizer pressure" or more cryptically "Nebulizer Pressure Duty Cycle Exceeded" that happened around the time the high vacuum sensor turns off.

Solutions:

- Power Cycle the QTOF by pressing the power button to turn off, waiting 5 seconds, and then pressing the power button again to turn ON.
- Check and record vacuum levels in the operation and maintenance excel log sheet. If the error continues, immediately stop your work and notify a BioCORE Technician.

Case 4: QTOF Tuning "Bad Coefficients Returned"

Symptoms:

- Tuning the QTOF fails in a given mode and gives the error message "Bad Coefficients Returned"
- Error message in tuning dialog box gives "Error-1: Error 438 object"
- The tuning mix ions in the MS spectrum appear at very different m/z than expected.

Cause:

- The tune file that is currently loaded in DA is corrupted, resulting in bad m/z assignments that are too far away from the theoretical m/z for the tune to succeed.

Solutions:

- Load an older tune file that was successful and is not corrupt.
- Repeat the QTOF tune and when complete, save the tune file with a unique name for future reference if needed.

Case 5: Binary Pump 2 not loading in DA

Symptoms:

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- Loading DA gives various error messages for Method Editor pane failure and time failures
- DA loads only a single “Binary Pump”, instead of “Binary Pump 2”

Cause:

- The Voyager Engine fails to recognize the correct S/N for the Binary Pump 2 module.

Solutions:

- Put QTOF on Standby, Close DA software, Turn off Voyager Engine, Power Cycle PC, Power Cycle all LC modules.
- Open DA software: both Binary Pump 1 and Binary Pump 2 should be recognized. This may show errors, but ignore these.
- Turn off Binary Pump 1 module (bottom-most module), close DA, and Turn off Voyager Engine.
- Restart Voyager Engine first, then open DA software. No errors should occur during DA loading and the Binary Pump 2 should be the only pump module present.
- If this does not work, cease operations and notify a BioCORE Technician immediately.

Case 6: Column Compartment will not go to “Ready”

Symptoms:

- Column Compartment state will not reach the “Ready” state, despite reaching temperature.

Cause:

- The compartment door sensor is not recognizing the door as closed.

Solutions:

- Apply some pressure to the top of the column compartment door and check for the Ready state in DA and a green indicator light on the module.

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Case 7: Reference Ion abundances/ratio suddenly change

Symptoms:

- MS spectra in DA show a sudden, unexpected, large difference in the Reference Ion abundances and/or ratio (high m/z / low m/z).
- This may happen after completing a worklist, despite the LC and QTOF setting being the same as before the worklist was started.

Cause:

- Unknown as of yet, but suspected that the LC-QTOF settings are switched after completing a worklist such that the different ion abundances are due to different ionization efficiencies.

Solutions:

- Select the Method of choice for conditioning the LC-QTOF system. Hit Apply for the Method, and for the Use Reference Ions. Change LC Stream so that it is going to waste, and hit Apply. Change flow rate to 5 mL/min for 1 minutes. Reduce flow rate to the original flow rate for the Method. Switch the LC stream back to MS, and hit Apply. Check that the Reference Ion abundances and ratio are fixed.
- If this does not work, inform BioCORE Technician for assistance.

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