LC-QTOF General Maintenance
Standard Operating Procedure
Document ID: ACHBC00010

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Location	*ACH B0-103*	Revision #:	1.2
		Revision Date:	October 18 2019
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Purpose

This document provides basic maintenance instructions for the Agilent 6550 LC-QTOF mass spectrometer.

Applicability & Authorization

Only trained BioCORE staff and trained researchers are allowed to perform general maintenance on the LC-QTOF. 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 in both the LC and the QTOF, and will be used in this SOP.

Background

General maintenance procedures on the LC-QTOF include A) replacing solvents used in LC, and B) cleaning the source spray chamber in the QTOF. These procedures will be outlined below.

Procedure

A) Instructions for Changing or Replacing LC Solvent

When: Any time i) the solvent volume is low, ii) fresh solvent is needed, iii) a different solvent is replacing old solvent in the same line.

1. In the Data Acquisition (DA) software, turn off LC by pressing the red button.
2. Remove lid from the solvent bottle and carefully place the LC line & lid in such a way that they are hanging in the air without touching anything to prevent dust contamination.
3. If replacing solvent due to low volume or old solvent, then the same bottle can be used.
4. Discard solvent containing Acetonitrile into a special organic waste container in the fume hood in B0-108. Discard other solvents down the drain with running water.
5. Rinse the bottle 3 times with a small volume of MS-grade H2O, discarding each time.
6. Rinse a 500 mL volumetric flask 3 times with a small volume of MS-grade solvent.
7. Fill the volumetric flask approximately half way with MS-grade solvent.
8. Use a sterile pipette tip and pipet to deliver an accurate volume of MS-grade additives (ex. 0.5 mL Formic Acid, MS-grade) into the volumetric flask.

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9. Continue to fill the volumetric flask up to the accurate 500 mL line with MS-grade solvent (ex. H2O), this way allowing mixing of the formic acid or other additive.
    a. NEVER USE PARAFILM TO PREPARE OR MIX SOLVENTS FOR MASS SPECTROMETRY.
10. Rinse the solvent bottle 3 times with a small volume of fresh solvent and discard.
11. Fill the solvent bottle with the fresh solvent and replace the LC line and lid in the new solvent.
12. In the DA software, right click the LC pump and select Bottle Fillings.
13. Update the new volume in each solvent bottle. This must be done manually.
14. After replacing low or old solvent, or adding a new solvent, there will be air in the LC line and the line must be "Purged".
15. In DA software, right click the LC pump and select Purge. Select the appropriate solvent line at 100% and purge for 5 minutes at 5 mL per minute.
16. Perform Purge for each solvent that was replaced.

LC Prime

Prime the LC Pump if lines have run dry. This process allows the pump to run at maximum to pull solvent into lines. This process is made easier if the user temporarily replaces one solvent bottle with 100% IPA for the Prime procedure as IPA is easier to pull into the pump.

LC Condition

Condition the LC Pump if there are any micro air bubbles inside the pump head. The user will know if there are air bubbles in the pump head because the pressure chromatogram will have regular blips/spikes above a baseline. The user should condition the pump for 15 minutes to remove the air bubbles.

2) Instructions for Cleaning the ESI Source Spray Chamber

When:

i) Daily if using dirty samples (ex. diluted plasma) or running multiple samples each day

ii) Weekly if using irregularly

iii) Anytime if the background spectra is higher than usual and/or if any other weird signal is observed. The cleanliness of the source can be checked and cleaned if needed.

1. In DA software, turn off the LC and the MS by pressing the red buttons.
2. Prepare materials including a fresh Agilent microfiber cloth, Kim wipes, tweezers, 50% IPA/H2O, 100% Methanol, and a can of pressurized air.
3. Put on new nitrile gloves.
4. Remove LC line by unscrewing red inlet from source piece.
5. Open the source latch at the right side of the source chamber.
6. Place two Kim wipes on the counter for placing the source cone.
7. The Source Cone is **very hot**! Be very careful when removing the source cone to not let it fall on the floor!
8. Use the Agilent microfiber cloth to carefully twist the Source Cone counter-clockwise until it removes from the instrument, and place it on the kim wipes.
9. Holding a beaker below to collect rinse below the source nozzle, use the 50% IPA spray bottle to spray only the metal (silver) area and the black surrounding area. Be careful not to spray the golden or white colored plastic near the top of the nozzle piece.
10. Use kim wipes to spot clean any large drops remaining in the black area.
11. No beaker is needed to clean the source plate area. Use 50% IPA spray bottle to carefully spray the metal source plate and the black surrounding area.
    a. NEVER spray into or touch the center hexabore capillary.
12. Wet kim wipes with 50% IPA and carefully wipe the metal source plate
    a. If the source cone lip (lip surrounding the hexabore capillary) appears dirty, carefully use wet kim wipes to wipe the lip only, being careful not to touch the capillary.
    b. If the hexabore capillary face appears to be dirty, let a BioCORE technician know immediately, and/or continue the source cleaning and tell a BioCORE technician as soon as possible.
13. After the source cone has cooled and is safe to handle, use 3-4 cycles of spraying with 50% IPA and wiping down with kim wipes.
14. Use tweezers rinsed with 50% IPA and carefully grip the source cone on the threading, being careful not to damage the threads. Spray the cone with 50% IPA, then 100% Methanol, then air dry using compressed air.
15. Screw the source cone onto the source plate using gloved hands until it is only finger tight, so that it can be easily removed with a slight pressure.
16. Spray the entire source chamber with compressed air, then close the source chamber and latch.
17. Remove the source nozzle carefully by turning counter clockwise and pulling straight up, being careful not to damage the nozzle (the tip).

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18. Inspect the tip of the nozzle using a magnifying glass and make sure the two sheath areas around the nozzle are clear of debris or buildup. These should look like two thin black circles around the nozzle tip. Nitrogen gas flow typically prevents buildup.
    a. The nozzle should be flush with the source or sticking out a maximum of 0.003 inches. Ask a BioCORE Technician for help if you think the nozzle is not flush with the source.
19. Wet kim wipes with 50% IPA and clean from the base towards the tip, with a twisting motion at the tip to clean the nozzle, and repeat twice.
20. Spray nozzle tip with compressed air, then very carefully, using two hands, insert the capillary into the source chamber making sure not to touch the nozzle tip against any surfaces. Twist clockwise to secure nozzle.
21. Replace the red LC line into the source chamber.
22. User must always perform a Check Tune/Mass Calibration after cleaning the Source. See SOP for Tuning & Calibration.

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3) Instructions for Capillary Cleaning – BioCORE personnel only

1. In DA software, turn off all modules. Ensure QTOF is on standby.
2. Vent QTOF by right clicking in QTOF module and choosing vent.
    a. This will take ~20 minutes to vent the instrument.
    b. At the end of venting, an audible whooshing sound can be heard when the QUAD and TOF chambers are filled with Nitrogen gas to prevent ambient air entering.
3. Do not turn off QTOF, leave on during next steps. This ensures N2 stays in Quad + TOF.
4. Put on clean gloves.
5. Open source chamber.
6. Use microfiber cloth to remove source cone and place onto kim wipes on top of Q-TOF.
7. Remove capillary cap and place onto kim wipes on top of Q-TOF.
8. Carefully grasp end of capillary with gloves and apply pulling pressure with slight twisting motion to pull out capillary. Place on kim wipes on top of Q-TOF.
9. Close source chamber.
10. Place cut 1 mL pipette tips on each end of the capillary to protect the ends from rubbing.
11. In MS-cleaning dedicated glassware (1000 mL glass beaker) prepare a 0.5% Alconox solution: add 5g Alconox powder to 1000 mL mqH2O, and mix with spin bar for 5 minutes.

12. Place capillary with protected ends into dissolved Alconox solution, ensuring it is completely submerged. Sonicate in water bath for 15 minutes.
13. Use cut 1 mL pipette tips to manually flush capillary hexabores with Alconox solution.
    a. Will have to temporarily remove pipette end protectors.
14. Flush capillary with hot H2O tap water first by placing capillary inside water tubing for 10 minutes.
15. Replace capillary end protecting pipettes.
16. Flush capillary with hot H2O tap water for another 20 minutes by placing water tubing flow into beaker containing protected capillary.
17. Sonicate in hot H2O 10 minutes in water batch, then flush manually with cut pipette tip.
18. Sonicate in mqH2O 10 minutes, then flush manually with cut pipette tip.
19. Sonicate in IPA 15 minutes, then flush manually.
20. Add small capillary cap to the beaker containing the capillary and sonicate in MeOH 15 minutes, then flush manually. Remove capillary protectors.
21. Purge capillary hexabores and capillary cap until dry using N2 gas.
22. Soak microfiber lint-free cloth with IPA then wet the entire outside of the capillary.
23. Insert wetted capillary, with black stripe towards the front of the source (must be this direction), into position using a twisting motion to prevent springs from popping into funnel.
24. Replace capillary end cap over capillary, then replace source cone
25. If necessary, clean source chamber following protocol located earlier in this SOP.
26. Power cycle the QTOF instrument quickly (Turn OFF, wait 5 seconds, then Turn ON).
    a. The rough pumps turning on will make a loud noise in the exhaust location when they power on. This is normal.
27. Wait 3-5 minutes for QTOF instrument to make a beep sound, indicating that it can now communicate with the computer software.
28. Open QTOF Diagnostics program. Click Connect> Connect to MS>IP = 192.168.294.1 (default). Once connected, open parameters tab. Monitor Turbo pumps to make sure they are powering on (>10% power after 5 minutes is expected).
29. Wait overnight to 24 hours for vacuums to reach operational levels and for electronics to equilibrate. DO NOT TURN QTOF ON IN SOFTWARE UNTIL OPERATING VACUUM IS REACHED:
    a. QUAD = 5.3 e-5 (approximately)
    b. TOF = 2.3 e-7 (approximately)
    c. Rough = 2.8 e0 (approximately)
30. Perform Initial Autotune of Q+TOF in Both Polarities at 3200 m/z (use previous slicer position). Once passed, perform Standard Autotune at 1700 m/z.
31. Record tune ion abundances of Initial and Standard tune in (+) and (-) in excel sheet.

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