Technical Overview

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Simplified Background Reduction Protocol for Agilent Triple Quadrupole LC/MS

Introduction

This technical overview describes how to thoroughly clean an Agilent triple quadrupole LC/MS system before beginning method development work or for a general instrument checkup. This method can be used for instrument troubleshooting as well as reagent purity.

Materials

Notes

- Use clean bottles only.
  - Use borosilicate glass bottle only.
  - Rinse bottle with desired solvent before refilling it.
  - Bottles can get contaminated with detergents form the dishwasher.
- Use only LC/MS-grade solvents and water filtered through 0.2 µm filters.
  - Residues or contaminations may block filters or capillaries.

Materials needed

- Four clean one-liter bottles are needed
  - **Bottle 1**: LC/MS grade water
  - **Bottle 2**: LC/MS grade isopropanol
  - **Bottle 3**: 50/50 LC/MS grade methanol/LC/MS grade acetonitrile
  - **Bottle 4**: LC/MS grade methanol
- Agilent HPLC Flushing Solution (part number G1969-85026)
- Two squirt bottles
  - **Bottle 1**: 50/50 LC/MS grade water/LC/MS grade methanol
  - **Bottle 2**: 100% LC/MS grade isopropanol
  - Zero Dead Volume Union (part number UCU402) (This is only needed if no bypass has been setup in the column compartment)
- 500 to 1,000 mL beaker
- Waste container
- Paper towels
- Kim wipes
- Lint free cloth (part number 05980-60051)
- 4,000 grit paper
- 10 to 20 mL graduated cylinder
- Gloves

Definitions

- **Purging** = 5.0 mL/min
- **Flushing** = 0.5 mL/min

Initial tasks

1. Disconnect the exhaust polyethylene tube connected at the bottom of the source and place the beaker underneath the opening so the excess solvent will drain into the beaker.
2. Open the source and rinse everything well, first with a squirt bottle of water/methanol followed by isopropanol. The surfaces can be wiped down with a lint-free laboratory tissue

Warning: Take care not to squirt solvent into the MS inlet hole.

3. Remove the shield and capillary cap using the Agilent Lint Free Cloth taking extra care to not touch the metal with bare hands or gloves. Clean the shield and cap with isopropanol. If needed, use 4,000 grit paper with water only to remove discolorations. Sonicate the shield and capillary cap in isopropanol for 5 to 10 minutes and rinse with methanol.

Warning: Wait for the shield and capillary cap to cool before handling.

4. Inspect the nebulizer with magnifying tool and if needed, clean the nebulizer barrel with 50/50 water/methanol followed by isopropanol, use 4,000 grit paper if discoloration is observed. See application note G1960-90470 for nebulizer adjustment.
5. Sonicate for 5 to 10 minutes by submersing the nebulizer tip in a 10 to 20 mL graduated cylinder filled with 90/10 isopropanol/water.

6. Reinstall the nebulizer, capillary cap, and shield using the Agilent Lint Free Cloth for handling. Rinse with methanol and close the source.
7. Reconnect the exhaust tube.
8. Disconnect the LC line from the MS and put in into waste container or switch MS diverter valve to waste.

LC/MS cleaning protocol

1. Remove the column and replace with a zero dead volume union or switch the valve to bypass mode (no column).
2. Put both mobile phase lines A and B into one bottle of water and purge for 8 minutes.
3. Switch both mobile phase line to the isopropanol bottle. Purge the lines for 8 minutes and flush for 40 to 60 minutes.
4. After flushing all the lines, reconnect the LC line to the MS or switch MS diverter valve to MS.
5. Switch both lines to the Agilent HPLC Flushing Solvent bottle and flush for 3 to 4 hours.
6. Switch both lines to the isopropanol bottle and flush for 1 to 2 hours.
7. Switch both lines to the 50/50 methanol/acetonitrile bottle. Purge the lines for 8 minutes and flush for 40 to 60 minutes.
8. Switch both lines to the methanol bottle. Purge the lines for 8 minutes and flush for 40 to 60 minutes.
9. Switch both lines to the water bottle. Purge the lines for 8 minutes and flush for 40 to 60 minutes.
10. Put each solvent line into freshly prepared current method solvents. Purge the lines for 8 minutes, and flush overnight (12 hours minimum).
11. Check the total ion chromatogram (TIC) background level with the "Background Method".

Background method

Use Agilent MassHunter Acquisition to create a positive mode MS2 Scan Background Method from *m/z* 40 to 1,000 and save for future use. Name the datafile as the current date as a future reference for instrument cleanliness.

After acquisition of the MS2 Scan, open the datafile using Agilent MassHunter Qualitative Analysis software. Review the total ion chromatogram (TIC) and observe the Y-axis for total number or counts compared to Table 3.

Table 1. LC Configuration and Parameters.

Configuration	
Injection Volume	No Injection
Analytical Column	Zero Dead Volume Union
Column Temperature	Not Controlled
Mobile Phase A	Water (without current method additives)
Mobile Phase B	Methanol or Acetonitrile (without current method additives)
Flow Rate	0.35 to 0.5 mL/minute
Isocratic	50/50
Stop Time	1 minute

Table 2. LC/TQ Mass Spectrometer Configuration and Parameters.

Configuration	
Agilent Triple Quadrupole Mass Spectrometer	
Ionization Mode	Positive
Scan Type	MS2 Scan (Ultivo = Scan mode) Range 40 to 1,000 *m/z*
Drying Gas Temperature	250 to 350 °C
Drying Gas Flow	11 L/min
Nebulizer Pressure	30 to 50 psi
Sheath Gas Temperature	300 to 400 °C
Sheath Gas Flow	11 L/min
Nozzle Voltage	0 V
Capillary Voltage, Positive	4,000 V
Delta EMV, Positive	0 V

Table 3. TIC reference ranges from extra clean to dirty for Agilent triple quadrupole instrument platforms. (These are estimated ranges and will vary by instrument.)

LC/TQ	Extra Clean	Clean	Working Range	Dirty
Ultivo	<1M	1M to 2M	2M to 6M	>6M
6470	<1M	1M to 2M	2M to 6M	>6M
6495	<20M	20M to 30M	30M to 100M	>100M

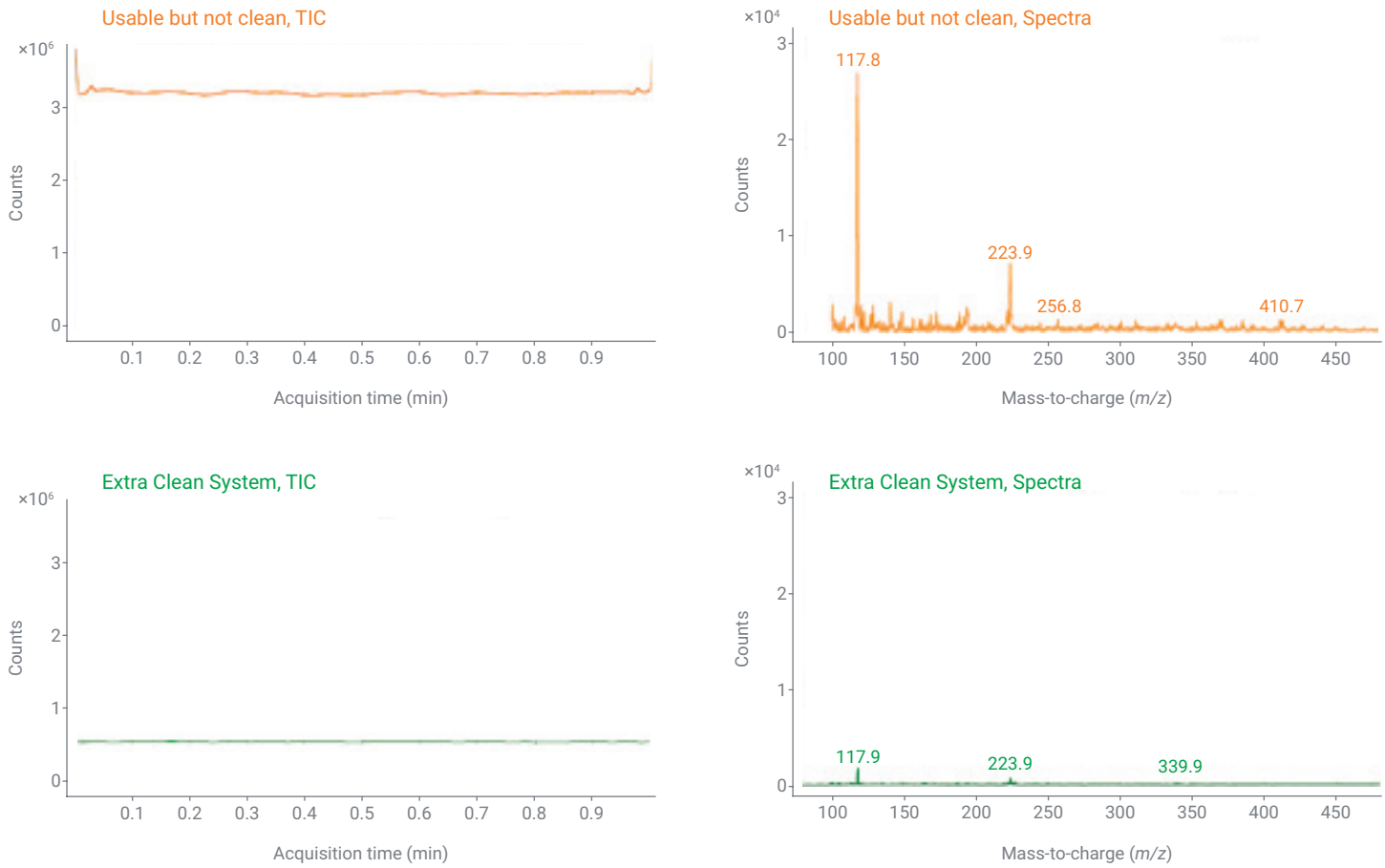

Figure 1. Example of Agilent 6470 triple quadrupole LC/MS system background counts before the cleaning protocol (orange text) and after the cleaning protocol (green text).

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