

QTrap 6500+ LC/MS Performance Monitoring and Maintenance

Table of Contents

1	INTRODUCTION	2
2	SCOPE	2
3	EQUIPMENT	2
4	STANDARDS AND CONTROLS	3
4.1	Testmix	3
4.2	Mass Calibration Solutions	3
4.2.1	Preparation of PPG Dilution Solvent	3
4.2.2	Preparation of Diluted PPG Standard Solution (Positive Mode)	3
4.2.3	PPG 3000 Standard (Negative Mode)	3
5	PROCEDURE	3
5.1	Daily Checks	3
5.2	Yearly Scheduled Maintenance/Checks	4
5.2.1	Mass Spectrometer	4
5.2.2	Liquid Chromatograph	4
5.3	As Needed Maintenance/Checks	5
5.3.1	Mass Spectrometer	5
5.3.2	Liquid Chromatograph	5
5.4	Mass Calibration Check	5
5.4.1	Q1 or Q3 Quadrupole in Positive Mode	5
5.4.2	Q1 or Q3 Quadrupole in Negative Mode	5
5.4.3	Completion of the Calibration Check	6
6	INSTRUMENTAL CONDITIONS	6
6.1	Testmix (Positive Mode)	6
6.1.1	Liquid Chromatograph	6
6.1.2	Mass Spectrometer	6
7	ACCEPTANCE CRITERIA	6
7.1	Testmix	6
7.2	Mass Calibration Check	7
7.2.1	Positive Mode	7
7.2.2	Negative Mode	7
8	LIMITATIONS	7
9	SAFETY	7
10	REVISION HISTORY	7

QTrap 6500+ LC/MS Performance Monitoring and Maintenance

1 INTRODUCTION

This document addresses the performance monitoring and maintenance of the QTrap 6500+ LC/MS system consisting of a Sciex QTrap 6500+ Mass Spectrometer (MS) and a Liquid Chromatograph (LC) or Ultra-High Performance Liquid Chromatograph (UPLC). The QTrap can be used as a Triple-Stage Quadrupole or as a Linear Ion Trap (in MS or MS/MS mode). The instrument is configured with an Atmospheric Pressure Ionization (API) source that is capable of both electrospray (ESI) and atmospheric pressure chemical (APCI) ionization. Currently, the instrument is primarily used in the ESI mode. However, it can be used for APCI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines are outlined in IOSS-701.

2 SCOPE

This document applies to personnel using the associated instrument(s)/equipment in the Toxicology discipline.

3 EQUIPMENT

- Instrumentation
 - Sciex QTrap 6500+ MS with API Source and "Analyst" software (or equivalent)
 - Shimadzu Prominence LC (or equivalent)
 - Waters Acquity UPLC (or equivalent)
- Materials
 - Nitrogen, 99.99% (high purity or equivalent)
 - Methanol (Optima grade or equivalent)
 - Deionized Water, 18.2 MΩ-cm (Milli-Q or equivalent)
 - Water (Optima Grade or equivalent)
 - Acetonitrile (HPLC grade or equivalent)
 - Formic Acid - ~98% (Fluka or equivalent)
 - Ammonium Acetate (Reagent grade or equivalent)
 - Caffeine (Sigma or equivalent)
 - Codeine (Sigma or equivalent)
 - Brucine (Sigma or equivalent)
 - Reserpine (Sigma or equivalent)
 - 10 to 500 µL LC syringe (Hamilton or equivalent)
 - Sciex PPG (Polypropylene Glycol) 3000 (include in Part # 401936)
 - Sciex Mass Spectrometer Solution Kit (Part # 401936)
 - General laboratory supplies

4 STANDARDS AND CONTROLS

4.1 Testmix

The stock testmix is prepared by weighing 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. The testmix is further diluted by using 50 μ L of the stock testmix and diluted to 10-mL volumetric flask with methanol. Store the solution in the refrigerator. It has a shelf life of three years. The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system.

4.2 Mass Calibration Solutions

The mass calibration solutions are used for coarse tuning and calibrating the mass spectrometer over the entire mass range. Using this instrument as a Triple-Stage Quadrupole requires it to be calibrated both in the positive and negative mode. As a result, two calibration solutions are needed to calibrate this instrument.

4.2.1 Preparation of PPG Dilution Solvent

This solution can be purchased directly from Sciex or prepared as follows:

- A. Dissolve 15.4 milligrams of ammonium acetate in 49.9 mL of deionized water.
- B. It is essential to dissolve the ammonium acetate in deionized water first.
- C. To 49.9 mL of methanol, add 0.1 mL of formic acid and 0.1 mL of acetonitrile.
- D. Mix the two solutions together to make the final PPG Dilution Solvent. Store the solution in the refrigerator. It has a shelf life of three years.

4.2.2 Preparation of Diluted PPG Standard Solution (Positive Mode)

The Diluted PPG Standard Solution is used to calibrate the Q1 (Quadrupole) and Q3 in the positive mode. The PPG Standard (in Mass Spectrometer Solution Kit) purchased from Sciex is diluted with the prepared PPG Dilution Solvent (listed above) or the one supplied from the purchase kit at a ratio of 1:50.

- A. Put 20 mL of the PPG Dilution Solvent in a clean vial and remove 0.4 mL leaving 19.6 mL.
- B. Add 0.4 mL of PPG Standard (1×10^{-4} M) to the 19.6 mL of PPG Dilution Solvent and mix well. Store the solution in the refrigerator. It has a shelf life of three years.

4.2.3 PPG 3000 Standard (Negative Mode)

The PPG 3000 Standard is used to calibrate the Q1 (Quadrupole) and Q3 in the negative mode. The standard is purchased directly from Sciex and no dilution is needed.

5 PROCEDURE

5.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the instrument log.

IOSS-723-03: QTrap 6500+ LC/MS	Page 3 of 7	Issue Date: 09/30/2022
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- A. Record the remaining disk space on the hard drive. Verify that the hard disk has at least 1GB of free disk space. Do not use if less than 1GB remain.
- B. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 60 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.
- C. Verify that the system is under vacuum and the ion gauge is reading less than 6.0×10^{-5} torr.
- D. Check the curtain/orifice plate for residue. Wipe clean if needed.
- E. Prepare the instrument for analysis of the testmix. Verify that the instrument has the correct source probe installed (ESI), positive ion mode selected, and centroid data being collected.
- F. Start the LC pump and inspect tubing connections to verify there are no leaks. Engage the ESI probe and turn on the MS.
- G. Set the instrument in scanning mode for 15 min with the appropriate method loaded to allow the temperature and gas flow to stabilize.
- H. Perform an analysis of the testmix prior to the analysis of samples using parameters listed in the 'Instrumental Conditions' section. Start an acquisition using a filename such as 'testmix' (or equivalent). Make three 5 μ L injections of the testmix solution approximately 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Acceptance Criteria' section. If the results are acceptable, print the TIC and extracted ions for all components in the testmix.
- I. If all requirements are within specification, prepare the documentation as outlined in IOSS-701. If any requirements fail, contact appropriate instrument support personnel.

5.2 Yearly Scheduled Maintenance/Checks

The following steps will be performed once in a calendar year. Enter the information in the appropriate instrument log to indicate completion. Refer to IOSS-701 for more information on instrument maintenance and documentation.

5.2.1 Mass Spectrometer

- A. Clean curtain/orifice plate.
- B. Check air filter. Clean or replace if needed.
- C. Check level and color of rough pump oil. Top off or change oil as needed.
- D. Check the mass resolution and calibration. Perform a full calibration if warranted.

5.2.2 Liquid Chromatograph

- A. Inspect pump plungers, diaphragms and check valves. Replaced if needed.
- B. Inspect oven fan.
- C. Inspect degasser filters, replace if needed.
- D. Check autosampler injection count and performance. Replace components (seals, rotors, stators, needle) if warranted.

5.3 As Needed Maintenance/Checks

The following steps are to be performed as needed. Enter the information in the appropriate instrument log to indicate completion. Refer to IOSS-701 for more information on instrument maintenance and documentation.

5.3.1 Mass Spectrometer

- A. Check the curtain/orifice plate for debris, clean as needed.
- B. Replace the heaters if needed.
- C. Clean the source if needed.
- D. Clean the ion guide/lenses/rods if need is indicated by tune/calibration evaluation.

5.3.2 Liquid Chromatograph

- A. Replaced solvent frits as needed.
- B. Replace drain valve as needed.
- C. Replace degasser pump as needed.

5.4 Mass Calibration Check

The mass calibration check should be performed when scheduled or as needed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

5.4.1 Q1 or Q3 Quadrupole in Positive Mode

- A. Load a 250 μ L syringe with the Diluted PPG Standard.
- B. Connect the syringe to the ESI probe assembly, and place in the syringe pump.
- C. Set the syringe pump to the correct syringe type and set the pump rate to 10 μ L/minute.
- D. Activate the 'LM MSONLY' instrument configuration.
- E. Select the 'API Instrument' project.
- F. Enter the 'Manual Tuning' window.
- G. Load the tune file 'LM Q1 Pos PPGs.dam' ('LM Q3 Pos PPGs.dam' for Q3).
- H. Check that instrument is in POSITIVE ION mode.
- I. Ensure that 'MCA' is checked and 'Scans' are set to 10.
- J. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- K. Click 'Start'.
- L. Once the MS icon shows 'Idle' right click and 'Open File' in the mass spectrum pane.
- M. On the data panes, right click and 'List Data'.
- N. Click the 'Calibration Peak List' tab.
- O. Evaluate the results using the 'Acceptance Criteria' section.

5.4.2 Q1 or Q3 Quadrupole in Negative Mode

- A. Load a 250 μ L syringe with the Diluted PPG Standard.
- B. Connect the syringe to the ESI probe assembly, and place in the syringe pump.

- C. Set the syringe pump to the correct syringe type and set the pump rate to 10 $\mu\text{L}/\text{minute}$.
- D. Activate the 'LM MSONLY' instrument configuration.
- E. Select the 'API Instrument' project.
- F. Enter the 'Manual Tuning' window.
- G. Load the tune file 'LM Q1 Neg PPGs.dam' ('LM Q3 Neg PPGs.dam' for Q3).
- H. Check that instrument is in NEGATIVE ION mode.
- I. Ensure that 'MCA' is checked and 'Scans' are set to 10.
- J. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- K. Click 'Start'.
- L. Once the MS icon shows 'Idle' right click and 'Open File' in the mass spectrum pane.
- M. On the data panes, right click and 'List Data'.
- N. Click the 'Calibration Peak List' tab.
- O. Evaluate the results using the 'Acceptance Criteria' section.

5.4.3 Completion of the Calibration Check

If all requirements are within specification, prepare the documentation as outlined in IOSS-701. If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

6 INSTRUMENTAL CONDITIONS

6.1 Testmix (Positive Mode)

6.1.1 Liquid Chromatograph

Mobile Phase:	(From discipline-specific procedure)
Flow Rate:	0.3 mL/min (HPLC), 0.15 mL/min (UPLC)
Column:	N/A
Injection Volume:	5 μL
Number of Injections:	3

6.1.2 Mass Spectrometer

Ionization:	ESI
Polarity:	Positive
Scan Mode:	SIM
Source Temperature:	600°C
Masses:	95, 300, 395, 609

7 ACCEPTANCE CRITERIA

7.1 Testmix

Verify the results of the testmix. The following ions should be observed in the three testmix injections:

- Caffeine 195 m/z
- Codeine 300 m/z

- Brucine 395 m/z
- Reserpine 609 m/z

7.2 Mass Calibration Check

Verify the results of the mass calibration. The calibration procedure will indicate if the procedure was successful.

7.2.1 Positive Mode

The mass calibration check in positive mode for (Q1 and Q3) confirms the presence of ions m/z 59.1, 175.1, 616.5, 906.7. For each, verify the peak width is between 0.6 – 0.8 amu, and the mass shift is ≤ 0.1.

7.2.2 Negative Mode

The mass calibration check in negative mode (for Q1 and Q3) confirms the presence of ions m/z 45.0, 585.4, 933.6. For each, verify the peak width is between 0.6 – 0.8 amu, and the mass shift is ≤ 0.1.

8 LIMITATIONS

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

9 SAFETY

Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

10 REVISION HISTORY

Revision	Issued	Changes
02	10/04/2018	Section 1- Updated scope to include applicable disciplines/categories of testing. Section 2- Removed 'Spark Holland Symbiosis from Sections 1 & 2 and added Shimadzu LC. Section 7- Removed 'Manufacturer's Maintenance Agreement'. Section 8- Changed to discipline-specific SOP. Section 14- Updated to 'Instrument Operation and Systems Support' Added 'appropriate instrument support personnel' throughout.
03	09/30/2022	Revised to match new format requirements. Section 3- Updated instrument model 6500+ and added UPLC. Section 5- Added yearly maintenance and expanded as-needed maintenance. Updated mass calibration check for 6500+. Section 7- Updated criteria for 6500+.