

## **Performance Monitoring Protocol (QA/QC) for the AB Sciex QTRAP LC/MS**

### **1 Scope**

This document addresses the performance monitoring (QA/QC) of the AB Sciex QTRAP LC/MS system consisting of an AB Sciex QTRAP Mass Spectrometer (MS) and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Toxicology.

### **2 Principle**

The QTRAP system is comprised of a Shimadzu LC and an AB Sciex QTRAP MS that 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 as a Triple-Stage Quadrupole in the ESI mode. However, this protocol can also be used for APCI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

### **3 Equipment/Materials/Reagents**

- a. Instrumentation – AB Sciex QTRAP MS, API Source, Shimadzu LC, and data system with “Analyst” software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Collision Gas & Ion Trap Gas - Nitrogen, 99.999% (high purity or equivalent)
- d. Methanol, HPLC grade or equivalent
- e. Deionized Water, 18 MΩ·cm Milli-Q or equivalent
- f. Optima Grade Water or equivalent
- g. Acetonitrile, HPLC grade or equivalent
- h. Formic Acid - ~98% (Fluka or equivalent)
- i. AB Sciex PPG (Polypropylene Glycol) 3000 (include in Part # 401936)
- j. AB Sciex Mass Spectrometer Solution Kit (Part # 401936)

- k. Caffeine (Sigma or equivalent)
- l. Agilent ESI Tuning Mix
- m. Ammonium Acetate, reagent grade
- n. Codeine (Sigma or equivalent)
- o. Brucine (Sigma or equivalent)
- p. Reserpine (Sigma or equivalent)
- q. Volumetric glassware
- r. Infusion Syringe - 10 to 500  $\mu$ L LC syringe (Hamilton or equivalent)

## **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  $\mu$ 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 Calibration Solutions**

The calibration solution is 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 AB 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 AB 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 ( $1 \times 10^{-4}M$ ) 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 AB Sciex and no dilution is needed.

### **5 Calibration**

The calibration procedure should be performed 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.

#### **5.1 Calibration of Q1 Quadrupole in the Positive Mode**

- a. Load a 250  $\mu$ 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  $\mu$ L/minute.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q1 Pos PPGs.dam' reference file.
- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
  - Correct tune file is loaded.
  - 'PPGs Pos' is loaded under the dialog box label 'Standard'.

- Q1 and Unit are checked under 'Quad/Resolution'.
  - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA (Multi-Channel Averaging) mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Save the calibration and print the calibration report.

## 5.2 Calibration of Q3 Quadrupole in the Positive Mode

- a. Load a 250  $\mu$ 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  $\mu$ L/minute.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q3 Pos PPGs.dam' reference file.
- f. Check that instrument is in POSITIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
- Correct tune file is loaded.
  - 'PPGs Pos' is loaded under the dialog box label 'Standard'.
  - Q1 and Unit are checked under 'Quad/Resolution'.
  - Search range is set to 3 amu with threshold at 200 cps (counts per seconds) under the 'Peak Search Parameter'.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.

- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- l. Save the calibration and print the calibration report.

### **5.3 Calibration of Q1 Quadrupole in the Negative Mode**

- a. Load a 250  $\mu$ L syringe with the PPG 3000 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  $\mu$ L/minute.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q1 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
  - Correct tune file is loaded.
  - 'PPGs Neg' is loaded under the dialog box label 'Standard'.
  - Q1 and Unit are checked under 'Quad/Resolution' section.
  - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.

- l. Save the calibration and print the calibration report.

#### **5.4 Calibration of Q3 Quadrupole in the Negative Mode**

- a. Load a 250  $\mu$ L syringe with the PPG 3000 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  $\mu$ L/minute.
- d. Enter the 'Manual Tuning' window.
- e. Load the tune file 'Q3 Neg PPGs.dam' reference file.
- f. Check that instrument is in NEGATIVE ION mode.
- g. Click on 'Resolution Optimization' and verify that the following are correct:
  - Correct tune file is loaded.
  - 'PPGs Neg' is loaded under the dialog box label 'Standard'.
  - Q1 and Unit are checked under 'Quad/Resolution' section.
  - Search range is set to 3 amu with threshold at 200 cps under the 'Peak Search Parameter' section.
  - Positive mode is checked. NOTE: VERIFY THAT MASS CALIBRATION UPON COMPLETION IS UNCHECKED.
- h. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
- i. In the 'Optimization' window, click start.
- j. When the calibration is complete, the software will display whether or not the calibration was successful. If the procedure passes, evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- k. Acquire 10 scans to disk in the MCA mode and click on the icon in the menu bar labeled 'Calibrate from Spectrum' to mass calibrate the instrument.
- m. Save the calibration and print the calibration report.

#### **5.5 Completion of Calibration**

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

## 6 Sampling or Sample Selection

Not applicable.

## 7 Procedures

### 7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows Explorer program to 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  $6.0 \times 10^{-5}$  torr.
- d. 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.
- e. Perform an analysis of the testmix prior to the analysis of samples using parameters listed in the 'Instrumental Conditions' section of this protocol. Start the LC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'testmix' (or equivalent). Make three 5  $\mu$ 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 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC and extracted ions for all components in the testmix.
- f. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

### 7.2 As Needed Checks

- a. Replace heaters.

- b. Clean or replace the heated capillary.

## 8 Instrumental Conditions

### 8.1 Testmix (Positive Mode)

#### Liquid Chromatograph

Mobile Phase:	From discipline specific SOP
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	5 $\mu$ L
Number of Inj:	3

#### Mass Spectrometer

Ionization:	ESI
Scan Mode:	SIM
Source Temp:	600°C
Mass:	95, 300, 395 and 609 m/z

## 9 Decision Criteria

### 9.1 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

### 9.2 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. Calibration in the positive mode for Q1 and Q3, confirms the presence of ions m/z 59.1, 175.1, 616.5, 906.7, 1254.9, 1545.1, 2010.5, and 2242.6. In addition, verify that the sensitivity and peak width are within range as specified below.



### Positive Mode Q1 and Q3

	Sensitivity (cps) m/z 906.7	Peak width (amu)	Sensitivity (cps) m/z 2242	Peak width (amu)
Q1	> 2.0e7	0.6 – 0.8	> 1.0e6	0.6 – 0.8
Q3	> 2.0e7	0.6 – 0.8	> 8.0e5	0.6 – 0.8

Calibration in the negative mode for Q1 and Q3, confirms the presence of ions m/z 45.0, 585.4, 933.6, 1223.8, 1572.1, 1863.3, 2037.4, and 2211.6. In addition, verify that the sensitivity and peak width are within range as specified below.

### Negative Mode Q1 and Q3

	Sensitivity (cps) m/z 933.6	Peak width (amu)	Sensitivity (cps) m/z 2037.4	Peak width (amu)
Q1	> 2.0e7	0.6 – 0.8	3.0e6	0.6 – 0.8
Q3	> 1.0e7	0.6 – 0.8	N/A	0.6 – 0.8

## 10 Calculations

Not applicable.

## 11 Measurement Uncertainty

Not applicable.

## 12 Limitations

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

## 13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis. 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.

## 14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual*.

*FBI Laboratory Safety Manual*.

Rev. #	Issue Date	History
0	12/14/07	New document.
1	03/14/12	Updated instrument manufacturer to 'AB Sciex' in Title and Sections 1, 2, 3, 4.2.1, 4.2.2 and 4.2.3. Removed ammonium hydroxide and added Optima grade water to Section 3. Changed testmix dilution in Section 4.1. Added storage and stability information to Section 4.2.1.d. Added molar concentration in Section 4.2.2.b. Removed the word "case" from Section 7.1e. Changed scan mode to SIM, added source temperature and masses to Section 8.1. Changed scan mode to MRM and added mass reference to Section 8.2. Added instrument manual to Section 14.
2	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Removed 'Spark Holland Symbiosis from Sections 1 & 2 and added Shimadzu LC to Section 2. Added 'appropriate instrument support personnel' to Sections 5.5 and 7.1 b & f. Removed 'Manufacturer's Maintenance Agreement' Section 7.3. Updated heading in Section 6. Changed to discipline specific SOP in Section 8.1. Removed Section 8.2 (covered in Section 5). Updated 'Instrument Operation and Systems Support' in Section 14 and header.

**Approval**

Redacted - Signatures on File

Toxicology Technical  
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Date: 09/28/2018

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**QA Approval**

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