

Performance Monitoring Protocol (QA/QC) for the Thermo LTQ LC/MS and Shimadzu HPLC

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo Linear Trap Quadrupole (LTQ) LC/MS system consisting of a Thermo Electron LTQ Mass Spectrometer (MS) and a Shimadzu Prominence High Performance Liquid Chromatography (HPLC) system. This document applies to personnel using the associated instrument(s)/equipment in the following discipline/category of testing: Explosives (chemistry) examinations performed at the Huntsville facility.

2 Principle

The LTQ system is comprised of a Shimadzu Prominence HPLC system and a Thermo Electron Linear Ion Trap LTQ MS. The Shimadzu Prominence HPLC system is configured with at least two LC-20AD binary pumps, an autosampler injection system, and an optional column oven. The Thermo LTQ is configured with an Atmospheric Pressure Ionization (API) source that is capable of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). When this protocol is used for APCI, the method of ionization must be 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 - Thermo Electron LTQ MS, API Source, Shimadzu Prominence LC, and Data System with XCalibur software (or equivalent)
- b. Instrumentation – Shimadzu Prominence HPLC comprised of at least two LC-20AD pumps, SIL-20AC HT Autosampler, CBM-20A Communications Bus Module, DGU-20A 5R Degasser, and/or CTO-20AC Column oven (or equivalent)
- c. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- d. Ion Trap Gas - Helium, 99.99% (high purity or equivalent)
- e. Methanol (Optima grade or equivalent)
- f. Deionized Water, 18.2 MΩ Milli-Q (or equivalent)
- g. Acetone (HPLC grade)

- h. Ammonium Nitrate (NH_4NO_3) (reagent grade or equivalent)
- i. Pierce LTQ ESI Positive Ion Calibration Solution (Thermo or equivalent)
- j. Caffeine (Sigma or equivalent)
- k. Pierce ESI Negative Ion Calibration Solution (Thermo or equivalent)
- l. Reserpine (Sigma or equivalent)

Redacted

- o. Volumetric glassware
- p. Infusion Syringe - 10 to 500 μL LC syringe (Hamilton or equivalent)
- q. C-18 Column (Thermo Hypersil Gold or equivalent)
- r. 3.125 mM Ammonium Nitrate Mobile Phase (250 mg to 1 Liter water)
- s. Autosampler vials - 2 mL vials, crimp or screw top, with or without 100-500 μL inserts (Thermo or equivalent)
- t. Analytical balance

4 Standards and Controls

4.1 Testmix

The Testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system.

4.1.1 Explosives by ESI

Redacted

4.1.2 Explosives by APCI

Redacted

4.2 Calibration Solution

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. This procedure only needs to be performed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

The calibration solution is purchased from Thermo Fisher Scientific or equivalent.

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.

- a. Load infusion syringe with the appropriate calibration solution.
- b. Connect the infusion 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 to an appropriate flow rate.
- d. Load the appropriate tune file.
- e. Check that the instrument is in the appropriate ion mode.
- f. Set the detector using the parameters listed in the 'Instrumental Conditions' section of this protocol.
- g. Engage the ESI probe and turn on the MS.
- h. Turn on the syringe pump and verify that the solution is flowing.
- i. Backup current calibration file.

- j. In the Tune page, open the Calibrate dialog box, choose the 'Semi-Automatic' tab and check the individual tests and then 'Start.'
- k. When the calibration is complete, it will display whether or not the calibration was successful.
 - If the procedure fails, repeat the calibration. If calibration continues to fail, contact the appropriate instrument support personnel.
 - When the procedure passes, print the report and evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- l. Repeat for opposite ion mode.

6 Sampling

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps are to 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 to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 and 100psi. If it cannot maintain this pressure, contact the appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 250 psi remain.
- c. Record the line pressure of the building helium supply (ion trap gas). The regulator should read between 20 and 40 psi (30 and 60 psi if two instruments are to be run off the same regulator). If it cannot maintain this pressure, contact the appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 psi remain.
- d. Check the Vacuum Pressure to ensure that no significant leaks are present in the system. Do not use if the Convectron Gauge reads above 2 torr, or the Ion Gauge (if present) reads above 20 microtorr.

- e. Prime each Shimadzu LC solvent line to be used that day. Open the prime valve on the front of each pump module to be used by turning the valve handle ninety degrees counter clockwise and press the “PURGE” button on the module. If the pump does not start priming, disengage remote control by pressing the “PUMP” button and then pressing the “PURGE” button again. After the prime cycle finishes, close the prime valves.
- f. Prime the autosampler solvent wash by pressing the “PURGE” button on the front of the autosampler module.
- g. Prepare the instrument for analysis of the appropriate Testmix. Verify that the instrument has the correct source probe installed, the correct tune file loaded for the selected method (exp_tune or equivalent), positive ion or negative ion mode selected, and that centroid data is being collected. Begin scanning.
- h. Allow the column to equilibrate.
- i. Check the pressure on the LC pump while in use. If the pressure is higher than 2,800psi., do not use, and notify the appropriate instrument support personnel.
- j. Conduct a performance verification of the appropriate Testmix through the column. Evaluate the results using the ‘Decision Criteria’ section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the Testmix.
- k. If all requirements are within specification, prepare the documentation as outlined in the “General Instrument Maintenance Protocol.” If any requirements fail, contact the appropriate instrument support personnel.

7.2 As Needed Checks

- a. Replace the metal needle as needed.
- b. Clean or replace the heated capillary as needed.
- c. Clean solvent reservoirs.
- d. Clean filters.
- e. Replace seals

8 Instrumental Conditions

Refer to the “General Instrument Maintenance Protocol” for procedures on minor deviations.

8.1 Testmix

8.1.1 Explosives by ESI

Liquid Chromatograph

Mobile Phase: 60% Methanol : 40% 3.125 mM Ammonium Nitrate
Flow Rate: 0.3 mL/min
Program: Isocratic
Column: C-18
Inj Volume: 5 µL

Mass Spectrometer

Ionization: ESI
Polarity: Negative
Tune File: exp_tune (or equivalent)
Sheath Gas Flow: 20 (arb)
Aux Gas Flow: 5 (arb)
Sweep Gas Flow: 0 (arb)
Scan Mode: Full Scan
Scan Range: 200-400 m/z (minimum)

8.1.2 Explosives by APCI

Liquid Chromatograph

Mobile Phase: Methanol with 1.25 mM Ammonium Nitrate / DI H₂O with 1.25 mM Ammonium Nitrate
Flow Rate: 0.3 mL/min
Program: Gradient: 90% Water at 0-2 min, 50% Water at 12-14 min, 90% Water at 17-20 min
Column: C-18
Inj Volume: 10 µL

Mass Spectrometer

Ionization: APCI
Polarity: Positive
Tune File: **Redacted** (or equivalent)
Sheath Gas Flow: 35 (arb)
Aux Gas Flow: 15 (arb)
Sweep Gas Flow: 0 (arb)
Scan Mode: Full Scan

Scan Range: 150-250 m/z (minimum)

8.2 Calibration

Mass Spectrometer

Ionization: ESI
Tune File: esi_tune
Scan Mode: Full Scan
Scan Range: 100-2000 m/z

9 Decision Criteria

9.1 Testmix

9.1.1 Explosives by ESI

Verify the results of the Testmix. RICs should show contemporaneous signals for components at
Redacted

9.1.2 Explosives by APCI

Verify the results of the Testmix. RICs should show contemporaneous signals for components at
Redacted

9.2 Calibration (Positive Mode)

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

- Caffeine 195 m/z
- MRFA 524 m/z
- Ultramark 1022 m/z
1122 m/z
1222 m/z
1322 m/z

1422 m/z
1522 m/z
1622 m/z
1722 m/z
1822 m/z
1922 m/z

9.3 Calibration (Negative Mode)

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

- Sodium dodecyl sulfate 265 m/z
- Sodium taurocholate 517 m/z
- Ultramark 1280 m/z
1380 m/z
1480 m/z
1580 m/z
1680 m/z
1780 m/z

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” (IOG 001) *Instrument Operations Group SOP Manual*.

“Liquid Chromatograph General Maintenance Protocol” (IOG 003) *Instrument Operations Group SOP Manual*.

“Mass Spectrometer General Maintenance Protocol” (IOG 004) *Instrument Operations Group SOP Manual*.

FBI Laboratory Safety Manual.

<u>Rev #</u>	<u>Issue Date</u>	<u>History</u>
0	10/04/18	New document which specifies instrument protocol for the Huntsville Laboratory.

Approval

Redacted - Signatures on File

Scientific Analysis
Unit Chief

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TL Approval

Explosives (Chemistry)
Technical Leader

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QA Approval

Quality Manager

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