Orbitrap LC/MS Performance Monitoring and Maintenance

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1 INTRODUCTION

This document addresses the performance monitoring and maintenance of the Orbitrap LC/MS system consisting of a Thermo Electron Orbitrap Mass Spectrometer (MS) and a Shimadzu Liquid Chromatograph (LC). This system can be used for both unit mass and high resolution accurate mass chemical analyses. The instrument is configured with an API source that is capable of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or atmospheric pressure photoionization (APPI). The instrument is primarily used in ESI mode. However, in can be used for APCI or APPI 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 following disciplines/subdisciplines: Toxicology, General Chemistry, and Seized Drugs.

3 EQUIPMENT

- Instrumentation
  - Thermo Electron Orbitrap XL MS, API Source, and Xcalibur software (or equivalent)
  - Shimadzu Prominence LC (or equivalent)
- Materials
  - Nitrogen, 99.99% (high purity or equivalent)
  - Helium, 99.99% (high purity or equivalent)
  - Methanol (Optima grade or equivalent)
  - Deionized Water, 18.2 MΩ·cm (Milli-Q or equivalent)
  - Acetonitrile (HPLC grade or equivalent)
  - Ammonium Formate (reagent grade)
  - Formic Acid (reagent grade)
  - Ammonium Hydroxide (NH_4OH) (reagent grade or equivalent)
  - Caffeine (Sigma or equivalent)
  - Codeine (Sigma or equivalent)
  - Brucine (Sigma or equivalent)
  - Reserpine (Sigma or equivalent)
  - γ-Aminobutyric Acid (GABA),(Sigma or equivalent)
  - 10 µL LC syringe (Hamilton or equivalent)
  - 250 or 500 µL LC syringe (Hamilton or equivalent)
  - Pierce LTQ ESI Positive Ion Calibration Solution (Thermo or equivalent)
  - Pierce ESI Negative Ion Calibration Solution (Thermo or equivalent)
  - General laboratory supplies

4 STANDARDS AND CONTROLS

4.1 Testmix

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record preparations in the appropriate discipline/subdiscipline reagent log.

- Stock Solution
  Weigh 1.5 mg GABA, 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. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled up.
- Testmix Solution
  Pipette 4.0 mL of the Stock Solution into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled.

4.2 Calibration Solutions

The calibration solutions are 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 solutions are purchased from Thermo Fisher Scientific or equivalent.

5 PROCEDURE

5.1 Daily Checks

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

A. Record the remaining disk space on the hard drive. 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 100 psi. 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 250 psi remaining.
C. Record the line pressure of the building helium supply (ion trap gas). The regulator should read between 20 and 40 psi (30 - 60 psi if two instruments are to be run off the same regulator). If it cannot maintain this pressure, contact 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 remaining.
D. Check the oil level in the vacuum pumps housed in the compartment directly below the LTQ portion of the instrument. If a significant amount of oil is present in the mist filter, then ballast pumps individually by temporarily rotating the ballast switch until the oil has been evacuated.

E. 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.
F. Prime each 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 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 (about three minutes), close the prime valves.
G. If using the Shimadzu LC System, the autosampler solvent wash can be primed by pressing the "PURGE" button on the front of the autosampler module.
H. Prepare the instrument for analysis of testmix. Verify that the instrument has the correct source probe installed, the correct tune file loaded (esi_tune or equivalent), positive ion mode selected, and centroid data being collected.
I. Select the proper analyzer. In Tune Plus, select 'Ion Trap' analyzer for unit mass resolution using the LTQ. For accurate mass analysis using the Orbitrap, select 'FTMS' analyzer and a resolution of 60,000.
J. Perform an analysis of the testmix prior to the analysis of case samples. For testmix analysis, use parameters listed in the 'Instrumental Conditions' section. If a column is installed, remove it from that system and replace it with a zero-dead-volume union. Start the LC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'TMyymmdd' (or equivalent). Make three 5 µL injections of the testmix solution at least 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 spectra for components in the testmix.
K. 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 Orbitrap

A. Clean faceplate.
B. Change the capillary.
C. Check level and color of rough pump oil. Top off or change oil as needed.
D. Check water chiller filters, clean if needed.
E. Perform a full calibration evaluation/check. If any steps do not pass, repeat. 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 Orbitrap

A. Check the faceplate for debris, clean as needed.
B. Check water level in chiller, top off as needed.
C. Bakeout the analyzer as needed.

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

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

A. Load a 250 or 500 µL 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 rate to 10 µL/minute. The pump rate can be adjusted as needed.
D. Load the tune file "calibration_solution_positive" (or equivalent).
E. Check that instrument is in the appropriate ionization mode (positive or negative) and collecting centroid data.
F. Set the detector using the parameters listed in the 'Instrumental Conditions' section.
G. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
H. Engage the ESI probe and turn on the MS.
I. To perform an accurate mass calibration for the Orbitrap only, open the 'Calibrate' dialog box, choose the 'Semi-Automatic' tab and check ONLY 'Mass Calibration' for FT and then 'Start.'
J. To perform a unit mass calibration for the LTQ only, open the 'Calibrate' dialog box, choose the 'Semi-Automatic' tab and check ONLY 'Select All - Ion Trap' and then 'Start.'

K. To perform a calibration check, open the 'Calibrate' dialog box, choose the ‘Check' tab and check all that apply, and then 'Start.' Individual items may need to be repeated to pass.
L. If needed based on ‘Check’ results, a full calibration can be performed by opening the 'Calibrate' dialog box, choosing the ‘Semi-Automatic' tab and check all that apply, and then 'Start.' Individual items may need to be repeated to pass.
M. When the calibration is complete, it will display whether or not the calibration items were successful.
    - If the procedure fails, repeat the calibration.
    - When the procedure passes, print the report and evaluate the calibration solution spectrum using the 'Acceptance Criteria' section. If the results are acceptable, print the spectrum of the calibration solution.
N. If all requirements are within specification, prepare the documentation as outlined in IOSS-701. If any requirements fail, contact appropriate instrument support personnel.

6 INSTRUMENTAL CONDITIONS

6.1 Testmix

6.1.1 Liquid Chromatograph

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

6.1.2 Mass Spectrometer

Ionization:	ESI
Polarity:	Positive
Tune File:	testmix_pos (or equivalent)
Sheath Gas Flow:	14 (arb)
Aux Gas Flow:	3 (arb)
Sweep Gas Flow:	3 (arb)
Scan Mode:	Full Scan
Scan Range:	100–650 m/z

6.2 Mass Calibration

6.2.1 Mass Spectrometer

Ionization:	ESI
Polarity:	Negative
Tune File:	calibration_solution_positive (or equivalent)
Scan Mode:	Full Scan
Scan Range:	100-2000 m/z (minimum)

7 ACCEPTANCE CRITERIA

7.1 Testmix

7.1.1 Unit Mass

Verify the results of the testmix. The following ions should be observed in the three testmix injections when using the system for unit mass resolution. RICs should show contemporaneous signals for components at the following masses:

- Caffeine 195 m/z
- Codeine 300 m/z
- Brucine 395 m/z
- Reserpine 609 m/z

7.1.2 Accurate Mass

When using the Orbitrap analyzer for accurate mass analysis, the testmix components should be observed within ±3 mmu of their expected monoisotopic masses:

	Formula	Expected Mass	Acceptable Mass Range
Caffeine	$C_8H_{11}O_2N_4$	195.0877	195.0847 - 195.0907
Codeine	$C_{18}H_{22}O_3N$	300.1594	300.1564 - 300.1624
Brucine	$C_{23}H_{27}O_4N_2$	395.1965	395.1935 - 395.1995
Reserpine	$C_{33}H_{41}O_9N_2$	609.2807	609.2777 - 609.2837

7.2 Mass Calibration

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

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
01	10/04/2018	Section 1- Updated scope to include applicable disciplines/categories of testing. Section 2- Removed Waters Alliance LC Section 7- Added targeted analytes. Section 8- Changed to discipline-specific mobile phase. Section 9- Reduced decimal places from five to four. Section 14- Updated to 'Instrument Operation and Systems Support' Added 'appropriate instrument support personnel' throughout.
02	09/30/2022	Revised to match new format requirements. Section 5- Expanded as-needed maintenance.