ID-X Tribrid LC/MS Performance Monitoring and Maintenance

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1 INTRODUCTION

This document addresses the performance monitoring and maintenance of the ID-X Tribrid LC/MS system consisting of a Thermo Fisher Scientific Orbitrap ID-X Tribrid (MS) and a Waters Ultra-High Performance Liquid Chromatograph (UPLC). This system can be used for high resolution accurate mass analyses and unit mass 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, it 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: General Chemistry and Seized Drugs.

3 EQUIPMENT

- Instrumentation
    - Thermo Fisher Scientific Orbitrap ID-X Tribrid MS, API Source, Xcalibur and TraceFinder software (or equivalent)
    - Waters Acquity I-Class UPLC (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)
    - Caffeine (Sigma or equivalent)
    - Codeine (Sigma or equivalent)
    - Brucine (Sigma or equivalent)
    - Reserpine (Sigma or equivalent)
    - Testmix mobile phase (80:20 methanol:water with 0.1% formic acid)
    - 10 µL LC syringe (Hamilton or equivalent)
    - 100, 250 or 500 µL LC syringe (Hamilton or equivalent)
    - Pierce FlexMix calibration solution (Thermo or equivalent)
    - General laboratory supplies

4 STANDARDS AND CONTROLS

4.1 Testmix

The Testmix Solution is used to assess daily operating performance, mass assignment, and continued integrity of the system. Alternatively, a procedure-specific positive control (e.g., insulin, hGH) may be used to assess instrument performance. 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 pipetting 4.0 mL of the stock testmix into a 100-mL volumetric flask and diluting to the mark with methanol, mixing well, to produce the Testmix Solution. 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 Solution

The FlexMix 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 warranted based on system performance or if the system has been significantly moved, down for a long period of time, or undergone a major repair. The calibration solution is 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 (for API source, marked “High Pressure Line”). 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 nitrogen supply (for higher energy collision-induced dissociation (HCD), internal calibration, and ion-routing multipole, marked “Low Pressure Line”). The regulator should read between 30 and 60 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 100 psi remaining.
D. Record the line pressure of the building helium supply (ion trap gas). The regulator should read between 30 and 60 psi. 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.
E. Check the vacuum pressure under instrument status on the tune page. If a green circle with a white check mark is present, the system is ready.
F. Prime the UPLC system:
    1. Open the Acquity UPLC Console and select ‘System’ in the menu on the left side of the screen.
    2. From the control drop down menu select ‘Start Up System.’
    3. Confirm for the sample manager (SM) that the strong wash, weak wash, and sample syringe are checked and 3 is entered in the cycles box.

4. Confirm for the binary solvent manager (BSM) that all the boxes are checked and set the duration time to 5 minutes.
5. Click the start button to start priming the system.

G. If a column is installed, remove it from the system and replace it with a zero-dead-volume union. Perform an analysis of the appropriate testmix prior to the analysis of case samples. For Testmix Solution, use parameters listed in the 'Instrumental Conditions' section. Start the LC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'yyyymmddTM' (or equivalent). Make three 2 µL injections of the testmix solution at least 10 seconds apart if using the manual loop injector, and then stop the data collection. The testmix may also be injected using the autosampler rather than by manual injection. For targeted analysis, a procedure-specific positive control can be substituted for the testmix. Use the appropriate column, mobile phase, and instrument conditions as specified in the discipline/subdiscipline procedure. Evaluate the results using the 'Acceptance Criteria' section. If the results are acceptable, print the TIC and spectra for components in the testmix.
H. 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 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.

5.2.1 Mass Spectrometer

A. Check the faceplate for debris, clean as needed.
B. Bakeout the analyzer as needed.
C. Change the capillary.
D. Check the level and color of the rough pump oil. Top off or change the oil as needed.
E. Check/clean air filter.

5.2.2 UPLC

A. Replace solvent frits as needed.
B. Replace drain valve as needed.
C. Replace degasser pump as needed.
D. Replace seals, rotors, stators, and/or needles if appropriate.

5.3 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 100, 250 or 500 µL syringe with the Pierce FlexMix 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 5 µL/minute. The flow rate can be adjusted as needed.
D. Verify that the ESI heater is turned off.
E. Monitor spray stability to ensure a stable spray cutoff is achieved (2 < % RSD <15)
F. On the tune page Calibration tab:
    - Select “Check” to perform a calibration check, or “Calibrate” to perform a calibration (the “Check, Calibrate if required” option may also be used).
    - Select “Positive” Ionization for Positive Mode, or “Negative” Ionization for Negative Mode.
    - Select “Orbitrap Mass” to perform only a mass calibration, or “Orbitrap Mass & System” to perform a full check/calibration.
G. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.
H. Ensure the ESI probe is engaged and turn on the MS.
I. Click the "Start" button at the bottom of the Calibration tab to start the calibration.
J. When the calibration is complete, it will display whether or not the calibration was successful.
    - If the procedure fails, repeat the calibration.
    - When the procedure passes, save the report and evaluate the calibration solution spectrum using the 'Acceptance Criteria' section.
K. 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 UPLC

Recommended Mobile Phase:	Testmix mobile phase (or from discipline-specific procedure)
Flow Rate:	0.150 mL/min
Column:	N/A
Injection Volume:	2 µL (volume of sample loop)
Number of Injections:	3 (if using manual loop injector)

6.1.2 Mass Spectrometer

Ionization:	ESI
Polarity:	Positive
Tune File:	testmix_pos (or equivalent)
Sheath Gas Flow:	25 (arb)
Aux Gas Flow:	4 (arb)
Sweep Gas Flow:	0 (arb)
Scan Mode:	Full Scan
Scan Range:	100–650 m/z
Resolution:	60,000

6.2 Mass Calibration

6.2.1 Mass Spectrometer

Ionization:	ESI
Polarity:	Positive or Negative (select as appropriate)
Scan Mode:	Full Scan
Scan Range:	100-2000 m/z

7 ACCEPTANCE CRITERIA

7.1 Testmix

A. The testmix components should be observed within the ranges list below from 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 FlexMix calibration solution are listed below for each mode.

7.2.1 Positive Mode

- Triethylamine 102.1277 m/z
- Tetramethylpiperidine 142.1590 m/z
- Caffeine 195.0877 m/z
- Hexamethoxyphosphazine 322.0481 m/z
- MRFA 524.2650 m/z
- Hexakis(2,2-difluoroethoxy)phosphazene 622.0290 m/z
- Hexakis(2,2,3,3-tetrafluoropropoxy)phosphazene 922.0098 m/z
- Ultramark 1022.0033 m/z
  1121.9969 m/z
  1221.9905 m/z
  1321.9841 m/z
  1421.9777 m/z
  1521.9713 m/z
  1621.9649 m/z
  1721.9585 m/z
  1821.9521 m/z

7.2.2 Negative Mode

○ Trifluroacetic acid	112.9845 m/z
○ Pentafluropropionic acid	162.9813 m/z
○ Perfluoroheptanoic acid	362.9685 m/z
○ [2,4,6-Tris(heptafluoropropyl)-1,3,5-triazine + OH]$^-$	601.9779 m/z
○ [Hexakis(2,2,3,3-tetrafluoropropoxy)phosphazene + TFA]$^-$	1033.9870 m/z
○ Ultramark	1133.9816 m/z
	1233.9752 m/z
	1333.9688 m/z
	1433.9624 m/z
	1533.9560 m/z
	1633.9496 m/z
	1733.9432 m/z
	1833.9368 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. High voltages are present in the API source, use caution when handling while connected.

10 REVISION HISTORY

Revision	Issued	Changes
00	09/01/2023	New document
01	02/18/2025	Added General Chemistry and Seized Drugs and removed Toxicology from Scope. Added analyte specific check to Testmix section to match LTQ procedure. Added Testmix mobile phase definition. Removed yearly scheduled maintenance and moved to as needed maintenance.