# Performance Monitoring Protocol (QA/QC) for the Thermo Exactive OrbiTrap LC/MS (ESI)

## 1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo Exactive OrbiTrap LC/MS system consisting of a Thermo Electron Exactive OrbiTrap 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: Drug chemistry, toxicology, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

## 2 Principle

The Exactive OrbiTrap system is comprised of a Waters LC and a Thermo Electron Exactive OrbiTrap High Resolution MS. This system can be used for high resolution accurate mass chemical analyses. The instrument is configured with an atmospheric pressure ionization (API) source that is capable of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). The instrument is primarily used in ESI mode. However, this protocol can also be used for APCI and APPI provided the method of ionization is clearly labeled in the resulting data. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

## 3 Equipment/Materials/Reagents

a. Instrumentation - Thermo Electron Exactive OrbiTrap MS, API Source, Waters Acquity UPLC (or equivalent), and Data System with Xcalibur software (or equivalent)

b. API Gas - Nitrogen, 99.99% (high purity or equivalent)

c. Caffeine (Sigma or equivalent)

d. Codeine (Sigma or equivalent)

e. Brucine (Sigma or equivalent)

f. Reserpine (Sigma or equivalent)

g. γ-Aminobutyric Acid (GABA), (Sigma or equivalent)

h. Optima Grade Methanol (or equivalent)

i. LTQ Velos OrbiTrap ESI Positive Ion Calibration Solution (Thermo or equivalent)

j. LTQ ESI Negative Ion Calibration Solution (Thermo or equivalent)

k. Infusion Syringe - 10 to 500 µL LC syringe (Hamilton or equivalent)

l. Deionized Water, 18 MΩ·cm Milli-Q or equivalent

m. Acetone, HPLC grade

n. Volumetric glassware

o. Ammonium Nitrate ($NH_4NO_3$), reagent grade

p. HMX, RDX, Tetryl, NG, PETN standards at 1000 µg/mL (Cerilliant or equivalent)

q. 3.125 mM Ammonium Nitrate Mobile Phase (250 mg to 1 Liter water)

r. Waters Cortecs UPLC C18 1.6 µm, 2.1 mm X 50 mm, or equivalent

## 4 Standards and Controls

### 4.1 Testmix (Toxicology/General Chemistry)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record all preparations in the Reagent Log. To prepare:

a. 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.

b. Testmix Solution - Pipet 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 Testmix (Explosives Chemistry)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record stock solution preparations in the Reagent Log. To prepare:

a. 100 µg/mL Stock Solution - Pipette 1 mL of each 1000 µg/mL of HMX, RDX, Tetryl, NG and PETN standards in a separate 10 mL volumetric flask and dilute to the mark with acetone to achieve a final concentration of 100 µg/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.

b. 10 µg/mL Stock Solution - Pipette 1 mL of each 100 µg/mL stock solution of HMX, RDX, Tetryl, NG, and PETN into a 10 mL volumetric flask and dilute to the mark with acetone to achieve a concentration of 10 µg/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.

c. Testmix Solution - For daily use, dilute 20 µL of the 10 µg/mL stock solution to 1 mL with a 50:50 solution of methanol/water.

### 4.3 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

### 5.1 Calibration (Positive Mode)

a. Load an infusion syringe with the LTQ Velos OrbiTrap ESI positive ion 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.

d. On the tune page click “Calibrate” and confirm that MS Mass Calibration (pos) is checked.

e. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.

f. Engage the ESI probe and turn on the MS.

g. Click the “Calibrate” button to start the calibration.

h. When the calibration is complete, it will display whether or not the calibration was successful. If the procedure fails, repeat the calibration.

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

### 5.2 Calibration (Negative Mode)

a. Load an infusion syringe with the LTQ ESI negative ion 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.

d. On the tune page click “Calibrate” and confirm that MS Mass Calibration (neg) is checked.

e. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.

f. Engage the ESI probe and turn on the MS.

g. Click the “Calibrate” button to start the calibration.

h. When the calibration is complete, it will display whether or not the calibration was successful. If the procedure fails, repeat the calibration.

i. If all requirements are within specification, prepare records 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 either the Windows Explorer or Xcalibur program to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain. If analysis consists of multiple samples in a sequence, ensure that there is additional sufficient storage space.

b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 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 250 p.s.i. remaining.

c. Check the oil level of the vacuum pump.

d. Check the vacuum pressure under instrument status on the tune page. If a green circle with a white check mark in it is present, the system is ready.

e. To prime LC system:

1. Open up the Acquity UPLC Console and select Acquity UPLC system in the menu on the left side of the screen.

2. From the control drop down menu select system start up.

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 that the duration time is set to 5 minutes.

5. Click the start button to start priming the system.

f. For Toxicology/General Chemistry Testmix: If a column is installed, remove it from that system and replace it with a zero-dead-volume union.

g. For Toxicology/General Chemistry Testmix: Perform an analysis of the appropriate testmix prior to the analysis of case samples. For targeted analytes, a positive control can be substituted for the testmix. Use parameters listed in the 'Instrumental Conditions' section of this protocol. Select the appropriate mobile phase. Start the HPLC 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 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the testmix.

h. For Explosives Chemical Analysis: 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.

i. If all requirements are within specification, prepare records as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact 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 the ion sweep cone (the heated interface front plate) as needed.

## 8 Instrumental Conditions

### 8.1 Testmix (Toxicology/General Chemistry)

Liquid Chromatograph

| | |
|---|---|
| Mobile Phase: | From discipline-specific SOP |
| Flow Rate: | 0.15 mL/min |
| Column: | None |
| Inj Volume: | 5 µL |

Mass Spectrometer

| | |
|---|---|
| Ionization: | ESI |
| Tune File: | testmix_pos |
| Sheath Gas Flow: | 9 (arb) |
| Aux Gas Flow: | 3 (arb) |
| Sweep Gas Flow: | 0 (arb) |
| Scan Mode: | Full Scan |
| Scan Range: | 100-650 m/z |
| Resolution: | 75000 |

### 8.2 Testmix (Explosives Chemistry)

Liquid Chromatograph

| | |
|---|---|
| Mobile Phase: | From discipline-specific SOP |
| Flow Rate: | 0.5 mL/min |
| Column: | Waters Cortecs UPLC C18 1.6 µm, 2.1 mm X 50 mm |
| Inj Volume: | 8 µL |

Mass Spectrometer

| | |
|---|---|
| Ionization: | ESI |
| Tune File: | exp_tune |
| 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) |
| Resolution: | 17500 |

### 8.3 Calibration

Mass Spectrometer

| | |
|---|---|
| Ionization: | ESI |
| Scan Mode: | Full Scan |
| Scan Range: | 100-2000 m/z |

## 9 Decision Criteria

### 9.1 Testmix (Toxicology/General Chemistry)

When using the OrbiTrap analyzer for accurate mass analysis, the testmix components should be observed within the range 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 |

### 9.2 Testmix (Explosives Chemistry)

When using the OrbiTrap analyzer for accurate mass analysis, the testmix components should be observed within the range below from their expected monoisotopic masses:

| | Formula | Expected Mass | Acceptable Mass Range |
|---|---|---|---|
| HMX(+$NO_3$) | $C_4H_8O_{11}N_9$ | 358.0338 | 358.0288-358.0388 |
| RDX(+$NO_3$) | $C_3H_6O_9N_7$ | 284.0222 | 284.0172-284.0272 |
| Tetry(+$NO_3$) | $C_7H_5O_{11}N_6$ | 349.0011 | 348.9961-349.0061 |
| NG(+$NO_3$) | $C_3H_5O_{12}N_4$ | 288.9898 | 288.9848-288.9948 |
| PETN(+$NO_3$) | $C_5H_8O_{15}N_5$ | 378.0011 | 377.9961-378.0061 |

### 9.3 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 |

### 9.4 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 (electronic or hardcopy).

"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.*

"Preparation of Chemical Reagents" (Tox 103) *Toxicology SOP Manual.*

“Solid Phase Extraction of Opioids from Biologicals with Analysis by LC-Tandem MS” (Tox 418) *Toxicology SOP Manual.*

*FBI Laboratory Safety Manual.*

| Rev. # | Issue Date | History |
|---|---|---|
| 2 | 05/23/17 | Changed 'Stock Solution' to '100 µg/mL Stock Solution' in 4.2.a. Changed 'Testmix Solution' to '10 µg/mL Stock Solution' in 4.2.b. Added 'Testmix Solution' section 4.2.c, dilution for daily use. |
| 3 | 10/04/18 | Updated Section 1 Scope to include disciplines/categories of testing. Changed Section 3 q to 250 mg. Changed 'all' to 'stock solution' in Section 4.2. Added 'appropriate instrument support personnel' to Sections 5.1 i. 5.2 I, and 7.1 b & i. Updated heading in Section 6. Changed 'subunit' to 'discipline' mobile phase in section 8.1. Added '(minimum)' to scan range in 8.2. Updated mobile phase and flow rate in section 8.2. Reduced decimal places from five to four in Section 9.1. Corrected typos in Section 9.2. Updated 'Instrument Operation and Systems Support' in Section 14 and header. |

## Approval

Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader: Date: 09/28/2018

Toxicology
Technical Leader: Date: 09/28/2018

Explosives (Chemistry)
Technical Leader: Date: 09/28/2018

IOSS Manager: Date: 09/28/2018

Chemistry Unit Chief: Date: 09/28/2018

## QA Approval

Quality Manager: Date: 09/28/2018