UPLC, Sample Manager, and Q-TOF System Performance Check

NPC.SOP.MS002 Version 2.1

Effective Date: April 2019

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1. Purpose

The purpose of this standard operating procedure (SOP) is to document the Ultra Performance Liquid Chromatography (UPLC) Quadrupole Time of Flight (Q-TOF) Mass Spectrometer (MS) system performance check to confirm that the UPLC-QTOF is calibrated, performing to predefined acceptance criteria prior to sample analysis or following any repairs or any stoppages during analysis.

2. Scope

The scope of this SOP will cover Waters Acquity UPLC visual inspection and routine maintenance and Waters Xevo G2-XS Q-TOF MS visual inspection, detector and lock mass set up and resolution check and calibration.

This scope of this SOP does not cover sample analysis or sample batch preparation.

3. Materials

Consumables

- LCMS grade methanol
- LCMS grade isopropanol
- Leucine enkephaline (LeuEnk) standard (NPC.SOP.MS001)
- Sodium formate calibration solution (NPC.SOP.MS001)
- RP-SSTM solution (reversed phase system suitability test mix)
- Foil
- Seal wash

Personal Protective Equipment

- Laboratory coats
- Nitrile gloves (when handling chemicals)
- Safety glasses (when handling chemicals)

This procedure involves the handling of chemicals which must be handled in accordance with the chemicals safety data sheets and risk assessments

4. Procedure

4.1. UPLC-QTOF system check

Perform the procedures outlined below for the UPLC system check and record any results required in the proforma (NPC.PRO.MS002). If the system fails the checks, label the system out of use, contact Waters to place an iRequest and do not use the system.

Seal wash will be replaced weekly regardless of volume.

Expiration dates for strong and weak wash are 3 months and 1 month from preparation date.

UPLC and Sample Manager - Visual inspection

- **Check for the following:**
  - Non-essential materials have been removed from the LC tray on top of the system.
  - Signs of leaks or corrosion around binary solvent manager pumps, fittings and instrument waste outlets.
  - Sample manager for damage or leaks. Remove any samples not required.
  - Solvent bottles on top of the LC system are adequately filled and that the solvent lines are fully submerged. Replace if expired.
  - All mobile phase solvent bottles are securely capped or covered with foil.

4.1.1. UPLC system and Sample Manager - Routine maintenance

4.1.1.1. Perform visual inspection (see respective box).

4.1.1.2. Remove back pressure regulator (BPR) line from isopropanol recirculation and connect to waste.

4.1.1.3. Remove solvent lines A and B from storage in isopropanol and place in 100% methanol.

4.1.1.4. In system set up, prime all lines for 4 minutes to ensure that there is no air in the lines.

4.1.1.5. Set the initial UPLC conditions to 50% A and 50% B at a flow rate of 0.05 mL/min and allow to equilibrate until the pressure has stabilised (ΔPSI <20 PSI).

4.1.1.6. Perform the dynamic leak test for both pumps A and B at 14000 PSI Accumulator pressure and record results in the proforma. If the test fails, consider repair or use different instrument.

4.1.1.7. Once the leak test is complete replace solvent lines A and B into storage in fresh isopropanol and prime for 4 minutes.

4.1.1.8. Set the flow to 50% A and 50% B at 0.05 mL/min and allow the flow to waste for 5 minutes before returning the BPR line back to the isopropanol storage for recirculation.

4.1.1.9. Purge weak and strong needle wash lines by activating the wash station valves for 30 seconds.

4.1.1.10. Rinse the sample manager syringe 9 times with weak wash, 9 times with strong wash followed by 9 times with weak wash.

4.1.1.11. Rinse the sample loop with weak wash, strong wash, and weak wash.

4.1.1.12. Check and record sample manager temperature.

4.2. Q-TOF system check

Perform the procedures outlined below for the MS system check and record any results required in the proforma (NPC.PRO.MS002). If the system fails the checks, label the system out of use, contact Waters to place an iRequest and do not use the system.

MS - Visual inspection

- **Check for the following**:
  - The volume of the LeuEnk solution. Top up if below 100 mL and record in proforma.
  - Place the MS into operate mode and load the correct tune file - TOF XX.ipr *(XX = instrument number)* and enter tune page in Proforma.
  - All settings correspond with the NPC standard setting outlined in Table 2.
  - Vacuum read back values - record results in proforma.

4.2.1. Detector and lockspray set-up

4.2.1.1. Perform detector set up using stable LeuEnk signal at an intensity of 5 e^5 via the IntelliStart option. Record the results in the proforma NPC.PRO.MS002.

4.2.1.2. Uncalibrate the instrument in IntelliStart normal mode.

4.2.1.3. Check the nominal mass of the LeuEnk model peak and adjust V_{eff} if necessary.

4.2.1.4. Perform lockspray set up as outlined in NPC.SOP.MS001. Record all results in the proforma NPC.PRO.MS002.

N.B.: If the instrument is in the opposite ion mode than required, switch the instrument polarity and leave for 1 hour to settle before preforming the set-up steps.

4.2.2. Preparation of system suitability test mix (SSTM)

4.2.2.1. If performing a resolution check on a MS designated for RPOS, RNEG or HPOS assays, an aliquot of the SSTM is required. Remove a prepared sample from storage at -80 °C and allow to defrost before use.

4.2.2.2. Add the defrosted SSTM aliquot (10 mL) to a 100 mL volumetric flask and make up to volume with LCMS grade water. This is now the working concentration of the SSTM.

4.2.2.3. If there is no pre-prepared SSTM follow steps as given below in 4.2.2.4 – 4.2.2.7.

4.2.2.4. Weigh the quantities of each dry chemical standard detailed in Table 1 into individual weighing boats and rinse into a 1 L volumetric flask with high purity water.

4.2.2.5. Make the 1 L volumetric flask up to volume with LCMS grade water and sonicate for 40 minutes at 25 °C or until the contents have fully dissolved.

4.2.2.6. Aliquot the SSTM into 15 mL corning centrifuge tubes at a volume of 10 mL.

4.2.2.7. Label the SSTM aliquots and store at -80 °C until required.

Table 1. SSTM composition

RP-SSTM	SIGMA Cat #	Target weight (g)	Max (g)	Min (g)
L-Glutamine	49419	0.09134	0.09225	0.09043
L-Glutamic Acid	49449	0.07356	0.0743	0.07283
Creatinine	C4255	0.05656	0.05712	0.05599
Cytidine	C122106	0.24322	0.24565	0.24078
Citric Acid	251275	0.04803	0.04851	0.04755
L-Isoleucine	58879	0.04919	0.04968	0.0487
L-Leucine	61819	0.04919	0.04968	0.0487
L-Tryptophan	93659	0.07658	0.07735	0.07582
Benzoic Acid	242381	0.12212	0.12334	0.1209
Octanoic Acid	C2875	0.14421	0.14565	0.14277
Hippuric Acid	112003	0.11198	0.1131	0.11086
L-Phenylalanine	78019	0.13215	0.13347	0.13083

4.2.3. MS system – Resolution check

4.2.3.1. If resolution check is required in the opposite ion mode, switch the instrument polarity and leave for 1 hour to settle before preforming the next steps.

4.2.3.2. Adjust the "Tune page" settings to the parameters outlined in Table 2 corresponding to the method type to be used on the system.

4.2.3.3. Purge the fluidics, either line A containing SSTM (RPOS/RNEG/HPOS) or line B containing LeuEnk (LPOS/LNEG), start infusing at 15 µL/min

4.2.3.4. For a stable signal with an intensity of $2e^4$ of the selected m/z peak adjust gas flow, capillary voltage and/or desolvation temperature, respectively. *N.B. Recommended ions for SSTM: Cytidine $[M+H]^+$ =244.0928 / citric acid $[M-H]^-$ = 191.0197. If necessary, adjust V_{eff}.*

4.2.3.5. Perform a "Tune page" acquisition for 3 minutes of the infusion and open up the ResCal tool.

4.2.3.6. Open up the TIC, combine the spectra for 30 second intervals (e.g. 0.5-1 min, 1-1.5 min, etc.) and measure the resolution over the respective peak. Repeat this for 5 independent intervals and calculate the average resolution value.

4.2.3.7. Record all results in the proforma NPC.PRO.MS002.

4.2.3.8. Repeat the acquisition (steps 4.2.3.4 – 4.2.3.7) at an intensity of $2e^5$.

4.2.3.9. Enter the average resolution value of the $2e^4$ in the acquisition setting.

4.2.4. MS system – Calibration and final checks

4.2.4.1. Purge the fluidics line C with calibration solution (sodium formate) twice.

4.2.4.2. Perform calibration as outlined in NPC.SOP.MS001 and record the results in the proforma NPC.PRO.MS002.

4.2.4.3. Purge lines A and C with wash solvent twice and infuse until SSTM and sodium formate have been purged from the system (typically 2-3 minutes).

4.2.4.4. Save the system settings to PC and backup system settings to the EPC.

4.2.4.5. Ensure MS settings match assay specific NPC standards, major assays are given in Table 2.

4.2.4.6. Save the tune file TOFXX.ipr (both tuning parameters and system settings).

4.2.4.7. Create a system report for the respective mode and save it as YYYYMMDD_TOFXX_SR_POS.xps (or for negative mode respectively)

4.2.4.8. Reset ADC-Results.csv from EPC via telnet Putty.exe.

Table 2. NPC standard MS settings for major assays.

Parameter	RPOS, HPOS	RNEG	LPOS	LNEG
Mode	Sensitivity, MS	Sensitivity, MS	Sensitivity, MS	Sensitivity, MS
Quad Profile	Auto Profile	Auto Profile	Auto Profile	Auto Profile
Gas	API: on COL: on	API: on COL: on	API: on COL: on	API: on COL: on
Sample Probe Position (mm)	7	7	7	7
Capillary Voltage (kV)	1.5	1.0	2.0	1.5
Sampling Cone Voltage	20	20	25	25
Source Offset	80	80	80	80
Source Temperature (ºC)	120	120	120	120
Desolation Temperature (ºC)	600	600	600	600
Cone Gas Flow (L/hr)	150	150	150	150
Desolvation Gas Flow (L/hr)	1000	1000	1000	1000
Collision Energy (kV)	Off/4	Off/4	Off/6	Off/6
Stepwave 2 Offset	Auto/10	Auto/10	Auto/15	Auto/15
Automatic Gain control (Instrument Detector Control in Tune Page)	ON	ON	ON	ON
Optimise Detector Gain Usage (System Auto Detector Gain Control in System View)	Background Ions Settings	Background Ions Settings	Background Ions Settings	Background Ions Settings
Ions Settings: Timeout (sec)	120	120	70	70
Use Max Voltage Adjust (V) - ticked	1.0	1.0	1.0	1.0
Lockspray Infusion Rate (µL/min)	≤15	≤15	≤15	≤15
Lockspray Capillary Voltage (kV)	3	2	3	2

Table 3. NPC MS calibration settings

Parameter	Calibration POS	Calibration NEG
Capillary (kV)	3	2
Cone (V)	150	100
Desolvation temp (C)	450	450
Cone gas (L/hr)	0	0
Desolvation gas (L/hr)	500	500

5. Related Documents

Document Number	Title
NPC.PRO.MS002	UPLC and Q-TOF system performance check - Proforma
NPC.SOP.MS001	Calibration and LockMass

6. Version History

Current version

Version number	Author	Changes and justification	Section(s) updated
V2.1	VHS	Revision and minor corrections	all

Previous versions

Version number	Author	Changes and justification	Section(s) updated
V2.0	VHS/SC/ BC	Changed for new diluted MS protocols	all
V1.1	VHS/SC/ BC	Revision and minor corrections	all
V1	DB	New SOP	N/A

7. Responsibilities

Centre management is responsible for ensuring that laboratory technical personnel are appropriately qualified to perform the procedures outlined in this SOP. The appointed laboratory personnel are in turn responsible for conducting the procedure as outlined in accordance with health and safety standards.

Health and safety statement: before commencing any activities described in this document personnel must be adequately trained e.g. staff having completed local institution Chemical Safety Training and staff having read and understood the relevant risk assessments. Chemical, biological and general waste should be disposed of according to local policies.

8. Approval

Prepared by Dr Verena Horneffer-van der Sluis	Date
Reviewed by Dr Maria Gomez-Romero	Date
Authorised by Dr Matthew Lewis	Date

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