Calibration and LockMass

National Phenome Centre

NPC.SOP.MS001 Version 2.1

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1. Purpose

The purpose of this standard operating procedure (SOP) is to document the following procedures:

- Preparation of sodium formate and subsequent calibration of Q-ToF mass analysers
- Preparation of leucine enkephaline (LeuEnk) and subsequent lock mass acquisition of Q-ToF analysers

2. Scope

Calibration of a Q-ToF mass analyser as well as acquisition and application of a suitable lockmass is crucial to ensure accurate and consistent analytical results.

Calibration, or at the very minimum a calibration check of the system prior to any analysis, verifies that the instrument is accurately reading analyte masses prior to the acquisition of any data.

Lockmass acquisition and application during the course of a run ensures that should any factors affect the calibration of the quad or flight tube – such as atmospheric or temperature fluctuations – that these changes are accounted for and do not alter the observed analyte mass.

This is particularly important when working with unknown samples where anlayte ID is desired, as small changes in the observed mass can potentially lead to analyte misidentification.

3. Materials

Consumables

- LCMS grade sodium hydroxide solution
- LCMS grade formic acid
- Alternative: LCMS grade sodium formate
- LCMS grade high purity water
- LCMS grade 2-propanol (IPA)
- LCMS grade acetonitrile
- Leucine Enkephaline standards (Waters P/N: 700008842-1)

Equipment

- 200 µL pipette
- 1 mL pipette
- 10 mL pipette

Personal Protective Equipment

- White laboratory coats
- Nitrile gloves
- Eye protection

4. Calibration

4.1. Preparation of sodium formate calibration solution – fresh on day of use

This solution should be prepared on day of use and can be stored between 2-4ºC for up to one week or at -20ºC for one month before discarding.

Alternative 1:

1. Dilute the sodium hydroxide solution to 0.05% v/v in water. E.g. dilute 10 µL sodium hydroxide 50% v/v to 10 mL in water.
2. Combine: 19 mL of IPA, 1 mL of 0.05% sodium hydroxide and 5 µL pure formic acid.

Alternative 2:

1. Prepare a 50 mM stock solution of sodium formate in water. E.g. dilute 0.0170025g sodium formate in 50 mL LCMS grade water. This solution can be stored between 2-4ºC for up to one month.
2. Combine: 9 mL of 2-propanol with 1 mL of sodium formate stock solution.

4.2. Calibration Procedure - To be conducted before running an assay

4.2.1. Place instrument into operate mode in the polarity to be calibrated. Leave for 60 minutes ±5 minutes for electronics to settle.

4.2.2. Purge the sample fluidics with calibration solution twice.

4.2.3. Adjust tune page settings to calibration settings:

Parameter	Calibration setting (pos mode)	Calibration setting (neg mode)
Capillary (kV)	3.0	2.0
Cone (V)	150	100
Desolvation temp (ºC)	450	450
Cone gas (L/hr)	0	0
Desolvation gas (L/hr)	500	500

4.2.4. Calibrate using the Intellistart function.

4.2.5. Using Intellistart in “normal mode”, clear the last calibration profile by loading the “uncal” profile.

4.2.6. In “configuration mode”, calibrate in sensitivity mode for the intended acquisition polarity.

4.2.7. Ensure the profile is as follows:

- Mass range: 50-1200 Da (RPOS/RNEG/HPOS), or 50-2000 Da (LPOS/LNEG)
- Type of calibration: assisted
- Use "sodium formate" as the calibrant

4.2.8. Data acquisition options:

- "MS mode"
- Collision energies "off"
- Cone and capillary voltages: "Tune page" settings
- Fluidics: "Tune page" settings

4.2.9. RMS and 95% confidence interval values should be <1 ppm and all peaks should have been found and picked properly.

4.2.10. Return instrument tune page settings back to required assay settings. See respective SOP.

4.2.11. Purge fluidics lines with wash solution twice and flush through sample probe for 30 seconds.

5. LockMass

5.1. Preparation of leucine enkephaline (LeuEnk)

To prepare a 400 ng/µL LeuEnk stock solution:

1. Add 7.5 mL of ultrapure water to Waters 3 mg/bottle LeuEnk standard.
2. Mix to dissolve the LeuEnk.
3. Label appropriately.
4. Store at -20ºC for up to 1 year.

To prepare LeuEnk working solution:

1. For reversed phase (RP) and HILIC methods prepare a 200 pg/µL solution of LeuEnk diluted in 50:50 water: acetonitrile 0.1% formic acid.
2. For lipid methods prepare a 600 pg/µL solution of LeuEnk diluted in 50:50 water: acetonitrile.
3. Please see table below for recommended volumes.

Assay	Final Concentration (pg/µL)	LeuEnk stock needed (µL)	Solvent A (250 mL)	Solvent B (250 mL)
RP & HILIC	200	500	Water	Acetonitrile + 0.1% formic acid
Lipids	600	1500	Water	Acetonitrile

4. Sonicate for 5 minutes.
5. Label appropriately.
6. Place on instrument.

5.2 LockMass setup - To be conducted before running an assay

1. Load tune page for desired analytical method.
2. Purge the line B containing LeuEnk lock mass solution.
3. Set sprayer position to “lock spray”, and infuse at 15 µL/min.
4. Adjust the lock spray capillary voltage to achieve a stable signal of:
   - m/z 556.2771 at 2 e^4 for positive mode
   - m/z 554.2615 at 2 e^4 for negative mode.
5. Set up only the mode (pos OR neg) to be used for the assay.
6. In Intellistart “configuration mode”, select “lock spray set up”.
7. In the “LockSpray Profile editor” select new.
8. Name the methods e.g. “Leu_Enk_POS_Sens” / “Leu_Enk _NEG_Sens”.
9. As “Reference compound” choose “Detector setup (Leucine Enkephalin)”
10. In MS mode: for positive mode: include mass 556.2771 with Collision Energy off.
    for negative mode: include mass 554.2615 with Collision Energy off.
11. Use custom setup to display report.
12. For cone voltage and analyser transmission set up use appropriate method “Tune page” settings.
13. For fluidics setup use appropriate method “Tune page” settings.
14. Once lockspray setup is complete, a successful outcome is indicated in the report by the following:
    - DRE Lens setting of 99.9%.
    - Minimum recommended total Lock spray time below 0.150 seconds.

N.B.: When repeating lockspray setups, the method created in step 7 can be overwritten and the respective lockspray method chosen from the drop down row.

6. Related Documents

Document Number	Title
NPC.SOP.MS002	UPLC & Q-ToF System Performance Check

7. Version History

Current Version

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

Previous Version

Version number	Author	Changes and justification	Section(s) updated
V2.0	VHS	Changed for new diluted MS protocols	all
V1.1	VHS/BC	Revision and minor corrections	all
V1.01	DJB	Transfer to new SOP template	N/A
V1	DJB	New SOP	N/A

8. 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.

9. Approval

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

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