STANDARD OPERATING PROCEDURE

Title: Optimizing Mass Spectrometer Performance for Experiments 1 and 2

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1. PURPOSE

The purpose of this document is to describe the procedures for calibrating and optimizing the performance of the TripleTTOF® 5600+ mass spectrometer for electrospray mass spectrometry with *nano*-LC-MS.

2. SCOPE

This procedure encompasses the i) preparation of benchmark and calibrant solutions; ii) spectral data acquisition to assess state of the instrument prior to tuning; iii) optimization procedure; and iv) assessment of instrument performance after calibration and tuning.

3. RESPONSIBILITIES

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures and the user manual for the instrument. The interpretation of results must be done by a person with expertise in mass spectrometry and familiar with such interpretation. It is the responsibility of the primary instrument operator to perform these procedures when the system has failed to meet daily specification requirements for LC-MS as given in WU-SOP-MS2-01.

4. EQUIPMENT

Source: New Objective Digital Picoview for PV-450
Emitter tip: New Objective PicoTips® emitter silica tips (FS360-20-10N-20-C20)
LC-to-source connection: Fused silica tubing (Polymicro Technologies,1068150009)
Mass spectrometer: TripleTOF® 5600+ (Sciex)

Microcentrifuge, Eppendorf 5415D
Rainin™ Pipet-lite XLS, P20, P200, P1000

5. MATERIALS

Syringe (Hamilton 8175) (250 µL)
Fused Silica Tubing (100 µm)
Water containing 0.1% formic acid (Honeywell Burdick & Jackson, LC452-2.5)
Acetonitrile containing 0.1% formic acid (Honeywell Burdick & Jackson, LC441-2.5)
Glu-Fib (Sigma (F3261- 0.1)
Installation Kit for TTOF System™ (Cat No. 4456736, reserpine and tuning solution)
Methanol (SIGMA, 34860)
Graduated cylinders (10 mL and 100 mL)
Glass media bottle (Pyrex)
Microtubes: Axygen® MCT-175-C (311-04-051)
Axygen® MAXYmum™ recovery tips;
P200 and P20: T-200-C-L-STK,
P1000: T-1000-C-L-R

6. REAGENTS

Preparation Of Calibrant And Benchmark Solutions.

A. Preparation Of Diluent Solution (DS) (90% Water, 10% Acetonitrile, 0.1% formic acid)

1) Measure 90 mL of water containing 0.1% formic acid in a 100 mL graduated cylinder.
2) Transfer to a 100 mL glass media bottle.
3) Measure 10 mL of acetonitrile containing 0.1% formic acid in a 10 mL graduated cylinder.
4) Add to the water/formic acid solution and mix by swirling.

B. Preparation Of The Glu-Fib Solutions

1) Add 900 µL of DS to the Glu-Fib vendor vial (50 pmol/µL) and vortex 3 x 30s.
2) Rinse the microfuge tubes with methanol three times.
3) Dilute the stock solution by adding 180 µL of methanol to the washed microfuge tubes using a Pipetman®.
4) Dilute the primary stock solution by adding 100 µL to a washed tube containing 900 µL of IS and mix by vortexing as in Step 1.
5) Prepare two working solutions from the Glu-Fib secondary stock use the washed microfuge tubes and mix as described above (Step 1):
    i) Glu-Fib (500 fmol/µL), 100 µL of secondary stock and 900 µL of DS;

ii) Glu-Fib (150 fmol/µL), 350 µL of secondary stock and 500 µL of DS.

C. Preparation Of Instrument Performance Solution

1) Add the following quantities to a washed microfuge vial:
2) DS (50 µL),
3) Tuning solution (50 µL),
4) Reserpine (150 µL)
5) Glu-Fib, 150 fmol/µL (150 50 µL).

D. Preparation of Final Standards

The final concentration of standards is 75 and 56 fmol/µL of Glu-Fib, respectively, in a 1:10 dilution of stock tuning solution.

- This is a 1:10 dilution of Tuning Sol'n
- 56 fmol/µl Reserpine *m/z* 609.4
- 75 fmol/µl GluFib *m/z* 785.8

7. PROCEDURES

A. MS Setup Parameters

1) Source/Gas Parameters:
    a) Curtain Gas (CUR): 25
    b) IonSpray Voltage (IS): 3200
    c) Ion Source Gas 1 (GS1): 30
    d) Ion Source Gas 2 (GS2): 0
    e) Interface Heater Temperature (IHT): 175ºC

2) Scheduled PRM Parameters:
    a) PRM detection window (sec): 600
    b) Target Scan Time (sec): 1.5

3) MS Parameters:
    a) Declustering Potential (DP): 70
    b) Collision Energy (CE): From Skyline (default ABI TTOF 5600 algorithm)
    c) Resolution Q1: Unit
    d) Resolution TOF MS1: >25000
    e) Resolution TOF MS2 >15000
    f) Intensity threshold (total count): 50
    g) Settling time (ms): 0
    h) Pause between mass ranges (ms): 1.038

B. New Objective Stage Positioning and Analyst® TF Parameters

NOTE: values will vary slightly after installing a new tip. Infuse the Instrument Assessment Solution to determine the best tip position and Analyst® TF Parameters.

1) **New Objective Source Stage Position**

Axis	Value	Range
X (left-right)	10500	10,000 - 11,500
Y (in-out)	1500	FIXED
Z (up-down)	4300	4000 – 5000

2) **Analyst TF® Parameters**

Source/Gas Tab	Value	Range
GS1	3	5-15
GS2	0	Not used for nanospray
CUR	40	Fixed
ISFV	3600	3400 – 3800 typical (start new tip at 3000)
IHT	150	75 – 150

Compund Tab	Value	Range
DP	100	flat in 80 – 110 range; same value in TOFMS & MSMS
CE	10	FIXED in TOFMS

NOTE: Use rolling Collision Energy for IDA data acquisition.

NOTE: In most cases, parameters under the Resolution or Detector tabs should not be adjusted. These values are set by the Sciex field service engineers running the 'Instrument Optimization' function

NOTE: FIXED is based on various infusion tests with 50 fmol/ul GluFib

C. Assessing Instrument Status Prior to Optimization

1) Double click on Manual Tuning
2) Set up the following parameters:
    a) Drop down menu: Syringe Pump Method
        Diameter: 2.3 (250 µL ul syringe)
        Flow: 800 nL/min
    b) Drop down menu: MS Method
        Source/Gas Tab: see above
        MS tab: Duration: 1 sec
        TOF Masses: min 400 max 1800
3) Click Start and go to drop down menu View / Graph Information Window

4) Zoom in on 785.8 and highlight the first peak, monitoring the TIC and adjusting the Source/Gas parameters until obtaining the highest Resolution and Intensity possible.
5) Click Stop.
6) Get a screen cap that includes the Graph Information Window and paste into Powerpoint, labeling as GluFib Baseline TOFMS PreClean/Opts

7) Click on Acquire Sample Name 2014xxxx_GluFib_75 fmol_TOFMS
Data File Name 2014xxxx_GluFib_75fmol
(this goes to Analyst Data\Projects\January 2014\Data)

8) Click Start
9) Acquire for ~2 min then click Stop
10) Zoom in on the TIC for about a 0.5 min range
11) Double click in the box, bringing up a new window
12) Zoom in on 785 and set threshold arrow up so it only gets the first 785 pea
13) RIGHT click in window and click on List Data, adding a new window
14) Get a screen cap, paste in Powerpoint and label as Glu Fib MS Centroid

At Spec:
Res ≥30k Centroid Intensity: xxxx4000 (Glu-Fib)

15) In the MS tab:
a) Set Type to Product Scan
b) Click High Resolution
c) Parent mass: 785.8
d) Duration: 1 sec
e) TOF Masses: min 100 max 1800

16) In the Compound Tab: Set the Collision Energy to 45

17) Click on Acquire Sample Name 2014xxxx_GluFib_66fmol_HR
Data File Name 2014xxxx_GluFib_66fmol
18) Click Start
19) Acquire for ~2 mins, then click Stop
20) Zoom in on the TIC for about a 0.5min range
21) Double click in the box, bringing up a new window
22) Set threshold arrow up so it only just catches the 187.07 peak
23) Right click in the window and click on List Data, adding a new window
24) Adjust the windows sizes so that the values for 187.07, 480.25, 813.39 and 1056.48 are visible. Get a screen capture, paste into PowerPoint and label as Glu Fib Product Ion – HR

At Spec:

Mass	Resolution	Centroid Intensity: xxxx
187.07	>20k	>40
480.25	>20k	>130

813.39	>20k	>250
1056.48	>20k	>130

25) In the MS tab: Click High Sensitivity
NOTE: no other parameters need to be changed.
26) Click on Acquire Sample Name 2014xxxx_GluFib_75fmol_HS
Data File Name 2014xxxx_GluFib_75fmol
27) Click Start
28) Acquire for ~2 mins, then click Stop
29) Zoom in on the TIC for about a 0.5 min range
30) Double click in the box, bringing up a new window
31) Set threshold arrow up so it only just catches the 187.07 peak
32) Right click in the window and click on List Data, adding a new window
33) Adjust the windows sizes so that the values for 187.07, 480.25, 813.39 and 1056.48 are visible.
34) Get a screen cap, paste in Powerpoint and label as Glu Fib Product Ion – HS
At Spec:

Mass	Resolution	Centroid Intensity
187.07	>12k	>xxxx120
480.25	>12k	>425
813.39	>12k	>750
1056.48	>12k	>450

D. Instrument Optimization

1) Confirm the following parameters:

2) Drop down menu: Syringe Pump Method
   a. Diameter: 2.3 (250ul syringe)
   b. Flow: 0.8 ul/min
3) Drop down menu: MS Method
   a. Source/Gas Tab: 2-25
   b. MS tab: Duration: 1 sec
   c. TOF Masses: min 100 max 1800
4) Click Start and go to drop down menu View / Graph Information Window
5) Zoom in on 829.5 and highlight the first peak, monitoring the TIC and adjusting the Source/Gas parameters until obtaining the highest Resolution and Intensity possible. Click Stop.
   i. Resolution: ~20k Intensity: ~65k
6) Get a screen cap that includes the Graph Information Window and paste into Powerpoint, labeling as Instrument Opts Baseline TOFMS PostClean/Opts
7) Close Manual tuning and double click Instrument Optimization
8) Check all boxes, click ok
9) Enter GS1, ISFV, Syringe diameter and flow rate
10) Click OK
11) Copy the Optimization Summary Table into a Word doc

E. Post-Optimization Assessment With Glufib Standard Infusion

1) Repeat the Pre-Optimization GluFib Infusion, labeling all screen captures as 'Post Instr Opts'.
2) The instrument should meet the specifications as shown above for GluFib standard. If the instrument fails these specifications the entrance section of the instrument should be cleaned and the status again assessed as above. If instrument continues to fail specifications the mass spectrometer vendor should be consulted.

F. Procedure To Clean Q-JET

1) Turn the MS off.
2) Turn the roughing pump off
3) Remove nano source.
4) Allow source heater to cool and MS to vent
5) Remove source cover and heater
6) Remove Q-Jet and cover opening with foil
7) Take the lens off of the Q-Jet assembly and scrub both it and the rails with 1% Alconox
8) RINSE WITH TAP WATER THOROUGHLY
9) RINSE WITH deionized WATER THOROUGHLY
10) Immerse the Q-Jet in 100% Methanol, suspending it with spatulas
11) Sonicate for 20 mins, flip ends and sonicate for 20 mins
12) Blow dry with nitrogen
13) Re-install in instrument
14) Pump down overnight (green light on instrument indicates adequate vacuum has been achieved. Value can be viewed within the software.

8. REFERENCED DOCUMENTS

WU-SOP-MS2-01- Mass Spectrometry Using Parallel Reaction Monitoring for Experiments 1 and 2

9. ABBREVIATIONS

DS, instrument compatible diluent solution for preparation of benchmark solutions