Evaluating Nanospray Ruggedness on a Curtain Gas-Triple Quadrupole MS Equipped with Emitter Rinsing

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Introduction

Nanospray has become an essential tool in high-sensitivity MS, but limited robustness and reproducibility have historically challenged the adoption of nanospray in quantitative applications with triple quadrupole mass spectrometry. MS-based biomarker quantitation places strict requirements on the analytical performance of nanobore LC-MS not only in the need for reproducibility and robustness but also due to the intrinsically complex nature of the samples used in these workflows. Automated emitter positioning with rinsing has been previously demonstrated to improve spray stability and analyte response on a 3D ion trap MS[1]. Here we investigate the utility of automated tip rinsing to improve emitter spray stability and data quality on a hybrid triple quadrupole/LIT MS equipped with a heated interface blanketed by a laminar flow of nitrogen gas.

Methods - QQQ/LIT

Instrumentation

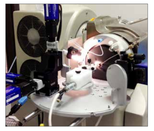
- Mass Spectrometer: 4000 Q TRAP (AB SCIEX)
  - Scan Settings: Q1 MS Scan; 400 – 1000 Da; profile mode; unit resolution
  - Compound parameters: Declustering potential – 70; Entrance potential - 10
  - Source/Gas parameters: Curtain gas - 10.0; Ion spray voltage - 2200 kV, Ion source gas - 3.0; Interface heater temperature -150°C
- Source: Digital PicoView DPV-450 nanospray source (New Objective, Inc.)
- Emitter: uncoated, 360 µm OD x 20 µm ID x 10 µm tip (New Objective, Inc.)
- HPLC: Eksigent nanoLC-2D (AB SCIEX)
- Autosampler: HTC PAL (Leap Technologies)

Reagents

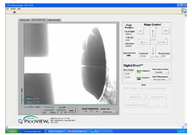
- Tip Rinse: 50% water/50% methanol, gravity flow (~50 µL/min.)
- Mobile Phase A: 0.1% formic acid in water (JT Baker)
- Mobile Phase B: 0.1 formic acid in acetonitrile (JT Baker)
- Peptides: Angiotensin I, Angiotensin II, [Glu[1]]-Fibrinopeptide B, Insulin Chain B (Sigma-Aldrich)

Flow Injection

- Flow Rate: 300 nl/min.; 90% mobile phase A/10% mobile phase B
- Analyte: 500 fmol/µL 4 peptide mixture in 70% mobile phase A; 30% mobile phase B
- Injection: 1 µL loop



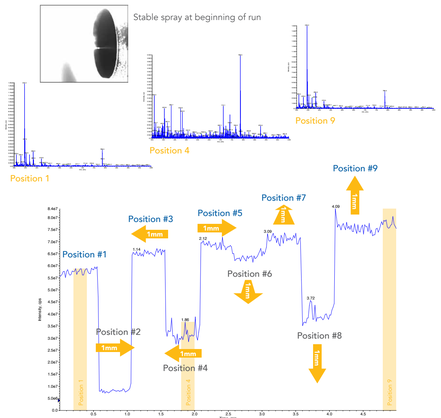
Digital PicoView DPV-450 nanospray source installed on an AB SCIEX 4000 Q TRAP instrument.



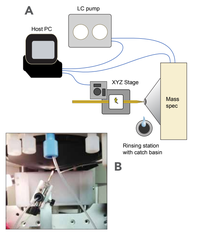
PV Acquire v2.0.0 software package which accompanies the Digital PicoView system. The automated Digital Divert mode is active, as shown in the image.

1. Berg, AL; Marshall-Waggett, CJ; Valaskovic, GA; Proceedings of the 57th Conference on Mass Spectrometry and Allied Topics, Philadelphia, PA, May 29 - June 5, 2009

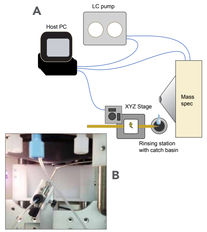
Effects of Positioning on Signal



The automated XYZ stage and software control of Digital PicoView enables facile tuning for optimal signal and spray stability on the 4000 QTrap interface.

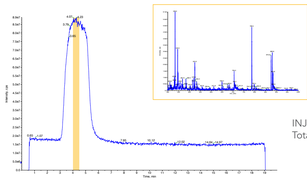


A) System schematic during run (spraying) B) Picture of emitter position relative to curtain plate inlet on instrument during data collection.

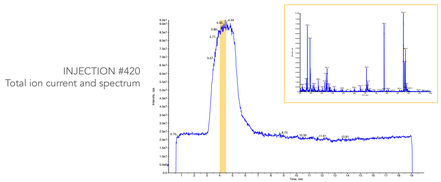


A) System schematic between injections (not spraying). A contact closure from the LC triggers the movement of the XYZ stage to the rinse station position. B) Picture of emitter diverted from the inlet and parked under rinse station in between injections.

Results With Emitter Rinsing



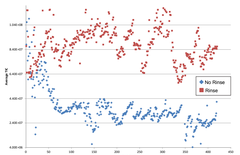
INJECTION #20
Total ion current and spectrum



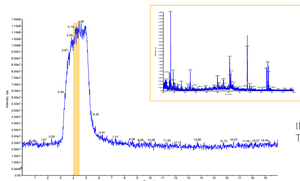
INJECTION #420
Total ion current and spectrum

A) Spray image collected at injection 20; B) Spray image collected at injection 420; C) Photo of emitter at 32X magnification taken after 420 injections (140 hours run time)

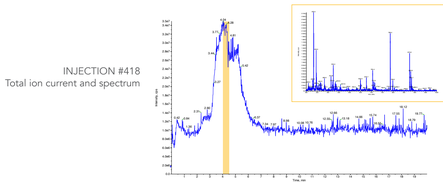
Plot of average total ion current (TIC) over 418 replicate injections for two data sets: with tip rinsing and without tip rinsing. The average total ion current was calculated for a 0.5-minute time segment (4.0 – 4.5 minutes).



Results Without Emitter Rinsing



INJECTION #30
Total ion current and spectrum

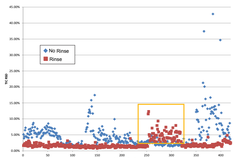


INJECTION #418
Total ion current and spectrum

A) Spray image collected at injection 1; B) Spray image collected at injection 420; C) Photo of emitter at 32X magnification taken after 418 injections (139 hours run time)

Plot of total ion current relative standard deviation (RSD) over 418 replicate injections for two data sets: with tip rinsing and without tip rinsing.

SPRAY INSTABILITY The TIC RSD showed a change beginning at injection 252 reflecting an increase in spray instability. The autosampler was injecting air. Spray stability was restored, as indicated by the TIC RSD, once the air was flushed from the system.



Ion Trap Methods & Results

Instrumentation

- Ion-trap mass spectrometer equipped with Harvard Biosciences syringe pump (LCQ Deca, Thermo Scientific)
- Nanospray source (Digital PicoView 150, New Objective)
- 1100 Capillary pump operated in Normal Mode (Agilent)
- PicoFrit emitter (360 µm OD, 75 µm ID, 15 µm tip ID, New Objective)

Photo of PicoFrit emitter taken after 650 injections without tip rinsing using optical microscopy

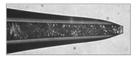
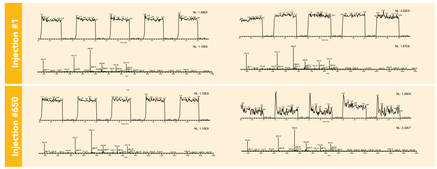


Photo of PicoFrit emitter taken after 650 injections with rinsing using optical microscopy.

Reagents

- 0.1% Formic acid in water (J.T. Baker)
- 0.1% Formic acid in acetonitrile (J.T. Baker)
- Methanol (J.T. Baker)
- 250 ng/µL Caffeine, MW 194.19 Da (Sigma-Aldrich)
- 5 pmol/µL Bradykinin fragment 1-7, MW 756.39 Da (Sigma-Aldrich)
- 5 pmol/µL Angiotensin I, MW 1296.48 Da (Sigma-Aldrich)
- 5 pmol/µL Neurotensin, MW 1672.92 Da (Sigma-Aldrich)
- tert-Butyl methyl ether (MTBE, Sigma-Aldrich)
- Liquid-liquid extracted canine plasma with heparin (Harlan Bioproducts)



Left: Comparative data plot of average intensity per injection for two data sets, one collected with rinsing and one without. The average intensity for two different ions, neurotensin MH2+, 837.2 Da and caffeine MH+, 195.2 Da, is plotted for each data set.

Conclusions

- Demonstrated benefits of automated tip rinsing on two different instrument platforms
- Validated regular monitoring of TIC RSD as a good indicator of system performance
- Observed finite and variable analyte recovery and emitter performance when regular automated tip rinsing is not enabled
- Enabled robustly stable nanospray and reproducible data using remote controlled automated tip rinsing at regular intervals