Evaluation of Nanospray Voltage and Spray Stability and their Impact on Chromatographic Peak Area

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Introduction

State-of-the-art liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis uses a constant electrospray (ESI) voltage for data acquisition. Modern qualitative and quantitative LC-MS/MS methods depend on highly efficient gradient elution chromatography. The changing chemical composition of mobile phase during gradient elution results in an inherent disconnect with single point ESI voltage optimization. Using a nanospray source equipped with a digitally controlled stage and software for precise and reproducible emitter positioning for data acquisition we investigate the relationship between spray stability and data quality.

Reagents

• MBFA 519 Da MH\textsuperscript{2+} Mass Range 509.2–519.2 Da (Sigma Aldrich) 
• Blackbrin 1-7 fragment: 379 Da MH\textsuperscript{2+} Mass Range 369.0–379.0 Da 
• Blackbrin 1-7 fragment: 531 Da MH\textsuperscript{2+} Mass Range 521.0–531.0 Da 
• Neurotensin 837 Da MH\textsuperscript{2+} Mass Range 827.2–837.2 Da

Methods & Materials

Mass Spectrometer

• LTQ Linear Ion Trap (Thermos)
  - Full Scan: 150 – 2000 Da
  - Spray Voltage: fixed per file, variable across replicated injections.
  - Analytic Specific Targeted MS Scans
    - MRM 524 Da MH\textsuperscript{2+} Mass Range 514.3–534.3 Da
    - Blackbrin 1-7 fragment: 379 Da MH\textsuperscript{2+} Mass Range 369.4–389.4 Da
    - Blackbrin 1-7 fragment: 531 Da MH\textsuperscript{2+} Mass Range 521.0–541.0 Da
    - Neurotensin 837 Da MH\textsuperscript{2+} Mass Range 827.2–847.2 Da

Chromatography

• Agilent 1100 Capillary Pump
  - 20 µl flow meter, Operated in MicroMode
  - Flow Rate: 1.0 µl/min
  - Mobile Phase A: 0.1% formic acid in water
  - Mobile Phase B: 0.1% formic acid in acetonitrile
  - Gradient: 10 minutes 20%-B

Columns: Pico-spray columns (150 µm i.d. x 75 µm id x 15 µm tip) packed to 10 cm with BioBasic C18 (5µm, 100 Å, C18, Thermos).

HTC PDL Autosampler (Leap Technologies)

• 6-port injection valve (OIC) Video Instruments, Inc., 3.0 µl loop

Future Work

• Evaluate effect of spray stability on data quality peak area for quantitation calculators (RSDs)
• Investigate effect of spray stability on data quality at lower (200 nl/min) and higher (1-10 µl/min) flow rates
• Incorporate an orthogonal (frequency based) feedback control system via the digitally controlled nanospray source (Digital Picoview)