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. Repetitive on-column injections at different (fixed) target ESI voltage settings were executed for four separate analytes with a wide range of gradient elution times. Plotting the chromatographic peak area for selected ion currents yields an apparent compound dependant response curve in which a total maximum value is observed. Image capture enabled by the Digital PicoView software program reveals a direct correlation between the observed spray mode, spray stability and chromatographic quality.

Methods & Materials

Mass Spectrometer

- LTQ Linear Ion Trap (Thermo)
  - Full Scan MS: 150 -2000 Da
  - Spray Voltage: fixed per data file, variable across replicated injections
  - Analyte Specific Targeted MS Scans
    - MRFA 524 Da MH^+: Mass Range 514.3–534.3 Da
    - Bradykinin 1-7 Fragment 379 Da MH2+: Mass Range 374.4–384.4 Da
    - Bradykinin 531 Da MH^{2+}: Mass Range 521.0–541.0 Da
    - Neurotensin 837 Da MH^{2+}: Mass Range 827.2–847.2 Da
    - DPV-550 Digital PicoView nanospray source (New Objective, Inc.)

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: 30 minutes 2-50% B
- Column: PicoFrit column (360 µm OD x 75 µm ID x 15 µm tip) slurry packed to 10 cm with BioBasic C18 (5µm, 300 Å, C18, Thermo)
- HTC Pal Autosampler (Leap Technologies)
  - 6-port injection valve (VICI Valco Instruments, Inc.)
  - 1.0 µL loop

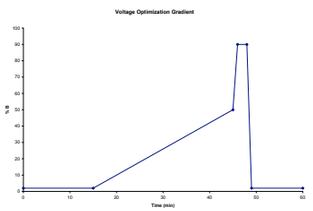
Reagents

- MRFA: 500 fmol/µL, MW 523 Da (Sigma Aldrich)
- Bradykinin 1-7 fragment: 500 fmol/µL, MW 756.4 Da (Sigma Aldrich)
- Bradykinin: 500 fmol/µL, MW 1060.2 Da (Sigma Aldrich)
- Neurotensin: 500 fmol/µL, MW 1672.0 Da (Sigma Aldrich)
- 0.1% Formic acid in water (JT Baker)
- 0.1% Formic acid in acetonitrile (JT Baker)

Conclusions

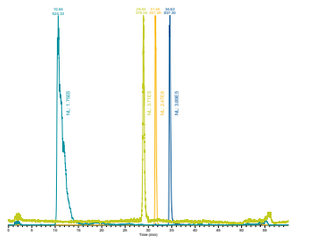
- Effect of voltage related spray stability on data quality across a gradient demonstrated
  - Gradient associated changes in spray stability observed
  - Spray voltage optimum according to maximum peak area observed
  - Increased noise and decreased peak area observed for poor spray stability
  - Ion signal stability appears to correlate with periodic (1-10 Hz) events associated with electrospray (voltage/flow rate) characteristics

FIGURE 1



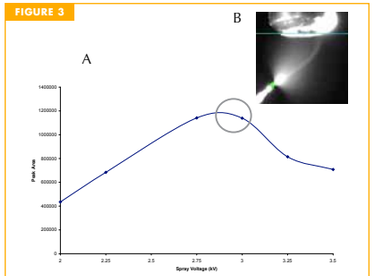
Plot of 60 minute gradient program with a 30 minute gradient of 2-50% B.

FIGURE 2



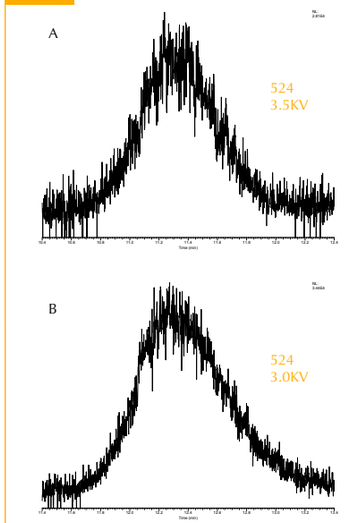
Base peak chromatograms of (left to right) MRFA, Bradykinin 1-7 Fragment, Bradkinin and Neurotensin highlighting their elution order relative to the 60 minute gradient program

FIGURE 3



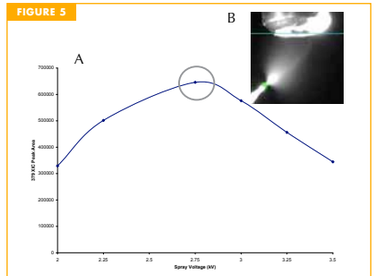
(A) Plot of spray voltage versus peak area for MRFA ion 524. A peak area maximum at 3.0 kV is observed, as indicated. (B) Captured spray image for this data point.

FIGURE 4



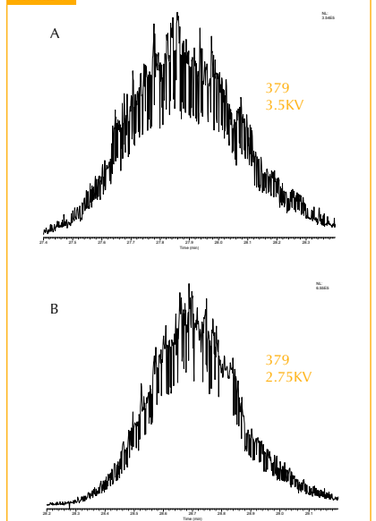
(A) Extracted ion chromatogram for MRFA at a spray voltage of 3.5kV; (B) Extracted ion Chromatogram for MRFA at a spray voltage of 3.0 kV. A decrease in noise for the XIC at 3.0 kV is observed as well as an increase in peak area.

FIGURE 5



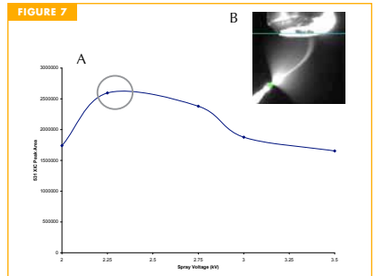
(A) Plot of spray voltage versus peak area for Bradykinin 1-7 Fragment 379 Da MH^{2+} ion. A peak area maximum at 2.75 kV is observed, as indicated. (B) Captured spray image for this data point.

FIGURE 6



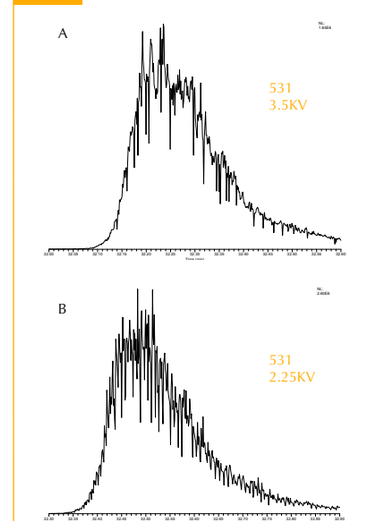
(A) Extracted ion chromatogram for Bradykinin 1-7 Fragment 379 Da MH^{2+} ion at a spray voltage of 3.5kV; (B) Extracted ion Chromatogram for Bradykinin 1-7 Fragment 379 Da MH^{2+} ion at a spray voltage of 2.75 kV. A decrease in noise for the XIC at 2.75 kV is observed as well as an increase in peak area.

FIGURE 7



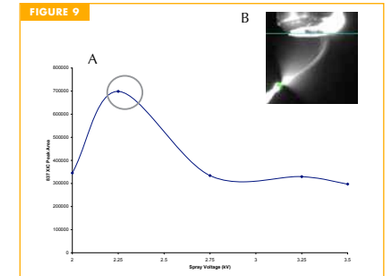
(A) Plot of spray voltage versus peak area for Bradykinin 531 Da MH^{2+} ion. A peak area maximum at 2.25 kV is observed, as indicated. (B) Captured spray image for this data point.

FIGURE 8



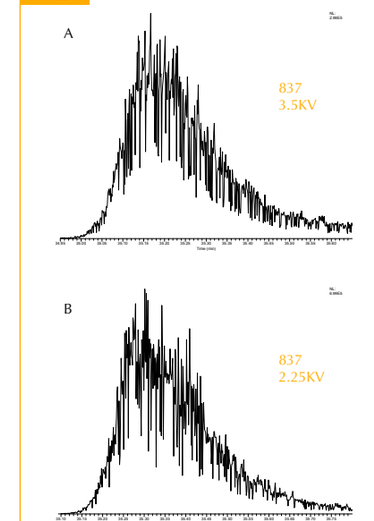
(A) Extracted ion chromatogram for Bradykinin 531 Da MH^{2+} ion at a spray voltage of 3.5kV; (B) Extracted ion Chromatogram for Bradykinin 531 Da MH^{2+} ion at a spray voltage of 2.25 kV. A decrease in noise for the XIC at 2.25 kV is observed as well as an increase in peak area.

FIGURE 9



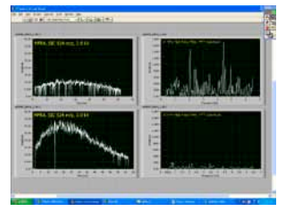
(A) Plot of spray voltage versus peak area for Neurotensin 837 Da MH^{2+} ion. A peak area maximum at 2.25 kV is observed, as indicated. (B) Captured spray image for this data point.

FIGURE 10



(A) Extracted ion chromatogram for Bradykinin 531 Da MH^{2+} ion at a spray voltage of 3.5kV; (B) Extracted ion Chromatogram for Bradykinin 531 Da MH^{2+} ion at a spray voltage of 2.25 kV. A decrease in noise for the XIC at 2.25 kV is observed as well as an increase in peak area.

FIGURE 11



Fourier transform analysis of MRFA 524 Da MH^+ ion at 2.0 kV spray voltage and 3.0 kV spray voltage. The plot of frequency (Hz) in the panels on the right indicate a much lower level of noise—indicating spray fluctuation—at the 3.0KV spray voltage. The decreased noise corresponds to more accurate peak area integration.

Future Work

- Evaluate effect of spray stability on data quality (peak area) for quantitative calculations (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)

References:
Valaskovic, G.A, Murphy, J.P., Lee, M.S. *"Automated Orthogonal Control System for Electrospray Ionization"*, J. Am. Soc. Mass Spectrom., 2004, 15, 1201-1215.