Improving Quantitative Nanospray LC-MSMS Workflows through Voltage Control

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Introduction

Qualitative and quantitative LC-MSMS work flows rely on gradient elution chromatography to reduce analyte complexity and maximize detector response. While parameters for efficient gradient elution and sensitive MS detection can be tightly controlled and optimized for targeted analysis, ESI is typically limited to a constant voltage for data acquisition. The changing chemical composition of mobile phase during gradient elution results in an inherent disconnect with single-point ESI voltage optimization. Efficient ESI is highly dependent on a number of variables (spray voltage, flow rate, composition, gas parameters) and requires tight control for optimal results and reproducibility.

Using a nanospray source equipped with a digitally controlled stage and software for precise and reproducible emitter positioning for data acquisition, the relationship between spray stability and data quality was investigated. Repetitive on-column injections at different (fixed) target ESI voltage settings were executed resulting in a compound-dependent response curve for chromatographic peak area at selected ion currents in which a total maximum value was consistently observed. Using a custom software program generating a time-dependent linear voltage gradient, we evaluated the effect of optimized spray voltage on ion current and peak area for multiple reaction monitoring (MRM). Incorporating a linear voltage gradient improved peak area RSDs and enabled stable spray with minimal or no nebulization gas.

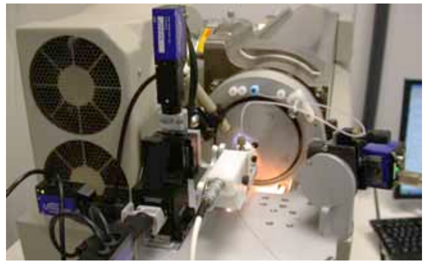
Methods & Materials

LTQ Linear Ion Trap Experiments (Thermo Scientific)

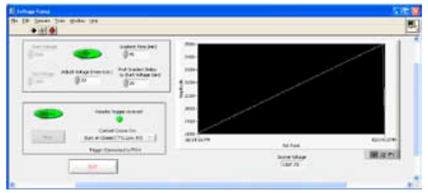
- Spray voltage: Fixed per data file, variable as indicated
- Analyte-specific targeted MS Scans (500 fmol/µL)
  - Enolase phosphopeptide (Waters) T18 1P 432.2 MH2+: 427.3–437.3 Da
  - Enolase Phosphopeptide (Waters) T19 1P 407.2 Da MH2+: 402.5–412.5 Da
  - Bradykinin 1-7 fragment (Sigma) 378.7 Da MH2+: 374.4–384.4 Da
  - [Hyp3]-Bradykinin (Sigma) 538.6 Da MH2+: 534.2–544.2 Da
  - Enolase phosphopeptide (Waters) T43 2P 684.8 MH2+: 680.2-690.2 Da
  - Neurotensin (Enzo Life Sciences) 558.3 Da MH3+: 553.8–563.8 Da
  - ACTH fragment (Sigma) 18-39 822.4 MH3+: 818.0–828.0 Da
  - Insulin chain B oxidized (Sigma) 1166.6 MH3+: 1161.2–1171.2 Da
- DPV-550 Digital PicoView nanospray source (New Objective)
- Eksigent nanoLC·Ultra 2D plus
  - Mobile Phase A: 0.1% formic acid in water (JT Baker)
  - Mobile Phase B: 0.1% formic acid in acetonitrile (JT Baker)
  - Gradient: 30 minutes 2-50% B, 200 nL/min.
- Column: PicoFrit column (360 µm OD x 75 µm ID x 15 µm tip, New Objective) slurry packed to 10 cm with BioBasic 5µm, 300 Å, C18 (Thermo Scientific)
- HTC Pal Autosampler with 1.0 µl loop (Leap Technologies)

4000 Q TRAP Experiments (AB SCIEX)

- Scan type: MRM, unit resolution, 23 peptides, 5 transitions per peptide, 10 mS dwell time
- Source parameters: Curtain gas 10, 150°C Interface Heater Temperature, Ion Spray Voltage and Ion Source Gas 1 (GS1) variable
- Compound parameters: Entrance Potential 10, Collision Cell Exit Potential 15.0, Declustering Potential and Collision Energy optimized for individual peptides
- Eksigent nanoLC•2D
  - Mobile Phase A: 0.1% Formic acid in water (JT Baker)
  - Mobile Phase B: 0.1% Formic acid in acetonitrile (JT Baker)
  - Gradient: 300 nL/min. 80 minutes, 3 min. 2.7–13.5 %B, 34 min. 13.5–31.5% B
- HTC Pal autosampler with 1.0 µL loop (Leap Technologies)
- Analyte: 50 fmol/µL equimolar 6 bovine protein digest (Michrom Bioresources)
- DPV-450 Digital PicoView nanospray source (New Objective)
  - PS350/5000V - 25W high voltage power supply (Stanford Research Systems, Inc.)
  - Automated tip rinsing enabled
  - Custom linear voltage gradient software
- Column: PicoFrit column (360 µm OD x 75 µm ID x 10 µm tip, New Objective) slurry packed to 12 cm with Reprosil-PUR 3 µm, 120 Å, C18-AQ (Dr. Maisch)



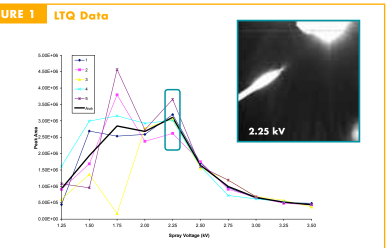
DPV-450 Digital PicoView nanospray source installed on the AB SCIEX 4000 Q TRAP



Custom voltage control software enabling a time based linear voltage gradient

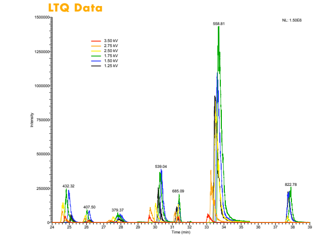
1 Valaskovic, G.A, Murphy, J.P., Lee, M.S. "Automated Orthogonal Control System for Electrospray Ionization", *J. Am. Soc. Mass Spectrom.*, 2004, 15, 1201-1215

FIGURE 1 LTQ Data



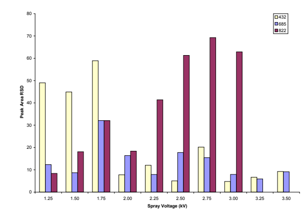
Plot of extracted peak area for five replicate injections at ten different spray voltage settings for enolase phosphopeptide T18 1P (MH^{2+} 432)

FIGURE 2 LTQ Data



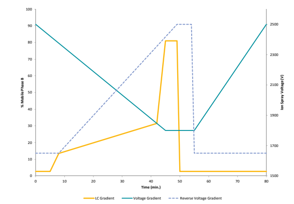
Overlay of six base peak chromatograms collected at different spray voltages. Changes in peak area can be observed for the absolute intensity scale.

FIGURE 3 LTQ Data



Extracted peak area RSD for three different peptides at different spray voltages. The lowest RSD value for each analyte directly correlates with the maximum peak area plots.

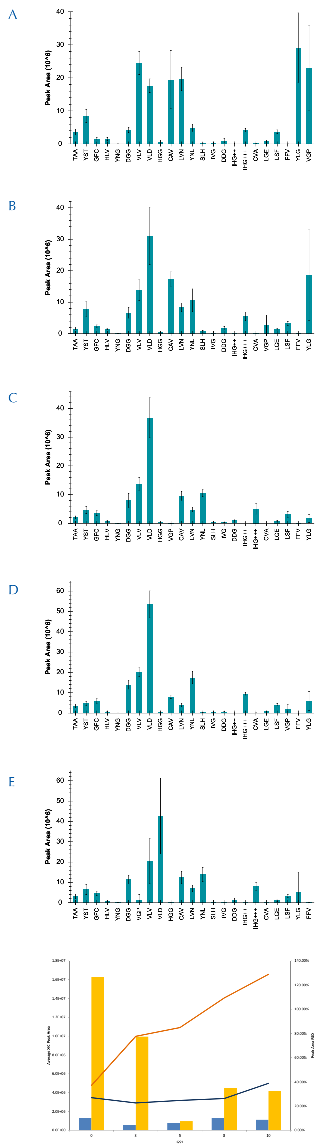
FIGURE 4 Q TRAP Data



Plot of gradients used for 4000 Q TRAP LC-MSMS data collection A) mobile phase B, B) linear voltage gradient, C) reverse voltage gradient

FIGURE 5 VOLTAGE GRADIENT: 2500 V - 1800V

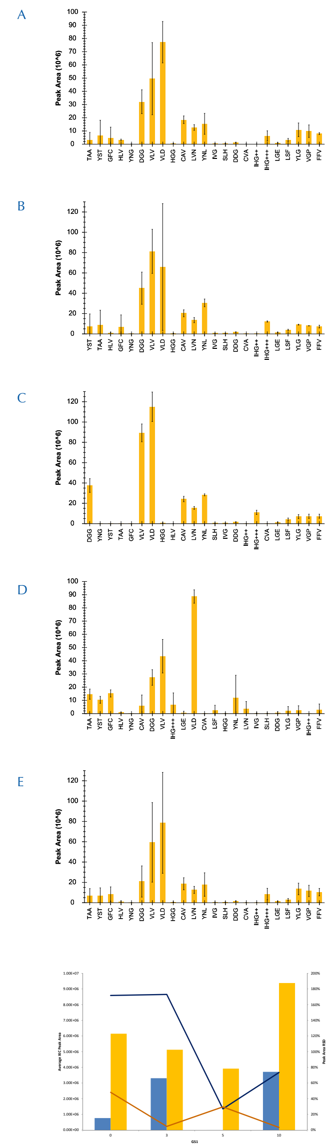
Peptide specific peak areas collected using a 45-minute linear voltage gradient of 2500 to 1800 volts collected at varying Ion Source Gas 1 settings of A) 0 GS1, B) 3 GS1, C) 5 GS1, D) 8 GS1, and E) 10 GS1



Average XIC peak area and peak area RSD plotted against varying Ion Sourge Gas 1 settings for two different peptide transitions, glutamate dehydrogenase MH^{2+} 540 Da peptide Y8 transition and alpha casein MH^{2+} 634 DA peptide Y8 transition. Data was collected using a 45-minute linear voltage gradient of 2500 to 1800 volts.

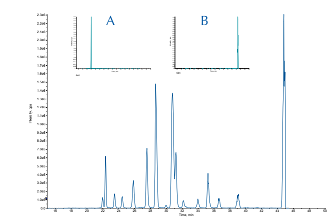
FIGURE 6 VOLTAGE GRADIENT: 1650V - 2500 V

Peptide specific peak areas collected using a 41-minute linear voltage gradient of 1650 to 2500 volts collected at varying Ion Source Gas 1 settings of A) 0 GS1, B) 3 GS1, C) 5 GS1, D) 8 GS1, and E) 10 GS1



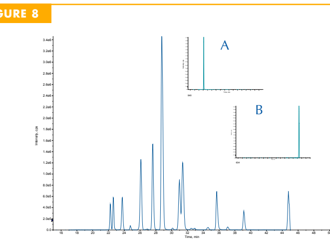
Average XIC peak area and peak area RSD plotted against varying Ion Source Gas 1 settings for two different peptide transitions, glutamate dehydrogenase MH^{2+} 540 Da peptide Y8 transition and alpha casein MH^{2+} 634 Da peptide Y8 transition. Data was collected using a 41-minute reverse linear voltage gradient of 1650 to 2500 volts.

FIGURE 7



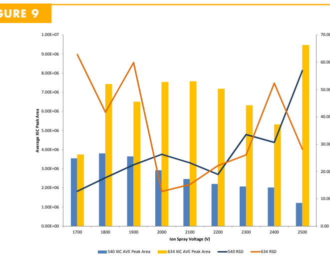
Total ion chromatogram of equimolar 6-protein digest collected using a reverse linear voltage gradient of 1650 to 2500 volts at an Ion Source Gas 1 setting of 3. A) Extracted ion chromatogram for glutamate dehydrogenase MH^{2+} 540 Da peptide Y8 transition. B) Extracted ion chromatogram for alpha casein MH^{2+} 634 Da peptide Y8 transition.

FIGURE 8



Total ion chromatogram of equimolar 6-protein digest collected using a reverse linear voltage gradient of 1650 to 2500 volts at an Ion Source Gas 1 setting of 8. A) Extracted ion chromatogram for glutamate dehydrogenase MH^{2+} 540 Da peptide Y8 transition. B) Extracted ion chromatogram for alpha casein MH^{2+} 634 Da peptide Y8 transition.

FIGURE 9



Average XIC peak area and peak area RSD plotted against varying ion spray voltage for two different peptide transitions, glutamate dehydrogenase peptide MH^{2+} 540 Da peptide Y8 transition and alpha casein MH^{2+} 634 Da peptide Y8 transition. Data was collected using an constant Ion Source Gas 1 setting of 10.

Conclusions

- Gradient-associated changes in spray stability were observed at fixed spray voltages
- Compound-dependent maximum peak area were observed
- Observed increase in noise and decreased peak area were the results of poor spray stability
- Voltage gradient allowed for lower nebulization gas values without compromising spray quality
- Demonstrated the value of optimizing source parameters and their effect on data quality