Improving Quantitative Nanospray LC-MSMS Workflows through Voltage Control

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Introduction

Quantitative and linear AB-ESI work flows rely on gradient elution chromatography to reduce analyte complexity and maintain detector responses. While parameters for optimal gradient elution and sensitivity are derived, the relation between spray stability and spray stability may be traced. Reprocessive rejections injection at different (fixed) target ESI voltage sets were associated with a component-dependent improvement in chromatographic peak area at selected ion current at which a total maximum value was consistently observed. Using a custom program generating a time-dependent linear spray gradient, we evaluated the effect of optimized spray voltages on ion current and peak area for multiple-mass monitoring (MSM) incorporating a linear spray gradient increased peak area RSDs and enabled stable spray with minimal or no nebulization gas.

Methods & Materials

LC Gradient

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Eksigent nanoLC·Ultra 2D plus DPV-550 Digital PicoView nanospray source (New Objective)

Mobile Phase A: 0.1% formic acid in water (JT Baker)

Mobile Phase B: 0.1% Formic acid in acetonitrile (JT Baker)

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Analyte-specific targeted MS Scans (500 fmol/µL) (Thermo Scientific)

HTC Pal autosampler with 1.0 µL loop (Leap Technologies)

Column: PicoFrit column (360 µm OD x 75 µm ID x 10 µm tip, New Objective, Inc., Woburn, MA; 2 Milestone Development Services, Newtown, PA)

Column: C18 (Thermo Scientific)

Compound parameters: Entrance Potential 10, Collision Cell Exit Potential 15.0, Declustering Potential and Collision Energy optimized for individual peptides

Scan type: MRM, unit resolution, 23 peptides, 5 transitions per peptide, 10 mS dwell time

Source parameters: Curtain gas 10, 150°C Interface Heater

- Bradykinin 1-7 fragment (Sigma) 378.7 Da MH2+: 374.4–384.4 Da

- Insulin chain B oxidized (Sigma) 1166.6 MH3+: 1161.2–1171.2 Da

- ACTH fragment (Sigma) 18-39 822.4 MH3+: 818.0–828.0 Da

- Gas 1 settings for two different peptide transitions, glutamate dehydrogenase 437.3 Da and alpha casein MH2+ 540 Da peptide Y8 transition. Data was collected using a 45-minute linear voltage gradient of 2500 V/minute from 1.75 kV to 2.50 kV.

- Peptide specific peak areas collected using a 41-minute linear voltage gradient of 2000 V/minute from 1.75 kV to 3.00 kV (see Fig. 7A–D).

- Conclusions

- Gradient associated changes in stability likely occurred at fixed target ESI voltage

- Comparative dependent maximum peak area was observed

- Optimized increase in spray and determined peak area were correlated

- 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

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