Characterization of the Key Tuning Parameters in NanoESI Analysis using an Automated Positioning System

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Introduction

Establishing optimal spray conditions for nanoLC-MS is a key part of method development. Nano-ESI is dependent on a number of factors, including the effective voltage, specific dimensions of a given spray tip, effective flow rate, and mobile phase composition. All of these parameters can be tightly controlled through instrumentation software and commercially available nano-ESI emitters. The position of the emitter relative to the MS orifice plays an important role in spray optimization but is a highly subjective variable lacking in the control enabled by LC and MS instrument vendors for flow rate, applied voltage, and gas parameters. The implementation of a feedback-controlled nano-electrospray source where either the spray voltage or nanospray emitter position is under feedback control has been previously reported[1]. The utility of a digital-control system to map the spray current of the ESI plume under a variety of experimental conditions has been demonstrated on an LCQ DECA mass spectrometer[2]. Here we investigate the utility of a digital-control system for spray optimization on a 4000 Q TRAP instrument.

The effects of a laminar flow of heated nitrogen gas from the inlet and a coaxial sheath gas on spray stability and analyte signal were evaluated using commercially available peptides via syringe infusion at 300 nL/min. flow rates. Proprietary software controlling an automated XYZ translation stage was modified with a custom program written to initiate an MS acquisition. Each MS acquisition was triggered by movement to a defined set of XYZ coordinates. The custom program was used to generate a raster scan pattern of the nanospray emitter XYZ coordinates relative to the MS orifice, enabling the ability to correlate analyte signal with emitter position for a specific set of nano-ESI parameters. Using this information, a parameter and position dependent map can be generated and used for reproducible and robust nano-ESI, minimizing the need for manual optimization.

Figure 1: DPV-450 Digital PicoView nanospray source installed on an AB SCIEX 4000 Q TRAP

Figure 2: The PV Acquire user interface can be used to reproducibly measure the emitter protrusion from the sheath gas nozzle. The blue cursor reads relative to the green cursor and generates a read-out in mm. The ~1.6 mm emitter protrusion shown here was used for all data acquisition.

Methods

Mass Spectrometer

- AB SCIEX 4000 Q TRAP™
  - Scan type: Q1 MS Scan
    - Mass range: 400 – 1000 Da
    - Scantime: 0.1801 (s)
    - Scan mode: Profile
    - Step size: 0.1 amu
    - Resolution Q1: Unit
  - Compound parameters
    - Declustering potential (DP): 100
    - Entrance potential (EP): 10
  - Source/Gas parameters
    - Curtain gas (CUR): 10.0 or 20.0 (as stated)
    - Ion spray voltage (IS): 1,800, 2,200 or 2,500 kV (as stated)
    - Ion source gas (GS1): 3.0
    - Interface heater temperature (IHT): 150°C
- Digital PicoView® DPV-450 nanospray source (New Objective, Inc.) modified for scanned spray
  - PV Acquire™ 1.5.1 software with scanning module
  - Raster scanning step size: 700 µm
  - Image size: 8 x 8 pixels; 4.9 x 4.9 mm typical

Continuous Infusion Conditions

- Syringe pump (Harvard Apparatus, PHD Model)
- 250 µL Gastight syringe (Hamilton)
- Flow Rate: 300 nL/min.
- Emitter: 360 µm OD x 20 µm ID x 10 µm tip, 15 cm long, non-coated (New Objective, Inc.)
- Analyte: 6-Peptide mixture (AB SCIEX) diluted according to instructions
- Composition: 0.1% formic acid / 50% water / 50% acetonitrile (JT Baker)

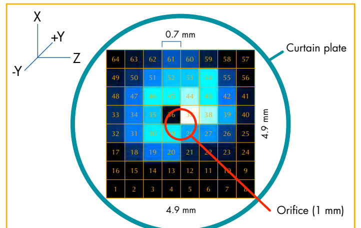

Figure 3: Schematic detailing the X and Z coordinates used to generate the intensity maps relative to the opening of the 4000 curtain plate. For each intensity map, 64 data files were collected. Orifice size relative to data collection grid shown to scale.

Emitter Position vs. Signal Intensity: Curtain Gas 10

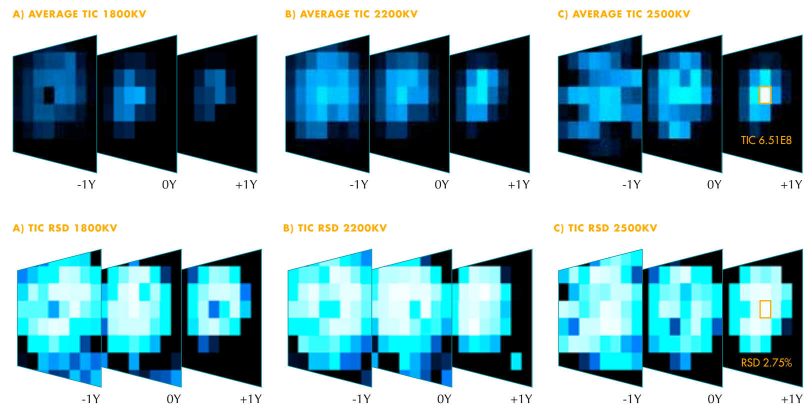

Figure 5: Average TIC and TIC RSD intensity maps at a curtain gas setting of 10; Spray voltages of A) 1,800 kV B) 2,200 kV and C) 2,500 kV for 64 data points

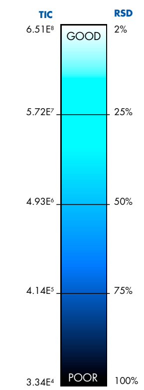

Figure 7: Chart correlating intensity map color to TIC and RSD values. Average TIC data was scaled to a maximum value of 6.51E8 (highest average TIC calculated)–indicated in white–and a minimum value of 3.34E4 (minimum signal detectable on the 4000)–indicated in black for all data points. The TIC RSD data was scaled to a minimum value of 2.0% (2.06% minimum RSD calculated) and a maximum value of 100%. The same color-value scale was used for all TIC intensity maps.

Emitter Position vs. Signal Intensity: Curtain Gas 20

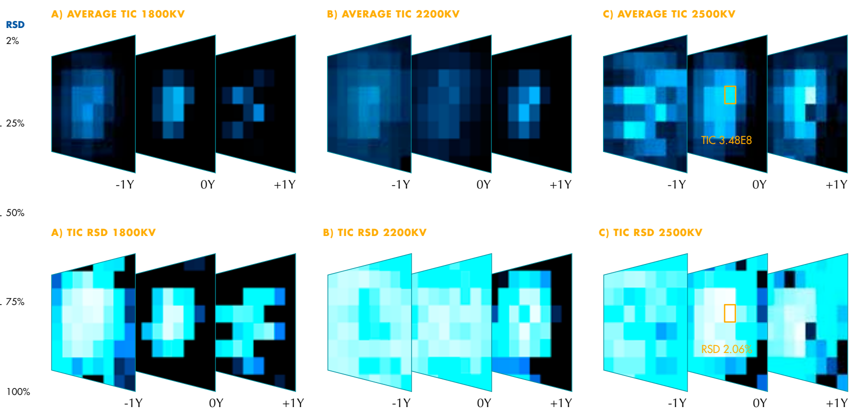

Figure 6: Average TIC and TIC RSD intensity maps at a curtain gas setting of 20; Spray voltages of A) 1,800 kV B) 2,200 kV and C) 2,500 kV for 64 data files

Optimum Average TIC Observed with 1 mm Shift

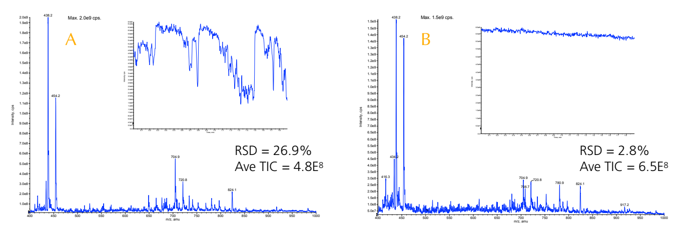

Figure 8: Spectra and TIC for data collected at 2,500 kV and a curtain gas of 10 for the same X and Z coordinates (point 37). A) At a Y-axis coordinate of 0, the average TIC is 4.8E8 with an RSD of 26.9%, indicating spray instability. B) When the emitter moves 1 mm to a Y-axis coordinate of +1, the average TIC increases to 6.5E8 with an RSD of 2.8%. The spray stability improved 10-fold by moving the emitter 1 mm.

Optimum TIC RSD Observed with 1 mm Shift

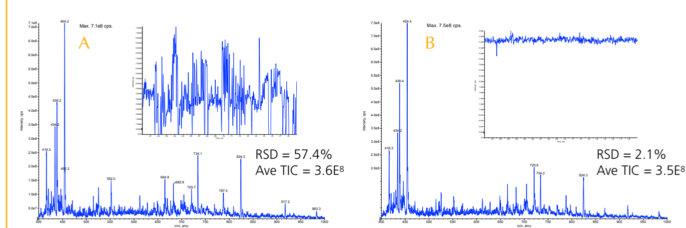

Figure 9: Spectra and TIC for data collected at 2,500 kV and a curtain gas of 20. A) For point 27 at a Y-axis coordinate of -1, the average TIC is 3.6E8 with an RSD of 57.4%, indicating spray instability. B) For point 37 at a Y-axis coordinate of 0 the average TIC stays constant but the RSD decreases to 2.1%. The spray stability improved 25-fold by moving the emitter 1 mm.

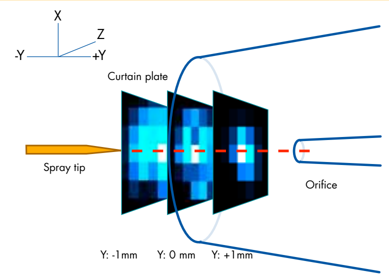

Figure 4: Schematic detailing emitter position relative to the curtain plate inlet on the 4000 Q TRAP. The emitter was positioned on-axis (Y-axis), perpendicular to the MS orifice. The Y-axis positions indicated refer to the emitter positions; 1 mm inside the curtain plate (+1 mm), flush with the curtain plate (0 mm) and 1mm away from the curtain plate (-1 mm).

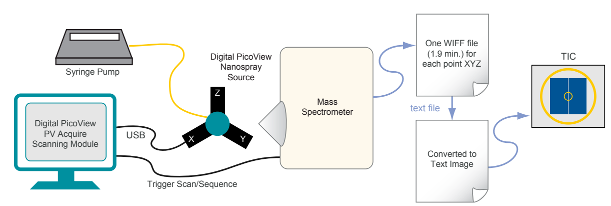

Figure 10: Schematic of the intensity mapping experiment. The nanospray emitter mounted on the XYZ stage of the source is scanned in front of the MS orifice in a raster pattern. The source generates a contact closure after each stage movement, triggering the acquisition of a 1.9 min. WIFF file for that point. After a 64-point scan (8x8 image) the TIC was extracted out to a text file and the average TIC and TIC RSD for each point was calculated.

Conclusions

- Characterized the spatial relationship of the nanospray emitter on a 4000 Q TRAP LC-MSMS system at different spray voltage settings (1,800 kV, 2,200 kV, 2,500 kV) and curtain gas settings (10, 20)
- Observed a maximum average TIC (6.5E8) and RSD value (2.8%) at 2,500 kV and curtain gas setting of 10 at position 37, +1Y
- Coordinates which deliver high values for TIC do not always have good spray stability
- Emitter coordinates at +1Y can achieve higher TIC, but will have a much smaller 'sweet spot'
- Precise and reproducible positioning of the spray emitter is enabled by the digital stage control
- Data collected at -1Y coordinates generate stable spray and acceptable TIC over a much wider range of coordinates than the 0Y and +1Y coordinates
- Higher curtain gas settings require higher voltage settings for stable spray

1 Valaskovic, et. al. JASMS, 2004, 15, 2001. 2 Valaskovic, GA; Lee, MS, Proceedings of the 56th Conference on Mass Spectrometry and Allied Topics, Denver, CO June 1-5, 2008.