# Novel and Robust ESI Source for Efficient Bioanalytical Workflowsat Capillary LC Flow Rates

Amanda Berg, Gary Valaskovic

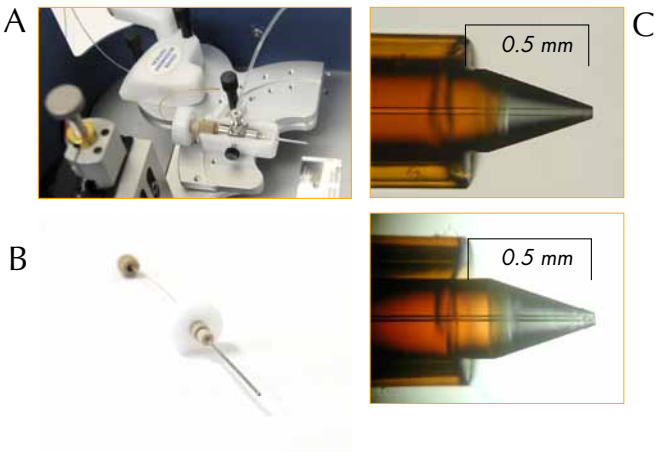
New Objective, Woburn, MA

## Introduction

The diverse requirements of small and large molecule analyses have driven the bifurcation of electrospray ionization (ESI) into high (mL/min) and ultra-low (nL/min) flow regimes. Small molecule analysis has predominantly relied on high flow rates using millimeter diameter liquid chromatography (LC) columns. Fast gradient elution on short (≤ 5 cm) columns with sample-to-sample injection times measured in 1-5 minute intervals dominates the workflow. Large molecule (protein and peptide) analysis has relied on ultra-low flow ESI (nanospray) and the use of nanobore (≤ 75 um) LC columns. Workflows based on nanopsray/nanobore LC enable exceptional sensitivity and high separation power for complex mixtures. Long gradient elution LC with injection cycles of greater than 30-60 min. dominates the workflow.

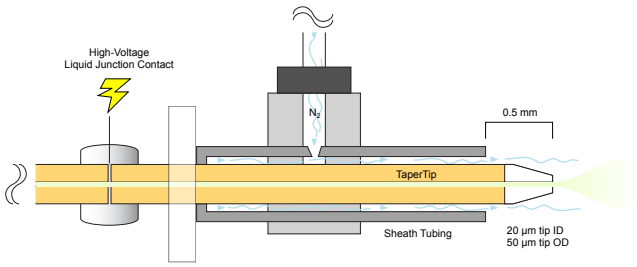
Biomarker validation of (endogenous) proteins and peptides demands that biomolecular LC-MS/MS transitions from qualitative to quantitative analysis. Traditional small molecule workflows typically lack sufficient sensitivity and the selectivity required for biomarker validation. On the other hand, large molecule workflows lack sufficient throughput for the quantification of large sample sets. Enabling efficient ESI in an intermediate flow range, 1-20 µL/min., allows for the facile implementation of capillary scale (0.18 to 0.5 mm ID) columns. Capillary columns enable higher sensitivity than mm scale columns while delivering higher throughput, robustness, and ease-of-use than nanobore LC. Two different novel ESI source designs, based on high precision fused-silica components and microfluidic connectors, enable the integration of the concepts used in both high-flow ESI and nanospray. This precision emitter assembly enables stable ESI at flow rates ranging from 1 to 20 µL/min. at low (<2%) and high (>90%) organic mobile phase composition. Continuous flow and flow injection experiments demonstrate relative standard deviation values of 5% and better for absolute ion intensity on a 3D ion trap mass spectrometer.

**FIGURE 1**




*Capillary spray hardware: A) Photo of TaperTip emitter mounted onto source stage showing sheath gas tubing connection and high-voltage liquid-junction connection; B) TaperTip spray emitter assembly for error-free, easy to use plug-and-spray analyses; C) Before and after photos of 20 µm ID TaperTip emitter used to collect flow injection data over 3,200 replicate injections. The 0.5 mm delta between the end of the sheath tubing and the TaperTip emitter is indicated.*

**FIGURE 2**




*Schematic of TaperTip emitter spray assembly indicating high-voltage liquid-junction connection and $N_2$ sheath gas inlet. The delta between the termini of the sheath tubing and TaperTip emitter, indicated at 0.5mm, is fixed for each spray assembly.*

## Methods & Materials

### Instrumentation

- Mass Spectrometer: LCQ Deca (Thermo Fisher Scientific)
  - 3 microscans/spectra
  - 300.00 – 550.00 Da mass range
  - N2 sheath gas with a setting of 35
  - 160° C capillary temperature
  - 2.5 kV spray voltage
- HPLC: nanoLC•2D Channel 1 (Eksigent)
  - Mobile Phase A = 0.1% Formic Acid in 100% Water
  - Mobile Phase B = 0.1% Formic Acid in 100% Acetonitrile
  - Flow Rate: 10 ul/min
  - Composition: Isocratic 50% B
- Autosampler: HTC Pal (Leap Technologies)
  - 50 µL syringe
  - 6-port micro valve (VICI)
  - 2.0 µl sample loop
- ESI Source: CSP-150, custom capillary spray source (New Objective)
  - TaperTip emitter, 360 µm OD x 20 µm ID

### Analyte

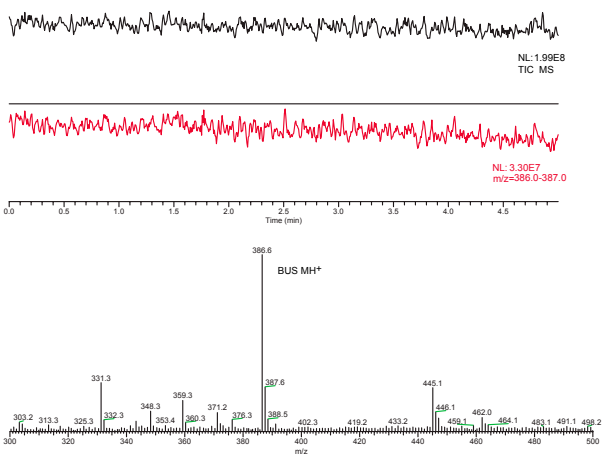
- Matrix: 50% Mobile Phase A/50% Mobile Phase B
- 1 µM Buspirone Hydrochloride,$MH^+$ 386 Da (Sigma)
- 1 pmol/µL Angiotensin I, $MH3^+$ 433 Da (Sigma)
- 1 pmol/µL Angiotensin II, $MH2^+$ 524 Da (Sigma)

### Flow Injection

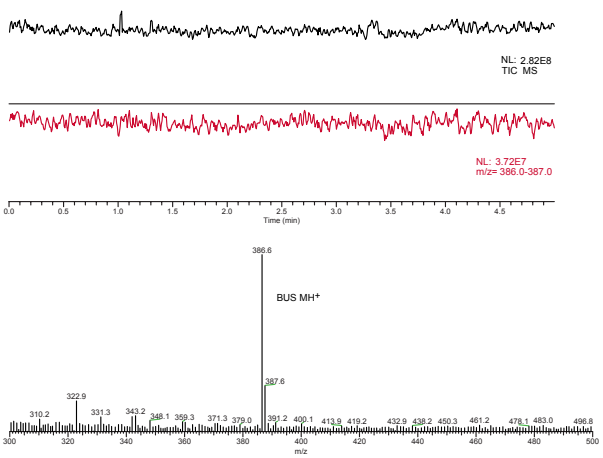
Using Channel 1 on the Eksigent nanoLC•2D to deliver a constant isocratic flow of 50% B at 10 µl/min, 10 minute data files were collected successively in XCalibur. 2 µl of analyte were flow injected for 30 seconds each successively at 30 second intervals with a total of 9 injections per ten minute data file. A 20µm ID TaperTip emitter was precisely positioned at a fixed position on-axis 2mm away from the inlet of the LCQ ion transfer tube. Voltage was delivered via a high-voltage liquid junction maintained at 2.5 kV. A constant flow of N2 sheath gas was delivered at an arbitrary setting of 35, established within the LCQ Tune file. The TaperTip emitter protruded from the sheath gas tubing precisely 500 µm. All of these conditions were held constant throughout the collection of 360 data files, which translates to 3240 replicate injections of analyte.

**FIGURE 3**

A *1 µL/Minute flow rate*




B *20 µL / Minute flow rate*

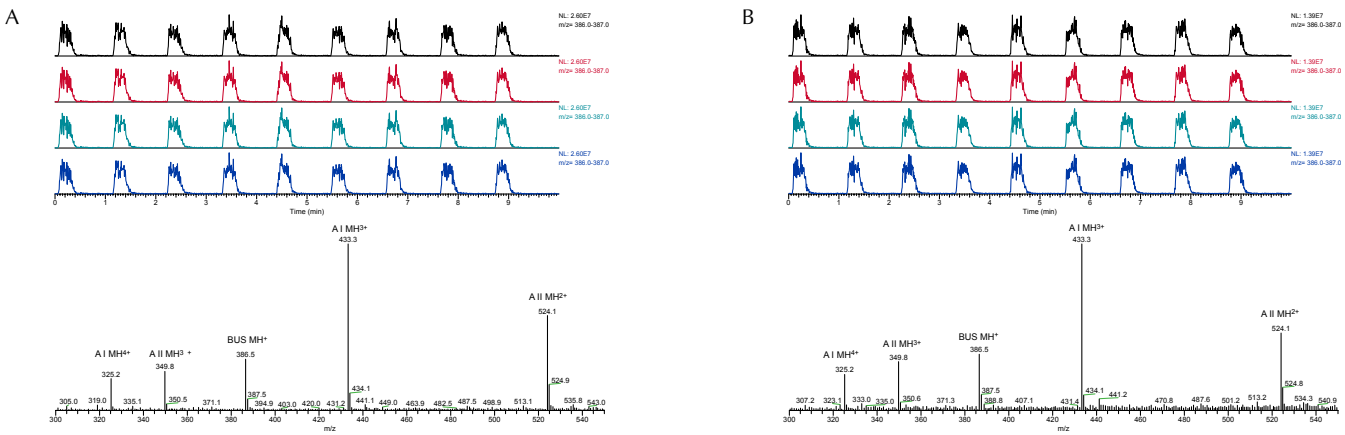



*Direct infusion of 1 µM buspirone in 30% ACN at A) 1 µL/min. and B) 20 µL/min. Normalized intensities for the TIC and XIC are very similar for both flow rates.*

## FIGURE 4

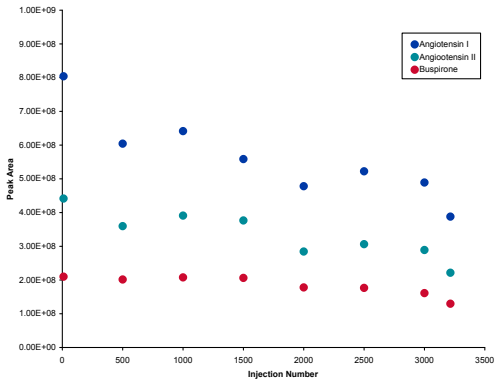
*Injection #10 - #18*

*Injection #3,214 - #3,222*



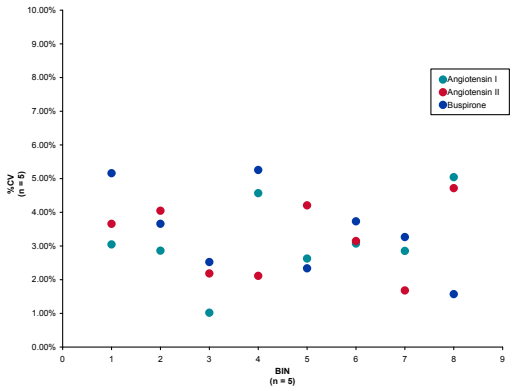

*Normalized intensities for the TIC and XIC of each analyte at the A) beginning and B) end of data collection for 3,200 replicate injections.*

## FIGURE 5 Peak Area vs. Injection Number



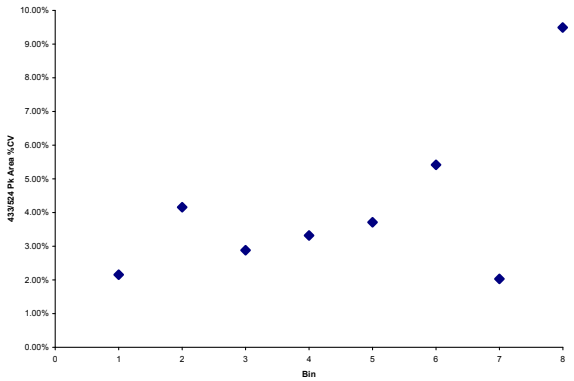

*Change in peak area over 3200 replicate injections for each analyte*

## FIGURE 6



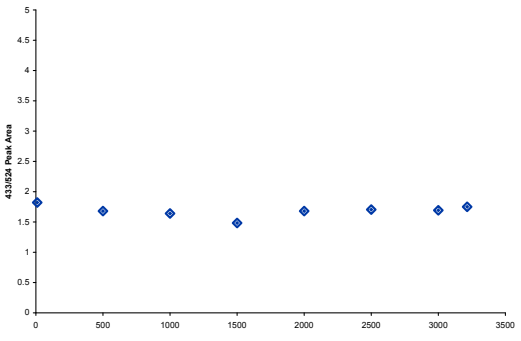

*Plot of %CV with n=5 for each analyte over 3,200 injections*

## FIGURE 7




*Plot of Angiotensin peak area ratio %CV with n=5 for each data point across 3,200 replicate injections*

## FIGURE 8 Angiotension Peak Area Ratio vs Injection




*Change in the peak area ratio of Angiotensin I (MH3+, 433 Da) to Angiotensin II ($MH^{2+}$, 524 Da) over 3,200 injections.*

**FIGURE 9**

| BIN | n | INJECTION | ANGIOTENSIN I 433 MH3+ | | ANGIOTENSIN II 524 MH2+ | | PEAK AREA RATIO 433/524 | |
|---|---|---|---|---|---|---|---|---|
| | | | *Area* | *Data Analysis* | *Area* | *Data Analysis* | *Ratio* | *Data Analysis* |
| 1 | 1 | 10 | 8.04E+08 | **AVE** 8.30E+08 | 4.42E+08 | **AVE** 4.70E+08 | 1.82E+00 | **AVE** 1.77E+00 |
| | 2 | 11 | 8.09E+08 | **STD** 2.53E+07 | 4.69E+08 | **STD** 1.72E+07 | 1.72E+00 | **STD** 3.81E-02 |
| | 3 | 12 | 8.39E+08 | **%CV** 3.05% | 4.81E+08 | **%CV** 3.66% | 1.75E+00 | **%CV** 2.16% |
| | 4 | 13 | 8.67E+08 | | 4.85E+08 | | 1.79E+00 | |
| | 5 | 14 | 8.31E+08 | | 4.74E+08 | | 1.75E+00 | |
| 2 | 1 | 496 | 6.26E+08 | **AVE** 6.01E+08 | 3.58E+08 | **AVE** 3.62E+08 | 1.75E+00 | **AVE** 1.66E+00 |
| | 2 | 497 | 5.84E+08 | **STD** 1.72E+07 | 3.51E+08 | **STD** 1.47E+07 | 1.66E+00 | **STD** 6.90E-02 |
| | 3 | 498 | 5.85E+08 | **%CV** 2.86% | 3.55E+08 | **%CV** 4.05% | 1.65E+00 | **%CV** 4.16% |
| | 4 | 499 | 6.04E+08 | | 3.88E+08 | | 1.56E+00 | |
| | 5 | 500 | 6.04E+08 | | 3.60E+08 | | 1.68E+00 | |
| 3 | 1 | 1000 | 6.41E+08 | **AVE** 6.38E+08 | 3.91E+08 | **AVE** 4.00E+08 | 1.64E+00 | **AVE** 1.59E+00 |
| | 2 | 1001 | 6.38E+08 | **STD** 6.51E+06 | 4.03E+08 | **STD** 8.75E+06 | 1.59E+00 | **STD** 4.60E-02 |
| | 3 | 1002 | 6.46E+08 | **%CV** 1.02% | 3.93E+08 | **%CV** 2.19% | 1.64E+00 | **%CV** 2.88% |
| | 4 | 1003 | 6.36E+08 | | 4.13E+08 | | 1.54E+00 | |
| | 5 | 1004 | 6.29E+08 | | 4.02E+08 | | 1.56E+00 | |
| 4 | 1 | 1495 | 5.76E+08 | **AVE** 5.71E+08 | 3.65E+08 | **AVE** 3.67E+08 | 1.58E+00 | **AVE** 1.56E+00 |
| | 2 | 1496 | 5.61E+08 | **STD** 4.54E+07 | 3.57E+08 | **STD** 7.75E+06 | 1.57E+00 | **STD** 5.17E-02 |
| | 3 | 1497 | 5.33E+08 | **%CV** 7.95% | 3.63E+08 | **%CV** 2.11% | 1.47E+00 | **%CV** 3.32% |
| | 4 | 1498 | 6.01E+08 | | 3.76E+08 | | 1.60E+00 | |
| | 5 | 1499 | 5.87E+08 | | 3.73E+08 | | 1.57E+00 | |
| 5 | 1 | 1999 | 4.87E+08 | **AVE** 4.94E+08 | 2.68E+08 | **AVE** 2.88E+08 | 1.81E+00 | **AVE** 1.72E+00 |
| | 2 | 2000 | 4.78E+08 | **STD** 1.24E+07 | 2.85E+08 | **STD** 1.21E+07 | 1.68E+00 | **STD** 6.37E-02 |
| | 3 | 2001 | 5.12E+08 | **%CV** 2.51% | 2.95E+08 | **%CV** 4.21% | 1.74E+00 | **%CV** 3.71% |
| | 4 | 2002 | 4.93E+08 | | 2.99E+08 | | 1.65E+00 | |
| | 5 | 2003 | 5.00E+08 | | 2.94E+08 | | 1.70E+00 | |
| 6 | 1 | 2494 | 4.97E+08 | **AVE** 5.06E+08 | 2.95E+08 | **AVE** 3.06E+08 | 1.68E+00 | **AVE** 1.66E+00 |
| | 2 | 2495 | 4.96E+08 | **STD** 1.49E+07 | 3.14E+08 | **STD** 9.63E+06 | 1.58E+00 | **STD** 8.97E-02 |
| | 3 | 2496 | 4.92E+08 | **%CV** 2.95% | 3.16E+08 | **%CV** 3.15% | 1.56E+00 | **%CV** 5.42% |
| | 4 | 2497 | 5.28E+08 | | 2.97E+08 | | 1.78E+00 | |
| | 5 | 2498 | 5.18E+08 | | 3.07E+08 | | 1.68E+00 | |
| 7 | 1 | 2998 | 5.03E+08 | **AVE** 5.06E+08 | 2.92E+08 | **AVE** 2.96E+08 | 1.72E+00 | **AVE** 1.71E+00 |
| | 2 | 2999 | 5.11E+08 | **STD** 1.44E+07 | 2.98E+08 | **STD** 4.97E+06 | 1.71E+00 | **STD** 3.47E-02 |
| | 3 | 3000 | 4.89E+08 | **%CV** 2.85% | 2.89E+08 | **%CV** 1.68% | 1.69E+00 | **%CV** 2.03% |
| | 4 | 3001 | 5.27E+08 | | 3.00E+08 | | 1.76E+00 | |
| | 5 | 3002 | 4.98E+08 | | 3.00E+08 | | 1.66E+00 | |
| 8 | 1 | 3214 | 3.74E+08 | **AVE** 3.92E+08 | 2.23E+08 | **AVE** 2.12E+08 | 1.68E+00 | **AVE** 1.85E+00 |
| | 2 | 3215 | 3.88E+08 | **STD** 1.98E+07 | 2.22E+08 | **STD** 1.00E+07 | 1.75E+00 | **STD** 1.76E-01 |
| | 3 | 3216 | 3.88E+08 | **%CV** 5.04% | 2.09E+08 | **%CV** 4.72% | 1.86E+00 | **%CV** 9.47% |
| | 4 | 3217 | 3.84E+08 | | 2.08E+08 | | 1.84E+00 | |
| | 5 | 3218 | 4.26E+08 | | 1.99E+08 | | 2.14E+00 | |

*Data table for figure 7 and figure 8. Each bin is equal to 5 injections over which the %CV was calculated. The change in %CV can be correlated to the robustness of the analysis.*

## Conclusions

- Reproducible XIC intensity of buspirone at 1 µL/min. and 20 µL/min. indicates a wide range of system stability
- Reproducible %CV values consistently below 5% for all three analytes demonstrates system robustness
- Angiotensin peak area ratio values indicates changes in ion intensity are sample related and not system related

*Originally published at the 2009 Chemical and Pharmaceutical Structure Analysis Symposium in Langhorne, PA*