Bridging the Gap Between Nanospray and ESI with Capillary Spray

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Introduction

Diverse requirements for small and large-molecule analysis have split ESI into two distinct flow regimes: high (mL/min.) and ultra-low (nL/min.) High-flow-rates using mm-diameter columns enable fast elution on short columns. Fast cycle times and robust analyses are the hallmarks of the workflow employed in small-molecule analysis. On the other end of the spectrum, nanobore-chromatography has provided the qualitative field of proteomics with the sensitivity required to identify biomolecules from complex matrices. Maximizing sensitivity for protein identification has been the trademark of the workflow employed in large-molecule analysis.

Biomarker validation of endogenous proteins and peptides demands that biomolecular LC-MS/MS makes the transition from qualitative to quantitative analysis, but presents unique challenges. It requires the throughput and robustness of small molecule workflows to efficiently quantify large sample sets as well as the sensitivity realized with nanobore-chromatography. Enabling efficient ESI in an intermediate flow range, 1–20µL/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 and deliver higher throughput and robustness than nanobore LC. A novel approach to ESI based on high-precision fused-silica components and microfluidic-connectors enables the integration of concepts used in both high-flow ESI and nanospray. Here we investigate a capillary-spray-source employing a novel spray-probe incorporating these features. System stability between flow rates of 1 µL/min. and 20 µL/min. is demonstrated by reproducible buspirone XIC intensity from continuous flow and flow injection experiments, as well as across a gradient separating a BSA digest on a 300 µm ID column.

Methods & Materials

Instrumentation

- Mass Spectrometer: LCQ Deca (Thermo Fisher Scientific)
  - 3 microscans/spectra
  - 300.00 – 550.00 Da mass range (flow injection)
  - 300.00 – 2,000.00 Da mass range (chromatography)
  - N_2 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 Rates: 5 µL/min., 10 µL/min., and 20 µL/min.
  - Composition: Isocratic 50% B (flow injection)
  - Gradient: 2–34% B over 20 minutes (chromatography)
- Autosampler: HTC Pal (Leap Technologies)
  - 50 µL syringe
  - 6-port micro valve (VICI)
  - 2.0 µL sample loop
- ESI Source: Custom capillary spray source (New Objective)
  - Spray probe; TaperTip emitter, 360 µm OD x 20 µm ID
  - Column: 300 µm ID x 50 mm bed, 5µm 300 Å C18, ProteoPep II (New Objective)

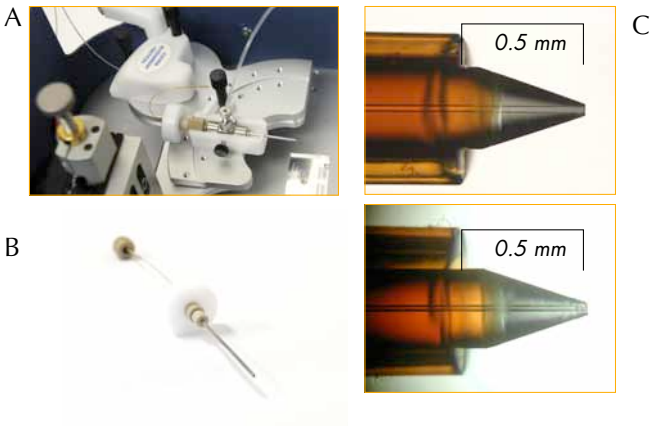
Reagents

- 62.5 ng/µL BSA (MassPrep, Waters)
- 0.1% Formic acid in water (JT Baker)
- 0.1% Formic acid in acetonitrile (JT Baker)
- 1 µM buspirone hydrochloride, m/z 386 Da (Sigma)
- 1 pmol/µL angiotensin I, MH^{3+} 433 Da (Sigma)
- 1 pmol/µL angiotensin II, MH^{2+} 524 Da (Sigma)

Flow Injection

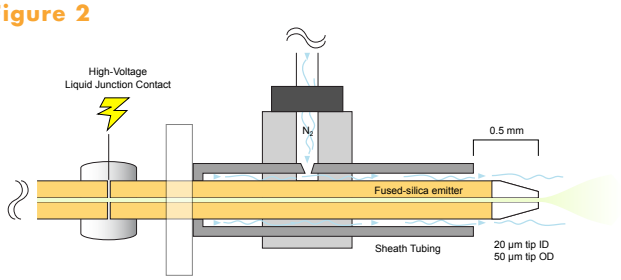
Using Channel 1 on the Eksigent nanoLC•2D to deliver a constant isocratic flow of 50% B at 10 µL/min., 10-minute data files were collected in XCalibur. Successive injections of 2 µL of analyte each were delivered at 30 second intervals resulting in a total of 9 injections per 10-minute data file. The capilaary spray probe containing a 20 µm ID TaperTip emitter was precisely positioned at a fixed point on-axis 2 mm 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 N_2 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 of the probe assembly precisely 500 µm. All of these conditions were held constant throughout the collection of 360 data files, which translates to 3,240 replicate injections of analyte.

Figure 1



Capillary spray hardware: A) Photo of capillary spray probe mounted onto source stage showing sheath gas tubing connection and high-voltage liquid-junction connection; B) capillary spray probe assembly for error-free, easy to use 'plug and spray' analyses; C) Before and after photos of capillary spray probe used to collect flow injection data over 3200 replicate injections. The 0.5 mm delta between the end of the sheath gas tubing and the spray probe emitter is indicated.

Figure 2



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

Figure 3 *Direct infusion*

Direct infusion: 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.

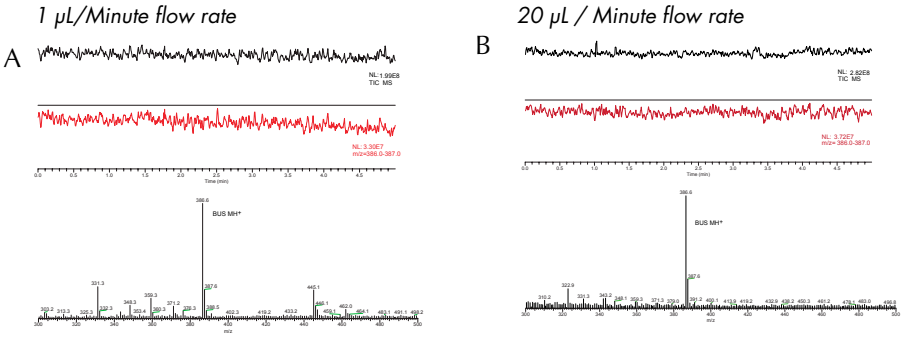


Figure 4 *Flow injection*

Flow Injection: 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.

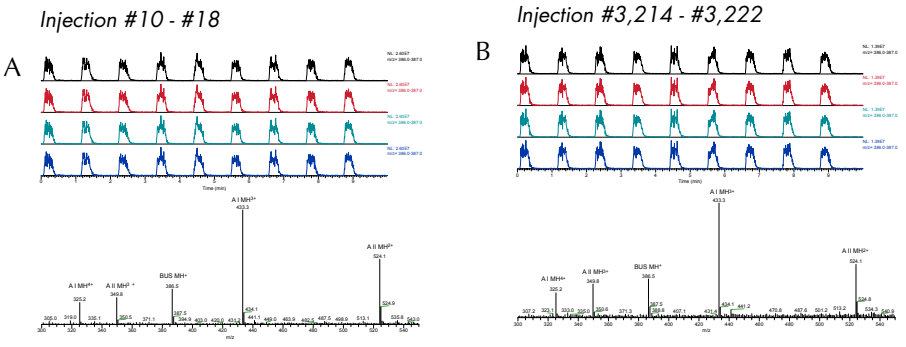
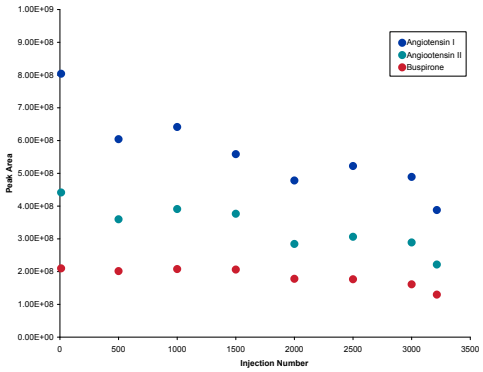
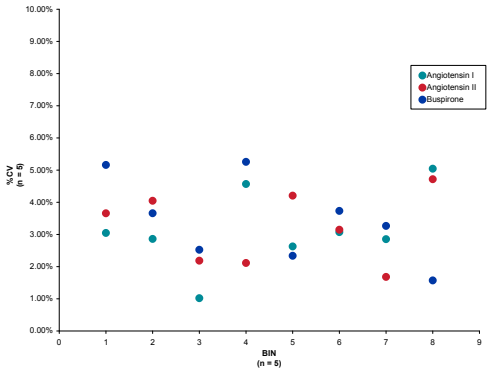


Figure 5 *Peak Area vs. Injection Number*



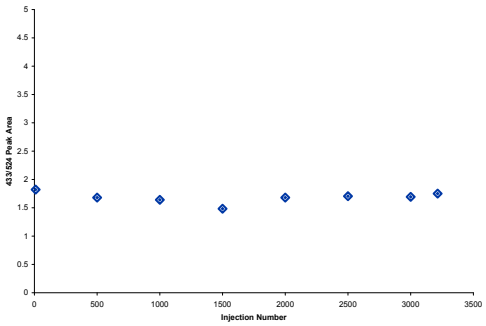
Flow injection experiment: Change in peak area over 3,200 replicate injections for each analyte

Figure 6



Flow injection experiment: Plot of %CV with n=5 for each analyte over 3,200 injections

Figure 7 *Angiotension Peak Area Ratio vs Injection*



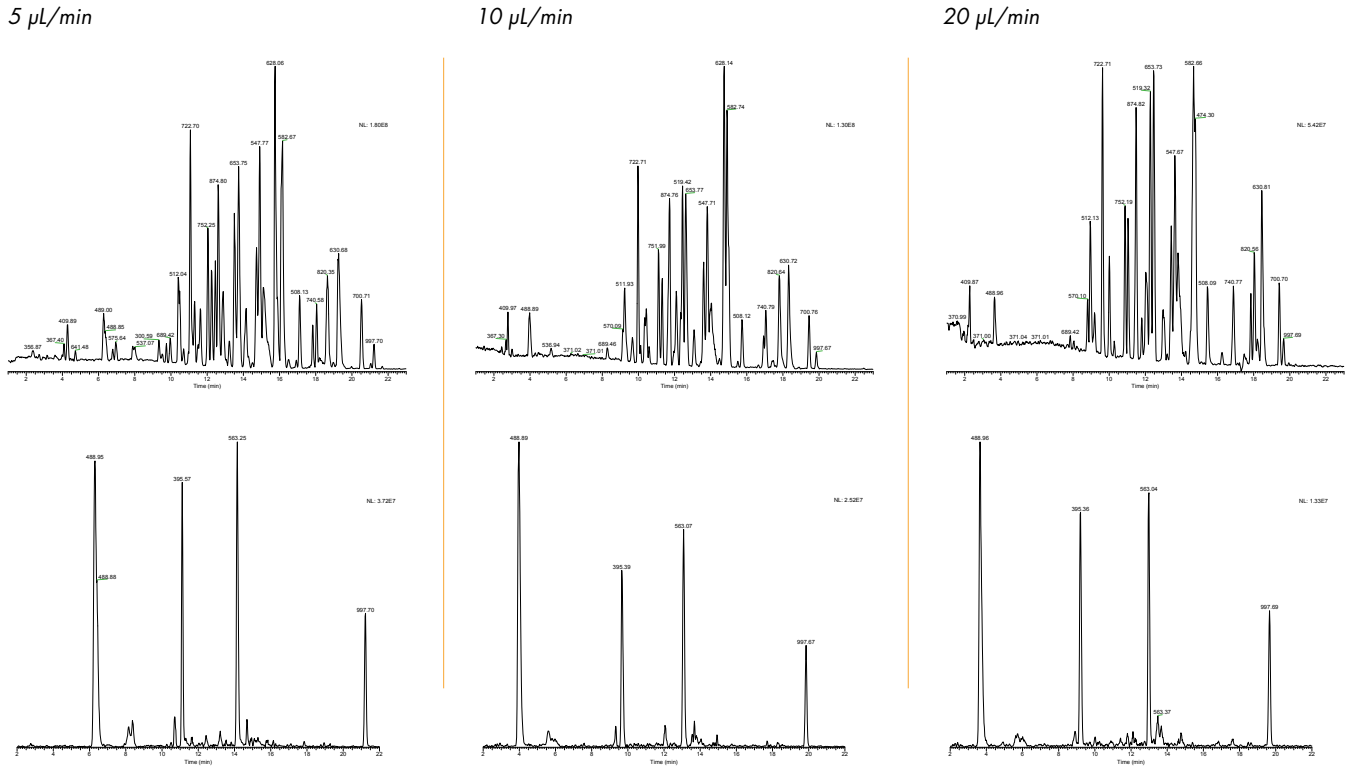
Flow injection experiment: Change in the peak area ratio of angiotensin I (MH3+, 433 Da) to Angiotensin II (MH_{2+}, 524 Da) over 3,200 injections.

Figure 8

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. 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.

Figure 9 *Gradient chromatography*



Chromatography: separation of 125ng on-column injection of a commercial BSA digest on a 300 µm ID x 50 mm C18 column at 5, 10 and 20 µL/min. XIC of four BSA peptides of similar ion intensity at all three flow rates are highlighted to demonstrated spray stability across a gradient and range of capillary flow rates.

Conclusions

- Reproducible XIC intensity of buspirone for direct infusion at 1 µL/min. and 20 µL/min. indicates a wide range of system stability
- Reproducible %CV values calculated for flow injection experiments consistently below 5% for all three analytes demonstrates system robustness
- In flow injection experiments, angiotensin peak area ratio values indicates changes in ion intensity are sample related and not system related
- Chromatography at 5, 10 and 20 µL/min. further supports a wide range of system stability and demonstrates robust spray stability throughout a gradient

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