Increasing Operable Flow Range of a Nanospray Source for High-Performance Microspray on a Curtain Gas Equipped Triple–Quadrupole API-MS

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Introduction

Qualitative workflows have employed nanoflow LC-MS for sample-limited analysis of complex mixtures. The intrinsic complexity of these samples requires long, slow gradients to maximize separation capacity. Quantitative applications requiring higher throughput are traditionally run at conventional LC flow rates (100 µL/min. and higher), limiting the ability to analyze small sample volumes. High-performance microspray, defined here as flow rates of 1 – 10 µL/min. delivers much of the sensitivity realized by nanoflow LC-MS while enabling efficient duty cycle and throughput. Nanospray source hardware has traditionally been used at flows ranging from 20 - 1,000 nL/min. We investigate the capability of a modified nanospray source, using conventional nanospray emitters and columns to operate at 1 µL/min. to 10 µL/min. by evaluating spray stability and analyte response.

Methods

Infusion Experiments

- Instrumentation
  - Mass spectrometer: 4000 QTRAP (SCIEX)
  - Scan Settings: EMS Scan; 300-1200 m/z, Scan rate: 1000 amu/s
  - Compound parameters: Declustering potential – 70; Collision Energy – 10
  - Source/Gas parameters: Curtain gas - 10.0; ion spray voltage - 2300 kV,
  - Nebulizing gas – 3, 10, 20; interface heater temperature -150°C
  - Source: Digital PicoView DPV-450 nanospray source (New Objective, Inc.)
  - Emitters:
  - Uncoated, 360 µm OD x 20 µm ID x 10 µm tip (New Objective, Inc.)
  - Uncoated, 360 µm OD x 50 µm ID x 8 µm tip (New Objective, Inc.)
  - Syringe pump: Pump 11 Elite (Harvard Apparatus)
  - Syringe: 1 mL (Hamilton)
  - Flow rates: 0.25, 0.5, 1, 2, 3, 4 and 5 µL/min
- Samples
  - 10 fmol/µL of Buspirone (European Pharmacopoeia Reference Standard), 100 fmol/µL of angiotensin II (Sigma Aldrich) and 100 fmol/µL of insulin chain B (Sigma Aldrich) in 50% water, 50% acetonitrile, 0.1% formic acid

DPV-450 Digital PicoView nanospray source

Chromatographic Experiments

- Instrumentation
  - Mass Spectrometer: 4000 QTRAP (SCIEX)
  - Scan Settings: EMS Scan 300 – 1200 m/z; Scan rate 4000 amu/s
  - Compound Parameters: Declustering potential – 70; Collision Energy - 5
  - Source/Gas Parameters: Curtain gas - 10.0; ion spray voltage - 2300 kV,
  - Nebulizing gas: 20 (0.5, 1 µL/min) and 30 (2-10 µL/min)
  - Interface heater temperature: 150°C
- Source: Digital PicoView DPV-450 nanospray source (New Objective, Inc.)
- Column: Packed tip column, 150 µm ID x 10 µm tip (PicoFrit, New Objective, Inc.), 5 and 10 cm bed packed with Reprosil-PUR C18-AQ 3 µm 120 Å (Dr. Maisch GmbH)
- Eksigent nanoLC-Ultra 2D plus (SCIEX)
- HTC Pal autosampler (Leap Technologies)
  - 6-port injection valve (VICI Valco Instruments, Co., Inc.)
  - 1 µL Loop, overfill
- Chromatography
  - Flow rates: 0.5, 1, 3, 5, 9 and 10 µL/min
  - Mobile phase: A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile
  - Gradient: 2-35% B in 5 and 10 min
- Samples
  - PicoSure™ standard (New Objective, Inc.)
  - 500 fmol/µL in water + 0.1% formic acid
  - BSA digest (Waters) with angiotensin II, buspirone and insulin spikes
  - 200 fmol/µL BSA digest in water + 0.1% formic acid
  - 25 fmol/µLspike of angiotensin II (Sigma Aldrich)
  - 10 fmol/µLspike of buspirone (European Pharmacopoeia Reference Standard)
  - 100 fmol/µLspike of insulin chain B (Sigma Aldrich)

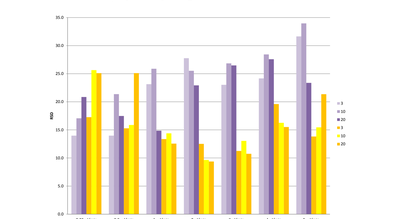
Conclusions

- Demonstrated microspray capability of a modified nanospray source at 1 – 10 µL/min.
- Reduced cycle time 3X: 25 min at 0.5 µL/min. to 8 min at 3 µL/min.
- Generated lower RSD values 8-21% using 50 µm ID emitter at 1-10 µL/min.
- Observed a -0.86 fold signal decrease from 1 uL/min. to 10 uL/min. for angiotensin II

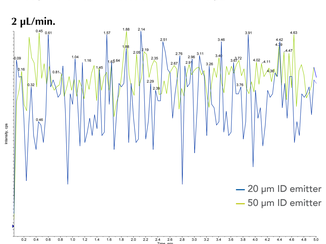
Effects of Emitter Size on Spray Stability

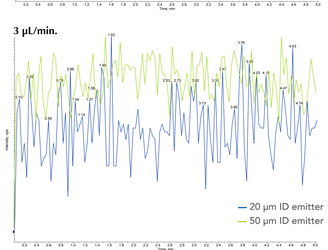
		Buspirone (m/z 386.5 Da)				Angiotensin II (m/z 524.0 Da)				Insulin (m/z 1166 Da)			
		20 µm ID, 10 µm tip		50 µm ID, 8 µm tip		20 µm ID, 10 µm tip		50 µm ID, 8 µm tip		20 µm ID, 10 µm tip		50 µm ID, 8 µm tip	
Flow (µL/min)	NG	Average XIC Intensity	RSD	Average XIC Intensity	RSD	Average XIC Intensity	RSD	Average XIC Intensity	RSD	Average XIC Intensity	RSD	Average XIC Intensity	RSD
0.25	3	1.14E+07	14.0	9.04E+06	17.3	1.11E+07	15.5	2.14E+07	10.1	8.32E+06	18.9	8.93E+06	19.3
	10	5.45E+06	17.1	5.46E+06	25.6	8.50E+06	16.9	1.29E+07	15.3	1.46E+07	15.4	6.51E+06	21.8
	20	5.23E+06	20.9	4.76E+06	25.1	5.99E+06	19.7	1.06E+07	17.3	4.05E+06	26.3	6.20E+06	21.1
0.5	3	1.14E+07	14.0	1.33E+07	15.3	1.12E+07	14.7	2.49E+07	11.2	1.07E+07	21.9	9.34E+06	18.4
	10	6.21E+06	21.4	1.30E+07	15.9	7.80E+06	18.0	2.20E+07	13.8	6.31E+06	20.2	9.87E+06	15.8
	20	7.23E+06	17.5	4.26E+06	25.1	7.71E+06	20.2	8.80E+06	17.8	6.92E+06	21.0	5.36E+06	24.5
1	3	7.72E+06	23.1	1.15E+07	13.4	1.03E+07	27.4	2.15E+07	11.7	9.57E+06	18.8	1.21E+07	18.3
	10	4.51E+06	25.9	1.34E+07	14.4	6.35E+06	23.1	2.08E+07	9.9	5.37E+06	26.8	1.20E+07	15.1
	20	9.23E+06	34.9	1.53E+07	12.6	1.07E+07	14.4	2.18E+07	9.9	9.47E+06	18.0	1.24E+07	16.2
2	3	5.68E+06	27.8	1.46E+07	12.5	7.24E+06	28.8	2.26E+07	10.8	6.50E+06	24.2	1.28E+07	12.9
	10	4.71E+06	25.5	2.24E+07	9.6	6.10E+06	23.8	1.84E+07	11.8	5.64E+06	25.8	2.02E+07	11.9
	20	5.33E+06	22.9	2.40E+07	9.4	7.53E+06	21.3	2.80E+07	8.4	7.90E+06	20.1	2.42E+07	10.0
3	3	5.08E+06	23.0	1.81E+07	11.2	5.47E+06	26.1	2.54E+07	10.6	7.60E+06	32.7	1.39E+07	15.1
	10	3.73E+06	26.8	1.42E+07	13.0	4.33E+06	29.6	1.48E+07	12.3	5.28E+06	25.7	1.52E+07	14.0
	20	4.83E+06	26.5	1.99E+07	10.7	7.57E+06	20.3	1.61E+07	12.1	7.98E+06	21.0	1.82E+07	13.2
4	3	4.43E+06	24.2	8.82E+06	19.6	5.39E+06	24.8	9.33E+06	15.9	4.90E+06	28.7	8.77E+06	18.6
	10	3.78E+06	28.4	9.70E+06	16.3	4.96E+06	28.3	1.00E+07	16.3	6.87E+06	27.6	1.02E+07	16.2
	20	4.05E+06	27.6	9.66E+06	15.5	6.15E+06	24.7	9.52E+06	17.8	7.06E+06	21.8	1.06E+07	15.7
5	3	2.96E+06	31.6	9.96E+06	13.8	3.69E+06	32.8	1.03E+07	15.7	5.46E+06	34.4	1.17E+07	17.7
	10	2.81E+06	34.0	9.04E+06	15.5	3.19E+06	37.0	9.89E+06	17.3	4.85E+06	31.0	1.03E+07	16.1
	20	3.93E+06	23.4	6.92E+06	21.4	4.06E+06	29.7	7.24E+06	20.9	6.57E+06	22.0	8.57E+06	19.8

Relative standard deviation (RSD) and signal intensity calculated for extracted ion chromatograms of buspirone (m/z 386.5 Da), angiotensin II MH2+ ion (524.0 Da) and insulin chain B MH3+ (1166.5 Da) at different flow rates and sheath gas settings. The spray stability at low flow rates of 0.25 and 0.5 µL/min is better with the 20 um ID emitter while the 50 µm ID emitter improved the spray stability at higher flow rates (1-5 µL/min).



Plot of buspirone RSD calculated from 5 min. long direct infusion files collected at different flow rates and nebulizer gas setting. Data were collected with 20 µm ID emitter (purple) and with 50 µm ID emitter (yellow). The data show improved spray stability when the 50 µm ID emitter was used at flow rates 1-5 µL/min.



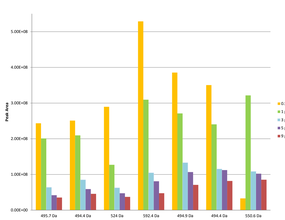


Extracted ion chromatogram of buspirone ion 386.5 Da. The data show significant improvement in spray stability when using the 50 µm ID emitter at 2 and 3 µL/min.

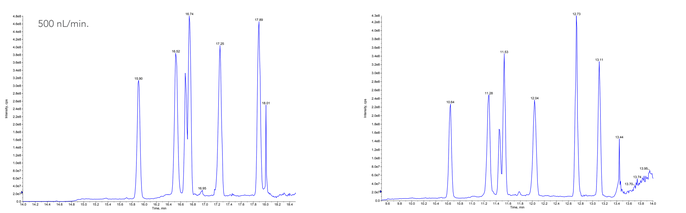
Improved Cycle Time at Higher Flow Rates

m/z	Flow Rate (µL/min)	Peak Area	Peak Height	RT (min)	Start Time (min)	Stop Time (min)	FWHM (min)	Asymmetry
495.7 Da	0.5	2.44E+08	6.33E+07	15.9	15.8	16.1	0.06	2.09
	1	2.05E+08	5.97E+07	10.6	10.6	10.7	0.06	0.97
	3	6.38E+07	2.27E+07	3.5	3.4	3.5	0.05	0.84
	5	4.19E+07	1.31E+07	2.6	2.5	2.6	0.05	0.84
	9	3.54E+07	1.12E+07	2.0	1.9	2.0	0.05	0.70
494.5 Da	0.5	2.51E+08	5.58E+07	16.5	16.4	16.7	0.08	1.43
	1	2.50E+08	5.26E+07	11.3	11.2	11.4	0.07	1.01
	3	8.31E+07	2.33E+07	4.0	3.9	4.1	0.06	0.87
	5	5.91E+07	1.68E+07	3.2	3.1	3.2	0.06	0.80
	9	4.56E+07	1.38E+07	2.5	2.5	2.6	0.05	0.74
524.0 Da	0.5	2.90E+08	5.05E+07	16.7	16.6	16.8	0.04	1.08
	1	1.27E+08	4.74E+07	11.5	11.4	11.5	0.04	1.01
	3	6.26E+07	2.18E+07	4.2	4.1	4.3	0.05	1.36
	5	4.72E+07	1.80E+07	3.4	3.3	3.4	0.04	1.34
	9	3.71E+07	1.31E+07	2.7	2.7	2.8	0.05	1.46
592.4 Da	0.5	5.29E+08	1.55E+08	16.7	16.7	16.9	0.06	1.88
	1	3.10E+08	1.15E+08	11.5	11.5	11.6	0.04	1.07
	3	1.05E+08	3.78E+07	4.3	4.2	4.4	0.04	1.36
	5	8.10E+07	3.08E+07	3.4	3.4	3.5	0.04	1.48
	9	4.75E+07	1.80E+07	2.8	2.8	2.9	0.04	1.22
494.9 Da	0.5	3.86E+08	9.37E+07	17.2	17.2	17.3	0.07	0.71
	1	2.71E+08	6.83E+07	12.0	11.9	12.1	0.06	0.64
	3	9.33E+08	3.20E+07	4.8	4.6	4.9	0.06	0.52
	5	5.07E+08	2.67E+07	3.9	3.7	4.0	0.06	0.50
	9	7.08E+07	1.79E+07	3.2	3.1	3.3	0.06	0.46
494.4 Da	0.5	3.51E+08	9.11E+07	17.9	17.8	18.1	0.06	2.14
	1	2.41E+08	7.87E+07	12.7	12.7	12.8	0.05	1.37
	3	1.15E+08	3.38E+07	5.4	5.4	5.5	0.05	1.65
	5	7.12E+08	3.30E+07	4.6	4.5	4.7	0.05	1.55
	9	8.18E+07	2.41E+07	3.9	3.8	4.0	0.05	1.48
550.6 Da	0.5	3.29E+07	2.22E+07	18.0	17.9	18.0	0.04	1.18
	1	3.22E+08	9.68E+07	13.1	13.1	13.3	0.05	2.37
	3	1.08E+08	3.75E+07	5.8	5.7	5.9	0.05	1.41
	5	1.02E+08	3.50E+07	5.0	4.9	5.0	0.05	1.19
	9	8.53E+07	2.81E+07	4.3	4.2	4.4	0.05	1.12

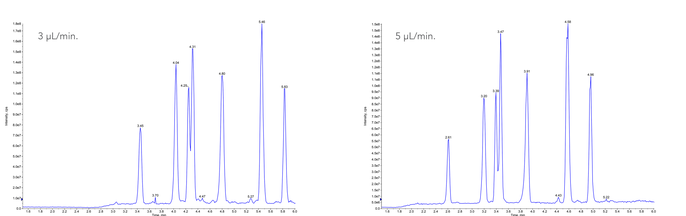
LEFT: Peptide specific peak area, peak width and retention time data calculated for different PicoSure peptides separated on a 5 cm long, 150 µm ID column. The sample separation was achieved by 2-35% acetonitrile gradient with flow varying from 0.5 to 9 µL/min. The values were calculated as the average from 2 consecutive injections (at 0.5 and 1 µL/min) and from 5 consecutive injections (at 3, 5 and 9 µL/min).

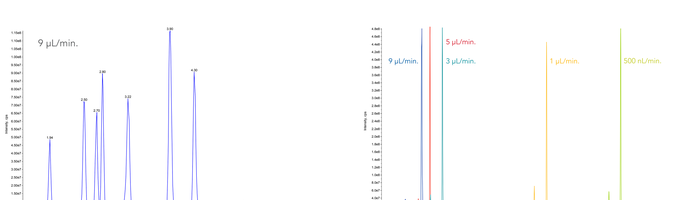


Plot of peptide specific peak area for PicoSure peptides. Data were collected at 5 cm long, 150 µm ID column with 5 min 2-35% gradient at flow rates from 0.5 to 9 µL/min.



TIC chromatographic separation of PicoSure standard. Data was collected on 5 cm long column at different flow rates ranging from 0.5 to 9 µL/min with 5 min gradient 2-35% B. The elution window shifted from 1.5 – 4.5 min at 9 µL/min to 15.6 – 18.2 min at 0.5 µL/min.



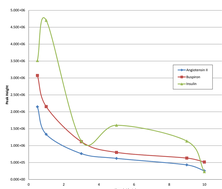


Extracted ion chromatograms of PicoSure peptide m/z 524.5 Da showing the retention time shift at different flow rates.

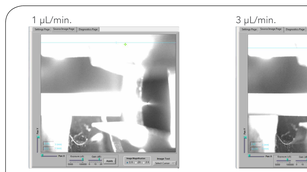
Evaluation of Signal Intensity at Higher Flow Rates

		5 cm long column					10 cm long column				
Analyte	Flow Rate (µL/min)	Peak Area	Peak Height	RT (min)	FWHM (min)	Asymmetry	Peak Area	Peak Height	RT (min)	FWHM (min)	Asymmetry
25 fmol/µL Angiotensin II	0.5	7.54E+06	2.15E+06	19.20	0.06	1.05	6.32E+06	1.89E+06	19.20	0.04	0.94
	1	6.07E+06	1.34E+06	13.70	0.08	1.09	5.01E+06	1.51E+06	13.95	0.05	0.67
	3	3.51E+06	7.64E+05	5.83	0.07	1.19	8.09E+06	1.89E+06	7.06	0.07	1.04
	5	2.94E+06	6.23E+05	4.96	0.08	1.13	7.73E+06	1.66E+06	6.02	0.08	0.93
	9	1.88E+06	4.30E+05	4.29	0.07	0.78	Not collected				
	10	8.39E+05	2.55E+05	4.15	0.06	0.65	Not collected				
10 fmol/µL Buspirone	0.5	1.54E+07	3.08E+06	21.10	0.07	1.25	1.61E+07	4.18E+06	21.60	0.05	1.02
	1	1.19E+07	2.16E+06	15.30	0.08	1.01	8.56E+06	1.86E+06	16.00	0.07	1.39
	3	5.73E+06	1.12E+06	6.98	0.08	0.88	3.10E+06	1.07E+06	8.52	0.05	0.88
	5	4.40E+06	8.00E+05	5.86	0.09	0.83	1.59E+06	5.44E+05	7.86	0.05	0.95
	9	3.45E+06	6.32E+05	4.98	0.09	1.88	Not collected				
	10	2.80E+06	5.15E+05	4.82	0.09	1.09	Not collected				
100 fmol/µL Insulin	0.5	1.28E+07	3.52E+06	21.85	0.06	0.81	3.04E+07	9.92E+06	21.70	0.05	1.09
	1	1.82E+07	4.71E+06	16.65	0.06	0.69	1.57E+07	5.66E+06	16.65	0.04	0.89
	3	4.22E+06	1.14E+06	9.05	0.06	0.81	3.48E+06	1.22E+06	5.67	0.04	1.02
	5	6.50E+06	1.66E+06	8.28	0.07	0.90	2.84E+06	1.50E+06	4.20	0.03	0.83
	9	4.48E+06	1.14E+06	7.70	0.06	1.53	Not collected				
	10	7.70E+05	2.29E+05	7.61	0.06	1.07	Not collected				

Signal intensity, retention time and peak width calculated for buspirone, angiotensin II and insulin spiked into 200 fmol/µL BSA. Data was collected on 5 and 10 cm long 150 µm ID column with 2-35%B in 10 min gradient. The values were calculated as the average from 2 consecutive injections (at 0.5 and 1 µL/min) and from 5 consecutive injections (at 3, 5, 9 and 10 µL/min).

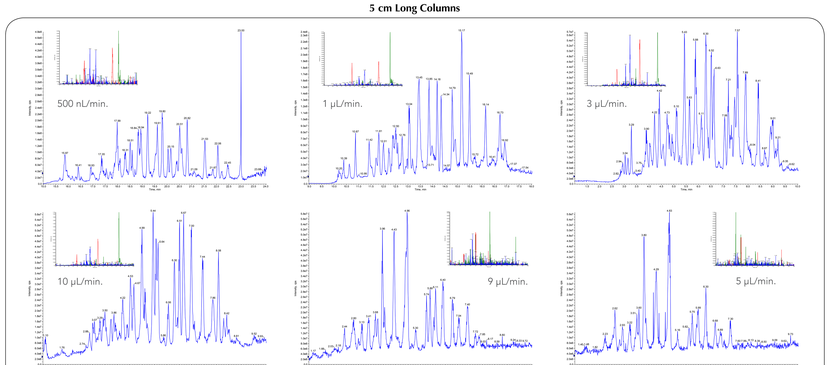


Plot of analyte specific peak height for buspirone, angiotensin II and insulin spiked into 200 fmol/µL BSA. Data was collected on 5 cm long PicoFrit columns at flow rate ranging from 0.5 to 10 µL/min.



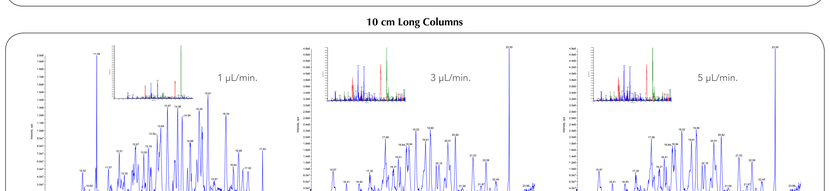
Photos of spray at 30% B at different flow rates. Spray conditions: 2.3 kV, 20 NG (1 µL/min) and 30 NG (3-10 µL/min).

5 cm Long Columns



TIC chromatograms of 200 fmol/ul BSA standard spiked with buspirone, angiotensin II and insulin standards. Sample separation was achieved with 10 min 2-35% B gradient on 5 cm long columns. The buspirone, angiotensin II and insulin XIC chromatograms show that the spike signal is clearly visible at flow rates up to 5 µL/min. The signal diminished in the background at flow rates of 9 and 10 µL/min.

10 cm Long Columns



BSA chromatograms collected on 10 cm long column run with 10 min. gradient 2-35%B at flow rates 1, 3 and 5 µL/min.