



Accessing High Performance Microflow on a Chip-Based Platform

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Introduction

Source hardware and consumables for nanoflow LC-MS have undergone many developments in recent years. Chip-based solutions capitalize on the sensitivity improvements enabled by nanoflow, while making this technique easier and accessible to a wider audience by minimizing the need for special expertise in handling. Existing chip-based technologies support long columns, temperature control and UPLC compatibility, but are targeted towards nanoflow and built on C18-based separations. Flexibility in packed column chemistry supports protein analysis, metabolomics, and small molecule quantitation. Temperature control and gas-enabled features expand the flow-rate based application window from nanoliter to microliter flow rates. Here, we present a chip-based solution which preserves ease-of-use and performance while facilitating application flexibility.

Methods

Mass Spectrometer

- LTQ Linear Ion Trap (Thermo)
- Full Scan MS: Infusion: 150-2000 Da; Chromatography: 300-1500 Da (BSA samples) or 400-600 Da (PicoSure samples)
- Voltage: 2.0, 2.1, 2.2, 2.3, 2.4, and 2.5 kV
- Sheath gas: 0, 5, 10, 15, 20, 25, and 30

Nanospray Source

- PicoChip source (New Objective, Inc.)
 - Gas-enabled
 - Thermal control: CSI32 series miniature benchtop controller (Omega)
 - 3-axes adjustable stage
 - Optimized emitter position: 2 mm from MS inlet
- PicoChip columns – gas and temperature enabled (New Objective, Inc.):
 - 360 µm OD x 150 µm ID x 15 µm tip
 - 5 cm ReproSil-Pur C18-AQ, 3 µm, 120 Å (Dr. Maisch HPLC GmbH), code H354
 - 5 cm HALO PEPTIDE ES C18, 2.7 µm, 160 Å (Advanced Material Technology), code H039
 - 5 cm HALO Peptide C18, 5 µm, 160 Å (Advanced Material Technology), code H044
 - 5 cm ACQUITY BEH C18, 1.7 µm, 130 Å (Waters), code H111
 - Emitter protrusion from gas nozzle: 1 mm

Chromatography

- Eksigent nanoLC-Ultra 2D plus (SCIEX)
 - Flow Rate: 5 µL/min. and 10 µL/min.
 - Mobile Phase A: 0.1% formic acid in water
 - Mobile Phase B: 0.1% formic acid in acetonitrile
 - Gradient: 2% -35% B in 1, 1.5, 2, 5 and 10 min.
- HTC Pal Auto-sampler (Leap Technologies)
 - 6-port injection valve (VICI Valco Instruments, Co., Inc.)
 - 1 µL loop, with overfill
 - Temperature: 50°C

Samples

- PicoSure Standard (New Objective)
 - 500 fmol/µL PicoSure in water with 0.1% formic acid
- BSA digest (Waters)
 - 500 fmol/µL in water with 0.1% formic acid
 - Spiked with 100 fmol/µL angiotensin II (Sigma Aldrich), 100 fmol/µL of buspirone (European Pharmacopoeia Reference Standard) and 500 fmol/µL of insulin (Sigma Aldrich)

Peptide #	Sequence	Molecular Weight (Da)	Calculated (Da) (20% error)
1	MGQGGGGGWR	888.4762	493.2436
2	ELIAGAGGWR	887.5138	443.7568
3	DRYVYDPP	1045.5345	522.7673
4	PRRWVGLRNC	1182.537	591.2688
5	ELIAGGWR	886.5169	443.2584
6	ELIPLRWR	885.5961	442.7981
7	ELIPLRWR	1098.6801	549.3401

List of peptides in PicoSure™ 7-peptide mixture

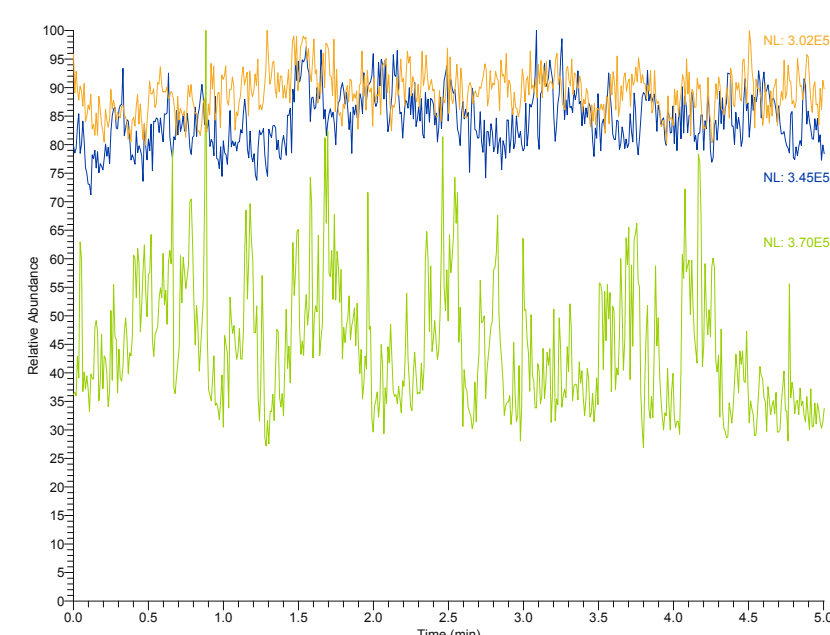


PicoChip GT nanospray source with Omega controller

Spray Stability

10% ACN		Sheath Gas Setting					
Voltage (kV)	0	5	10	15	20	25	30
2	11.8	12.3	5.2	3.9	4.2	2.1	4.8
2.1	5.5	5.1	4.9	5.4	4.7	4.2	4.2
2.2	11.5	8.7	6.6	4.7	5.1	5.5	6
2.3	12.8	8.8	7.5	5.2	5.7	5	5.4
2.4	14.7	9.5	5.9	4.5	4.1	6.7	4.8
2.5	9.6	8	6.7	5.7	4.5	5.2	4.7

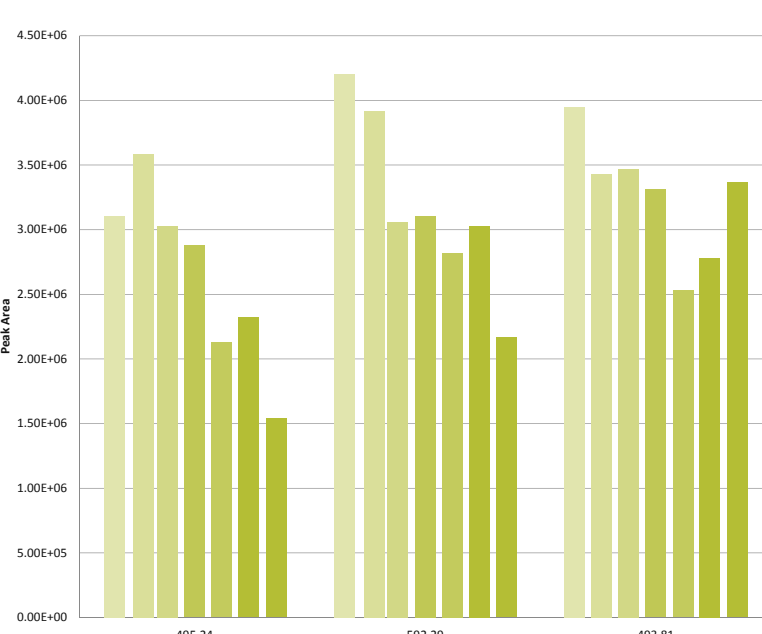
Relative standard deviation (RSD) of 5 min. long TICs collected at 10% and 30% acetonitrile in water with 0.1% formic acid at varying voltage and sheath gas settings. The spray stability was greatly improved with the addition of sheath gas. Significant improvement in spray stability was observed even with very low sheath gas settings.



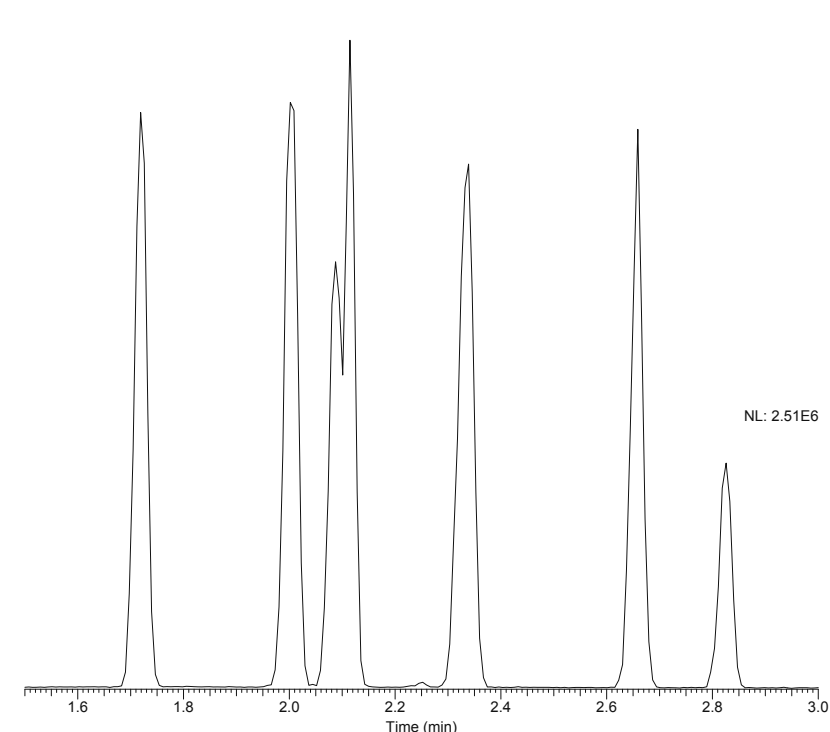
TICs collected at 30% acetonitrile at 2.2 kV. The data was collected at 0, 5 and 15 units of sheath gas.

Voltage	Sheath Gas	Peak (mV)						
		495.24	494.76	523.77	522.26	494.28	493.8	550.34
0	3.11E+06	4.33E+06	3.13E+06	4.21E+06	4.36E+06	3.95E+06	2.31E+06	
5	3.09E+06	3.29E+06	3.91E+06	3.70E+06	4.81E+06	3.44E+06	1.72E+06	
10	3.03E+06	3.43E+06	2.24E+06	3.07E+06	3.79E+06	3.47E+06	2.08E+06	
15	2.89E+06	3.31E+06	2.02E+06	3.12E+06	3.49E+06	3.32E+06	1.78E+06	
20	2.14E+06	3.29E+06	1.72E+06	3.20E+06	3.00E+06	2.54E+06	1.89E+06	
25	3.32E+06	2.95E+06	1.70E+06	3.03E+06	3.43E+06	2.80E+06	1.85E+06	
30	1.54E+06	2.32E+06	1.27E+06	2.16E+06	2.75E+06	3.36E+06	2.38E+06	

Peptide specific peak area was calculated for seven PicoSure peptides separated on a 5 cm long, 150 µm ID column, packed with ReproSil-PUR C18-AQ, 3 µm, 120 Å resin. The sample separation was achieved by 2 min, 2-35% acetonitrile gradient at 10 µL/min. with varying voltage and sheath gas settings. Most peptides show decreased signal intensity corresponding with an increased sheath gas setting.



Plot of peak areas for three different PicoSure peptides collected with 2 min. long gradient at 2.2 kV and varying sheath gas settings.

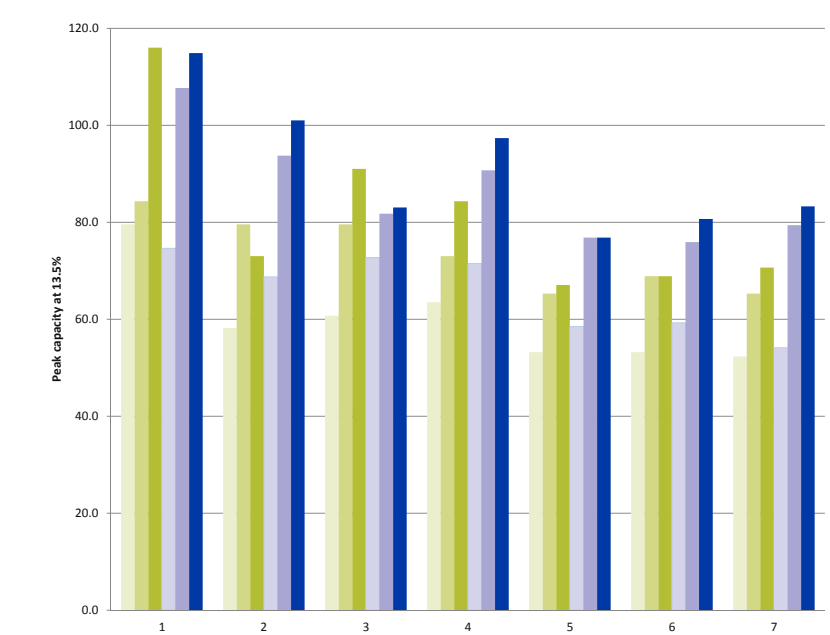


Example of base peak chromatogram of 500 fmol/µL PicoSure standard collected with 2 min. 2-35% Acetonitrile gradient at settings of 2.2 kV and 5 SG units. A 5 cm long, 150 µm ID column packed with H354 resin was used for the data collection.

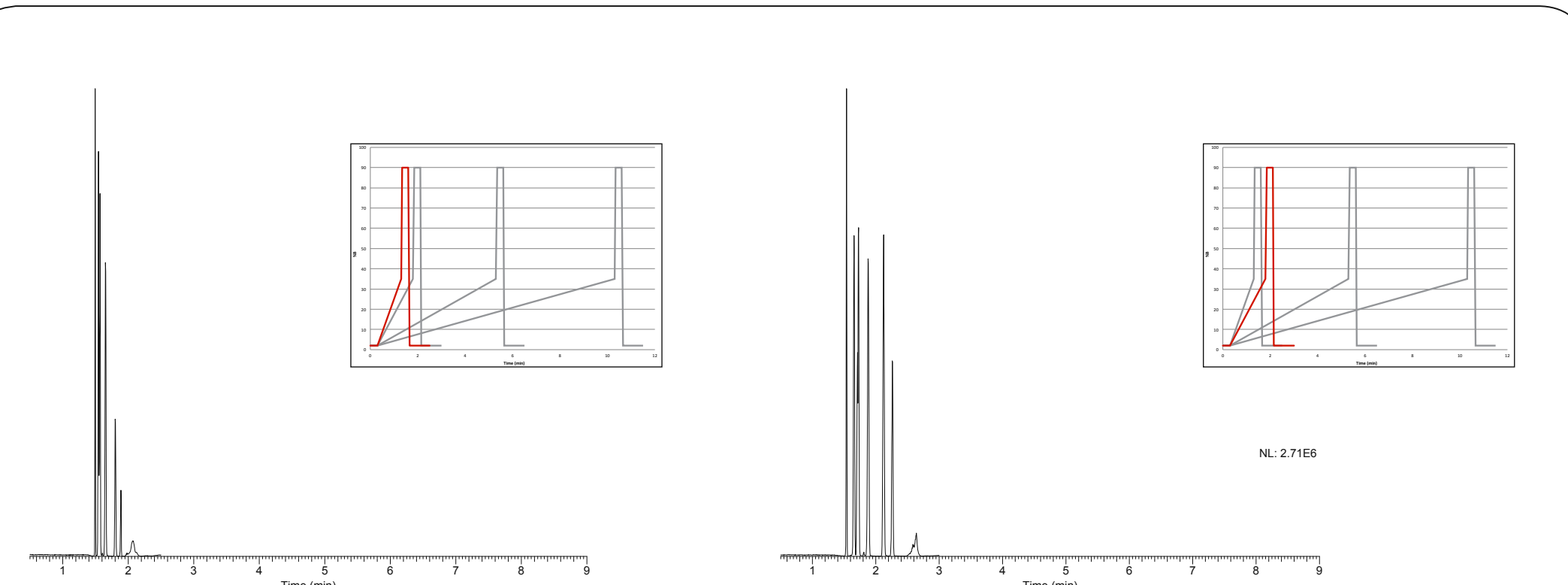
Gradient Optimization

Sample	Resin	Flow	10 min. Gradient					15 min. Gradient					20 min. Gradient					25 min. Gradient					30 min. Gradient				
			Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity
1	H111	10

Peptide specific retention time, peak area, peak width, and peak capacity calculated from data collected using different resins and gradient lengths varying from 1 min. to 10 min. Each data point is calculated as an average of 5 consecutive injections.

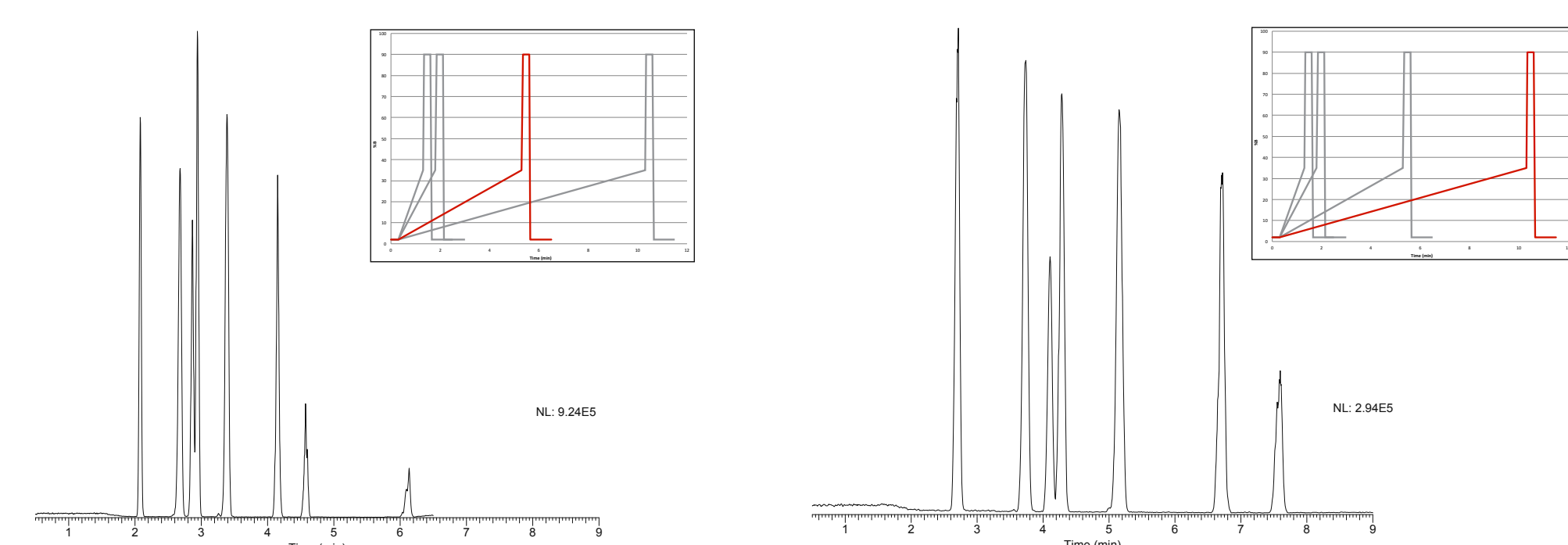


Plot of peptide specific peak capacity collected on 5 cm long columns at 10 µL/min. with 5 and 10 min. long gradients.



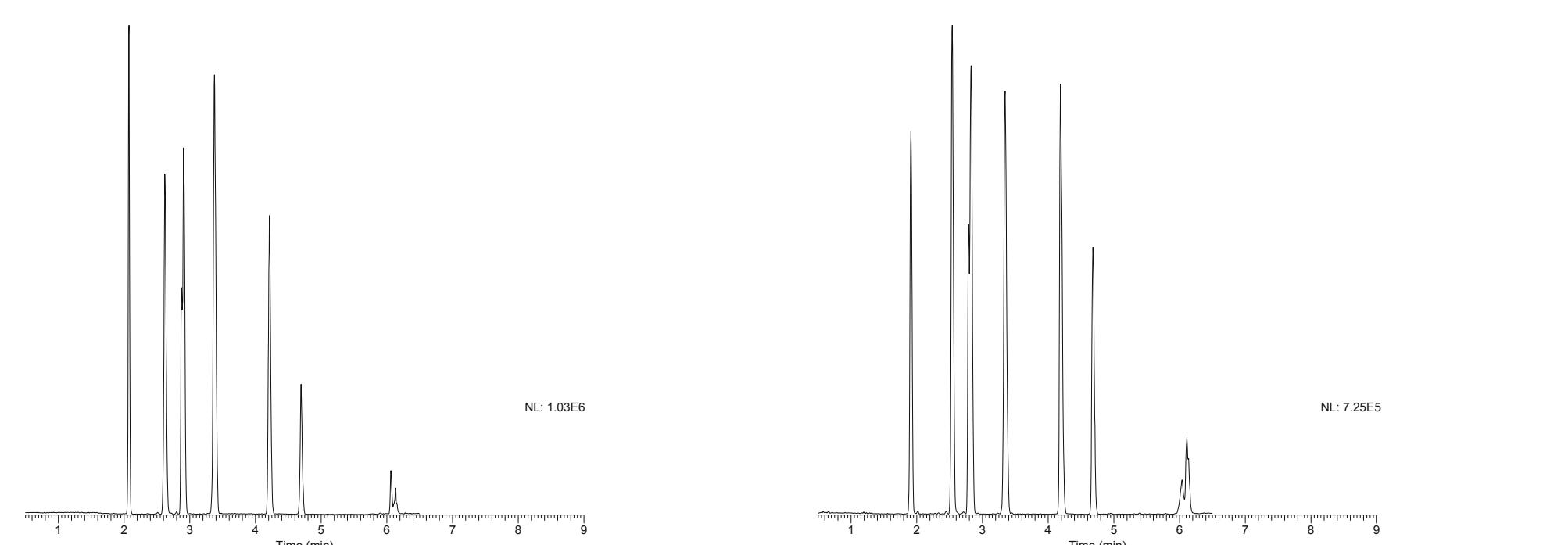
H354 - 1 min. Gradient

H354 - 1.5 min. Gradient



H354 - 5 min. Gradient

H354 - 10 min. Gradient



H039 - 5 min. Gradient

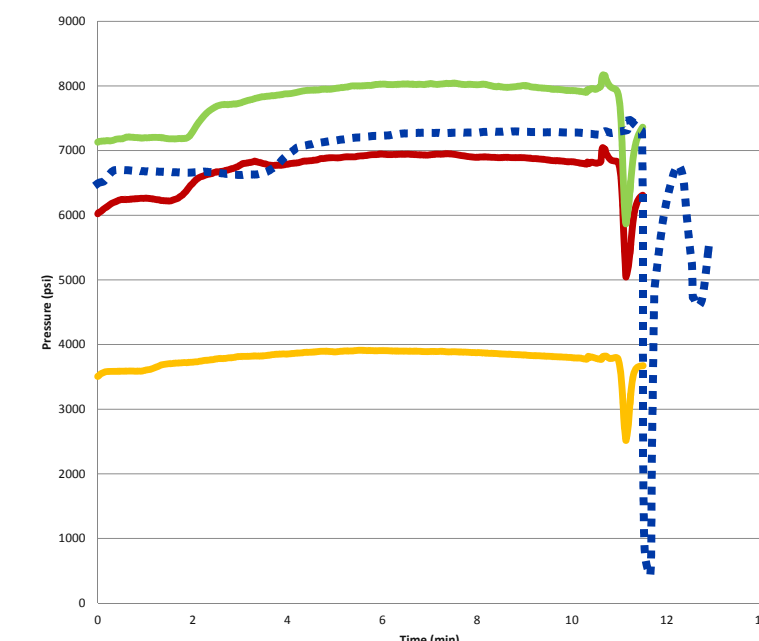
H044 - 5 min. Gradient

Chromatographic data collected of PicoSure standard at various gradient lengths and cycle times. All data was collected at 50°C and at a flow rate of 10 µL/min. Chromatograms are normalized to the longest run time (10 min.) for comparison purposes. The insets show gradient profiles for the different length gradients.

Complex Sample Analysis

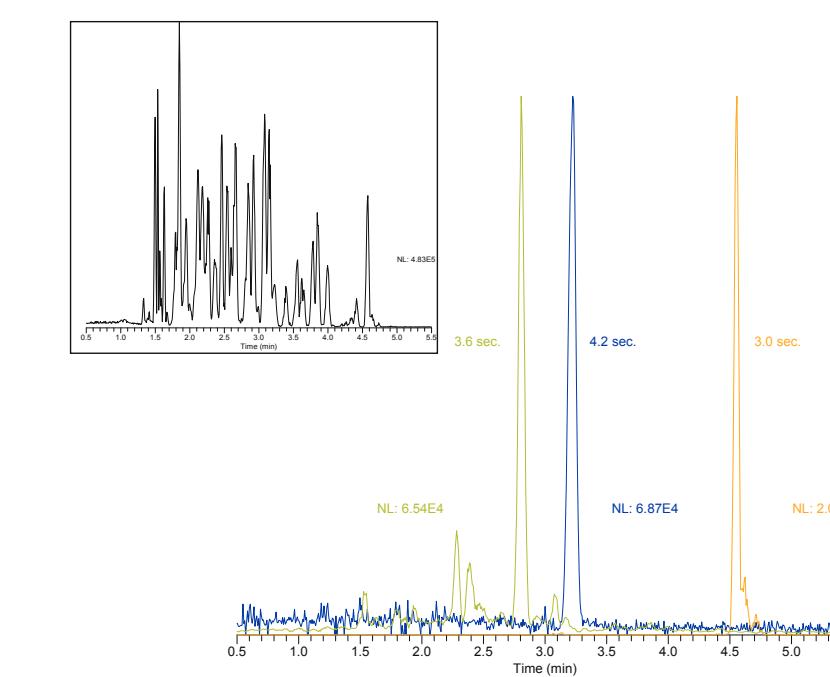
Sample	Resin	Flow	10 min. Gradient					15 min. Gradient					20 min. Gradient					25 min. Gradient					30 min. Gradient				
			Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity
1	H111	10

Signal intensity, retention time, and peak width for buspirone, angiotensin II, and insulin spiked into 500 fmol/µL BSA. Data was collected on 5 cm long, 150 µm ID column with 2-35%B varying length gradients. The values were calculated as the average of 10 consecutive injections.

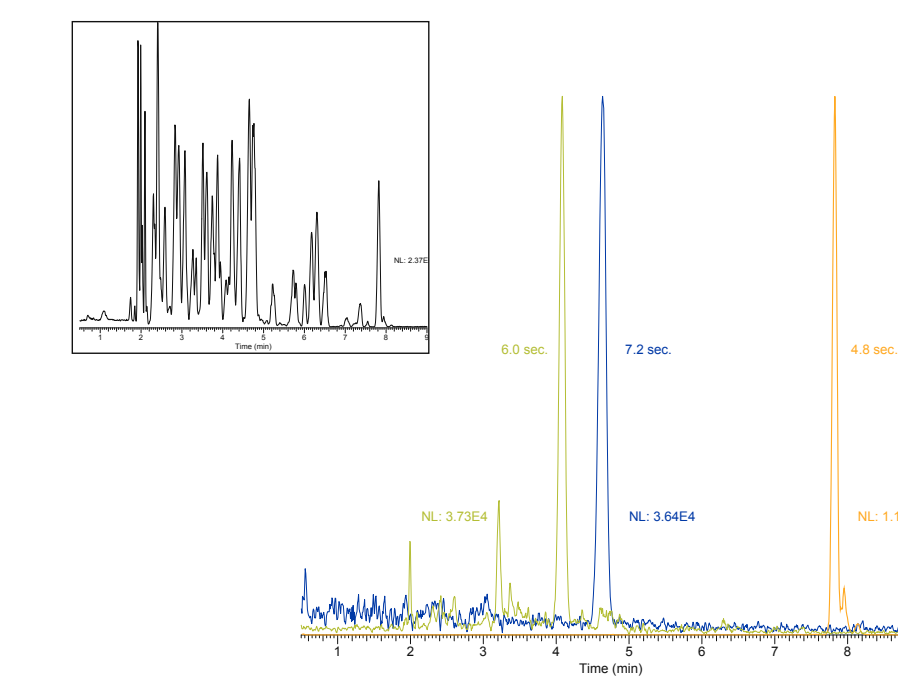


Pressure traces collected over a 10 min. long BSA gradient with column packed with different resins. The resin types and flow rates are indicated in the plot.

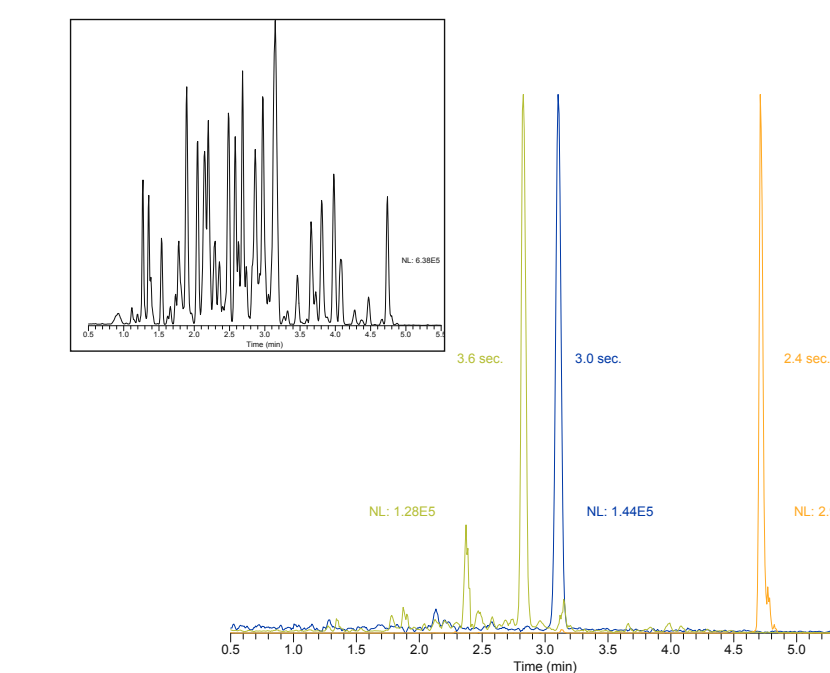
H354 - 5 min. Gradient



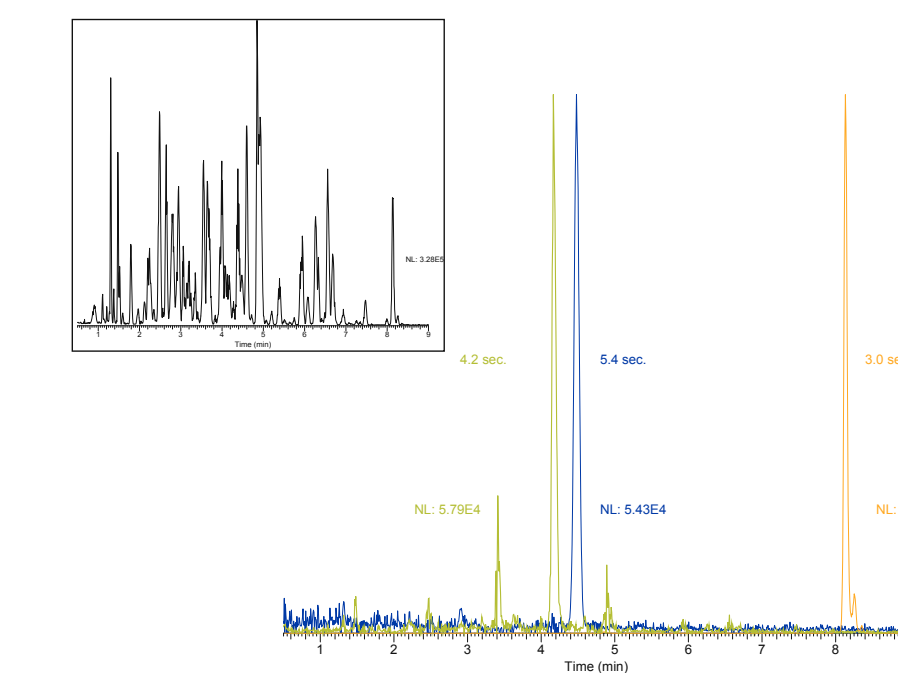
H354 - 10 min. Gradient



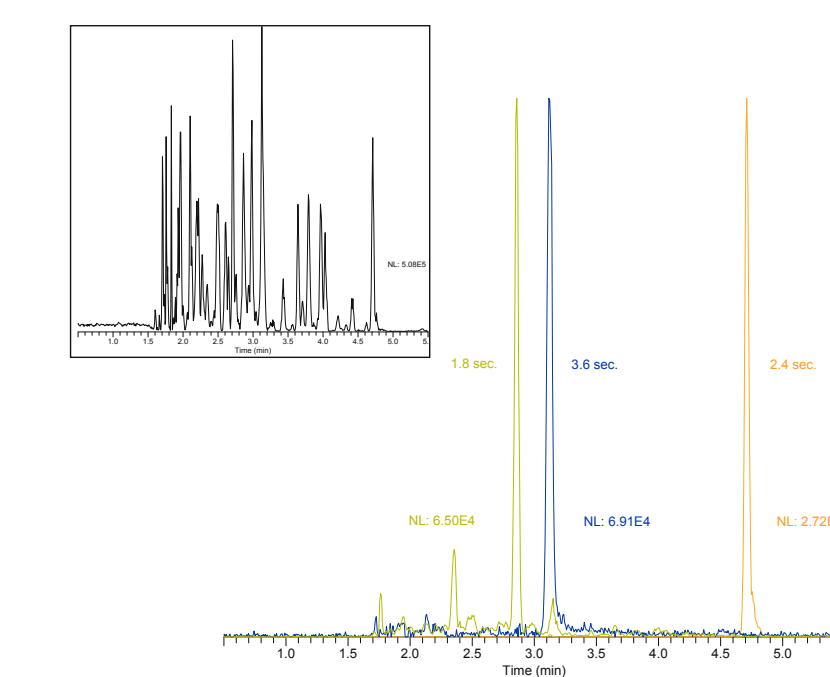
H044 - 5 min. Gradient



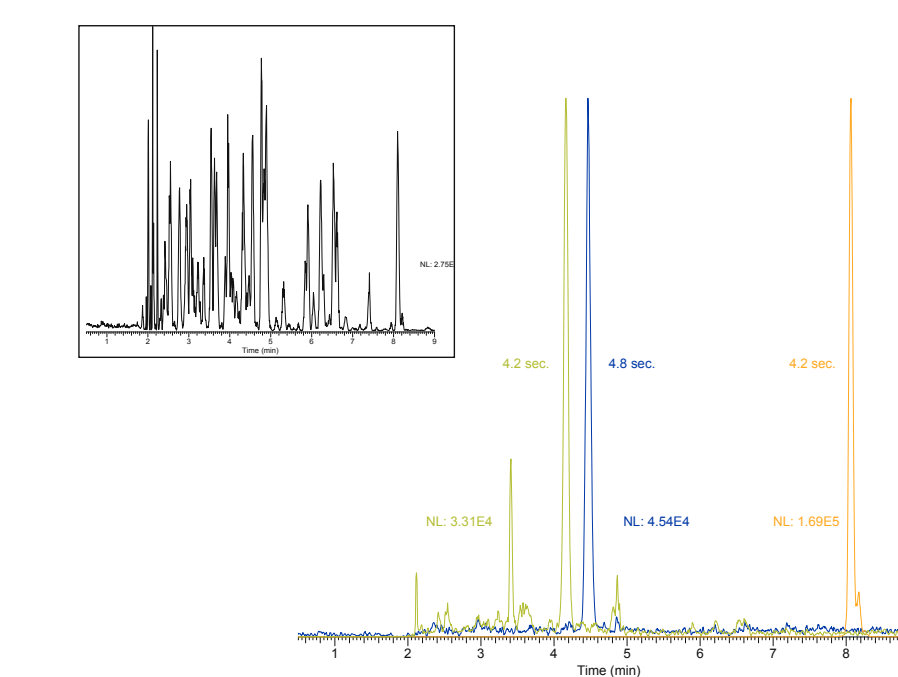
H044 - 10 min. Gradient



H039 - 5 min. Gradient



H039 - 10 min. Gradient

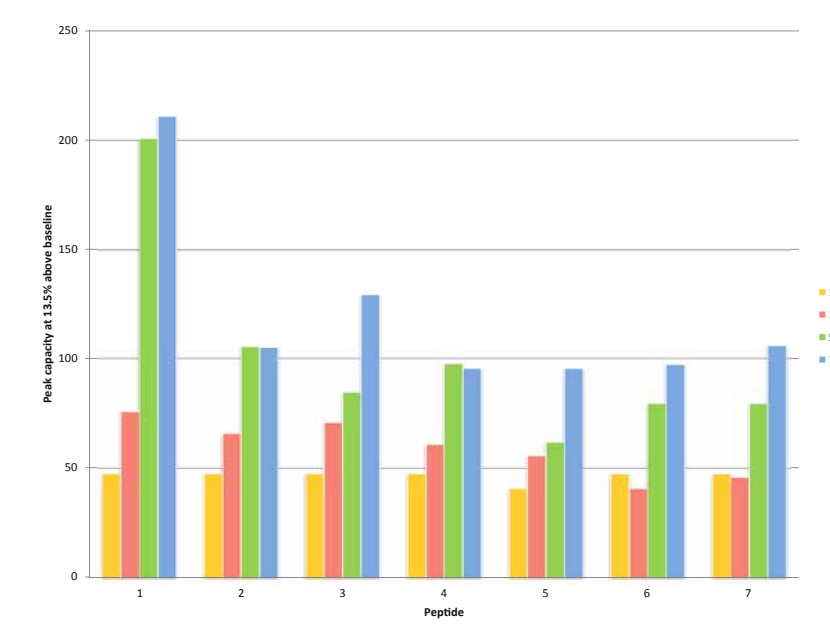


XICs for buspirone, angiotensin II, and insulin. Sample separation was achieved with 5 and 10 min. 2-35% B gradient on 5 cm long columns at 10 µL/min. and 50°C. FWHM is indicated for each peak. The insets show base peak chromatograms of 500 fmol/µL BSA standard spiked with buspirone, angiotensin II, and insulin standards.

Using Sub-2µm Particles

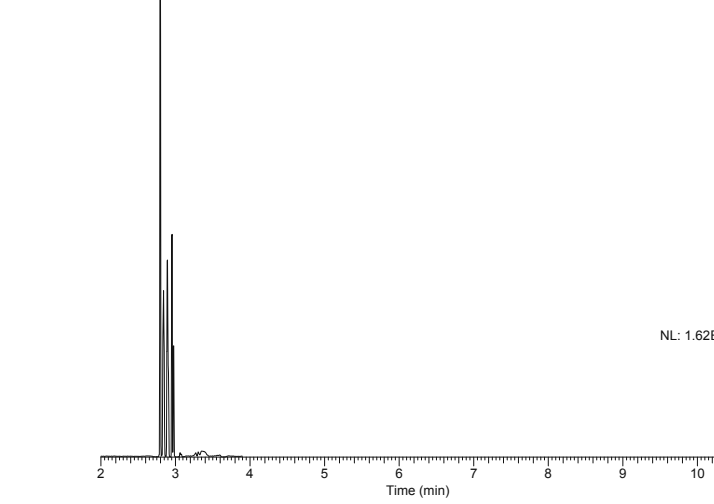
Sample	Resin	Flow	10 min. Gradient					15 min. Gradient					20 min. Gradient					25 min. Gradient					30 min. Gradient				
			Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity
1	H111	10

Peptide specific retention time, peak area, peak width and peak capacity calculated for data collected with column packed with BEH, 1.7 µm particle, 130 Å pore size. Each data point is calculated as an average of 5 consecutive injections.

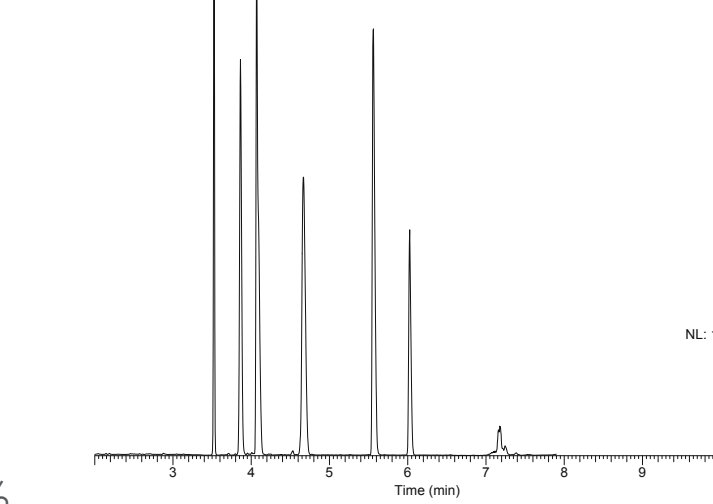


Right: Plot of peptide specific peak capacity collected on 5 cm long column packed with H111 resin. Gradient (2-35% acetonitrile) length was varied from 1 to 10 min.

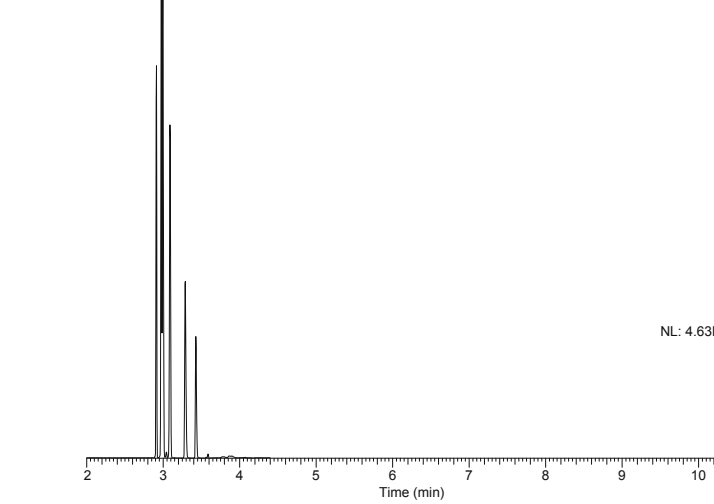
H111 - 1 min. Gradient



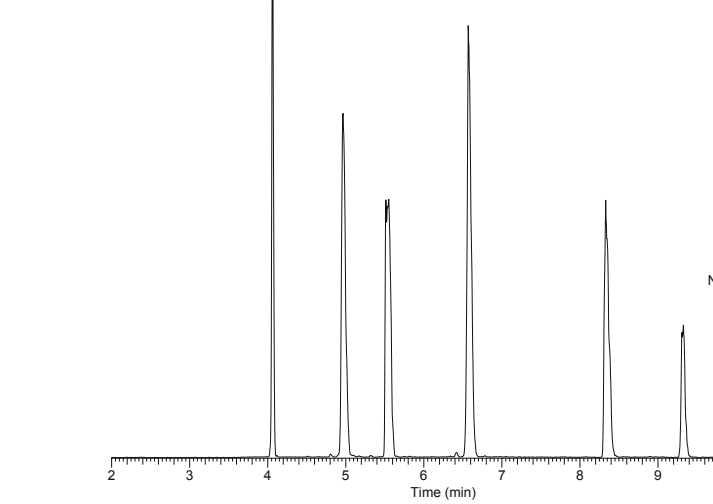
H111 - 5 min. Gradient



H111 - 1.5 min. Gradient



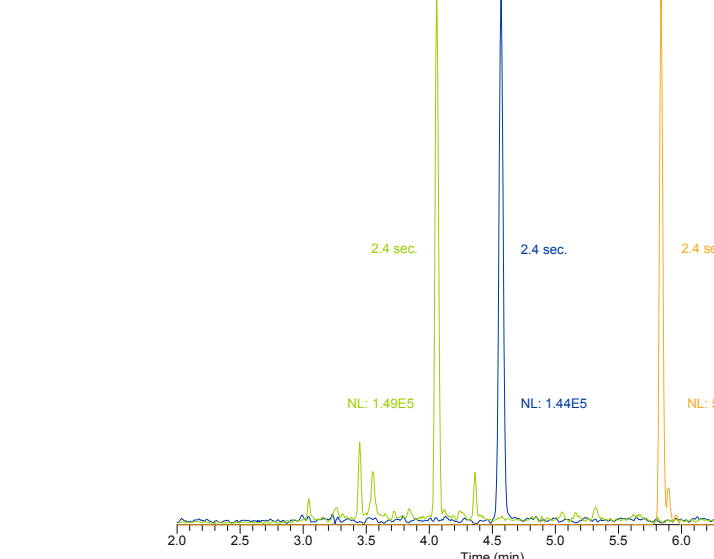
H111 - 10 min. Gradient



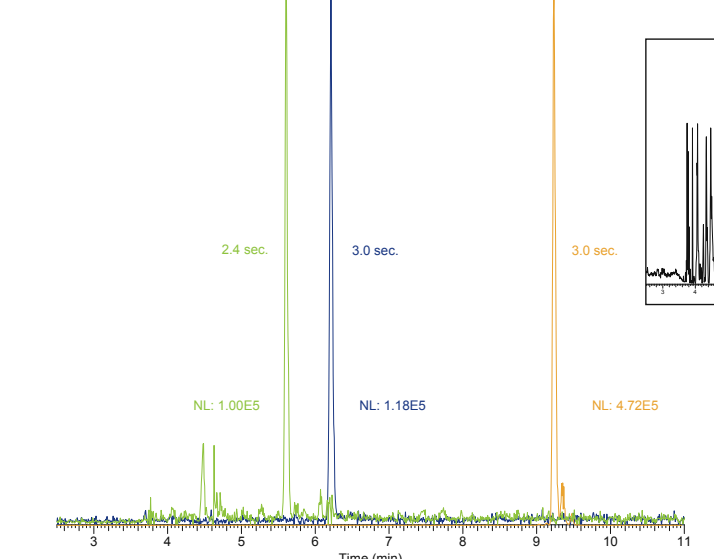
Sample	Resin	Flow	5 min. Gradient					10 min. Gradient						
			Area	Peak	FWHM	Retention	Capacity	Area	Peak	FWHM	Retention	Capacity		
1	H111	10

Signal intensity, retention time, and peak width calculated for buspirone, angiotensin II, and insulin spiked into 500 fmol/µL BSA. Data was collected on 5 cm long, 150 µm ID column, packed with BEH, 1.7 µm particle, 130 Å pore size with 2-35%B varying length gradients. The values were calculated as the average of 5 consecutive injections.

BSA - 5 min. Gradient



BSA - 10 min. Gradient



XICs for buspirone, angiotensin II, and insulin. Sample separation was achieved with 5 and 10 min. 2-35% B gradient on 5 cm long column packed with H111 resin at 5 µL/min. and 50°C. FWHM is indicated for each peak. The insets show base peak chromatograms of 500 fmol/µL BSA standard spiked with buspirone, angiotensin II, and insulin standards.

Conclusions

- Verified the performance of chip-based columns with 150 µm ID columns at 10 µL/min. flow rate.
- The maximum flow rate through the 150 µm ID, 5 cm long chip based columns is dictated by the resin size. Columns packed with 3 and 5 µm C18 resins can be used at 10 µL/min. while the maximum flow rate on columns packed with sub 2 µm particles was limited to 5 µL/min.
- Observed impact of the sheath gas on signal intensity. The peak area for most of the 7 PicoSure peptides decreased with the increasing sheath gas setting.
- Reduced the service part of the run (sample loading, column washing and column equilibration) to 1.5 min. per sample injection at 10 µL/min.
- Observed pressure was in line with the particle size of the resin. The pressure of the column packed with 2.7 µm fused core resin rose up the 8,000 psi during the gradient run while the pressure of the column packed with 5 µm fused core resin remained under 4,000 psi during the whole duration of the run.
- The highest peak capacity for buspirone and insulin was observed with H044 resin. The observed peak widths along with the low column pressure make this resin optimal for the 10 µL/min. flow rate.

Future Work

- Develop high flow chip based columns with 200 µm ID.
- Evaluate long term stability of different resin types at 10 µL/min.
- Interface the chip based technology with different MS platforms.