Performance Evaluation of a Flexible, Easy-to-Use Packed-Tip Column Device for Nanospray-Enabled LC-MS

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Introduction

Nanobore liquid chromatography is the method of choice for protein/peptide separation in the life sciences. Despite all the advances in nanospray product development, running experiments in nanospray mode is still very challenging and requires specialized training and in-depth knowledge of instrumentation. Here we test the performance of a newly developed integrated nanobore column which combines a packed tip column, high voltage liquid junction, column heater and transfer line into one easy-to-use device (PicoChip column). A self-guided positioning system ensures reproducible tip placement. Leak-free connections inside the integrated nanobore column guarantee good intra- and inter-column reproducibility while maintaining the sensitivity and separation efficiency of packed tip columns.

Methods

Heated PicoChip column with the Omega Benchtop controller

Mass Spectrometer

- LTQ Linear Ion Trap (Thermo Scientific)
  - Full Scan MS: 300-1500 Da
- Custom heated PicoChip source with preconfigured tip positioning (New Objective)
  - Omega Benchtop Controller (Omega Engineering)

Chromatography

- Eksigent nanoLC·Ultra 2D plus (AB SCIEX)
  - Flow Rate: 500 nL/min.
  - Mobile Phase A: 0.1% formic acid in water (J.T. Baker)
  - Mobile Phase B: 0.1% formic acid in acetonitrile (Sigma-Aldrich)
  - Load at 2% B: 5-minutes
  - Gradient: 30 minutes 2-50% B
- HTC Pal Autosampler (Leap Technologies)
  - 6-port injection valve (VICI Valco Instruments Co., Inc.)
  - 1.0 µL loop
  - Loop overfill
  - PicoChip columns (360 µm OD x 75 µm ID x 15 µm tip - New Objective) slurry-packed to 10.5 and 25 cm with ProteoPep II (C18, 5 µm, 300 Å - New Objective) or Reprosil-PUR (C18-AQ, 3 µm, 120 Å - Dr. Maisch)

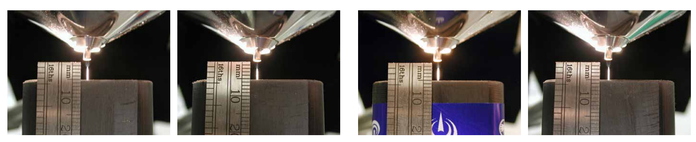
Ambient Temperature Monitoring

- Track-It RHT automated temperature and humidity recorder (Monarch Instruments)
- Room temperature recorder was positioned on the nanospray source next to the analytical column and the room temperature was recorded every minute throughout the duration of the analytical experiments.

Samples

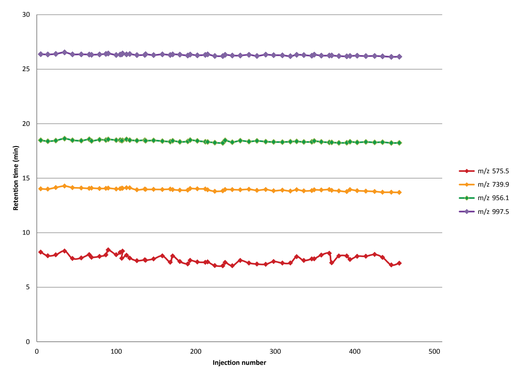
- BSA digest (Waters MassPrep)
  - 1 pmol/µL in water + 0.1% formic acid
- 6 Bovine protein digest equal molar mix (Michrom Bioresources)
  - 50 fmol/µL in water + 0.1% formic acid

Consistent Positioning Chip-to-Chip

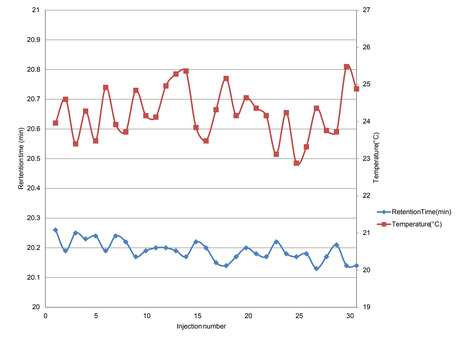


Injection Reproducibility

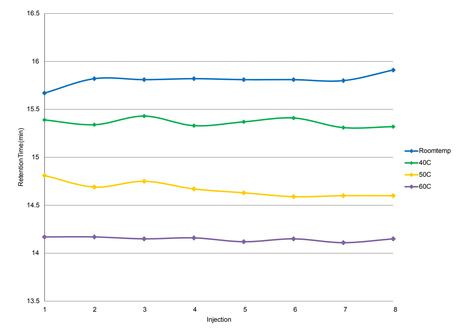
Chromatographic separation of 300 fmol/µL BSA digest on PicoChip column packed with Proteopep II (C18, 5 µm, 300 Å) during the first injection (A), and after 300 injections (B)



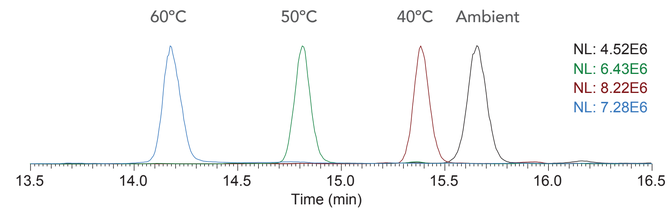
Plot of peptide specific retention times for four different BSA peptides with m/z 575.5 Da, 739.9 Da, 956.1 Da and 997.5 Da across 454 injections on the same PicoChip column



Example of temperature fluctuation in the lab environment and the retention time for BSA peptide m/z 508 Da. Despite more than 2°C fluctuation in the ambient temperature, the retention time RSD is less than 1% when using PicoChip column.



Left: Retention time shift for BSA peptide with m/z 643.8 Da. at different temperature settings. Retention time RSD for 8 consecutive injections at different temperature settings is less than 1% at each temperature setting.



Extracted ion chromatograms of BSA peptide m/z 643.8 Da at different temperatures

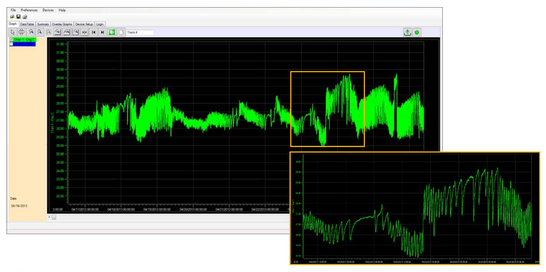
Conclusions

- Injection to injection reproducibility on the integrated nanobore column device was demonstrated on a set of over 400 injections on a single column
- Column back pressure was reduced 40% for 5 µm particle resin and 30% for 3 µm resin when the temperature was increased from ambient temperature to 60°C
- Change of elution order of certain BSA peptides at ambient and increased temperature was observed
- Significant improvement in peptide separation was demonstrated when using newly developed integrated column prototype with 25 cm long bed

Future Work

- Evaluate the temperature effect on different types of C18 resin
- Develop integrated nanobore column device with bed lengths longer than 25 cm
- Incorporate the temperature control for columns longer than 10.5 cm

ProteoPep II C18 5 µm 300 Å



Example of room temperature fluctuation near the column. The sharp rise and drop in temperature is caused by the air conditioning system and the heat emitted by the mass spectrometer, itself.

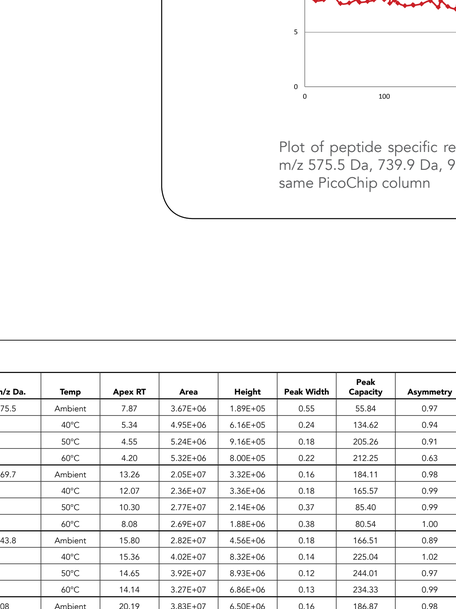
PicoChip column pressure recorded at different temperature settings

Resin Type	Particle Size (µm)	Pore size (Å)	Flow (nL/min)	%B in Mobile Phase	Pressure (psi)			
					Ambient	40°C	50°C	60°C
C18	5	300	1000	2	1185	912	871	719
C18-AQ	3	120	500	2	1819	1564	1434	1273

Extracted ion chromatogram of BSA peptides with m/z 474.3 Da, 582.5 Da and 628.0 Da., respectively. The elution order of these peptides changes at different temperature settings

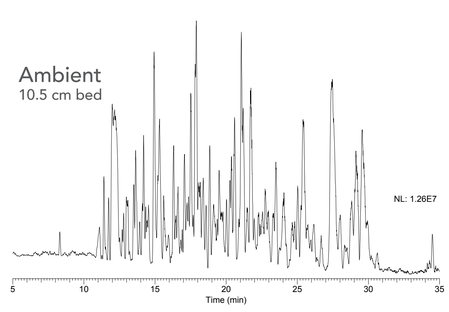
m/z Da.	Temp	Apex RT	Area	Height	Peak Width	Peak Capacity	Asymmetry
575.5	Ambient	7.87	3.67E+06	1.89E+05	0.55	55.84	0.97
	40°C	5.34	4.95E+06	6.16E+05	0.24	134.62	0.94
	50°C	4.55	5.24E+06	9.16E+05	0.18	205.26	0.91
	60°C	4.20	5.32E+06	8.00E+05	0.22	212.25	0.63
569.7	Ambient	13.26	2.05E+07	3.32E+06	0.16	184.11	0.98
	40°C	12.07	2.36E+07	3.36E+06	0.18	165.57	0.99
	50°C	10.30	2.77E+07	2.14E+06	0.37	85.40	0.99
	60°C	8.08	2.69E+07	1.88E+06	0.38	80.54	1.00
643.8	Ambient	15.80	2.82E+07	4.56E+06	0.18	166.51	0.89
	40°C	15.36	4.02E+07	8.32E+06	0.14	225.04	1.02
	50°C	14.65	3.92E+07	8.93E+06	0.12	244.01	0.97
	60°C	14.14	3.27E+07	6.86E+06	0.13	234.33	0.99
508	Ambient	20.19	3.83E+07	6.50E+06	0.16	186.87	0.98
	40°C	20.07	4.46E+07	8.45E+06	0.14	224.54	1.01
	50°C	19.45	4.88E+07	9.07E+06	0.14	217.53	0.97
	60°C	18.99	4.98E+07	9.55E+06	0.14	228.56	1.01

Peptide specific peak capacity data calculated for 4 different BSA peptides separated on Proteopep II (C18, 5 µm, 300 Å) PicoChip column at different temperature settings

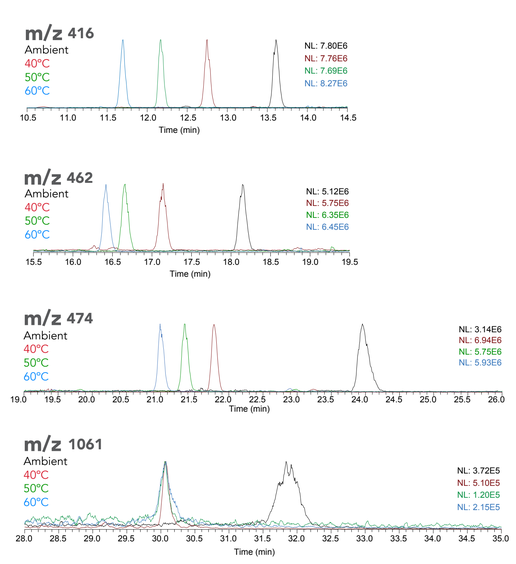


Chromatographic separation of 1 pmol/µL BSA digest on PicoChip columns packed with ProteoPep II (C18, 5 µm, 300 Å)

Reprosil-PUR C18 AQ 3 µm 120 Å



Chromatographic separation of 50 fmol/µL 6 bovine protein digest equal molar mix on PicoChip columns packed with 10.5 cm and 25 cm of Reprosil-Pur (C18-AQ, 3 µm, 120 Å)



Extracted ion chromatograms of 4 different bovine digest peptides at different temperature settings

Extracted Ion m/z (Da)	Bed Length (mm)	Temp	Apex RT (min)	Area	Height	Peak Width (min)	Peak Capacity	Asymmetry
416.4	105	Ambient	13.59	3.75E+07	6.97E+06	0.15	195.7	0.94
	105	40°C	12.74	3.51E+07	7.76E+06	0.13	235.9	0.83
	105	50°C	12.17	3.20E+07	7.69E+06	0.11	269.1	1.08
	105	60°C	11.71	3.56E+07	8.17E+06	0.13	226.4	0.94
	250	Ambient	22.52	1.93E+07	4.47E+06	0.12	246.4	0.94
462	105	Ambient	18.18	2.95E+07	4.47E+06	0.19	162.1	0.98
	105	40°C	17.12	3.41E+07	5.97E+06	0.15	196.5	0.98
	105	50°C	16.66	3.55E+07	6.71E+06	0.15	198.9	0.95
	105	60°C	16.44	3.52E+07	6.35E+06	0.15	195.4	1.03
	250	Ambient	25.81	4.51E+07	8.78E+06	0.14	210.2	1.07
474.6	105	Ambient	24.06	3.28E+07	3.50E+06	0.24	125.5	0.99
	105	40°C	21.78	4.13E+07	7.50E+06	0.15	199.3	1.01
	105	50°C	21.35	3.56E+07	6.18E+06	0.16	190.7	1.03
	105	60°C	21.01	3.18E+07	5.57E+06	0.16	190.3	1.06
	250	Ambient	29.64	4.18E+07	7.72E+06	0.16	192.5	0.96
1061.7	105	Ambient	32.18	8.17E+06	4.48E+05	0.53	56.6	0.91
	105	40°C	30.10	4.08E+06	6.75E+05	0.18	167.3	0.94
	105	50°C	30.08	8.99E+05	1.20E+05	0.25	121.3	0.81
	105	60°C	30.06	1.78E+06	1.87E+05	0.30	109.9	0.95
	250	Ambient	36.01	7.03E+05	1.32E+05	0.15	198.9	1.06

Peptide specific peak capacity data calculated for 4 different bovine digest peptides separated on Reprosil-PUR (C18 AQ, 3 µm, 120 Å) PicoChip column at different temperature settings

Acknowledgement
Sincere thanks is extended to Catherine Tremblay, Northeastern University, for her contributions in extracting and compiling data for this presentation