Development of a Chip-Based Nanobore Column Platform with Universal Connectivity, Column Heating and Sheath Gas Capability

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Introduction

Nanobore column chromatography has become a method of choice when analyzing a wide variety of peptide and protein samples. The complex nature of biological samples requires frequent testing of different separation methods on a variety of nanobore columns to determine the best separation conditions. Here we test a newly developed easy-to-use chip based system enabled with different column lengths, column heating and universal connectivity. The flexible design of the chip columns facilitates method development, providing columns with different inner diameters, bed lengths and a wide selection of stationary phase materials. The PicoChip® column has sheath gas capability which further expands the possible use of PicoChip columns for higher flow rates (> 1 µL/min.) and on different MS platforms (data not included)[1].

Methods

Mass Spectrometer

- LTQ Linear Ion Trap (Thermo)
  - Full scan MS: 300-1500 Da
- Heated PicoChip source with preconfigured tip positioning (New Objective, Inc.)
  - PicoChip column heating is controlled by Omega Benchtop Controller

Chromatography

- Eksigent nanoLC-Ultra 2D plus (AB Sciex)
  - Flow Rate: 300 nL/min.
  - Mobile Phase A: 0.1% formic acid in water
  - Mobile Phase B: 0.1% formic acid in acetonitrile
  - 5 Min. load at 2% B
  - Gradient: 2-35% B in 30, 60, 90 and 120 min.
  - 2 Min. column wash at 90% B
  - 23 Min. column equilibration at 2% B
- HTC Pal Autosampler (Leap Technologies)
  - 6-port micro-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, Inc.), slurry packed to 10.5 and 25 cm with Reprosil-PUR C18-AQ, 3 µm, 120 Å (Dr. Maisch, GmbH)

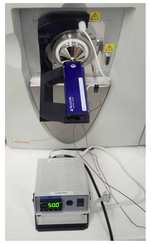
Ambient Temperature Monitoring

- 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

- PicoSure™ standard (New Objective, Inc.)
  - 500 fmol/µL in water + 0.1% formic acid
- BSA digest (Waters MassPrep)
  - 1 pmol/µL in water + 0.1% formic acid
- HeLa Digest (Thermo Scientific)
  - 200 ng/µL in water + 0.1% formic acid

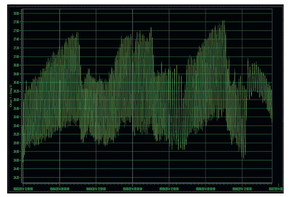
A



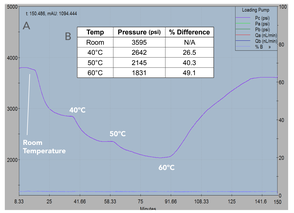
B

A) PicoChip 2 nanospray system with column temperature control mounted on Thermo Scientific LTQ; B) PicoChip nanospray columns with nanoViper connection (green) and fused-silica pigtail (blue)

Temperature, Pressure, Reproducibility



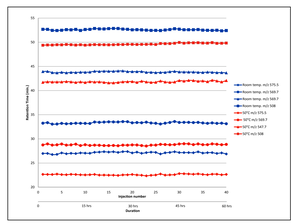
Example of temperature fluctuation in the room next to the PicoChip column. The sharp spikes in temperature are caused by the air conditioning system.



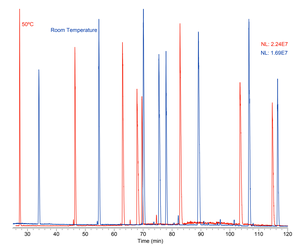
Temp	Pressure (psi)	% Difference
Room	3595	N/A
40°C	2642	26.5
50°C	2145	40.3
60°C	1831	49.1

A) Pressure of the 25 cm PicoChip column recorded during the temperature changes from room temperature to 40, 50 and 60°C. The column pressure stabilizes within 20 min. from changing the temperature.

B) Column pressure values for a 25 cm PicoChip column at room, 40°, 50° and 60°C temperatures.



Plot of peptide specific retention times for four BSA peptides selected across the gradient. Forty consecutive injections (gradient 2-35%B in 60 min.) were collected at two different temperature settings - room temperature and 50°C. The retention time RSD is less than 1% for all four peptides.

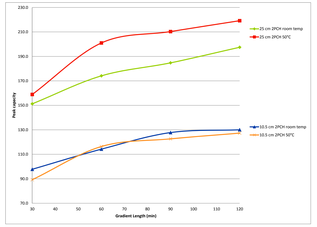


Base peak chromatograms of 500 fmol/µL PicoSure standard separated using a 25 cm long PicoChip column at room temperature (blue) and at 50°C (red).

Effect of Gradient Length on Separation Quality

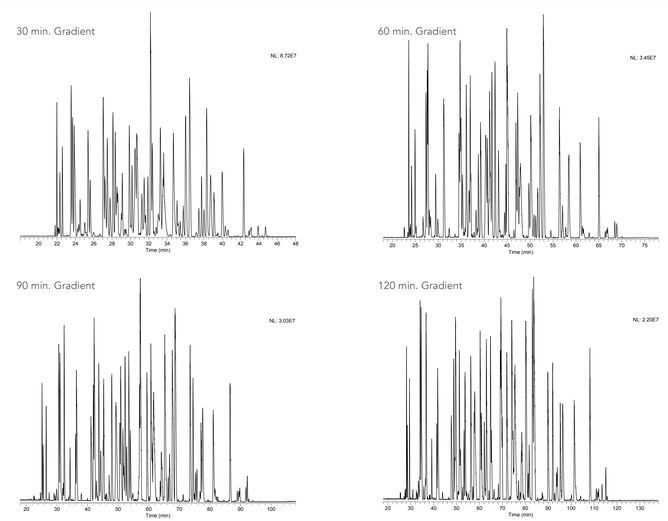
m/z (Da)	Temp	Gradient Length (min.)	10.5 cm PicoChip column				25 cm PicoChip column			
			Apex RT (min.) (±0.5%)	FWHM (min.)	Peak Capacity (±0.5%)	Asymmetry (±0%)	Apex RT (min.) (±0.5%)	FWHM (min.)	Peak Capacity (±0.5%)	Asymmetry (±0%)
575.5	Room	30	20.09	0.13	143.9	0.9	24.5	0.09	320.8	1.3
		60	21.82	0.18	188.5	1.1	29.7	0.13	244.6	1.3
		90	26.92	0.24	231.8	1.0	34.2	0.17	339.3	1.5
		120	29.73	0.27	251.0	1.0	39.1	0.19	388.4	1.5
	50°C	30	18.01	0.11	188.5	1.6	22.06	0.08	301.0	1.4
		60	19.7	0.16	188.5	1.6	25.1	0.11	334.3	1.3
		90	21.2	0.26	196.7	1.2	27.6	0.14	361.0	1.6
		120	22.4	0.28	227.4	1.0	29.8	0.20	329.3	2.0
569.7	Room	30	23.1	0.16	116.4	1.1	28.1	0.10	183.1	1.9
		60	29.35	0.22	154.8	0.9	36.3	0.16	220.6	2.2
		90	34.7	0.28	167.7	1.9	43.6	0.22	240.0	2.7
		120	39.7	0.36	188.5	1.1	51.0	0.27	262.0	2.9
	50°C	30	21.4	0.12	131.4	0.7	25.7	0.09	201.0	2.0
		60	25.7	0.23	147.3	1.2	31.8	0.14	253.0	2.1
		90	29.5	0.24	184.7	1.4	37.0	0.18	273.7	3.4
		120	32.8	0.35	180.1	1.9	42.2	0.24	280.1	2.5
508	Room	30	32.6	0.17	97.8	1.1	38.3	0.12	151.3	2.6
		60	47.6	0.29	114.2	2.1	56.7	0.19	174.1	2.3
		90	61.5	0.37	127.8	1.7	73.5	0.24	184.7	3.7
		120	74.6	0.42	130.0	2.3	89.9	0.34	197.5	4.1
	50°C	30	31.77	0.18	89.2	0.9	37.0	0.11	158.9	2.2
		60	45.6	0.28	116.4	2.5	53.4	0.18	201.0	4.3
		90	58.3	0.41	122.6	2.7	69.0	0.26	210.3	2.8
		120	70.3	0.51	127.3	3.6	84.0	0.30	219.2	6.0

Peptide specific peak capacity and peak width data calculated for 3 different BSA peptides separated on a 25 cm Picochip column. The sample separation was achieved by 2-35% acetonitrile gradient with gradient lengths varying from 30 to 120 min. Data was collected at room temperature and at 50°C.



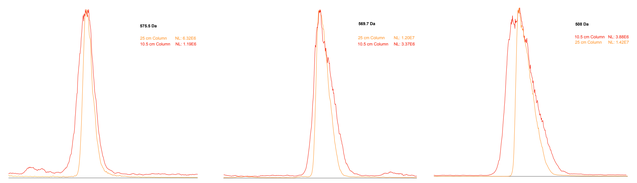
Peak capacity and gradient length plot for BSA peptide m/z 508. The data was collected on10.5 cm and 25 cm PicoChip columns

25 cm PicoChip Column, BSA Digest



Base peak chromatographic separation of 1 pmol/µL BSA on a 25 cm PicoChip column at different gradients (2-35% B in 30, 60, 90 and 120 min.)

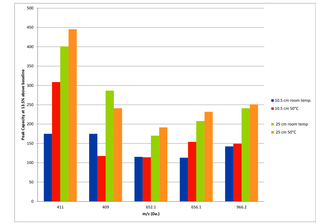
BSA Digest, Extracted Ion Chromatograms



Extracted ion chromatograms of BSA peptides m/z 575.5 Da, 569.7 Da and 508 Da. Data was collected on 10.5 and 25 cm PicoChip columns using a 2-35% B in 120 min. gradient at 50°C.

Effects of Column Length and Temperature on Sample Separation

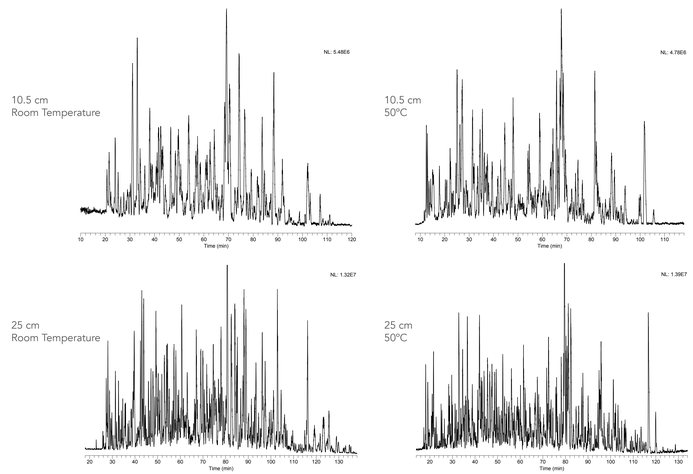
Extracted Ion (Da)	Column Length (cm)	Room temperature					50°C				
		Apex RT (min.) (±0.5%)	Start RT (min.) (±0.5%)	End RT (min.) (±0.5%)	FWHM (min.)	Peak Capacity (±0.5%)	Asymmetry (±10%)	Apex RT (min.) (±0.5%)	Start RT (min.) (±0.5%)	End RT (min.) (±0.5%)	FWHM (min.)	Peak Capacity (±0.5%)	Asymmetry (±10%)
411	10.5	20.70	20.35	21.04	0.40	174.5	0.9	12.49	12.30	12.69	0.24	328.7	1.0
	25	27.32	27.18	27.48	0.19	401.0	1.2	18.17	18.08	18.35	0.17	445.4	2.1
409	10.5	42.99	42.65	43.34	0.38	174.9	1.1	27.07	26.93	27.58	0.53	117.5	1.0
	25	43.00	42.85	43.27	0.25	286.7	1.8	36.61	36.45	36.95	0.27	241.0	1.9
652.1	10.5	69.22	68.68	69.73	0.61	115.3	1.0	47.71	47.39	48.45	0.61	114.2	2.1
	25	80.68	80.42	81.13	0.45	170.0	1.7	79.52	79.38	80.01	0.38	181.5	3.4
656.1	10.5	88.42	87.88	88.95	0.41	113.1	1.0	81.38	81.14	81.92	0.3	154.8	2.0
	25	102.84	102.70	103.28	0.28	207.9	2.6	95.77	95.65	96.17	0.29	231.8	3.1
966.2	10.5	108.36	107.79	108.64	0.38	142.2	0.6	105.53	105.10	105.91	0.46	149.5	0.9
	25	121.69	121.53	122.01	0.23	241.0	2.0	119.95	119.72	120.20	0.3	251.0	1.2



ABOVE: Peptide specific peak capacity and peak width calculated for 5 different HeLa peptides separated on 10.5 and 25 cm PicoChip columns

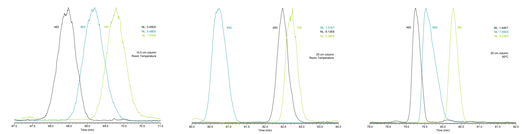
RIGHT: Plot of peptide specific peak capacity data calculated for five different HeLa peptides. Data was collected on 10.5 and 25 cm PicoChip columns at both room temperature and at 50°C.

HeLa Cell Digest



Base peak chromatographic separation of 200ng of HeLa digest on 10.5 and 25 cm PicoChip columns; gradient 2-35% B in 120 min. collected at room temperature and at 50°C.

Column Length and Temperature for Method Development



Extracted ion chromatograms of HeLa peptides m/z 492.3 Da, 652.6 Da and 765.2 Da. The elution order of these peptides is changing at the increased temperature and at different column lengths.

Conclusions

- Utilized PicoChip column format for different column lengths
- Enabled column heating for PicoChip columns with different bed lengths
- Demonstrated injection to injection retention time reproducibility at room temperature and at 50°C. The retention time RSD is less than 1% despite the significant temperature fluctuation in the room
- Up to 50% reduction of column pressure was observed on the 25 cm long PicoChip columns heated to 60°C
- Elution order of certain HeLa digest peptides is changing at the increased temperature

[1]Future Work

- Evaluate the temperature stability of different types of resin
- Use the sheath gas capability to run PicoChip columns at flow rates higher than 1 µL/min.
- Enable the use of PicoChip column technology on different MS platforms

Acknowledgement

Sincere thanks is extended to Aaron Dewberry for his contributions in extracting and compiling data for this presentation