Temperature Control Effects in Packed Emitter Nanobore LC-MS/MS for Protein Digest Analysis

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Overview

- Controlled heating of nanobore LC packed-tip emitter
- Demonstrated enhancement of chromatography at elevated temperatures
- Demonstrated operation of sub-2 µm material below 5,000 psi

Introduction

Column temperature control is a common parameter for method development in conventional (mm diameter) HPLC[1]. Although there are numerous known advantages to elevated-temperature nanobore LC[2] (improved chromatographic speed, peptide recovery) the widespread adoption of this platform has been limited by interface issues. The common packed-tip emitter used in proteomics applications offers challenges of integration of temperature control and application of high voltage. Commonly employed fused-silica tubing, however, has excellent thermal-transfer characteristics due to the high surface area/volume ratio. The effects of higher than ambient temperature (50-80 °C) on the separation and column back-pressure characteristics of a packed-tip emitter are investigated.

Materials & Methods

Instrumentation

- 3-D ion-trap mass spectrometer (LCQ Deca™; Thermo Fisher)
- Customized nanospray source equipped with ceramic cartridge heater assembly (Digital PicoView®)
- Direct flow capillary-LC pump (Agilent®)
- nano LC-2D pump (Eksigent®)
- Autosampler (Leap CTC™) equipped with 6-port micro-valve (VICI®) containing 1.0 µL loop
- Column #1 - PicoFrit® column (360µm OD x 75 µm ID x 15 µm tip) packed with 20 cm ProteoPep II (5 µm, 300 Å C18, New Objective)
- Column #2 - PicoFrit column (360µm OD x 75 µm ID x 15 µm tip) packed with 10 cm ProteoPep™ III (1.8 µm 90 Å C18, New Objective)
- Platinum microelectrode embedded in Teflon® elastomer (PicoClear™ conductive union, New Objective)

Reagents

- BSA digest (Waters®)
- 0.1% Formic Acid in water (JT Baker®)
- 0.1% Formic Acid in Acetonitrile (JT Baker)

Conditions

- Gradient: 8 or 20 minutes; 2%B to 50% B
- Flow rate: 300 or 500 nL/min.
- On-column injection: 300 fmol BSA digest

TABLE 1 - *Changes in Column Pressure at Elevated Temperature*

Flow Rate (nL/min)	Temp (°C)	Column Pressure PSI (bar)	Temp (°C)	Column Pressure PSI (bar)	%Change
200	23	1750(121)	50	1350(93)	23%
300	23	2650(183)	50	1940(134)	27%
400	23	3446(238)	50	2530(174)	27%
500	23	4270(294)	50	3170(219)	26%

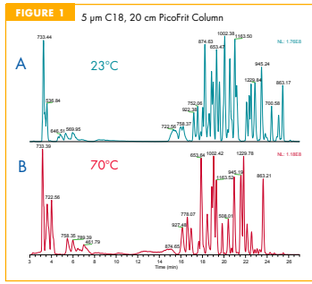
Column = 1.8 µm C18, 75 µm x 10 cm
Mobile phase = 2% ACN, 0.1% formic acid

Flow Rate (nL/min)	Temp (°C)	Column Pressure PSI (bar)	Temp (°C)	Column Pressure PSI (bar)	%Change
200	23	686(47)	50	580(40)	15%
300	23	990(68)	50	838(58)	15%
400	23	1280(88)	50	1094(75)	15%
500	23	1575(109)	50	1365(94)	13%
600	23	1880(130)	50	1621(112)	14%
700	23	2169(150)	30	1884(130)	13%

Column = 3.5 µm C18, 75 µm x 10 cm
Mobile phase = 2% ACN, 0.1% formic acid

Recorded column pressure for two different columns at increasing flow rates. Column #1, packed with a 1.8 µm C18, exhibits an average 26% decrease in column pressure at an elevated temperature of 50°C as compared to 23°C. Column #2, packed with a 3.5 µm C18, exhibits an average 14% decrease in column pressure at an elevated temperature of 50°C.

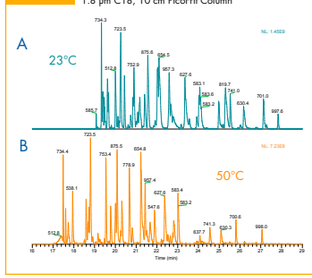
FIGURE 1 5 µm C18, 20 cm PicoFrit Column



Column #1
Base peak chromatograms of BSA peptides:

(A) 23°C
(B) 70°C

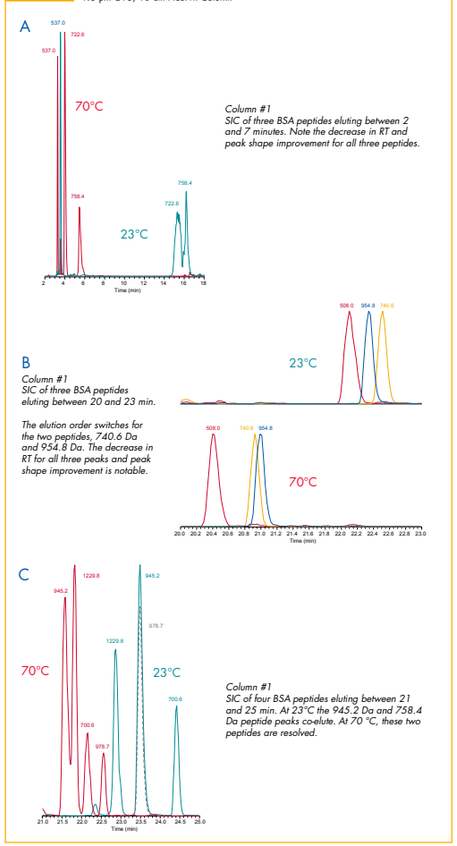
FIGURE 2 1.8 µm C18, 10 cm PicoFrit Column



Column #2
Base peak chromatograms of BSA peptides:

(A) 23°C
(B) 50°C

FIGURE 3 1.8 µm C18, 10 cm PicoFrit Column



Column #1
SIC of three BSA peptides eluting between 2 and 7 minutes. Note the decrease in RT and peak shape improvement for all three peptides.

Column #1
SIC of three BSA peptides eluting between 20 and 23 min.

The elution order switches for the two peptides, 740.6 Da and 954.8 Da. The decrease in RT for all three peaks and peak shape improvement is notable.

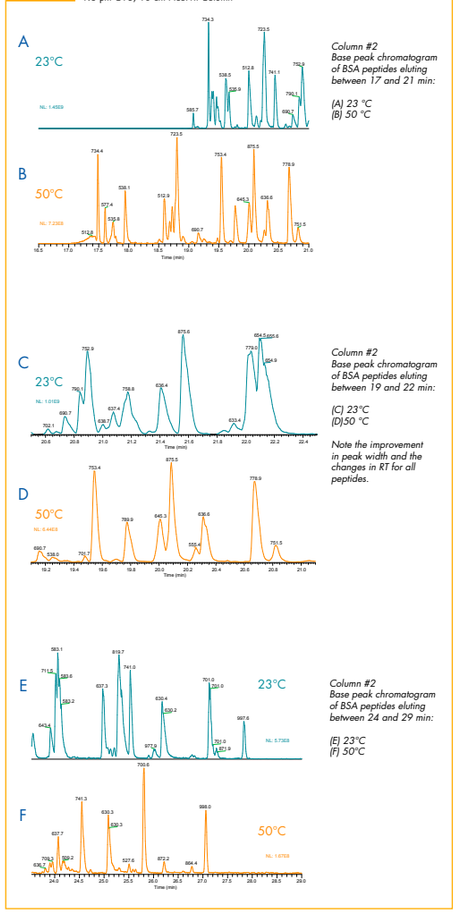
Column #1
SIC of four BSA peptides eluting between 21 and 25 min. At 23°C the 945.2 Da and 758.4 Da peptide peaks co-elute. At 70 °C, these two peptides are resolved.

TABLE 2 5 µm C18, 20 cm Column Data

m/z (Da)	23 °C RT (min)	Area	Height	FWHM (m)	FWHM (s)	width 13% (min)	pk capacity	70 °C RT (min)	Area	Height	FWHM (m)	FWHM (s)	width 13% (min)	pk capacity	Data Analysis ΔRT (min)	ΔFWHM (m)	ΔFWHM (s)
508.0	22.1	4.39E+08	6.55E+07	0.16	9.6	0.22	91.91	20.39	3.01E+08	4.63E+07	0.15	9.0	0.24	84.33	1.69	0.01	0.60
537.0	3.6	3.72E+08	7.79E+07	0.11	6.6	0.17	118.65	3.28	2.23E+08	6.17E+07	0.08	4.8	0.12	167.67	0.29	0.03	1.80
700.6	24.42	3.63E+08	5.43E+07	0.16	9.6	0.22	91.91	22.14	2.51E+08	3.70E+07	0.15	9.0	0.22	91.91	2.28	0.01	0.60
722.6	15.3	6.70E+08	1.39E+07	0.70	42.0	0.95	22.05	3.99	4.97E+08	6.84E+07	0.37	10.2	0.28	72.43	11.3	0.53	31.80
740.6	22.5	5.79E+08	9.60E+07	0.13	7.8	0.13	154.85	20.92	4.39E+08	7.31E+07	0.13	7.8	0.22	91.91	1.6	0.00	0.00
758.5	16.2	3.07E+08	2.70E+07	0.26	15.6	0.69	29.99	5.47	1.94E+08	1.94E+07	0.22	13.2	0.45	45.44	10.74	0.04	2.40
945.2	23.5	8.38E+08	1.39E+08	0.13	7.8	0.26	77.92	21.57	6.81E+08	1.05E+08	0.15	9.0	0.26	77.92	1.92	-0.02	-1.20
954.8	22.3	5.05E+08	9.90E+07	0.14	8.4	0.22	91.91	20.99	3.73E+08	7.23E+07	0.13	7.8	0.2	101.00	1.35	0.01	0.60
978.6	23.5	1.66E+08	2.17E+07	0.18	10.8	0.30	67.67	22.56	1.39E+08	3.32E+07	0.09	5.4	0.18	112.11	0.95	0.09	5.40
1229.5	22.9	5.04E+08	1.12E+08	0.11	6.6	0.22	91.91	21.83	6.18E+08	1.39E+08	0.11	6.6	0.17	118.65	1.04	0.00	0.00
		Average Peak Capacity					93.36				Average Peak Capacity						96.41

Data for 10 different BSA peptides separated on a 20 cm PicoFrit column packed with 5 µm C18 at 23°C and 70°C. Data collected at 70°C demonstrates decrease RT. The average peak capacity is consistent at both temperatures.

FIGURE 4 1.8 µm C18, 10 cm PicoFrit Column



Column #2
Base peak chromatogram of BSA peptides eluting between 17 and 21 min:

(A) 23 °C
(B) 50 °C

Column #2
Base peak chromatogram of BSA peptides eluting between 19 and 22 min:

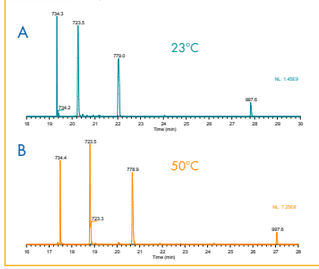
(C) 23°C
(D)50 °C

Note the improvement in peak width and the changes in RT for all peptides.

Column #2
Base peak chromatogram of BSA peptides eluting between 24 and 29 min:

(E) 23°C
(F) 50°C

FIGURE 5 1.8 µm C18, 10 cm PicoFrit Column



Column #2
SIC of four BSA peptides:

(A) 23°C
(B) 50°C

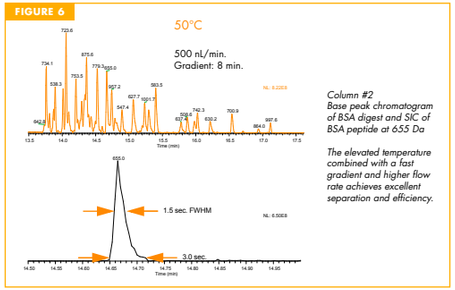
The SIC at 50°C exhibits demonstrated improvement in the peak width, as well as decreased RT.

TABLE 3 1.8 µm C18, 10 cm Column Data

m/z (Da)	23 °C RT (min)	Area	Height	FWHM (m)	FWHM (s)	width 13% (min)	pk capacity	50 °C RT (min)	Area	Height	FWHM (m)	FWHM (s)	width 13% (min)	pk capacity	Data Analysis ΔRT (min)	ΔFWHM (m)	ΔFWHM (s)
734.4	19.3	1.69E+09	1.45E+09	0.02	1.2	0.06	334.33	17.49	8.42E+08	6.07E+08	0.03	1.8	0.06	334.33	1.84	-0.01	-0.6
723.5	20.3	3.74E+09	1.32E+09	0.06	3.6	0.09	223.22	18.81	1.47E+09	7.23E+08	0.04	2.4	0.08	251.00	1.45	0.02	1.2
778.9	22.0	3.47E+09	8.41E+08	0.08	4.8	0.12	167.67	20.67	1.50E+09	5.21E+08	0.06	3.6	0.09	223.22	1.37	0.02	1.2
997.6	27.8	5.04E+08	2.04E+08	0.04	2.4	0.09	223.22	27.07	2.31E+08	8.71E+07	0.05	3.0	0.09	223.22	9.76	-0.01	-0.6
		Average Peak Capacity					237.11				Average Peak Capacity						257.94

Data for 4 different BSA peptides separated on a 10 cm PicoFrit column packed with 1.8 µm C18 at 23°C and 70°C. The average peak capacity is consistent at both temperatures.

FIGURE 6



Column #2
Base peak chromatogram of BSA digest and SIC of BSA peptide at 655 Da

The elevated temperature combined with a fast gradient and higher flow rate achieves excellent separation and efficiency.

Conclusions

- Column back pressure (50° C) is reduced by more than 14% and 26% for 3.5 µm and sub-2 µm-packed columns respectively
- Elevated temperature enables sub-2 µm-packed column compatibility with conventional pressure (<5,000 psi) HPLC pumps
- Elevated temperature improves peak resolution for hydrophobic peptides, lengthening the chromatogram
- Elevated temperature can cause peptide elution order to change significantly
- Hydrophilic peptides may be poorly retained at high temperature, requiring highly aqueous (< 1% B) column conditioning

References

1 Hayward, MJ; Budac, DP; Clark, J "Fast Gradient LC Seperations Using "standard" HPLC Systems" *LC-GC* 2007, Feb. 2007, 2.

2 Blackler, AR; Speers, AE; Wu, CC "Chromatorgraphic Benefits of Elevated Temperature for the Proteomic Analysis of Membrane Proteins" *Proteomics* 2008, 8, 3956-3964.