NanoLC-MS Column Heating for Improved Peptide Separations on a PST µAutoNano LC Dual Column Nanospray/Microspray Source

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Goal

To significantly increase nanospray LC-MS resolution of a peptide mixture by implementing novel PST capillary column heaters at 50°C versus room temperature (20ºC).

Introduction

The use of LC column heaters is an established practice for analytical scale LC-MS for the purposes of increasing throughput and resolution, reducing backpressure, and increasing mass transfer to eliminate carryover from previous analyses. Column heaters for nanospray LC-MS, however, are not commonly utilized. Conventional column heater ovens are not a viable option for nanospray LC-MS as the extra pre-column and post-column tubing introduces unacceptable dead volumes that lead to band-broadening and lower sensitivity. Phoenix S&T (PST) has developed a compact nano-LC column heater that employs dynamic feedback control to maintain steady and uniform temperatures up to 100ºC. This technical note demonstrates improvement in resolution of the separation of peptides by comparing analyses performed with columns at both room temperature (~20ºC) and heated to 50ºC.

Experimental Conditions

Sample

A 63-synthetic human peptide mixture was prepared in 95% water / 5% acetonitrile at a concentration of 80 fmol / µL.

MS Source, Column Heaters, Columns

The PST µAutoNano LC dual-column system positions the columns in front of the mass spectrometer inlet. While one column (A) encased in the column heater performed a gradient separation with nanospray directly into the MS inlet, the second column (B) performed column equilibration with an aqueous buffer, sample loading and wash while off-axis.

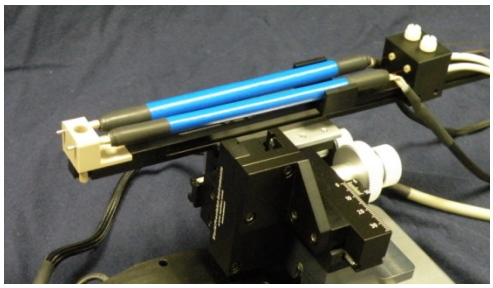

Figure 1: The µAutoNano LC automated source with dual column heaters (columns enclosed), the nanospray emitter assembly with purging gas, the column heater support on a motorized platform, and the high voltage liquid junction block. The µAutoNano LC system is equipped with Active Spray Control that ensures the column is ready for the nanoLC-MS as soon as the column has been positioned at the mass spectrometer inlet.

The columns were heated at 50ºC with 25 cm long column heaters (PST-CH-25). The temperature was maintained to within 0.1° C of the set temperature with a temperature controller (PST-CHC). Two fused silica capillary columns (75 µm inside diameter, 360 µm outside diameter) with integral nanospray emitters were packed with 5µm C18 particles to a depth of 31 cm and 27 cm, respectively. The emitters were placed directly in front of the mass spectrometer. Alternatively, the column heaters can be used with capillary columns placed closer to the LC.

Sample Analysis

2 µL partial loop injections were made off-axis with an additional 10 minutes of isocratic flow to provide a wash of the sample. The mobile phases were pumped by a 2-D splitless nanoflow LC at 270 nL/minute. Mobile Phase A was water with 0.2% formic acid and mobile phase B was acetonitrile with 0.2% formic acid. The compound was eluted using a gradient as follows: 0-2 min. 95% A; 2-12 min. 95-80% A; 12-42 min. 80-58% A; 42-52 min. 58-30% A; 52-62 min. 30-95% A; 62-82 min. 95% A. Isocratic conditions for column equilibration and sample loading: 95% A. The MS analysis was performed on a Thermo LCQ Advantage instrument scanning a mass range of 395-1500 amu.

Results

Applying column heating produced dramatic improvements in peak resolution. Resolution was calculated using the half-height method:

$$Rs = \frac{2(t2 - t1)}{1.7\,(w_{0.5,1} + w_{0.5,2})}$$

Table 1 shows the resolution values for six peptides. The resolution results are based on the nearest eluting peptide.

	Column A		Column B	
[MH+2]2+	Nearest Peak Res. at Room Temp (~20ºC)	**Nearest Peak Res. Heated to 50ºC**	Nearest Peak Res. Room Temp (~20ºC)	**Nearest Peak Res. Heated to 50ºC**
651.3	1.34	**19.06**	1.68	**14.49**
m/z 1095	1.34	**19.06**	1.68	**14.49**
583.8	0.67	**19.29**	3.58	**24.04**
855.5	0.67	**17.34**	3.58	**24.04**
947.5	2.09	**17.34**	17.93	**26.39**
800.0	8.13	**43.24**	43.34	**45.26**

Table 1: Resolution Data for Columns A and B, Peptide Standard Mix, at room temperature (~20ºC) and 50ºC.

For Column A with the 31 cm column bed, only two peptides had a > 1.5 Rs value (baseline resolution). An Rs of 2.0 or greater is generally desired to accommodate minor chromatographic changes throughout a large sample set.

Heating this column to 50°C increased the resolution dramatically from 432% to 2657% with Rs values of 17 to 43. For Column B, the shorter 27 cm bed column, all of the peaks at room temperature had baseline resolution or Rs values > 1.5. At 50°C, the increases in resolution ranged from 4% to 762% with Rs values of 15 to 45.

Column A had the greatest increase in resolution when heated to 50°C. Figure 2 shows the analysis of the 63-peptide mix with no column heat. This analysis shows little to no separation of the selected peptides at room temperature. Figure 3 shows the same analysis but heated to 50°C. All the selected peptides, which closely eluted at room temperature, are well separated at 50°C.

Column heating also guarantees stable column temperatures for lengthy or overnight analyses. Experiments using the column heaters with this peptide mix, neat as well as spiked into plasma, were conducted over five different days with the longest set run for over 18 hours, totaling over 75 hours of long term, continuous analysis. The dual column approach enabled the unique capability of equilibrating one column while analyzing a second column, producing a75% increase in throughput over a conventional single column method. For the neat peptide standard mix run, retention time RSDs of ~0.5% were observed for one column using the column heaters at 50°C.

Conclusion

The PST nanoLC column heaters make the benefits of column heating available to nanospray LC-MS users. Increased resolution is advantageous for quantitation, peptide identifications, as well as reduced run times which translate to higher throughput. Stable column temperatures assure reproducibility over long sample set analyses. Lower backpressures accommodate smaller particle size resins for greater resolution and sensitivity or higher flow rates for higher throughput. Finally greater mass transport allows thorough washing of analytes from the stationary phase pores for minimizing sample carry-over.

The results of these experiments demonstrate that utilizing PST nano-LC column heaters during nanoLC-MS analysis dramatically increase resolution over analyses run at room temperature. One resolution (Rs) comparison showed an increase of 0.7 to 19.3 or ~2700%.

Acknowledgements

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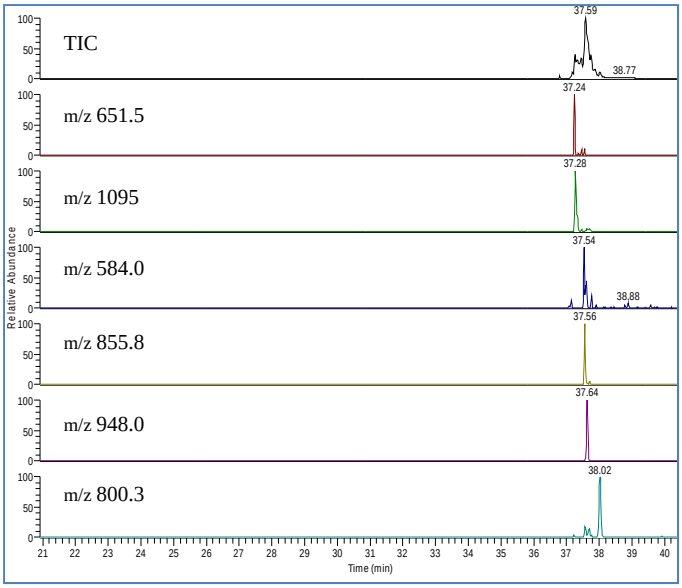

Figure 2: Column A at Room Temperature (20°C)

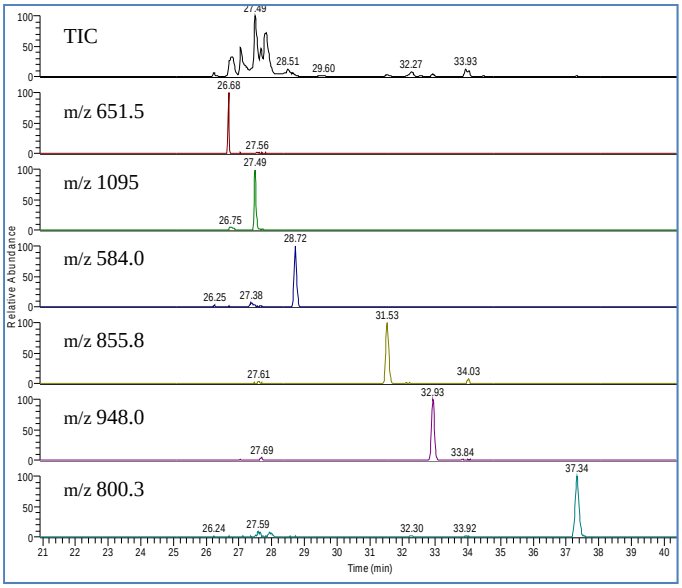

Figure 3: Column A at 50°C

Figures 2 and 3: Selected peptides show little separation at room temperature. When the column is heated to 50°C, baseline resolution is achieved for all selected peptides.