Optimal NanoLC-MS Performance Utilizing an Integrated Heated Dual- Column Automated Source

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Goal

To present a practical demonstration of nanospray LC-MS that addresses typical problems with nanospray systems and illustrates improved robustness and throughput, along with excellent resolution and precision.

Introduction

Nanospray LC-MS offers greater sensitivity then standard LC methodologies and therefore is a desirable technique for many biological assays. However, this technique has been challenging to implement because lower flow rates create difficulty in initiating consistent spray, and problems can be encountered with spray disruption and stability. This method has also required lengthy equilibration times and subsequent sample loading and washing steps that have reduced sample throughput considerably. The results presented here demonstrate how these challenges are comprehensively resolved with the Phoenix S&T µAutoNano LC dual-column source with temperature control.

The µAutoNano LC system allows greater throughput by performing equilibration, sample loading and washing on one column off axis of the mass spectrometer while the other column runs a sample analysis. The use of offline column equilibration in conjunction with the precise column temperature control yields excellent retention time reproducibility and method stability. Additionally, the column heaters improve peak resolution, critical for both component identification and quantification. Other significant commonly encountered issues are spray disruption and instability caused by air bubbles, surface tension changes in the gradient and tip degradation during the gradient analysis. These problems are eliminated by the built-in proprietary Active Spray Control technologies of emitter motion, TipGuard™, nitrogen purging and high voltage programmability, thus allowing for hours of continuous spray and uninterrupted, unattended analyses.

Experimental Conditions

Sample

A custom-made mixture of 63 synthetic peptides at 80 fmol/µL (5% ACN / 95% H_2O) was used either neat or spiked in human plasma (previously cleaned of high molecular weight proteins and particulates by a C18 SPE cartridge). The spiking was done by evaporating 50 µL of the peptide standard mix to dryness under nitrogen and reconstituting with 50 µL plasma (40 µg plasma/ 500 µL 99.5/0.5 H_2O / ACN) to make a 80 fmol/µL Peptide standard mix in 80 ng/µL plasma.

MS Source with Dual Columns and Column Heaters

The PST µAutoNano LC dual-column system positioned 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 washing while off-axis.

The columns were heated at 50°C with 25 cm long column heaters (PST-CH-25). 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 laser-pulled ends acting as integrated 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.

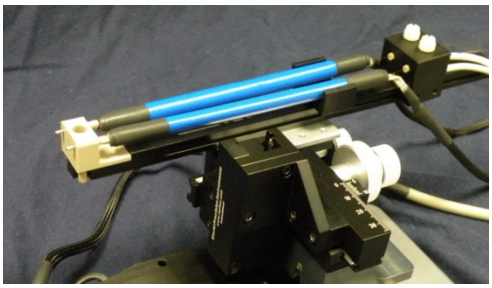

Figure 1: The µAutoNano LC automated source with dual column heaters (columns enclosed). The nanospray emitter assembly with purging gas, the column heater support on a motorized platform, and the high voltage liquid junction block. The µAutoNano LC is equipped with Active Spray Control to enable continuous uninterrupted spray.

Sample Analysis

2 µL partial loop sample 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 compounds were 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 loading: 95% A. The MS analysis was performed on a Thermo LCQ Advantage, scanning a mass range of 395-1500 amu.

Results

Unattended analysis of both a peptide standard mix and spiked plasma samples were completed without a spray failure for 76 hours of experiments. The dual column system increased the throughput over a conventional single column system by 75%. The ability to implement long equilibration times while providing a constant temperature for the separation produced an exceptional retention time precision of ~0.5 RSD on nine peptides in one of the columns. Peptide resolution was

significantly increased up to 2600% through the application of the integrated column heaters.

Spray Robustness

Figure 2 shows the robust performance of the experiments, both for the peptide standard mix and for the spiked plasma samples. The individual experiments ranged from 9.5 to 18.5 hours each. There were no spray disruptions from the initiation of each gradient run to the end for each analysis.

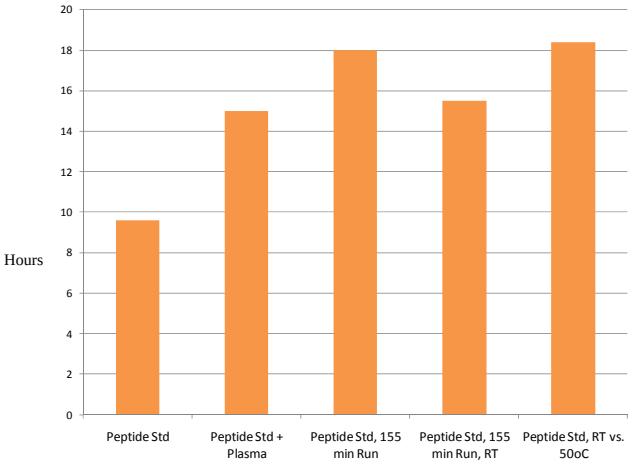

Figure 2: Hours of continuous, unattended analysis

Retention Time Reproducibility

In order to obtain reproducibility in nanospray, long column equilibration times are necessary due to the low flow rates combined with the mixer, tubing, and column volumes. The dual column approach makes these longer equilibration times possible, and also increases throughput from 75 to 100% over single column methods. The ion traces in Figure 3 are of the same peptide on four different analyses of the same column. The retention time RSD of the four runs is 0.30%.

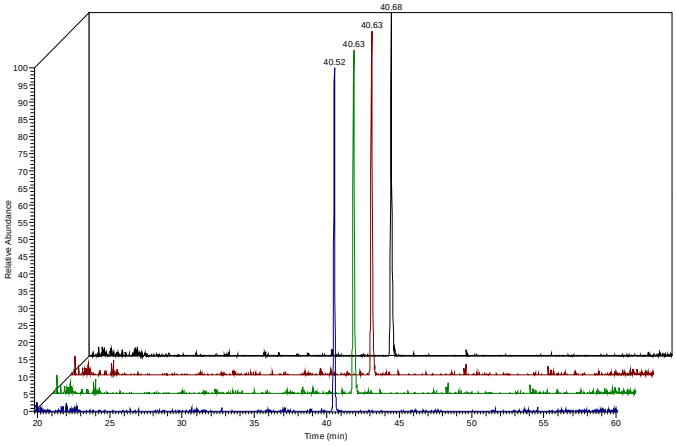

Figure 3: Exceptional retention time reproducibility of peptide m/z 800 in four different runs.

Improved Resolution

Column heating is typically used to assure uniform analysis temperatures over an extended period of time. Column heating has additional benefits such as lowering of column pressures, reducing carryover, and increasing resolution. Figure 4 shows the analysis of the 63-peptide mix with no column heat. Little to no separation of the selected peptides can be observed at room temperature. Figure 5 shows the same analysis but heated to 50°C. The previously co-eluting peptides at room temperature are well separated at 50°C.

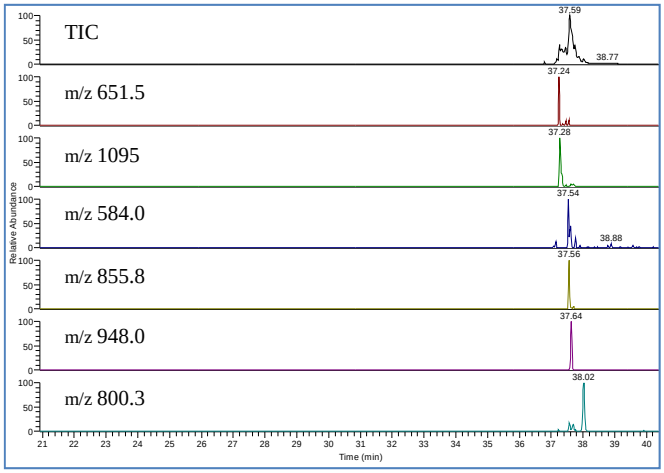

Figure 4: Column A at Room Temperature (20°C)

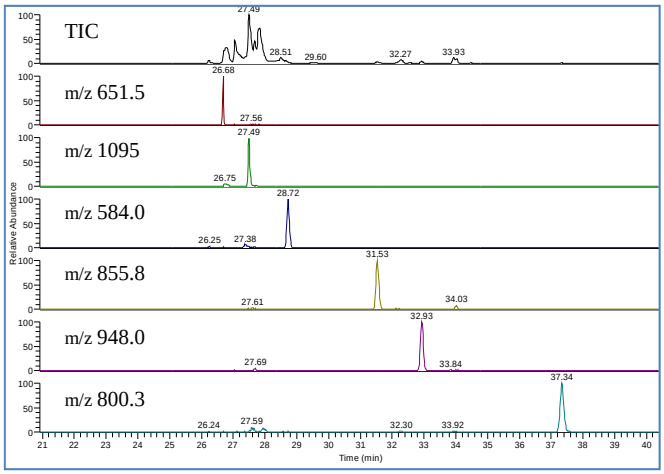

Fig 5: Significantly improved resolution of Column A peaks at 50°C

Conclusion

The PST µAutoNano LC source successfully performed nanospray LC-MS analyses with a 75% increase in throughput over single-column methodology. Retention time precision as high as ~0.5% was observed. Column heating provided uniform temperatures for improved reproducibility, and also significantly increased peptide resolution up to 2,600%. The integrated technologies in Active Spray Control successfully initiated and maintained continuous uninterrupted spray for over 76 hours of analysis on two columns. The µAutoNano LC source is the key instrument for improving the robustness, throughput, and precision for successful nanoLC-MS experiments, especially those involving complex biological matrices.

Acknowledgements

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