

High Resolution Dual nano-LC/MS Source for Increasing Sample Throughput of Gradient LC Methods

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Overview

Many factors make nanospray-LC/MS an attractive alternative technique to analytical scale LC/MS, from increased resolution and sensitivity to reduced sample and solvent consumption. A significant drawback to the use of nanoliter flowrates in nano-LC is the lengthy column re-equilibration times required before a second analysis can be performed. By incorporating a novel automated nanospray-LC/MS source with the dual column capability for performing a gradient analysis on one capillary column while a second column is equilibrating, the equilibration time was reduced 67% with overall injection-to-injection time reduced by 42% for flow rates of 300 nL/minute.

Introduction

Nanospray-LC/MS has rapidly become the technique of choice for analysis in life sciences, in particular proteomics and drug and biomarker discovery. The increased resolution and sensitivity offered by nanospray-LC/MS provides confidence for peptide identifications. This report shows the use of a novel automated nanospray-MS source with a dual column configuration to reduce wait time before the mass spectrometer due to mixer, transfer tubing, and column equilibration times. For nanoflow rates of 300 nL/min., equilibration times of 30-40 minutes are common depending on the LC system and the valve/tubing configuration. A previously published two-column, valve-based system for analytical scale LC applications has disadvantages for nanospray-LC/MS because substantial dead volume exists between the columns, post-column valve, and a single spray emitter. For nanospray-LC, this excessive post-column dead volume increases peak-broadening thus reducing resolution. By spraying directly from the columns to the MS inlet, increased throughput is possible while maintaining high peak resolution. In this paper, the use of the dual column technique and instrumentation is demonstrated through experiments to optimize a separation technique for seven closely eluting peptides on two similar C18 nano-LC columns.

Method

Columns: Two 75 µm I.D. capillaries were packed with 5 µm C18 particles to a depth of 10 cm. The column ends were fritted and tapered and were used to spray directly to the mass spectrometer. The columns were placed 14.3 mm apart on an automated motorized axis that alternates the positions of the columns on-axis to the MS inlet.

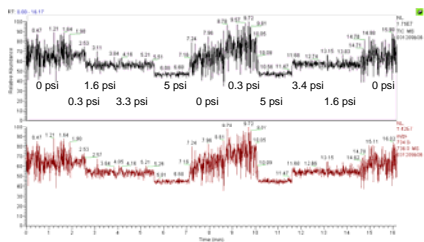
Source Interface Design: Each column end is placed 14.3 mm apart by tees that introduce nitrogen sheath to either purge the emitter of excess eluent before analysis or as a nebulizing gas for higher flow rates. Motors and software control the positioning of the column before the mass spectrometer. While one column sprays before the mass spectrometer, a second column equilibrates with starting mobile phase without high voltage present. When the gradient analysis of the first column is complete, a N₂ purge is applied to the second column followed by a placement of the column before the mass spectrometer inlet. A high voltage spike and a final X-axis movement of the column toward and back of the MS inlet occur to remove any remaining eluent from the equilibrated column thus establishing steady spray conditions for the next analysis. This process is continuous alternating between the two columns.



Dual Column T-Assembly for N₂ purge or sheath gas options



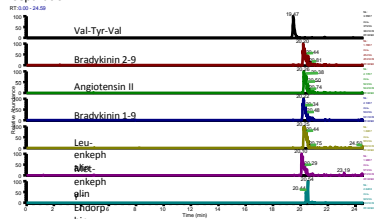
Nano-LC column with fritted, tapered end. The column exit is the spray emitter.



Effects of N₂ Sheath Gas on micro-flow rate (1 µL/min) Erythromycin by Infusion. At flowrates > 1 µL/min, 3-5 psi of sheath gas dramatically improves the signal to noise ratio.

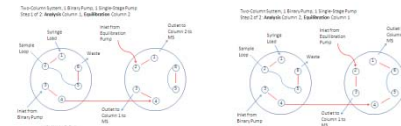
LC/MS Experimental

The time saving capability utilizing the dual column technology is demonstrated by performing a simple method development experiment. The C18 resin used is known to have difficulty separating small peptides. By incrementally increasing the starting organic concentration of the mobile phase, a best set of conditions is sought for optimum peak width as well as analysis speed and component separation.

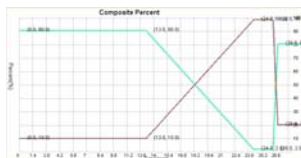


An initial, weak mobile phase (98% A for 3 minutes) followed by a steep gradient (2% A at 14 min.) shows tailing and broad peaks with retention times over 20 minutes.

Using the dual column source, repeated analyses were performed with different starting mobile phase compositions. The end of each analysis had mobile phase conditions of the next experiment for a correct transition of mobile phases when the columns switched from the isocratic equilibration pump to the gradient analysis pump.



Simultaneous gradient and equilibration flows are established using one gradient pump, one isocratic pump, and two six-port valves.



Gradient runs have a 10 minute equilibration after column switching, direct-drive syringe pumps to refill, and repressurization at the start of a run. After the 10 minute equilibration, the sample is injected and the MS acquisition begins. This process reduces analysis time by 30 minutes. As the dead volume from mixer to column exit is ~10 minutes, the previous MS analysis continues to run during the first 10 minute equilibration period of the subsequent run.

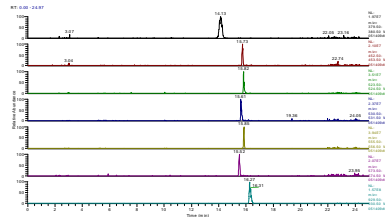
LC/MS Conditions

- MicroTech Scientific XtremeSimple Dual Binary System
 - One binary system used for gradient analysis
 - One binary system used for isocratic equilibration
- 300 nL/min flow rate
- 200 nL partial loop injection
- Mobile Phases
 - Reservoir A: 98% H₂O/2% ACN/0.1% formic
 - Reservoir B: 98% ACN/2% H₂O/0.1% formic
- Thermo Finnigan LCQ Advantage Ion Trap Mass Spectrometer
- Full scan 200- 1200 amu

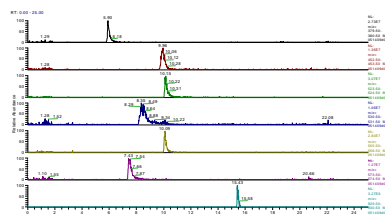
7 Component Sample Mixture

500 ng/mL of Val-Tyr-Val, Angiotensin II, Leu-enkephalin, Met-enkephalin (100 pg each injected with a 200 nL injection) 1 µg/mL of γ-Endorphin, Bradykinin 1-9, Bradykinin 2-9 (200 pg each injected with a 200 nL injection)

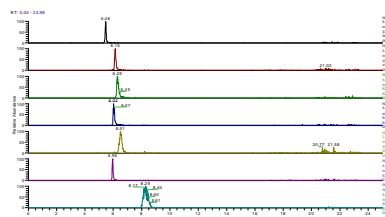
Results



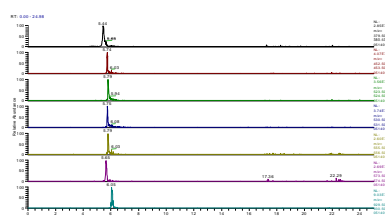
Starting mobile phase of 90% A shows sharper, earlier peaks for all but Val-Tyr-Val



Starting mobile phase of 80% A shows good peak separation, early retention times, but excessively tailing and broad peaks.

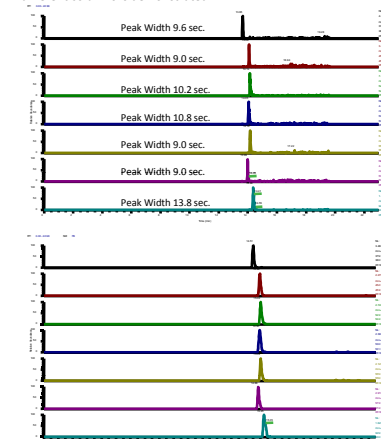


Starting mobile phase of 70% A shows a reasonable compromise of peak shape, analysis speed, and separation.



Starting mobile phase of 60% A shows very sharp peaks and early retention times, but little to no peak separation.

Below are two analyses on the C18 columns using the dual column interface. The gradient program is further refined to optimize only for peak width of all components. Good reproducibility of analysis between two different columns is demonstrated.



The gradient program for the above analyses was 98% A for 3 min, 30% A at 4 min, 2% at 13 min, 2% A 13-15 min, 98% A at 15.1 min. An ~10 min delay of gradient from mixer to column exit is present.

Summary and Discussion

A dual column MS interface is presented and demonstrated for increasing nano-LC/MS sample throughput.

- A 67% reduction in equilibration time and a 42% reduction of total analysis time was obtained without sacrificing peak width resolution. This was accomplished by the design of a MS dual column interface that did not increase post-column dead volume.
- The decrease in equilibration time can be utilized to improve sample throughput or, as demonstrated in this paper, scouting for method development.
- The results of the method development indicate other resins or mobile phases should be tried as the conditions tested cannot provide both sharp, symmetrical peaks and good peak separation.
- The two columns produced similar results when run in a dual column experiment with the same mobile phase gradient.

Nanoflow gradient liquid chromatography requires extensive equilibration due to the low flow rates combined with mixer and tubing volumes as well as the column volume. The vendor of the nano-LC used for these experiments recommends 10 minutes of equilibration time just for equilibrating the mixer alone. Analytical-scale dual column set-ups designed to equilibrate one column while another is undergoing analysis utilize a switching valve post-column to a single spray emitter. For nanoflow applications this approach introduces an unacceptable amount of dead volume which would result in peak broadening. As narrow peak widths are integral to the improved sensitivity and resolution of nanospray-LC/MS, the reduction or elimination of post-column dead volume is important. The technique described in this poster allows higher throughput with no post-column dead volume as the exit of the column is the nanospray emitter. This is demonstrated by the 9 second peaks observed in the work presented.

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References: 1) Oertel, R., Richter, K., Fauler, J., Kirch, W., J. Chromatography A 2002 948, 187-192.