Methods enabling high throughput, high sensitivity nanoLC-MS with a trap column

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Introduction

Many methods for proteomic analysis with nano- LC-MS utilize a trap column for sample loading that is in-line with the analytical column. The trap column allows the sample to be rapidly loaded (flow rate ~2 µL/min) and also allows for the sample to be washed from salts. If debris are in the sample, the analytical column is protected by the trap column. Recent advances in analysis have included the use of dual column methods to increase throughput by equilibrating the column and then loading the sample off-line while another sample is being analyzed. While loading sample with dual columns is typically done at a higher flow rate), the down time can be from sufficiently cleaning and equilibrating the column after clinical samples to ensure that there is no carry over to the subsequent sample analysis.

We present the set-up and method for a dual trap column system to increase sample throughput that will allow sufficient time for cleaning and equilibrating the column prior to analysis. Since the analytical columns have integrated nanospray emitters, there is no post-column dead volume.

Methods

•A dual column, Nimbus, source (Phoenix S&T) was paired to a LTQ ion trap (Thermo) for analysis.
•Two eFrit® columns (Phoenix S&T's capillary column with an integrated nanospray emitter and an embedded frit) were packed to 20 cm with 4 µm, Jupiter Proteo particles (Phenomenex). Both columns were heated to 60ºC.
•500 fmol of a protein mix was loaded and subsequently separated on the column. The protein mix was a tryptic digestion of the following 4 proteins: bovine serum albumin, cytochrome c, α-lactalbumin and myoglobin.
•A 90 min gradient was performed with an Eksigent nano-LC system from 3% acetonitrile in 0.1% formic acid to 60% acetonitrile in 0.1% formic acid. After each run a 10 min wash was performed with high organic (95% acetonitrile in 0.1% formic acid) and then the column was put to initial conditions prior to switching

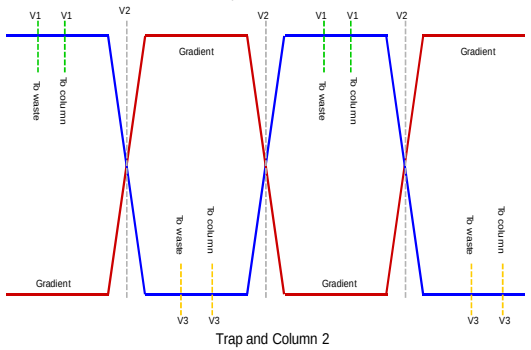


Figure 1. Diagram showing approximately when the valves are switching to control the flow of the pumps to the columns. Valve 1 (V1) and Valve 3 (V3) directs the flow through the trap to either waste when loading sample at a high flow rate or to the column. Valve 2 (V2) controls which column receives the gradient. All valves were controlled through the Eksigent software.

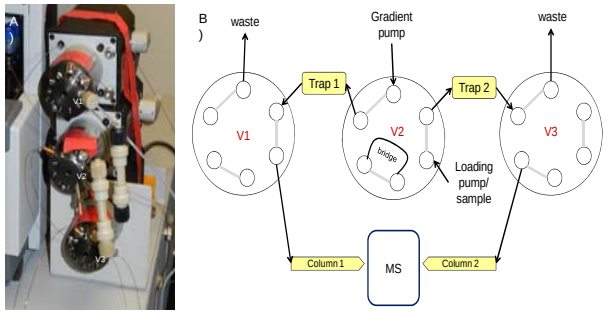


Figure 2. A) Picture of the valve configuration on the Eksigent system. B) Diagram of the plumbing for the dual trap system.

Results

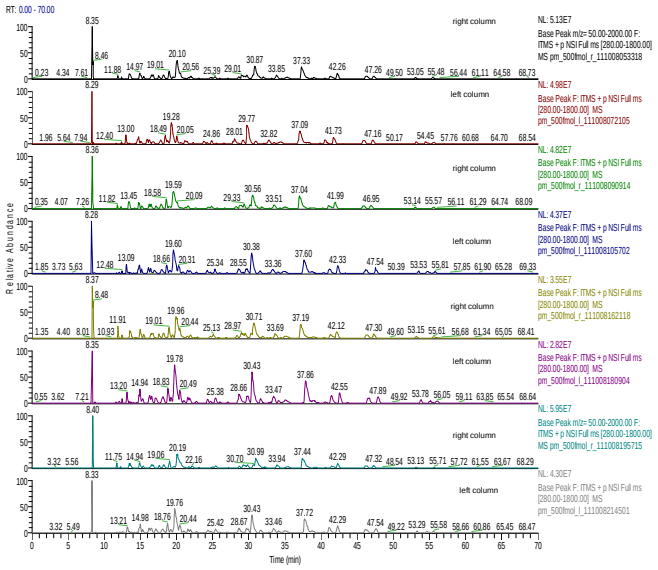
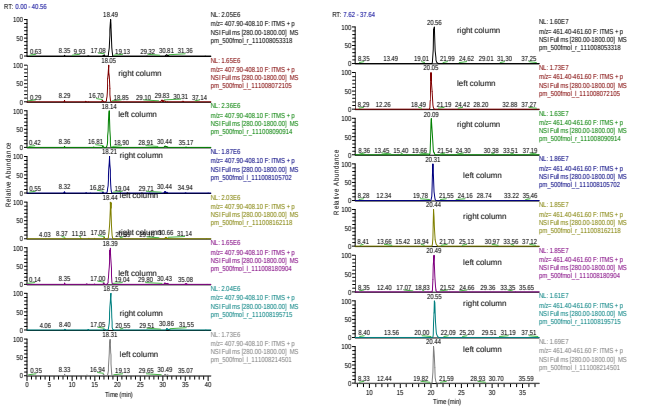


Figure 3. Base peak chromatograms of the protein mix demonstrating 8 continuous runs using the Nimbus dual column source with a dual trap column set-up.



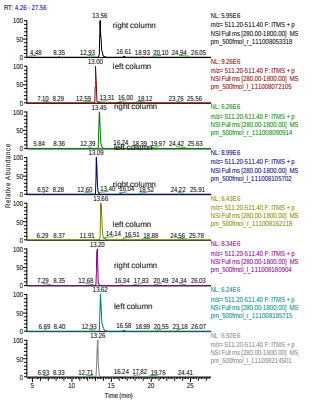


Figure 4. Chromatograms for three different peptides shown during eight consecutive runs between two columns.

Conclusions:

•An automated dual trap column method was presented using a relatively simple 3-valve system (as opposed to the 4-valve system previously reported) to allow for off-line column equilibration and sample loading while simultaneously analyzing a different sample on a second analytical column, thus dramatically increasing throughput and reducing potential of the column wash dirtying the MS inlet.
•Excellent reproducibility was shown between both the two different columns and traps.
•This set-up in this presentation does not introduce post-column dead volume thus compensating for the unavoidable peak broadening due to the use of trap columns.

Acknowledgments

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