Factors Influencing Chromatographic Performance in Nano-flow LC/MS/MS for Proteomics Applications

Steven A Cohen*, Chris L Stumpf, Jeffrey W Finch, Wensheng Xu and Robert Plumb, Life Sciences R&D, Waters Corporation, Milford MA, USA

Introduction

The success of proteomic LC/MS studies is dependent upon the quality of the chromatographic separation processes employed. These processes are typically very complicated involving 2 dimensional peptide separations often employing ion-exchange and reversed-phase chromatography. The analytical reversed-phase chromatography is typically performed on capillary columns having internal dimensions of 100µm or less. At these dimensions, the quality of the separation produced is highly dependent upon the inter-column connections. This dependency is exaggerated when employing a two dimensional system with a 300µm i.d. ion-exchange column and a C18 trapping column.

In addition to the effect of the connections the efficiency of the column packing significantly effect the results obtained. Other factors that can have a deleterious effect on the chromatography are the valving and the direction of trapping column loading and elution.

In this poster we will discuss procedures to reduce peak broadening. We will show how chromatographic performance can be improved by the careful control of fittings and optimization of the mode of operation. The results we have obtained with the nano-scale separations will be compared to those achieved with a 2mm i.d analytical column.

Methodology

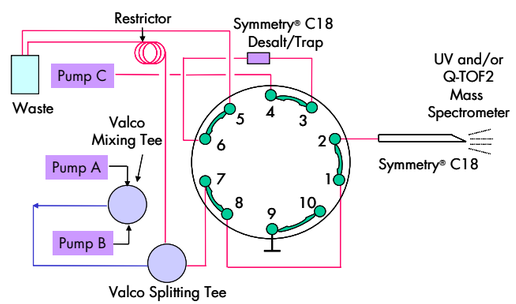
Conditions	Analytical	Nano-LC
Sample	Apo-myoglobin tryptic digest	Apo-myoglobin tryptic digest
Column	15cm x 2mm Symmetry® C18 3.5µm	15cm x 75µm Symmetry® C18 3.5µm
Injection volume	10µL	5µL
Gradient	5-40% B over 30 mins	5-40% B over 30 mins
Flow rate	250µL/min	200nL/min
Detection	+ve ion ESI	+ve ion nanospray
Scan range	50-950m/z	250-1800m/z

Where A = aqueous formic acid (0.1%) and B = acetonitrile
Analytical experiments were performed on a using a Waters 2796 and a MicroMass QuattroMicro and nano-scale experimanets were performed on a using a Waters modular Cap-LC™ system connected to a MicroMass Q-TOF II .

Trapping experiments

The trapping experiments were performed on a Waters C18 trapping column 1cm x 300µm i.d and connected to a 15cm x 75µm separation column. A 5µL injection of Apo-Myoglobin digest was loaded onto the ion-exchange column with 0.1% aqueous formic acid at 5µL/min. These peptides were then eluted from the trapping column in a back-flush mode onto the analytical column with the nano-scale gradient described before. A schematic diagram of the fluidics is shown below.

Methodology



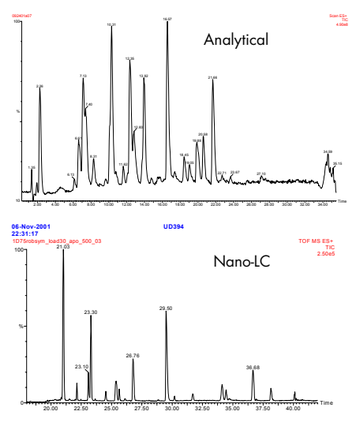
Valve position 1: Sample trapped on RP desalt/trap column
Valve position 2: Sample eluted from RP desalt/trap column and analytical RP column

Results and discussion

Comparison of nano-scale and analytical scale HPLC

The chromatographic performance of the nano-scale HPLC was compared to that achieved with the 2mm i.d. column. The results obtained are shown in Figure 1. This data indicates that the it is possible to achieve separations at the nano-scale with the same efficiency as those obtained with a 2mm i.d column. However, it is only possible to obtain these separations when the connections are properly managed. To this end the use of a connector, such as Teflon, which allows the capillary connection to be visually checked is recommended. However these connections will not hold back-pressures greater than approximately 100psi.

Figure 1 Comparison of analytical and nano-LC performance

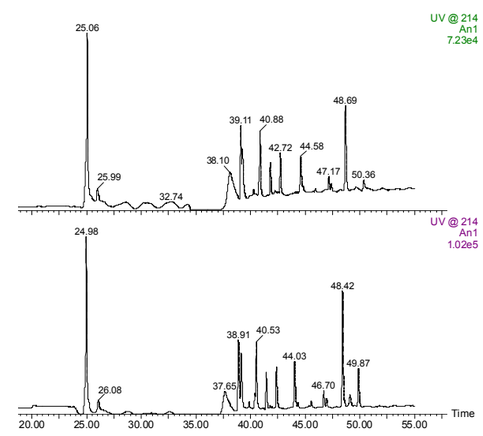


The high pressure connections were only made when necessary, thus the nano columns were directly connected to the injection or switching valve. This entailed using columns with relatively long lengths, (approx 10cm) of open fused silica prior to the packed bed. All connection or transfer tubing was fused silica with internal dimensions of 50µm or less All the trapping columns were connected directly into the valving.

Column to column variation

A important requirement for the successful operation of nano-scale separations is column to column reproducibility. The data shown below in Figure 2 shows two columns packed on separate days. It is our experience that nano-columns are now very reproducible and offer comparable performance to regular analytical columns.

Figure 2 Comparison of two analytical columns



Trapping columns

The availability of good quality trapping columns is essential to facilitate 2 dimensional chromatography. Large sample volumes of (5-10µL) can be loaded in a reasonable period of time by using trapping columns with an internal diameter of 300µm. With these columns it is necessary to minimize the number of column connections. Therefore we have developed a trapping column which connects directly into the switching valve, (see Fig 3), thus reducing the number of tubing connectors required and minimizing band spreading.

Figure 3 Nano trapping column connected directly into switching valve

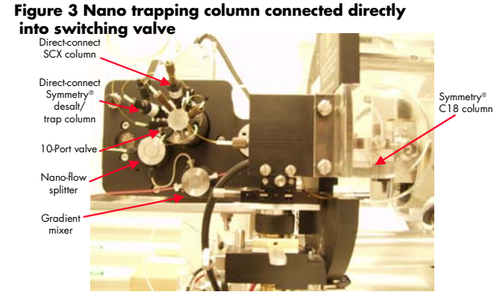
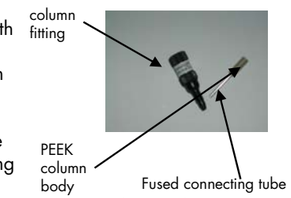


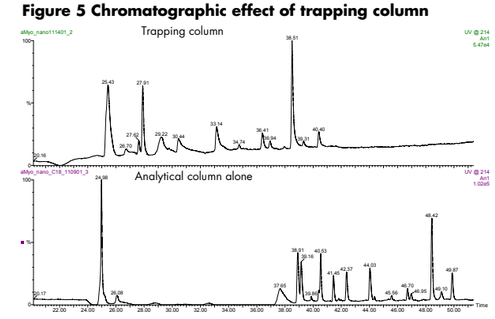
Figure 4 Trapping column

The trapping column design is one piece with the inlet tube fused directly into the column body, (Figure 4). This inlet tube has been configured to minimize peak broadening during elution.



The data shown below in Figure 5 indicates that some band broadening was observed when the trapping column was used with a high efficiency 75µm column compared to using the column alone. This broadening is however a reasonable trade off for increased MS sensitivity (provided by higher volume loading) with a small loss in chromatographic performance.

Figure 5 Chromatographic effect of trapping column



Conclusion

- Nano-LC columns can be produced and operated with efficiencies similar to analytical columns.
- These columns show good reproducibility.
- Trapping columns for high volume sample loading and 2 dimensional chromatography have been produced.
- Direct connection trapping columns have been developed to minimize peak dispersion and band broadening.
- The effect of column connections and valving has been investigated. By reducing the number of connections and internal valve volume the intrinsic chromatographic efficiency can be maintained.