

Approaching 100% LC-MS uptime for peptide analyses using matched chip columns

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Overview

- Robust, high performance separations are difficult to achieve in nanoflow.
- Microchip technologies can be utilized to build LC-MS tools that are optimized at nanoflow.
- Run-to-run retention time reproducibility is very high, with RSD < 1% over multiple columns.

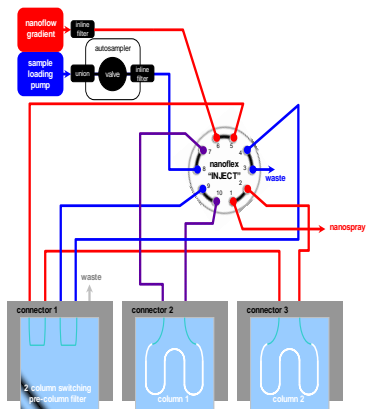
Introduction

- Variability in performance between nanoLC columns makes it difficult to standardize proteomic methods.
- Part of this variability is the highly skill dependent nature of making fluidic connections for nanoliter/min separations.
- Traditional nanoLC columns vary greatly, in packed bed length and quality.
- Microchip devices have inherent appeal as a chromatographic medium as the microchannels are defined lithographically and are highly reproducible.
- Microfluidic devices require a chip-to-world interface which does not sacrifice the performance gains from the microdevices themselves.
- Presented here is how microchips used in an advantageous workflow can yield a large increase in productivity.

Conclusions

- Microchip columns give highly similar performance
- Microchip similarity can be used in two-column switching workflow
- Workflow allows for almost 100% analysis time

Microchip LC/MS Platform



The cHiPLC-nanoflex platform can be configured to perform a variety of workflows, based on the type of pre-column filter chip that is installed. In this case, two-column switching is represented.

Other workflows are possible, including direct injection, and trap & elute.

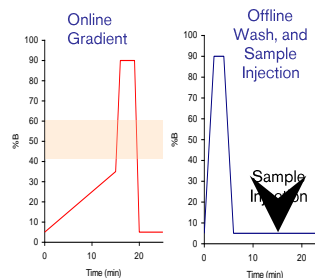
Methods

- Sample: BSA, reduced and alkylated, followed by overnight trypsin digestion at 37°C. BSA obtained from Sigma Aldrich, DTT and Iodoacetamide from Sigma Aldrich, trypsin (sequence modified to resist autolysis) obtained from Invitrogen.
- LC separations performed using an Eksigent NanoLC Ultra 2D+ nanoflow system and a cHiPLC-nanoflex microchip system.
- Gradient test method: gradient rate set to 2% B/min, from 5% to 35% B followed by a step to 90% B. A: 0.1% formic acid in H₂O, and B: 0.1% formic acid in ACN.
- Column phase: Microchip devices were packed with ChromXP, 5 micron particles, 120 Angstrom pore.
- Column dimensions: 75 micron x 150 mm
- MS analysis acquired using a Thermo LTQ. Data acquired in full scan mode, 200-1000 m/z.
- MS data analysis was performed using Quan and Qual Browser.

Results and Discussion

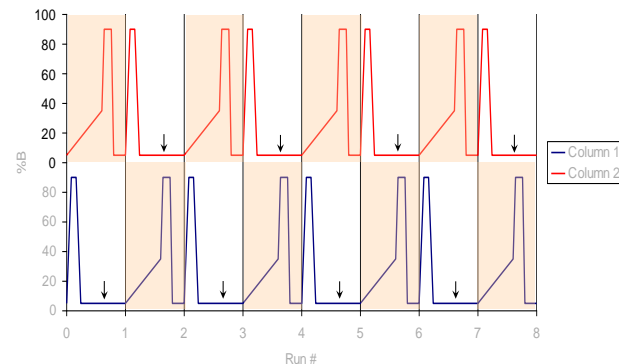
How Two Column Switching Works

- As Column 1 runs a gradient, Column 2 is being washed and loaded with a Sample.



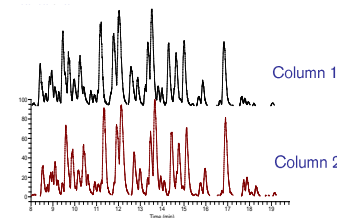
- When the gradient is finished, a valve turns and the columns are switched.

A gradient begins on Column 2, and simultaneously, Column 1 is washed and loaded with new sample.



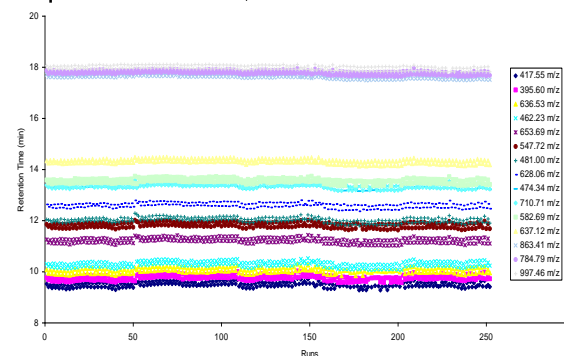
Column-to-Column Performance

Microchip columns are manufactured very reproducibly, resulting in a very high degree of column-to-column interchangeability.



Two-Column Performance Over ~320 Consecutive Runs (258 Sample Replicates + 64 Blanks)

Retention times of 15 ions monitored over 6 different days, and 2 different columns



For entire data set RSD on retention time is < 1%.

This data set includes LC-MS runs that span 6 days of continuous operation.

Long term stability of retention time on different days and columns means that methods for your assays remain relevant for longer.

Ultimately, for the core facility or clinical user this kind of retention time stability means less troubleshooting and more "up-time".