

Improved reproducibility in nanoLC using chip based columns.

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Overview

- High performance separations at nanoflow
- Reproducible and robust
- Routine operation using microchip technology

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 packing 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 are recent developments in our microchip work.

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 2D+ Ultra 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 (Eksigent), 3 micron particles, 120 Angstrom pore.
- Microchips: Chips were fabricated lithographically in fused silica using an anisotropic method that produces spherical channels.
- MS analysis acquired using a Thermo LTQ. Data acquired in full scan mode, 200-1200 m/z.
- MS data analysis was performed using Quan and Qual Browser.

Microchips



The microchips start life as a quartz wafer that undergoes lithography to form the ideal circular channels for chromatography.

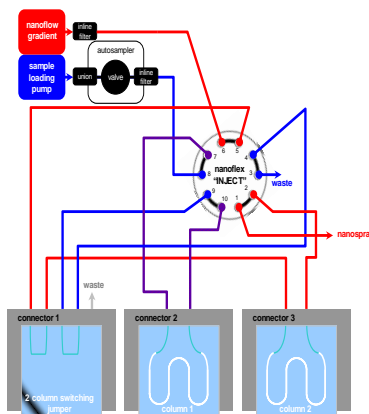
Rather than create an integrated "lab-on-a-chip", we use a special Eksport interface to combine chips with different functions together.

Chip Connector



The Eksport connector is the chip-to-world interface allowing the trap and column chips to be quickly removed and replaced. The connector forms the basis for a plug-and-play microfluidic platform. The connectors are also temperature controlled for long-term retention time stability.

Microchip LC/MS Platform



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

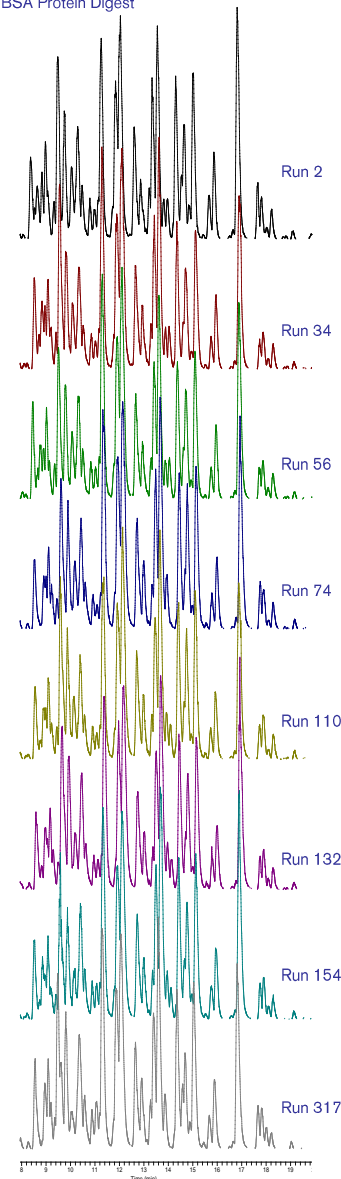
Other workflows are possible, including direct injection, and trap & elute.

Results

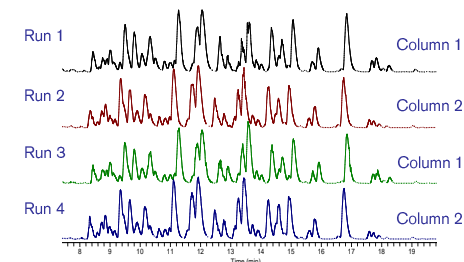
Stable Retention Times

In conjunction with a NanoLC Ultra pump, run-to-run stability is incredibly stable. The NanoLC Ultra pump is capable of exquisitely accurate flow control, which allows the microchip system to perform at its best.

BSA Protein Digest



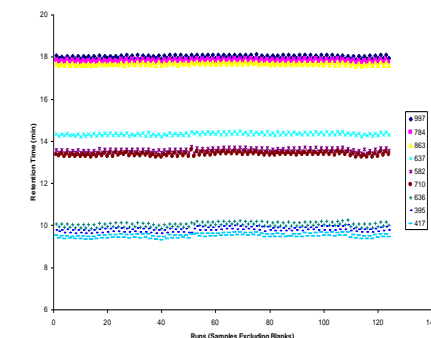
Inter-Column Performance



Microchip columns are produced with a very high degree of similarity. Closely matched performance means retention time stability across multiple columns.

Chip-to-chip reproducibility is achieved as a result of the fluidic design and manufacture, in addition to the proprietary packing process.

Below is a summary of long-term performance for a two-column switching workflow. Monitored are 9 ions from a BSA digest.



Conclusions

- 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.
- Chip-to-chip reproducibility is extremely good due to design.
- Run-to-run retention time reproducibility is very high, with RSD < 1% over multiple columns.