

Increased long term retention time stability in scheduled MRM peptide assays using chip based chromatography

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Overview

- Scheduled MRM assays require better retention time stability
- Reproducibility at nanoflow a major hurdle
- 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 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 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 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 Reprosil, 3 micron particles, 120 Angstrom pore.
- Column dimensions: 75 micron I.D. x 150 mm length.
- Microchips: Chips were fabricated lithographically in fused silica using an isotropic method that produces spherical channels.
- MS analysis acquired using a Thermo TSQ. Data acquired in MRM mode.
- MS data analysis was performed using Quan and Qual Browser.

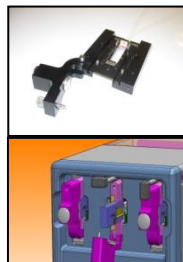
Microchips



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

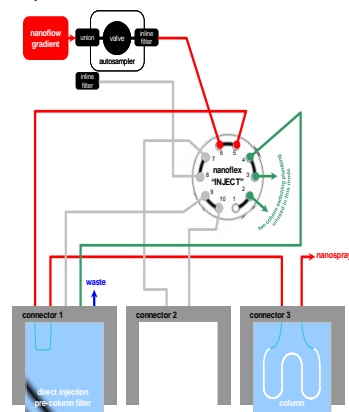
Rather than create an integrated "lab-on-a-chip", we use a special Eksport interface to combine chips with different functions together.

Eksport 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



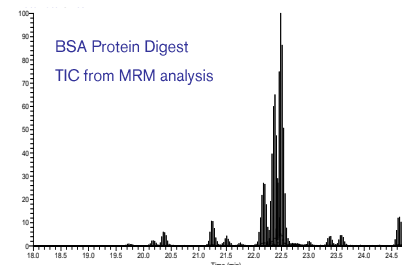
The cHiPLC-nanoflex platform can be configured to perform a variety of workflows, based on the type of 'jumper' chip that is installed. In this case, direct injection is represented.

Other workflows are possible, including two-column switching, 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.

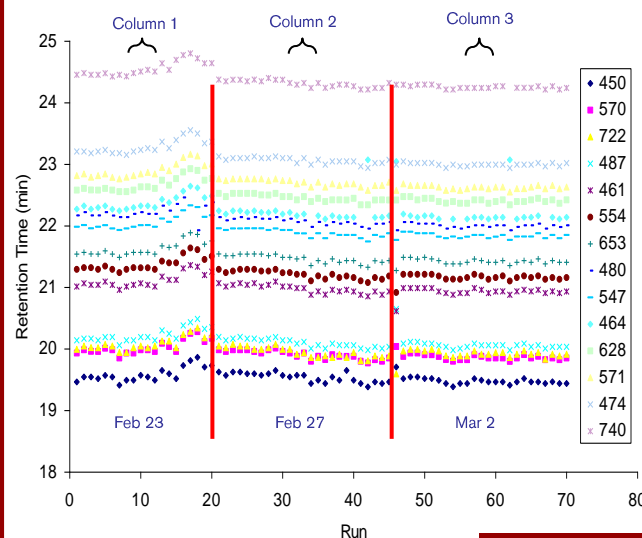


Inter-Column Performance

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

Implications for MRM Assays in a Clinical Setting

Retention times of 14 ions monitored over 3 different days, and 3 different columns



For entire data set RSD on retention time is < 1%.
This data set includes LC-MS runs from different days, with a new column used on each day.
Long term stability of retention time on different days and columns means that scheduled MRM assays can remain relevant for longer.
Ultimately, for the clinical environment this kind of retention time stability means less troubleshooting and more "up-time".

Multi-Site Data Collection

There is great potential for standardization of MRM assays across multiple data collection sites.

With careful analytical method design, the advantages of retention time stability from this microchip platform can be extended to other labs, even ones with different mass spectrometers.

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.