Highly reproducible data from a microfluidic separation platform for application in proteomics

V30-S1

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Overview

- **Demonstration of the cHiPLC nanoLC platform as a high performance chromatographic tool for proteomics with the advantages of microfluidic design elements.**
- **Chromatographic performance on par with conventional packed capillary/spray tip combinations.**
- **High run-to-run reproducibility, and low inter-column variation.**

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.
- Microfluidic 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.
- In this poster, we will introduce a new microfluidic platform for nanoliter separations on microchips and demonstrate that robust, high quality separations enhances the reproducibility of proteomic experiments.

Microfluidic Columns

The columns used in this work are microfabricated on fused silica using standard lithographic techniques.

- Circular channels in cross-section
- Packed at high pressure
- 3 µm particle packing

- Chip Column dimensions: 75 µm i.d. x 150 mm
- Packing material: Eksigent ChromXP C18 3µm 120Å

Methods

- Separations were carried out on an Eksigent NanoLC Ultra 2Dplus LC system and a cHiPLC-nanoflex microfluidic system.

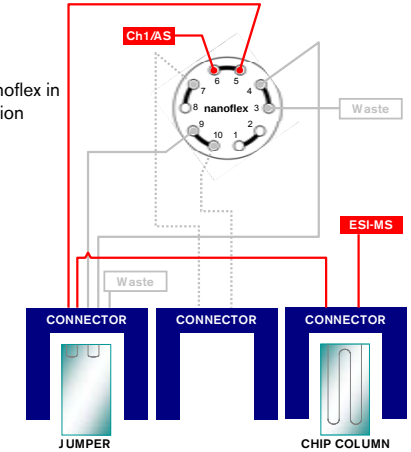
- A: 0.1% formic acid in H_2O, and B: 0.1% formic acid in ACN.
- Gradient test method: 5 – 40%B at 2%/minute.
- Nanospray source from New Objective, using FS360-20-10, non-coated, pulled tips.
- MS data was collected using a Thermo LTQ ion trap.
- Data treatment and interpretation was performed using Mascot Distiller and MASCOT

- Samples in this work include:
  - •Sigma Aldrich Peptide Mixture at 50 pg/ul
  - •BSA, reduced, alkylated (carbamidomethyl), and digested with Trypsin

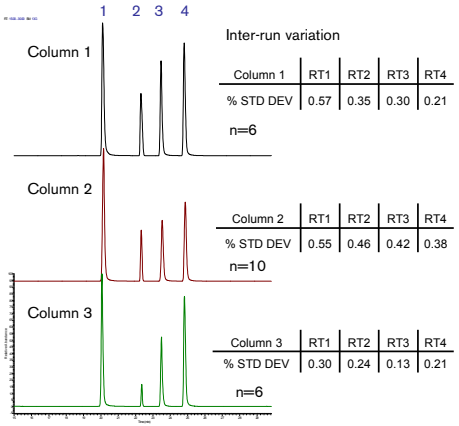
Method Workflow

- cHiPLC nanoflex platform is operated in direct injection mode
  - •Main valve is toggled in the "inject" position
  - •Column eluate is plumbed directly into a nanospray source

cHiPLC-nanoflex in direct injection mode



Results



Column 1	RT1	RT2	RT3	RT4
% STD DEV	0.57	0.35	0.30	0.21
n=6				

Column 2	RT1	RT2	RT3	RT4
% STD DEV	0.55	0.46	0.42	0.38
n=10				

Column 3	RT1	RT2	RT3	RT4
% STD DEV	0.30	0.24	0.13	0.21
n=6				

- Represented to the left are 3 columns, each demonstrating a gradient separation of standard peptide mixture.
- Peaks 1 – 4 have retention times which are represented by RT1 – RT4.
- Inter-run variation is summarized for each column
- The table below summarizes the inter-column retention time reproducibility.

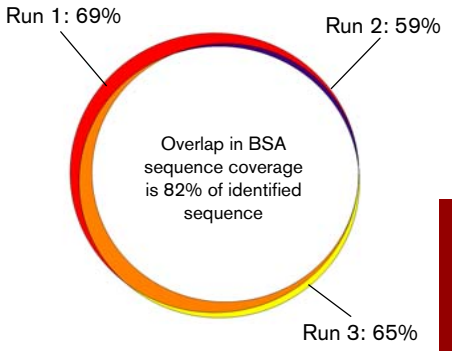
Inter-column reproducibility

Inter-column	RT1	RT2	RT3	RT4
%STD DEV	0.60	1.26	0.76	0.82

- Inter-column reproducibility is key to experiments that run over long periods of time (months to years). e.g. validation of biomarkers.

Inter-Run Sequence Coverage

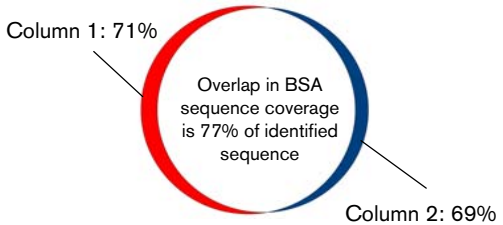
- A high degree of inter-run reproducibility is important in establishing robust proteomic methods.
- Below is a Venn diagram that describes the inter-run overlap in sequence coverage.



71% Total Sequence Coverage.

Inter-Column Sequence Coverage

- Two microfluidic columns are compared.
- Below is a Venn diagram that describes the inter-column overlap in sequence coverage



- 82 % Total Sequence Coverage.

Conclusions

- **Microfluidic columns offer high inter-column reproducibility**
- **Inter-run repeatability is a measure of method robustness**
- **High quality, reproducible separations enable standardized methods for proteomics**