

High capacity separations for complex proteomic mixtures using multiple microfluidic chip columns in series

J. Bryce Young, David Wyrick, Erika Lin, Remco van Soest, Nicole Hebert
 Eksigent Technologies 5875 Arnold Road, Dublin, CA 94568



Overview

- Flexible, high performance microchip platform allows plumbing of columns in series.
- Analysis of single protein, multi-protein, and cell lysate digests.
- Demonstration of advantages in doubling peak capacity for highly complex mixtures.

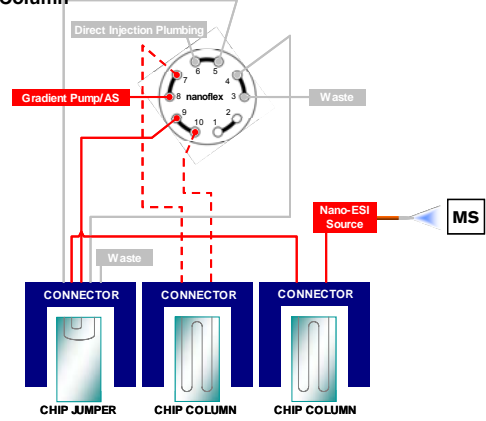
Introduction

- Standard separations on 15 cm packed beds are typically limited to peak capacities of 300-600.
- Longer capillary columns can achieve greater capacities, but can be inconsistent batch-to-batch.
- Connecting shorter columns in series scales the peak capacity with the linear addition of total column length (with a corresponding increase in gradient length).
- Performance of the serial column is greatly dependent on the quality of the connection between the individual columns.
- chPLC nanoflex uses a self-aligning, high pressure edge connector.
- Low dead volume connections allow for the use of 2 microfluidic columns in series without sacrificing performance.

Methods

- Gradient Rate. 1 column: 1%/min. 2 column: 0.5%/min. A: 0.1% formic acid in H₂O, and B: 0.1% formic acid in ACN.
- LC-MS analysis carried out using Eksigent NanoLC 2D Ultra Plus system with a chPLC Nanoflex microfluidic chip platform and a Thermo LTQ with a New Objective PicoView nanospray source.
- chPLC nanoflex was fitted with a Trap&Elute jumper chip and two 15 cm column chips packed with ChromXP C18 3µm 120A.
- Data Dependent Analysis: 1 survey/4 dependent scans, 10,000 count threshold, dynamic exclusion after 2 repeats.
- Data files were analysed using Mascot Distiller (Matrix).
- Proteomic analysis carried out using Protein Pilot (Applied Biosystems/Life Technologies). All searches specified the Uniprot/Swissprot database, with biological modifications. False Discovery Rate analysis was used for all data sets, with a specified maximum FDR of 1%.

Nanoflex Microfluidic LC/MS Platform – Serial 2 Column



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 120A

Samples

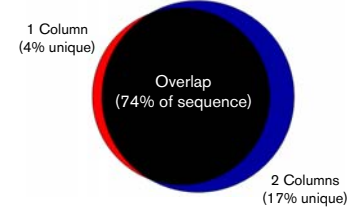
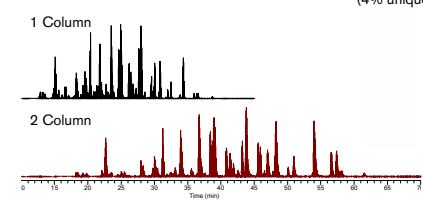
- Bovine serum albumin (Sigma Aldrich), 20 Protein digest and *E. coli* cell lysate (Applied Biosystems/Life Technologies). Reduced and alkylated (iodoacetamide) followed by trypsin digestion.

Conclusions

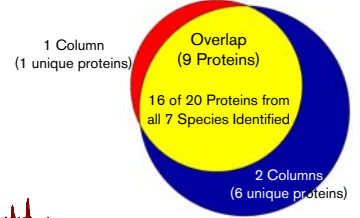
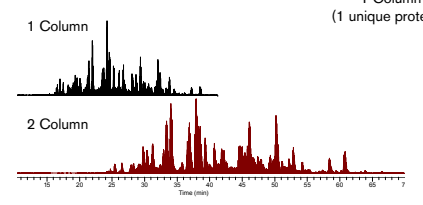
- Low dead volume edge connector allows microfluidic columns can be plumbed in series with good results.
- Analysis of complex mixtures benefits from increased peak capacity.
- Serial columns can obtain better sequence coverage and greater numbers of peptides identified.

Results

BSA Digest

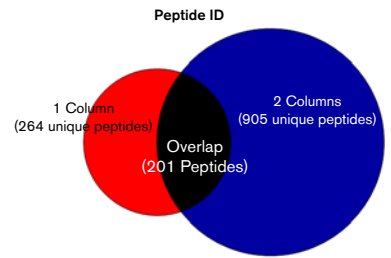
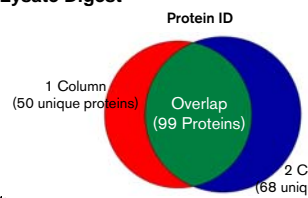
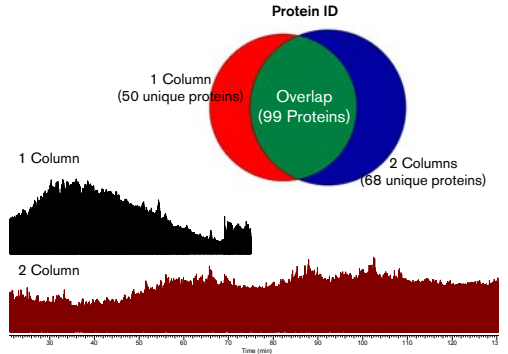


20 Protein Mixture Digest



Species represented include: Cow, Horse, Chicken, Rabbit, Bacillus, Pig, and Horseradish

E. coli Cell Lysate Digest



Discussion

For simple mixtures like BSA digest, there is only a small advantage to using 2 columns.

The 20 protein mixture is somewhat more complex, and the proteomic analysis is made more difficult by the number of species represented in the protein list. While virtually all proteins have been identified, not all were assigned the correct species.

The water soluble fraction of *E. coli* cell lysate digest is much more complex. The peak capacity advantage of the 2 column setup is now much more obvious. Though protein id is only modestly better, the number of peptides identified is ~2.5X greater using the serial column.

The implication of this is that quantitative applications and biomarker validation experiments can benefit from greatly increased peak capacity.

Acknowledgements

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