

The use of dual cHiPLC columns to increase throughput in quantitative peptide MRM analyses

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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.

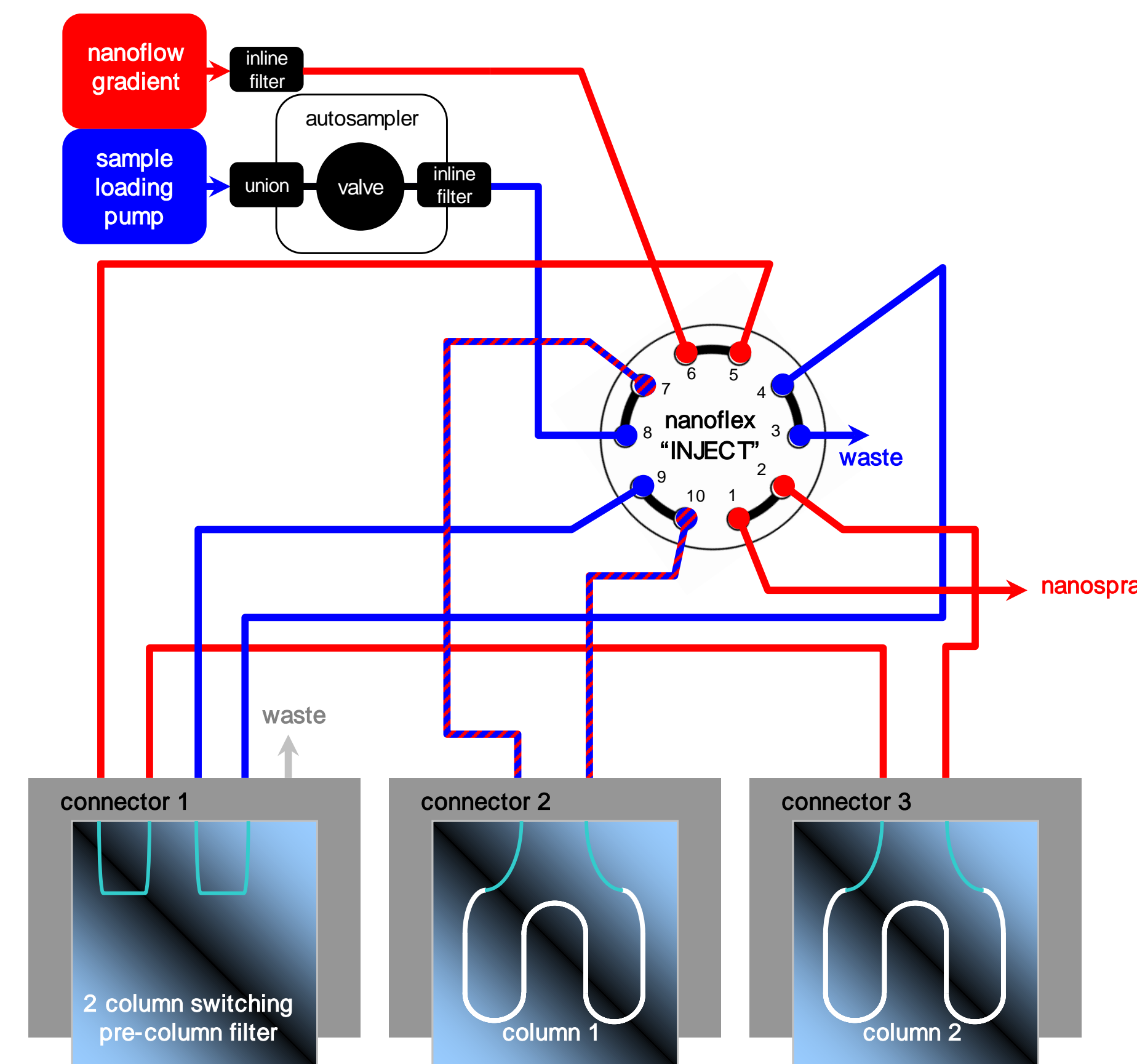
Introduction

- 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
- Although RT stability of cHiPLC columns are guaranteed within 2% RSD, we observe < 1% RSD.

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.

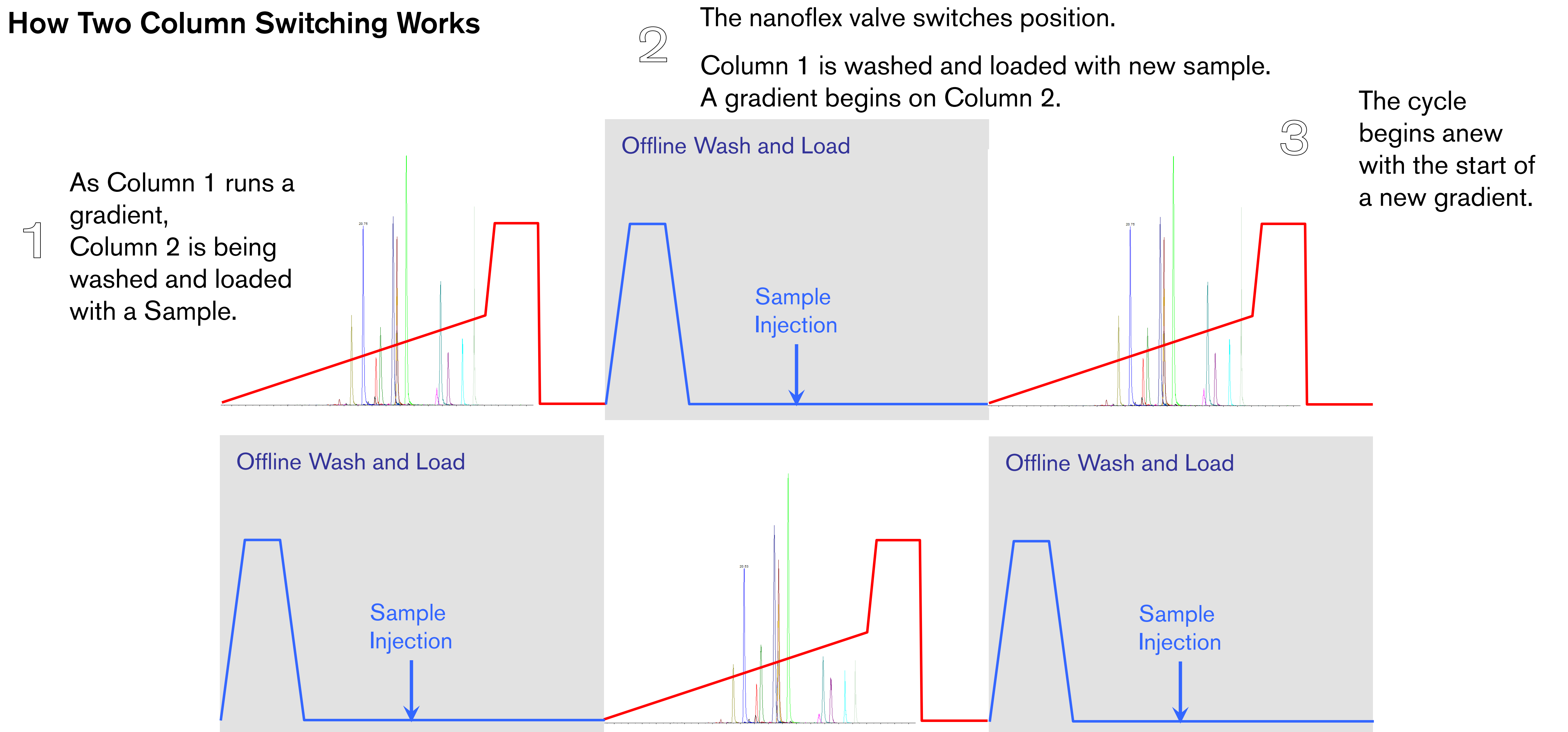
Other workflows are possible, including direct injection, and trap & elute.

Methods

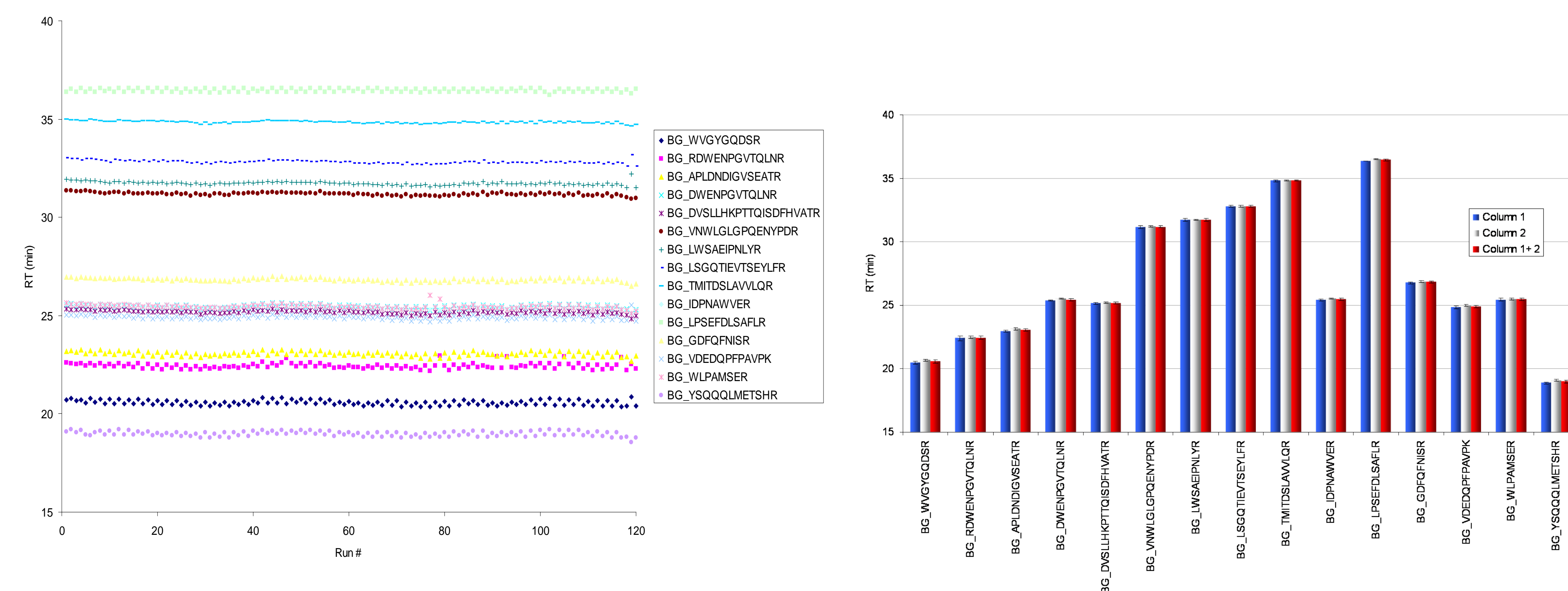
- Sample: 100 fmol Beta-galactosidase digest in a matrix of 100 nl/μl digested Yeast protein extract.
- Sample preparation: Proteins reduced and alkylated, followed by overnight trypsin digestion at 37°C. BSA, DTT and Iodoacetamide (Sigma Aldrich, St. Louis, MO), sequence modified trypsin (Invitrogen, Carlsbad, CA).
- LC separations performed using an Eksigent™ NanoLC Ultra 2D+ nanoflow system and a cHiPLC™-nanoflex microchip system.
- Gradient test method: gradient rate set to 1% 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-Pur C18AQ, 3 micron particles, 120 Angstrom pore (Dr. Maisch, Germany).
- Column dimensions: 75 micron x 150 mm
- MS analysis acquired using a AB SCIEX™ 4000 QTRAP®. Data acquired in MRM mode, monitoring 15 ions, 30 ms per transition.
- Data analysis was performed using MultiQuant™.

Results and Discussion

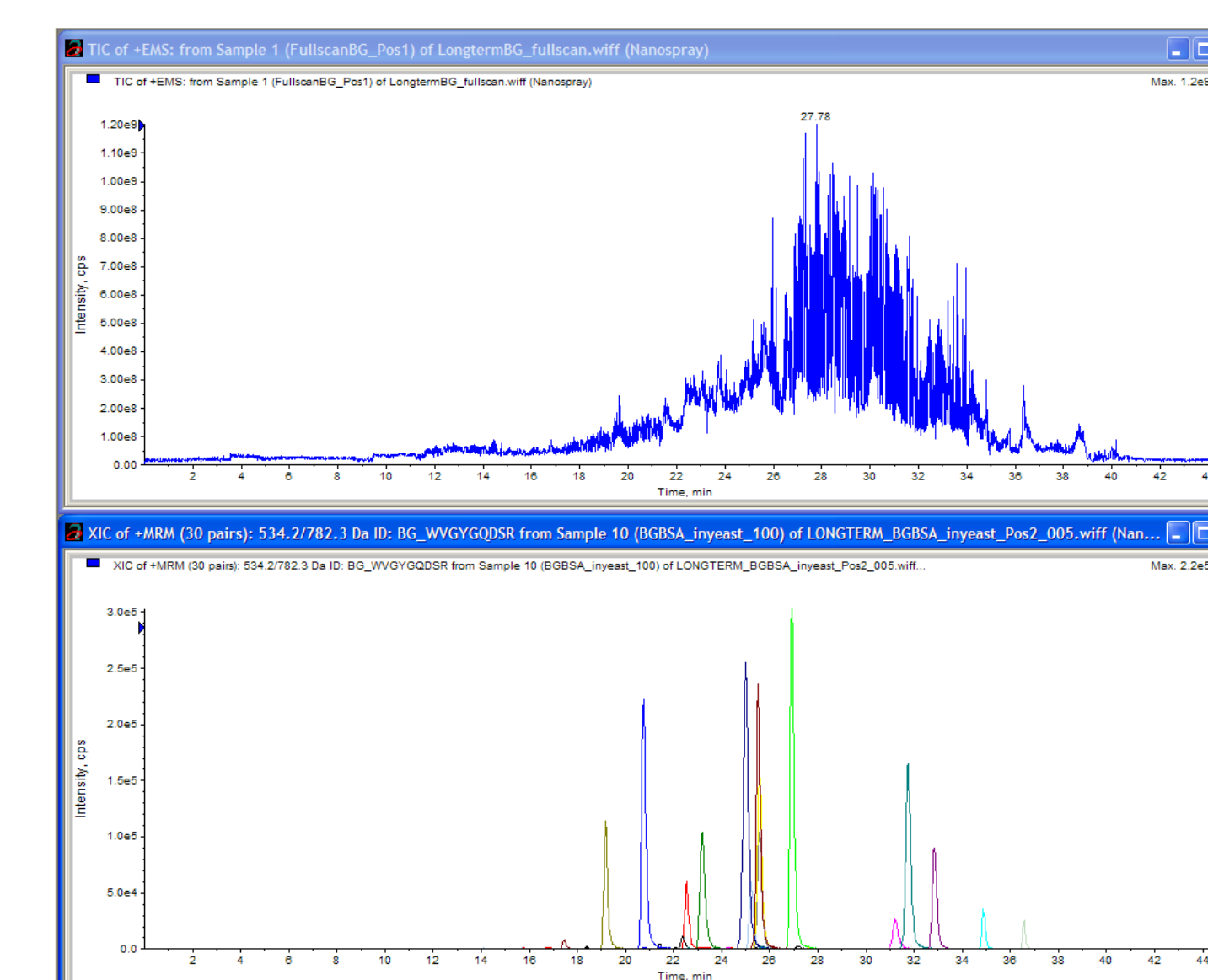
How Two Column Switching Works



Two-Column Performance Over 120 Consecutive Runs



Complex Sample Matrix



100 fmol beta-galactosidase in 100 ng/ul digested yeast protein extract.

The complex mixture provides a more realistic sample environment for evaluating method robustness.

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

This data set includes LC-MS runs that span 5 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, clinical user, or multi-lab collaborations this kind of retention time stability can be utilized for developing standardized methods for proteomics.

Acknowledgements

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