Improving Proteomics Reproducibility using Chip-Based Nanoflow Chromatography

Two-Column Switching Workflows Enabled by the cHiPLC® System

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Nanoelectrospray LC-MS remains a dominant technique in proteomics due to its high sensitivity and specificity. The blueprint for success using this technique includes the use of pulse-free nanoflow gradient delivery, high performance chromatographic separations, a bright ion source and a fast scanning, sensitive mass spectrometer.

However, nanoflow LC has a number of challenges that has limited its adoption in higher throughput environments. Making low dead volume connections remains a technique-dependent art for many users. Reproducibility of nanoflow columns has traditionally been poor limiting the utility across large sample sets. And finally the low solvent flow rate means that loading sample, running gradients and re-requilibrating the columns takes a long time, limiting throughput.

In this application note, we will describe the Two-Column Switching multiplexed workflow, and explain how the connector system and microfluidic chip columns are integral to the ultimate reproducibility and reliability of the workflow in the context of peptide quantification and standardized higher throughput proteomics.

Key Features of the cHiPLC® System

- The cHiPLC® system is a microchip platform that allows columns and traps to be easily installed for plug-and-play simplicity, without any sacrifice in chromatographic fidelity.
- This flexible system allows for direct injection, trap-elute, two-column switching and serial two-column nanoLC-MS workflows
  - Microfabrication technology for producing the microfluidic chips ensures excellent column-to-column reproducibility (<2%)
  - Unique connection system makes changing columns and traps easy, precise low dead volume connections are made easily every time
  - Easily enables a multiplexed two-column switching workflow that maximizes MS duty cycle by putting autosampler, washing and regeneration steps offline.

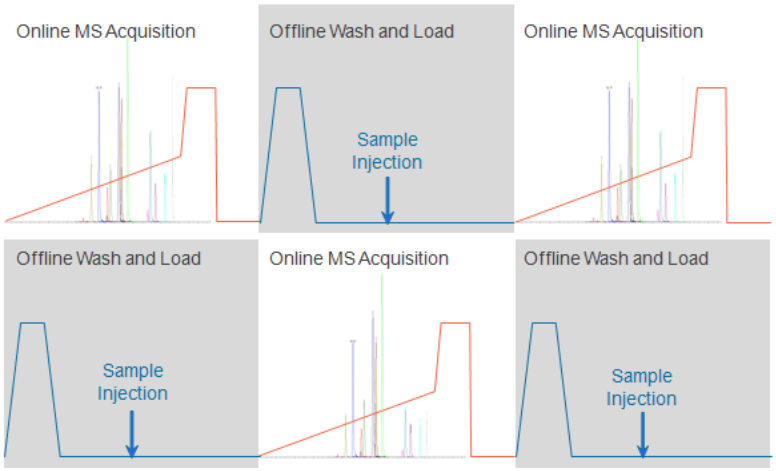

Figure 1. Two-Column Switching Workflow using cHiPLC® system. This easy-to-setup workflow provides up to a 2-fold increase in throughput. One column is used for running the biological sample, while the other column is regenerated offline.

Methods

Sample Preparation: A yeast lysate was isolated and digested. Protein and peptide standards were purchased.

Chromatography: The sample was analyzed using the nanoLC Ultra® 2Dplus System combined with the cHiPLC® system in Two-Column mode with two nano chip columns (75 µm x 15 cm ChromXP™ C18-CL 3 µm 300 Å). The elution gradients consisted of 10-30% acetonitrile (0.1% formic acid) of varying gradient lengths. The concept for the Two-Column Switching workflow for multiplexing two columns is represented in Figure 1 as a three run snapshot. Column 1 is represented by the top trace, and Column 2 is on the bottom. In the first run, Column 1 is online and Column 2 is being washed, equilibrated and loaded in the offline position. As soon as the run 1 gradient is over, Column 2 is switched to the online position, and run 2 begins. Now Column 1 is offline being washed, equilibrated and loaded. At the end of run 2, the columns are switched, and run 3 begins immediately.

Mass Spectrometry: Analysis was done using either a 4000 QTRAP® or a QTRAP® 5500 LC-MS/MS systems equipped with a NanoSpray® III source. Data was acquired in either full scan EMS mode or using MRM analysis.

Data Processing: All data was processed using MultiQuant™ Software.

Reproducibility of Chip Manufacturing

An important challenge to achieving reproducible nanoLC separations is in the production of nanoflow columns. By traditional packing methods, a typical set of nanoflow columns packed in capillaries can vary in total packed bed length by several millimeters, or more. This will result in analyte retention times that vary greatly and provide little in terms of column-to-column reproducibility. By contrast, cHiPLC column chips are all exactly the same length because the chips are produced lithographically, using the same techniques as in the semiconductor industry. Combined with SCIEX's proprietary packing process, columns are produced that achieve analyte retention time reproducibility of < 2% column-to-column (Figure 2).

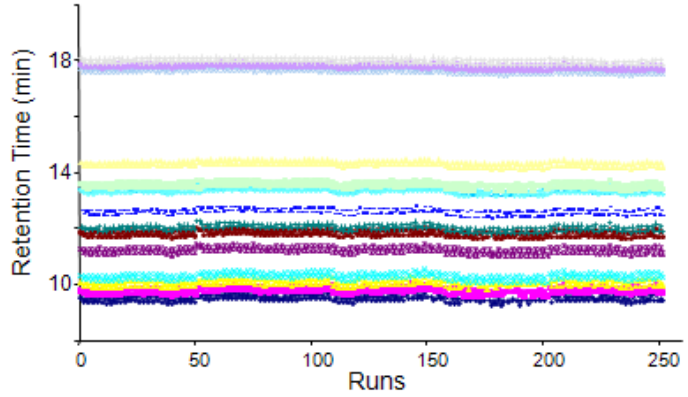

Figure 3. Retention Time Reproducibility of Simple BSA Mixture. The stability in retention time was assessed using replicate injections of a BSA digest. Shown is the measured retention time on both columns across 320 consecutive runs (64 were blanks).

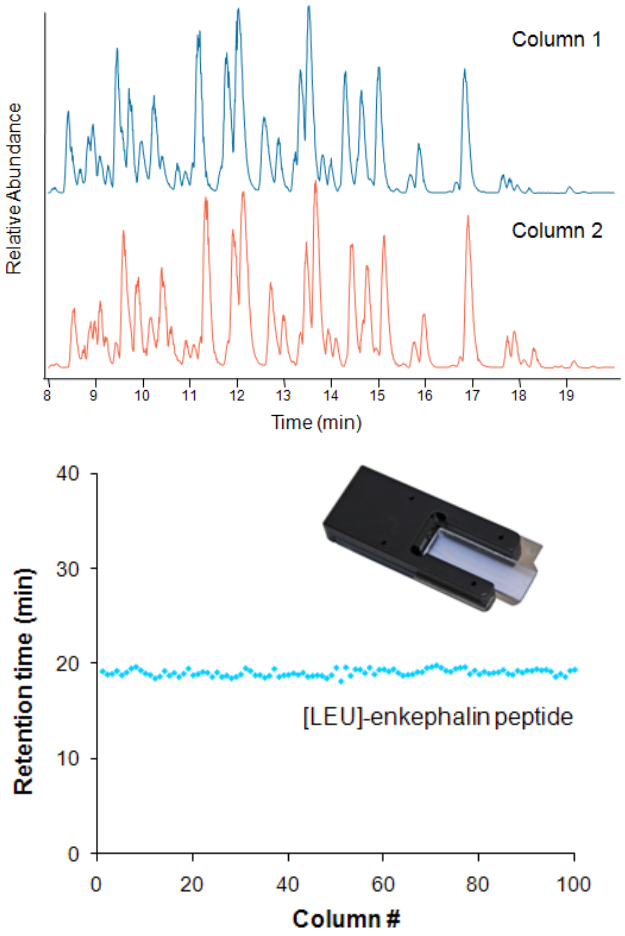

Figure 2. Matched Columns. (Top) BSA reproducibility between columns. (Bottom) Testing peptide RT on 100 separate chip columns. <2% RSD.

cHiPLC chips operate as a part of the Eksport™ connector system. The Eksport connector is a high performance chip-to-world interface that operates essentially as the socket for plug-and-play cHiPLC chips. The Eksport forms a very low-delay volume connection with cHiPLC chips, and the connection can be made and remade without any change in performance, and without any user adjustments. This kind of set-and-forget convenience is the hallmark of the cHiPLC system, and sets the stage for truly standardized proteomics workflows.

Long Term Retention Time Stability in Complex Matrix

To test the long term reproducibility of this Two-Column Switching workflow, a stability study using a simple BSA protein digest was run. Using a 30 min linear gradient and offline column regeneration, 320 total injections (including 64 blanks) were run (Figure 3). Very high retention time reproducibility was demonstrated.

In order to demonstrate the retention time reproducibility of this workflow a complex matrix, Beta-Galactosidase (BGal) digest was spiked into a 100 ng/µL yeast extract digest to a concentration of 100 fmol/µL. Using a 45 min gradient program, and offline column regeneration, the run-to-run cycle time was 45 minutes. A total of 100 injections were run, for a total acquisition time of 3 days and 3 hours. Figure 4 shows a TIC of a yeast extract digest, followed by a XIC of the MRMs for BGal, from the same sample.

Figure 5 plots the retention times of MRM transitions to some of the yeast and BGal peptides over the course of 100 runs using the Two-Column Switching workflow. The yeast / BGal sample is analyzed with excellent retention time reproducibility, showing that increased sample complexity has little or no effect on the effectiveness of separations on the cHiPLC® system.

Figure 6 is a representation of the yeast peptide retention times with error bars that represent the standard deviation of the replicate runs as achieved with Two-Column Switching workflow.

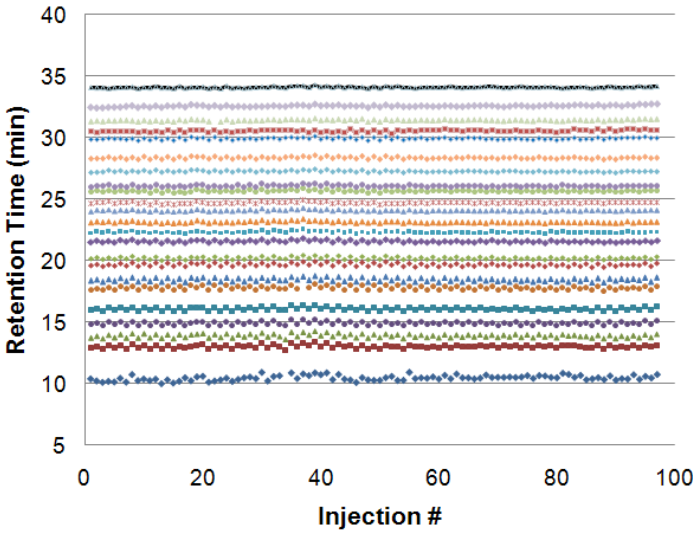

Figure 5. Retention Time Reproducibility in Complex Matrices. The retention times of yeast and Beta galactosidase peptides over the course of 100 nanoflow LC runs using the Two-Column Switching workflow are plotted.

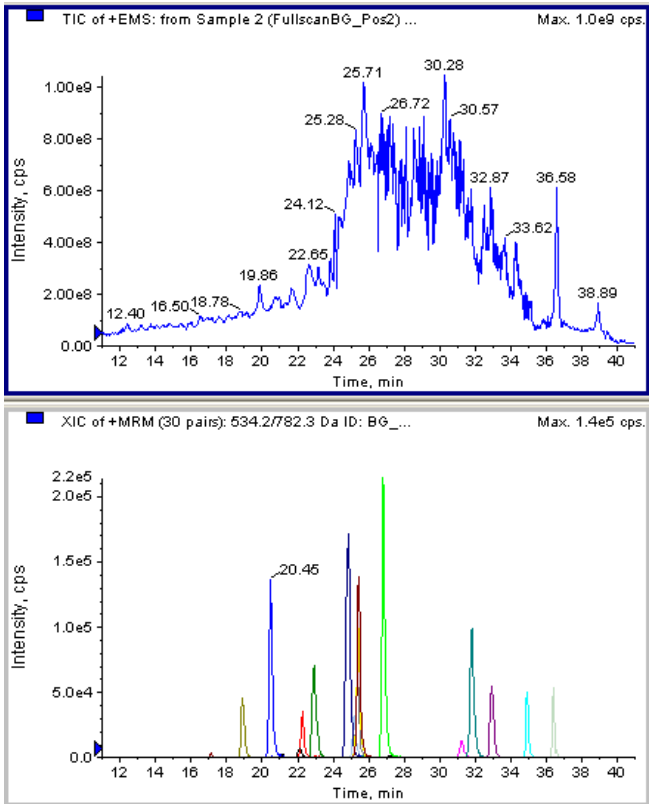

Figure 4. Two-Column Switching Workflow in Complex Matrices. Protein digest (BGal) was spiked into a digested yeast cell lysate. (Top) Total ion chromatogram of the EMS scan shows the complexity of the sample on column. (Bottom) MRM data on the spiked protein shows the quality of the chromatography within this complex mixture.

The Throughput Advantage

The true value in the Two Column Switching workflow is the time it saves, by putting the time-consuming steps offline (sample loading, column washing and regeneration). These steps are easily accommodated off-line during Two-Column Switching. In a traditional single column workflow, 47 mins for sample pickup, injection and loading would be required in addition to the 45 min linear gradient analysis, giving an inject-to-inject cycle time of 92 mins; 100 samples analyzed in this way would take 6.4 days.

By contrast, the same linear gradient used now in a Two-Column Switching workflow is limited to just 45 min total cycle time; 100 samples analyzed using this workflow takes 3.1 days, less than half of the time needed in the traditional single column workflow. In addition, column washing (for reduced carryover) can be incorporated into the offline column regeneration, which is a further time savings, reducing the number of blanks that are required for low level quantitative work.

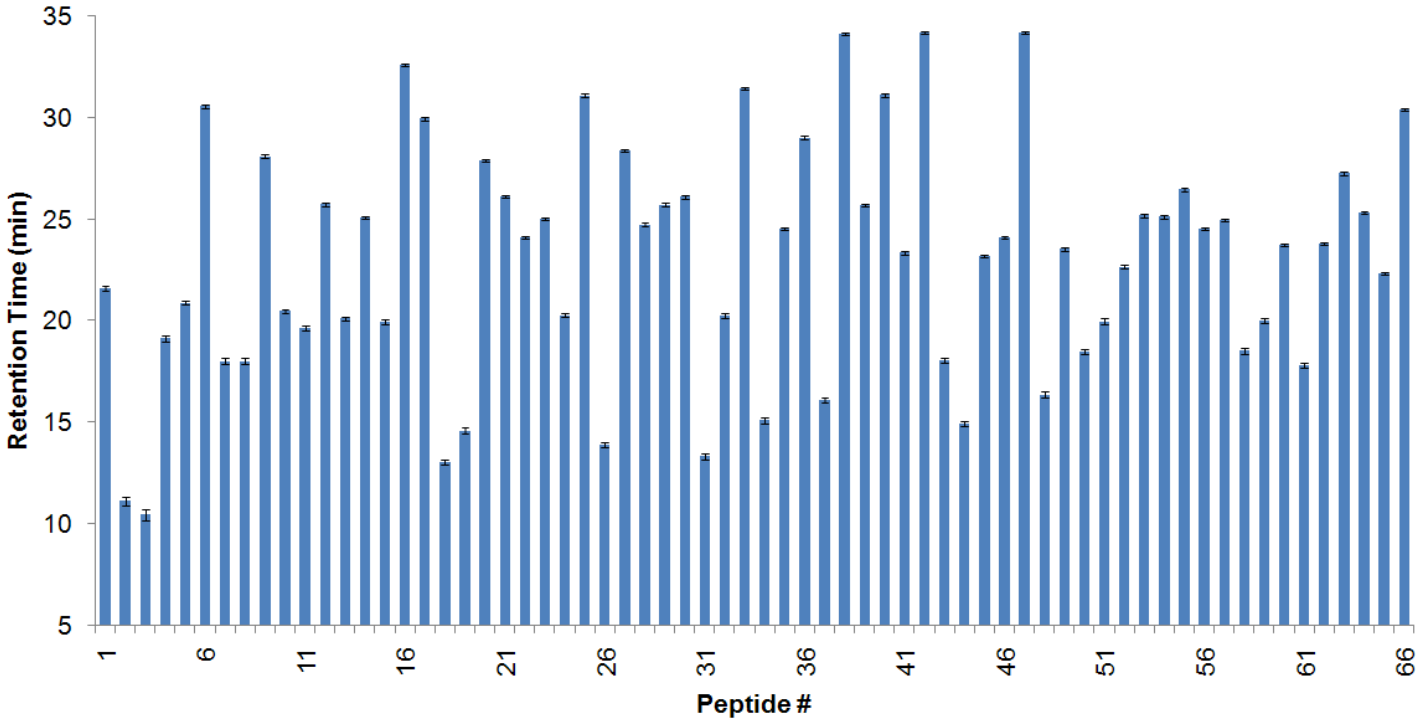

Figure 6. Retention Time Reproducibility Across the Gradient. The retention times for yeast and BGal peptides are plotted with error bars indicating the standard deviation across 100 nanoflow LC runs.

Conclusions

- The flexibility of the cHiPLC® system provides users with many different workflows, to suit the chromatographic needs of their proteomic experiments.
- The Two-Column Switching workflow is a powerful workflow for nanoflow LC, uniquely enabled by the performance attributes of the cHiPLC® system.
- Column-to-column reproducibility of <2% RSD means all columns are matched and the same methods can be run on each column and easily compared.
- Coupled with the SCIEX nanoflow LC systems, the stability of the system enables very long batches.
- Finally, the throughput enabled by this efficient workflow means the MS time is approaching 100% utilization and many more samples can be run per day.

References

1. Increasing Depth of Coverage using Serial Two-Column Workflows. SCIEX Technical Note, Publication RUO-MKT-02-2888-A.

Document number: RUO-MKT-02-2893-A

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