

cHiPLC™-nanoflex system:

Two-column switching using microfluidic LC chips increases throughput for proteomic LC-MS analysis

AUTHORS:

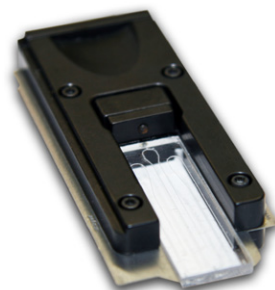
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Eksigent's new cHiPLC-nanoflex, in combination with the nanoLC-Ultra system, delivers superior sensitivity, column-to-column reproducibility, and exceptional ease of operation.

Introduction

75 μm x 150 μm cHiPLC nano column



Increasing throughput in LC-MS experiments by alternating between two columns is an idea initially used by the pharmaceutical industry, typically at microbore and analytical flow rates. These are highly time-saving methods, where one column is online with the MS while the other column is switched off-line, and available to be washed, re-equilibrated and loaded with sample. This innovative approach can achieve up to a twofold increase in throughput by placing the time consuming re-equilibration and sample loading off-line from the MS.

However, one of the challenges is that columns made for use at nL/min flow rates have not typically exhibited very good reproducibility. What is needed are reproducible columns that can be used interchangeably and can operate in this mode for long periods of time.

Eksigent offers a nanoLC separation system that encompasses a nanoflow LC pump and a microfluidic separations module called the cHiPLC-nanoflex system. Columns are produced in quartz using techniques from the semiconductor industry. The resulting cHiPLC columns are highly reproducible and exhibit inter-column retention time stability within 2% RSD for proteomic separations.

With the cHiPLC-nanoflex separations module, these microfluidic columns offer the ability to perform column switching workflows with great reproducibility. The key advantages of two-column switching workflows will be discussed in this note, and include:

- Increased throughput, up to 2x for short proteomic gradients.
- Lower carryover: persistent, sticky peptides can be washed using offline gradients.
- Retention time reproducibility: Peptides will have inter-column retention time reproducibility of < 2% RSD.

Experimental

- Sample: 100 fmol Beta-galactosidase (BG) digest in a matrix of 100 ng/ μ l digested Yeast protein extract.
- LC separations performed using an Eksigent NanoLC Ultra 2D+ nanoflow system and a cHiPLC-nanoflex system.
- LC Gradient: gradient rate set to 1% B/min from 5% to 35% B, followed by a step to 90% B. Total flow rate was 300 nl/min. A: 0.1% formic acid in Water, and B: 0.1% formic acid in Acetonitrile.
- LC column: Microchips were packed with ReproSil-Pur C18AQ, 3 μ m particles, 120 Angstrom pore (Dr. Maisch, Ammerbuch, Germany). Column dimensions: 75 μ m x 150 mm.
- MS data was acquired using an AB SCIEX™ 4000 QTRAP®. Data acquired in MRM mode, monitoring 15 ions, 30 ms per transition.
- Data analysis was performed using MultiQuant™.

Results & Discussion

Two-column switching using the cHiPLC-nanoflex system: high throughput and carryover

A plumbing diagram of the cHiPLC-nanoflex system is shown in Figure 1. For the two column switching workflow, a workflow jumper chip and two column chips are loaded into the Nanoflex. The columns are used in alternating fashion: while one column is online with the LC-MS, the other is offline being washed, equilibrated and loaded with the next sample.

Figure 2 illustrates these events with a three-run snapshot taken from a typical multi-run sequence. Two traces, top and bottom, represent how the Nanoflex switches between the two chip columns:

- Column 1 (top trace): gradient elution, followed by column equilibration and loading, then another gradient elution (online-offline-online).
- Column 2 (bottom trace): this channel is undergoing the opposite cycle of events (offline-online-offline).

Essentially, the Nanoflex switches between the two columns as the sample queue advances; by the time the first LC-MS run is complete, the second column is ready for the next LC-MS analysis.

The two-column switching workflow can significantly reduce sample carryover between runs. By programming gradients into the offline equilibration/sample loading method, the column can be very thoroughly washed [1].

Stable chromatography for scheduled quantitation methods

For biomarker validation studies requiring accurate peptide quantitation, peptide Multiple Reaction Monitoring (MRM) and MIDAS™ (MRM Initiated Detection and Sequencing) workflows are the natural routes to take when verification by antibody techniques are unavailable or impractical.

These studies can sometimes require the quantitation of many hundreds of MRM transitions in a single run. For the best sensitivity, these MRM scans can be scheduled such that the MRM channel is only open for a small window around the time period that the peptide is expected to elute. This reduces the concurrency of MRM scans during the time period of any group of co-eluting peptides, enhancing the sensitivity of the peptide quantitation.

This approach, however, requires very reproducible chromatography. In accelerating peptide quantitation studies using two-column switching, it is of the utmost importance that the columns exhibit optimal column-to-column reproducibility[2].

Because the Eksigent cHiPLC columns are factory tested and are guaranteed to match in peptide retention time performance to within 2% RSD, two-column switching workflows become practical for accelerating scheduled MRM methods.

In addition, the cHiPLC-nanoflex system provides a plug-and-play interface that combines ease of use with an industry leading, high performance microfluidic chip interface.

Stable performance with samples in complex matrices

A sample containing 100 ng/ μ l of a digest of yeast cell lysate was spiked with 100 fmol/ μ l beta-galactosidase (BG) digest and analyzed; the separation was carried out using the two-column switching workflow, and the BG peptides were monitored by MRM.

Over the course of 2.5 days, the sample was analyzed in 120 consecutive runs. The complexity of the sample is represented in Figure 3, which shows a screen shot of the Total Ion Chromatogram (TIC) of the LC-MS analysis; below this is an Extracted Ion Chromatogram (XIC) of the BG peptides monitored by MRM. Despite the complexity of the sample matrix and the short gradient, the BG peptides have good peak shapes, i.e., are narrow and symmetrical.

The stability of the peptide retention times are represented in Figure 4, which graphs the retention times of each peptide for each of the 120 runs. The RSD for the entire data set is less than 1% RSD for each peptide in this two-column switching workflow.

Conclusions

Two-column switching with the cHiPLC-nanoflex system can increase throughput in LC-MS based proteomic studies

The cHiPLC-nanoflex system provides a robust, plug-and-play platform on which to build proteomic workflows; the zero dead volume microfluidic channels yield minimal peak dispersion and high efficiency LC separations.

As demonstrated for a complex sample, the two-column switching strategy permits automated, unattended multi-run sequences to be performed with excellent retention time precision.

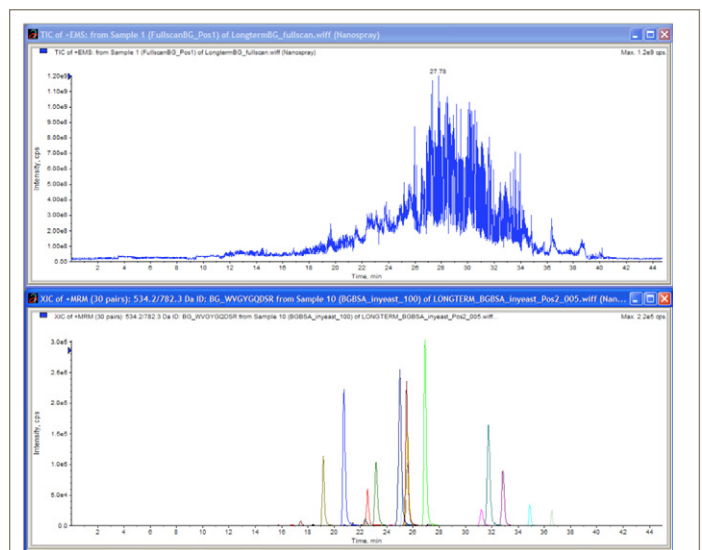
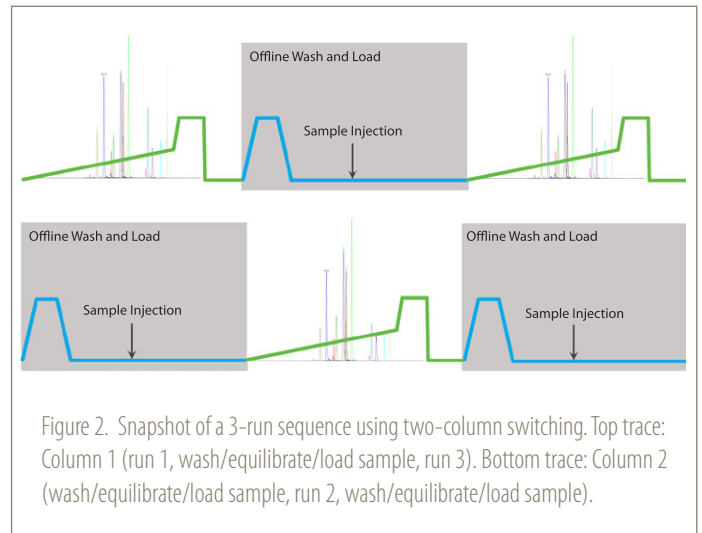
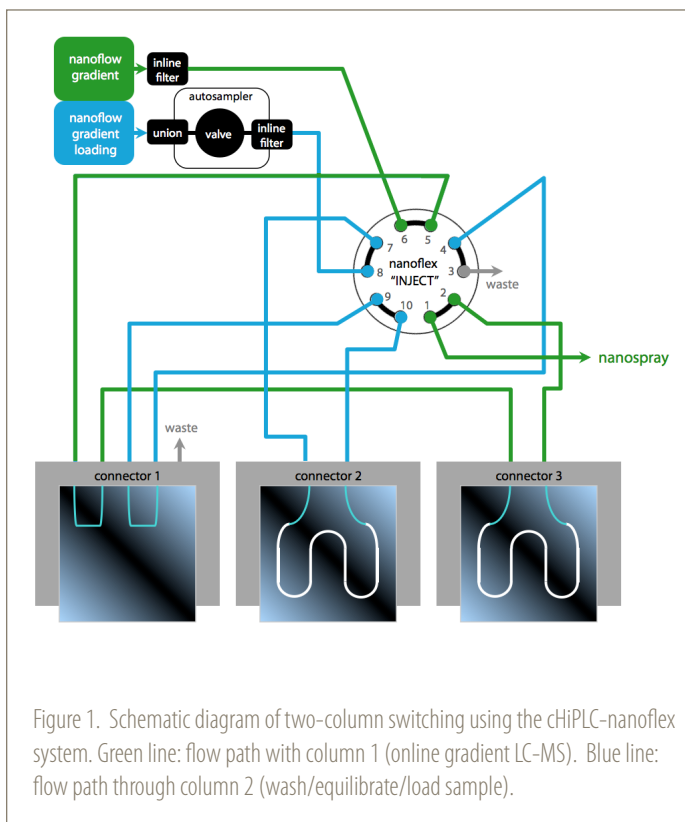


Figure 3. Total Ion Chromatogram (TIC) of a yeast cell lysate protein/spiked BG digest sample (top). Extracted Ion Chromatogram (XIC) of the BG peptides.

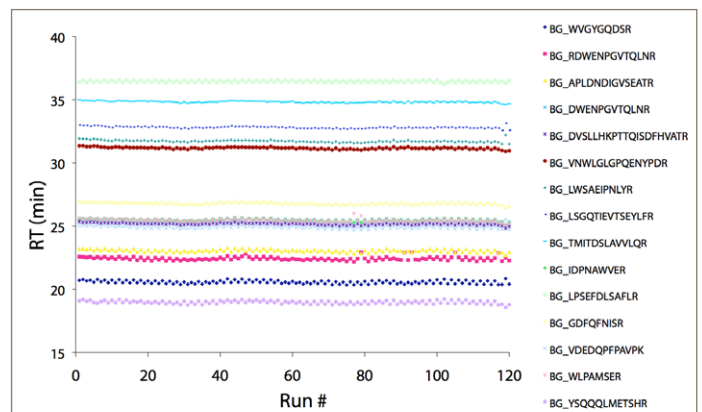


Figure 4. Retention times of BG peptides for 120 consecutive runs using the two-column switching workflow.

References

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About Eksigent Technologies

Eksigent is creating new possibilities for life science research and drug discovery & development with its innovative MicroFlow™ and NanoFlow™ fluid delivery systems. Eksigent's LC systems deliver dramatic increases in analysis speed, throughput, and sensitivity. Today, leading research, pharmaceutical, and biotechnology firms around the world use Eksigent's innovative solutions.

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