200 µm ID C18 Reverse Phase Micro Columns for the cHiPLC®-Nanoflex System

Robust, Higher Throughput Separations by cHiPLC nanoflow LC MS

Nanoflow liquid chromatography coupled with mass spectrometry (nanoLC MS) is the method of choice for sensitive peptide and protein identification and quantification. While providing excellent sensitivity, the low flow rate reduces sample throughput due to gradient delay in the nanoLC system itself, delay in the autosampler and sample loop, and the delay caused by the connecting tubing.

One way to address this is to use a larger inner diameter column at a proportionally higher flow rate. While this will reduce sensitivity when the total sample amount is kept equal, the delay times can be greatly reduced and sample throughput accelerated. Reducing the column and gradient lengths can further reduce the analysis time required per sample.

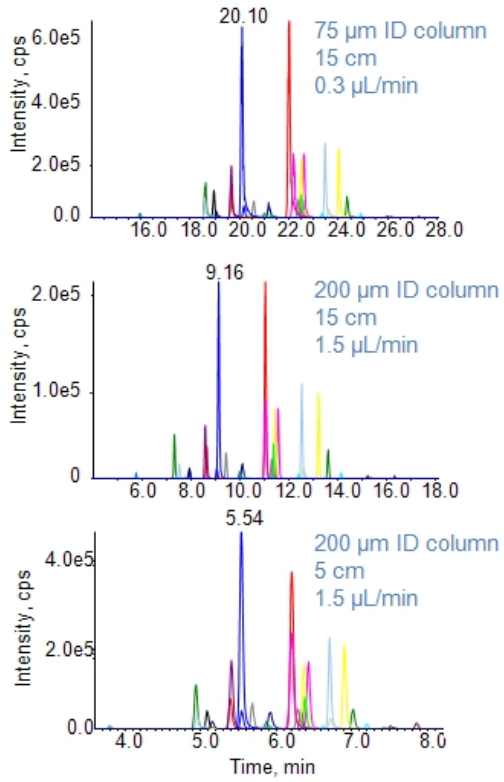

Figure 1. Comparison of Column Length and Diameter of cHiPLC Columns. BSA and Beta Galactosidase tryptic peptides were separated on 15 cm columns of different ID's using a 15 minute gradient using a AB SCIEX QTRAP® 5500 System. Using the same flow rate of 1.5 µL/min on a 200 µm ID column, the analysis time can be significantly shortened by using a 5 cm column and running a 5 minute gradient.

Advantages of the cHiPLC®-NanoFlex and the cHiPLC Technology

- **Easy:** Plug & play chip simplicity
- **Extendable:** Flexibility to switch between workflows and projects rapidly in multi-user labs
- **Every time:** Reproducible results from day-to-day, column-to-column and lab-to-lab

Decrease Run Times and Increase Throughput with Larger Diameter 200 µm ID micro cHiPLC columns

Using a mixture of bovine serum albumin (BSA) and Beta Galactosidase (BetaGal) digests, the chromatographic performance of a 200 µm ID micro cHiPLC column was compared with a 75 µm ID chip (Figure 1). Using the higher flow rates, it is possible to decrease the total run time of the analysis from 28 to 18 minutes due to faster sample loading and reduced delay for peak elution. The improved chromatography at the higher flow rates (narrower peaks) helps to reduce the loss in peak height to only ca. 3x in going from 0.3 µL/min with the 75 µm ID column to1.5 µL/min with the 200 µm ID column.

Table 1. C18 Reverse Phase Micro cHiPLC®-Nanoflex Chip Options.

Description	Dimension	Stationary Phase	Flow Range	Part Number
C18 cHiPLC column	*15 cm x 200 µm*	*ChromXP C18-CL 3µm, 120 Å*	*1 – 2.5 µL/min*	*5015840*
C18 cHiPLC column	*5 cm x 200 µm*	*ChromXP C18-CL 3µm, 120 Å*	*1 – 2.5 µL/min*	*5015839*
C18 cHiPLC trap	*6 mm x 200 µm*	*ChromXP C18-CL 3µm, 120 Å*	*1 – 5 µL/min*	*5015841*

Further improvement in throughput can be obtained by using a 5 cm column in conjunction with running a shorter gradient (Figure 1, bottom). However, because of the shorter column length, peak capacity is reduced. While peak area is reduced for all analyses run at 1.5 µL/min versus 300 nL/min, the use of a short column and fast gradient leads to narrower peaks, resulting in an overall loss of peak height of only a factor of ~2-3 (Figure 1).

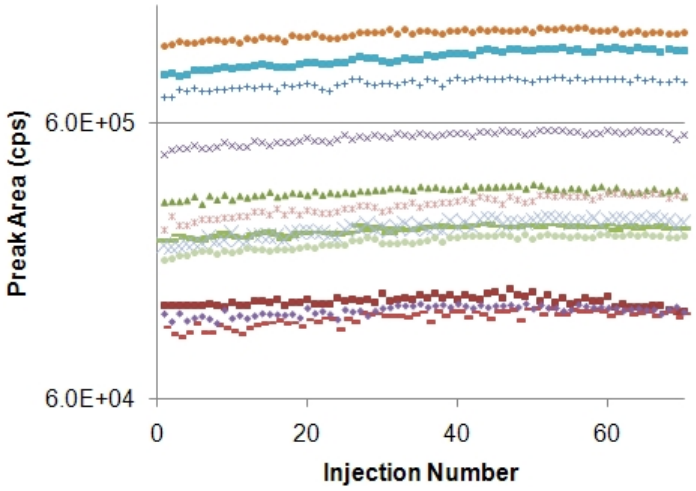

Figure 2. Reproducibility of Peptide Quantitation. Replicate injections of BetaGal peptides analyzed by MRM in a complex E.*coli* digest were analyzed across 80 injections using a trap-elute methods with a short 5 min gradient on a 5 cm column at 1.5 µL/min. Reproducibility of peak areas were measured and found have minimal variation, with average CV across the 15 peaks was ~7 %.

Robust Quantitation using Trap and Elute with 200 µm ID Chips

A standard digest of BetaGal was spiked into a digested *E.coli* cell lysate and analyzed on the 200 µm ID x 5 cm column at 1.5 µL/min in trap elute mode. The reproducibility across 70 injections was analyzed. The average variation seen in retention time across the 12 peaks analyzed was 0.2% RSD and the average variation in peak area was ~6% (Figure 2), demonstrating excellent robustness of the cHiPLC system for peptide quantitation in complex matrices.

Summary

- The 200 µm ID nanoflex chip allows samples to be run at higher flow rates and therefore with higher throughput
- The higher diameter column also provides increased robustness, ease of use and sample loading capacity over the traditional 75 µm ID format of nanoflow LC
- Reducing the column length from 15 cm to 5 cm, in conjunction with faster gradients, decreases run times further at the cost of a loss in peak capacity, but results in only a minimal loss in peak height compared to using 75 µm ID chips.
- For many applications, the increased throughput could outweigh the reduced peak capacity and sensitivity

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