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Micro-flow Peptide Monitoring Using a Kinetex™ XB-C18 Core-Shell Column to Improve Sensitivity

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Introduction

While mass spectrometry coupled to liquid chromatography has proven to be a powerful tool in proteomics studies, proteomics research is often challenged by the complex nature of its samples. The identification and quantification of peptides of interest can prove particularly difficult when dealing with small amounts of samples, small volumes, and complex sample matrices. Liquid chromatography column miniaturization can overcome these challenges. Reducing column inner diameter results in a reduction in chromatographic dilution and increased sensitivity allowing for more efficient MS/MS sampling and thus a higher number of molecule identifications. Furthermore, another way to improve ion sensitivity is to use a core-shell particle. These particles consist of a solid non-porous core surrounded by a porous silica layer containing the chromatographic stationary phase. By using core-shell technology, the peptide diffusion path is reduced which leads to sharper chromatographic peaks and thus increased ion intensities and higher peak capacities. Most of the studies done on core-shell are focused on analytical flow chromatography. In this technical note, we investigate the impact of a sub-2 µm fully porous particle and a core-shell particle in micro-LC-MS/MS to see the effect they have on peptide separations using a commercially available mixture of 20 synthetic peptides with a wide mass and retention time range coverage (**Table 1**).

LC Conditions

Columns: Kinetex 2.6 µm XB-C18 (core-shell)
Waters® nanoEase™ M/Z Peptide BEH 1.7 µm C18 (sub-2 µm fully porous)
Thermo Scientific® Acclaim™ PepMap™ 100 2 µm C18 (fully porous)

Dimensions: 50 x 0.3 mm (Kinetex, Waters, Thermo Scientific)

Part No.: 00B-4496-AC

Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	2
20	40
20.1	80
25	80
25.1	2
30	2

Flow Rate: 10 µL/min

Injection: 1 µL

Temperature: 40 °C

Detector: Q Exactive™ Plus Orbitrap™

System: NanoLC™ 425 (SCIEX®)

Detection: MS

Sample: SCIEX PepCalMix (20 fmol/µL)

MS/MS Conditions

Scan Type: Full MS SIM

Polarity: Positive

Resolution: 70, 000

Scan Range: 400 to 1650 m/z

AGC Target: 3E6

Maximum IT 200 ms

Table 1. SCIEX PepCalMix Peptide Amino Acid Sequences Used and Their Respective Monitored M+H Species.

Peptide Sequence	Analyte - m/z
AETSELHTSLK	408.5501+++
GAYVEVTAK	473.2602++
IGNEQGVSR	485.2530++
LDSTSIPVAK	519.7997++
AGLIVAEGVTK	533.3233++
LVGTPAEER	491.2656++
LGLDFDSFR	540.2734++
GFTAYYIPR	549.2863++
SGGLLWQLVR	569.8340++
AVGANPEQLTR	583.3136++
SAEGLDASASLR	593.8005++
VFTPLEVDVAK	613.3495++
VGNEIQYVALR	636.3527++
YIELAPGVDNSK	657.3450++
DGTFAVDGPGVIAK	677.8583++
YDSINNTEVSGIR	739.3615++
SPYVITGPGVVEYK	758.9105++
ALENDIGVPSDATVK	768.9034++
AVYFYAPQIPLYANK	589.3183+++
TVESLFPEEAETPGSAVR	643.6540+++

Results and Discussion

Bottom-up proteomics LC-MS/MS workflows heavily rely on LC chromatographic separation of peptides/peaks to acquire quality mass spectrometric data. In the proteomics world, it is common knowledge that the sharper chromatographic peaks (that have reduced peak widths and higher intensities) leads to high mass spectrometry quality data. It has been long known and successfully established that core-shell particles reduce an analyte's peak width due to less diffusion in them, which in turn results in narrower, sharper peaks and high ion intensities. In contrast, sub-2 µm fully porous particle columns have also been known to provide the same effect in ion intensity and peak shapes as core-shell particles, but with the disadvantage of generating much higher pressures due to the fully porous smaller particle size (**Table 2**).

A comparison of ion intensities and peak widths was made among a core-shell column, a fully porous column, and a sub-2 µm column. As observed in **Figure 1**, the core-shell column and sub-2 µm column produced peptide peak ion intensities of similar height for most peptides. Nonetheless, for this sample, significant peak tailing was observed for various peptides using the sub-2µm column. Different columns, blanks, and column cleaning steps were done in order to remove any possible contamination that could cause these peaks to tail. Peak tailing was reproducible and possibly due to the inherited chemistry of the column. Consequently, in order to compare peak widths and ion intensities, only those peptides that showed symmetrical peak shape were chosen (**Figure 2**).

As expected, the core-shell column and the sub-2 µm column produced total ion chromatograms with a higher maximum ion intensity than the fully porous particle column (**Figures 1** and **2**) for symmetrical peaks. Also, average peak widths were narrower in the core-shell column and the sub-2 µm column with increased peak capacities than in the fully porous column (**Table 2**). However, the sub-2 µm column's pressure was twice as much in comparison to the core-shell column's pressure, restricting the modification of flow rates if required (**Table 2**).

Figure 1. Extracted Ion Chromatogram (XIC) of Peptides on a Kinetex™ 2.6 µm XB-C18 column (Top), a nanoEase™ M/Z Peptide BEH 1.7 µm C18 Column (Middle), and an Acclaim™ PepMap™ 100 2.0 µm C18 Column (Bottom).

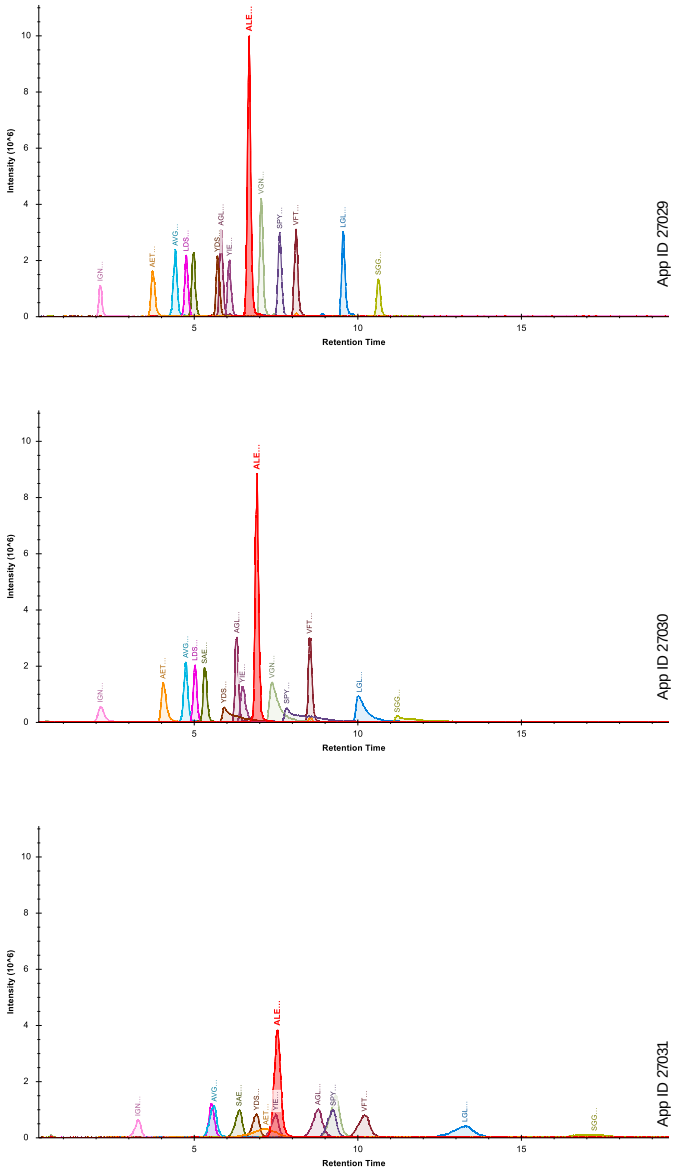

Figure 2. Ion Intensity Comparison for 8 Representative SCIEX® PepCalMix Peptides.

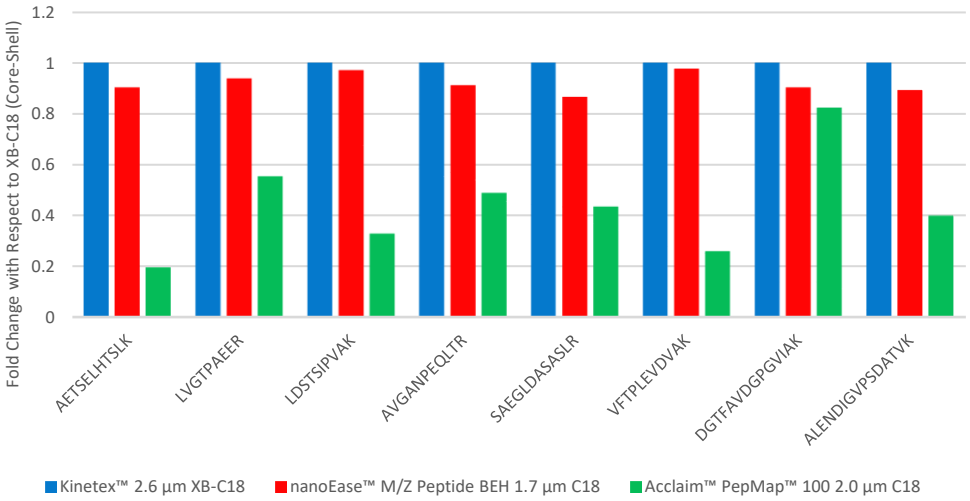

Table 2. Average Peak Widths, Peak Capacities and Corresponding Pressures for a 20 Minute Gradient Run of Peptide Mix.

Column	Average Peak Width	Peak Capacity	Pressure (bar)
Kinetex 2.6 µm XB-C18 (core-shell)	0.38	23.55	172
Acclaim PepMap 100 2.0 µm C18 (fully porous)	1.43	10.72	170
nanoEase M/Z Peptide BEH 1.7 µm C18 (sub-2 µm fully porous)	0.63	15.44	345

Conclusions

The selected core-shell column chemistry of the Kinetex 2.6 µm XB-C18 with its ability to reduce analyte diffusion path and silanol activity resulted in improved peak shapes and significantly increased intensities using 20 fmol of peptide on column. Additionally, its ability to resolve peptides with various molecular weights and inherited retention times makes the Kinetex 2.6 µm XB-C18 an appropriate selection for analyte characterization for proteomics studies.

Kinetex™ Micro LC Columns Ordering Information

2.6 µm Micro LC Columns (mm)						
Phases	**30 x 0.3**	**50 x 0.3**	**100 x 0.3**	**150 x 0.3**	**50 x 0.5**	**150 x 0.5**
XB-C18	00A-4496-AC	00B-4496-AC	00D-4496-AC	00F-4496-AC	00B-4496-AF	00F-4496-AF
Biphenyl	—	00B-4622-AC	—	00F-4622-AC	00B-4622-AF	—
C18	00A-4462-AC	00B-4462-AC	—	00F-4462-AC	00B-4462-AF	—
EVO C18	—	00B-4725-AC	—	00F-4725-AC	00B-4725-AF	—
F5	—	00B-4723-AC	00D-4723-AC	00F-4723-AC	00B-4723-AF	—

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