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Micro-flow Bottom-up Proteomics or Detection of Peptides at Trace Levels Using a Kinetex™ XB-C18 Column

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Introduction

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 trace amounts of samples, small volumes, and a variety of complex biological sample matrices. While mass spectrometry (MS) coupled to liquid chromatography (LC) has proven to be a powerful tool in the area of discovery proteomics, miniaturization of liquid chromatography columns can further overcome the sensitivity challenges associated with smaller sample volumes and complex sample matrices. Reducing the inner-diameter of the LC column results in reduction in the chromatographic diffusion. This, in turn, can increase the method's sensitivity up to 60X in comparison to analytical dimensions, allowing for more efficient MS/MS sampling and thus a higher number of unique peak identifications. Another way to improve ion sensitivity is to select a core-shell particle. These particles consist of a compact non-porous core surrounded by a porous silica layer and bonded with the stationary phase primary and secondary ligands. By using core-shell technology, the peptide diffusion path is significantly reduced. This results in sharper chromatographic peaks leading to increased ion intensities and higher peak capacities. Most of the proteomics studies done on core-shell particles are focused on analytical flow chromatography which unfortunately does not truly display the benefits of core-shell particles. Therefore, in this technical note we have compared and investigated sub-2 µm fully porous particles with core-shell particles in a micro-LC format to demonstrate the benefits of core-shell particles in peptide separation performance as well as relative peptide and protein identifications in a commercially available HeLa cell lysate tryptic digest.

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 2.0 µm C18 (fully porous)

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

Part Nos.: 00B-4496-AC (50 mm)
00F-4496-AC (150 mm)

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

MS/MS Conditions

Source Type: Ion Max Source with HESI-II probe and 50 µm ID needle

Scan Type: Full MS

Polarity: Positive

Resolution: 70, 000

Scan Range: 400 to 1650 m/z

AGC Target: 3e6

Maximum IT: 15 ms

Scan Type: dd-MS^2

Resolution: 35, 000

AGC Target: 1e5

Maximum IT: 45 ms

CE: 29

Isolation Window: 1.2 m/z

Results and Discussion

Bottom-up proteomics LC-MS/MS workflows rely on the fragmentation by parent ions to provide MS2 spectra containing enough peptide sequence information that can be assigned to a particular protein. Through increasing the parent ion intensity in the MS1 spectrum, higher fragment ion intensities (MS2s) will result (**Figure 2**). This provides high quality spectra which, in turn, generate larger numbers of unique peptide and protein identifications of any proteomics sample. It has been successfully established that core-shell particles reduce analytes' peak widths due to reduced diffusion in them. Narrower, sharper peaks and high ion intensities are the result. 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 1**).

As was expected, the core-shell column and sub-2 µm columns produced Total Ion Chromatograms (TICs) with a higher maximum ion intensity than the fully porous particle column (**Figure 1**). Also, the average peak widths were narrower in the core-shell column and the sub-2 µm column with almost identical increased peak capacities than in the fully porous column (**Table 1**). However, the sub-2 µm column's pressure was twice as much in comparison to the core-shell particle column's pressure, restricting the modification of flow rates if required. Moreover, the relative number of peaks that were repeatedly sequenced was significantly higher for the fully porous column due to its higher average peak widths, which leads to increased redundant MS2 fragment data generation (**Table 1**).

Figure 3 shows the relative number of unique peptides and proteins identified when using the core-shell, sub-2 µm, and the fully porous column. Again, as was expected, the core-shell and sub-2 µm columns resulted in a higher number of protein and peptide identifications. It is worth pointing out that the absolute number of unique groups found using the core-shell column, loading only 200 ng of digested protein, was 411. To improve protein IDs, a longer column can be used. Here, we also compared the number of protein IDs obtained with a 5 and a 15 cm long core-shell Kinetex™ 2.6 µm XB-C18 column. The number of IDs increased by almost 50% by using a column three times longer (**Table 2**).

Figure 1. Total Ion Chromatograms of 200 ng of HeLa Cell Tryptic Digest. Maximum Ion Intensity is Shown.

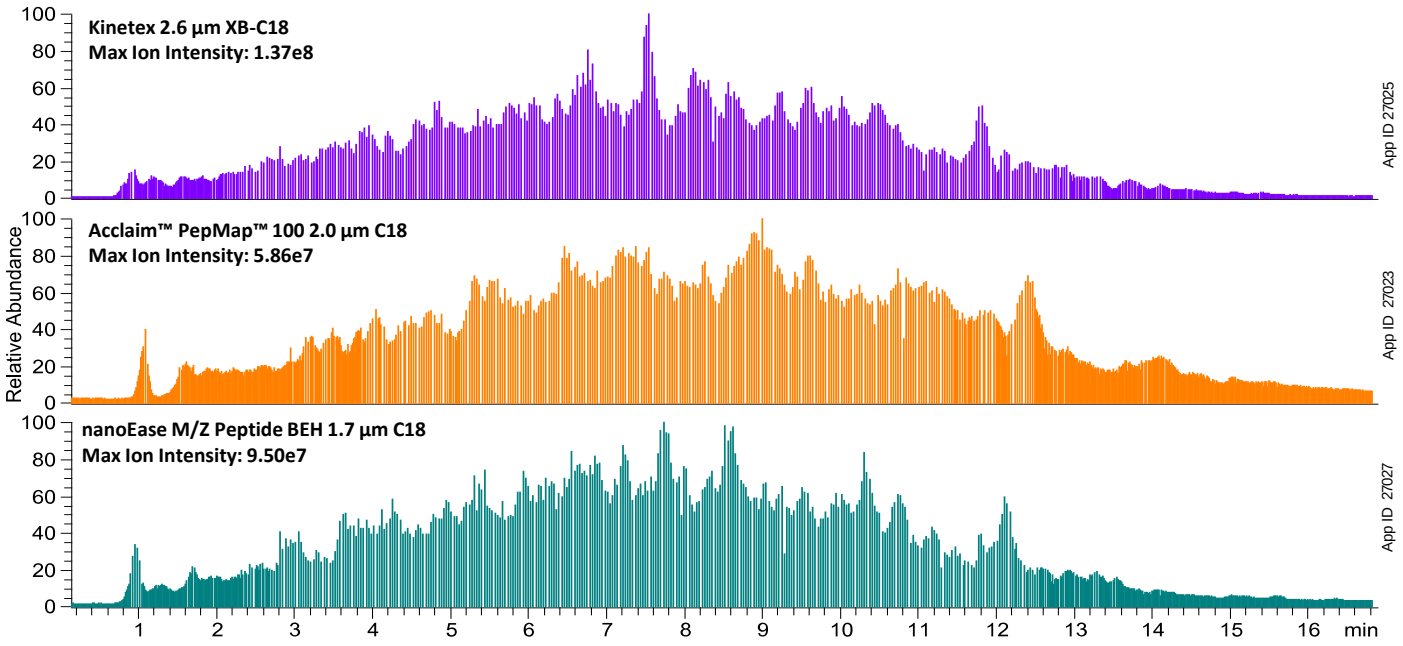

Table 1. Average Peak Widths, Peak Capacities and Corresponding Column Pressures (bar) for a 20 Minute Gradient Run of 200 ng of HeLa Lysate Tryptic Digest.

Column	Average Peak Width	Peak Capacity	Column Pressure
Kinetex™ 2.6 µm XB-C18 (core-shell)	0.55	29	172
nanoEase M/Z Peptide BEH 1.7 µm C18 (sub-2 µm)	0.64	29	345
Acclaim™ PepMap™ 100 2.0 µm C18 (fully porous)	0.75	21	170

Figure 2. MS/MS Fragmentation of a 19 Amino Acid Long HeLa Peptide with m/z 934.9887+2, when Using a Kinetex XB-C18 Core-shell Column (Top), a nanoEase M/Z Peptide BEH C18 sub-2 µm Column (Middle) and an Acclaim PepMap 100 C18 Fully Porous Column (Bottom).

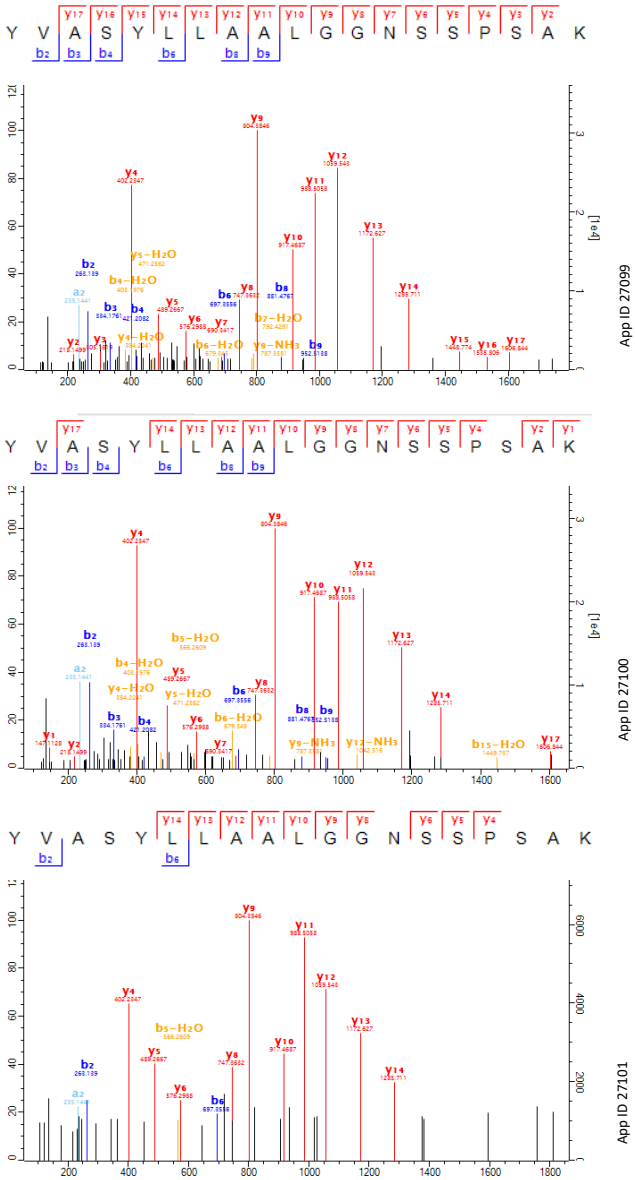

Figure 3. Relative Percent of Unique Peptide Identifications, Repeatedly Sequenced Peaks, and Unique Protein Identifications Found in a 20 min Gradient Run of 200 ng of HeLa Lysate Tryptic Digest.

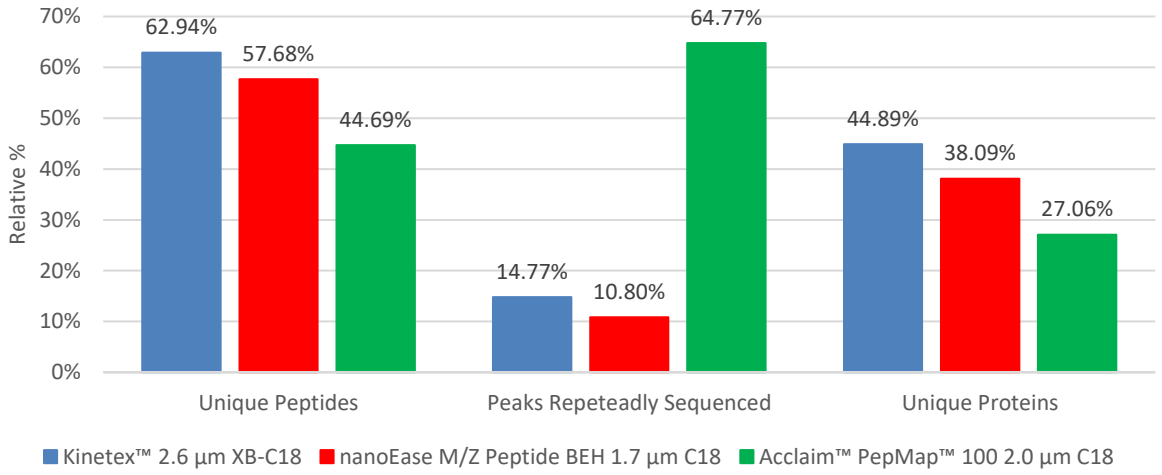

Table 2. Number of Unique Protein Identifications Found Using a 5 cm Long Kinetex 2.6 µm XB-C18 Column and a 5 cm Long Kinetex 2.6 µm XB-C18 Column with 200 ng of HeLa Lysate Tryptic Digest and the % Protein ID Increase Found by Using a Longer Column.

Column	Protein Groups
5 cm Kinetex 2.6 µm XB-C18	411
15 cm Kinetex 2.6 µm XB-C18	901
% Protein ID Increase	45.62%

Conclusions

Column miniaturization (reduction of column inner diameter) can be a powerful tool to increase ion intensities and thus the number of peptides and proteins identified in a particular sample. Apart from the smaller column inner diameters, core-shell technology can also further improve the peak shapes. This leads to improved ion intensities which enhances the number of unique IDs that can be detected in MS, as compared to what can be achieved through a conventional fully porous particle column. Another major advantage of core-shell technology is that the generated back pressure is almost half of what is generated with a sub-2 µm particle column. This is highly desirable in a micro-LC setting where complex sample components tend to accumulate in the column, further increasing the back pressures and leading to reduced column lifetimes.

In summary, it can be implied that miniaturized core-shell technology provides a useful medium to improve the identification of peptides and proteins in bottom-up proteomics workflows, while still maintaining the practical back pressure in the column.

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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