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Peptide Calibration Mix using Kinetex® 2.6 µm XB-C18 Core-Shell Column Compared with 1.7 µm Fully Porous Column

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Introduction

The Kinetex 2.6 µm XB-C18 micro-UHPLC columns provide exceptional peak efficiency with which to resolve and evaluate the many peaks of a complex peptide mixture without generating the corresponding backpressure. The superficially-porous nature of the Kinetex core-shell particles feature shorter pore pathways that mimic the efficiency of smaller fully porous particles, while also mitigating the backpressure associated with UHPLC efficiency. In this application note we show that a peptide calibration standard demonstrates higher peak capacity, higher efficiency, and lower backpressure using the Kinetex 2.6 µm XB-C18 media when compared with competing fully porous micro-UHPLC columns that share the same column dimensions.

ID	Q1 m/z	Q3 m/z	CE	Conc. (Value and Unit)
PepCalMix.AETSELHTSLK.+3y5.heavy	408.55	593.35	24.3	fmol/µL
PepCalMix.AGLIVAEGVTK.+2y7.heavy	533.32	711.41	26.5	fmol/µL
PepCalMix.ALENDIGVPSDATVK.+2y7.heavy	768.9	725.39	35.6	fmol/µL
PepCalMix.AVGANPEQLTR.+2y6.heavy	583.31	753.41	31.5	fmol/µL
PepCalMix.AVYFYAPQIPLYANK.+2y6.heavy	883.47	713.41	47.9	fmol/µL
PepCalMix.DGTFVAVDGPVIAK.+2y7.heavy	677.86	649.41	33.6	fmol/µL
PepCalMix.GAYVEVTAK.+2y5.heavy	473.26	555.32	23.9	fmol/µL
PepCalMix.GFTAYIYPR.+2y5.heavy	549.29	721.39	26.9	fmol/µL
PepCalMix.IGNEQGVSR.+2y8.heavy	485.25	856.41	26.7	fmol/µL
PepCalMix.LDSTSIPVAK.+2y4.heavy	519.8	422.29	34	fmol/µL
PepCalMix.LGLDFDFSR.+2y6.heavy	540.27	796.35	26.7	fmol/µL
PepCalMix.LVGTPEAER.+2y7.heavy	491.27	769.37	23.4	fmol/µL
PepCalMix.SAEGLDASASLR.+2y7.heavy	593.8	729.38	30	fmol/µL
PepCalMix.SGGLLWQLVR.+2y5.heavy	569.83	711.42	29.6	fmol/µL
PepCalMix.SPYVITGPGVVEYK.+2y9.heavy	758.91	957.51	37.8	fmol/µL
PepCalMix.TVESLFPEEAETPGSAVR.+2y6.heavy	964.98	596.34	42.4	fmol/µL
PepCalMix.VFTPLEVDVAK.+2y8.heavy	613.35	878.51	26.3	fmol/µL
PepCalMix.VGNEIQVALR.+2y6.heavy	636.35	759.44	33.5	fmol/µL
PepCalMix.YDSINNTEVSGIR.+2y9.heavy	739.36	999.51	40.3	fmol/µL
PepCalMix.YIELAPGVDNSK.+2y7.heavy	657.34	724.37	29	fmol/µL

Results and Discussion

The average peak width for a peptide calibration mixture was 0.136 min when evaluated on a Kinetex 2.6 µm XB-C18 (50 x 0.3 mm) column, allowing for a peak capacity of 47 (**Figure 1**). The 1.7 µm Waters® column generated a wider average peak width of 0.138 min when packed in the same column dimensions, yielding a lower peak capacity of 43 (**Figure 2**). Peak capacity was calculated with the equation:

$$PC = (T/PW)+1$$

where PC is the peak capacity, T is the retention time of the last eluting peak, and PW is the average peak width. The Kinetex 2.6 µm XB-C18 is shown to generate higher efficiencies and peak capacities than columns packed with fully porous 1.7 µm media like the Waters nanoEase™ M/Z Peptide CSH C18 column when using the same column dimensions. Correspondingly, the Kinetex 2.6 µm XB-C18 column generated a lower backpressure of 1900 psi, compared with the 4000 psi of the Waters 1.7 µm fully porous column, when packed into the same column dimensions of 50 x 0.3 mm.

LC Conditions

Column: Kinetex 2.6 µm XB-C18
Waters 1.7 µm nanoEase M/Z Peptide CSH C18
Dimensions: 50 x 0.3 mm
Part Number: 00B-4496-AC (Kinetex)
Pressure (bar): 1900 bar (Kinetex)
4000 bar (Waters)
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile
Gradient:

Time (min)	% B
0	3
10	40
12	80
14	80
15	3
20	3

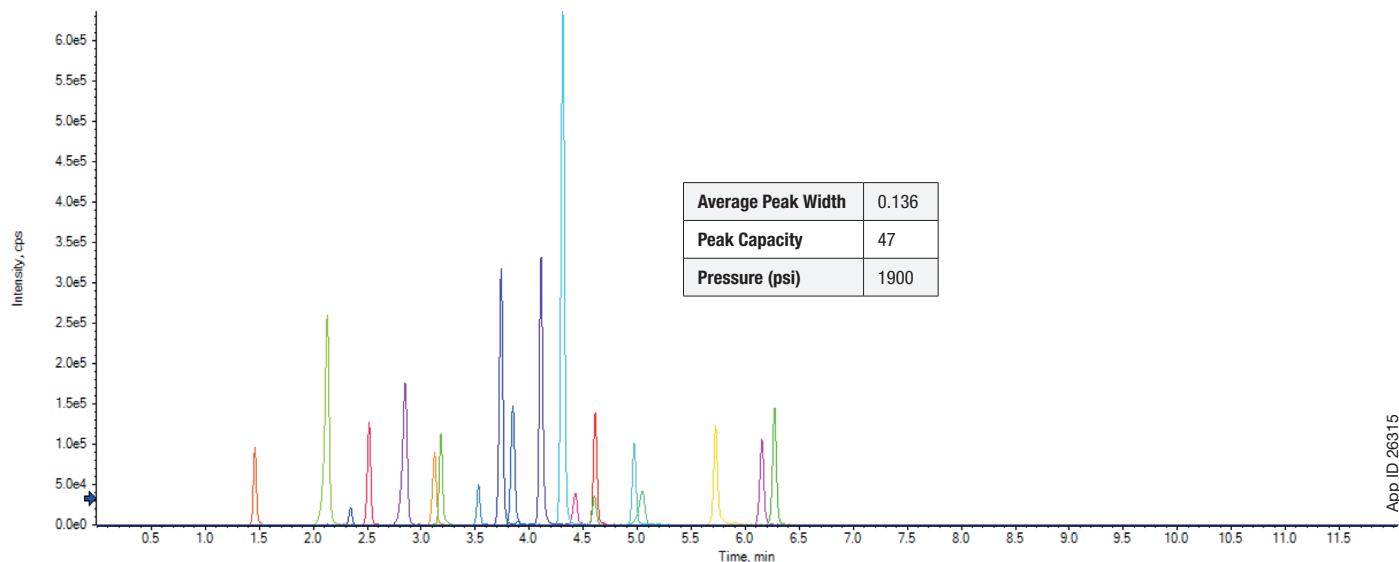
Flow Rate: 10 µL/min
Temperature: 30 °C
LC System: NanoLC™ (SCIEX®)
Detection: nanoESI
Detector: 6500 QTRAP® (SCIEX)
Injection Volume: 1 µL



Roxana Eggleston-Rangel, Application Scientist

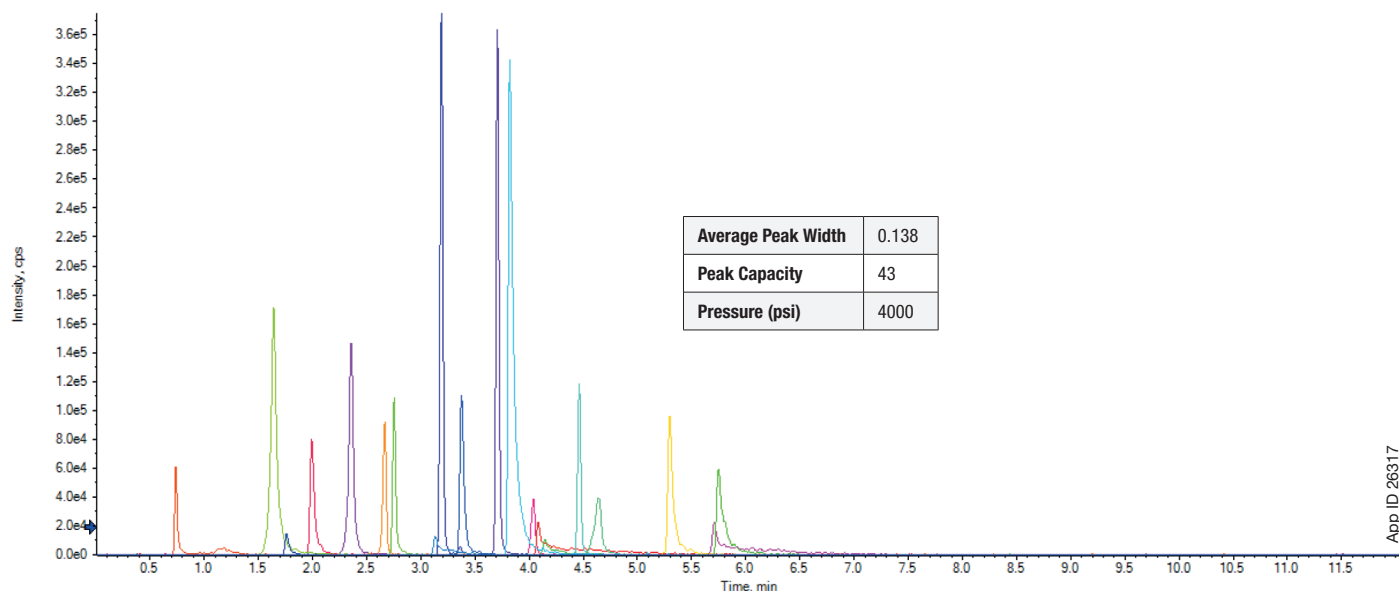
Roxana likes to spend time with her dog and family. She has a German Shepherd mix named Cobi who was named after the official mascot of the 1992 Summer Olympics and not the basketball player. Besides the lab, you might find Roxana in old episodes of the Ghost Whisperer, MAD TV, The L word and others as she used to be a TV extra during her school years.

Figure 1.
PepCalMix run on the Kinetex® 2.6 µm XB-C18 (50 x 0.3 mm).



App ID 26315

Figure 2.
PepCalMix run on the Waters® 1.7 µm nanoEase™ M/Z Peptide CSH C18 (50 x 0.3 mm).

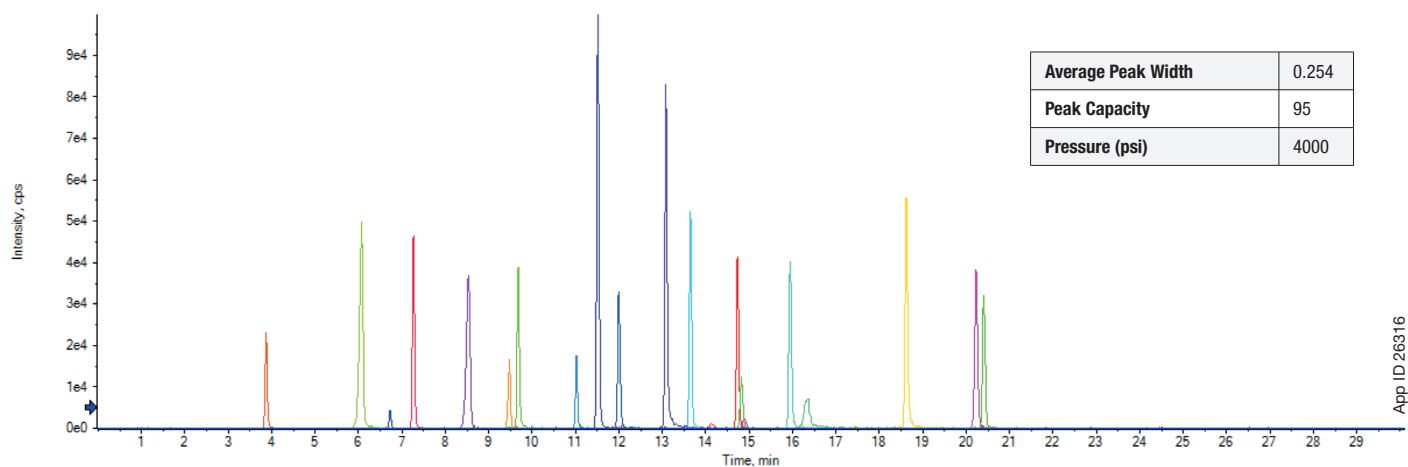


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Chromatographic efficiency and resolution is proportional to the length of a column, but longer columns also generate higher backpressure. A longer 150 x 0.3 mm Kinetex 2.6 µm XB-C18 produced average peak widths of 0.254 min, yielding a peak capacity of 95 peaks (**Figure 3**). The corresponding backpressure of the analysis was a modest 4000 psi at 25 °C. The longer 150 mm Kinetex 2.6 µm XB-C18 generates even higher efficiency levels and peak capacity while performing within the pressure limitations of most micro-UHPLC instrumentation.



Figure 3.
PepCalMix run on the Kinetex® 2.6 µm XB-C18 (150 x 0.3 mm).



App ID 26316

LC Conditions

Column: Kinetex 2.6 µm XB-C18
Dimensions: 150 x 0.3 mm
Part Number: [00F-4496-AC](#)
Pressure (bar): 1900 bar
Mobile Phase: A: 0.1 % Formic Acid in Water
 B: 0.1 % Formic Acid in Acetonitrile
Gradient:

Time (min)	% B
0	3
30	40
36	80
42	80
45	3
60	3

Flow Rate: 10 µL/min
Temperature: 30 °C (Kinetex)
LC System: NanoLC™ (SCIEX®)
Detection: nanoESI
Detector: 6500 QTRAP® (SCIEX)
Injection Volume: 1 µL

Conclusion

The Kinetex 2.6 µm XB-C18 micro-UHPLC columns generate the efficiency and resolution necessary to optimize peptide characterization within a single chromatogram. Exceedingly high peak capacities may be achieved with the Kinetex 2.6 µm XB-C18 columns on a wide range of micro-UHPLC instrumentation while operating well within the capabilities of the instrument. Ultimately, the Kinetex 2.6 µm XB-C18 media expands opportunities for micro-UHPLC peptide characterization.



Kinetex 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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