

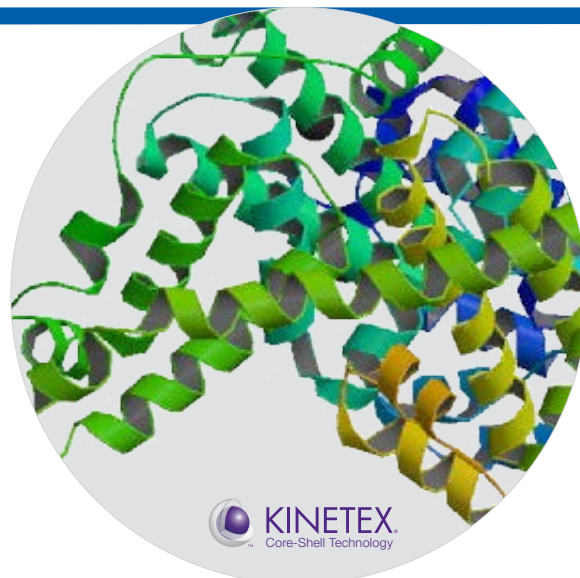
TN-1285

# Enhancing Sensitivity and Peak Capacity for Protein Digest using Micro-LC and the Power of 2.6 μm Kinetex® Core-Shell Columns

Roxana Eggleston-Rangel<sup>1</sup>, Dr. Jason Anspach<sup>1</sup>, Dr. Helen Whitby<sup>2</sup>, and Dr. Bryan Tackett<sup>1</sup>

<sup>1</sup>Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501, USA

<sup>2</sup>Phenomenex Ltd., Queens Avenue, Hurdsfield Ind. Est., Macclesfield, Cheshire SK10 2BN UK



## Introduction

Microflow LC uses columns with smaller inner diameters and a reduced flow rate to achieve an improvement in ionization efficiency for LC-MS and as a result, greater sensitivity. In this tech note we show that coupling the ultra-high efficiency provided by the Kinetex core-shell 2.6 μm columns with these narrow column ID's can drive efficiency and peak capacity to improve what is seen for sub-2 μm fully porous UHPLC columns. High efficiency (narrower peaks) provides higher peak capacity, and this is something regarded as essential when looking at protein digest samples which are complex in nature and require detailed peak identification and profiling. When studying proteins, using a bottom-up proteomics approach, high efficiency reversed phase offers a significant advantage over other techniques by coupling high resolving power with mass spectrometry. Performance is measured by assessing peak capacity and this is defined as the number of peaks that can be separated or dispersed into the gradient time frame with a defined resolution.

The key to high efficiency with core-shell particles comes from their unique particle morphology and nature which minimizes efficiency losses and offers an increase in mass transfer rates through decreasing the effects of diffusion.

In this technote we compare the performance of the Kinetex 2.6 μm core-shell packed into 0.3 mm ID columns to a 1.7 μm fully porous UHPLC alternative and demonstrate the average peak widths and peak capacities seen between a 2.6 μm core-shell material are comparable to a sub-2 μm fully porous whilst operating at a significantly lower backpressure. Furthermore, the reduction in backpressure can facilitate the use of longer columns, something which has the potential to be extremely beneficial when the goal is maximising peak capacity.

**Figure 1.**

Comparison of average peak width and peak capacity for BSA tryptic digest

Column	Kinetex 2.6 μm XB-C18	Waters CSH M/Z Peptide 1.7 μm
<b>Average Peak Width</b>	0.184	0.191
<b>Peak Capacity</b>	54	45
<b>Pressure (psi)</b>	1900	4000

## Results and Discussion

We compared the peak capacity and average peak width of the 2.6 μm Kinetex column to the 1.7 μm Waters® CSH and when using the 50 mm column length we found the average peak width to be lower and total peak capacity higher with the core-shell material (**Figure 1**). 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. **Figures 2 and 3** demonstrate an improved peak shape for many of the later eluting peptides when working with the Kinetex core-shell material leading to this increase in peak capacity. The lower backpressure observed when a 2.6 μm column is used allows additional column length to be investigated, unfortunately as a result of pressure in excess of 10,000 psi when using the 1.7 μm column, we were unable to obtain comparative data for 150 mm length using the Waters 1.7 μm nanoEase™ M/Z Peptide CSH C18 column. When using the longer Kinetex column the gradient was adjusted accordingly to maintain a comparable gradient slope.

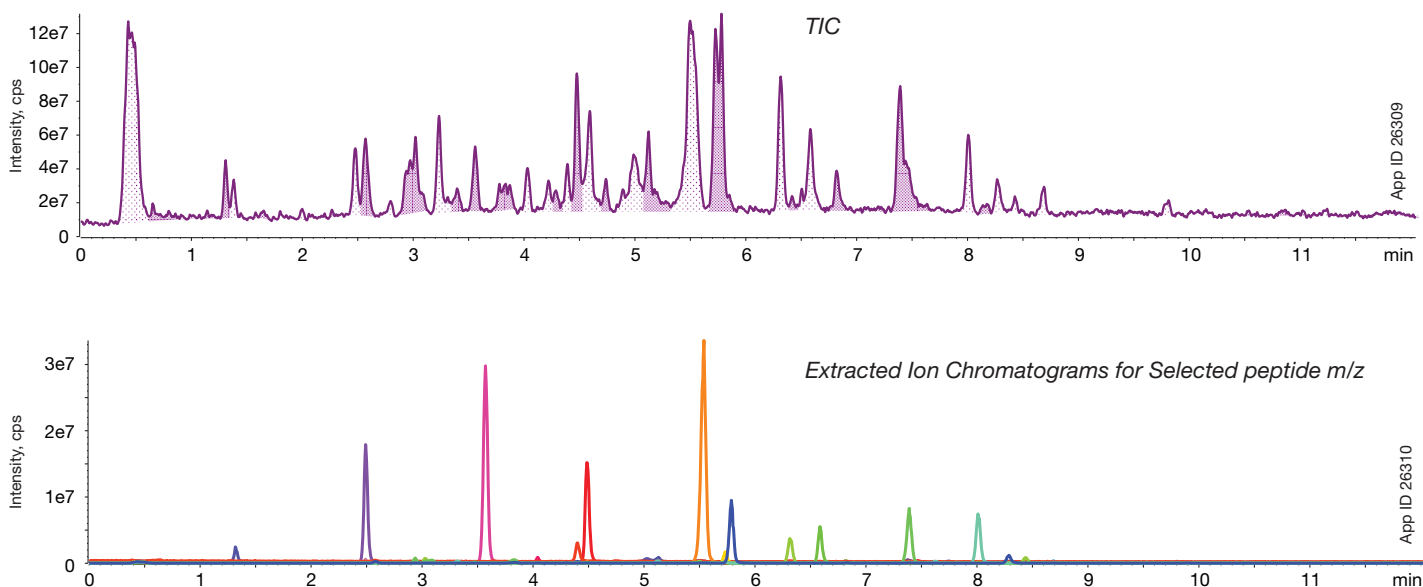
The 150 mm Kinetex 2.6 μm XB-C18 column allowed us to increase peak capacity from 54 to 105 when comparing it with the 50 mm alternative packed with the same media. There was a slight increase in peak width with increasing column length, however the overall increase in plate count because of a longer column affords significantly greater resolution across the gradient (**Figure 4**). Under these running conditions the pressure observed with the 150 x 0.3 mm 2.6 μm (4000 psi) column was comparable to the 50 x 0.3 mm 1.7 μm fully porous alternative (4000 psi), further highlighting the design space created when working with high efficiency core-shell particles.



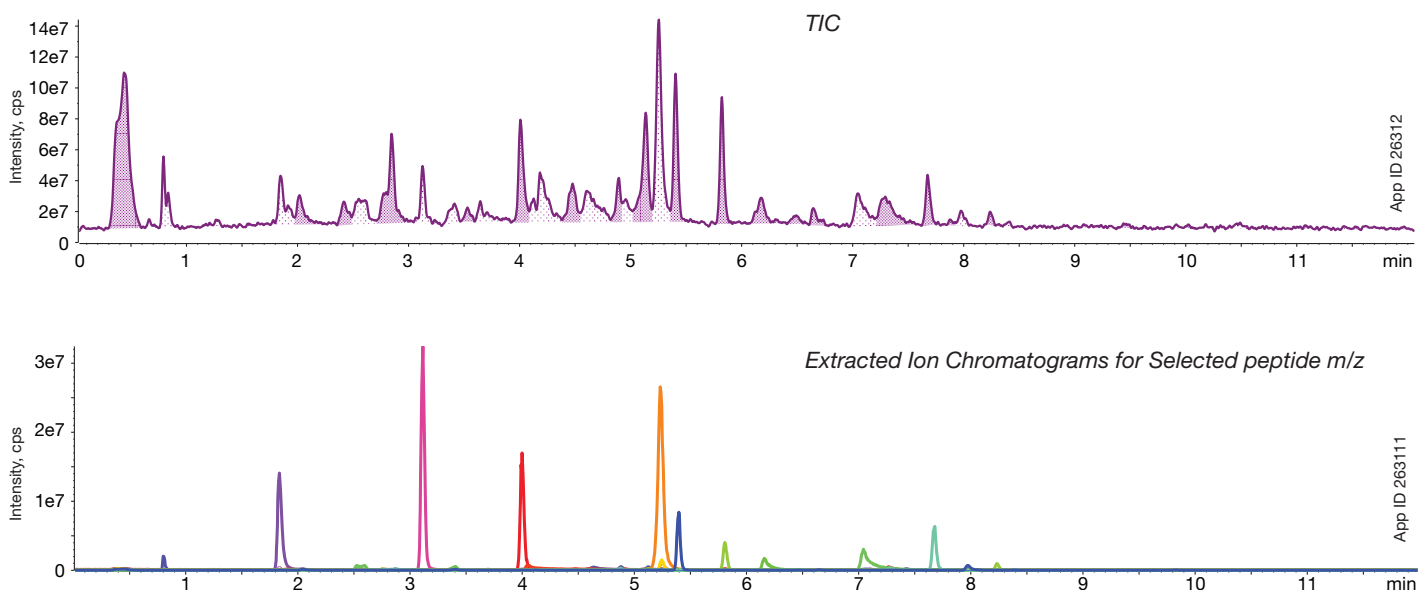
### Roxana Eggleston-Rangel, Application Scientist

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 2.**  
Kinetex® 2.6µm XB-C18 50 x 0.3 mm, BSA tryptic digest



**Figure 3.**  
Waters® 1.7 µm nanoEase™ M/Z Peptide CSH C18 column 50 x 0.3 mm, BSA tryptic digest



### LC Conditions

**Column:** Kinetex 2.6µm XB-C18  
Waters 1.7µm M/Z Peptide CSH C18  
**Dimensions:** 50 x 0.3 mm  
**Part No.:** 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

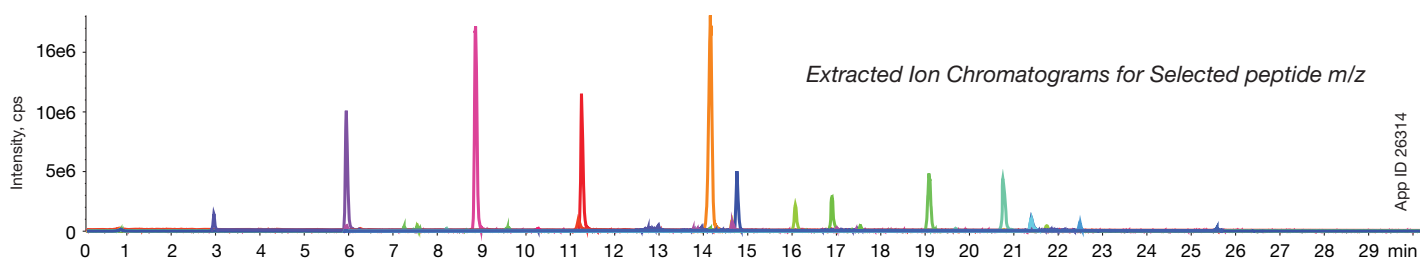
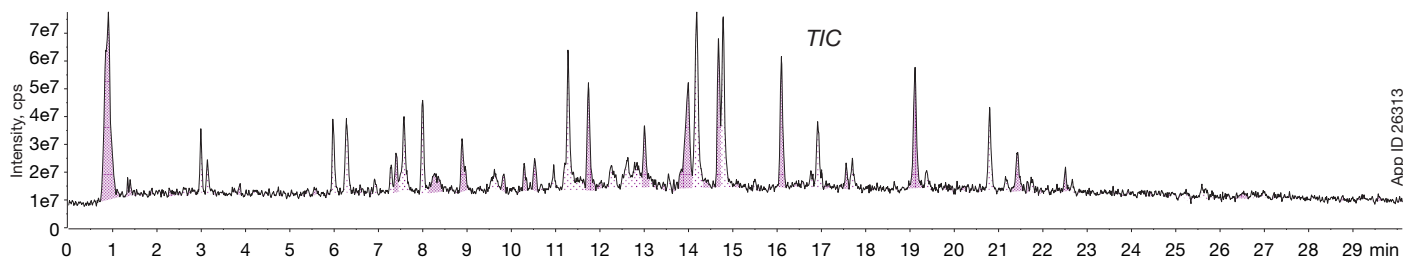
**Gradient:**

Time (min)	% B
0	3
11.7	36
12.4	95
15.41	95
15.42	3
21	3

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



**Figure 4.**  
Kinetex® 2.6µm XB-C18 150 x 0.3 mm, BSA tryptic digest



### LC Conditions

**Column:** Kinetex 2.6µm XB-C18  
**Dimensions:** 150 x 0.3 mm  
**Part No.:** [00F-4496-AC](#)  
**Pressure (bar):** 4000 bar  
**Mobile Phase:** A: 0.1 % Formic Acid in Water  
 B: 0.1 % Formic Acid in Acetonitrile

Gradient: Time (min)	% B
0	3
35.1	36
37.2	95
46.23	95
46.26	3
63	3

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

### Conclusions

Kinetex columns packed with 2.6µm core-shell particles offer comparable efficiency and lower backpressure to fully porous 1.7µm columns with the same column dimension. The lower backpressure produced from a larger particle can be utilized to

allow longer columns to be used at flow rates which in many cases would be unattainable on sub-2µm UHPLC columns due to pressure restrictions.



## Ordering Information

## 2.6µm 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
<b>XB-C18</b>	<a href="#">00A-4496-AC</a>	<a href="#">00B-4496-AC</a>	<a href="#">00D-4496-AC</a>	<a href="#">00F-4496-AC</a>	<a href="#">00B-4496-AF</a>	<a href="#">00F-4496-AF</a>
<b>Biphenyl</b>	—	<a href="#">00B-4622-AC</a>	—	<a href="#">00F-4622-AC</a>	<a href="#">00B-4622-AF</a>	—
<b>C18</b>	<a href="#">00A-4462-AC</a>	<a href="#">00B-4462-AC</a>	—	<a href="#">00F-4462-AC</a>	<a href="#">00B-4462-AF</a>	—
<b>EVO C18</b>	—	<a href="#">00B-4725-AC</a>	—	<a href="#">00F-4725-AC</a>	<a href="#">00B-4725-AF</a>	—
<b>F5</b>	—	<a href="#">00B-4723-AC</a>	<a href="#">00D-4723-AC</a>	<a href="#">00F-4723-AC</a>	<a href="#">00B-4723-AF</a>	—

**BE-HAPPY™**  
GUARANTEE

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

[www.phenomenex.com/behappy](http://www.phenomenex.com/behappy)

**Australia**

t: +61 (0)2-9428-6444  
auinfo@phenomenex.com

**Austria**

t: +43 (0)1-319-1301  
anfrage@phenomenex.com

**Belgium**

t: +32 (0)2 503 4015 (French)  
t: +32 (0)2 511 8666 (Dutch)  
beinfo@phenomenex.com

**Canada**

t: +1 (800) 543-3681  
info@phenomenex.com

**China**

t: +86 400-606-8099  
cninfo@phenomenex.com

**Czech Republic**

t: +420 272 017 077  
cz-info@phenomenex.com

**Denmark**

t: +45 4824 8048  
nordicinfo@phenomenex.com

**Finland**

t: +358 (0)9 4789 0063  
nordicinfo@phenomenex.com

**France**

t: +33 (0)1 30 09 21 10  
franceinfo@phenomenex.com

**Germany**

t: +49 (0)6021-58830-0  
anfrage@phenomenex.com

**Hong Kong**

t: +852 6012 8162  
hkinfo@phenomenex.com

**India**

t: +91 (0)40-3012 2400  
indiainfo@phenomenex.com

**Ireland**

t: +353 (0)1 247 5405  
eireinfo@phenomenex.com

**Italy**

t: +39 051 6327511  
italiainfo@phenomenex.com

**Luxembourg**

t: +31 (0)30-2418700  
nlinfo@phenomenex.com

**Mexico**

t: 01-800-844-5226  
tecnicomx@phenomenex.com

**The Netherlands**

t: +31 (0)30-2418700  
nlinfo@phenomenex.com

**New Zealand**

t: +64 (0)9-4780951  
nzinfo@phenomenex.com

**Norway**

t: +47 810 02 005  
nordicinfo@phenomenex.com

**Poland**

t: +48 22 104 21 72  
pl-info@phenomenex.com

**Portugal**

t: +351 221 450 488  
ptinfo@phenomenex.com

**Singapore**

t: +65 800-852-3944  
sginfo@phenomenex.com

**Slovakia**

t: +420 272 017 077  
sk-info@phenomenex.com

**Spain**

t: +34 91-413-8613  
espinfo@phenomenex.com

**Sweden**

t: +46 (0)8 611 6950  
nordicinfo@phenomenex.com

**Switzerland**

t: +41 (0)61 692 20 20  
swissinfo@phenomenex.com

**Taiwan**

t: +886 (0) 0801-49-1246  
twinfo@phenomenex.com

**Thailand**

t: +66 (0) 2 566 0287  
thaiinfo@phenomenex.com

**United Kingdom**

t: +44 (0)1625-501367  
ukinfo@phenomenex.com

**USA**

t: +1 (310) 212-0555  
info@phenomenex.com

🌐 **All other countries/regions**

**Corporate Office USA**  
t: +1 (310) 212-0555  
info@phenomenex.com

**www.phenomenex.com**

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at [international@phenomenex.com](mailto:international@phenomenex.com)

**Terms and Conditions**

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

**Trademarks**

Kinetex is a registered trademark and BE-HAPPY is a trademark of Phenomenex. SCIEEX and QTRAP are registered trademarks and NanoLC is a trademark of AB SCIEEX Pte. Ltd. Waters is a registered trademark and NanoEase is a trademark of Waters Technologies Corporation.

**Disclaimer**

Comparative separations may not be representative of all applications. Phenomenex is not affiliated with Waters Technologies Corporation.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2021 Phenomenex, Inc. All rights reserved.



Have questions or want more details on implementing this method? We would love to help!  
Visit [www.phenomenex.com/Chat](http://www.phenomenex.com/Chat) to get in touch with one of our Technical Specialists

phenomenex®