

## Leveraging Ultra-High Efficiency Kinetex<sup>®</sup> Core-Shell 5 $\mu$ m Technology for Lab-Scale Preparative LC

Jeff Layne, J Preston, Marc Jacob, and J.T. Presley III  
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

*Kinetex 5  $\mu$ m is the first and only core-shell media available for lab-scale preparative purification. Chromatographers can now scale their ultra-high efficiency Kinetex core-shell analytical columns directly to Kinetex 5  $\mu$ m Axia<sup>™</sup> packed preparative columns. This solution is well suited for small-scale purification (10s of milligrams to gram levels).*

### Introduction

In analytical chromatography, the goal is to separate target analytes from matrix components so that they can be accurately identified and quantified, i.e. qualitative and quantitative determination. In contrast, the goal of preparative chromatography is to isolate and purify a given component from its matrix, i.e. purification.

It is well-established in the literature that core-shell columns such as Kinetex provide higher efficiency than columns packed with fully porous particles of comparable diameter. Until very recently, core-shell columns were only available in sub-3  $\mu$ m particle sizes (e.g. Kinetex 2.6  $\mu$ m and 1.7  $\mu$ m), and generated too much pressure to be applied to conventional preparative LC systems. However, with the Kinetex 5  $\mu$ m particle, preparative chromatographers can now take advantage of a higher performance HPLC particle that operates at 5  $\mu$ m pressures and is packed in the award winning Axia hardware. Under analytical conditions, columns packed with Kinetex 5  $\mu$ m media provide the same or better efficiency as columns packed with fully porous 3  $\mu$ m particles, but operate at backpressures that are consistent with 5  $\mu$ m media. Thus, Kinetex core-shell technology provides a distinct chromatographic advantage over traditional fully porous media for analytical separations. This study is to confirm that the Kinetex core-shell advantage is also applicable under preparative conditions.

To investigate this aspect of core-shell performance, the performance of a Kinetex core-shell 5  $\mu$ m column (Axia packed C18 50 x 21.2 mm) and a fully porous 5  $\mu$ m column (XBridge<sup>™</sup> Prep 5  $\mu$ m C18 OBD<sup>™</sup> 50 x 19 mm) were compared under identical conditions for some strongly basic test probes (amitriptyline and doxepin).

### Material and Methods

All chemicals were obtained from Sigma Chemical Co. (St. Louis, Missouri). Solvents were purchased from EMD (San Diego, California). The Kinetex 5  $\mu$ m C18 Axia packed 50 x 21.2 mm column was obtained from Phenomenex (Torrance, California) and the Waters<sup>®</sup> XBridge<sup>™</sup> Prep C18 5  $\mu$ m OBD<sup>™</sup> 50 x 19 mm was obtained from Waters (Milford, MA.). All analytical methods were performed using an Agilent HP 1100 system equipped with a quaternary pump, variable wavelength detector, and autosampler and analyzed using ChemStation software (Rev. A.10.02). Preparative chromatography was performed using a Shimadzu<sup>®</sup> LC-20AP preparative chromatography system.

### Results and Discussion

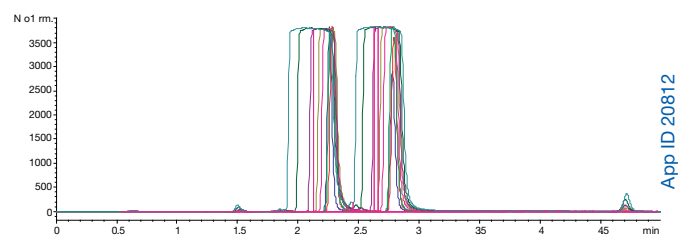
With the release of the new Kinetex 5  $\mu$ m media, Phenomenex can now offer chromatographers a core-shell particle that can be scaled directly from analytical to preparative levels. A simple scaling experiment is illustrated in **Figure 1**, using caffeine and theophylline as the model test probes. The process begins

with an analytical loading experiment in which successively larger amounts (from 4  $\mu$ g to 2.4 mg) are loaded onto an analytical column (Kinetex 5  $\mu$ m C18 50 x 4.6 mm). The resulting overlaid chromatograms are shown in **Figure 1a**, and then the final upper level limit of 2.4 mg on-column is shown in **Figure 1b**. This final chromatogram is then directly scaled-up to a preparative format, in this case a Kinetex 5  $\mu$ m C18 Axia packed 50 x 21.2 mm column (**Figure 1c**). We scaled-up the sample load proportional to the increase in bed mass, thus allowing us to load 48 mg on-column while preserving chromatography.

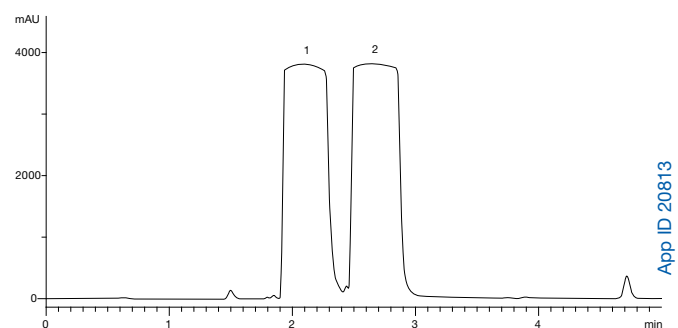
**Figure 1.**

Analytical scale-up loading experiment using a Kinetex core-shell 5  $\mu$ m C18 50 x 4.6 mm.

a. Analytical scale-up using a Kinetex 5  $\mu$ m C18; 4  $\mu$ g – 2.4 mg on-column



b. Final analyte loading of 2.4 mg injection



Conditions for Figure 1a and 1b:

Column: Kinetex 5  $\mu$ m C18  
Dimensions: 50 x 4.6 mm  
Part No.: 00B-4601-E0  
Mobile Phase: A: Water with 0.1% TFA  
B: Acetonitrile with 0.1% TFA  
Gradient: 

Time (min)	% B
0	3
5	35

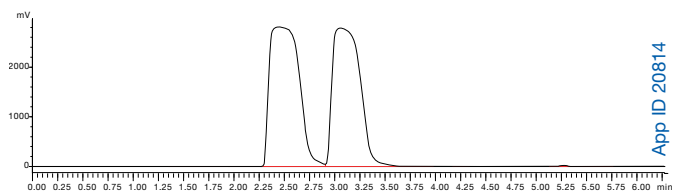
  
Flow Rate: 1.0 mL/min  
Temperature: 22 °C  
Detection: UV @ 254 nm  
Sample Concentration: 20 mg/mL in 80/20 water/methanol  
Sample: 1. Caffeine  
2. Theophylline

XBridge and OBD are trademarks and Waters is a registered trademark of Waters Corporation (Milford, MA, USA). Phenomenex is not affiliated with Waters Corporation. Comparative separations may not be representative of all applications.

# TN-1136

## APPLICATIONS

c. Preparative scale-up using a Phenomenex Kinetex 5 µm C18 Axia™ packed 50 x 21.2 mm with 48 mg on-column



App ID 20814

### Conditions for Figure 1c:

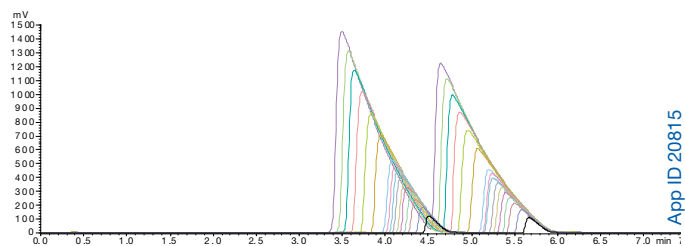
<b>Column:</b> Kinetex 5 µm C18 Axia packed	<b>Flow Rate:</b> 20 mL/min
<b>Dimensions:</b> 50 x 21.2 mm	<b>Temperature:</b> 22 °C
<b>Part No.:</b> 00B-4601-PO-AX	<b>Detection:</b> UV @ 254 nm
<b>Mobile Phase:</b> A: Water with 0.1 % TFA	<b>Injection Volume:</b> 2.4 mL
B: Acetonitrile with 0.1 % TFA	<b>Sample Concentration:</b> 20 mg/mL in 80/20 water/methanol
<b>Gradient:</b> Time (min) % B	<b>Sample:</b> 1. Caffeine
0 3	2. Theophylline
5 35	

To determine how the Kinetex 5 µm media would perform for more challenging analytes, we evaluated the loading performance of the Phenomenex Kinetex 5 µm C18 Axia packed column and compared it to a fully porous 5 µm alternative (Waters® XBridge™ Prep 5 µm C18 OBD™ 50 x 19 mm) for two very strongly basic analytes – doxepin and amitriptyline. **Figures 2a** and **2b** show overlays of successive injections of the drug mixture onto the Kinetex 5 µm C18 Axia packed 50 x 21.2 mm column (**2a**) and a fully porous XBridge Prep 5 µm C18 OBD 50 x 19 mm (**2b**). When these basic analytes are loaded using a TFA-modified mobile phase (0.1 %), the peaks are relatively well-behaved, even at high sample loads on both columns. Visually, there is very little difference in the chromatographic elution profiles.

### Figure 2.

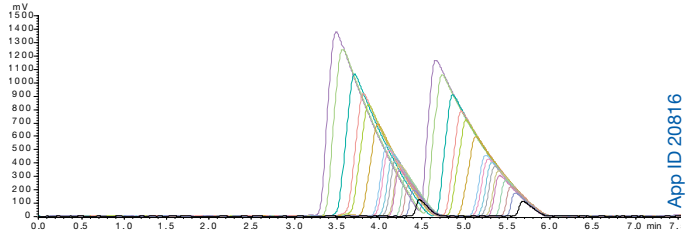
Preparative load of doxepin and amitriptyline using a mobile phase containing 0.1 % TFA

a. Phenomenex Kinetex 5 µm C18 Axia packed 50 x 21.2 mm



App ID 20815

b. Waters XBridge Prep 5 µm C18 OBD™ 50 x 19 mm



App ID 20816

### Conditions for Figure 2:

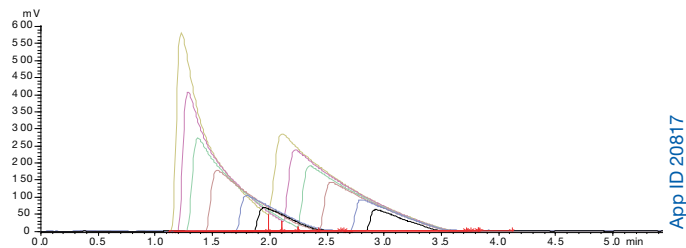
<b>Column:</b> Kinetex 5 µm C18 Axia packed	<b>Flow Rate:</b> 30 mL/min
XBridge Prep 5 µm C18 OBD	<b>Temperature:</b> 22 °C
<b>Dimensions:</b> 50 x 21.2 mm	<b>Detection:</b> UV @ 254 nm
50 x 19 mm	<b>Sample Concentration:</b> 200 mg/mL in DMSO
<b>Mobile Phase:</b> A: Water with 0.1 % TFA	<b>Sample:</b> 1. Doxepin
B: Acetonitrile with 0.1 % TFA	2. Amitriptyline
<b>Gradient:</b> Time (min) % B	
0 20	
8 50	

If we perform this same evaluation, but substitute formic acid (0.1 %) for the TFA, we get a much different picture (**Figure 3**). Without the presence of the ion-pairing capacity of the TFA, the chromatographic behavior of these strong bases is compromised, resulting in more peak distortion for both columns. However, as you can clearly see, at higher loads, the chromatography is much better preserved on the Kinetex® 5 µm C18 Axia™ packed 50 x 21.2 mm column as the peaks become grossly distorted on the Waters® XBridge™ Prep 5 µm C18 OBD™ 50 x 19 mm column. Thus, under this set of conditions, the Kinetex 5 µm C18 core-shell Axia packed column actually vastly outperforms the fully porous 5 µm alternative (this experiment was repeated on 3 different Waters XBridge Prep 5 µm C18 OBD 50 x 19 mm columns with similar results).

### Figure 3.

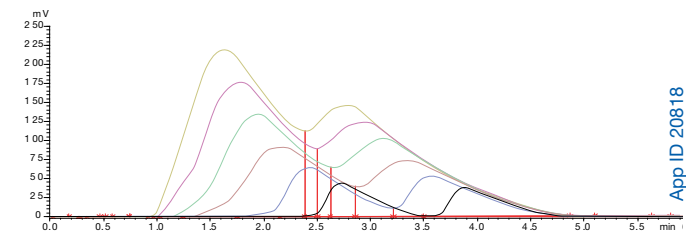
Preparative load of doxepin and amitriptyline using a mobile phase containing 0.1 % formic acid

a. Phenomenex Kinetex 5 µm C18 Axia packed 50 x 21.2 mm



App ID 20817

b. Waters XBridge Prep C18 5 µm OBD 50 x 19 mm



App ID 20818

### Conditions for Figure 3:

<b>Column:</b> Kinetex 5 µm C18 Axia packed
XBridge Prep 5 µm C18 OBD
<b>Dimensions:</b> 50 x 21.2 mm
50 x 19 mm
<b>Mobile Phase:</b> A: Water with 0.1 % Formic acid
B: Acetonitrile with 0.1 % Formic acid
<b>Gradient:</b> Time (min) % B
0 20
8 50
<b>Flow Rate:</b> 30 mL/min
<b>Temperature:</b> 22 °C
<b>Detection:</b> UV @ 254 nm
<b>Sample Concentration:</b> 200 mg/mL in DMSO
<b>Sample:</b> 1. Doxepin
2. Amitriptyline

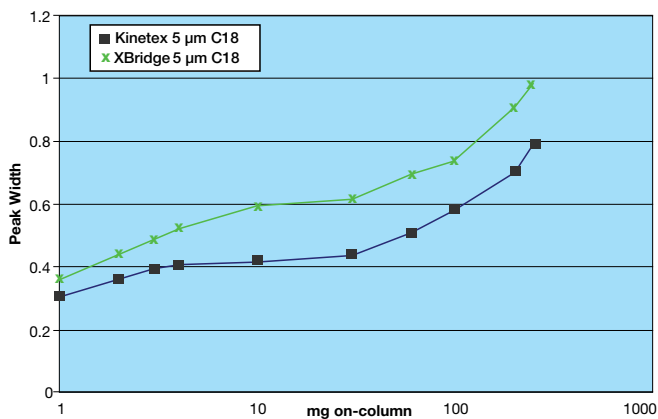
XBridge and OBD are trademarks and Waters is a registered trademark of Waters Corporation (Milford, MA, USA). Phenomenex is not affiliated with Waters Corporation. Comparative separations may not be representative of all applications.

# TN-1136

## APPLICATIONS

If we look closely at the peak shape for one of the bases (amitriptyline; **Figure 4**), you can clearly see that the peak width for amitriptyline is narrower on the Kinetex Axia packed column, and stays narrower across every single sample load. This data may reflect the higher efficiency of the Kinetex 5  $\mu\text{m}$  media, resulting in narrower peak widths for the analytes. In addition, note that the rate of peak distortion (shown by the slope of the increase in peak width as a function of sample load) increases at approximately the same rate on both media. Since peak distortion is a function of sample overloading, we can conclude that, for these analytes and these conditions, the Kinetex 5  $\mu\text{m}$  Axia packed core-shell column has a very similar loading capacity to the fully-porous XBridge Prep 5  $\mu\text{m}$  C18 OBD 50 x 19mm column. But, because the core-shell Kinetex 5  $\mu\text{m}$  Axia packed column starts out with narrower peaks due to the increased efficiency of the core-shell particle morphology, it is able to provide slightly better performance across the entire loading spectrum.

**Figure 4.** Plots of peak width as a function of sample load for amitriptyline using the 0.5% formic acid modified mobile phase. Data obtained from the chromatograms shown in Figure 3.



### Conclusion

Our investigation indicates that, at least for these test probes (doxepin and amitriptyline) and under these conditions (water/acetonitrile gradient using either 0.1% TFA or 0.1% formic acid), the Kinetex 5  $\mu\text{m}$  C18 Axia packed column is able to perform as good or better than preparative columns packed with fully porous 5  $\mu\text{m}$  media (Waters XBridge Prep 5  $\mu\text{m}$  C18 OBD™ 50 x 19). Peak widths for these two probes were actually more narrow on the Axia packed column across the entire range of sample loads evaluated (5 to 500 mg on-column for the 50 x 21.2 mm Kinetex 5  $\mu\text{m}$  C18 Axia packed column and the 50 x 19 mm Waters XBridge Prep 5  $\mu\text{m}$  C18 OBD column). This initial data suggests that the increased efficiency that core-shell materials exhibit as compared to fully porous particles of equivalent diameter offer performance gains that may apply to both analytical and small-scale preparative applications.

XBridge and OBD are trademarks and Waters is a registered trademark of Waters Corporation (Milford, MA, USA). Phenomenex is not affiliated with Waters Corporation. Comparative separations may not be representative of all applications.

# TN-1136 APPLICATIONS

## Ordering Information

### Kinetex<sup>®</sup> HPLC Columns

NEW! 5µm Columns (mm)	SecurityGuard <sup>™</sup> ULTRA Cartridges*					SecurityGuard ULTRA Cartridges*			
	50 x 2.1	3/pk			50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
<b>Phases</b>									
<b>XB-C18</b>	00B-4605-AN	AJO-8782	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0			AJO-8768
<b>C18</b>	00B-4601-AN	AJO-8782	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0			AJO-8768
<b>C8</b>	00B-4608-AN	AJO-8784	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0			AJO-8770
<b>PFP</b>	00B-4602-AN	AJO-8787	00B-4602-E0	00D-4602-E0	00F-4602-E0	00G-4602-E0			AJO-8773
<b>Phenyl-Hexyl</b>	00B-4603-AN	AJO-8788	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0			AJO-8774

for 2.1 mm ID

for 4.6 mm ID

\* SecurityGuard ULTRA cartridges require holder, Part No. AJO-9000.

### NEW! 5µm Axia<sup>™</sup> Packed Preparative Columns (mm)

Phases	SecurityGuard PREP Cartridges**				
	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2	ea
<b>XB-C18</b>	00B-4605-P0-AX	00D-4605-P0-AX	00F-4605-P0-AX	00G-4605-P0-AX	AJO-9145
<b>C18</b>	00B-4601-P0-AX	00D-4601-P0-AX	00F-4601-P0-AX	00G-4601-P0-AX	AJO-9145
<b>PFP</b>	00B-4602-P0-AX	00D-4602-P0-AX	00F-4602-P0-AX	00G-4602-P0-AX	AJO-9146
<b>Phenyl-Hexyl</b>	00B-4603-P0-AX	00D-4603-P0-AX	00F-4603-P0-AX	00G-4603-P0-AX	AJO-9147

\*\* SecurityGuard PREP 21.2 mm ID cartridges require holder, Part No. AJO-8223.

for 21.2 mm ID

#### Australia

t: 02-9428-6444  
f: 02-9428-6445  
auiinfo@phenomenex.com

#### Austria

t: 01-319-1301  
f: 01-319-1300  
anfrage@phenomenex.com

#### Belgium

t: 02 503 4015 (French)  
t: 02 511 8666 (Dutch)  
f: +31 (0)30-2383749  
beinfo@phenomenex.com

#### Canada

t: (800) 543-3681  
f: (310) 328-7768  
info@phenomenex.com

#### Denmark

t: 4824 8048  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### Finland

t: 09 4789 0063  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### France

t: 01 30 09 21 10  
f: 01 30 09 21 11  
franceinfo@phenomenex.com

#### Germany

t: 06021-58830-0  
f: 06021-58830-11  
anfrage@phenomenex.com

#### India

t: 040-3012 2400  
f: 040-3012 2411  
indiainfo@phenomenex.com

#### Ireland

t: 01 247 5405  
f: +44 1625-501796  
eireinfo@phenomenex.com

#### Italy

t: 051 6327511  
f: 051 6327555  
italiainfo@phenomenex.com

#### Luxembourg

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

#### Mexico

t: 001-800-844-5226  
f: 001-310-328-7768  
tecnicomx@phenomenex.com

#### The Netherlands

t: 030-2418700  
f: 030-2383749  
nlinfo@phenomenex.com

#### New Zealand

t: 09-4780951  
f: 09-4780952  
nzinfo@phenomenex.com

#### Norway

t: 810 02 005  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### Puerto Rico

t: (800) 541-HPLC  
f: (310) 328-7768  
info@phenomenex.com

#### Sweden

t: 08 611 6950  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### United Kingdom

t: 01625-501367  
f: 01625-501796  
ukinfo@phenomenex.com

#### United States

t: (310) 212-0555  
f: (310) 328-7768  
info@phenomenex.com

#### All other countries: Corporate Office USA

t: (310) 212-0555  
f: (310) 328-7768  
info@phenomenex.com



If you are not completely satisfied with Kinetex core-shell columns, send in your comparative data to a similar product with the Kinetex column within 45 days for a FULL REFUND.

#### 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

Axia and SecurityGuard are trademarks and Kinetex is a registered trademark of Phenomenex. ChemStation is a trademark of Agilent Technologies, Inc. Waters is a registered trademark and XBridge and OBD are trademarks of Waters Corporation. Shimadzu is a registered trademark of Shimadzu Corporation.

#### Disclaimer

Phenomenex is in no way affiliated with Agilent or Waters Corporation. Dimensions and chromatographic conditions are the same for all columns unless otherwise noted. Comparative separations may not be representative of all applications.

Axia is patented by Phenomenex. U.S. Patent No. 7,674,383

© 2013 Phenomenex, Inc. All rights reserved.