Leveraging Ultra-High Efficiency Kinetex® Core-Shell 5 µm 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 µm 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 µm Axia™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 the analytes 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 µm particle sizes (e.g. Kinetex 2.6 µm and 1.7 µm), and generated too much pressure to be applied to conventional preparative LC systems. However, with the Kinetex 5 µm particle, preparative chromatographers can now take advantage of a higher performance HPLC particle that operates at 5 µm pressures and is packed in the award winning Axia hardware. Under analytical conditions, columns packed with Kinetex 5 µm media provide the same or better efficiency as columns packed with fully porous 3 µm particles, but operate at backpressures that are consistent with 5 µm 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 µm column (Axia packed C18 50 x 21.2 mm) and a fully porous 5 µm column (XBridge™ Prep 5 µm C18 OBD™ 50 x 19 mm) were compared under identical conditions for some strongly basic test probes (amitriptyline and doxepin).

Material and Methods

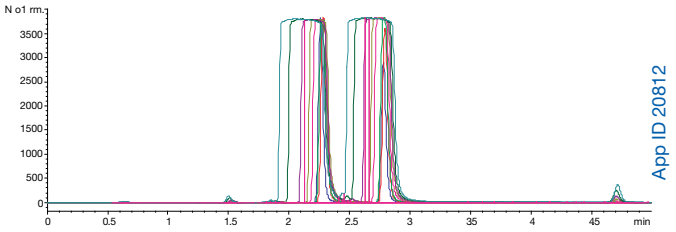
All chemicals were obtained from Sigma Chemical Co. (St. Louis, Missouri). Solvents were purchased from EMD (San Diego, California). The Axia-packed Kinetex 5 µm C18 column (50 x 21.2 mm) was obtained from Phenomenex (Torrance, California) and the Waters® XBridge™ Prep C18 5 µm OBD™ 50 x 19 mm was obtained 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 LC-20AP preparative chromatography system.

Results and Discussion

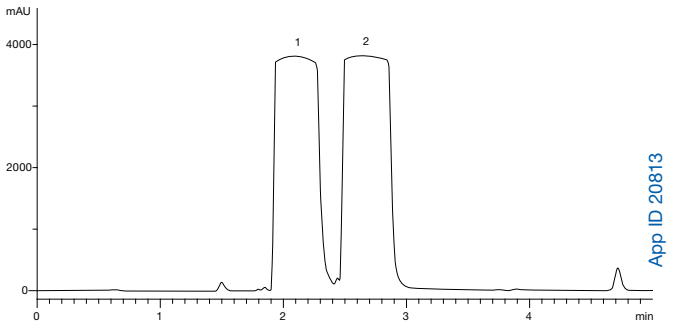
With the release of the new Kinetex 5 µm media, Phenomenex can now offer chromatographers a core-shell particle that can be scaled directly from analytical to preparative levels. A simple scaling experiment is illustrated in **Figure 1**, using caffeine and theophylline as the model test probes. The process begins with an analytical loading experiment in which successively larger amounts (from 4 µg to 2.4 mg) are loaded onto an analytical column (Kinetex 5 µm 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 an Axia packed Kinetex 5 µm C18 50 x 21.2 mm column (**Figure 1c**). In this case, we can scale-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 µm C18 50 x 4.6 mm.

a. Analytical scale-up using a Kinetex 5 µm C18; 4 ug – 2.4 mg on-column



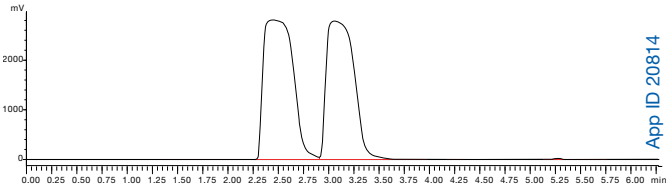
b. Final analyte loading of 2.4 mg injection



Conditions for Figure 1a and 1b:

Column:	Kinetex 5 µm C18
Dimensions:	50 x 4.6 mm
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:	1. Caffeine
	2. Theophylline
Sample Concentration:	20 mg/mL in 80/20 water/methanol

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



Conditions for Figure 1c:

Column: Kinetex 5 µm C18 Axia packed
Dimensions: 50 x 21.2 mm
Mobile Phase: A: Water with 0.1 % TFA
B: Acetonitrile with 0.1 % TFA
Gradient:

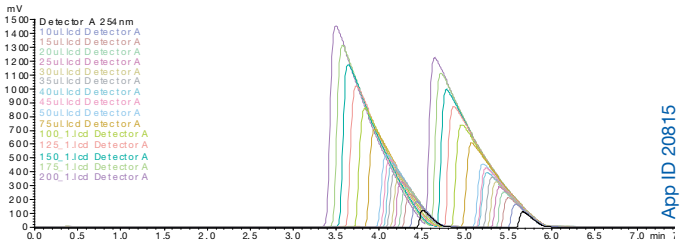
Time (min)	% B
0	3
5	35

Flow Rate: 20 mL/min
Temperature: 22 °C
Detection: UV @ 254 nm
Injection Volume: 2.4 mL
Sample: 1. Caffeine
2. Theophylline
Sample Concentration: 20 mg/mL in 80/20 water/methanol

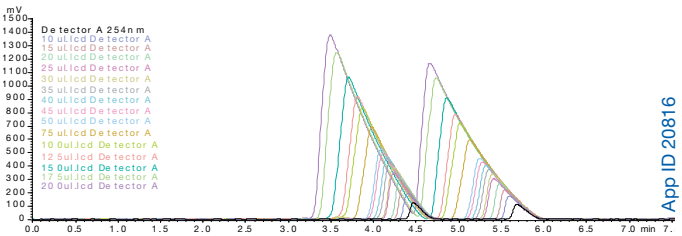
To determine how the Kinetex 5 µm media would perform for more challenging analytes, we evaluated the loading performance of the Phenomenex Axia packed Kinetex 5 µm C18 column and compared it to a fully porous 5 µm alternative (Waters® XBridge™ Prep 5 µm C18 OBD™ 50 x 19 mm) for two very strongly basic analytes – doxepin and amitriptyline. **Figures 2a** and **2b** show overlays of successive injections (5 - 500 mg on-column) of the drug mixture onto the Axia packed Kinetex 5 µm C18 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. However, if we look closely at the peak shape for one of the bases (amitriptyline; **Figure 3**), you can clearly see that the peak width for amitriptyline is narrower on the Axia-packed Kinetex column, and stays narrower across every single sample load. This data may reflect the higher efficiency of the Kinetex 5 µm 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 Axia-packed Kinetex 5 µm core-chell column has a very similar loading capacity to the fully-porous XBridge Prep 5 µm C18 OBD 50 x 19 mm column. But, because the core-shell Kinetex 5 µm 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 2.
Preparative load of doxepin and amitriptyline (5 to 500 mg on-column) using a mobile phase containing 0.1 % TFA

a. Phenomenex Kinetex 5 µm C18 50 x 21.2 mm



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



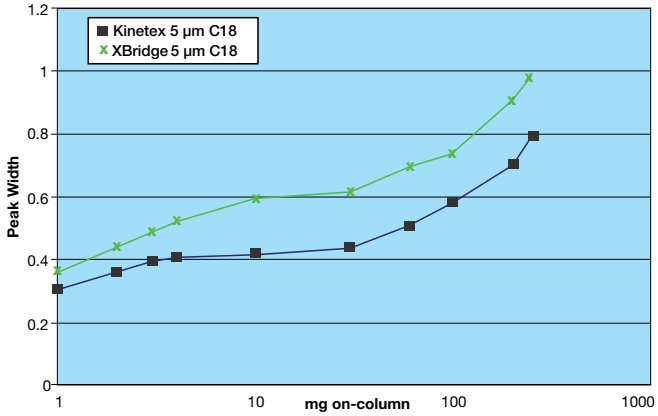
Conditions for Figure 2:

Column: Kinetex 5 µm C18 Axia packed
XBridge Prep 5 µm C18 OBD
Dimensions: 50 x 21.2 mm
50 x 19 mm
Mobile Phase: A: Water with 0.1 % TFA
B: Acetonitrile with 0.1 % TFA
Gradient:

Time (min)	% B
0	20
8	50
11	50

Flow Rate: 30 mL/min
Temperature: 22 °C
Detection: UV @ 254 nm
Sample: 1. Doxepin
2. Amitriptyline
Sample Concentration: 200 mg/mL in DMSO

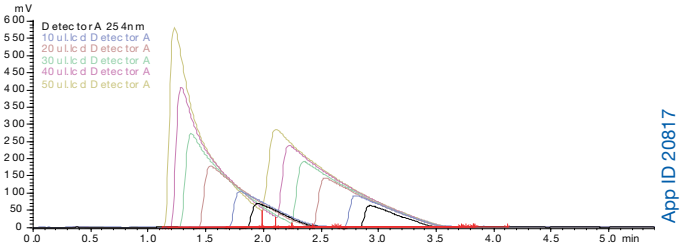
Figure 3.
Plots of peak width as a function of sample load for amitriptyline using the 0.1 % TFA modified mobile phase. Data obtained from the chromatograms shown in Figure 2.



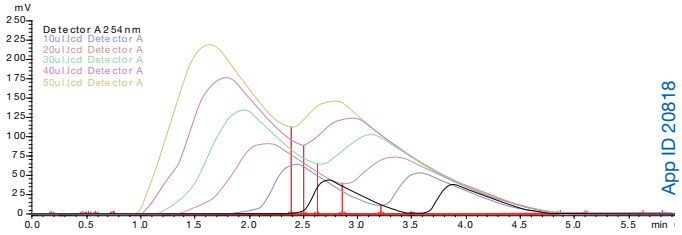
If we perform this same evaluation, but substitute formic acid (0.1%) for the TFA, we get a much different picture (**Figure 4**). Without the presence of the ion-pairing capacity of the TFA, the chromatographic behavior of these strong bases is compromised, resulting in more peak distortion for both columns. However, as you can clearly see, at higher loads, the chromatography is much better preserved on the Axia packed Kinetex 5 µm C18 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 packed Axia 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 4.
Preparative load of doxepin and amitriptyline (5 to 500 mg on-column) using a mobile phase containing 0.1 % formic acid

a. Phenomenex Kinetex 5 µm C18 50 x 21.2 mm



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



Conditions for Figure 4:

Column:	Kinetex 5 µm C18 Axia packed XBridge Prep 5 µm C18 OBD
Dimensions:	50 x 21.2 mm 50 x 19 mm
Mobile Phase:	A: Water with 0.1 % TFA B: Acetonitrile with 0.1 % TFA
Gradient:	**Time (min) % B** 0 20 8 50 11 50
Flow Rate:	30 mL/min
Temperature:	22 °C
Detection:	UV @ 254 nm
Sample:	1. Doxepin 2. Amitriptyline
Sample Concentration:	200 mg/mL in DMSO

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 Axia packed Kinetex 5 µm C18 column is able to perform as good or better than preparative columns packed with fully porous 5 µm media (Waters XBridge Prep 5 µm 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 Axia packed Kinetex 5 µm C18 column and the 50 x 19 mm Waters XBridge Prep 5 µm 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.

TN-1136

APPLICATIONS

NEW! 5µm Columns (mm)	50 x 2.1	SecurityGuard™ ULTRA Cartridges* 3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard ULTRA Cartridges* 3/pk
XB-C18	00B-4605-AN	AJ0-8782	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-AN	AJ0-8782	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
PFP	00B-4602-AN	AJ0-8787	00B-4602-E0	00D-4602-E0	00F-4602-E0	00G-4602-E0	AJ0-8773
Phenyl-Hexyl	00B-4603-AN	AJ0-8788	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
		for 2.1 mm ID					for 4.6 mm ID

* SecurityGuard ULTRA cartridges require holder, Part No. AJ0-9000.

NEW! 5µm Axia Packed Preparative Columns (mm)	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2	SecurityGuard PREP Cartridges** ea
XB-C18	00B-4605-P0-AX	00D-4605-P0-AX	00F-4605-P0-AX	00G-4605-P0-AX	AJ0-9145
C18	00B-4601-P0-AX	00D-4601-P0-AX	00F-4601-P0-AX	00G-4601-P0-AX	AJ0-9145
PFP	00B-4602-P0-AX	00D-4602-P0-AX	00F-4602-P0-AX	00G-4602-P0-AX	AJ0-9146
Phenyl-Hexyl	00B-4603-P0-AX	00D-4603-P0-AX	00F-4603-P0-AX	00G-4603-P0-AX	AJ0-9147
					for 21.2 mm ID

** SecurityGuard PREP 21.2 mm ID cartridges require holder, Part No. AJ0-8223.

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@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

guarantee

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.

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN96740812_W