Kinetex® 5 µm Core-Shell Columns Deliver Significant Improvements in Chromatographic Efficiency and Resolution versus Traditional Fully Porous 5 µm Columns

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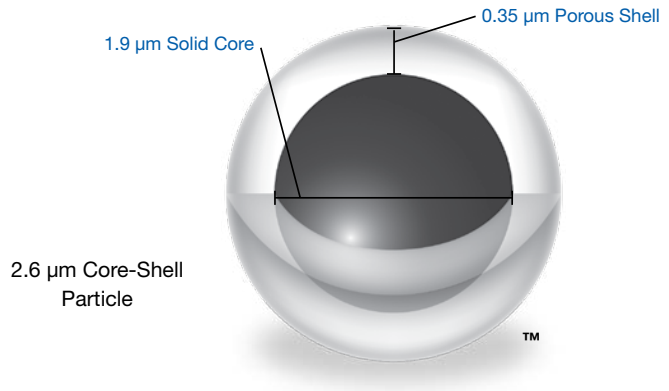
Kinetex 5 µm core-shell technology columns provide chromatographers a simple solution for dramatically improving the performance of their methods developed on 5 µm fully porous columns. This newly introduced core-shell media delivers backpressures of a fully porous 5 µm particle at efficiencies equal to or better than a fully porous 3 µm particle. Without the need for extensive method development, replacing the fully porous 5 µm column with the Kinetex 5 µm core-shell column results in improved chromatographic resolution and sensitivity. In addition, the lower backpressure can provide many benefits such as longer column lifetime, higher throughput, and increased system compatibility.

Introduction

Over the last several years, UHPLC/HPLC columns that can provide previously unheard of column efficiencies, in excess of 250,000 plates/meter, have dominated the market. Smaller particle diameter (<2 µm) versions of the traditional 5 and 3 µm media based on fully porous silica are now widely available, but due to the inherent increase in pressure that accompanies this decrease in particle diameter, UHPLC systems capable of operating in the range of 600 to 1200 bar (9000 to 17,500 psi) must be used to fully realize the performance benefits of the columns packed with these sub-2 µm particles. More recently, 2.6 µm core-shell particles, which consist of a non-porous silica core surrounded by a thin layer of porous silica **(Figure 1)**, have been introduced and widely adopted. These 2.6 µm core-shell particles can deliver UHPLC-like efficiencies and chromatographic performance, but in contrast to fully porous sub-2 µm silica particles, core-shell particle columns can be utilized on conventional HPLC systems. This can extend benefits of UHPLC performance to a wider audience without necessitating a major capital expenditure in new UHPLC systems. This technical note focuses on the most recently introduced 5 µm core-shell particle technology and the efficiency and resolution benefits it provides.

Figure 1.
Kinetex 2.6 µm Core-Shell Particle. Ratio (ρ) of solid core diameter to core-shell particle diameter remains the same for the new Kinetex 5 µm core-shell particle.



Materials and Methods

Unless stated otherwise, all chemicals and standards were obtained from Sigma Chemical Co. (St. Louis, Missouri). Standards for the analysis of paroxetine and its impurities were obtained from the European Directorate for the Quality of Medicines and Healthcare (EDQM). Solvents were purchased from EMD (San Diego, California). The Kinetex 5 µm XB-C18 and C18 columns were obtained from Phenomenex (Torrance, California). Non-Phenomenex brand columns were purchased from the original equipment manufactures as noted in the figures. The column dimensions and running conditions for the various assays are detailed in the figure captions. Chromatograms in **Figure 3** were obtained using an Agilent® 1200SL HPLC system equipped with a micro flow cell. Chromatograms for **Figures 4** and **6** were obtained using an Agilent 1100 HPLC system equipped with a standard flow cell, and chromatograms in **Figure 5** were obtained using an Agilent 1100 HPLC system which had been optimized to reduce extra-column dead volume. Data was collected using ChemStation software (Agilent, Santa Clara, California).

Results and Discussion

We have recently released a core-shell media, Kinetex 5 µm, which generates the same backpressure as traditional fully porous 5 µm columns **(Figure 2)**, while providing chromatographic efficiencies in the range of 180,000 plates/meter. This is about 2x greater than the typical efficiencies achieved with fully porous 5 µm columns (ca. 100,000 plates/meter, **Figure 3**) and comparable to the efficiencies attained with fully porous 3 µm columns (ca. 160,000 plates/meter, Figure 3). Thus, the new Kinetex 5 µm media is able to deliver efficiencies that are as good, or better, than fully porous 3 µm media at the typical operating pressures of a 5 µm column. The Kinetex 5 µm core-shell particle is scaled up directly from the original Kinetex 2.6 µm core-shell particle, maintaining the same ratio of core diameter to core-shell particle diameter (ρ = 0.73).

(continued on page 2)

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Figure 2.
Backpressures Delivered by 5 µm Core-shell Particle

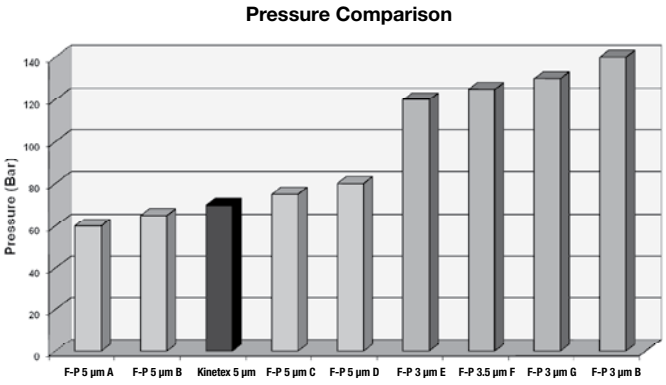


Figure 3.
Kinetex® 5 µm Core-Shell Columns Deliver Efficiencies ≥ Fully Porous 3 µm Columns

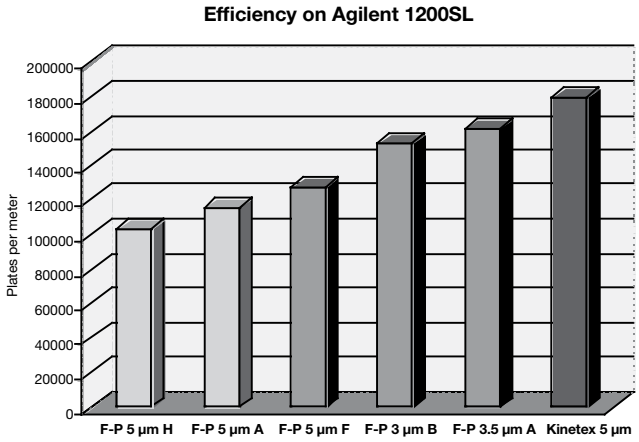


Figure 4 illustrates how the increased efficiency provided by Kinetex 5 µm core-shell columns can significantly improve chromatographic resolution for a complex separation. Here a stability-indicating method for paroxetine, an anti-depressant drug, was run using a 250 x 4.6 mm column packed with fully porous 5 µm C18 particles. In this method, one must be able to quantify four principle degradants, labeled a-d. In addition, there is also an unidentified degradant which elutes between degradant c and the paroxetine API peak that must be resolved.

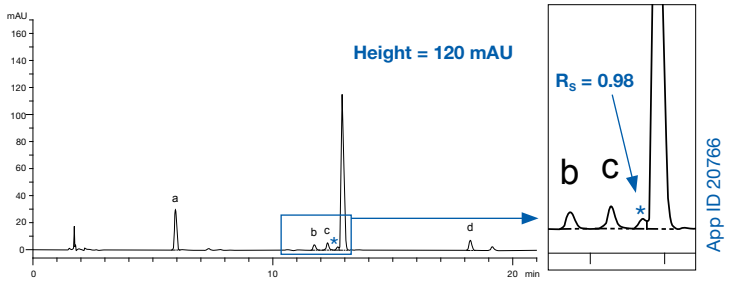
Zooming on the region around the base of the API peak, we can see that the resolution between paroxetine and the unidentified degradant was 0.98 using the fully porous 5 µm column.

Keeping all of the conditions constant and simply replacing the fully porous 5 µm column with a Kinetex 5 µm C18 column, we see an immediate benefit of higher efficiency expressed by the Kinetex column. The resolution between paroxetine and the unidentified degradant has increased from 0.98 to 2.23, an increase of 127%. The increase in resolution cannot be fully attributed to the increase in column efficiency however, but also due to a small difference in selectivity. Note that while the overall selectivities of the core-shell and fully porous 5 µm columns are very similar, the retention on the Kinetex column is reduced slightly. This is due to the reduced surface area of the core-shell particle.

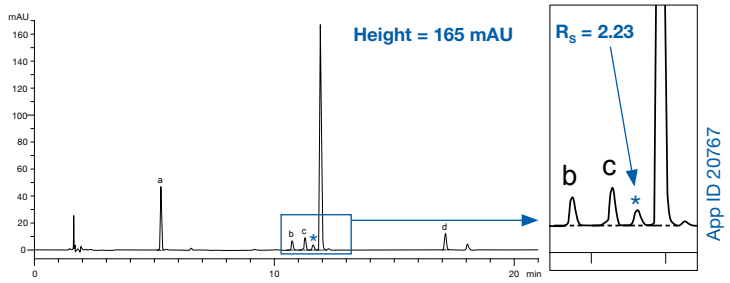
It should also be noted that operating these columns under the same conditions on a conventional HPLC system (Agilent® 1100) generates the same pressure, so this increase in efficiency and resolution does not require changes to the system or investing in a higher pressure UHPLC system.

Figure 4.
Improved Resolution and Sensitivity for Paroxetine Stability-indicating Assay on Kinetex 5 µm Core-shell Versus Fully Porous 5 µm Column on standard HPLC system

a. Agilent® ZORBAX® 5 µm XDB-C18 250 x 4.6 mm



b. Phenomenex Kinetex 5 µm XB-C18 250 x 4.6 mm



Conditons for both columns

Column:	Kinetex 5 µm C18 ZORBAX 5 µm XDB-C18
Dimensions:	250 x 4.6 mm
Mobile Phase:	A: TFA/Water/THF (5/900/100) B: TFA/Acetonitrile/THF (5/900/100
Gradient:	**Time (min)** **% B** 0 10 40 80
Flow Rate:	1.5 mL/min
Temperature:	40 °C
Detection:	UV @ 295 nm
Sample:	1. Impurity A 4. Unidentified 2. Impurity B 5. Paroxetine 3. Impurity C 6. Impurity D

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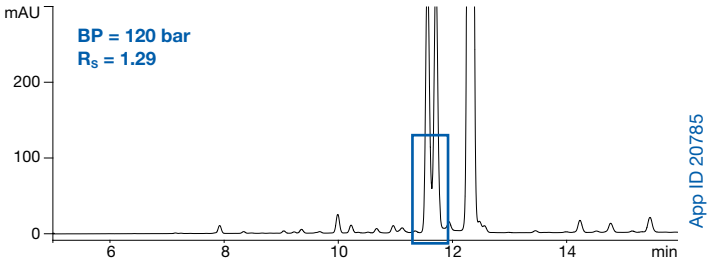
In **Figure 5** we illustrate another example of the benefits of the Kinetex® 5 µm core-shell media. In this method, the resolution between the two degradant peaks is 1.29 on the Agilent® ZORBAX® 5 µm SB-C18 column. Under the identical operating conditions, and at the same backpressure, the Kinetex 5 µm C18 column provides a 22 % increase in resolution. Simply by replacing the fully porous 5 µm column with the Kinetex core-shell 5 µm column, and without any additional method development or optimization, the chromatographic separation has been improved. This can be invaluable from a validation perspective since in most cases, simply changing from one column to a similar column (same length, particle size, bonded phase), a full re-validation of the method is not necessary. You might just need to perform an equivalency study depending upon the regulations of your particular company and/or group.

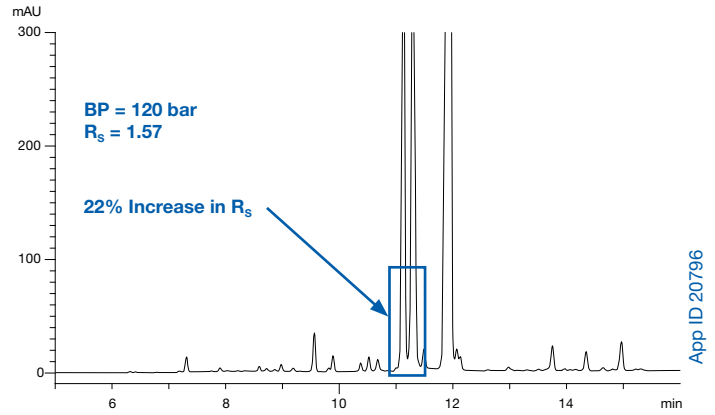
For methods that have a critical pair (or several critical pairs) of compounds to resolve, it can be difficult to meet system suitability requirements for resolution. In such cases one may find that instrument-to-instrument or column batch-to-batch variability can cause the method to fail suitability, resulting in valuable analysis time spent troubleshooting to resolve the issue. In such cases, simply switching to a Kinetex 5 µm core-shell column may provide a sufficient increase in resolution to overcome the variability that may cause sensitive methods to sometimes fail suitability. In the example illustrated in Figure 5, if additional resolution was required (and assuming the increase in analysis time was not a barrier) a longer 250 x 4.6 mm Kinetex 5 µm core-shell column could be used.

Figure 5.
Improved Resolution and Sensitivity for Mupirocin Degradation Analysis with Kinetex 5 µm versus Fully-Porous 5 µm Column on optimized HPLC system

a. Agilent® ZORBAX® 5 µm SB-C18 150 x 4.6 mm



b. Phenomenex Kinetex 5 µm XB-C18 150 x 4.6 mm



Conditions for all columns:

Columns:	Kinetex 5 µm XB-C18 ZORBAX 5 µm SB-C18
Dimension:	150 x 4.6 mm
Mobile Phase:	A: Water with 0.1% TFA B: Acetonitrile with 0.1% TFA
Gradient:	**Time (min) % B** 0 10 20 70
Flow Rate:	1.2 mL/min
Temperature:	Ambient
Detection:	UV @ 210 nm
Sample:	Mupirocin degradants

*Agilent 1100 was optimized with a Core-Shell Performance Enhancement Kit (AQ0-8892).

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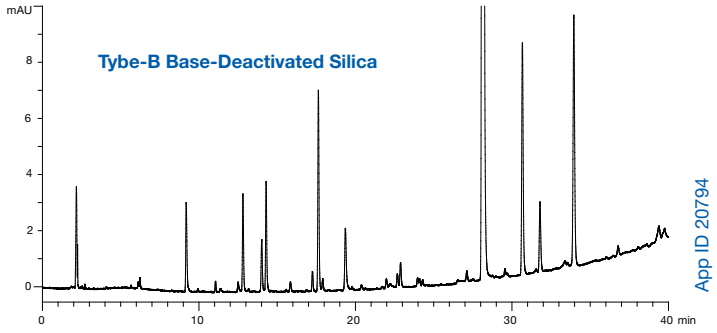
The final example of the improved chromatographic performance possible with Kinetex® 5 µm is shown in **Figure 6**. This is a stability-indicating assay for degradants of the proton-pump inhibitor, omeprazole. Here we ran the same sample on Kinetex 5 µm core-shell column and a column made with an older generation of fully porous silica, Thermo-Keystone® HYPERSIL® 5 µm ODS. What is immediately obvious in the chromatograms is the significant improvement in peak shape for the basic omeprazole peak on the core-shell column **(Figure 6A)**. Zooming out to full-scale **(Figure 6B)**, one can really appreciate the difference in performance between the two columns. With the y-axis scaled the same, the effect of the excessive peak tailing on the intensity of the omeprazole peak is apparent.

The reason for this vast difference in performance is related to the nature of the silica that is used to make the particles. Older generations of HPLC columns were based on Type A silica that contains a higher concentration of metal ions (>1000 ppm) such as iron or aluminum, which activate free silanols on the silica surface, making them more acidic. These silanols can then interact with basic analytes via ion-exchange, resulting in significant peak tailing. Kinetex core-shell particles are manufactured from high purity Type B silica, which is much lower in metal content (<< 50 ppm); this results in much less secondary silanol interactions with basic analytes and much sharper, more symmetrical peaks, which improves analyte quantitation.

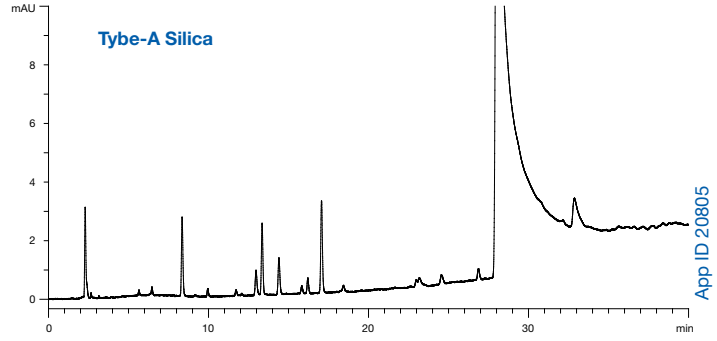
Figure 6.
Improved Peak Shape for Basic Analytes on Kinetex 5 µm XB-C18 versus HYPERSIL 5 µm ODS

6A - Zoom view

a. Phenomenex Kinetex 5 µm XB-C18 250 x 4.6 mm



b. Thermo-Keystone® HYPERSIL® 5 µm ODS 250 x 4.6 mm



Conditons for both columns:

Column: Kinetex 5 µm C18
HYPERSIL 5 µm ODS
Dimensions: 250 x 4.6 mm
Mobile Phase: A: 20 mM Potassium Phosphate, pH 7
B: 50/50 Metthanol Acetonitrile
Gradient:

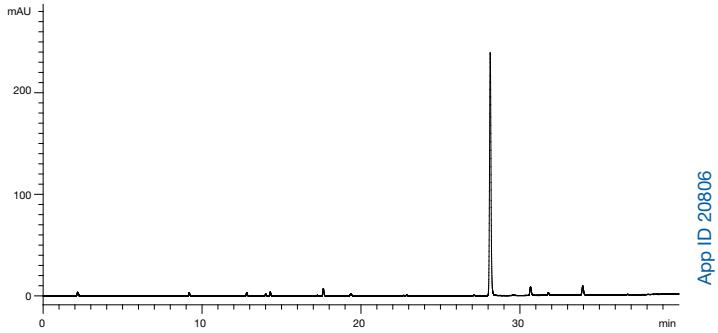
Time (min)	% B
0	5
40	70

Flow Rate: 1.2 mL/min
Temperature: Ambient
Detection: UV @ 280 nm
Sample: Omeprazole degradants

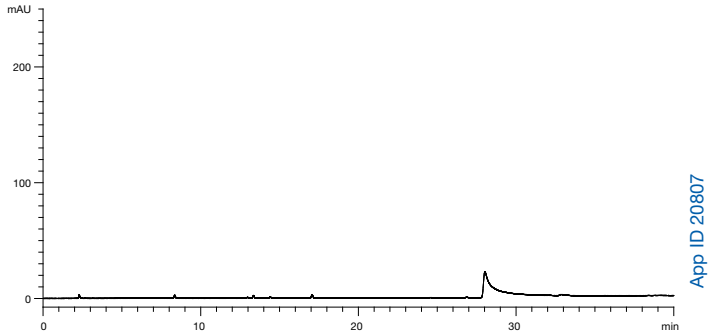
Hypersil is a registered trademark of Thermo Hypersil-Keystone (Waltham, Ma, USA). Phenomenex is not affiliated with Thermo Hypersil-Keystone. Comparative separations may not be representative of all applications.

6B - Full view

a. Phenomenex Kinetex® 5 µm XB-C18 250 x 4.6 mm



b. Thermo-Keystone® HYPERSIL® 5 µm ODS 250 x 4.6 mm



Conditons for both columns:

Column: Kinetex 5 µm C18
HYPERSIL 5 µm ODS
Dimensions: 250 x 4.6 mm
Mobile Phase: A: 20 mM Potassium Phosphate, pH 7
B: 50/50 Methanol/Acetonitrile
Gradient:

Time (min)	% B
0	5
40	70

Flow Rate: 1.2 mL/min
Temperature: Ambient
Detection: UV @ 280 nm
Sample: Omeprazole degradants

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Conclusion

- The new Kinetex® 5 µm core-shell columns operate at pressures that are consistent with typical fully porous 5 µm columns, but with efficiencies that are equal to, or greater than, fully porous 3 µm columns.

- For methods currently using fully-porous 5 µm columns, Kinetex 5 µm core-shell columns will provide improved chromatography, in terms of improved efficiency, resolution, and sensitivity, without the need for extensive method development.

Ordering Information

5 µm Analytical 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 for Part No. AJ0-9000.

5 µm Axia™ Packed Preparative Columns (mm)	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2	SecurityGuard ULTRA 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.

guarantee

If Kinetex core-shell columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase, and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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