Dramatically Improve Existing 5 µm and 3 µm Fully Porous Methods with Kinetex® 5 µm Core-shell Technology

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With virtually no development effort or investment required chromatographers running traditional methods can instantly improve resolution, sensitivity, and productivity by simply replacing the 5 µm and 3 µm fully porous columns currently used with a Kinetex 5 µm core-shell column. This newly introduced core-shell technology is able to achieve efficiency values that are equal or greater than fully porous 3 µm columns, but operates at a pressure that is consistent with typical fully porous 5 µm columns.

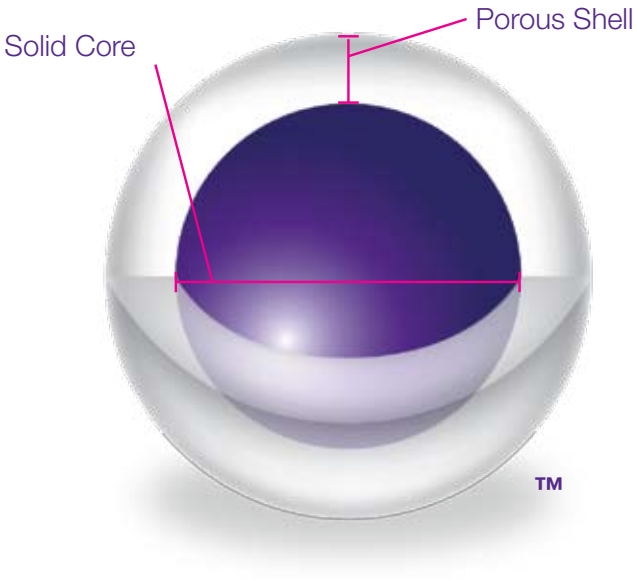
Introduction

Since the term "high performance liquid chromatography" was coined by Csaba Horvath in 1970, the dominant trend in HPLC media manufacturing has been the drive to create chromatographic media capable of delivering increasing efficiency values. Up until recently, this increase in column efficiency was achieved primarily through decreasing the mean diameter of the particles. Thus, from the 10 µm media that was prevalent pre-1970s, we have seen a steady migration to 5 µm which give efficiency values in the range of 90,000 -100,000 plates per meter, 3 µm particles giving efficiencies of about 150,000 plates per meter, and more recently sub-2 µm UHPLC (ultra high performance liquid chromatography) particles capable of achieving efficiency values in the range of almost 300,000 plates per meter. Of course, the drawback of these small particles was the accompanying increase in backpressure. In fact, the column backpressures generated by UHPLC particles was so great that specially designed UHPLC instrumentation, capable of operating at pressures of 600-1000 bar was required. When coupled with sub-2 µm packed columns, these UHPLC systems were able to deliver amazing performance, but the high cost of these systems, typically costing 2-3 times as much as conventional HPLC systems, placed them out of reach of many potential end-users.

The development of core-shell media initially served as a counterpoint to the miniaturization of chromatographic media. In contrast to fully-porous silica particles which, as the name implies, consist of a porous silica microsphere, core-shell particles consist of an impermeable inner core surrounded by a layer of porous silica (**Figure 1**). We have recently released a core-shell product, Kinetex 5 µm, which generates the same backpressure as traditional fully porous 5 µm columns, but is able to generate efficiency values in the range of 170,000 -180,000 plates per meter. To put this into perspective, typical efficiencies for fully-porous 5 µm packed columns are in the range of 90,000 -100,000 plates per meter and average 3 µm fully-porous columns typically provide 150,000 -170,000 plates per meter. Thus, the new Kinetex 5 µm core-shell media is able to deliver efficiencies that are as good as or better than 3 µm media at the pressure of a 5 µm particle.

Of course, efficiency values by themselves are only useful as bragging rights for HPLC column manufacturers. To the typical chromatographer, the true value of core-shell particles is the improved resolution, sensitivity, and productivity that they can provide when compared to fully porous particles of similar size. In this study, we present data generated using columns packed with Kinetex 5 µm core-shell particles and show how they provide improved chromatographic performance when compared to fully-porous 5 µm and 3 µm packed columns.

Figure 1.
Structure of Kinetex particle.



Material 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. Data from **Figures 2** and **3** were obtained using an Agilent 1200SL HPLC system equipped with a micro flow cell. All other chromatograms were obtained using an Agilent 1100 HPLC system equipped with a standard flow cell. Data was collected using ChemStation software (Agilent, Santa Clara, California).

Results and Discussion

Kinetex 5 µm core-shell media provides improved resolution and sensitivity compared to traditional 5 µm fully porous media

As shown in **Figure 2**, Kinetex 5 µm core-shell particles can provide efficiency values that are as good or greater than conventional 5 µm and 3 µm columns, while operating at the same pressure as fully porous 5 µm columns (**Figure 3**). For demanding chromatographic methods such as stability-indicating assays and impurity profiles, the increased efficiency of Kinetex 5 µm can provide substantial improvements in both resolution and sensitivity when compared with columns packed with fully porous 5 µm

Figure 2.
Comparison of the efficiency of fully porous (F-P) 5 µm and 3 µm media with Kinetex 5 µm core-shell media. Running conditions: 65/35 Acetonitrile/Water; 1ml/min; ambient temperature; efficiency determined from naphthalene peak.

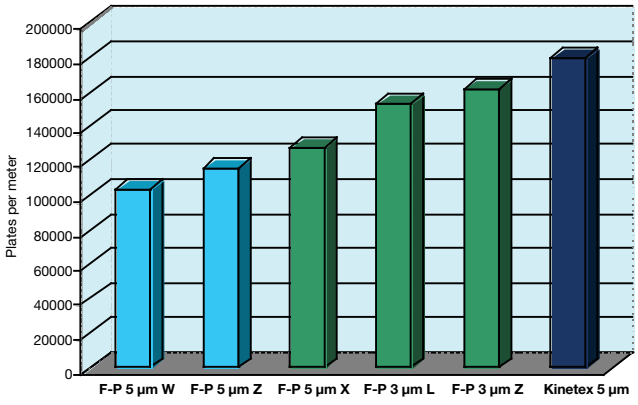


Figure 3.
Comparison of the backpressure of fully porous 5 µm and 3 µm media with Kinetex core-shell 5 µm media. Running conditions: 65/35 Acetonitrile/Water; 1ml/min; ambient temperature; all columns 150 x 4.6 mm

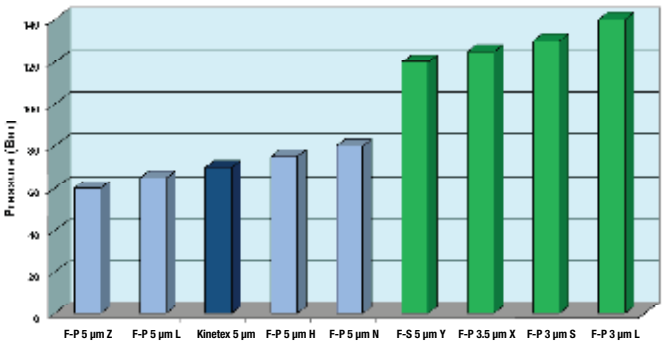


Figure 4 shows a stability-indicating assay developed for the antidepressants drug paroxetine. When the degraded drug sample is analyzed using a fully porous 5 µm packed column (Agilent®ZORBAX® 5 µm XDB-C18 250 x 4.6 mm) four principle degradants are identified and labeled as a-d (**Figure 4a**). There is also a small, unidentified degradant peak that is partially co-eluting with the main paroxetine peak (Rs = 0.98; indicated in the zoomed-in view). If we run this same method and sample using the Kinetex 5 µm XB-C18 column (250 x 4.6 mm; **Figure 4b**), we first note that the peak height of all of the components is dramatically increased. For example, the peak height of the main paroxetine is 120 mAU on the fully-porous 5 µm ZORBAX column and 165 mAU on the Kinetex 5 µm XB-C18 column, a ~37 % increase in sensitivity. In addition, the unidentified impurity peak is completely resolved on the Kinetex 5 µm XB-C18 column (Rs = 2.23; a 127 % increase). At least some of that increase in efficiency may also be attributable to differences in selectivity, not just the improved efficiency of the Kinetex 5 µm XB-C18 column, but a significant portion of that improved resolution can also be attributable to the peak widths being much narrower on the Kinetex 5 µm XB-C18 column.

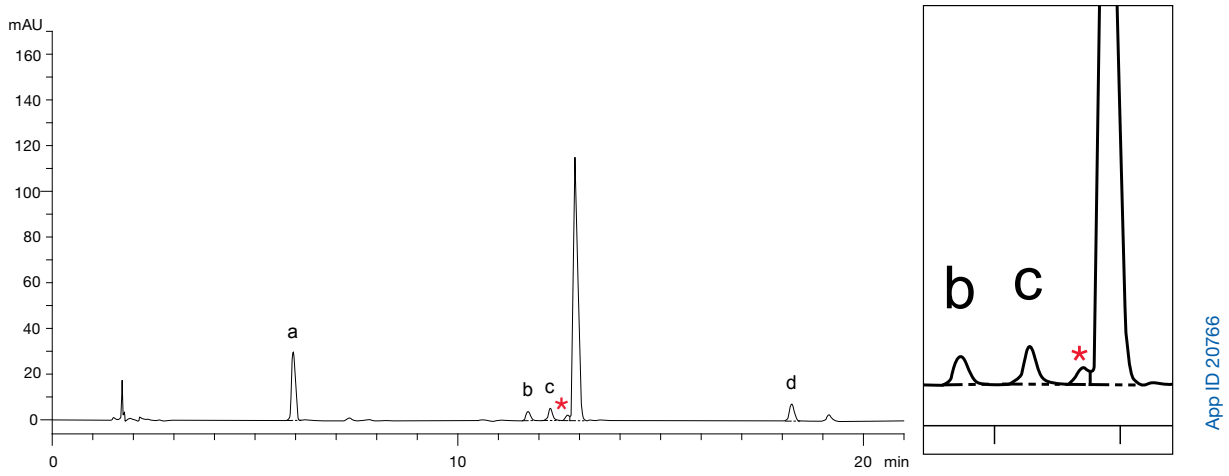
Thus, without making any significant changes to the method itself, the Kinetex 5 µm XB-C18 column was able to provide a significant increase in resolution (127 %) and sensitivity (37 %) when compared to the fully porous 5 µm column.

In **Figure 5**, we present another comparison of the performance of a Kinetex core-shell 5 µm column versus a fully porous 5 µm

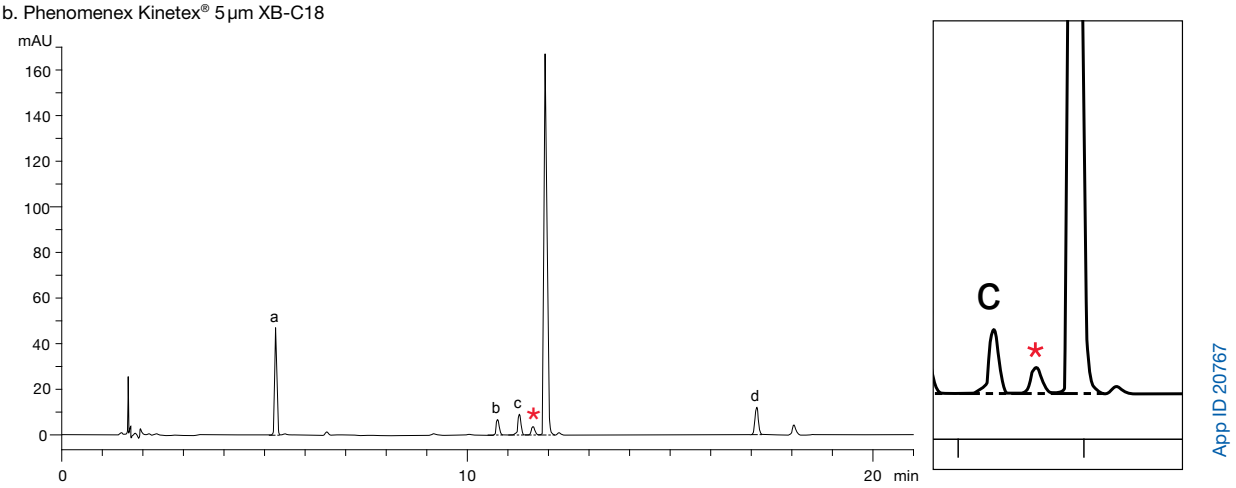
Comparative separations may not be representative of all applications.

Figure 4.
Stability-indicating assay for paroxetine on a fully porous 5 µm column and Kinetex core-shell 5 µm column.

a. Agilent® ZORBAX® 5 µm XDB-C18



b. Phenomenex Kinetex® 5 µm XB-C18

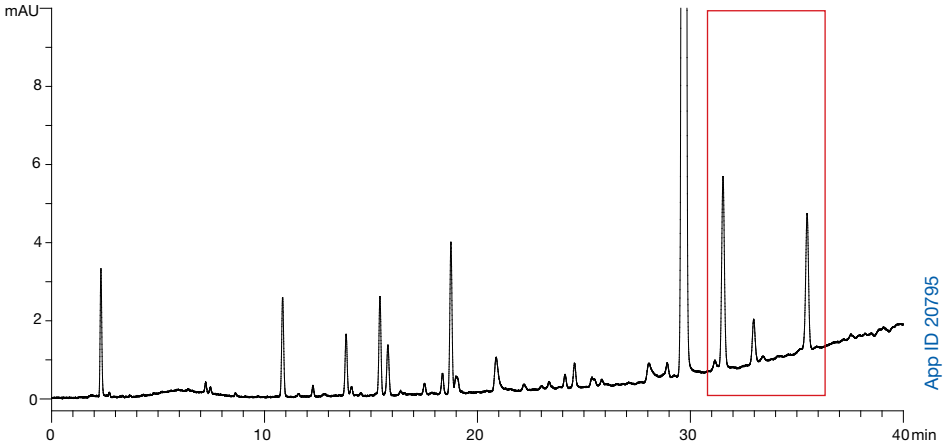


Conditons for both columns

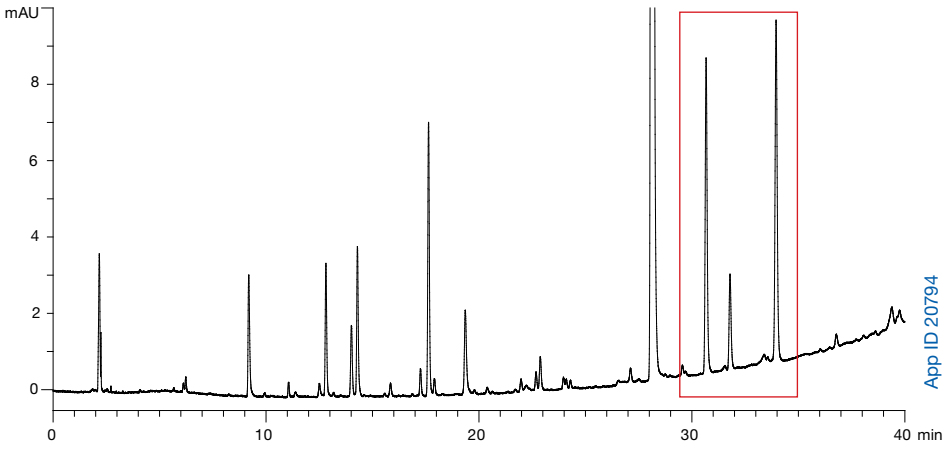
Column:	Kinetex 5 µm C18	
	ZORBAX 5 µm XDB-C18	
Dimensions:	250 x 4.6 mm	
Mobile Phase:	A: TFA/Water/THF (5/900/100)	
	B: TFA/Acetonitrile/THF (5/900/100	
Gradient:	**Time (min)**	**% B**
	0	10 %
	40	80 %
Flow Rate:	1.5 mL/min	
Temperature:	40 °C	
Detection:	UV @ 295 nm	
Sample:	1. Impurity A	4. Unidentified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

Figure 5.
Impurity profile for omeprazole using a fully porous 5 µm column and Kinetex 5 µm core-shell column.

a. Waters® Symmetry® 5 µm C18 250 x 4.6mm; Backpressure = 190 Bar



b. Phenomenex Kinetex® 5 µm C18 250 x 4.6 mm; Backpressure = 188 Bar



Conditons for both columns

Column:	Kinetex 5 µm C18 Symmetry 5 µm XDB-C18
Dimensions:	250 x 4.6 mm
Mobile Phase:	A: mm Potassium Phosphate, pH 7 B: Acetonitrile
Gradient:	**Time (min) % B** 0 5 % 40 70 %
Flow Rate:	1.2 mL/min
Temperature:	Ambient
Detection:	UV @ 295 nm
Sample:	Omeprazole degradants

packed column, in this case Waters Symmetry® 5 µm C18. This is an impurity profile for the drug omeprazole. Overall, the selectivity between the two columns is fairly similar. However, the sensitivity, in terms of peak height response, is much greater on the Kinetex core-shell 5 µm C18 column. This is immediately apparent if you look at the peak height response of the three impurity peaks in the red boxes – the peak heights are almost double on the Kinetex 5 µm column as compared to the fully porous Symmetry 5 µm C18 column.

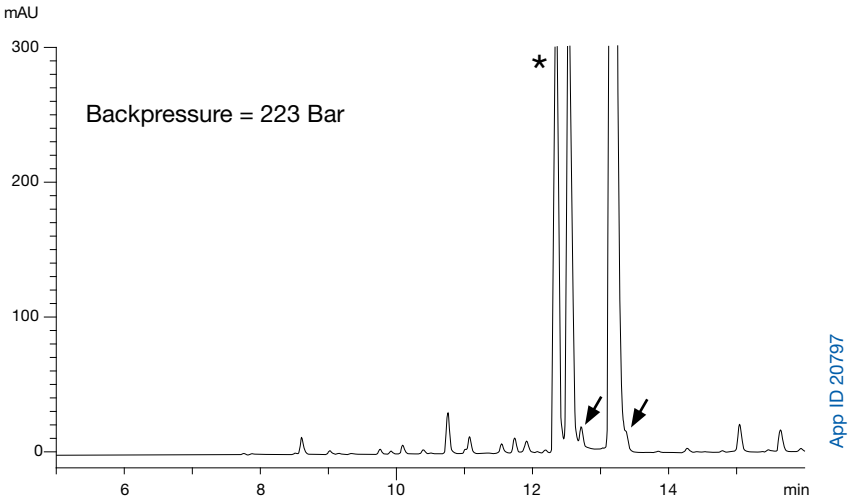
Kinetex® 5µm provides equivalent or better performance compared to a 3µm column, but at a fraction of the pressure

In the previous section we showed that, with its improved efficiency, the Kinetex 5µm core-shell column was able to provide significant improvements in resolution and sensitivity when compared to fully porous 5µm columns. However, Kinetex 5µm core-shell media is also an excellent alternative to fully porous 3µm columns as it is able to provide equivalent or better efficiency compared to a 3µm column (**Figure 2**), but operates at a much lower backpressure. This is well suited for pressure-limited operating systems and also gives the chromatographer the opportunity to either (a) run at higher flow rates to decrease run time and increase productivity or (b) use a longer column to gain further resolution without introducing excessive backpressure to the system.

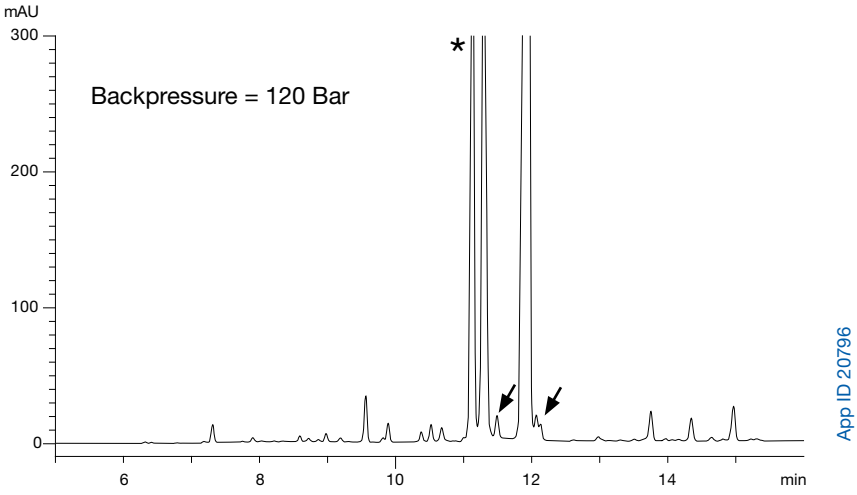
Figure 6 shows a stability-indicating assay developed for a proprietary API and run on a fully porous 3µm packed column (CERI L-Column 3µm ODS 150 x 4.6mm) and on the Kinetex core-shell 5µm C18 column (150 x 4.6mm). The resolution between the two primary degradants of interest, indicated by the asterisk, are virtually identical on both columns (Rs = 1.59 on the 3µm fully po-

Figure 6.
Stability-indicating assay for a proprietary API using a fully-porous 3um column and Kinetex 5µm C18.

A. CERI L-Column 3µm ODS 150 x 4.6mm



B. Phenomenex Kinetex 5µm C18 150 x 4.6mm



Conditons for both columns

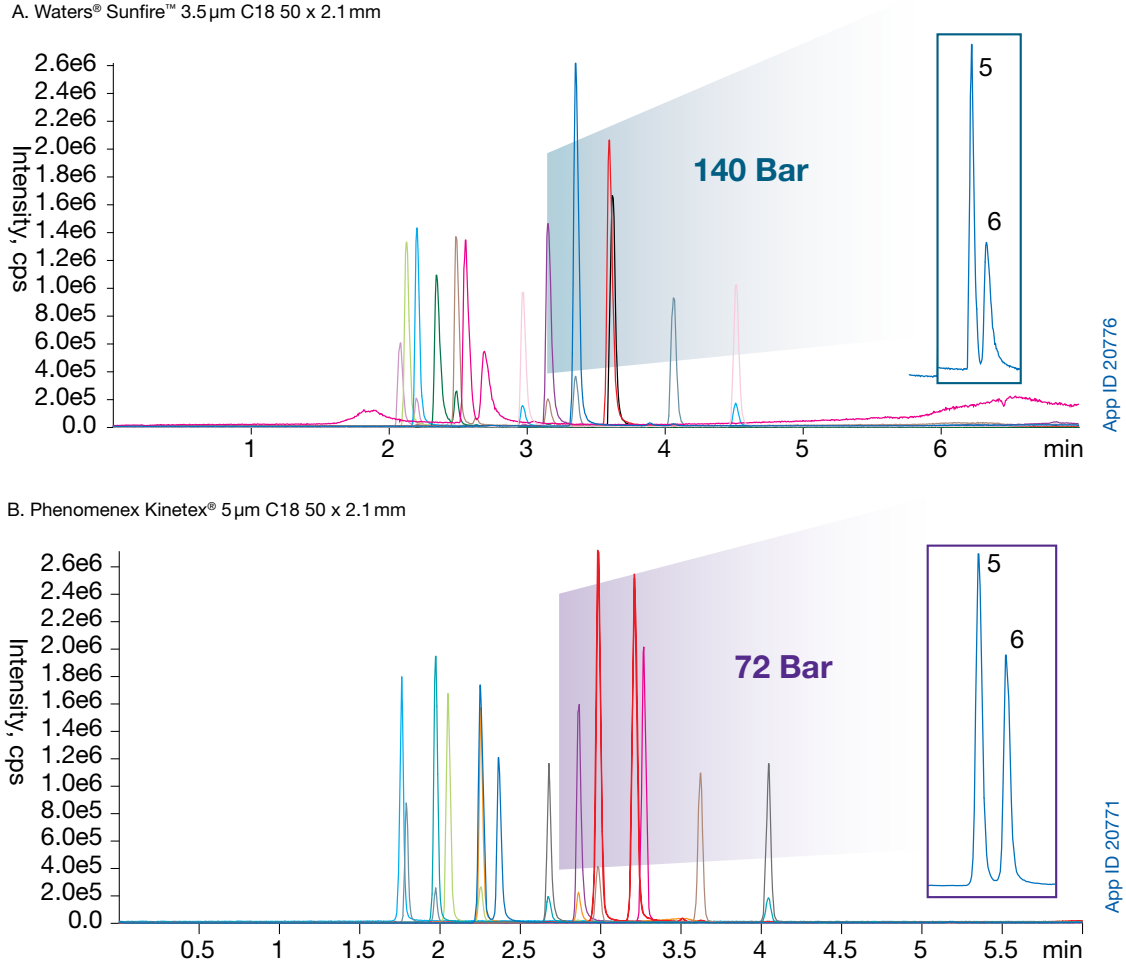
Column:	Kinetex 5µm C18 L-Column 3 µm ODS
Dimensions:	150 x 4.6 mm
Mobile Phase:	A:Water with 0.1% TFA B: Acetonitrile with 0.1% TFA
Gradient:	**Time (min) % B** 0 10 % 20 70 %
Flow Rate:	1.0 mL/min
Temperature:	22 °C
Detection:	UV @ 210 nm
Sample:	Proprietary API degradants

L-Column is a trademark of CERI (Chemicals Evaluation and Research Institute, Japan). Phenomenex is not affiliated with CERI. Comparative separations may not be representative of all applications.

rous column and 1.57 on the Kinetex 5 µm core-shell column). However, the fully porous column is operating at almost double the backpressure that is generated by the Kinetex 5 µm core-shell column (223 Bar versus 120 Bar). In addition, several of the smaller impurities, indicated by the arrows, are actually better resolved on the Kinetex 5 µm core-shell column.

Thus far, we have focused primarily on LC/UV applications run using longer (150 mm and 250 mm) column formats. However, the benefits of Kinetex 5 µm also extend to shorter lengths that are typically associated with LC/MS analysis. In **Figure 7** we compare the performance of Kinetex 5 µm core-shell media packed in a 50 x 2.1mm format against a fully porous 3.5 µm column of equivalent dimension (Waters® Sunfire™ 3.5 µm C18). As is apparent from the chromatograms, the Kinetex core-shell 5 µm C18 column provides much better performance than the fully porous 3.5 µm column, with narrower peak widths and improved peak shape for these strongly basic analytes. For this method, the critical isobaric pair that needs to be resolved are 4,8- and 7,8-dimethyl IQx, which are extracted from the rest of the components in

Figure 7.
LC/MS/MS analysis of heterocyclic amines extracted from salmon tissue using a fully porous 3.5 µm column and the Kinetex 5 µm core-shell column.



Conditons for both columns

Column:	Kinetex 5 µm C18 Sunfire 3.5 µm C18	
Dimensions:	50 x 2.1 mm	
Mobile Phase:	A: 30 mm Ammonium formate B: Acetonitrile	
Gradient:	**Time (min)**	**% B**
	0	5 %
	5	60 %
	5.1	100 %
	6	100 %
	7	5 %
Flow Rate:	1.2 mL/min	
Temperature:	Ambient	
Detection:	MS/MS (API 4000)	
Sample:	1. DMIP	10. PhIP
	2. Qx	11. AaC
	3. IQ	12. MeAaC
	4. 8-MeIQ	13. MeIQ
	5. 4,8-DiMe-Iqx	
	6. 7,8-DiMe-Iqx	
	7. Trp-P-2	
	8. Harman	
	9. Norharman	

the bottom ion chromatogram. This critical isobaric pair is much better separated on the Kinetex® 5µm core-shell media than on the fully porous 3.5 µm media. Also note that the backpressure of the Kinetex 5 µm core-shell column is about one-half of the fully porous 3.5 µm column.

Conclusion

- Kinetex 5 µm core-shell media is able to achieve efficiency values that are equal or greater than fully porous 3 µm columns, but operates at a pressure that is consistent with typical fully porous 5 µm columns
- For methods using fully porous 5 µm columns, Kinetex 5 µm core-shell media has the potential to provide improved chromatography, in terms of improved resolution and sensitivity, without the need for extensive method development.
- When compared to fully porous 3 µm columns, Kinetex 5 µm has the capacity to deliver equivalent or greater performance at about one-half of the pressure, allowing the chromatographer to take advantage of increasing flow rates to shorten analysis times or, alternatively, to use longer column lengths to further improve resolution. In addition, because of the low backpressure generated by the Kinetex 5 µm columns, there is also the potential for increased column lifetime when compared to column packed with smaller particles.

Ordering Information

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.

5µm Axia Packed Preparative Columns (mm)

	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2
XB-C18	00B-4605-P0-AX	00D-4605-P0-AX	00F-4605-P0-AX	00G-4605-P0-AX
C18	00B-4601-P0-AX	00D-4601-P0-AX	00F-4601-P0-AX	00G-4601-P0-AX
PFP	00B-4602-P0-AX	00D-4602-P0-AX	00F-4602-P0-AX	00G-4602-P0-AX
Phenyl-Hexyl	00B-4603-P0-AX	00D-4603-P0-AX	00F-4603-P0-AX	00G-4603-P0-AX

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.

APPLICATIONS

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