Increased Efficiency and Resolution with Kinetex™ Core-Shell Technology

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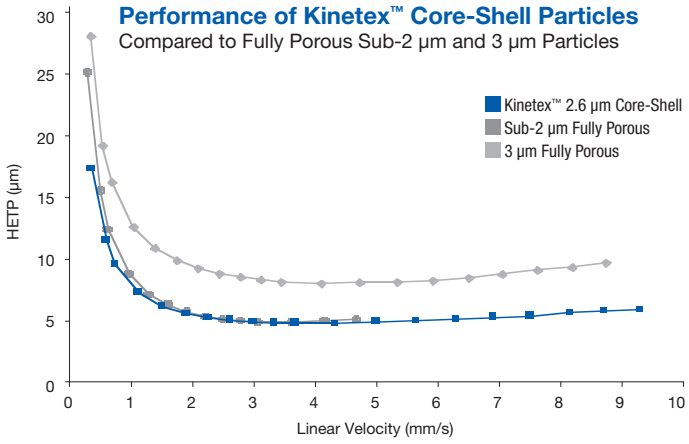
The combination of the small particle size and narrow particle size distribution coupled with the significantly shorter diffusion path provided by the Kinetex™ core-shell particle results in a material that yields significantly increased column efficiencies and chromatographic resolution. This is a significant benefit for separation scientists looking to increase efficiency and chromatographic resolution, especially for complex separations containing many compounds and very closely eluting compounds so that accurate identification and quantitation can be achieved.

Introduction

Over the past several years column manufacturers have been introducing columns packed with smaller particle sizes – sub-2 µm and 3 µm – to take advantage of the improvements that such small particle size columns offer. The benefits offered by these columns include faster separations (see Technical Note TN-1057) due to the reduction in the resistance to mass transfer term (C-term) in the van Deemter equation (**Graph 1**) allowing for higher efficiency over a wider range of linear velocity.

Graph 1



As known, from the familiar resolution equation (below), an increase in column efficiency directly results in an increase in resolution that is equal to the square root of the increase in efficiency.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

k = the average value for the two peaks
α = the measurement of the spacing between the two peaks, expressed as:
$\alpha = k_2/k_1$

A comparison of the efficiency of Kinetex™ column across multiple instrument platforms was performed. In addition, other small fully porous particle columns were compared as a reference against the performance observed for Kinetex™ core-shell technology.

Results and Discussion

Part 1: Increased Efficiency

Fast LC on Traditional HPLC System

The first examples (**Figures 1a & 1b**) focus on how Kinetex™ 2.6 µm compares with a high efficiency 3 µm material in a commonly used analytical column dimension (150 x 4.6 mm) on a traditional HPLC system (Agilent 1100).

One can observe that the column efficiency, as tested by injecting a standard reversed phase column test mixture, for Kinetex™ column is dramatically increased versus a Luna® 3 µm C18(2) column. The Kinetex™ column exhibits column efficiencies greater than 225,000 plates/meter (p/m) – more than 60 % greater than the high efficiency Luna® 3 µm 150 x 4.6 mm column under the same isocratic mobile phase conditions. The peaks are significantly narrower and the analysis time much shorter on the Kinetex™ column, indicating that the expected benefits provided by smaller particle size HPLC columns (higher efficiency, faster separations, and improved resolution) can be obtained even when using older model HPLC systems.

Figure 1a.
Kinetex™ 2.6 µm C18
Efficiency (based on Naphthalene) = 225,900 p/m

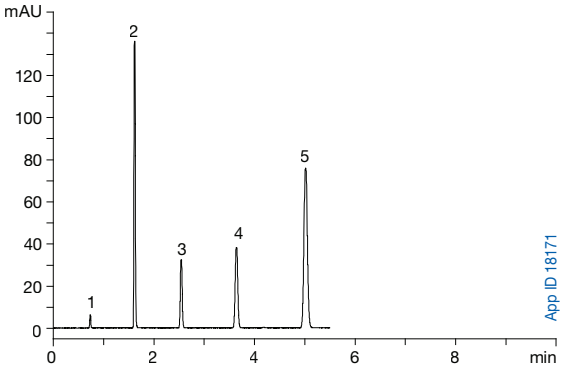
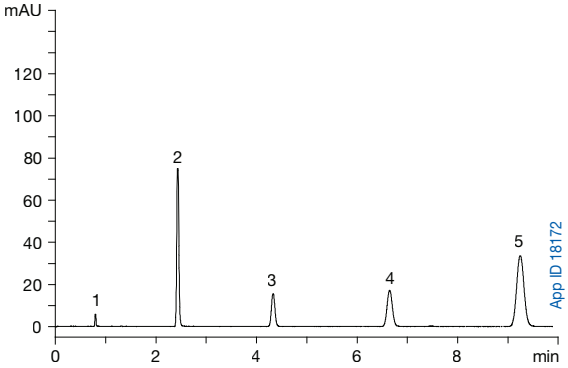


Figure 1b.
Luna® 3 µm C18(2)
Efficiency (based on Naphthalene) = 140,240 p/m



Conditions for both columns:
Columns: material as noted
Dimensions: 150 x 4.6 mm
Mobile Phase: Acetonitrile / Water (50:50)
Flow Rate: 1.7 mL/min
Temperature: 25 °C
Detection: UV @ 254 nm

Instrument: Agilent 1100
Sample: 1 µL test mixture
1. Uracil
2. Acetophenone
3. Benzene
4. Toluene
5. Naphthalene

APPLICATIONS

Part 1: Increased Efficiency (cont'd)

Fast LC on Agilent 1200 SL RRLC System

The next example (**Figures 2a & 2b**) looks at how Kinetex™ columns compare with other materials packed in a commonly used "fast LC" column dimension (50 x 4.6 mm) on a Rapid Resolution HPLC system (Agilent 1200SL) that has a higher pressure limit (600 bar) and significantly reduced dead volume as compared to the more widely available traditional HPLC systems having 400 bar pressure limits.

The Kinetex™ core-shell media provides notably higher column efficiencies than the fully porous Agilent Technologies ZORBAX® 1.8 µm media, despite a larger particle size (2.6 µm vs. 1.8 µm). This illustrates the significant benefits provided by the shorter diffusion path for the superficially porous Kinetex™ media on the C-term in the van Deemter equation, and ultimately on the overall column efficiency. Kinetex™ delivers superior column efficiencies in a shorter analysis time with significantly narrower and taller peaks, which also result in an increase in sensitivity.

Figure 2a.
Kinetex™ 2.6 µm C18
Efficiency (based on Naphthalene) = 264,700 p/m

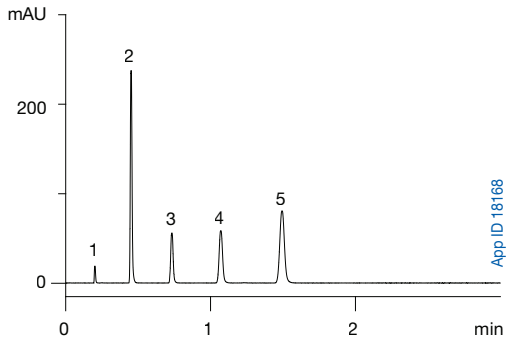
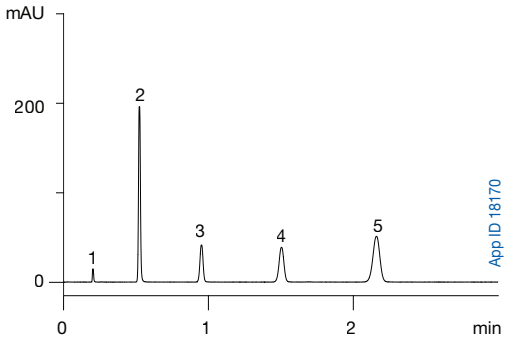


Figure 2b.
Agilent Technologies ZORBAX® 1.8 µm C18
Efficiency (based on Naphthalene) = 250,880 p/m



Conditions for both columns:
Columns: material as noted
Dimensions: 50 x 4.6 mm
Mobile Phase: Acetonitrile / Water (50:50)
Flow Rate: 2.16 mL/min
Temperature: 25 °C
Detection: UV @ 254 nm

Instrument: Agilent 1200SL RRLC
Sample: 0.5 µL test mixture
1. Uracil
2. Acetophenone
3. Benzene
4. Toluene
5. Naphthalene

Fast LC on Waters® ACQUITY® UPLC® System

In this example, (**Figures 3a & 3b**) a comparison of the performance of Kinetex™ 1.7 µm C18 with the Waters® ACQUITY® BEH 1.7 µm C18 as tested on the Waters® ACQUITY® UPLC® system. The Waters® UPLC® system was specifically designed for operation at high pressures, allowing for the use of sub-2 µm particles packed into short, narrow ID columns to obtain very fast separations while maintaining chromatographic resolution.

The chromatograms show that the Kinetex™ 1.7 µm column provides a significantly higher column efficiency than the Waters® ACQUITY® 1.7 µm column (>318,000 p/m vs. >272,080 p/m) under the same isocratic conditions for the column test mixture. Once again, this highlights the benefits of the shorter diffusion path associated with the Kinetex™ core-shell technology, which result in a significant improvement in column efficiency, peak shape and overall analysis time.

Figure 3a.
Kinetex™ 1.7 µm C18
Efficiency (based on Naphthalene) = 318,680 p/m

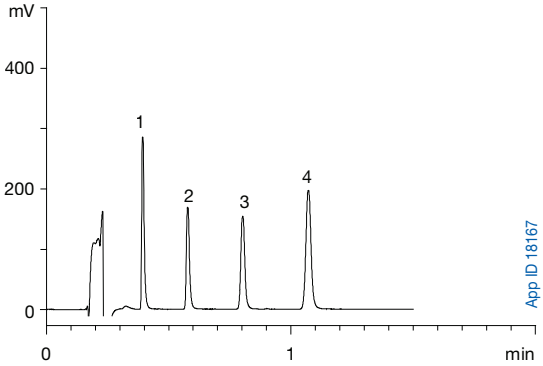
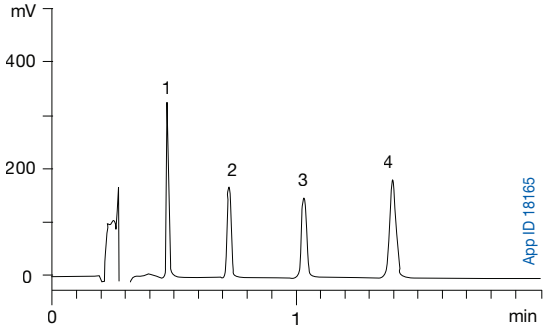


Figure 3b.
Waters® ACQUITY® BEH 1.7 µm C18
BP = 450 bar, Efficiency (based on Naphthalene) = 272,080 p/m



Conditions for both columns:
Columns: material as noted
Dimensions: 50 x 2.1 mm
Mobile Phase: Acetonitrile / Water (50:50)
Flow Rate: 0.6 mL/min
Temperature: 25 °C
Detection: UV @ 254 nm

Instrument: Waters® ACQUITY® UPLC®
Sample: 0.5 µL test mixture
1. Acetophenone
2. Benzene
3. Toluene
4. Naphthalene

APPLICATIONS

Part 2: Increased Resolution

Increased Column Efficiencies Using a Traditional HPLC System

The following examples (**Figures 4a & 4b**) show how the increased efficiency of Kinetex™ columns leads to improved resolution of closely eluted analytes. The first example shows the effect of increased column efficiencies using Kinetex™ on a standard HPLC system (Agilent 1100) for the isocratic separation of Aflatoxins. This separation can be performed with good resolution of all four aflatoxins (G2, B2, G1, and B1) on a Luna® 3 µm C18(2) 150 x 4.6 mm column (**Figure 4b**) in about 8 minutes. The critical pair, B2 and G1, is baseline resolved with resolution (Rs) = 1.98. Substituting Kinetex™ 2.6 µm C18 150 x 4.6 mm for the Luna® column, on the same system, and maintaining the mobile phase composition and flow rate, one can see (**Figure 4a**) the increase in efficiency (142,240 to 227,233 plates/meter) obtained with the Kinetex™ column results in an increase in resolution from 1.98 to 2.46.

Figure 4a.
Kinetex™ 2.6 µm C18

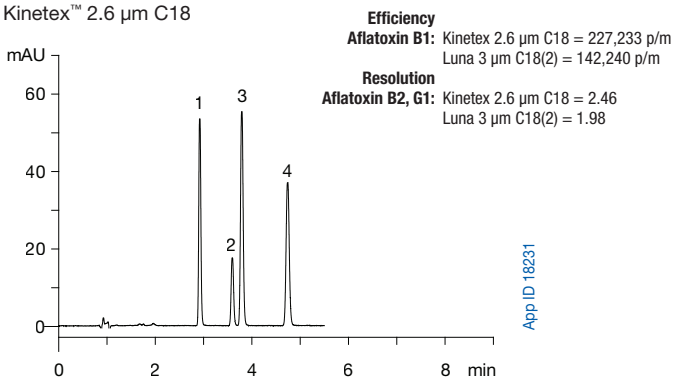
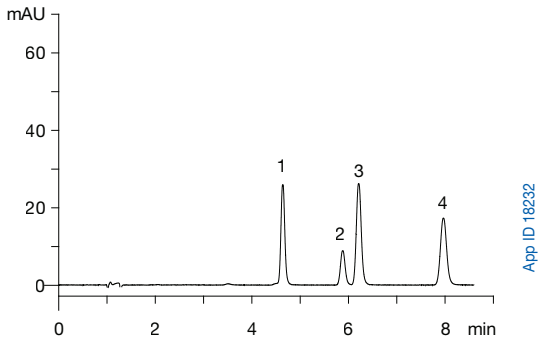


Figure 4b.
Luna® 3 µm C18(2)



Conditions for both columns:
Columns: material as noted
Dimensions: 150 x 4.6 mm
Mobile Phase: Acetonitrile / Water (30:70)
Flow Rate: 1.5 mL/min
Temperature: 25 °C
Detection: UV @ 254 nm

Instrument: Agilent 1100
Sample: 1. Aflatoxin G2
2. Aflatoxin B2
3. Aflatoxin G1
4. Aflatoxin B1

Increased Column Efficiencies on Waters® ACQUITY® UPLC® System

A final example shows the improvement on resolution and peak capacity using Kinetex™ provides on a high pressure capable LC system (Waters® ACQUITY® UPLC®).

The separation of the sulfa drugs can be performed with baseline resolution of all four analytes in less than 1.5 minutes on a Waters® ACQUITY® BEH 1.7 µm C18 50 x 2.1 mm column (**Figure 5b**). The measured average peak capacity is 137. The critical pair sulfathiazole and sulfamerazine are baseline resolved with Rs = 3.52. Under the same conditions with the Kinetex™ 1.7 µm C18 50 x 2.1 mm (**Figure 5a**), the separation is also baseline resolved in less than 1.5 minutes. However, the higher efficiency provided by the Kinetex™ 1.7 µm column results in an increased average peak capacity (148 vs. 137) and an increase in resolution from 3.52 to 3.97.

Figure 5a.
Kinetex™ 1.7 µm C18

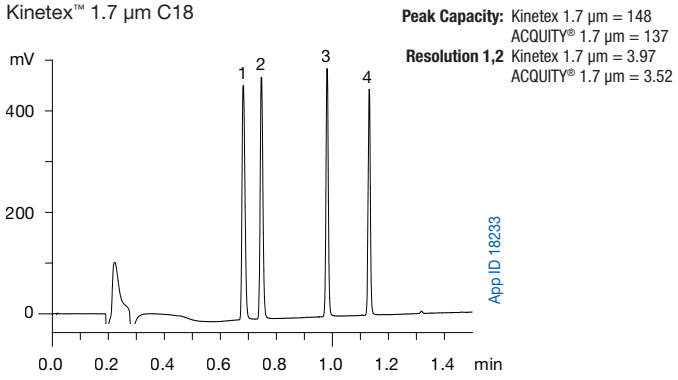
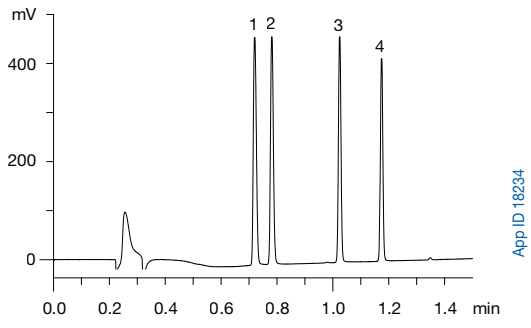


Figure 5b.
Waters® ACQUITY® BEH 1.7 µm C18



Conditions for both columns:
Columns: material as noted
Dimension: 50 x 2.1 mm
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile

Gradient:	**Time (min)**	**% B**
	0	5
	2.33	5
	2.36	95
	3.37	5

Flow Rate: 0.6 mL/min
Temperature: 45 ºC
Detection: UV @ 260 nm
Injection: 0.2 µL
HPLC System: Waters® ACQUITY® UPLC®
Sample 1. Sulfathiazole
2. Sulfamerazine
3. Sulfamethoxazole
4. Sulfaquinoxaline

APPLICATIONS

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In this case there is only a marginal improvement in sample throughput, as both columns provide the desired separation of the four analytes in about the same time. The real benefit is in the incremental improvement in resolution provided by Kinetex™ 1.7 µm particle because of the higher efficiency and average peak capacity.

Conclusion

The examples provided in this technical note illustrate the significant benefits offered by the shorter diffusion path and uniform particle size of the Kinetex™ core-shell media. Kinetex™ columns result in significantly higher column efficiencies than traditional fully porous 3 µm and sub-2 µm columns in different dimensions (4.6 and 2.1 mm ID) and across different HPLC and UHPLC system platforms. This increase in column efficiencies provided by the Kinetex™ technology results in sharper, narrower peaks, and a significant increase in chromatographic resolution.

Kinetex™ Ordering Information

Part No.	Description	Dimensions	Unit
00B-4475-AN	Kinetex 1.7 µm C18	50 x 2.1 mm	ea
00D-4475-AN	Kinetex 1.7 µm C18	100 x 2.1 mm	ea
00F-4475-AN	Kinetex 1.7 µm C18	150 x 2.1 mm	ea
00B-4462-AN	Kinetex 2.6 µm C18	50 x 2.1 mm	ea
00D-4462-AN	Kinetex 2.6 µm C18	100 x 2.1 mm	ea
00F-4462-AN	Kinetex 2.6 µm C18	150 x 2.1 mm	ea
00B-4462-Y0	Kinetex 2.6 µm C18	50 x 3.0 mm	ea
00D-4462-Y0	Kinetex 2.6 µm C18	100 x 3.0 mm	ea
00F-4462-Y0	Kinetex 2.6 µm C18	150 x 3.0 mm	ea
00B-4462-E0	Kinetex 2.6 µm C18	50 x 4.6 mm	ea
00D-4462-E0	Kinetex 2.6 µm C18	100 x 4.6 mm	ea
00F-4462-E0	Kinetex 2.6 µm C18	150 x 4.6 mm	ea

Other phases available, contact your Phenomenex technical consultant.

KrudKatcher™ Ultra In-Line Filter Ordering Information

Part No.	Description	Unit
AF0-8497	KrudKatcher Ultra In-Line Filter, 0.5 µm Porosity x 0.004 in. ID	3/pk

Installation wrench not provided. KrudKatcher Ultra requires $^{5}/_{16}$ in. wrench.

guarantee

If Kinetex™ analytical 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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