Increased Speed of Analysis and Sample Throughput with Kinetex™ Core-Shell Technology

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The introduction of the new Kinetex™ core-shell technology provides a significant benefit to all separation scientists looking to increase sample throughput by reducing chromatographic analysis times while maintaining chromatographic resolution. This is especially important for QC labs, which typically have limited resources and can benefit the most from faster separations and higher sample throughput, allowing them to make the best use of their limited resources. However, the benefits derived from faster separations are equally important to discovery and method development departments as well.

Introduction

Current economic conditions have brought even more intense focus on the need of chromatographers to do more with less. Increased throughput – translated in the laboratory as the need to analyze more samples in a given time period without the addition of people or instruments – brings the focus squarely on speed of analysis. One of the significant advantages that has drawn the attention of chromatographers to small (sub-2 µm) particle size columns is the ability of these products to provide faster chromatographic separations without sacrificing much, if any, chromatographic resolution.

Kinetex™ core-shell technology satisfies this need for faster sample analysis and increased sample throughput. The Kinetex™ core-shell technology particle provides a reduced diffusion path in comparison with porous particles, thereby reducing the resistance to mass transfer (C-term in a van Deemter plot). One can run at faster mobile phase flow rates without concurrent loss of efficiency. These faster flow rates result in shorter analysis times, meeting the growing requirement that researchers have for reduced chromatographic analysis and increased sample throughput.

Experimental Conditions

Columns: Kinetex™ 2.6 µm C18 (Core-Shell particles)
Fully Porous 5 µm C18
Dimensions: as noted
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile
Gradient: Gradient profile as noted in each chromatogram
Sample: Acid/Base/Neutral (ABN) test mixture
1. Pyridine
2. Acetaminophen
3. Quinine
4. Acebutolol
5. Chlorpheniramine
6. Triprolidine
7. Prednisolone
8. 4-Chlorobenzoic acid
9. 4-Chlorocinnamic acid
10. Diazepam
11. Diflunisal
12. Hexanophenone

Instrument: Agilent 1200SL

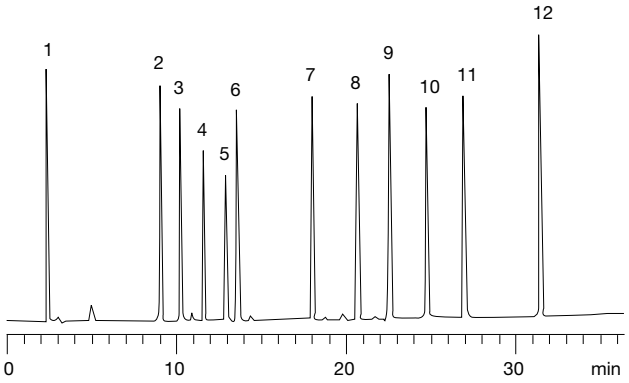
Results and Discussion

Examples of how Kinetex™ can help researchers realize increased sample throughput without compromising the quality of the chromatographic separation are shown in the accompanying figures.

The test mixture used in these experiments is a mixture of acids, bases, and neutral compounds which allows one to monitor the performance of the Kinetex™ HPLC columns across a wide range of analytes. Further, the different interactions of the analyte with the bonded phase and silica substrate can be studied using this complex mixture. The separation was run under gradient conditions and the conditions adjusted for column volume as column dimensions changed.

The first two chromatograms (**Figures 1 & 2**) show the results obtained under conditions typically used for analytical chromatographic separation of a mixture of compounds. A wide baseline separation of the 12 compounds in the mixture is achieved on both the 250 x 4.6 and 150 x 4.6 mm Fully Porous columns; however, the overall analysis time is lengthy. Efforts were undertaken to reduce run time while maintaining chromatographic resolution, especially for peaks 5 and 6 which are the two closest eluting peaks in the sample mixture.

Figure 1.
Fully Porous 5 µm C18 250 x 4.6 mm at 1 mL/min



Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile

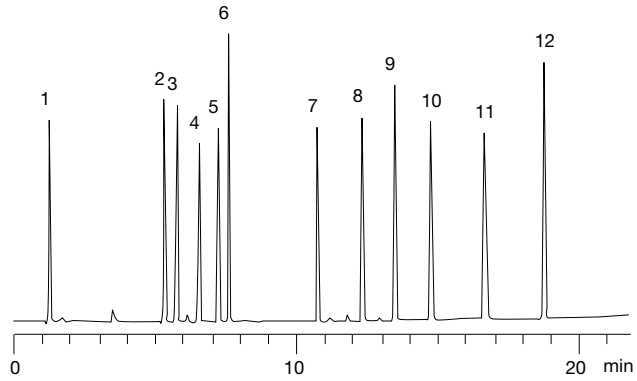
Gradient Profile:

Step No.	Time (min)	% A	% B
1	0	95	5
2	2.79	95	5
3	36.15	5	95
4	36.38	95	5

Flow Rate: 1.0 mL/min
Temperature: 45 ºC
Detection: UV @ 254 nm
Injection: 5 µL
Comment: Backpressure = 106 bar
Resolution 5,6 = 3.4

APPLICATIONS

Figure 2.
Fully Porous 5 µm C18 150 x 4.6 mm at 1 mL/min



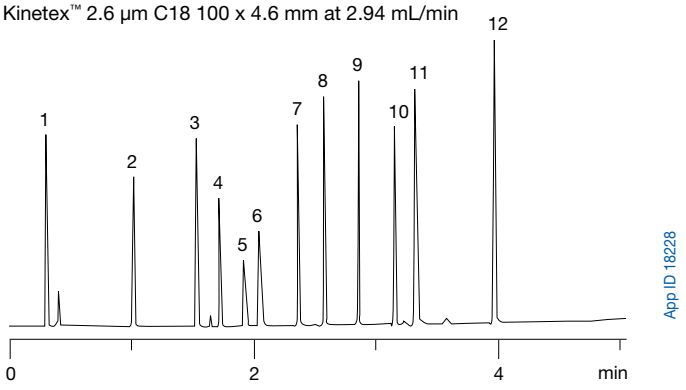
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile

Gradient Profile:

Step No.	Time (min)	% A	% B
1	0	95	5
2	1.67	95	5
3	21.69	5	95
4	21.83	95	5

Flow Rate: 1.0 mL/min
Temperature: 45 ºC
Detection: UV @ 254 nm
Injection: 3 µL
Comment: Backpressure = 80 bar
Resolution 5,6 = 5.0

Figure 3.
Kinetex™ 2.6 µm C18 100 x 4.6 mm at 2.94 mL/min



Part No.: 00D-4462-E0
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile

Gradient Profile:

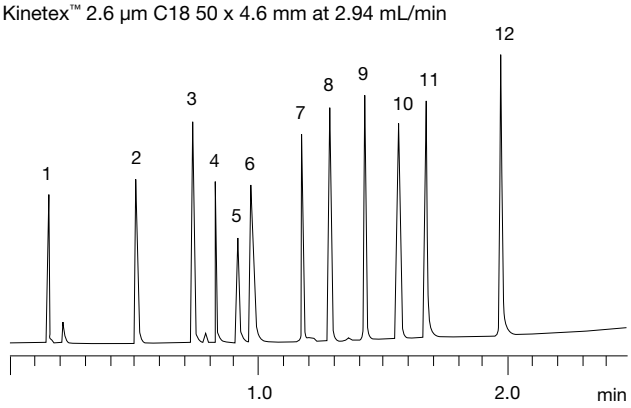
Step No.	Time (min)	% A	% B
1	0	95	5
2	0.48	95	5
3	5.02	5	95
4	5.05	95	5

Flow Rate: 2.94 mL/min
Temperature: 45 ºC
Detection: UV @ 254 nm
Injection: 1 µL
Comment: Backpressure = 480 bar
Resolution 5,6 = 3.2

The separation achieved on the Kinetex™ 2.6 µm C18 100 x 4.6 mm column shows a significant improvement in overall separation time (reduced to 4 minutes), with resolution maintained at 3.2 between peaks 5 and 6, and all 12 compounds are fully resolved. The flow rate was increased to almost 3 mL/min to reduce run time; while such a high linear velocity might compromise performance, the Kinetex™ column demonstrates good performance even under such extreme conditions. One notable change is the increase in backpressure to 480 bar – this necessitates the use of a higher pressure capable UHPLC system (an Agilent 1200SL was used for all separations shown here).

APPLICATIONS

Figure 4.
Kinetex™ 2.6 µm C18 50 x 4.6 mm at 2.94 mL/min



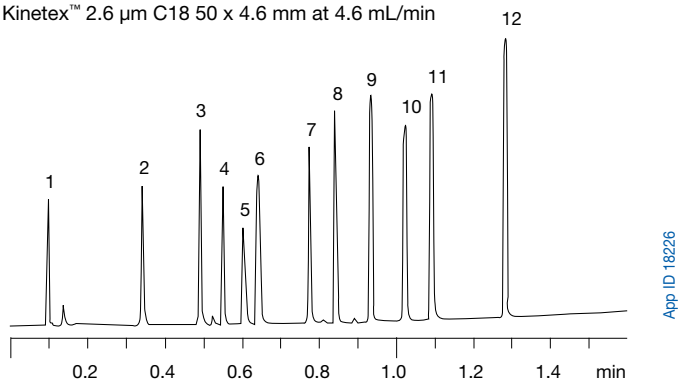
Part No.: 00B-4462-E0
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile
Gradient Profile:

Step No.	Time (min)	% A	% B
1	0	95	5
2	0.2	95	5
3	2.47	5	95
4	2.48	95	5

Flow Rate: 2.94 mL/min
Temperature: 45 ºC
Detection: UV @ 254 nm
Injection: 0.5 µL
Comment: Backpressure = 300 bar
Resolution 5,6 = 3.2

The use of a shorter 50 x 4.6 mm Kinetex™ column also resulted in a fully resolved baseline separation, with resolution between peaks 5 and 6 of 3.2 – essentially the same as that achieved on the Fully Porous 5 µm 150 x 4.6 mm column (**Figure 2**), but in less than one-tenth of the time. The backpressure in this case (300 bar) was maintained below the pressure limit (400 bar) for a traditional analytical HPLC system; this would therefore not necessitate the use of a UHPLC system in order to obtain this faster separation.

Figure 5.
Kinetex™ 2.6 µm C18 50 x 4.6 mm at 4.6 mL/min



Part No.: 00B-4462-E0
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile
Gradient Profile:

Step No.	Time (min)	% A	% B
1	0	95	5
2	0.14	95	5
3	1.59	5	95
4	1.60	95	5

Flow Rate: 4.6 mL/min
Temperature: 45 ºC
Detection: UV @ 254 nm
Injection: 0.5 µL
Comment: Backpressure = 485 bar
Resolution 5,6 = 3.0

Increasing the flow rate on the Kinetex™ 50 x 4.6 mm column from 2.94 to 4.6 mL/min results in a reduction in the overall run time to less than 1.4 minutes, while resolution between peaks 5 and 6 only decreases slightly to 3.0. The peaks are still fully resolved – this separation represents a 15-fold decrease in analysis time, while maintaining chromatographic resolution.

APPLICATIONS

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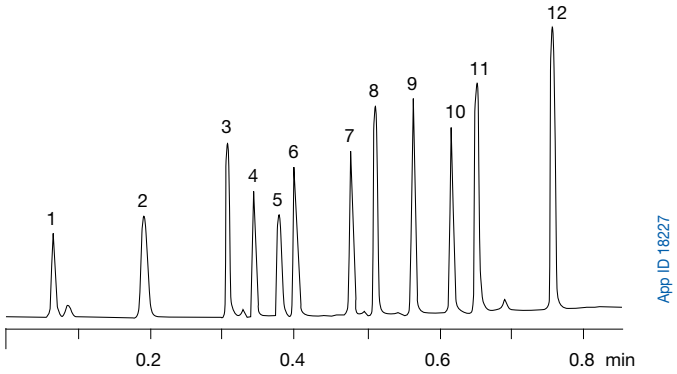
Figure 6.
Kinetex™ 2.6 µm C18 50 x 2.1 mm at 1.7 mL/min



Part No.: 00B-4462-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile

Gradient Profile:

Step No.	Time (min)	% A	% B
1	0	95	5
2	0.02	95	5
3	0.84	5	95
4	0.85	95	5

Flow Rate: 1.7 mL/min
Temperature: 45 °C
Detection: UV @ 254 nm
Injection: 0.1 µL
Comment: Backpressure = 520 bar
Resolution 5,6 = 2.3

If one is looking for even faster analysis, a Kinetex™ 50 x 2.1 mm column run at 1.7 mL/min delivers a full baseline resolution of all 12 compounds in the mixture in less than 1 minute!

Conclusions

The examples shown in this technical note illustrate the significant reduction in sample analysis time, and the resulting improvement in sample throughput that can be achieved using Kinetex™ core-shell technology columns. This improvement in sample throughput and reduction in chromatographic analysis time can be obtained without loss in chromatographic resolution, thereby maintaining the quality of the analytical results.

Ordering Information

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

Other phases available, contact your Phenomenex technical consultant.

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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