Chromatography Focus

Increased Sensitivity, Improved Resolution and Faster Analysis Times For Compendial Methods

Determination of Impurities and Related Substances Using Newly Developed 2.6 µm Core-Shell Kinetex™ LC Columns

Presently, HPLC methods for the determination of impurities and related substances of drug products specified in monographs by the various Pharmacopoeial agencies typically employ LC columns packed with fully-porous 3 or 5 micron spherical silica chromatographic media. Due to the performance limitations of fully-porous 3 and 5 micron spherical silica chromatographic media, these analytical methods commonly require long analysis times. Additionally, accurate quantitation of low-level impurities in routine LC-UV applications may be challenging due to the low intensity peaks generated by these columns.

In recent years, smaller fully-porous LC particles (sub-2 micron diameter) have been introduced that offer faster analysis times and generate higher intensity peaks for better sensitivity. Unfortunately, widespread adoption of this sub-2 micron HPLC column technology has been slowed since the smaller particle columns generate system backpressures that require specialised ultra-high pressure capable LC instrumentation.

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Recently a newly developed Kinetex™ 2.6 m Core-Shell chromatographic particle has been commercialised that offers the performance benefits of sub-2 µm fully-porous particles but at substantially lower operating pressures. To demonstrate the performance benefits of this new core-shell technology, a Kinetex™ 2.6 µm Core-Shell C18 column was compared with a fully-porous 5 µm C18 column referenced in EP ([Ph. Eur.] European Pharmacopoeia) Monograph 0703 for Atenolol and related substances on a conventional HPLC instrument with an upper pressure limit of 400 bar.

First, to demonstrate equivalency, a Kinetex™ column of similar dimension to the column referenced was operated under the conditions specified in the monograph. Then the Kinetex™ column was operated at a higher flow rate still within the + -50% adjustment allowed by the EP for meeting system suitability. The Kinetex™ column achieved a 65% faster analysis time (3x productivity improvement) and significantly improved resolution and sensitivity versus the EP referenced fully-porous 5 µm column.

OVERVIEW OF KINETEX™ 2.6 µm CORE-SHELL TECHNOLOGY

Precision Core-Shell Manufacturing

The Kinetex™ technology is comprised of a nearly monodispersed 1.9 µm solid silica core and a 0.35 µm porous silica shell. This particle design results in a very stable and homogeneous packed column bed that significantly reduces peak dispersion due to eddy diffusion (the 'A' term of the van Deemter equation). Additionally, the short diffusion path of the 0.35 µm porous silica shell allows for faster kinetics of diffusion, thereby minimising peak dispersion due to resistance to mass transfer (the 'C' term in the van Deemter equation).

Ultra-High Efficiency Particle

Columns packed with Kinetex™ 2.6 µm core-shell silica particles are capable of maintaining ultra-high efficiencies across an extended range of mobile phase linear velocity. In *Figure 2*, van Deemter plots of plate height versus mobile phase linear velocity are presented for the Kinetex™ 2.6 µm column and a leading sub-2 µm column. Data was generated on an Agilent 1200SL instrument with an upper pressure limit of 600 bar. Note that the Kinetex™ 2.6 µm column achieved plate heights equivalent to the sub-2 µm column and was able to be operated at a higher flow rate before the upper system pressure limit was reached. Also note that there is not a significant increase in plate height as mobile phase velocity is increased. This is due to the very low resistance to mass transfer of analytes in-to and out-of the porous shell containing the stationary phase that surrounds the solid silica core (minimising the contribution of the 'C' term to plate height).

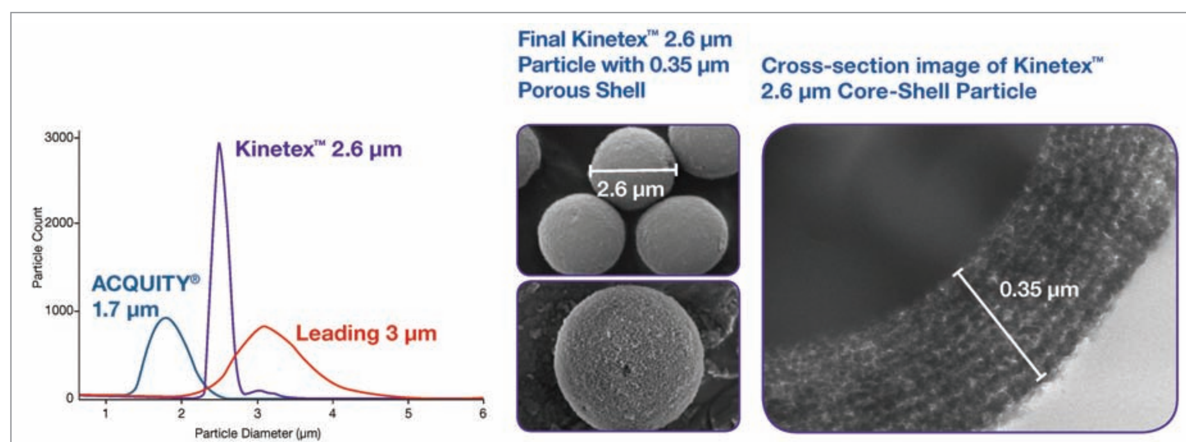

Figure 1: Uniform Particle Size Distribution

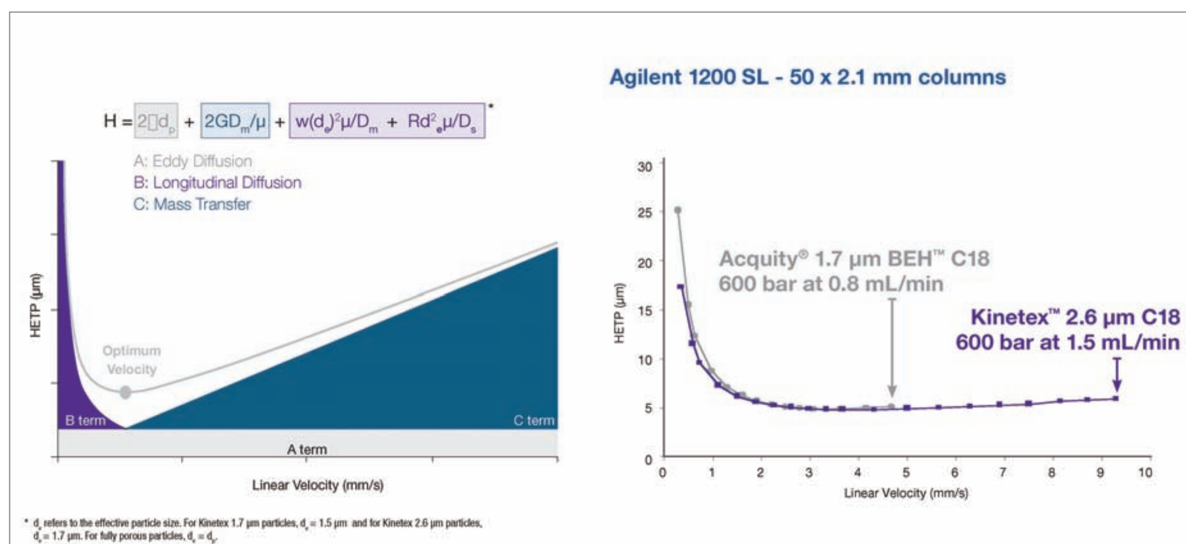

Figure 2: van Deemter Plot Equation and van Deemter Data

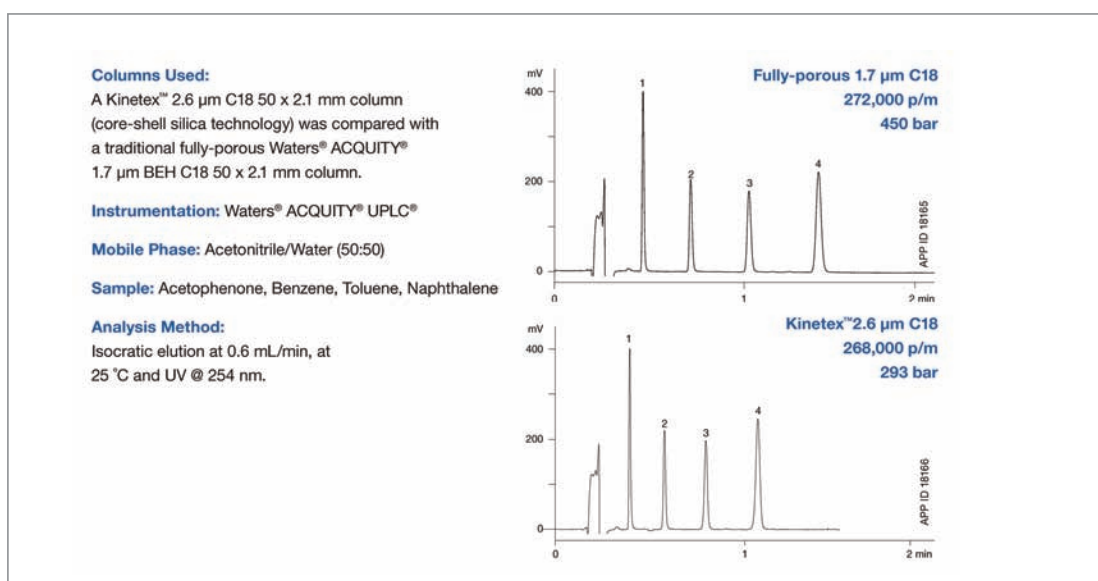

Figure 3

Reasonable LC System Operating Pressures

The comparison results in *Figure 3* demonstrate the ability of the Kinetex™ 2.6 µm core-shell technology to achieve chromatographic efficiencies comparable to those of fully-porous sub-2 µm columns at substantially lower system backpressures. The lower pressures generated by columns packed with Kinetex™ 2.6 µm particles allow them to be used on conventional LC instruments for routine analysis under 400 bar whereas traditional fully-porous sub-2 µm particles have limited utility below 400 bar and therefore require specialised ultra-high pressure capable LC instrumentation. This capability eliminates the challenges associated with the transfer of ultra-high performance methods across various LC system platforms, and makes Ultra-High Performance LC accessible to more scientists and laboratories.

ATENOLOL AND RELATED SUBSTANCES: EUROPEAN MONOGRAPH 0703

Experimental

Columns Used:

A fully-porous 5 µm C18 125 x 4.0 mm column (as specified by the monograph) was compared with a Kinetex™ 2.6 µm C18 100 x 4.6 mm column (The closest available dimension).

Instrumentation:

Agilent 1100 LC System (Agilent Technologies Inc, Palo Alto, CA, USA) equipped with a Quaternary gradient pump, autosampler, column oven, and variable wavelength detector.

Mobile Phase Preparation:

A: Dissolve 3.4 g of Potassium Dihydrogen Phosphate in 1 L of DI water, adjust the pH to 3.0 using Phosphoric acid

B: Mix 180 mL of Methanol and 20 mL of Tetrahydrofuran and 800 mL of A

C: Dissolve 1.0 g of Sodium Octanesulphonate and 0.4 g of Tetrabutyl Ammonium Hydrogen Sulphate in 1 L of B.

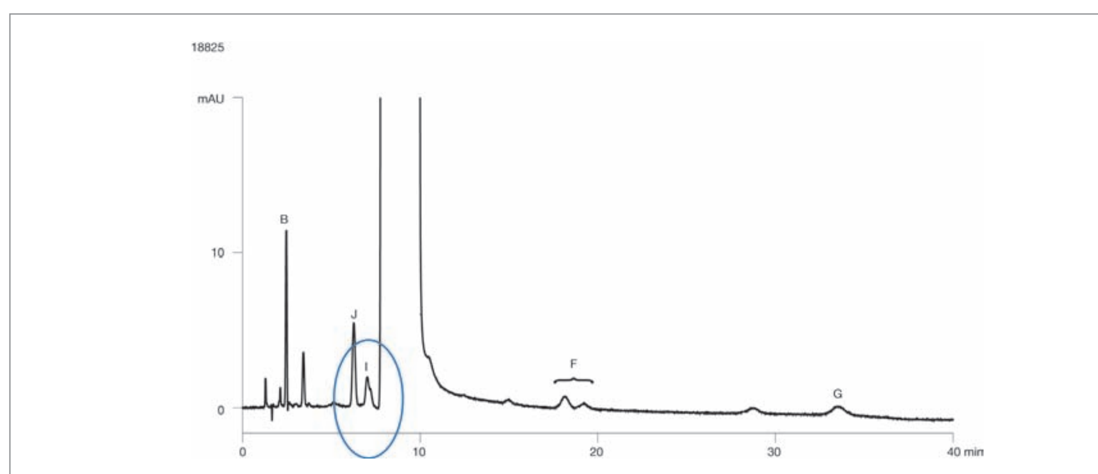

Figure 4: Atenolol CRS: Fully-porous 5 µm C18

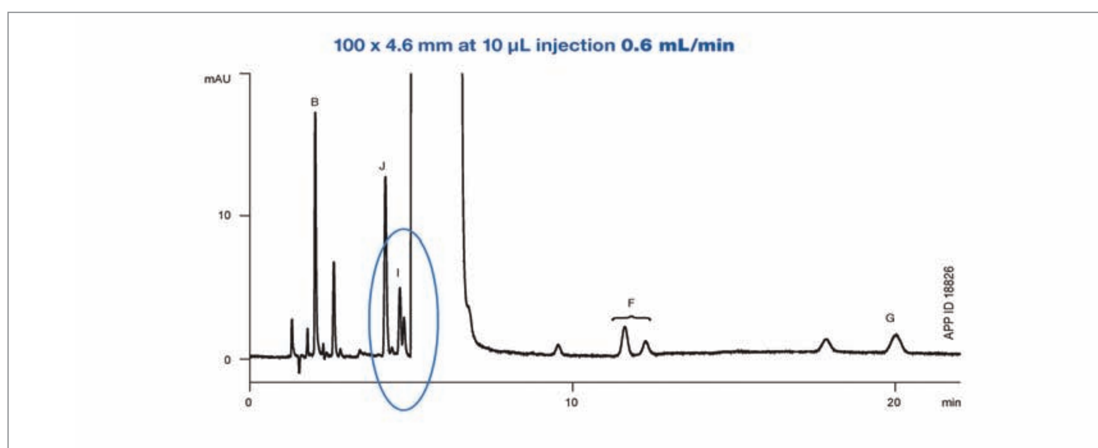

Figure 5: Atenolol CRS: Kinetex™ 2.6 µm C18

Table 1: Equivalency Study

	Fully-porous 5 µm	Kinetex 2.6 µm C18
Column Dimensions	125 x 4.0 mm	100 x 4.6 mm
Particle Size	5 µm fully porous	2.6 µm core-shell
Flow Rate	0.6 mL/min	0.6 mL/min
Backpressure	168 Bar	180 Bar
Resolution of Impurities J & I	1.95	2.26
S/N ratio for Impurity J	40	82.7
S/N ratio for Impurity I	12.3 (% RSD 2.77)	28.9 (% RSD 1.61)
S/N ratio for Impurity G	3.31	8.67
N of Impurity J	8,206	20,372
Elution time of last peak	33.3 min	20.0 min

Sample Preparation:

Atenolol Certified Reference Standard (CRS) for system suitability (containing atenolol and impurities B, F, G, I and J) was obtained from the European Pharmacopoeia. 5 mg of Atenolol CRS was dissolved in 2.5 mL of the mobile phase

Atenolol Analysis Method:

The monograph calls for isocratic elution with 100 % of mobile phase as prepared above at 0.6 mL/min. Column temperature kept at ambient and UV detection wavelength set at 226 nm.

RESULTS AND DISCUSSION

Following the methodology described in European Pharmacopoeia Monograph 0703 and using a fully-porous 5 µm C18 125 x 4.0mm column as referenced in the method, a chromatogram similar to that of the specimen chromatogram provided with the Atenolol CRS was obtained (*Figure 4*).
A Kinetex™ 2.6 µm C18 100 x 4.6mm column (the closest available dimension) was used according to the conditions specified in the monograph. The resulting chromatogram demonstrated equivalency for selectivity and also demonstrated significantly improved sensitivity (*Figure 5*).
Table 1 summarises the data comparing the Kinetex™ column to the fully-porous 5 um column at the specified flow rate of 0.6mL/min. The monograph requires resolution between impurities I and J of at least 1.4. Due to the significantly narrower peaks generated by the higher efficiency Kinetex™ column, a substantial improvement in resolution between impurities I and J was achieved with Kinetex™ .
Sensitivity was also significantly improved for all impurities as a result of the Kinetex™ column generating narrower and taller peaks. Signal-to-noise ratios for the early eluting impurities were increased by roughly a factor of 2 and by roughly a factor of 3 for later eluting impurities.
With 10 µL of the Atenolol CRS injected, the fully-porous 5 µm C18 column generated a signal-to-noise ratio of 12.3 for Impurity I. Multiple 10 µL injections were performed on the fully-porous 5 µm C18 column and the resulting %RSD value for peak area of Impurity I was 2.77.
In comparison, with 10 µL of the Atenolol CRS sample injected on the Kinetex™ 2.6 µm core-shell C18 column, a signal-to-noise ratio of 28.9 was observed for Impurity I. Multiple 10 µL injections were performed on the Kinetex™ column and the resulting %RSD value for peak area of Impurity I was 1.61.
Considering that LOQ (defined by the European Pharmacopoeia as corresponding to a signal-to-noise ratio of 10 for a peak) can be one of the most challenging parameters to meet in routine operation, the higher signal-to-noise ratios and subsequently lower %RSD values observed with the Kinetex™ core-shell technology represent a significant performance advantage.
It should be noted that the Kinetex™ column generated a system pressure comparable to the 5 µm column under these conditions.

ATENOLOL AND RELATED SUBSTANCES: EUROPEAN MONOGRAPH 0703 (cont'd)

Significantly Faster Analysis Times with Kinetex™

As demonstrated in *Figure 2*, columns packed with Kinetex™ 2.6 µm core-shell particles are capable of maintaining high efficiencies (low plate heights) with increasing mobile phase flow rates. This is due to favourable physical, kinetic, and thermodynamic properties attributed to core-shell particles. Shorter analysis times may be achieved with Kinetex™ either by reducing the length of the column or increasing the mobile phase flow rate (or a combination of both) without significantly compromising chromatographic performance.

Following European Pharmacopoeia guidelines, the extent to which the various parameters of a

Table 2: Acceptable Modifications for Meeting System Suitability

Method Parameter	Acceptable Modification	Monograph 0703 Atenolol	Kinetex™ 2.6 µm Fast Method	Modification
Mobile phase pH	± 0.2 units	3	No Change	--
Concentration of salts in buffer	± 10 %	as specified	No Change	--
Ratio of components in mobile phase	± 30 % of the minor component(s), or 2% absolute of that component, whichever is greater, but a change in any component cannot exceed ± 10 % absolute.	as specified	No Change	--
Wavelength of UV-Detector	no deviations permitted	226 nm	No Change	--
Injection volume	Increased to as much as twice the volume specified, provided no adverse effects - must be within stated linearity range of the method	10 µL	No Change	--
Column temperature	± 10 °C	ambient	No Change	--
Column length	± 70 %	125 mm	100 mm	- 20%
Column inner diameter	± 50 %	4.0 mm	4.6 mm	+ 15%
Particle size	- 50%	5 µm	2.6 um	- 48%
Flow rate	± 50 %	0.6 ml/min	0.9 ml/min	+ 50%

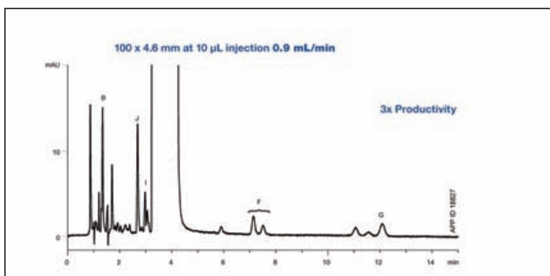

Figure 6: Atenolol CRS: Kinetex™ 2.6 µm C18

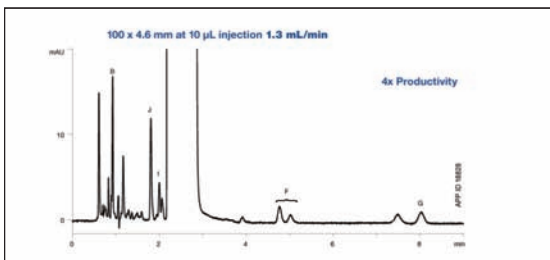

Figure 7: Atenolol CRS: Kinetex™ 2.6 µm C18

chromatographic test may be adjusted to satisfy system suitability (when replacing one column with another of the same type, for example) is summarised in *Table 2*. Staying within these guidelines, the Kinetex™ 2.6 µm core-shell C18 column was run according to the conditions specified in the monograph, but with 50% increase in the flow rate (from 0.6 mL/min to 0.9 mL/min).

With a flow rate of 0.9 mL/min, the total analysis time was shortened from over 33 minutes to just under 12 minutes (*Figure 6*). Resolution, efficiency and sensitivity remained substantially higher with the Kinetex™ column at the higher flow rate (*Table 3*). The reduction in analysis time achieved with Kinetex™ in this example represents roughly a 3-fold increase in sample throughput capability. Operating outside of the allowed adjustments and using a flow rate of 1.3mL/min on the Kinetex 2.6 µm C18 100 x 4.6mm column resulted in an analysis time of 8 minutes with substantially higher resolution, efficiency and sensitivity as compared to the fully-porous 5 µm 125 x 4.0mm column (*Figure 7*). This represents a 4-fold increase in throughput capability for this analysis. Note that this performance was achieved using an Agilent 1100 LC system with an upper pressure capability of 400 bar.

A flow rate of 1.3mL/min generated a system pressure of 380 bar. Further optimisation would be possible with a shorter Kinetex™ column.

Table 3: Improvements to the Monograph

	Fully-porous 5 µm	Kinetex 2.6 µm C18 0.9 mL/min	Kinetex 2.6 µm C18 1.3 mL/min
Column Dimensions	125 x 4.0 mm	100 x 4.6 mm	100 x 4.6 mm
Particle Size	5 µm fully porous	2.6 µm core-shell	2.6 µm core-shell
Flow Rate	0.6 mL/min	0.9 mL/min	1.3 mL/min
Backpressure	168 Bar	270 Bar	380 Bar
Resolution of Impurities J & I	1.95	3.72	3.65
S/N ratio for Impurity J	40	78.1	78.7
S/N ratio for Impurity I	12.3	30.2	28.7
S/N ratio for Impurity G	3.31	9.38	9.33
N of Impurity J	8,206	21,118	19,473
Elution time of last peak	33.3 min	11.9 min	8.0 min

CONCLUSIONS

Newly developed Kinetex™ 2.6 µm core-shell particles are capable of achieving chromatographic performance equivalent to columns packed with traditional fully-porous sub-2 µm particles at substantially lower operating pressures that are compatible with conventional HPLC instrumentation.

Laboratories performing routine API and related substance analysis with traditional fully-porous LC columns can benefit from the increased speed, resolution and sensitivity that Kinetex™ 2.6 µm columns provide without having to replace existing instrumentation with ultra-high pressure capable LC systems.

Faster analysis times resulting in higher throughput and productivity can be achieved with Kinetex™ columns with minimal changes to validated methods by employing shorter length columns and/or higher mobile phase flow rates without sacrificing performance. Improved resolution and higher sensitivity resulting from narrower and taller chromatographic peaks generated by Kinetex™columns allow for more precise detection and quantitation of low level impurities in routine operation.

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