Determination of Impurities and Related Substances for Metoprolol Tartrate (Ph. Eur. Monograph 1028): Increased Sensitivity, Improved Resolution and Faster Analysis Using Kinetex™ 2.6 µm Core-Shell LC Columns

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- *Methods can be improved for ultra-high performance without the need for higher-pressure capable instrumentation*
- *Within allowable modifications for system suitability, methods can be improved for better resolution, higher sensitivity, and significantly faster analysis time*

Introduction

HPLC methods for the determination of impurities and related substances of drug products specified in monographs by the various Pharmacopoeia agencies typically employ LC columns packed with fully porous 3 and 5 µm spherical silica chromatographic media. Due to the performance limitations of fully porous 3 and 5 µm spherical silica chromatographic media, these analytical methods commonly require long analysis times to provide the required chromatographic resolution for the impurities present. 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 µm diameter) have been introduced that offer faster analysis times and generate higher intensity peaks for better sensitivity. Unfortunately, since the smaller particle columns generate system backpressures that require specialized ultra-high pressure capable LC instrumentation, widespread adoption of this sub-2 µm HPLC column technology has been slow.

Recently, a newly developed Kinetex 2.6 µm core-shell chromatographic particle has been commercialized that offers the performance benefits of fully porous sub-2 µm 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 European Pharmacopoeia [Ph. Eur.] Monograph 1028 for Metoprolol Tartrate and related substances on a conventional HPLC instrument with an upper pressure limit of 400 bar.

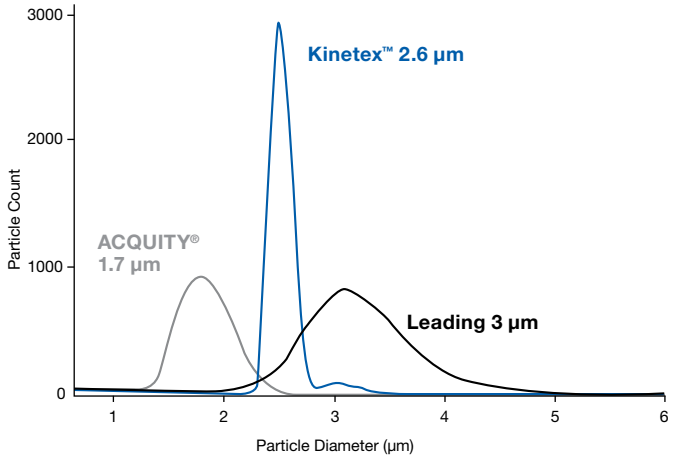
First, to demonstrate equivalency, a Kinetex column of the closest available dimension to the column referenced was operated under the conditions specified in the monograph. Then, in order to illustrate the extent of the performance benefits of the Kinetex column, a shorter Kinetex column (by one-third) was operated at a 50 % faster flow rate; with both column length and flow rate maintained within the adjustments allowed by the Ph. Eur. for meeting system suitability. The Kinetex column achieved 60 % shorter analysis time (greater than 2.5x productivity improvement) and significantly improved resolution and sensitivity versus the EP referenced fully porous 5 µm column, while meeting the system suitability requirements. Finally, a further reduction in the column length, again within the allowable adjustments, was operated at the flow rate specified in the monograph. Analysis time was 65 % shorter (3x productivity improvement) and solvent consumption per analysis was reduced by about 67 % relative to the specified column and conditions of the Ph. Eur. monograph.

Overview of Kinetex 2.6 µm Core-Shell Technology

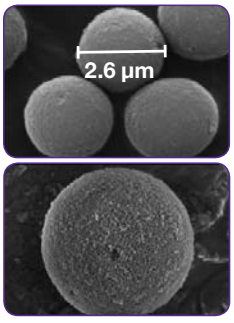
Precision Core-Shell Manufacturing

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 minimizing peak dispersion due to resistance to mass transfer (the "C" term in the van Deemter equation) **(Figure 1 & 2).**

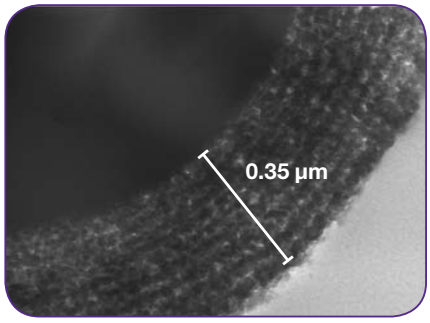
Figure 1.
Uniform Particle Size Distribution



Kinetex 2.6 µm Particle with 0.35 µm Porous Shell



Cross-section Image of Kinetex 2.6 µm Core-Shell Particle



APPLICATIONS

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 into and out of the porous shell containing the stationary phase that surrounds the solid silica core (minimizing the contribution of the "C" term to plate height).

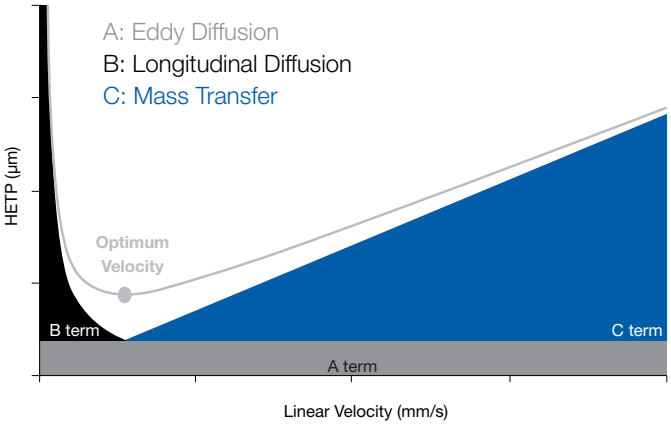
Reasonable LC System Operating Pressures

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 specialized 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.

Figure 2.

van Deemter Equation

$$H = 2\lambda d_p + 2GD_m/\mu + w(d_e)^2\mu/D_m + Rd^2_e\mu/D_s \;^*$$



* d_e refers to the effective particle size. For Kinetex 1.7 µm particles, d_e = 1.5 µm and for Kinetex 2.6 µm particles, d_e = 1.7 µm. For fully porous particles, $d_e = d_p$.

van Deemter Data
Agilent 1200 SL - 50 x 2.1 mm columns

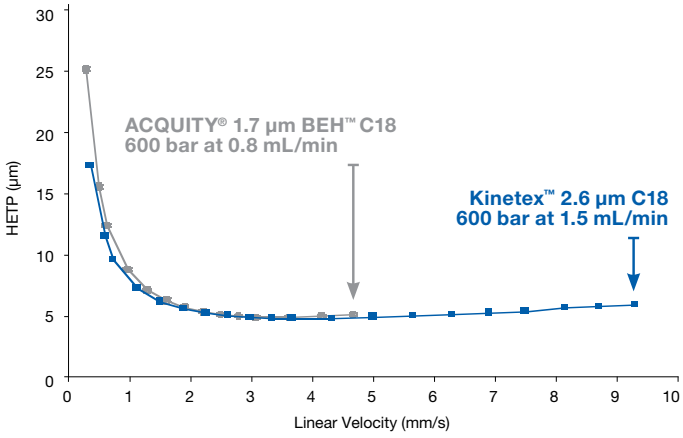
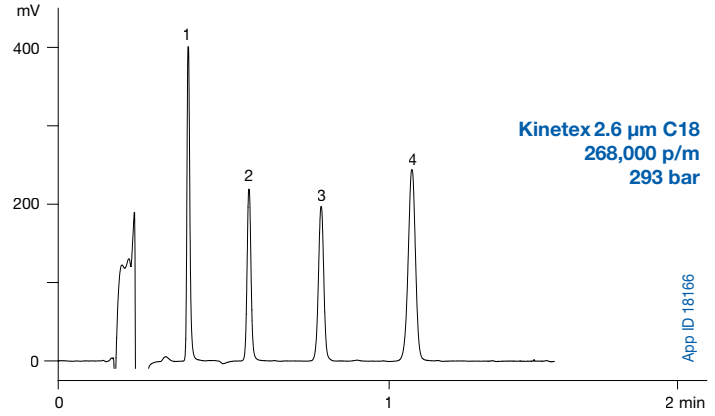
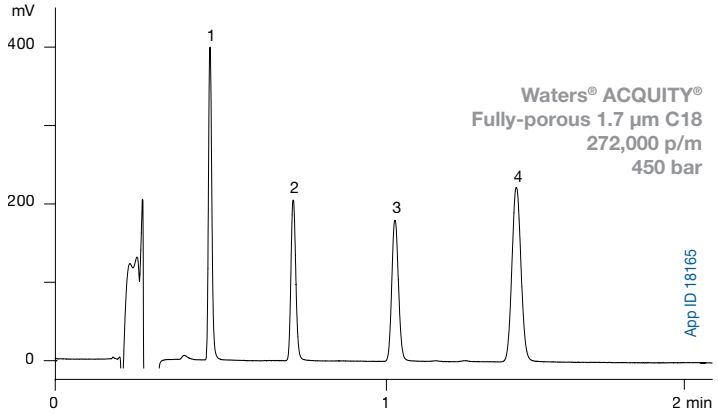


Figure 3.





Conditions for both columns:

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: 1. Acetophenone
2. Benzene
3. Toluene
4. Naphthalene

Experimental

Metoprolol Tartrate and Related Substances:
European Monograph 1028

Columns Used:
A fully-porous 5 µm C18 150 x 3.9 mm column (as specified by the monograph) was compared with a Kinetex 2.6 µm C18 150 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:
Dissolve 3.9 grams of ammonium acetate in 810 mL of water, add 2.0 mL of triethylamine (TEA), 10.0 mL of glacial acetic acid, 3.0 mL of phosphoric acid, and 146 mL of acetonitrile.

Sample Preparation:
Metoprolol Tartrate Certified Reference Standard (CRS) for system suitability was obtained from the European Pharmacopoeia. Reference Solution (a) was prepared by dissolving 5.0 mg of Metoprolol Tartrate CRS, 3.0 mg of Metoprolol Tartrate Impurity A CRS, and 3.0 mg of Metoprolol Tartrate Impurity G in mobile phase then diluted to 100 mL with mobile phase.

Metoprolol Tartrate Analysis Method:
The monograph calls for 20 µL of sample to be injected with isocratic chromatographic separation using 100 % of mobile phase as prepared above at 1.0 mL/min. Column temperature maintained at 25 °C and UV detection wavelength set at 280 nm.

Results and Discussion

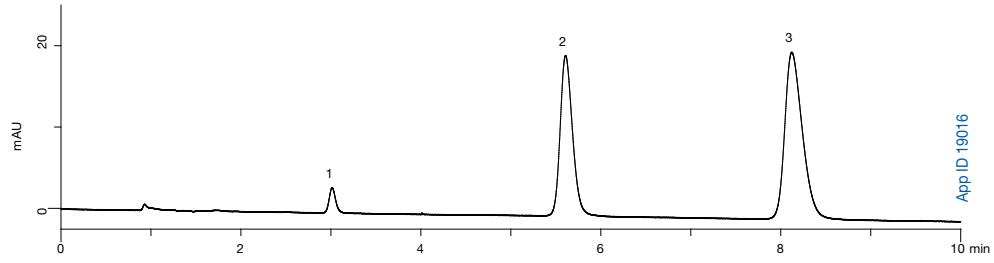
Following the methodology described in Ph. Eur. Monograph 1028, and using a fully porous 5 µm C18 150 x 3.9 mm column as referenced in the method, a chromatogram similar to that of the specimen chromatogram provided with the Metoprolol Tartrate CRS was obtained (**Figure 4**).

A Kinetex 2.6 µm C18 150 x 4.6 mm 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 summarizes the data comparing the Kinetex column to the fully porous 5 µm column at the specified flow rate of 1.0 mL/min. The monograph requires resolution between Acetylsalicylic acid CRS and Salicylic acid (Impurity C) of least 6.0. Due to the significantly narrower peaks generated by the higher efficiency Kinetex column, a substantial improvement in resolution between Acetylsalicylic acid and Salicylic acid 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 both impurity G and impurity A were increased by almost a factor of 2. Injecting 20 µL of reference solution (a) on the fully porous 5 µm C18 column generated a signal-to-noise ratio of 62 for impurity G. By comparison injecting 20 µL of reference solution (a) on the Kinetex 2.6 µm core-shell C18 column, a signal-to-noise ratio of 118 was observed for impurity G.

Figure 4.
Metoprolol Tartrate CRS: 20 µL injection on fully porous 5 µm C18 150 x 3.9 mm at 1.0 mL/min



Column: Waters® Symmetry® 5 µm C18
Dimensions: 150 x 3.9 mm
Mobile Phase: 3.9 g Ammonium acetate in 810 mL Water / 2.0 mL Triethylamine / 10 mL Glacial acetic acid / 3.0 mL Phosphoric acid / 146 mL Acetonitrile
Flow Rate: 1 mL/min
Temperature: 25° C
Detection: UV @ 280 nm
Sample: 1. Metoprolol Impurity G
2. Metoprolol Impurity A
3. Metoprolol tartrate CRS

Meeting the LOQ (defined Ph. Eur. as corresponding to a signal-to-noise ratio of 10 for a chromatographic peak) requirement can be one of the most challenging parameters in routine operation; however, the higher signal-to-noise ratios observed with the Kinetex core-shell technology represent a significant performance advantage – allowing one to easily achieve the required LOQ with improved precision.

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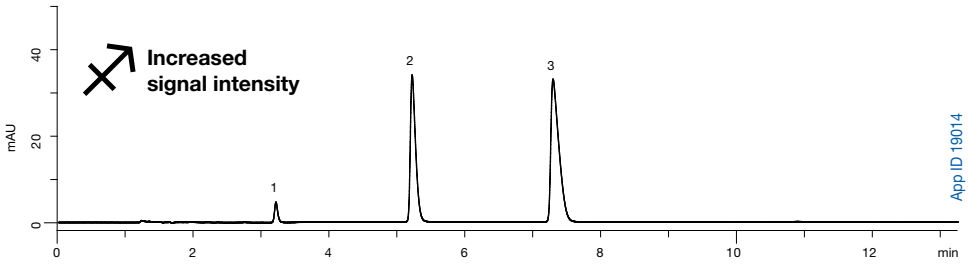
Table 1. Equivalency Study

	Fully porous 5 µm	Kinetex™ 2.6 µm C18	Comment
Column Dimensions	150 x 3.9 mm	150 x 4.6 mm	
Particle Size	5 µm fully porous	2.6 µm core-shell	
Flow Rate	1.0 mL/min	1.0 mL/min	
Backpressure	139 bar	310 bar	
Resolution of Impurity A and Metoprolol Tartrate	8.43	11.82	40 % increase
S/N ratio for Metoprolol Tartrate	410	825	101 % increase
S/N ratio for Impurity A	382	850	122 % increase
S/N ratio for Impurity G	62	118	90 % increase
N for Metoprolol Tartrate (p/m)	57,260	121,651	112 % increase
Elution time of Metoprolol Tartrate	8.12 min	7.28 min	10 % faster

Figure 5.
Metoprolol Tartrate CRS: 20 µL injection on Kinetex 2.6 µm C18 150 x 4.6 mm at 1.0 mL/min; backpressure = 310 bar



Column: Kinetex 2.6 µm C18
Dimensions: 150 x 4.6 mm
Mobile Phase: 3.9 g Ammonium acetate in 810 mL Water / 2.0 mL Triethylamine / 10 mL Glacial acetic acid / 3.0 mL Phosphoric acid / 146 mL Acetonitrile
Flow Rate: 1 mL/min
Temperature: 25° C
Detection: UV @ 280 nm
Sample: 1. Metoprolol Impurity G
2. Metoprolol Impurity A
3. Metoprolol tartrate CRS

Significantly Faster Analysis Times with Kinetex Columns
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 favorable 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.

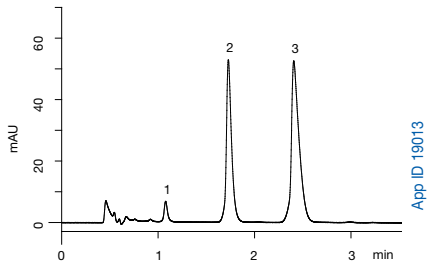
Following Ph. Eur. guidelines, the extent to which the various parameters of a chromatographic test may be adjusted to satisfy system suitability (when replacing one column with another of the same type, for example) is summarized in **Table 2**. Staying within these guidelines, a shorter Kinetex 2.6 µm core-shell C18 column (100 x 4.6 mm, representing a decrease in column length of 33 % relative to the monograph) was run according to the conditions specified in the monograph, but with 50 % increase in the flow rate (from 1.0 mL/min to 1.5 mL/min).

With a flow rate of 1.5 mL/min, the total analysis time was shortened from about 9 minutes to less than 3.5 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 represents roughly a 2.5-fold increase in sample throughput capability. Note that this performance was achieved using an Agilent 1100 LC system with an upper pressure capability of 400 bar. A flow rate of 1.5 mL/min generated a system pressure of 305 bar.

Table 2.
Acceptable Modifications for Meeting System Suitability

Method Parameter	Acceptable Modification	Monograph 1028 Metoprolol Tartrate	Kinetex 2.6 µm Fast Method	Modification
Mobile phase pH	± 0.2 units	as specified	No Change	--
Concentration of salts in buffer	± 10 %	as specified	No Change	--
Ratio of components in mobile phase	± 30 % relative 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	280 nm	No Change	--
Injection volume	May be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory.	20 µL	No Change	--
Column temperature	± 10 %, to a maximum of 60 °C	25 °C	No Change	--
Column length	± 70 %	150 mm	100 mm	**- 33 %**
Column inner diameter	± 25 %	3.9 mm	4.6 mm	**+ 18 %**
Particle size	- 50 %	5 µm	2.6 µm	**- 48 %**
Flow rate	± 50 %	1.0 mL/min	1.5 mL/min	**+ 50 %**

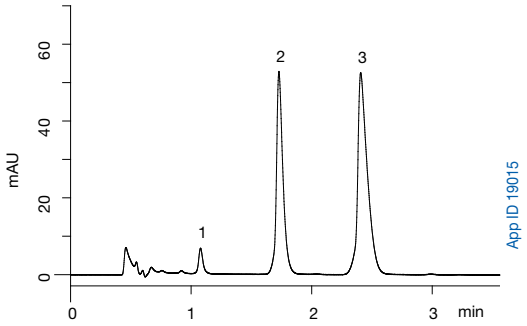
Figure 6.
Metoprolol Tartrate CRS: 20 µL injection on Kinetex 2.6 µm C18 100 x 4.6 mm at 1.5 mL/min; backpressure = 305 bar.



Column: Kinetex 2.6 µm C18
Dimensions: 100 x 4.6 mm
Mobile Phase: 3.9 g Ammonium acetate in 810 mL Water / 2.0 mL Triethylamine / 10 mL Glacial acetic acid / 3.0 mL Phosphoric acid / 146 mL Acetonitrile
Flow Rate: 1.5 mL/min
Temperature: 25° C
Detection: UV @ 280 nm
Sample: 1. Metoprolol Impurity G
2. Metoprolol Impurity A
3. Metoprolol tartrate CRS

A further reduction in analysis time while operating within the allowed adjustment for column length was obtained using a Kinetex 2.6 µm C18 50 x 4.6 mm column at the flow rate of 1.0 mL/min specified in the monograph. These conditions resulted in a further reduction in analysis time (<3 minutes) with substantially higher efficiency and sensitivity as compared to the fully porous 5 µm 150 x 3.9 mm column **(Figure 7)**. Resolution between Impurity A and Metoprolol Tartrate was 6.0, which meets the system suitability requirement. Using the shorter Kinetex column represents a 3-fold increase in throughput capability for this analysis.

Figure 7.
Metoprolol Tartrate CRS: 20 µL injection on Kinetex 2.6 µm C18 50 x 4.6 mm at 1.0 mL/min; backpressure = 103 bar.



Column: Kinetex 2.6 µm C18
Dimensions: 50 x 4.6 mm
Mobile Phase: 3.9 g Ammonium acetate in 810 mL Water / 2.0 mL Triethylamine / 10 mL Glacial acetic acid / 3.0 mL Phosphoric acid / 146 mL Acetonitrile
Flow Rate: 1 mL/min
Temperature: 25° C
Detection: UV @ 280 nm
Sample: 1. Metoprolol Impurity G
2. Metoprolol Impurity A
3. Metoprolol tartrate CRS

Table 3.
Improvements To The Monograph

	Fully porous 5 µm	Kinetex 2.6 µm C18	Kinetex 2.6 µm C18
Column Dimensions	150 x 3.9 mm	100 x 4.6 mm	50 x 4.6 mm
Particle Size	5 µm fully porous	2.6 µm core-shell	2.6 µm core-shell
Flow Rate	1.0 mL/min	1.5 mL/min	1.0 mL/min
Backpressure	95 bar	305 bar	103 bar
Resolution of Impurity A and Metoprolol Tartrate	8.43	9.21	6.0
S/N ratio for Metoprolol Tartrate	410	672	751
S/N ratio for Impurity A	382	705	754
S/N ratio for Impurity G	62	98	99
N for Metoprolol Tartrate (p/m)	57,260	109,810	98,580
Elution time of Metoprolol Tartrate	8.12 min	3.30 min	2.41 min
Solvent Usage	< 10 mL	< 6 mL	< 3 mL

Conclusion

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.

For this monograph two options are illustrated for providing significant improvement in sample throughput while meeting the system suitability requirement, and operating within the allowable adjustments specified by the Ph. Eur. Reducing column length by two-thirds to 50 mm while maintaining the flow rate specified in the monograph offers comparable improvements in sample throughput to a one-third reduction in column length (100 mm) and 50 % increase in flow rate. An additional, and not insignificant, benefit to using the shorter 50 x 4.6 mm Kinetex column at the flow rate specified in the monograph is the approximate 70 % reduction in solvent usage, while operating at a backpressure comparable with the fully porous 5 µm column specified in the monograph.

APPLICATIONS

Ordering Information

1.7 µm Minibore Columns (mm)

Phases	50 x 2.1	100 x 2.1	150 x 2.1
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN
HILIC	00B-4474-AN	—	—

2.6 µm Minibore Columns (mm)

Phases	50 x 2.1	100 x 2.1	150 x 2.1
C18	00B-4462-AN	00D-4462-AN	00F-4462-AN
PFP	00B-4477-AN	00D-4477-AN	00F-4477-AN
HILIC	00B-4461-AN	00D-4461-AN	00F-4461-AN

2.6 µm Solvent Saver MidBore™ Columns (mm)

Phases	50 x 3.0	100 x 3.0	150 x 3.0
C18	00B-4462-Y0	00D-4462-Y0	00F-4462-Y0
PFP	00B-4477-Y0	00D-4477-Y0	00F-4477-Y0
HILIC	—	—	00F-4461-Y0

2.6 µm Analytical Columns (mm)

Phases	50 x 4.6	100 x 4.6	150 x 4.6
C18	00B-4462-E0	00D-4462-E0	00F-4462-E0
PFP	00B-4477-E0	00D-4477-E0	00F-4477-E0
HILIC	00B-4461-E0	00D-4461-E0	00F-4461-E0

KrudKatcher™ Ultra In-line Filter

The KrudKatcher Ultra filter body houses an integrated 0.5 µm 316 stainless steel filter element that efficiently removes microparticulates from the flow stream without contributing to system backpressure or dead volume (<0.2 µL).

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

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

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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