Determination of Impurities and Related Substances for Glibenclamide (EP Monograph 0718). Increased Sensitivity, Improved Resolution and Faster Analysis Using Kinetex™ 2.6 µm Core-Shell LC Columns

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- *Within allowable modifications for system suitability, the EP method for the analysis of Glibenclamide, a sulfonylurea used to treat Type II diabetes is improved for better resolution higher sensitivity and increased sample throughput*
- *Ultra-high performance results are achieved on a conventional HPLC by utilizing Kinetex core-shell technology*

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 to 5 micron (µm) spherical silica chromatographic media. Due to the performance limitations of fully porous 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 lower peak heights 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, widespread adoption of this sub-2 µm HPLC column technology has been slowed since the smaller particle columns generate exponentially higher system backpressures that require specialized ultra-high pressure capable LC instrumentation.

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 EP ([Ph. Eur.] European Pharmacopoeia) Monograph 0718 for Glibenclamide and related substances on a conventional HPLC instrument with an upper pressure limit of 400 bar.

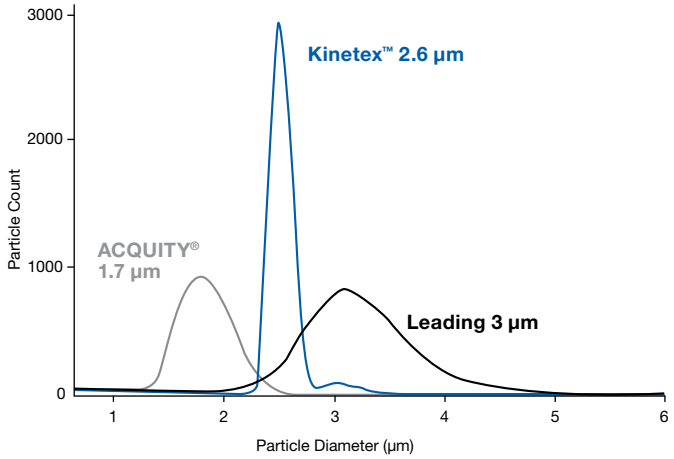
First, to demonstrate equivalency, a Kinetex column of the same dimension as the column referenced was operated under the conditions specified in the monograph. Then under the conditions of the monograph, a shorter Kinetex column was used; the 50 % decrease in column length being within the adjustment range allowed by the EP for meeting system suitability. Finally, the shorter Kinetex column was operated at a faster flow rate, but still within the ±50 % adjustment range allowed by the EP. This column achieved greater than 50 % faster analysis time (greater than 2x productivity improvement) and significantly improved resolution and sensitivity versus the EP referenced fully-porous 3 µm column.

Overview of Kinetex™ 2.6 µm Core-Shell Technology

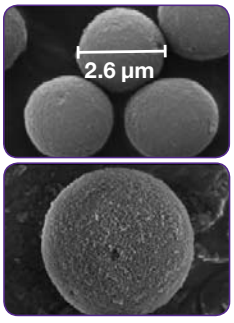
Precision Core-Shell Manufacturing

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

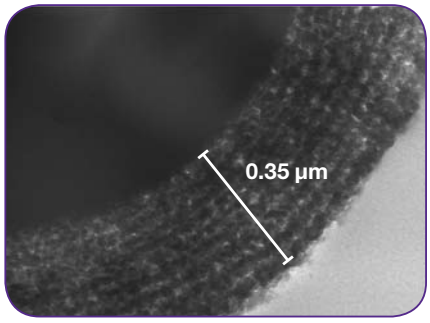
Figure 1.
Uniform Particle Size Distribution



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 velocities. 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 linear velocity is increased. This is due to the very low resistance to mass transfer of analytes into and out of the porous shell stationary phase that surrounds the solid silica core (minimizing the contribution of the "C" term to plate height).

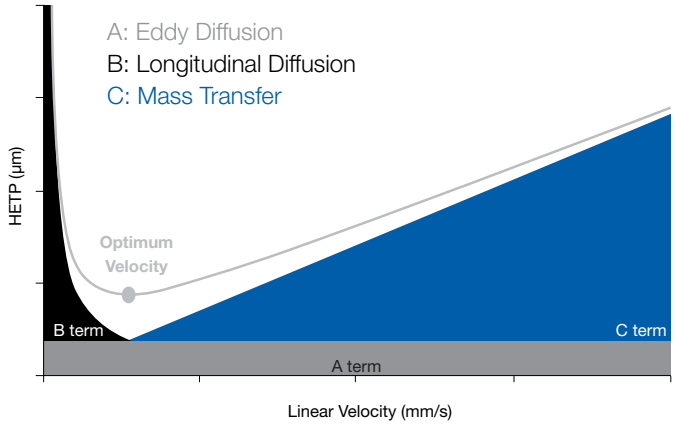
Reasonable LC System Operating Pressures

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 requiring 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 and useful 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

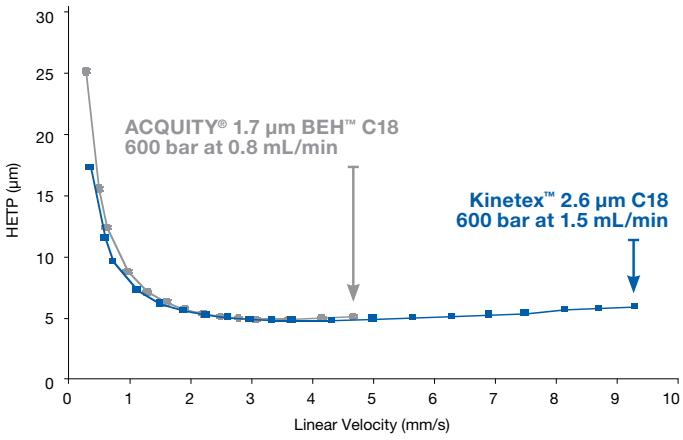
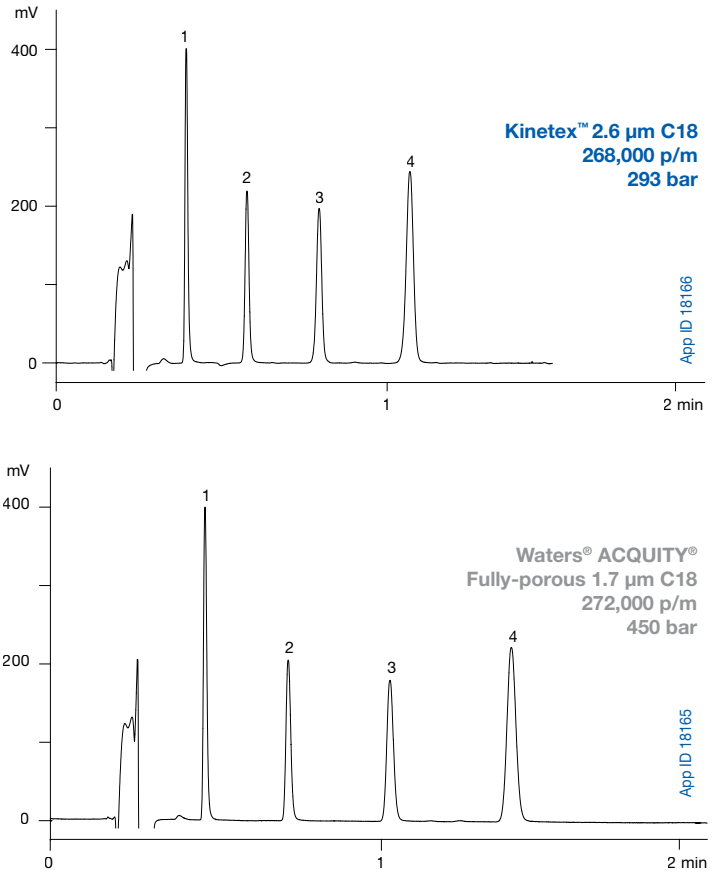


Figure 3.



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

Glibenclamide and Related Substances:
European Monograph 0718

Columns Used:
A fully porous 3 µm C18 100 x 4.6 mm column (as specified by the monograph) was compared with a Kinetex 2.6 µm C18 100 x 4.6 mm column.

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: Mix 20 mL of a 101.8 g/L solution of freshly distilled triethylamine (TEA) adjusted to pH 3.0 using phosphoric acid (H_3PO_4) and 50 mL of acetonitrile; dilute to 1000 mL with water.

B: Mix 20:65:915 (v/v/v) of mobile phase A/water/acetonitrile

Sample Preparation:
Glibenclamide Certified Reference Standard (CRS), Glibenclamide Impurity A CRS, Glibenclamide Impurity B CRS, and Gliclazide CRS for system suitability was obtained from the European Pharmacopoeia.

Reference Solution (a) was prepared by dissolving 5.0 mg of Glibenclamide Impurity A CRS and 5.0 mg of Glibenclamide Impurity B CRS in methanol and diluting to 100 mL with the same solvent. Dilute 5.0 mL of this solution to 20.0 mL with methanol.

Reference Solution (c) was prepared by dissolving 5 mg of Gliclazide CRS in methanol and diluting to 100 mL with the same solvent. Dilute 1 mL of this solution to 10 mL with methanol.

Glibenclamide Analysis Method:
The monograph calls for 10 µL of sample to be injected under gradient chromatographic conditions (see table below) at 0.8 mL/min using mobile phase as prepared above. Column temperature maintained at 35 °C and UV detection wavelength set at 230 nm.

Gradient Time (min)	Flow Rate (mL/min)	A (%)	B (%)
0.0	0.8	45	55
15	0.8	45	55
30	0.8	5	95
40	0.8	5	95
41	0.8	45	55
55	0.8	45	55

Results and Discussion

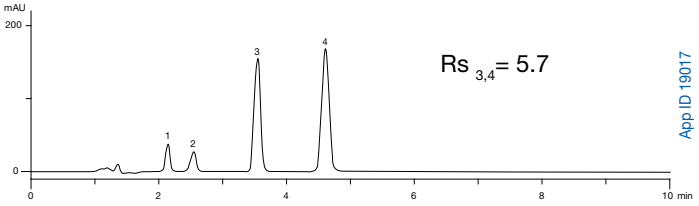
Equivalency Study:
Following the methodology described in European Pharmacopoeia Monograph 0718, and using a fully porous 3 µm C18 100 x 4.6 mm column as referenced in the method, a chromatogram similar to that of the specimen chromatogram provided with the Glibenclamide CRS was obtained **(Figure 4)**. Although the monograph indicates gradient mobile phase conditions are used, the separation of the analytes of interest occurs during the initial isocratic portion of the gradient.

A Kinetex 2.6 µm C18 100 x 4.6 mm column 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 3 µm column at the specified flow rate of 0.8 mL/min. The monograph requires resolution between Glibenclamide and Gliclazide of least 5.0. Due to the significantly narrower peaks generated by the higher efficiency Kinetex column, a substantial improvement in resolution between Glibenclamide and Gliclazide was achieved with Kinetex.

Sensitivity was also significantly improved for all impurities as a result of the Kinetex column generating narrower and taller peaks.

Figure 4.
Glibenclamide CRS: 10 µL injection on fully porous 3 µm C18 100 x 4.6 mm at 0.8 mL/min; column and conditions as specified in monograph.



Column: HyperClone BDS 3 µm C18
Dimensions: 100 x 4.6 mm
Mobile Phase: A: 101.8 g/L Triethylamine solution / Acetonitrile / Water (20:50:930)
B: Mobile phase A / Acetonitrile / Water (20:915:65)

Gradient:

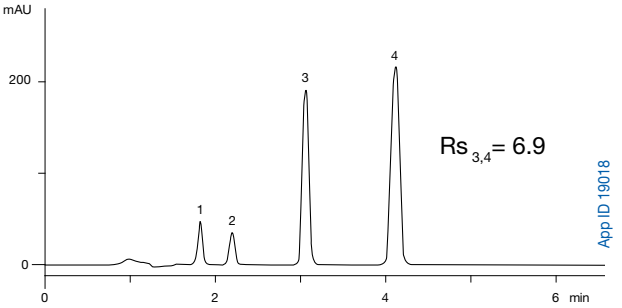
Time (min)	% A	% B
0	45	55
15	45	55
30	5	95
40	5	95
41	45	55
55	45	55

Flow Rate: 0.8 mL/min
Temperature: 35 °C
Detection: UV @ 230 nm
Sample: 1. Glibenclamide impurity A
2. Glibenclamide impurity B
3. Glibenclamide
4. Gliclazide

With 10 µL of the Glibenclamide CRS injected, the peak height for impurity A on the Kinetex 2.6 µm core-shell C18 column was 47 % greater than on the fully porous 3 µm C18 column.

Considering that LOQ (defined by the European Pharmacopoeia as corresponding to a signal-to-noise ratio of 10 for a chromatographic peak) can be one of the most challenging parameters to meet in routine operation, the more intense peaks observed with the Kinetex core-shell technology will result in higher signal-to-noise ratio and provide a significant performance advantage – allowing one to easily achieve the required LOQ.

Figure 5.
Glibenclamide CRS: 10 µL injection on Kinetex 2.6 µm C18 100 x 4.6 mm at 0.8 mL/min; backpressure = 124 bar



Column: Kinetex 2.6 µm C18
Dimensions: 100 x 4.6 mm
Mobile Phase: A: 101.8 g/L Triethylamine solution / Acetonitrile / Water (20:50:930)
B: Mobile phase A / Acetonitrile / Water (20:915:65)

Gradient:

Time (min)	% A	% B
0	45	55
15	45	55
30	5	95
40	5	95
41	45	55
55	45	55

Flow Rate: 0.8 mL/min
Temperature: 35 °C
Detection: UV @ 230 nm
Sample: 1. Glibenclamide impurity A
2. Glibenclamide impurity B
3. Glibenclamide
4. Gliclazide

Table 1. Equivalency Study

	Fully porous 3 µm C18	Kinetex 2.6 µm C18	Comments
Column Dimensions	100 x 4.6 mm	100 x 4.6 mm	
Particle Size	3 µm fully porous	2.6 µm core-shell	
Flow Rate	0.8 mL/min	0.8 mL/min	
Backpressure	72 bar	124 bar	
Resolution of Glibenclamide and Gliclazide	5.7	6.9	21 % increase
Peak Height ratio for Impurity A	33.1	48.8	47 % increase
Peak Height ratio for Impurity B	25.3	35.8	42 % increase
N for Glibenclamide (p/m)	58840	77,510	32 % increase
Elution time of Glibenclamide	3.55 min	3.06 min	

Fast Method
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.

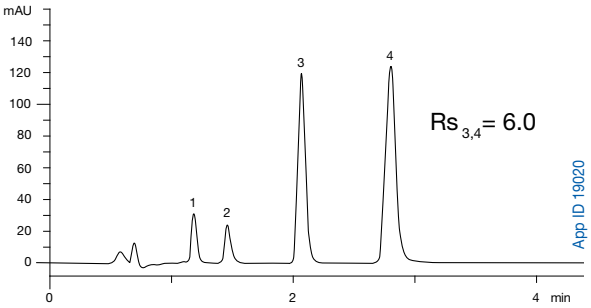
Following European Pharmacopoeia guidelines, the extent to which the various parameters of a chromatographic test may be adjusted to satisfy system suitability (when replacing one column with another of the same type, for example) is summarized in **Table 2**. Staying within these guidelines, a shorter Kinetex 2.6 µm core-shell C18 column (50 x 4.6 mm, representing a decrease in column length of 50 % relative to the monograph) was run at the same flow rate as specified in the monograph.

With the shorter Kinetex column the retention time for the last peak of interest (Gliclaxide) was reduced from 4.6 minutes to just under 3 minutes **(Figure 6)**. Resolution, efficiency and sensitivity remained substantially higher with the shorter Kinetex column, relative to the specified column, operated under the conditions specified in the monograph **(Table 3)**. The reduction in retention time achieved with Kinetex in this example represents roughly a 1.5-fold increase in sample throughput capability. It should be noted that decreasing the length of the Kinetex column from 100 to 50 mm resulted in a faster separation, with only a marginal decrease in resolution.

Table 2.
Acceptable Modifications for Meeting System Suitability

Method Parameter	Acceptable Modification	Monograph 0718 Glibenclamide	Kinetex 2.6 µm Fast Method	Modification
Mobile phase pH	± 0.2 units	3.0	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	230 nm	No Change	--
Injection volume	May be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory.	10 µL	No Change	--
Column temperature	± 10 %, to a maximum of 60 °C	35 °C	No Change	--
Column length	± 70 %	100 mm	50 mm	**- 50 %**
Column inner diameter	± 25 %	4.6 mm	4.6 mm	--
Particle size	- 50 %	3 µm	2.6 µm	**- 13 %**
Flow rate	± 50 %	0.8 mL/min	0.8 mL/min	--

Figure 6.
Glibenclamide CRS: 10 µL injection on Kinetex 2.6 µm C18 50 x 4.6 mm at 0.8 mL/min; backpressure = 43 bar



Column: Kinetex 2.6 µm C18
Dimensions: 50 x 4.6 mm
Mobile Phase: A: 101.8 g/L Triethylamine solution / Acetonitrile / Water (20:50:930)
B: Mobile phase A / Acetonitrile / Water (20:915:65)

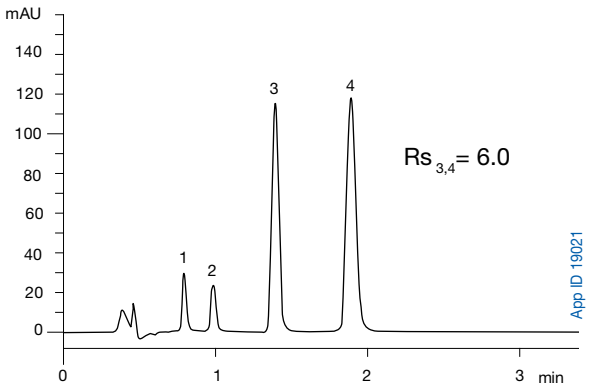
Gradient:

Time (min)	% A	% B
0	45	55
15	45	55
30	5	95
40	5	95
41	45	55
55	45	55

Flow Rate: 0.8 mL/min
Temperature: 35 °C
Detection: UV @ 230 nm
Sample: 1. Glibenclamide impurity A
2. Glibenclamide impurity B
3. Glibenclamide
4. Gliclazide

A further reduction in analysis time, while operating within the allowed adjustments, was obtained using the shorter 50 x 4.6 mm Kinetex 2.6 µm C18 column at a flow rate of 1.2 mL/min. This represents a 50 % increase in flow rate relative to the monograph. These conditions resulted in a further reduction in analysis time to less than 2 minutes with substantially higher efficiency and sensitivity as compared to the fully porous 3 µm 100 x 4.6 mm column **(Figure 7)**. Resolution between Glibenclamide and Gliclaxide was 6.0, which meets the system suitability requirement.

Figure 7.
Glibenclamide CRS: 10 µL injection on Kinetex 2.6 µm C18 50 x 4.6 mm at 1.2 mL/min; backpressure = 64 bar.



Column: Kinetex 2.6 µm C18
Dimensions: 50 x 4.6 mm
Mobile Phase: A: 101.8 g/L Triethylamine solution / Acetonitrile / Water (20:50:930)
B: Mobile phase A / Acetonitrile / Water (20:915:65)

Gradient:

Time (min)	% A	% B
0	45	55
15	45	55
30	5	95
40	5	95
41	45	55
55	45	55

Flow Rate: 1.2 mL/min
Temperature: 35 °C
Detection: UV @ 230 nm
Sample: 1. Glibenclamide impurity A
2. Glibenclamide impurity B
3. Glibenclamide
4. Gliclazide

Table 3.
Improvements To The Monograph

	Fully porous 3 µm C18	Kinetex 2.6 µm C18	Kinetex 2.6 µm C18
Column Dimensions	100 x 4.6 mm	50 x 4.6 mm	50 x 4.6 mm
Particle Size	3 µm fully porous	2.6 µm core-shell	2.6 µm core-shell
Flow Rate	0.8 mL/min	0.8 mL/min	1.2 mL/min
Backpressure	72 bar	43 bar	64 bar
Resolution of Glibenclamide and Gliclazide	5.7	6.0	6.0
Peak Height ratio for Impurity A	33.1	38.6	39.2
Peak Height ratio for Impurity B	25.3	29.6	30.0
N for Glibenclamide (p/m)	58,840	126,280	118,680
Elution time of Glibenclamide	3.55 min	2.07 min	1.39 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.

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 EP. Reducing column length by one-half to 50 mm while maintaining the flow rate specified in the monograph provides a 1.5x improvement in sample throughput, while increasing the flow rate by 50 % on the shorter column results in an overall 2x improvement in sample throughput relative to the original monograph.

APPLICATIONS

Kinetex 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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