Using Core-Shell Kinetex® XB-C18 HPLC Columns as a Solution for Analyzing Fluoroquinolone Antibiotics by LC/MS

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Additional Kinetex core-shell media research and product development has culminated in the introduction of unique bonded phases, such as Kinetex XB-C18. Kinetex XB-C18 is used for developing a rapid LC/MS method for separating fluoroquinolones; when compared to other competitive phases, the Kinetex XB-C18 demonstrates good peak shape and separation of closely related compounds.

Introduction

Kinetex core-shell columns were introduced in the fall of 2009 and now include five different bonded phase chemistries: XB-C18, C8, C18, PFP and HILIC. The geometry of Kinetex core-shell particles are designed to deliver ultra-high performance, approaching 300K plates per meter, at pressures amenable to operation on standard HPLC systems with 400 bar backpressure limits[1]. Kinetex XB-C18, with its di-isobutyl C18 ligand, offers an alternate selectivity with different retention characteristics, especially for bases at low pH, to Kinetex C18 and other traditional C18 phase columns.

Fluoroquinolones are a class of antibiotics that have become very popular over the last decade due their broad spectrum effectiveness on both gram positive and gram negative bacteria. Despite toxicity issues with several members of the drug class (that have been removed from the market) the most notable active pharmaceutical ingredient (API) in the class, Ciprofloaxin (Cipro®), continues to grow in popularity as a front line antibiotic for G.I. track infections. Growing popularity requires an improved high-speed LC/MS method for quantitating such drugs. Fluoroquinolones present some unique separation challenges related to their chemical structure; they are polyaromatic basic compounds with an attached fluorine atom and carboxcylic acid group resulting in some zwitterionic properties[2]. Separations of fluoroquinolones were performed to evaluate if the reduced silanol activity of Kinetex XB-C18 column offered a better analysis solution compared to other UHPLC columns on the market.

Materials and Methods

All chemicals were purchased from Sigma Chemical (St. Louis, MO), except for USP test standards, which were purchased from USP (Rockville, MD). Solvents were obtained from EMD (San Diego, CA). Kinetex XB-C18, 2.6 µm, 50 x 2.1 mm dimension column (Phenomenex, Inc., Torrance, CA) was compared against columns from a different manufacturer (ACQUITY® 1.7 µm CSH™ C18, Waters®, Milford, MA) to demonstrate performance differences. All analyses were run on an Agilent® 1200 HPLC system (Palo Alto, CA) with autosampler and column oven. MS detection was performed using an AB SCIEX API 4000™ mass spectrometer (Foster City, CA) with an ESI+ interface operating in MRM mode.

LC/MS compatible mobile phase was used for the separation (A: 0.1 % formic acid in water, B: methanol) with a rapid gradient from 15 % B to 90 % B in 5 minutes. The flow rate was 0.4 mL/min and column temperature was maintained at 40 °C. Standard solutions were diluted to 50 ng/mL with 15 % B prior to injection.

Results and Discussion

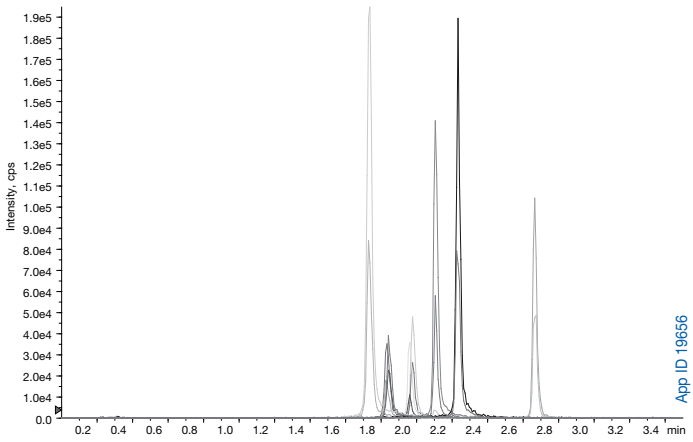
Fluoroquinolones present a unique separation challenge because of the unique chemistry of the compounds. They contain multiple aromatic amines, a carboxcylic acid functionality, and at least one fluorine molecule.

Kinetex XB-C18 media offers a possible solution for separating fluoroquinolones because the di-isobutyl C18 ligands offer significant shielding of silanol interactions, which lead to peak tailing. This is especially important in the volatile buffers preferred for LC/MS separations in order to maximize MS sensitivity. An example separation of a fluoroquinolone mixture is shown in **Figure 1.** Note the high efficiency and good peak shape with minimal tailing, as well as the near-baseline resolution of every component in the mixture. The high efficiency and good selectivity that Kinetex XB-C18, 2.6 µm core-shell media delivers allow for a rapid separation of this mixture in less than 5 minutes. A fully porous sub-2 µm, C18 media column (ACQUITY CSH 1.7 µm C18) was shown in comparison in **Figure 2.** Note the significant peak tailing for all components in the mixture and the lower efficiency achieved in this application.

For the fluoroquinolone application the Kinetex XB-C18, 2.6 µm column offered the better solution; the good peak shape in the Kinetex chromatogram suggests a more inert solution for this application. The unique geometry of the XB-C18 ligand may also offer some selectivity benefits over other C18 phases, aside from the silanol-shielding effects. While not a focus of this application, the core-shell particle also offers method portability advantages over fully porous sub-2 µm media. The Kinetex 2.6 µm column shown here operates at significantly lower backpressure than sub-2 µm media, making it suitable for both UHPLC and standard HPLC systems.

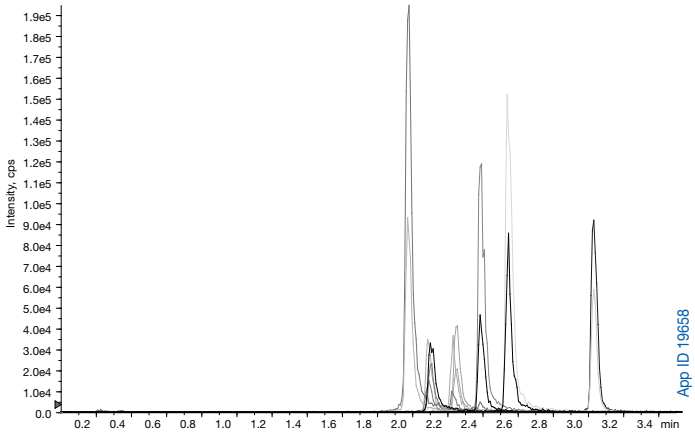
APPLICATIONS

Figure 1.



Fluoroquinolones analyzed by LC/MS using a Kinetex® 2.6 µm XB-C18 column. Note the good peak shape, high efficiency, and peak resolution for the mixture.

Figure 2.



Fluoroquinolones analyzed by LC/MS using an ACQUITY® CSH™ 1.7 µm, C18 column. Note the significant peak tailing and reduced efficiency compared to the Kinetex XB-C18 column.

Conditions for both columns:

Columns: Phenomenex® Kinetex® 2.6 µm XB-C18
Waters® ACQUITY® CSH™ 1.7 µm C18

Dimensions: 50 x 2.1 mm

Mobile Phase: A: 0.1 % Formic acid
B: Methanol

Gradient:

Time (min)	% B
0	15
3	15
5	90
6	90

Flow Rate: 0.4 mL/min

Temperature: 40 °C

Detection: MS

Instrument: API 4000™

Sample: 1. Ofloxacin
2. Norfloxacin
3. D5-Norfloxacin
4. Ciprofloxacin
5. D8-Ciprofloxacin
6. Enrofloxacin
7. Difloxacin
8. Sparfloxacin

Conclusion

Data from this technical note demonstrate the utility of the Kinetex XB-C18, 2.6 µm column for separating fluoroquinolones, a difficult class of basic pharmaceutical compounds. The excellent peak shape for basic compounds in volatile buffers makes the Kinetex XB-C18 phase an ideal choice for separating basic compounds for LC/MS applications where non-ion pairing buffers are used.

References

1. Phenomenex® Technical Note TN-1058: “Increased Efficiency and Resolution with Kinetex Core-Shell Technology”
2. “Quinolone” - Wikipedia web site (www.wikipedia.com)

APPLICATIONS

Ordering Information

Kinetex® 2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard™ Ultra Cartridges‡ /3pk	KrudKatcher™ Ultra In-Line Filter* /3pk
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768	AF0-8497
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768	AF0-8497
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770	AF0-8497
PFP	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0	AJ0-8773	AF0-8497
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJ0-8772	AF0-8497

for 4.6 mm ID

Kinetex 2.6 µm MidBore™ Columns (mm)

	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard Ultra Cartridges‡ /3pk	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	—	00B-4496-Y0	—	00D-4496-Y0	—	AJ0-8775	AF0-8497
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775	AF0-8497
C8	—	00B-4497-Y0	—	00D-4497-Y0	—	AJ0-8777	AF0-8497
PFP	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0	AJ0-8780	AF0-8497
HILIC	—	—	—	—	00F-4461-Y0	AJ0-8779	AF0-8497

for 3.0 mm ID

Kinetex 2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard Ultra Cartridges‡ /3pk	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782	AF0-8497
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782	AF0-8497
C8	00A-4497-AN	00B-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784	AF0-8497
PFP	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN	AJ0-8787	AF0-8497
HILIC	—	00B-4461-AN	00D-4461-AN	00F-4461-AN	AJ0-8786	AF0-8497

for 2.1 mm ID

Kinetex 1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard Ultra Cartridges‡ /3pk	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00B-4498-AN	00D-4498-AN	—	AJ0-8782	AF0-8497
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782	AF0-8497
C8	00B-4499-AN	00D-4499-AN	—	AJ0-8784	AF0-8497
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN	AJ0-8787	AF0-8497
HILIC	00B-4474-AN	—	—	AJ0-8786	AF0-8497

for 2.1 mm ID

‡SecurityGuard Ultra cartridges require holder, Part No.: AJ0-9000.
Check for availability in your country.
*KrudKatcher Ultra requires $^{5}/_{16}$ in. wrench. Wrench not provided.

UHPLC / HPLC Sure-Lok™ High Pressure PEEK™ Male Nut Fittings

Part No.	Description	Unit
AQ0-8503	Sure-Lok High Pressure PEEK 1-Pc Nut 10-32, for $^{1}/_{16}$ in. Tubing, 12,000 psi (827 bar)	10/pk
AQ0-8530	Sure-Lok Fitting Tightening Tool, Aluminum	ea

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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