

Complementary Selectivities of Kinetex[®] Core-Shell Phases: C18, XB-C18, C8 and PFP

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Further research and product development of Kinetex core-shell media has culminated with the introduction of two additional bonded phases, Kinetex XB-C18 and Kinetex C8. These new phases offer complementary selectivities to the existing Kinetex phases (C18, PFP and HILIC) giving chromatographers additional solutions for their ultra-high performance separations, especially useful for basic compounds and for LC/MS conditions.

Introduction

The geometry of Kinetex core-shell particles are designed to deliver ultra-high performance, approaching and in some cases exceeding 300K plates per meter, at pressures amenable to operation on standard HPLC systems with 400 bar backpressure limits.¹ A key to the utility of the product line was the introduction of three different bonded phases, providing options for method developers. The C18 and PFP offer complementary reversed phase selectivities: C18 for separations primarily based on differences in analyte hydrophobicities, and PFP based on a complement of aromatic, hydrophobic, and polar selectivities. The silica HILIC phase offers an orthogonal separation mode based on polar differences between analytes. While such phases offer solutions to a large percentage of the methods developed in the industry, other complementary phases have now been developed to offer extra solutions for chromatographer's needs. The XB-C18, with its cross-butyl ligand bonding, offers different retention characteristics, especially for bases at low pH. The C8 offers a less hydrophobic alternative to the Kinetex C18 phases with minimal secondary interactions (due to greater bonding density) resulting in improved peak shape for particularly troubling analytes, such as basic drug metabolites. This technical note will cover some general observations regarding the selectivity of the new Kinetex phases and where they complement the other selectivities in the Kinetex product line.

Material 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 columns of various phases (C18, XB-C18, C8, PFP and HILIC) used were either 50 x 2.1 mm or 100 x 4.6 mm dimensions (Phenomenex, Torrance, CA). All analyses were run on either an Agilent 1100 or 1200 HPLC system (Palo Alto, CA) with autosampler, column oven, and multi-wavelength detector. Specific method conditions are listed with each application.

Results and Discussion

Kinetex XB-C18 versus Kinetex C18

With a cross-butyl C18 ligand instead of a traditionally bonded C18 ligand, Kinetex XB-C18 offers a unique selectivity over the Kinetex C18 phase. While the XB-C18 is only a slightly more hydrophobic phase than the Kinetex C18 phase, the selectivity differences for basic and acidic compounds are significant, especially under non-ion pairing acid mobile phase conditions, e.g. 0.1 % formic

acid. Basic compounds will tend to be less retained by Kinetex XB-C18, and acids will be slightly more retained. An example of this is shown in **Figure 1** where a mixture of acids, bases, and neutral compounds are used to highlight selectivity differences under gradient conditions. When Kinetex C18 (**Figure 1A**) is compared to Kinetex XB-C18 (**Figure 1B**) one observes a significant elution order change for this sample mixture. Basic compounds elute significantly earlier on the XB-C18 and acidic compounds elute slightly later. It is believed that this is due to differences in silanol interactions between the sterically hindered cross-butyl C18 ligand compared to the traditionally bonded C18. Increased silanol-based retention on the Kinetex C18 phase would indicate the phase has greater selectivity for basic compounds, especially when TFA based buffers are used. For applications where formic acid is used (e.g. LC/MS conditions) the Kinetex XB-C18 would be the preferred starting point for improved peak shape and reduced basic compound retention. However, such rules are not straightforward, as is shown in **Figures 2A and 2B** when looking at a steroid mixture. In this case there are no basic compounds present, yet elution order differences are seen between the Kinetex C18 and Kinetex XB-C18 phases. While one might explain cortisone-21-acetate peak shifts between the phases due to it being an ester, shifts in the 21-hydroxyprogesterone and estrone point to another mechanism impacting selectivity differences between the phases. In the end, both phases have unique and complementary selectivities making both useful in a method development column screen.

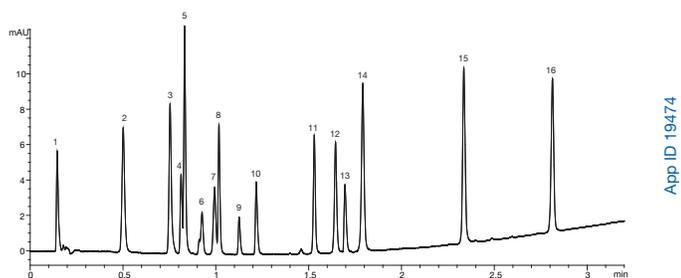
Kinetex C8

While most consider a C8 phase a less hydrophobic version of a C18 phase, examination of the selectivity differences between Kinetex C18 and Kinetex C8 point out that is not the complete story. The Kinetex C8 phase offers users the opportunity to upgrade their USP methods using fully-porous C8 columns to high-throughput and high performance with core-shell media. Additionally when one looks at the pharmaceutical mixture run on the Kinetex C8 (**Figure 3**) one quickly notices that the phase selectivity is not just a reduction in hydrophobicity versus the Kinetex C18 phase. Basic components shift relative to neutral and acidic analytes; while not as drastic as the comparison between XB-C18 and C18, these shifts give Kinetex C8 selectivity that is "in the middle" compared to the other phases. This unique selectivity makes Kinetex C8 an additional choice for a method development column screen, especially for one looking to update an existing USP method using a L7 column. When one looks at "real-life" methods the C8 phase can be a far superior choice as shown in **Figures 4A and 4B** where veterinary drugs are analyzed from horse urine. In these applications, also run on the Kinetex C18 and XB-C18, several contaminants from the urine co-migrate with analyte peaks in the chromatogram (**Figure 4A**.) However, when the Kinetex C8 is used, all the analyte peaks are easily resolved from matrix contaminants. Such results further indicate the usefulness of the Kinetex C8 phase as a unique selectivity that should be considered in method development.

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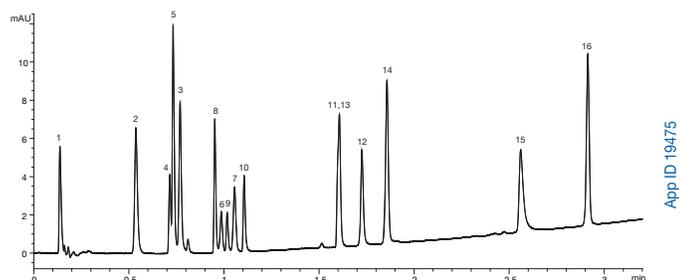
APPLICATIONS

Figure 1A.
Pharmaceutical Mixture on Kinetex 2.6µm C18 Column



App ID 19474

Figure 1B.
Pharmaceutical Mixture on Kinetex 2.6µm XB-C18 Column



App ID 19475

Column: As noted
Dimensions: 50 x 2.1 mm
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Acetonitrile
Gradient:

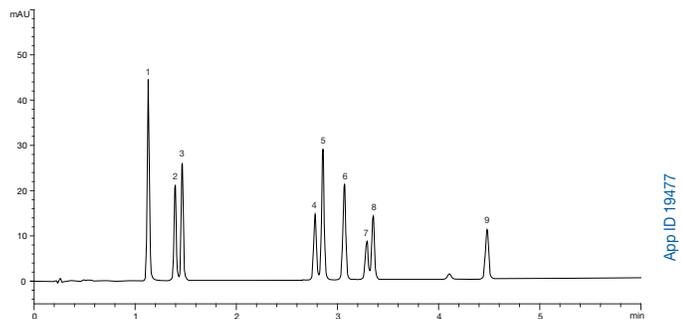
Time (min)	% B
0	5
0.2	5
4	95

Flow Rate: 0.8 mL/min
Temperature: Ambient
Detection: UV @ 254 nm
Sample:

1. Pyridine	9. Chlorpheniramine
2. Acetaminophen	10. Triprolidine
3. Sulfathiazole	11. Prednisolone
4. Pindolol	12. 3-Methyl, 4-nitrobenzoic acid
5. Quinidine	13. Nortriptyline
6. Benzyl Alcohol	14. 2-Hydroxy, 5-methylbenzaldehyde
7. Phenol	15. Diflunisal
8. Acebutolol	16. Hexanophenone

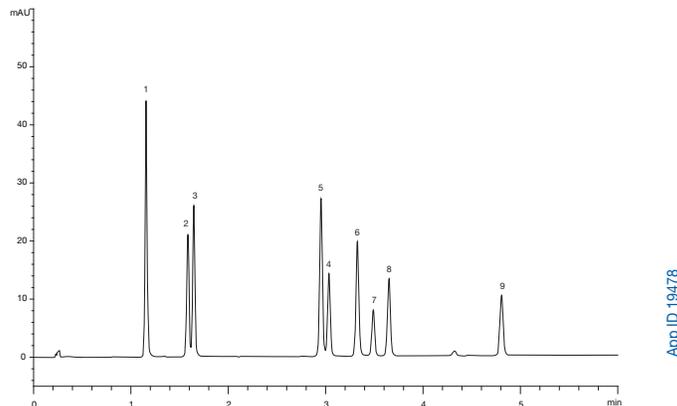
Note the decreased retention of basic compounds on the Kinetex XB-C18 column versus the Kinetex C18 column. The different selectivity of the two phases make them complementary for a method development column panel.

Figure 2A.
Steroid Mixture on Kinetex 2.6µm C18 Column



App ID 19477

Figure 2B.
Steroid Mixture on Kinetex 2.6µm XB-C18 Column

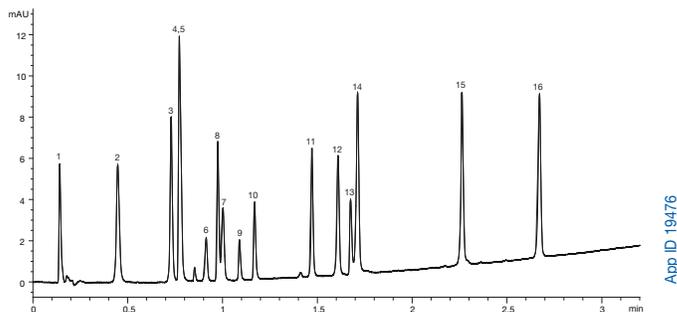


App ID 19478

Column: As noted
Dimensions: 50 x 2.1 mm
Mobile Phase: Water / Acetonitrile (20:60)
Flow Rate: 0.5 mL/min
Temperature: Ambient
Detection: UV @ 220 nm
Sample:

1. Estriol	6. 21-Hydroxyprogesterone
2. Hydrocortisone	7. Estrone
3. Cortisone	8. 17-Hydroxyprogesterone
4. Cortisone-21-acetate	9. Deoxycorticosterone
5. Estradiol	

Figure 3.
Pharmaceutical Mixture on Kinetex 2.6µm C8



App ID 19476

Note the reduced overall retention of most analytes on the Kinetex C8 column. Also note the selectivity for basic compounds indicating the Kinetex C8 as a good intermediate selectivity between Kinetex C18 and Kinetex XB-C18. See **Figure 1** for running conditions.

Figure 4A.
Veterinary Drugs in Horse Urine on Kinetex 2.6 μ m C18

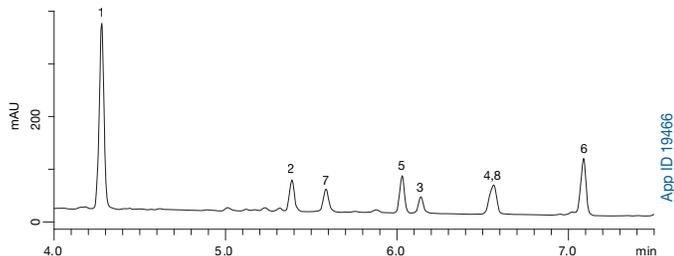
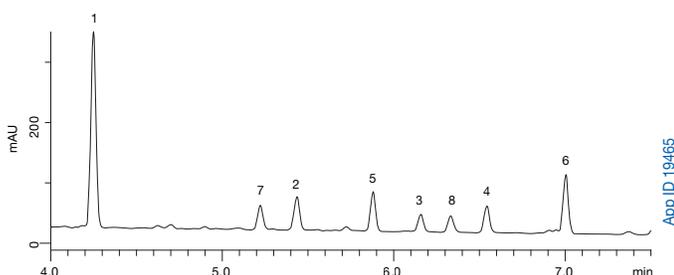


Figure 4B.
Veterinary Drugs in Horse Urine on Kinetex 2.6 μ m C8



Column: As noted
Dimensions: 100 x 4.6 mm
Mobile Phase: A: 0.1% Phosphoric acid in Water
B: 0.1% Phosphoric acid in Acetonitrile

Gradient	Time (min)	% B
	0	30
	4	50
	7.5	70
	9	95
	10	30

Flow Rate: 1.5 mL/min

Temperature: 25 °C

Detection: UV @ 230 nm

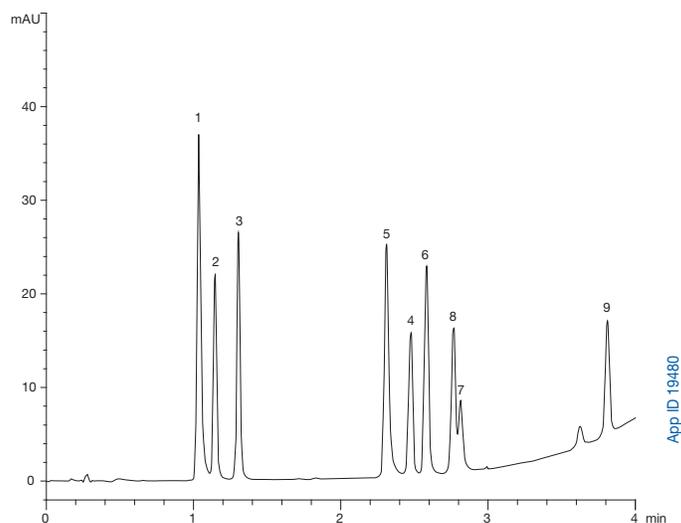
Sample	1. Naproxen	5. Indomethacin
	2. Fenoprofen	6. Meclofenamic Acid
	3. Ibuprofen	7. Matrix Impurity
	4. Phenylbutazone	8. Matrix Impurity

Note the reduced overall retention of most analytes on the C8 column. Also note the selectivity for basic compounds make the Kinetex C8 a good intermediate selectivity between Kinetex C18 and Kinetex XB-C18, and the first choice for this application because of its ability to resolve matrix contaminants from analytes.

Linking XB-C18 and C8 With Other Kinetex Phases

In addition to the selectivity differences between Kinetex C18, XB-C18, and C8, Kinetex PFP is also a necessary tool for many method developers. The PFP phase is both polar and aromatic, offering a very unique selectivity versus all purely alkyl-based phases. An example of such selectivity difference is demonstrated in **Figure 5** when one looks at the steroid mixture on the Kinetex PFP column. In this case selectivity differences are seen across the entire chromatogram (peaks 2, 3, 4, and 5 for example). Such an elution order could be further modulated with the use of methanol as the organic mobile phase instead of acetonitrile, thus increasing the influence of aromatic interactions². In the end, analyte characteristics like basicity, polarity, and aromaticity should be evaluated in making a first choice of an HPLC column with the understanding that alternate phases are available for optimizing a separation.

Figure 5.
Steroid Mixture on Kinetex 2.6 μ m PFP column



Note the significantly different selectivity for several compounds throughout the chromatogram (as compared to Figures 2A and 2B). Such different selectivity offers the possibility of using a different stationary phase to improve resolution between closely eluting components in a mixture. See **Figure 2** for running conditions.

Conclusion

With the addition of the new XB-C18 and C8 phases the Kinetex core-shell HPLC columns offer additional solutions for developing optimized ultra-high performance separations. Comparing analyte composition as well as the mobile phase conditions dictated by the type of analysis required (LC/UV versus LC/MS) allow one to choose a good starting point in phase selection with the understanding that other phases in the portfolio are available if the resolution of a key component in a mixture is required.

References

1. Phenomenex Technical Note TN-1058 *Increased Efficiency and Resolution with Kinetex Core-Shell Technology*
2. Phenomenex Technical Note TN-1029 *Using Aromatic Selectivity with Gemini C6-Phenyl for Difficult Separations*

TN-1088 APPLICATIONS

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XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJO-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJO-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJO-8770
PFP	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0	AJO-8773
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJO-8772

†SecurityGuard Ultra cartridges require holder, Part No.: AJO-9000. Check for availability in your country.

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†
XB-C18	—	00B-4496-Y0	—	00D-4496-Y0	—	AJO-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJO-8775
C8	—	00B-4497-Y0	—	00D-4497-Y0	—	AJO-8777
PFP	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0	AJO-8780
HILIC	—	—	—	—	00F-4461-Y0	AJO-8779

†SecurityGuard Ultra cartridges require holder, Part No.: AJO-9000. Check for availability in your country.

2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	KrudKatcher Ultra In-Line Filter
					/3pk
XB-C18	—	00B-4496-AN	00D-4496-AN	—	AF0-8497
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AF0-8497
C8	—	00B-4497-AN	00D-4497-AN	—	AF0-8497
PFP	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN	AF0-8497
HILIC	—	00B-4461-AN	00D-4461-AN	00F-4461-AN	AF0-8497

KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	KrudKatcher Ultra In-Line Filter
				/3pk
XB-C18	00B-4498-AN	00D-4498-AN	—	AF0-8497
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN	AF0-8497
C8	00B-4499-AN	00D-4499-AN	—	AF0-8497
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN	AF0-8497
HILIC	00B-4474-AN	—	—	AF0-8497

KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

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