APPLICATIONS

A Screen of 22 Common Antibiotics that Demonstrates the Unique Reversed Phase Selectivity and Improved Chromatographic Performance for Bases using a Kinetex® PS C18 HPLC/UHPLC Column

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Zeshan loves to collect watches and the Back to the Future Trilogy. He has twin boys who drive him crazy! He is an Apple Fanboy for life and he likes being in the lab more than anywhere else.

Overview

The Kinetex PS C18 is a USP classified L1 column, that provides both a unique polar/hydrophobic selectivity, and is 100 % aqueous stable. The column demonstrates enhanced selectivity and peak shape for basic compounds under typical reversed phase conditions. In addition, the solid support is a Kinetex core-shell (superficially porous) particle morphology that provides ultra-high column efficiency on any HPLC or UHPLC system.[1]

The mobile phase program chosen was a routine gradient of Acetonitrile with 0.1 % Formic Acid as the strong organic solvent and Water with 0.1 % Formic Acid as the weak solvent. The flow rate of 0.5 mL/min was used, and the column heater was set to ambient temperature (25 °C).

Introduction

In this application, 22 antibiotics were analyzed to demonstrate the Kinetex PS C18 HPLC/UHPLC column's unique multi-modal selectivity and improved chromatographic performance for polar bases. The unique selectivity and performance benefits were compared to another reversed phase C18 column of similar superficially porous (core-shell) particle structure under the identical system and method conditions. The selected antibiotics were chosen due to their associated challenges when analyzed under reversed phase conditions. These challenges are primarily due to a wide variety of analyte polarity, and are demonstrated by the compounds' wide range of LogP values, from -0.52 to 2.32. In addition, several of these antibiotics were selected because they are polar basic compounds.

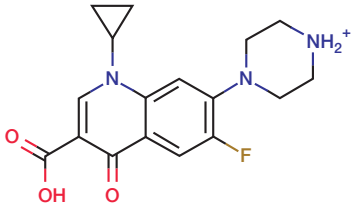
Following the analysis of the 22 antibiotic mixture, three individual antibiotic comparisons were run using ciprofloxacin, florfenicol, and difloxacin to better demonstrate the relative peak shape performance and selectivity of the Kinetex PS C18. For comparison, a core-shell C18 column was also run under identical method conditions for the three individual antibiotics to validate stationary phase selectivity differences.

Experiment

Analytical reference standards for the 22 antibiotics and individual compounds (ciprofloxacin, florfenicol, and difloxacin) were obtained through Sigma-Aldrich® (Saint Louis, MO). An Agilent® 1200 Infinity HPLC system was used for this investigation and a SCIEX™ 4000 MS/MS was used for detection.

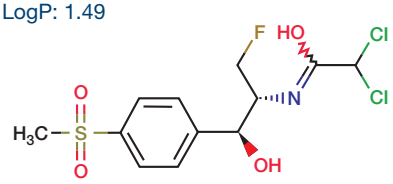
Ciprofloxacin

Molecular Formula: $C_{17}H_{19}FN_3O_3$
Basic p*K*a: 8.77
Acidic p*K*a: 5.56
LogP: -0.86



Florfenicol

Molecular Formula: $C_{12}H_{14}Cl_2FNO_4S$
Basic pKa: 13.6
Acidic pKa: 8.49
LogP: 1.49



Difloxacin

Molecular Formula: $C_{21}H_{19}F_2N_3O_3$
Basic p*K*a: 6.99
Acidic p*K*a: 5.52
LogP: 1.75

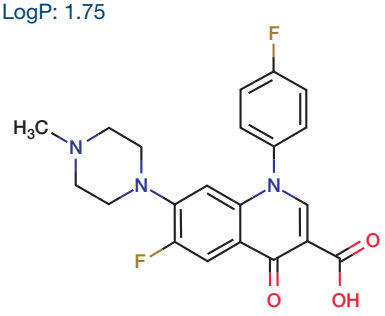
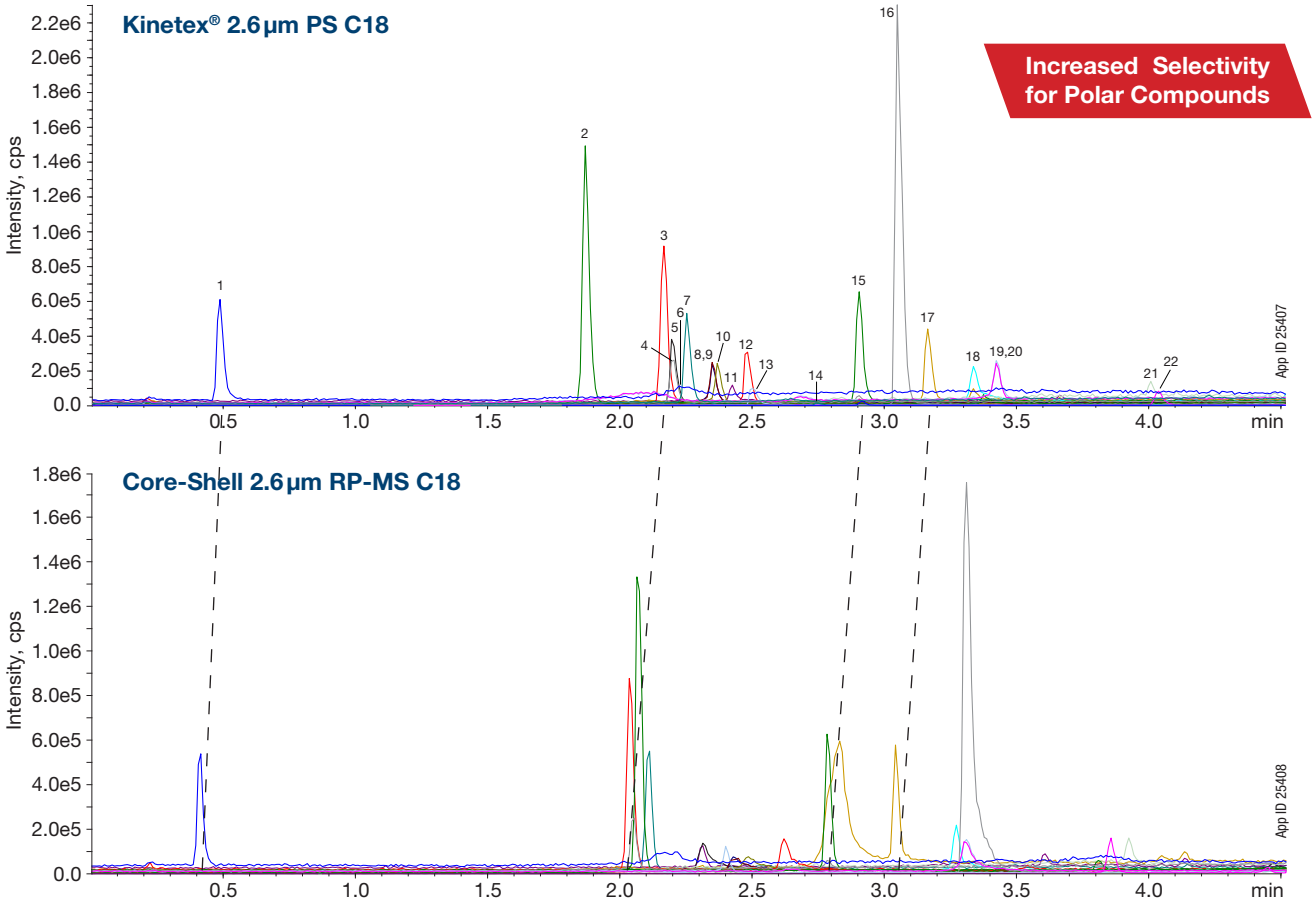


Figure 1.
A Screen of 22 Antibiotics.



Conditions for all examples:

Column: Kinetex 2.6 µm PS C18
Core-Shell 2.6 µm RP-MS C18
Dimensions: 50 x 2.1 mm
Mobile Phase: A: Water with 0.1 % Formic Acid
B: Acetonitrile with 0.1 % Formic Acid
Gradient:

Time (min)	% B
0	3
0.5	3
5	100
5.1	3
8	3

Flow Rate: 0.5 mL/min
Temperature: Ambient
Detector: MS/MS SCIEX™ API 4000™

Sample: **22 Antibiotics** (Retention on Kinetex PS C18)

1. Sulfaguanidine (0.48 min)
2. Lincomycin (1.86 min)
3. Sulfapyridine (2.16 min)
4. Sulfathiazole (2.19 min)
5. Marbofloxacin (2.19 min)
6. Sulfamerazine (2.25 min)
7. Ciprofloxacin (2.28 min)
8. Florfenicol (2.34 min)
9. Danofloxacin (2.34 min)
10. Enrofloxacin (2.36 min)
11. Sulfamonomethoxine (2.42 min)
12. Difloxacin (2.47 min)
13. Valnemulin (2.49)
14. Tylosin A (2.74 min)
15. Sulfamethoxazole (2.9 min)
16. Tiamulin (3.04 min)
17. Sulfaquinoxaline (3.16 min)
18. Trimethoprim (3.33 min)
19. Oxolinic Acid (3.42 min)
20. Flumequine (3.42 min)
21. Sulfadimethoxine (4.0 min)
22. Rifaximin (4.03 min)

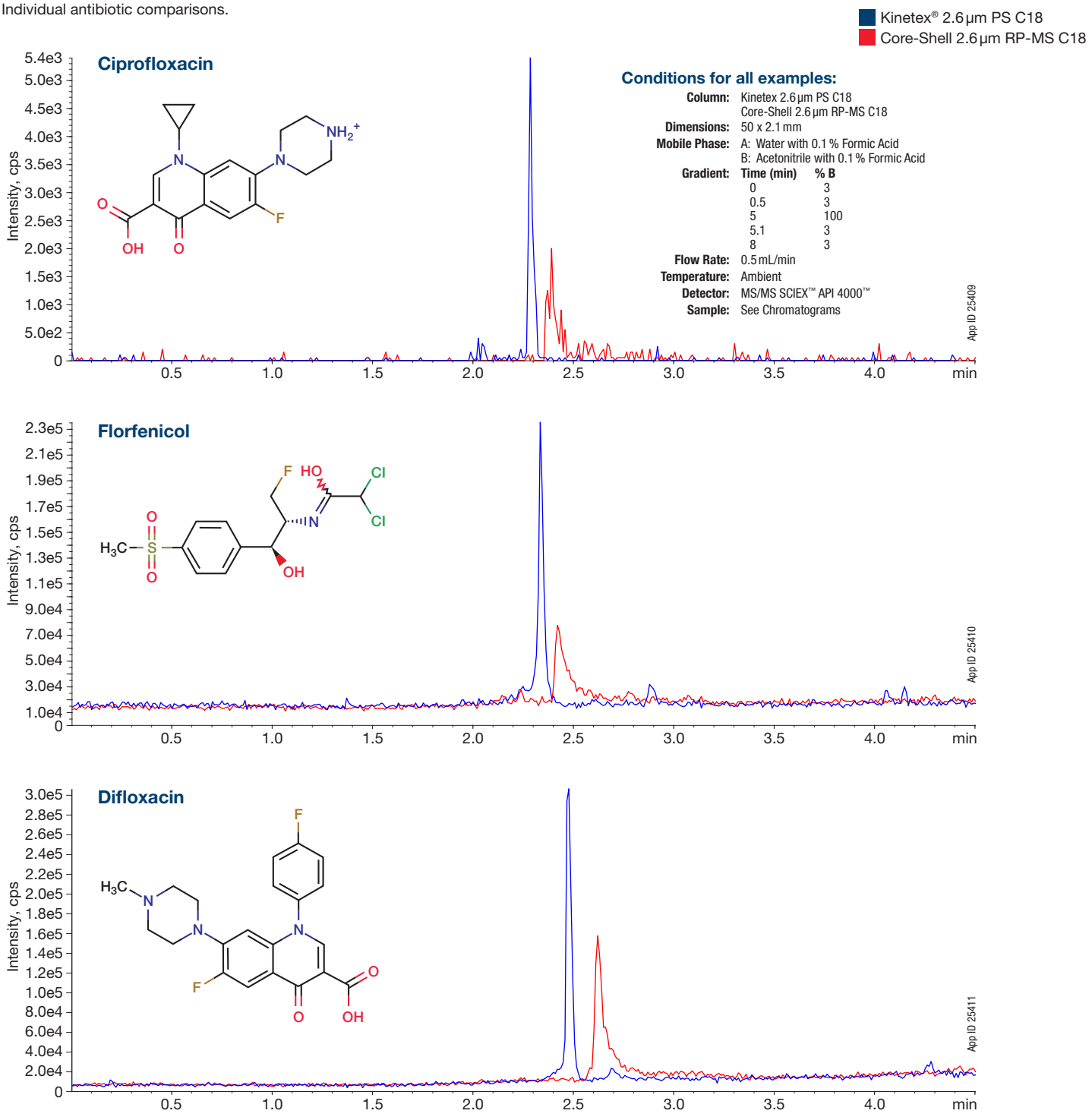
Discussion

In **Figure 1** the Kinetex PS C18 displayed improved selectivity and several peak shape gains for the 22 polar basic antibiotics in comparison to a similar core-shell column with a C18 stationary phase.

The positive surface charge and C18 ligand of the Kinetex PS C18 demonstrates multi-mode interaction mechanisms which drive the apparent chromatographic improvements and separation of the 22 polar antibiotics.

Under the routine and MS compatible mobile phase conditions used in this example, several of the antibiotics were either fully/partially positively ionized chemically, which can contribute to peak shape problems. In these cases, the positive surface charge of the Kinetex PS C18 takes advantage of ionic repulsion to deliver improved polar basic peak shape.

Figure 2.
Individual antibiotic comparisons.



Conclusion

Following the 22 antibiotic mixture, individual compound comparisons were evaluated with the antibiotics ciprofloxacin, florfenicol, and difloxacin to better demonstrate the relative peak shape performance and selectivity of the Kinetex PS C18. As before, the performance of the new stationary phase was compared to a C18 with a similar particle morphology. **Figure 2** depicts chromatographic overlays, offset in order to aid in the visual comparison of the relative and corresponding chromatographic peak shape under identical conditions. In all three examples, the Kinetex PS C18 displayed demonstrates peak shape.

Kinetex® Core-Shell LC Column Ordering Information

2.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
PS C18	00A-4780-AN	00B-4780-AN	00D-4780-AN	00F-4780-AN	AJ0-8951
					for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
PS C18	00B-4780-Y0	00D-4780-Y0	00F-4780-Y0	AJ0-8950
				for 3.0 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
PS C18	00B-4780-E0	00D-4780-E0	00F-4780-E0	00G-4780-E0	AJ0-8949
					for 4.6 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000.

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References

1. Fabrice Gritti, Georges Guiochon. Facts and Legends About Columns Packed with sub-3-µm Core-Shell Particles. *LC-GC North America. 2012, 30(7)*, 586-595.

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