Food Safety Analysis: LC/MS/MS Applications Using New Kinetex® Core-Shell Technology HPLC Columns

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The availability of alternative bonded phases based on the ultra-high efficiency Kinetex core-shell technology provides orthogonal selectivity that is shown to be useful for the separation challenges presented in food safety analysis.

Introduction

The safety of our food supply has come under increasingly intense scrutiny with recent episodes of food products found tainted with melamine, antifreeze, salmonella, and potentially harmful antibiotics, to name a few of the higher profile examples.

The chemistry and structure of these potential food contaminants and the potential for the presence of multiple contaminants presents a significant separation challenge. A single bonded phase, such as C18, is unlikely to offer the selectivity required to chromatographically resolve these potentially complex mixtures. Therefore, the availability of orthogonal bonded phases that provide alternative selectivity through additional modes of interaction is important for the separation of this broad spectrum of analytes. Increased testing mandated by government regulations for an ever expanding list of contaminants in food and beverages has driven the need for increased sample throughput. Additionally, the very complex sample matrices present unique sample preparation challenges.

Over the last several years, smaller fully-porous LC particles (sub-2 µm diameter) have been introduced and sparked much interest because they provide higher efficiency and resolution, which results in significantly shorter analysis times and increased sensitivity. However, the widespread adoption of sub-2 µm HPLC column technology has been slow because these smaller particle size columns generate system backpressures that require the use of specialized ultra-high pressure capable LC instrumentation.

A newly developed, commercialized Kinetex 2.6 µm core-shell chromatographic particle offers the performance benefits of fully-porous sub-2 µm particles (increased chromatographic efficiency and resolution, shorter analysis times, and increased sensitivity) but at substantially lower operating pressures. The benefits provided by the core-shell technology are illustrated in three food safety LC/MS applications (antibiotics in meat, aflatoxins in peanut butter, and melamine and cyanuric acid in baby formula) on the three Kinetex phases currently available.

Kinetex Core-Shell Technology

The Kinetex technology comprises a nearly monodisperse 1.9 µm solid silica core and a 0.35 µm porous silica shell (**Figure 1**). This particle design results in a very stable and nearly 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 2A** shows a FE-SEM of 2.6 µm Kinetex particles under 2,500x magnification highlighting the monodisperse nature of the porous shell particle, and **Figure 2B** shows a FE-SEM of a single 2.6 µm particle under 100,000x magnification highlighting the 100 Å porous surface of the shell.

Figure 1.
Kinetex 2.6 µm Core-Shell Technology

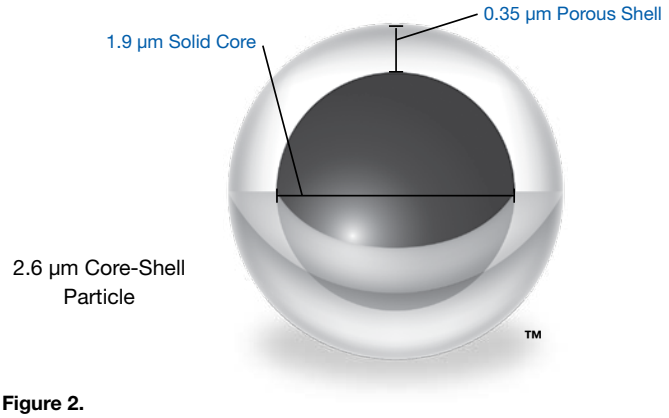
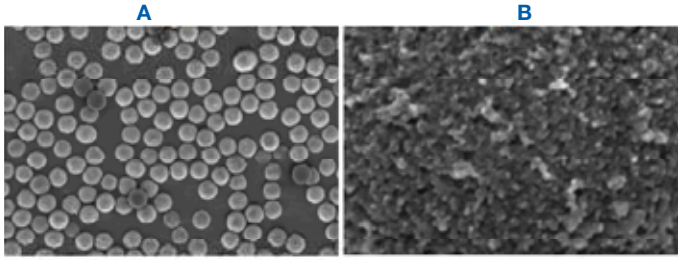


Figure 2.
Kinetex 2.6 µm Core-Shell Technology



A. FE-SEM of 2.6 µm Kinetex particle 2500x magnification showing the monodisperse nature of the porous shell particle.

B. FE-SEM of a single 2.6 µm particle 100,000x magnification showing the 100Å porous surface of the shell.

The core-shell technology columns provide an increase in chromatographic efficiency which allows faster analysis through the use of shorter columns without compromising resolution. This will significantly improve sample throughput for food safety laboratories where government regulations mandate increased sample testing. In addition, the sharper chromatographic peaks obtained with core-shell columns result in increased sensitivity, making it easier to achieve the required lower limits of detection. Kinetex core-shell columns are currently available in three different (orthogonal) bonded phases and each is highlighted here to illustrate the benefits of the core-shell technology for specific food safety applications. Antibiotics in meat were analyzed using Kinetex C18, aflatoxins in peanut butter were analyzed using Kinetex PFP, and melamine and cyanuric acid in baby formula were analyzed using Kinetex HILIC.

APPLICATIONS

The C18 bonded phase is arguably the most widely used column chemistry because of its ability to retain many compounds through hydrophobic interactions and provide the desired chromatographic resolution. Despite the popularity and widespread use of C18 columns, there are instances when an alternative bonded phase can prove extremely beneficial for resolving compounds that may not be easily separated on C18 columns. Phenyl phases provide aromatic (π-π) interactions in addition to hydrophobic interactions; this additional aromatic interaction provides alternative selectivity for the separation of aromatic compounds. In addition to the aforementioned aromatic and hydrophobic interactions, pentafluorophenyl (PFP) phases also introduce the potential for hydrogen-bonding and dipole-dipole interactions due to the presence of the very electronegative fluorine substituents on the phenyl ring. The potential for interactions between analytes and these alternative bonded phases — in addition to hydrophobic interactions provided by C18 phases — results in a different selectivity compared to C18, which can be extremely beneficial for chromatographic resolution of complex samples.

Highly polar and extremely water soluble analytes provide an additional separation challenge as they do not interact very strongly, if at all, with the more hydrophobic C18 and PFP bonded phases, resulting in little or no retention and resolution. HILIC phases allow for very polar analytes to be separated without the use of complex mobile phase additives such as ion-pair reagents, while using conditions (a high percentage of acetonitrile) that are ideally suited for MS detection. The primary interactions between polar analytes and HILIC phases involve dipole-dipole and hydrogen-bonding.

Mode of Interaction	Modes of Interaction	Modes of Interaction
1 Hydrophobic	1 Hydrogen Bonding	1 Hydrophillic
	2 Dipole-Dipole	2 Hydrogen Bonding
	3 Aromatic Pi-Pi	3 Dipole-Dipole
	4 Hydrophobic	4 Ionic

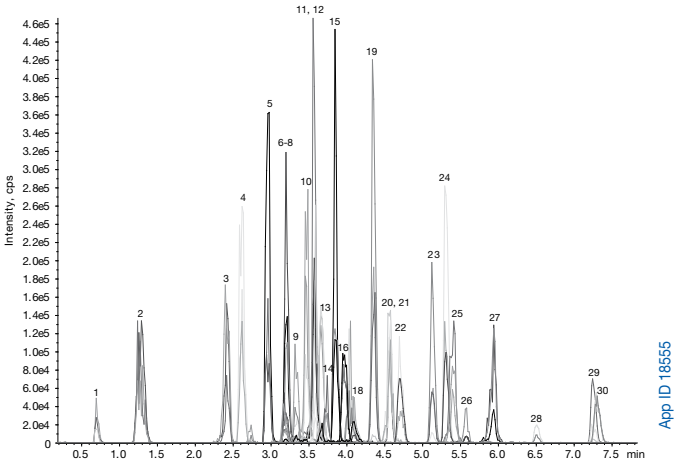
Results and Discussion

Antibiotics in Meat using Kinetex C18

Antibiotics are an indispensable part of food animal production in countries throughout the world and help maintain the optimal health of the animals. However, the residue of antibiotics remaining in animal-derived human foods may pose potential human health hazards. Many countries have implemented a series of regulations governing the use, dosage, and withdrawal times for many of these antibiotics in animal production.

This application illustrates the screening of thirty compounds encompassing seven classes of antibiotics (beta-lactam, tetracycline, sulfonamide, macrolide, amphenicol, fluoroquinolone, and flunixin) by LC/MS/MS using Kinetex C18. The narrow, sharp peaks obtained with the Kinetex column (**Figure 3**) simplify the simultaneous analysis of multiple analytes by providing increased chromatographic resolution and increased sensitivity.[1]

Figure 3.
30 Antibiotics in Meat using Kinetex® C18



For most of the antibiotics present, the desired sensitivity can be achieved by monitoring in positive ion mode; however, negative ion mode is better able to deliver the required sensitivity for several of the antibiotics.

Column: Kinetex 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Methanol

Gradient:

Time (min)	A (%)	B (%)
0	98	2
0.3	98	2
7.27	20	80
7.37	1	99
8.27	1	99
13	98	2

Flow Rate: 0.5 mL/min
Temperature: 40 °C
Detection: Mass Spectrometer (MS)
Injection Volume: 10 µL
Sample: Antibiotics standard mixture (100 ng/mL)

1. Sulfanilamide	16. Florfenicol
2. Amoxicillin	17. Spiramycin
3. Lincomycin	18. Chlorotetracycline
4. Sulfadiazine	19. Sulfadoxine
5. Sulfathiazole	20. Clindamycin
6. Ampicillin	21. Tilmicosin
7. Thiamphenicol	22. Chloramphenicol
8. Sulfamerazine	23. Sulfadimethoxine
9. Tetracycline	24. Sulfaquinoloxaline
10. Ciprofloxacin	25. Erythromycin
11. Enrofloxacin	26. Tylosin
12. Danofloxacin	27. Josamycin
13. Sulfamethazine	28. Penicillin G
14. Sarafloxacin	29. Cloxacillin
15. Sulfamethoxypyridazine	30. Flunixin

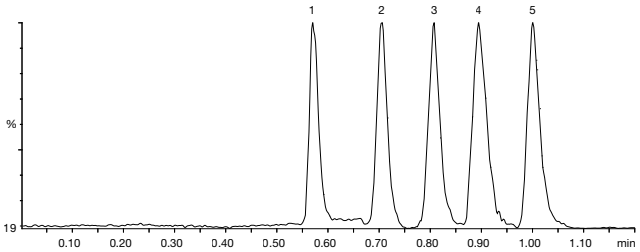
Aflatoxins in Peanut Butter using Kinetex PFP

Aflatoxins are a subclass of mycotoxins which can contaminate a wide spectrum of foodstuffs. Because of their high toxicity and carcinogenicity, aflatoxins are of major concern in the food industry. Maximum permissible limits for aflatoxins have been set in the low µg/kg for food matrices. Using a two-stage SPE cleanup method removed a majority of potential contaminants.[2]

APPLICATIONS

Most of the published separations of aflatoxins use C18 phases; however, Aflatoxin B2 and G1 are usually not fully resolved. Kinetex® PFP is a pentafluorophenyl phase that provides electrophilic interactions due to the delocalized electrons in π orbitals above and below the planar ring. Solutes containing aromatic rings participate in a stacking interaction with the PFP ligand resulting in increased interaction and resolution. The aflatoxins are well retained and baseline resolved on Kinetex PFP.

Figure 4.
Aflatoxins in Peanut Butter using Kinetex PFP



Column: Kinetex 2.6 µm PFP
Dimensions: 50 x 2.1 mm
Part No.: 00B-4477-AN
Mobile Phase: A: 0.1 % Formic acid and 1 mM Ammonium formate in Water
B: 0.1 % Formic acid and 1 mM Ammonium formate in Methanol

Gradient:

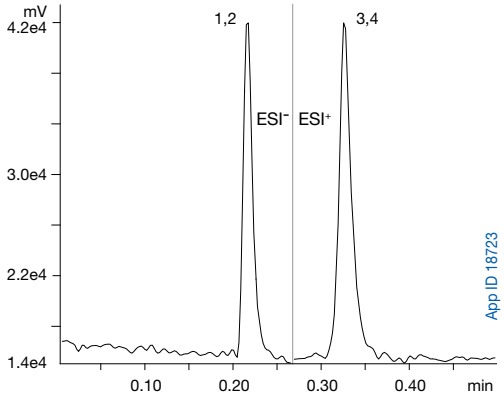
Time (min)	A (%)	B (%)
0.0	50	50
1.1	40	60
1.11	5	95
1.6	5	95
1.61	50	50
2.6	50	50

Flow Rate: 0.6 mL/min
Temperature: 25 °C
Detection: Mass Spectrometer (MS), ESI⁻, MRM
Injection Volume: 20 µL
Sample: 1. Aflatoxin M1, 329.3-273.2 (IS)
2. Aflatoxin G2, 331.3-313.2
3. Aflatoxin G1, 329.2-234.3
4. Aflatoxin B2, 315.3-287.3
5. Aflatoxin B1, 313.2-285.3

Melamine and Cyanuric Acid in Baby Formula using Kinetex HILIC
Over the last few years, increased instances of processed food contaminated with melamine and cyanuric acid has led to increased government regulation and required testing. When present at concentrations exceeding 2 µg/mL, melamine and cyanuric acid crystallize in a 1:1 ratio to form melamine cyanurate, a very poorly water-soluble complex. Consumption of melamine and cyanuric acid contaminated food products can result in adverse health effects, including kidney failure and death. Melamine and cyanuric acid are very polar, water-soluble compounds that are challenging to extract simultaneously out of different food samples. These compounds retain poorly on reversed phase columns, but retain very well under HILIC conditions on Kinetex HILIC.[3]

The MS ionization mode was initially set for negative ionization to facilitate the detection of cyanuric acid, but following elution of the cyanuric acid peak, the source had to be switched to positive ionization mode in order to allow melamine detection.

Figure 5.
Cyanuric Acid and Melamine in Baby Formula using Kinetex HILIC



Column: Kinetex 2.6 µm HILIC
Dimensions: 50 x 2.1 mm
Part No.: 00B-4461-AN
Mobile Phase: Acetonitrile/100 mM Ammonium acetate, pH 5.8 (90:10)
Flow Rate: 1.0 mL/min
Temperature: 25 °C
Detection: Mass Spectrometer (MS)
Switch from Negative Ion Mode (Cyanuric acid) to Positive Ion (for Melamine) at 0.26 min until 1 min; MRM
Sample: 1. Cyanuric acid 128-85.0 (quant ion), 128.0-42.0 (qualifier ion)
2. Cyanuric acid 13C3 (ISTD) 131.1-87.0
3. Melamine 127.1-85 (quant ion), 127.1-68 (qualifier ion)
4. Melamine-13C3.15N3 (ISTD) 133.2-89.1

Conclusions

The availability of several alternative Kinetex bonded phase chemistries allows for the separation and analysis of a wide variety of potential contaminants in food products. For the analysis of antibiotics in meat, the ultra-high efficiency Kinetex C18 column provided narrow, sharp peaks which simplified the simultaneous analysis of thirty antibiotics by providing increased chromatographic resolution and sensitivity. In analyzing aflatoxins in peanut butter, the Kinetex PFP column's selectivity and ultra-high efficiency provided baseline resolution in less than 1.2 minutes following a two-step SPE cleanup protocol. In analyzing melamine and cyanuric acid in baby formula, Kinetex HILIC provides resolution of these very polar analytes in less than 0.4 minutes following the simultaneous sample extraction and cleanup of melamine and cyanuric acid using Strata® Melamine.[3]

References

1. Phenomenex Technical Note TN-1074: Rapid LC/MS/MS Analysis of Antibiotics in Meat for Human Consumption Using Kinetex 2.6 µm Core-Shell LC Column.
2. Phenomenex Technical Note TN-0022: Rapid, High Resolution Analysis of Aflatoxin Extracts from Peanut Butter Using Kinetex Core-Shell Technology and Strata® SPE.
3. Phenomenex Technical Note TN-0021: Simultaneous Extraction of Melamine and Cyanuric Acid from Food Products Using Strata® Melamine SPE and Ultra-fast LC/MS/MS Analysis Using Kinetex HILIC, Rapid LC/MS/MS Analysis on Luna® HILIC, or Rapid GC/MS Analysis on Zebron™ ZB-XLB HT

Acknowledgement

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APPLICATIONS

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Kinetex® Ordering Information

1.7 µm Minibore Columns (mm)

	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)

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

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
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0
PFP	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0
HILIC	—	—	—	—	00F-4461-Y0

2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	100 x 4.6	150 x 4.6
C18	00A-4462-E0	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

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

UHPLC / HPLC Sure-Lok High Pressure PEEK male nut fittings are recommended for installation of Kinetex columns. The convenient one-piece design (AQ0-8503) is pressure rated to 12,000 psi (827 bar). A handy fitting tightening tool (AQ0-8530) is available to facilitate achievement of a leak-free connection.

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

KrudKatcher™ Ultra In-line Filter

The KrudKatcher Ultra filter body houses an integrated 0.5 µm 316 stainless steel depth filter 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 Depth Filter 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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