Rapid LC/MS/MS Analysis of Antibiotics in Meat for Human Consumption Using Kinetex™ 2.6 µm Core-Shell LC Column

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- *Rapid analysis of 7 classes of antibiotics from meat products in less than 8 minutes results in shorter cycle times and improved productivity*
- *Ultra-high efficiency Kinetex core-shell columns provide narrower peaks and increased sensitivity*

Introduction

In modern mass production operations, many animal feeds, which are used in beef, pork, chicken and turkey production, contain antibiotics as prophylactics.[1] The beta-lactam, macrolide, sulfonamide, tetracycline, and other antibiotics are an indispensable part of food animal production and help to maintain the optimal health of the animals. However, the residues of antibiotics remaining in animal-derived human foods may pose potential human health hazards toxicologically, microbiologically or immunopathologically.[1, 2, 3, 4] Many countries have implemented a series of regulations governing the use, dosage, and withdrawal times for many of these antibiotic compound classes in animal production.

While there are several methods to determine antibiotic residues including bioassays, immunoassay, thin layer chromatography, and so on, LC/MS/MS is much more compelling due to its higher specificity and sensitivity, which leads to better quantitation and identification.[1, 2] The method described here is for screening of seven classes of antibiotics: beta-lactam, tetracycline, sulfonamide, macrolide, amphenicol, fluoroquinolone, and flunixin. HPLC method development issues are explored and a newly introduced high efficiency core-shell particle is used as HPLC media to optimize retention and provide higher sensitivity of analysis.

Experimental

Sample Preparation

1 gram of homogenized beef kidney, kidney juice, or serum was extracted using 10 mL of a 2:8 (v/v) mixture of water and acetonitrile and vortexed for 5 minutes. The sample was then centrifuged for 5 minutes and the supernatant decanted into a 50 mL tube containing 500 mg Strata® C18E SPE sorbent. The sample was again briefly vortexed, shaken for 30 seconds and centrifuged for 1 minute. A 5 mL aliquot of the resulting supernatant was transferred to a graduated tube and the contents evaporated down to less than 1 mL. The sample was then brought up to 1 mL total volume with water, and filtered (0.45 µm PVDF) prior to LC/MS/MS analysis.[5]

Chromatographic Conditions

The chromatographic system consisted of an Agilent 1100 series binary pump equipped with on-line solvent degasser, autosampler, and column temperature module (Palo Alto, California); interfaced with an Applied Biosystems 4000 QTRAP® LC/MS/MS system with Turbo V™ ion source. Positive polarity and negative polarity were monitored separately. The system was controlled using Analyst® software version 1.4.1.

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
Inj. Volume: 10 µL
Flow Rate: 0.5 mL/min
Temperature: 40 ºC
Detection: Mass spectrometer (MS)

Gradient Time (min)	Flow Rate (mL/min)	A (%)	B (%)
0.0	0.5	98	2
0.3	0.5	98	2
7.27	0.5	20	80
7.37	0.5	1	99
8.27	0.5	1	99
13	0.5	98	2

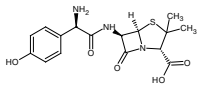
Analytes	Retention Time (min)	Ionization	MRM Transition
1. Sulfanilamide	0.57	Positive	173.1 to 92.1
2. Amoxicillin	2.08	Positive	366.1 to 349.1
3. Lincomycin	3.19	Positive	407.4 to 126.1
4. Sulfadiazine	2.51	Positive	251.1 to 156.0
5. Sulfathiazole	2.90	Positive	256.1 to 156.1
6. Ampicillin	3.56	Negative	348.0 to 207.0
7. Thiamphenicol	2.78	Negative	354.0 to 289.9
8. Sulfamerazine	3.18	Positive	265.1 to 92.2
9. Tetracycline	3.71	Positive	445.2 to 410.1
10. Ciprofloxacin	4.05	Positive	332.2 to 314.2
11. Enrofloxacin	4.13	Positive	360.3 to 342.2
12. Danofloxacin	4.16	Positive	358.2 to 340.2
13. Sulfamethazine	3.67	Positive	279.2 to 92.1
14. Sarafloxacin	4.35	Positive	386.3 to 368.1
15. Sulfamethoxypyridazine	3.79	Positive	281.1 to 155.9
16. Florfenicol	3.66	Negative	356.1 to 185.0
17. Spiramycin	4.90	Positive	422.5 to 174.1
18. Chlorotetracycline	4.52	Positive	479.3 to 444.0
19. Sulfadoxine	4.29	Positive	311.2 to 156.2
20. Clindamycin	5.29	Positive	425.4 to 126.1
21. Tilmicosin	5.44	Positive	435.6 to 695.7
22. Chloramphenicol	4.28	Negative	321.1 to 152.0
23. Sulfadimethoxine	5.03	Positive	311.1 to 156.2
24. Sulfaquinoloxaline	5.18	Positive	301.1 to 156.1
25. Erythromycin	6.10	Positive	734.6 to 158.2
26. Tylosin	6.16	Positive	916.7 to 174.3
27. Josamycin	6.68	Positive	828.7 to 109.1
28. Penicillin G	5.68	Negative	333.0 to 192.4
29. Cloxacillin	6.41	Negative	434.1 to 292.9
30. Flunixin	6.88	Negative	295.1 to 191.0

APPLICATIONS

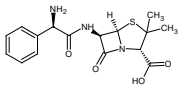
Experimental Conditions

Beta-Lactams

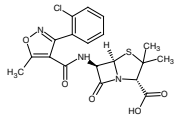
Amoxicillin: $C_{16}H_{19}N_3O_5S$
CAS: 26787-78-0 MW: 365.10



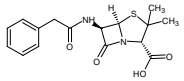
Ampicillin: $C_{16}H_{19}N_3O_4S$
CAS: 69-53-4 MW: 349.11



Cloxacillin: $C_{19}H_{18}ClN_3O_5S$
CAS: 61-72-3 MW: 435.07

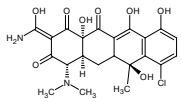


Penicillin G: $C_{16}H_{18}N_2O_4S$
CAS: 61-33-6 MW: 334.10

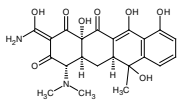


Tetracyclines

Chlorotetracycline: $C_{22}H_{23}ClN_2O_8$
CAS: 57-62-5 MW: 478.11

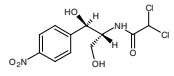


Tetracycline: $C_{22}H_{24}N2O_8$
CAS: 60-54-8 MW: 444.15

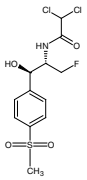


Amphenicols

Chloramphenicol: $C_{11}H_{12}C_{l2}N_2O_5$
CAS: 56-75-7 MW: 322.01



Florfenicol: $C_{12}H_{14}Cl_2FNO_4S$
CAS: 73231-34-2 MW: 357.01



Thiamphenicol: $C_{12}H_{15}C_{l2}NO_5S$
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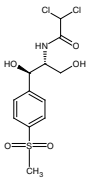
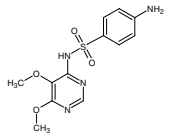
MW: 355.00

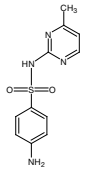


Sulfonamides

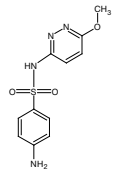
Sulfadoxine: $C_{12}H_{14}N_4O_4S$
CAS: 2447-57-6
MW: 310.07



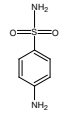
Sulfamerazine: $C_{11}H_{12}N_4O_2S$
CAS: 127-79-7
MW: 264.07



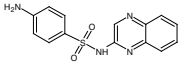
Sulfamethoxypyridazine:
$C11H_{12}N_4O_3S$ CAS: 80-35-3
MW: 280.06



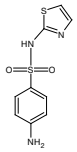
Sulfanilamide: $C_6H_8N_2O_2S$
CAS: 63-74-1 MW: 172.03



Sulfaquinoxaline: $C_{14}H_{12}N_4O_2S$
CAS: 59-40-5 MW: 300.07

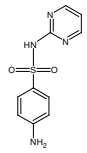


Sulfathiazole: $C_9H_9N_3O_2S_2$
CAS: 72-14-0 MW: 255.01

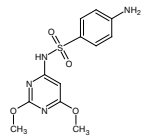


Sulfonamides *(con't)*

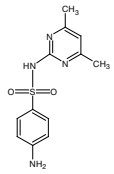
Sulfadiazine: $C_{10}H_{10}N_4O_2S$
CAS: 68-35-9 MW: 250.05



Sulfadimethoxine: $C_{12}H_{14}N_4O_4S$
CAS: 122-11-2 MW: 310.0736

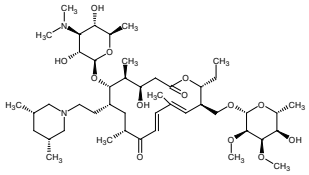


Sulfamethazine: $C_{12}H_{14}N_4O_2S$
CAS: 57-68-1 MW: 278.08

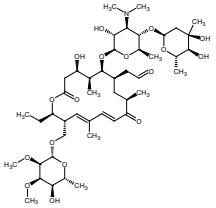


Macrolides

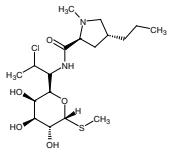
Tilmicosin: $C_{46}H_{80}N_2O_{13}$
CAS: 108050-54-0 MW: 868.57



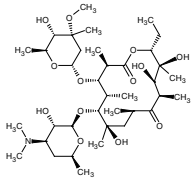
Tylosin: $C_{46}H_{77}NO_{17}$
CAS: 1401-69-0 MW: 915.52



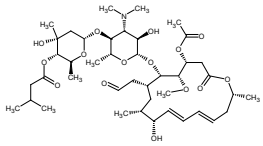
Clindamycin: $C_{18}H_{33}ClN_2O_{5S}$
CAS: 18323-44-9 MW: 424.18



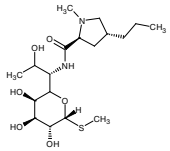
Erythromycin: $C_{37}H_{67}NO_{13}$
CAS: 114-07-8 MW: 733.46



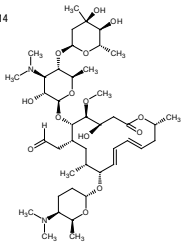
Josamycin: $C_{42}H_{69}NO_{15}$
CAS: 16846-24-5
MW: 827.47



Lincomycin: $C_{18}H_{34}N_2O_6S$
CAS: 154-21-2 MW: 406.21

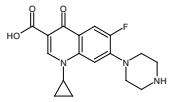


Spiramycin: $C_{43}H_{74}N_2O_{14}$
CAS: 8025-81-8
MW: 842.51

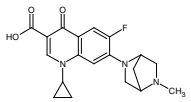


Fluoroquinolones

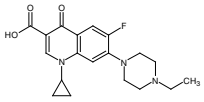
Cipprofloxacin: $C_{17}H_{18F}N_3O_3$
CAS: 852721-33-1 MW: 331.13



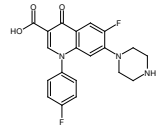
Danofloxacin: $C_{19}H_{20}FN_3O_3$
CAS: 112398-08-0 MW: 357.15



Enrofloxacin: $C_{19}H_{22}FN_3O_3$
CAS: 93106-60-6 MW: 359.16

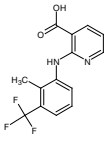


Sarafloxacin: $C_{20}H_{17}F_2N_3O_3$
CAS: 98105-99-8 MW: 385.12

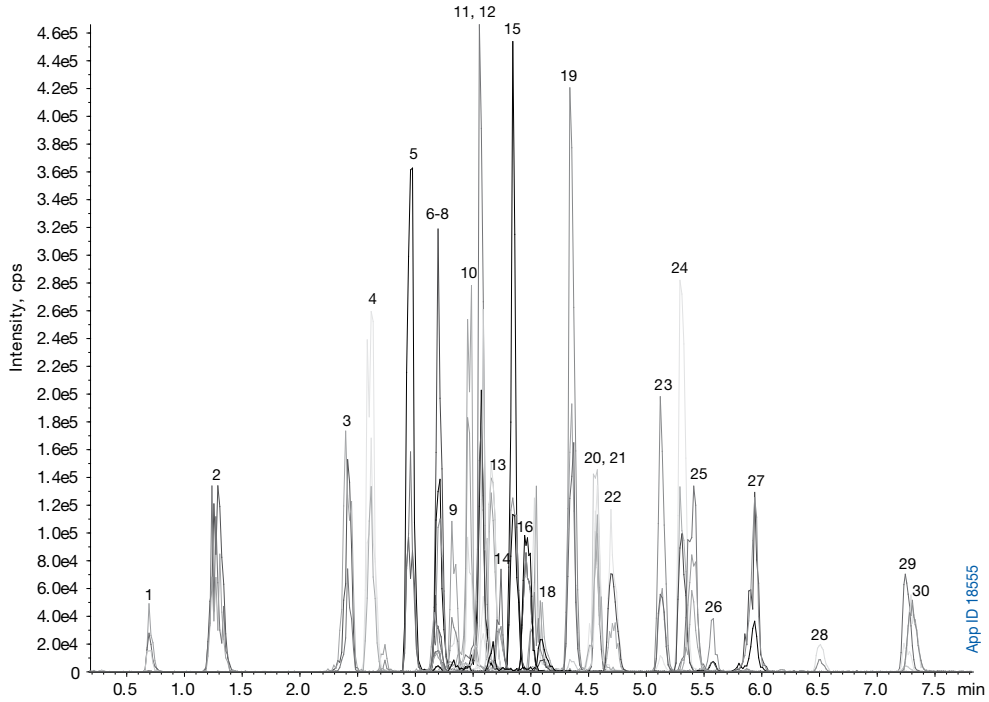


Flunixin

Flunixin: $C_{14}H_{11}F_3N_2O_2$
CAS: 38677-85-9 MW: 296.08



Extracted Ion Chromatogram of Antibiotics Standard Mixture (100 ng/mL)



Results and Discussion

The simultaneous analysis of 30 antibiotics in meat samples presents a significant analytical challenge. The sample matrix is rather complex suggesting that an extensive sample cleanup might be required in order to reduce potential matrix interferences. However, it was determined that minimal sample preparation involving liquid-liquid extraction and centrifugation was adequate to obtain the desired sensitivity. This simplified and minimal sample preparation procedure did not result in any performance issues, with either the LC separation or with MS/MS detection.

Kinetex columns are based on a new 2.6 µm core-shell particle technology. The particle is comprised of a nearly monodisperse 1.9 µm solid (non-porous) silica core surrounded by 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). In addition, the short diffusion path defined by the 0.35 µm porous shell reduces resistance to mass transfer as the analyte molecules diffuse from the mobile phase into and out of the porous shell containing the stationary phase, thereby minimizing peak dispersion (the "C" term in the van Deemter equation). The improved mass transfer kinetics significantly improves chromatographic resolution, providing sub-2 µm performance but at backpressures that are compatible with conventional HPLC instruments.

The narrow, sharp peaks obtained with the Kinetex column simplify the simultaneous analysis of multiple analytes by providing increased chromatographic resolution and also increased sensitivity. 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. The very narrow chromatographic peaks provided with the Kinetex core-shell C18 column on a conventional HPLC system enables the 4000 QTRAP® system to easily switch between positive ion and negative ion mode as needed to optimize detection for each of the thirty antibiotics monitored in the meat samples.

Conclusions

LC/MS/MS has the capabilities for screening multiple classes of antibiotics quickly and accurately from a complex sample matrix. Kinetex core-shell columns deliver ultra-high efficiencies on a conventional HPLC system. For the separation of these complex mixtures, ultra-high efficiencies result in narrower and taller peaks, providing a 2-fold increase in signal intensity versus fully porous 3 µm columns that ensures a corresponding increase in sensitivity. Kinetex 2.6 µm core-shell columns and 4000 QTRAP® LC/MS/MS systems provide a high efficiency, high sensitivity platform for screening complex mixtures of antibiotics. This platform is optimal for use in regulatory screening of antibiotics in animal production.

References

1. Francis, F. J. *Wiley Encyclopedia of Food Science and Technology, 2nd Edition*, Vol. 1. Wiley-Interscience **1999**.
2. Martins-Júnior, H.A. Kussumi, T. A. Wang, A. Y.; Lebre, D. T. A Rapid Method to Determine Antibiotic Residues in Milk using Liquid Chromatography Coupled to Electrospray Tandem Mass Spectrometry. *J. Braz. Chem. Soc.,* **2007** *18* (2), 397-405.
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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

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