A Rapid Screening Method for Analysis of Multi-Class Antibiotics from Ground Meat (sausage) using QuEChERS and LC/MS/MS

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The purpose of this study was to develop a rapid, robust, and sensitive multi-class screening method for the detection of antibiotics in ground meat samples at maximum residue limit levels defined by Commission Regulation (EU) No 37/2010

Introduction

Antibiotics consist of many different classes of compounds such as sulfa drugs, penicillins, tetracyclines, and cephalosporins, etc. These agents are used to treat infectious diseases for well over 70 years in humans. This usage has also been applied to food animals to control the bacterial harmful effect. In addition to this therapeutic use in food animals, antibiotics have been proven to promote growth when administered in small daily doses[1]. The mechanism of this phenomenon is unclear, but the use of antibiotics for growth promotion is on the rise and not well-publicized. According to US FDA, over 13 million kilograms of antibiotics approved for use in food animals were sold in the US and distributed to other countries in 2009. Over time, the daily use of low-dose antibiotics as feed supplements will promote antibiotic resistant bacteria. Furthermore, the subsequent consumption of the meat from these food animals can create the same phenomenon in humans and hamper the treatment of drug-resistant bacteria by conventional antibiotics. This improper use of antibiotics in food animals is an enormous concern to public health and safety. Many countries in the European Union and Canada have already banned sub-therapeutic use of antibiotics in food animals.

In order to regulate this practice, a sensitive and accurate screening method is required to detect antibiotics in meat produced from food animals. In this work, we demonstrate a rapid sample preparation and LC/MS/MS method for multi-class antibiotic screening from pork sausage using roQ™ QuEChERS dSPE (dispersive) cleanup kit and Kinetex® XB-C18 2.6 µm core-shell HPLC column. Limit of detection of 50 ppb was achieved with excellent signal-to-noise ratios, which is the maximum residue limit for a number of antibiotics per Commission Regulation (EU) No 37/2010.

Material and Methods

Reagents and Chemicals

Antibiotic standards were purchased from Sigma-Aldrich Co (St Louis, MO, USA) and Toronto Research Chemicals (Toronto, ON, Canada). All solvents were obtained from Sigma-Aldrich Co (St Louis, MO, USA).

Experimental Conditions

Sample Preparation

Extraction from Ground Meat (Sausage)

2 mL of 1 % formic acid solution was added to 2 g of ground sausage. The sample was mixed well and further homogenized using an Omni TH hand homogenizer. 8 mL of methanol was added to the mixture. The sample was placed on a mechanical shaker for 30 minutes at high setting and then centrifuged at 4000 g for 5 minutes.

dSPE Cleanup

5 mL of supernatant from the extraction step was transferred to a roQ QuEChERS dSPE tube containing 900 mg of $MgSO_4$, 150 mg of PSA and 150 mg of C18E (p/n KS0-8921). The sample was shaken vigorously for 1 minute and centrifuged at 4000 g for 5 minutes.

Reconstitution

2 mL of supernatant from the dSPE step was evaporated over a stream of nitrogen at 60 °C to dryness. The sample was reconstituted in 1 mL of 0.1 % formic acid/acetonitrile-methanol 50:50 with 0.1 % formic acid (95:5) for analysis.

LC/MS/MS Conditions

LC/MS/MS was performed using a Kinetex 2.6 µm XB-C18 100 x 2.1 mm HPLC column (p/n 00D-4496-AN) on an Agilent® 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 400 bar, equipped with a binary pump, autosampler and interfaced with an API 5000™ triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA). The ionization source was electrospray ionization (ESI) analyzed in positive ion modes (**Table 1**). At least two to three MRM transitions were developed for each analyte. The primary MRM was chosen for quantitative purposes and the other additional MRM channels served for confirmatory purpose.

LC/MS/MS Conditions

Column: Kinetex 2.6 µm XB-C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4496-AN
Security guard: AJ0-8768
Mobile Phase: A: 0.1 % Formic acid in water
B: Methanol/Acetonitrile (50:50) with 0.1 % Formic acid
Gradient:

Time (min)	% B
0.00	5
1.00	5
4.00	50
6.00	95
7.50	95
7.51	5
10.0	5

Flow Rate: 0.45 mL/min
Injection Volume: 10 µL
Temperature: 50 °C
Detection: API 5000 (AB SCIEX)

Table 1.
Analyte identification, retention time, and S/N ratio from a meat extract

Analyte Peak Name	Analyte Retention Time (min)	Analyte Signal-To-Noise (S/N) Ratio at 50 ppb
AMOXICILLIN	1.52	236
SULFADIAZINE	2.72	1006
SULFATHIAZOLE	3.07	162
SULFAPYRIDINE	3.15	701
4-EPITETRACYCLINE	3.17	1411
TETRACYCLINE	3.47	1739
TRIMETHOPRIM	3.15	344
MARBOFLOXACIN	3.28	668
SULFAMERAZINE	3.31	649
CEFQUINOME	3.34	179
AMPICILLIN	3.39	699
CEFALONIUM	3.45	311
4-EPIOXYTETRACYCLINE	3.17	831
OXYTETRACYCLINE	3.47	530
CIPROFLOXACIN	3.50	117
CEFAPIRIN	3.55	21
DANOFLOXACIN	3.64	435
ENROFLOXACIN	3.66	405
SULFAMETHAZINE	3.72	1386
DIFLOXACIN	3.83	733
SARAFLOXACIN	3.85	118
NEOSPIRAMYCIN	3.83	1700
SPIRAMYCIN	4.07	299
SULFAMETHOXAZOLE	4.21	66
DOXYCYCLINE	4.36	1807
TILMICOSIN	4.48	161
CEFOPERAZONE	4.72	545
SULFAQUINOXALINE	4.87	558
TIAMULIN	5.10	141
TYLOSIN A	5.17	599
VALNEMULIN	5.50	2949
OXACILLIN	5.91	173
CLOXACILLIN	6.05	635
DICLOXACILLIN	6.12	408
NAFCILLIN	6.16	79

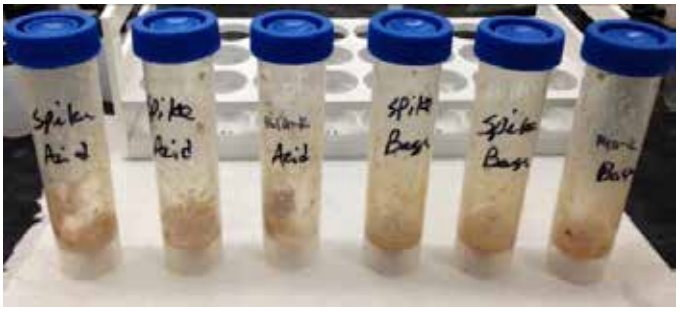
Results and Discussion

In this study, a screening method for multi-class antibiotics from fatty ground meat matrix (sausage) was developed. For the pretreatment of meat, both acidic and basic pretreatment conditions were examined. However, acidic pretreatment produced higher sensitivity for a larger group of analytes. Sample cleanup using roQ™ QuEChERS dSPE kit (p/n KS0-8921) containing PSA/C18E successfully removed interferences from the meat matrix to furnish excellent recoveries and signal-to-noise ratios (**Table 1**). **Figure 1** shows meat samples during various stages of sample preparation. Pretreatment under acidic conditions produced superior extraction results from meat/sausage matrix, which in turn, increased extraction efficiency for the subsequent solvent extraction using methanol (**Figure 1a**). During dSPE cleanup, matrix interferences such as lipids and pigment were eliminated by loose SPE sorbents (**Figure 1b-c**). The resulting extracts were visibly clear and ready for injection after solvent switching (**Figure 1d**).

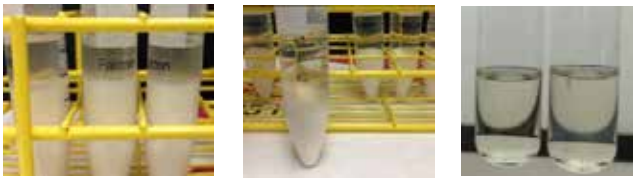
Kinetex® 2.6 µm XB-C18 Core-Shell Technology column provided excellent peak shape and high efficiency. All analytes eluted in less than 7 minutes and run time was only 10 minutes, including column cleaning and re-equilibration. **Figure 2** shows a representative ion chromatogram of an unspiked extract from ground meat (sausage). **Figure 3** and **4** show the extracted ion chromatograms of meat samples with antibiotics spiked at 50 and 800 ppb, respectively. High sensitivity and signal-to-noise ratios were achieved at the low spike concentration of 50 ppb (**Table 1**). Based on this data, good signal-to-noise ratios can be expected at even lower analyte concentrations. Note the presence of two peaks (identified by arrows) in the unspiked meat sample were initially suspected to be endogenous antibiotics. However, they lack both the proper retention time and a secondary confirmatory MRM transitions and hence are considered isobaric impurities.

The current method is extremely versatile for the multi-class screening of antibiotics from meat matrices, notably a high fat content sample. Undoubtedly, similar analyses from other matrices may require slight modifications in the sample preparation step, i.e. choice of acidic or basic digestion method depending on sample, sample homogenization procedure, consideration of using different dSPE sorbents based on matrix interferences presented by sample, etc. For quantitation of specific antibiotics, a more selective method using solid phase extraction (SPE) followed by analysis with the same Kinetex 2.6 µm XB-C18 column can be employed.

Figure 1.
Meat samples (sausage) at various stages of sample preparation



a) Prior to extraction after the initial pretreatment



b) During dSPE cleanup c) After dSPE cleanup d) Prior to dry down

Figure 2.
Representative chromatogram of an unspiked meat extract, note that peaks at 4 and 5 min (identified by arrows) are isobaric impurities and are not representative of any of the tested antibiotics

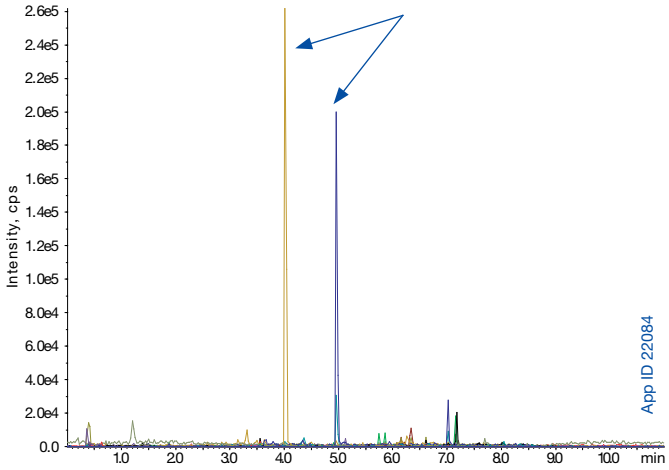


Figure 3.
Representative chromatogram of meat fortified with 50 ppb antibiotic mixture

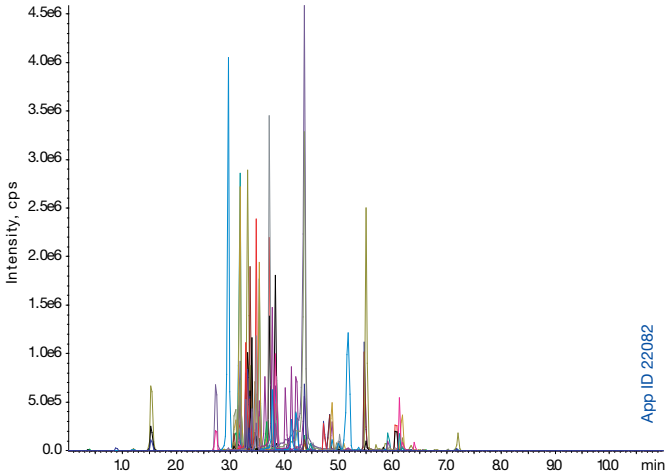
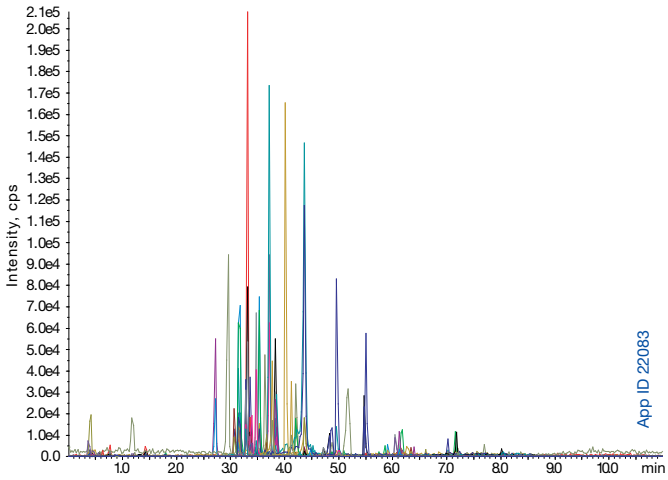


Figure 4.
Representative chromatogram of meat fortified with 800 ppb antibiotic mixture



Conclusion

In this study, we presented a rapid and sensitive multi-class screening method for the detection of multiple classes of antibiotics in ground meat samples at maximum residue limit levels defined by Commission Regulation (EU) No 37/2010. Samples were prepared using a simple, yet effective extraction and cleanup procedure. Extracts were analyzed using a core-shell technology Kinetex® 2.6 µm XB-C18 HPLC column. Excellent signal-to-noise ratios were obtained at low spike concentration of 50 ppb and based on a small volume, 10 µm sample injection. This method was proven to be powerful for the detection of antibiotics in meat produced from food animals.

References

1. FDA's Strategy on Antimicrobial Resistance - Questions and Answers". U.S. FDA, April 11, 2012.

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
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJ0-8772
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774
						for 4.6 mm ID

*SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

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
XB-C18	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
C8	00A-4497-AN	00B-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784
HILIC	00A-4461-AN	00B-4461-AN	00D-4461-AN	00F-4461-AN	AJ0-8786
Phenyl-Hexyl	00A-4495-AN	00B-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788
					for 2.1 mm ID

Ordering Information

roQ™ Extraction Kits

Extraction Kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
EN 15662 Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/PK	KS0-8909
AOAC 2007.01 Method Extraction Kits		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/PK	KS0-8911
Original Non-buffered Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl	50/PK	KS0-8910
6.0 g $MgSO_4$, 1.5 g NaCl	50/PK	KS0-8912

roQ dSPE Kits

dSPE Kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

Description	Unit	Part No.
2 mL dSPE Kits		
150 mg $MgSO_4$, 25 mg PSA, 25 mg C18-E	100/PK	KS0-8913
150 mg $MgSO_4$, 25 mg PSA, 2.5 mg GCB	100/PK	KS0-8914
150 mg, $MgSO_4$, 25 mg PSA, 7.5 mg GCB	100/PK	KS0-8915
150 mg $MgSO_4$, 25 mg PSA	100/PK	KS0-8916
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18-E, 50 mg GCB	100/PK	KS0-8917
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18-E	100/PK	KS0-8918
150 mg $MgSO_4$, 50 mg PSA, 50 mg GCB	100/PK	KS0-8919
150 mg $MgSO_4$, 50 mg PSA	100/PK	KS0-8920
15 mL dSPE Kits		
900 mg $MgSO_4$, 150 mg PSA, 150 mg C18-E	50/PK	KS0-8921
900 mg $MgSO_4$, 150 mg PSA, 15 mg GCB	50/PK	KS0-8922
900 mg $MgSO_4$, 150 mg PSA, 45 mg GCB	50/PK	KS0-8923
900 mg $MgSO_4$, 150 mg PSA	50/PK	KS0-8924
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18-E, 400 mg GCB	50/PK	KS0-8925
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18-E	50/PK	KS0-8926
1200 mg $MgSO_4$, 400 mg PSA, 400 mg GCB	50/PK	KS0-8927
1200 mg $MgSO_4$, 400 mg PSA	50/PK	KS0-8928

Bulk roQ QuEChERS Sorbents

Phases	10 g	100 g
C18-E	–	04G-4348
GCB (Graphitized Carbon Black)	04D-4615	04G-4615
PSA	–	04G-4610

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