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Screening of multi-class antibiotics in pork meat by LC-Orbitrap-MS with modified QuEChERS extraction

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The quantification capability of high resolution mass spectrometry is of great interest to analysts. We described a method for analysis of multi-class antibiotics in pork meat by UPLC-quadrupole (Q)-Orbitrap-MS. The QuEChERS approach with a clean-up step using a sorbent of primary-secondary amine (PSA) and C18 was adopted for sample preparation, and 37 antibiotics including beta-lactams, tetracyclines, sulfonamides, fluoroquinolones and macrolides were analyzed. The Q-Orbitrap method showed high sensitivity with limits of detection (LODs) ranging from 0.8 μg kg^{-1} to 2.9 μg kg^{-1}. The method was further validated by intra and inter-day tests with fortified samples. Recovery (85–105.6%) and precision values (RSDs < 15%) for all analytes were obtained. The result indicates that UPLC-Q-Orbitrap-MS coupled with QuEChERS preparation can serve as a routine method for multi-class antibiotic analysis in pork meat.

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Introduction

In recent years, fast increases in meat production have occurred in developing countries and this will continue, especially for poultry and pork. China alone produces and consumes roughly half the world's pigs.[1] Meanwhile, the use of veterinary antibiotics as feed additives for growth promotion has reached approximately 8000 tons annually in China.[2] Considering the negative effect of antibiotic usage, potential health risks for pork consumption become a public concern. Actually, some studies have shown that some antibiotics such as sulphonamides, tetracyclines and fluoroquinolones are found in waterways and manure from Chinese pig farms[2,3]

The abuse of antibiotics has two major adverse impacts on human, bacterial resistance and toxicological effects resulting from their residues. A recent study based on research in East China finds evidence that exposure to different antibiotics is a possible cause for obesity in children.[4] From the legal perspective, European Community Regulation (EU) no 470/2009 established antibiotic maximum residue limits (MRLs) in foodstuff of animal origin, considering toxicological risks and pharmacological effects of residues.[5] Chinese Ministry of Agriculture also published announcement (no. 235) for MRLs. Furthermore, the Chinese government has recently launched a pilot program that aims to eliminate the use of antibiotics in livestock feed by 2020.

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Besides establishing regulation for antibiotic addition, comprehensive surveillance of targeted antibiotics in pork muscle is necessary. Accordingly, methods for antibiotics determination are required with satisfactory qualitative and quantitative results at trace level in muscle matrix. Recently, a growing number of reports have focused on separation and detection of antibiotics with liquid chromatography tandem mass spectrometry (LC-MS/MS), which have been widely applied in quantitative target analysis.[6–8] Some new kinds of mass spectrometry have also been used for screening and confirmation of drug residues, such as time-of-flight (TOF), Orbitrap, and hybrid mass spectrometer of quadrupole-time-of-flight (Q-TOF) or Q-Orbitrap.[9–15] Comparing to triple-quadrupole MS, these MS with high resolution has more precise criteria for mass accuracy and mass resolution.

In our previous work,[9] Orbitrap MS technology was proven to be selective and sensitive for the qualitative analysis of some β-lactams in chicken muscle. The limits of detection (LOD) of β-lactam (methicillin) can reach 0.01 μg kg^{-1}. However, we usually cannot suspect certain kind of antibiotic residue in routine test. Accordingly, methods for multi-class antibiotics determination are of great interests for the analysts.

This paper aims to develop a multi-residue analysis method using LC-quadrupole-Orbitrap and QuEChERS pre-treatment. Thirty-seven antibiotics including beta-lactams, tetracyclines, sulfonamides, fluoroquinolones and macrolides were determined in pork meat. Modified QuEChERS-based preparation was chosen as a best compromise in terms of analytes recoveries and quantification limits achieved. Stable-isotope-labeled antibiotics were adopted as internal standards to compensate the loss of sample preparation and matrix effect.

Table 1 Retention time and *m/z* ions selected for the confirmation of selected antibiotics[a]

Antibiotics	Analyte	Formula	Theoretical precursor (*m/z*)	Retention (min)	Confirmation fragment (*m/z*)
Beta-lactams	Penicillin G	$C_{16}H_{17}N_2O_4S$	334.0982	2.53	160.0430/176.0710
	Penicillin G-d7	$C_{16}H_{17}D7N_2O_4S$	341.1530	2.54	160.0430/183.1120
	Ampicillin	$C_{16}H_{19}N_3O_4S$	350.1169	3.20	106.0723/160.0428
	Penicillin V	$C_{16}H_{17}O_5N_2S$	350.0931	3.20	106.07/114.00/160.0428
	Amoxicillin	$C_{16}H_{19}N_3O_5S$	366.1118	3.05	114.0429/160.0428
	Oxacillin	$C_{19}H_{19}N_3O_5S$	402.1118	3.39	144.0415/160.0428
	Cloxacillin	$C_{19}H_{18}ClN_3O_5S$	436.0729	4.36	160.0430/178.01/277.04
Tetracyclines	Tetracycline	$C_{22}H_{24}N_2O_8$	445.1605	2.48	410.1242/154.0502
	Tetracycline-d6	$C_{22}H_{18}D6N_2O_8$	451.1982	2.47	416.1541
	Doxycycline	$C_{22}H_{24}N_2O_8$	445.1605	3.01	428.1349/154.0502
	Oxytetracycline	$C_{22}H_{24}N_2O_9$	461.1555	2.54	426.1190/201.0550
	Chlortetracycline	$C_{22}H_{23}ClN_2O_8$	479.1216	3.51	444.0849/462.0954/154.0502
Sulfonamids	Sulfadiazine	$C_{10}H_{10}N_4O_2S$	251.0597	1.87	156.0116/108.0450
	Sulfadoxine	$C_{12}H_{14}N_4O_4S$	311.0809	3.29	156.0116/108.0450
	Sulfadoxine-d3	$C_{12}H_{11}D3N_4O_4S$	314.0997	3.20	159.0295
	Sulfadimidine	$C_{12}H_{14}N_4O_2S$	279.0910	1.70	156.0116/108.0450
	Sulfamerazine	$C_{11}H_{12}N_4O_2S$	265.0754	2.23	156.0116/108.0450
	Sulfamonomethoxine	$C_{11}H_{12}N_4O_3S$	281.0703	2.77	156.0116/126.0666
	Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	254.0594	3.49	156.0116/108.0450
	Sulfamethoxypyridazine	$C_{11}H_{12}N_4O_3S$	281.0703	3.13	156.0116/126.0666
	Sulfapyridine	$C_{11}H_{11}N_3O_2S$	250.0645	2.08	156.0116/108.0450
	Sulfathiazole	$C_9H_9O_2N_3S_2$	256.0209	2.09	156.0116/108.0450
	Sulfadimethoxin	$C_{12}H_{14}N_4O_4S$	311.0809	4.00	156.0116/108.0450
Fluoroquinolones	Enoxacin	$C_{15}H_{17}FN_4O_3$	321.1358	2.4	234.1041/206.0729
	Enrofloxacin	$C_{19}H_{22}FN_3O_3$	360.1718	2.77	316.1825/245.1089
	Enrofloxacin-d5	$C_{19}H_{17}D5FN_3O_3$	365.2032	2.72	365.2321/347.2537
	Fleroxacin	$C_{17}H_{18}F_3N_3O_3$	370.1373	2.60	326.1480/269.0901
	Flumequine	$C_{14}H_{12}FNO_3$	262.0874	4.81	238.0515/244.0766
	Gatifloxacin	$C_{19}H_{22}FN_3O_4$	376.1667	2.97	332.1771/261.1037
	Lomefloxacin	$C_{17}H_{19}F_2N_3O_3$	352.1467	2.79	265.1152/308.1576
	Marbofloxacin	$C_{17}H_{19}FN_4O_4$	363.1463	2.43	319.1653/261.1039
	Norfloxacin	$C_{16}H_{18}FN_3O_3$	320.1405	2.44	276.1511/233.1089
	Ofloxacin	$C_{18}H_{20}FN_3O_4$	362.1511	2.44	261.1039/318.1618
	Oxolinic acid	$C_{13}H_{11}NO_5$	262.0710	3.87	234.0401/244.0602
	Sparfoxacin	$C_{19}H_{22}F_2N_4O_3$	393.1733	3.18	349.1840/292.1260
Macrolides	Tilmicocin	$C_{46}H_{80}N_2O_{13}$	869.5733	3.95	174.1126/696.4690
	Rosamicin	$C_{31}H_{51}NO_9$	582.3637	4.81	158.1178/116.0711
	Roxithromycin	$C_{41}H_{76}N_2O_{15}$	837.5319	5.34	158.1179/679.4365
	Roxithromycin-d7	$C_{41}H_{70}D7N_2O_{15}$	844.5758	5.32	686.5002/158.1179
	Clarithromycini	$C_{38}H_{69}NO_{13}$	748.4842	5.12	158.1182/495.9654
	Eprinomectin	$C_{50}H_{75}NO_{14}$	914.5260	8.97	186.1130/199.1122
	Tylosin	$C_{46}H_{77}NO_{17}$	916.5264	8.90	154.0866/186.1130

[a] The Δ ppm between the exact precursor and theoretical was no more than 2; the two fragment irons were used for quantification.

Materials and methods

Materials and reagents

Penicillin G, ampicillin, penicillin V, amoxicillin, oxacillin, cloxacillin, tetracycline, doxycycline, oxytetracycline, chlortetracycline, sulfadiazine, sulfadoxine, sulfadimidine, sulfamerazine, sulfamonomethoxine, sulfamethoxazole, sulfamethoxypyridazine, sulfapyridine, sulfathiazole, sulfadimethoxin, enoxacin, enrofloxacin, fleroxacin, flumequine, gatifloxacin, lomefloxacin, marbofloxacin, norfloxacin, ofloxacin, oxolinic acid, sparfoxacin, tilmicocin,

Table 2 Comparison for the recovery of spiking isotope-labeled antibiotics using different sorbents in the pretreatment

	Recovery% (spiking 100 μg kg^{-1}, $n = 3$)					
Sorbent for clean-up	Penicillin G-d7	Tetracycline-d6	Sulfadiazine-d4	Enrofloxacine-d5	Roxithromycin-d7	Average recovery (%)
PSA (100 mg)	71.5	66.2	75.2	69.4	65.7	69.6
C18 (100 mg)	55.6	65.2	66.4	70.2	58.5	63.2
PSA/C18 (50 mg : 50 mg)	88.7	85.2	84.2	83.6	84.5	85.2
PSA/C18 (80 mg/20 mg)	74.2	69.5	75.5	72	78.7	74.0
PSA/C18 (20 mg/80 mg)	72.2	67.5	70.5	68.5	69.8	69.7

rosamicin, roxithromycin, clarithromycin, eprinomectin, tylosin were all purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). Roxithromycin-d7 and tetracycline-d6 were purchased from J&K Chemistry (Beijing, China). Penicillin G-d7, Enrofloxacin-d5, and Sulfadoxine-d3 were obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile, methanol, and formic acid and acetic acid were HPLC gradient grade and purchased from Merck (Darmstadt, Germany). Ethylenedinitrilotetra-acetic acid disodium salt (EDTA) from were obtained from Merck. Double-deionized water was obtained with a Milli-Q Gradient water system (Millipore, Bedford, MA).

Stock standard solutions of all analytes were prepared at 100 mg L^{-1} by dissolving the compounds in methanol. These standard solutions were stored at −20 °C in dark glass bottles

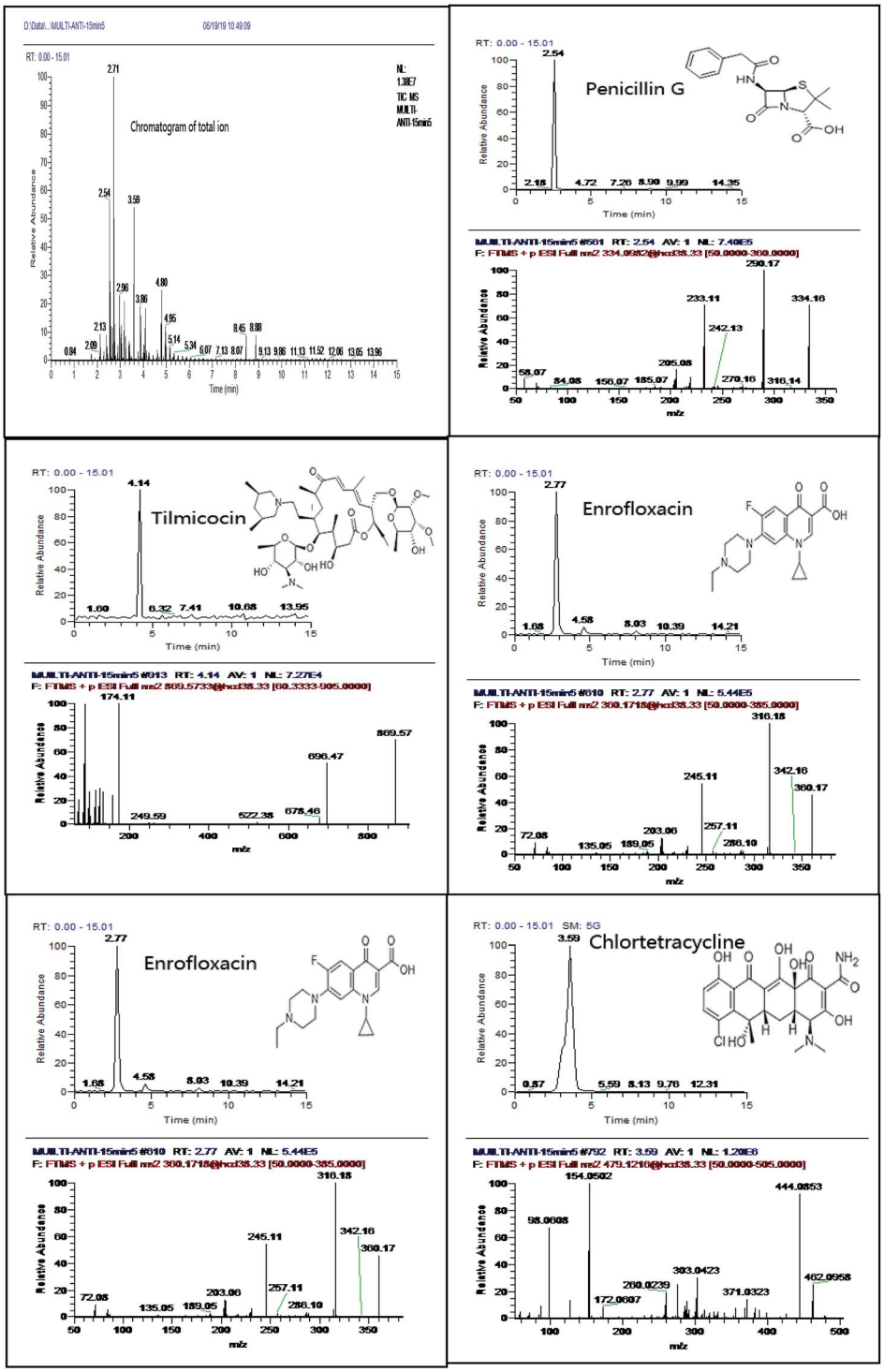

Fig. 1 Chromatogram of total ion with PRM scan mode and five typical extracted ion chromatogram and their spectrum of fragments in spiked sample (10 μg kg^{-1}).

during the three-month validity period and diluted with acetonitrile or methanol to prepare working solutions. The working solutions were kept at −20 °C in dark glass bottles for a month, after which they were replaced with fresh solutions.

Sample preparation

QuEChERS-based method. The sample preparation was based on our previous report[9] with some modifications. In brief, 5 g homogenized tissues spiked with 100 μL of internal standard (200 μg L^{-1}) were added to 15 mL extraction tube containing 4.5 mL acetonitrile, 0.3 mL water and 0.2 mL of Na_2EDTA water solution (200 mmol L^{-1}). The samples were vortexed for 10 min and sonicated for 20 min at 37 °C. Subsequently, they were centrifuged at 8000*g* for 10 min. The supernatant was transferred to a tube containing 4 g $MgSO_4$, and vortexed for 1 min. The upper layer was decanted into 15 mL tube with 50 mg PSA, 50 mg C_{18} and 150 mg of anhydrous $MgSO_4$ and vortexed for 10 min. Finally, samples were centrifuged again and the supernatant was transferred to glass tube and dried under nitrogen flow at 30 °C. The residue was reconstituted into 2 mL of acetonitrile/water (10 : 90, v/v). The final solution was filtered through a 0.22 μm nylon membrane for LC-HRMS analysis.

HPLC conditions. A Vanquish UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) including a quaternary pump, an autosampler and a column oven was coupled by a HESI-II electrospray source to a Q-Exactive Orbitrap™-based mass spectrometer. Chromatographic separation was performed on a HSS T3 C18 column (1.7 μm, 2.1 × 100 mm) (Waters Corporation, MA, USA) at 37 °C. Mobile phase A (water) and B (acetonitrile) both contained 0.1% formic acid. Gradient elution program was: 0–1.0 min 8% B; 1.0–8.0 min 80% B; 8.0–11 min 100% B; 11.0–14 min 100% B; 14.0–14.1 min 10% B; 14.1–15 min 8% B; the flow rate was 0.3 mL min^{-1}. The sample injection volume was 5 μL.

HRMS/MS conditions. Q-Exactive™ high resolution tandem mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with heated electrospray ionization (HESI-II) was used for identification and quantification of target analytes. Mass spectrometer was operated in the PRM scan mode. Full spectral information served for identification and quantification and MS/MS data for confirmation. An inclusion list of all target analytes with their information was implemented into the instrumental method including target analytes names, precursor ions, and retention times (Table 1). The following ionization parameters were applied: electrospray voltage 3.8 kV for positive mode, capillary temperature 340 °C, heater temperature 250 °C, sheath gas (N_2) 40 arbitrary units (arb), auxiliary gas (N_2) 15 (arb), and S-Lens RF level at 50. The instrument was calibrated in positive mode every 7 days using the Pierce LTQ Velos ESI positive-ion calibration solutions (Thermo Scientific, San Jose, CA, USA).

The MS parameters of PRM were: default charge 1, inclusion on, ms^2 resolution 17 500, maximum IT 100 ms, AGC target 2.0 × 10^6, isolation window 2.0 *m*/*z*, and NCE/stepped 25, 35, 55. For the method development and data evaluation, operational software of Xcalibur and TraceFinder was used (Thermo Scientific, San Jose, CA, USA). As an additional criterion for confirmation of the presence of particular analytes in positive samples, spectral library of target analytes MS/MS fragments was created using Thermo Library Manager application (Thermo Scientific, San Jose, CA, USA).

Method validation. Validation of the method was performed with blank pork meat matrix. Target analytes recoveries were determined in six replicates by using spiking samples by a composite mixture of analytical standards. Three spiking levels of 10, 50, and 150 μg kg^{-1} were designed. Five stable-isotope-labeled antibiotics were adopted as the internal standards. The relative standard deviations (RSD) of particular analytes were then calculated in 6 consecutive days for inter-day assay.

Results and discussion

Optimization of the extraction procedure

The selected solvent used for extraction must recover all analytes from the matrix and preserve co-elution of interfering compounds. In terms of the solubility of these antibiotics, the acetonitrile was used for extraction. However, when the concentration of acetonitrile in solvent was too high, it was difficult to extract some highly polar components, such as β-lactams. So, 10% water was added to extraction solvent. Furthermore, Na_2EDTA was used to prevent chelation complexes of multivalent cations with antibiotics, especially tetracyclines and fluoroquinolones.[16,17] The chelation can interfere in the protonation of target compounds for MS

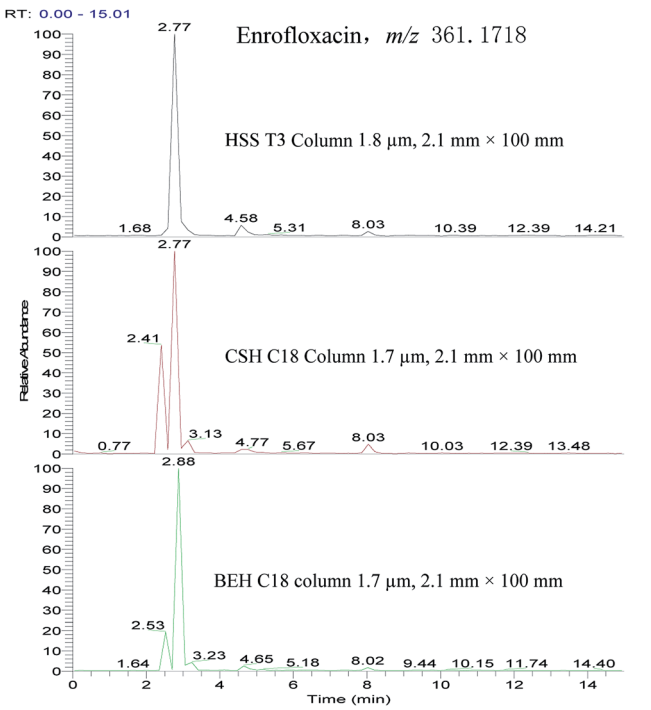

Fig. 2 Extracted ion chromatogram of enrofloxacin performed in different columns in spiked sample (10 μg kg^{-1}).

Table 3 Analytical performance (method trueness and precision) data for antibiotics in pork meat

Analyte	LODs (μg kg^{-1})	LOQs (μg kg^{-1})	Spiking recovery (RSD, %, $n = 6$)			Inter-day (%, $n = 6$)
			10 μg kg^{-1}	50 μg kg^{-1}	150 μg kg^{-1}	
Penicillin G	0.8	2.4	94.5 (3.5)	99.6 (4.1)	100.1 (4.2)	7.2
Ampicillin	1.0	3	98.4 (7.1)	100.1 (5.4)	96.8 (5.6)	9.4
Penicillin V	1.1	3.3	96.3 (4.3)	96.6 (4.9)	97.5 (5.1)	6.9
Amoxicillin	1.5	4.5	97.2 (3.9)	97.6 (4.1)	96.5 (3.4)	5.2
Oxacillin	0.9	2.7	89.5 (8.9)	96.2 (9.5)	95.1 (8.5)	12.7
Cloxacillin	1.2	3.6	96.6 (4.5)	97.3 (4.3)	95.2 (4.1)	6.9
Tetracycline	1.6	4.8	94.8 (2.9)	99.2 (2.5)	95.8 (2.0)	2.2
Doxycycline	1.5	4.5	96.7 (3.9)	105.5 (3.1)	97.2 (1.5)	7.2
Oxytetracycline	1.8	5.4	97.6 (4.8)	99.6 (4.2)	96.9 (7.1)	9.5
Chlortetracycline	1.7	5.1	93.2 (6.2)	98.5 (6.1)	95.8 (3.9)	5.8
Sulfadiazine	0.8	2.4	88.7 (3.4)	97.9 (3.0)	97.1 (2.9)	5.1
Sulfadoxine	1.1	3.3	92.6 (4.5)	96.5 (4.1)	94.8 (7.1)	4.1
Sulfadimidine	0.8	2.4	93.6 (1.9)	98.6 (1.6)	96.2 (2.4)	3.6
Sulfamerazine	0.9	2.7	92.4 (6.4)	95.9 (6.1)	95.6 (3.5)	6.2
Sulfamonomethoxine	1.3	3.9	96.1 (5.5)	94.9 (4.3)	92.4 (2.4)	7.1
Sulfamethoxazole	1.4	4.2	87.6 (8.8)	96.3 (4.7)	95.5 (4.6)	11.2
Sulfamethoxypyridazine	1.5	4.5	94.8 (5.7)	95.9 (2.5)	96.8 (3.4)	5.9
Sulfapyridine	0.9	2.7	96.2 (4.1)	98.8 (3.5)	94.8 (2.6)	3.6
Sulfathiazole	1.0	3.0	94.3 (8.6)	99.5 (6.7)	100.5 (6.1)	7.8
Sulfadimethoxin	1.2	3.6	96.8 (2.5)	97.9 (2.8)	95.2 (3.2)	8.2
Enoxacin	1.8	5.4	99.3 (1.7)	102.5 (1.9)	99.8 (2.1)	3.2
Enrofloxacin	1.6	4.8	93.5 (5.4)	97.8 (3.7)	95.7 (4.1)	6.9
Fleroxacin	2.1	6.3	90.5 (4.7)	96.9 (4.6)	95.8 (4.9)	8.5
Flumequine	2.4	7.2	97.7 (4.6)	99.9 (5.1)	96.4 (4.6)	9.7
Gatifloxacin	2.6	7.8	97.5 (3.2)	98.5 (3.4)	96.2 (3.9)	9.1
Lomefloxacin	2.2	6.6	96.6 (2.9)	99.2 (3.2)	105.6 (4.1)	4.5
Marbofloxacin	2.8	8.4	94.6 (7.6)	96.8 (4.4)	96.4 (5.2)	11.3
Norfloxacin	2.0	6.0	98.2 (4.5)	97.4 (4.1)	99.8 (3.7)	10.5
Ofloxacin	2.3	6.9	96.1 (2.2)	99.1 (2.6)	94.9 (2.0)	6.3
Oxolinic acid	1.5	4.5	95.3 (6.1)	102.5 (5.4)	96.8 (7.1)	5.2
Sparfoxacin	1.6	4.8	86.8 (5.8)	101.3 (3.6)	96.7 (3.6)	8.4
Tilmicocin	2.9	8.7	93.1 (4.3)	99.4 (4.1)	95.9 (3.4)	7.5
Rosamicin	2.5	7.5	94.2 (4.6)	96.8 (5.2)	96.8 (5.9)	10.2
Roxithromycin	2.4	7.2	91.5 (5.7)	95.5 (6.6)	96.1 (8.2)	9.2
Clarithromycin	3.5	10.5	85 (7.5)	94.1 (4.1)	97.7 (2.9)	11.5
Eprinomectin	2.6	7.8	91.6 (9.6)	95.8 (3.8)	98.5 (5.4)	10.7
Tylosin	2.4	7.2	93.2 (6.7)	96.2 (4.7)	96.5 (3.2)	9.7

analysis. Considering tetracyclines, macrolides and β-lactams are stable in neutral or low alkaline solution, while quinolones and sulfonamides are less affected by pH, we do not change the pH value of extraction solvent.

In the clean-up step of QuEChERS preparation, various sorbents are used for co-extractives removal depending on the different sample type. Previous reports evaluated more than 50 sorbents in the terms of their selectivity and applicability.[18–20] Among these kind of sorbents the most commonly used in the QuEChERS methods is PSA with main function to remove co-extracted constituents such as NH_2–organic acids, fatty acids, sugars and ionic-lipids. Moreover, octadecyl silica (C_{18}) provides good results in the purification of samples with significant fat. Accordingly, we selected both PSA and C_{18} as sorbents, and optimized the ratio using five isotope-labeled standards. The results were obtained by the external standard calibration. It showed that supplement with ratio of 1 : 1 had the high recovery (Table 2).

Optimization of LC-Orbitrap-MS conditions

In the chromatographic separation, formic acid was added into mobile phase to protonate antibiotics between mobile phase composition and MS response for selected xx antibiotics. Regarding the organic solvent, acetonitrile showed better sensitivity (S/N) and peak shape than methanol. We adopt water and acetonitrile containing 0.1% formic acid as the mobile phase. The separation was performed over a run time of 30 min with gradient elution (Fig. 1). The initial mobile phase with high water phase content was used to elute hydrophilic compounds causing matrix interference and, therefore, avoid co-elution with targeted analytes. Later, a high percentage of organic reagents (95–100%) at the end of the gradient and relatively long washing intervals avoided carry-over phenomena.[21] In addition, we investigated the performance of different columns (BEH C18 column 1.7 μm, 2.1 mm × 100 mm; CSH C18 column 1.7 μm, 2.1 mm × 100 mm; HSS T3 column 1.8 μm, 2.1 mm × 100 mm) for the separation of selected antibiotics.

HSS T3 column provided satisfactory separation and peak shapes for selected antibiotics. For example, enrofloxacin can be fully separated in HSS T3 column (Fig. 2).

Using hybrid quadrupole-Orbitrap mass spectrometry, qualification and quantification of complicated compounds can be performed in one analysis. For confirmation of targeted analytes, four identification points must be obtained and, therefore, at least two ions must be included in the high-resolution mass spectrometric method. In present study, we adopted parallel reaction monitoring (PRM) scan mode for selected antibiotics. PRM, basically similar with MRM or SRM in triple quadrupole MS is novel scan strategy that can be utilized on high-resolution MS platforms.[22] In this scan mode, targeted precursor ion is isolated in Q1, and then all generated MS/MS fragment ions are recorded in parallel with characteristics of full scan, accurate mass and high-resolution.[23] One of the well-known drawbacks of the LC-Orbitrap methodology is co-elution matrix signals may suppress analyses at very low concentrations. This problem was resolved successfully in our method by using PRM scan mode, which only monitored targeted precursor ion (Fig. 1).

In Q-Exactive Orbitrap, the resolving power of is divided to four different levels as medium (17 500), enhanced (35 000), high (70 000) and ultra-high (140 000), but increased resolution decreased the scanning speed. Consequently, the choice of this parameter was balanced against the quality of peak shapes where insufficient numbers of scans are plotted, resulting in reduced quantitative capacity.[24] For the fragmentation purposes, the relative high dynamic range C-trap setting (1×10^6) and an injection time of 150 ms were selected to combine high detection sensitivity with an extended linear range for quantification. These parameters controlled the capacity of the ion trap to regulate the ion population within it. Sensitivity can be improved by increasing either the C-trap dynamic range value or injection time. Three-step NCE (values adjusted on 25, 35, and 45 eV) was applied in MS^2 acquisition mode, which meant the center energy was 35 eV (plus 10 above and below). Most of fragments of selected antibiotics can be obtained with three-step NCE. All fragments created in these steps were collected sequentially in the HCD and sent to the Orbitrap analyser.

Method validation

The validation was carried out with the purpose to ensure the adequate identification and quantification of the analytes. Matrix effect was evaluated by the response comparison of analytes in initial mobile phase and matrix extraction. The ratios of mass response for analytes in matrix extraction to those in mobile phase were all less than 90%. Hence, stable-isotope labeled-antibiotics were used as internal standards for compensation of the matrix effect. Additionally, the mass spectrometry only scans the targeted precursors in PRM scan mode, which reduces related interfering ions, and no interfering peaks appeared in our results. Sensitivity was evaluated by limits of detection (LODs) and limits of quantification (LOQs). LOD were then calculated based on a minimal accepted value of the signal to-noise ratio (S/N) of 3, and LOQ for S/N = 10. The LODs of all antibiotic were ranged from 0.8 μg kg^{-1} to 2.9 μg kg^{-1}. The calibration curves showed good linearity with regression coefficients (r^2) of each analytes greater than 0.99 in the range of 2–250 μg L^{-1}. As shown in Table 3, average recoveries of analytes at three spiking levels ranged from 85% to 105.6%. There was no significant deviation in intra and inter-day test, where relative standard deviations (RSDs) were all less than 15%. It is obvious that the high selectivity and sensitivity of Q-Orbitrap mass detection provides an excellent method for complex sample analysis.

Application to real samples

To our knowledge, there are few reports about multi-class antibiotic residues in pork from China. A report from

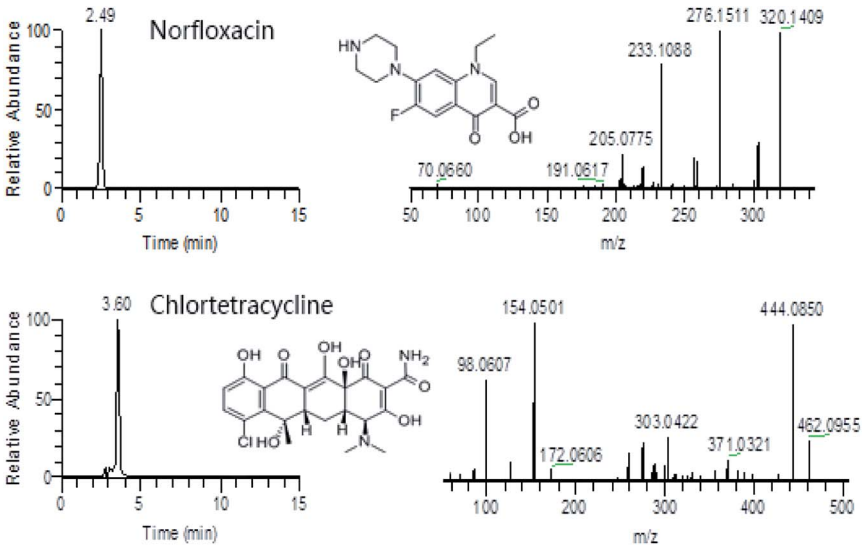

Fig. 3 Extracted ion chromatogram and spectrum of fragments of norfloxacin (45.6 μg kg^{-1}) and chlortetracycline (12.56 μg kg^{-1}) from two real pork samples.

Shanghai, China investigated antibiotic residues in meat, milk and aquatic products by LC-Q-TOF-MS. It revealed that some samples contained 27.0 μg kg^{-1} norfloxacin, 3.4 μg kg^{-1} roxithromycin, 4.0 μg kg^{-1} chlortetracycline in pork.[25] In this study, we applied the established LC-Q-Orbitrap-MS method to analysis 15 pork samples collected from the Chinese market in Hangzhou. Two samples were detected with 45.6 μg kg^{-1} norfloxacin and 12.56 μg kg^{-1} chlortetracycline, separately (Fig. 3). According to the guideline of Chinese Ministry of Agriculture, norfloxacin is not allowed to use in animals, and the maximum residue limit of chlortetracycline is 100 μg kg^{-1}.

Conclusion

Multi-residue determination of antibiotics in pork meat has been successfully established based on LC-Q-Orbitrap-MS with QuEChERS pretreatment. The compromise in analytical conditions obtained satisfied recoveries, sensitivities and linear dynamic ranges for the vast majority of the antibiotics. All fragment ions resulting from the precursor ions recorded in the mode of PRM workflow served as an indispensable tool for analysis in line with the official requirements.[26]

Author contributions statement

X.-D. P. and Q. C. conceived the experiment(s), X.-D. P. and Q. C. conducted the experiment(s), X.-D. P., B. Z., J.-L. H. and B.-F. H. analyzed the results. All authors reviewed the manuscript.

Conflicts of interest

The authors declare no competing financial interests.

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