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A rapid multiclass method for antibiotic residues in goat dairy products by UPLC-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry

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Abstract

Background: Sulfanilamides, quinolones, nitroimidazoles, tetracyclines, cephalosporins, macrolides, and β-lactam are common tools in agriculture and can be found in animal-based foods such as goat milk and goat dried milk. To evaluate the risk of these species, reliable analytical methods are needed for accurate concentration determination, especially in goat milk and goat dried milk.

Method: We describe a method based on PRiME extraction coupled with UPLC-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry to accomplish this task.

Result: Under optimal conditions, the limit of quantification for all antibiotics was 0.5–100 μg/L in goat milk and goat dried milk samples. The recoveries were 60.6–110.0% for goat milk and 60.1–109.6% for goat dried milk with a coefficient of variation less than 15%. The detection limits were 0.5–1.0 μg/kg. The limits of quantification for the analytes were 5.0–10.0 μg/kg. Finally, the method was used to screen veterinary antibiotics in 50 local goat milk and goat dried milk samples; metronidazole and enrofloxacin were detected in goat milk.

Conclusion: This method offers good reliability and the capacity for simultaneous detection can be used to detect residual contents and evaluate health risks in goat milk and goat dried milk.

Introduction

Veterinary antibiotics are widely used to prevent infections, increase reproduction, and improve animal husbandry (Han et al. 2015; Javorska et al. 2017; Li et al. 2016; Tran et al. 2016; Reinholds et al. 2016; Serra-Compte et al. 2017; Cámara et al. 2013). However, these drugs are often used in discriminately in cattle and goat feeding (Zorraquino et al. 2011), which can lead to adverse human health effects, especially for infants and children who consume large amounts of dairy products (Li et al. 2019; Li et al. 2017).

To ensure the safety of human food, several countries have established stringent food safety regulations for these antibiotics in animal-based foods such as eggs, milk, kidney, liver, fat, and muscle (Han et al. 2012). For example, the maximum residue limits (MRLs) of benzylpenicillin, chlortetracycline, and danofloxacinin bovine milk are 4 μg/kg, 100 μg/kg, and 30 μg/kg, respectively, via the European Commission (Directives2006/141/ECand2003/89/EC). China's MRL are published (GB 31650-2019 National food safety standard-Maximum residue limits for veterinary drugs in foods 2020) and set the MRLs for benzylpenicillin, ampicillin, and moxicillin at 4 μg/kg. Other MRLs in bovine milk include 25 μg/kg sulfadimidine; 30 μg/kg oxacillin, danofloxacin, and cloxacillin; 40 μg/kg erythromycin; 50 μg/kg flumequine, trimethoprim, tilmicosin, and sulfonamides (parent drug);

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100 μg/kg sulfonamides (expect sulfadimidine), tylosin, enrofloxacin, and ceftiofur; 150 μg/kg lincomycinas; and 200 μg/kg spiramycin. These MRLs are quite low; thus, a sensitive and selective analytical method is needed.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a common tool in the analysis of veterinary residues. Most studies of trace antibiotic levels are based on triple quadrupole (TQ) mass spectrometry (Heller et al. 2006; Zorraquino et al. 2011; De Almeida et al. 2015; Liu et al. 2016; Li et al. 2017; Zhang et al. 2016; Forgacsova et al. 2019; Oyedeji et al. 2019; Kazakova et al. 2018; Socas-Rodríguez et al. 2017; Li et al. 2018). Multiple reaction monitoring (MRM) and selected reaction monitoring (SRM) are usually the standard quantification method. However, matrix effects and ion interference in TQ-MS remain due to complicated food composites. For better confirmation at ion and higher throughout in analysis of multi-residue veterinaries, liquid-chromatography-high resolution mass spectrometry (LC-HRMS) has become increasingly popular, specifically time-of-flight mass spectrometry (Li et al. 2016; Li et al. 2016; Berendsen et al. 2017; Zhang et al. 2015; Liu et al. 2019; Fu et al. 2018; Emhofer et al. 2019; Weng et al. 2020; Moreno-González et al. 2017; Pan et al. 2016; Saito-Shida et al. 2018) and quadrupole/electrostatic field orbitrap mass spectrometry (Jia et al. 2014b; Hu et al. 2019; Zhao et al. 2017; Jia et al. 2014a; Casado et al. 2018; Paepe et al. 2019; Jia et al. 2017; Casado et al. 2018; Jia et al. 2018a, 2018b; Jia et al. 2018a, 2018b; Casado et al. 2019; Rusko et al. 2019; Abdallah et al. 2019; López-García et al. 2017; Paepe et al. 2018; Kim et al. 2018; Jia et al. 2017).

Recently, new pre-treatment methods have been proposed for extraction and clean-up of each class of veterinary antibiotic residues in food samples. These include liquid-liquid extraction (LLE) for macrolides extraction from milk samples (Şanli et al. 2011) as well as a modified QuEChERS and solid-phase extraction (SPE) or dispersive SPE for clean-up of complex food samples (Junza et al. 2011; Jia et al. 2014a; Kaufmann and Widmer 2013; Dubreil-Chéneau et al. 2014; Heller et al. 2006; Chen et al. 2017). More recently, a novel phospholipids-removing SPE column–PriME HLB was developed based on the specific adsorption for phospholipids carrying fatty acid chains. In contrast to traditional SPE methods, this procedure removes interferences, fats, and phospholipids while simultaneously extracting multiple veterinary residues from milk and dried milk in one loading step; the method is convenient, fast, affordable, and green.

The objective of this study is to establish an effective method to simultaneously determine 60 selected veterinary antibiotic residues, including 17 sulfanilamides, 16 quinolones, 7 nitroimidazoles, 3 tetracyclines, 2 cephalosporins, 8 macrolides, and 7 β-lactams, in milk and dried milk samples by UPLC-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry. The resulting method was then successfully used to screen veterinary antibiotic residues in local goat milk and goat dried milk samples.

Experimental

Chemicals and reagents

We obtained the following from Dr. Ehrenstorfer GmbH (Augsburg, Germany): sulfamerazine (SMZ), sulfathiazole, trimethoprim (TMP), sulfamethizole, sulfisoxazole (SIZ), sulfadiazine (SD), sulfachlorpyridazine, sulfamethoxydiazine, sulfadimethoxypyrimidine, sulfaquinoxaline, sulfadimoxine (SDM), sulfamethoxypyridazine, sulfamethazine, sulfapyridine (SPD), sulfamethoxazole (SMX), sulfaguanidine, sulfaphenazole, lomefloxacin (LOM), ciprofloxacin (CIP), enrofloxacin (ENR), ofloxacin (OFX), norfloxacin (NOR), orbifloxacin (ORB), danofloxacin (DAN), sparfloxacin (SPA), sarafloxacin (SAR), marbofloxacin (MAR), enoxacin (ENO), flumequine (FLU), fleroxacin (FLE), difloxacin (DIF), pefloxacin (PEF), nalidixic acid, erythromycin, lincomycin (LIN), spiramycin, roxithromycin, tilmicosin (TIL), tylosin (TYL), clindamycin, kitasamycin, dimetridazole, hydroxymetronidazole, ipronidazole-OH, ipronidazole, ornidazole, metronidazole, 2-methyl-5-nitroimidazole, chlortetracycline (CLT), doxycycline (DOX), demeclocycline (DEM), ceftiofur (TIL), cefapirin, oxacillin (OXAC), dicloxacillin (DICL), cloxacillin (CLOX), nafcillin (NAFC), ampicillin (AMPI), penicillin G (PEG), and penicillin V (PEV).

Acetonitrile and methanol were HPLC gradient grade and purchased from Merck (Darmstadt, Germany). Formic acid and acetic acid were purchased from Anpu (Shanghai, China), and doubly deionized water was obtained from a Milli-Q gradient water system (Milipore, Bedford, MA).

Stock solutions of individual compounds were prepared in methanol (1000 mg L^{-1}) and stored at − 20 °C in dark glass bottles during the three-month validity period. The working mixed standard solution was then diluted with 0.1% formic acid solution and kept at − 20 °C in dark glass bottles for one month. PRiME HLB solid phase extraction cartridges (60 mg, 3CC) were obtained from Waters (Milford, USA).

Sample preparation

Goat milk sample

The target analytes were extracted from 1 g of milk sample with 4 mL of 0.2% formic acid/acetonitrile solution and vortexed for 30 s. The mixture was then shaken for 30 min and centrifuged at 10,000 r/min for 10 min at 4 °C. The total supernatant fraction was directly loaded on a PRiME HLB. All elutes were collected in a centrifugal tube and evaporated under nitrogen gas at 40 °C. The

Table 1 UPLC-quadrupole/electrostatic field orbitrap parameters of the 60 veterinary antibiotic residues

No.	Compound	RT (min)	Elemental composition	Ionization mode	Theoretical precursor (*m/z*)	Measured precursor (*m/z*)	Accuracy(△ppm)	Production1 (m/z)	Production2 (m/z)	NCD
1	Sulfaguanidine	1.13	$C_7H_{10}N_4O_2S$	$[M+H]^+$	215.05972	215.05916	− 2.60	158.02707	149.02336	20
2	Metronidazole-OH	1.47	$C_6H_9N_3O_4$	$[M+H]^+$	188.06658	188.06615	− 2.29	123.05563	126.03013	35
3	2-Methyl-5-nitroimidazole	1.50	$C_4H_5N_3O_2$	$[M+H]^+$	128.04545	128.04562	1.33	98.04795	111.04313	90
4	Metronidazole	1.83	$C_6H_9N_3O_3$	$[M+H]^+$	172.07167	172.07126	− 2.38	128.04578	–	20,40, 60
5	Cefapirin	2.02	$C_{17}H_{17}N_3O_6S_2$	$[M+H]^+$	424.06315	424.06180	− 3.18	292.0575	320.05334	16
6	Sulfadiazine	2.34	$C_{10}H_{10}N_4O_2S$	$[M+H]^+$	251.05972	251.05910	− 2.47	156.01151	98.98463	30
7	Dimetridazole	2.50	$C_5H_7N_3O_2$	[M+H]+	142.0611	142.06088	− 1.55	95.06079	56.05011	70
8	Sulfathiazole	2.77	$C_9H_9N_3O_2S_2$	[M+H]+	256.02089	256.02014	− 2.93	156.01149	108.04473	20,40, 60
9	Sulfapyridine	2.81	$C_{11}H_{11}N_3O_2S$	[M+H]+	250.06447	250.06380	− 2.68	156.01157	184.08719	35
10	Lincomycin	2.86	$C_{18}H_{34}N_2O_6S$	[M+H]+	407.22103	407.21994	− 2.68	126.12802	–	20,40, 60
11	Sulfamerazine	2.96	$C_{11}H_{12}N_4O_2S$	$[M+H]^+$	265.07537	265.07462	− 2.83	156.01152	190.02831	35
12	Ampicillin	2.97	C16H19N3O4S	[M+H]+	350.1169	350.11603	− 2.48	106.0655	192.04791	20
13	Penicillin G	2.98	$C_{16}H_{18}N_2O_4S$	$[M+H]^+$	335.106	335.10513	− 2.60	128.05305	91.05464	50
14	Trimethoprim	3.01	$C_{14}H_{18}N_4O_3$	$[M+H]^+$	291.14517	291.14441	− 2.61	123.06679	261.09839	50
15	Enoxacin	3.02	$C_{15}H_{17}FN_4O_3$	$[M+H]^+$	321.13575	321.13498	− 2.40	206.07185	250.06224	60
16	Marbofloxacin	3.04	C17H19FN4O4	[M+H]+	363.14631	363.14532	− 2.73	72.08136	261.10376	20,40, 60
17	Norfloxacin	3.05	C16H18FN3O3	[M+H]+	320.1405	320.13971	− 2.47	205.0784	233.10873	60
18	Ofloxacin	3.07	C18H20FN3O4	[M+H]+	362.15106	362.15015	− 2.51	318.16141	261.10361	35
19	Pefloxacin	3.08	$C_{17}H_{20}FN_3O_3$	$[M+H]^+$	334.15615	334.15521	− 2.81	233.1087	205.07704	60
20	Fleroxcain	3.08	$C_{17}H_{18}F_3N_3O_3$	$[M+H]^+$	370.1373	370.13626	− 2.81	326.14755	269.08969	35
21	Ciprofloxacin	3.08	$C_{17}H_{18}FN_3O_3$	$[M+H]^+$	332.1405	332.13953	− 2.92	231.05676	98.9846	60
22	Danofloxacin	3.12	$C_{19}H_{20}FN_3O_3$	$[M+H]^+$	358.15615	358.15521	− 2.62	82.06564	96.08123	60
23	Sulfadimethoxine	3.11	$C_{12}H_{14}N_4O_2S$	$[M+H]^+$	279.09102	279.09018	− 3.01	204.04398	124.08715	35
24	Lomefloxacin	3.13	$C_{17}H_{19}F_2N_3O_3$	$[M+H]^+$	352.14672	352.14584	− 2.50	265.1149	308.15735	35
25	Sulfamethizole	3.16	$C_9H_{10}N_4O_2S_2$	$[M+H]^+$	271.03179	271.03110	− 2.55	156.01154	108.04475	20,40, 60
26	Ipronidazole-OH	3.16	$C_7H_{11}N_3O_3$	$[M+H]^+$	186.08732	186.08690	− 2.26	168.07701	82.06566	20,40, 60
27	Sulfamethoxydiazine	3.16	$C_{11}H_{12}N_4O_3S$	$[M+H]^+$	281.07029	281.06946	− 2.95	156.01154	126.06644	30
28	Enrofloxacin	3.17	$C_{19}H_{22}FN_3O_3$	$[M+H]^+$	360.1718	360.17081	− 2.75	245.10895	203.06152	60
29	Sulfamethoxypyridazine	3.19	$C_{11}H_{12}N_4O_3S$	$[M+H]^+$	281.07029	281.06949	− 2.85	156.01152	108.04471	30

Table 1 UPLC-quadrupole/electrostatic field orbitrap parameters of the 60 veterinary antibiotic residues *(Continued)*

No.	Compound	RT (min)	Elemental composition	Ionization mode	Theoretical precursor (*m/z*)	Measured precursor (*m/z*)	Accuracy(△ppm)	Production1 (m/z)	Production2 (m/z)	NCD
30	Ornidazole	3.21	$C_7H_{10}ClN_3O_3$	$[M+H]^+$	220.04835	220.04778	− 2.59	128.04572	–	35
31	Orbifloxacin	3.22	$C_{19}H_{20}F_3N_3O_3$	$[M+H]^+$	396.15295	396.15186	− 2.75	295.10544	352.16312	35
32	Demethylchlortetracycline	3.28	$C_{21}H_{21}ClN_2O_8$	$[M+H]^+$	465.10592	465.10463	− 2.77	448.07971	430.06918	20
33	Sparfloxacin	3.32	C19H22F2N4O3	[M+H]+	393.17327	393.17212	− 2.92	292.12558	349.18362	35
34	Sarafloxacin	3.30	$C_{20}H_{17}F_2N_3O_3$	$[M+H]^+$	386.13107	386.13007	− 2.59	299.09912	342.14148	35
35	Spiramycin	3.34	$C_{43}H_{74}N_2O_{14}$	$[M+H]^+$	843.52128	843.51898	− 2.73	174.11266	142.12254	20
36	Difloxacin	3.34	$C_{21}H_{19}F_2N_3O_3$	$[M+H]^+$	400.14672	400.14578	− 2.35	299.0993	356.1571	40
37	Sulfachlorpyridazine	3.34	$C_{10}H_9ClN_4O_2S$	$[M+H]^+$	285.02075	285.02002	− 2.56	156.01147	108.04472	20,40, 60
38	Nafcillin	3.36	$C_{21}H_{22}N_2O_5S$	$[M+H]^+$	415.13222	415.13104	− 2.84	128.0531	256.0975	40
39	Chlorotetracycline	3.34	$C_{22}H_{23}ClN_2O_8$	$[M+H]^+$	479.12157	479.12003	− 3.21	462.095	444.08493	20
40	Sulfamethoxazole	3.40	$C_{10}H_{11}N_3O_3S$	$[M+H]^+$	254.05939	254.05869	− 2.76	156.01154	108.04475	35
41	Clindamycin	3.41	$C_{18}H_{33}ClN_2O_5S$	$[M+H]^+$	425.18715	425.18610	− 2.47	126.12799	–	20,40, 60
42	Sulfadimethoxypyrimidine	3.41	$C_{12}H_{14}N_4O_4S$	$[M+H]^+$	311.08085	311.07993	− 2.96	156.0769	108.04472	35
43	Sulfisoxazole	3.49	$C_{11}H_{13}N_3O_3S$	$[M+H]^+$	268.07504	268.07425	− 2.95	156.01155	113.07126	20
44	Ipronidazole	3.58	$C_7H_{11}N_3O_2$	$[M+H]^+$	170.0924	170.09193	− 2.76	109.07641	84.08131	60
45	Doxycycline	3.50	$C_{22}H_{24}N_2O_8$	$[M+H]^+$	445.16054	445.15936	− 2.65	428.13425	359.02859	20
46	Ceftiofur	3.50	$C_{19}H_{17}N_5O_7S_3$	$[M+H]^+$	524.03629	524.03522	− 2.04	241.03911	210.02065	20
47	Sulfadimoxine	3.65	$C_{12}H_{14}N_4O_4S$	$[M+H]^+$	311.08085	311.07993	− 2.96	156.01155	108.04476	35
48	Sulfaquinoxaline	3.66	$C_{14}H_{12}N_4O_2S$	$[M+H]^+$	301.07537	301.07458	− 2.62	156.0112	108.0445	35
49	Sulfaphenazole	3.68	$C_{15}H_{14}N_4O_2S$	$[M+H]^+$	315.09102	315.09003	− 3.14	265.1149	308.15735	35
50	Tilmicosin	3.69	$C_{46}H_{80}N_2O_{13}$	$[M+H]^+$	869.57332	869.57434	1.17	174.11258	694.46985	30
51	PenicillinV	3.69	$C_{16}H_{18}N_2O_5S$	$[M+H]^+$	351.10092	351.09982	− 3.13	229.06467	257.05951	19
52	Erythromycin	3.83	$C_{37}H_{67}NO_{13}$	$[M+H]^+$	734.46852	734.46637	− 2.93	158.11775	576.37396	15
53	Tylosin	3.91	$C_{46}H_{77}NO_{17}$	$[M+H]^+$	916.52643	916.52716	0.80	174.11261	88.07617	20,40, 60
54	Nalidixic acid	3.99	$C_{12}H_{12}N_2O_3$	$[M+H]^+$	233.09207	233.09232	1.07	205.06107	187.05046	60
55	Oxacillin	4.01	$C_{19}H_{19}N_3O_5S$	$[M+H]^+$	402.11182	402.11185	0.07	160.04289	243.07666	17
56	Flumequine	4.06	$C_{14}H_{12}NO_3F$	$[M+H]^+$	262.0874	262.08774	1.30	220.04066	238.05119	70
57	Cloxacillin	4.18	$C_{19}H_{18}ClN_3O_5S$	$[M+H]^+$	436.07285	436.07355	1.61	178.00571	220.01628	20
58	Dicloxacillin	4.43	$C_{19}H_{17}Cl_2N_3O_5S$	$[M+H]^+$	470.03387	470.03674	6.11	160.04286	310.99814	15
59	Roxithromycin	4.46	$C_{41}H_{76}N_2O_{15}$	$[M+H]^+$	837.53185	837.5387	8.18	679.43811	158.11763	15
60	Josamycin	4.79	$C_{42}H_{69}NO_{15}$	$[M+H]^+$	828.474	828.47449	0.59	174.11267	109.06515	20

residue was added to 1 mL acetonitrile: 0.1% formic acid solution (1:9, v/v) and filtered with a 0.22-μm filter membrane. The final extract solution was transferred to vial and injected into UPLC-quadrupole/electrostatic field orbitrap mass spectrometer system under full ms/dd-ms^2 optimized conditions for each compound.

Goat dried milk

The goat dried milk (0.45 g) was weighed in a centrifuge tube (50 mL) and dissolved with 3 mL water (40–50 °C). Next, 7 mL of acetonitrile with 0.2% formic acid was added as an extraction solvent, and the tube was vigorously mixed for 30 s. The tube was then immediately shaken for 30 min and then centrifuged for 20 min at 10,000 r/min at 4 °C. The upper layer was submitted to a PRiME HLB. All elutes were collected into a centrifugal tube and evaporated under nitrogen gas at 40 °C. The residue was added with 1 mL acetonitrile: 0.1% formic acid solution (1:9, v/v), and filtered with a 0.22-μm filter membrane. The final extract solution was analyzed like the goat milk samples.

UPLC-quadrupole /electrostatic field orbitrap mass analysis

The analytes were measured with an ultra-high performance liquid chromatography system (Ultimate 3000, USA) coupled with a quadrupole/electrostatic field orbitrap mass spectrometer (Thermo &Fisher Q Exactive, USA). A Thermo Hypersil GoldaQ (2.1 × 100 mm, 1.9 μm) column was used for separation. Mobile phase consisting of elute A (water, 0.1% formic acid) and elute B (acetonitrile) was used at a flow rate of 0.3 mL/min. All analytes were separated using gradient method: 0–1 min: 10% B; 1–6 min: 10% B to 80% B; 6–8 min: 80% B; 8.1–12 min: 10% B. The optimized sample injection volume was set at 10 μL. All 62 target analytes ware eluted over 0–6 min while the last 6 min were used for column cleaning and re-equilibration.

The quadrupole/electrostatic field orbitrap was equipped with a heated electrospray ionization (HESI) source. The temperature of the HESI was 350 °C, the capillary temperature was 320 °C, and the spray voltage was 3.8 kV

Table 2 Matrix effect (ME) for 60 veterinary antibiotic residues spiked in blank milk and milk power sample for individual donors (n = 5)

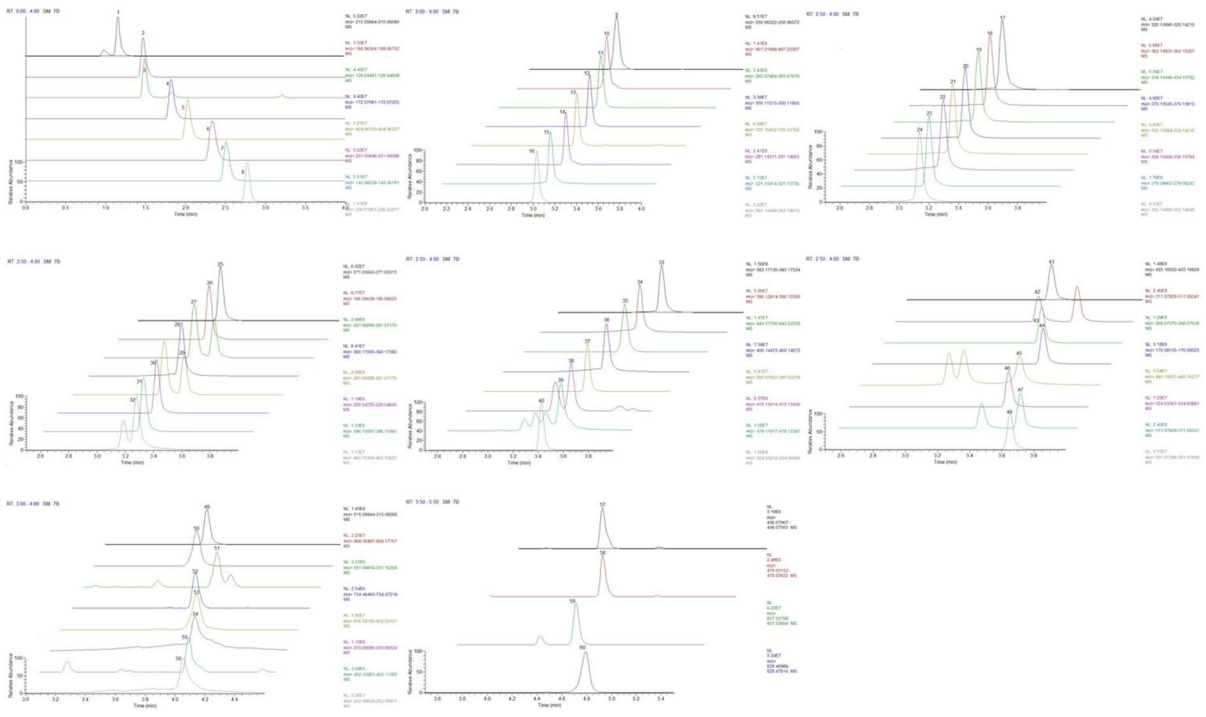
No.	Compound	Sample type		No.	Compound	Sample type		No.	Compound	Sample type	
		Milk	Milk power			Milk	Milk power			Milk	Milk power
		10 μg/kg (ME %)	10 μg/kg (ME %)			10 μg/kg (ME %)	10 μg/kg (ME %)			10 μg/kg (ME %)	10 μg/kg (ME %)
1	Sulfaguanidine	115.2	90.6	21	Sulfamethizole	107.6	88.8	41	Ornidazole	106.1	88.8
2	2-Methyl-5-nitroimidazole	101.5	115.3	22	Sulfamethoxydiazine	93.5	99.9	42	Sulfamethoxazole	115.0	97.6
3	Metronidazole	100.9	117.0	23	Ciprofloxacin	93.5	92.8	43	Sulfisoxazole	114.0	99.6
4	Cefapirin	90.2	87.5	24	Dimetridazole	99.2	108.7	44	Ipronidazole	101.8	100.2
5	Sulfadiazine	118.8	115.0	25	Marbofloxacin	101.8	101.9	45	Doxycycline	102.6	114.1
6	Sulfapyridine	117.5	115.0	26	Fleroxcain	89.7	101.1	46	Ceftiofur	110.1	114.0
7	Lincomycin	104.8	107.6	27	Orbifloxacin	91.5	109.3	47	Sulfaquinoxaline	114.1	95.1
8	Sulfathiazole	109.3	114.5	28	Enrofloxacin	106.2	97.0	48	Sulfaphenazole	106.1	112.5
9	Sulfamerazine	94.4	114.0	29	Sulfamethoxypyridazine	85.7	104.3	49	Sulfadimoxine	105.6	97.2
10	Ampicillin	114.4	92.6	30	Sparfloxacin	96.1	114.0	50	Erythromycin	113.0	100.3
11	Penicillin G	109.4	111.1	31	Demethylchlortetracycline	102.3	114.8	51	Tilmicosin	101.3	111.5
12	Trimethoprim	101.4	104.5	32	Sulfachlorpyridazine	97.0	109.6	52	Penicillin V	107.2	112.0
13	Metronidazole-OH	110.4	113.0	33	Difloxacin	95.5	115.4	53	Tylosin	82.1	110.0
14	Norfloxacin	89.7	100.5	34	Chlorotetracycline	92.6	114.0	54	Flumequine	90.8	111.2
15	Lomefloxacin	106.5	94.4	35	Spiramycin	90.2	92.8	55	Nalidixic acid	86.3	102.1
16	Enoxacin	112.8	100.2	36	Ipronidazole-OH	104.3	99.4	56	Oxacillin	114.0	106.2
17	Ofloxacin	92.5	96.1	37	Sulfadimethoxypyrimidine	104.7	84.3	57	Cloxacillin	102.2	92.3
18	Pefloxacin	98.5	99.2	38	Sarafloxacin	92.5	110.0	58	Dicloxacillin	101.6	110.6
19	Danofloxacin	91.2	98.3	39	Nafcillin	98.8	94.5	59	Roxithromycin	100.7	106.4
20	Sulfadimethoxine	103.6	97.6	40	Clindamycin	105.4	113.0	60	Josamycin	96.3	107.7

for positive mode. All other quantitative data were acquired in full scan mode. Full MS/dd-MS^2 was used for qualitative analysis. Precursor ions were selected by the quadrupole sent to the S-Lens in consideration of the detection of target analytes. The productions were then obtained from fragmented precursor ions via normalized collision energy (NCE).

The MS parameters of full MS/dd-MS^2 were as follows: Full MS, inclusion on, resolution 70,000, maximum IT 200 ms, and AGC target $3.0e^6$. The dd-MS^2 settings were as follows: inclusion on, resolution17,500, maximum IT 6 ms, AGC target $2.0e^5$, and isolation window 2.0 *m/z*. The accurate masses for the precursor ions and productions are shown in Table 1.

Validation

The method was validated according to the EU Commission 2002/657/EC. The blank milk matrix samples were carefully selected to account for the possible variation within a given matrix(e.g., fat content, protein content, and other organics). The method was evaluated for linearity, limit of detection (LOD), precision, and accuracy. In the experiment, matrix-matched instead of internal standard was used because of the level of matrix effects can by significantly reduced by matrix-matched calibration curve (Table 2). At the same time, internal standard can be found in a few antibiotics. A matrix-matched calibration curve was established for each target antibiotics separately. Six calibration levels were prepared by spiking the blank matrix with each antibiotic. The coefficients of determination (r^2) were higher than 0.99 in all matrices. The veterinary antibiotics were divided into two groups according to the response value of each target analyte to mass spectrometry. Group1 included erythromycin, spiramycin, roxithromycin, TIL, TYL, clindamycin, CLT, DEM, ceftiofur, cefapirin, OXAC, DICL, CLOX, NAFC, AMPI, PEG, and PEV with the following spiking levels: 10, 20, and 50 μg/kg. Group 2 included SMZ, sulfathiazole, TMP, sulfamethizole, SIZ, SD, sulfachlorpyridazine, sulfamethoxydiazine, sulfamethazine, sulfaquinoxaline, SDM, sulfamethoxypyridazine, sulfadimethoxypyrimidine, SPD, SMX, sulfaguanidine, sulfaphenazole, LOM, CIP ENR, OFX, NOR, ORB, DAN, SPA, SAR, MAR, ENO, FLU, FLE, DIF, PEF, nalidixic acid, LIN, dimetridazole, metronidazole-OH, ipronidazole-OH, ipronidazole, ornidazole, metronidazole, 2-methyl-5-nitroimidazole, doxycycline, and josamycin with the following spiking levels: 5, 10, and 20 μg/kg. The accuracy was determined with recovery experiments using blank samples at LOQ spiking levels in triplicate. The repeat ability was evaluated via the relative standard deviation (RSD, %). The limits of detection



1.Sulfaguanidine, 2.Metronidazole-OH, 3.2-Methyl-5-nitroimidazole, 4.Metronidazole, 5.Cefapirin, 6.Sulfadiazine, 7.Dimetridazole, 8.Sulfathiazole, 9.Sulfapyridine, 10.Lincomycin, 11.Sulfamerazine, 12.Ampicillin, 13.Penicillin G, 14.Trimethoprim, 15.Enoxacin, 16.Marbofloxacin, 17.Norfloxacin, 18.Ofloxacin, 19.Pefloxacin, 20.Fleroxcain, 21.Ciprofloxacin, 22. Danofloxacin, 23.Sulfadimethoxine, 24.Lomefloxacin, 25.Sulfamethizole, 26.Ipronidazole-OH, 27.Sulfamethoxydiazine, 28.Enrofloxacin, 29.Sulfamethoxypyridazine, 30.Ornidazole, 31.Orbifloxacin, 32.Demethylchlortetracycline, 33.Sparfloxacin, 34.Sarafloxacin, 35.Spiramycin, 36.Difloxacin, 37.Sulfachlorpyridazine, 38.Nafcillin, 39.Chlorotetracycline, 40.Sulfamethoxazole, 41.Clindamycin, 42.Sulfadimethoxypyrimidine, 43.Sulfisoxazole, 44.Ipronidazole, 45.Doxycycline, 46.Ceftiofur. 47.Sulfadimoxine, 48.Sulfaquinoxaline, 49. Sulfaphenazole, 50.Tilmicosin, 51.PenicillinV, 52.Erythromycin, 53.Tylosin, 54.Nalidixic acid, 55.Oxacillin, 56.Flumequine, 57.Cloxacillin, 58.Dicloxacillin, 59.Roxithromycin, 60.Josamycin

Fig. 1 Separation of 60 veterinary antibiotic residues in Thermo Hypersil GOLD aQ

(LOD) and quantification (LOQ) were defined as lowest concentrations with a signal-to-noise (S/N) ratio of 3 for LOD or 10 for LOQ.

The matrix effect (ME) was investigated by comparing the peak area of each antibiotic spiked in blank sample after extraction procedure at same concentration level, with peak area of each antibiotic in water (without matrix matched) at the same concentration. The peak area of each antibiotic in water was set at 100% (Javorska et al. 2017).

Results and discussion

Optimization of the UPLC-quadrupole/electrostatic field orbitrap conditions

Ultra-performance chromatography columns with sub-2-μm particles have outstanding separation capacity. They have facilitated the development of quantification methods for multi-residues within a short run time. Here, different types of chromatographic columns were investigated. Under the same determination conditions, DICL and PEV were weak retention on a Waters ACQUITY UPLC® BEH Shield RP 18 (100 mm × 2.1 mm, 1.7 μm), and CLOX and OXAC were unreserved on a Waters ACQUITY UPLC® HSS T3 column (100 mm × 2.1 mm,1.8 μm). However, the Thermo Hypersil GOLD aQ (100 mm × 2.1 mm, 1.9 μm) showed good performance in the separation of 60 veterinary antibiotics. The analysis process was completed within 12 min (Fig. 1).

Different solvents were tested to achieve better separation and retention of target analytes including cetonitrile, methanol, and 0.1% formic acid aqueous solutions. There needed to be some compromise between mobile phase composition and MS response for the 60 selected veterinary antibiotics. Consideration of the sensitivity (S/N) and the peak shape showed that the target analytes had better performance in acetonitrile than in methanol. When the aqueous solution was water, the peaks of quinolones, macrolides, and tetracyclines were asymmetrical and heavy-trailed. However, the shape of peak and the retention were well when formic acid was added into the aqueous solution. This is because the addition of formic acid improved the ionization efficiency. Therefore, acetonitrile and 0.1% formic acid were selected as the mobile phase.

The optimum mass spectrometric parameters for the identification and quantification of 60 veterinary antibiotics were obtained after analyzing the compounds by flow injection analysis. The sensitivity of target analytes was investigated via the chromatograms in full scan mode in positive ionization mode. Due to adduct formation with formic acid, all analytes showed strong formic/hydrogen adduct species ($[M + H]^+$); these species appear to be the precursor ions in the mass spectrum. The target analytes could achieve better base separation with the interference peak. This was more efficient and lowered the matrix effects, thereby leading to a resolution of 70,000 versus 17,500.

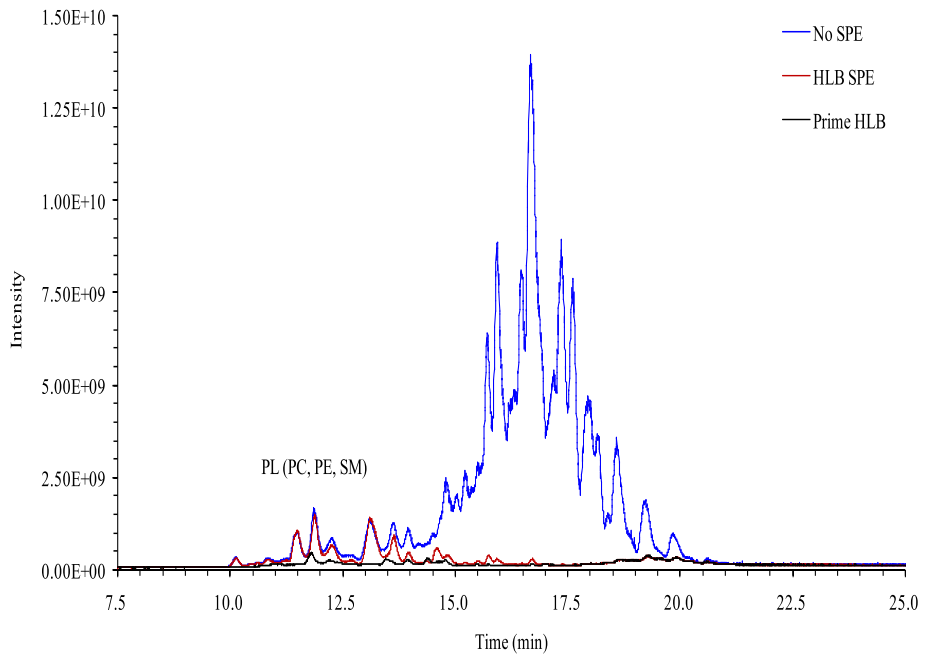


Fig. 2 Comparison the effect of different SPE on phospholipids

The full MS/dd-MS^2 mode led to a production spectrum with accurate mass measurement according to the inclusion list (a list of targeted accurate masses). This was defined as a data-dependent acquisition (dd-MS^2). After full scan analysis, specific mass windows were extracted to screen the data for the presence of analytes. The effect of the isolation window on analyte selectivity was tested. The best results were achieved when an isolation window of 2.0 ppm was employed. Table 1 shows the optimal parameters of the UPLC-quadrupole/electrostatic field orbitrap.

Optimization of the extraction procedure

According to these reports, milk and dried milk contained a great deal of phospholipids. Two different solid-phase extraction (SPE) columns (PRiME HLB and Oasis-HLB) were compared to reduce the phospholipids of the milk samples. Twelve blank milk samples were prepared following the "Sample preparation" section; four of the samples were not treated with solid phase extraction columns, four were treated with HLB, and the last four were purified with PRiME HLB. All of these samples were injected into a UPLC-quadrupole/electrostatic field orbitrap analysis in full MS mode to acquire identifies phospholipids in milk.

Although the high-resolution quadrupole/electrostatic field orbitrap is selective, the complicated matrix can still affect target analyte ionization; this leads to ion suppression or enhancement. The recovery of veterinary antibiotics in the Oasis HLB column tailed off at 25% versus the PRiME HLB. Many components, such as phospholipids, aminoacids, and fat, in milk can lead to interference of mass response. As such, these components were not effectively removed by the PRiME HLB column.

Figure 2 shows that the peak intensities of phospholipids were significantly different among the three treatment modes. The peak intensities of these compounds were not influenced by HLB purification in milk samples versus untreated milk samples. The peak intensities of phospholipids significantly decreased, which confirmed that one step of pretreating milk samples by PRiME HLB led to effective removal of phospholipids for the high-throughput detection of multiple veterinary antibiotic residues.

Previous studies showed that PRiME HLB removes phospholipids from milk via a single pretreatment step. There are no pre-equilibration and washing steps before eluting from the SPE. The effects of purifying the phospholipids including via absorption were compared for the SPE.

Here, different extraction solvents (pure acetonitrile, acetonitrile acidified with formic acid, or water) were evaluated, considering the acidic or basic character of these veterinary antibiotics. Commission Decision 2002/657/EC and GB/T 27404-2008 were used as guidelines to calculate recoveries and matrix effects (Fig. 3). Many target analytes had low recovery with 80% aqueous acetonitrile. Probably,

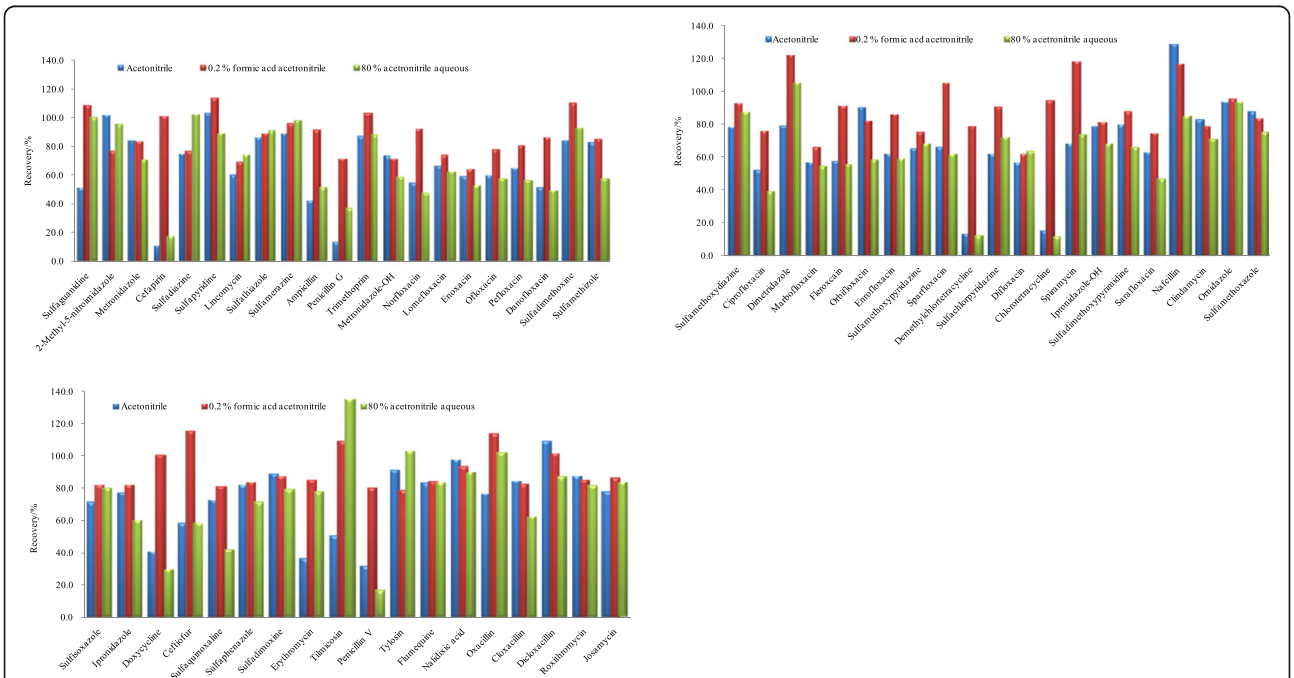


Fig. 3 Effect of different extraction solutions on the extraction properties (experiments were performed at spike levels of 10 g/kg for 60 veterinary antibiotic residues)

Table 3 Validation parameters for 60 veterinary antibiotic residues at three concentration levels in blank milk samples and milk powder samples.

Peak	Compound	Sample	Linear range (μg/L)	Spiking level (μg/kg)			Recovery (Mean ± SD%)			Intra-day (RSD, n = 6)			Inter-day (RSD, n = 3)			LOD (μg/kg)	LOQ (μg/kg)	R^2
1	Sulfaguanidine	Milk	0.5–20	5	10	20	105.4 ± 8.6	101.8 ± 6.5	99.4 ± 5.4	8.5	9.2	6.6	9.2	9.0	5.5	0.5	5.0	0.9957
		Milk powder					81.2 ± 8.6	91.2 ± 6.6	98.2 ± 2.3	8.6	6.4	2.5	9.5	7.5	3.1	0.5	5.0	0.9901
2	2-Methyl-5-nitroimidazole	Milk	0.5–20	5	10	20	104.4 ± 5.5	107.1 ± 4.2	90.6 ± 0.5	5.4	4.4	0.4	6.0	2.4	2.0	0.5	5.0	0.9965
		Milk powder					78.0 ± 7.8	87.8 ± 6.9	97.6 ± 2.2	7.5	6.4	2.0	8.9	7.1	3.3	0.5	5.0	0.9959
3	Metronidazole	Milk	0.5–20	5	10	20	62.1 ± 7.5	86.4 ± 3.8	90.3 ± 2.5	8.0	4.2	3.2	9.7	5.9	4.8	0.5	5.0	0.9932
		Milk powder					70.5 ± 4.6	95.5 ± 4.5	97.2 ± 1.7	4.9	4.4	2.1	5.8	5.7	2.9	0.5	5.0	0.9930
4	Cefapirin	Milk	1–100	10	20	50	101.2 ± 7.3	97.6 ± 5.8	94.0 ± 4.2	6.5	5.4	4.8	8.4	6.1	5.2	1.0	10.0	0.9919
		Milk powder					65.9 ± 9.0	80.5 ± 6.2	86.4 ± 3.8	9.5	6.8	4.2	10.1	7.4	5.1	1.0	10.0	0.9919
5	Sulfadiazine	Milk	0.5–20	5	10	20	82.3 ± 9.5	110.0 ± 7.4	97.7 ± 7.4	9.5	8.2	7.8	9.4	9.7	8.4	0.5	5.0	0.9955
		Milk powder					74.7 ± 9.2	88.6 ± 8.0	101.5 ± 3.7	9.5	8.9	4.5	9.8	9.5	5.2	0.5	5.0	0.9992
6	Sulfapyridine	Milk	0.5–20	5	10	20	103.5 ± 8.6	109.3 ± 8.8	98.2 ± 8.5	8.5	9.2	8.5	9.8	9.5	9.5	0.5	5.0	0.9968
		Milk powder					80.8 ± 8.2	103.7 ± 3.5	93.7 ± 4.9	8.8	3.9	3.2	8.5	4.4	6.0	0.5	5.0	0.9937
7	Lincomycin	Milk	0.5–20	5	10	20	106.1 ± 9.1	108.9 ± 7.0	87.6 ± 7.0	9.5	9.2	8.4	9.1	8.8	9.0	0.5	5.0	0.9925
		Milk powder					73.6 ± 3.8	89.7 ± 3.8	97.8 ± 1.6	4.5	4.1	2.3	5.9	6.2	3.4	0.5	5.0	0.9917
8	Sulfathiazole	Milk	0.5–20	5	10	20	96.0 ± 9.5	106.3 ± 8.1	98.2 ± 5.4	8.9	7.5	5.1	10.5	9.2	6.4	0.5	5.0	0.9935
		Milk powder					75.6 ± 3.2	83.6 ± 1.6	98.2 ± 3.4	4.2	2.5	4.1	5.9	3.1	5.5	0.5	5.0	0.9906
9	Sulfamerazine	Milk	0.5–20	5	10	20	93.6 ± 7.9	103.6 ± 3.7	98.8 ± 8.6	8.9	3.0	7.8	9.5	4.0	9.5	0.5	5.0	0.9936
		Milk powder					82.2 ± 7.9	94.3 ± 3.9	97.2 ± 2.4	8.4	4.9	1.5	9.4	5.2	2.6	0.5	5.0	0.9947
10	Ampicillin	Milk	1–100	10	20	50	64.7 ± 9.8	70.3 ± 9.3	91.6 ± 7.2	9.1	9.5	8.2	10.2	10.1	9.5	1.0	10.0	0.9945
		Milk powder					63.0 ± 8.8	66.9 ± 4.5	107.4 ± 0.9	9.0	5.1	1.1	10.1	5.4	2.6	1.0	10.0	0.9902
11	Penicillin G	Milk	1–100	10	20	50	61.3 ± 9.1	62.3 ± 9.2	71.5 ± 4.1	9.4	9.5	5.4	10.7	10.2	6.5	1.0	10.0	0.9929
		Milk powder					60.7 ± 4.9	64.1 ± 3.5	66.4 ± 1.3	5.2	3.9	2.1	6.2	5.1	3.8	1.0	10.0	0.9912
12	Trimethoprim	Milk	0.5–20	5	10	20	92.4 ± 5.8	108.4 ± 4.7	98.1 ± 5.0	6.8	5.4	4.5	7.4	6.8	5.5	0.5	5.0	0.9933
		Milk powder					108.4 ± 5.6	97.5 ± 3.5	96.7 ± 2.4	5.9	4.2	3.0	6.9	5.1	4.0	0.5	5.0	0.9946
13	Metronidazole-OH	Milk	0.5–20	5	10	20	103.7 ± 8.5	109.1 ± 7.4	87.2 ± 9.2	8.5	7.4	10.5	9.5	8.5	11.1	0.5	5.0	0.9958
		Milk powder					75.2 ± 3.8	77.1 ± 3.5	102.0 ± 1.7	4.8	4.1	2.3	5.9	4.5	3.1	0.5	5.0	0.9948
14	Norfloxacin	Milk	0.5–20	5	10	20	92.2 ± 6.3	101.6 ± 4.5	85.6 ± 3.1	7.3	5.5	3.2	8.1	5.6	4.8	0.5	5.0	0.9945
		Milk powder					79.4 ± 6.2	95.7 ± 5.5	101.0 ± 4.7	6.8	5.9	4.8	7.5	6.9	5.4	0.5	5.0	0.9942
15	Lomefloxacin	Milk	0.5–20	5	10	20	106.1 ± 6.5	107.1 ± 5.7	91.8 ± 4.7	7.1	6.5	5.7	8.5	7.8	6.5	0.5	5.0	0.9937
		Milk powder					82.7 ± 8.9	84.5 ± 4.5	99.5 ± 3.1	9.0	4.8	3.2	9.2	6.1	4.5	0.5	5.0	0.9913
16	Enoxacin	Milk	0.5–20	5	10	20	83.1 ± 8.7	100.6 ± 5.0	95.6 ± 5.0	9.8	6.8	4.5	9.1	7.2	6.4	0.5	5.0	0.9946
		Milk powder					103.4 ± 8.4	104.7 ± 4.6	98.9 ± 3.4	8.9	5.2	3.9	9.8	6.5	4.8	0.5	5.0	0.9951

Table 3 Validation parameters for 60 veterinary antibiotic residues at three concentration levels in blank milk samples and milk powder samples. *(Continued)*

Peak	Compound	Sample	Linear range (μg/L)	Spiking level (μg/kg)			Recovery (Mean ± SD%)			Intra-day (RSD, n = 6)			Inter-day (RSD, n = 3)			LOD (μg/kg)	LOQ (μg/kg)	R^2
17	Ofloxacin	Milk	0.5–20	5	10	20	105.3 ± 6.3	104.4 ± 3.3	94.6 ± 3.0	7.4	5.6	4.5	7.8	6.2	5.2	0.5	5.0	0.9938
		Milk powder					108.3 ± 5.3	103.2 ± 3.2	98.0 ± 3.0	5.8	4.2	3.5	6.9	5.2	4.5	0.5	5.0	0.9976
18	Pefloxacin	Milk	0.5–20	5	10	20	100.3 ± 8.6	109.5 ± 7.8	93.2 ± 3.6	9.1	8.8	4.5	9.2	9.2	5.4	0.5	5.0	0.9948
		Milk powder					94.3 ± 4.8	96.5 ± 1.9	99.3 ± 1.2	5.0	2.1	1.5	6.5	3.5	2.9	0.5	5.0	0.9972
19	Danofloxacin	Milk	0.5–20	5	10	20	87.2 ± 9.7	98.2 ± 6.6	83.7 ± 8.5	9.5	7.8	7.5	8.1	7.0	8.0	0.5	5.0	0.9948
		Milk powder					77.3 ± 7.8	85.5 ± 6.3	95.2 ± 5.5	8.0	6.5	5.7	9.5	8.7	6.3	0.5	5.0	0.9937
20	Sulfadimethoxine	Milk	0.5–20	5	10	20	93.1 ± 5.8	108.1 ± 3.8	101.4 ± 4.2	5.5	4.1	4.5	6.0	4.5	5.2	0.5	5.0	0.9937
		Milk powder					85.9 ± 9.1	90.6 ± 7.7	94.7 ± 4.5	9.5	7.9	4.9	9.8	9.4	7.1	0.5	5.0	0.9914
21	Sulfamethizole	Milk	0.5–20	5	10	20	106.7 ± 8.0	109.4 ± 5.3	98.2 ± 4.0	9.5	6.4	5.1	9.8	7.0	5.5	0.5	5.0	0.9920
		Milk powder					90.1 ± 9.3	102.4 ± 7.2	99.6 ± 9.0	9.8	7.4	8.9	9.2	9.5	9.1	0.5	5.0	0.9908
22	Sulfamethoxydiazine	Milk	0.5–20	5	10	20	101.9 ± 9.3	107.6 ± 7.7	94.1 ± 8.0	9.9	8.1	7.9	9.5	8.2	9.0	0.5	5.0	0.9936
		Milk powder					86.5 ± 9.8	95.9 ± 4.6	105.3 ± 3.6	9.8	5.0	3.5	9.9	6.5	4.1	0.5	5.0	0.9911
23	Ciprofloxacin	Milk	0.5–20	5	10	20	98.3 ± 5.8	92.9 ± 4.2	109.6 ± 3.2	6.8	5.4	4.2	7.4	5.9	5.1	0.5	5.0	0.9947
		Milk powder					98.0 ± 8.2	99.4 ± 4.4	107.5 ± 2.6	8.4	4.7	3.0	9.8	6.4	4.9	0.5	5.0	0.9961
24	Dimetridazole	Milk	0.5–20	5	10	20	102.8 ±7.5	108.3 ± 8.9	92.3 ± 9.5	8.5	8.5	9.8	8.5	9.5	9.2	0.5	5.0	0.9909
		Milk powder					81.6 ± 4.4	108.6 ± 5.5	100.9 ± 2.9	4.9	5.7	3.1	5.7	6.5	4.1	0.5	5.0	0.9948
25	Marbofloxacin	Milk	0.5–20	5	10	20	90.8 ± 8.5	108.0 ± 5.1	94.8 ± 5.2	9.1	6.5	5.4	11.5	7.5	6.8	0.5	5.0	0.9940
		Milk powder					93.5 ± 6.7	95.6 ± 6.5	100.6 ± 4.6	7.0	6.8	4.9	8.9	7.9	5.8	0.5	5.0	0.9970
26	Fleroxcain	Milk	0.5–20	5	10	20	71.8 ± 7.7	104.0 ± 5.5	95.4 ± 6.5	8.9	6.5	7.4	8.9	8.4	7.9	0.5	5.0	0.9938
		Milk powder					94.0 ± 8.6	95.2 ± 8.9	98.3 ± 3.6	8.4	8.7	4.1	9.9	9.5	5.2	0.5	5.0	0.9942
27	Orbifloxacin	Milk	0.5–20	5	10	20	79.9 ± 9.8	102.3 ± 6.3	99.5 ± 9.0	10.5	7.4	9.4	9.5	8.5	9.8	0.5	5.0	0.9938
		Milk powder					75.4 ± 9.2	85.7 ± 7.7	95.7 ± 4.9	9.5	7.9	5.1	9.8	9.1	6.8	0.5	5.0	0.9908
28	Enrofloxacin	Milk	0.5–20	5	10	20	91.2 ± 9.2	106.4 ± 9.6	88.5 ± 9.3	9.5	9.8	9.9	9.1	9.9	9.5	0.5	5.0	0.9936
		Milk powder					80.2 ± 8.0	99.7 ± 3.7	99.7 ± 1.7	9.7	4.1	1.9	9.9	5.4	2.8	0.5	5.0	0.9968
29	Sulfamethoxypyridazine	Milk	0.5–20	5	10	20	66.5 ± 8.9	103.9 ± 6.0	90.6 ± 7.6	9.1	6.9	7.0	9.2	8.9	7.5	0.5	5.0	0.9911
		Milk powder					77.2 ± 8.0	97.6 ± 7.8	108.5 ± 8.0	9.2	9.4	8.5	9.1	9.5	9.4	0.5	5.0	0.9945
30	Sparfloxacin	Milk	0.5–20	5	10	20	60.6 ± 7.8	73.9 ± 6.5	86.7 ± 5.6	8.2	6.9	6.5	9.5	7.2	7.0	0.5	5.0	0.9936
		Milk powder					83.8 ± 4.5	94.7 ± 4.0	105.2 ± 3.6	4.9	4.2	4.0	5.8	5.2	5.0	0.5	5.0	0.9925
31	Demethylchlortetracycline	Milk	1–100	10	20	50	60.6 ± 8.6	65.5 ± 7.1	78.9 ± 6.5	9.5	9.5	8.4	9.1	9.5	8.1	1.0	10.0	0.9958
		Milk powder					75.2 ± 8.4	77.7 ± 9.0	76.1 ± 8.2	8.9	10.5	9.4	9.1	9.5	7.9	1.0	10.0	0.9954
32	Sulfachlorpyridazine	Milk	0.5–20	5	10	20	87.9 ± 9.4	98.5 ± 7.8	99.8 ± 7.7	9.5	8.2	7.9	9.1	8.0	8.0	0.5	5.0	0.9956
		Milk powder					68.0 ± 8.2	73.1 ± 8.7	90.7 ± 8.3	8.8	8.9	9.0	9.5	9.7	9.8	0.5	5.0	0.9980

Table 3 Validation parameters for 60 veterinary antibiotic residues at three concentration levels in blank milk samples and milk powder samples. *(Continued)*

Peak	Compound	Sample	Linear range (μg/L)	Spiking level (μg/kg)			Recovery (Mean ± SD%)			Intra-day (RSD, n = 6)			Inter-day (RSD, n = 3)			LOD (μg/kg)	LOQ (μg/kg)	R^2
33	Difloxacin	Milk	0.5–20	5	10	20	87.4 ± 8.1	90.5 ± 9.8	87.1 ± 9.1	8.5	9.1	9.5	9.0	9.5	9.5	0.5	5.0	0.9944
		Milk powder					85.2 ± 3.4	97.0 ± 2.9	103.6 ± 2.5	3.9	2.8	2.9	4.5	3.4	3.0	0.5	5.0	0.9942
34	Chlorotetracycline	Milk	1–100	10	20	50	70.3 ± 9.1	72.8 ± 8.3	94.7 ± 5.1	9.5	8.7	5.6	9.7	9.2	6.4	1.0	10.0	0.9955
		Milk powder					73.4 ± 9.0	88.0 ± 9.1	85.4 ± 5.7	9.5	8.7	6.5	9.7	8.9	6.8	1.0	10.0	0.9901
35	Spiramycin	Milk	1–100	10	20	50	71.7 ± 9.6	76.6 ± 9.2	107.8 ± 8.5	9.6	8.2	9.5	9.7	8.8	9.7	1.0	10.0	0.9928
		Milk powder					63.6 ± 6.7	102.6 ± 5.8	93.7 ± 3.0	6.8	5.7	3.2	7.5	6.9	4.1	1.0	10.0	0.9998
36	Ipronidazole-OH	Milk	0.5–20	5	10	20	65.9 ± 9.7	105.2 ± 7.8	89.8 ± 3.0	9.9	8.8	4.0	10.9	9.4	5.5	0.5	5.0	0.9907
		Milk powder					71.9 ± 9.4	97.9 ± 9.1	102.3 ± 7.5	9.5	9.1	8.0	10.9	10.5	9.0	0.5	5.0	0.9924
37	Sulfadimethoxypyrimidine	Milk	0.5–20	5	10	20	109.6 ± 9.4	106.9 ± 9.3	96.0 ± 8.7	9.4	9.6	9.0	10.5	10.3	9.5	0.5	5.0	0.9939
		Milk powder					73.1 ± 7.6	84.1 ± 6.4	107.5 ± 6.1	7.8	6.8	6.5	8.5	7.2	7.0	0.5	5.0	0.9951
38	**Sarafloxacin**	**Milk**	**0.5–20**	**5**	**10**	**20**	**82.6 ± 8.9**	**104.1 ± 9.1**	**98.5 ± 9.2**	**8.7**	**8.9**	**8.7**	**9.5**	**9.1**	**9.5**	**0.5**	**5.0**	**0.9942**
		Milk powder					**94.9 ± 6.5**	**97.5 ± 6.0**	**103.5 ± 6.0**	**6.8**	**6.2**	**5.9**	**7.8**	**7.1**	**7.0**	**0.5**	**5.0**	**0.9976**
39	Nafcillin	Milk	1–100	10	20	50	60.5 ± 8.6	64.7 ± 4.5	87.7 ± 3.6	8.5	5.1	3.7	9.5	5.5	4.5	1.0	10.0	0.9943
		Milk powder					60.4 ± 1.0	60.1 ± 1.2	60.8 ± 1.3	1.5	1.4	1.1	2.5	2.3	2.1	1.0	10.0	0.9904
40	Clindamycin	Milk	1–100	10	20	50	60.1 ± 8.8	71.7 ± 7.9	89.9 ± 4.9	8.1	8.7	4.7	8.5	8.0	5.9	1.0	10.0	0.9951
		Milk powder					80.6 ± 5.5	94.3 ± 3.7	92.3 ± 2.5	6.0	4.0	3.0	7.8	5.4	4.2	1.0	10.0	0.9928
41	Ornidazole	Milk	0.5–20	5	10	20	95.1 ± 9.8	108.5 ± 8.7	89.2 ± 9.6	9.5	9.1	8.9	10.5	9.9	9.8	0.5	5.0	0.9937
		Milk powder					75.4 ± 8.6	73.9 ± 7.4	103.5 ± 5.6	8.5	7.3	6.1	9.2	8.2	7.5	0.5	5.0	0.9998
42	Sulfamethoxazole	Milk	0.5–20	5	10	20	81.2 ± 8.5	103.8 ± 8.5	89.5 ± 9.1	8.1	8.9	9.2	9.1	9.5	9.2	0.5	5.0	0.9927
		Milk powder					67.2 ± 9.0	81.9 ± 6.4	101.0 ± 2.6	9.8	6.9	3.2	9.1	7.8	4.5	0.5	5.0	0.9953
43	Sulfisoxazole	Milk	0.5–20	5	10	20	60.7 ± 9.5	108.4 ± 4.6	94.0 ± 5.8	9.4	5.4	5.0	9.9	6.5	6.0	0.5	5.0	0.9910
		Milk powder					95.0 ± 9.2	90.0 ± 5.6	96.2 ± 3.3	9.5	6.1	3.4	9.9	7.8	4.5	0.5	5.0	0.9962
44	Ipronidazole	Milk	0.5–20	5	10	20	79.3 ± 9.8	103.8 ± 9.5	95.1 ± 9.2	9.4	9.1	9.8	9.2	9.5	9.0	0.5	5.0	0.9933
		Milk powder					84.6 ± 5.6	104.6 ± 4.8	96.1 ± 3.2	5.9	5.1	3.5	7.1	6.4	4.1	0.5	5.0	0.9915
45	Doxycycline	Milk	0.5–20	5	10	20	81.4 ± 8.8	100.5 ± 8.1	95.6 ± 8.4	8.2	8.5	8.4	9.1	9.5	9.1	0.5	5.0	0.9961
		Milk powder					91.0 ± 7.8	89.3 ± 6.6	102.4 ± 4.6	8.0	6.9	5.1	9.5	7.8	5.9	0.5	5.0	0.9910
46	Ceftiofur	Milk	1–100	10	20	50	91.5 ± 9.5	98.2 ± 7.6	105.1 ± 5.5	9.6	8.1	5.6	9.7	9.1	6.1	1.0	10.0	0.9931
		Milk powder					93.2 ± 2.7	104.7 ± 1.8	100.1 ± 1.3	2.9	1.9	1.5	3.5	2.9	4.1	1.0	10.0	0.9916
47	Sulfaquinoxaline	Milk	0.5–20	5	10	20	82.9 ± 8.7	89.0 ± 6.6	105.4 ± 5.5	9.1	6.8	6.1	9.8	7.2	6.5	0.5	5.0	0.9953
		Milk powder					81.8 ± 9.0	93.1 ± 8.8	98.3 ± 7.9	9.5	9.0	8.0	9.5	9.8	9.0	0.5	5.0	0.9960
48	Sulfaphenazole	Milk	0.5–20	5	10	20	98.5 ± 6.9	93.1 ± 4.5	86.9 ± 5.1	7.9	5.0	5.6	8.2	6.2	5.9	0.5	5.0	0.9937
		Milk powder					87.4 ± 9.6	94.0 ± 6.4	101.3 ± 4.8	9.8	6.5	5.0	10.2	7.0	6.5	0.5	5.0	0.9958

Table 3 Validation parameters for 60 veterinary antibiotic residues at three concentration levels in blank milk samples and milk powder samples. *(Continued)*

Peak	Compound	Sample	Linear range (μg/L)	Spiking level (μg/kg)			Recovery (Mean ± SD%)			Intra-day (RSD, n = 6)			Inter-day (RSD, n = 3)			LOD (μg/kg)	LOQ (μg/kg)	R^2
49	Sulfadimoxine	Milk	0.5–20	5	10	20	82.6 ± 8.9	95.5 ± 7.4	88.8 ± 8.2	8.5	8.4	8.1	9.2	9.2	8.7	0.5	5.0	0.9954
		Milk powder					87.1 ± 8.7	91.2 ± 6.3	96.9 ± 2.9	8.8	6.5	3.1	8.9	7.5	4.3	0.5	5.0	0.9937
50	Erythromycin	Milk	1–100	10	20	50	62.1 ± 8.4	64.3 ± 7.4	84.8 ± 5.4	8.1	8.4	5.5	9.0	8.9	5.5	1.0	10.0	0.9906
		Milk powder					78.8 ± 8.2	71.5 ± 7.9	78.0 ± 6.7	8.9	8.0	6.9	9.9	9.1	7.8	1.0	10.0	0.9903
51	Tilmicosin	Milk	1–100	10	20	50	77.3 ± 8.5	80.0 ± 7.5	108.6 ± 7.1	8.1	7.5	8.1	9.1	7.5	8.9	1.0	10.0	0.9928
		Milk powder					85.3 ± 8.2	109.2 ± 6.0	92.8 ± 6.2	8.5	6.5	6.0	9.7	7.8	7.1	1.0	10.0	0.9931
52	PenicillinV	Milk	1–100	10	20	50	61.9 ± 8.3	69.8 ± 7.3	79.7 ± 5.5	8.6	7.1	6.1	9.5	9.5	6.8	1.0	10.0	0.9971
		Milk powder					71.5 ± 9.4	76.9 ± 7.9	94.8 ± 3.0	9.5	9.1	3.2	9.5	9.9	4.5	1.0	10.0	0.9903
53	Tylosin	Milk	1–100	10	20	50	63.0 ± 5.5	67.8 ± 4.1	94.5 ± 3.8	5.4	4.2	3.9	6.8	5.9	4.9	1.0	10.0	0.9943
		Milk powder					88.2 ± 8.6	98.2 ± 7.4	90.8 ± 3.4	8.8	7.8	3.5	9.9	8.5	4.0	1.0	10.0	0.9948
54	Flumequine	Milk	0.5–20	5	10	20	73.4 ± 5.4	103.4 ± 4.9	85.1 ± 3.6	6.1	5.1	3.9	7.8	6.9	5.5	0.5	5.0	0.9944
		Milk powder					88.7 ± 6.0	101.0 ± 4.5	109.1 ± 2.6	6.5	4.9	3.1	7.8	5.9	5.0	0.5	5.0	0.9961
55	Nalidixicacid	Milk	0.5–20	5	10	20	89.3 ± 9.9	90.5 ± 8.6	85.2 ± 3.3	10.1	8.7	3.4	9.1	9.2	3.5	0.5	5.0	0.9959
		Milk powder					86.6 ± 8.4	88.8 ± 3.3	102.2 ± 2.1	9.0	3.8	2.5	9.9	4.8	3.1	0.5	5.0	0.9938
56	Oxacillin	Milk	1–100	10	20	50	90.8 ± 7.7	93.7 ± 5.2	103.3 ± 4.5	7.8	5.3	4.9	9.8	6.5	5.9	1.0	10.0	0.9950
		Milk powder					64.7 ± 9.5	85.1 ± 9.8	102.7 ± 1.8	9.9	9.1	2.5	9.4	9.0	3.5	1.0	10.0	0.9919
57	Cloxacillin	Milk	1–100	10	20	50	70.3 ± 7.9	82.2 ± 6.5	83.7 ± 3.5	7.5	6.4	3.2	8.9	7.2	4.9	1.0	10.0	0.9959
		Milk powder					63.9 ± 6.3	64.5 ± 6.0	82.5 ± 3.9	6.8	6.5	4.1	7.8	7.0	5.4	1.0	10.0	0.9910
58	Dicloxacillin	Milk	1–100	10	20	50	94.7 ± 9.4	103.1 ± 7.2	101.5 ± 7.2	9.5	8.2	8.1	9.9	9.5	9.0	1.0	10.0	0.9996
		Milk powder					62.5 ± 9.7	68.5 ± 8.1	94.0 ± 5.6	9.9	8.4	5.8	9.1	9.8	6.4	1.0	10.0	0.9906
59	Roxithromycin	Milk	1–100	10	20	50	82.9 ± 9.2	86.4 ± 7.7	103.8 ± 6.5	9.8	7.8	6.4	9.5	8.5	7.2	1.0	10.0	0.9946
		Milk powder					77.8 ± 4.7	100.1 ± 2.6	102.1 ± 2.0	5.1	2.9	2.5	6.5	3.8	3.1	1.0	10.0	0.9905
60	Josamycin	Milk	0.5–20	5	10	20	66.8 ± 8.5	108.1 ± 6.3	96.0 ± 3.8	9.5	7.5	4.2	8.7	8.9	6.1	0.5	5.0	0.9948
		Milk powder					78.1 ± 4.0	86.7 ± 2.0	95.7 ± 3.0	4.5	2.5	3.9	5.9	3.8	4.8	0.5	5.0	0.9944

the effect of precipitation of protein was weakened in acetonitrile aqueous solution, in which caused higher matrix effect and lower extraction recoveries. Acidic acetonitrile and pure acetonitrile both had good recoveries for most veterinary antibiotics. These results indicated that these solvents could prevent the interference of proteins and phospholipids. However, certain antibiotics (e.g., cefapirin, penicillin G, demethylchlortetracycline, chlorotetracycline, doxycycline, erythromycin, and penicillin V) had recoveries that were too low (below 50%) with pure acetonitrile. It was difficult to extract some highly polar components such as β-lactams when the concentration of acetonitrile in the solvent was too high. Therefore, acidic acetonitrile could be used for extraction. The 0.2% formic acid acetonitrile extracted more than 95% veterinary antibiotics spiked into blank milk samples and precipitated protein in milk sample; these results were better than extracted by pure acetonitrile.

Method validation

PRiME HLB could eliminate the matrix effects, and matrix-matched calibration was also used to reduce the impact of matrix effects on precision and accuracy of the UPLC-quadrupole/electrostatic field orbitrap mass method.

The ME was calculated via the method in the "Validation" section. The result showed that the ME was established for each antibiotic spiked into milk and dried milk sample was not higher than 15%. Therefore, the matrix-matched calibration was applied for these matrices instead of internal standard. The results showed the matrix matched calibration can corrected the level of matrix effects (Table 2).

The resultant matrix-matched calibration curves using the instrument response were linear from 0.5 to 20 μg/L for sulfanilamides, quinolones, and nitroimidazoles. The range was 1–100 μg/L for tetracyclines, macrolides, and β-lactams. The response function was linear with a coefficient (r^2) of 0.9906–0.9971 for milk samples and 0.9901–0.9998 for dried milk samples (Table 3).The sensitivity was evaluated via the limit of detection (LOD) and limit of quantification (LOQ). The LOQs were calculated at a signal-to-noise ratio (S/N) of 10; LODs used S/N of 3. These data are shown in Table 3.The LODs were 0.5 to 1.0 μg/kg and the LOQs ranged from5.0 to 10.0 μg/kg.

The intra-day and inter-day relative standard deviations (RSDs) were adopted for precision validation. The intra-day precision was evaluated via three repeated analyses at different concentrations on three sequential runs with six replicates. The inter-day precision was performed by analyzing spiked samples over five days. The RSDs were 0.4% to 10.5% for intra-day and 2.0% to 11.5% for inter-day experiments; these values were all less than 15%. It indicated that the developed method was reliable and reproducible within its analytical range.

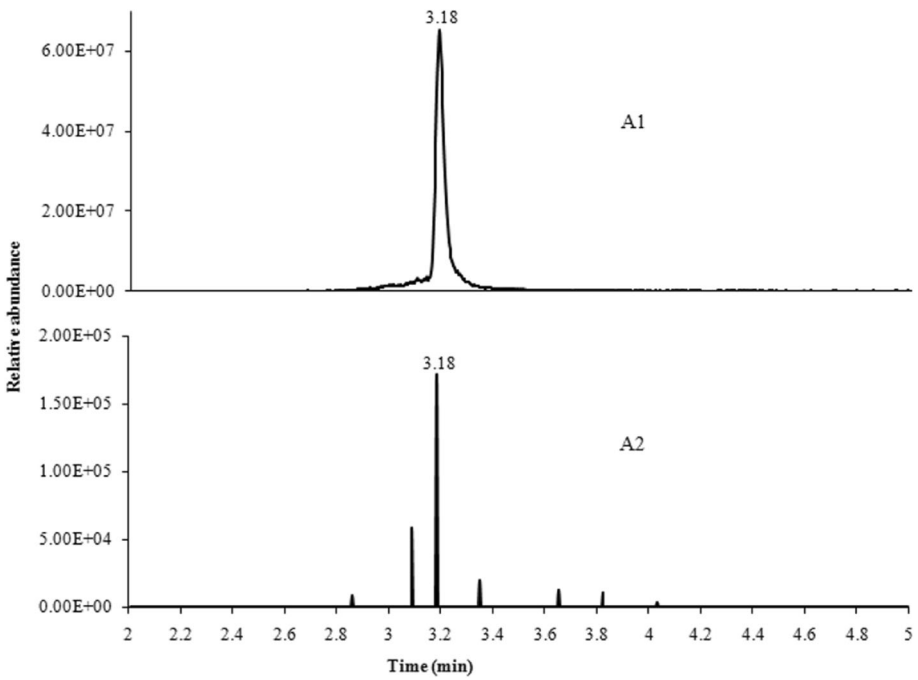


Fig. 4 Example of typical chromatography and spectra from a full MS/dd-MS^2 experiment: (A1) extracted ion chromatogram of enrofloxacin $[M + H]^+$ m/z 360.17081 in sample N0. 17; (A2) dd-MS^2 total ion chromatogram of enrofloxacin of $[M + H]^+$ m/z 245.10895 in sample No. 17

The recoveries were assessed by spiking blank dairy samples at three concentration levels (LOQs, 2×LOQs, 4×LOQs) with six replicate sat each level. The average recoveries were 60.6–110.0% for milk samples in all fortification levels; the values were 60.1–109.6% for dried milk samples.

Figure 4 shows the typical chromatograms from a full MS/dd-MS^2 experiment of analytes detected in positive samples. With the UPLC-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry method, not only accuracy was enhanced but also the low concentration antibiotic residues; this suggests that the UPLC-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry method was appropriate for the screening of antibiotic residues in milk and dried milk samples.

Method applications

Next, 25 goat milk and 35 dried milk samples were collected from local dairy farms in Shaanxi province, China. Traces of three veterinary antibiotic residues over allowable levels were detected in six samples: 2.45 μg/kg, 5.02 μg/kg of metronidazole in sample No. 3 (goat milk) and No. 15 (goat milk), and 112.4 μg/kg of enrofloxacin (goat milk) in sample No.17. These results suggest that one-step extraction by PRiME HLB combined with UPLC-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry for milk products is a simple and effective method for analyses in goat milk and goat dried milk samples.

Conclusions

A methodology for the analysis of veterinary antibiotic residues in goat milk products based on PRiME HLB extraction combined with UPLC-quadrupole/electrostatic field orbitrap high-resolution mass spectrometry. The method can achieved the simultaneous analysis of sixty-two veterinary antibiotics belong to six different classification. The method showed good performance on recoveries, precision, accuracy, MDL, and MQL, proving the effectiveness of the methodology for analysis these compounds. Compared with traditional methods, the sensitivity was enhanced, and the accuracy was improved, leading to effective method for screening antibiotic residues in milk products.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-021-00268-4.

Additional file 1. National food safety standard-Maximum residue limits for veterinary drugs in foods.

Abbreviations

SMZ: Sulfamerazine; TMP: Trimethoprim; SIZ: Sulfisoxazole; SD: Sulfadiazine; SDM: Sulfadimoxine; SPD: Sulfapyridine; SMX: Sulfamethoxazole; LOM: Lomefloxacin; CIP: Ciprofloxacin; ENR: Enrofloxacin; OFX: Ofloxacin; NOR: Norfloxacin; ORB: Orbifloxacin; DAN: Danofloxacin; SPA: Sparfloxacin; SAR: Sarafloxacin; MAR: Marbofloxacin; ENO: Enoxacin; FLU: Flumequine; FLE: Fleroxacin; DIF: Difloxacin; PEF: Pefloxacin; LIN: Lincomycin; TIL: Tilmicosin; TYL: Tylosin; CLT: Chlortetracycline; DOX: Doxycycline; DEM: Demeclocycline; TIL: Ceftiofur; OXAC: Oxacillin; DICL: Dicloxacillin; CLOX: Cloxacillin; NAFC: Nafcillin; AMPI: Ampicillin; PEG: Penicillin G; PEV: Penicillin V

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Authors' contributions

LZ contributed to the conception of the study and data analyses and wrote the manuscript. LS performed the experiment. QH contributed significantly to analysis and manuscript preparation. YL helped perform the analysis with constructive discussions. The authors read and approved the final manuscript.

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Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files.

Declarations

Competing interests

The authors declared that they have no competing interests.

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