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Generic and rapid determination of veterinary drug residues and other contaminants in raw milk by ultra performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A generic, rapid and simple analytical method able to identify 255 veterinary drug residues and other contaminants in raw milk had been developed. The method was based on two-step simple precipitation and ultra performance liquid chromatography coupled with electrospray ionization and tandem mass spectrometry (UPLC–ESI–MS/MS) operating both in positive and negative multiple reaction mode (MRM). For most of the target analytes, the optimized pretreatment processes led to no significant interference on analysis from complicated sample matrix. For quantification, matrix-fortified calibration curves were performed to compensate for the matrix effect and loss in sample preparation. Competent linearity was found for over 90% of target compounds with linear regression coefficients (R) higher than 0.99. Detection limits ranged from 0.05 to 10 μg/kg. Average recoveries spiked into raw milk were in the range from 63% to 141% with associated RSD values from 1% to 29% under the selected conditions. The method had been validated for its extraction sensitivity, linearity, recoveries and precision. The results clearly demonstrated the feasibility of the approach proposed. Application of this method, which improved efficiency and coverage of residues, would imply a drastic reduction of both effort and time in routine monitoring programs.

1. Introduction

Good-quality raw milk is required by dairy plants to make good-quality dairy products. Once raw milk is defective, it cannot be improved during processing, and defects may become more pronounced. The possible presence of a variety of low-molecular veterinary drug residues and other contaminants in raw milk is one of the key issues for milk safety due to the risk of direct toxic effects on consumers, allergic reaction in hypersensitive individuals, and the development of antibiotic-resistant pathogens [1–3]. Moreover, during the manufacture of cheese and cultured dairy products, antibiotics can inhibit dairy starter cultures used to develop acid (e.g. lactic acid bacteria), which may result in the loss of significant amounts of product and milk [2]. In routine process of monitoring programs, all the testes of raw milk in tank trucks should be completed as soon as possible (usually in less than 3 h) before it is unloaded to be processed, undoubtedly, rapid and generic determinative analytical methods for determination of veterinary drug residues and other contaminants in raw milk are urgently needed for the process of monitoring and controlling in dairy plants [4]. Up to now, some multi-class analytical methods by LC–MS/MS or LC–TOF MS concerning milk or raw milk had been described in the literatures for determination of undesirable chemicals, such as β-lactams, macrolides, tetracyclines, quinolones, sulfonamides, peptides, hormones, non-steroidal anti-inflammatory, anthelmintic drugs, mycotoxins, pesticides, and so on [1–3,5–15]. Yet most of those methods determined for several classes without generic characteristics as proposed by Mol et al. [16], and most of them could not offer satisfactory recoveries for a large range of compounds with different polarity simultaneously [14,17]. Furthermore, some methods, in spite of allowing for the simultaneous analysis of >100 analytes, were based on tedious procedures such as solid phase extraction that increased the time of the analysis [2,15]. In addition, numerous steps of some methods might result in losses of the target compounds [17].

The different chemical groups, the amphoteric properties of many compounds, and the large polarity range pose difficulties

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for extraction, cleanup, and the analytical separation [18]. When developing multi-class residue methods, more generic sample preparation should be applied to allow high recoveries of all the analytes while minimizing the presence of interferences [16–19]. In addition, the number of sample preparation steps should be kept as low as possible to improve the efficiency and reduce errors [17]. This paper described the validation of a simple, rapid and generic quantification method based on UPLC–MS/MS for the analysis of 255 veterinary drug residues and other contaminants from a variety of classes in raw milk, and the preparation methodology and main characteristics were described and discussed in detail.

2. Experimental

2.1. Chemicals and reagents

The reference standards were purchased from Dr. Ehrenstorfer, Sigma–Aldrich, Witega and EU pharmacopoeia. Every individual standard stock solution was prepared in acetonitrile–water (50:50, v/v), acetonitrile–ethanol (50:50, v/v), or acetonitrile–dimethyl sulfoxide (DMSO) (50:50, v/v) at 0.5 mg/mL based on its dissolubility and purity. A diluted mixed working standard at 20 ng/mL (for agonists and chloramphenicol), 2000 ng/mL (for bacitracin, pimaricn, β-lactams, and tetracyclines) or 200 ng/mL (for all other analytes) were prepared in acetonitrile from the relevant stock solutions. All these solutions were kept at −18 °C. Working solutions were renewed every month. HPLC-grade acetonitrile, ethanol, methanol, formic acid and ammonium acetate were supplied by Tedia (USA). All other solvents and chemicals used in this study were of analytical grade. Water was purified from a Milli-Q deionization unit (Millipore, Molsheim, France).

A mixed internal standard including d_9-clenbuterol, d_3-iprondazole, d_8-ciprofloxacin, d_8-sulfadoxine, d_8-progesterone, d_5-chloramphenicol, d_8-diethylstilbestrol was prepared in acetonitrile at concentration of 400 ng/mL, and they were not used for recovery corrections but for monitoring the efficiency of the extraction procedure and to monitor the run-to-run differences in retention times.

2.2. Samples

All milk samples tested were raw (not pasteurized) milk samples collected by the Ningbo Dairy Group (Ningbo, China) from transport trucks. The samples were kept frozen (−18 °C) until analysis.

2.3. Sample preparation

4.0 mL of raw milk (approximately 4.0 g) was transferred into a 50 mL tube containing 150 mg EDTA–Na_2 and 40 μL of the internal standards, and mixed with 5 mL of ethanol–acetonitrile (1:5, v/v), and the mixture was homogenized with a high-speed blender (PT 2000, Polytron, Switzerland) for 30 s at 20,000 rpm. Then, the samples were centrifuged (Type 3K-18, Sigma, Germany) at 4500 rpm (4000 × *g*) for 10 min at 0 °C. The supernatant was filter through a plug of absorbent cotton into a 15 mL tube, and 4.5 mL of the clear filtrate was transferred into another 50 mL tube, then 4 mL alcohol and 35 mL of acetonitrile were added in turn. Then the mixtures were again vortexed, and centrifuged at 4500 rpm for 10 min at 0 °C. All the supernatant was transferred into pear-flask and evaporated to nearly dryness with a rotatory evaporator (Buchi Rota vapor R210, Buchi Laboratories, Switzerland) set at 40 °C, and the residues obtained were dissolved in 1.25 mL of water–ethanol–acetonitrile (6:3:1, v/v/v) and vortexed for 20 s. The solutions were filtered simultaneously through a 0.22 μm PTEF filter fixed with a multi-filter apparatus (Superb20-40, Ningbo Sciences, China) directly into vials (LC Run 2 and LC Run 3), and 150 μL of the filtrate was diluted with 450 μL water (LC Run 1).

2.4. The parameters of the instrument

The ultra performance liquid chromatography (UPLC) and mass spectrometry system consisted of a Acquity UPLC™ system equipped with a XEVO triple quadrupole mass spectrometer (Waters, USA). The injection volume was full-loop (10 μL) and the chromatographic separation was performed at 40 °C using an Acquity HSS-T_3 column (Waters, 100 mm × 2.1 mm internal diameter, 1.8 μm particle size), and flow rate was set at 400 μL/min. The mass spectrometer was operated in the both positive and negative ESI mode, with the capillary voltage of 3.5 and 3.0 kV, respectively. The temperature of source and desolvation were 145 and 450 °C, respectively. Gas desolvation and nebulization were carried out using nitrogen at flow rates of 750 and 50 L/h, respectively. In the collision cell, argon was used as collision gas at a pressure of 3.3×10^{-3} mbar. The signal acquisition was performed by multiple reaction monitoring mode (MRM). The divert valve was programmed to send the LC flow to waste for the first 0.5 min after injection and again after all the analytes of interest had eluted. The gradient parameters were presented in Table 1.

2.5. Matrix-matched calibration

Matrix-fortified calibration curves were prepared and used for quantification. Blank samples (4.0 mL for each) were used for each calibration standard level. A six-point series of matrix fortified calibration was prepared by addition of 0, 40, 80, 160, 320, and 480 μL of the diluted mixed working standard. After fortification, samples were held for 10 min prior to extraction as above (Section 2.3).

2.6. LOQs, recovery and precision

The recovery and precision were calculated at three different concentration levels of low, medium and high QC samples by addition of 80, 160, and 320 μL of the diluted mixed working standard. The limits of quantification (LOQs) were determined from spiked samples, as the minimum detectable amount of analyte with a signal-to-noise ratio (S/N) of 10. The precision of the method was determined by repeated spiked samples, expressing it as the relative standard deviation (RSD) of six replicate measurements.

3. Results and discussion

3.1. Optimization of chromatography and mass spectrometry

To obtain satisfactory separation and high sensitivity of the target analytes, an optimization of the liquid chromatography and mass spectrometer conditions was performed by the injection of standard solutions of the mixture of all analytes. The column chosen was a Acquity HSS-T_3 C_{18} that achieved superior resolution, speed, and sensitivity as compared to HPLC column. The time of each run was not more than 12 min in this study. Moreover, the characteristics of the column that could be compatible with 100% aqueous mobile phase was advantageous since the very hydrophilic analytes required the proportion of organic solvent in initial mobile phase as low as possible to achieve competent retention. Therefore, it was suitable for separation the hydrophobic and hydrophilic compounds simultaneously.

The precursor ion selection strategies in LC–MS mainly choose the most prominent signals based on the S/N for MS/MS analysis. The optimization of ionization was performed by a serial of preliminary experiments, testing for example different modifiers in the mobile phase such as formic acid, acetic acid and ammonium

Table 1
Gradient UPLC method.

Positive ESI mode (LC Run 1 and Run 2)				Negative ESI mode (LC Run 3)			
Time (min)	A_1[a]	B_1[b]	Curve	Time (min)	A_2[c]	B_2[d]	Curve
0	99	1	0	0	99	1	0
2.5	80	20	6	6	0	100	6
8	0	100	6	10	0	100	6
10	0	100	6	10.5	99	1	1
10.5	99	1	1	12	99	1	1
12	99	1	1				

[a] 0.1% (v/v) formic acid in water containing 0.5 mmol/L (v/v) ammonium acetate, [b] methanol containing 0.1% (v/v) formic acid, [c] water containing 2.5 mmol/L (v/v) ammonium acetate, [d] methanol.

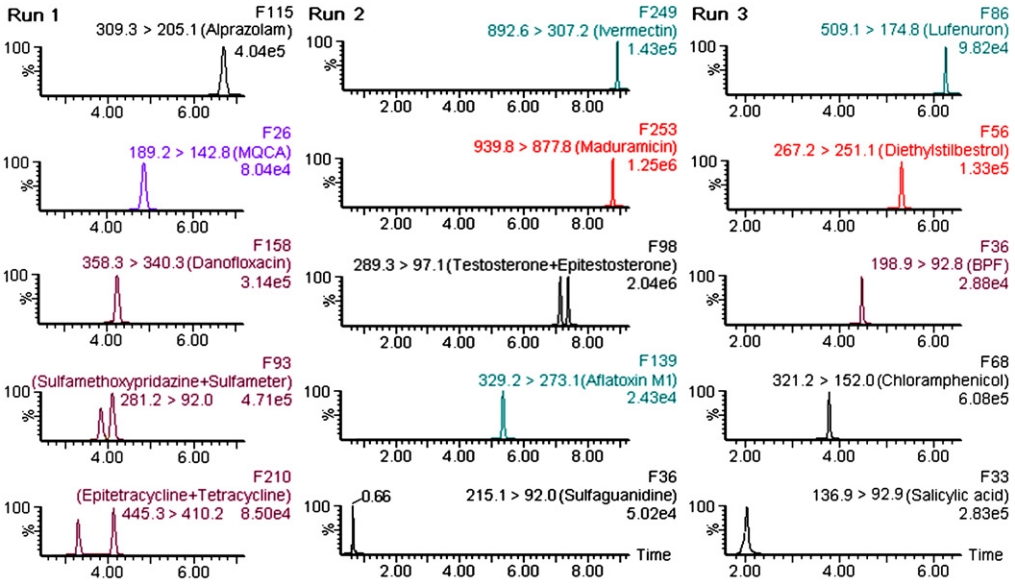

Fig. 1. The chromatograms of the most representative compounds (the earliest eluting compound, the latest eluting compound and compounds known to be difficult to be separated) with three different chromatographic runs (Run 1, Run 2 and Run 3).

acetate at various concentrations. The results indicated that the addition of 0.1% formic acid and 2.5 mmol/L ammonium acetate gave the best sensitivity in positive mode and negative mode, respectively. The injection solvent played an important role in the chromatographic method [7]. In this study, the difference between Run 1 and Run 2 was the content of the organic solvent of the final injection and two different analyses (Run 1 and Run 2) in positive ion mode were necessary. On the one hand, the high proportion of water (water–ethanol–acetonitrile (36:3:1, v/v/v, about 90%) was prefer to the hydrophilic analytes (Run 1, listed in Table 2) because some peaks might split or show bad shape—mainly the more polar compounds when the high proportion of organic solvent in the sample extract was used. On the other hand, the high proportion of organic solvent (water–ethanol–acetonitrile (6:3:1, v/v/v, about 40%) was suitable for the hydrophobic analytes (Run 2, listed in Table 3) because it was more difficult for those analytes to be dissolved in the solution with low proportion of organic solvent (10%). The chromatograms of the most representative compounds were shown in Fig. 1.

As for glucocorticoids, when 0.1% formic acid was used as the additive, $[M+H]^+$, $[M-CH_3O]^-$ and $[M+HCOO]^-$ were found in the mass spectra, and the peak of $[M+HCOO]^-$ was more abundant than that of $[M+H]^+$ and $[M-CH_3O]^-$ [3]. Nevertheless, with ammonium acetate used as the additive, $[M-CH_3O]^-$, resulted from the loss of the of a formaldehyde group resulting from cleavage of the C_{20}–C_{21}, was better than $[M+HCOO]^-$ as it yielded a much cleaner background trace. The comparison of the S/N from different transitions of glucocorticoids was shown in Fig. 2. Therefore, $[M-CH_3O]^-$ was chosen as the precursor ion due to the unusually low level required for the satisfactory analysis of glucocorticoids. The similar results had been given by Malone et al. [20].

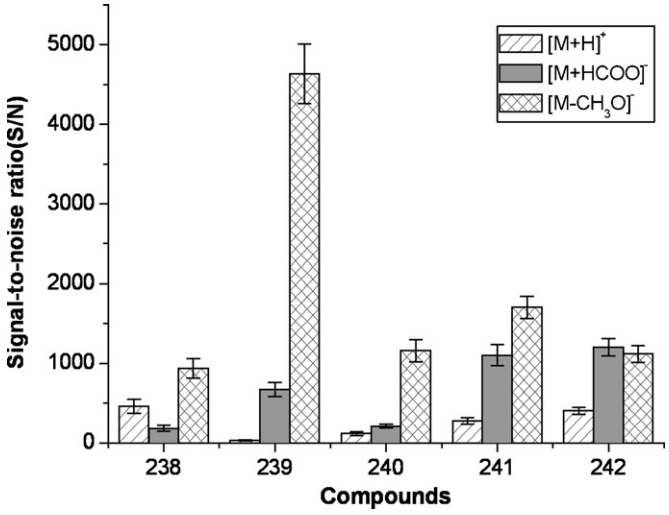

Fig. 2. The comparison of the signal-to-noise ratio (S/N) from different transitions of glucocorticoids (238: prednison; 239: cortisone; 240: prednisolone; 241: dexamethasone; 242: methylprednisolone).

Table 2
The retention time, transitions, linearity, range of average recovery, range of RSD and LOQs of 107 compounds in raw milk by LC Run 1 in ESI(+) mode ($n = 6$).

Compounds		RT (min)	Transition 1[a]	Transition 2[b]	r[c]	Range of average R (%)[d]	Range of RSD (%)	LOQs (μg/kg)
Food additives (1)								
1	Coffein	3.96	195.2[e] > 110.1	195.2 > 42.0	0.999	100–112	3–9	0.5
Pesticides (5)								
2	Chlordimeform	3.94	197.1[e] > 117.0	197.1 > 46.1	1.000	99–106	5–6	0.2
3	Dicyclanil	2.23	191.2[e] > 41.0	191.2 > 109.0	0.999	87–92	8–9	0.5
4	Trichlorfon	4.78	257.0[e] > 109.0	257.0 > 79.0	1.000	96–104	5–16	0.5
5	Dimethoate	4.81	229.9[e] > 124.8	229.9 > 198.9	0.999	96–105	7–9	0.5
6	Dichlorvos	5.93	221.0[e] > 109.0	221.0 > 79.0	0.994	103–114	6–23	0.5
Agonists (17)								
7	Cimaterol	2.15	220.2[e] > 143.0	220.2 > 160.1	1.000	94–102	6–11	0.1
8	Terbutaline	2.27	226.2[e] > 152.0	226.2 > 107.1	0.997	83–94	5–22	0.1
9	Salbutamol	2.41	240.2[e] > 148.1	240.2 > 222.2	0.999	101–104	2–10	0.1
10	Cimbuterol	2.81	234.2[e] > 143.1	234.2 > 160.1	0.999	87–105	5–9	0.1
11	Fenoterol	3.04	304.3[e] > 107.0	304.3 > 135.1	0.993	94–109	11–12	0.5
12	Ractopamine	4.09	302.3[e] > 107.1	302.3 > 164.1	0.995	98–124	7–18	0.1
13	Clenbuterol	4.40	277.2[e] > 132.0	277.2 > 167.9	0.997	101–103	5–12	0.05
14	Formoterol	4.51	345.2[e] > 121.1	345.2 > 149.0	0.998	92–98	7–9	0.05
15	Tulobuterol	4.71	228.1[e] > 154.1	228.1 > 118.7	1.000	92–106	7–9	0.05
16	Bromobuterol	4.75	367.0[e] > 293.0	367.0 > 349.0	0.997	88–112	6–13	0.1
17	Isoprenaline	4.76	212.0[e] > 132.2	212.0 > 184.0	0.995	107–116	3–10	0.5
18	Mabuterol	4.81	311.2[e] > 237.1	311.2 > 217.1	0.992	102–124	11–16	0.2
19	Isoxsuprine	4.85	302.2[e] > 284.1	302.2 > 107.1	0.993	100–129	12–17	0.2
20	Fenfluramine	5.23	232.1[e] > 159.0	232.1 > 109.0	0.999	94–98	5–11	0.1
21	Diphenhydramine	5.61	256.2[e] > 167.1	256.2 > 152.0	0.997	96–106	5–9	0.1
22	Propanolol	5.51	260.2[e] > 116.1	260.2 > 183.1	0.998	89–103	3–7	0.1
23	Betaxolol	5.65	308.4[e] > 116.1	308.4 > 98.1	0.990	92–127	6–12	0.2
β-Lactams (4)								
24	Amoxicillin	1.98	366.3[e] > 114.0	366.3 > 134.1	0.995	83–97	10–13	4.0
25	Cephalonium	3.53	459.2[e] > 152.0	459.2 > 337.2	0.999	83–93	2–10	4.0
26	Cefapirin	3.12	424.2[e] > 124.1	424.2 > 292.2	0.998	82–93	5–16	1.0
27	Cefazolin	4.33	455.2[e] > 156.1	455.2 > 323.2	0.998	90–85	6–10	2.0
Thyreostats (2)								
28	Methylthiouracil	1.87	143.0[e] > 84.0	143.0 > 126.1	0.995	88–123	5–11	5.0
29	Propyl thiouracil	4.03	171.2[e] > 154.0	171.2 > 112.1	1.000	86–127	5–11	2.0
Quinoxalines (9)								
30	Olaquindox	2.59	264.1[e] > 143.0	264.1 > 102.0	0.995	74–95	7–20	0.5
31	MQCA[f]	4.77	189.2[e] > 171.0	189.2 > 142.8	0.999	82–101	6–12	5.0
32	3-Methyl-2-quinoxalinol	3.40	161.0[e] > 133.1	161.0 > 92.1	0.995	88–118	9–28	1.0
33	Maquinoxalines	4.24	219.2[e] > 143.1	219.2 > 160.0	0.998	78–95	7–20	0.5
34	Pyrazino-2,3-quinoxaline	4.43	183.1[e] > 129.1	183.1 > 102.1	1.000	93–117	11–17	0.5
35	Carbadox	4.46	263.2[e] > 231.1	263.16 > 129.2	0.996	71–102	8–13	0.5
36	2-QCA[g]	4.65	175.2[e] > 102.0	175.2 > 129.1	0.997	65–105	5–29	5.0
37	Difurazone	5.66	361.3[e] > 154.0	361.3 > 302.2	1.000	90–113	8–22	0.5
38	2,3-Dimethylquinoxaline	5.80	159.1[e] > 77.0	159.1 > 118.1	0.983	94–110	10–21	2.0
Lincosamides (2)								
39	Lincomycin	3.65	407.3[e] > 126.1	407.3 > 359.3	0.999	71–97	6–16	0.05
40	Clindamycin	5.58	425.3[e] > 126.1	425.3 > 377.3	0.999	93–101	1–9	0.2
Nitroimidazoles (7)								
41	Metronidazole	2.53	172.1[e] > 128.1	172.1 > 82.0	0.998	97–105	5–7	1.0
42	Metronidazole-OH	1.88	188.2[e] > 126.0	188.2 > 123.1	0.998	85–108	6–12	1.0
43	Dimetridazole	2.79	142.1[e] > 96.0	142.1 > 81.0	0.999	94–122	2–12	1.0
44	Dimetridazole-2-OH	2.34	158.1[e] > 140.0	158.1 > 55.1	0.997	96–106	6–12	1.0
45	Teridazole	2.49	128.1[e] > 82.0	128.1 > 111.1	0.999	101–133	10–27	1.0
46	Ternidazole	3.43	248.0[e] > 121.0	248.0 > 128.0	0.999	89–99	6–11	1.0
47	Iprondazole	4.88	170.1[e] > 124.1	170.1 > 109.1	0.999	89–100	2–7	0.1
Benzimidazole (4)								
48	2-Aminobenzimidazole	2.57	134.1[e] > 92.1	134.1 > 65.1	0.999	72–96	3–24	0.2
49	Carbendazim	3.40	192.1[e] > 160.1	192.1 > 132.1	1.000	92–107	3–15	0.05
50	Thiabendazole	3.88	202.1[e] > 131.0	202.1 > 175.0	0.999	98–111	5–7	0.1
51	Thiabendazole-5-OH	3.47	218.1[e] > 147.1	218.1 > 191.1	1.000	78–94	7–10	0.2
Quinolones (17)								
52	Pipemidic acid	3.49	304.3[e] > 286.1	304.3 > 189.1	0.998	66–117	12–16	0.2
53	Marbofloxacin	3.63	363.2[e] > 72.0	363.2 > 320.2	1.000	84–90	8–11	0.2
54	Ofloxacin	3.87	362.1[e] > 261.1	362.1 > 318.1	0.998	90–109	7–23	0.1
55	Pefloxacin	3.88	334.3[e] > 316.3	334.3 > 233.2	0.998	66–81	5–12	0.1
56	Enoxacin	3.89	321.3[e] > 303.2	321.3 > 232.1	0.999	69–98	12–13	0.1
57	Norfloxacin	3.97	320.4[e] > 302.2	320.4 > 276.3	1.000	67–124	12–16	0.1
58	Ciprofloxacin	4.07	332.3[e] > 231.1	332.3 > 314.2	0.996	94–127	11–21	0.1
59	Enrofloxacin	4.13	360.3[e] > 316.3	360.3 > 245.2	0.994	78–90	3–19	0.1
60	Danofloxacin	4.14	358.3[e] > 340.3	358.3 > 82.1	1.000	81–108	5–13	0.1
61	Lomefloxacin	4.21	352.3[e] > 265.2	352.3 > 308.3	0.996	81–113	4–7	0.1
62	Orbifloxacin	4.26	396.3[e] > 295.3	396.3 > 352.3	0.998	85–100	7–15	0.1
63	Difloxacin	4.28	400.3[e] > 382.3	400.3 > 299.3	0.999	83–113	5–13	0.05
64	Sparfloxacin	4.67	393.3[e] > 251.2	393.3 > 349.3	0.999	85–103	1–12	0.5
65	Cinoxacin	5.05	263.1[e] > 189.0	263.1 > 245.1	0.999	69–99	3–16	0.2

Table 2 (*Continued*)

Compounds		RT (min)	Transition 1[a]	Transition 2[b]	r^2[c]	Range of average R (%)[d]	Range of RSD (%)	LOQs (μg/kg)
66	Oxolinic acid	5.30	262.1[e] > 244.1	262.1 > 160.1	0.999	65–117	14–19	0.05
67	Nalidixic acid	5.92	233.2[e] > 187.1	233.2 > 215.2	1.000	86–101	4–13	0.05
68	Flumequine	6.08	262.2[e] > 202.1	262.2 > 244.1	0.999	70–102	9–19	0.05
Sulfonamides (23) and pyrimidines (1)								
69	Sulfadiazine	2.70	251.1[e] > 92.0	251.1 > 156.0	1.000	96–102	3–4	0.2
70	Sulfisomidin	2.73	279.2[e] > 92.1	279.2 > 186.1	0.999	94–106	3–5	0.2
71	Sulfathiazole	3.04	255.9[e] > 92.1	255.9 > 156.0	0.998	93–117	5–26	0.2
72	sulfapyridine	3.23	250.1[e] > 92.0	250.1 > 108.1	1.000	93–103	4–9	0.2
73	Sulfamerazine	3.43	265.1[e] > 92.0	265.1 > 156.0	1.000	66–105	9–13	0.5
74	Sulfamethoxypyridazine	3.83	281.2[e] > 92.0	281.2 > 108.1	0.990	97–108	4–10	0.5
75	Sulfamethizole	3.92	271.1[e] > 92.0	271.1 > 65.0	0.999	97–108	4–7	0.5
76	Sulfamethazine	3.95	279.2[e] > 92.1	279.2 > 186.1	0.998	88–109	1–16	0.1
77	Sulfameter	4.08	281.2[e] > 92.0	281.2 > 108.1	0.999	84–116	3–9	0.1
78	Sulfamethoxazole	4.36	254.1[e] > 92.1	254.1 > 108.0	0.998	96–104	4–8	0.5
79	Sulfadimethoxin	4.54	311.1[e] > 92.0	311.1 > 156.0	0.992	94–117	6–11	0.1
80	Sulfisoxazole	4.56	268.2[e] > 92.0	268.2 > 156.0	1.000	88–105	5–12	0.2
81	Sulfabenzamide	4.77	277.2[e] > 92.0	277.2 > 156.0	0.999	94–127	5–22	0.2
82	Sulfaphenzole	4.96	315.2[e] > 158.2	315.2 > 92.0	0.997	76–90	7–19	0.2
83	Sulfachloropyridazine	5.04	285.1[e] > 92.0	285.1 > 156.0	0.997	89–101	8–15	1.0
84	Pyrimethamine	5.15	249.1[e] > 177.0	249.1 > 198.0	0.997	82–95	8–12	0.1
85	Sulfadoxine	5.16	311.2[e] > 92.0	311.2 > 156.0	0.993	91–104	9–14	0.2
86	Sulfaquinoxaline	5.31	301.2[e] > 92.0	301.2 > 108.1	0.998	74–111	8–14	0.2
87	Trimethoprim	3.70	291.2[e] > 110.1	291.2 > 261.2	0.999	93–119	4–19	0.1
Tetracyclines (10)								
88	Methacycline	3.55	443.2[e] > 426.2	443.2 > 127.2	0.996	87–97	6–12	2.0
89	Minocycline	3.76	458.2[e] > 441.2	458.2 > 283.1	0.994	85–98	3–21	2.0
90	Epitetracycline	3.15	445.3[e] > 410.2	445.3 > 98.0	0.992	83–95	7–12	2.0
91	Tetracycline	4.05	445.3[e] > 410.2	445.3 > 154.0	0.984	70–113	13–25	2.0
92	Doxycycline	5.44	445.2[e] > 428.1	445.2 > 98.0	0.988	86–93	9–18	2.0
93	Epioxytetracycline	4.15	461.3[e] > 426.2	461.3 > 201.0	0.999	96–106	7–17	2.0
94	Oxytetracycline	4.15	461.3[e] > 426.2	461.3 > 201.0	0.999	86–94	12–17	2.0
95	Epichlortetracycline	4.09	479.2[e] > 444.1	479.2 > 462.1	0.989	70–104	3–11	2.0
96	Chlortetracycline	4.89	479.2[e] > 444.1	479.2 > 462.1	0.990	78–100	4–23	2.0
97	Demeclocycline	4.48	465.3[e] > 448.3	465.2 > 289.1	0.986	87–122	11–28	2.0
Sedatives (11)								
98	Lorazepam	3.89	321.2[e] > 303.1	321.2 > 275.1	0.999	63–96	8–9	0.2
99	Xylazine	4.36	221.1[e] > 90.0	221.1 > 164.0	1.000	89–115	4–22	0.1
100	Midazolam	5.58	326.2[e] > 291.1	326.2 > 249.3	0.996	78–96	6–8	0.1
101	Chlordiazepoxide	5.68	300.2[e] > 283.1	300.2 > 227.2	0.996	87–96	14–27	0.2
102	Promethazine	5.99	285.2[e] > 86.1	285.2 > 198.1	1.000	93–117	5–22	0.1
103	Acepromazine	6.00	327.3[e] > 86.01	327.3 > 58.0	0.999	90–99	3–8	0.1
104	Clonazepam	6.31	316.3[e] > 270.1	316.3 > 214.1	0.994	94–108	6–11	0.5
105	a-Hydroxyalprazolam	6.45	325.3[e] > 216.2	325.3 > 297.2	0.998	92–101	6–10	0.5
106	Chlorpromazine	6.55	319.2[e] > 214.0	319.16 > 239.2	0.984	83–102	9–19	0.5
107	Alprazolam	6.61	309.3[e] > 205.1	309.3 > 281.1	1.000	91–128	5–7	0.1

[a] Quantification transition; [b] qualification transition; [c] regression coefficient; [d] recovery; [e] $[M+H]^+$; [f] 2-quinoxalinecarboxylic acid; [g] 3-methyl-quinoxaline-2-carboxylic acid.

3.2. Optimization of sample preparation

Milk has high content of lactose (4.6%), protein (3.2%) and fat (3.9%) and special focus should be placed on sample preparation. In this study, we abandoned the concept of partitioning that has always been the core of pesticide multi-class methods and applied less specific sample preparation approaches [6,16]. To avoid partitioning, various water-soluble extraction organic solvents with generic properties were investigated, including the use of acetone, alcohol, methanol, acetonitrile and acetonitrile–alcohol (5:1, v/v). With respect to matrice precipitation, acetonitrile and acetonitrile–alcohol (5:1, v/v) were better than other solvents. Extracting only with acetonitrile might generate binary phases between an aqueous and an acetonitrile phase. Alcohol was used to avoid generating binary phases. Therefore, acetonitrile–alcohol (5:1, v/v) instead of acetonitrile was used as the extract solvent. Comparing with the intensity of the response of the analytes, acetonitrile–alcohol was better than alcohol, acetone and methanol in most of the cases in this study. In the first extraction step, the percentage of organic solvent was about 55.6%, which could be employed to extract non-polar analytes (such as ionophores and steriods) in milk, while the content of water in milk could ensure the recoveries of water-soluble analytes (such as tetracyclines and β-lactams). Under this condition, though most of protein and fat was eliminated, some water-soluble matrices and salts were co-extracted. In the next step, with the addition of 4 mL of alcohol and 35 mL of acetonitrile, the percentage of organic solvent reached about 95.1%, most of the remaining water-soluble matrices and salts were precipitated by centrifugation, because the solubility of those matrices in the high proportion of organic solvent remarkably decreased. In addition, the presence of EDTA–Na_2 was vital important to ensure recovery and linearity of tetracyclines and macrolides [6]. To prevent the remaining hydrophilic matrices in the final extract from entering into the source of mass spectrometer, it was necessary to switch the LC flow to waste by switching valve from 0 to 0.7 min.

By contrast, the sample preparation method for determination anticoccidials reported by Thompson et al. was extremely simple with only one extraction step by 5 mL of acetonitrile, without additional cleanup or concentration of the resulting extract [1]. Yet it was not suitable for multi-class method. The proportion of organic solvent in the final injection (about 66.7%) was too high for most of the hydrophilic analytes. Moreover, after dilution 3 times with acetonitrile, the following analytical method might be sufficiently sensitive to quantify part of compounds but not for the analytes with weak intensity of response in trace amounts.

Table 3
The retention time, transitions, linearity, range of average recovery, range of RSD and LOQs of 99 compounds in raw milk by LC Run 2 in ESI(+) mode ($n = 6$).

Compounds		RT (min)	Transition 1[a]	Transition 2[b]	r[c]	Range of average R (%)[d]	Range of RSD (%)	LOQs (μg/kg)
Amphenicols (1)								
108	Florfenicol amine	0.89	248.1[i] > 230.1	248.1 > 130.2	0.986	81–104	9–27	2.0
Sulfonamides (1)								
109	Sulfaguanidine	0.65	215.1[e] > 92.0	215.1 > 156.0	0.998	94–98	8–18	2.0
β-Lactams (7)								
110	Ampicillin	4.33	350.2[e] > 106.0	350.2 > 192.1	0.996	87–95	4–9	1.0
111	Piperacillin	5.88	518.3[e] > 143.1	518.3 > 114.9	0.998	92–98	2–10	1.0
112	Penicilline V	6.43	351.2[e] > 114.1	351.2 > 160.1	1.000	93–96	1–11	1.0
113	Oxacillin	6.43	402.2[e] > 114.1	402.2 > 160.1	1.000	92–96	1–6	1.0
114	Cloxacilline	6.54	436.2[e] > 114.1	436.2 > 160.1	0.999	91–98	2–7	2.0
115	Nafcillin	6.77	415.2[e] > 171.1	415.2 > 115.1	0.985	92–102	7–27	1.0
116	Dicloxacillin	6.78	470.2[e] > 114.1	470.2 > 160.1	0.999	79–95	3–6	1.0
Benzimidazole (10)								
117	Mebendazole-amine HMEB	5.02	238.2[e] > 77.0	238.2 > 105.1	0.996	83–97	1–9	0.2
118	Albendazole sulfone	5.17	298.2[e] > 266.1	298.2 > 159.0	1.000	91–99	1–14	0.2
119	Aminoflubendazol	5.19	256.1[e] > 95.0	256.1 > 123.1	0.998	91–103	5–10	0.2
120	Oxibendazole	5.55	250.2[e] > 176.1	250.2 > 218.2	0.997	91–105	1–8	0.1
121	Oxfenbendazol	5.62	316.2[e] > 159.0	316.2 > 191.1	0.998	85–103	3–13	0.1
122	Flubendazole	6.46	314.2[e] > 123.1	314.2 > 282.2	0.999	87–95	4–13	0.2
123	Albendazole	6.48	266.2[e] > 234.1	266.2 > 191.2	0.997	70–91	2–11	0.1
124	Albendazole sulfoxide	6.48	282.2[e] > 95.0	282.2 > 123.1	0.999	88–95	8–13	0.1
125	Triclabendazole	7.96	359.0[e] > 274.1	359.0 > 171.0	1.000	87–97	1–14	0.2
126	Febantel	7.42	447.3[e] > 383.2	447.3 > 415.2	0.998	89–100	3–11	0.1
Anabolic steroids (20)								
127	Estradiol benzoate	8.54	377.4[e] > 105.0	377.4 > 135.1	0.997	76–86	4–9	0.2
128	Mifeprisone	6.83	430.3[e] > 134.0	430.3 > 159.1	0.998	90–100	5–11	0.5
129	Norethisterone	6.96	299.4[e] > 91.0	299.4 > 79.0	0.999	87–102	8–14	0.5
130	D-Norgestrel	7.27	313.3[e] > 91.0	313.3 > 109.0	0.998	93–107	6–11	0.5
131	Medroxyprogesterone	7.50	345.4[e] > 123.1	345.4 > 97.0	0.999	92–101	3–10	0.2
132	Megestrol actate	7.53	385.5[e] > 209.2	385.5 > 224.3	0.997	93–107	6–10	0.5
133	Chlormadinone acetate	7.56	405.1[e] > 309.2	405.1 > 267.2	1.000	88–99	5–9	0.5
134	Medroxyprogesterone17-actate	7.59	387.4[e] > 123.1	387.4 > 327.4	0.996	95–108	1–6	0.5
135	Melengestrol acetate	7.64	397.4[e] > 221.2	397.4 > 279.3	0.999	85–103	3–9	0.5
136	Progesterone	7.69	315.4[e] > 97.0	315.4 > 109.1	0.997	76–105	8–10	0.1
137	Hyroxyprogesterone caproate	8.24	429.6[e] > 313.4	429.6 > 109.0	0.998	75–101	6–17	0.5
138	Trenbolone	6.77	271.4[e] > 199.1	271.4 > 165.2	0.998	92–99	13–23	0.5
139	Boldenone	6.84	287.4[e] > 121.1	287.4 > 135.1	0.999	88–100	3–15	0.1
140	19-Nortestosterone	6.94	275.3[e] > 109.0	275.3 > 257.3	0.999	84–94	8–20	0.5
141	Epitestosterone	7.15	289.3[e] > 109.1	289.3 > 97.1	0.999	89–100	2–9	0.5
142	Testosterone	7.40	289.3[e] > 97.1	289.3 > 109.1	0.999	92–103	2–12	0.2
143	Methyltestosterone	7.35	303.4[e] > 97.0	303.4 > 109.0	0.998	91–101	3–10	0.5
144	Trenbolone acetate	7.60	313.3[e] > 253.3	313.3 > 91.0	0.998	92–101	3–8	0.2
145	Testosterone7-propionate	8.24	345.5[e] > 97.0	345.5 > 109.0	0.999	81–98	3–6	0.2
146	Nandrolone phenylpropionate	8.53	407.5[e] > 105.1	407.5 > 257.3	0.998	79–82	8–12	0.2
Macrolides (10)								
147	Spiramycin	4.91	843.4[e] > 173.9	843.4 > 142.0	0.997	88–94	7–14	1.0
148	Tilmicosin	5.47	869.6[e] > 174.1	869.6 > 696.4	0.998	89–102	7–8	1.0
149	Oleandomycin	5.77	688.7[e] > 158.1	688.7 > 544.5	0.999	90–101	7–9	1.0
150	Tiamulin	6.03	494.4[e] > 192.2	494.4 > 119.0	1.000	94–105	2–6	0.1
151	Tylosin	6.12	916.7[e] > 174.1	916.7 > 83.1	0.999	89–93	2–10	1.0
152	Leucomycin	6.17	772.7[e] > 109.1	772.7 > 174.2	1.000	86–99	2–11	1.0
153	Josamycin	6.54	828.7[e] > 109.1	828.7 > 174.1	0.999	93–102	3–6	0.1
154	Roxithromycin	6.69	837.7[e] > 158.1	837.7 > 116.0	0.999	93–100	2–8	0.1
155	Erythromycin	6.70	734.6[e] > 158.1	734.6 > 83.0	0.999	86–115	5–16	0.1
156	Valnemulin	6.71	565.4[e] > 263.2	565.4 > 147.1	0.998	91–95	8–19	0.5
Mycotoxins (6)								
157	Aflatoxin G_2	5.29	331.2[e] > 245.1	331.2 > 257.1	0.998	87–98	7–26	1.0
158	Aflatoxin M_1	5.31	329.2[e] > 229.2	329.2 > 273.2	0.999	77–104	2–14	2.0
159	Aflatoxin G_1	5.48	329.2[e] > 243.1	329.2 > 283.1	1.000	86–105	1–9	1.0
160	Aflatoxin B_2	5.66	315.2[e] > 259.1	315.2 > 287.2	0.998	94–101	6–11	1.0
161	Aflatoxin B_1	5.83	313.2[e] > 241.2	313.2 > 285.0	0.999	88–97	7–11	1.0
162	Ochratoxin A	7.22	404.2[e] > 101.9	404.2 > 239.1	0.998	65–92	16–28	2.0
NSAIDs (15)								
163	N-acetyl dapsone	4.52	291.2[e] > 156.0	291.2 > 92.2	0.996	91–103	4–13	1.0
164	Ethopabate	5.72	238.2[e] > 136.1	238.2 > 206.2	0.982	80–106	3–5	0.2
165	Indapamide	5.85	366.2[e] > 132.1	366.2 > 117.1	0.999	92–94	5–14	0.5
166	Piroxicam	6.15	332.2[e] > 121.0	332.2 > 78.0	0.997	86–102	5–7	0.5
167	Sulindac	6.72	357.2[e] > 233.2	357.2 > 248.0	0.999	93–105	2–9	0.2
168	Tolmetin	6.78	258.3[e] > 91.0	258.3 > 119.1	0.998	67–98	5–11	0.5
169	Bumetanide	6.79	365.3[e] > 240.2	365.3 > 284.2	0.998	89–97	5–12	1.0
170	Ketoprofen	6.80	255.1[e] > 77.0	255.1 > 209.2	0.989	76–90	19–24	0.5
171	Naproxene	6.95	231.1[e] > 185.1	231.1 > 170.1	0.999	81–96	10–11	0.5
172	Praziquantel	6.98	313.3[e] > 203.2	313.3 > 83.1	0.995	85–89	9–15	0.5
173	Nabumetone	7.16	229.3[e] > 171.1	229.3 > 128.0	0.997	76–92	2–10	0.5
174	4-Phenylbutazone	7.30	309.3[e] > 92.1	309.3 > 120.1	0.998	92–101	5–10	1.0

Table 3 (*Continued*)

Compounds		RT (min)	Transition 1[a]	Transition 2[b]	r[c]	Range of average *R* (%)[d]	Range of RSD (%)	LOQs (μg/kg)
175	Flunixin meglumine	7.41	297.2[e] > 279.2	297.2 > 264.2	0.998	82–101	6–9	0.2
176	Decoquinate	8.55	418.5[e] > 204.1	418.5 > 372.3	0.996	84–90	4–7	0.1
Pesticides (12)								
177	Methidathion	6.72	303.0[e] > 145.0	303.0 > 85.1	1.000	86–94	7–15	0.1
178	Malathion	7.13	331.0[e] > 99.0	331.0 > 127.0	0.992	92–98	6–10	0.5
179	Triazophos	7.23	314.1[e] > 161.9	314.1 > 118.9	0.999	98–104	8–12	0.1
180	Coumaphos	7.66	363.0[e] > 307.0	363.0 > 289.0	0.998	85–103	4–19	0.5
181	Fenthion	7.68	279.1[e] > 169.1	279.1 > 247.1	0.999	82–99	8–15	1.0
182	Phoxim	7.74	299.0[e] > 129.0	299.0 > 153.0	0.998	82–100	1–16	1.0
183	Phorate	7.84	261.0[e] > 75.0	261.0 > 97.0	0.999	103–107	11–19	1.0
184	Disulfoton	7.90	275.1[e] > 89.0	275.1 > 61.0	0.997	83–110	13–27	1.0
185	Chlorpyriphos-methyl	7.95	321.8[e] > 125.0	321.8 > 289.9	0.998	80–100	5–22	1.0
186	Chlorpyrifos	8.33	349.9[e] > 97.0	349.9 > 198.0	0.999	85–98	4–14	1.0
187	Amitraz	8.47	294.2[e] > 163.1	294.2 > 122.1	0.989	62–99	5–8	1.0
188	Carbofuran	6.01	222.1[e] > 165.1	222.1 > 123.0	1.000	67–87	6–12	1.0
Anticoccidials (6)								
189	Clopidol	3.66	192.1[e] > 87.0	192.1 > 101.0	0.986	74–87	5–21	1.0
190	Maduramicin	8.84	939.8[e] > 877.7	939.8 > 895.8	0.998	89–100	6–12	0.1
191	Salinomycin	8.96	773.6[g] > 431.4	768.7[f] > 733.7	0.999	89–98	2–14	0.5
192	Nigericin	9.04	742.8[g] > 107.1	742.8 > 149.1	0.999	91–93	1–7	0.2
193	Narasin	9.15	787.7[g] > 431.4	782.8[f] > 747.7	0.998	84–104	9–13	0.1
194	Lasalocid A	9.25	613.5[g] > 377.3	608.7[f] > 237.3	0.989	79–90	4–9	0.5
Anthelmintics (7)								
195	Emamectin benzate	7.89	886.6[e] > 82.0	886.6 > 158.1	0.999	93–101	2–4	0.2
196	Eprinomectin B1a	8.52	914.8[e] > 112.2	914.8 > 186.1	0.996	83–89	7–11	1.0
197	Abamectin (B1a)	8.61	890.8[e] > 305.3	890.8 > 567.5	0.997	81–95	8–16	2.0
198	Doramectin	8.77	916.8[f] > 331.4	916.8 > 593.5	1.000	90–98	1–13	2.0
199	Moxidectin	8.85	640.7[g] > 528.4	640.7 > 498.4	0.998	78–88	6–13	2.0
200	Ivermectin	8.99	892.6[e] > 569.5	892.6 > 307.2	0.998	81–95	5–15	2.0
Triphenylmethane dyes (3)								
201	Leucomalachite green	6.80	331.2[e] > 239.1	331.2 > 223.1	0.998	96–110	2–20	0.5
202	Malachite green	5.80	329.4[e] > 313.4	329.3 > 165.0	0.999	84–101	5–7	0.2
203	Crystal violet	6.77	372.2[e] > 356.2	372.2 > 340.2	0.998	91–101	6–14	0.2
Quinoxalines (1)								
204	Ethoxyquin	6.28	218.2[e] > 160.1	218.2 > 148.0	0.999	106–126	5–26	0.5
Preservative (1)								
205	Pimaricn	6.60	666.6[e] > 503.5	666.6 > 467.4	0.998	75–94	6–24	5.0
Peptides (1)								
206	Bacitracin	6.05	712.3[h] > 110.2	712.3 > 86.1	0.988	89–103	7–12	10

[a] Quantification transition; [b] qualification transition; [c] regression coefficient; [d] recovery; [e] $[M+H]^+$; [f] $[M+NH_4]^+$, [g] $[M+Na]^+$, [h] $[M/2+H]^+$.

An another possible way to get rid of the matrix interferences from milk would be sample cleanup by reversed-phase SPE (Oasis HLB or Strata-X cartridges) after the milk proteins was precipitated by acetonitrile [2,14]. The SPE cleanup generally led to a loss in the number of analytes that could be analyzed in the method due to its inherent selectivity. For example, very hydrophilic drugs (e.g. salbutamol) were difficult to be retained, while very hydrophobic drugs (e.g. ionophores) were too strongly retained to be eluted by acetonitrile or methanol [17]. Therefore, those drugs were not quantitatively recovered in this way [4]. Moreover, such tedious procedures increase the time and cost of the analysis.

More recently, QuEChERS (quick, easy, cheap, effective, rugged and safe) methodology that employed PSA and C_{18} had further been adapted for the cleanup of animal food extracts [18,21,22]. But our experiment found that this kind of QuEChERS was defective because the tetracyclines, β-lactams would be lost markedly with

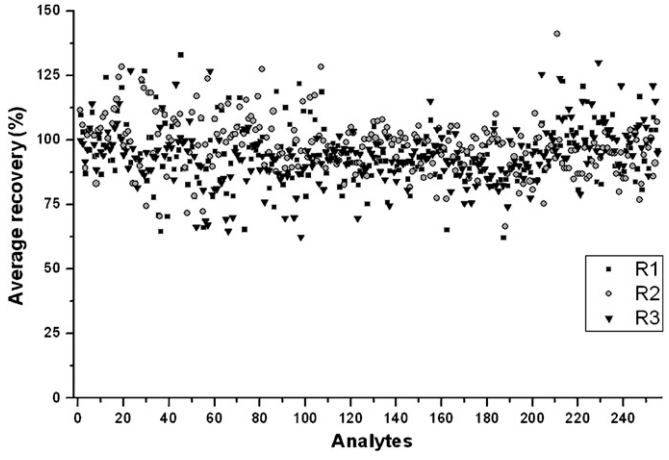

Fig. 3. The average recovery of 255 analytes in raw milk ($n = 6$).

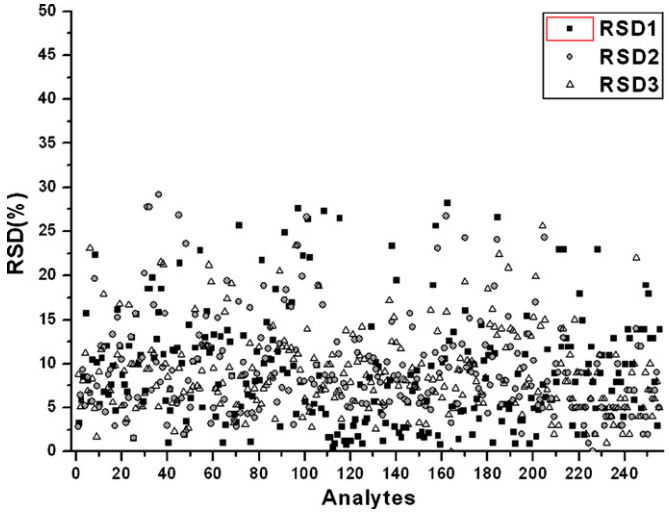

Fig. 4. The RSD of 255 analytes in raw milk ($n = 6$).

Table 4
The retention time, transitions, linearity, range of average recovery, range of RSD and LOQs of 39 compounds in raw milk by LC Run 3 in ESI(−) mode ($n = 6$).

Compounds		RT (min)	Transition 1[a]	Transition 2[b]	r[c]	Range of average R (%)[d]	Range of RSD (%)	LOQs (μg/kg)
Amphenicols (3)								
207	Thiamphenicol	2.77	354.1[e] > 289.9	354.1 > 184.8	0.999	91–123	13–19	0.5
208	Florfenicol	3.23	356.1[e] > 335.8	356.1 > 184.9	0.996	84–103	14–22	0.5
209	Chloramphenicol	3.79	321.2[e] > 152.0	321.2 > 257.2	0.995	94–121	6–15	0.2
Sulfonamides (1)								
210	Sulfanitran	4.22	334.3[e] > 136.2	334.3 > 270.0	0.998	93–98	3–9	0.5
NSAIDs (11)								
211	Salicylic acid	2.16	136.9[e] > 92.9	136.9 > 64.9	0.999	94–141	11–22	4.0
212	3,5-Dinitro-o-toluamide	3.34	224.0[e] > 150.9	224.0 > 180.9	0.982	98–124	10–14	2.5
213	Nitromide	3.53	210.1[e] > 42.0	210.1 > 167.0	0.996	91–123	13–23	2.5
214	Phenylbutazone	4.39	307.3[e] > 279.2	307.3 > 131.0	0.995	103–114	7–10	0.5
215	Diclofenac	4.86	250.1[e] > 214.1	252.2 > 178.0	0.986	89–109	8–12	0.5
216	Ibuprofen	4.95	205.2[e] > 161.1	–	0.990	94–101	2–13	0.5
217	Indomethacine	4.95	356.3[e] > 312.2	356.3 > 297.2	0.996	97–109	3–6	0.5
218	Carprofen	4.98	228.1[e] > 226.1	–	0.986	85–102	5–15	0.5
219	Mefenamic	5.01	240.1[e] > 196.1	240.1 > 179.9	0.984	96–103	2–9	0.5
220	Tolfenamic acid	5.14	260.1[e] > 216.1	262.1 > 218.1	0.997	81–97	5–18	0.5
221	Tribromosalicylanilide	5.28	450.0[e] > 250.8	450.0 > 80.7	0.995	79–105	8–15	0.5
Anticoccidials (6)								
222	Diflunisal	4.39	249.3[e] > 205.1	249.3 > 157.0	0.998	115–121	2–5	0.5
223	Toltrazuril-sulfoxide	5.01	440.3[e] > 440.3	440.3 > 371.2	0.997	97–103	2–9	0.5
224	Ponazuril	5.38	456.3[e] > 456.3	–	0.998	85–115	6–10	0.5
225	Diclazuril	5.38	407.2[e] > 336.1	405.2 > 334.1	0.997	86–97	6–12	0.5
226	Nicarbazine	5.47	301.3[e] > 136.9	301.3 > 106.9	0.997	92–114	6–12	0.5
227	Toltrazuril	5.78	424.3[e] > 424.2	–	0.997	99–109	2–5	0.5
Enviromental hormones (2)								
228	Bisphenol F (BPF)	4.42	198.9[e] > 92.8	198.9 > 104.9	0.990	84–96	9–23	0.5
229	Bisphenol A (BPA)	4.90	227.3[e] > 212.1	227.3 > 133.1	0.991	97–130	9–11	0.5
Anabolic steroids (6)								
230	Estriol	4.32	287.3[e] > 171.1	287.2 > 145.1	0.989	83–94	5–11	1.0
231	Zeranol	5.28	321.4[e] > 277.1	321.4 > 303.0	0.998	97–109	3–6	0.5
232	Diethylstilbestrol	5.30	267.2[e] > 251.1	267.2 > 237.1	0.997	96–107	1–4	0.5
233	Estrone	5.31	269.3[e] > 145.1	269.3 > 143.0	0.992	97–104	5–11	0.5
234	Dienoestrol	5.34	265.2[e] > 93.0	265.2 > 249.1	0.997	95–104	4–7	0.5
235	Hexestrol	5.43	269.2[e] > 134.1	269.2 > 119.0	0.999	101–110	3–13	0.5
Mycotoxins (2)								
236	Zearalanone	5.41	319.4[e] > 275.1	319.4 > 205.0	0.996	95–110	2–11	0.25
237	Zearalenone	5.47	317.4[e] > 130.9	317.4 > 174.9	0.999	97–104	4–10	0.25
Glucocorticoids (5)								
238	Prednison	6.05	327.4[f] > 149.1	327.4 > 299.2	0.999	90–96	5–11	2.0
239	Cortisone	6.11	329.3[f] > 137.0	329.3 > 311.2	0.998	87–100	5–10	2.0
240	Prednisolone	6.29	329.3[f] > 280.2	329.3 > 295.2	0.994	80–91	3–8	2.0
241	Dexamethasone	5.01	361.3[f] > 307.1	361.3 > 325.2	0.998	87–121	2–4	0.5
242	Methylprednisolone	6.69	343.4[f] > 394.2	343.4 > 309.3	1.000	85–90	5–10	2.0
Pesticides (13)								
243	Gibberellic acid	2.80	345.3[e] > 239.1	345.3 > 142.9	1.000	97–104	4–10	0.5
244	Cloprop	3.58	198.8[e] > 126.8	198.8 > 70.8	0.986	90–97	5–6	0.5
245	2,4-D	3.92	218.6[e] > 160.7	218.6 > 124.8	0.985	84–103	14–22	2.0
246	Dichlorprop	4.30	232.9[e] > 160.9	232.9 > 124.9	0.992	89–105	4–14	0.5
247	Cyclanilide	4.39	272.2[e] > 159.8	272.2 > 227.9	0.997	77–117	4–12	0.5
248	Tralkoxydim	4.59	328.4[e] > 254.0	328.4 > 284.4	0.996	81–97	2–14	0.5
249	2,4,5-T	4.60	254.5[e] > 212.1	254.5 > 133.9	0.985	92–108	9–19	2.0
250	Acifluorfen	4.80	360.2[e] > 315.9	360.2 > 194.8	0.996	99–105	2–18	0.5
251	MCPB	4.80	227.4[e] > 140.8	–	0.985	86–97	7–13	2.0
252	Fomesafen	4.82	437.2[e] > 194.8	437.2 > 221.9	0.999	100–104	4–13	0.5
253	Dicloran	5.03	205.1[e] > 174.8	205.1 > 168.8	0.989	86–121	6–8	0.5
254	Hexaflumuron	6.01	459.2[e] > 438.8	459.2 > 174.8	0.999	91–115	3–9	0.5
255	Lufenuron	6.30	509.1[e] > 174.8	509.1 > 338.9	0.999	95–107	5–14	0.5

[a] Quantification transition; [b] qualification transition; [c] regression coefficient; [d] recovery; [e] $[M-H]^-$, [f] $[M-CH_3O]^-$.

PSA as QuEChERS sorbents in pure acetonitrile, the similar results had given by Boscher et al. [18] and Mastovska and Lightfield [22].

Therefore, the sample preparation method employed in this study was superior to previously reported analytical methods and the characteristics of generality, simplicity, and speed allowed to achieve more rapid and high-throughput analysis.

3.3. Method validation

3.3.1. Selectivity, linearity, and LOQs

According to the analysis of 10 blank raw milk samples, this UPLC–MS/MS method provided clean and background-free mass traces for the matrix studied, demonstrating that the method had satisfactory selectivity.

Quantitative analysis was carried out using an external standard. Matrix-fortified standard calibration curves used to compensate for the matrix effects and losses in sample preparation achieved satisfactory accuracy of the method. The range of linearity was from 0.982 to 1.000, good linearity was found for over 90% of target compounds with linear regression coefficients (R) higher than 0.99. The limits of quantification (LOQs) were from 0.05 to 10 μg/kg, which were usually sufficient to verify compliance of products with EU legal tolerances for most analytes. The results of linearity and LOQs were summarized in Tables 2–4.

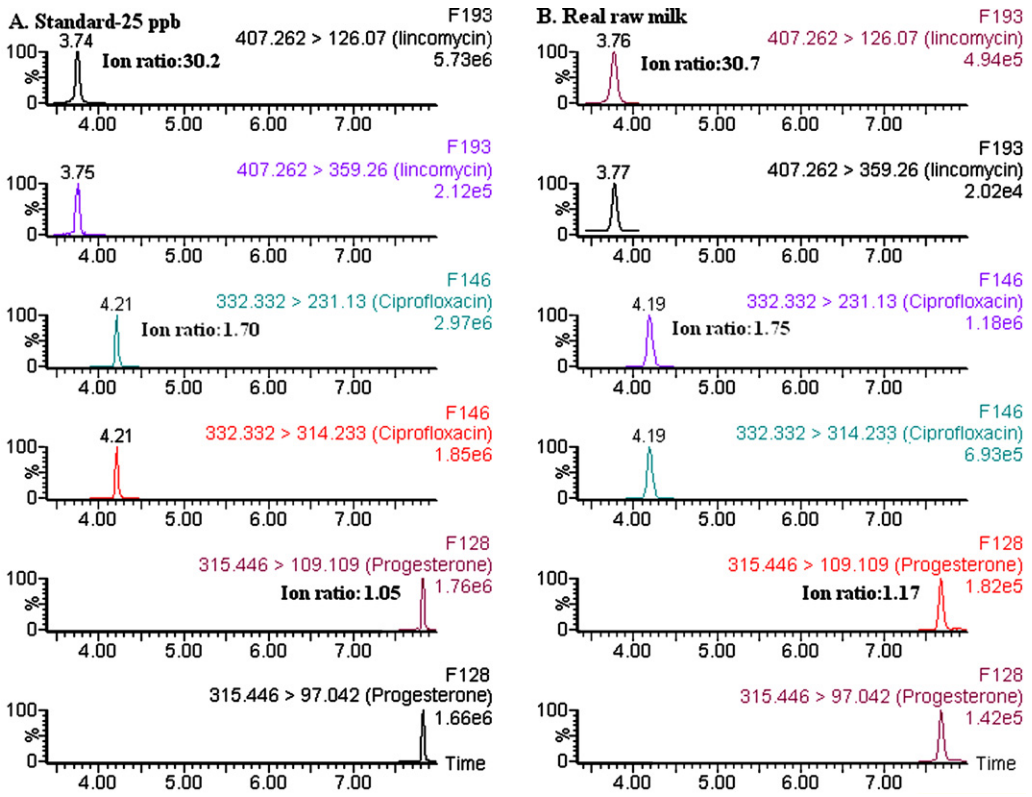

Fig. 5. Chromatograms of standard solution (A), a real raw milk sample positive for lincomycin, ciprofloxacin and progesterone (B).

3.3.2. *Precision and recovery*

The average relative recoveries of three different concentration levels, calculated based on matrix-fortified calibration curves, were in the range of 63–141% (Fig. 3). The range of the corresponding precision was from 1% to 29% (Fig. 4). For over 80% of the analytes, recoveries were between 70% and 120% and precision were mostly in the range of 1–15%. The overall recovery and precision measurements obtained by three fortified levels were summarized in Tables 2–4.

3.4. *Application of the method to real samples*

The proposed method was applied to the analysis of 20 real raw milk samples. Analysis showed that endogenous progesterone was presented in each sample with the range of concentration from 2.7 to 10.3 μg/kg. Five samples were found containing lincomycin (0.14–13.7 μg/kg). Fourteen samples were found containing ciprofloxacin (0.59–25.4 μg/kg). The values were much lower than the EU MRL for them (150 and 100 μg/kg). Confirmatory analysis was performed according to the revised EU criteria (2002/657/EC) [23], and the applying MRM of 2 fragment ion transitions (except for carprofen, MCPB, ibuprofen, toltrazuril, ponazuril with only one transition for each) could reduce the risk of false positives. Moreover, the ratio between different product ions and relative retention time provided additional identification and confirmation, as shown in Fig. 5.

4. Conclusions

This work proposed a generic multi-class analysis to extract and quantify 255 veterinary drug residues and other contaminants with low molecule weight in raw milk. The developed extraction and cleanup steps were easy and rapid, and the straightforward sample preparation also could reduce error sources. Furthermore, utilizing the UPLC technology shortened analysis times for all analytes. Therefore, application of the analytical method employed in this study, which improved efficiency and coverage of residues, would imply a drastic reduction of both effort and time in routine monitoring programs.

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