Note

Development and Validation of Rapid Analysis Method for Multi-Class Veterinary Drugs in Livestock Products by LC-MS/MS

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A method for rapid analysis of multi-class residual veterinary drugs in livestock products was developed and validated in accordance with the Japanese guideline for pesticides. Using LC-MS/MS, 43 multi-class veterinary drugs, including sulfonamides, quinolones, coccidiostats and antiparasites, could be analyzed simultaneously in only 18 minutes. The extraction process was developed by modifying the QuEChERS approach to provide faster and less expensive extraction. The samples were extracted by using two kinds of solvent, acetonitrile and acetonitrile including 1 vol% formic acid, and salted out with magnesium sulfate, trisodium citrate and sodium chloride. Using these two extractants, 40 out of 43 drugs satisfied the guideline criteria in bovine muscle and swine muscle, 39 drugs were found in chicken muscle, and 37 drugs were found in eggs. The limit of quantification was less than the MRL for all analytes.

Key words: veterinary drug; livestock product; LC-MS/MS; multi-class analysis; QuEChERS approach

Veterinary drugs are often used on farms to treat and prevent diseases. However over-dosing and noncompliance with the discontinuation period could cause the accumulation of drugs in animal tissues; therefore there is a risk of human consumption of drug-contaminated livestock products that could lead to emergence of drug-resistant bacteria, allergic reaction and toxicity¹⁾.

Veterinary drugs are divided into many classes, including synthetic antibacterials, antibiotics, antiparasites, hormones and pesticides. Each class covers a wide variety of chemical and physical properties from hydrophilic to lipophilic, and acidic to basic; therefore it is difficult to simultaneously analyze many drugs even in the same class. Although many reports have described simultaneous analysis of multiple veterinary drugs in livestock products²⁾⁻⁷⁾, they do not cover the most commonly used drugs on farms, *i.e.*, the drugs most frequently detected and reported at testing institutions such as quarantine stations. Furthermore, the sample solutions in the previous analytical methods require considerable time to prepare and are expensive, because they require a range of solvents, reagents and experi-

mental instruments.

In recent years, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach has attracted great interest⁸⁾⁻¹⁵⁾. However, there are few reports on residual veterinary drugs in livestock samples¹⁶⁾⁻²¹⁾. In addition, the reported methods could not analyze multi-class drugs, such as polyethers and macrolides that are frequently used and detected in livestock.

The aim of this study was to develop a rapid and easy analytical method for residual veterinary drugs in livestock products by modifying the QuEChERS approach, and to validate the method in accordance with the Japanese guidelines for pesticides^{*1)}.

Materials and Methods

Samples

Samples (bovine muscle, swine muscle, chicken muscle and eggs) were purchased from local supermarkets in Tokyo and were confirmed to be free of the targeted drugs. The tissues were minced with an electric household food processor and stored at -20°C .

Chemicals and reagents

Powdered standards for testing residual veterinary drugs were purchased from Dr Ehrenstorfer (Augsburg, Germany), Hayashi Pure Chemical Industries (Osaka, Japan), Kanto Chemical Company (Tokyo, Japan), LKT Laboratories (St. Paul, U.S.A.), Sigma-Aldrich (St.

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*¹⁾ Guidelines for the Validation of Analytical Method for Residual Agricultural Chemicals in Food (November 15, 2007, Notification No. 1115001). The Ministry of Health, Labour and Welfare of Japan.

Louis, U.S.A.) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Stock standard solutions of individual compounds were prepared as described below. Portions of approximately 5 mg of fenbendazole, flubendazole, nicarbazine, oxfendazole and oxfendazole sulfone powder were weighed and dissolved in 5 mL of dimethylformamide (HPLC grade), then diluted with methanol (HPLC grade) to 50 mL. Decoquinatone was dissolved in 5 mL chloroform (analytical grade) and then diluted with methanol. Oxolinic acid, pyrimethamine and sulfadimidine were dissolved in 50 mL of acetonitrile (HPLC grade), and other compounds were dissolved in 50 mL of methanol. These solutions were stored at -20°C . A working standard solution was prepared by mixing 500 μL of all stock standard solutions and diluting the mixture with acetonitrile to 50 mL. When stored at 4°C , this solution was stable for three months.

Acetonitrile, distilled water (HPLC grade), methanol, formic acid (LC/MS grade), ammonium acetate (LC/MS grade), magnesium sulfate (analytical grade) and sodium chloride (analytical grade) were purchased from Wako Pure Chemical Industries. Trisodium citrate dehydrate (analytical grade) and dimethylformamide were purchased from Kanto Chemical Company.

LC-MS/MS conditions

LC-MS/MS conditions are shown in Table 1.

Optimization was performed by direct infusion of 1 $\mu\text{g}/\text{mL}$ of each standard solution, and the optimized conditions for selective reaction monitoring (SRM) transitions are presented in Table 2.

Sample preparation

A 5 g aliquot of each minced sample was weighed into a 50 mL polypropylene centrifuge tube and then 5 mL of distilled water and 15 mL of 1 vol% formic acid in acetonitrile (method A) or acetonitrile (method B) were added. The mixture was homogenized thoroughly using an Ultra-Turrax T25 (IKA), then 4 g of magnesium sulfate, 1.5 g of trisodium citrate dehydrate and 2 g of sodium chloride were added, and the tube was vortexed immediately for 1 min. The mixture was centrifuged at $1,800\times g$ for 10 min with a multipurpose refrigerated centrifuge

AX-320 (Tomy), and the supernatant was transferred into a 20 mL volumetric flask. After dilution with 1 vol% formic acid in acetonitrile or acetonitrile to volume, a portion of the solution was transferred to a 1.5 mL microtube and centrifuged at $16,000\times g$ for 10 min with a microcentrifuge 5415R (Eppendorf). Three μL of supernatant was injected into the LC-MS/MS.

Quantification

Matrix-matched calibration curves were prepared. Blank samples were prepared as above, and then matrix-matched standard solutions were prepared by mixing 900 μL of blank solution and 100 μL of an appropriately diluted working solution. The final concentrations of matrix-matched standard solutions were 1, 2.5, 10, 25 and 50 $\mu\text{g}/\text{L}$.

Method validation

The method was validated according to the "Guidelines for the Validation of Analytical Methods for Residual Agricultural Chemicals in Food" by the Ministry of Health, Labour and Welfare, Japan. Accuracy, repeatability and within-laboratory reproducibility were determined by means of fortified recovery tests at using two different concentrations, 10 and 100 $\mu\text{g}/\text{kg}$, performed with two samples per day on five different days. The results were evaluated by one-way analysis of variance. Limits of quantification (LOQ) were calculated by analyzing blank samples fortified at 0.25, 0.5, 1, 2 and 2.5 $\mu\text{g}/\text{kg}$ and they were determined as the lowest concentration of an analyte for which the signal-to-noise ratio was over 10. Selectivity was determined by analyzing blank samples.

Results and Discussion

LC-MS/MS conditions

With reference to previous reports^{1)-4),22)}, a mobile phase consisting of 0.1 vol% formic acid in 10 mmol/L ammonium acetate and acetonitrile was chosen. The ammonium-adduct ions were most sensitively detected for ivermectin and doramectin, and the deprotonated ions for diclazuril, florfenicol, nicarbazine, triclabendazole sulfone and triclabendazole sulfoxide, while for the oth-

Table 1. LC-MS/MS conditions

HPLC system	Prominence series (Shimadzu Corporation)
HPLC column	Syncronis aQ (2.1 mm i.d. $\times$ 100 mm, 5 μm , Thermo Scientific)
Column temperature	40°C
Flow rate	0.3 mL/min
Mobile phase	(A) 0.1 vol% formic acid in 10 mmol/L ammonium acetate (B) acetonitrile
Gradient	(A% : B%) = 0 min (95 : 5) $\rightarrow$ 7 min (5 : 95) $\rightarrow$ 12 min (5 : 95) $\rightarrow$ 12.1 min (95 : 5) $\rightarrow$ 17 min (95 : 5)
Injection volume	3 μL
MS system	TSQ Quantum Access MAX (Thermo Scientific)
Ionization	ESI positive, negative
Analysis mode	Selective reaction monitoring (SRM)
Ion-spray voltage	(positive) 3,000 V (negative) 2,500 V
Vaporizer temperature	460°C
Capillary temperature	220°C

Table 2. MS/MS parameters for each analyte

Analyte	R.T. (min)	SRM transition				Tube lens (V)
		ESI	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Collision energy (eV)	
Clopidol	2.58	+	192	101	24	122
5-Hydroxythiabendazole	2.67	+	218	108	37	105
Levamisole	2.80	+	205	91	35	102
5-Propylsulfonyl-1 <i>H</i> - benzimidazole-2-amine	2.83	+	240	133	30	82
Sulfathiazole	2.83	+	256	92	26	109
Trimethoprim	3.01	+	291	123	26	94
Sulfamerazine	3.07	+	265	92	29	93
Ciprofloxacin	3.11	+	332	314	20	119
Ormethoprim	3.17	+	275	123	21	107
Enrofloxacin	3.31	+	360	316	18	116
Thiabendazole	3.33	+	202	65	40	106
Sulfadimidine	3.33	+	279	186	16	93
Sarafloxacin	3.60	+	386	368	26	138
Sulfamonomethoxine	3.60	+	281	108	25	82
Sulfamethoxazole	3.87	+	254	92	25	96
Florfenicol	3.91	—	356	336	13	—87
Oxfendazole	3.91	+	316	159	31	120
Pyrimethamine	4.03	+	249	177	28	114
Oxolinic acid	4.05	+	262	244	19	106
Sulfaquinoxaline	4.27	+	301	108	26	95
Sulfadimethoxine	4.27	+	311	156	20	96
Oxfendazole sulfone	4.43	+	332	300	22	118
Nalidixic acid	4.68	+	233	215	13	97
Flubendazole	4.84	+	314	123	34	123
Fenbendazole	5.51	+	300	268	19	103
Nicarbazin	5.94	—	301	137	16	—65
Triclabendazole sulfoxide	5.90	—	373	181	46	—78
Nequinat	6.18	+	366	145	47	100
Buquinolate	6.22	+	362	204	35	95
Diclazuril	6.23	—	405	334	22	—70
Triclabendazole sulfone	6.25	—	391	312	29	—102
Triclabendazole	6.65	+	359	198	25	124
Decoquinat	7.20	+	418	204	41	107
Eprinomectin	7.20	+	915	112	55	113
Semduramicin	7.95	+	891	630	22	126
Doramectin	8.10	+	917	331	24	138
Lasalocid	8.25	+	609	237	23	112
Moxidectin	8.39	+	641	499	12	113
Ivermectin	8.49	+	893	570	13	113
Maduramicin	8.73	+	935	630	21	132
Salinomycin	8.79	+	769	734	17	94
Monensin	8.84	+	689	461	23	119
Narasin	9.16	+	783	748	17	114

ers, protonated forms were most sensitively detected.

Next, HPLC columns were examined, because analysis of multi-class drugs from hydrophilic compounds such as like sulfonamides to hydrophobic compounds such as polyethers is difficult, particularly from the viewpoints of peak shapes and good separation. L-column2 ODS (Chemicals Evaluation Research Institute), Atlantis T3 (Waters), Accucore RP-MS (Thermo Scientific) and Synchronis aQ (Thermo Scientific) were compared. Though they all basically have ODS chains, except for Accucore RP-MS (which has optimized alkyl chain length and slightly weaker retention than ODS),

Atlantis T3 and Synchronis aQ were the most suitable for highly polar compounds because they are more retentive than other conventional ODS columns.

Using L-column2 ODS, the peak shapes of sulfadimidine and sulfamerazine were poor, showing splitting. Although Atlantis T3 produced good peak shapes for sulfonamides, polyethers such as lasalocid and narasin could not be eluted from the column. In contrast, Accucore RP-MS produced good chromatograms of macrolides and polyethers, but hydrophilic analytes were not retained in the column. On the other hand, Synchronis aQ showed good retention of polar analytes, and it could

also release lipophilic analytes, as well as providing good separation and peak shapes. Therefore, we adopted Synchrois aQ.

Extraction process

The extraction process was developed using chicken muscle that was confirmed not to contain the target analytes. All studies below were performed by fortifying 50 μ L of 1 μ g/mL working standard mixture into this sample, and acetonitrile was used as the extraction sol-

vent. With reference to the previous report¹⁶⁾, the method was modified as described below, and we also changed the clean-up step from filtration to high-speed centrifugation.

First, buffering effects were compared between sodium acetate buffer and sodium citrate buffer. Using acetate buffer, less than 40% of the target analytes were recovered; especially, quinolones, polyethers and macrolides showed very low recoveries. On the other hand, more than 60% of all analytes, except fluoroquinolones,

Fig. 1. Effect of the concentration of formic acid in the extraction solvent on recovery of 10 μ g/kg standard spiked into chicken muscle

Fig. 2. Effect of different amounts of sodium chloride for salting out on recovery of 10 μ g/kg standard spiked into chicken muscle

Table 3. Validation results of method A

Analyte	Bovine muscle				Swine muscle			
	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility (%)	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility (%)
Clopidol	0.01	59.9	6.1	7.5	0.01	57.4	12.2	17.5
	0.1	64.4	9.2	21.8	0.1	64.4	9.7	9.1
5-Hydroxythiabendazole	0.01	43.8	18.0	22.9	0.01	51.7	31.3	37.8
	0.1	51.6	16.5	17.0	0.1	61.5	9.9	10.8
Levamisole	0.01	79.1	6.5	7.0	0.01	74.1	4.0	13.4
	0.1	76.7	4.5	9.8	0.1	74.4	3.6	5.8
5-Propylsulfonyl-1 <i>H</i> -benzimidazole-2-amine	0.01	60.0	6.6	9.5	0.01	57.6	11.6	14.8
	0.1	63.8	4.9	5.3	0.1	66.6	5.5	7.2
Sulfathiazole	0.01	67.1	11.6	12.5	0.01	76.0	7.1	8.9
	0.1	78.6	4.8	12.8	0.1	85.8	3.6	6.5
Trimethoprim	0.01	70.4	3.6	4.7	0.01	68.6	7.2	8.0
	0.1	74.9	5.7	9.1	0.1	76.3	3.6	4.1
Sulfamerazine	0.01	74.4	5.6	11.7	0.01	77.7	12.4	14.7
	0.1	82.3	2.3	9.6	0.1	85.3	3.4	4.9
Ciprofloxacin	0.01	46.7	11.6	11.9	0.01	45.1	10.0	11.9
	0.1	42.9	10.6	10.8	0.1	50.0	5.8	8.1
Ormethoprim	0.01	71.9	5.5	5.9	0.01	71.1	6.3	7.9
	0.1	72.7	4.4	12.0	0.1	77.7	3.4	3.9
Enrofloxacin	0.01	73.3	1.6	2.1	0.01	65.8	5.7	5.8
	0.1	70.9	3.5	9.5	0.1	73.0	3.0	3.7
Thiabendazole	0.01	59.7	8.0	12.7	0.01	63.2	11.0	11.6
	0.1	67.0	6.0	13.3	0.1	59.8	6.1	7.2
Sulfadimidine	0.01	83.1	2.4	3.1	0.01	82.1	4.8	7.8
	0.1	83.2	2.0	5.3	0.1	84.3	2.5	4.1
Sarafloxacin	0.01	66.0	6.1	9.0	0.01	70.0	8.0	9.1
	0.1	73.3	3.7	6.2	0.1	70.9	1.6	3.5
Sulfamonomethoxine	0.01	81.6	2.3	3.5	0.01	79.9	4.0	6.7
	0.1	79.5	1.8	9.5	0.1	87.2	2.0	3.9
Sulfamethoxazole	0.01	80.9	5.5	5.7	0.01	85.5	8.6	11.3
	0.1	85.1	3.5	9.3	0.1	93.6	1.8	3.8
Florfenicol	0.01	78.0	9.8	10.2	0.01	78.1	5.6	7.0
	0.1	87.0	5.4	7.0	0.1	85.0	8.3	8.3
Oxfendazole	0.01	86.4	1.3	3.2	0.01	77.4	4.8	7.6
	0.1	79.2	1.4	4.2	0.1	79.3	2.2	5.2
Pyrimethamine	0.01	75.7	1.4	2.3	0.01	73.5	4.7	7.9
	0.1	75.8	2.9	6.3	0.1	76.6	2.1	3.8
Oxolinic acid	0.01	81.5	1.6	3.1	0.01	80.9	3.6	7.1
	0.1	81.9	2.5	7.4	0.1	79.8	1.2	3.5
Sulfaquinoxaline	0.01	80.3	7.4	8.7	0.01	92.9	7.0	8.2
	0.1	81.3	2.0	11.0	0.1	101.7	2.7	5.1
Sulfadimethoxine	0.01	81.7	4.2	4.2	0.01	86.2	4.2	6.4
	0.1	86.3	2.5	4.9	0.1	93.8	2.6	4.9
Oxfendazole sulfone	0.01	86.0	2.2	3.2	0.01	84.1	4.1	6.8
	0.1	84.4	4.5	5.1	0.1	84.3	3.3	5.0
Nalidixic acid	0.01	82.5	2.5	3.9	0.01	81.2	4.6	5.9
	0.1	82.8	2.5	7.2	0.1	82.2	1.6	4.2
Flubendazole	0.01	81.9	2.5	2.6	0.01	81.5	3.4	6.8
	0.1	81.2	9.0	9.3	0.1	81.4	3.7	6.1
Fenbendazole	0.01	70.4	3.0	5.6	0.01	81.4	4.0	9.0
	0.1	78.3	11.5	11.9	0.1	75.8	4.1	8.4
Nicarbazin	0.01	75.7	4.8	7.2	0.01	80.9	5.4	5.5
	0.1	85.4	2.6	2.8	0.1	70.4	2.2	4.0
Triclabendazole sulfoxide	0.01	72.3	5.0	8.2	0.01	71.6	7.0	11.3
	0.1	80.4	2.6	5.1	0.1	71.0	5.3	5.9
Nequinat	0.01	70.4	3.7	6.3	0.01	74.9	3.0	5.4
	0.1	72.0	7.5	9.4	0.1	74.1	2.2	4.6
Buquinolate	0.01	77.8	3.2	3.9	0.01	78.2	3.6	6.8
	0.1	75.0	4.7	6.6	0.1	77.1	2.4	5.6
Diclazuril	0.01	84.6	7.6	10.0	0.01	82.5	12.1	14.1
	0.1	88.5	4.4	6.4	0.1	77.7	3.3	4.3
Triclabendazole sulfone	0.01	80.6	5.9	7.0	0.01	84.5	4.6	4.7
	0.1	91.5	5.3	6.2	0.1	79.3	2.5	3.3
Triclabendazole	0.01	62.9	6.2	10.4	0.01	79.5	5.2	8.5
	0.1	69.6	10.1	15.4	0.1	64.7	13.4	13.6
Decoquinat	0.01	95.9	4.8	7.0	0.01	87.5	7.6	10.6
	0.1	87.8	5.0	9.2	0.1	108.2	4.7	12.9
Eprinomectin	0.01	66.1	13.8	19.3	0.01	75.9	25.2	65.7
	0.1	84.0	12.2	15.0	0.1	62.4	18.4	24.6
Semduramicin	0.01	58.0	24.5	34.5	0.01	83.6	5.6	8.7
	0.1	94.8	10.9	11.4	0.1	47.1	24.8	36.6
Doramectin	0.01	45.1	42.2	50.6	0.01	71.2	22.5	27.2
	0.1	72.6	6.8	18.0	0.1	43.2	22.0	30.1
Lasalocid	0.01	58.2	13.7	14.5	0.01	73.0	6.5	11.5
	0.1	70.0	5.9	8.7	0.1	55.2	7.0	20.7
Moxidectin	0.01	47.3	17.2	17.8	0.01	80.1	18.1	18.4
	0.1	77.1	6.5	14.1	0.1	41.4	18.1	24.7
Ivermectin	0.01	63.6	8.3	10.7	0.01	82.2	9.9	11.2
	0.1	80.6	8.9	11.7	0.1	55.2	8.3	19.3
Maduramicin	0.01	91.3	5.1	6.4	0.01	81.2	5.7	11.1
	0.1	89.9	5.2	9.3	0.1	81.8	6.4	8.1
Salinomycin	0.01	66.0	5.2	8.6	0.01	79.3	7.2	12.0
	0.1	93.2	4.2	8.4	0.1	69.8	5.6	8.8
Monensin	0.01	71.8	7.6	7.8	0.01	81.5	9.5	11.9
	0.1	88.2	7.6	7.6	0.1	67.1	9.6	12.9
Narasin	0.01	78.4	11.9	13.3	0.01	82.3	5.8	6.9
	0.1	93.2	3.3	6.5	0.1	75.7	2.9	7.2

Table 3. Continued

Analyte	Chicken muscle				Egg			
	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility (%)	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility (%)
Clopidol	0.01	63.6	7.1	7.7	0.01	76.6	11.9	21.5
	0.1	71.6	7.9	22.0	0.1	89.2	9.8	27.4
5-Hydroxythiabenzazole	0.01	52.7	15.9	16.6	0.01	71.1	17.7	20.1
	0.1	43.5	17.3	21.6	0.1	80.4	12.8	13.2
Levamisole	0.01	76.3	8.2	9.1	0.01	78.3	8.7	12.8
	0.1	81.4	3.9	6.8	0.1	76.4	6.1	8.2
5-Propylsulfonyl-1 <i>H</i> -benzimidazole-2-amine	0.01	54.2	10.8	14.0	0.01	67.2	10.1	10.2
	0.1	60.0	3.0	6.8	0.1	63.9	7.3	8.0
Sulfathiazole	0.01	81.2	11.4	11.6	0.01	110.8	5.2	19.9
	0.1	87.3	5.5	8.3	0.1	127.1	3.8	16.8
Trimethoprim	0.01	68.7	3.5	5.9	0.01	80.3	3.5	8.1
	0.1	74.8	3.4	5.7	0.1	85.2	3.8	6.2
Sulfamerazine	0.01	79.7	11.9	13.4	0.01	90.3	6.7	9.8
	0.1	87.7	3.8	5.4	0.1	93.1	3.5	7.6
Ciprofloxacin	0.01	44.9	4.0	7.1	0.01	55.3	14.6	17.0
	0.1	35.2	8.5	9.2	0.1	44.1	5.0	6.4
Ormethoprim	0.01	70.3	5.3	6.7	0.01	87.3	3.3	10.0
	0.1	77.7	4.7	5.4	0.1	94.9	2.3	7.5
Enrofloxacin	0.01	70.4	4.3	7.8	0.01	86.7	3.5	4.8
	0.1	71.3	3.4	6.0	0.1	84.4	4.9	7.4
Thiabenzazole	0.01	70.2	5.4	7.3	0.01	59.3	16.2	17.2
	0.1	66.5	7.6	10.6	0.1	66.5	5.1	8.9
Sulfadimidine	0.01	83.4	2.8	4.5	0.01	93.3	2.5	4.2
	0.1	90.2	1.9	2.8	0.1	88.8	1.8	4.3
Sarafloxacin	0.01	68.1	7.5	7.8	0.01	110.8	8.2	10.7
	0.1	68.3	4.5	7.2	0.1	92.7	4.8	7.9
Sulfamonomethoxine	0.01	83.1	3.8	4.6	0.01	114.4	3.6	13.5
	0.1	88.3	2.3	3.2	0.1	100.4	2.6	12.3
Sulfamethoxazole	0.01	80.9	4.1	9.2	0.01	108.0	7.0	13.1
	0.1	93.2	3.1	3.2	0.1	108.0	2.8	11.2
Florfenicol	0.01	74.1	7.2	8.6	0.01	153.9	10.4	29.0
	0.1	84.9	2.7	11.6	0.1	128.9	4.0	18.2
Oxfendazole	0.01	84.4	11.3	11.4	0.01	103.9	4.3	11.8
	0.1	89.8	2.3	2.7	0.1	95.5	2.2	8.2
Pyrimethamine	0.01	73.2	5.5	5.7	0.01	82.5	2.4	4.2
	0.1	80.1	3.3	3.6	0.1	75.3	2.6	4.9
Oxolinic acid	0.01	83.4	1.6	2.2	0.01	91.2	2.5	3.7
	0.1	87.5	2.6	3.2	0.1	82.8	1.8	4.0
Sulfaquinoxaline	0.01	94.8	3.5	5.4	0.01	135.4	5.7	22.1
	0.1	109.0	3.0	3.0	0.1	187.8	2.7	22.6
Sulfadimethoxine	0.01	90.9	1.6	2.1	0.01	99.5	2.6	8.8
	0.1	103.3	2.6	2.8	0.1	132.0	1.4	8.6
Oxfendazole sulfone	0.01	92.7	2.2	4.4	0.01	104.8	3.8	9.2
	0.1	95.6	2.7	3.0	0.1	235.2	2.9	7.2
Nalidixic acid	0.01	85.6	2.2	4.3	0.01	109.2	1.6	10.0
	0.1	90.7	2.1	4.4	0.1	184.3	2.8	12.5
Flubendazole	0.01	92.5	4.8	4.8	0.01	121.5	1.8	14.6
	0.1	96.6	2.3	4.9	0.1	221.0	1.9	15.6
Fenbendazole	0.01	91.7	2.2	3.0	0.01	99.4	2.1	8.9
	0.1	95.5	2.9	4.2	0.1	91.8	1.7	8.3
Nicarbazin	0.01	67.6	4.6	7.5	0.01	89.4	4.0	8.6
	0.1	81.1	3.3	4.7	0.1	86.3	2.6	5.8
Triclabendazole sulfoxide	0.01	67.2	17.0	17.5	0.01	85.8	7.2	10.3
	0.1	76.5	7.6	7.9	0.1	87.9	4.0	5.1
Nequinat	0.01	77.1	4.7	6.7	0.01	105.8	3.4	12.0
	0.1	81.5	1.4	4.8	0.1	97.7	3.1	9.5
Buquinolate	0.01	79.2	2.7	8.5	0.01	113.0	3.1	11.8
	0.1	83.9	2.3	5.0	0.1	103.9	2.3	8.9
Diclazuril	0.01	72.0	7.5	7.5	0.01	124.7	6.8	23.7
	0.1	77.9	3.8	7.5	0.1	119.0	5.6	16.4
Triclabendazole sulfone	0.01	72.5	5.3	10.2	0.01	118.9	1.3	14.3
	0.1	79.7	5.2	9.3	0.1	112.1	2.2	7.1
Triclabendazole	0.01	79.6	2.5	5.1	0.01	98.2	14.6	15.2
	0.1	85.3	4.3	6.6	0.1	103.1	6.4	9.9
Decoquinat	0.01	71.9	5.2	28.5	0.01	194.1	7.0	25.6
	0.1	75.8	8.1	21.8	0.1	284.3	9.5	19.7
Eprinomectin	0.01	73.2	36.0	40.1	0.01	81.9	16.7	18.0
	0.1	83.0	29.2	33.3	0.1	135.8	26.9	30.4
Semduramicin	0.01	65.8	21.3	24.2	0.01	85.7	6.9	21.3
	0.1	86.4	16.0	18.4	0.1	139.3	9.7	22.7
Doramectin	0.01	53.5	37.3	50.3	0.01	165.5	23.3	42.8
	0.1	97.7	15.7	31.3	0.1	222.9	14.0	20.5
Lasalocid	0.01	55.5	6.6	10.9	0.01	94.1	10.3	13.2
	0.1	79.2	4.2	13.9	0.1	126.1	7.0	12.8
Moxidectin	0.01	59.8	32.6	33.5	0.01	93.5	22.8	24.6
	0.1	84.7	3.8	14.0	0.1	144.0	10.2	14.7
Ivermectin	0.01	80.3	5.6	12.0	0.01	113.1	12.1	20.5
	0.1	94.5	4.8	10.7	0.1	173.4	14.9	18.8
Maduramicin	0.01	82.8	6.4	6.9	0.01	92.2	2.4	15.9
	0.1	95.4	6.3	6.7	0.1	119.7	3.4	14.4
Salinomycin	0.01	59.0	14.5	16.6	0.01	68.7	1.5	31.5
	0.1	67.4	12.8	13.6	0.1	86.1	6.6	31.0
Monensin	0.01	75.9	22.9	27.5	0.01	103.3	4.6	15.0
	0.1	89.3	17.7	21.2	0.1	149.2	10.3	14.1
Narasin	0.01	72.2	2.3	11.4	0.01	90.4	3.9	26.8
	0.1	78.3	2.2	7.3	0.1	108.2	4.3	23.6

not meeting the criteria of the guideline

Table 4. Validation results of method B

Analyte	Bovine muscle				Swine muscle			
	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility (%)	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility (%)
Clopidol	0.01	87.1	20.9	23.2	0.01	83.3	9.5	10.0
5-Hydroxythiabenzazole	0.1	88.5	10.4	13.1	0.1	75.5	8.2	18.5
	0.01	66.5	35.3	44.2	0.01	60.8	27.6	39.3
Levamisole	0.1	90.3	11.4	13.2	0.1	69.0	8.9	16.7
	0.01	95.6	5.5	10.8	0.01	97.9	2.2	9.4
5-Propylsulfonyl-1 <i>H</i> -benzimidazole-2-amine	0.1	89.7	7.5	8.0	0.1	87.2	3.8	10.8
	0.01	79.9	15.0	20.4	0.01	70.7	10.4	14.9
Sulfathiazole	0.1	85.6	11.0	11.6	0.1	70.3	4.2	7.7
	0.01	93.1	7.9	8.1	0.01	93.7	8.7	10.5
Trimethoprim	0.1	94.5	4.6	7.0	0.1	93.3	3.0	14.6
	0.01	89.1	9.3	12.4	0.01	85.0	10.7	13.1
Sulfamerazine	0.1	87.8	4.9	5.9	0.1	79.2	5.0	5.5
	0.01	96.2	8.7	12.2	0.01	113.8	5.0	6.2
Ciprofloxacin	0.1	90.4	6.3	7.9	0.1	92.8	1.9	9.3
	0.01	—	—	—	0.01	14.6	4.1	4.2
Ormethoprim	0.1	4.5	20.9	24.0	0.1	2.9	8.3	13.1
	0.01	89.8	5.2	10.9	0.01	81.2	3.4	5.4
Enrofloxacin	0.1	93.3	7.1	8.5	0.1	80.1	3.1	9.0
	0.01	17.8	28.8	29.0	0.01	12.3	9.9	25.5
Thiabenzazole	0.1	21.1	14.9	16.2	0.1	11.7	8.8	15.1
	0.01	84.3	8.5	9.2	0.01	98.4	7.4	11.7
Sulfadimidine	0.1	90.9	5.3	7.7	0.1	89.3	2.1	11.5
	0.01	93.7	5.9	6.4	0.01	101.9	2.9	4.6
Sarafloxacin	0.1	92.0	3.7	6.6	0.1	93.3	2.4	9.5
	0.01	20.0	40.1	43.5	0.01	14.8	13.4	20.6
Sulfamonomethoxine	0.1	19.7	15.3	15.4	0.1	6.7	14.0	56.6
	0.01	94.2	5.7	10.6	0.01	100.9	2.4	6.2
Sulfamethoxazole	0.1	91.9	5.9	6.3	0.1	93.3	2.5	10.6
	0.01	96.5	2.0	4.2	0.01	106.4	9.3	10.8
Florfenicol	0.1	97.7	3.2	5.2	0.1	98.1	2.0	9.9
	0.01	92.6	11.3	33.0	0.01	78.3	12.2	13.3
Oxfendazole	0.1	103.0	13.2	32.3	0.1	77.1	6.9	15.3
	0.01	97.1	18.2	20.8	0.01	107.9	5.3	30.0
Pyrimethamine	0.1	91.6	9.7	10.9	0.1	86.5	1.5	6.8
	0.01	82.4	7.3	7.4	0.01	78.4	15.2	19.5
Oxolinic acid	0.1	86.3	4.3	4.8	0.1	84.6	1.8	7.3
	0.01	65.8	20.4	21.8	0.01	66.4	2.7	23.0
Sulfaquinoxaline	0.1	59.2	4.8	5.5	0.1	51.9	2.8	4.7
	0.01	114.3	12.8	13.4	0.01	120.2	4.2	36.5
Sulfadimethoxine	0.1	111.1	10.9	12.7	0.1	96.3	3.4	8.7
	0.01	102.2	14.4	15.4	0.01	114.6	1.5	23.8
Oxfendazole sulfone	0.1	97.6	3.2	4.4	0.1	96.2	1.9	9.0
	0.01	95.8	15.2	18.5	0.01	114.4	10.7	33.1
Nalidixic acid	0.1	89.9	6.7	7.0	0.1	87.8	1.7	4.7
	0.01	58.3	17.8	20.2	0.01	55.0	4.0	22.8
Flubendazole	0.1	52.5	6.0	7.9	0.1	43.7	2.3	5.0
	0.01	99.0	19.1	20.3	0.01	115.5	7.9	34.0
Fenbendazole	0.1	89.0	7.0	7.6	0.1	87.1	1.8	7.6
	0.01	94.5	15.5	17.5	0.01	120.5	3.5	35.6
Nicarbazin	0.1	90.4	2.4	5.9	0.1	91.6	0.7	8.8
	0.01	95.3	7.3	8.5	0.01	89.1	5.3	8.8
Triclabendazole sulfoxide	0.1	88.2	3.0	5.8	0.1	78.9	2.4	7.6
	0.01	82.8	10.6	14.7	0.01	76.2	13.9	15.0
Nequinat	0.1	82.4	7.7	12.3	0.1	77.4	4.0	7.5
	0.01	108.5	8.0	9.3	0.01	99.1	1.7	7.0
Buquinolate	0.1	95.7	8.2	8.3	0.1	82.0	2.3	7.0
	0.01	101.8	6.7	8.3	0.01	100.3	2.2	8.7
Diclazuril	0.1	91.6	5.2	7.8	0.1	84.3	2.1	6.5
	0.01	94.4	8.5	12.7	0.01	83.8	6.4	13.6
Triclabendazole sulfone	0.1	94.0	9.4	16.9	0.1	81.3	1.8	4.5
	0.01	87.6	12.7	25.2	0.01	90.8	4.0	24.7
Triclabendazole	0.1	92.5	12.1	15.5	0.1	95.7	6.1	8.5
	0.01	108.2	21.9	22.6	0.01	114.6	4.8	24.0
Decoquinat	0.1	97.6	6.7	12.8	0.1	89.9	2.5	11.5
	0.01	126.7	15.7	18.5	0.01	89.9	2.2	19.2
Eprinomectin	0.1	103.7	8.0	22.8	0.1	75.5	3.5	8.1
	0.01	98.9	22.0	22.1	0.01	100.2	4.3	22.6
Semduramicin	0.1	99.2	6.0	6.5	0.1	85.0	4.0	6.9
	0.01	94.3	9.1	11.5	0.01	108.3	8.4	8.7
Doramectin	0.1	83.7	7.7	8.5	0.1	94.5	6.5	10.7
	0.01	110.0	17.5	17.6	0.01	101.4	10.6	16.1
Lasalocid	0.1	87.8	14.7	17.9	0.1	93.1	5.5	7.4
	0.01	81.0	15.5	16.7	0.01	81.9	8.0	13.5
Moxidectin	0.1	71.9	8.2	9.1	0.1	78.9	3.9	12.0
	0.01	101.1	16.5	16.7	0.01	104.9	6.7	8.7
Ivermectin	0.1	88.6	12.9	14.2	0.1	90.8	6.4	8.5
	0.01	96.7	23.5	25.8	0.01	108.3	6.2	9.0
Maduramicin	0.1	83.7	5.1	12.5	0.1	97.7	3.3	12.7
	0.01	94.0	19.2	23.1	0.01	110.3	2.2	2.9
Salinomycin	0.1	91.9	6.6	12.0	0.1	97.1	1.6	10.3
	0.01	91.4	14.4	16.7	0.01	95.6	2.8	6.1
Monensin	0.1	87.8	5.1	7.8	0.1	82.1	2.2	5.6
	0.01	91.3	20.9	25.0	0.01	95.5	2.0	4.9
Narasin	0.1	83.4	5.2	8.7	0.1	85.0	2.2	5.9
	0.01	84.3	11.7	9.2	0.01	89.1	4.0	6.9
	0.1	83.5	9.6	10.8	0.1	80.1	0.8	5.7

Table 4. Continued

Analyte	Chicken muscle				Egg			
	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility(%)	Fortified (µg/g)	Accuracy (%)	Repeatability (%)	Within laboratory reproducibility (%)
Clopidol	0.01	86.1	8.5	17.2	0.01	62.9	8.9	14.1
	0.1	91.4	15.0	17.7	0.1	70.8	8.6	10.3
5-Hydroxythiabenzazole	0.01	83.4	56.1	63.7	0.01	57.0	17.9	39.2
	0.1	77.3	14.2	16.1	0.1	80.5	12.7	13.9
Levamisole	0.01	95.2	11.1	17.0	0.01	78.4	8.4	8.4
	0.1	90.5	1.0	3.3	0.1	86.1	6.0	11.2
5-Propylsulfonyl-1 <i>H</i> -benzimidazole-2-amine	0.01	70.9	8.9	13.0	0.01	80.8	15.0	19.0
	0.1	77.4	4.2	9.5	0.1	87.3	2.1	14.4
Sulfathiazole	0.01	91.8	10.2	15.7	0.01	91.2	15.7	20.3
	0.1	90.1	4.9	7.4	0.1	87.0	5.8	18.7
Trimethoprim	0.01	84.8	10.7	11.5	0.01	79.5	7.9	9.4
	0.1	84.0	2.4	3.7	0.1	83.2	3.2	10.1
Sulfamerazine	0.01	94.1	4.5	11.5	0.01	92.7	7.3	11.2
	0.1	97.5	3.1	9.0	0.1	88.4	3.3	10.8
Ciprofloxacin	0.01	—	—	—	0.01	—	—	—
	0.1	3.5	6.8	36.4	0.1	—	—	—
Ormethoprim	0.01	79.8	8.1	8.9	0.01	83.7	6.5	9.3
	0.1	85.5	3.6	4.1	0.1	85.4	1.8	11.6
Enrofloxacin	0.01	8.7	24.9	28.7	0.01	—	—	—
	0.1	11.4	8.7	18.4	0.1	—	—	—
Thiabenzazole	0.01	91.2	10.0	12.9	0.01	79.6	6.0	9.0
	0.1	90.6	4.3	6.5	0.1	83.0	4.5	8.3
Sulfadimidine	0.01	97.3	4.1	7.7	0.01	90.7	3.0	4.5
	0.1	96.5	2.9	8.0	0.1	91.2	2.6	6.4
Sarafloxacin	0.01	8.8	19.9	22.6	0.01	—	—	—
	0.1	5.8	10.1	46.6	0.1	2.0	15.5	35.3
Sulfamonomethoxine	0.01	105.5	7.3	12.3	0.01	94.5	14.6	20.5
	0.1	96.0	2.4	11.0	0.1	89.6	3.7	18.6
Sulfamethoxazole	0.01	98.5	7.6	8.7	0.01	98.2	16.2	17.0
	0.1	98.2	2.3	8.3	0.1	89.4	4.1	16.2
Florfenicol	0.01	88.5	6.3	13.0	0.01	118.1	12.5	39.3
	0.1	102.4	7.3	15.5	0.1	93.6	3.2	27.8
Oxfendazole	0.01	88.6	7.4	7.9	0.01	99.4	5.6	15.8
	0.1	90.5	2.0	3.8	0.1	96.9	4.2	12.4
Pyrimethamine	0.01	78.5	11.5	12.9	0.01	85.3	1.1	3.5
	0.1	86.1	2.3	7.2	0.1	85.3	3.1	6.0
Oxolinic acid	0.01	51.5	10.3	10.5	0.01	8.6	15.8	20.9
	0.1	50.4	2.7	8.9	0.1	11.4	2.6	18.7
Sulfaquinoxaline	0.01	91.3	6.8	8.0	0.01	104.2	24.9	29.9
	0.1	99.2	4.9	5.4	0.1	98.6	3.2	29.4
Sulfadimethoxine	0.01	94.1	5.1	7.9	0.01	98.0	9.7	11.9
	0.1	97.6	2.9	8.4	0.1	98.0	2.9	10.7
Oxfendazole sulfone	0.01	89.4	12.9	15.1	0.01	97.3	3.2	10.5
	0.1	89.0	1.5	6.0	0.1	97.6	2.8	10.0
Nalidixic acid	0.01	41.7	5.5	9.4	0.01	6.3	2.9	6.7
	0.1	40.9	3.4	7.6	0.1	9.0	3.2	16.4
Flubendazole	0.01	88.3	8.6	8.9	0.01	100.5	11.7	19.2
	0.1	88.2	2.7	4.8	0.1	110.5	5.6	30.2
Fenbendazole	0.01	100.4	9.8	13.3	0.01	94.5	12.2	18.1
	0.1	92.8	1.1	6.9	0.1	93.0	5.3	12.0
Nicarbazin	0.01	83.3	7.1	5.5	0.01	84.9	3.1	6.1
	0.1	84.6	3.0	4.4	0.1	81.1	1.8	5.6
Triclabendazole sulfoxide	0.01	74.6	4.3	6.0	0.01	85.2	4.8	9.6
	0.1	74.4	2.8	6.0	0.1	87.4	5.1	11.7
Nequinat	0.01	84.3	5.1	6.5	0.01	87.4	11.5	13.5
	0.1	83.1	2.2	2.2	0.1	82.1	2.6	10.0
Buquinolate	0.01	87.9	5.8	6.7	0.01	89.8	10.8	12.4
	0.1	86.2	2.6	3.0	0.1	83.4	2.4	10.5
Diclazuril	0.01	91.9	8.8	9.4	0.01	95.4	7.0	13.6
	0.1	84.3	2.3	9.0	0.1	82.3	1.9	16.2
Triclabendazole sulfone	0.01	99.3	8.9	14.8	0.01	100.8	5.8	13.9
	0.1	93.8	4.3	5.8	0.1	104.3	4.1	18.6
Triclabendazole	0.01	96.7	13.1	18.0	0.01	86.4	9.5	13.3
	0.1	89.4	3.2	7.3	0.1	79.2	7.5	12.4
Decoquinat	0.01	81.0	4.0	11.7	0.01	90.5	11.5	15.8
	0.1	80.2	1.3	18.4	0.1	73.9	3.3	18.8
Eprinomectin	0.01	112.5	11.7	14.5	0.01	102.5	7.6	17.2
	0.1	99.9	3.8	7.2	0.1	93.4	7.5	17.0
Semduramicin	0.01	93.1	13.4	16.0	0.01	88.7	14.3	16.1
	0.1	90.2	5.9	8.3	0.1	86.0	12.2	14.0
Doramectin	0.01	102.2	16.6	17.2	0.01	79.4	7.9	29.2
	0.1	105.2	7.5	8.9	0.1	79.6	3.9	19.7
Lasalocid	0.01	83.6	4.4	24.7	0.01	70.3	8.3	9.5
	0.1	82.8	12.1	14.0	0.1	71.3	10.3	13.5
Moxidectin	0.01	109.2	14.9	20.6	0.01	85.1	11.6	12.6
	0.1	101.2	11.2	12.6	0.1	86.4	4.8	5.4
Ivermectin	0.01	95.3	13.3	14.6	0.01	89.3	12.0	17.6
	0.1	89.4	5.2	8.1	0.1	83.3	5.1	6.5
Maduramicin	0.01	98.8	8.1	10.4	0.01	95.7	6.2	10.3
	0.1	95.6	6.8	7.3	0.1	85.5	2.9	7.3
Salinomycin	0.01	88.8	2.4	10.5	0.01	87.2	5.1	8.4
	0.1	82.9	5.9	6.4	0.1	85.9	3.0	5.4
Monensin	0.01	89.8	4.5	9.2	0.01	98.9	11.7	19.0
	0.1	90.1	7.2	8.0	0.1	87.8	3.6	13.0
Narasin	0.01	83.8	6.0	10.0	0.01	87.1	17.2	17.7
	0.1	79.6	6.5	7.4	0.1	107.3	12.3	17.4

— not recovered at all

not meeting the criteria of the guideline

Table 5. Validated method and LOQ of each analyte

Analyte	Bovine muscle		Swine muscle		Chicken muscle		Egg	
	Validated method	LOQ (µg/g)	Validated method	LOQ (µg/g)	Validated method	LOQ (µg/g)	Validated method	LOQ (µg/g)
Clopidol	B	0.002	B	0.002	—	0.001	—	0.001
5-Hydroxythiabendazole	—	0.01	—	0.005	—	0.005	A	0.005
Levamisole	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
5-Propylsulfonyl-1 <i>H</i> -benzimidazole-2-amine	B	0.002	B	0.002	B	0.002	B	0.002
Sulfathiazole	B	0.001	A, B	0.001	A, B	0.001	B	0.001
Trimethoprim	A, B	0.001	B	0.001	B	0.001	A, B	0.001
Sulfamerazine	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Ciprofloxacin	—	0.005	—	0.005	—	0.005	—	0.005
Ormethoprim	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Enrofloxacin	A	0.002	—	0.002	A	0.002	A	0.002
Thiabendazole	B	0.01	B	0.01	B	0.01	B	0.01
Sulfadimidine	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Sarafloxacin	—	0.005	A	0.005	—	0.005	A	0.005
Sulfamonomethoxine	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Sulfamethoxazole	A, B	0.002	A, B	0.002	A, B	0.002	A, B	0.002
Florfenicol	A	0.005	A, B	0.005	A, B	0.005	—	0.005
Oxfendazole	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Pyrimethamine	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Oxolinic acid	A	0.001	A	0.001	A	0.001	A	0.002
Sulfaquinoxaline	A, B	0.001	A	0.001	A, B	0.001	—	0.001
Sulfadimethoxine	A, B	0.001	A, B	0.001	A, B	0.001	B	0.001
Oxfendazole sulfone	A, B	0.001	A	0.001	A, B	0.001	B	0.001
Nalidixic acid	A	0.002	A	0.002	A	0.002	—	0.002
Flubendazole	A, B	0.001	A	0.001	A, B	0.001	—	0.001
Fenbendazole	A, B	0.001	A	0.001	A, B	0.001	A, B	0.001
Nicarbazin	A, B	0.001	A, B	0.001	B	0.001	A, B	0.001
Triclabendazole sulfoxide	A, B	0.005	A, B	0.005	B	0.005	A, B	0.005
Nequinat	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Buquinolate	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Diclazuril	A, B	0.005	A, B	0.005	A, B	0.005	B	0.005
Triclabendazole sulfone	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Triclabendazole	B	0.002	B	0.002	A, B	0.002	A, B	0.002
Decoquinat	A	0.001	A, B	0.001	B	0.001	B	0.001
Eprinomectin	B	0.002	B	0.002	B	0.002	B	0.005
Semduramicin	B	0.001	B	0.001	B	0.001	B	0.001
Doramectin	B	0.001	B	0.001	B	0.002	B	0.002
Lasalocid	B	0.001	B	0.001	B	0.001	B	0.001
Moxidectin	B	0.001	B	0.001	B	0.001	B	0.001
Ivermectin	B	0.001	B	0.001	A, B	0.001	B	0.001
Maduramicin	A, B	0.001	A, B	0.001	A, B	0.001	A, B	0.001
Salinomycin	B	0.001	B	0.001	B	0.001	B	0.001
Monensin	A, B	0.001	B	0.001	B	0.001	B	0.001
Narasin	A, B	0.001	A, B	0.001	A, B	0.001	B	0.001

— not meeting the criteria of the guideline with both methods

were recovered using citrate buffer. It was considered that the lower pH favored transfer to the acetonitrile layer. Therefore, to allow analysis of as many drugs as possible, the combination of magnesium sulfate, trisodium citrate and sodium chloride was adopted for the buffering.

Second, acidic conditions were studied by comparing no additive, 0.1 and 1 vol% formic acid as added acids to acetonitrile. The results are shown in Fig. 1. Recoveries of quinolones and fluoroquinolones were increased as the acidity increased, but the mean recoveries of polyethers and macrolides were less than 70%. It was con-

sidered that the unionized forms of quinolones increased as the acidity increased, but cleavage of large molecules such as polyethers and macrolides was enhanced²³⁾. In egg also, matrix effects were not sufficiently corrected even when using matrix-matched calibration curves, because of the larger amounts of co-extracted matrices derived from the samples. Therefore we used two methods of extraction; method A with an acidic solvent for quinolones, and method B with only acetonitrile, a non-acid solvent, for lipophilic analytes.

Third, the effect of salting-out was studied because the amount of sodium chloride influenced the recovery

rate and ionization effect from the matrices^{8),24)}. Studies used 1.0, 2.0 and 5.0 g of sodium chloride; however the operation could not be continued in the case of 5.0 g of sodium chloride because the sample solution formed an emulsion, so only the results for 1.0 and 2.0 g are shown in Fig. 2. Although there were no differences with more soluble drugs such as sulfonamides, quinolones and fluoroquinolones, the recoveries of polyethers and macrolides were improved.

Method validation

To validate the method, recovery tests using both method A and method B were performed in accordance with the Japanese guidelines, and the results are shown in Tables 3 and 4. Quantification values were obtained from matrix-matched calibration curves where the correlation coefficient (r^2) was over 0.999. In method A, the number of analytes that satisfied the criteria of the guidelines was 27 analytes out of 43 in bovine and swine muscle, 26 in chicken muscle and 21 in egg. In method B the results were 35 analytes in bovine muscle, 33 in swine muscle and egg, and 36 in chicken muscle.

Quinolones and fluoroquinolones showed higher accuracy in method A, acidic extraction. However, some fluoroquinolones were not sufficiently recovered even using method A because they show amphoteric characteristics, which made them more soluble in the water layer than in the acetonitrile layer. In contrast, polyethers and macrolides were well validated using method B. Highly hydrophilic analytes such as 5-hydroxythiabendazole and 5-propylsulfonyl-1*H*-benzimidazole-2-amine (both metabolites) were difficult to recover in either method because they are highly soluble in water, and were not transferred to the organic layer.

Selectivities were confirmed by analyzing blank samples, and there were no interfering peaks at the same retention times as the target analytes.

LOQs are shown in Table 5, and they were less than the MRLs.

Conclusion

In this study, new methods for rapid analysis of multi-class residual veterinary drugs in livestock products were developed, and were validated according to the Japanese validation guidelines. Analytical methods were developed using LC-MS/MS and results were achieved in only 18 min to analyze 43 analytes simultaneously. Sample preparation processes were developed by modifying the QuEChERS approach and using two extraction solvents; method A which included 1 vol% formic acid in acetonitrile obtained higher recoveries of quinolones, and method B which did not include acid was used for polyethers and macrolides. Using these methods, the number of analytes that were validated in accordance with the Japanese validation guidelines was 40 analytes out of 43 in bovine and swine muscle, 39 in chicken muscle, and 37 in egg.

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