Validation Study

Single-Laboratory Validation Study of Rapid Analysis Method for Multi-Class Veterinary Drugs in Milk, Fish and Shellfish by LC-MS/MS

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A method of rapid analysis of multi-class residual veterinary drugs in milk, fish and shellfish was validated in accordance with Japanese guidelines for the validation of analytical methods for residual agricultural chemicals in food. Using LC-MS/MS, 43 multi-class veterinary drugs, including sulfonamides, quinolones, coccidiostats and antiparasites, could be analyzed in one injection. Analytes were extracted from samples with two kinds of solvent, acetonitrile containing 1 vol% formic acid and anhydrous acetonitrile, and salted out with 4.0 g of magnesium sulfate, 1.5 g of trisodium citrate and 2.0 g of sodium chloride. This method was assessed by performing recovery tests in retail milk and 4 kinds of fresh cultured fish and shellfish (salmon, tiger shrimp, red sea bream and bastard halibut) spiked with the 43 target analytes at the levels of 10 and 100 µg/kg. Using this method, 40 out of 43 drugs satisfied the guideline criteria in milk, 37 drugs in salmon, 42 drugs in tiger shrimp, 41 drugs in red sea bream and 39 drugs in bastard halibut.

Key words: veterinary drug, milk; fish and shellfish; LC-MS/MS, multi-class analysis; single-laboratory validation study

Introduction

Veterinary drugs are used worldwide for treatment and prevention of animal diseases, and as food additives for growth promotion. However, some of those drugs can remain and accumulate in animal muscle due to over-administration or noncompliance with withdrawal period guidelines. Residual veterinary drugs pose a potential risk of allergic reactions, pathogen resistance and cytotoxicity in humans¹⁾; therefore, it is important to monitor foods for residual veterinary drugs.

There are two major analytical strategies for the analysis of residual substances, namely, multi-class analysis²⁾⁻⁷⁾ and individual analysis of each compound⁸⁾⁻¹⁰⁾. However, quick and easy multi-class analysis has become more and more attractive because of the development of sophisticated analytic equipment, the diversification of medicines used, and increasing governmental requests for the analysis of foods available on the market. In addition, the analytic methodologies used by the inspection institutes have to be validated by 13/12/2013 in line with a notice issued by the Ministry of Health, Labour and Welfare of Japan. Therefore, a simple, rapid and simultaneous analysis of veterinary drugs is highly

desirable.

However, there are some issues that have yet to be resolved in order to validate the simultaneous analysis of veterinary drugs:

- 1) A wide variety of drugs must be simultaneously extracted, ranging from hydrophilic compounds, such as antibiotics and fluoroquinolones, to lipophilic compounds, such as macrolide antiparasites and polyether antibiotics.
- 2) The exact drugs that are detected in both domestic and foreign inspection institutes have to be confirmed.
- 3) Many consumable supplies such as reagents, solvents, SPEs and HPLC columns are required.
- 4) It is hard to secure sufficient time and human resources to perform validation study, and it is difficult to guarantee the precision of the analytical method, especially inter-day repeatability, if the extraction process is complicated.

We developed a simple, rapid and simultaneous analytical method for residual veterinary drugs that covered the inspection items of the Tokyo Metropolitan Institute of Public Health by modifying the QuEChERS methodology¹¹⁾. In that previous study, the new methodology was successfully applied and validated for bovine muscle, swine muscle, chicken muscle and avian eggs. Therefore,

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in this study, our multi-class analysis was applied to a wider range of food samples and validated in accordance with the Japanese guidelines ^{*1}.

Materials and Methods

Chemicals and Reagents

Powdered standards for testing residual veterinary drug grades were used: ciprofloxacin hydrochloride (purity: 98.0%), clopidol (98.7%), closantel sodium (99.0%), decoquinate (97.0%), fenbendazole (98.0%), florfenicol (98.0%), 5-hydroxythiabendazole (98.0%), oxfendazole (98.0%), oxfendazole sulfone (99.9%), sulfadimethoxine (99.0%), sulfamethoxazole (99.0%), triclabendazole (99.0%), triclabendazole sulfone (98.0%), triclabendazole sulfoxide (98.0%) and trimethoprim (99.0%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Danofloxacin mesylate (99.5%), enrofloxacin (99.8%), levamisole hydrochloride (99.0%), nalidixic acid (99.8%), nicarbazin (99.8%), oxolinic acid (98.8%), pyrimethamine (99.8%), sulfadimidine (99.4%), sulfamerazine (99.5%), sulfathiazole (99.9%), sulfaquinoxaline sodium (99.6%) and thiabendazole (99.0%) were purchased from Kanto Chemical Co. (Tokyo, Japan). Diclazuril (99.8%), eprinomectin (99.3%), flubendazole (99.9%) and 5-propylsulfonyl-1*H*-benzimidazole-2-amine (99.1%) were purchased from Hayashi Pure Chemical Industries (Osaka, Japan). Doramectin (94.8%), monensin sodium (86.3%), moxidectin (97.1%), narasin (97.0%) semduramicin sodium (96.1%), salinomycin (93.8%), sarafloxacin hydrochloride (97.3%) and 100 µg/mL solution of lasalocid A sodium were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ivermectin (96.0%), maduramicin ammonium (85.5%) and ormethoprim (99.0%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

A stock standard solution of danofloxacin was prepared as below: transfer about 5 mg of powdered standard, accurately weighed, to a 50 mL of round-bottom flask, and then dilute with methanol to the concentration of 100 µg/mL. A closantel solution was prepared by dissolving about 5 mg of powder, accurately weighed, in 5 mL of tetrahydrofuran, and diluting the resulting solution with methanol to the concentration of 100 µg/mL. Other analytes were prepared in accordance with the method outlined in the previous study¹¹⁾. These solutions were stored at 4°C for up to one year. A working standard solution was prepared by mixing 500 µL of all stock standard solutions and diluting with acetonitrile to 50 mL. This solution was stored at 4°C for up to three months.

Acetonitrile (HPLC grade), formic acid (LC/MS grade), ammonium acetate (LC/MS grade), magnesium sulfate (analytical grade), trisodium citrate dehydrate (analytical grade) and sodium chloride (analytical grade) were

purchased from Wako Pure Chemical Industries. Methanol (HPLC grade), tetrahydrofuran (analytical grade) and dimethylformamide (analytical grade) were purchased from Kanto Chemical Co. Ultrapure water was obtained from a Milli-Q water system (Merck Millipore, Billerica, MA, USA).

Samples

Retail milk and 4 kinds of fresh cultured fish and shellfish (salmon, tiger shrimp, red sea bream and bastard halibut) were purchased from local supermarkets in Tokyo and were confirmed to be free of the targeted analytes. The tissue samples were minced with an electric food processor and stored at -20°C. Milk was stored at 4°C.

LC-MS/MS conditions

The LC-MS/MS conditions basically followed those of the previous study¹¹⁾. Briefly, LC separation was performed using a Prominence 20A series (Shimadzu Corporation, Kyoto, Japan), and Synchronis aQ (2.1 mm i.d. × 100 mm, 5 µm, Thermo Fisher Scientific, Waltham, MA, USA) as an LC column at 40°C. The gradient was constructed from 0.1% formic acid in 10 mmol/L ammonium acetate (A) and acetonitrile (B). The initial condition was (A) : (B) = 95 : 5. The acetonitrile concentration was increased to 95% for 7 min, and this was maintained for 5 min. Then, the mobile phases were re-equilibrated to the initial condition for 5 min. The flow rate was 0.4 mL/min. The injection volume was 3 µL.

MS detection was performed using a TSQ Quantum Access MAX (Thermo Fisher Scientific), and positive and negative electrospray ionization with voltages of 3.0 kV and 2.5 kV, respectively. The vaporizer temperature was 460°C and the capillary temperature was 220°C. Optimization of MS/MS parameters was performed by direct infusion of 1 µg/mL of each standard solution, and the optimized conditions for selective reaction monitoring (SRM) transitions are shown in Table 1.

Sample preparation

Five grams of each sample was placed into a 50 mL polypropylene centrifuge tube, then 50 µL or 500 µL of working standard solution was added. After waiting for more than 30 min, 5 mL of water (except in the case of milk) and 15 mL of 1 vol% formic acid in acetonitrile (Method A) or acetonitrile alone (Method B) were added. Tissue samples were homogenized thoroughly and the milk was well shaken. Next, 4 g of magnesium sulfate, 1.5 g of trisodium citrate dehydrate and 2 g of sodium chloride were added to the tube, which was immediately shaken by hand for 1 min. The mixture was centrifuged at 1,800 × *g* for 10 min, and the supernatant was transferred into a 20-mL volumetric flask. After dilution to volume with extraction solvent, a portion of the solution was transferred to a 1.5 mL microtube and centrifuged at 16,000 × *g* for 10 min. Three µL of the supernatant was injected into the LC-MS/MS.

^{*1} Guidelines for the Validation of Analytical Method for Residual Agricultural Chemicals in Food (December 24, 2010, Notification 1224, No. 1). The Ministry of Health, Labour and Welfare of Japan

Table 1. MS/MS parameters of each analyte

Analyte	M.R.W.* (min)	SRM transition				
		ESI	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Collision energy (eV)	Tube lens (V)
Clopidol	2.2–3.0	+	192	101	24	122
5-Hydroxythiabendazole	2.4–3.1	+	218	108	37	105
Levamisole	2.5–3.3	+	205	91	35	102
5-Propylsulfonyl-1 <i>H</i> -benzimidazole-2-amine	2.5–3.3	+	240	133	30	82
Sulfathiazole	2.5–3.3	+	256	92	26	109
Trimethoprim	2.7–3.5	+	291	123	26	94
Sulfamerazine	2.8–3.6	+	265	92	29	93
Ciprofloxacin	2.8–3.6	+	332	314	20	119
Danofloxacin	2.9–3.7	+	358	82	35	133
Ormethoprim	2.9–3.7	+	275	123	21	107
Enrofloxacin	3.0–3.9	+	360	316	18	116
Thiabendazole	3.0–3.9	+	202	65	40	106
Sulfadimidine	3.0–4.0	+	279	186	16	93
Sarafloxacin	3.4–4.1	+	386	368	26	138
Sulfamonomethoxine	3.4–4.1	+	281	108	25	82
Sulfamethoxazole	3.5–4.3	+	254	92	25	96
Florfenicol	3.5–4.3	—	356	336	13	—87
Oxfendazole	3.6–4.3	+	316	159	31	120
Pyrimethamine	3.8–4.8	+	249	177	28	114
Oxolinic acid	3.8–4.8	+	262	244	19	106
Sulfaquinoxaline	3.9–4.7	+	301	108	26	95
Sulfadimethoxine	3.9–4.7	+	311	156	20	96
Oxfendazole sulfone	4.0–4.8	+	332	300	22	118
Nalidixic acid	4.2–5.4	+	233	215	13	97
Flubendazole	4.5–5.3	+	314	123	34	123
Fenbendazole	5.2–6.0	+	300	268	19	103
Nicarbazin	5.4–6.6	—	301	137	16	—65
Triclabendazole sulfoxide	5.4–6.4	—	373	181	46	—78
Diclazuril	5.9–6.6	—	405	334	22	—70
Triclabendazole sulfone	6.0–6.7	—	391	312	29	—102
Triclabendazole	6.3–7.1	+	359	198	25	124
Decoquinate	7.0–8.0	+	418	204	41	107
Eprinomectin	7.0–8.0	+	915	112	55	113
Closantel	7.5–8.2	—	661	345	37	106
Semduramicin	7.5–8.5	+	891	630	22	126
Doramectin	7.5–8.6	+	917	593	10	138
Lasalocid	7.5–8.7	+	609	237	23	112
Moxidectin	7.9–8.9	+	641	499	12	113
Ivermectin	8.2–8.9	+	893	570	13	113
Maduramicin	8.5–9.1	+	935	630	21	132
Salinomycin	8.5–9.2	+	769	734	17	94
Monensin	8.6–9.3	+	689	461	23	119
Narasin	8.7–9.7	+	783	748	17	114

* Monitoring retention window

Table 2. Comparison of matrix effects in each extracted solution derived from food samples

Response ratio*	Milk		Salmon		Tiger shrimp		Red sea bream		Bastard halibut	
	A	B	A	B	A	B	A	B	A	B
>1.2	0	2	3	2	1	1	3	6	3	2
1.1–1.2	4	4	5	3	3	2	2	12	6	3
0.9–1.1	36	35	34	36	34	35	35	25	31	29
0.8–0.9	3	2	1	1	4	2	1	0	2	8
<0.8	0	0	0	1	1	3	2	0	1	1

* Peak area ratio of matrix-matched standard solution/extraction solvent standard solution
 All solutions were fortified at 25 µg/L

Table 3. The results of performance characteristics of method A

Analyte	Fortified (µg/kg)	Milk				Salmon				Tiger shrimp				Red sea bream				Bastard halibut			
		Trueness* (%)	RSDr** (%)	RSDwtr*** (%)	Trueness* (%)	RSDr** (%)	RSDwtr*** (%)	Trueness* (%)	RSDr** (%)	RSDwtr*** (%)	Trueness* (%)	RSDr** (%)	RSDwtr*** (%)	Trueness* (%)	RSDr** (%)	RSDwtr*** (%)	Trueness* (%)	RSDr** (%)	RSDwtr*** (%)		
Clopidol	10	81.3	4.7	5.5	66.3	2.4	8.2	69.5	7.2	12.0	77.6	2.4	9.3	68.9	5.1	8.7					
	100	83.8	1.7	5.5	68.4	2.8	4.3	75.3	3.4	4.7	76.0	2.8	10.2	71.4	2.7	3.4					
	10	75.7	11.2	12.4	52.3	6.4	11.7	48.5	11.9	28.5	60.1	15.7	20.2	69.6	14.7	16.8					
	100	80.3	4.3	7.7	54.0	6.3	6.6	67.7	3.0	6.4	68.7	6.1	7.6	66.4	5.4	7.5					
Levamisole	10	87.3	1.7	3.9	78.1	6.4	7.2	77.1	4.8	10.8	83.8	6.1	9.8	80.7	3.8	6.1					
	100	87.0	2.1	2.5	77.2	2.9	3.8	77.4	2.5	5.7	84.6	2.9	8.0	79.2	3.1	4.6					
5-Propylsulfonyl-1H-benzimidazole-2-amine	10	82.4	7.7	19.1	54.6	8.1	9.9	63.1	9.4	23.0	57.5	21.0	23.3	65.1	16.7	19.1					
	100	86.5	2.5	8.5	63.2	8.2	9.6	72.1	5.2	5.7	67.7	4.5	5.2	65.7	2.8	4.7					
Sulfathiazole	10	90.5	3.9	5.5	76.7	6.4	17.3	79.4	7.7	9.5	83.4	7.5	11.0	83.3	5.7	11.0					
100	92.8	2.8	5.5	5.5	78.8	7.4	11.8	87.1	2.5	5.8	86.5	5.3	6.3	81.9	2.8	4.7					
Trimethoprim	10	84.6	7.1	8.0	66.7	2.9	7.5	74.8	7.9	8.5	81.1	4.1	9.9	74.2	8.6	8.8					
100	84.5	0.8	3.0	3.0	73.8	1.9	4.2	79.9	4.9	6.4	78.7	5.0	6.4	75.7	2.4	3.3					
Ciprofloxacin	10	254.6	5.1	16.3	105.8	3.9	21.3	92.4	5.0	11.4	101.6	6.7	12.2	113.7	3.4	5.8					
100	187.1	3.9	12.1	12.1	78.6	5.9	14.4	66.8	3.5	4.2	71.3	2.2	7.4	77.0	6.1	6.8					
Danofloxacin	10	177.7	17.9	21.8	87.0	12.3	17.5	113.2	5.3	26.0	93.3	11.3	13.3	148.5	6.8	15.4					
100	120.2	1.8	7.3	7.3	70.4	5.9	11.2	78.4	4.9	7.3	80.7	8.0	13.0	77.9	9.1	10.4					
Ornethoprim	10	83.4	2.5	10.0	69.1	3.4	8.1	71.4	6.2	12.5	76.6	5.6	9.3	71.4	4.8	8.3					
100	87.4	2.1	4.3	4.3	75.4	2.6	5.2	79.4	3.3	3.6	80.9	3.1	7.1	75.5	2.6	2.9					
Sulfamerazine	10	89.5	6.3	12.0	79.2	5.1	16.0	76.7	13.6	15.1	91.7	3.9	8.7	79.2	8.0	8.6					
100	96.5	2.2	6.6	6.6	89.8	4.7	11.0	89.7	2.1	7.7	91.3	3.2	6.4	85.6	4.6	4.6					
Enrofloxacin	10	93.4	3.0	6.5	75.6	3.5	8.9	81.2	3.4	7.5	82.4	3.9	8.6	102.2	4.9	5.8					
100	94.3	1.4	6.2	6.2	73.3	2.9	8.0	75.1	3.1	5.0	77.8	2.7	3.4	77.6	2.2	2.3					
Thiabendazole	10	86.2	4.4	5.5	74.0	6.3	11.3	76.6	8.3	15.8	80.6	8.5	9.3	73.6	6.4	8.4					
100	85.4	3.4	3.5	3.5	76.0	5.1	7.8	78.0	4.3	5.3	77.2	2.8	6.4	74.8	3.6	4.4					
Sarafloxacin	10	136.7	3.2	25.5	105.6	6.5	15.6	79.3	9.7	10.6	90.2	8.5	13.2	144.1	9.7	12.0					
100	116.3	1.6	13.3	13.3	83.5	10.6	13.5	77.1	3.8	5.4	81.2	2.0	4.3	98.6	3.1	3.1					
Sulfadimidine	10	88.1	4.1	12.9	84.7	5.9	26.2	83.6	10.0	10.5	86.3	2.8	12.2	78.4	3.1	10.0					
100	92.5	3.1	6.2	6.2	93.5	5.8	18.5	89.3	3.0	8.3	88.3	5.2	6.2	82.3	1.6	3.4					
Sulfamonomethoxine	10	90.2	6.9	10.3	86.6	5.1	8.3	76.0	7.7	8.9	83.8	11.7	12.3	86.5	6.9	7.1					
100	86.4	3.5	5.6	5.6	87.3	5.9	12.4	91.5	6.6	9.3	88.7	3.6	4.5	83.6	2.9	4.5					
Pyrimethamine	10	84.5	3.8	7.7	61.6	6.6	17.5	69.1	4.1	11.9	79.1	3.7	6.1	73.8	4.6	9.0					
100	88.3	1.2	3.1	3.1	78.1	1.9	9.3	78.3	3.0	6.7	82.5	2.0	5.9	77.0	2.7	3.2					
Sulfamethoxazole	10	91.3	4.9	9.6	77.5	5.7	11.3	88.3	15.6	16.4	101.5	13.6	15.3	80.5	7.7	18.5					
100	90.5	2.7	8.5	8.5	78.2	7.3	22.0	88.0	9.5	10.0	87.9	8.0	9.8	83.4	4.1	8.1					
Florfenicol	10	82.0	2.4	30.1	80.3	20.1	26.2	79.0	11.3	33.6	90.9	23.8	27.2	80.7	10.2	19.6					
100	91.1	9.9	11.8	11.8	86.5	7.9	10.1	84.5	5.3	6.1	91.0	6.7	10.8	86.4	2.4	4.9					
Oxfendazole	10	94.8	3.0	10.4	78.8	6.9	7.3	72.6	5.8	21.6	85.9	4.0	8.7	80.6	4.8	9.1					
100	87.7	1.5	5.8	5.8	78.9	18.3	21.3	82.6	4.7	5.5	80.1	5.6	6.2	79.0	2.6	4.0					
Oxolinic acid	10	89.8	1.9	6.4	83.1	1.1	3.7	81.5	6.5	6.8	96.8	5.7	7.5	92.7	10.0	11.9					
100	90.6	0.8	7.4	7.4	84.5	2.5	7.0	83.3	1.8	4.7	90.2	1.4	10.3	87.5	2.5	3.2					
Sulfaquinoxaline	10	95.7	4.5	8.5	85.3	6.4	18.1	88.8	10.3	10.9	84.8	3.5	6.7	82.4	13.3	13.3					
100	93.8	5.8	6.3	6.3	90.7	3.1	9.1	88.9	5.3	5.6	92.0	6.7	7.4	87.1	1.9	5.1					
Sulfadimethoxine	10	86.7	5.8	11.0	78.3	14.9	16.6	74.5	5.9	8.0	83.4	4.1	8.6	77.5	4.6	10.1					
100	94.6	2.3	5.1	5.1	93.8	3.4	15.0	87.4	2.6	6.1	90.9	1.2	5.9	86.6	2.7	3.2					

Table 3. Continued

Analyte	Fortified (µg/kg)	Milk			Salmon			Tiger shrimp			Red sea bream			Bastard halibut		
		Trueness (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)
Oxendazole sulfone	10	96.1	3.0	9.1	81.2	11.9	22.7	77.0	5.0	11.2	85.9	5.0	12.6	82.7	4.3	10.0
	100	90.0	3.1	7.5	86.4	4.5	9.8	83.7	2.9	7.2	86.6	1.9	5.6	82.9	3.2	3.7
Nalidixic acid	10	88.2	2.3	5.3	84.0	2.2	7.9	81.2	5.4	9.4	93.0	3.0	5.1	91.8	1.8	8.9
	100	87.2	0.8	2.1	85.0	1.5	11.2	83.6	1.9	5.3	89.7	0.7	8.4	87.2	5.3	6.2
Flubendazole	10	76.9	2.2	15.8	85.7	2.8	11.2	77.5	5.0	9.7	82.0	3.1	12.9	78.5	11.3	12.5
	100	81.6	3.2	8.3	91.7	3.3	25.1	83.4	2.5	4.7	83.3	2.4	7.3	83.7	2.1	4.2
Fenbendazole	10	88.7	4.1	9.6	83.3	3.4	21.2	78.5	3.5	11.9	80.1	1.8	11.0	77.0	3.0	4.3
	100	88.9	2.6	3.1	85.8	6.7	21.5	88.0	4.3	9.1	84.3	2.2	6.0	81.1	1.2	4.0
Nicarbazin	10	86.2	2.6	5.2	81.7	3.2	11.9	76.1	3.8	10.6	85.3	5.4	8.1	75.2	4.3	9.3
	100	85.2	1.1	1.8	79.8	1.5	17.8	79.6	3.8	6.9	84.7	3.6	8.5	79.9	2.7	5.7
Triclabendazole sulfoxide	10	85.6	3.8	6.8	79.7	10.8	16.3	60.0	13.1	13.4	78.6	5.8	17.4	82.1	15.2	17.6
	100	89.8	5.0	9.6	73.6	9.8	12.0	67.6	4.4	9.2	80.7	5.7	8.3	74.6	5.4	8.4
Diclazuril	10	79.1	7.6	11.1	80.0	4.4	15.1	78.6	17.4	24.4	79.8	14.3	17.5	97.5	16.3	16.6
	100	88.1	6.0	7.0	89.2	8.7	13.9	90.1	4.8	8.4	99.4	7.4	13.5	89.4	5.5	20.3
Triclabendazole sulfone	10	97.0	5.2	8.6	85.9	3.2	15.3	80.2	9.1	13.2	76.2	18.6	19.1	79.8	2.8	8.7
	100	93.7	3.5	6.7	87.9	14.7	14.9	84.2	6.8	12.0	83.6	1.7	10.8	80.9	2.8	5.6
Triclabendazole	10	78.7	5.9	9.4	57.4	14.4	17.9	78.1	4.1	17.7	66.8	10.4	46.2	47.1	9.9	42.0
	100	79.5	0.9	8.9	61.4	4.8	11.4	98.8	7.7	8.8	73.5	16.3	23.2	84.5	2.6	33.1
Decoquinat	10	94.1	2.5	7.5	66.1	2.0	9.9	84.8	9.5	9.9	92.7	5.2	13.0	76.4	2.5	32.6
	100	88.4	1.2	2.4	70.3	9.9	11.7	93.8	4.1	10.8	75.7	3.9	14.7	81.6	2.4	15.7
Eprinomectin	10	68.7	7.9	27.2	64.4	10.0	19.3	87.6	16.6	22.6	76.4	23.3	27.4	70.4	11.9	16.4
	100	80.8	3.9	6.6	72.9	12.5	14.3	109.0	9.0	20.9	70.3	8.8	15.9	77.6	11.5	24.7
Closantel	10	81.7	7.2	10.2	83.0	5.5	9.4	83.2	6.7	14.8	97.8	11.1	12.6	74.1	9.9	16.8
	100	76.1	2.6	5.9	77.8	3.3	11.2	86.2	7.2	11.8	84.2	6.5	9.6	84.9	3.2	12.6
Semduramicin	10	112.8	5.2	12.9	89.6	6.3	10.9	99.9	7.1	13.1	109.4	7.1	11.0	82.6	6.5	20.5
	100	109.5	2.1	6.1	95.8	6.1	6.6	110.7	4.9	10.0	100.4	1.6	10.2	89.6	4.1	11.2
Doramectin	10	98.4	3.4	39.8	67.2	9.1	24.2	72.6	18.6	23.3	56.3	20.9	30.5	74.1	16.3	18.1
	100	89.2	2.1	8.8	69.9	8.9	9.9	113.1	8.5	17.6	74.8	4.4	12.1	72.9	7.3	15.0
Lasalocid	10	85.7	6.1	8.9	50.1	3.6	11.4	69.2	7.1	12.3	55.0	10.3	31.7	54.7	7.1	19.7
	100	88.2	2.5	12.3	62.0	6.2	6.8	85.2	6.5	10.1	66.1	4.0	12.5	64.9	2.5	8.7
Moxidectin	10	74.9	11.5	45.1	70.2	14.6	36.2	96.0	9.0	14.5	70.0	9.3	27.7	72.4	6.5	8.7
	100	71.0	3.3	25.0	67.6	7.4	24.2	102.7	9.0	13.1	69.5	2.9	13.3	75.6	1.6	5.9
Ivermectin	10	91.2	8.4	37.8	73.8	22.4	54.6	96.1	11.2	18.3	79.0	24.1	25.3	74.7	15.9	38.1
	100	86.1	4.9	20.3	60.2	7.8	29.0	113.2	18.2	20.4	70.3	2.4	9.5	72.5	2.1	5.9
Maduramicin	10	110.6	6.2	14.0	108.3	10.0	19.7	100.6	7.1	12.1	128.3	2.3	3.5	89.3	3.9	18.3
	100	103.5	2.5	12.7	101.5	5.3	15.1	113.6	9.6	10.5	115.2	3.0	10.4	94.0	3.8	8.9
Salinomycin	10	65.7	4.6	6.0	71.4	2.5	16.7	69.6	4.7	6.9	81.3	4.2	8.9	72.7	5.6	13.5
	100	67.6	2.0	10.6	67.7	5.6	11.5	80.6	7.7	8.3	74.8	4.3	6.7	73.3	3.5	6.1
Monensin	10	82.2	5.0	17.5	75.9	4.3	17.2	86.5	5.7	16.2	92.4	6.8	13.7	85.7	5.9	11.3
	100	82.3	3.4	11.4	74.8	3.7	6.9	96.3	10.5	14.1	88.8	4.7	9.2	85.7	4.5	8.2
Narasin	10	78.1	7.9	11.1	80.2	2.5	19.8	68.8	2.8	7.7	73.5	4.0	18.3	71.2	2.5	6.6
	100	77.7	1.7	19.7	72.6	4.7	7.5	77.4	3.4	8.2	72.7	4.1	5.9	74.4	2.6	3.0

Did not meet the criteria of the guideline

* The mean recovery rates

** RSD of repeatability

*** RSD of within-laboratory reproducibility

Table 4. The performance characteristics of method B

Analyte	Fortified (µg/kg)	Milk			Salmon			Tiger shrimp			Red sea bream			Bastard halibut		
		Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)
Clotidol	10	77.6	5.1	9.2	69.8	9.0	9.6	78.8	8.6	10.2	78.7	10.0	11.7	77.8	9.2	10.6
	100	81.4	2.4	4.6	72.5	1.8	2.7	84.9	2.0	6.8	77.9	2.2	3.9	80.3	1.8	3.2
5-Hydroxythiabendazole	10	92.8	4.0	14.6	67.4	5.7	18.5	79.2	12.0	16.9	78.5	10.1	18.0	71.1	13.1	22.9
	100	80.9	2.7	6.0	71.4	5.0	6.0	84.6	5.2	11.6	77.5	4.5	6.1	81.4	2.1	11.1
Levamisole	10	86.2	6.4	7.1	75.5	1.9	7.0	86.8	6.1	7.0	90.0	5.5	11.0	83.2	5.3	12.0
	100	85.3	2.2	2.6	78.9	3.3	3.9	88.9	2.9	6.8	82.8	3.3	4.8	84.5	2.2	7.7
5-Propylsulfonyl-1H-benzimidazole-2-amine	10	82.1	7.8	7.8	72.7	6.8	11.6	82.5	12.5	16.1	84.5	4.8	8.8	81.2	10.8	13.0
	100	83.7	2.9	5.4	72.2	3.0	4.0	85.8	3.7	7.2	76.3	6.7	9.2	76.2	2.9	6.9
Sulfathiazole	10	93.1	7.4	9.9	73.2	6.8	13.3	77.8	11.4	12.7	88.3	8.9	11.7	83.0	8.4	8.8
	100	90.0	3.5	6.8	77.6	7.3	8.3	92.9	5.4	6.3	82.2	6.8	7.3	85.8	2.5	10.8
Trimethoprim	10	81.0	3.9	9.3	69.2	8.0	8.1	90.0	3.8	14.4	81.4	3.7	7.3	79.4	10.3	12.2
	100	82.7	3.4	4.3	73.8	2.2	3.0	89.7	1.9	5.8	80.1	1.5	2.8	79.8	4.5	5.2
Ciprofloxacin	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	100	32.8	12.2	40.7	12.4	3.5	28.1	—	—	—	9.5	7.3	49.0	11.9	3.5	45.4
Danofloxacin	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	100	48.2	10.1	23.5	15.4	2.5	40.9	—	—	—	16.8	6.6	22.1	16.1	7.0	21.3
Ormethoprim	10	82.7	5.7	8.1	69.9	4.5	7.8	89.7	2.7	8.1	82.3	3.0	6.0	79.9	3.5	9.4
	100	84.7	0.9	4.0	76.1	3.7	3.9	89.4	1.6	7.8	81.1	3.3	3.9	80.6	2.3	6.5
Sulfamerazine	10	85.9	6.4	7.4	71.6	8.9	9.1	93.2	5.5	18.3	89.7	4.7	8.4	83.9	7.5	10.0
	100	90.8	2.9	5.4	84.1	3.6	6.4	96.1	4.2	6.2	91.8	2.7	5.0	88.9	2.0	4.6
Enrofloxacin	10	42.1	6.2	21.9	8.9	4.2	74.0	22.9	3.0	44.0	21.0	9.5	39.7	30.7	3.7	8.7
	100	50.9	3.6	11.8	7.0	6.3	11.1	—	—	—	15.0	4.6	21.2	12.5	5.2	7.6
Thiabendazole	10	81.0	6.6	13.6	80.9	10.9	12.8	97.6	8.3	11.8	82.2	9.0	10.1	80.2	7.6	8.9
	100	88.1	3.3	3.3	77.1	3.5	3.9	94.4	5.6	8.1	83.4	6.3	6.5	85.9	1.4	5.9
Sarafloxacin	10	66.0	7.3	47.8	—	—	—	—	—	—	15.9	19.2	99.5	—	—	—
	100	64.1	2.9	17.5	13.8	10.0	44.9	—	—	—	14.0	9.4	22.7	16.9	10.1	12.7
Sulfadimidine	10	82.5	3.5	9.3	82.5	4.8	10.0	95.9	4.3	8.2	92.5	4.7	4.7	84.8	4.4	7.4
	100	91.6	2.8	6.6	84.5	5.6	7.3	96.5	3.9	7.9	89.3	3.5	5.7	91.6	2.4	6.0
Sulfamonomethoxine	10	84.0	8.7	20.2	76.7	9.8	18.2	84.4	11.3	16.3	91.0	8.7	20.4	89.7	3.5	6.6
	100	87.4	5.8	6.3	82.1	3.6	9.2	92.2	3.3	6.4	87.8	6.1	6.5	87.5	2.7	9.8
Pyrimethamine	10	83.6	7.3	8.8	71.5	2.8	5.9	92.2	2.3	12.2	82.5	6.6	8.4	83.0	6.0	18.8
	100	86.1	1.5	4.2	78.1	3.0	3.1	90.9	1.7	10.9	81.0	2.6	3.3	85.6	2.1	6.0
Sulfamethoxazole	10	91.6	5.3	7.6	77.6	3.3	13.2	91.6	15.1	17.8	90.1	6.5	10.9	85.1	5.7	18.6
	100	86.5	4.5	8.5	83.8	4.0	4.2	95.8	5.7	13.7	86.9	3.1	7.2	91.6	3.5	8.0
Florfenicol	10	100.5	9.2	17.3	77.5	8.6	17.8	109.7	19.2	22.6	75.1	13.7	18.9	79.2	23.8	26.3
	100	85.9	6.9	7.7	80.1	4.6	11.2	93.4	6.4	8.3	87.5	4.0	10.2	84.0	7.2	7.8
Oxfendazole	10	84.6	5.9	11.1	75.2	10.0	14.3	90.5	7.3	14.0	89.3	6.3	9.4	89.3	4.5	12.2
	100	85.8	2.9	6.2	80.6	2.8	8.0	95.9	2.6	11.8	84.7	2.4	6.2	88.2	2.1	9.0
Oxolinic acid	10	80.0	2.0	4.0	35.4	3.3	7.9	20.3	8.7	55.9	55.9	5.5	15.0	45.1	4.1	17.7
	100	83.2	2.6	5.4	36.4	5.7	11.2	14.7	8.9	47.8	52.4	2.9	4.0	44.8	1.7	10.7
Sulfaquinoxaline	10	92.3	3.5	11.7	77.5	7.9	8.8	91.5	10.7	10.9	92.8	2.7	13.4	94.6	7.7	18.6
	100	95.9	2.9	8.9	82.8	3.5	6.0	95.2	3.1	11.9	90.4	4.5	5.2	94.9	2.3	8.6
Sulfadimethoxine	10	87.1	3.0	5.7	81.8	4.7	6.0	89.9	7.3	9.4	91.7	5.0	12.9	91.5	4.4	21.1
	100	94.0	2.1	5.8	88.5	5.6	6.0	97.1	5.6	10.5	90.3	1.9	4.4	98.7	1.0	8.5
Oxfendazole sulfone	10	88.8	7.3	11.0	82.6	7.0	13.3	98.3	3.7	5.0	94.3	4.3	11.1	89.6	4.9	17.0
	100	88.5	3.5	8.3	84.1	3.4	12.0	96.0	3.8	9.8	86.7	2.2	3.0	96.7	2.1	20.3
Nalidixic acid	10	75.8	2.8	3.9	29.5	4.2	12.3	17.3	7.3	61.8	47.7	4.0	16.8	37.3	5.5	10.0
	100	79.1	0.6	4.6	30.1	8.2	12.2	11.1	7.2	68.5	43.6	3.9	5.7	36.9	5.7	10.7

Table 4. Continued

Analyte	Fortified (µg/kg)	Milk			Salmon			Tiger shrimp			Red sea bream			Bastard halibut		
		Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)	Trueness* (%)	RSDr** (%)	RSDwr*** (%)
Flubendazole	10	86.3	5.4	11.8	81.4	3.7	6.8	94.2	4.2	9.0	91.4	2.9	9.3	76.9	6.0	11.8
	100	87.4	3.4	13.8	84.4	2.4	4.7	93.9	2.3	6.5	88.9	2.3	5.0	86.8	9.5	11.3
Fenbendazole	10	85.7	4.6	11.0	92.5	3.4	12.7	93.6	1.9	10.8	94.0	1.6	6.6	88.6	3.6	14.3
	100	89.0	3.5	5.0	90.0	6.6	12.7	95.1	2.3	9.6	90.5	0.6	3.7	99.6	20.5	23.8
Nicarbazin	10	82.2	2.3	4.2	79.1	9.7	10.8	98.1	4.9	16.2	87.8	6.6	8.8	80.2	10.5	10.8
	100	83.1	2.5	4.4	81.2	5.0	18.3	94.0	5.5	11.0	84.1	5.2	5.3	84.6	8.7	10.3
Triclabendazole sulfoxide	10	76.8	5.5	8.7	70.2	10.0	33.8	116.0	11.2	29.5	82.2	12.0	16.0	90.9	10.8	29.7
	100	92.9	7.0	9.2	79.7	7.2	19.1	85.1	10.4	17.8	78.1	4.0	7.4	94.6	7.4	19.6
Diazuril	10	83.2	10.2	14.2	86.8	11.8	23.2	93.6	10.9	35.2	83.5	21.2	24.4	86.4	18.9	25.3
	100	86.3	5.8	8.8	94.6	2.8	4.6	92.7	7.7	17.2	81.6	5.3	9.2	92.2	6.9	23.6
Triclabendazole sulfone	10	87.6	5.8	10.0	78.7	11.9	13.9	99.4	20.9	31.9	89.4	10.7	11.7	115.9	6.7	35.7
	100	88.4	2.0	6.9	91.5	11.4	16.7	90.9	7.3	15.1	85.1	9.0	10.1	104.0	4.3	20.8
Triclabendazole	10	89.0	6.4	9.0	91.3	7.1	17.8	100.2	2.1	6.2	108.1	18.6	20.9	89.1	14.5	31.0
	100	82.9	4.4	4.5	70.3	4.2	21.9	97.0	5.2	16.0	86.0	12.8	30.5	92.8	9.4	21.7
Decoquinat	10	90.4	1.4	5.6	79.4	5.0	8.2	107.5	2.2	13.6	123.3	4.9	5.1	93.0	2.7	13.3
	100	85.6	1.7	3.7	85.5	3.7	5.0	96.5	2.2	9.9	94.6	2.8	10.4	92.7	1.3	6.9
Eprinomectin	10	82.8	11.8	13.9	82.8	9.3	11.5	94.2	12.0	17.7	95.3	12.1	13.3	93.2	16.9	25.5
	100	90.4	4.7	6.4	90.2	3.5	11.7	105.5	7.1	11.7	107.2	4.5	9.2	106.2	5.6	8.8
Closantel	10	43.7	7.8	16.5	74.4	7.3	8.8	87.3	9.8	13.1	90.2	15.1	21.9	92.2	3.3	11.7
	100	56.3	5.6	22.0	78.9	3.5	4.3	92.5	4.6	10.8	82.9	6.9	17.2	89.5	2.9	8.0
Semduramicin	10	85.8	4.7	15.7	97.7	6.4	8.4	103.5	4.5	14.8	105.7	5.3	8.2	98.0	5.7	18.0
	100	88.0	1.6	7.9	90.5	1.9	5.4	97.7	2.8	6.7	93.3	5.6	8.7	93.4	3.5	10.7
Doramectin	10	86.1	10.7	15.1	90.6	7.5	11.0	102.6	10.3	11.8	100.5	26.0	29.2	90.6	23.6	26.0
	100	94.2	5.7	6.8	89.4	3.0	8.8	100.1	5.5	16.4	101.0	2.7	10.0	89.2	8.4	13.7
Lasalocid	10	65.8	7.2	8.2	69.5	4.7	11.9	90.7	5.5	15.0	80.4	8.8	12.3	85.1	4.6	8.2
	100	75.4	2.5	6.5	72.9	4.3	4.7	85.2	1.5	6.3	80.6	5.3	9.7	79.5	3.5	11.2
Moxidectin	10	89.0	11.3	27.1	87.9	7.7	11.5	106.8	6.2	8.6	104.4	5.1	14.0	91.0	9.5	15.0
	100	82.3	3.1	10.1	89.8	2.9	3.9	102.4	2.9	6.6	91.6	3.3	10.5	88.8	1.7	11.4
Ivermectin	10	92.4	2.5	18.2	98.4	15.1	16.2	111.6	6.1	14.9	93.5	15.7	18.1	96.7	8.1	18.7
	100	86.9	3.4	3.5	92.0	10.3	12.0	100.5	2.9	10.6	93.2	4.3	9.3	91.7	2.0	10.5
Maduramicin	10	81.4	5.0	7.1	95.8	5.8	20.4	98.6	2.2	17.9	105.0	6.2	13.1	97.5	3.8	21.2
	100	83.0	1.7	12.2	92.7	4.4	4.7	94.8	1.6	6.5	90.1	3.7	3.7	91.9	1.3	9.2
Salinomycin	10	85.3	2.1	10.7	84.2	6.5	7.3	98.5	2.3	14.7	93.6	6.6	15.2	96.3	4.2	21.2
	100	84.8	3.5	4.8	89.7	2.8	14.4	92.5	2.0	7.8	84.6	3.9	4.6	89.6	1.7	8.4
Monensin	10	84.4	4.2	9.9	88.1	7.6	14.5	103.2	4.5	14.1	93.2	4.5	17.1	100.0	2.8	16.9
	100	82.1	3.4	7.0	93.8	2.7	3.2	93.6	3.1	7.9	86.8	4.6	5.9	90.3	3.1	8.7
Narasin	10	84.5	5.3	10.3	89.7	9.6	18.6	100.3	3.6	11.7	96.5	1.6	12.4	97.8	3.0	15.1
	100	87.2	3.4	8.8	97.2	2.8	16.0	93.3	0.8	7.5	87.0	2.0	3.4	88.8	0.5	8.4

Did not meet the criteria of the guideline

— Not recovered at all

* The mean recovery rates

** RSD of repeatability

*** RSD of within-laboratory reproducibility

Table 5. Validated method and LOQ of each analyte

Analyte	Milk		Salmon		Tiger shrimp		Red sea bream		Bastard halibut	
	Validated method	LOQ (µg/kg)	Validated method	LOQ (µg/kg)	Validated method	LOQ (µg/kg)	Validated method	LOQ (µg/kg)	Validated method	LOQ (µg/kg)
Clopidol	A, B	2	—	—	B	2	A, B	4	B	4
5-Hydroxythiabendazole	A, B	10	—	—	B	10	B	10	B	10
Levamisole	A, B	4	A, B	4	A, B	4	A, B	4	A, B	4
5-Propylsulfonyl-1 <i>H</i> -benzimidazole-2-amine	A, B	2	B	2	B	2	B	4	B	2
Sulfathiazole	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Trimethoprim	A, B	1	—	—	A, B	1	A, B	1	A, B	1
Ciprofloxacin	—	—	A	10	—	—	A	4	A	10
Danofloxacin	—	—	A	10	A	10	A	10	—	—
Ormethoprim	A, B	1	—	—	A, B	1	A, B	1	A, B	1
Sulfamerazine	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Enrofloxacin	A	1	A	2	A	1	A	1	A	2
Thiabendazole	A, B	4	A, B	2	A, B	2	A, B	2	A, B	4
Sarafloxacin	—	—	A	10	A	2	A	2	—	—
Sulfadimidine	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Sulfamonomethoxine	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Pyrimethamine	A, B	1	B	1	B	1	A, B	1	A, B	1
Sulfamethoxazole	A, B	2	B	2	A, B	2	A, B	2	A, B	2
Florfenicol	B	4	A, B	4	B	2	A, B	4	A, B	4
Oxfendazole	A, B	1	B	1	A, B	1	A, B	1	A, B	1
Oxolinic acid	A, B	2	A	2	A	1	A	1	A	2
Sulfaquinoxaline	A, B	2	A, B	1	A, B	1	A, B	1	A, B	1
Sulfadimethoxine	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Oxfendazole sulfone	A, B	1	A, B	1	A, B	1	A, B	1	A	1
Nalidixic acid	A, B	2	A	2	A	2	A	1	A	2
Flubendazole	A, B	1	B	1	A, B	1	A, B	1	A, B	1
Fenbendazole	A, B	1	B	1	A, B	1	A, B	1	A	1
Nicarbazin	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Triclabendazole sulfoxide	A, B	4	A	4	B	2	A, B	2	A, B	4
Diclazuril	A, B	4	A, B	2	A	2	A, B	2	—	—
Triclabendazole sulfone	A, B	1	A, B	1	A	1	A, B	1	A	1
Triclabendazole	A, B	4	—	—	A, B	4	—	—	—	—
Decoquinate	A, B	1	B	1	A, B	1	A	1	B	1
Eprinomectin	B	2	B	2	B	4	A, B	2	B	2
Closantel	A	1	A, B	1	A, B	4	A, B	2	A, B	1
Semduramicin	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Doramectin	B	2	B	8	A, B	4	—	—	A, B	2
Lasalocid	A	1	—	—	B	1	B	1	B	1
Moxidectin	B	2	B	1	A, B	1	B	1	A, B	1
Ivermectin	B	2	B	10	B	10	A, B	10	B	10
Maduramicin	A, B	1	A, B	1	A, B	1	B	2	A, B	1
Salinomycin	B	4	B	4	B	1	A, B	1	A, B	2
Monensin	A, B	1	A, B	1	A, B	1	A, B	1	A, B	1
Narasin	A, B	1	A, B	1	B	1	A, B	1	A, B	1

— Did not meet the criteria of the guideline with both methods, so LOQ was not evaluated.

Quantification

Calibration curves were obtained from the matrix-matched calibration samples. Blank samples were prepared as described 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 the matrix-matched standard solutions were 1, 2, 4, 10, 20, 25, 50, 100 µg/L.

Performance characteristics

The accuracy of the method was evaluated according to the “Guidelines for the Validation of Analytical Methods for Residual Agricultural Chemicals in Food” notified by the Ministry of Health, Labour and Welfare in

Japan. The trueness, repeatability and within-laboratory reproducibility were determined by fortified recovery tests using two different concentrations (10 and 100 µg/kg), performed with two samples per day on five different days. The obtained results were evaluated by one-way analysis of variance. Selectivity was determined by analyzing blank samples. The limits of quantification (LOQs) were estimated by analyzing blank samples fortified at the levels of 0.25, 0.5, 1 and 2.5 µg/kg, and the lowest concentration of an analyte for which the signal-to-noise ratio was over 10 was determined.

Results and Discussion

Analytical procedures, LC-MS/MS conditions and extraction processes basically followed those of previous

studies¹¹⁾. The target analytes were selected in accordance with the inspection items of Tokyo Metropolitan Institute of Public Health. However in the extraction process for milk analysis, the addition of water to the sample was skipped because the recovered amount of extraction solvent and the recoveries of each analyte were almost equal to those obtained when water was added.

Matrix effects were studied by comparing the peak areas of 25 µg/L matrix-matched standard solutions versus solvent standard solutions (Table 2). It had been considered that a large number of analytes could be quantified by the use of external calibration curves. However, matrix-matched calibration curves were adopted due to the fact that some analytes were greatly affected by matrices derived from food samples. Specifically, levamisole, ormethoprim, oxfendazole sulfone, 5-propylsulfonyl-1*H*-benzimidazole-2-amine, pyrimethamine, sulfadimidine, sulfamerazine, sulfamonomethoxine, sulfaquinoxaline, thiabendazole and trimethoprim were only slightly affected by each matrix derived from food samples in both Method A and B. In contrast, fluoroquinolones (especially ciprofloxacin, danofloxacin and sarafloxacin) and macrolide antiparasites (especially doramectin, eprinomectin and moxidectin) were greatly affected and enhanced in both Methods A and B. Polyethers (especially maduramicin, salinomycin and semduramicin) tended to be suppressed by food matrices in Method A.

To validate the method, recovery tests using both Methods A and B were performed in accordance with 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 0.991. In Method A, the number of analytes that satisfied the criteria of the guidelines was 33 out of 43 in milk, 25 in salmon, 30 in tiger shrimp, 36 in red sea bream and 32 in bastard halibut. In Method B, the results were 37 analytes in milk, 30 in salmon, 35 in tiger shrimp, 34 in red sea bream and 32 in bastard halibut. The pattern of recovery rates was similar to that in the previous study¹¹⁾. Specifically, fluoroquinolones were not sufficiently recovered even when Method A was used, while polyethers and macrolides were well recovered using Method B. This indicates that this QuEChERS-based methodology is not suitable for analyzing amphoteric and highly polar compounds such as fluoroquinolones and antibiotics^{11), 12)}. This is because those compounds are much more soluble in water than acetonitrile, even though sodium chloride is used for salting out.

The selectivity of each analyte was confirmed by analyzing blank samples. There were no interfering peaks or peaks over one-third of MRL at the same retention times as the target analytes.

The estimated LOQs are shown in Table 5. The values are those of the methods that exhibited higher recoveries. They were lower than or equal to the MRL in each case.

Conclusion

In this study, the rapid analysis of multi-class residual veterinary drugs in milk, fish and shellfish was validated according to Japanese validation guidelines. Analytical procedures were performed using LC-MS/MS, and it took only 18 min to analyze 43 analytes. Sample preparation procedures were adopted from the QuEChERS methodology using two extraction solvents; Method A, which uses 1 vol% formic acid in acetonitrile, provided higher recoveries of quinolones, and Method B, which did not include acid, was used for polyethers and macrolides. Using these methods, the numbers of analytes that were validated in accordance with Japanese guidelines were 40 analytes out of 43 in milk, 37 in salmon, 42 in tiger shrimp, 41 in red sea bream and 39 in bastard halibut.

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